

THE INFLUENCE OF FERTILISER NITROGEN ON SOIL NITROGEN AND ON
THE HERBAGE OF A GRAZED KIKUYU PASTURE IN NATAL.

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ABSTRACT

The work reported in this thesis was designed to develop a better understanding of the fate of fertiliser nitrogen applied to a tropical pasture under field conditions, with the eventual objective of improving the economy of livestock production off such pastures. This involved an examination of the concentrations of soil total nitrogen, ammonium nitrogen and nitrate nitrogen at different depths within the soil profile following the application of different levels of fertiliser nitrogen to a grazed kikuyu (Pennisetum clandestinum) pasture, as well as the influence of such applications on pasture yield and some elements of pasture quality. The trial was conducted over a two year period at Broadacres in the Natal Mistbelt.

A labelled $^{15}\text{NH}_4\text{NO}_3$ fertiliser experiment was also conducted to ascertain how the labelled ammonium ion moved through the soil, roots and herbage after being applied in spring onto a kikuyu pasture.

In the absence of fertiliser N, a total of 15.45 t/ha of soil N was recorded at an average concentration of 0.15%. More than 30% of the soil total N was, however, situated within the top 10cm of soil. Organic matter (OM) content in the top 0-10cm of the profile was high (4.75%), reflecting an accumulation of organic matter in this zone. However, as organic C (and thus C:N ratios) declined with depth, so too did soil total N concentration.

Not surprisingly, fertiliser N applications did not measurably increase soil total N, but indirectly may have

affected soil N dynamics by increasing net mineralisation (due to its "priming" effect) thereby stimulating plant growth and thus increasing the size of the organic N pool through greater plant decay.

Total soil N concentration did not change significantly from the first to the second season. This could be attributed to the fact that N gains and losses on the pastures, being over 15 years old, were probably in equilibrium. Generally similar trends in soil total N down the profile over both seasons was further confirmation of this.

Before the application of any fertiliser, 331.9 kg $\text{NH}_4\text{-N}$ was measured in the soil to a depth of 1m, on average, over both seasons. This amount represented only 2.1% of the soil total N in the profile. The concentration of $\text{NH}_4\text{-N}$ followed a quadratic trend down the soil profile, irrespective of the amount of fertiliser N applied, with the largest concentrations accumulating, on average, in the 0-10cm and 75-100cm depth classes and lowest concentrations in the 50-75cm depth class. Laboratory wetting/drying experiments on soil samples collected from a depth of 75-100cm showed that $\text{NH}_4\text{-N}$ concentrations declined only marginally from their original concentrations. A high organic C content of 1.44% at this depth was also probable evidence of nitrification inhibition. Analysis of a similar Inanda soil form under a maize crop did not exhibit the properties eluded to above, suggesting that annual turn-over of the soil was causing mineralisation-immobilisation reactions to proceed normally.

Addition of fertiliser N to the pasture significantly

increased the amount of $\text{NH}_4\text{-N}$ over that of the control camps. Furthermore, the higher the application rate, the greater the increase in $\text{NH}_4\text{-N}$ accumulation within the soil profile. As N application rates increased, so the $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$ ratio narrowed in the soil complex. This was probably due to $\text{NH}_4\text{-N}$ being applied in excess of plant requirements at the high N application rates.

On average, 66.7 kg more $\text{NH}_4\text{-N}$ was present in the soil in the first season than in the second after fertilisation. Although this amount did not differ significantly from spring through to autumn, during early spring and late summer/autumn concentrations were higher than in mid-summer. Observed soil $\text{NH}_4\text{-N}$ trends were also very similar to the soil total N trends within both seasons, suggesting that soil total N concentrations might well play an important role in determining soil $\text{NH}_4\text{-N}$ concentrations.

Before fertilisation, only 45.6 kg $\text{NO}_3\text{-N}$, representing 0.29% of the soil total N, was on average, found in the profile to a depth of 1m. The highest concentration of $\text{NO}_3\text{-N}$ was lodged in the top 10cm of the soil. Nitrate-N declined, on average, with depth down the profile. However, during the second season, even though the concentration of $\text{NO}_3\text{-N}$ declined down the profile, it increased with depth during relative to that of the first season, suggesting the movement of $\text{NO}_3\text{-N}$ down the profile during this period.

Fertilisation significantly increased the concentration of $\text{NO}_3\text{-N}$ above that of the control camps. Concentrations increased as fertiliser application rates increased, as did $\text{NO}_3\text{-N}$ concentrations with depth. This has important implications

regarding potential leaching of $\text{NO}_3\text{-N}$ into the groundwater, suggesting that once applications reach levels of 300 kg N/ha/season or more, applications should become smaller and more frequent over the season in order to remove this pollution potential.

On average, 94.3 kg $\text{NO}_3\text{-N}$ /ha was present down to a depth of 1m over both seasons. However, significantly more $\text{NO}_3\text{-N}$ was present in the second season than in the first. This result is in contrast to that of the $\text{NH}_4\text{-N}$, wherein lower concentrations were found in the second season than in the first.

No specific trends in $\text{NO}_3\text{-N}$ concentration were observed within each season. Rather, $\text{NO}_3\text{-N}$ concentrations tended to vary inconsistently at each sampling period. Nitrate N and ammonium N concentrations within each month followed a near mirror image.

A DM yield of 12.7 t/ha, averaged over all treatments, was measured over the two seasons. A progressive increase in DM yield was obtained with successive increments of N fertiliser. The response of the kikuyu to the N applied did, however, decline as N applications increased.

A higher yield of 1.8 t DM/ha in the first season over that of the second was difficult to explain since rainfall amount and distribution was similar over both seasons.

On average, 2.84% protein N was measured in the herbage over both seasons. In general, protein N concentrations increased as N application rates increased.

On average, higher concentrations of protein-N were measured within the upper (>5cm) than in the lower (<5cm) herbage stratum, irrespective of the amount of N applied.

Similar bi-modal trends over time in protein-N concentration were measured for all N treatments and within both herbage strata over both seasons, with concentrations tending to be highest during early summer (Dec), and in early autumn (Feb), and lowest during spring (Oct), mid-summer (Jan) and autumn (March). Spring and autumn peaks seemed to correspond with periods of slower growth, whilst low mid-summer concentrations coincided with periods of high DM yields and TNC concentrations.

The range of $\text{NO}_3\text{-N}$ observed in the DM on the Broadacres trial was 0.12% to 0.43%. As applications of fertiliser N to the pasture increased, $\text{NO}_3\text{-N}$ concentrations within the herbage increased in a near-linear fashion.

On average, higher concentrations of $\text{NO}_3\text{-N}$, irrespective of the amount of fertiliser N applied, were measured within the upper (>5cm) than the lower (<5cm) herbage stratum.

A similar bi-modal trend to that measured with protein-N concentrations was observed in both seasons for $\text{NO}_3\text{-N}$ in the herbage. High concentrations of $\text{NO}_3\text{-N}$ were measured during spring (Nov) and autumn (Feb), and lower concentrations in mid-summer (Dec & Jan), very early spring (Oct) and early autumn (March). During summer, declining $\text{NO}_3\text{-N}$ concentrations were associated with a corresponding increase in herbage DM yields.

A lack of any distinctive trend emerged on these trials in the response of TNC to increased fertilisation with N suggests that, in kikuyu, applied N alone would not materially alter TNC concentrations.

Higher concentrations of TNC were determined in the lower (<5cm) height stratum, on average, than in the corresponding

upper (>5cm) stratum. This may be ascribed to the fact that TNCs tend to be found in higher concentrations where plant protein-N and $\text{NO}_3\text{-N}$ concentrations are low.

A P concentration of 0.248% before N fertilisation, is such that it should preclude any necessity for P supplementation, at least to beef animals. Herbage P concentrations did, however, drop as N fertiliser application rates were increased on the pasture, but were still high enough to preclude supplementation.

Even though no significant difference in P concentration was measured between the two herbage strata, a higher P content prevailed within the lower (<5cm) herbage stratum.

On average, 2.96% K was present within the herbage material in this trial. The norm for pastures ranges between 0.7 and 4.0%.

On these trials, applications of fertiliser N to the camps did not significantly affect K concentrations within the herbage.

The lower (<5cm) herbage stratum, comprising most of the older herbage fraction, was found to contain the highest K concentration in the pasture.

The presence of significantly (although probably biologically non-significantly) less K within the herbage in the second season than in the first may be linked to depletion of reserves of this element in the soil by the plant and/or elemental interactions between K and other macro-nutrients.

An average Ca content of 0.35% within the herbage falls within the range of 0.14 to 1.5% specified by the NRC (1976) as being adequate for all except high-producing dairy animals.

Increasing N application rates to the pasture increased the

Ca content within the herbage.

No significant differences in Ca concentration were found between the upper (>5cm) and lower (<5cm) herbage strata over both seasons, even though the lower stratum had a slightly higher Ca concentration, on average, than the upper stratum.

Calcium concentrations did not vary between seasons, probably because concentrations tend rather to vary according to stage of plant maturity, season or soil condition. However, higher concentrations of the element were measured in the second season than in the first. The reason for this is unknown.

On average, 0.377% Mg was present within the herbage over both seasons. This compares favourably with published data wherein Mg concentrations varied from 0.04 to 0.9% in the DM, with a mean of 0.36%.

All camps with N applied to them contained significantly more Mg in their herbage than did the material of the control camps.

On these trials, the Ca:Mg ratio is 0.92:1, which is considered to be near the optimum for livestock and thus the potential for tetany to arise is minimal.

Magnesium concentrations remained essentially similar within both herbage strata, regardless of the rate of fertiliser N applied.

As in the case of Ca, Mg concentrations within the herbage were significantly higher in the second season than in the first.

Calcium:phosphate ratios increased, on average in the herbage, as N application rates increased. This ratio was high in spring, dropped off in summer and increased again into autumn,

suggesting that the two ions were following the growth pattern of the kikuyu over the season.

The K/Mg+Ca ratios were nearly double that of the norm, suggesting that the pasture was experiencing luxury K uptake which may be conducive to tetany in animals grazing the pasture. This ratio narrowed as N application rates were increased, probably as a result of ion dilution as the herbage yields increased in response to these N applications. The ratio was low in spring (October), but increased to a peak in December, before declining again to a low in March.

DECLARATION

I hereby declare that the research work in this thesis is my own original work except for assistance which is acknowledged, or where due reference has been made in the text.

A handwritten signature in dark ink, appearing to read 'G.D. Hefer.' with a stylized, cursive script.

G.D. Hefer.

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GENERAL INTRODUCTION

With the population explosion, and associated decrease in agricultural land area per unit of population, man has been confronted with a major shortage of plant products, not only to feed himself, but also to maintain his animals. In order for him to alleviate this problem, he has attempted to develop a better understanding of the principles involved in plant nutrition. In South Africa the problem of feed shortages for livestock is compounded by the fact that the veld of high rainfall areas is "sour" and so does not produce useful feed for livestock during the winter months. Tropical pastures have been seen as a means of alleviating this problem and today more than 30 000 ha of land is under kikuyu grass (Pennisetum clandestinum) in Natal alone. This makes it the most important summer pasture species in the Province (Dept. of Agriculture and Fisheries, 1981).

Historically, heavy fertilisation of these tropical pastures has been practised in order to increase herbage yields, and thus animal production per hectare. This practice was, prior to the 1970's, economically viable, albeit wasteful, but the energy crisis of the 1970's caused farmers to reappraise this management practice. Unfortunately, little was known about the fundamental principles of fertiliser use and uptake by tropical species in South Africa at the time of this crisis. Farmers were forced to apply their own management experience in order to decrease their input costs.

Inflation and the high cost of living resulted in farmers having to become more astute in terms of their financial management, especially in the light of large amounts of N being applied to pastures, in order to maintain or increase farm returns. Currently, recommended applications of fertiliser for an average yield target of 10 t/ha are 50 kg N/ha for dryland kikuyu establishment and 225 kg N/ha/ann for pasture maintenance. At an N cost of R2.08 per kg, limestone ammonium nitrate (LAN 28) fertiliser now costs the farmer R583.00 per ton (Anon. 1993). At this price, fertiliser costs attributed to dairy farmers alone currently account for almost 15% of their production costs (Whitehead N. Pers. Comm. 1991. Dept. of Agric. Pvt. Bag X9059 Cedara).

Tropical pastures, and their performance under different fertiliser regimes, need to be researched under field conditions in South Africa to allow farmers to identify ways of lowering their costs by utilising fertiliser more efficiently. To do this requires an understanding of the processes involved. What happens to fertiliser nitrogen when it is applied to a pasture? How much of it is likely to be volatilised? What is its pattern of movement down the soil profile? How much is taken up by plants and how much is leached through the soil profile? Is fertiliser being wasted in agricultural systems? What is the environmental impact associated with any perceived fertiliser wastage? Answers to these questions will assist in the design of nitrogen fertiliser regimes which will hopefully lead to an improvement in the efficiency of nitrogen use and at the same

time also reduce the pollution hazard associated with the wasteful use of nitrogen fertilisers.

Current knowledge regarding N dynamics, both within the soil and the plant, has to a large extent been limited to temperate species growing in temperate environments. Little data exist on N dynamics associated with temperate pastures in South Africa, and even less are available on tropical perennial species.

Plants can effectively utilise both nitrate N and ammonium N in the soil. The ammonium N is derived largely from mineralisation of the soil organic matter or by addition of ammoniacal fertilisers or urea, and is readily oxidised by soil micro-organisms to nitrate N by nitrification reactions. However, soil colloids tend to bind the ammonium ion, making it less directly available for plant uptake. Nitrate N is not bound to the clay particles, and therefore is the predominant form of nitrogen available to pasture plants for normal plant metabolism.

Losses of both nitrate N and ammonium N within the soil complex do arise in a number of ways. Potential pathways of N loss include those of leaching, volatilisation and denitrification. Nitrate N, because it is not bound to soil colloids, is more susceptible to leaching than is ammonium N. Recent work by Mckenzie (1991) has shown that losses through volatilisation of fertiliser nitrogen (LAN and urea) from kikuyu pastures, at least under the conditions under which he worked, were generally less than 5% of the nitrogen applied. Denitrification, the process

whereby nitrate N is transformed into N_2 and nitrous oxide, also a potentially important pathway for N loss, was initially thought to occur only in waterlogged soils. However, large N_2O fluxes from apparently well aerated soils can be attributed to anoxic microsites which could regulate the relative concentrations of NO_3^- and NH_4^+ bacteria, and thus the mineralisation-immobilisation reactions in these regions (Davidson *et al.* 1990; Sierra, 1992; Vermes & Myrold, 1992).

Leaching, therefore, would seem to be the most likely process leading to nitrogen loss from pastures. Such loss will be determined by the pattern of movement of nitrogen through the soil profile and the extent to which this is adsorbed by soil colloids or taken up by plants. Clearly, it is the efficiency of use by the plant of the applied nitrogen which determines the biological efficiency of the practice, whether this uptake is directly from the nitrogen applied as fertiliser or indirectly from the nitrogen, derived from that fertiliser, which is subsequently returned in the excreta of animals grazing the pasture.

Nitrogen is usually, but not exclusively, taken up by the plant in the NO_3^- ion form as this is usually the most readily available supply of N within the soil profile. Once taken up, NO_3^- ions as well as any NH_4^+ ions present within the herbage are converted, via specific catalytic pathways, into compounds such as amide and amino nitrogen, protein nitrogen and carbohydrates. Excess NO_3^- may be stored in the plant. These fractions are used by the

plant for growth, and are important constituents in a grazing animal's diet. Carbohydrates, and more specifically non structural carbohydrates, are also important nutrients regulating plant growth and development, especially when under stress. Moreover, concentrations of these nutrients seem to be linked to concentrations of protein N and nitrate N within the plant.

Historically, the nitrogen relationships of pastures have been determined from cutting trials where between 55 and 80% of the applied N has been recovered from the material harvested (Ball & Ryden, 1984). The relatively high apparent efficiency of N usage measured in the herbage suggests an efficient uptake of fertiliser N by the plant. As previously mentioned, grazing animals may increase this efficiency further by recycling the applied nitrogen. The pathways associated with N use and nitrogen loss are therefore very different in cut and grazed swards. For example, 75-90% of the N consumed by animals may be returned to the sward by way of excreta (Ball & Ryden, 1984). This return would enable some of the applied fertiliser N to be utilised more than once during a growing season, so that it is likely that the overall response to fertiliser N might be considerably greater under grazing than under a "controlled removal" regime. However, recirculation of N by grazing animals may involve large losses, and it is speculated that perhaps no more than about 40% of the nitrogen contained in animal excreta is again made available for plant use. However, data for tropical pastures under grazing are limited and such measurements need to be made under field conditions appropriate to farming conditions.

Another factor frequently affected by fertiliser N application to pastures is the availability of macronutrients. This is particularly important in kikuyu whose herbage, even when grown on soils with an abundant supply of macronutrients, provides an imbalanced diet to animals. Because of this, it is important to understand the effects of fertiliser application upon these nutrients under grazing, as these effects may result in decreased animal performance, either as a result of reduced intake because of the presence of excessively high concentrations of such macronutrients in the plant, or because of an imbalance of the concentrations of the different macronutrients. Research will hopefully clarify the influence of applying N to the pasture on the macronutrient content of the herbage produced.

The questions that this work then hopes to address include the following :

1. what is the influence of applying nitrogen fertiliser on the concentrations of total soil N, soil ammonium N and soil nitrate N at various depths within the soil profile, and what is the effect of varying nitrogen application rates on these concentrations?
2. what are the concentrations of protein N, nitrate N and carbohydrate in the herbage, and what is the effect of varying N application rates on these concentrations, and on dry matter yields?

- 3 does the application of fertiliser N to the pasture influence the relative concentrations of the macronutrients within the herbage over the season, and if so, what effect might this have on animal health and productivity?

The successful appraisal of these questions, amongst others, will enable researchers in future to better understand the role which applied fertiliser nitrogen plays in tropical grassland systems subjected to grazing. These answers will help one gain an insight into some of the mechanisms involved in the dynamics of N in pastures, thereby assisting researchers in their task of providing useful and practical management recommendations to the farmer; whether this is to maximise the efficiency of use of applied N, or to minimise adverse environmental effects.

1. STUDY METHODOLOGY AND LABORATORY TECHNIQUES

1.1 Introduction

In this chapter, the layout of the trial, the materials utilised and the methods adopted are outlined. In addition, laboratory techniques used in determinations are documented.

Considerations in arriving at the investigative techniques were ease of management, cost effectiveness, simplicity, reliability and availability at the institution. Any perceived problems and/or shortcomings of each technique were identified, and the best precautions to limit error, as a result of these perceived inadequacies, were taken. Unless otherwise stated, all analyses were performed by the author and staff at Cedara Agricultural College.

1.2 Methods and materials

1.2.1 Site

The initial trial, as well as a subsequent labelled $^{15}\text{NH}_4\text{NO}_3$ trial (see Ch. 1.6) were conducted on Broadacres, which is part of the Cedara Research Station, situated some 15 km north west of Pietermaritzburg. Located at $29^{\circ}32'S$ and $30^{\circ}17'E$ and at an altitude of approximately 1 150m above sea level, the area has a mean annual rainfall (72 years) of 877mm and an annual evaporation of 1577 mm. A plan of Broadacres, indicating the

experimental area, is presented in Fig. 1. The whole farm is situated, according to Acocks (1988), in bioclimate region 3, the Natal Mist-belt. The experiment was situated on an east facing slope of 10 to 15%. The initial trial was conducted over two seasons¹ (1988-1990), and the labelled $^{15}\text{NH}_4\text{NO}_3$ over one season (1991/92).

The investigation was conducted on a dryland kikuyu (Pennisetum clandestinum) pasture that had been established approximately fifteen years previously (Fig. 1). Originally, the pastures were used as night camps for dairy herds. After acquisition of the farm by Cedara, the camps were used for research work and for fattening beef animals.

The soil on the experimental area is of the Inanda form (MacVicar, 1991). Soil particle size analysis, determined by the pipette method and ultrasound (Gee & Bauder, 1986), indicated that the soil had a clayey texture (74% clay on average to a depth of 1m, but increasing from 68% in the 0-10cm depth class to 82% in the 75-100cm depth class). Organic carbon was analysed by the Walkley-Black procedure which measures the readily oxidisable organic carbon within the soil (Walkley & Black, 1934). On average, the soil contained 2.27% organic carbon down to a depth of 1m (Table 1). However, organic carbon content

¹Season, as defined in the context of the thesis (and as commonly used in South Africa), corresponds to the growing period of a kikuyu pasture during summer. Kikuyu becomes dormant during winter. For the purpose of these trials then, the term "season" encompasses the months from September through to April, respectively.

declined sharply with depth.

Soil chemical analyses (Fertiliser Advisory Service, Cedara) revealed that the pH (KCl) increased from 4.63 at a depth of 0-10 cm to 5.61 at a depth of 75-100cm. Concentrations² of "available" phosphorus (P) were highest in the top 10 cm, and decreased with increasing depth. Exchangeable potassium (K) and calcium (Ca) levels decreased to a depth of 50 to 75 cm, but tended to increase at a greater depth (Table 1), although this increase was marginal for Ca. (In the light of these analytical data, no maintenance P and K were applied to the experiment since levels of these nutrients were deemed to be adequate). Soil sample bulk densities were also measured by the Fertiliser Advisory Service, Cedara, and for the purpose of this investigation, an average bulk density of 1.0 was assumed (Manson A.D. 1991. Pers. comm. Dept. Agric. Development, Cedara. Pvt. Bag X9059 RSA).

²Concentration and content, in their definitions and context of this thesis, should be taken to be synonymous with each other.

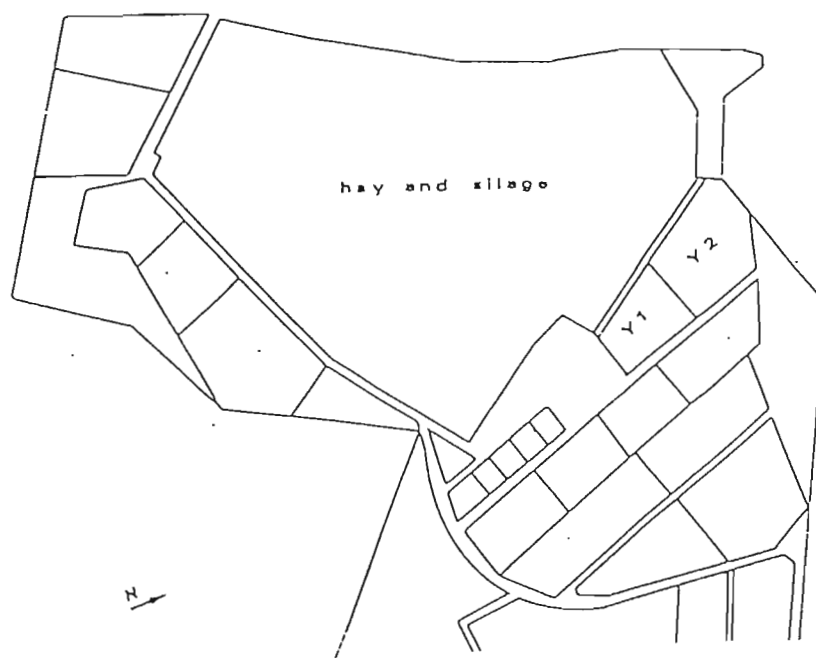


Figure 1. Diagrammatical representation of the experimental area on Broadacres (after Bartholomew, 1985). (Y1 & Y2 = trial camps).

Table 1. Selected physical and chemical properties over the experimental site (samples collected prior to the commencement of experimentation).

Soil depth (cm)	P	K (mg/l)	Ca	Mg	pH (KCl)	Clay %	Org. C (%)	Total N (%)	C:N ratio
0-10	12.0	332	1518	615	4.63	68	4.57	0.471	9.7
10-25	2.0	186	1499	603	4.85	70	2.16	0.222	9.7
25-50	0.5	74	1071	575	4.99	73	2.03	0.142	14.2
50-75	0.2	43	786	581	5.26	78	1.17	0.081	14.4
75-1m	0.3	123	827	586	5.61	82	1.44	0.072	20.0

1.2.2 Layout and design

The trial was laid out in two blocks (replications). Each was sub-divided into eight equal sized camps, with dimensions of 36m x 36m (Fig. 2). The upper four camps in both blocks (P0 to P3) were designated 'pre-treatment' camps. Each 'pre-treatment' and its corresponding 'treatment' camp (T0 to T3) were subjected to a specific fertiliser treatment. The aim of having 'pre-treatment' camps identical to the 'treatment' camps was to allow only excrement containing N representative of that specific treatment, to be passed onto the 'treatment' area.

REP 1				REP 2			
PRE-TREATMENT CAMPS							
P1	P3	P0	P2	P3	P1	P2	P0
TREATMENT CAMPS							
T1	T3	T0	T2	T3	T1	T2	T0

P = pretreatment camps
 T = treatment camps
 0 = 0 kg N/ha/season
 1 = 150 kg N/ha/season
 2 = 300 kg N/ha/season
 3 = 450 kg N/ha/season

Camp dimensions 36m x 36m = 1296m²

Figure 2. Diagrammatical representation of the trial design on Broadacres.

Nitrogen was applied as limestone ammonium nitrate (LAN, 28%) at four rates: 0, 150, 300 and 450 kg N/ha/season. These amounts were applied in three equal split applications (0, 50, 100 and 150 kg N/ha/application) over the season (September, November and January), to both 'pre-treatment' and 'treatment' camps. All camps received fertiliser applications on the same date on each occasion. Fertiliser was applied by means of a tractor mounted 'spandicar' fitted with screens in order to prevent fertiliser contamination of adjacent camps.

1.2.3 Animals

For purposes outlined in the introduction, the trial was carried out under grazing, with beef animals being the grazers. No measurements were taken of animal performance, and stocking rate was gauged by the farm manager, according to area and the abundance of herbage present in each 'treatment' camp. The objective was to graze the camps to a uniform level (approximately 7-10 cm) within the specified time of occupancy. Because of the variable N rates imposed, herbage availability at the start of each grazing period was variable and therefore herd sizes varied across the trial camps at each grazing.

The movement of animals on and off the trial was strictly controlled. Animals were initially grazed in the 'pre-treatment' camps for three days in order to remove any trace of foreign nutrients from the digestive tract, and to adapt the animals to the specific treatment applying to that camp. The animals were

then moved to the 'treatment' camps for a further four days. They were then removed from the entire system for three weeks, before again being returned to the 'pre-treatment' camps for the next rotation. Fertiliser was applied, when appropriate, immediately the animals left the 'treatment' camps. This cycle proceeded for the duration of two consecutive grazing seasons, each season running from September to April, respectively.

1.3 Sampling

For the purpose of herbage and soil sampling, each 'treatment' camp was divided into three equal strata (each 432 m²) in order to ensure a representative coverage of the whole treated camp. In each stratum, sampling was carried out in a randomly selected square (1m x 1m), with new sampling squares being selected on each sampling date. One such square was chosen to be sampled from within each 432 m² stratum (ie 3 per 'treatment' camp) on each sampling occasion.

Sampling was performed at approximately monthly intervals (every 28 days) for the duration of the two growing seasons (Table 2). Sampling was carried out immediately before animals were introduced to the camps.

Two types of sampling were carried out within the selected squares of each stratum. Firstly, a herbage sample was removed in each of two height strata - that above 5 cm, and that from ground level to a height of 5 cm above ground level. These two

samples were kept separate from one another. The three separate samples for each height stratum were later combined in order to form a composite sample for each of the two height strata from each treatment. This procedure was repeated for each 'treatment' camp over both replications. Sampling was carried out at the same time during the morning on all occasions in order to reduce potential variability in the chemical composition due to fluctuations that may have been induced by diurnal changes in the environment.

Once the herbage samples had been removed, a hydraulic auger was employed to remove a soil core from the same sampling square to a depth of 1 m. The three soil cores, one from each stratum, and each measuring 5 cm in diameter, were then separated into five depth classes. These five depth classes were 0-10 cm, 10-25 cm, 25-50 cm, 50-75 cm and 75-100 cm. Composite samples for each depth class from the three sampling points within each 'treatment' camp were obtained once sampling was completed.

The final measurement carried out on each 'treatment' camp was that of herbage disc metering (Bransby & Tainton, 1977), in order to obtain a measurement of dry matter (DM) yields in the different treatments. To do this, 25 disc meter readings were taken over each 'treatment' camp in each replication, avoiding sampling points used for herbage and soil sampling. The disc meter readings (again 25 points) were repeated once the animals had been removed from the 'treatment' camps four days later, the purpose being to obtain an estimate of the amount of herbage

consumed.

Calibration of the disc meter was not necessary as Bartholomew (1985) had previously developed a calibration for kikuyu from some 9300 samples. The yield equation he developed is as follows:

$$y = 749.5 + 242.79 (\pm 10.37)d$$

where: y = pasture yield (kg DM/ha)
 d = disc meter height (cm)

($r = 0.816^{**}$ for 9300 disc meter calibration pairs (after Bartholomew, 1985)).

1.3.1 Sample analyses

Both the herbage and soil samples were removed from the field as soon after collection as possible. The herbage samples were oven dried for 24 hours in a convection oven at 70°C, and the soil samples air dried at room temperature in open trays.

After drying, the herbage and soil samples were milled through 1 mm sieves. Both sets of samples were then bottled and marked, ready for laboratory analysis. In all laboratory analyses, control ("known") samples were included in order to ensure a high standard of accuracy.

1.4 Herbage analyses

1.4.1 Nitrate N

Nitrate N in herbage was determined colorimetrically using the procedure of Cataldo *et al.* (1975) (see Ch. 3).

1.4.2 Protein N and total non structural carbohydrate determinations

Protein nitrogen and total non-structural carbohydrate (TNC) concentrations of the herbage were analysed using near-infrared reflectance spectrometry (NIRS) according to the method used by Norris et al. (1976), Schenk et al. (1979) & Day & Fearn, (1980) all in Eckard, (1986). The theory of operation of NIRS is basically the correlation of bond vibrational frequencies (ie N-H bond of ammoniated N) in the NIR region, to the known concentration of constituent in the sample. A regression analysis between the reflectance of a number of standards at various wavelengths, and the known concentration of protein N or TNC of the standards are measured to obtain regression coefficients for the sample (Day & Fearn, 1982).

1.4.3 Plant macro-nutrient analyses

Plant macro-nutrients (P, K, Ca and Mg) were determined for each date of sampling and each height stratum. The macro-nutrients were analysed at the South African Sugar Experimental Station at Mount Edgecombe using colorimetry (P) and atomic absorption techniques (K, Ca and Mg) (Burrows & Meyer, 1976; Anon. 1977; Wood et al. 1985) (see Ch. 5).

1.5 Soil analyses

1.5.1 Soil macro-nutrient analyses

In soil samples, Ca and Mg determinations were performed by

atomic absorption after extraction in 1 M KCl, using a soil solution ratio of 1:10 (v/v) and a stirring time (400 r.p.m.) of 10 min. Phosphorus and K were extracted in 0.25 M NH_4HCO_3 + 0.01 M NH_4F + 0.01 M EDTA at pH 8.0 (1:10 soil:solution; 10 min stirring time), with K being determined by flame emission and P colorimetrically (Miles, 1986).

1.5.2 Total soil N

Soil samples from all depths were analysed for total nitrogen using the AOAC (1980) modified Kjeldahl method which included the addition of zinc dust in order to allow for the complete reduction of NO_2^- and NO_3^- in the samples (Bremner & Yeomans, 1987, in Wilson, 1987) (see Ch. 2).

1.5.3 Inorganic soil N fractions

An autoanalyser was used in the determination of both soil ammonium and nitrate N after extraction with 1 M KCl (1:10 soil:solution ratio) (Willis & Gentry, 1987). In the case of nitrate determinations, a cadmium wire was used as the reducing agent (Milham et al. 1970; Willis & Gentry, 1987) (see Ch. 2).

1.6 Labelled ^{15}N analysis

The aim of this investigation was to determine and confirm the relative proportions of N in the roots, herbage and within different depth strata within the soil over a season after a

spring application of labelled ^{15}N ammonium nitrate fertiliser (see Ch. 4).

1.6.1 Plot dimensions

The ^{15}N study ran for one season, this being October 1992 through to March 1993, and was performed on the same kikuyu pasture as the initial trials. The trial consisted of four plots, each representing one replication of the treatment to be applied. The plots were circular in dimension, with a diameter of 1.1m. This resulted in an enclosed area of 0.950332m^2 . A circular metal sleeve of diameter 1.1m was driven into the soil to a depth of 30cm around the perimeter of each plot to lower the possibility of N loss through lateral movement, at least down to this depth. The possibility of going deeper was considered, but was constrained by the difficulty of embedding the sleeve without disturbing the profile. Each metal sleeve protruded 3cm above the soil surface to prevent surface flow of added ^{15}N , in the event of heavy rainfall. A 10cm border within each plot was excluded from sampling, but cognisance of this fact was taken into account in the calculations (see 2(a) in appendix 1 for details). The plots were also secured from animals by placing exclosures over them for the duration of this study.

A framework of 12 squares was placed over the circular plot on each sampling occasion. A random number, pertaining to the month of sampling (i.e. 1=October; 5=February) had earlier been allocated to each of the squares for each replication. Samples

corresponding to the particular month in question were removed from those squares allocated the same random number for that month (Fig. 3):

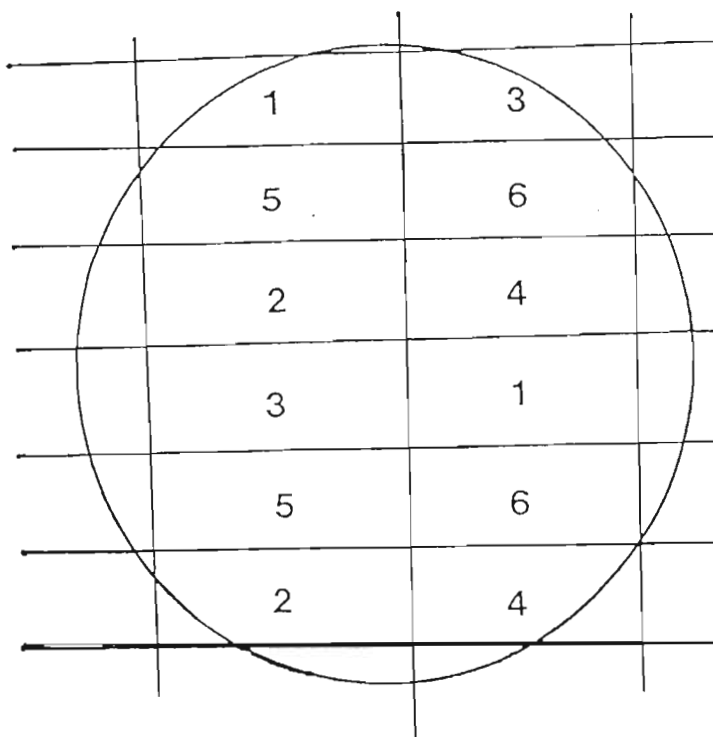


Figure 3. A diagrammatical representation of a circular plot, including its allocated random numbers.

1.6.2 Fertiliser applications

Singly labelled ammonium nitrate ($^{15}\text{NH}_4\text{NO}_3$), obtained from Premier Technologies, Cape Town RSA, was applied to all four replications at the start of the season i.e. October. Thereafter, non-labelled NH_4NO_3 was applied to all four replications in December and February.

Nitrogen was applied at a level equivalent to 225 kg N/ha/season, in three equal applications of 75 kg N/ha/dressing. Two forms of N were used on the plots, these being non-labelled NH_4NO_3 and

labelled $^{15}\text{NH}_4\text{NO}_3$. However, only the spring application of 75 kg N/ha contained ^{15}N . The N in the two subsequent dressings was not labelled. For full details of calculations pertaining to this trial, see Appendix 1.

1.6.3 Sampling procedure

Samples were taken from all plots at monthly intervals. These comprised two soil cores, root samples and herbage samples. The soil cores were then divided up into 3 soil depth classes, namely 0-30cm, 30-60cm and 60-100cm and air dried for one week. Roots were then separated from the dry soil samples with the use of sieves. Herbage and root samples were dried in a convection oven for one day at 70°C, before being milled to allow them to also pass through a 40 mesh sieve. Soil samples were then milled to the consistency of face powder.

The three samples collected from each of the soil depth classes, along with the two root and herbage samples, were then pooled to form composite samples³ for that month for each replication.

A Carlo Erba-NA 1500, coupled to a VG iso-mass SIRA series II

³Composite samples were deemed to give a sufficient level of precision, judging from preliminary work using a high performance liquid chromatogram (HPLC) (Bye, BSc Consultants-see acknowledgements). To confirm these results, an autoanalyser measured the presence of ammonia in the samples, as per 1.5.3 (Willis & Gentry, 1987). Nitrogen concentrations did not fluctuate to any extent within plots (as determined by their low CV%), leading one to conclude that within-plot variability was sufficiently low to warrant pooling the within-plot samples. (Note: the high cost of sample analysis also imposed a restriction on the number of samples submitted for analysis).

mass spectrometer was then used to determine the presence and concentration of the initially-labelled NH_4^+ ion over the season within all the samples being analysed. Sample preparation was via the Dumas combustion method, which ensured total catalysis and reduction of N fractions to pure N_2 . Principles elluding to the method of ^{15}N determination by mass spectrometer are documented in VG ISOGAS Application note No.1 and VG ISOGAS Application note No.3. The use of mass spectrometry in this trial was to endeavour to determine the recovery of ^{15}N (%) in the herbage, roots and soil of the pasture over the season.

Climatic data were also obtained for the duration of the trial (see Ch. 1.7 & Table 2a).

1.7 Meteorological data collection

All meteorological data used in this thesis were supplied by Cedara's Department of Meteorology. Data collected included temperature, rainfall and evaporation for the years 1988 to 1990.

Data for the 28 days (animals out for 21 days and in for 7 days) up to and including the day of sampling were calculated for temperature, rainfall and evaporation. These figures were determined at each sampling date for both seasons (Table 2).

Table 2. Rainfall, temperature and evaporation data for Cedara for both seasons over 28 days up to and including the day of sampling.

Sampling date	Total rainfall (mm)	Av. Temp. (°C)	Pan evap. (mm)
03/09/1988	22.7	21.3	116.7
31/10/1988	57.7	22.6	122.3
28/11/1988	38.1	22.0	144.0
26/12/1988	119.2	23.4	123.1
23/01/1989	233.7	23.1	113.4
20/02/1989	95.4	24.3	132.9
20/03/1989	669.8	23.9	114.5
03/09/1989	8.3	22.1	158.9
31/10/1989	33.7	22.3	148.0
28/11/1989	170.7	20.5	170.7
26/12/1989	206.9	23.4	136.5
23/01/1990	206.9	25.5	93.3
20/02/1990	41.2	25.4	134.2
20/03/1990	843.5	23.3	114.3

Table 2a. Climatic data for the labelled N trial in 1992/93 season.

Months	Total monthly rainfall (mm)	Mean max temperatures (°C)	Mean min temperatures (°C)
Oct	34.2	24.2	11.1
Nov	82.6	24.5	12.4
Dec	68.7	26.1	15.3
Jan	69.2	26.7	15.3
Feb	108.1	25.1	14.9
Mar	114.9	25.0	13.7

2. THE INFLUENCE OF FERTILISER APPLICATIONS ON NITROGEN FRACTIONS WITHIN THE SOIL PROFILE

2.1 Introduction

Environmental factors such as radiation, temperature and moisture often impose severe limitations on pasture yields. These influences may be direct, affecting plant growth per se, or indirect, for instance by moderating the dynamics of N in the soil-plant complex and thereby influencing plant growth. Because of this latter limitation, the farmer's most effective means of reliably increasing yield is to apply fertiliser nitrogen (N) to the system when conditions generally favour rapid growth (Prins & Arnold, 1980). It is precisely this input of fertiliser N to pastoral systems that has allowed the progressive intensification of this section of the agricultural industry (Dowdell et al. 1980, in Prins & Arnold, 1980).

Pastoral systems rely heavily on soil reserves to meet N requirements if adequate fertiliser N is not forthcoming. There is also some recycling of N from the excreta of grazing animals, but this appears to be rather inefficient because of "aggregation" and attendant N losses (Ball & Ryden, 1984). In the natural state, unfortunately, the N reserves in the soil are largely contained in the organic matter (OM), and are thus largely unavailable to plants. Even though total N in a pasture topsoil generally exceeds 5 000 kg/ha, only limited amounts are readily available for plant uptake in the mineral forms, nitrate (NO_3^-) and exchangeable ammonium (NH_4^+) (Stevenson, 1982). If high yields are to be maintained, it is necessary to either

supply readily-available mineral N to the system or, alternatively, to stimulate the release of N from the organic pool.

In most pastoral systems today it is customary to subsidise, in part, the organic N pool by using inorganic N fertilisers in order to increase yields (Jansson & Persson, 1982, in Stevenson, 1982). Traditionally, research into the effects of fertiliser N on pastures has concentrated largely on yield responses, with little effort being directed at aspects such as N transformations and losses. However, as indicated in the General Introduction, losses of N from pastoral systems may occur through a number of avenues. The purpose of the investigations discussed in this chapter was to study the distribution of organic (plant unavailable) and inorganic (plant available) N fractions in the soil profile, and to give an indication of the possible importance of leaching as a pathway for N loss under the various treatments. A study of this nature would hopefully complement the work done by McKenzie (1991), who found that volatilisation losses following N fertilisation were relatively insignificant on kikuyu pastures in this environment. The present investigation may also go some way to explaining N movement and utilisation, thereby enhancing our ability to increase the efficiency of fertiliser use.

In essence, therefore, the following question will be addressed in this chapter : what are the concentrations and amounts of soil total N, soil ammonium N and soil nitrate N at various depths

within the soil profile, and what is the effect of varying N fertiliser rates on these concentrations and amounts?

2.2 Methodology

Refer to Chapter 1 for methods pertaining to this chapter.

2.3 Results

2.3.1 Soil total N

An amount of 15.45 tons of total N/ha was present to a depth of 1 m (averaged over two seasons) in the control (unfertilised) camps (Table 3a & 3b). This was not distributed evenly throughout the soil profile, with 30.3% being lodged within the top 10 cm of the profile (Table 3b). The 0-10 cm depth class contained 0.471% total N (in both seasons) (Table 3a). Total N concentration declined, on average, to 0.077% at a depth of 75-100 cm. The N present in the 75-100cm depth class represented only 11.9% of the soil total N present in the soil profile. Averaged over both seasons, the concentration of N in the soil of the 0-10cm depth class was some 0.4% greater than that in the 75-100cm depth class (0.471% vs 0.077% N) in the control camps (Table 3b).

Although the influence of fertiliser N applications on soil total N was inconsistent, during the first season the application of 150 and 450 kg N/ha/season each increased the concentration of soil total N above that of the control camps and the camps treated with 300 kg N/ha/season (Table 3a). Further, the application of 150 kg N/ha in the first season resulted in an

increase in total N of 1400 kg/ha over that of the control. This aspect will be expanded upon in the discussion and conclusions.

In the second season, however, camps treated with 300 kg N/ha/season showed the largest increase in soil total N over all treatments, whilst in the camps to which 450 kg N/ha/season was applied, the total N concentration declined by 1.9 t N/ha in comparison to season 1. A similar, albeit slightly smaller concentration decline in total N occurred after an application of 150 kg N/ha (1.1 t N/ha) in the second season.

On average, 15.9 tons of N were measured over both seasons to a depth of 1m, averaged over all fertiliser N treatments (Table 3b). Soil total N, regardless of soil depth and the treatment applied, did not change significantly from the first to the second season. Furthermore, both seasons' data followed generally similar trends down the profile (Table 3a).

Table 3a. Effects of different fertiliser N rates on soil total N (%) within each depth class over two seasons. Bold figures represent the total amount of soil N (t N/ha) within each depth class. Data are averages from 7 samplings undertaken at monthly intervals during each of the 2 growing seasons. (Average soil density to a depth of 1m = 10^3 kg/m³).

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
Season 1						
0	0.471 4.7	0.222 3.3	0.142 3.5	0.081 2.0	0.072 1.9	0.154 15.4
150	0.509 5.0	0.247 3.6	0.157 3.9	0.094 2.4	0.072 1.9	0.168 16.8
300	0.479 4.8	0.251 3.7	0.142 3.5	0.081 2.0	0.081 2.0	0.160 16.0
450	0.509 5.0	0.247 3.6	0.157 3.9	0.094 2.4	0.072 1.9	0.168 16.8
Depth Means	0.492 4.9	0.241 3.6	0.149 3.7	0.087 2.2	0.074 1.9	16.3
%	30.0	22.0	22.6	13.4	11.6	
Season 2						
0	0.471 4.7	0.222 3.3	0.142 3.5	0.081 2.0	0.081 2.0	0.155 15.5
150	0.479 4.8	0.222 3.3	0.142 3.5	0.093 2.3	0.071 1.8	0.157 15.7
300	0.550 5.5	0.222 3.3	0.142 3.5	0.081 2.0	0.081 2.0	0.164 16.4
450	0.430 4.3	0.222 3.3	0.142 3.5	0.081 2.0	0.071 1.8	0.149 14.9
Depth Means	0.482 4.8	0.222 3.3	0.142 3.5	0.084 2.1	0.076 1.9	15.6
%	30.7	21.1	22.4	13.4	12.1	

LSDs for N (%) :

depth classes season 1	5%=0.010	1%=0.013
N levels within season 1	5%=0.011	1%=0.015
body of the table for season 1	5%=0.022	1%=0.031
depth classes for season 2	5%=0.009	1%=0.012
N levels within season 2	5%=0.010	1%=0.014
body of the table for season 2	5%=0.019	1%=0.028
season 1 vs season 2	5%=0.854	1%=0.946

Table 3b. Soil total N concentrations (%) and total amounts of soil N (t N/ha) meaned over both seasons and all depths.

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
0	0.471 4.7	0.222 3.3	0.142 3.5	0.081 2.0	0.072 1.9	0.154 15.4
150	0.495 4.9	0.234 3.4	0.149 3.7	0.093 2.4	0.071 1.8	0.162 16.2
300	0.514 5.1	0.236 3.5	0.142 3.5	0.081 2.0	0.081 2.0	0.162 16.1
450	0.469 4.7	0.234 3.4	0.149 3.7	0.087 2.2	0.071 1.8	0.158 15.8
Depth Means	0.487 4.9	0.231 3.4	0.145 3.6	0.086 2.1	0.073 1.9	15.9
%	30.3	21.6	22.5	13.4	11.9	

2.3.1.1 seasonal patterns of soil total N

2.3.1.1.1 season 1 (1989/90)

The concentration of soil N observed during the first season (0.20%), this was not significantly different from that present in the second season (0.20%) (Table 4). Soil total N concentrations showed no conclusive trends during the season.

2.3.1.1.2 season 2 (1990/91)

The second sampling season again showed no conclusive pattern over the seven month period (Table 4).

Table 4. Seasonal variations in soil total N (%), meaned over all soil depths and all fertiliser N applications.

Month	soil total N (season 1) %	soil total N (season 2) %	Means
September	0.21	0.19	0.20
October	0.20	0.21	0.20
November	0.21	0.19	0.20
December	0.21	0.21	0.21
January	0.20	0.20	0.20
February	0.19	0.17	0.18
March	0.21	0.21	0.21
Means	0.20	0.20	

LSD (body of the table) 5% = 0.40 1% = 0.45
 LSD (monthly means) 5% = 0.25 1% = 0.27

Results observed above for both seasons showed no real trends because difficulties involved in the sampling of soil total N, especially in terms of spatial variations, even on trials spanning a number of years (Ball, 1979), would in all likelihood make presentation of data at monthly intervals nearly impossible to interpret. Sampling error is likely to be the major factor giving significant differences between months, especially when averaged over two years. Although these monthly differences are statistically different between months, this phenomenon is probably not biologically significant.

2.3.2 Soil ammonium N

In control camps, an amount of 82.7 kg more $\text{NH}_4\text{-N}$ was measured in the soil in the first season than in the second (Table 5a). Averaged over both seasons, the soil in the control camps contained 331.9 kg $\text{NH}_4\text{-N/ha}$ to a depth of 1m (Table 5b). This constituted 2.1% of the total N contained in the soil to a depth of 1m. This amount, as in the case of the soil total N, was not distributed evenly through the soil profile, with similar trends in $\text{NH}_4\text{-N}$ concentration evident in both seasons (Tables 5a & 5b). However, unlike the pattern of distribution of soil total N down the profile, a considerably greater percentage (28.7% or 115.3 kg/ha) of the $\text{NH}_4\text{-N}$ occurred within the 75-100cm depth class (Table 5b) on average over all N treatments, compared to only 11.9% in the case of total N (Table 3b). The percentage of $\text{NH}_4\text{-N}$ occurring within the 0-10cm depth class was only 13.7% (55.0 kg/ha) (averaged over two seasons), compared to 30.3% of the total N (Tables 5b & 3b, respectively).

Ammonium N concentrations (mg/kg) showed a distinct quadratic trend with depth down the profile, irrespective of the rate of N applied to each of the camps (Table 5b). This trend was characterised by the highest average concentrations of $\text{NH}_4\text{-N}$ within the 0-10cm and 75-100cm depth classes, and the lowest in the 50-75cm class. Within the 10-25cm and 25-50cm depth classes, $\text{NH}_4\text{-N}$ concentrations were a little higher than in the 50-75cm class, although not significantly so in both seasons. Such similar quadratic trends were evident in both seasons (Table 5a).

The addition of fertiliser N, irrespective of application rate, to the 'treatment' camps significantly increased the concentration of soil $\text{NH}_4\text{-N}$ above that of the control camps in both seasons (Table 5a). Furthermore, as N application rates increased, so too did the concentrations of $\text{NH}_4\text{-N}$ (Table 5b), but this trend was significant only during the first year.

In the second season, even though the application of 450 kg N/ha/season resulted in the highest $\text{NH}_4\text{-N}$ concentration within the soil, this concentration was not significantly greater than was measured after application of 150 or 300 kg N/ha/season. Applications of only 150 kg N/ha/season also increased $\text{NH}_4\text{-N}$ concentrations above those in camps to which no N was applied (Table 5a).

On average, 424.4 kg $\text{NH}_4\text{-N}$ was measured to a depth of 1m in those camps to which N was applied (Table 5b). However, the amount of $\text{NH}_4\text{-N}$ was 66.6 kg higher in the first than in the second season. This resulted from a decline in the amount of $\text{NH}_4\text{-N}$ throughout the soil profile (Table 5a).

Table 5a. Effects of different fertiliser N rates on soil $\text{NH}_4\text{-N}$ (mg/kg of dry soil) within each depth class over two seasons. Bold figures represent the total amount of soil $\text{NH}_4\text{-N}$ (kg $\text{NH}_4\text{-N/ha}$) within each specific depth class. Data are averages from 7 samplings undertaken at monthly intervals during each of the 2 growing seasons. (Average soil density to a depth of 1m = 10^3 kg/m^3).

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
Season 1						
0	47.1	32.9	33.9	32.1	45.0	37.3
	47.1	49.3	84.7	80.2	112.0	373.3
150	54.3	38.2	39.3	33.9	52.7	42.6
	54.3	57.3	98.2	84.7	131.0	426.0
300	63.3	43.6	43.2	40.9	46.3	45.4
	63.3	65.4	108.0	102.0	115.0	453.7
450	66.3	48.5	47.6	43.1	52.7	49.7
	66.3	72.7	119.0	108.0	131.0	497.0
Depth Means	57.8	40.8	41.0	37.5	49.1	437.2
	57.8	61.1	102.4	93.7	122.2	
%	13.2	14.0	23.4	21.4	27.9	
Season 2						
0	41.2	26.7	21.7	23.8	38.3	29.0
	41.2	40.0	54.2	59.5	95.7	290.6
150	46.0	32.7	30.7	25.7	40.5	36.9
	46.0	81.7	76.7	64.2	101.0	369.6
300	56.2	38.6	31.2	35.2	45.4	39.3
	56.2	57.9	78.0	88.0	113.0	393.1
450	66.3	48.5	47.6	43.1	52.7	40.0
	66.3	63.3	78.0	82.5	124.0	414.1
Depth Means	52.4	36.6	32.8	31.9	44.2	366.8
	52.4	60.7	71.7	73.6	108.4	
%	14.2	16.5	19.5	20.0	29.5	

LSDs for $\text{NH}_4\text{-N}$ (mg/kg):

depth classes for season 1	5% = 4.7	1% = 5.9
N levels within season 1	5% = 4.2	1% = 5.6
body of the table for season 1	5% = 6.4	1% = 8.8
depth classes for season 2	5% = 4.9	1% = 6.1
N levels within season 2	5% = 4.5	1% = 5.7
body of the table for season 2	5% = 7.0	1% = 8.9
season 1 vs season 2	5% = 68.4	1% = 74.3

Table 5b. Soil $\text{NH}_4\text{-N}$ concentrations (mg/kg) and total amounts of soil $\text{NH}_4\text{-N}$ (kg/ha) meaned over both seasons and all depths.

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
0	44.1	29.8	27.8	27.9	41.6	33.1
	44.1	44.7	69.4	69.9	103.8	331.9
150	50.1	35.4	35.0	29.8	46.6	39.8
	50.1	69.5	87.4	74.4	116.0	397.4
300	59.8	41.1	37.2	38.0	45.8	42.3
	59.8	61.6	93.0	95.0	114.0	423.4
450	66.3	48.5	47.6	43.1	52.7	71.2
	63.3	68.0	98.5	95.2	127.5	452.5
Depth Means	55.0	38.7	36.9	34.7	46.7	401.8
	55.0	60.9	87.0	83.6	115.3	
%	13.7	15.2	21.4	20.7	28.7	

2.3.2.1 seasonal patterns of soil ammonium N

2.3.2.1.1 season 1 (1989/90)

Higher concentrations of $\text{NH}_4\text{-N}$ were found within each month of this season than in the second season (Table 6). This pattern was recorded also in the concentration of soil total N observed within the first season (Table 4). Ammonium N concentrations declined in this season from an initial peak in September to a low in November (26.4 mg/kg), before rising, albeit non-significantly, again in January (30.0 mg/kg). February, however, showed a significantly lower $\text{NH}_4\text{-N}$ concentration over that of January and March, where concentrations were again relatively high.

2.3.2.1.2 season 2 (1990/91)

A very similar $\text{NH}_4\text{-N}$ trend to that of the first season was observed in the second (Table 6). However, in all months $\text{NH}_4\text{-N}$ concentrations were lower, although not always significantly so.

Table 6. Seasonal variations in soil $\text{NH}_4\text{-N}$ (mg/kg), meaned over all soil depths and all fertiliser N applications.

Month	soil $\text{NH}_4\text{-N}$ (season 1) mg/kg	soil $\text{NH}_4\text{-N}$ (season 2) mg/kg	Means
September	49.8	36.2	43.0
October	33.5	26.0	29.8
November	26.4	22.1	24.2
December	29.7	23.2	26.4
January	30.0	21.4	25.7
February	18.4	11.2	14.8
March	38.3	24.3	31.3
Means	32.3	23.4	

LSD (body of the table) 5% = 13.6 1% = 19.9
 LSD (monthly means) 5% = 10.2 1% = 15.6

2.3.3 Soil nitrate N

An indication of the extent of possible nitrate leaching is an important aspect of this investigation. This is especially so in the light of current efforts to eliminate, or at least reduce to a minimum, stream and lake contamination, and thereby eutrophication.

Of the soil total N present to a depth of 1m in the soils of the control camps (15.9 tons/ha), only 0.30% (45.6 kg $\text{NO}_3\text{-N}$ /ha) was

recovered in the form of $\text{NO}_3\text{-N}$ (Table 7a & 7b). Averaged over both seasons, the highest concentration of soil $\text{NO}_3\text{-N}$ in the control camps was found in the 0-10cm depth class (6.9 mg/kg), and the lowest in the 50-75cm depth class (3.4 mg/kg) (Table 7b).

Significantly more $\text{NO}_3\text{-N}$ in each season was found throughout the profile after fertilisation than in the control camps (Table 7a), suggesting that this ion may be readily leached down the profile when fertiliser is applied to the pasture.

Not unexpectedly, $\text{NO}_3\text{-N}$ concentrations increased as N fertiliser levels increased, so that the application of 450 kg N/ha/season increased the amount of soil $\text{NO}_3\text{-N}$ by 88.9 kg/ha (195%) above that of the control camps (Table 7a & 7b). However, in the first season, concentrations of $\text{NO}_3\text{-N}$ were not significantly different in plants fertilised with 300 and 450 kg N/ha/season. This was not so in the second season, when the application of 450 kg N/ha/season increased the $\text{NO}_3\text{-N}$ concentration significantly above that of the camps treated with 300 kg N/ha/season (Table 7a). The trends in concentration of $\text{NO}_3\text{-N}$ over time within each season (Table 8) were more variable than those of $\text{NH}_4\text{-N}$ (Table 6).

Unlike the very definite quadratic trend in $\text{NH}_4\text{-N}$ concentrations with depth down the soil profile, no distinct pattern was evident in the distribution of $\text{NO}_3\text{-N}$, except for an increasing concentration of $\text{NO}_3\text{-N}$ at depth during the second season, especially after treatment of the camps with 300 and 450 kg N/ha/season. This suggests a movement of this ion down the

profile at these high rates of N application (Table 7a), thus creating a higher potential for it to be leached out of the profile (see discussion).

Table 7a. Effects of different fertiliser N rates on soil NO₃-N (mg/kg of dry soil) within each depth class over two seasons. Bold figures represent the total amount of soil NO₃-N (kg NO₃-N/ha) within each specific depth class. Data are averages from 7 samplings undertaken at monthly intervals during each of the 2 growing seasons. (Average soil density to a depth of 1m = 10³ kg/m³).

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
Season 1						
0	7.1	4.7	4.5	2.6	3.7	4.1
	7.1	7.1	11.1	6.5	9.3	41.1
150	11.9	8.1	7.1	5.6	4.2	6.6
	11.9	12.0	18.0	14.0	10.4	66.3
300	17.0	16.5	12.3	9.6	5.5	11.0
	17.0	24.8	30.3	24.1	14.8	111.0
450	18.8	14.3	12.8	7.8	7.0	11.1
	18.8	22.5	32.5	19.6	17.6	111.1
Depth Means	13.7	10.9	9.1	6.4	5.1	82.2
	13.7	16.6	22.9	16.0	13.0	
%	16.7	20.1	27.8	19.4	15.8	
Season 2						
0	6.7	4.5	5.3	4.2	5.2	5.0
	6.7	6.9	13.2	10.3	13.1	50.2
150	11.2	8.4	8.5	9.1	9.0	9.0
	11.2	12.6	21.2	22.8	22.5	90.3
300	21.4	11.3	12.2	13.0	11.2	12.9
	21.4	17.0	30.2	32.4	28.0	129.0
450	19.4	16.4	14.9	15.0	15.8	15.8
	19.4	24.7	37.2	37.4	39.3	158.0
Depth Means	14.7	10.1	10.2	10.3	10.3	106.8
	14.7	15.3	25.4	25.7	25.7	
%	13.7	14.3	23.8	24.1	24.1	

LSDs for NO₃-N (mg/kg):

depth classes for season 1	5%=2.3	1%=3.6
N levels for season 1	5%=1.9	1%=2.6
body of the table for season 1	5%=3.9	1%=4.8
depth classes for season 2	5%=2.6	1%=3.2
N levels for season 2	5%=2.2	1%=3.0
body of the table for season 2	5%=3.8	1%=4.7
season 1 vs season 2	5%=24.8	1%=25.3

Table 7b. Soil NO₃-N concentrations (mg/kg) and total amounts of soil NO₃-N (kg/ha) meaned over both seasons and all depths.

Fertiliser N levels (kg N/ha)	Depth (cm)					Means & Totals
	0-10	10-25	25-50	50-75	75-100	
0	6.9	4.6	4.9	3.4	4.4	4.5
	6.9	7.0	12.1	8.4	11.2	45.6
150	11.6	8.2	7.8	7.3	6.6	7.8
	11.6	12.3	19.6	18.4	16.4	78.3
300	19.2	13.9	12.2	11.3	8.3	11.9
	19.2	20.9	30.2	28.2	21.4	120.0
450	19.1	15.3	13.8	11.4	11.4	13.4
	19.1	23.6	34.8	28.5	28.5	134.5
Mean	14.2	10.5	9.7	8.3	7.7	94.3
Depths	14.2	15.9	24.1	20.8	19.3	
%	15.0	16.8	25.5	22.0	20.4	

2.3.3.1 seasonal patterns of soil nitrate N

2.3.3.1.1 season 1 (1989/90)

Soil NO₃-N, not only during this season, but also in the second season, tended to show trends that were mirror images of the soil NH₄-N concentrations observed on the trial. Furthermore, a lower concentration of NO₃-N was observed within this season than in the second (Table 8). Nitrate N concentrations generally remained within a relatively narrow range, except for an extremely low value in January and a relatively high value in February.

2.3.3.1.2 season 2 (1990/91)

Nitrate N trends between the first and second season were very similar, even though a higher concentration, on average, was obtained during this season than in the first (Table 8).

Table 8. Seasonal variations in soil $\text{NO}_3\text{-N}$ (mg/kg), meaned over all soil depths and all fertiliser N applications.

Month	soil $\text{NO}_3\text{-N}$ (season 1) mg/kg	soil $\text{NO}_3\text{-N}$ (season 2) mg/kg	Means
September	9.3	14.9	12.1
October	11.1	15.3	13.2
November	11.0	12.8	11.9
December	10.2	9.5	9.9
January	3.8	8.0	5.9
February	17.3	15.2	16.2
March	11.5	7.9	9.7
Means	10.6	11.9	

LSD (body of the table) 5% = 6.0 1% = 7.8

LSD (monthly means) 5% = 2.9 1% = 4.1

No marked increase in $\text{NO}_3\text{-N}$ concentration were detected in the 75-100 cm depth class in each month, after fertiliser N applications in September, November and January of both seasons. However, as mentioned above, movement of this ion was detected, to some extent, as fertiliser application rates were increased on the pasture (Table 7a).

2.4 Discussion and conclusions

2.4.1 Soil total N

2.4.1.1 effects of fertiliser N on soil total N

In the absence of fertiliser N, the pasture soil on Broadacres was found to contain, on average, 15.45 t N/ha to a depth of 1m, at an average concentration of 0.15%. These results compare favourably with those of Ball & Crush (1980) (7-15 t N/ha),

Whitehead (1986, in Van Der Meer et al. 1986) and Bacon (undated) (0.50 - 4.0% N in the topsoil). The reason why such a large decline in total N of 1.1 t/ha and 1.9 t/ha after application of 150 and 450 kg N/ha/season, respectively, were observed is unknown, but may have arisen in response to immobilisation reactions, resulting from fertilisation.

The total N pool is an important constituent of the soil complex. It acts as a major reservoir for any N inputs, be they organic or inorganic. Jackman (1964) stated that total N remained essentially constant below a permanent pasture. In effect, in permanent pastures an equilibrium exists between the addition of organic matter, to form organic N initially, and inorganic N release by mineralisation. Almost 98% of the total N pool on the Broadacre trial was comprised of organic N. This value concurs with that of Titlyanova (1986). Most of the organic N found within the total N pool arises from death and decay of roots and herbage. Furthermore, most plant residues are deposited on or found in the topsoil and thus it is not surprising that over 30% of the soil total N found on the Broadacre trial was present within the 0-10cm depth class. The high organic carbon (C) content of 4.57% (Table 1) in the topsoil reflects the accumulation of organic matter in this zone. Not surprisingly, therefore, total N concentrations declined with depth down the profile in concert with the decline in organic C content (Table 1). These results concur with those of Stevenson & Dhariwal (1959), Nommik (1967), Khan & Sowden (1971), Meints & Peterson (1977) and Stevenson (1982).

A carbon:nitrogen (C:N) ratio of 9.7:1 in the 0-10cm depth class, because of its narrowness (i.e. no serious imbalance in accumulation of C and N), infers that in the process of OM breakdown there will be sufficient N to satisfy the needs of the microbial organisms utilising the OM, while still allowing some N to be released for plant uptake. As the C:N ratio increases with depth, one would expect that decreasing amounts of N are likely to be released for plant use (Table 1) (Miller & Donahue, 1990).

2.4.1.2 variations in soil total N with depth

If the C:N ratio is narrow, as observed within the 0-10cm depth class on the Broadacre trial, then the application of fertiliser N may not measurably influence the soil total N pool because it will become diluted in the very large N pool within this depth class (Macvicar et al. 1950; Broadbent, 1966; Russell, 1973; Miller & Donahue, 1990). Any suggestion that when the C:N ratio is narrow, a large N pool is to be expected may not hold true, however, if C levels are low. This would not be the case in this trial though, as C levels were high throughout the profile (Table 1).

Attempts have been made to use C:N ratios to predict mineralisation and hence fertiliser requirements; these studies have, however, met with limited success (Oien & Selmer-Olsen, 1980; Hong et al. 1990). Furthermore, sampling methods such as that of Walkley - Black (1934), which determines organic C concentrations, are known to be inefficient, although quick and

easy to use, and this should be borne in mind (Russell, 1973).

Apart from the C:N ratio, other factors such as moisture, heat, pH, aeration, residual N concentration and texture are important determinants of mineralisation of organic N (Miles & Manson, 1992).

2.4.1.3 seasonal patterns of soil total N

The application of fertiliser N to the pasture on Broadacres elicited a varied soil total N concentration response. In the first season, applications of 150 and 450 kg N/ha/season appeared to significantly influence the amount of total N present in the soil (150 kg N/ha during the first season illicited 1400 kg/ha more N than the control). This increase cannot be fully explained. However, it is probably a result of the fertiliser stimulating the microbes in the total N pool through a form of 'priming' effect, resulting in increased mineralisation-immobilisation reactions within the soil complex.

However, in the second season, application of 300 kg N/ha/season to the pasture resulted in the largest increase in soil total N, and camps treated with 450 kg N/ha/season the smallest, but these differences were not significant. The reasons for these inconsistencies are not clear. However, as there was no consistent pattern to these results, in terms of N rate and season, the likely explanation is one of error of measurement. The observed inconsistencies may have resulted from the difficulty inherent in showing the influence of such small

additions of N to an existing large N pool. They could have resulted, therefore, from relatively small errors in sampling and in N analysis or, alternatively, from spatial variation(s) in soil total N (Ball, 1979), or some combination of these.

Fertiliser N applications, whilst not measurably increasing total N, may, however, still affect N dynamics by increasing net mineralisation via chemical and physical phenomena such as salt effects, pH changes and other side effects which may result in increased or decreased activity within the soil complex (Stevenson, 1982). Dowdell et al. (1980, in Prins & Arnold, 1980) and Miller & Donahue (1990) contended that it was very difficult to trace the path of fertiliser N, and its effect on the total N pool. However, they stated that it was likely that most of the N, added as fertiliser or mineralised in a pasture, was recycled (immobilised) into organic materials, mostly as protein in higher plants and microbes. Other researchers have concluded, however, that the application of moderate to high amounts of fertiliser N had little effect on organic N accumulation because its addition seemed to stimulate both immobilisation and mineralisation phases within the total N fraction of the soil (Whitehead, 1970; Stevenson, 1982). Alternatively, increased mineralisation, resulting from fertiliser N applications, may stimulate plant growth, thereby adding to and swelling the organic N pool.

Conversely, researchers such as Jansson (1963), Broadbent (1967), Simpson & Freney (1967) and Stevenson (1982) intimated that there

was appreciable incorporation of the added fertiliser N itself into organic forms. This, they concluded, led to immobilisation of the fertiliser N within the soil complex.

Neither of the two seasons showed significant differences in soil total N concentrations. This could be attributed to the fact that the pastures on Broadacres are over 15 years old and losses and gains in total N are therefore probably at equilibrium (Jackman, 1964). Furthermore, both season's data followed generally similar trends down the profile, suggesting once again that total N was in an equilibrium phase.

It is difficult to ascertain exactly why the soil total N trend within both seasons followed the pattern alluded to in Ch. 2.3.1.1 and Table 4. It is feasible that soil temperature, and moisture to a lesser extent, promoted the fluctuations of soil N over both seasons (Stanford *et al.* 1973). Furthermore, the action of inorganic fertiliser as a "priming" agent for the release of organic N from the N pool was not conclusively shown in the results, even though Table 14 (Ch. 3.5.6) may go some way to show this phenomenon. The fact that autumn and summer (March and December) (Table 4) of both seasons showed an accumulation of soil total N may suggest that plant growth had declined because temperatures had dropped into autumn, or conversely, rainfall in summer had not been conducive to plant growth and/or microbial activity. Other months showed erratic changes in soil total N concentrations, suggesting that N was being metabolised and utilised during this period in summer.

Jackman (1964) also showed that total N did not vary to any extent when assessed in mature permanent pastures over a number of seasons. The theory pertaining to this observation relates to the fact that permanent pastures become very stable, and the losses and gains in N reach an equilibrium within the soil. This may have been the case with the permanent pastures used for this trial on Broadacres, as they have been established for a number of years.

2.4.1.4 conclusions

It may be concluded from these data then that a large amount of total N is tied up in the surface soil layer, and especially within the OM. However, spatial and some temporal variations in soil total N, and the difficulty of sampling adequately when working in field situations, make it difficult to identify specific trends. It is for this reason that N balance and cycling models are not easily developed. Measuring the contributions of each component with sufficient precision for a reliable N balance to be struck would require a prohibitively large and expensive sampling programme (Ball, 1979). Furthermore, mineralisation reactions are affected by a large number of different organisms with a wide range of optima for environmental variables. Therefore, this process is not too greatly affected by weather vagaries. In this respect then, significant monthly differences in soil total N has probably no major biological significance within the system as a whole and should be taken only at face value.

2.4.2 Soil ammonium N

2.4.2.1 introduction

The inorganic N portion of the total N pool consists almost entirely of the NH_4^+ cation and the NO_3^- anion, except in some very specialised situations beyond the scope of this review. These ions may be produced by the decomposition of soil organic matter (mineralisation), or be supplied as N fertiliser. The former process is effected by soil micro-organisms (Russell, 1973; Miller & Donahue, 1990; Miles & Manson, 1992).

2.4.2.2 variation in soil ammonium N with depth

Analyses from control camps revealed the presence of 331.9 kg $\text{NH}_4\text{-N/ha}$ to a depth of 1m, on average, over both seasons. In effect then, only 2.1% of the total N pool of 15.45 t N/ha to 1m was present in the $\text{NH}_4\text{-N}$ form, on average, over the whole trial period. Indications are that the small quantity liberated from the total N pool during this trial was similar to the amounts (between 1 and 8.5%) obtained by researchers such as Stevenson (1982), Whitehead (1986), Ball & Field (1987) and Miller & Donahue (1990). However, the $\text{NH}_4\text{-N}$ measured in the profile should not be taken solely as an indication of the amount of N mineralised. Residual $\text{NH}_4\text{-N}$ and/or fertiliser N applied the previous season may also have been included in the amount calculated above (Jarvis & Barraclough, 1991). Furthermore, plant uptake of this N fraction has also been discounted in the calculation.

The largest concentrations of $\text{NH}_4\text{-N}$, on average, within the soil profile were found in the 0-10cm and 75-100cm depth classes, and the lowest concentrations, on average, in the 50-75cm depth class. This produced a distinct quadratic trend with depth down the soil profile, irrespective of the amount of fertiliser N applied. A result of this nature suggests that mineralisation and/or residual $\text{NH}_4\text{-N}$ concentrations were highest in the 0-10cm and 75-100cm classes and lowest in the 50-75cm depth class. This may well be true with respect to the 0-10cm depth class, where the return of plant litter and the dung and urine of grazing animals, high soil temperatures, combined with high organic matter, microbial activity and aeration would together have resulted in increased rates of mineralisation and thus high concentrations of $\text{NH}_4\text{-N}$. This, coupled with the addition of fertiliser N, along with the residual $\text{NH}_4\text{-N}$ from previous fertiliser applications, may have enhanced the concentration of this N fraction in this depth class. Accumulation of $\text{NH}_4\text{-N}$ at depth is, however, difficult to explain in terms of mineralisation alone, unless nitrification, (the process whereby NH_4^+ ions are oxidised to NO_3^- ions) was inhibited. This may have resulted in a cationic (NH_4^+) build-up in this lower region of the soil profile over a number of years. Alternatively, plant uptake may have been minimal at this depth, resulting in accumulation of this N fraction.

Analysis of a similar Inanda soil in a maize land situated 50m upslope of the trial pasture showed no $\text{NH}_4\text{-N}$ accumulation at depth, suggesting that mineralisation-nitrification reactions

were probably proceeding normally in this region of the soil profile (Fig. 4).

2.4.2.3 the perceived fate of soil ammonium N

To determine whether or not nitrification had been curtailed in the soil of the pasture at this depth, a laboratory wetting/drying experiment was performed on selected soil samples from the 75-100cm depth class. After four wetting and drying cycles, $\text{NH}_4\text{-N}$ concentrations had decreased only slightly from the original concentration of 36.2 mg/kg to 33.7 mg/kg, on average.

The conclusion drawn from this experiment was that nitrification at this depth was indeed restricted, resulting in $\text{NH}_4\text{-N}$ accumulation. Further confirmation of nitrification inhibition, and subsequent $\text{NH}_4\text{-N}$ build-up, could be related to the presence of a relatively high organic C content at this depth (Table 1) (2.2%, on average, below the pasture; 1.3% on the maize land). This suggests that $\text{NH}_4\text{-N}$ may be bound strongly to soil colloids and/or that a paucity of microbial activity, conducive to the nitrification process, had arisen probably as a result of soil compaction and poor aeration, although this could be disputed from this result because the aeration of the soil in this experiment still did not effect nitrification and so another mechanism was probably causing $\text{NH}_4\text{-N}$ accumulation.

The wider C:N ratio at this depth, relative to the shallower depth classes, infers the presence of an abundance of organic C

relative to N at this depth class. The colour of the pasture soil (Plate 1), was much darker than that of the maize land (Plate 2), a further indication of the difference in organic matter contents between the two soils.

This feature is likely to reflect differences in past management employed on these two soils. In the case of the maize land, annual ploughing and soil turnover has occurred, resulting in increased aeration and rapid organic matter breakdown, even at depth. Conversely, on the pasture, a lack of ripping, a permanent cover and high nutrient recycling have combined to form a very large organic matter pool within the soil complex (4.2% OM, on average on the pasture; 2.7% OM on the maize land). High OM contents were also observed by Williams & Clement (1965) on their trials under similar management regimes.

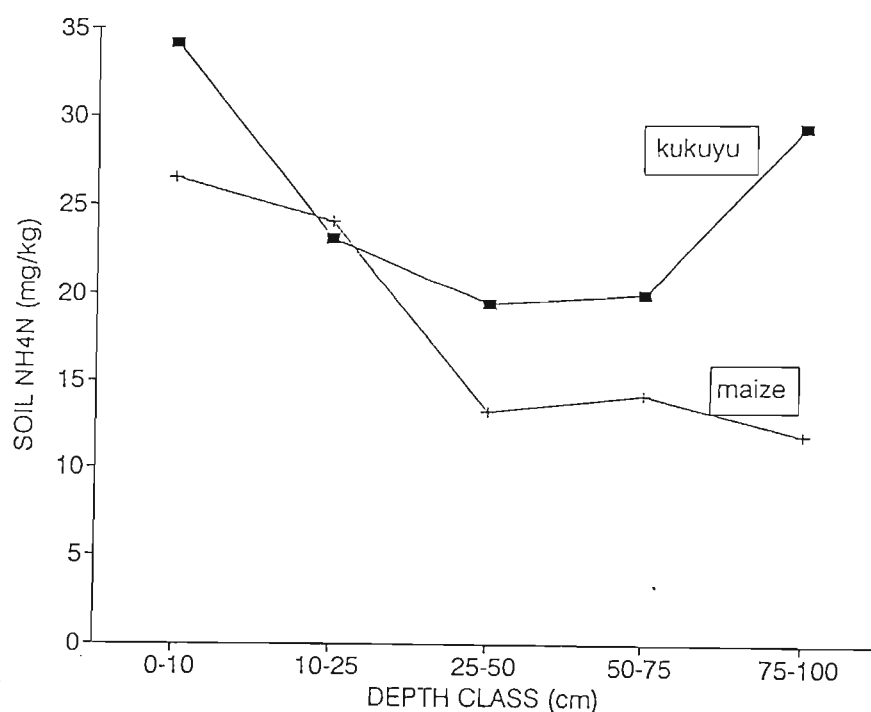


Figure 4. Soil $\text{NH}_4\text{-N}$ trends (mg/kg) on kikuyu pasture and maizeland with depth (cm) down the profile.

Root growth and form through the soil profile may also have influenced $\text{NH}_4\text{-N}$ concentrations at depth. The maize had a far shallower and lower root density with depth (Plate 3), whilst the kikuyu root system was denser and the roots penetrated deeper down the profile (Plate 4). This concurs with the findings of Russell (1973) who stated that grasses had very extensive fine roots that may have great length, and could add over 2.5 t/ha dry matter to the soil per annum. The fact that the roots of kikuyu were shown to penetrate to a depth of at least 1m, and yet $\text{NH}_4\text{-N}$ concentrations remained high, suggests that the roots may either have preferentially excluded the uptake of this ion (Theron, 1963), or alternatively act only as conduits for water uptake at this depth.



Plate 1. The dark coloured Inanda soil form, signifying a high organic matter content under the kikuyu pasture.



Plate 2. The lightly coloured Inanda soil form, prevalent under the maize as a result of annual ploughing which led to the subsequent breakdown of organic matter.



Plate 3. Shallow, low rooting densities prevalent within the maizeland.



Plate 4. Very dense, deep, high rooting density as observed under the kikuyu pasture.

2.4.2.4 soil ammonium N and other cations

Of particular interest is that cations other than $\text{NH}_4\text{-N}$ also showed a similar, albeit less pronounced trend to that of the $\text{NH}_4\text{-N}$ with depth down the profile (Table 1). Clay content is probably the major factor influencing this quadratic trend with depth down the profile (Table 1). Furthermore, as the NH_4^+ ion is cationic in nature, it will be influenced in a similar manner to the other cations (Miller & Donahue, 1990). Clay concentration (texture) could, therefore, be the most important factor influencing the $\text{NH}_4\text{-N}$ trend down the profile.

2.4.2.5 the effect of fertiliser N addition on soil ammonium N

The application of fertiliser N to the pasture, averaged over all depths, significantly increased the amount of $\text{NH}_4\text{-N}$ over that of the control camps. Furthermore, the higher the application rate, the greater the increase in $\text{NH}_4\text{-N}$ accumulation within the soil profile. This may have been due to the fact that at lower N rates, the grass sward may have acted as a sink for NH_4^+ ions, competing, to an extent, with the nitrifying organisms (Jarvis & Barraclough, 1991). This would have led to a lower $\text{NH}_4\text{-N}$ concentration at these rates, relative to those at the higher rates of N application. Conversely, at the high N application rates (450 kg N/ha/season), there is potential for a considerable excess of input over removal (uptake by microbes and plants), with the opportunity for nitrification and accumulation, followed by a perceived substantial loss of $\text{NO}_3\text{-N}$ (see Ch. 3.5.3).

Furthermore, as N application rates increased, so the $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$ ratio narrowed in the soil complex (Table 9). This could have arisen because $\text{NH}_4\text{-N}$ at the high N application rate was then in excess of plant requirements. This abundance of $\text{NH}_4\text{-N}$ would be expected to lead to increased nitrification by nitrifiers, leading to an accumulation of $\text{NO}_3\text{-N}$ in the profile (Jarvis & Barraclough, 1991). This could have serious repercussions in that if appropriate climatic and physical soil conditions predominate, leaching of excess $\text{NO}_3\text{-N}$ may result (see Ch. 3.5.3).

Table 9. Ammonium N, nitrate N and their ratio (kg N/ha),
meaned over both seasons and all depth classes.

N levels kg/ha/season	NH ₄ -N (kg N/ha)	NO ₃ -N	Ratio NH ₄ -N:NO ₃ -N
0	331.9	45.6	7.2
150	397.4	78.3	5.0
300	423.4	120.0	3.5
450	452.5	134.5	3.3

Corrective fertiliser N applications are important for sustained plant growth. However, N should be applied regularly in small amounts, especially if heavy annual application rates are planned. If this does not occur, a build-up of NH₄-N may lead to pasture induced animal toxicity and/or excessive NO₃-N accumulation, resulting in increased potential for leaching and subsequent pollution of groundwater.

2.4.2.6 seasonal patterns of soil ammonium N

On average, 66.7 kg/ha more NH₄-N was present in the soil in the first season than in the second after fertilisation. This could not be related to climatic conditions because, on the whole, these conditions remained similar over both seasons. However, nitrification, being a two-step process via nitrite to nitrate, is accomplished by only a few species with quite defined environmental optima. Thus, this process is far more susceptible to weather and seasonal changes than micro-organisms associated with the initial process of mineralisation.

Mineralisation may have been proceeding at an accelerated pace during the first season (probably due to an increase in microbial activity as a result of favourable external factors), which may

have resulted in an increase in $\text{NH}_4\text{-N}$ concentrations during this period. Alternatively, these high concentrations may also have been induced by the high N applications previous to this study, resulting in high $\text{NH}_4\text{-N}$ levels initially. This appears to be the most likely explanation, because the decline in mineral N throughout the profile was greatest in the control swards; total mineral N declined by 75 kg N/ha, while the decline was considerably less than this when fertiliser input continued. However, nitrification appeared to play a more important role during the second season (see Ch. 3.5.3), as the residual $\text{NH}_4\text{-N}$ declined in the soil profile. Macduff & White (1984) experienced a similar contrast in their trials, but ascribed it to spatial and temporal variations in soil mineral N, which was exacerbated under field conditions.

Whilst the amount of $\text{NH}_4\text{-N}$ observed in the upper 1m of the soil profile during both seasons was not significantly different, factors such as temperature may have exerted an influence on mineralisation and immobilisation reactions, and thus soil $\text{NH}_4\text{-N}$ concentrations (Table 6). At the start (September) and end (March) of both seasons, $\text{NH}_4\text{-N}$ concentrations were high (Fig. 5). These periods correspond to early spring and late summer/autumn when pasture growth is relatively slow. Temperatures then are typically cooler. Because of this, mineralisation may decline, thereby reducing ammonification, and ultimately other processes such as nitrification. This may result in higher NH_4^+ concentrations during these periods. During summer, however, mineralisation and plant uptake of N proceed vigorously.

This scenario is similar to that proposed by Whitehead (1970), wherein he suggested that during periods of rapid growth, $\text{NH}_4\text{-N}$ would be rapidly taken up, but during drought or cold, this process would be delayed, resulting in a build-up of $\text{NH}_4\text{-N}$.

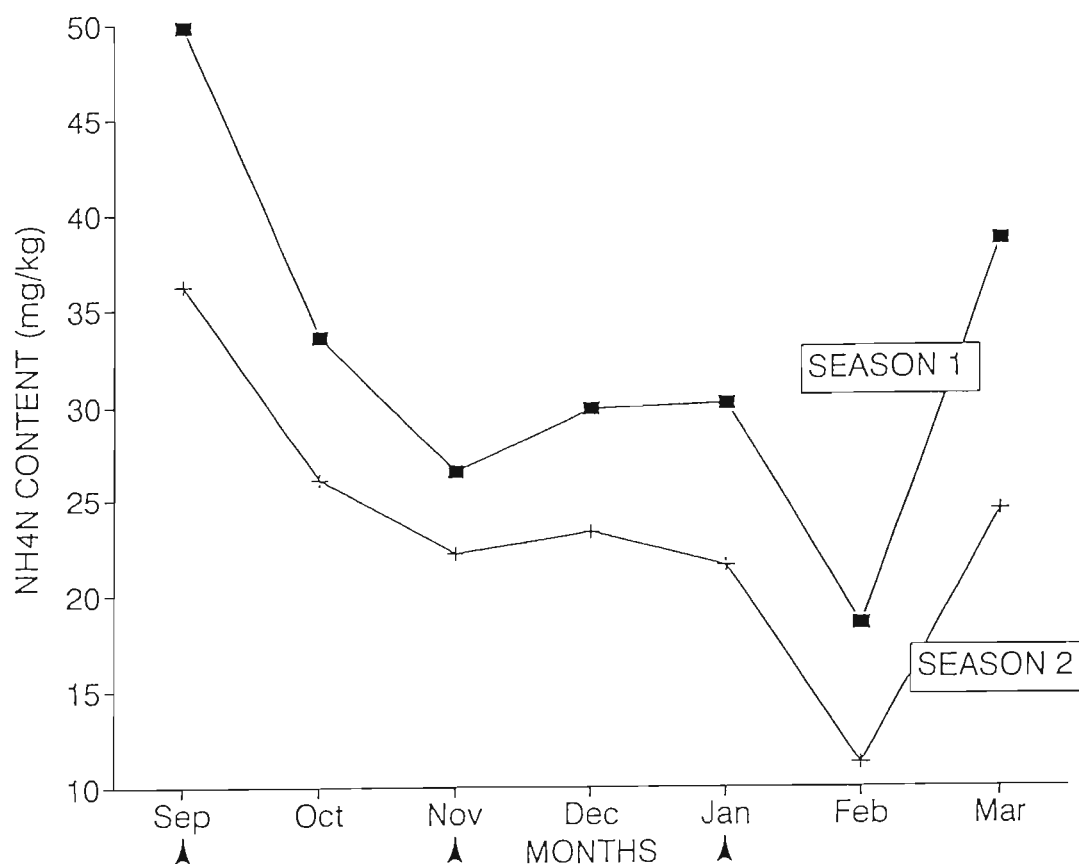


Figure 5. Monthly soil ammonium-N concentrations (mg/kg) for both seasons over all N treatments (arrows indicate fertiliser applications).

2.4.2.7 conclusions

Although the conclusions drawn from these results show specific trends, the seasonal and depth variations in ammonium and nitrate-N soil values are merely used to indicate the levels of these compounds relative to total nitrogen as the methods used

in this study do not allow for any further interpretation. If further interpretation is required, mineralisation techniques will need to be used to predict the levels of ammonium and nitrate-N in the soil.

Because many of the answers pertaining to $\text{NH}_4\text{-N}$, and more specifically the soil $\text{NH}_4\text{-N}$ quadratic effect, were felt to be unsatisfactory, a further trial, utilising labelled ^{15}N , was undertaken in order to determine the fate of this ion. The methodology appears in Chapter 1 and results appear in Chapter 4.

2.4.3 Soil nitrate N

2.4.3.1 introduction

Once mineralisation of organic N into NH_4^+ ions is complete, and given the right environmental conditions, oxidation of this ion into the NO_3^- ion occurs. This process is termed nitrification.

2.4.3.2 variations in soil nitrate N with depth

Only 45.6 kg $\text{NO}_3\text{-N/ha}$, on average, was found in the control camps to a depth of 1m on the Broadacre trial (Table 7b). This represented only 0.29% of the soil total N found within the profile down to this depth. The highest concentration of $\text{NO}_3\text{-N}$ was located in the 0-10cm depth class, probably because all N inputs, whether from natural or fertiliser sources (eg. wash-down from upslope lands, either by naturally occurring processes or through agricultural practices), and the large quantities of N recycled in plant residues and the excreta of the animals grazing

these pastures, all arrive at the soil surface. This portion of the soil profile is where biological transformations and interaction between environmental factors and N inputs and removal are likely to be at a maximum and therefore mineralisation and nitrification is likely to be most rapid. From the upper soil profile, $\text{NO}_3\text{-N}$ declined, on average, with depth down the profile (Table 7b) (Linville & Smith 1971; Gast et al. 1978), but there was an increase in $\text{NO}_3\text{-N}$ concentration with depth during the second season, suggesting the movement of this N fraction down the profile over this time. Diminished forage yields in the second year (see Ch. 3.3.1) meant less uptake of soil N by the pasture, which probably accounts for the greater accumulation of $\text{NO}_3\text{-N}$ throughout the soil profile as observed during the second season.

2.4.3.3 effects of fertiliser N addition on soil nitrate N

Fertilisation of the pasture significantly increased $\text{NO}_3\text{-N}$ concentrations above those of the control camps. These concentrations increased as fertiliser application rates increased (294.9% increase in $\text{NO}_3\text{-N}$ concentration between control and 450 kg N/ha/season application). Apart from the presence of NO_3^+ ions in the fertiliser, these increases could probably be directly related to the nitrification of some of the applied $\text{NH}_4\text{-N}$, contained in the fertiliser, to $\text{NO}_3\text{-N}$. Conversely, low $\text{NO}_3\text{-N}$ concentrations on the control, and low concentrations of NO_3^- ions at low N application rates, were probably the result of a decline in nitrification and/or an increase in plant uptake, mainly as

a result of the pasture acting as a major $\text{NH}_4\text{-N}$ sink and thus competing for these ions against the nitrifying organisms, and more complete uptake of $\text{NO}_3\text{-N}$ by these swards. Dalal & Mayer (1986) and Ladd & Amato's (1986) conclusions from their trials were similar to those intimated above for the Broadacre trial.

2.4.3.4 variations in soil nitrate N with depth

As fertiliser N application rates increased, so too did $\text{NO}_3\text{-N}$ concentrations with depth. This again relates to the fact that, in addition to the $\text{NO}_3\text{-N}$ contained in the fertiliser, nitrification reactions were probably stimulated by the additional N applied at the higher application rates, consequently releasing more $\text{NO}_3\text{-N}$ into the soil profile (Mallarino & Wedin, 1990; Jarvis & Barraclough, 1991).

Although the degree of leaching was not measured on this trial, results pertaining to the movement of $\text{NO}_3\text{-N}$ down the profile do not show it to be a potential problem on these pastures at present. However, because $\text{NO}_3\text{-N}$, unlike $\text{NH}_4\text{-N}$, is mobile, it may be leached out of the solum. With increased application rates, the potential for leaching also increases because of the higher concentrations of $\text{NO}_3\text{-N}$, resulting in larger quantities being found at depth. This has important implications for fertiliser use on pastures in that it would probably be more effective, and thus more economical in terms of spreading the fertiliser costs and increasing the efficiency of use of the fertiliser, to apply the fertiliser N in small quantities, and at frequent intervals

over a season, even though on this trial $\text{NO}_3\text{-N}$ movement did not seem to occur to any extent after application. This would hopefully then also reduce the potential for groundwater contamination because high levels of $\text{NO}_3\text{-N}$ would then not accumulate within the soil complex.

Alternatively, if high fertiliser N rates are to be applied over the season, then a slow release form of fertiliser or nitrification inhibitors in conjunction with an ammoniacal fertiliser, could be advocated, although the exercise may be costly. Inhibitors control the oxidation of NH_4^+ to NO_3^- ions within the soil, thereby removing the potential for leaching of excess NO_3^- ions into the groundwater. Inhibitors currently being marketed include nitrapyrin and ammonium thiosulphate. These additives are, however, still under investigation as they have been shown to provide inconsistent results under some environmental conditions (Miller & Donahue, 1990; Hefer *et al.* 1992).

2.4.3.5 seasonal patterns of soil nitrate N

On average, 94.3 kg $\text{NO}_3\text{-N/ha}$ was present down to a depth of 1m over both seasons (Table 7b). However, significantly more $\text{NO}_3\text{-N}$ was present in the second season than in the first. This result is in contrast to that of the $\text{NH}_4\text{-N}$, wherein lower concentrations were found in the second season than in the first. They also suggest that in the first season the lack of $\text{NO}_3\text{-N}$ may have been a direct result of nitrification inhibition, whilst in the second season this was not so. The presence of a higher concentration

of $\text{NH}_4\text{-N}$ in the profile during the first season should, however, have stimulated nitrification reactions, resulting in a more rapid formation of $\text{NO}_3\text{-N}$. The reasons that nitrification reactions may have predominated in the second and not the first season are unclear. However, these results suggest that soil conditions during the first season may have limited the nitrification process, with mineralisation, and even ammonification reactions having the ability to proceed more favourably than nitrification. Changes in soil conditions such as pH, amongst others, may reverse these reactions in a matter of hours, days or even weeks, let alone in a season. This fact alone makes it difficult to explain changes in the status of compounds within the soil over time (Fey M. 1992. Pers. Comm. University of Natal, PO Box 375 Pietermaritzburg 3200 RSA). However, reference has already been made to the smaller herbage yields in the second season, and that this reduced uptake of N by the sward would be expected to enhance $\text{NO}_3\text{-N}$ levels in the soil profile.

The predominance of $\text{NO}_3\text{-N}$ in the second season relative to the first, which may also have been linked to the higher rainfall during this season, may have increased the potential for this ion to be leached. Furthermore, at the higher N application rates (300 & 450 kg N/ha/season) in the second season, increased concentrations of $\text{NO}_3\text{-N}$ at depth were observed when compared with similar applications in the first season. This suggested that a build-up of NO_3^- ions may have taken place over the two seasons at the high N application rates. No such result occurred at the

lower N application rates and the control over both seasons, thus reflecting the greater potential for $\text{NO}_3\text{-N}$ leaching over time at high application rates.

No specific trends in $\text{NO}_3\text{-N}$ concentration were observed within each season. Rather, $\text{NO}_3\text{-N}$ concentrations tended to vary inconsistently at each sampling period. This was probably due, in part, to varying environmental, physical and microbial activities taking place together, or alone, within the soil profile.

Nitrate N concentrations within each month during both seasons followed a near mirror image of $\text{NH}_4\text{-N}$ (Tables 6 & Table 8; Figs. 5 & 6). This suggests that $\text{NO}_3\text{-N}$ concentrations were influenced by temperature, especially at the beginning and end of both seasons, where lower temperatures may have depressed nitrification. The higher $\text{NH}_4\text{-N}$ concentrations prevalent at these times during the season, further suggests that nitrification was inhibited, resulting in an accumulation of this N fraction, and a corresponding decline in soil $\text{NO}_3\text{-N}$.

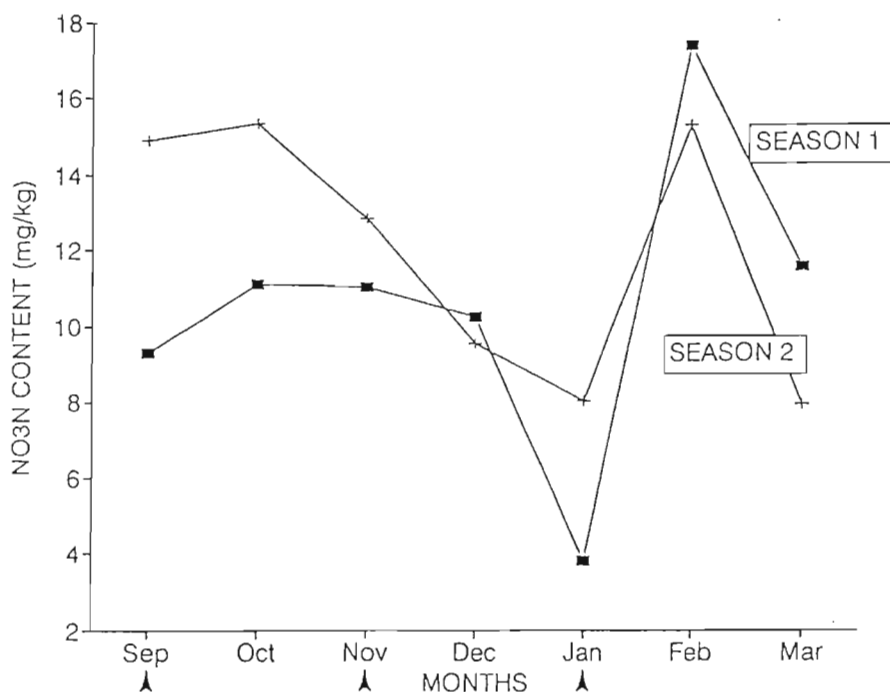


Figure 6. Monthly soil nitrate-N concentrations (mg/kg) for both seasons over all N treatments (arrows indicate fertiliser applications).

2.4.3.6 conclusion

The dynamic nature of $\text{NO}_3\text{-N}$ within the soil is the major reason why it has been so difficult to formulate an accurate N test that would be able to predict fertiliser N application rates from $\text{NO}_3\text{-N}$ concentrations within the soil. Also, soil moisture status, although not measured in these trials, may have an important bearing on $\text{NO}_3\text{-N}$ movement within a profile, with an upward movement of the ion into the root zone in dry periods, and their leaching during wet periods (Ball *et al.* 1978). Furthermore,

this may also then influence the relative concentration of this ion within the herbage (O'Hara & Fraser, 1975).

The amounts of fertiliser recommended to maintain a kikuyu pasture, as noted from this chapter, may be in excess of the plant's requirements, especially on heavy textured soils. Further, potential leaching and groundwater contamination should be observed more closely especially in terms of simulation modelling to improve fertiliser recommendations and to pinpoint conditions under which there would be substantial leaching. This would hopefully then improve the nutrient use efficiency of pastures.

3. THE EFFECT OF N FERTILISATION ON DRY MATTER YIELDS, PROTEIN NITROGEN, NITRATE NITROGEN AND TOTAL NON-STRUCTURAL CARBOHYDRATES IN THE HERBAGE

3.1 Introduction

Different species of plants will take up different amounts and proportions of nutrients from a soil, but all require N in either the $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ form. Nitrogen is required for protein synthesis and is therefore involved in all enzymatic activities during plant growth. It is taken up from the soil mainly as the NO_3^- ion, but also as the NH_4^+ ion. Most plants seem to prefer the former (Lewis, 1986). The reason for this is that NO_3^- ions are usually more readily available to plants within the soil profile because they are not normally bound to the soil colloids, as NH_4^+ ions sometimes are (Haynes, 1986). Because of this, NO_3^- ions tend to be taken up by the roots far more readily than their cationic counterparts. However, temperate grasses grew equally well with $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ as sources of N; although they did better when both forms were supplied together (Ball P.R. 1993. Pers. Comm. Massey Univ. NZ).

Fertilisation of the pasture with N stimulates sward growth by increasing the amounts of NO_3^- and/or NH_4^+ ions available for plant uptake, but this may be dependent upon the availability of other plant requirements (eg: water & temperature), and the time of N application relative to the development of the plant (Russell, 1973). Furthermore, environmental factors can also play a role in determining $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ uptake by the pastures (Syrett, 1954). These factors may ultimately, then, influence

carbohydrate concentrations within the plant.

Herbage from the kikuyu pastures on Broadacres was analysed after fertilisation in order to determine the relative concentrations of the inorganic N fractions and total non-structural carbohydrates (TNC), within the herbage. Since these components also influence dry matter yields, assessments relating to their impact on yield were also undertaken. Herbage was sampled at two heights representing different strata within the sward. These strata were the lower (<5cm; assumed to represent material which would have limited availability to the grazing animal) and the upper (>5cm; representing material available to the animal) herbage strata.

In effect then, the following questions are addressed in this chapter :

what are the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and soluble carbohydrate in the two height strata within the sward; and

what is the effect of varying N application rates on these concentrations, and on dry matter yields?

Answers to these questions will hopefully contribute to the development of strategies for increasing forage yield and improving quality through improved N fertiliser use in the future.

3.2 Methodology

Refer to Chapter 1 for methods pertaining to this chapter.

Because of the high cost of analyses, only two composite samples were taken per camp. The variance of each mean value on each of the graphs was large and thus could not conveniently be graphically shown. Instead then, the two composite sample data for each point are depicted about their means to provide some illustration of their dispersion about their mean (Figs. 9a & 9b; 10a & 10b; 11a & 11b; 12a & 12b; 13a & 13b; 14a & 14b).

3.3 Results

3.3.1 Herbage dry matter yields

On average, 10.85 t dry matter (DM) per ha was produced from the control (no nitrogen) treatments (i.e. sum of the >5cm and <5cm strata yields). This was less than the DM yields obtained from any of the fertilised camps over the two seasons. The application of 450 kg N/ha/season resulted in the largest yield of DM, on average, over the two seasons (14.2 t DM/ha). This was 3.35 t more DM/ha than that produced by the control camps (Table 10 & Fig. 7).

Total yield of dry matter, per unit of N applied, declined as N fertiliser rates increased from 150 to 450 kg N/ha/season (Table 10), as a result of a decline in the mean response per unit of N applied to the pasture. Looking at the average effects, at the 150 kg N/ha/season level, 11.00 kg of additional DM was produced for each kilogram of N applied. This declined to 4.30 kg for

each additional kilogram of N applied in excess of 150 kg when 300 kg N was applied, and increased to 7 kg DM for each kilogram of N applied above 300 kg/ha when 450 kg was applied. This non-linear response per unit of N applied over the range 150 to 450 kg N/ha/season can be attributed to the negative response, in the 1989/90 season, to the increase in N fertiliser rate from 150 to 300 kg N/ha/season (Fig. 7), a pattern which was not duplicated in the second season (1990/91), when the 300 kg N/ha/season treatment produced 1.5 t/ha more DM than the 150 kg N/ha/season treatment (a response of 10 kg DM/kg N in excess of 150 kg was recorded).

Table 10. Mean yields for the 1989/90 and 1990/91 seasons, and the yield response to nitrogen fertiliser.

Treatment N (kg/ha/seas)	Mean yield (t DM/ha)	Mean yield response (t DM/ha)	Mean marginal response (kg DM/kg N applied)
0	10.85	----	----
150	12.50	1.65	11.00
300	13.15	0.65	4.30
450	14.20	1.05	7.00

During the first season of the trial, 13.6 t DM/ha, on average, was produced off the 'treatment' camps (meaned over all fertiliser N treatments). This was 1.8t DM/ha more ($P < 0.01$) than was produced in the second season off the same camps (2nd season = 11.8 t DM/ha).

Even though less DM was produced off the trial in the second season than the first, a generally similar response pattern (Fig. 7) and seasonal trend (Fig. 8) was observed in both seasons. In both seasons, November growth rates were significantly lower than

in any other month (1.5 t DM/ha on average, over both seasons). Conversely, DM yields in January of the first season and October and January of the second were higher than in any of the other months. Yield trends seemed to bear a close relationship to $\text{NO}_3\text{-N}$, protein-N and TNC concentrations at these times (see later in this chapter).

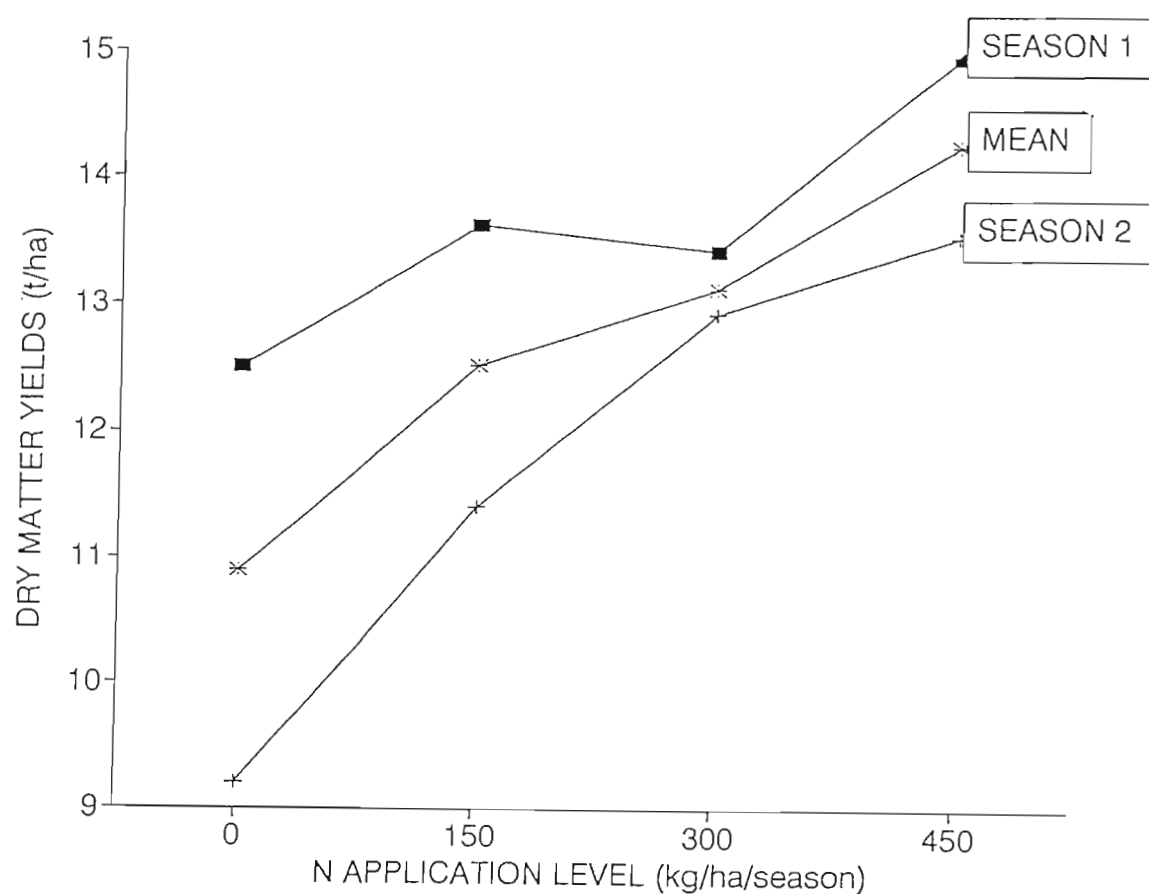


Figure 7. Kikuyu dry matter yields (t/ha) for both seasons on Broadacres, averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

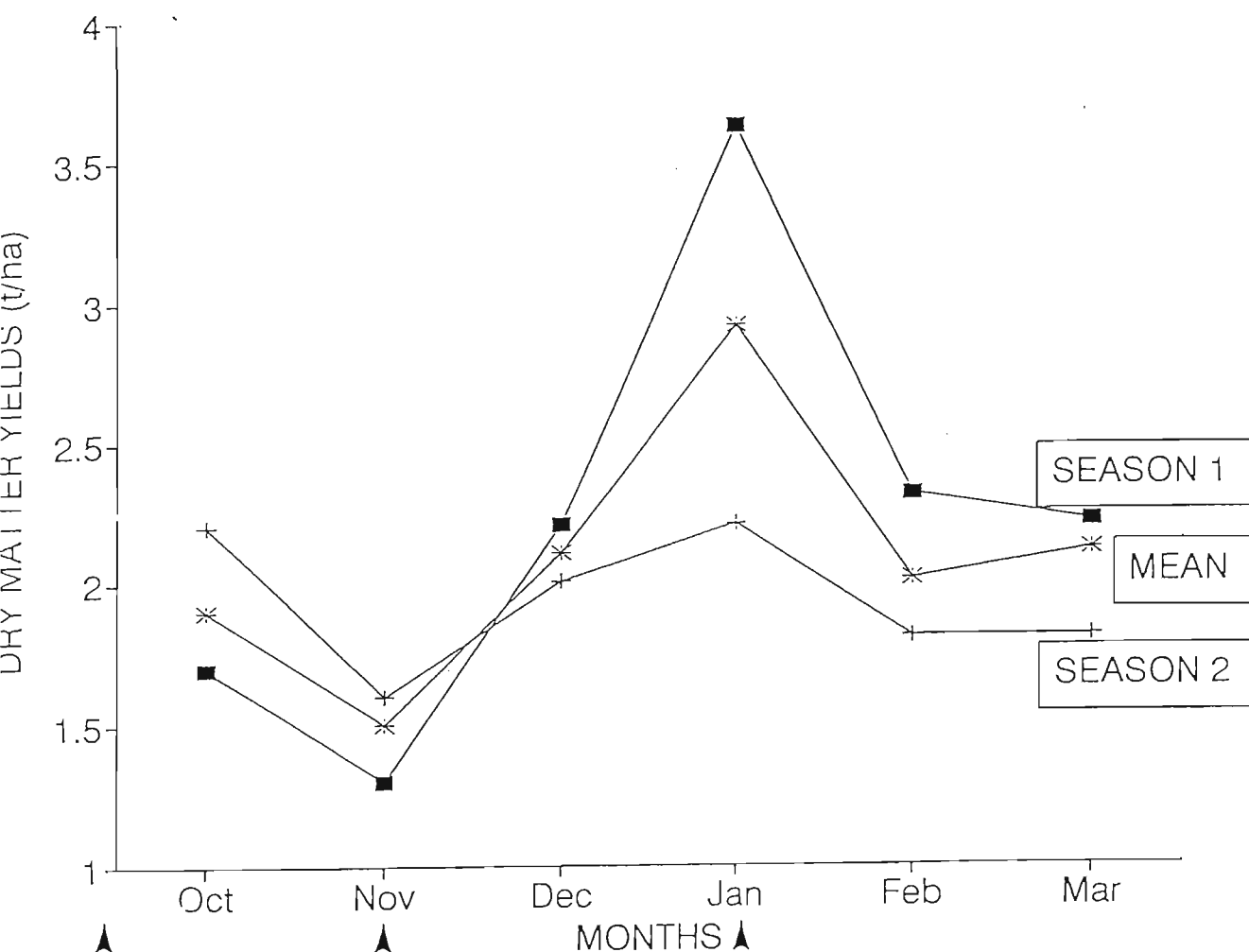


Figure 8. Seasonal patterns of kikuyu dry matter yields (t/ha) meaned over all treatments (arrows indicate fertiliser applications), averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

3.3.2 Plant protein nitrogen

3.3.2.1 seasonal protein-N yields

3.3.2.1.1 season 1 (1989/90)

The protein-N concentration of the combined herbage of the two sampling strata increased from 2.43% in the control (no nitrogen) treatment to 2.97% where 300 kg N/ha/season was applied. No further increase was recorded when 450 kg N/ha/season was applied

(Table 11).

Throughout season 1, herbage of the <5cm stratum contained more protein-N per unit tissue, both on average and at each level of N applied, than that of the >5cm sampling stratum. However, while herbage N levels increased throughout the N application range in the <5cm herbage stratum, it increased to only the 300 kg N/ha/season level in the >5cm sampling stratum, before again declining, although not significantly.

3.3.2.1.2 season 2 (1990/91)

The response in season 2 was somewhat different. Herbage protein-N again increased in the combined herbage of the two sampling strata throughout the range of N applied. In this season, this pattern was also exhibited in each individual stratum, but in this season significantly more protein-N accumulated in the >5cm stratum than in the <5cm stratum (Table 11). These differences in protein-N concentration between the first and second season could not be accounted for, although there was more $\text{NO}_3\text{-N}$ throughout the soil profile in the second season.

Table 11. Kikuyu protein-N concentrations (%) within each herbage stratum for both seasons and under varying N fertilisation levels, averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

Fert N levels kg N/ha	Season 1 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	2.65	2.22	2.43
150	2.96	2.59	2.78
300	3.02	2.92	2.97
450	3.15	2.78	2.96
Means %	2.94	2.62	2.79
Fert N levels kg N/ha	Season 2 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	2.26	2.55	2.40
150	2.32	2.97	2.64
300	2.84	3.49	3.16
450	3.02	3.72	3.37
Means %	2.61	3.18	2.89

	<u>5%</u>	<u>1%</u>
LSDs (N level means for season 1)	0.14	0.16
LSDs (herbage strata means for season 1)	0.09	0.12
LSDs (body of the table for season 1)	0.25	0.38
LSDs (N level means for season 2)	0.20	0.24
LSDs (herbage strata means for season 2)	0.10	0.13
LSDs (body of the table for season 2)	0.27	0.39
LSDs season 1 vs season 2	0.12	0.19

3.3.2.2 seasonal patterns of protein-N concentration

3.3.2.2.1 season 1 (1989/90)

In this season, protein-N concentration within the total herbage of the combined sampling strata showed a distinct bi-modal trend. Levels rose from low values in October to reach an initial peak,

both in the control and in the three fertiliser treatments, in November. Protein-N levels then declined before rising again to reach a second peak in February, before again declining at the March sampling (Fig. 9a) (NB: in some cases, however, variances of sampled data about their means for the fertiliser N applied, were large, suggesting data variability).

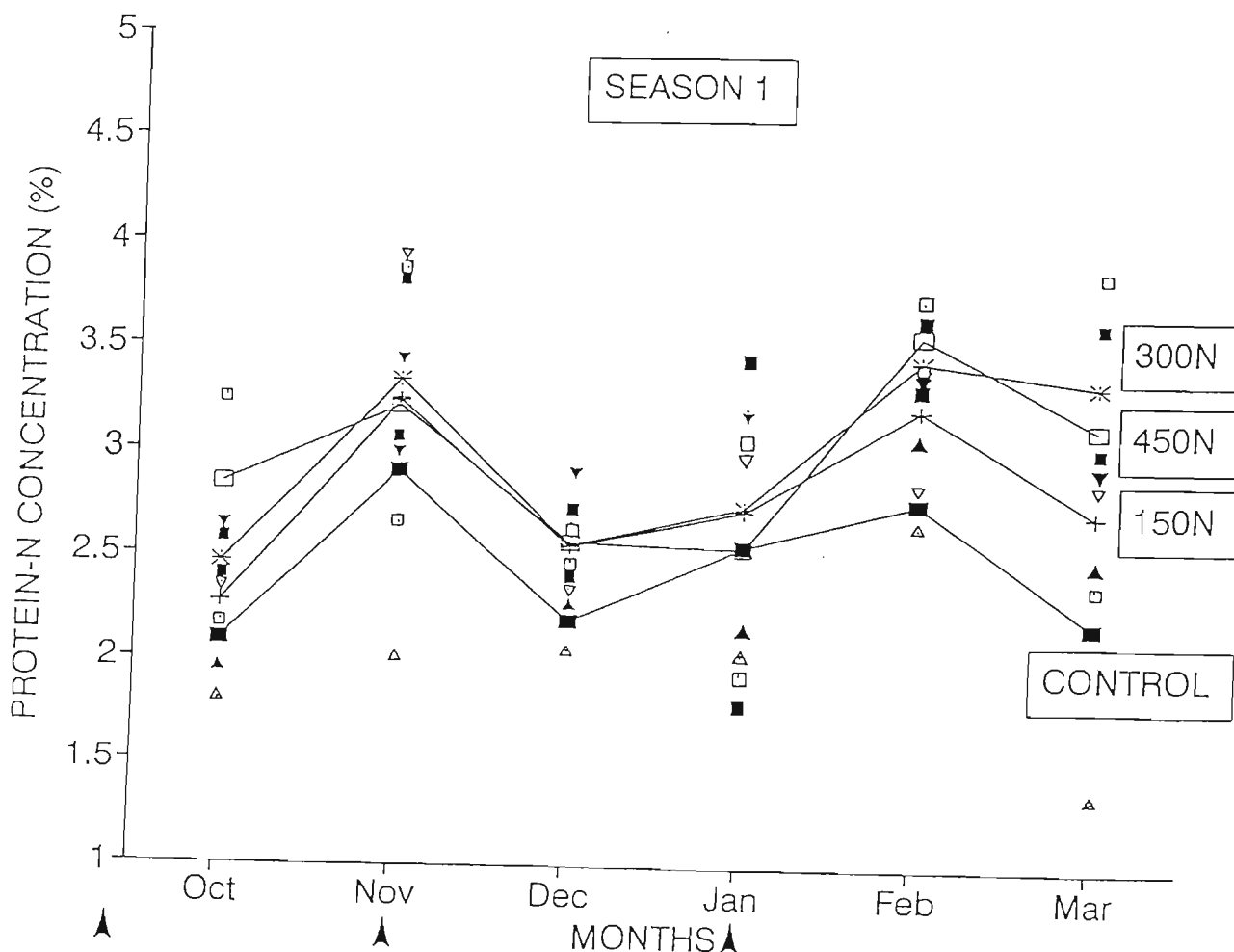


Figure 9a. Kikuyu protein-N concentrations (%) at monthly intervals during the first season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control= ▽ 150N = + 300N = ▼ 450N = ■

3.3.2.2.2 season 2 (1990/91)

In this season, the bi-modal pattern of protein-N accumulation, while still apparent, was less accentuated than in the first season (although variability of the data about the mean was again present under some fertiliser application rates, as observed in season 1) (Fig. 9b). Protein-N levels initially rose through the spring period to reach a peak in December, declined marginally in January, and then rose to a second peak in February. Protein-N levels then declined marginally in March. The reasons for these differences, when compared to the first season, were not apparent and could not be attributed to climatic patterns (see Table 2).

Protein-N levels at each sampling occasion were generally, but not always, higher in those treatments to which N fertiliser was applied, than in the control treatment.

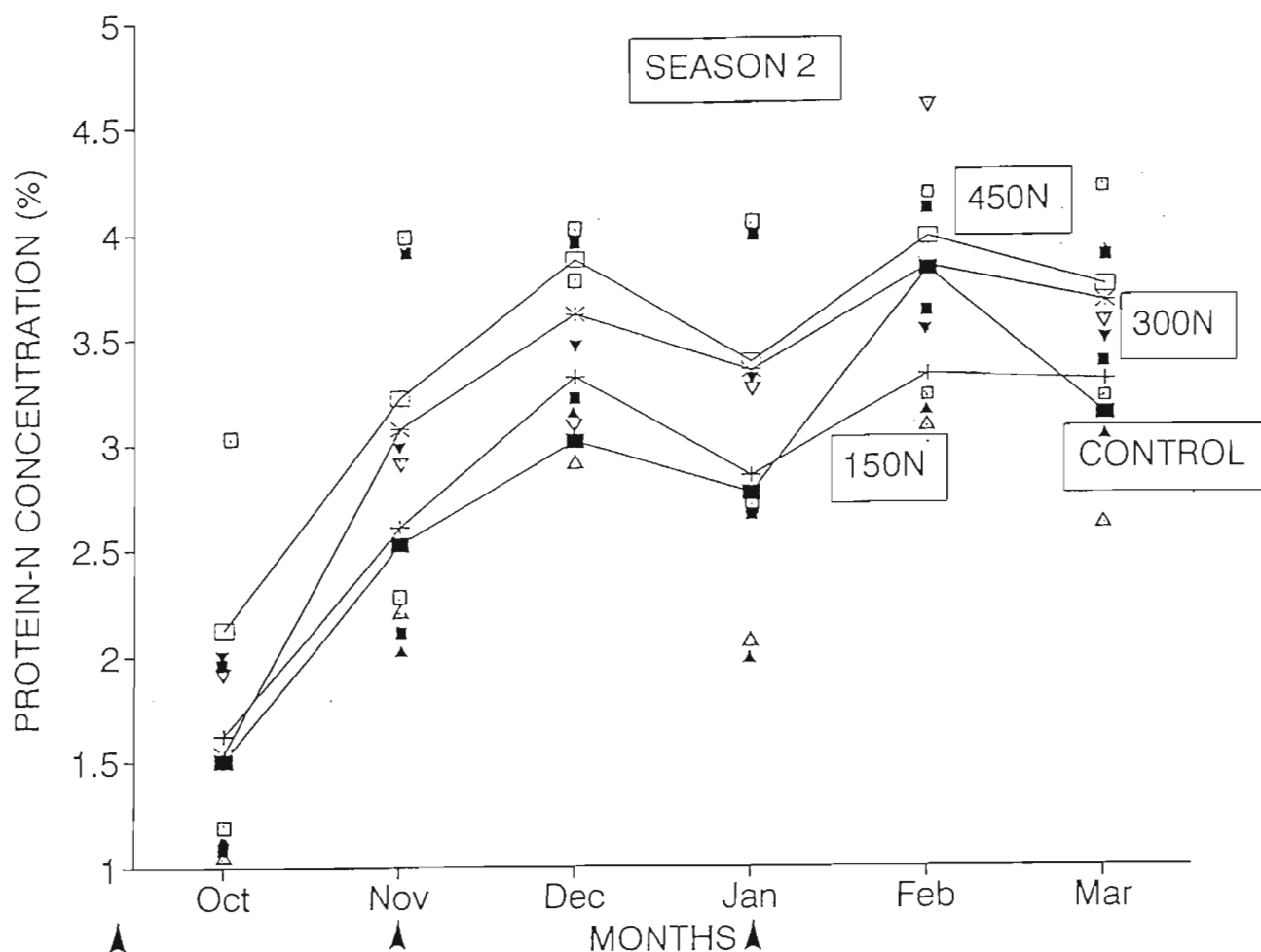


Figure 9b. Kikuyu protein-N concentrations (%) at monthly intervals during the second season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control=▽ 150N =▼ 300N =■ 450N =□

3.3.2.3 comparison of seasonal trends in protein N concentrations in the different sampling strata

3.3.2.3.1 season 1 (1989/90)

During early spring (Oct/Nov), no differences could be detected in the protein-N concentration within the herbage of the two sampling strata (Fig. 10a). In both, protein-N levels increased

considerably during this period, but then declined in both sampling strata to minimum values in January. This decline was, however, more dramatic in the material of the upper stratum than in that of the lower stratum. When these levels again rose in the late summer to reach a peak in February, the difference between the two strata were maintained, so that throughout the summer period protein levels remained higher in the material of the lower stratum than in that of the upper stratum (Average monthly data were used because of the great variability of sample data about the mean within each month (Fig. 10a)).

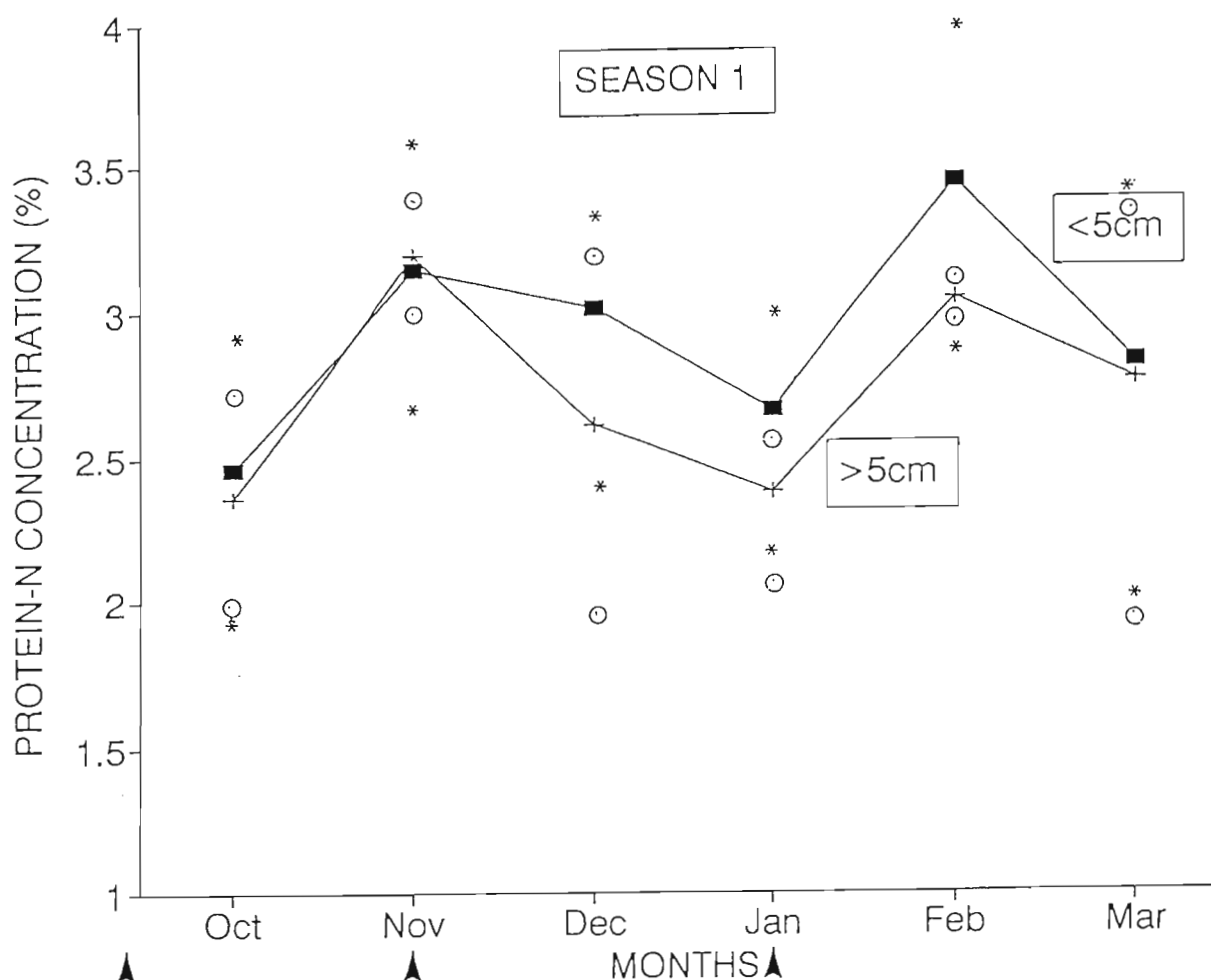


Figure 10a. Kikuyu protein-N concentrations (%) within the upper (>5cm) and lower (<5cm) herbage strata within the first season, meaned over all N treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: <5cm= * >5cm= O .

3.3.2.3.2 season 2 (1990/91)

The pattern associated with protein-N within the sampling strata in the second season was essentially similar to that of the first season (Fig. 10b). During early spring (Nov), levels of protein-N within both sampling strata increased considerably from those initially observed in October, and declined to a minimum within both strata in January. The decline was marginally more

prominent in the upper stratum than in the lower stratum. As the season progressed, protein-N concentrations in both sampling strata increased once again to a peak in February, followed by a decline in concentration in March (large sample variability existed within certain months eg november, and so data means were used to determine trends over the season (Fig. 10b)).

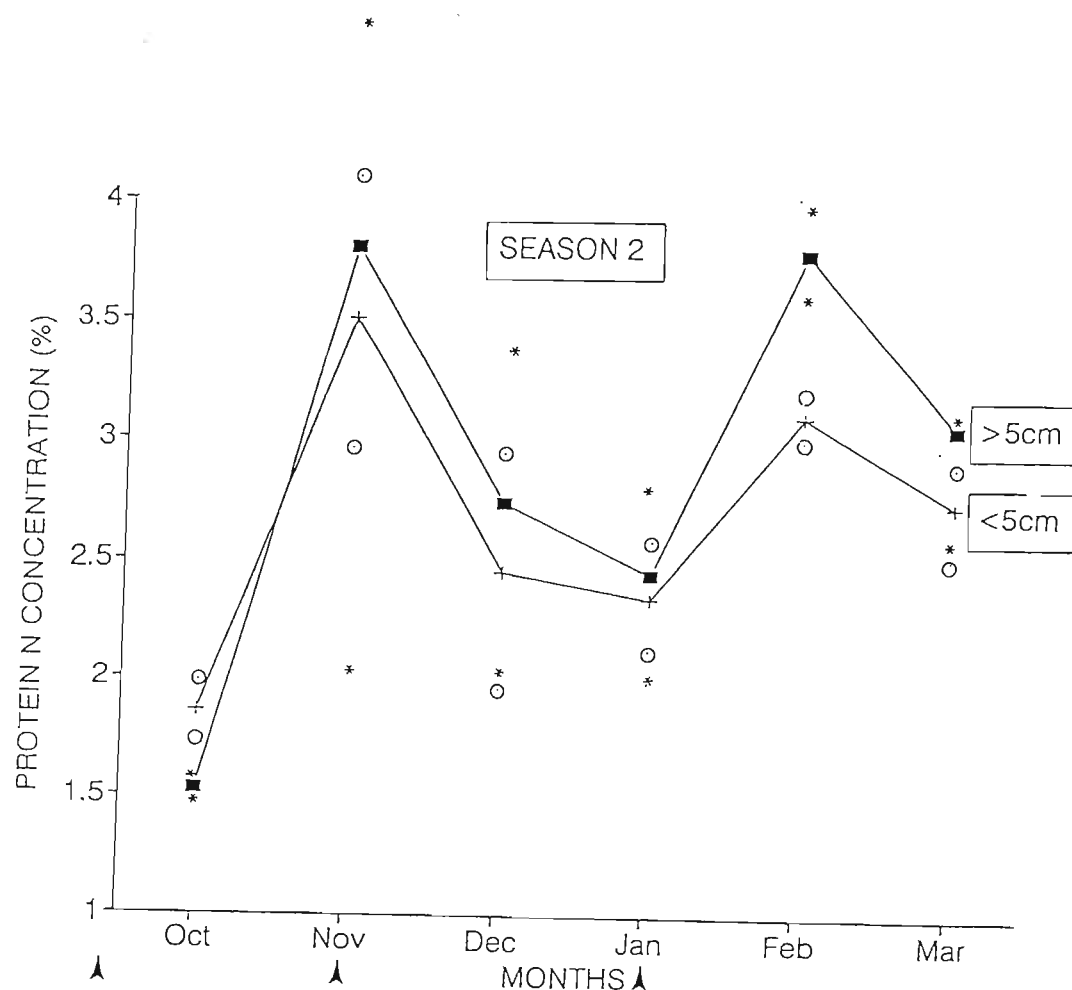


Figure 10b. Kikuyu protein-N concentrations (%) within the upper (>5cm) and lower (<5cm) herbage strata within the second season, meaned over all N treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: <5cm= * >5cm= O .

3.3.3 Plant nitrate nitrogen

3.3.3.1 seasonal nitrate nitrogen trends

3.3.3.1.1 season 1 (1989/90)

The mean $\text{NO}_3\text{-N}$ concentration of the combined herbage of the two sampling strata increased from 0.11% in the control (no nitrogen) treatment to 0.59% where 300 kg N/ha/season was applied to the pasture. No significant change was recorded when an additional 150 kg N/ha/season (450 kg N/ha/season) was applied to the pasture (Table 12).

Within sampling strata, the herbage of the >5cm stratum accumulated more $\text{NO}_3\text{-N}$, both on average and at each level of N applied, than that of the <5cm sampling stratum. In both cases, herbage $\text{NO}_3\text{-N}$ levels increased to the 300 kg N/ha/season level, before again declining (at the 450 kg N/ha/season level).

3.3.3.1.2 season 2 (1990/91)

Nitrate N levels within the combined herbage of the two sampling strata increased, although not always significantly, throughout the range of N applied. Furthermore, in this season, this pattern was observed within each individual stratum. As was the case in the first season, significantly more $\text{NO}_3\text{-N}$ accumulated, on average, within the >5cm stratum than in the <5cm stratum (Table 12).

Table 12. Kikuyu $\text{NO}_3\text{-N}$ concentrations (%) within each herbage stratum for both seasons and under varying N fertilisation levels, averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

Fert N levels kg N/ha	Season 1 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	0.10	0.12	0.11
150	0.29	0.36	0.32
300	0.59	0.60	0.59
450	0.49	0.57	0.53
Means %	0.37	0.41	0.39
Fert N levels kg N/ha	Season 2 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	0.12	0.14	0.13
150	0.14	0.18	0.16
300	0.26	0.31	0.28
450	0.30	0.36	0.33
Means %	0.21	0.24	0.22

5% 1%

LSDs (N level means for season 1)	0.05	0.07
LSDs (herbage strata means for season 1)	0.02	0.03
LSDs (body of the table for season 1)	0.08	0.10
LSDs (N level means for season 2)	0.03	0.05
LSDs (herbage strata means for season 2)	0.01	0.03
LSDs (body of the table for season 2)	0.06	0.08
LSDs season 1 vs season 2	0.14	0.18

3.3.3.2 seasonal patterns of nitrate N accumulation

3.3.3.2.1 season 1 (1989/90)

Nitrate N concentrations within the herbage of the combined sampling strata showed a bi-modal trend within this season (Fig. 11a). However, the degree of bi-modality within each of the

fertiliser application levels declined from the 300 kg N/ha/season application rate to the control treatment.

Nitrate levels rose from low values during October for all treatments, to reach an initial peak in November in those camps to which 150 and 300 kg N/ha/season had been applied. For the rest, initial peaks in $\text{NO}_3\text{-N}$ concentration were in December. In all treatments, $\text{NO}_3\text{-N}$ levels declined to low values in January before again increasing, to reach a second peak in February. Nitrate levels then either changed very little or declined (150 kg N/ha) in the March sampling (Fig. 11a) (unlike with protein N, sample data pertaining to fertiliser N applications about the mean were not greatly variable in the case of $\text{NO}_3\text{-N}$ (Figs. 11a & 11b)).

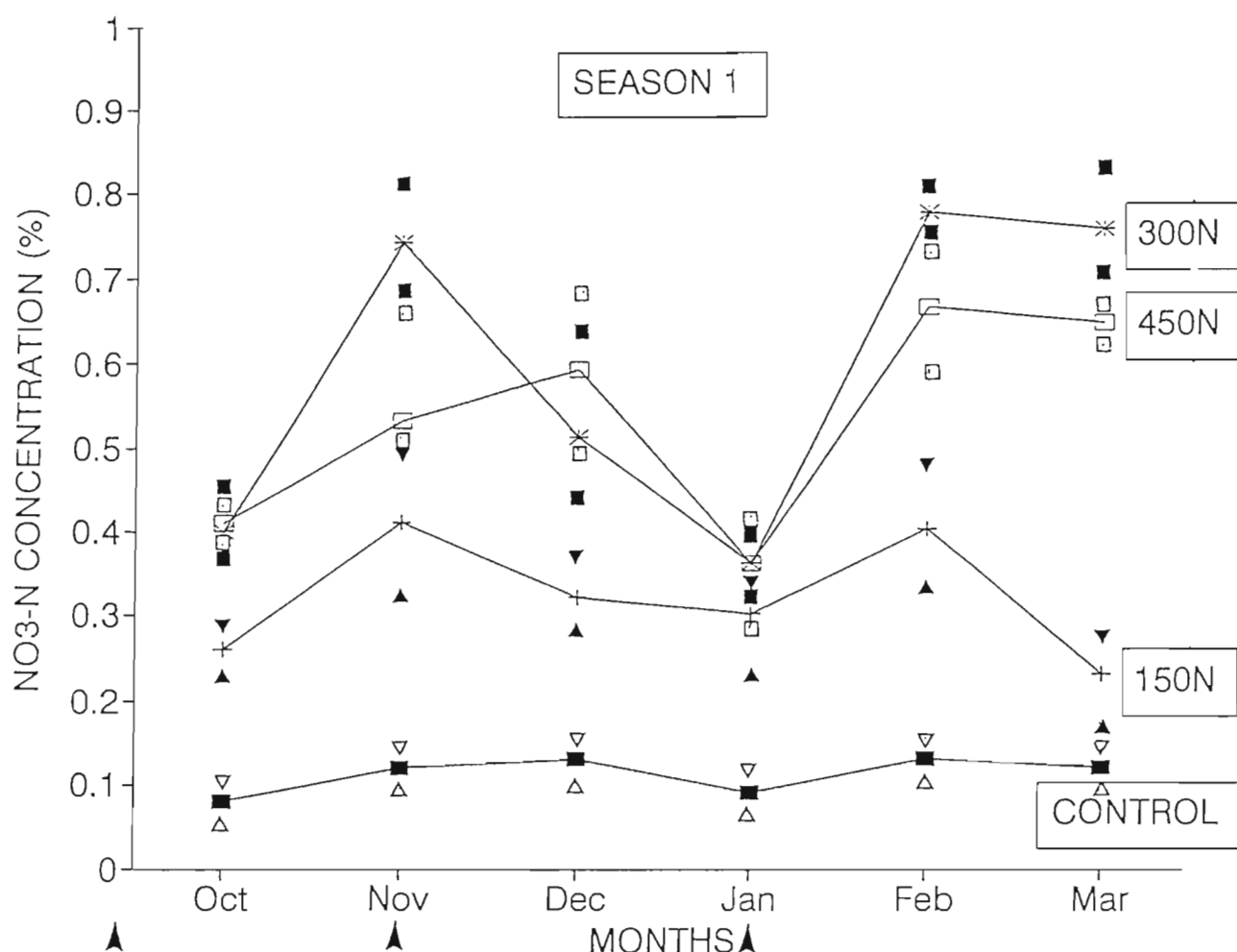


Figure 11a. Kikuyu NO₃-N concentrations (%) at monthly intervals during the first season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control=▽ 150N=▼ 300N=■ 450N=□ .

3.3.3.2.2 season 2 (1990/91)

In this season, the bi-modal pattern of NO₃-N accumulation was still apparent, but the initial increase in NO₃-N concentration in the fertilised camps between October and November was more pronounced than that between January and February (Fig. 11b). The exception to this was the concentration of NO₃-N in the

control swards, which rose slightly in November, before declining to a low in December, and then again rising to its highest level in February. The reason for this pattern is not apparent, although herbage yields were relatively low during the same period (Fig. 8). Alternatively, this result may have been a simple "concentration effect", the slower growth resulting in less dilution of the $\text{NO}_3\text{-N}$ absorbed by the plant, in the control sward in particular.

Nitrate N levels were generally, but not on all occasions, higher in those treatments to which N fertiliser had been applied, than in the control treatment.

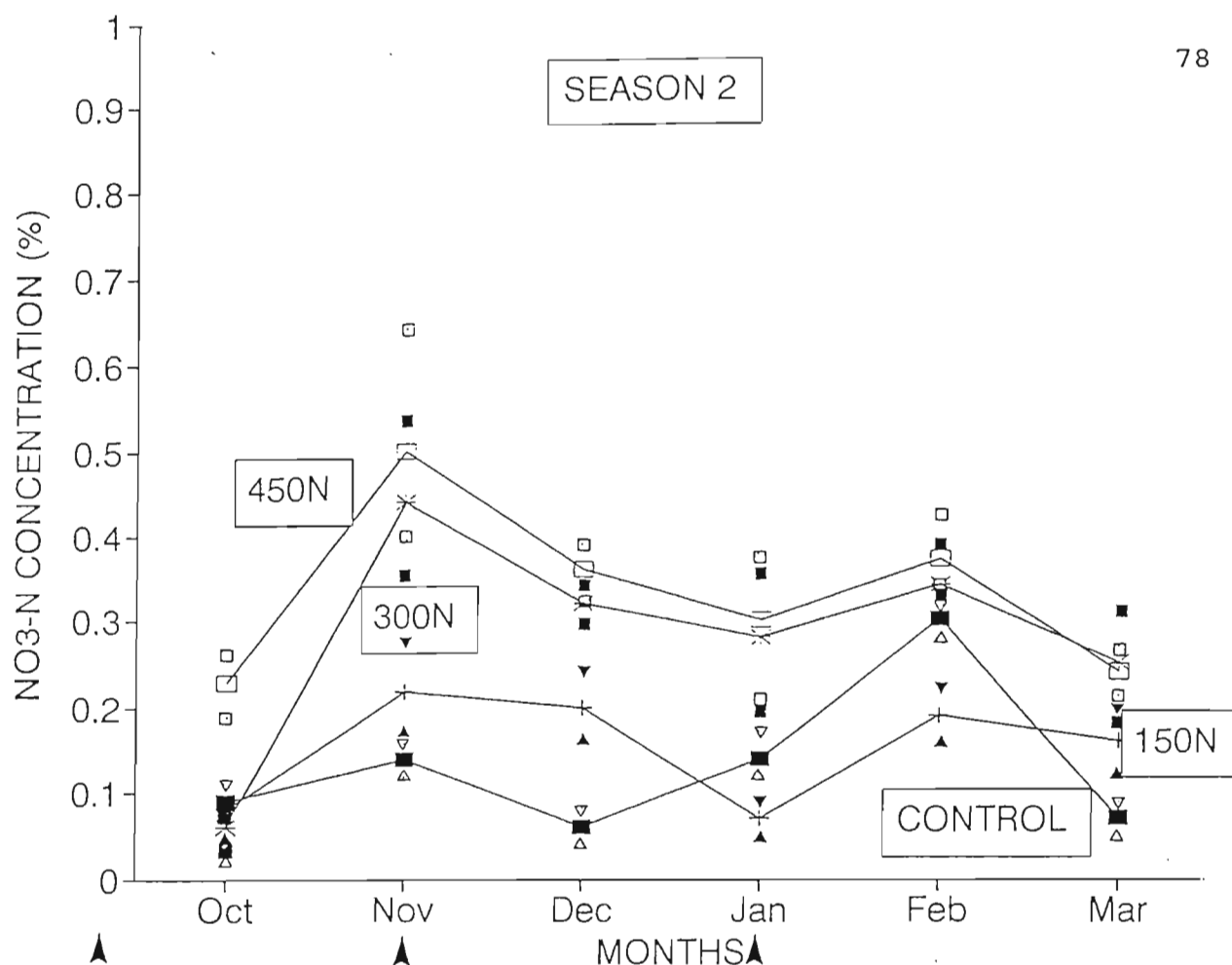


Figure 11b. Kikuyu NO₃-N concentrations (%) at monthly intervals during the second season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control= ∇ 150N= \blacktriangledown 300N= \blacksquare 450N= \square .

3.3.3.3 comparison of seasonal trends in NO₃-N concentrations in the different sampling strata for seasons 1 and 2

Bi-modal patterns of NO₃-N concentration in the herbage material, although less distinct in season 2 than in season 1, were observed individually in both strata in both seasons (Fig. 12a & 12b). However, sample data about the mean during season 1 were more variable than those in season 2).

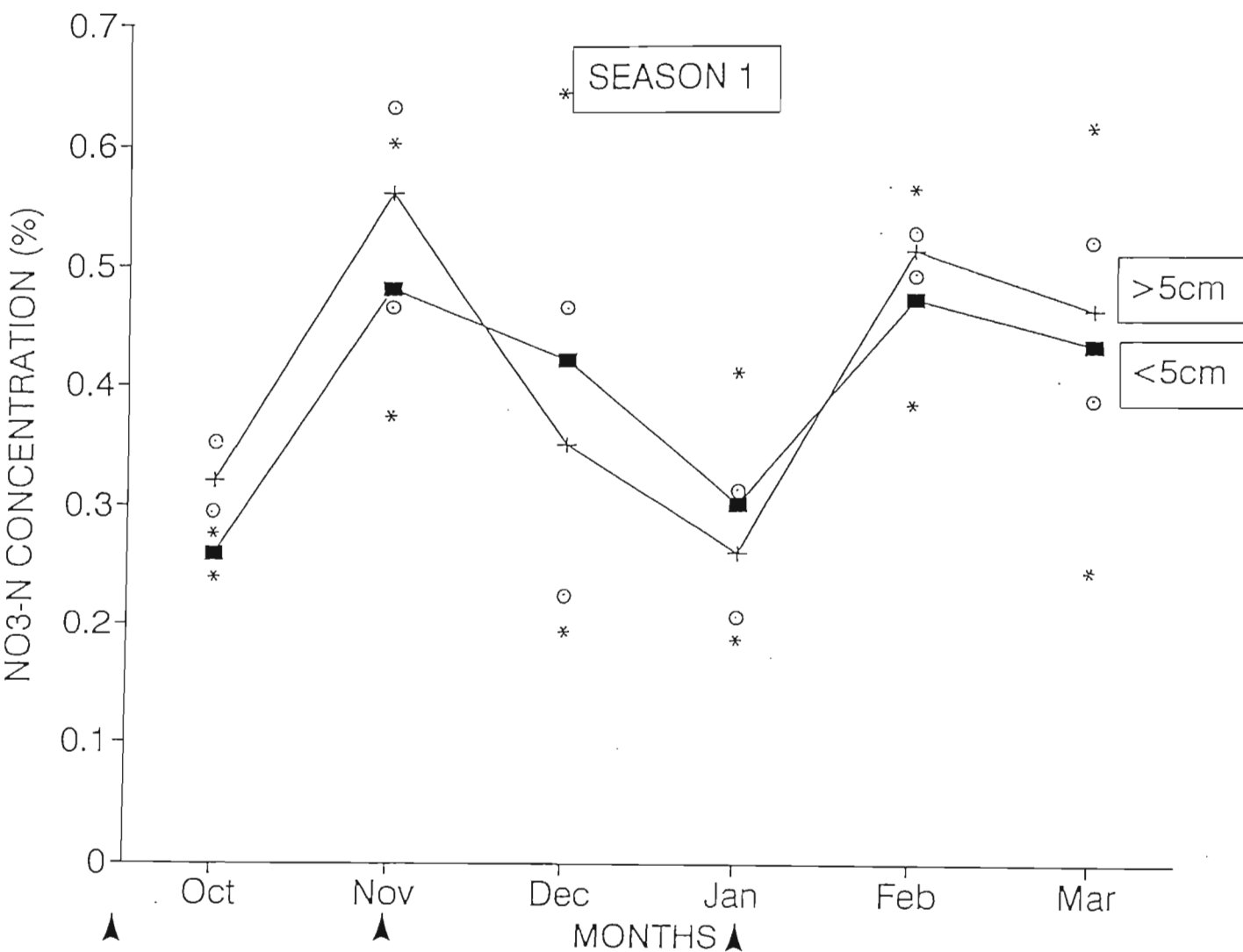


Figure 12a. Kikuyu NO₃-N concentrations (%) within the upper (>5cm) and lower (<5cm) herbage strata within the first season, meaned over all N treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: <5cm= * >5cm= O .

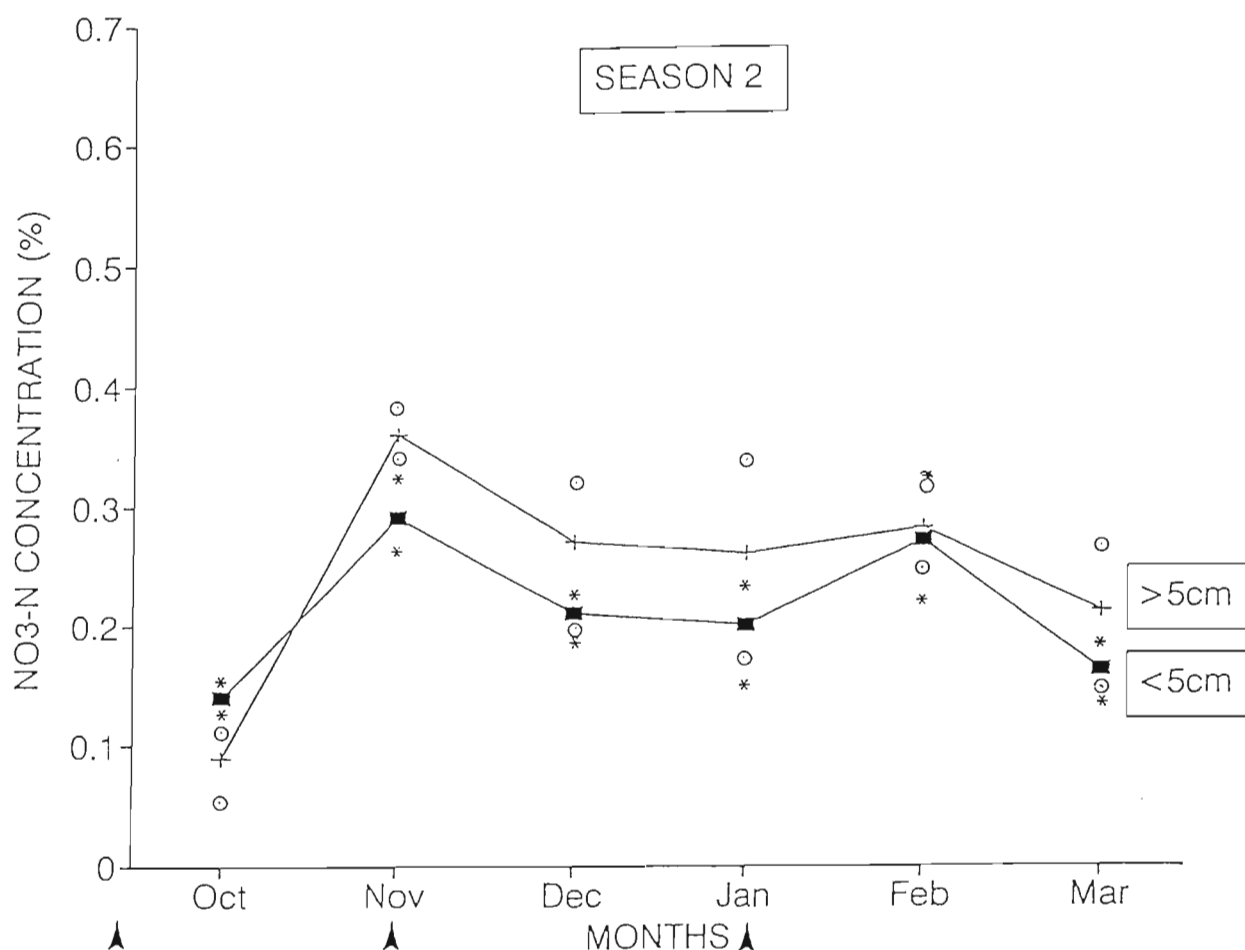


Figure 12b. Kikuyu NO₃-N concentrations (%) within the upper (>5cm) and lower (<5cm) herbage strata within the second season, meaned over all N treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: <5cm= * >5cm= O .

3.3.4 Plant total non-structural carbohydrates (TNC)

3.3.4.1 seasonal TNC yields

3.3.4.1.1 season 1 (1989/90)

Differences in TNC concentrations across N treatments were not

large, with only the levels recorded in the combined herbage of the two sampling strata from the 150 kg N/ha/season treatment significantly less than those of all other treatments (Table 13). This result was, however, probably still spurious in that an inter-seasonal interaction may have influenced this result to a large extent.

Within sampling strata, herbage of the upper (>5cm) stratum accumulated more TNC, both on average, and at each level of N applied, than that of the lower (<5cm) stratum. However, herbage TNC levels throughout the N application range in both strata showed no consistent trend.

3.3.4.1.2 season 2 (1990/91)

Here again, there were no consistent trends in the TNC concentrations across N treatments. In contrast to the data of the first season, however, levels were higher in the <5cm stratum than in the >5cm stratum.

Table 13. Kikuyu TNC concentrations (%) within each herbage stratum for both seasons and under varying N fertilisation levels, averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

Fert N levels kg N/ha	Season 1 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	5.1	6.3	5.7
150	5.1	5.8	5.4
300	5.5	6.0	5.8
450	5.2	6.3	5.8
Means %	5.2	6.1	5.7
Fert N levels kg N/ha	Season 2 Herbage Strata		Means %
	Lower (<5cm)	Upper (>5cm)	
0	5.8	4.1	4.9
150	6.3	4.7	5.5
300	6.0	4.2	5.1
450	5.6	3.8	4.7
Means %	5.9	4.2	5.0

	<u>5%</u>	<u>1%</u>
LSDs (N level means for season 1)	0.23	0.32
LSDs (herbage strata means for season 1)	0.14	0.19
LSDs (body of the table for season 1)	0.34	0.44
LSDs (N level means for season 2)	0.21	0.27
LSDs (herbage strata means for season 2)	0.12	0.15
LSDs (body of the table for season 2)	0.30	0.40
LSDs season 1 vs season 2	0.62	0.85

3.3.4.2 seasonal patterns of TNC accumulation

3.3.4.2.1 season 1 (1989/90)

The general seasonal TNC concentration pattern was one of a rapid decline, irrespective of N fertiliser treatment, from October to November, and a recovery of TNC levels only at the end of the season (February to March) (Fig. 13a) (composite sample data

about the mean tended to be variable over all months, fertiliser applications and herbage heights (Figs. 13a, 13b 14a & 14b).

3.3.4.2.2 season 2 (1990/91)

In this season, TNC levels again declined in the early spring (October to November) and again recovered in the autumn (Fig. 13b). Recovery in this season was apparent one month earlier (February) than the first season.

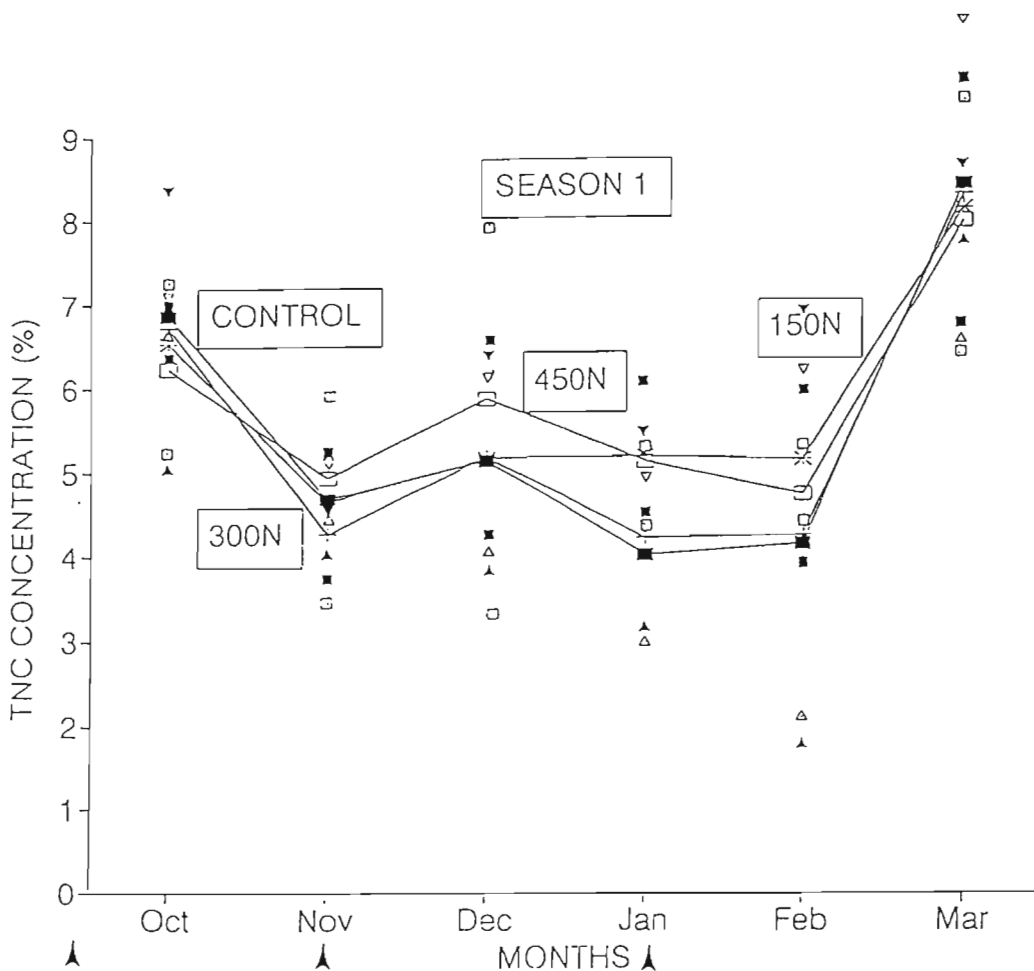


Figure 13a. Kikuyu TNC concentrations (%) at monthly intervals during the first season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control=▽ 150N=▼ 300N=■ 450N=□ .

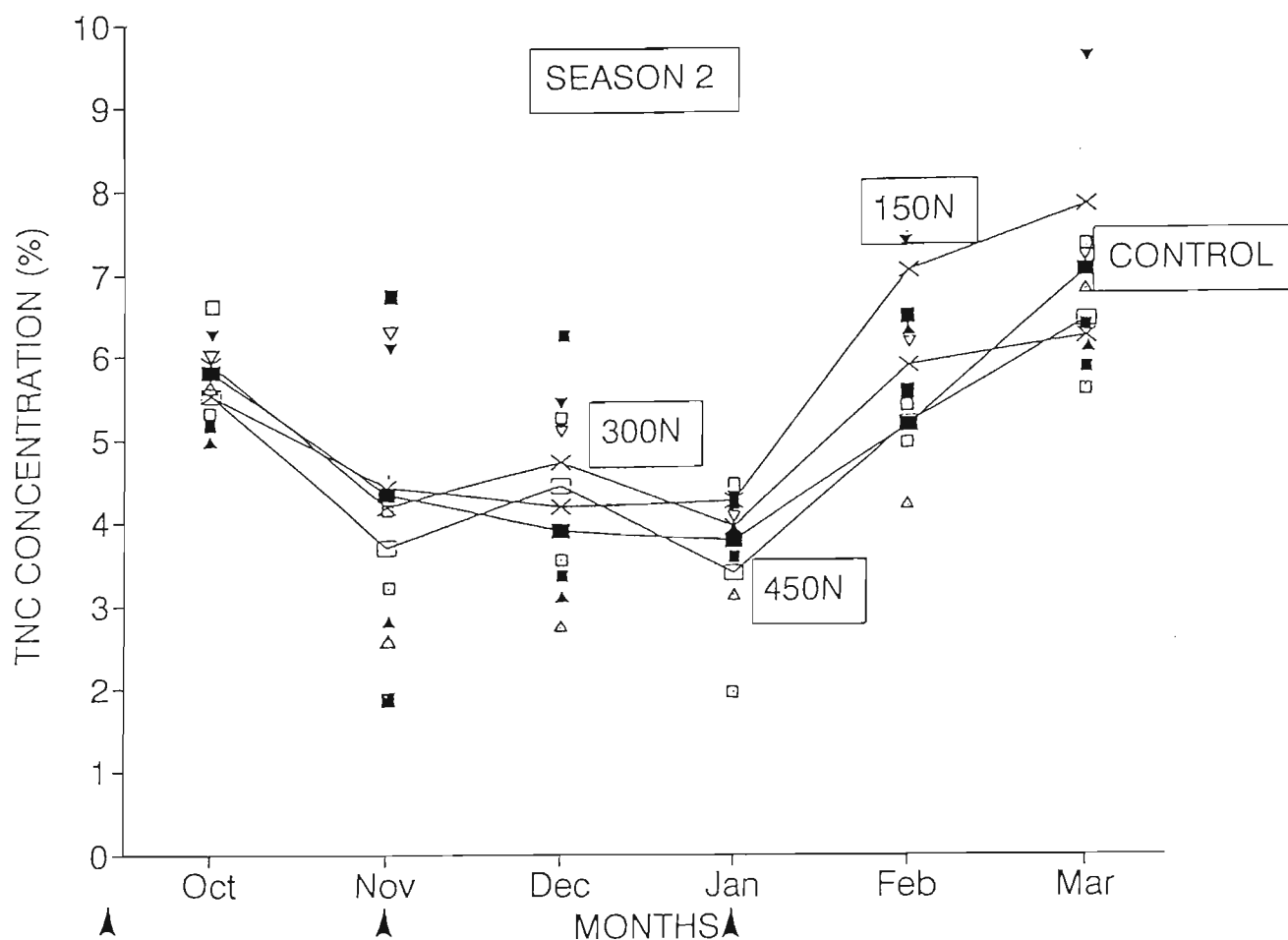


Figure 13b. Kikuyu TNC concentrations (%) at monthly intervals during the second season after the application of different rates of fertiliser N to the camps, meaned over both height strata (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: control=▽ 150N=▼ 300N=■ 450N=□ .

3.3.4.3 comparison of seasonal trends in TNC concentrations in the different sampling strata

Trends within the herbage of the two sampling strata were inconsistent between the two seasons (Fig. 14a & 14b). However, a rise in TNC concentration within both herbage strata at the end of each season (March), suggested that the pasture was storing carbohydrates prior to the winter period. Also, in both strata,

a relatively high concentration of TNC was also observed in spring (October). This was in contrast to both protein-N and $\text{NO}_3\text{-N}$ concentrations, which were relatively low at this time. Some concern must, however, be expressed with respect to the value of these data because of the general lack of any pattern between N treatments and seasons, and between the two herbage strata. Perhaps the only real value of the data are reflected in the distinct seasonal trends which emerged, particularly when compared with the seasonal patterns of N concentration. The inverse relationship between these two is of some physiological interest, and clearly may have a bearing on the seasonal quality changes in the kikuyu herbage.

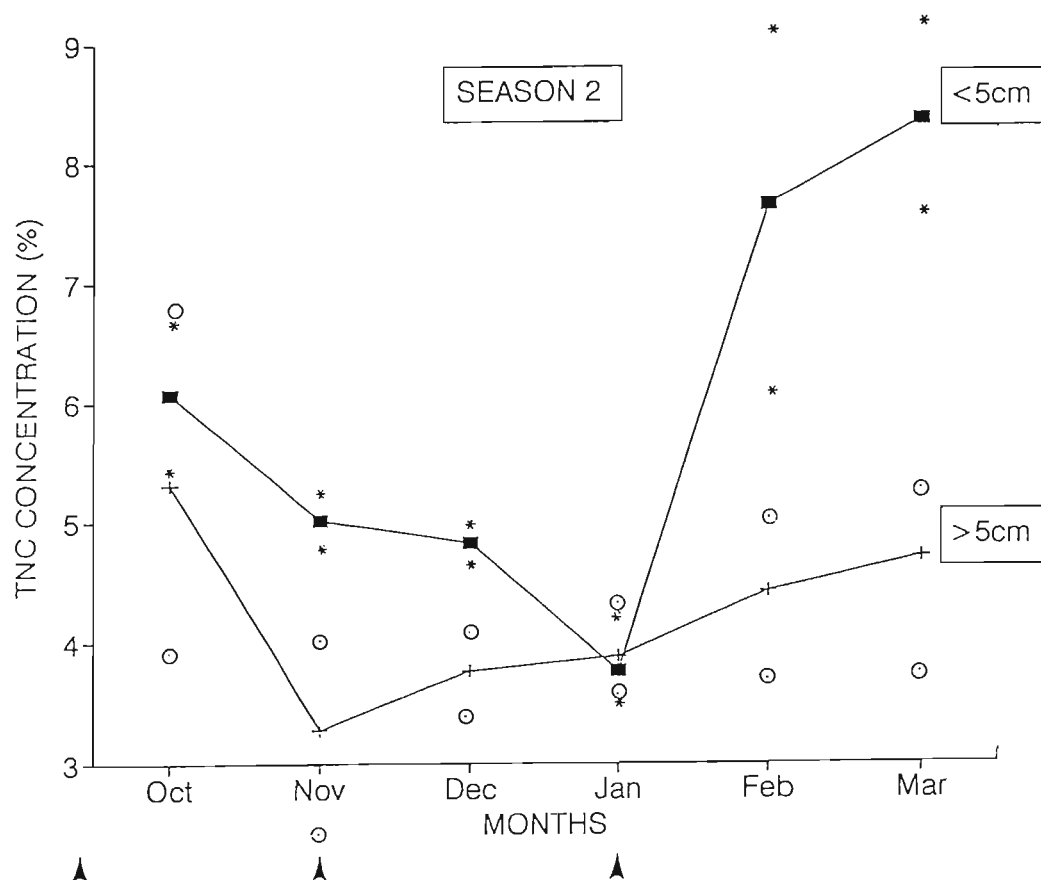


Figure 14b. Kikuyu TNC concentrations (%) within the upper (>5cm) and lower (<5cm) herbage strata within the second season, meaned over all N treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during the growing season. Composite sample points: <5cm= * >5cm= O .

3.4 Relationships between DM yields, protein-N, nitrate N and TNCs

In examining seasonal patterns of DM yield, protein-N and $\text{NO}_3\text{-N}$ concentrations, it is clear that yield was inversely related to both protein-N (Fig. 15a) $\text{NO}_3\text{-N}$ concentration (Fig. 15b). This was highlighted by the fact that during mid-summer (January),

when DM yields were high, the concentration of both N fractions, and particularly of $\text{NO}_3\text{-N}$, was relatively low. Conversely, high concentrations of the two N fractions corresponded to low growth periods. This may be related to plant utilisation of these two N fractions for growth, and so a dilution of both protein-N and $\text{NO}_3\text{-N}$ in the herbage material at times of rapid growth. At the same time, these patterns suggest that some factor(s) other than simple $\text{NO}_3\text{-N}$ or protein N concentration in the herbage was regulating kikuyu growth. In this respect, similar trends emerged in the soil total N, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations wherein these were found to be high during spring and autumn, and low during mid-summer over the two seasons (Ch. 2), suggesting that they may have influenced these herbage N fraction concentrations to some extent.

Data variability in protein N and TNC levels were large, but reasons for this could not be accounted for.

DM yields did not seem to be related directly to TNC concentrations (Fig. 16), as was the case for the two N fractions. Instead TNC concentrations were low during the summer months and were high only in early spring and autumn (March). This suggested that the kikuyu may have started storing TNCs in late summer as growth slowed, and that some of the TNC was carried through the winter until growth commenced in the spring.

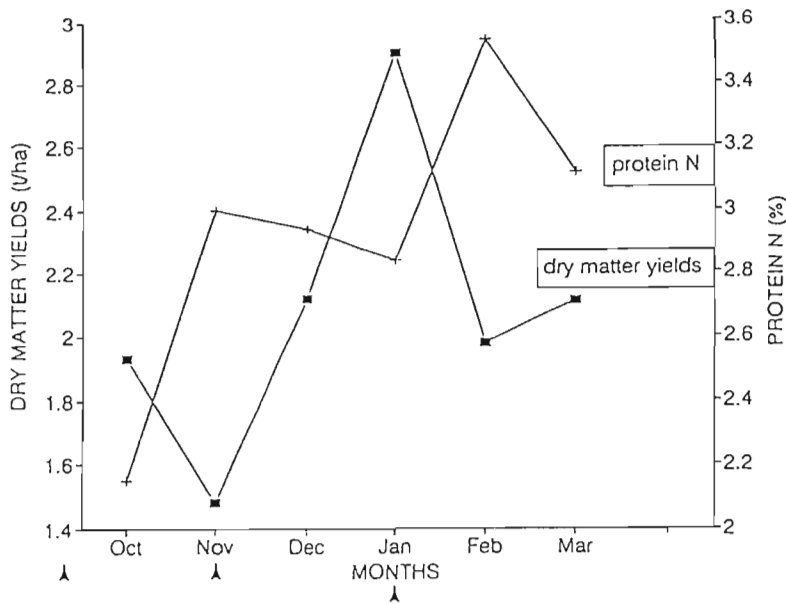


Figure 15a. The relationship between DM yields (t/ha) and protein-N concentration (%) on the kikuyu pasture, meaned over both seasons and all treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

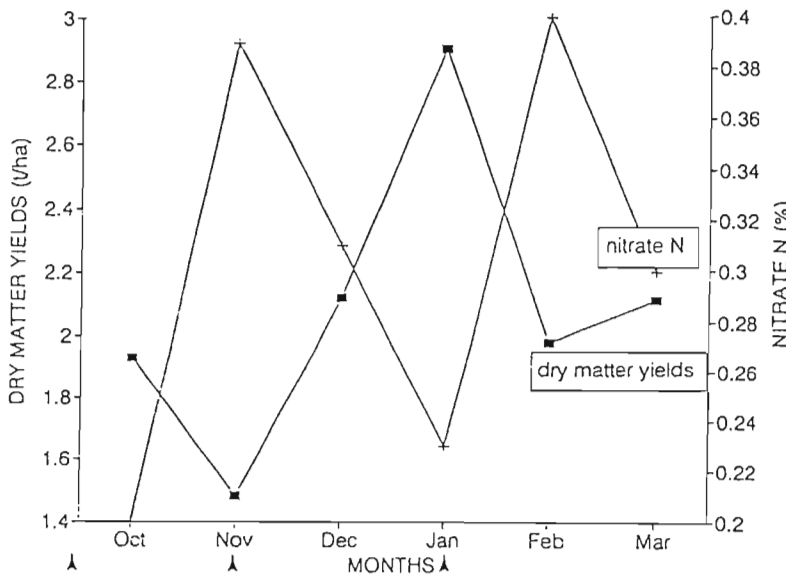


Figure 15b. The relationship between DM yields (t/ha) and NO₃-N concentration (%) on the kikuyu pasture, meaned over both seasons and all treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

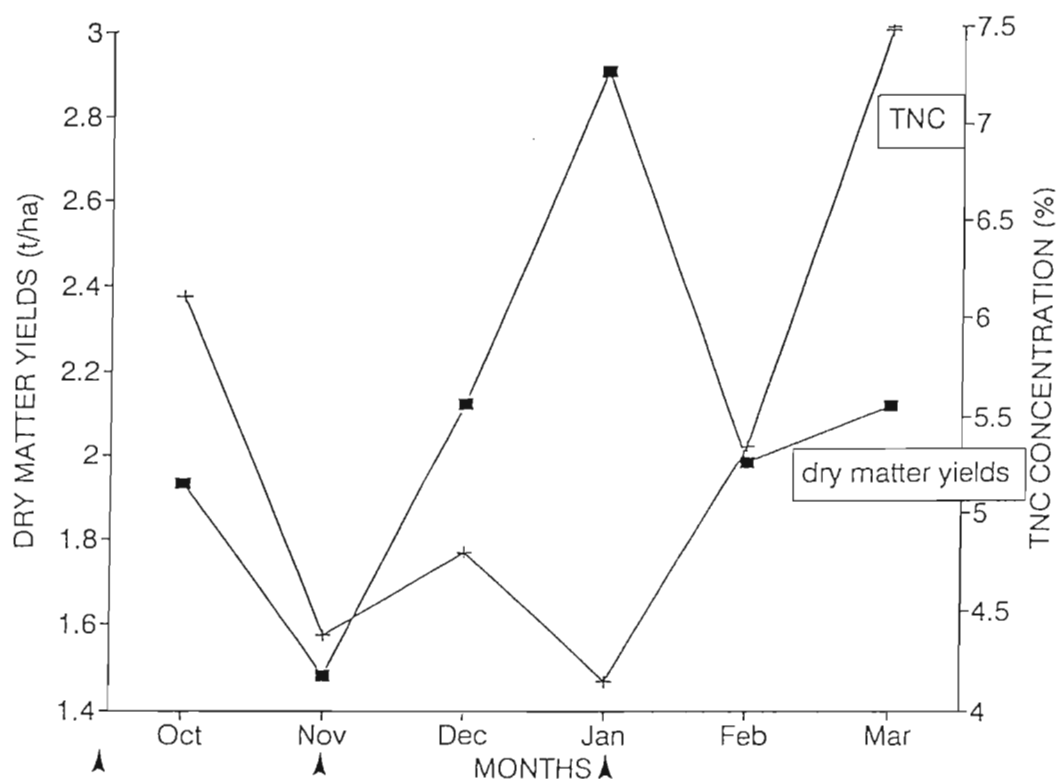


Figure 16. The relationship between DM yields (t/ha) and TNC concentration (%) on the kikuyu pasture, meaned over both seasons and all treatments (arrows indicate fertiliser applications) and averaged over the 6 assessments undertaken at monthly intervals during each of the 2 growing seasons.

3.5 Discussion and conclusions

3.5.1 Herbage DM yields

A mean DM yield of 12.7 t/ha for both seasons, averaged over all fertiliser N treatments and the control (Table 10), is not considered unreasonable from a pasture situated in the mist belt region of Natal. However 10.9 t DM, on average, off the control camps was deemed to be surprisingly high, especially since rainfall was below average for both seasons (Table 2). However, residual N from previous fertiliser applications, as alluded to

below, may have caused this to be so, thereby negating the probable negative effects of the lower rainfall.

Dry matter yields on the Broadacre trial increased progressively with successive increments of N fertiliser. Although the classical response curve is an asymptote, which is an effect well documented (Reid et al. 1966; Rhykerd et al. 1966; Cowling & Lockyer, 1967; Mengel & Kirkby, 1978, Ball, 1979 and Crowder & Chheda, 1982). A trend of this nature arises because the efficiency of fertiliser utilisation by the plant is normally relatively constant with increasing rates of N up to the level at which near-maximum yields are attained. If supra-optimal rates were investigated, eventually no further yield response would probably be observed; and ultimately yield would be then more than likely depressed.

The efficiency of N fertilisation of grasses is usually estimated in terms of kilograms of DM produced per kilogram of N applied. On the Broadacre trial, the response of the kikuyu to the N applied declined as N applications increased. Similar responses have been observed by Blue (1970), Henzell (1971) & Crowder & Chheda (1982). The efficiency of fertiliser use declines as N rates increase because the plant tends to reach a point of "saturation" where uptake by the plant of fertiliser-derived N fractions are no longer large. In general, the first increments of applied N increase growth rate, whilst further increments raise the N content of the grass without necessarily affecting the growth rate to any appreciable extent so N continues to be

taken up (Crowder & Chheda, 1982).

An increased yield of 1.8 t DM/ha in the first season over that of the second is difficult to explain in light of the fact that 174mm more rain fell, in total, during the second than during the first season. Notwithstanding this fact, rainfall distribution and volumes recorded each month were very similar for both seasons. However, below-average rainfall prior to the initiation of this trial (1987 season) may have resulted in a very dry soil at the start of the first season of the trial (1988 season). This, coupled with early rains in the 1988 season, may have caused mineralisation to proceed at an accelerated rate. Alternatively, rainfall at the end of the 1988 season was unusually high, which may have resulted in a high soil moisture status at the end of that season. Rainfall in the spring of the second trial season (1989 season), may then not have initiated mineralisation reactions to the extent that it had in the previous season because soil moisture was still high from the that season's late rainfall.

Conversely, residual N resident in the soil profile from previous dressings over the years may also have influenced this result.

Soil NH_4^+ data were higher during the first season, especially at the 300 kg N/ha/season and lower rates of fertiliser application, which tends to verify the premise that residual N build-up was indeed present in the soil profile during this time (NO_3^- concentrations do not show this trend, probably because the pasture was using these ions up as fast as they could be produced

in the soil). Thus, the response to N fertilisation in season 1 was limited, but by season 2, the residual N, especially at the lower N application rates (0 and 150 kg N/ha/season) had been largely utilised, hence the very much lower yields at these lower N levels in the second season. Higher NO_3^- levels in the herbage during the first season are also consistent with this hypothesis.

The higher yields in season 1 may also reflect a perceived response only to very high N levels of fertilisation (i.e. DM yields situated at or near the apex of the DM response curve), whilst plants during the second season showed a response that was lower down the response curve than the previous season's yields. Rainfall patterns, being similar for both seasons, influenced DM yields in a similar manner. Typically, spring growth of the pasture should have been rapid, but due to a low rainfall in October and November of the first season, and October of the second, growth was slow. As rainfall increased from December onwards in both seasons, DM yields responded accordingly, reaching a peak in January of both seasons. After January DM yields declined even though rainfall remained high until March of both seasons. Pasture senescence is likely to have become a factor limiting any further increased yields after January into autumn.

From these results, one might conclude that these trends are influenced to a large degree by environmental factors, including those of rainfall, daylength and soil moisture status, as well as previous management practices, and that the

mineralisation/immobilisation reactions governing the presence/absence of readily-available soil N for plant use would have been regulated by them.

3.5.2 Plant protein nitrogen

The relatively toxic NH_4^+ ion present within the plant from either the reduction of the NO_3^- ion and/or uptake of any unbound NH_4^+ ions from the soil by the plant, is immediately converted by the enzyme glutamate synthetase/synthase into non toxic derivatives (amines) for use by the plant (Haynes, 1986). Once conversion has occurred, these products contribute towards protein-N synthesis in the herbage (Haynes, 1986; Lewis, 1986).

The bulk of the N in plants is contained in protein-N, with leaf material containing 2% protein-N, on average (Beevers, 1976). This figure is lower than that recorded in the herbage on the Broadacre trial (2.84%, on average). Ball (1979), working in a ryegrass-white clover association, observed an average N content throughout the year of 3.5%.

In forage crops, an important effect of fertiliser N is its enhancement of protein-N content, and thus plant quality (Olson & Kurtz, 1982). This is particularly important with tropical species (Henzell, 1971). In general, protein-N concentrations increased in this trial as N application rates were increased. Mays (1974), Benzian & Lane (1981), Benzian *et al.* (1983) and Wilman & Wright (1983) also observed a similar result on their trials and concluded that protein-N content increased linearly

(as observed on the Broadacre trial) with N rate, irrespective of the N rate which gave the maximum yield.

On average, over both seasons, higher concentrations of protein-N were measured within the upper (>5cm) than in the lower (<5cm) herbage stratum, irrespective of the amount of N applied. This may be related to the fact that N is translocated to newer younger leaves in order for growth to proceed in this region of the plant. The importance of this result is that it suggests that the newer leaves are of better quality than the more mature lower stratum tissues.

Similar bi-modal trends over time in protein-N concentration were measured for all N treatments and within both herbage strata over both seasons (Figs. 9a & 9b). Concentrations tended to be highest during early summer (Dec), and in early autumn (Feb), and lowest during spring (Oct), mid-summer (Jan) and autumn (March). Spring and autumn peaks seemed to correspond with periods of slower growth, whilst low mid-summer concentrations pertained to periods of high DM yields and TNC concentrations. The high protein-N concentrations measured at the beginning and end of the season are important to note as they suggest that during these periods protein-N would probably be adequate for animal growth and/or maintenance. Furthermore, applications of fertiliser N might then be delayed or even withheld so that plants are forced to draw upon and use the high protein-N levels prevalent within the herbage at these times, thereby obtaining the maximum benefit from previously applied fertiliser N. Fertiliser N additions to

the pasture at these times might also result in plant induced NH_3 toxicosis to animals because of the ready availability of N within the plant/soil complex.

3.5.3 Plant nitrate nitrogen

In general, the main source of plant N is derived from the uptake of NO_3^- ions from the soil. Nitrate ions tend to be absorbed preferentially to the NH_4^+ ion because the NH_4^+ ions, unlike the NO_3^- ions, tend to be bound by the soil colloids. Nitrate can move freely towards plant roots by mass flow in the 'stream' of water created by transpiration. Once absorbed by the plant, NO_3^- ions are reduced to NH_4^+ ions before being assimilated into organic compounds. This process is called "assimilatory nitrate reduction" and uses the enzyme nitrate reductase (Lewis, 1986).

Not all of the NO_3^- ions are immediately converted to the NH_4^+ ion within the plant and excess NO_3^- ions are translocated to vacuoles for storage and subsequent use (Lewis, 1986). This reduces the potential for NO_3^- toxicity within its cells. Herbage containing high concentrations of unconverted NO_3^- ions are a potential health hazard to animals grazing the pasture.

Critical limits of $\text{NO}_3\text{-N}$ toxicity in animals from the DM seem to vary from author to author and between temperate and tropical species. Vanderlip & Pesek (1970) considered 0.07% to be the critical value in brome grass (Bromus inermis), whilst Ryan et al. (1972) considered safe levels to be 0.15% in perennials. Leeuwen (1972) quoted ranges from 0.14% to 1.5%, while Dickson &

Macpherson (1976) suggested a range of 0.5 to 0.7% as the LD_{50} for NO_3-N in the DM. Coombe & Hood (1980) demonstrated that grazing cattle were able to tolerate levels of 0.52% NO_3-N in the DM. The range of NO_3-N observed in the DM on the Broadacres trial was 0.12% to 0.43%. These figures therefore compare favourably with published data and suggest the possibility of causing NO_3^- toxicity at the higher N levels; at least as observed during the first season.

As applications of fertiliser N to the pasture increased, NO_3-N concentrations within the herbage increased in a near-linear fashion (Table 12 & Figs. 11a & 11b). Similar results were obtained by Ferguson & Terry (1956), Hanway & Englehorn (1958), Sumner et al. (1965), Lovelace et al. (1968), George et al. (1973), Wilman & Wright (1981) and Ehlig & Hagemann (1982). They concluded that as the abundance of the NO_3^- ion increased within the soil, primarily as a result of increased N application rates, so plant uptake of the ion also increased. This, they intimated, led to higher concentrations of NO_3-N being measured at higher N application rates than at the lower rates.

On average, higher concentrations of NO_3-N , irrespective of the amount of fertiliser N applied, were measured within the upper (>5cm) than the lower (<5cm) herbage stratum. The presence of NO_3-N within the younger portion of the sward suggests that the plant is actively translocating this N fraction to these regions for subsequent synthesis into NH_4^+ ions, and thus protein-N (Deinum & Sibma, 1980; Lewis, 1986). However, Deinum & Sibma

(1980) and Prins (1983) found that under a cutting regime, $\text{NO}_3\text{-N}$ accumulation rarely occurred with an N supply of less than 400 kg/ha/annum, because under these conditions most of the absorbed $\text{NO}_3\text{-N}$ became reduced in the roots and thus never reached the leaves. Problems of $\text{NO}_3\text{-N}$ toxicity might, however, still arise within the upper herbage stratum in the presence of high concentrations of soil $\text{NO}_3\text{-N}$, especially where high N rates are applied in succession, and/or where the plant is unable to convert all the NO_3^- ions absorbed by the plant to NH_4^+ ions all at once (see 3.5.4 TNC discussion).

A higher concentration of $\text{NO}_3\text{-N}$ was measured in the herbage during the first season than in the second on the Broadacre trials, meaned over all treatments and both height strata. The higher concentration in the first season may have resulted from the presence of residual N within the soil which could have increased herbage uptake of $\text{NO}_3\text{-N}$ above that of the second season (see 3.3.1). Alternatively, $\text{NO}_3\text{-N}$ within the soil complex may have become leached down the profile, as a result of the higher rainfall during this season (Table 2), and thus out of reach of the plant roots (Table 7a). Furthermore, O'Hara & Fraser (1975) and Ross *et al.* (1978) showed that under dry conditions, more $\text{NO}_3\text{-N}$ accumulated in the plant than under wetter conditions. This would then probably explain this result, as 173.6mm less rain fell during this season than in the second season (see Table 2).

O'Hara & Fraser (1975) also suggested that, unlike under wet

conditions, $\text{NO}_3\text{-N}$ accumulation within the root zone during dry periods was common, and this ultimately led to an accumulation of this N fraction within the herbage. Results on this trial were similar, in that $\text{NO}_3\text{-N}$ concentrations within the profile in the second season were higher and more concentrated at depth, especially at the higher N application rates, than in the first season. This probably corresponded to the higher rainfall during this season which resulted in a lower uptake of these ions into the herbage because they were leached out of the root zone by water percolating down through the profile (see Table 7a).

A similar bi-modal trend to that measured with protein-N concentrations was observed in both seasons for $\text{NO}_3\text{-N}$ in the herbage. High concentrations of $\text{NO}_3\text{-N}$ were measured during spring (Nov) and autumn (Feb), and lower concentrations in mid-summer (Dec & Jan), very early spring (Oct) and early autumn (March). These results suggest that N fertiliser should be withheld when $\text{NO}_3\text{-N}$ concentrations are high, at least until such time as plant growth is about to be impaired by a shortage of available N for plant uptake. Potential toxicosis may also become problematic at these times if fertiliser N were to be applied at high application rates. During summer, declining $\text{NO}_3\text{-N}$ concentrations are associated with a corresponding increase in herbage DM yields. Furthermore, $\text{NO}_3\text{-N}$ and protein-N concentrations follow similar trends over the season, which confirms that these two N fractions are linked to growth functions in the plant.

3.5.4 Plant total non structural carbohydrates

Non structural carbohydrates (TNCs) are the readily metabolisable source of energy needed for growth and survival, both in plants and animals (Ross et al. 1978). They include water soluble carbohydrates, but exclude the celluloses, hemicelluloses and lignin, these being structural in nature. The TNCs are stored in the plant and are used for maintenance during periods of stress and for growth (Butler & Bailey, 1973).

Applications of fertiliser N to the kikuyu revealed inconsistencies over the two seasons that were monitored. Lee & Smith (1972a & b) and Ross et al. (1978) encountered similar variability on their trials, ascribing it to changes in the time of day when sampling was performed on the pasture. However, authors such as Burton et al. (1959), Metson et al. 1966, Nowakowski & Cunningham (1966), Balasko & Smith (1971) and Ross et al. (1978) all measured a decline in TNC concentration with increased N application rates. A low TNC content within the herbage during the season might also be ascribed to climate, frequent defoliation, as well as an abundant supply of available N, or combinations of these and other factors (Ross et al. 1978). A lack of any distinctive trend emerging among these trials in response of TNC to increased fertilisation with N, suggests that, in kikuyu, applied N alone would not materially alter TNC concentrations.

Higher concentrations of TNC were determined in the lower (<5cm) height stratum, on average, than in the corresponding upper

(>5cm) stratum. This may be ascribed to the fact that TNCs tend to be found in higher concentrations where plant protein-N and $\text{NO}_3\text{-N}$ concentrations are low. Work performed by Jones et al. (1961), Mays (1974), Ball et al. (1978) and Ross et al. (1978) showed this to be so; a combination which would favour $\text{NO}_3\text{-N}$ poisoning in animals consuming forages of low TNC content with correspondingly high NO_3 and protein N levels (Barnett & Bowman, 1957; Wright & Davison 1964). This inverse relationship between both protein-N and $\text{NO}_3\text{-N}$ and TNC levels in temperate species has been well documented, and these results now suggest that tropical species such as kikuyu follow similar patterns to the temperates (Nowakowski, 1962; Bryant & Ulyatt, 1965; Ross et al. 1978).

It is also interesting to note that the increased concentration of TNC in autumn suggests that in kikuyu, TNCs may indeed accumulate. Furthermore, the high concentration of TNCs in spring suggests that TNC concentrations remain high over winter and only decline once protein-N and $\text{NO}_3\text{-N}$ concentrations begin to increase in the summer. However, more extensive measurements are needed to confirm this result.

3.5.5 The relationship between protein-N, $\text{NO}_3\text{-N}$, TNCs and DM yields

As alluded to earlier, a number of relationships were found to exist between the two N fractions and TNCs. It is also useful to determine what relationship, if any, exists between these three components and DM yields since they are important determinants of pasture growth.

An inverse relationship existed between DM yields and the two N fractions. When DM yields were high (characteristically in summer), these two N fractions were low. These two N fractions were probably being actively utilised for growth and thus concentrations were low at this time. As DM yields declined into autumn, or before the spring regeneration period when the pasture was essentially dormant, protein-N and $\text{NO}_3\text{-N}$ concentrations were high because they were not being utilised for growth.

3.5.6 Conclusions

Of the 10.85 t DM/ha at a total herbage N percent of 2.53, measured on average on the control camps over the two seasons, 274.5 kg N/ha/season was calculated to have been present within the soil, either as residual N and/or from OM breakdown (Table 14). After application of 150 kg N/ha/season, the proportion of total N in the herbage was 368.7 kg N/ha/season. Response to this N application, coupled with the presence of the residual N as a result of past N applications and resultant OM breakdown through the "priming" effect (274.5 kg N/ha), was 94.2 kg N/ha/season. This response increased with each N application level (Table 14).

Table 14. Proportions of N taken up by the herbage as fertiliser N (kg/ha) and response to N applications, averaged over both seasons and all fertiliser application rates.

Fert N levels kg/ha	DM yields t/ha	Herbage protein N %	Herbage NO ₃ -N %	Total herbage N %	Proportion of total N from herbage (1) and response to N applications (2) kg N/ha/season	
					(1)	(2)
0	10.85	2.41	0.12	2.53	274.5	0
150	12.50	2.71	0.24	2.95	368.7	94.2
300	13.15	3.06	0.43	3.49	458.9	184.4
450	14.20	3.16	0.43	3.59	509.8	235.2

The response of 94.2 kg N/ha/season, as a result of the application of 150 kg N/ha/season, accounted for 62.8% of the fertiliser N applied to the herbage. This declined to 61.8% after the application of 300 kg N/ha/season, and 52.2% after the application of 450 kg N/ha/season, and may go some way to showing the response of OM and/or residual N through the "priming" effect as a result of fertiliser N applications to the pasture.

4. THE FATE OF LABELLED AMMONIUM ^{15}N IONS APPLIED IN SPRING AS AMMONIUM NITRATE FERTILISER TO A KIKUYU PASTURE.

4.1 Introduction

Of the six known isotopes of N, only those having mass numbers of 14 and 15 are stable and occur naturally. Their use as tracers is based on the fact that ^{14}N and ^{15}N occur naturally in an almost constant ratio of 272:1 (i.e. naturally occurring N contains about 0.3663 atom % ^{15}N) (Hauck & Bremner, 1976).

The value of tracer techniques in studying the absorption of plant nutrients, and in detecting changes in the distribution of various elements among fractions of the soil and in the herbage, has long been recognised. Materials with unusually high or low concentrations of ^{14}N and ^{15}N can be used as tracers, provided that their N isotope composition is measurably different from the unlabelled N at the time the herbage or soil material under investigation is sampled. The change in N isotope ratio in samples obtained from the system permits study of the transformations of the added tracer and thus calculation of the extent to which the tracer has interacted with and become part of the system (Hauck & Bremner, 1976). In order to formulate recommendations for the more efficient use of fertiliser N, it is important to understand the fate of fertiliser N applied to a pasture. In this respect, ^{15}N labelled fertilisers are often used in experiments to determine the fate of fertiliser N within the soil/plant continuum over time (Jenkinson et al. 1985). In previous studies, little attention has been given to the

measurement of N in the roots, herbage and soil of pastures in humid subtropical regions, apart from investigations by Macvicar et al. (1950) and Dowdell & Webster (1980). Furthermore, in South Africa most labelled N studies have been restricted to controlled environments, using plants grown in pots, due mainly to the extremely high cost of the isotopes (Jokela & Randall, 1987; Sanchez et al. 1987; Stumpe et al. 1989).

Results obtained earlier in these investigations (Ch. 2) showed that no appreciable change in $\text{NH}_4\text{-N}$ levels could be detected in pasture soils following the application of limestone ammonium nitrate (28) fertiliser. Data showed that the soil initially contained relatively large amounts of $\text{NH}_4\text{-N}$, making it difficult to detect the relatively small amounts which were applied through the fertiliser. A decision was therefore taken to use labelled ^{15}N to investigate this matter further.

The aims of the investigation were to determine the uptake of labelled $^{15}\text{NH}_4^+$ ions by the roots, its accumulation in herbage and movement between different depth strata in the soil, and to then measure the subsequent recovery of ^{15}N by using a mass spectrometer, as a percentage of that applied as $^{15}\text{NH}_4\text{NO}_3$ (35) fertiliser, after a spring application.

4.2 Methodology

The experiment was undertaken on a dryland kikuyu pasture on the Broadacre trials. The trial ran for one growing season (October

1992 through to March 1993). (Refer to Ch. 1.6 for full details of methodology and calculations).

Before $^{15}\text{NH}_4\text{NO}_3$ fertiliser was applied in October, samples were analysed from the herbage, roots and each soil depth class to determine the abundance of naturally occurring ^{15}N in the N of these components. Under normal circumstances, naturally occurring N contains about 0.366 atom % ^{15}N (Hauck & Bremner, 1976). However, in these trials, and probably as a result of the combination of a high organic C and clay content (Table 1) as well as the age of the pasture, naturally occurring N contained more ^{15}N than the 0.366 atom % alluded to above. This phenomenon is not uncommon and is related to the slightly higher mass and smaller size of the ^{15}N atoms in relation to that of the ^{14}N atoms. Over time, ^{15}N atoms will form stronger bonds in the soil-plant continuum, and will therefore be more prone to movement in the soil. This tendency will explain the slow enrichment of N, common to all soils. This problem can be overcome by analysing the control plot to ascertain these relative abundances. For the purpose of this trial and for the sake of accuracy, the following ^{15}N atom %, calculated from the October analyses, were utilised in the respective calculations to determine the recovery of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ for each component:

herbage:	0.370440 atom % ^{15}N ;
roots:	0.371228 atom % ^{15}N ;
soil:	
(0-30cm):	0.373090 atom % ^{15}N ;
(30-60cm):	0.373080 atom % ^{15}N ;
(60-100cm):	0.376240 atom % ^{15}N .

The thick layer of organic matter on the soil surface, and common to a sub-tropical perennial pasture such as kikuyu, was not fully analysed in this trial as some of the undecomposed material was removed from the soil at sieving. Due to the size and spongy nature of many of the stolons, they could not pass through the designated sieve diameter (40 mesh) and so would have been removed along with the other debris such as rocks and clods. This could lead to inaccuracies in the amount of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ recovered, especially between samples of the same treatment, because some of the fertiliser N contained in the organic layer could be discarded and therefore not accounted for in the calculations, although in the case of this trial a recovery of nearly 100% suggests that this was not so. This is recognised as a potential deficiency of the technique used (note that the analytical procedure required that all material pass through the designated sieve) and methods such as milling/ashing will need to be adopted to circumvent this problem in future work.



Plate 5. Labelled $^{15}\text{NH}_4\text{NO}_3$ trial layout showing the 4 metal sleeves on the kikuyu pasture, each acting as a single replication.



Plate 6. Exclosures were placed over each cylindrical replication to protect them from animals.

4.3 Results and discussion

4.3.1 ^{15}N recorded in the herbage

Of the ^{15}N applied as an $^{15}\text{NH}_4\text{NO}_3$ spring fertiliser dressing, 52.24295% was recovered in the herbage harvested through the season. Of the total amount applied, 37.3232% was recovered in the months of November and December alone. Similar recoveries were reported by Dowdell & Webster (1980), Whitehead & Dawson (1984) and Bristow *et al.* (1987) in their trials on ryegrass pastures.

As might have been expected, the highest percentage of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ (20.733% of that applied) was measured in the herbage one month after application (i.e. November). This percentage subsequently declined (significantly so ($P < 0.01$) in some cases) in a near linear fashion over time, which is according to Terman & Brown (1968) and Jenkinson *et al.* (1985), a common observation, and is probably related to a series of factors including those of plant uptake, immobilisation, pool substitution and/or dilution as a result of the "priming" or "added nitrogen interaction (ANI)" effect of fertilisation (Jenkinson *et al.* 1985; Hart *et al.* 1986) (Table 15 & Fig. 17). The accumulated recovery of initially applied $\text{NH}_4\text{-}^{15}\text{N}$, as a percentage of that applied, showed a curvi-linear trend with depth following fertiliser N application (Fig. 17).

Table 15. The average monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that initially applied, within the herbage after a spring application of labelled $^{15}\text{NH}_4\text{-NO}_3$ fertiliser.

Month	Average $\text{NH}_4\text{-}^{15}\text{N}$ within each month %	Significance ($P < 0.05$)	Significance ($P < 0.01$)
Nov	20.7330	a	a
Dec	16.5902	a	a
Jan	8.2068	b	a
Feb	5.2120	b c	b
Mar	1.5008	c	b
Total	52.2429		

4.3.2 ^{15}N recorded in the roots

The highest amount of ^{15}N recovered in the roots was in December (3.6237%). This was significantly ($P < 0.01$) more than was recovered in the months of November, March ($P < 0.01$) and February ($P < 0.05$) (Table 16 & Fig. 17). Accumulated labelled N within the roots of the plant is not presented, as was done for the herbage, because N is transitory in the roots, which act only as conduits for its uptake from the soil and subsequent movement into the herbage. Whitehead & Dawson (1984) stated that there was little information on the extent to which fertiliser N is retained in the roots, but Clark (1977) found in his trials that at least some of the N was resident in the roots for a long period. This amount could not be quantified from this trial on Broadacres.

Again, the decline in ^{15}N recorded over time is probably accounted for by the labelled ions becoming diluted within the soil complex from subsequent fertiliser N applications, nitrification reactions (leading to leaching) as well as by its removal into

the herbage. The low amount recorded in November, when the highest amount might have been expected, could not be accounted for.

Table 16. The average monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that initially applied, within the roots after a spring application of labelled $^{15}\text{NH}_4\text{-NO}_3$ fertiliser.

Month	Average $\text{NH}_4\text{-}^{15}\text{N}$ within each month %	Significance ($P < 0.05$)	Significance ($P < 0.01$)
Nov	0.8813	a	a b
Dec	3.6237	c	c
Jan	2.6407	b c	b c
Feb	1.3179	a b	a b c
Mar	0.2724	a	a

4.3.3 ^{15}N recorded from the soil profile

4.3.3.1 ^{15}N recorded within the 0-30cm depth class

On analysis of these data, a discrepancy was observed in February wherein more labelled N was recovered in the 0-30cm depth class, on average, than was applied to the plots (124.2233%). Repeat analyses of the soil could not reveal the source of this error which seemed, therefore, to have arisen from some sampling problem. A regression analysis was then performed in order to obtain a theoretical amount of labelled N recovered in February (Dicks H. 1993. Pers. Comm. University of Natal, PO Box 375 Pietermaritzburg 3200 RSA). An equation, as shown below, for the model accounted for 84% of the variance:

$$y = -8.0126x + 72.216 \quad (R^2=0.84)$$

Where :

y = labelled ^{15}N recovered in February

The F ratio for this equation was significant (10.7), suggesting that the linear arrangement of the remaining points was not due to chance, once again verifying Terman & Browns' (1968) and Jenkinson *et al.* (1985) results which depicted linearity from similar earlier experiments. From this equation, an amount of 38.1723% ^{15}N , on average, was calculated as having been resident in February in this soil depth class.

The amount of ^{15}N recovered within each month declined from 69.4300% (of that applied) in November to 35.1447% in March, probably again reflecting the dilution effect of subsequent fertiliser applications and/or microbial action, as well as plant uptake over the season (Table 17 & Fig. 17).

Table 17. The average monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that initially applied, within the 0-30cm soil depth class after a spring application of labelled $^{15}\text{NH}_4\text{-NO}_3$ fertiliser.

Month	Average $\text{NH}_4\text{-}^{15}\text{N}$ within each month %	Significance ($P < 0.05$)	Significance ($P < 0.01$)
Nov	69.4300	a	a
Dec	53.2600	a	a
Jan	39.2478	a	a
Feb	38.1723	a	a
Mar	35.1447	a	a

4.3.3.2 ^{15}N recorded within the 30-60cm depth class

The highest amount of ^{15}N recorded at this depth class was 10.2459%, on average, in December. In general, in each month thereafter, ^{15}N recorded in this depth class declined linearly to reach a minimum in March of 3.1994%. Of interest is that less

labelled N was recorded at this depth in November, one month after application, than in December. In this month only 4.4565% of the applied ^{15}N was recorded as being resident in the depth class. This was the second lowest amount determined over the season (this may give some indication of the rate at which the fertiliser N penetrated to this depth) (Table 18 & Fig. 17).

Table 18. The average monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that initially applied, within the 30-60cm soil depth class after a spring application of labelled $^{15}\text{NH}_4\text{-NO}_3$ fertiliser.

Month	Average $\text{NH}_4\text{-}^{15}\text{N}$ within each month %	Significance ($P < 0.05$)	Significance ($P < 0.01$)
Nov	4.4565	a b	a
Dec	10.2459	c	b
Jan	7.5809	b c	a b
Feb	5.7404	a b	a b
Mar	3.1994	a	a

4.3.3.3 ^{15}N recorded within the 60-100cm depth class

A weighted average of the ^{15}N recorded in the 60-100cm depth class revealed that the least labelled N was found at this depth, compared to the other two depth classes, at all times during the season (Table 19). The highest amount of labelled N was recorded in January (4.5670%), this being significantly ($P < 0.05$) more than in March (1.4356%). Unlike in the two depth classes above, no distinct pattern in the decline of ^{15}N was observed from the beginning to the end of the season. However, a slight decline from January through February and March suggests that some of the labelled N may have been leached downwards out of the system (Fig. 17), assuming that the pasture was not extracting N from

this soil depth.

Table 19. The average monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that initially applied, within the 60-100cm soil depth class after a spring application of labelled $^{15}\text{NH}_4\text{-NO}_3$ fertiliser.

Month	Average $\text{NH}_4\text{-}^{15}\text{N}$ within each month %	Significance ($P < 0.05$)	Significance ($P < 0.01$)
Nov	2.7260	a b	a
Dec	3.7446	a b	a
Jan	4.5670	b	a
Feb	2.6105	a b	a
Mar	1.4356	a	a

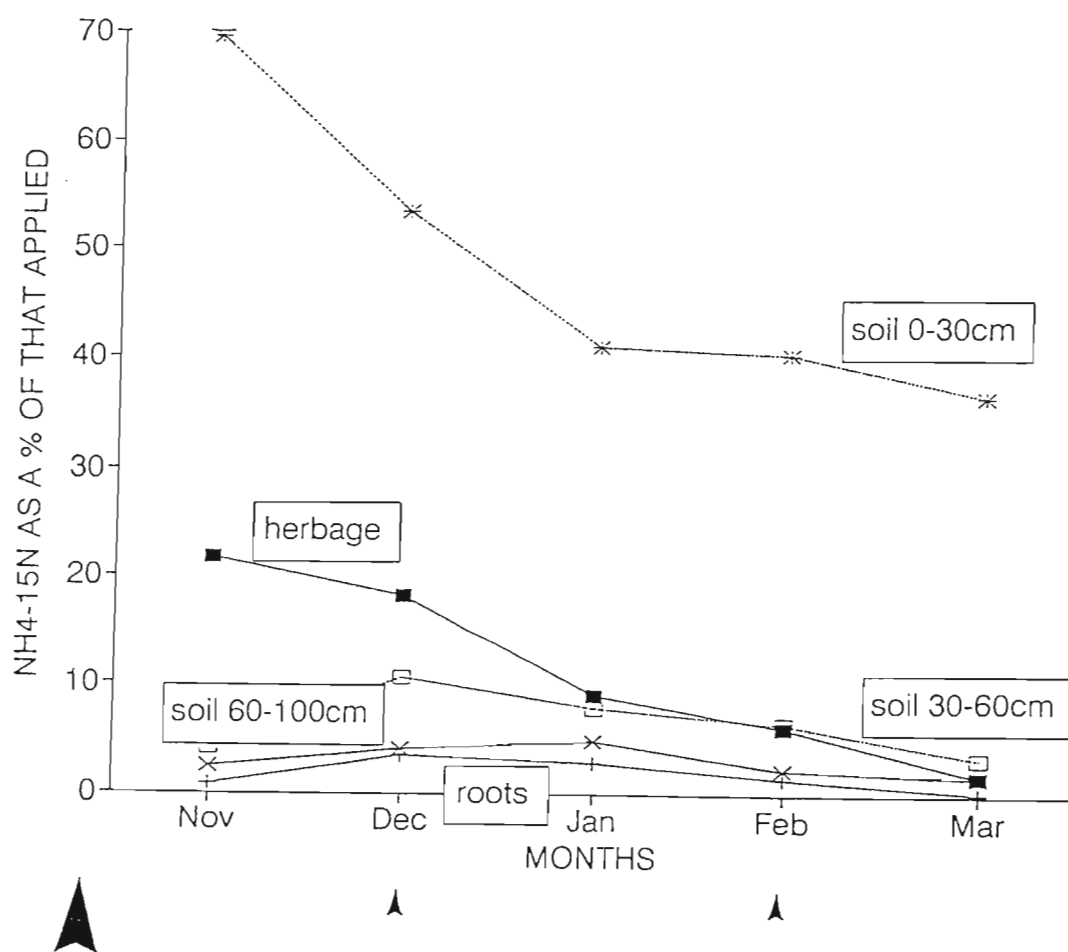


Figure 17. Mean monthly amounts of ^{15}N recovered, as a % of that applied, in the herbage, roots and soil over the season (Arrows indicate fertiliser applications (large arrow depicts labelled N application)).

4.3.3.4 leaching potential

The initially applied NH_4^+ ion did not seem to have been too prone to leaching down the profile during the season. However, as the amount of ^{15}N that was recorded in the 0-30cm depth class declined from November to December, so the amount of ^{15}N increased in the 30-60cm and 60-100cm depth classes at this time. Also, in January, the 60-100cm depth class was the only one to show an increase in ^{15}N over the previous month, before these rates also declined at this depth in February. This suggested some movement from the upper depth classes into this depth class, as well as some movement out of the soil at this depth, probably as a result of nitrification, followed by leaching. Rainfall in November and December may have contributed to the movement of the ^{15}N (probably in the $^{15}\text{NO}_3$ form) into the 30-60cm and 60-100cm depth classes from the 0-30cm depth class. During these two months 151.3 mm fell, 54% of it in November (Table 2a). The high rainfall later in the season (February and March) (Table 2a) may not have influenced, to any extent, the movement of ^{15}N down the profile from the 0-30cm depth class because a large percentage of the initially applied $\text{NH}_4\text{-}^{15}\text{N}$ had, by this time, been taken up in the herbage and roots.

4.3.4 Total ^{15}N recovered during the season

Monthly ^{15}N recovered, as a percentage of that applied within the herbage, roots and soil to a depth of 1m ranged from 98.2769% in November to 108.1974% in December (Table 20). Shown also in

Table 20 is the recovery in the herbage, presented as a percentage of the residual amount of ^{15}N within the system at the start of each growth cycle. As the season progressed from December to March, so the amount of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ recovered, as a percentage of the residual amount in the soil and roots, declined.

Bearing in mind that this was a field trial, the labelled N recovery is considered satisfactory as a margin of error of approximately 10% is generally considered acceptable in this type of work. The percentage of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ recovered in the herbage relative to that in the roots and soil to a depth of 1m, declined from 23.264% in December to 6.040% in March (Table 20), probably as a result of this initially applied $\text{NH}_4\text{-}^{15}\text{N}$ being bound to soil colloids and organic matter, thereby necessitating uptake of N by the plant from other non-bound sources within the soil. As the season progressed, the decline in residual $\text{NH}_4\text{-}^{15}\text{N}$ may have been a result of displacement by subsequent fertilisations from the colloids and their uptake by the roots, and/or nitrification processes occurring within these structures.

The total amount of ^{15}N recovered, on average, in the herbage, roots and soil to 1m over the season was 98.069%. This high recovery rate suggests that the NH_4^+ ion is not easily moved out of the soil profile, and is therefore available to be utilised by the plant for growth during the season. Furthermore, the extraction of the organic matter layer during the soil sieving process did not seem to materially affect the overall recovery

of ^{15}N in the trial, although it did apparently have an influence on recovery at the sampling one month after its application. The data also suggest, however, that this labelled N was subsequently incorporated into the soil profile.

Table 20. Mean monthly amounts of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ recorded, as a % of that applied, in the herbage, roots and soil to a depth of 1m over the season.

Months	Mean monthly $\text{NH}_4\text{-}^{15}\text{N}$ recorded in total in each month (%)	Initially applied $\text{NH}_4\text{-}^{15}\text{N}$ recovered in the herbage as a percentage of the residual amount in the soil and roots (%)
Nov	98.2269	
Dec	108.1974	23.264
Jan	99.5665	18.133
Feb	98.5833	8.717
Mar	92.2951	6.040

4.4 Conclusions

In conclusion, the results show that spring applied fertiliser N containing labelled $^{15}\text{NH}_4^+$ ions can indeed be taken up into the herbage. However, as the season progressed, so the amount of labelled N declined within the herbage. Roots, being the interface between the soil and herbage, had similar, albeit smaller, quantities of labelled $^{15}\text{NH}_4^+$ ions present throughout the season, suggesting that the roots merely transferred the ions from the soil to the herbage and did not themselves accumulate large amounts of N.

Soil within the 0-30cm depth class contained the highest amount of labelled $^{15}\text{NH}_4^+$ ions, on average, throughout the profile,

although this amount declined in a similar manner to that in the herbage over time. In general, the $^{15}\text{NH}_4^+$ ions seemed to remain essentially within the 0-30cm and 30-60cm depth classes, suggesting that these ions may have been adsorbed, to a large extent, onto soil colloids in this region of the profile. Even so, a small amount of downward movement of the ion was still detected during the season, again probably as a result of nitrification reactions leading to the formation of labile $^{15}\text{NO}_3$ compounds, followed by leaching.

The ability of researchers to recover most of the applied labelled N on a field trial has been shown here. However, future research should include the use of control plots and the analysis of the thick organic matter layer normally present under a kikuyu sward to determine the extent to which it inhibits or accelerates fertiliser penetration into the soil. Labelled N should also be applied later in the season to examine the extent of uptake during these times, and the use of double labelled N (NH_4 and NO_3) fertilisers should be considered so that transformation reactions, including those of nitrification to $^{15}\text{NO}_3$, can be studied.

5. EFFECT OF NITROGEN FERTILISATION ON PLANT MACRO-NUTRIENT CONCENTRATIONS, IN TERMS OF ANIMAL REQUIREMENTS.

5.1 Introduction

Up to 150 years ago it was still a matter of scientific controversy as to whether mineral elements functioned as nutrients for plant growth. The recognition of their importance is largely due to Justus Von Liebig who concluded, mostly through conjecture, that the mineral elements N, P, K, Ca and Mg, amongst others, were essential for plant growth (Marschner, 1986).

The elements found in plant material may be divided into two categories. The first are micro (non-essential) elements, these being nutrients not required by the plant in order to carry out life preserving functions. The second are the macro (essential) elements, which are those that are deemed necessary in order to allow for the functioning of metabolic pathways in order for the plant to live, and are required by the plant at all times. Furthermore, depending upon how great the growth requirement is for a given nutrient, the nutrient is, again, either referred to as a macro or micro-nutrient. This division of the macro elements was based upon the work done by Arnon & Stout (1939). The macro-nutrients are found and are required in relatively higher amounts than are the micro-nutrients.

Macro-nutrients vary in importance from species to species, but in the main C, N, O, H, P, S, K, Ca and Mg are found in this category. The metallic macro-nutrients such as K, Ca and Mg function mainly as constituents of charge-balancing reactions

within organic structures, whilst non-metal macro-nutrients such as N, P and S, in general, tend to serve as constituents of protein and nucleic acid structures (Marschner, 1986).

Macro-nutrients also play an important role in animal health and metabolism, even though the animal body contains less than 5% by mass of these mineral elements (Groenewald & Boyazoglu, 1980; Holmes & Wilson, 1987). The balance of the different components is important to ensure that deficient and toxic levels that might adversely affect animal performance, do not occur. Fertilisation may influence the relative concentrations of these macro-nutrients, resulting in toxicity-related problems in ruminants. Thus, it is important to establish the influence of fertiliser applications on the relative concentrations of these macro-nutrients within herbage.

For the purposes of this chapter, only the macro-nutrients P, K, Ca and Mg will be investigated (see Ch. 3 - herbage N discussion). The main objectives of measuring herbage levels of these elements was to monitor possible plant nutrient deficiencies or excesses, and to examine possible N fertiliser implications, and to determine, under different fertiliser application levels, whether animal nutrient requirements would be met.

Apart from main treatment differences, the design of the experiment was not suited to allowing separation of the many possible influences which may have been involved in determining

mineral composition. These include species, season, temperature, soil moisture availability, herbage maturity and their possible interactive effects (Whitehead, 1966).

The main issue to be considered in this chapter then is: Does the application of fertiliser N to pasture influence the relative concentration of macro-nutrients (subject to the other nutrients being non-limiting) within the herbage over the season, and, if so, what effects might this have on animal health and productivity?

5.2 Methodology

See Chapter 1 for methodology pertaining to the assessment of each of the plant macro-nutrients.

5.3 Results

5.3.1 Herbage phosphorous

Herbage P levels were significantly ($P < 0.05$) affected by the application of N. The higher N application rates induced lower concentrations of herbage P at all times during both seasons. The average herbage P concentration, meaned over all treatments, was 0.248% (Table 21). Only 0.003% (not significant) more P was found within the lower herbage stratum than within the upper stratum (Table 21).

Table 21. Phosphorous concentrations within herbage strata, meaned over all fertiliser N applications and both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level) %	upper herbage stratum (5cm and above) %	Means
0	0.267	0.258	0.262
150	0.252	0.254	0.253
300	0.243	0.247	0.245
450	0.239	0.230	0.234
Means	0.250	0.247	

LSD (body of the table) 5% = 0.021 1% = 0.029
 LSD (N levels) 5% = 0.007 1% = 0.011

The first season's herbage contained 0.01% more P ($P < 0.05$) (but of doubtful biological significance), on average, than the 2nd season's growth (Table 22). The presence of a low concentration of P in the early part of the season (October) is noteworthy and probably relates to the fact that there is limited uptake of this nutrient by kikuyu during early spring growth. While monthly and seasonal interactions in herbage P were significant, they did not follow a consistent pattern over both seasons.

Table 22. Seasonal variations in herbage phosphorous, meaned over both herbage strata and all fertiliser N applications.

Month	herbage P (season 1) %	herbage P (season 2) %	Means
October	0.194	0.131	0.162
November	0.273	0.160	0.216
December	0.297	0.279	0.288
January	0.263	0.294	0.278
February	0.275	0.303	0.303
March	0.242	0.318	0.289
Means	0.257	0.247	

LSD (body of the table) 5% = 0.210 1% = 0.421
 LSD (monthly means) 5% = 0.009 1% = 0.014

5.3.2 Herbage potassium

No significant differences in K concentration were found between the control and treated plots over the two seasons of growth. An average of 2.96% K was present in the herbage material (Table 23).

The lower herbage stratum, regardless of the amount of fertiliser N applied, contained significantly more K than the herbage within the upper stratum (Table 23). The herbage within the lower stratum had a K concentration of 3.12%, on average, this being 0.32% more than the herbage K concentration within the upper stratum (2.80%).

On average over the two seasons, significantly more K was found

in the herbage after application of 300 kg N/ha/season than after application of 150 kg N/ha/season (Table 23).

Table 23. Potassium concentrations within herbage strata, meaned over all fertiliser N applications and both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level) %	upper herbage stratum (5cm and above) %	Means
0	3.16	2.70	2.93
150	3.09	2.69	2.89
300	3.24	2.93	3.08
450	2.98	2.88	2.93
Means	3.12	2.80	

LSD (body of the table) 5% = 0.31 1% = 0.46

LSD (N levels) 5% = 0.16 1% = 0.24

Herbage during the first season of growth contained significantly higher concentrations of K than the second season's growth (Table 24). These results were consistent over all treatments applied to the plots. Uptake of K seemed to be restricted during the early spring period, but as plant growth progressed over both seasons, the uptake of this nutrient increased.

Table 24. Seasonal variations in herbage potassium, meaned over both herbage strata and all fertiliser N applications.

Month	herbage K (season 1) %	herbage K (season 2) %	Means
October	2.32	1.67	1.99
November	3.30	1.29	2.29
December	4.22	3.29	3.76
January	3.14	2.91	3.02
February	3.39	3.73	3.56
March	3.07	3.15	3.11
Means	3.24	2.67	

LSD (body of the table) 5% = 0.45 1% = 0.91
 LSD (monthly means) 5% = 0.29 1% = 0.74

5.3.3 Herbage calcium

Herbage from the control plot contained lower concentrations of Ca than from any of the treatment plots, and significantly so except for the plot that had 150 kg N/ha/season LAN applied to it. Calcium concentrations increased, on average, as N application rates rose on the pasture (Table 25). Increased Ca concentrations with increasing N level could have important consequences for animal performance on kikuyu, where Ca levels are notoriously low, and tend to be below the animal requirements of 0.54% for a dairy cow producing between 17-23kg milk (Gilchrist & Mackie, 1984).

Differences in herbage Ca concentrations, on average, between the upper and lower strata of the herbage were not significant, with

the lower stratum containing only 0.003% more Ca than the upper stratum (Table 25). Calcium concentrations showed no consistent patterns within each of the strata in response to different levels of N.

Table 25. Calcium concentrations within herbage strata, meaned over all fertiliser N applications and both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level) %	upper herbage stratum (5cm and above) %	Means
0	0.331	0.313	0.322
150	0.323	0.341	0.332
300	0.382	0.362	0.372
450	0.374	0.380	0.377
Means	0.352	0.349	

LSD (body of the table) 5% = 0.028 1% = 0.036
 LSD (N levels) 5% = 0.011 1% = 0.020

Significantly more Ca was observed in the herbage during the second season than in the first, averaged over all N treatments (0.306% vs 0.393%; Table 26). Calcium levels tended to decline a little from October through to mid-summer, before again increasing markedly into the autumn.

Table 26. Seasonal variations in herbage calcium, meaned over both herbage strata and all fertiliser N applications.

Month	herbage Ca (season 1) %	herbage Ca (season 2) %	Means
October	0.301	0.401	0.351
November	0.273	0.411	0.342
December	0.282	0.404	0.343
January	0.284	0.392	0.338
February	0.343	0.353	0.348
March	0.350	0.401	0.376
Means	0.306	0.393	

LSD (body of the table) 5% = 0.071 1% = 0.152
 LSD (monthly means) 5% = 0.002 1% = 0.007

5.3.4 Herbage magnesium

As N applications were increased on the pasture, herbage Mg concentrations also increased, on average, over the two seasons (Table 27). Significantly higher concentrations of Mg were obtained from fertilised herbage than from the control camps, and herbage treated with 450 kg N/ha/season had the highest concentration of Mg. This concentration was also significantly different from the concentration of Mg found in the herbage after application of 150 and 300 kg N/ha/season. These results were consistent over both seasons of growth.

No significant differences in Mg concentrations were measured within the two herbage strata, with the lower stratum containing 0.002% less Mg than the upper stratum (Table 27). In general, Mg concentrations increased within each stratum as fertiliser N

rates were increased. This trend was, however, more consistent in the lower (<5cm) than the upper (>5cm) stratum over the treatments applied to the pasture.

Table 27. Magnesium concentrations within herbage strata, meaned over all fertiliser N applications and both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level) %	upper herbage stratum (5cm and above) %	Means
0	0.310	0.322	0.316
150	0.361	0.401	0.381
300	0.411	0.390	0.400
450	0.423	0.400	0.411
Means	0.376	0.378	

LSD (body of the table) 5% = 0.020 1% = 0.036
 LSD (N levels) 5% = 0.010 1% = 0.022

Herbage from the second season of growth contained a higher Mg concentration, on average, than the herbage of the first season. Magnesium concentrations for each month, averaged over both seasons, were inconsistent and showed no distinct trends (Table 28).

Table 28. Seasonal variations in herbage magnesium, meaned over both herbage strata and all fertiliser N applications.

Month	herbage Mg (season 1) %	herbage Mg (season 2) %	Means
October	0.31	0.40	0.36
November	0.36	0.37	0.37
December	0.36	0.37	0.37
January	0.36	0.43	0.40
February	0.36	0.41	0.39
March	0.40	0.41	0.41
Means	0.36	0.40	

LSD (body of the table) 5% = 0.009 1% = 0.152
 LSD (monthly means) 5% = 0.002 1% = 0.007

5.4 Discussion and conclusions

5.4.1 Phosphorous

Phosphorous, even though deficient in most dystrophic soils in South Africa because of its adsorption onto soil colloids, is important in many physiological functions within the plant, including carbohydrate and energy transformations and its presence within cell nuclear genetic material. In this respect then, P is an essential macro-nutrient, not just for plant life, but for all organisms. In plants, P is absorbed by the roots mainly in the di-hydrogen phosphate ion (H_2PO_4^-) form.

The P content of the kikuyu pastures averaged 0.248%. Published data shows tropical pastures to contain P in the range from 0.02 to 0.58% in the DM, with a mean of 0.22%, although the

concentration does tend to vary between species and is also dependent on growth stage (Skerman & Riveros, 1990). The P concentrations measured on the Broadacre trial are such that they should preclude any necessity for P supplementation at least for beef farming, as the recommended level of P in the diet for a 450 kg beef animal is 0.17% (NRC, 1968), whilst that of a dairy cow producing 15 l/day should be approximately 0.34% of the herbage dry matter (Stewart G. 1993, Pers Comm) (see also Table 1 showing soil P results).

The reported effects of N application on P content within the herbage has been found to be variable. Walker et al. (1952) concluded that N treatment on soils low in inherent P actually decreased herbage P, whilst the opposite is true on P rich soils. Conversely, Stewart et al. (1953) found that the addition of fertiliser N had no effect on P concentration within the herbage. Similar results were also observed by Reith et al. (1964) and Whitehead (1966).

Ammonium ions seems to be the precursor for increased P uptake. Several sources maintain that it is this ion and not the NO_3^- ion that enhances P movement across the root symplast and into the xylem (Olson et al. 1956; Rennie et al. 1958; Leonce et al. 1966; Blair et al. 1970; Mamaril et al. 1970). Conversely, an increased NO_3^- -N presence has been found to decrease P uptake within the plant (Hay et al. 1953; Hageman et al. 1960).

Arnon (1939) concluded that the reason why P uptake was enhanced

in the presence of the NH_4^+ ion was because it would stimulate anion absorption (such as the H_2PO_4^- ion), whereas the NO_3^- ion would stimulate cation uptake.

Herbage P concentrations dropped as N fertiliser application rates were increased on the pasture. This result was similar to that observed by Mays (1974) and Ball (1979), and is most likely due to dilution of the P within the herbage as DM yields increased in response to higher N application rates (Miles N. 1992, Pers Comm).

Even though applications of 300 and 450 kg N/ha/season elicited similar concentrations of P within the herbage, the zero N camp contained well in excess of animal P requirements, and thus application of fertiliser P is not justified on the pasture.

Even though no significant difference in P concentration was measured between the two herbage strata, a higher P content prevailed within the lower (<5cm) herbage stratum. However, only 0.003% more P was present within the lower stratum, and this is probably of questionable biological significance. This difference, although negligible, was contrary to that observed by Michael (1939, cited by Mengel et al. 1976), Hartt (1972) and Skerman & Riveros (1990). They concluded that higher levels of P were commonly more prevalent within the younger fractions of a pasture (corresponding to the upper (>5cm) stratum). This is so because P is an essential element in energy transduction, in the orthophosphate form, and is commonly found as such in the

newer portions of the plant (Glass, 1989).

The inconsistent P concentrations observed within each month over the two seasons suggests that P concentrations are variable and dynamic in nature. The stage of plant growth during the season and/or temperatures (see Table 2) are probably major factors determining P variability and availability over the season (Tainton, 1981; Skerman & Riveros, 1990). In this respect, Miles (1986) and Miles *et al.* (1991) showed that Italian Ryegrass (*Lolium multiflorum*) was more responsive to P application in winter than in the summer, and kikuyu had lower P concentrations in spring/early summer than in mid-summer, when growth was at a peak. Practically then, in October when P concentrations are low, early spring additions of phosphatic fertiliser may enhance pasture growth at that time. Furthermore, the addition of early spring fertiliser N applications may result in a favourable N-P interaction which could increase pasture yields (Summer & Farina, 1986).

5.4.2 Potassium

Potassium, besides N, is quantitatively the most utilised nutrient derived from soils by pastures. It is highly immobile in the soil complex in the non-exchangeable form but is quite mobile on the soil exchange complex, and is mobile within plant tissues and is the most abundant element in the cytoplasm (Mengel & Kirkby, 1978; Marschner, 1986). It is not found in structural components, but is important as an ion activator, photosynthetic

translocator and plays a role in guard cell turgidity (Blanchet et al. 1962; Evans & Sorger, 1966; Hartt, 1970; Mengel et al. 1976; Marschner, 1986; Glass, 1989). Potassium deficiencies restrict responses to fertiliser N, and furthermore, reduce cold tolerance and drought hardiness, whilst increasing susceptibility to disease and lodging in pasture plants (Miles, 1991).

On average, 2.96% K was present within the herbage material in this trial. The norm for pastures ranges between 0.7 and 4.0%, with the concentration of this ion greatly exceeding that of most other elements derived from the soil, except that of N which is normally of the same magnitude as K (Evans & Sorger, 1966; Mays, 1974; Tainton, 1981; Miles, 1991). As the concentration of K measured off these pastures lies within these range limits, it is extremely unlikely that well fed ruminants, whose dietary K requirements range from 0.5 to 0.80%, will suffer from a deficiency of this element on this pasture (Gilchrist & Mackie, 1984; NRC, 1984).

The N status of plants has been widely shown to have a marked influence on requirements for K (Kresge & Younts 1963; Gartner 1969; Heathcote 1972 (cited by Mengel and Kirkby, 1978); Prins et al. 1985; Smith et al. 1985). Under conditions of adequate K supply, uptake of K increases with increasing N supply, and the required K concentration for maximum yield (critical level) has been found to increase with increasing N concentration. However, on these trials applications of fertiliser N to the crops did not significantly increase K concentrations within the herbage.

Exactly why this should be so is difficult to ascertain, but it may be because K within this soil form was not limiting (151.6 mg/l, on average, to a depth of 1m (Table 1)), even on the control camps; or antagonistic reactions between other macro-nutrients may have affected K uptake. Kemp (1960) indicated that N applications actually caused a decline in K content when herbage contained a K concentration of less than 2%, and an increase when it was above 2%. Other researchers remain divided over the issue, although Talibudeen et al. (1976) concluded that plants limit K uptake when N is deficient and also limit N uptake when K is deficient.

Another explanation, although unlikely, for the lack of response in K with increasing N in this study may have been the additional di-basic material that accompanied the N in the LAN. Subsequent investigations revealed that the limestone fraction of the LAN did contain a mixture of both calcitic and dolomitic lime, the relative proportions of which are 3.2% calcitic and 2.4% dolomitic lime (Anon., 1993). However, it could be concluded that these concentrations are too small to influence macro-nutrient concentrations, at least in the short term.

Stage, and thus age, of plant growth has a major bearing upon K concentrations within the herbage, with concentrations declining with maturity. The lower (<5cm) herbage stratum, being the older herbage fraction, was found to contain the highest K concentration in the pasture. As no deficiency was detected in the plant or the soil, K presumably remained stored within the

older plant tissue and would probably only have been translocated from these regions towards meristematic tissue to supplement the younger, actively growing herbage in times of deficiency (Greenway & Pitman, 1965). Haeder & Mengel (1969, cited by Mengel & Kirkby, 1978) also found that uptake and transport of K to younger plant parts was influenced by the presence of N. Why the translocation of K^+ ions is affected in this fashion is still not fully understood, but Jacoby *et al.* (1973) postulated that there were relationships between K movement, N fertilisation, protein N synthesis, growth rate and cytokinin supply within the meristematic regions.

Although probably biologically non-significant, the presence of significantly less K (0.57%) within the herbage in the second season than in the first may be linked to depletion of reserves of this element in the soil by the plant and/or elemental interactions between K and other macro-nutrients (Anderson, 1973; Mengel & Kirkby, 1978). Alternatively, removal of the herbage by the animal, combined with the short period of stay, may have resulted in K being excreted elsewhere other than on the camps from which the herbage was removed, or by "aggregation" of the K whereby animals eat from the larger area, but excrete over a restricted area (During, 1972). Soil and environmental factors such as soil aeration and temperature, may well also contribute to herbage K variability (Mays, 1974).

5.4.3 Calcium

Calcium normally functions extracellularly at the cell wall and at the external surface of the plasmalemma. It is also essential for normal cell membrane permeability (Glass, 1989). Calcium also has an important bearing on the mechanical strength of tissues and along with P, plays an important role in growth and bone formation. It must, therefore, be present in adequate amounts in an animal's diet. Yearling beef animals with a mass of approximately 300 kg and gaining 0.5 kg per day require 0.22% Ca in their diets (NRC, 1976), whilst a 600 kg cow with a daily milk yield of 14 to 21 kg requires approximately 0.48% Ca (NRC, 1978). In this respect then, an average Ca content of 0.35% within the herbage falls within the range of 0.14 to 1.5% specified by the NRC (1976), Tainton (1981), Marschner (1986) and Skerman & Riveros (1990) for beef animals, but not for high-producing dairy animals.

Calcium concentrations in most tropical grass species exceed that of P by a factor of 1.5 to 2 (McDowell et al. 1983). Kikuyu is, however, a notable exception to this rule (Heard, 1971, cited by Tainton, 1981). In this grass species, Ca:P ratios have normally tended to be close to unity or the plant tissue may even contain more P than Ca. This was not the case on these trials, with the average Ca:P ratio of 1.40:1 (Table 29) tending to follow the norms associated with most other tropical species. In this respect then, Ca supplementation for livestock grazing these pastures would not seem to be necessary, provided Ca does not

bind with oxalic acid in the plant to form insoluble Ca oxalate, because the Ca:P ratio lies within the norms for dairy animal requirements (greater than 1.5 with 1.0 as a minimum (Tainton & Eckard, 1992)). Various authors have, however, suggested that high oxalate levels in kikuyu severely restrict the availability to animals of Ca in this grass (Reason *et al.* 1989; Marais 1990). Further, luxury K uptake, as suggested by the high K/Mg+Ca ratio (Table 31 & 32) in these trials, suggest that Ca and Mg supplementation might be necessary on these pastures.

Table 29. Ca:P ratios at different N application rates and at two herbage heights within a kikuyu sward, averaged over both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level)	upper herbage stratum (5cm and above)	Means
0	1.23:1	1.21:1	1.22:1
150	1.28:1	1.34:1	1.31:1
300	1.57:1	1.47:1	1.51:1
450	1.56:1	1.65:1	1.61:1
Means	1.40:1	1.40:1	

The Ca:P ratios increase in the herbage, on average over both height strata, as the N application rates increased from 0 to 450 kg N/ha/season. In effect, Ca concentrations tended to increase when N levels were increased (Table 25), whilst P concentrations declined (Table 21).

Table 30. Seasonal variations in herbage Ca:P ratios, meaned over both herbage strata and all fertiliser N applications.

Month	(season 1)	(season 2)	Means
October	1.55:1	3.06:1	2.17:1
November	1:1	2.57:1	1.58:1
December	0.94:1	1.44:1	1.19:1
January	1.07:1	1.33:1	1.21:1
February	1.24:1	1.16:1	1.14:1
March	1.44:1	1.26:1	1.30:1
Means	1.19:1	1.59:1	

The trend, on average over the two seasons (Table 30), wherein the ratio declined from a high in October (2.17:1) to a low in December (1.19:1), before increasing again to 1.30:1 in March, is probably indicative of the growth pattern of kikuyu.

Increasing N application rates to the pasture increased the Ca content within the herbage. Both the application of 300 and 450 kg N/ha/season produced similar concentrations of Ca within the herbage. Authors such as Whitehead (1966), Reid *et al.* (1966), Loneragan & Snowball (1969) and Mengel & Kirkby (1978) found similar increases in Ca concentrations after N fertilisation. However, they found it difficult to ascertain fully whether or not this increase in Ca concentration could be attributed directly to the N applied (Ca from the limestone in the LAN may have influenced these results). Nielsen & Cunningham (1964) did determine that the Ca concentration varied in the herbage depending upon whether the fertiliser contained $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ in

greater or lesser amounts. High concentrations of $\text{NO}_3\text{-N}$ were found to increase Ca contents, whilst high concentrations of $\text{NH}_4\text{-N}$ lowered them. Results from these trials showed that $\text{NO}_3\text{-N}$ concentrations did indeed increase in both the soil and herbage as N application rates were increased (Ch. 2 & 3), results that may have influenced the relative abundance of Ca within the herbage.

Even though N applications increased Ca concentrations within the herbage, it is by no means certain that the increased Ca concentration within the herbage would benefit animals grazing the pasture. This is because a high N content, synonymous with increased N applications, increases the concentration of insoluble Ca oxalate within the herbage (Marais, 1990). Thus, even though N increased the concentration of Ca, the increased oxalate concentration may have rendered it unavailable to the ruminant grazing at the time. Thus, as N application rates were increased on the Broadacre trial, the potential for increased Ca oxalate manufacture in the plant would also have increased. Animals may, therefore, still have experienced Ca deficiencies at the higher N rates.

Marais et al. (1992) also noted that the higher the Ca concentration in the plant, the higher the DM yields. Since the trends for DM yield and Ca concentration in the presence of increasing N applications were similar, it might be concluded that Ca may indeed be involved in increasing potential yields on these pastures, although the Ca effect is more than likely only

co-incident. Similar effects to those observed above could have arisen from the presence of lime within the fertiliser, although the percentages are probably too small to be of any consequence, at least in the short term.

No significant differences in Ca concentration were found in the upper (>5cm) and lower (<5cm) herbage strata over both seasons, even though the lower stratum had a slightly higher Ca concentration (0.003%), on average, than the upper stratum. Calcium is generally immobile within the herbage, normally in the insoluble Ca oxalate form, and for this reason concentrations may have remained stable and similar within both strata. Furthermore, unlike P and K, Ca does not tend to discriminate in favour of younger, newer growth, but rather tends to accumulate in higher concentrations within the leaves than in the stems of the herbage (Marais, 1990; Skerman & Riveros, 1990).

Calcium concentrations within the herbage showed no distinct trend over both seasons, probably because concentrations tend rather to vary according to stage of plant maturity and season (Tainton, 1981). However, higher concentrations of the element were measured in the second season than in the first. The reason for this is unknown, but may be related to the decline in K concentration during this season. Unlike results obtained by Skerman & Riveros (1990), Ca concentrations seemed to increase marginally as the herbage matured. This suggested that the kikuyu, especially towards the end of the season, may have either been increasing its Ca uptake prior to the onset of winter, or

alternatively, that DM yield at this late stage (March) increased slightly over the previous month's yield in response to external factors such as rainfall (Table 2). Alternatively, the increase in rainfall during March may have allowed the plant to increase its uptake of the Ca ion.

5.4.4 Magnesium

The most familiar function of the Mg ion is its role as the central atom of the chlorophyll molecule (Mengel & Kirkby, 1978). Epstein (1972) and Glass (1989) showed that Mg was also an activator of many plant enzymes, especially those involved in phosphorylation metabolism. Magnesium levels are generally marginal or deficient with respect to animal requirements.

On average, 0.377% Mg was present within the herbage over both seasons. This compares favourably with published data wherein Mg concentrations varied from 0.04 to 0.9% in the DM, with a mean of 0.36% (Tainton, 1981; Skerman & Riveros, 1990). In terms of animal nutrition, the required Mg concentration in the diet of a dairy cow with a daily milk yield of 25 litres are approximately 0.2% (Kemp & Geurink 1978). The concentration of Mg measured within the herbage was, therefore, above the minimum recommended level in the diet (NRC, 1965).

All camps with N applied to them contained significantly more Mg in their herbage than on the control camps. Significantly higher concentrations of Mg were found in the herbage after application

of 450 kg N/ha/season than after applications of 150 and 300 kg N/ha/season respectively. Hemingway (1961) and Black & Richards (1965) also found that Mg concentrations increased within the herbage in response to applied N. The mechanism involved in this process is complex, but similar to that described for Ca. Uptake of Mg is dependent upon the form of the N fraction. Increased $\text{NO}_3\text{-N}$ levels enhance uptake, whilst $\text{NH}_4\text{-N}$ decreases Mg levels in the herbage because the NH_4^+ ions actively compete with the Mg^{2+} ions. This is especially noteworthy in season 1, when soil NH_4^+ was higher (Table 5a), plant Mg was lower (Table 22). Also, the addition of LAN, and more specifically the lime in the fertiliser mix (as Gardner et al. 1960 and Mortensen et al. 1964 found that NH_4NO_3 alone did not affect Mg concentrations in the herbage), may have been a precursor for increased Mg uptake into the plant. Similar conclusions pertaining to the presence of lime in the fertiliser, and its potential for enhancing macro-nutrient uptake were also drawn in the earlier discussions on K and Ca.

Antagonistic effects also occur between K and Mg, especially where K concentrations are high. This frequently results in Mg deficiencies within the herbage. "Luxury consumption" of K by pastures, especially in kikuyu, is usually also associated with a decline in the concentration of Mg. Because K and Mg are differentially absorbed by the ruminant, an excess of K in the herbage may inhibit Mg uptake by the animal, thus affecting its health (Reid & Jung 1974; Wilkinson & Stuedeman 1979). Mengel & Kirkby (1978) also stated that the presence of Ca in the soil/plant complex enhanced Mg uptake, which increased the

importance of the link between these two elements. To this end, the Ca:Mg ratio in pastures is well documented. This ratio, or the K/Mg+Ca ratio which acts as an indicator of the tetany potential of a pasture, may be of some importance when predicting imbalances of these elements in herbage and their subsequent effect on animal health. In this respect then, the following are the ratios of K/Mg+Ca found in the kikuyu under different N application rates and over both seasons on the Broadacre trial (Tables 31 & 32).

Table 31. K/Mg+Ca ratios at different N application rates and at two herbage heights within a kikuyu sward, averaged over both seasons.

N applied kg N/ha/season	lower herbage stratum (ground level to 5cm above ground level)	upper herbage stratum (5cm and above)	Means
0	4.92	3.78	4.35
150	4.51	3.62	4.06
300	4.08	3.89	3.98
450	3.73	3.69	3.71
Means	5.38	3.74	

Table 32. Seasonal variations in herbage K/Mg+Ca ratios, meaned over both herbage strata and all fertiliser N applications.

Month	(season 1)	(season 2)	Means
October	3.79	2.05	2.92
November	5.20	1.65	3.42
December	6.57	4.25	5.41
January	4.87	3.54	4.20
February	4.82	4.89	4.86
March	4.09	3.88	3.78
Means	4.89	3.37	

The K/Mg+Ca ratio in an animal's diet off a tropical pasture should be less than 2.2 (Tainton & Eckard, 1992). Figures on this trial, however, are nearly double the norm, as observed by Tainton & Eckard (1992), even at the zero N level and within both strata of the herbage, again suggesting luxury consumption of K in the herbage. The ratios declined, on average, as the N application levels on the trial were increased (Table 31). This resulted from an inherently high soil K content (Table 1), even on the control camps (see Table 23, 24, 25, 26, 27 & 28), and/or dilution of these ions within the herbage as yields increased as N application rates increased.

Also, this ratio was higher in the <5cm herbage stratum than in the >5cm stratum, on average, suggesting that older herbage may have a higher tetany potential than younger herbage, and that farmers, in their grazing strategy, should then not allow pastures of this type to be grazed to below 5cm without first

checking on this ratio. Ameliorating with Ca or Mg fertilisers, or cutting back on K fertilisers, may also be an option in order to manipulate this ratio.

Monthly variations in K/Mg+Ca ratios (Table 32) within each season were variable and difficult to explain. However, the ratio did show a more general trend over the two seasons, from an initial low in October (2.92) to a peak in December (5.41), before declining into 3.98 in March. The overriding factor influencing this trend over time seems to be the high soil K concentration. Thus, generally, as the pasture begins to grow in spring, luxury consumption and therefore, potential tetany is low. This potential toxicity increases into summer, when active growth occurs, before declining into autumn. The high ratio in February could not be accounted for, but may have resulted from an increase in soil K uptake due to high rains the previous month (Table 2) which are known to enhance the uptake of this ion (Bartholomew P. Pers. Comm. 1993. Dept. of Agric. Pvt Bag X9059 Cedara). In the case of this trial though, K/Mg+Ca ratios are still higher than the norm on average, over the two seasons, even in spring, and therefore toxicity related problems could still be envisaged if animals are grazed on these pastures at any time during the season. Thus, some form of Mg and/or Ca supplement (eg. di-calcium phosphate or lime) should be fed to animals grazing on these pastures at all times during the season.

Wolten (1963) stated that the application of high rates of fertiliser N may result in the Ca:Mg ratio becoming distorted due

to the lowering of the Mg concentration within the herbage. This, they stated, would result in hypomagnesaemia (grass tetany) in animals. On these trials, the Ca:Mg ratio is 0.92:1 which is considered to be near the optimum for livestock. Therefore, the potential for a deficiency in Mg on this pasture is remote.

Magnesium concentrations remained essentially similar within both herbage strata, with only 0.002% more Mg being observed in the upper herbage stratum, regardless of the rate of fertiliser N applied. This may be so because Mg is readily translocated to new tissue, which may enhance Mg deficiency symptoms, as other authors have also found (Underwood, 1966; Mengel & Kirkby, 1978). Furthermore, the lack of any major difference in average concentration between Ca and Mg within the strata is probably related to the similar transport mechanism utilised by these two ions (Mengel & Kirkby, 1978). Magnesium content in the herbage seems, however, to be extremely variable, and therefore further comment would be speculative.

As in the case of Ca, Mg concentrations within the herbage were significantly higher in the second season than in the first. This could again be related to the similarity in the uptake mechanisms of both ions by the herbage, and/or the decline in K concentration during this season and resulted in a decline in the K/Mg+Ca ratio during this time. This increase was, however, not enough to stop the herbage exhibiting signs of luxury consumption (see Table 24 & 32). Trends over each season were, in general, inconsistent. Tainton (1981) suggested that this may be related

to the stage of plant maturity, interactive effects between other elements and/or season.

A high Mg concentration in March of both seasons suggests that mature herbage may be bulking up Mg prior to the winter. This phenomenon was observed by Underwood (1966) and Mengel & Kirkby (1978), who stated that this arose probably as a result of plant maturity, because Mg tended to increase in concentration as the plant senesced.

6. GENERAL SUMMARY AND CONCLUSIONS

6.1 Soil N

6.1.1 Soil total N

In the absence of fertiliser N, 15.45 t/ha of N was measured to a depth of 1m. The average concentration of N in the soil was 0.15%. More than 30% of the soil total N was, however, located in the upper 0-10cm of the soil. Organic matter (OM) content in the top 0-10cm of the profile was also high (4.75%), reflecting an accumulation of organic matter in this zone. However, as organic C declined with depth, so too did soil total N concentration.

Carbon:Nitrogen (C:N) ratios increased with depth down the profile from 9.7 to 20.0, inferring that in the process of OM breakdown, more N would be available in the topsoil regions than at depth to satisfy the requirements of both the micro-organisms and the plant.

A varied response of soil total N concentration to fertiliser N applications arose over both seasons of the study. Not surprisingly, they did not measurably increase soil total N, but indirectly may have affected soil N dynamics by increasing both the mineral N pool and the net rate of mineralisation (due to its "priming" effect), thereby stimulating plant growth.

The total N concentration of the two seasons was not significantly different and could be attributed to the fact that

N gains and losses on the 15 year old pastures were probably balanced. Generally similar trends in soil total N down the profile over both seasons was further evidence to suggest that this was the case.

6.1.2 Soil ammonium N

Before the application of any fertiliser, 331.9 kg $\text{NH}_4\text{-N}$ was measured in the soil to a depth of 1m, on average, over both seasons. This amount represented only 2.1% of the total N in the profile. This is likely to have been derived both from recently mineralised N as well as from residual $\text{NH}_4\text{-N}$ derived from the fertiliser N applied the previous season.

The concentration of $\text{NH}_4\text{-N}$ followed a quadratic trend down the soil profile, irrespective of the amount of fertiliser N applied, with the largest concentrations accumulating, on average, in the 0-10cm and 75-100cm depth classes and the lowest concentrations in the 50-75cm depth class. Accumulation of this N fraction in the 0-10cm depth class may be explained by mineralisation reactions induced by high soil temperatures and wetness in this region of the profile, resulting in increased OM breakdown. Surface fertiliser additions would also have enhanced the surface N levels.

The accumulation of $\text{NH}_4\text{-N}$ at depth is difficult to explain in terms of mineralisation alone, unless nitrification was restricted at this depth. This would have resulted in cationic

build-up over a number of years, associated particularly with the high clay concentration found at this depth. Plant uptake at depth may also have been limited due to a lack of roots at this depth.

Laboratory wetting/drying experiments on soil samples from the 75-100cm depth class induced only a small decline in $\text{NH}_4\text{-N}$ concentration, leading one to conclude that nitrification was probably not inhibited, to any extent, by a lack of aeration from compaction in this depth class. Rather, the presence of a high clay content in this region probably resulted in increased cation exchange capacities which also influenced the NH_4^+ to some extent. A relatively high organic C content of 1.44% at this depth was further evidence of nitrification inhibition.

A similar Inanda soil form under a maize crop did not exhibit the properties eluded to above, suggesting that annual turn-over of the soil allowed mineralisation-immobilisation reactions to proceed at a relatively rapid rate.

Not unexpectedly, addition of fertiliser N to the pasture significantly increased the amount of $\text{NH}_4\text{-N}$ over that of the control camps. Furthermore, the higher the application rate, the greater the increase in $\text{NH}_4\text{-N}$ accumulation within the soil profile. Literature relates this to the fact that at low N application rates, the sward may act as a sink for NH_4^+ ions, so that the pasture plants would have had to compete with the nitrifying organisms, leading to low $\text{NH}_4\text{-N}$ concentrations at

these rates relative to those at the higher application rates. At the high N application rates, potential would exist for an excess of input over removal, with the opportunity for nitrification and accumulation, followed by a probable loss of $\text{NO}_3\text{-N}$ through leaching.

On average, 66.7 kg more $\text{NH}_4\text{-N}$ was present in the soil in the first season than in the second after fertilisation. These results could not be related to climatic conditions, because these remained essentially similar throughout both seasons. The most likely explanation is that high concentrations of $\text{NH}_4\text{-N}$ initially during the first season may have been induced by high N application rates previous to this study.

Although the amount of $\text{NH}_4\text{-N}$ did not differ significantly from spring through to autumn, during early spring and late summer/autumn concentrations were relatively high. During these times temperatures were cooler, and mineralisation reactions may, to an extent, have been inhibited. This may have effectively reduced nitrification reactions as well, resulting in an increase in the presence of $\text{NH}_4\text{-N}$ at these times during the season. During mid-summer, the opposite held true. Observed soil $\text{NH}_4\text{-N}$ trends were also very similar to the soil total N trends within both seasons.

6.1.3 Soil nitrate N

Before fertilisation, only 45.6 kg $\text{NO}_3\text{-N}$, representing 0.29% of

the soil total N, was found in the profile to a depth of 1m, on average. The highest concentration of $\text{NO}_3\text{-N}$ was lodged at a depth of 0-10cm, probably because this portion of the soil profile is where biological transformations and interaction between environmental factors and N inputs and removal are likely to be most rapid. From the upper soil profile, $\text{NO}_3\text{-N}$ declined, on average, with depth. However, during the second season there was an increase in $\text{NO}_3\text{-N}$ concentration with depth, suggesting the movement of this fraction down the profile during this period. This pointed to the likelihood of leaching losses, particularly at the higher N rates.

Fertilisation significantly increased the concentration of $\text{NO}_3\text{-N}$ above those of the control camps. Since the fertiliser contained NO_3^- ions, it is probably not surprising that concentrations of this ion increased as fertiliser application rates increased.

As fertiliser N application rates increased, so too did $\text{NO}_3\text{-N}$ concentrations with depth. This relates to the fact that, in addition to the $\text{NO}_3\text{-N}$ contained in the fertiliser, nitrification reactions may have been stimulated by the additional N applied at the higher application rates, consequently releasing more $\text{NO}_3\text{-N}$ into the soil profile. This has important implications for potential leaching of $\text{NO}_3\text{-N}$ into the groundwater, in that once total annual applications reach levels of 300 kg N/ha or more, applications should become smaller and more frequent over the season in order to hopefully limit this pollution potential.

Following the application of N fertilisers, 94.3 kg $\text{NO}_3\text{-N}$ /ha was present, on average, down to a depth of 1m over both seasons. However, significantly more $\text{NO}_3\text{-N}$ was present in the second season than in the first. This result is in contrast to that of the $\text{NH}_4\text{-N}$, where lower concentrations were found in the second season than in the first. This is likely to be attributable to differences in the rate of nitrification in the two seasons eg. the higher rainfall during the second season.

At the higher N application rates (300 & 450 kg N/ha/season), increased concentrations of $\text{NO}_3\text{-N}$ were observed at depth in the second season when compared with similar applications in the first season. This suggested that a build-up of NO_3^- ions may have taken place over the two seasons at the high N application rates. No such build-up was recorded at the lower N application rates and in the control over the two seasons, thus again reflecting the greater potential for $\text{NO}_3\text{-N}$ leaching over time at high application rates.

No specific trends in $\text{NO}_3\text{-N}$ concentration were observed within each season. Rather, $\text{NO}_3\text{-N}$ concentrations tended to vary inconsistently at each sampling period. This was probably due, in part, to varying environmental, physical and microbial activities taking place together, or alone, within the soil profile.

Nitrate N concentrations within each month during both seasons followed a near mirror image of $\text{NH}_4\text{-N}$ concentrations. This

suggests that $\text{NO}_3\text{-N}$ concentrations may have been influenced by temperature, especially at the beginning and end of both seasons, where lower temperatures may have depressed nitrification.

6.2 Herbage N and DM yields

6.2.1 Herbage DM yields

A DM yield of 12.7 t/ha, averaged over all treatments, was obtained over the two seasons. However, a DM yield of 10.9 t/ha off the control camp was deemed to be high, but may have resulted from high residual N from previous seasons or a rapid breakdown of accumulated OM and a subsequent release of nutrients sufficient for rapid plant growth.

A progressive increase in DM yield was obtained with successive increments of N fertiliser (Reid et al. 1966; Rhykerd et al. 1966; Cowling & Lockyer, 1967; Mengel & Kirkby, 1978, Ball, 1979 and Crowder & Chheda, 1982). However, the overall response of the kikuyu to the N applied was consistent with the law of "diminishing returns" (Blue 1970; Henzell 1971; Crowder & Chheda 1982).

A higher yield of 1.8 t DM/ha in the first season over that of the second, after fertilisation, was difficult to explain since rainfall amount and distribution was similar over both seasons. This also influenced DM trends in a similar manner over both seasons, with late spring rains retarding growth during this time in both seasons. As rainfall increased in mid-summer of both

seasons, DM yields responded accordingly. Dry matter yields declined into autumn, probably as a result of a decline in rainfall and/or temperatures as well as the onset of senescence in the kikuyu pasture during this time.

6.2.2 Plant protein N

Protein N content is important in that it is a major determinant of herbage quality. On average, 2.84% protein N was determined in the herbage over both seasons. In general, protein N concentrations increased as N application rates increased.

On average, higher concentrations of protein-N were measured within the upper (>5cm) than in the lower (<5cm) herbage stratum, irrespective of the amount of N applied. This may be related to the fact that N is translocated to newer younger leaves in order for growth to proceed in this region of the plant. The importance of this result is that it suggests that the newer leaves are of better quality than the more mature lower stratum tissues.

Similar bi-modal trends over time in protein-N concentration were measured for all N treatments and within both herbage strata over both seasons, with concentrations tending to be highest during early summer (Dec) and in early autumn (Feb), and lowest during spring (Oct), mid-summer (Jan) and autumn (March). Spring and autumn peaks seemed to correspond with periods of slower growth, whilst low mid-summer concentrations coincided with periods of high DM yields and TNC concentrations. The high protein-N

concentrations measured at the beginning and end of the season are important to note as they suggest that during these periods protein-N would probably be adequate for animal growth and/or maintenance. At these times, therefore, applications of fertiliser N might be withheld because of plant sufficiency.

6.2.3 Plant nitrate N

The $\text{NO}_3\text{-N}$ concentration observed in the DM on the Broadacres trial ranged from 0.12% to 0.43%. This range compares favourably with published data and suggest the possibility of NO_3^- toxicity at the higher N levels; at least in the data of the first season. As applications of fertiliser N to the pasture increased, $\text{NO}_3\text{-N}$ concentrations within the herbage increased in a near-linear fashion.

On average, higher concentrations of $\text{NO}_3\text{-N}$, irrespective of the amount of fertiliser N applied, were measured within the upper (>5cm) than the lower (<5cm) herbage stratum. The presence of $\text{NO}_3\text{-N}$ within the younger portion of the sward suggests that the plant is actively translocating this N fraction to these regions for subsequent synthesis into NH_4^+ ions, and thus protein-N. Problems of $\text{NO}_3\text{-N}$ toxicity might arise within the material of the upper herbage stratum in the presence of high concentrations of soil $\text{NO}_3\text{-N}$, especially where a succession of high N rates are applied, and/or where the plant is unable to convert all the NO_3^- ions absorbed by the plant to NH_4^+ ions as, for example, during periods of slow growth.

A higher concentration of $\text{NO}_3\text{-N}$ was measured in the herbage during the first season than in the second on the Broadacre trials, meaned over all treatments and both height strata. The lower concentration in the second season may have resulted from $\text{NO}_3\text{-N}$ within the soil complex becoming leached down the profile, as a result of the higher rainfall during this season. Results on this trial also showed that $\text{NO}_3\text{-N}$ concentrations within the profile in the second season were higher and more concentrated at depth, especially at the higher N application rates, than in the first season. This probably corresponded to the higher rainfall during this season. Alternatively, high $\text{NO}_3\text{-N}$ concentrations in the plant may also be directly related to high N concentrations within the herbage at these times.

A similar bi-modal trend to that measured with protein-N concentrations was observed in both seasons for $\text{NO}_3\text{-N}$ in the herbage. High concentrations of $\text{NO}_3\text{-N}$ were measured during spring (Nov) and autumn (Feb), and lower concentrations in mid-summer (Dec & Jan), very early spring (Oct) and early autumn (March). These results suggest that N fertiliser should be withheld when $\text{NO}_3\text{-N}$ concentrations are likely to be high, at least until such time as plant growth is about to be impaired by a shortage of available N for plant uptake. Potential toxicosis is likely to become accentuated by fertiliser N application at these times. During summer, declining $\text{NO}_3\text{-N}$ concentrations are associated with a corresponding increase in herbage DM yields.

Furthermore, $\text{NO}_3\text{-N}$ and protein-N concentrations follow similar

trends over the season, which confirms that these two N fractions are linked to growth functions in the plant.

6.2.4 Plant total non-structural carbohydrates

A lack of any distinctive trend emerging among these trials in the response of TNC to increased fertilisation with N suggests that, in kikuyu, applied N alone would not materially alter TNC concentrations.

Higher concentrations of TNC were recorded in the lower (<5cm) herbage stratum, on average, than in the corresponding upper (>5cm) stratum. This may be ascribed to the fact that TNC's tend to be found in higher concentrations where plant protein-N and $\text{NO}_3\text{-N}$ concentrations are low. This inverse relationship between both protein-N and $\text{NO}_3\text{-N}$ and TNC levels in temperate species has been well documented (Jones et al. 1961; Mays 1974; Ball et al. 1978; Ross et al. 1978), and these results now suggest that tropical species such as kikuyu follow a similar pattern.

It is also interesting to note that an increased concentration of TNC in autumn suggests that in kikuyu, TNCs may indeed accumulate. Furthermore, the high concentration of TNCs in spring suggests that their concentrations remain high over winter and decline when protein-N and $\text{NO}_3\text{-N}$ concentrations begin to increase in the summer. This effect may not, however, be directly and solely a result of protein-N and $\text{NO}_3\text{-N}$ concentration changes and thus more extensive measurements are needed to confirm this result.

6.2.5 Relationships between protein-N, $\text{NO}_3\text{-N}$, TNCs and DM yields

An inverse relationship existed between DM yields and the two N fractions. When DM yields were high (characteristically in summer), these two N fractions were low. These two N fractions were probably being actively utilised for growth and thus concentrations were low at this time. As DM yields declined into autumn, or before the spring regeneration period when the pasture was essentially dormant, protein-N and $\text{NO}_3\text{-N}$ concentrations were high because of active N uptake before the commencement of spring growth.

6.3 The fate of labelled ammonium ^{15}N

Of the ^{15}N applied as an $^{15}\text{NH}_4\text{NO}_3$ spring fertiliser dressing, 52.24295% was recovered in the herbage harvested through the season. Of the total amount applied, 37.3232% was recovered in the months of November and December alone. Similar recoveries were reported by Dowdell & Webster (1980), Whitehead & Dawson (1984) and Bristow *et al.* (1987) in their trials on ryegrass pastures.

As might have been expected, the highest percentage of initially applied $\text{NH}_4\text{-}^{15}\text{N}$ was measured in the herbage one month after application (i.e. November). This percentage subsequently declined (significantly so ($P < 0.01$) in some cases) in a near linear fashion over time, which is probably related to a series of factors including those of plant uptake (and so a reduced

amount of ^{15}N remaining in the soil for uptake), immobilisation, pool substitution and/or dilution as a result of the "priming" or "added nitrogen interaction (ANI)" effect of subsequent fertiliser applications (Jenkinson *et al.* 1985; Hart *et al.* 1986). The accumulated recovery of initially applied $\text{NH}_4\text{-}^{15}\text{N}$, as a percentage of that applied, showed a curvi-linear response following fertiliser N application.

The highest amount of ^{15}N recovered in the roots was in December. This was significantly ($P < 0.01$) more than was recovered in the months of November, March ($P < 0.01$) and February ($P < 0.05$). Again, the decline in ^{15}N recorded over time is probably accounted for by the dilution of labelled ions within the soil complex from subsequent fertiliser N applications, nitrification reactions (leading to leaching) as well as by its removal into the herbage. The low amount recorded in November, when the highest amount might have been expected, could not be accounted for.

The amount of ^{15}N recovered in the top 30cm of the soil within each month declined from 69.4300% (of that applied) in November to 35.1447% in March, probably again reflecting the dilution effect of subsequent fertiliser applications and/or microbial action, as well as plant uptake over the season. The highest amount of ^{15}N recorded at a depth of 30-60cm was 10.2459%, on average, in December. In general, in each month thereafter, ^{15}N recorded at this depth declined linearly to reach a minimum in March of 3.1994%. Of interest is that less labelled N was recorded at this depth in November, one month after application,

than in December. In this month only 4.4565% of the applied ^{15}N was recorded as being resident in the depth class. This was the second lowest amount determined over the season and may give some indication of the rate at which the fertiliser N penetrated to this depth. A weighted average of the ^{15}N recorded at a depth of 60-100cm revealed that the least labelled N was found at this depth, compared to the other two depth classes, at all times during the season. The highest amount of labelled N was recorded in January (4.5670%), this being significantly ($P < 0.05$) more than in March (1.4356%). Unlike in the two depth classes above, no distinct pattern in the decline of ^{15}N was observed from the beginning to the end of the season.

Monthly ^{15}N recovered, as a percentage of that applied as fertiliser, within the herbage, roots and soil to a depth of 1m ranged from 98.2769% in November to 108.1974% in December. The total amount of ^{15}N recovered, on average, in the herbage, roots and soil to 1m over the season was 98.069%. This high recovery rate suggests that the NH_4^+ ion is not easily moved out of the soil profile, and is therefore available to be utilised by the plant for growth during the season.

6.4 Herbage macro-nutrients

Macro-nutrients are important co-factors involved in plant growth. Each macro-nutrient has a specific function(s) within the plant that aides in the metabolic processes which ultimately allows the plant to grow.

The P content of the kikuyu pastures, before fertilisation, averaged 0.248%. This concentration is such that it should preclude any necessity for P supplementation at least for beef animals, but not for a dairy cow producing 15 l/day, where the P concentration should be approximately 0.34% of the herbage dry matter.

Herbage P concentrations dropped as N fertiliser application rates were increased on the pasture. This result is most likely due to dilution of the P within the herbage as DM yields increased in response to higher N application rates.

Even though no significant difference in P concentration was measured between the two herbage strata, a higher P content prevailed within the lower (<5cm) herbage stratum. However, only 0.003% more P was present within the lower stratum, and this is probably of questionable biological significance. This difference, although negligible, was contrary to that observed by other authors who concluded that higher levels of P were commonly more prevalent within the younger fractions of a pasture (corresponding to the upper (>5cm) stratum). This is so because P is an essential element in energy transduction, in the orthophosphate form, and is commonly found as such in the newer portions of the plant.

The inconsistent P concentrations observed within each month over the two seasons suggests that P concentrations are variable and dynamic in nature. The stage of plant growth during the season

and/or temperatures are probably major factors determining P variability and availability over the season. In this respect, kikuyu had lower P concentrations in spring/early summer than in mid-summer, when growth was at a peak. Practically then, in October when P concentrations are low, early spring additions of phosphatic fertiliser may enhance pasture growth at that time. Furthermore, the addition of early spring fertiliser N applications may result in a favourable N-P interaction which could increase pasture yields.

On average, 2.96% K was present within the herbage material in this trial. The norm for pastures ranges between 0.7 and 4.0%, with the concentration of this ion greatly exceeding that of most other elements derived from the soil, except that of N which is normally of the same magnitude as K. As the concentration of K measured off these pastures lies within these range limits, it is extremely unlikely that well fed ruminants, whose dietary K requirements range from 0.5 to 0.80%, will suffer from a deficiency of this element on this pasture.

The N status of plants has been widely shown to have a marked influence on requirements for K. Under conditions of adequate K supply, uptake of K increases with increasing N supply, and the required K concentration for maximum yield (critical level) has been found to increase with increasing N concentration. However, on these trials applications of fertiliser N to the camps did not significantly increase K concentrations within the herbage. Exactly why this should be so is difficult to ascertain, but it

may be because K within this soil form was not limiting, even on the control camps, or antagonistic reactions between other macro-nutrients may have affected K uptake. Another explanation, although unlikely, for the non response of herbage K to increasing N in this study may have been the additional di-basic material that accompanied the N in the LAN. Subsequent investigations revealed that the limestone fraction of the LAN did contain a mixture of both calcitic and dolomitic lime. However, it could be concluded that these concentrations are too small to influence macro-nutrient concentrations, at least in the short term.

Stage, and thus age, of plant growth has a major bearing upon K concentrations within the herbage, with concentrations declining with maturity. The lower (<5cm) herbage stratum, being the older herbage fraction, was found to contain the highest K concentration in the pasture. As no deficiency was detected in the plant or the soil, K presumably remained stored within the older plant tissue and would probably only have been translocated from these regions towards meristematic tissue to supplement the younger, actively growing herbage in times of deficiency. Uptake and transport of K to younger plant parts was found by a number of researchers to be influenced by the presence of N. The reason why translocation of K^+ ions is affected in this fashion is still not fully understood by researchers, but they have theorised that relationships might exist between K movement, N fertilisation, protein N synthesis, growth rate and cytokinin supply within the meristematic regions.

Although probably biologically non-significant, the presence of significantly less K within the herbage in the second season than in the first may be linked to depletion of reserves of this element in the soil by the plant and/or elemental interactions between K and other macro-nutrients. Alternatively, removal of the herbage by the animal, combined with the short period of stay, may have resulted in K being excreted elsewhere other than on the camps from which the herbage was removed, or by "aggregation" of the K whereby animals eat from the larger area, but excrete over a restricted area within individual camps. Soil and environmental factors such as soil aeration and temperature, may well also contribute to herbage K variability.

An average Ca content of 0.35% within the herbage falls within the range of 0.14 to 1.5% specified by the NRC (1976) as a general requirement for livestock, but not for high-producing dairy animals.

Increasing N application rates to the pasture increased the Ca content within the herbage. Both the application of 300 and 450 kg N/ha/season produced similar concentrations of Ca within the herbage. However, researchers found it difficult to ascertain fully whether or not it was the actual N (and more specifically the presence/absence of $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$) within the fertiliser that increased Ca concentrations. Results from these trials showed that $\text{NO}_3\text{-N}$ concentrations did indeed increase in both the soil and herbage as N application rates were increased, results that may have influenced the relative abundance of Ca within the

herbage. It should be noted that some Ca was contained in the LAN (28) applied as fertiliser.

In kikuyu, Ca:P ratios have normally tended to be close to unity or tissue may even contain more P than Ca. This was not the case on these trials, however, with the Ca:P ratio of 1.4:1 tending to follow the norms associated with most other tropical species. In this respect then, Ca supplementation for livestock grazing these pastures would not be necessary, provided Ca does not bind with oxalic acid in the plant to form insoluble Ca oxalate. Various authors have, however, suggested that high oxalate levels in kikuyu severely restrict the availability to animals of Ca in this grass.

Even though N applications increased Ca concentrations within the herbage, it is by no means certain that all the Ca is readily available to animals grazing the pasture because previous work reported that Ca content of kikuyu was dependent upon the N content of the grass. This was because a high N content, synonymous with increased N applications, increased the concentration of insoluble Ca oxalate within the herbage. Thus, even though a high concentration of Ca may have been measured, the oxalate may have rendered it unavailable to the ruminant grazing at the time. Thus, as N application rates were increased on the Broadacre trial, the potential for increased Ca oxalate manufacture in the plant would also have increased.

No significant differences in Ca concentration were found in the

upper (>5cm) and lower (<5cm) herbage strata over both seasons, even though the lower stratum had a slightly higher Ca concentration, on average, than the upper stratum. Calcium is generally immobile within the herbage, normally in the insoluble Ca oxalate form, and for this reason concentrations may have remained stable and similar within both strata. Furthermore, unlike P and K, Ca does not tend to discriminate in favour of younger, newer growth, but rather tends to accumulate in higher concentrations within the leaves than in the stems of the herbage.

Calcium concentrations within the herbage showed no distinct trend over both seasons, probably because concentrations tended rather to vary with stage of plant maturity and season. However, higher concentrations of the element were measured in the second season than in the first. The reason for this is unknown, but may be related to antagonistic effects experienced between K and Ca concentrations during this season.

On average, 0.377% Mg was present within the herbage over both seasons. This compares favourably with published data wherein Mg concentrations varied from 0.04 to 0.9% in the DM, with a mean of 0.36%.

All camps with N applied to them contained significantly more Mg in their herbage than on the control camps. Significantly higher concentrations of Mg were found in the herbage after application of 450 kg N/ha/season than after applications of 150 and 300 kg

N/ha/season respectively. The mechanism involved in this process is complex, but similar to that described for Ca. Uptake of Mg is dependent upon the form of the N fraction. Increased $\text{NO}_3\text{-N}$ levels enhance uptake, whilst $\text{NH}_4\text{-N}$ decreases Mg levels in the herbage because the NH_4^+ ions actively compete with the Mg^{2+} ions. Also, the addition of LAN, and more specifically the lime in the fertiliser mix, may have been a precursor for increased Mg uptake into the plant (Mg was also contained in the LAN (28) applied as fertiliser to the pasture).

On these trials, the Ca:Mg ratio is 0.92:1 which is considered to be near the optimum for livestock. Therefore, the potential for a deficiency in Mg, and/or a high tetany potential on this pasture is remote.

Magnesium concentrations remained essentially similar within both herbage strata, regardless of the rate of fertiliser N applied. Furthermore, the lack of any major difference in average concentration between Ca and Mg within the strata is probably related to the similar transport mechanism utilised by these two ions. Magnesium content in the herbage seems, however, to be extremely variable, and therefore further comment would be speculative.

As in the case of Ca, Mg concentrations within the herbage were significantly higher in the second season than in the first. This could again be related to the similarity in the uptake mechanisms of both ions by the herbage, and/or the decline in K

concentration during this season. Trends over each season were, in general, inconsistent and may be related to the stage of plant maturity, interactive effects between other elements and/or season.

The K/Mg+Ca ratio which acts as an indicator of the tetany potential of a pasture, may be of some importance when predicting imbalances of these elements in herbage and their subsequent effect on animal health. The K/Mg+Ca ratio in an animal's diet off a tropical pasture should be less than 2.2 (Tainton & Eckard, 1992). Figures on this trial, however, are nearly double the norm, as observed by Tainton & Eckard (1992), even at the zero N level and within both strata of the herbage, again suggesting luxury consumption of K by the pasture plants. The ratios declined, on average, as the N application levels on the trial were increased. This resulted from an inherently high soil K content, even on the control camps, and/or dilution of these ions within the herbage as yields increased as N application rates increased.

Also, this ratio was higher in the <5cm herbage stratum than in the >5cm stratum, on average, suggesting that older herbage may have a higher tetany potential than younger herbage, and that farmers, in their grazing strategy, should then not allow pastures of this type to be grazed to below 5cm without first checking on this ratio. Ameliorating with Ca or Mg fertilisers, or cutting back on K fertilisers, may also be an option in order to manipulate this ratio.

Monthly variations in K/Mg+Ca ratios within each season were variable and difficult to explain. However, the ratio did show a more general trend over the two seasons, from an initial low in October (2.92) to a peak in December (5.41), before declining into March (3.98). The overriding factor influencing this trend over time seems to be the high soil K concentration. Thus, generally, as the pasture begins to grow in spring, luxury consumption and therefore, potential tetany is low. This potential toxicity increases into summer, when active growth occurs, before declining into autumn. The high ratio in February could not be accounted for, but may have resulted from an increase in soil K uptake due to high rains the previous month, which are known to enhance the uptake of this ion. In the case of this trial though, K/Mg+Ca ratios are still higher than the accepted norm for animal requirement, even in spring, and so toxicity related problems could still be envisaged if animals are grazed on these pastures at any time during the season. Thus, some form of Mg and/or Ca supplement (eg. di-calcium phosphate or lime) should be fed to animals grazing on these pastures at all times during the season.

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APPENDIX 1

LABELLED $^{15}\text{NH}_4\text{NO}_3$ CALCULATIONS

1. Calculations for the determination of N applications per plot are as follows :

Nitrogen was applied at the rate of 75 kg N/ha, over each of three applications, to 0.950332 m² plots :

$$= 75 \text{ kg N/ha} \times 1000/\text{kg} \times 0.0001 \times 0.950332$$

$$= 7.1499 \text{ g N/application to a 1 m}^2 \text{ plot}$$

Therefore, 7.1499 g N represents the following number of moles of N :

$$\begin{aligned} \text{moles} &= \text{mass/molecular mass} \\ &= 7.1499/14.0067 \\ &= 0.510462 \text{ moles N/plot/application} \end{aligned}$$

It is necessary to determine the mass of NH_4NO_3 required to give a final quantity of 0.510462 moles N :

$$\begin{aligned} \text{molecular mass of } \text{NH}_4\text{NO}_3 &= 2(14.00670) + 4(1.00797) + 3(15.9994) \\ &= 28.0134 + 4.03188 + 47.9982 \\ &= 80.04348 \end{aligned}$$

$$\begin{aligned} \text{mass} &= \text{moles} \times \text{molecular mass} \\ &= 0.510462 \times 80.04348 \\ \text{mass} &= 40.8591 \text{ g } \text{NH}_4\text{NO}_3 \text{ per application} \end{aligned}$$

But, for every mole of NH_4NO_3 , 2 moles of N will be provided, since N is provided by both NH_4 and NO_3 . Therefore, only half the amount of NH_4NO_3 is required and so :

$$\text{mass of } \text{NH}_4\text{NO}_3 \text{ applied} = 20.4295 \text{ g AN per application}$$

If [^{15}N] enriched fertilizer is used, then the primary consideration is the level of [^{15}N] enrichment to be used.

Levels of 5 atom % are frequently quoted in the literature, but the IAEA and FAO (Anon, 1976) recommend that anything between 3 and 33 atom % is acceptable. The major considerations, then were the cost and the detection limits of the instrumentation used. Taking both these factors into account, it was decided to use an enrichment level of 10 atom %, for both detectability and cost-

efficiency.

So, in a fertilizer of 10 atom % enrichment, 90% will be unlabelled NH_4NO_3 and 10% will be $^{15}\text{NH}_4\text{NO}_3$.

The molecular mass of the enriched fertilizer was therefore the following :

$$\begin{aligned} &= 80.04348 + 2.00 \\ &= 82.04348 \end{aligned}$$

then molecular mass of the enriched $^{15}\text{NH}_4\text{NO}_3\text{N}$ is :

$$\begin{aligned} &= (90/100 \times 80.04348) + (10/100 \times 82.04348) \\ &= 72.039132 + 8.204348 \\ &= 80.24348 \end{aligned}$$

So if 0.510462 moles of N are required in total, the mass of the enriched fertilizer will be :

$$\begin{aligned} &= \text{moles} \times \text{molecular mass} \times 0.5 \\ &= 0.510462 \times 80.24348 \times 0.5 \\ &= 20.48062\text{g } ^{15}\text{NH}_4\text{NO}_3\text{N/plot/application} \end{aligned}$$

2. Calculations to determine the actual % recovery of ^{15}N in the herbage, roots and soil strata:

(a) corrected mass per plot:

$$E = D/0.9550332 \times 0.106029^A \times 1000 \times 1000$$

Where:

E = corrected mass per plot (mg/plot)

D = mass per plot ($\text{kg}/0.950332\text{m}^2$)

(b) total N per sample:

$$F = B/100 \times E$$

^A This figure corresponds to the effective area calculate after removing the 10cm circumference and dividing the area into 12 sectors

Where:

F = total N (mg/plot)

B = total N (%)

E = corrected mass per plot (mg/plot)

(c) ^{15}N present in each sample:

$$G = (C - \text{background}) * F/100$$

Where:

$G = ^{15}\text{N}$ (mg/plot)

$C = \text{atom } \% ^{15}\text{N}$

Background = all samples taken on 26/10 (ie at the beginning of the season & before fertilisation).

$F = \text{total N}$ (mg/plot)

(d) percent initially labelled $\text{NH}_4\text{-}^{15}\text{N}$ recovered:

$$H = G / (^{15}\text{N added}) * 100$$

Where:

$H = \% \text{NH}_4\text{-}^{15}\text{N}$ recovered

$G = ^{15}\text{N}$ (mg/plot)

$^{15}\text{N added} = 80.3112$ (1st mth after application)

83.3762 (2nd & 3rd mth after application)

86.4412 (2nd & 3rd mth after application)

Once each individual figure has been calculated, then the total ^{15}N recovered in the herbage, roots and soil to a depth of 1m can be calculated as follows:

Nov : herbage + roots + soil strata = 98.2269% ^{15}N recovered

Dec : herbage & roots from previous mth + this mth's herbage +
this mth's roots + this mth's soil strata = 108.1974% ^{15}N
recovered

Jan : herbage & roots from previous 2 mths + this mth's herbage
+ this mth's roots + this mth's soil strata = 99.5665% ^{15}N
recovered

Feb : herbage & roots from previous 3 mths + this mth's herbage
+ this mth's roots + this mth's soil strata = 60.4110% ^{15}N
recovered

Mar : herbage & roots from previous 4 mths + this mth's herbage
+ this mth's roots + this mth's soil strata = 92.2951% ^{15}N
recovered