THE EFFECT OF APPLICATION OF NITROGEN, PHOSPHORUS, POTASSIUM AND SULPHUR FERTILISERS TO A PERENNIAL RYEGRASS SWARD ON YIELD, QUALITY AND APPARENT INTAKE BY DAIRY COWS

by

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DECLARATION

I, Nicola Findlay, hereby declare that the work contained in this dissertation is my own original work. Where use has been made of the work of others it has been duly acknowledged in the text. It has not been previously submitted, either in its entirety or in part, at any other university for degree or examination purposes.

Signature: ……………………………

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ABSTRACT

Perennial ryegrass is an intensive, temperate pasture grass that responds well to applied fertiliser. The purpose of this project was to study the effects of fertiliser on the productivity and quality of perennial ryegrass in KwaZulu-Natal and how this impacts on animal intake. It was hypothesised that over-application of fertiliser to a perennial ryegrass pasture would negatively affect the quality of the herbage for grazing by dairy cattle and that intake would be affected. Thus the project aimed to assess the effects of applied fertiliser on yield, quality and intake of an established perennial ryegrass pasture.

The trial consisted of a set of six separate experiments. Each experiment focused on the interaction between two of the major nutrient elements nitrogen (N), phosphorus (P), potassium (K) and sulphur (S). The experiments (NxP, NxK, NxS, PxK, PxS and KxS) were managed separately to avoid possible transfer of nutrients during grazing, which would result in the contamination of treatments. Each factor had three levels (low, medium and high), giving a total of nine treatments per experiment. Each of the experiments was replicated three times in a randomised block design.

Increased fertiliser N application rates increased perennial ryegrass yield with a pattern of diminishing return, where split applications above 40 kg N ha^{-1} produced smaller increases in yield when compared with the response at lower applications of N. Applied P, K and S did not affect yield, suggesting that even the lowest application levels were sufficient to not limit production. Nitrogen application affected apparent intake, but it is suggested that this is due to the yield effect rather than a direct effect of N on apparent intake. The application of P, K and S did not affect apparent intake.

Results from this study showed that the quality of perennial ryegrass herbage, especially in terms of feed value to dairy cows, can be significantly affected by applied fertiliser. The extent of the response was affected by sampling date (i.e. time of year) and this must be taken into account when planning a fertiliser management strategy. This is particularly so with respect to N fertiliser recommendations.

Crude protein (CP) content of herbage increased with increasing levels of applied N and the extent of the response was influenced by season. P, K and S did not affect CP concentration in herbage, except in the PxK experiment where increased levels of K lowered herbage CP. Applied N considerably increased the concentration of non-protein nitrogen (NPN) in perennial ryegrass herbage. P and S did not affect NPN levels, whereas applied K decreased NPN content in the

NxK and PxK experiments. Non-structural carbohydrate (NSC) content of herbage was decreased by applied N but was unaffected by applications of P, K and S. Neither neutral detergent fibre (NDF) nor acid detergent fibre (ADF) was affected by applied fertiliser. In this study herbage P declined and herbage Ca increased with increasing levels of applied N. The addition of fertiliser K resulted in lower herbage Ca values. There was no herbage S response to applied fertiliser in this study.

Classification and regression tree (CART) analysis identified the primary determinant of apparent intake in experiments containing N as a factor as the amount of material available to be grazed and that NSC, NPN and ADF are also determinants of apparent intake. Cows do not regulate diet choice within the short-term time frame of a meal. Thus intake is determined by short-term needs rather than by meeting long-term nutrient requirements. Fibre creates physical fill within the rumen, thus restricting intake. High NPN content is associated with high nitrate values. The reduction in intake of herbage with high nitrate content may be due to reduced palatability or to a negative feedback system limiting the intake of nitrate and ammonium. Increased NSC content is associated with increased intake, possibly through the effect of sugar on herbage palatability.

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ABBREVIATIONS

ADF	Acid detergent fibre
ATP	Adenosine triphosphate
Ca	Calcium
CART	Classification and regression tree
CDMD	Cellular dry matter disappearance
CP	Crude protein
Cu	Copper
DM	Dry matter
K	Potassium
LSD	Least significant difference
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NH_4	Ammonium
NH_4HCO_3	Ammonium bicarbonate
NO_3	Nitrate
NPN	Non-protein nitrate
NSC	Non-structural carbohydrate
P	Phosphorus
S	Sulphur
Se	Selenium
Zn	Zinc

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CHAPTER 1

Introduction

1.1 General background

Ryegrass (*Lolium* spp) is the second most important irrigated pasture in South Africa after lucerne (Theron *et al.*, 2002). Its popularity, especially in the dairy industry, is largely due to its ability to meet the need for high-producing, high quality forage during the cooler months of the year. *Lolium multiflorum* Lam. (Italian ryegrass) has traditionally been favoured over *Lolium perenne* L. (perennial ryegrass) due to poor persistence exhibited by perennial ryegrass when grown in sub-tropical climates (McKenzie, 1994). However, rising costs of pasture establishment have resulted in the need for a perennial pasture which fulfils the same needs. *Lolium perenne* L. (perennial ryegrass) is a creeping perennial, which produces upright tufts of dark green, slender leaves. It is indigenous to Europe, northern Africa and temperate parts of Asia but has been introduced and cultivated over much of the world. In South Africa it grows best under irrigation in the cool, moist high altitudes of the country where the summers are mild. It has a high nutritive value (Table 1.1) and its dry matter content is higher than that of annual ryegrass, encouraging greater intake. The South African National Seed Organisation (Sansor) reported local market perennial ryegrass seed sales of 619 874 kg in 2008/09 (Sansor annual report 2008/09), a 46 % increase in sales for the 2006/07 period (Sansor annual report 2006/07). Perennial ryegrass pasture in South Africa is mostly for grazing in commercial and small-scale dairy operations, although it is also used for both fat lamb and beef production (McKenzie, 1994).

Perennial ryegrass has been widely researched, particularly in those parts of the world where it performs best, namely New Zealand, Australia, Britain and parts of the USA (Table 1.2). Until recently most ryegrass research in South Africa has been focused on annual ryegrass. Problems with persistence and production, as well as the susceptibility to rust, of perennial ryegrass lines imported from Europe meant that demand was minimal. Over the past decade or so there has been much work done on the genetic improvement of perennial ryegrass for warmer climates, especially in New Zealand and Australia. This, and the international research attention received by perennial ryegrass, has prompted South African farmers to again try perennial ryegrass as part of their pasture systems. With the increase in demand for suitable perennial ryegrass lines the need arose for South African-based research. McKenzie addressed the lack of research data in his PhD thesis (McKenzie, 1994), which focused primarily on the effects of grazing management on perennial ryegrass longevity. Since then, perennial ryegrass has received increasing research attention, notably in the Western Cape, where climatic conditions better support production and persistence than elsewhere in the country. However, in comparison with research conducted in major perennial ryegrass producing countries, published data on perennial ryegrass in South Africa is limited (Table 1.2). With information from other countries so readily accessible on the internet and the tendency of farmers to look to other countries for pasture management systems, it is essential that studies are conducted in South Africa that will identify management options suited to local conditions.

In the current economic climate of declining profit margins, farmers are placing increased production demands on pastures. Perennial ryegrass is an intensive pasture requiring high inputs in terms of fertiliser, irrigation and grazing management. It responds particularly well to applied fertiliser and increased nutrient supply has become evident as farmers seek to maximise pasture yields. Popular articles have often emphasised the positive effects of fertiliser, indirectly encouraging farmers to apply excessive quantities. However, the cost of nutrient oversupply may easily outweigh benefits. Apart from the environmental and financial implications of wasted fertiliser, excessive fertilisation costs the farmer in terms of pasture energy losses, loss of pasture palatability, increased risk of ammonia toxicity in animals and lowered animal production. This, in addition to the fact that fertiliser is often the most costly component in pasture production, underlines the importance of a sound approach to fertilisation of pastures.

Table 1.1. Composition of perennial ryegrass (DM basis)

Reference	CP % DM	ADF % DM	NDF % DM	NSC % DM	NPN	Total N	ME MJ kg DM^{-1}	S %	Ca %	P %	K %	Mg %	Na %
1	14.5-21.8				0.33 -0.58				0.49 -0.61	0.36 -0.42	2.71 -3.07	0.12 -0.15	0.1
2						2.1-3.5							
3	15.0-22.8		43.0-57.6	1.0-31.0			10.6-13.1	0.3					0.6-0.7
4	13.5-25.3		44.0-58.2	3.1-13.2			9.5- 11.9	0.2-0.4	0.3-0.7	0.2-0.6	1.9-4.5	0.2-0.4	0.2-0.6
5						4.1-4.5		0.3	0.3-0.4	0.3-0.4	3.9-4.4	0.2-0.3	0.1-0.4
6	15.0-27.7					2.6-4.6							
7								0.3-0.9		0.3-0.7	2.6-5.4		
8	9.3-24.7				0.19 -1.53	1.5-4.0		0.1-0.4					
9								0.3 -0.4	0.6-1.0	0.3-0.5	2.0-2.9	0.1-0.3	0.1-0.4
10				10.5									
11	12.5-30.0	22.0 -33.5	35.5-48.5	8.0-14.5									
12		25.9 -37.2		5.2-23.8		1.6-4.3							
13						2.8-2.9			0.4-0.5	0.3	3.3-3.5	0.2	0.1-0.2
14						1.6-4.9		0.2-0.6	0.3-1.1	0.3-0.7	1.9-4.9	0.1-0.3	0.1-0.4
15	11.8-25.0			13.9-28.8									
16	13.8-22.5		37.0-52.9	8.0-15.4									
17				6.5-35.9									
18			39.6-44.7	12.8-18.7		2.0-3.7	18.1- 19.4						
19						1.9		0.3	0.4		2.6		0.3
20			39.7	15.0		3.9			0.6	0.5	4.2	0.3	0.3
Mean (±SE)	**21.86 0.24**	**26.97 0.46**	**48.31 0.32**	**11.63 0.38**	**0.42 0.06**	**2.88 0.08**	**11.64 0.07**	**0.28 0.01**	**0.56 0.02**	**0.42 0.01**	**3.09 0.08**	**0.23 0.01**	**0.28 0.02**

[1] Hunt (1973)
[2] Alberda (1965)
[3] Jacobs *et al.* (2002)
[4] McKenzie *et al.* (1999)
[5] Thom *et al.* (1989)
[6] Fulkerson *et al.* (1993)
[7] Miles *et al.* (2005)
[8] Goh and Kee (1978)
[9] Hopkins *et al.* (1994)
[10] Deinum and Dirven (1975)
[11] Moller *et al.* (1996)
[12] Binnie *et al.* (2001)
[13] Crush *et al.* (1989)
[14] Whitehead *et al*. (1983)
[15] Humphreys (1989)
[16] Smit *et al.* (2005)
[17] Miller *et al.* (2001)
[18] Ulyatt *et al.* (1988)
[19] Waghorn *et al*. (1989)
[20] Wilman and Riley (1993)

Table 1.2 Number of papers reporting perennial ryegrass-related research, by country, conducted from 1990 to 2008

Country	**No of papers (1990 to 2008)**
Argentina	1
Australia	102
Bangladesh	1
Belgium	13
Canada	2
Chile	9
China	11
Czech Republic	7
Denmark	21
Falkland Islands	1
Finland	1
France	53
Germany	21
Greece	1
Ireland	11
Italy	4
Japan	38
Korea Rep.	9
Lithuania	4
Mexico	1
Netherlands	56
New Zealand	123
Norway	5
Pakistan	1
Poland	9
Russian Fed.	1
South Africa	8
Spain	5
Sweden	2
Switzerland	17
Tunisia	2
Turkey	2
United Kingdom	216
USA	132
Total	**890**

1.2 Project question

When considering all the implications of artificial nutrient supply, of importance to the farmer is the quantity of fertiliser necessary to meet the required levels of herbage production without negative effects on animal production, the environment and the economy of production. Unfortunately there is no universal answer to that question – while plant requirements remain relatively constant, the ability of the environment to meet those requirements is highly varied and dependent on soil type, climatic conditions, previous land use and current pasture management.

Work has been done on the effects of nutrient supply on herbage yield (e.g. nitrogen: van Burg, 1966; Eckard, 1989; McKenzie, 1996; Theron and van Rensburg, 1998; phosphorus: MacLusky and Holmes, 1963; Whitehead, 2000; potassium and sulphur: Morton et al., 1998; Miles and Beckerling, 2006). This has led to current fertiliser recommendations, which, in KwaZulu-Natal, are based on soil analyses (excluding nitrogen). Soil samples are taken to a depth of 15 mm for new and 10 mm for existing pastures. The Cedara Fertility and Analytical Services analyses samples for exchangeable cations, soil pH, exchangeable acidity, phosphorus, zinc, organic carbon and clay content and sample density. Phosphorus, potassium and sulphur are applied pre-planting or, in the case of maintenance of perennial pastures, as a topdressing according to the results of the soil analysis. Nitrogen recommendations are not based on soil analysis as the speed of N reactions in the soil make analyses unreliable. Instead, the Cedara Fertiliser Advisory Service bases its recommendations on the requirements of the crop to be grown. Three production levels are catered for when making N recommendations for pastures, *viz* low, medium and high. Expected production levels are dependent on numerous factors, primarily the availability of water (irrigated / dryland) and soil conditions. Based on the expected level of production, N recommendations for pastures range from 60 to 375 kg N ha^{-1} year^{-1} (Miles and Bartholomew, 1991). Adjustments to the N recommendation may be made to allow for residual mineral N and the mineralisation of crop residues and soil organic matter. This is based on the previous cropping history along with an estimate of soil organic matter (Manson *et al*., 2005). The current system recommends the application of 75 kg N ha^{-1} for pastures at germination and 50 kg N ha^{-1} after each defoliation event (cutting or grazing). When cutting and removing (e.g. for hay), it is recommended that 75 kg K ha^{-1} be applied after every second defoliation. Thus pasture fertiliser recommendations are fairly general and aim at maximising yield for all pasture types.

There is a need to understand the effects of fertiliser application on individual pasture types such as perennial ryegrass. Such research may allow conclusions to be drawn as to the applicability of current fertiliser recommendations for perennial ryegrass and if, by focusing on maximising yield, pasture farmers may be compromising other aspects of their livestock production systems.

1.3 Project objective

The purpose of this project was to improve on and add to current understanding regarding the effects of fertiliser on the quality and productivity of perennial ryegrass in KwaZulu-Natal and how this impacts on animal intake.

It was hypothesized that over-application of fertiliser to a perennial ryegrass pasture would negatively affect the quality of the herbage in terms of its usefulness for grazing by dairy cattle. In turn, questions were raised as to the effect this would have on intake and whether or not cows would select pasture with lower levels of applied nutrients. Thus the project aimed to assess the effects of applied fertiliser on:

a. yield,
b. quality and
c. intake

of an established perennial ryegrass pasture.

In order to address the objectives, the project first established the effects of fertiliser level on production, intake and post-grazing residual of a perennial ryegrass pasture by measurement of pre- and post-grazing pasture height. The pasture was used to establish qualitative differences in herbage between treatments. Finally, regression trees were used to establish the explanatory variables affecting intake and to determine whether the levels of fertiliser used in the project were sufficient to affect animal intake and therefore animal production.

Due to the nature of the analyses, it was possible to consider the results of the various aspects of the project as individual chapters. A chapter is dedicated to each section, containing the results and discussion relating to that section. The thesis thus comprises seven chapters including: an introductory chapter, a literature review, a methods chapter, three results chapters containing discussions and conclusions and finally a chapter containing a general conclusion.

It is hoped that this project will aid scientists, consultants and farmers in making decisions regarding fertiliser practices by giving a clearer understanding of the effects that fertiliser application has on perennial ryegrass pasture. It is expected that this understanding will result in practices that consider all aspects of a production system rather than just a focus on maximising pasture yield.

CHAPTER 2

Literature review

This chapter reviews the known effects of fertiliser supply on perennial ryegrass dry matter production and quality.

2.1 The effects of fertiliser supply on dry matter production

2.1.1 Nitrogen

In terms of scientific research, nitrogen (N) has received the most attention of all the elements essential to plant nutrition. It is one of the most widely distributed elements in nature and is required by all living organisms as it is a constituent of proteins and nucleic acids. Nitrogen supply is the major limiting factor in the production of biomass and as such is considered the most important management tool for manipulating dry matter production.

Nitrogen fertilisers can be broadly classified into four groups depending on the chemical form in which the nitrogen contained in the fertiliser is present. These four groups are: ammoniacal fertilisers (nitrogen in form of ammonium ions, NH_4^+), nitrate fertilisers (nitrate ions, NO_3^-), combined ammoniacal and nitrate fertilisers (both ammoniacal and nitrate ions) and amide fertilisers (simple organic compounds not readily available to plants, the most common form of which is urea) (FAO, 1984).

While their composition may vary slightly, nitrogen fertilisers used in southern Africa are generally based on urea, ammonium nitrate and ammonium sulphate (Miles and Manson, 2000). Eckard (1986; 1989) found variations of N source to have no significant effect on ryegrass dry matter production. Van Burg (1966) found no significant difference in yield of Italian ryegrass between limestone ammonium nitrate, calcium nitrate and ammonium sulphate. The fact that all kinds of fertiliser N are normally transformed to NO_3^- in the soil means that the initial form is not overly important and the choice of N carrier is therefore more economic than agronomic (Olsen and Kurtz, 1982).

Utilisation efficiency of N carriers may be decreased, however, if each carrier is not used in accordance with its limitations. At high levels of N application (above 300 kg N ha^{-1}) and high soil-temperatures, ammonium fertilisers give better growth than nitrate (Nowakowski *et al*., 1965). Cold and wet soils are essentially inactive with respect to nitrification (Schmidt, 1982). Thus, a nitrate carrier may be less efficient on poorly-drained soils than an ammonia carrier, due to the danger of denitrification. Nitrification is limited in acidic soils (Schmidt, 1982) and thus utilisation efficiency of nitrate fertilisers can also be affected if soils are not adequately limed (Eckard, 1986). Volatilisation can occur whenever free ammonia is present near the soil surface, where there is an excess of NH_4^+ ions in the soil (Nelson, 1982) or where the ammonium fertiliser remains on the soil surface for a period of time without being washed into the soil (Miles and Manson, 2000). Ammoniacal fertilisers, such as urea, should therefore not be used in sandy, shallow or near-neutral to alkaline soils.

The effects of increasing N supply on the production of ryegrass dry matter have been widely researched, both within and outside South Africa's borders (e.g. van Burg, 1966; George *et al*., 1973; Wilman and Wright, 1981; Eckard, 1989; Miles, 1991; McKenzie, 1996; Theron and van Rensburg, 1998). Results reflect largely similar patterns of response. The saturation-type curves show an initial near-linear response with increasing N fertilisation. Reports on the range of this linear response vary between 240 – 600 kg N ha^{-1} year^{-1}. Reid (1970) has shown a continuing increase in dry matter production at N fertilisation rates above 600 kg N ha^{-1} on perennial ryegrass. Ehlig and Hagemann (1982) reported increased dry matter yield with increases in N rate up to 1120 kg N ha^{-1} for irrigated Italian ryegrass in the South western United States. The N was applied in monthly applications of 90 to 112 kg N ha^{-1}. While the high fertilisation rate allowed for seven cuts with good yields, a high proportion of yield from the last cut was seed stalk and immature seed.

The yield response of ryegrass to applied N is quadratic in nature. The initial rapid response is thus followed by a phase of sharply diminishing yield response, per unit of applied N, up to a certain critical N application level. Above this critical level any increase in N application results in non-significant increases, and sometimes decreases, in dry matter production (Wilman and Wright, 1981; McKenzie, 1996).

The response curve described above is strongly dependent on season, climate conditions and defoliation level (Garstang, 1981; Eckard, 1989; McKenzie, 1996). For example, Garstang (1981) reported little benefit to dry matter production from applying extra N in low yield years. N response is also dependent on supply of other nutrients such as sulphur (S). Murphy and Quirke (1997) reported that on a S-deficient soil where no S was applied, even at the highest rate of applied N (480 kg ha^{-1}) it was only possible to achieve 60% of the maximum yield obtained when both N and S were applied. The addition of S to the high N treatment resulted in the production of an extra 5.8 t DM ha^{-1}.

It would be expected that recommendations for N fertilisation rates should be a net amount resulting from the consideration of several factors. These would include soil residual N, contributions from animal manures and from N fixation and removals and losses due to cropping, leaching and denitrification (Olson and Kurtz, 1982). However, in practice, fertilizer N recommendations are based on dry matter production requirements. Recommendations for fertiliser N requirements for planted pastures are based on three production levels: low, medium and high (Miles and Manson, 2000). Low production is reported to require 60 – 150 kg N ha^{-1} yr^{-1}, while medium and high production requires 175 – 250 kg and 275 – 375 kg N ha^{-1} yr^{-1} respectively. It is emphasised that these recommendations are general guidelines for pastures that do not have other factors (such as water or nutrient supply) limiting production potential.

2.1.2 Phosphorus

Phosphorus (P) has numerous biochemical functions in plants. One of its key functions is energy transfer through the synthesis and breakdown of ATP. Phosphorus is a constituent of nucleic acids, making it essential in cell division and therefore plant growth and reproduction. Seeds require relatively large amounts of phosphorus during germination. It is one of the primary structural components of cell membranes and is also involved in the synthesis of proteins and vitamins.

The importance of P in pasture dry matter production has long been appreciated (e.g. MacLusky and Holmes, 1963; Whitehead, 2000) and, after nitrogen, P is the element most likely to limit plant growth (Raven *et al*., 1992). In general, the decision on whether to apply fertiliser P, and if so at what rate, is based on soil analysis (Whitehead, 2000). The assessment of plant-available P depends on the measurement of the potentially soluble fraction (Whitehead, 2000). At the KwaZulu-Natal Department of Agriculture and Environmental Affairs (KZN DAE) Fertiliser Advisory Service, the recommended rate of application for a particular crop is based on soil P status, as assessed by an NH_4HCO_3 extracting solution (modified Isfei procedure; Hunter, 1975). The KZN DAE Fertiliser Advisory service recommends that topsoil P levels be maintained at 16 mg L^{-1} for the establishment and maintenance of perennial ryegrass.

Unlike nitrogen, phosphorus is relatively immobile in the soil/plant system (FAO, 1984). Phosphate in the soil solution is completely accessible, but this makes up a very small proportion of the total soil P (Mengel and Kirkby, 2001). More than 90 % of total soil phosphorus is present as insoluble and fixed forms (Mengel and Kirkby, 2001). The solubility of phosphate ions in the soil solution is very dependent on soil pH (FAO, 1984). In acidic soils phosphorus adsorption occurs, whereby phosphate ions replace the hydroxyl ions bound to elements such as iron, aluminium, clay minerals, calcite and allophanes (Parfitt and Smart, 1978, cited in Mengel and Kirkby, 2001). Soil pH also governs the availability of phosphorus in calcareous soils. Alkaline soils have a high concentration of calcium ions. Phosphate ions bind to the calcium ions, forming calcium (Ca) phosphates (FAO, 1984; Mengel and Kirkby, 2001). The lower the Ca/P ratio of the calcium phosphates the higher is their solubility in water (Mengel and Kirkby, 2001). Maintenance of soil pH at 6-7 allows greatest solubility of P ions (FAO, 1984). For example, the solubility of adsorbed phosphate can be greatly increased by liming (Haynes, 1982, cited in Mengel and Kirkby, 2001). However, at any soil pH added phosphorus is slowly converted to less soluble forms which are not available to plants (FAO, 1984; Mengel and Kirkby, 2001). To maintain a satisfactory level of P in the soil solution it is therefore usually desirable to apply P fertiliser for each crop and to maintain the soil P as near to the range 6-7 as possible (FAO, 1984).

The extent to which pasture shows a yield response to fertiliser P is influenced by soil type, past fertiliser application and by whether the sward is regularly cut or grazed (Whitehead, 2000). In his studies on Italian ryegrass, Miles (1986) found a highly significant yield response to P. The response was more marked in the first half of the growing season (March to June), which included the establishment phase.

With soils in pH range of about 5.0 – 7.5 that are deficient in P, application of fertiliser P will usually produce a cost-effective yield response. The regular application of fertiliser P for a number of years increases the soil supply of plant-available P and many pastures in intensively-farmed regions now show little response to current applications of fertiliser P. Yield response to fertiliser P is likely to be greater where herbage has been regularly cut and removed, rather than grazed (Whitehead, 2000). Highly acid or highly calcareous soils may show little response to fertiliser P due to it becoming rapidly insoluble. With acid soils, the problem may be overcome by liming, while in calcareous soils organic matter tends to increase the availability of P (Whitehead, 2000).

The extent to which grassland responds to fertiliser P is also influenced by supplies of other nutrients, particularly N and S (Whitehead, 2000). The application of a high rate of fertiliser N may increase the need for fertiliser P (Wolton et al., 1968) and a strong positive interaction between P and S has been noted in some regions (Whitehead, 2000).

2.1.3 Potassium

Potassium (K) is an essential element for all living organisms. It is taken up by plants in amounts only second to N (Kayser and Isselstein, 2005). In plant physiology it is the most important cation, not only in terms of its concentration in plant tissues but also with respect to its physiological and biochemical functions (Mengel and Kirkby, 2001). Potassium is vital for the water status of plants through the control of stomata and turgor. It influences the efficiency of photosynthesis and is involved in the translocation of carbohydrates, nitrogen assimilation and starch synthesis. Potassium also functions to activate various enzyme systems and plays an important role in the translocation of amino acids and synthesis and mobilisation of proteins. The effects of K deficiency include yield loss, reduced tolerance to drought and frost and increased susceptibility to fungal attack.

While soils generally contain large amounts of K relative to plant requirements, much of it is unavailable to plants (Manson *et al*., 2010). Soil K is grouped into three fractions according to its availability for plant growth. Non-exchangeable mineral K is found in the crystal lattice of relatively unweathered minerals or in the interlayers of clay minerals such as illite and vermiculite. It is available to plants only after weathering to the exchangeable form, but this occurs too slowly to meet the needs of field crops and pastures. Exchangeable K is held by electrostatic forces to negatively charged clay and organic matter surfaces. This form of K acts as a reservoir for readily available K found in the soil solution. Solution K is a small fraction present in the soil solution and is readily absorbed by plants.

Available K in soils is estimated by measuring the total of exchangeable and solution K. Soil analysis gives a K test value, reported in milligrams of K per litre of soil. At the Cedara Soil Fertility laboratory this involves the determination of the quantity of K extracted from the soil using the Ambic-2 extracting solution, which involves the displacement of K from the soil particles by ammonium ions (Manson and Roberts, 2000). Based on the K test value, a recommendation can be given as to the amount of K required to increase plant available K to meet the target K value. The target K value for perennial ryegrass is 140 mg K L^{-1}.

In comparison with N, few studies have been undertaken to quantify the effects of K fertiliser on perennial ryegrass yield. Although vital for plant growth, the general sufficiency of soil K has meant that plant response to applied K has been of limited research interest. Considering that K off take with harvested plant material can even exceed that of N, one would expect pasture yields to suffer with the progression of the growing season, exhibiting both reduced yield and reduced concentrations in the plant as described by Wedin (1974). However, in intensive dairy farming, surpluses of K arise from the input of concentrates and fertilisers and may lead to increasing K content in the soil (Kayser and Isselstein, 2005). In addition, grazing animals have a large influence on the fertility of pasture soils (Early *et al*., 1998). Williams (1988) reported that excretal returns of K can range from 180 kg ha^{-1} $year^{-1}$ on an intensively managed dairy farm to over 500 kg ha^{-1} $year^{-1}$ on a highly productive intensive dairy farm receiving K fertiliser inputs. Twenty percent of the urine-applied K in Early *et al*. (1998)'s study remained within the exchangeable fraction in the top 0-5 cm depth of soil, while Williams (1989) reported a K recovery by plant uptake of between 40 and 55 % of the amount applied during a urination event. This return and retention of K in pasture soils has meant that studies on intensively managed and grazed pasture often show limited, if any, yield response to applied K.

From the results of a cutting trial Tainton *et al*. (1999) reported a significant quadratic yield response of annual ryegrass to increasing amounts of applied K. Yield response in this study was most marked at soil test values of less than 250 mg L^{-1}. In his research using twenty-three sites in the United Kingdom, Damney (1992) found no herbage yield response to applied K when soil K was above 120 mg L^{-1}. Keady and O'Kiely (1998) conducted a study on the effect of K fertiliser application on a predominantly perennial ryegrass sward on K deficient soils in Ireland. They reported increased DM production ($P < 0.001$) with increasing levels of applied K, both of pasture primary growth and regrowth. For each additional kg of K applied to the primary growth, DM yield increased by 1.53 and 3.73 kg DM ha^{-1} for the early and late harvest dates respectively. There was no significant production response to K above an application rate of 180 kg ha^{-1}. In contrast, Morton *et al.* (1999) reported no significant pasture yield responses to K on four soil types in North Otago, New Zealand. The authors attributed the lack of response to the supply of exchangeable K associated with the 2:1 layer silicate clay minerals in the soil types used in the study. They did, however, note a decrease in soil K over time where K was not returned to the soil in the form of clippings.

The way in which crops respond to K depends to a considerable extent on the level of N nutrition (Kayser and Isselstein, 2005). An increasing N supply enhances growth and thus increases the demand for K (Wilkinson *et al.*, 2000). The demand for applied K may also be increased when large amounts of ammonia N are applied to the soil. Ammonia N can replace K^+ ions on the clay colloid, which can increase soil K losses through leaching.

2.1.4 Sulphur

Sulphur (S) is often considered to be the fourth most important plant nutrient after nitrogen, phosphorus and potassium. The S requirements of plants are approximately two-thirds of their P requirements, with legumes generally requiring more S than grasses (Miles and Beckerling, 2006). Sulphur affects both plant yield and quality as it is a constituent of the amino acids involved in protein synthesis and chlorophyll production. It is also contained in plant hormones that are involved in carbohydrate metabolism.

The main sources of S for plants are the soil, S-containing fertilisers and, in coastal and industrial areas, the atmosphere. The total S concentration in topsoils is often considerable. Miles and Beckerling (2006) reported S levels ranging between 100 kg and 1200 kg S ha^{-1} in topsoils in the eastern half of South Africa. However, the bulk of soil S is unavailable to plants as it is bound to carbon in the soil organic matter (Miles and Beckerling, 2006; Mengel and Kirkby, 2001). It is for this reason that soil tests showing total soil S are of limited value in determining levels of plant-available S in the soil. The mineralisation and immobilisation of S determines the amount of plant-available sulphur in the soil. Since these reactions are driven primarily by microbial activity, factors such as soil temperature and moisture affect the amount of plant-available S in the soil at any one time. The close relationship between soil carbon and sulphur (Miles and Beckerling, 2006) means that soil type also affects the availability of S. Soils with low organic matter tend to be poor suppliers of sulphur. Sandy soils are such an example. Their low organic matter content, added to the fact that sulphur readily leaches in sandy soils, means that S deficiencies are likely on sandy soils (Miles and Beckerling, 2006, Murphy and Boggan, 1988). Meyer (1985) found that, in South Africa, sugarcane growing in soils of the Cartref, Fernwood, Glenrosa, Longlands and Kroonstad forms is most prone to S deficiency. These soils are typically grey, light-textured soils with low clay content and below average organic matter percentage.

In general in South Africa, however, soils have historically contained adequate levels of plant-available S for high yields. Relatively little research was done into the S requirements of pastures in South Africa, mainly because plant S requirements were met incidentally through the use of single superphosphate (10% S), ammonium sulphate, ammonium sulphate nitrate (12% S) and generally low-grade, sulphur-containing fertilisers (Miles and Manson, 2000). In the 1970's and 1980's the fertiliser industry moved towards using high-grade, low-S fertilisers. It was predicted that the incidences of S deficiency would increase as greater use was made of the low-S fertilisers. In general, however, the S requirements of S-deficient soils continued to be satisfied through the application of superphosphate (Wedin, 1974, Morton *et al.*, 1998). The application of lime may also have assisted with meeting S requirements as a large proportion of adsorbed sulphate is released when lime is applied to a soil (Mengel and Kirkby, 2001). The predictions of S-deficient soils have therefore taken between 20 and 30 years to be realised (Miles and Beckerling, 2006; Brockett and Farina, 2003). It is only recently that several reports of severe S deficiency have been recorded. This may be an indication of a general decrease in soil S status in agricultural soils in KwaZulu-Natal as well as greater use being made of alternative fertiliser products to superphosphate. Miles and Manson (2000) believe there is little risk of S deficiency in KwaZulu-Natal soils that are tilled annually and that most loams and clays in KwaZulu-Natal are likely to be well-buffered against S deficiencies. S deficiencies can, however, arise in high-yielding permanent pastures that are heavily fertilised with nitrogen. These pastures tend to build up soil organic matter levels, which may then result in the immobilisation of S. This, compounded by the current increase in no-till practices, may point towards an increase in reports of soils deficient in plant-available S. Even so, annual application rates of 10 – 40 kg S ha^{-1} are usually sufficient to prevent S deficiencies (Manson *et al*., 2010).

Pasture yield responses to applied S are often quadratic in nature, as reported by Morton *et al*. (1998), who found a significant relationship between rates of S application and pasture growth. However, even on S-deficient soils that showed a significant S-response, the differences in pasture yield were not large. Morton *et al.* (1999) reported yield responses to 80 kg S ha^{-1} yr^{-1} on soils with an initial sulphate S level of 3 ppm. Smaller yield responses were obtained up to 40 kg S ha^{-1} yr^{-1} on soils with initial soil sulphate S levels of 8 and 4 ppm, while a yield response was only obtained up to 10 kg S ha^{-1} yr^{-1} on a soil with an initial soil sulphate S level of 9 ppm. According to research conducted in New Zealand by both Sinclair *et al.* (1996) and Morton *et al*. (1998), applications of 22 kg S ha^{-1} annually should result in near maximum pasture yields, while Gately (1994, cited in Murphy and Quirke, 1997) recommended 25 kg S ha^{-1} per annum for grazed pastures in Ireland. Miles & Beckerling (2006) suggest that, for management systems involving complete removal of the forage, annual S removals generally fall in the range 9 to 35 kg S ha^{-1}.

Pasture yield response to applied S increases as the season progresses (Gately and Murphy, 1983; Dampney and Unwin, 1993; Murphy and Quirke, 1997; Richards, 1999). Murphy and Quirke (1997) found that at the final two cuts of four over the season, S was responsible for more than doubling the herbage yield obtained from the first two cuts. Response to sulphur is also affected by level of fertiliser N. Gately and Murphy (1983) reported a production response to S at all levels of applied N, but the effect was much greater at higher levels. Similarly, Murphy and Quirke (1997) reported increasing S response with each level of N application. As Murphy and Quirke (1997) point out, correct S fertilisation allows the same level of production with greatly reduced levels of N fertiliser, which has both economic and environmental implications.

2.2 The effects of fertiliser supply on forage quality and intake

2.2.1 Nitrogen

Pasture quality is a function of nutrient content and voluntary intake (Meissner *et al.*, 1989, McKenzie *et al*., 1999b). It is often measured using CDMD (cellular dry matter disappearance), which is an indicator of digestibility. Studies have demonstrated increasing perennial ryegrass digestibility with increasing N application (McKenzie, 1996; van Vuuren *et al*., 1992). McKenzie (1996) reported that applied N did not significantly affect digestibility in early summer, when CDMD levels averaged 74 % regardless of level of applied N. Nitrogen application has been found to decrease herbage ADF and NDF levels (Moller *et al*., 1996), while increasing both ruminal and total tract digestibility of dietary ADF (Zhang *et al*., 1995). It was suggested that this is due to a reduction in the proportion of, and perhaps a delay in the development of, plant cell walls (Wilman *et al*., 1977). Based on his results, McKenzie considered there to be little justification in applying N levels in excess of 480 kg ha^{-1} yr^{-1} to improve digestibility. Some studies reported decreasing pasture digestibility with applied N (Stockdale and King, 1980; Whitehead, 1995). This appears to occur in mixed perennial pastures when changes in botanical composition result in a higher proportion of less digestible grass.

The N content of plant material increases with applied N (Zhang *et al*., 1995). Level of herbage N is equivalent to the difference between N uptake and N conversion to organic nitrogen (Prins, 1983), with uptake being dependent upon N supply (Darwinkel, 1976). At low fertilisation levels, there appears to be a linear relationship between applied N and that taken up by herbage. Thus low applications of fertiliser N will result in low N uptake (Sibma and Alberda, 1980; Prins, 1983). The result is very little effect on herbage N with low levels of applied nitrogen (Whitehead, 2000) as the demand for N is greater than the supply (Sibma and Alberda, 1980). The pattern of herbage N response changes with increasing rates of N application; Van Burg (1966) reported an increase in herbage N once a critical level of N application is reached. Sibma and Alberda (1980) suggested that at this level an equal amount of N is removed as is applied, with the critical level being closely correlated with maximum yield (van Berg, 1966). At this critical level conversion is most efficient, resulting in production and herbage N being optimised (Wolton *et al*., 1971). Various rates of N application have been proposed as being optimal for maximising production while maintaining low values of herbage N. Sibma and

Alberda (1980) suggested a value between 400 and 500 kg N ha^{-1} yr^{-1}. Van Berg (1966) suggested that, for maximum production, the nitrate concentration in the herbage should not be significantly less than 100 mmol kg^{-1}, which equates to an N application rate of 500 to 600 kg N ha^{-1} (Ruessink, 1969, quoted by Sibma and Alberda, 1980). Frame and Hunt (1971) reported herbage N levels of 2.2, 2.7 and 3.4 % with N applications of 100, 234, and 351 kg ha^{-1} respectively. McKenzie (1996) reported that levels of applied N above 480 kg ha^{-1} yr^{-1} did not produce significant increases in herbage N. Eckard (1990) reported studies by Griffith (1960) and Darwinkel (1975) that demonstrated increasing herbage N with applied N up to 3.2 to 3.5 and 2 to 4 % respectively, after which there was little increase in herbage N but rather a rapid accumulation of nitrate-N. Darwinkel (1975) attributed the accumulation of nitrate-N to uptake exceeding plant N demand for protein production. McKenzie (1996) suggested that, based on his and other studies, there to be little justification in applying N levels greater than 480 kg ha^{-1} yr^{-1} to improve perennial ryegrass herbage N content.

Increasing N applications result in marked increases in crude protein (CP) concentration (Garstang, 1981; van Vuuren *et al*., 1992; McKenzie, 1996; Moller *et al*., 1996; Wilkins *et al.,* 2000). Van Vuuren *et al*. (1992) observed increases of between 2.8 and 3.8 % CP (depending on season) when increasing N application from 275 to 500 kg N ha^{-1} $year^{-1}$. Wilkins *et al*. (2000) reported highly significant increases in mean N (and therefore CP) content of herbage of between 5 and 8 % N when increasing level of applied N from 100 to 600 kg N ha^{-1} $year^{-1}$. Excessive levels of applied N may result in plants taking up more N than is required for growth (luxury intake), leading to high CP levels in herbage (Eckard, 1990; Miles and Bartholomew, 1991; McKenzie, 1996). Peyraud and Astigarraga (1998) reported a CP increase of 50 – 90 g kg^{-1} DM per 100 kg ha^{-1} increase in N fertilisation. High herbage CP content leads to a high rate of CP degradation in the reticulorumen of dairy cows, causing high ruminal concentrations of NH_3 and substantial losses of N by urinary excretion (van Vuuren *et al*., 1992; Tas, 2006). Ferguson *et al*. (1993) reported metabolic problems in cows as a consequence of high herbage CP levels, eventually resulting in a loss of production and lowered fertility. With increasing levels of applied N, the composition of CP is altered. Peyraud and Astigarraga, (1998) reported a decreased proportion of true protein and increased proportion of non-protein N (NPN) in the herbage with increasing levels of applied N. Vendramini *et al*. (2008) showed that increases in applied fertiliser N resulted in herbage CP with greater concentrations of rumen degradable protein and increased effective CP degradability. The most noticeable cost of high herbage CP concentration is a reduction in milk production. Kolver (2003) attributes this to the energy used to synthesise and excrete urea from excess N. This is supported by Ferguson *et al*. (1988, in Moller *et al*., 1996), who reported that cows fed high protein diets (>18 % CP) had reduced milk production and sometimes lower conception rates than cows on diets with lower protein levels. For high yielding dairy cattle, Whitehead (1995) recommends the optimum CP concentration in the diet to be between 15 – 20 % of dry weight. This is supported by van der Merwe *et al*. (2001), who suggest herbage CP levels above 20 % DM to be sufficiently high as to consider monensin (an ionophore or antibiotic rumen manipulator) supplementation in order to reduce dairy cow blood and milk urea concentrations.

The nitrogen content of plant material can be divided into nitrogen fixed as protein (true protein, from which the CP content is derived) and non-protein nitrogen (NPN). The NPN fraction comprises amide-, ammonia- and nitrate-nitrogen. High NPN fractions in herbage can be toxic to ruminants. This occurs when the ammonia released from the NPN exceeds the rumen microbes' ability to convert it into protein. Rumen pH rises and, in severe cases, normal rumen function may eventually cease, causing muscular tremors, spasms, respiratory difficulty and death. In its milder form, ammonia toxicity depresses dry matter intake, restricts milk production and decreases reproductive ability.

Greenhill (1936) conducted one of the first experiments to determine the amounts and relative proportions of nitrogen compounds occurring in grass. He found that, while herbage concentrations of amide- and ammonium-nitrogen were unrelated to total N or to each other, higher proportions of NPN and nitrate-nitrogen were almost always associated with increases in total herbage N. Many authors have since reported similar results (Deinum and Dirven, 1974; Bartholomew and Chestnutt, 1977; Goh and Kee, 1978; Sibma and Alberda, 1980; Theron *et al*., 2002). Theron *et al*. (2002) described significant ($P<0.05$) increases in NPN as a percentage of total N with increasing levels of applied N, while Goh and Kee (1978) found consistent increases in NPN with each increment of N added. In fact, the increase in herbage N components is disproportionate, with the NPN fraction increasing to a greater extent than the true protein fraction with added fertiliser N (Hoffman and Brehm, 1999).

Increased levels of NPN translate into increased herbage nitrate concentrations in the plant (Greenhill, 1936). Nitrate, a component of NPN, is frequently reported in the literature due to the animal health issues (particularly grass tetany) that may result from cattle grazing high-nitrate herbage. Nitrate in the plant is converted to nitrite in the rumen. At high rumen nitrite levels, the nitrite diffuses into the blood and competes with oxygen for uptake by haemoglobin. This results in a decrease in the oxygen carrying capacity of the blood.

Although the effects of excessive levels of NPN or NO_3-N are well known, there is no general agreement on critical or toxic levels (Hanway *et al*., 1963). Based on a literature review, Eckard (1990) reported that toxicity could occur beyond a NO_3-N limit of 0.21 – 0.35 % DM. Prins (1983), however, suggested that NO_3-N limits for grazed grass are 2 % or even greater and van Burg (1966) reported that a NO_3-N content of 0.3 – 0.6 % DM is required just in order to achieve maximum grass yields. Unlike annual ryegrass, perennial ryegrass is not known to accumulate potentially toxic levels of NPN. In addition, levels of NPN and NO_3-N tend to fall rapidly within a few days of peaking. Hunt (1973) observed that, even at the highest level of N application, the risk of nitrate poisoning was over by day 16.

The increase in crude protein content is at the expense of non-structural carbohydrate (NSC) content, which decreases as the level of fertiliser N increases (Moller *et al*., 1996; Binnie *et al*., 2001;Tas, 2006). Carbohydrates are the major energy source in a ruminant diet and function to provide energy for both the rumen microbes as well as the host animal (National Research Council, 2001). Low NSC concentrations in herbage may decrease the efficiency of protein utilisation during autumn and winter and have the potential to limit animal production even when the digestibility of herbage is high (Dove and Milne, 1994). A study by Moller *et al*. (1993) found that lower producing herds had higher blood urea levels, higher pasture protein but lower pasture soluble carbohydrate concentrations compared with the higher producing herds. The inverse relationship that exists between CP and NSC appears to be related more to environmental factors than due to genetic effects. Research by Tas *et al*. (2006) showed that an increase in herbage NSC was not related with a decrease in CP content when comparing cultivars. Vinnie it al. (2001) noted a 28.6% reduction in NSC when N application level was increased from 0 to 90 kg N ha^{-1}.

In a review on the effect of N fertilisation on chemical composition, intake, digestion and nutritive value of fresh herbage, Peyraud and Astigarraga (1998) observed that studies on the response of acid and neutral detergent fibre (ADF and NDF) to increasing levels of applied N are varied in their findings. They concluded that the composition of the NDF fraction is unaffected by the level of N fertilisation (Wilman *et al*., 1977) and that any increase in digestibility is due rather to an increase in leaf material that is low in cell wall content (Waite, 1970).

Nitrogen application level also affects nutrient ratios within perennial ryegrass. Whitehead *et al*. (1978) reported that increasing levels of applied N resulted in increased N: S and K: (Ca + Mg) ratios. The efficiency with which dietary N is utilized by ruminants is affected by the N: S ratio (Whitehead *et al*., 1983), the optimum value being about 10: 1 (Bray and Hemsley, 1969). The K: (Ca + Mg) ratio is a useful indicator of the risk of hypomagnesaemia or grass tetany in cattle, with increasing risk as the ratio rises above 2.2: 1 (Grunes, 1970). Research by Hopkins *et al*. (1994) showed that the application of fertiliser N was associated with a decrease in the herbage content of Ca, Mn and Mo, but an increase in Mg, Na and Zn. The authors noted, however, that the changes in herbage mineral concentration were unlikely to be sufficient to cause animal health problems unless the soils were already deficient in elements such as Mg or Cu. Reports on the effects of applied N on herbage P are not consistent in their findings. Wolton et al. (1968) noted symptoms of phosphate deficiency where high levels of N were applied without fertiliser P. N application causes an increase in soil acidity, which may lead to a herbage P deficiency since uptake of P is influenced by soil pH (Whitehead, 2000). McKenzie *et al.* (1999b) reported that N fertilisers did not affect pasture P content in the short-term, while Gahoonia *et al*. (1992) found that fertiliser N applied in an ammoniacal form encourages P mobilisation and plant uptake through the acidification of the rhizosphere. The effect of N fertilisers on herbage K is also unclear. Jolley (1972) found applied N may decrease herbage K content, while Heddle and Crooks (1967) demonstrated that fertiliser N decreases pasture K when K content is below 2 %, but increases, or has no effect on, pasture K when K content is above 2 %. McKenzie *et al*. (1999b) reported that, in their study, pasture K content was not influenced by treatment.

The effects of applied N on perennial ryegrass quality may be influenced by season (McKenzie *et al*., 1999b), resulting in differing degrees of response in quality parameters over a single growing season.

2.2.2 Phosphorus

Phosphorus is an integral constituent of membrane phospholipids and thus forms an essential part of plant cell membranes (Bailey, 1991). Phosphorus deficiency is the most widespread and economically important nutrient deficiency in the higher-rainfall eastern parts of South Africa (Bainbridge *et al*., 1995).

Saul *et al*. (1999) found that higher rates of superphosphate increased the nutritive value of herbage. They reported increased perennial ryegrass CP with both higher average and current P rates. The concentration of N in the ryegrass was also increased, but it is uncertain whether this was due to the P, S or Ca component, as superphosphate contains all three elements. The authors suggested that increased clover production resulting from higher rates of applied P may also have increased CP and N concentrations in the mixed pasture. McKenzie *et al*. (1999b) showed that the application of P fertiliser had no effect on pasture nutritive value when no fertiliser N was applied.

McKenzie *et al*. (1999b) reported that P application had no effect on pasture NSC content. Bailey (1991) found that, on limed plots, increasing P rate from 0 to 50 kg P ha^{-1} significantly increased NSC concentration in plant shoots. Thereafter increases in P application resulted in a gradual decline in NSC level. On unlimed plots, however, increasing P rate caused a marked decline in shoot NSC content at low levels of applied P; at higher P rates there was no difference between limed and unlimed plots. As reported by Bailey (1991), a major consequence of inadequate P nutrition is a decline in the phospholipid content of root membranes and an increase in membrane permeability. The increase in permeability facilitates the movement of soluble amino acids, reducing sugars and mineral nutrients into the rhizosphere. This would explain the increase in shoot NSC concentration noted when the P deficiency was corrected.

Phosphorus application increases herbage P content, but decreases plant Zn and Mg concentration (Tainton *et al*., 1999, McKenzie *et al*., 1999b; Bailey, 1991). Smith *et al*. (1985) reported decreasing Zn concentrations as shoot P concentration increased. Bailey (1991) suggested that high rates of applied P hinder Zn diffusion to plant roots due to an interaction between P and Zn in the soil. Since Zn also plays an important part in cell membrane integrity, excessive levels of P in the soil may impair membrane integrity, also leading to a loss of solutes into the rhizosphere. This may ultimately lead to an energy drain and affect plant growth.

Smith *et al*. (1985) found a marked increase in the concentration of sulphur from 1.1 to 3.4 g kg^{-1} DM with increasing P concentrations in the shoots. Once the concentration of

P in the shoots exceeded 3 g kg^{-1} there was no further effect on herbage S concentrations. The authors reported that S was not efficiently absorbed by P-deficient plants despite non-limiting amounts of sulphur being applied. Hampp *et al*. (1980; in Smith *et al*., 1985) suggest that P plays an important role in the transport of sulphate into the chloroplast, the main site of sulphate assimilation (Schiff and Hodson, 1973). Thus an apparent S deficiency could be corrected by rectifying a P deficiency (Smith *et al*., 1985).

2.2.3 Potassium

Potassium is an essential element for both pasture and animal production. In spite of this, there is relatively little reported research on the effects of fertiliser K on perennial ryegrass nutritive value. This may be because, unlike N, P and most other essential nutrients, K does not become part of the chemical structure of the plant (Anon, 1998) and has no direct effect on pasture nutritive value. McKenzie *et al*. (1999b) reported that the application of K fertiliser had no effect on pasture nutritive value when no fertiliser N was applied.

While it appears that levels of K in the soil have little direct effect on herbage quality parameters such as CP and fibre, it often interacts with the availability and uptake of other nutrients. Morton *et al*. (1999) reported that pasture K concentration was significantly affected by rate of K application. Where past management has included fertiliser K applications at levels greater than required, pasture K content may be in excess. Potassium is required at a higher level by plants (2.5 – 3.0 % K in pasture; Roberts & Morton, 1999) than by animals (0.5 – 0.8 % K in their diet; Bredon & Dugmore, 1995). Grass exhibits the phenomenon of "luxury uptake" when more K is absorbed by the plant than is required for maximum growth (Dampney, 1992). Elevated pasture K content at calving has been associated with increased risk of metabolic disorders (hypomagnesaemia and hypocalcaemia) and, in mid lactation, may increase the risk of bloat (Morton *et al*., 2005). Dietary K in high concentrations (>2 % K) can reduce the absorption of magnesium, it is thought by interfering with the sodium-linked transport of Mg across the ruminal wall (NRC, 2001). This is in agreement with studies conducted by Dampney (1992) and Razmjoo & Kaneko (1993), which demonstrated that increasing levels of applied K resulted in decreased concentrations of Mg in perennial ryegrass. The study also found that K applications decreased the Ca content of herbage.

High-producing dairy cows find it difficult to meet their Ca requirements from dietary sources (Bredon & Dugmore, 1995). Decreased herbage Ca results in increased risk of animals displaying Ca deficiency symptoms and of altering the Ca: P ratio, which is recommended to be between 2:1 and 1:1 to avoid the negative effects of mineral imbalances.

Nowakowski and Byers (1972) found that K application affected the distribution of N-containing fractions in Italian ryegrass. The authors reported that applied K decreased the protein N to NPN proportion in herbage. They also found that K reduced the amount of nitrate N in the herbage. Whitehead (2000) reported a number of studies with similar findings (Reith *et al*., 1964; Laughlin *et al*., 1973; Banwart and Pierre, 1975), concluding that the application of K with N fertilisation may reduce herbage N.

Morton *et al.* (2005) studied the effect of increasing K application on dairy animal intake in response to reports of reduced intake of pastures with a high K content. The authors did not find a consistent pattern with respect to pasture K content and its effects on bite size or grazing time and concluded that high K application rates and the resulting increase in pasture K content has no effect on cow pasture preference.

2.2.4 Sulphur

Sulphur supply affects not only yield, but also the quality of the herbage (Goh and Kee, 1978), being essential for the formation of vitamin A, protein, carotene and chlorophyll (Tisdale, 1977). Herbage displaying signs of S deficiency is pale green or yellow in colour with stunted growth. Symptoms are similar to N deficiency except that S deficiency is first noticeable in younger leaves. Jones *et al*. (1982) reported that herbage quality may be improved by the addition of fertiliser S. This may be especially true in conditions where herbage S is less than animal requirements and where the increase in herbage S corrects a microbial S deficiency in the rumen (Spears *et al.,* 1985). McKenzie *et al*. (1999b) reported that the application of S fertiliser had no effect on pasture nutritive value when no fertiliser N was applied and Goh and Kee (1978) and Spears *et al.* (1985) both reported that herbage digestibility was not affected by S fertilisation. This may be explained by the role S plays in herbage, forming a part of the amino acids cysteine and methionine. As such, an S deficiency may result in decreased protein synthesis and an accumulation of nitrate-N, amides and free amino acids (Goh and Kee, 1978). Murphy and Quirke (1997) reported that the addition of S significantly reduced the N and nitrate-N content of herbage.

Brown *et al*. (1994) reported that sulphur fertilisation did not increase seasonal dry matter yield and plant uptake of S. In contrast, Goh and Kee (1978) found that total herbage S increased significantly with S application. The herbage S response to applied S appears to be dependent on the N: S ratio in the soil. When N availability is high and S is deficient, most of the herbage S is present in the protein fraction. As the ratio decreases, reducible S tends to increase (Stewart and Porter, 1969; Goh and Kee, 1978). Luxury uptake of S occurs when total S continues to increase but the protein S fraction remains constant. The critical level of S in herbage has been reported as 0.2 % (Jones *et al*., 1972), 0.23 % (Goh and Kee, 1978) and 0.28 % (Metson, 1973), while the optimum level of sulphur in the ruminant diet is between 0.16 and 0.24 % S (Bredon & Dugmore, 1995). The efficiency with which dietary N is utilised by ruminants is influenced by the N: S ratio (Whitehead *et al*., 1983).

There is evidence that high levels of soil S impair the adsorption of dietary Cu by ruminants (Whitehead *et al*., 1978; 1983). Suttle (1974) showed that increasing dietary sulphur from 0.1 to 0.3 or 0.4 % greatly reduced Cu availability in sheep. Mills (1975) suggested that as dietary S increases above 0.2 % the risk of impairment of Cu absorption increases, possibly through the formation of insoluble copper sulphide. Sulphur application may also reduce the uptake of selenium (Se) by plants (Murphy and Quirke, 1997). This may be due to the chemical and physical similarities between S and Se (Murphy and Quirke, 1997), as well as a dilution effect due to a dry matter response to applied S (Spencer, 1982). Selenium is an essential trace mineral, a deficiency of which causes a number of disorders in cattle, including white muscle disease, failure of reproductive function and a high incidence of retained placentas (Smart *et al.*, 1981).

2.2.5 Intake

Milk production in dairy cows is largely controlled by the quantity and quality of feed consumed (Reeves *et al*., 1996) and the relationship between voluntary food intake and animal productivity is well recognized (Allden and Whittaker, 1970). Many studies have investigated ruminant intake (Soest, 1964; Allden and Whittaker, 1970; Dougherty *et al*., 1992; Faverdin, 1999; Astigarraga et al., 2002; Rutter *et al*., 2004; Smit *et al*., 2005) in an attempt to establish the factors that determine preference and intake. Intake appears to be a complicated issue, starting with the amount of material on offer and ease of forage prehension and then being confounded by effects relating to the physical and chemical properties of the sward (Barre *et al*., 2006).

Herbage allowance is one of the most important factors affecting intake by ruminants (Dougherty *et al*., 1992), especially in a homogenous pasture where selection between grass species is not a concern. Gibb *et al*. (1997) considered sward height below 7 cm to be limiting for cattle grazing. Allden and Whittaker (1970) reported that plant height is much more closely related to the intrinsic availability of herbage than is yield per hectare. Limited availability of herbage restricts intake by decreasing bite size and bite rate rather than through any form of selection process. In a study on morphological characteristics of perennial ryegrass leaves influencing intake, Barre *et al.* (2006) found blade length to be important, explaining 49 % of the variation in fresh matter intake rate. Thus, in conditions where herbage availability is limited, the ease with which pasture is obtained will be the overriding factor influencing intake.

Fibre ferments and passes from the reticulorumen more slowly than the non-fibre constituents of feeds (Jung and Allen, 1995), consequently creating physical fill within the rumen. Meissner *et al*. (1989) and Smit *et al*. (2006) both reported negative effects on preference for NDF concentration. Waldo (1986) concluded that NDF is the best single chemical predictor of intake. Jung and Allen (1995), however, observed that intake was not limited exclusively by physical fill and concluded that NDF does not fully describe the filling effects of feeds. Non-structural carbohydrates have also been found to affect intake. Smit *et al*. (2006) conducted a study on preference among perennial ryegrass cultivars. They concluded that, of the four parameters they determined to significantly affect apparent intake (ash, NDF, NSC and DOM), NSC concentration showed the highest correlation with preference.

Research by Yeates *et al*. (2002) indicated that cows do not regulate diet choice within the short-term time frame of a meal. Rather than selecting for nutritive value, one must then conclude that cows are making preference decisions based on immediate feedback. Any indicator variables should therefore be able to be explained in terms of meeting immediate needs, such as herbage palatability and the time taken to feel full. In addition, it is important to note that preference for certain quality or structural factors do not increase intake when dairy cows are fed a homogenous pasture. In a grazing experiment where four perennial ryegrass cultivars were fed alone to the same dairy cows, Smit *et al.* (2005) found no differences in DM intake between cultivars, whereas when the cultivars were offered together the cows selected among them.

CHAPTER 3

Materials and Methods

3.1 Study Area

The trial was conducted at Cedara Agricultural Research Station (29° 32.255'S, 30° 15.764'E and altitude 1016 m) situated 15 km north west of Pietermaritzburg in the KwaZulu-Natal mistbelt. The 30-year mean annual rainfall is 880 mm (Agromet, 2005). Rain falls mainly from October to March. Winters (May to July) are cold with frost (mean minimum of 4.3 °C), while summers are warm (mean maximum of 25.1 °C). The soil of the experimental site is of the Hutton form (Soil Classification Working Group, 1991) on a slightly sloping (0.5 %) east-southeast facing aspect. The soil was sampled before the experiment commenced to determine nutrient status, exchangeable acidity, total cations, acid saturation, organic carbon and percentage clay (Table 3.1).

Perennial ryegrass (*Lolium perenne* L., cultivar Bronsyn) was established on 5 April 2004. Bronsyn, a variety developed in New Zealand, is widely grown by farmers in KwaZulu-Natal. Seed was planted at a rate of 30 kg ha^{-1}, higher than the recommended seeding rate of 20 – 25 kg ha^{-1} to ensure an optimum stand.

3.2 Experimental design and field lay-out

The trial consisted of a set of six distinct experiments, which were separated using electric fencing. Each experiment focused on the interaction between two of the major nutrient elements N, P, K and S (NxP, NxK, NxS, PxS, PxK and KxS). The experiments were managed separately to avoid possible transfer of nutrients during grazing, which would result in the contamination of treatments. Each factor had three levels (low, medium and high), giving a total of nine treatments per experiment. Each experiment was replicated three times in a randomised block design. Plots were laid out in the already-established ryegrass pasture (Table 3.1). Each plot measured 3 x 10 m, although within each plot only a central block of 1.4 x 7.2 m was harvested. This was to remove possible edge effects.

Table 3.1. Plot plan showing experimental design and plot sizes of trial

Fence

N

Rep 1			Rep 2			Rep 3		
N2P3	N3P2	N1P3	N2P2	N3P1	N3P2	N3P3	N1P1	N2P1
N3P1	N3P3	N2P2	N3P3	N2P1	N1P3	N2P2	N3P1	N3P2
N2P1	N1P2	N1P1	N1P2	N1P1	N2P3	N1P2	N2P3	N1P3
N1K1	N1K3	N3K1	N1K2	N3K1	N3K3	N1K2	N3K1	N3K2
N3K2	N2K1	N2K3	N1K1	N3K2	N2K3	N1K3	N3K3	N2K3
N1K2	N3K3	N2K2	N2K2	N1K3	N2K1	N2K2	N2K1	N1K1
N2S2	N3S3	N2S1	N2S1	N3S3	N2S2	N1S3	N2S1	N3S1
N3S2	N2S3	N1S2	N3S1	N3S2	N1S1	N1S2	N3S3	N3S2
N3S1	N1S1	N1S3	N2S3	N1S3	N1S2	N1S1	N2S3	N2S2
P1S1	P1S2	P2S2	P2S3	P1S1	P2S2	P3S3	P1S1	P2S2
P2S1	P3S1	P3S2	P1S2	P2S1	P3S1	P3S1	P1S3	P2S1
P1S3	P2S3	P3S3	P3S2	P3S3	P1S3	P2S3	P3S2	P1S2
P2K1	P1K3	P1K2	P1K3	P1K1	P3K3	P3K1	P2K1	P3K3
P3K2	P1K1	P2K2	P2K1	P2K2	P1K2	P2K1	P1K3	P2K3
P2K3	P3K1	P3K3	P3K1	P3K2	K2K3	P2K2	P1K2	P3K2
K2S2	K2S1	K1S1	K2S2	K2S3	K3S3	K3S1	K1S2	K1S1
K1S2	K3S1	K3S3	K3S2	K1S2	K1S3	K3S2	K2S2	K1S3
K2S3	K3S2	K1S3	K2S1	K1S1	K3S1	K3S3	K2S3	K2S1

ROAD

3 m

54 m

10 m

90 m

N – nitrogen levels (applied as urea)

N1 = 20 kg N ha^{-1} N2 = 40 kg N ha^{-1} N3 = 80 kg N ha^{-1}

P – phosphorus levels (applied as double superphosphate)

P1 = 0 kg P ha^{-1} P2 = 100 kg P P3 = 200 kg P ha^{-1}

K – potassium levels (applied as KCl)

K1 = 0 kg K ha^{-1} K2 = 250 kg K ha^{-1} K3 = 500 kg K ha^{-1}

S – sulphur levels (applied as sulphur95)

S1 = 0 kg S ha^{-1} S2 = 100 kg S ha^{-1} S3 = 200 kg S ha^{-1}

3.3 Treatments

Levels of nutrient application ranged from zero application to double the current Cedara Fertiliser Advisory Service recommendations for perennial ryegrass pastures. Levels per application were as follows:

N treatments: (as urea 46 % N)	N1	0 kg N ha^{-1} (later 20kg N ha^{-1})
	N2	50 kg N ha^{-1} (later 40kg N ha^{-1})
	N3	140 kg N ha^{-1} (later 80kg N ha^{-1})
P treatments: (as double	P1	0 kg P ha^{-1}
superphosphate 19.6 % P, 0 % S)	P2	100 kg P ha^{-1}
	P3	200 kg P ha^{-1}
K treatments: (as KCl 50 % K)	K1	0 kg K ha^{-1}
	K2	250 kg K ha^{-1}
	K3	500 kg K ha^{-1}
S treatments: (as sulphur95 100 % S)	S1	0 kg S ha^{-1}
	S2	100 kg S ha^{-1}
	S3	200 kg S ha^{-1}

Phosphorus, potassium and sulphur treatments were incorporated into the soil before planting and reapplied by broadcasting in April the following year. Application of N treatments commenced six weeks after establishment. Urea was broadcast onto each plot and the pasture then irrigated to ensure the N was washed into the soil. N treatments were subsequently applied within two days following each grazing episode (Table 3.2). The N treatments were adjusted after the second grazing episode. The 0 kg N ha^{-1} treatment was changed to 20 kg N ha^{-1} as the zero N plots appeared to be showing signs of N deficiency. The 50 and 140 kg N ha^{-1} treatments were decreased to 40 and 80 kg N ha^{-1} respectively, as they were considered to be excessively high.

Table 3.2. Fertiliser application schedule showing rates of N, P, K and S applied per date

Application date	Nitrogen (kg ha^{-1}) N1	N2	N3	Phosphorus (kg ha^{-1}) P1	P2	P3	Potassium (kg ha^{-1}) K1	K2	K3	Sulphur (kg ha^{-1}) S1	S2	S3
2004												
2 Apr 2004				0	100	200	0	250	500	0	100	200
28 Apr 2004	50	50	50									
2 Jun 2004	0	50	140									
19 Jul 2004	0	50	140									
1 Sep 2004	20	40	80									
3 Nov 2004	20	40	80									
14 Dec 2004	20	40	80									
Total	110	270	570	0	100	200	0	250	500	0	100	200
2005												
21 Jan 2005	20	40	80									
7 Apr 2005				0	100	200	0	250	500	0	100	200
18 May 2005	20	40	80									
23 Aug 2005	20	40	80									
5 Oct 2005	20	40	80									
22 Nov 2005	20	40	80									
Total	100	200	400	0	100	200	0	250	500	0	100	200

3.4 Experimental procedure

3.4.1 Soil sampling

The soil on the trial site was sampled six weeks before planting (Table 3.3). This soil sample was a composite sample of the entire trial area (depth 0 – 150 mm). The trial was resampled in March the following year (2005) and again in February 2006. The samples in 2005 and 2006 were taken on a per plot basis (composite samples of ten cores per plot) to depths of both 0 – 100 mm and 100 to 200 mm. Soils were analysed by the Cedara Soil Fertility and Analytical Services laboratories for soil moisture, pH, acid saturation and mineral elements. The methods of soil analysis are described in detail in Manson and Roberts (2001). The results of the soil analyses were used to determine P and K fertiliser rates for experiments where these nutrients were not treatment factors.

Table 3.3. Pre-trial soil chemical properties: mean of 40 samples at 0 – 100 mm depth (analysed by the Cedara Fertiliser Advisory Service)

P	K	Ca	Mg	Exch. acidity	Total cations	Acid sat.	pH	Zn	Mn	Cu	NIRS org. C	NIRS clay
mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	cmol l^{-1}	cmol l^{-1}	%	(KCl)	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
12	157	824	224	0.15	6.51	2	4.7	2.4	4	2.8	2.8	41

3.4.2 Pasture establishment and management

The site was ploughed, disced and rolled in February 2004 for initial land preparation. Seed was planted using a Connor-shea planter and subsequently rolled with a Cambridge-type roller.

The pre-trial soil analysis indicated that P was deficient (Table 3.2). In order to ensure satisfactory initial growth the soil P test value was increased from 12 to 16 mg l^{-1} by the addition of 40 kg P ha^{-1} to the entire pasture site during soil preparation. (This was applied in addition to the treatments on experiments where P was a factor.) Soil analyses at the beginning of the following season (March 2005) indicated that the NxK, NxS and KxS experiments did not require additional P for the second growing season.

Pre-trial soil K levels were in excess of the recommended levels for perennial ryegrass. K was therefore not added, apart from the application of K treatments during soil preparation. In April 2005 KCl was broadcast onto individual plots in the NxP, NxS and PxS experiments according to the results of soil analyses conducted on each plot in March of that year.

Nitrogen was broadcast onto all plots at a rate of 50 kg N $ha^{-}1$ at seedling emergence. Experiments without N as a factor were subsequently fertilised with 30 kg N ha^{-1} after each grazing episode.

Lime was not required in the first year. Soil analyses from individual plots in March 2005 indicated varying degrees of acidification. To facilitate ease of application, a blanket dressing of 1 t ha^{-1} of lime was therefore applied to the entire pasture.

N was applied to all plots at a rate of 75 kg N ha^{-1}. Nitrogen treatments were applied six weeks after establishment and again within two days following defoliation. It was decided to change the N treatments after the second grazing. The 0 kg N ha^{-1} treatment was changed to 20 kg N ha^{-1} as the zero N plots appeared to be showing signs of N deficiency. The 50 and 140 kg N ha^{-1} treatments were decreased to 40 and 80 kg N ha^{-1} respectively, as they were considered to be excessively high.

Supplemental irrigation was applied, using a dragline sprinkler system, to ensure a minimum application of 25 mm of water per hectare per week (rainfall of 5 mm and less was ignored). Turfweeder APM (broadleaf herbicide manuractured by Efekto) was applied in November 2004 at a rate of 1.3 l per 100 l water. It was applied again in February 2005 at a rate of 1 l per 100 l water.

A visual assessment indicated a severe deterioration of pasture vigour in the second season. The trial site was aerated with a Levy 40 aerator in June 2005 and reinforced with 9 kg seed ha^{-1}, which was broadcast onto the site.

3.4.3 Grazing management

Each experiment was grazed by ten Holstein Friesland heifers (Table 3.3). The free-selection (cafeteria) system described by Falkner and Casler (1998) and Casler and van Santen (2001) was followed. For this system, animals were free to graze all treatment plots within an experiment. The experiments were grazed when the pasture was at the 3-leaf stage. Leaf emergence rate is determined by temperature and soil moisture. Thus leaf emergence occurred at the same rate over all experiments, regardless of treatment. The experiments were grazed simultaneously, with each experiment being separated from adjacent experiments by an electric fence. Since animals were expected to display a preference for certain treatments over others, the chosen method was to leave the animals in an experimental block until one plot (the “preferred” plot) had been grazed to approximately 50 mm in height. This was assessed by monitoring the experiment throughout the day. This method presented some challenges. In some instances animal preference was not as clear as was hoped and plots were evenly grazed. On hotter days the cows were not eager to eat and were removed for milking before grazing a plot down to the required residual. It is not believed that these few instances affected the overall results.

Table 3.4. Grazing dates and data recorded for each date

Grazing date	Measurements NxP	NxK	NxS	PxK	PxS	KxS
5 Jul 2004	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield
23 Aug 2004	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield
19 Oct 2004	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield
7 Dec 2004						
18 Jan 2005	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield
1 Mar 2005	yield	yield	quality yield	quality yield	quality yield	quality yield
17 May 2005	yield	yield	quality yield	quality yield	quality yield	quality yield
22 Aug 2005	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield
3 Oct 2005						
21 Nov 2005	quality yield	quality yield	quality yield	quality yield	quality yield	quality yield

3.4.4 Apparent intake measurements

It is difficult to quantify forage intake in grazing systems (Burns et al., 1994; Reeves et al., 1996). Moore (1996, cited by Macoon *et al*., 2003) suggested that techniques based on disappearance of herbage mass, prediction from forage characteristics or calculations of energy requirements for observed animal performance are suitable estimates for groups of animals or a pasture. Intake predictions in this trial were based on herbage disappearance similar to that described in Macoon *et al*., 2003. Herbage disappearance is generally calculated as the difference between pre-grazing and post-grazing herbage mass. In this study, pasture height was used as a proxy for pasture mass. Twenty disc meter readings were taken from each plot within an experiment. These were used to calculate the pre-grazing average pasture height for each plot. In the same manner, , average post-grazing pasture height for each plot was determined once livestock had been removed. Since grazing periods were less than one day forage growth during the grazing period was not a significant factor (t'Mannetje, 1978). The entire trial site was mowed to a height of 50 mm after each grazing episode. This was to allow for a fair comparison of production between treatments at the subsequent grazing event. Apparent intake was calculated by subtracting post-grazing disc height from pre-grazing disc height.

3.4.5 Chemical analyses

Representative grab samples (approximately 500 g) were taken from each plot before grazing. Samples were oven dried to a constant weight at 70 °C to determine dry matter. The samples were milled (1 mm screen) and analysed by the Cedara Feed Laboratory.

Fat was measured using the ether extraction method (AOAC, 1980).

Acid detergent fibre and neutral detergent fibre were estimated following the methods of van Soest (1963).

Crude protein was measured using the Dumas combustion method (AOAC, 2002).

True protein and non-protein nitrogen were measured using the methods of Marais and Evemwell (1983).

Non-structural carbohydrates were measured using the acid-hydrolysis procedure (Marais, 1979).

Calcium, magnesium, potassium, phosphorus, sodium, zinc, copper, manganese and iron were measured using the Hunter method (Hunter, 1975), which was modified for inductively coupled plasma-mass spectrometry.

3.4.6 Data manipulation and statistical analyses

3.4.6.1 *Productivity and apparent intake analysis*

Due to the nature of the trial, plots within an experiment did not contain equal amounts of available herbage. In order to try and reduce any effects this may have had on the results and to make results comparable, average disc height values were converted to a percentage of available herbage consumed.

Data showing the effects of fertiliser on productivity and apparent intake were combined over two seasons within experiments for each level of the first factor, regardless of the level of the second factor. This was done after an analysis of variance (ANOVA) indicated no interaction between factors in terms of treatment effects on productivity and apparent intake. Treatment names were used in the graphical presentation of data to indicate a comparison between low, medium and high fertiliser application rates rather than to test the effects of actual fertiliser levels.

Productivity and apparent intake data were analysed using Statgraphics® *Centurion XV* for Microsoft Windows (Statpoint Technologies, 2005). Treatment means were subjected to LSD tests (Steel & Torrie, 1980).

3.4.6.2 *Quality analysis*

Pasture quality data were subjected to analysis of variance (ANOVA) and repeated measurements ANOVA to assess the effects of fertiliser on the chemical composition of the herbage. The data were separated according to sampling date for analysis but the sampling dates were not divided into seasons. Although this meant that seasonal fluctuations were not addressed, it is believed that combining the data allowed evaluation with less emphasis on seasonal effects, effectively obtaining a better assessment of treatment effects.

In order to obtain an idea of quality fluctuations over the growing season, each data value was allocated a number based on the day of the year on which sampling occurred, from one to 365. In this way the data from two seasons could be combined into a twelve month period.

Treatment means were subjected to LSD tests (Steel & Torrie, 1980). The statistical package 'GenStat' (2007) was used for all statistical analyses.

3.4.6.3 *Regression tree analysis*

Regression trees were constructed using CART4 (Steinberg & Colla, 1997) for apparent intake in each experiment. Classification and regression tree (CART) analysis is a non-parametric technique that can select from among a large number of variables those that are most important in determining the dependent variable to be explained (Yohannes and Hoddinott, 1999). It is a binary decision tree that shows the explanatory variable at each dichotomy that best divides the data into relatively homogenous groups (Morris and Fynn, 2003). A weakness of using CART is that there is no probability level or confidence interval associated with predictions derived from using it (Yohannes and Hoddinott, 1999). However, it was deemed to be useful for this study in order to graphically represent the complex set of factors that may have influenced apparent intake. Apparent intake values were tested against explanatory variables according to sampling date. Explanatory variables comprised applied nutrients, nutrient levels, replicate, herbage available for grazing, pasture age and elements included in the full feed analysis of the herbage.

CHAPTER 4

Yield and apparent intake

4.1 Results

The PxS, PxK and KxS experiments demonstrated no significant effects of fertilisation application on productivity or apparent intake after grazing (Table 4.1).

Table 4.1 Productivity and apparent intake in the PxS, PxK and KxS experiments as reflected by mean disc height[a].

	Disc height (cm)				
	PxS	PxK	KxS		P
	Productivity				
P1	7.4 ± 2.52	7.7 ± 2.47	—	7.6 ± 2.49	NS
P2	7.5 ± 2.52	7.9 ± 2.50	—	7.7 ± 2.51	NS
P3	7.7 ± 2.43	8.0 ± 2.73	—	7.8 ± 2.58	NS
S1	7.5 ± 2.46	—	7.6 ± 2.23	7.5 ± 2.34	NS
S2	7.5 ± 2.56	—	7.9 ± 2.28	7.7 ± 2.43	NS
S3	7.5 ± 2.46	—	7.3 ± 2.24	7.4 ± 2.35	NS
K1	—	7.7 ± 2.35	7.4 ± 2.23	7.5 ± 2.29	NS
K2	—	7.9 ± 2.63	7.8 ± 2.21	7.9 ± 2.42	NS
K3	—	7.9 ± 2.60	7.6 ± 2.33	7.7 ± 2.47	NS
	Intake				
P1	2.2 ± 1.77	2.1 ± 1.64	—	2.2 ± 1.70	NS
P2	2.2 ± 1.75	2.3 ± 1.87	—	2.2 ± 1.80	NS
P3	2.3 ± 1.62	2.3 ± 1.98	—	2.3 ± 1.80	NS
S1	2.2 ± 1.75	—	2.1 ± 1.51	2.1 ± 1.63	NS
S2	2.3 ± 1.71	—	2.3 ± 1.60	2.3 ± 1.65	NS
S3	2.2 ± 1.69	—	1.9 ± 1.47	2.0 ± 1.58	NS
K1	—	2.1 ± 1.61	2.0 ± 1.47	2.1 ± 1.54	NS
K2	—	2.4 ± 1.92	2.1 ± 1.50	2.2 ± 1.72	NS
K3	—	2.2 ± 1.95	2.2 ± 1.63	2.2 ± 1.79	NS

[a]NS = not significant

Data presented as mean ± standard deviation (SD).

There was a highly significant ($P<0.001$) response to the application of fertiliser N in all the experiments with applied N as a factor. Data for the N response are included in table 4.2. This is discussed under the separate variables measured.

Table 4.2. Nitrogen response in the NxP, NxK and NxS experiments, measured as disc height before and after grazing[a]

	Disc height (cm)		
	NxP	NxK	NxS
	Productivity		
N1	7.5[a]	6.9[a]	7.1[a]
N2	8.3[ab]	8.6[b]	8.3[b]
N3	8.8[b]	9.0[c]	9.1[c]
$LSD_{(0.05)}$	0.605	0.387	0.354
SE	0.292	0.187	0.171
	Intake		
N1	2.1[a]	1.8[a]	1.9[a]
N2	2.7[b]	2.8[b]	2.5[b]
N3	3.0[b]	3.3[c]	3.1[c]
$LSD_{(0.05)}$	0.484	0.357	0.369
SE	0.233	0.172	0.178

[a]LSD = least significant difference

4.1.1 Productivity

Relative production response to N is reflected in figure 4.2. The NxP experiment showed a significant effect of N ($F_{2,215}$ = 6.30, P=0.004) on herbage production at higher levels of N. Yield response to N in the NxK experiment was also highly significant ($F_{2,215}$ = 59.63, $P<0.001$), as was the response in the NxS experiment ($F_{2,215}$ = 71.73, $P<0.001$). In the NxK and NxS experiments, increases in production were significant between each level of applied N. Responses to P, K and S in these experiments were not significant and there were no significant interactions.

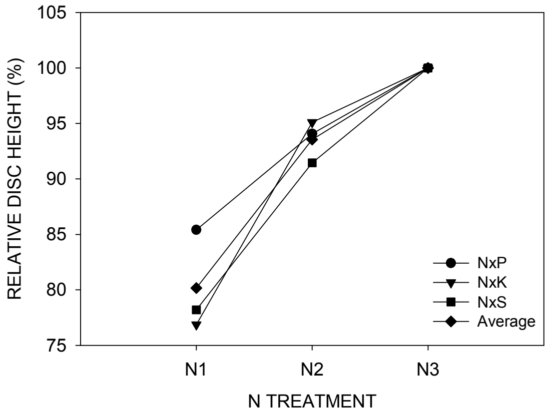


Figure 4.1. Relative production response to N in the NxP, NxK and NxS experiments, measured as pre-grazing disc height

4.1.2. Apparent intake

The NxP experiment demonstrated an effect of N ($F_{2,215}$ = 6.76, P=0.007) on apparent intake (Figure 4.2), with apparent intake being significantly greater at high N application (N3) compared with the lowest level of application (N1). In both the NxK and NxS experiments N had a highly significant effect ($F_{2,215}$ = 36.05, $P < 0.001$ and $F_{2,215}$ = 25.31, $P < 0.001$) on apparent intake.

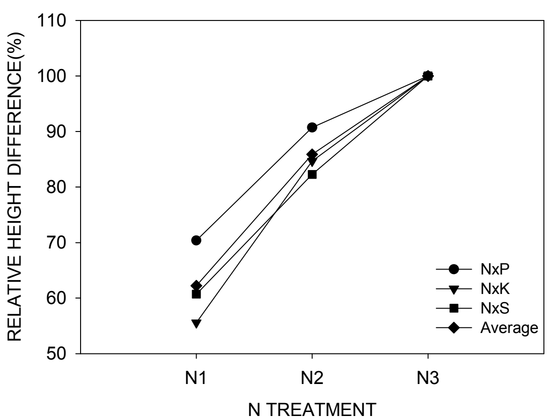


Figure 4.2. Relative apparent intake response to N in the NxP, NxK and NxS experiments, measured as difference between pre- and post-grazing disc height

4.2 Discussion

The objective of this study was to compare the production, intake and post-grazing residual of perennial ryegrass under different fertiliser treatments.

The only experiments that affected production to any significant extent were those containing N as a factor. The results in this study are similar to those obtained by McKenzie *et al*. (1999), who found that, even at low levels of soil P, K and S, application of these nutrients had non-significant effects on yield. Burkitt *et al*. (2010) also reported no yield response to applied P, either when applied as a large single dose or as multiple small applications, when soil extractable P concentrations were adequate. Whitehead (1995) suggested that yields can be improved with the addition of P, K and S to soils in which they are deficient. In this study, P, K and S were not considered to be limiting and therefore no yield response was expected.

Applied N significantly increased the amount of herbage produced. Such responses are well documented (Wolton *et al*., 1971; Frame and Boyd, 1987; McKenzie, 1996, Binnie *et al.*, 2001). In the NxP and NxK experiments in this study, yield response was only significantly different between the N1 (20 kg N ha^{-1}) and N3 (80 kg N ha^{-1}) N treatments. Yield differences between N2 and N3 were not significant, indicating that split applications of N greater than 40 kg N ha^{-1} may not be necessary. Such a quadratic pattern of N response has been reported in previous studies, including Wilman and Wright (1981), McKenzie (1996) and Eckard and Franks (1998). Reid (1970) showed a dry matter yield response in perennial ryegrass that increased linearly to 68 kg N ha^{-1} (per split application) and slowly decreased, becoming non-significant at around 112 kg N ha^{-1}. Ehlig and Hagemann (1982) reported increased dry matter yield with increases in N rate up to 1120 kg N ha^{-1} for irrigated Italian ryegrass in the south western United States. The N was applied in monthly applications of 90 to 112 kg N ha^{-1}. In the NxS experiment, yield response was significant at all three levels of applied N. While yield response to S itself is not significant, this response may be expected as herbage S requirement is closely linked with N metabolism (Goh and Kee, 1978). Increasing rates of N application therefore require corresponding increases in S for maximum yield to occur.

Apparent intake was positively correlated with N application, most likely due to the increased herbage availability in higher N plots. Burns and Mochrie (1981) suggested that applied N can stimulate intake due to the increased proportion of green leaf in the herbage and hence its palatability. Similarly, Da Silva and Carvalho (2005) found sward structural characteristics to be relatively more important than nutritional factors in terms of herbage intake regulation. Since N application affected yield (and therefore sward structure) to such an extent, this may explain why applied P, K and S did not significantly affect apparent intake in any way. It is worth noting that Burns and Mochrie (1981) reported the results of a trial in which animals preferred herbage treated with high N levels in spring but switched to a preference for lower N in very dry weather. Intake on perennial ryegrass may therefore be depressed with increased applied N if the pasture is not sufficiently irrigated.

4.3 Conclusions

This study has confirmed that increased fertiliser N application rates increase perennial ryegrass yield. The response follows the expected quadratic curve, indicating a pattern of diminishing return where split applications above 40 kg N ha^{-1} produce smaller increases in yield compared with an increase in application from 20 to 40 kg N ha^{-1}. Applied P, K and S did not affect yield, suggesting that even the lowest application levels were sufficient to not limit production.

Current recommendations for N application and scheduling are set at 50 kg N ha^{-1} after each grazing. The results from this study question the current practice and suggest that N applications may be reduced without affecting yield. Scheduling is not addressed in this chapter, but it is a pertinent issue as yield responses to N differ depending on season. The variation in response needs to be allowed for when devising a fertiliser management plan. The effect of season on plant response to N is considered in Chapter 5.

Nitrogen application affected apparent intake, but it is suggested that this is due to the yield effect rather than a direct effect of N on intake. Chapter 6 studies the effects of N on intake in more detail. The application of P, K and S did not affect apparent intake or post-grazing residual.

The study confirms that current fertiliser recommendations for P, K and S issued on the basis of soil analysis do not limit production. Applications in excess of current recommendations constitute an unnecessary expense as they neither improve yield nor affect intake.

CHAPTER 5

Chemical composition

5.1 Results

5.1.1 The effects of applied fertiliser N, P, K and S on crude protein and non-protein nitrogen in perennial ryegrass

5.1.1.1 *Crude protein*

5.1.1.1.1 *NxP*

In the NxP experiment, perennial ryegrass annual crude protein (CP) content was strongly linked to the level of applied N but was unrelated to the level of applied P (Figure 5.1). Highly significant increases in CP accompanied N fertiliser additions (P<0.001) at all levels of P.

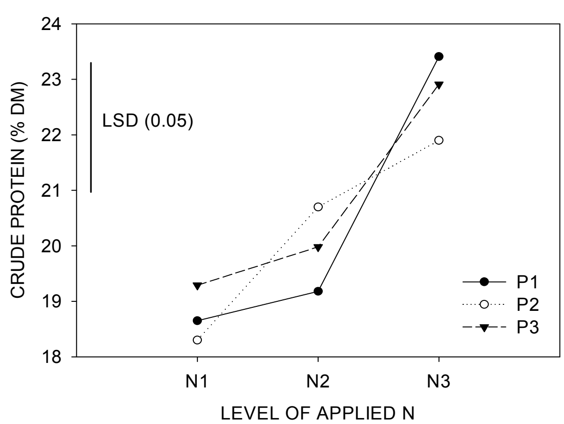


Figure 5.1. The effect of applied N and P on crude protein content of perennial ryegrass, averaged over all seasons.

Nitrogen application had a significant (P<0.001) effect on CP content at individual sampling dates (Figure 5.2). Sampling date had a significant quadratic effect on CP content in the NxP experiment (P<0.001). There was also a significant date-N interaction (P=0.04). Increasing levels of applied N resulted in higher herbage CP concentration, except in mid-spring, when intermediate levels of applied N produced the lowest CP values. Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.1.

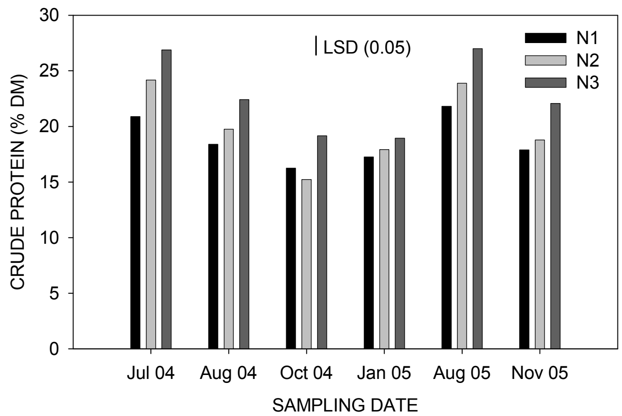


Figure 5.2. Fluctuations of CP over time for three levels of applied N

In general, CP appeared to peak in early spring (Figure 5.3), although there was variation between seasons. Annual variation in the levels of herbage CP was best described by the Gaussian 3-parameter equation:

$$y = 23.3806^{\left[-0.5\left(\frac{x-147.5077}{208.5525}\right)^2\right]}$$

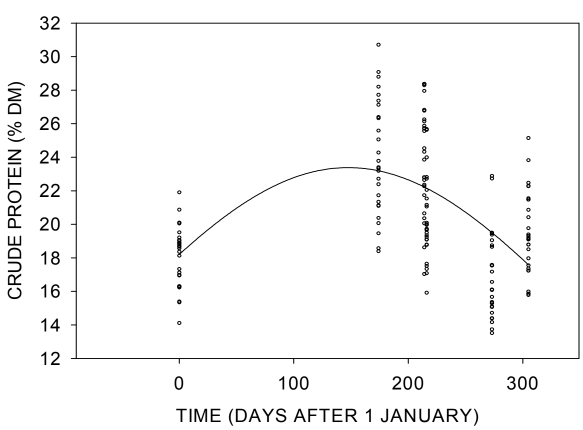


Figure 5.3. Annual variation in CP content over all NxP treatments ($P<0.001$, $R^2=0.304$, $F_{2,159} = 34.7582$)

Table 5.1. The influence of sampling date and applied N and P on the crude protein content of perennial ryegrass

Herbage CP content (% DM) - NxP experiment										
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	21.833	19.789	21.066	22.057	24.608	25.857	28.270	24.953	27.411	**23.983[cd*]**
24-Aug-04	18.465	18.120	18.599	19.553	20.800	18.905	23.590	21.256	22.390	**20.186[bc]**
20-Oct-04	16.459	17.227	15.046	14.821	15.383	15.470	19.688	17.979	19.799	**16.875[a]**
20-Jan-05	17.173	16.565	18.053	16.601	19.872	17.262	19.342	18.047	19.423	**18.038[a]**
22-Aug-05	19.913	20.692	24.807	24.152	24.290	23.193	27.000	26.413	27.594	**24.228[d]**
21-Nov-05	18.082	17.382	18.190	17.893	19.230	19.222	22.585	22.764	20.869	**19.580[b]**
Mean	**18.654[a*]**	**18.296[a]**	**19.293[ab]**	**19.179[ab]**	**20.697[c]**	**19.985[bc]**	**23.413[e]**	**21.902[d]**	**22.914[de]**	
LSD (0.05) Date = 1.169										
LSD (0.05) Treatment = 1.040										
LSD (0.05) Date x Treatment = 3.508 (NS)										
CV = 10.2										
	N1	**N2**	**N3**		**P1**	**P2**	**P3**			
13-Jul-04	20.896	24.174	26.878		24.053	23.117	24.778			
24-Aug-04	18.394	19.753	22.412		20.536	20.058	19.965			
20-Oct-04	16.244	15.225	19.155		16.989	16.863	16.772			
20-Jan-05	17.264	17.912	18.937		17.705	18.161	18.246			
22-Aug-05	21.804	23.878	27.002		23.688	23.799	25.198			
21-Nov-05	17.885	18.782	22.073		19.520	19.792	19.427			
Mean	**18.748[a*]**	**19.954[b]**	**22.743[b]**		**20.415[a*]**	**20.298[a]**	**20.731[a]**			
LSD (0.05) N = 0.789										
LSD (0.05) NxDate = 1.975 (NS, P = 0.058)**										
CV = 10.0										
LSD (0.05) P = 1.966 (NS)										
LSD (0.05) PxDate = 2.077 (NS)										
CV = 10.5										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.1.1.2 *NxK*

Application of N resulted in a significant increase in perennial ryegrass CP content ($P<0.001$) in the NxK experiment. Potassium applications did not affect the concentration of CP in the herbage (Figure 5.4).

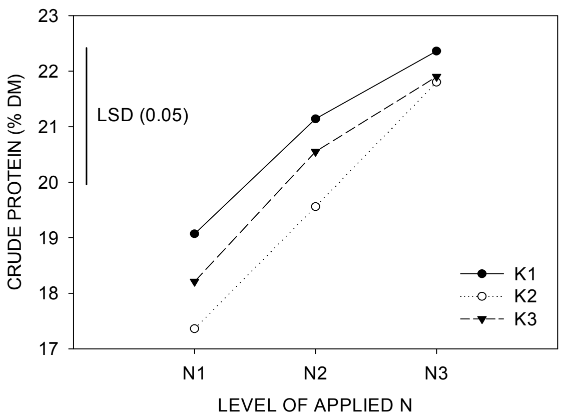


Figure 5.4. The effect of applied N and K on crude protein content of perennial ryegrass, averaged over all seasons.

Both date and level of applied N had a highly significant effect on CP content ($P<0.001$) when data were analysed on a per cut basis (Figure 5.5). The increase in CP was greatest at high levels of applied N, but differences were not significant at all cuts during the experiment. This is reflected in a significant date-N interaction ($P=0.023$). The data reflected a higher CP content in the first season than the second (Figure 5.5). Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.2.

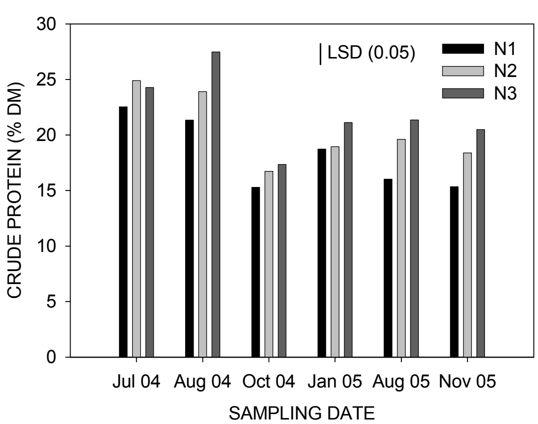


Figure 5.5. Fluctuations of CP over time for three levels of applied N

Annual variation in the levels of herbage CP (Figure 5.6) was best described by the Gaussian 3-parameter equation:

$$y = 23.7181^{\left[-0.5\left(\frac{x-126.7130}{208.8760}\right)^2\right]}$$

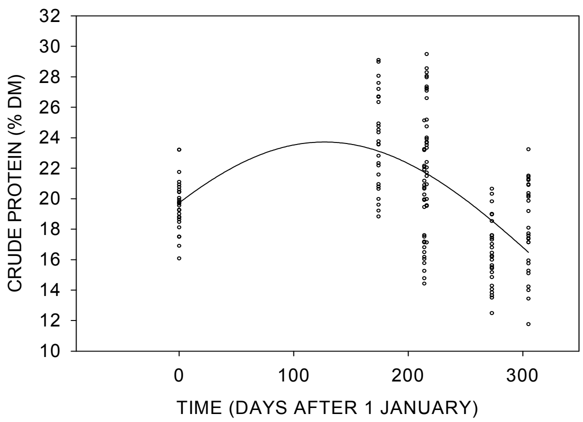


Figure 5.6. Annual variation in CP content over all NxK treatments ($P<0.001$, $R^2=0.304$, $F_{2,159} = 34.6997$)

Table 5.2. The influence of sampling date and applied N and K on the crude protein content of perennial ryegrass

Herbage CP content (% DM) - NxK experiment										
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	23.484	21.874	22.279	25.233	23.084	26.402	24.999	23.078	24.772	**23.912[d*]**
24-Aug-04	24.424	18.871	20.736	24.640	24.116	22.970	28.231	27.892	26.322	**24.245[e]**
20-Oct-04	15.343	14.618	15.890	17.857	16.407	15.935	17.956	17.862	16.222	**16.455[a]**
20-Jan-05	18.598	18.369	19.230	19.122	18.915	18.838	22.017	19.747	21.633	**19.608[c]**
22-Aug-05	15.985	16.190	15.905	21.074	17.430	20.326	19.735	22.589	21.757	**18.999[bc]**
21-Nov-05	16.605	14.256	15.205	18.898	17.421	18.855	21.193	19.617	20.667	**18.080[b]**
Mean	**19.073[bc*]**	**17.363[a]**	**18.208[ab]**	**21.137[def]**	**19.562[bcd]**	**20.554[cde]**	**22.355[f]**	**21.797[ef]**	**21.895[ef]**	
LSD (0.05) Date = 1.285										
LSD (0.05) Treatment = 1.595										
LSD (0.05) Date x Treatment = 3.855 (NS)										
CV = 10.8										
	N1	**N2**	**N3**		**K1**	**K2**	**K3**			
13-Jul-04	22.546	24.906	24.283		24.572	22.679	24.484			
24-Aug-04	21.344	23.909	27.482		25.765	23.626	23.343			
20-Oct-04	15.284	16.733	17.347		17.052	16.296	16.016			
20-Jan-05	18.732	18.958	21.132		19.912	19.010	19.900			
22-Aug-05	16.027	19.610	21.360		18.931	18.736	19.329			
21-Nov-05	15.355	18.391	20.492		18.899	17.098	18.242			
Mean	**18.215[a*]**	**20.418[b]**	**22.016[c]**		**20.855[a*]**	**19.574[a]**	**20.219[a]**			
LSD (0.05) N = 0.985										
LSD (0.05) NxDate = 2.091										
CV = 10.5										
LSD (0.05) K = 1.870 (NS)										
LSD (0.05) KxDate = 2.238 (NS)										
CV = 11.2										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.1.3 *NxS*

The concentration of CP in perennial ryegrass shows a linear response to increasing levels of applied N (Figure 5.7), with highly significant increases in CP accompanying N fertiliser additions (P<0.001). Increasing sulphur application did not affect CP concentration.

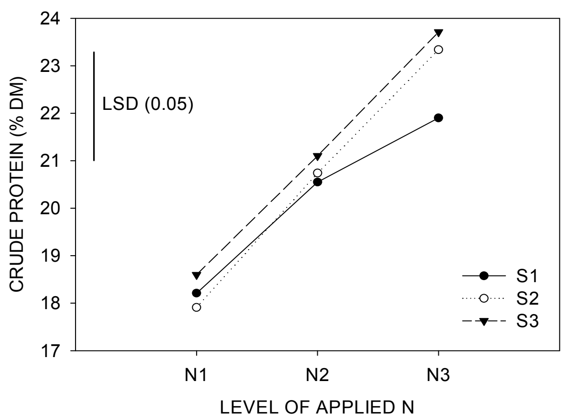


Figure 5.7. The effect of applied N and S on crude protein content of perennial ryegrass, averaged over all seasons.

The effects of N application were highly significant (P<0.001) at all except the October 2004 sampling date (Figure 5.8), where increasing the amount of applied N resulted in higher CP levels in the herbage. Date had a highly significant effect on CP (P<0.001) with CP levels generally higher in the first season than the second. Applied S did not affect CP content of herbage.

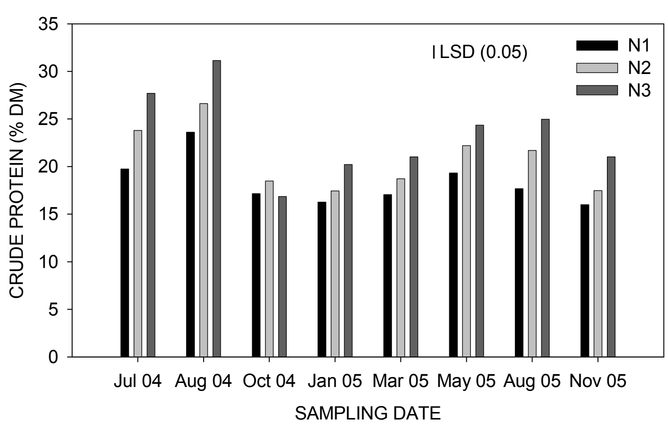


Figure 5.8. Fluctuations of CP over time for three levels of applied N

Annual variation in the levels of herbage CP (Figure 5.9) was best described by the Gaussian 3-parameter equation:

$$y = 23.8034^{\left[-0.5\left(\frac{x-158.3940}{191.1729}\right)^2\right]}$$

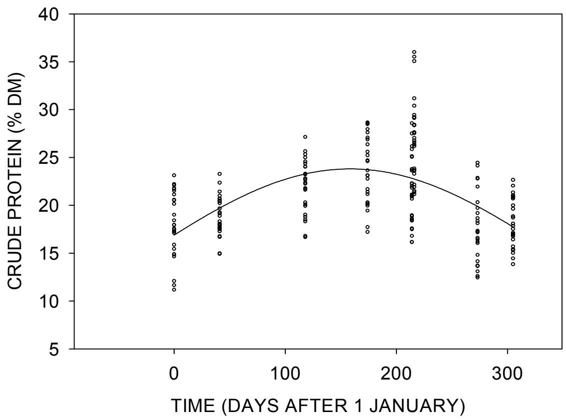


Figure 5.9. Annual variation in CP content over all NxS treatments (P<0.001, R^2=0.301, $F_{2,211}$ = 45.4363)

Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.3.

Table 5.3. The influence of sampling date and applied N and S on the crude protein content of perennial ryegrass

	Herbage CP content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	20.186	18.987	20.082	22.722	24.230	24.435	26.945	28.590	28.030	**23.801**[d*]
24-Aug-04	24.659	23.872	22.326	27.428	25.586	26.853	31.386	30.719	31.294	**27.125**[e]
20-Oct-04	16.309	18.719	16.478	18.298	19.470	17.702	18.902	15.678	15.440	**17.444**[a]
20-Jan-05	17.502	14.281	17.010	16.450	16.324	19.566	20.223	22.113	18.314	**17.976**[ab]
01-Mar-05	16.541	16.808	17.838	18.554	18.586	19.005	20.019	21.474	21.593	**18.935**[b]
17-May-05	19.773	17.795	20.422	22.171	21.402	23.014	24.534	23.343	25.187	**21.960**[c]
22-Aug-05	17.906	17.540	17.601	22.354	21.807	20.896	24.043	25.469	25.420	**21.448**[c]
21-Nov-05	15.704	15.255	17.027	16.559	18.552	17.321	20.644	21.043	21.360	**18.163**[ab]
Mean	**18.572**[a*]	**17.907**[a]	**18.598**[a]	**20.567**[b]	**20.744**[b]	**21.099**[b]	**23.337**[c]	**23.553**[c]	**23.330**[c]	
LSD (0.05) Date = 1.469										
LSD (0.05) Treatment = 1.256										
LSD (0.05) Date x Treatment = 4.406 (NS)										
CV = 11.7										
	N1	**N2**	**N3**		**S1**	**S2**	**S3**			
13-Jul-04	19.752	23.796	27.740		23.284	23.640	24.182			
24-Aug-04	23.619	26.622	31.133		27.824	26.725	26.824			
20-Oct-04	17.168	18.490	16.844		17.836	17.955	17.030			
20-Jan-05	16.264	17.446	20.217		18.058	17.573	18.297			
01-Mar-05	17.063	18.715	21.028		18.371	18.956	19.479			
17-May-05	19.330	22.196	24.354		22.159	20.846	22.874			
22-Aug-05	17.682	21.686	24.977		21.434	21.605	21.305			
21-Nov-05	15.995	17.477	21.016		17.636	18.283	18.570			
Mean	**18.359**[a*]	**20.803**[b]	**23.414**[c]		**20.825**[a*]	**20.698**[a]	**21.070**[a]			
LSD (0.05) N = 0.647										
LSD (0.05) NxDate = 2.397										
CV = 11.2										
LSD (0.05) S = 2.327 (NS)										
LSD (0.05) SxDate = 2.651 (NS)										
CV = 12.4										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.1.4 *PxS*

There were no significant effects of either P or S on CP concentration when data were averaged over all sampling dates. There were also no significant fertiliser effects at individual sampling dates. Crude protein showed a highly significant (P<0.001), quadratic response to sampling date. Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.4.

Annual variation in the levels of herbage CP (Figure 5.10) was best described by the Gaussian 3-parameter equation:

$$y = 22.9763^{\left[-0.5\left(\frac{x-141.5957}{189.4441}\right)^2\right]}$$

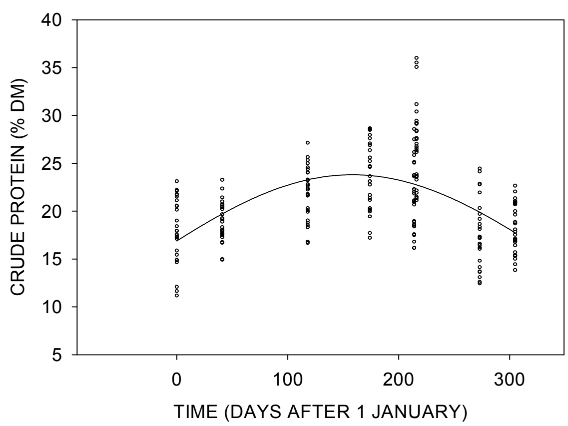


Figure 5.10. Annual variation in CP content over all PxS treatments (P<0.001, R^2=0.434, $F_{2,213}$ = 81.5187)

Table 5.4. The influence of sampling date and applied P and S on the crude protein content of perennial ryegrass

Herbage CP content (% DM) - PxS experiment										
	Treatment									
Cut date	P1S1	P1S2	P1S3	P2S1	P2S2	P2S3	P3S1	P3S2	P3S3	Mean
13-Jul-04	22.995	21.603	22.231	21.756	22.912	22.251	21.261	21.054	22.514	**22.064[e*]**
24-Aug-04	26.225	27.531	26.987	26.425	26.640	26.964	25.274	25.854	25.923	**26.425[f]**
20-Oct-04	15.683	16.492	15.636	17.121	16.782	15.636	14.246	16.578	13.888	**15.785[a]**
20-Jan-05	16.153	18.305	18.704	19.662	16.174	16.497	17.808	17.740	19.792	**17.871[b]**
01-Mar-05	20.765	19.766	20.650	19.857	20.033	19.112	21.367	20.369	19.873	**20.199[cd]**
17-May-05	20.383	20.094	20.778	21.285	20.400	18.904	21.035	22.401	22.774	**20.895[d]**
22-Aug-05	19.498	19.243	19.698	21.329	19.721	19.393	19.838	19.913	20.323	**19.884[c]**
21-Nov-05	15.505	15.107	15.841	17.729	14.602	16.129	16.693	16.360	16.338	**16.034[a]**
Mean	**19.651[a*]**	**19.768[a]**	**20.066[a]**	**20.646[a]**	**19.658[a]**	**19.361[a]**	**19.690[a]**	**20.034[a]**	**20.178[a]**	

LSD (0.05) Date = 0.952
LSD (0.05) Treatment = 1.341 (NS)
LSD (0.05) Date x Treatment = 2.856 (NS)
CV = 8.3

	P1	P2	P3	S1	S2	S3
13-Jul-04	22.276	22.306	21.610	22.004	21.856	22.332
24-Aug-04	26.915	26.677	25.683	25.975	26.675	26.625
20-Oct-04	15.937	16.513	14.904	15.683	16.617	15.053
20-Jan-05	17.721	17.445	18.446	17.874	17.406	18.331
01-Mar-05	20.394	19.667	20.536	20.663	20.056	19.878
17-May-05	20.418	20.197	22.070	20.901	20.965	20.819
22-Aug-05	19.480	20.148	20.025	20.222	19.626	19.805
21-Nov-05	15.484	16.153	16.464	16.643	15.356	16.103
Mean	**19.828[a*]**	**19.888[a]**	**19.967[a]**	**19.995[a*]**	**19.820[a]**	**19.868[a]**

LSD (0.05) P = 0.751 (NS)
LSD (0.05) PxDate = 1.562 (NS)
CV = 8.1
LSD (0.05) S = 0.749 (NS)
LSD (0.05) SxDate = 1.647 (NS)
CV = 8.3

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.1.5 *PxK*

Crude protein content was unaffected by both applied P and K fertiliser in the PxK experiment when data were averaged over all sampling dates. Potassium had a significant effect (P=0.002) on CP at individual sampling dates. This was especially noticeable in the second season, where higher levels of applied K depressed CP production (Figure 5.11). In general, CP content was lower in the second season compared with the first (Figure 11), with date having a significant effect on herbage CP (P<0.001).

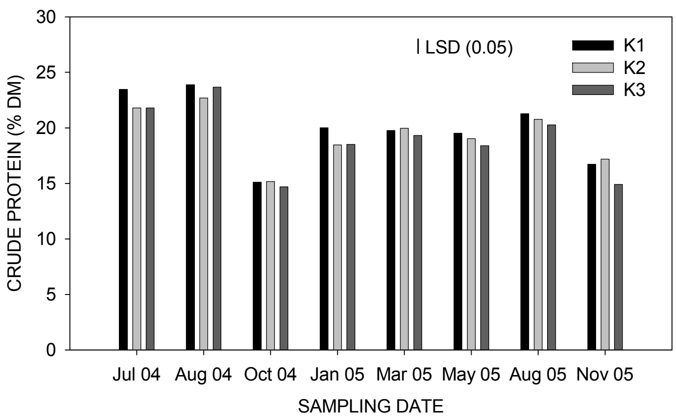


Figure 5.11. Fluctuations of CP over time for three levels of applied K

Annual variation in the levels of herbage CP (Figure 5.12) was best described by the cubic equation:

$$y = 19.1233 - 0.0145 + 0.0004x^2 - 1.228^{\exp-6}x^3$$

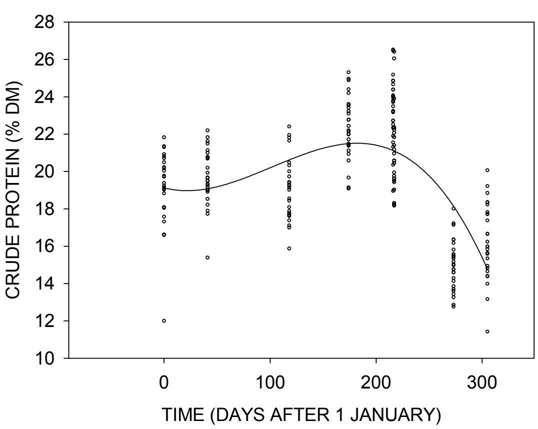


Figure 5.12. Annual variation in CP content over all PxK treatments (P<0.001, R^2=0.433, $F_{3,212}$ = 53.9446)

Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.5.

Table 5.5. The influence of sampling date and applied P and K on the crude protein content of perennial ryegrass

Herbage CP content (% DM) - PxK experiment										
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	23.146	21.202	20.080	23.477	21.875	22.709	23.766	22.316	22.589	**22.351[e*]**
24-Aug-04	23.582	21.969	23.731	23.654	23.726	22.389	24.418	22.358	24.869	**23.411[e]**
20-Oct-04	15.668	15.208	14.040	14.516	16.333	14.850	15.143	13.964	15.161	**14.987[a]**
20-Jan-05	19.717	18.551	19.099	20.705	19.706	18.679	19.589	17.157	17.771	**18.997[c]**
01-Mar-05	20.170	20.617	20.065	19.253	19.128	17.987	19.845	20.158	19.870	**19.677[c]**
17-May-05	19.157	19.435	18.941	19.879	18.653	16.824	19.530	18.993	19.432	**18.983[c]**
22-Aug-05	20.334	19.412	20.757	20.498	20.408	20.531	22.996	22.476	19.515	**20.770[d]**
21-Nov-05	18.434	16.786	16.543	16.776	17.052	12.992	14.956	17.703	15.199	**16.271[b]**
Mean	**20.026[b*]**	**19.148[ab]**	**19.157[ab]**	**19.845[b]**	**19.610[b]**	**18.370[a]**	**20.030[b]**	**19.391[b]**	**19.301[ab]**	
LSD (0.05) Date = 1.067										
LSD (0.05) Treatment = 0.914										
LSD (0.05) Date x Treatment = 3.200 (NS)										
CV = 9.1										
	P1	**P2**	**P3**		**K1**	**K2**	**K3**			
13-Jul-04	21.476	22.687	22.891		23.463	21.798	21.793			
24-Aug-04	23.094	23.256	23.882		23.885	22.685	23.663			
20-Oct-04	14.972	15.233	14.756		15.109	15.168	14.683			
20-Jan-05	19.122	19.697	18.172		20.003	18.471	18.517			
01-Mar-05	20.284	18.790	19.958		19.756	19.968	19.308			
17-May-05	19.178	18.452	19.318		19.522	19.027	18.399			
22-Aug-05	20.168	20.479	21.663		21.276	20.765	20.268			
21-Nov-05	17.254	15.607	15.953		16.722	17.180	14.911			
Mean	**19.443[a*]**	**19.275[a]**	**19.574[a]**		**19.967[b*]**	**19.383[a]**	**18.943[a]**			
LSD (0.05) P = 0.682 (NS)										
LSD (0.05) PxDate = 1.757 (NS)										
CV = 8.9										
LSD (0.05) K = 0.525										
LSD (0.05) KxDate = 1.789 (NS)										
CV = 9.1										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.1.6 *KxS*

Neither applied K nor S affected overall CP content in the KxS experiment. There was also no CP response to applied treatments at individual sampling dates. Again, date significantly affected the level of CP in the herbage, with second season CP levels not reaching those of the first season. Data reflecting the effects of treatment and sampling date on herbage CP are summarised in Table 5.6.

Annual variation in the levels of herbage CP (Figure 5.13) was best described by the waveform sine 4-parameter equation:

$$y = 19.4625 + 2.8747 \sin\left[\frac{2\prod x}{207.3078} + 2.5712\right]$$

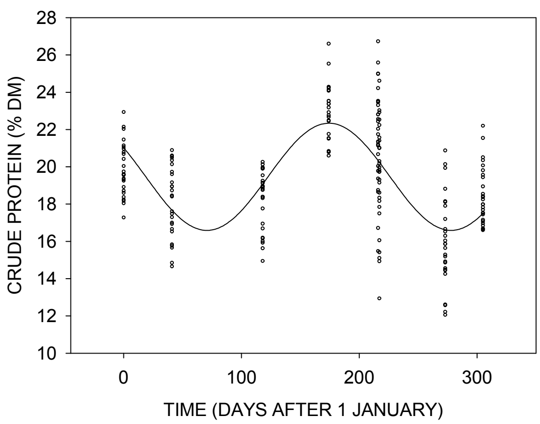


Figure 5.13. Annual variation in CP content over all KxS treatments (P<0.001, R^2=0.414, $F_{3,212}$ = 49.9382)

Table 5.6. The influence of sampling date and applied K and S on the crude protein content of perennial ryegrass

Herbage CP content (% DM) - KxS experiment										
	Treatment									
Cut date	**K1S1**	**K1S2**	**K1S3**	**K2S1**	**K2S2**	**K2S3**	**K3S1**	**K3S2**	**K3S3**	**Mean**
13-Jul-04	21.843	21.786	22.447	23.429	22.652	25.454	23.540	22.524	22.741	**22.935[e*]**
24-Aug-04	22.390	22.934	20.932	22.635	21.525	22.033	17.619	23.970	22.351	**21.821[e]**
20-Oct-04	14.226	17.898	18.359	14.241	17.427	14.593	14.053	16.944	16.055	**15.977[a]**
20-Jan-05	19.021	19.533	19.662	20.093	21.078	20.866	19.519	19.079	19.803	**19.850[d]**
01-Mar-05	18.232	17.752	18.127	19.991	18.851	17.965	16.967	18.098	17.235	**18.135[b]**
17-May-05	18.337	18.173	17.950	16.079	18.065	19.766	18.889	19.469	17.556	**18.254[bc]**
22-Aug-05	19.732	18.720	19.251	20.057	20.822	18.548	20.613	18.346	18.363	**19.384[cd]**
21-Nov-05	18.710	20.047	17.676	17.852	18.737	17.634	17.398	17.743	20.173	**18.441[bc]**
Mean	**19.061[ab*]**	**19.605[ab]**	**19.300[ab]**	**19.297[ab]**	**19.894[b]**	**19.607[ab]**	**18.575[a]**	**19.522[ab]**	**19.285[ab]**	

LSD (0.05) Date = 1.150
LSD (0.05) Treatment = 1.241 (NS)
LSD (0.05) Date x Treatment = 3.451 (NS)
CV = 10.0

	K1	**K2**	**K3**	**S1**	**S2**	**S3**
13-Jul-04	22.026	23.845	22.935	22.937	22.321	23.547
24-Aug-04	22.085	22.064	21.313	20.881	22.810	21.772
20-Oct-04	16.828	15.420	15.684	14.174	17.423	16.336
20-Jan-05	19.405	20.679	19.467	19.544	19.897	20.110
01-Mar-05	18.037	18.935	17.433	18.397	18.234	17.776
17-May-05	18.153	17.970	18.638	17.768	18.569	18.424
22-Aug-05	19.234	19.809	19.107	20.134	19.296	18.721
21-Nov-05	18.811	18.074	18.438	17.987	18.842	18.494
Mean	**19.322[a*]**	**19.600[a]**	**19.127[a]**	**18.978[a*]**	**19.674[b]**	**19.397[a]**

LSD (0.05) K = 0.681 (NS)
LSD (0.05) KxDate = 2.020 (NS)
CV = 10.4
LSD (0.05) S = 0.641 (NS, P = 0.099)**
LSD (0.05) SxDate = 1.980 (NS)
CV = 10.2

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.1.2 *Non-protein nitrogen*

5.1.1.2.1 *NxP*

Herbage non-protein nitrogen (NPN) increased significantly (P<0.001) with increasing levels of applied N but showed no response to applied P (Figure 5.14).

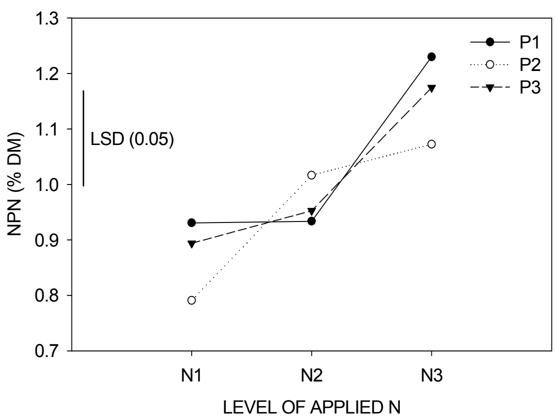


Figure 5.14. The effect of applied N and P on NPN content of perennial ryegrass, averaged over all seasons.

Nitrogen affected herbage NPN at individual sampling dates, with differences at the highest level of applied N being significant (P<0.001). Sampling date also had a significant (P<0.001) effect on NPN concentration, which, even at high levels of applied N, showed a decline in late summer (Figure 5.15).

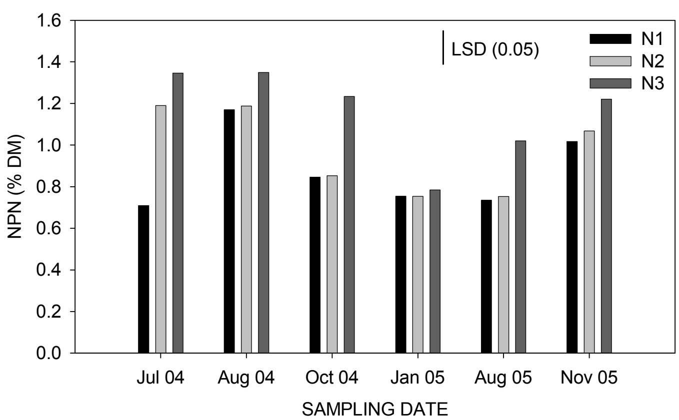


Figure 5.15. Fluctuations of NPN over time for three levels of applied N

Annual variation in the levels of herbage NPN (Figure 5.16) was best described by the Gaussian 3-parameter equation:

$$y = 1.0575^{\left[-0.5\left(\frac{x-254.7932}{321.7324}\right)^2\right]}$$

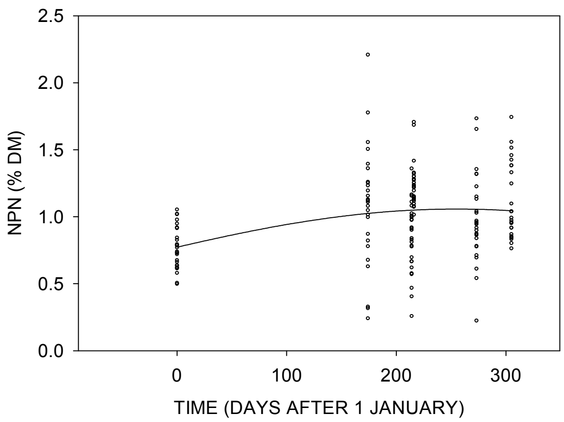


Figure 5.16. Annual variation in NPN content over all NxP treatments (P=0.002, R^2=0.101, $F_{2,159}$ = 8.9523)

Data reflecting the effects of treatment and sampling date on herbage NPN are summarised in Table 5.7.

Table 5.7. The influence of sampling date and applied N and P on the NPN content of perennial ryegrass

Herbage NPN content (% DM) - NxP experiment										
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	0.886	0.626	0.615	0.953	1.239	1.378	1.673	1.137	1.226	**1.081**[cd*]
24-Aug-04	1.171	1.162	1.176	1.164	1.196	1.204	1.424	1.237	1.387	**1.236**[d]
20-Oct-04	1.000	0.677	0.861	0.891	0.804	0.864	1.303	1.140	1.258	**0.978**[bc]
20-Jan-05	0.747	0.688	0.829	0.684	0.831	0.747	0.699	0.785	0.870	**0.764**[a]
22-Aug-05	0.573	0.694	0.937	0.737	0.872	0.649	1.090	0.890	1.082	**0.836**[ab]
21-Nov-05	1.208	0.897	0.945	1.172	1.158	0.873	1.191	1.246	1.226	**1.102**[cd]
Mean	**0.931**[bc*]	**0.791**[a]	**0.894**[ab]	**0.934**[bc]	**1.017**[cd]	**0.953**[bc]	**1.230**[f]	**1.072**[de]	**1.175**[ef]	
LSD (0.05) Date = 0.1562										
LSD (0.05) Treatment = 0.1108										
LSD (0.05) Date x Treatment = 0.4687 (NS)										
CV = 26.5										
	N1	**N2**	**N3**		**P1**	**P2**	**P3**			
13-Jul-04	0.709	1.190	1.345		1.171	1.001	1.073			
24-Aug-04	1.170	1.188	1.349		1.253	1.198	1.256			
20-Oct-04	0.846	0.853	1.234		1.065	0.874	0.994			
20-Jan-05	0.755	0.754	0.785		0.710	0.768	0.815			
22-Aug-05	0.735	0.753	1.020		0.800	0.819	0.889			
21-Nov-05	1.017	1.068	1.221		1.190	1.100	1.014			
Mean	**0.872**[a*]	**0.968**[b]	**1.159**[c]		**1.031**[a*]	**0.960**[a]	**1.007**[a]			
LSD (0.05) N = 0.0799										
LSD (0.05) NxDate = 0.2565										
CV = 25.6										
LSD (0.05) P = 0.1486 (NS)										
LSD (0.05) PxDate = 0.2747 (NS)										
CV = 27.2										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.2.2 *NxK*

There were no significant effects of either N or K on NPN when data were averaged over all sampling dates. There were also no significant fertiliser effects at individual sampling dates. There were, however, significant (P=0.006) treatment effects (Table 5.8), with trends shown of increased N progressively increasing herbage NPN and increased K application decreasing NPN. Non-protein nitrogen showed a highly significant (P<0.001) response to sampling date. Data reflecting the effects of treatment and sampling date on herbage NPN are summarised in Table 5.8.

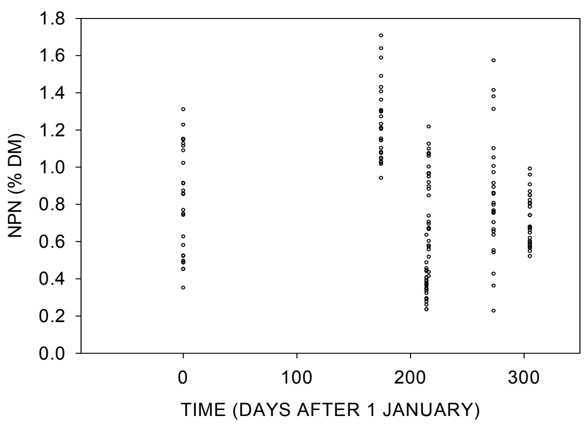


Figure 5.17. Annual variation in NPN content over all NxK treatments

Table 5.8. The influence of sampling date and applied N and K on the NPN content of perennial ryegrass

	Herbage NPN content (% DM) - NxK experiment									
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	1.205	1.096	1.200	1.270	1.168	1.391	1.373	1.194	1.262	**1.240^{c*}**
24-Aug-04	0.953	0.563	0.564	0.813	0.882	0.780	0.985	1.019	0.744	**0.812^{b}**
20-Oct-04	0.948	0.738	1.060	0.594	1.129	0.541	1.131	0.704	0.743	**0.843^{b}**
20-Jan-05	0.767	0.739	0.796	0.735	0.870	0.784	1.059	0.742	0.843	**0.815^{b}**
22-Aug-05	0.359	0.451	0.347	0.322	0.373	0.317	0.372	0.389	0.353	**0.365^{a}**
21-Nov-05	0.730	0.630	0.619	0.848	0.791	0.738	0.673	0.753	0.634	**0.713^{b}**
Mean	**0.827^{bc*}**	**0.703^{a}**	**0.765^{ab}**	**0.763^{ab}**	**0.869^{cd}**	**0.759^{ab}**	**0.932^{d}**	**0.800^{abc}**	**0.763^{ab}**	
LSD (0.05) Date = 0.1355										
LSD (0.05) Treatment = 0.0985										
LSD (0.05) Date x Treatment = 0.4066 (NS)										
CV = 28.9										
	N1	**N2**	**N3**		**K1**	**K2**	**K3**			
13-Jul-04	1.167	1.276	1.276		1.283	1.152	1.284			
24-Aug-04	0.693	0.825	0.916		0.917	0.821	0.696			
20-Oct-04	0.915	0.755	0.859		0.891	0.857	0.781			
20-Jan-05	0.767	0.796	0.882		0.854	0.784	0.808			
22-Aug-05	0.386	0.337	0.371		0.351	0.404	0.339			
21-Nov-05	0.660	0.792	0.687		0.750	0.725	0.664			
Mean	**0.765^{a*}**	**0.797^{a}**	**0.832^{a}**		**0.841^{b*}**	**0.791^{ab}**	**0.762^{a}**			
LSD (0.05) N = 0.0796 (NS)										
LSD (0.05) NxDate = 0.2266 (NS)										
CV = 28.6										
LSD (0.05) K = 0.0773 (NS)										
LSD (0.05) KxDate = 0.2320 (NS)										
CV = 29.0										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.2.3 *NxS*

When data were averaged over the entire experiment period, application of N resulted in a significant increase in NPN (P<0.001) in the NxS experiment. Sulphur applications did not affect NPN concentrations in the herbage (Figure 5.18).

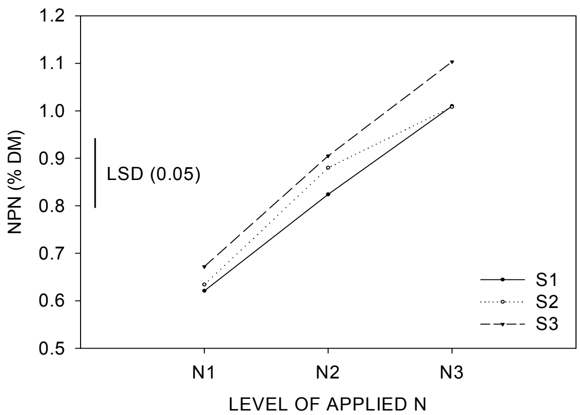


Figure 5.18. The effect of applied N and S on NPN content of perennial ryegrass, averaged over all seasons.

Effects of both date and N application on herbage NPN at individual sampling dates were highly significant (P<0.001). NPN in herbage was higher at high levels of N application, (Figure 5.19), with the exception of the cut in October 2004. There was a significant date-N interaction (P=0.004). The level of applied S did not affect the NPN concentration in the herbage. Data reflecting the effects of treatment and sampling date on herbage NPN are

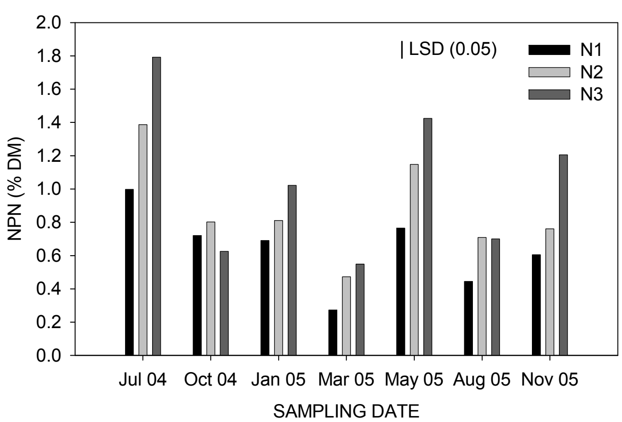


Figure 5.19. Fluctuations of NPN over time for three levels of applied N

Annual variation in the levels of herbage NPN (Figure 5.20) was best described by the quadratic equation:

$$y = 0.6542 + 0.0042x - 0.00001x^2$$

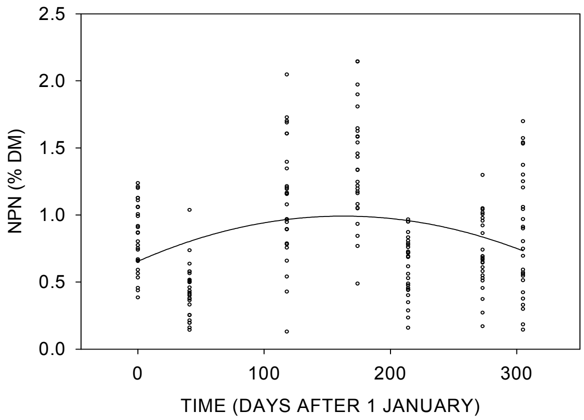


Figure 5.20. Annual variation in NPN content over all NxS treatments (P=0.0009, R^2=0.073, $F_{2,184}$ = 7.2784)

Table 5.9. The influence of sampling date and applied N and S on the NPN content of perennial ryegrass

	Herbage NPN content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	0.929	1.072	0.993	1.255	1.436	1.470	1.763	1.643	1.921	**1.387[e*]**
20-Oct-04	0.681	0.893	0.586	0.757	0.892	0.757	0.726	0.560	0.569	**0.713[bc]**
20-Jan-05	0.767	0.617	0.687	0.874	0.610	0.948	1.010	1.115	0.941	**0.841[c]**
01-Mar-05	0.291	0.223	0.305	0.463	0.444	0.511	0.499	0.727	0.419	**0.431[a]**
17-May-05	0.765	0.625	0.903	1.149	0.930	1.365	1.371	1.357	1.546	**1.112[d]**
22-Aug-05	0.480	0.539	0.316	0.627	0.778	0.721	0.645	0.800	0.653	**0.618[b]**
21-Nov-05	0.432	0.472	0.912	0.644	1.073	0.564	1.059	1.063	1.494	**0.857[cd]**
Mean	**0.621[a*]**	**0.634[a]**	**0.672[a]**	**0.824[ab]**	**0.880[bc]**	**0.905[bc]**	**1.010[bc]**	**1.038[c]**	**1.078[c]**	
LSD (0.05) Date = 0.1596										
LSD (0.05) Treatment = 0.2003										
LSD (0.05) Date x Treatment = 0.4787 (NS, P=0.069)**										
CV = 32.3										
	N1	**N2**	**N3**		**S1**	**S2**	**S3**			
13-Jul-04	0.998	1.387	1.792		1.316	1.383	1.461			
20-Oct-04	0.720	0.802	0.625		0.721	0.782	0.665			
20-Jan-05	0.690	0.810	1.022		0.884	0.780	0.859			
01-Mar-05	0.273	0.473	0.548		0.418	0.465	0.412			
17-May-05	0.765	1.148	1.425		1.095	0.971	1.272			
22-Aug-05	0.445	0.709	0.700		0.584	0.706	0.563			
21-Nov-05	0.605	0.760	1.205		0.712	0.869	0.990			
Mean	**0.642[a*]**	**0.870[b]**	**1.045[c]**		**0.818[a*]**	**0.851[a]**	**0.889[a]**			
LSD (0.05) N = 0.1013										
LSD (0.05) NxDate = 0.2792										
CV = 32.0										
LSD (0.05) S = 0.2024 (NS)										
LSD (0.05) SxDate = 0.3003 (NS)										
CV = 34.7										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.1.2.4 *PxS*

Herbage NPN levels were unaffected by both applied P and S fertilisers in the PxS experiment. Sampling date did, however, affect NPN concentration in the experiment (P<0.001). Data reflecting the effects of treatment and sampling date on herbage NPN are summarised in Table 5.10.

Annual variation in the levels of herbage NPN (Figure 5.21) was best described by the waveform damped sine 5-parameter equation:

$$y = 0.8872 + 0.9002e^{-\left(\frac{x}{122.8526}\right)} \sin\left(\frac{2\prod x}{168.3159} + 6.1179\right)$$

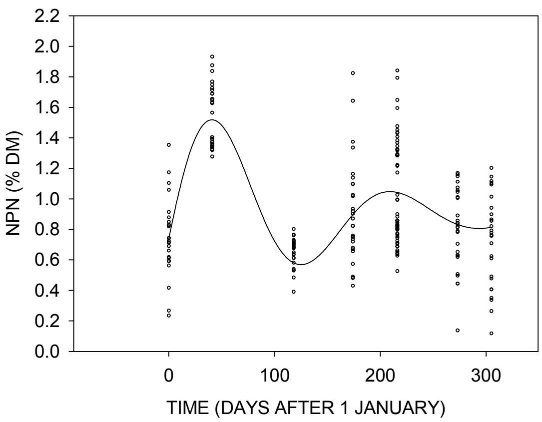


Figure 5.21. Annual variation in NPN content over all PxS treatments (P<0.0001, R^2=0.477, $F_{4,211}$ = 48.0470)

Table 5.10. The influence of sampling date and applied P and S on the NPN content of perennial ryegrass

Herbage NPN content (% DM) - PxS experiment										
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	1.082	0.930	1.073	0.983	0.955	1.045	0.875	0.641	0.726	**0.923[c*]**
24-Aug-04	1.270	1.505	1.423	1.351	1.083	1.498	1.135	1.202	1.280	**1.305[d]**
20-Oct-04	0.776	0.809	0.844	0.953	0.904	0.774	0.572	0.947	0.642	**0.802[bc]**
20-Jan-05	0.744	0.661	1.046	0.934	0.672	0.447	0.717	0.516	0.928	**0.741[ab]**
01-Mar-05	1.552	1.471	1.514	1.568	1.459	1.492	1.582	1.757	1.505	**1.545[e]**
17-May-05	0.722	0.523	0.709	0.629	0.642	0.633	0.633	0.662	0.733	**0.654[a]**
22-Aug-05	0.772	0.741	0.712	0.798	0.764	0.803	0.784	0.753	0.771	**0.766[ab]**
21-Nov-05	0.631	0.476	0.800	0.981	0.586	0.689	0.751	0.762	0.887	**0.729[ab]**
Mean	**0.944[a*]**	**0.890[a]**	**1.015[a]**	**1.025[a]**	**0.883[a]**	**0.923[a]**	**0.881[a]**	**0.905[a]**	**0.934[a]**	

LSD (0.05) Date = 0.1363

LSD (0.05) Treatment = 0.1860 (NS)

LSD (0.05) Date x Treatment = 0.4090 (NS)

CV = 24.8

	P1	**P2**	**P3**		**S1**	**S2**	**S3**
13-Jul-04	1.028	0.994	0.747		0.980	0.842	0.948
24-Aug-04	1.399	1.311	1.206		1.252	1.263	1.400
20-Oct-04	0.810	0.877	0.720		0.767	0.887	0.753
20-Jan-05	0.817	0.684	0.720		0.799	0.616	0.807
01-Mar-05	1.513	1.507	1.615		1.567	1.563	1.504
17-May-05	0.651	0.634	0.676		0.661	0.609	0.692
22-Aug-05	0.741	0.789	0.769		0.784	0.753	0.762
21-Nov-05	0.636	0.752	0.800		0.787	0.608	0.792
Mean	**0.949[a*]**	**0.943[a]**	**0.907[a]**		**0.950[a*]**	**0.893[a]**	**0.957[a]**

LSD (0.05) P = 0.1031 (NS)

LSD (0.05) PxDate = 0.2243 (NS)

CV = 24.3

LSD (0.05) S = 0.1004 (NS)

LSD (0.05) SxDate = 0.2295 (NS)

CV = 24.8

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.1.2.5 *PxK*

Neither applied P nor K affected overall herbage NPN in the PxK experiment. Potassium did, however, affect NPN content on a per cut basis (P=0.014), as did sampling date (P<0.001). Phosphorus application did not affect NPN concentrations at individual sampling dates (Figure 5.22). Data reflecting the effects of treatment and sampling date on herbage NPN are summarised in Table 5.11.

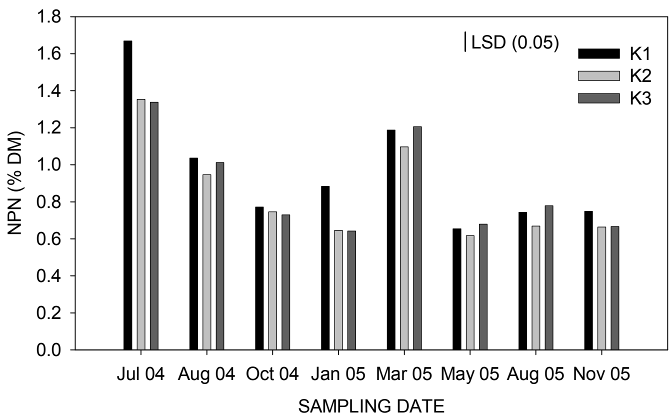


Figure 5.22. Fluctuations of NPN over time for three levels of applied K

Annual variation in the levels of herbage NPN (Figure 5.23) was best described by the Gaussian 3-parameter equation:

$$y = 1.0504^{\left[-0.5\left(\frac{x-131.5268}{184.1418}\right)^2\right]}$$

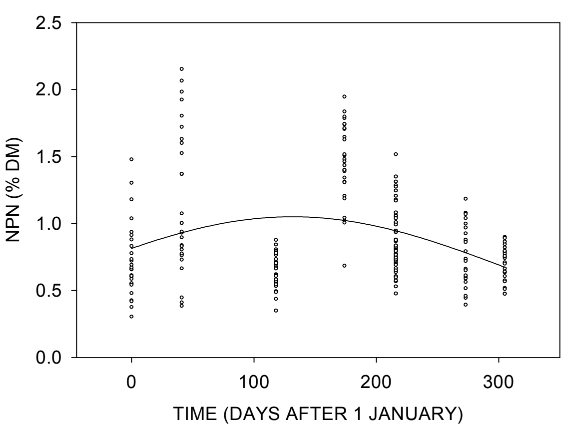


Figure 5.23. Annual variation in NPN content over all PxK treatments (P<0.0001, R^2=0.100, $F_{2,213}$ = 11.8832)

Table 5.11. The influence of sampling date and applied P and K on the NPN content of perennial ryegrass

	Herbage NPN content (% DM) - PxK experiment									
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	1.619	1.261	1.071	1.710	1.308	1.512	1.678	1.492	1.429	**1.453[c*]**
24-Aug-04	1.058	0.823	1.182	0.861	1.133	0.771	1.190	0.885	1.082	**0.998[b]**
20-Oct-04	0.756	0.733	0.649	0.745	0.927	0.726	0.813	0.577	0.815	**0.749[a]**
20-Jan-05	0.876	0.572	0.768	0.906	0.783	0.660	0.867	0.580	0.500	**0.724[a]**
01-Mar-05	1.117	1.211	1.452	0.995	1.111	0.967	1.451	0.969	1.198	**1.163[b]**
17-May-05	0.662	0.636	0.668	0.710	0.600	0.604	0.589	0.615	0.765	**0.650[a]**
22-Aug-05	0.638	0.661	0.811	0.803	0.639	0.804	0.789	0.708	0.721	**0.730[a]**
21-Nov-05	0.774	0.670	0.676	0.722	0.716	0.585	0.750	0.605	0.739	**0.693[a]**
Mean	**0.937[ab*]**	**0.821[a]**	**0.910[ab]**	**0.932[ab]**	**0.902[ab]**	**0.829[a]**	**1.016[b]**	**0.804[a]**	**0.906[ab]**	

LSD (0.05) Date = 0.1775
LSD (0.05) Treatment = 0.1350 (NS, P = 0.081)**
LSD (0.05) Date x Treatment = 0.5324 (NS)
CV = 33.2

	P1	**P2**	**P3**	**K1**	**K2**	**K3**
13-Jul-04	1.317	1.510	1.533	1.669	1.354	1.337
24-Aug-04	1.021	0.922	1.052	1.037	0.947	1.012
20-Oct-04	0.713	0.799	0.735	0.772	0.746	0.730
20-Jan-05	0.739	0.783	0.649	0.883	0.645	0.642
01-Mar-05	1.260	1.024	1.206	1.188	1.097	1.206
17-May-05	0.655	0.638	0.657	0.654	0.617	0.679
22-Aug-05	0.703	0.749	0.739	0.743	0.669	0.779
21-Nov-05	0.707	0.674	0.698	0.748	0.664	0.667
Mean	**0.889[a*]**	**0.887[a]**	**0.909[a]**	**0.962[b*]**	**0.842[a]**	**0.881[a]**

LSD (0.05) P = 0.0941 (NS)
LSD (0.05) PxDate = 0.2866 (NS)
CV = 31.3
LSD (0.05) K = 0.0779
LSD (0.05) KxDate = 0.2863 (NS)
CV = 31.4

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.1.2.6 *KxS*

Neither data averaged over all sampling dates nor that analysed at individual sampling dates provided evidence of significant effects or interactions to applied fertilisers in the KxS experiment. Sampling date was the only factor that had a significant effect on NPN in the herbage (P<0.001). Data reflecting the effects of treatment and sampling date on herbage NPN are summarised in Table 5.12.

Annual variation in the levels of herbage NPN (Figure 5.24) was best described by the Gaussian 3-parameter equation:

$$y = 1.0994^{\left[-0.5\left(\frac{x+168.3546}{465.2438}\right)^2\right]}$$

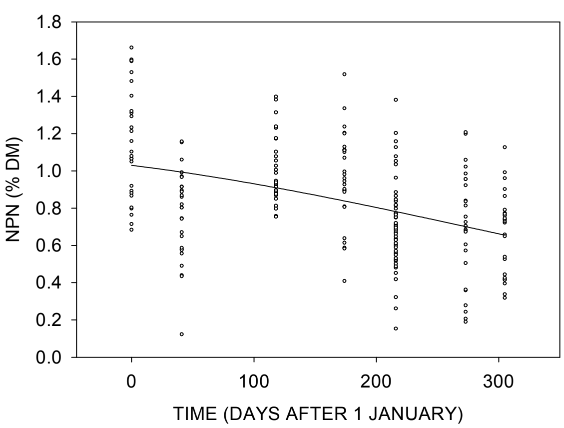


Figure 5.24. Annual variation in NPN content over all KxS treatments (P<0.0001, R^2=0.180, $F_{2,213}$ = 23.3893)

Table 5.12. The influence of sampling date and applied K and S on the NPN content of perennial ryegrass

Herbage NPN content (% DM) - KxS experiment										
	Treatment									
Cut date	**K1S1**	**K1S2**	**K1S3**	**K2S1**	**K2S2**	**K2S3**	**K3S1**	**K3S2**	**K3S3**	**Mean**
13-Jul-04	0.810	0.877	0.834	0.942	0.892	1.353	0.995	0.845	1.108	**0.962[b*]**
24-Aug-04	0.903	0.796	0.633	0.846	0.839	0.745	0.583	0.870	0.707	**0.769[a]**
20-Oct-04	0.799	0.878	0.897	0.386	0.725	0.589	0.553	0.822	0.697	**0.705[a]**
20-Jan-05	0.941	1.178	1.179	1.008	1.147	1.134	1.189	1.185	1.396	**1.151[c]**
01-Mar-05	0.725	0.688	0.757	0.867	0.765	0.717	0.775	0.758	0.836	**0.765[a]**
17-May-05	0.974	0.910	0.946	0.875	1.095	1.114	1.093	1.110	0.988	**1.012[bc]**
22-Aug-05	0.679	0.687	0.727	0.574	0.660	0.635	0.670	0.641	0.691	**0.663[a]**
21-Nov-05	0.753	0.793	0.606	0.645	0.799	0.615	0.451	0.493	0.818	**0.664[a]**
Mean	**0.823[a*]**	**0.851[a]**	**0.822[a]**	**0.768[a]**	**0.865[a]**	**0.863[a]**	**0.789[a]**	**0.840[a]**	**0.905[a]**	
LSD (0.05) Date = 0.1547										
LSD (0.05) Treatment = 0.1458 (NS)										
LSD (0.05) Date x Treatment = 0.4640 (NS)										
CV = 31.5										
	K1	**K2**	**K3**		**S1**	**S2**	**S3**			
13-Jul-04	0.840	1.062	0.983		0.916	0.871	1.098			
24-Aug-04	0.777	0.810	0.720		0.778	0.835	0.695			
20-Oct-04	0.858	0.567	0.690		0.579	0.809	0.728			
20-Jan-05	1.099	1.096	1.257		1.046	1.170	1.236			
01-Mar-05	0.723	0.783	0.790		0.789	0.737	0.770			
17-May-05	0.944	1.028	1.064		0.981	1.038	1.016			
22-Aug-05	0.698	0.623	0.667		0.641	0.663	0.685			
21-Nov-05	0.718	0.686	0.587		0.617	0.695	0.680			
Mean	**0.832[a*]**	**0.832[a]**	**0.845[a]**		**0.793[a*]**	**0.852[a]**	**0.863[a]**			
LSD (0.05) K = 0.818 (NS)										
LSD (0.05) KxDate = 0.2451 (NS)										
CV = 29.5										
LSD (0.05) S = 0.0750 (NS)										
LSD (0.05) SxDate = 0.2511 (NS)										
CV = 30.1										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.2 The effects of applied fertiliser N, P, K and S on non-structural carbohydrates in perennial ryegrass

5.1.2.1 *Non-structural carbohydrates*

5.1.2.1.1 *NxP*

Increasing N applications resulted in decreased NSC levels in perennial ryegrass significantly (P=0.01) when data were averaged over all sampling dates (Figure 5.25).

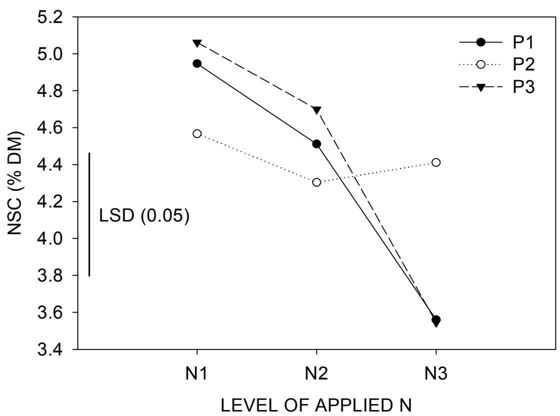


Figure 5.25. The effect of applied N and P on the NSC content of perennial ryegrass, averaged over all seasons.

Nitrogen application had a significant (P<0.001) effect on NSC content at individual sampling dates (Figure 5.26). NSC content also varied significantly (P<0.001) between sampling dates, with season 2 levels not reaching those attained in the first season after planting. Phosphorus did not affect NSC levels in the herbage.

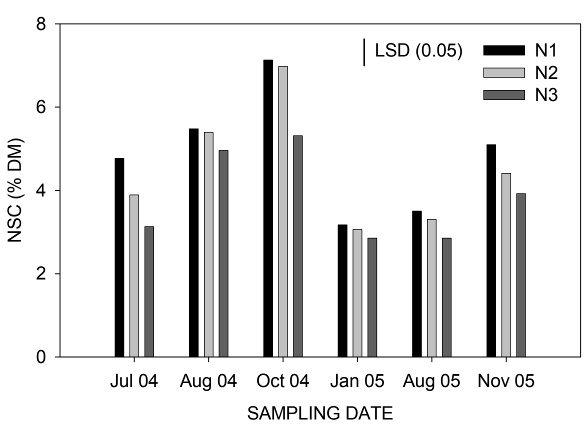


Figure 5.26. Fluctuations in NSC over time for three levels of applied N

Annual variation in the levels of herbage NSC was best described by the cubic equation:

$$y = 3.0460 - 0.0724x + 0.0007x^2 - 1.2996\exp - 6^3$$

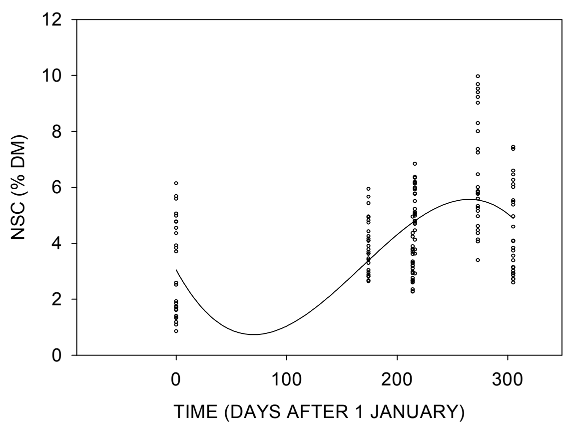


Figure 5.27. Annual variation in NSC content over all NxP treatments ($P<0.001$, $R^2=0.237$, $F_{3,158} = 16.4021$)

Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.13.

Table 5.13. The influence of sampling date and applied N and P on the NSC content of perennial ryegrass

Herbage NSC content (% DM) - NxP experiment										
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	4.666	5.069	4.578	4.065	3.499	4.104	3.203	3.408	2.778	**3.930^{bc*}**
24-Aug-04	5.876	5.636	4.922	4.714	5.581	5.869	4.119	6.029	4.730	**5.275^{d}**
20-Oct-04	6.985	5.885	8.515	7.362	6.978	6.584	4.625	6.644	4.667	**6.472^{e}**
20-Jan-05	3.064	2.682	3.770	2.850	2.063	4.276	2.607	3.716	2.245	**3.030^{a}**
22-Aug-05	3.788	3.668	3.051	3.087	3.291	3.531	3.123	2.647	2.794	**3.220^{ab}**
21-Nov-05	5.302	4.461	5.532	4.989	4.408	3.830	3.685	4.020	4.061	**4.476^{cd}**
Mean	**4.947^{b*}**	**4.567^{b}**	**5.061^{b}**	**4.511^{b}**	**4.303^{ab}**	**4.699^{b}**	**3.560^{a}**	**4.411^{b}**	**3.546^{a}**	

LSD (0.05) Date = 0.800
LSD (0.05) Treatment = 0.760
LSD (0.05) Date x Treatment = 2.399 (NS)
CV = 30.5

	N1	**N2**	**N3**	**P1**	**P2**	**P3**
13-Jul-04	4.771	3.890	3.130	3.978	3.992	3.820
24-Aug-04	5.478	5.388	4.959	4.903	5.749	5.174
20-Oct-04	7.128	6.975	5.312	6.324	6.502	6.589
20-Jan-05	3.172	3.063	2.856	2.840	2.821	3.431
22-Aug-05	3.502	3.303	2.855	3.333	3.202	3.125
21-Nov-05	5.098	4.409	3.922	4.658	4.296	4.474
Mean	**4.858^{b*}**	**4.505^{b}**	**3.839^{a}**	**4.339^{a*}**	**4.427^{a}**	**4.435^{a}**

LSD (0.05) N = 0.475
LSD (0.05) NxDate = 1.314 (NS)
CV = 29.2
LSD (0.05) P = 0.658 (NS)
LSD (0.05) PxDate = 1.338 (NS)
CV = 29.6

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.2.1.2 *NxK*

Nitrogen application significantly decreased NSC content of herbage (P=0.006) over the entire period of the trial (Figure 5.28). Potassium did not affect NSC levels.

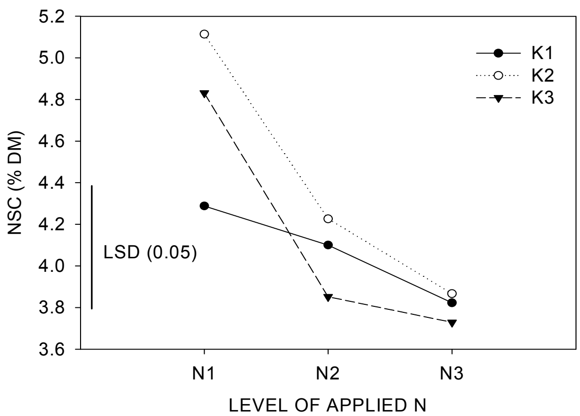


Figure 5.28. The effect of applied N and K on the NSC content of perennial ryegrass, averaged over all seasons.

Non-structural carbohydrate levels in the herbage decreased significantly (P<0.001) with increasing N application at individual sampling dates (Figure 5.29). Second-season levels of NSC did not reach those of the first season after planting.

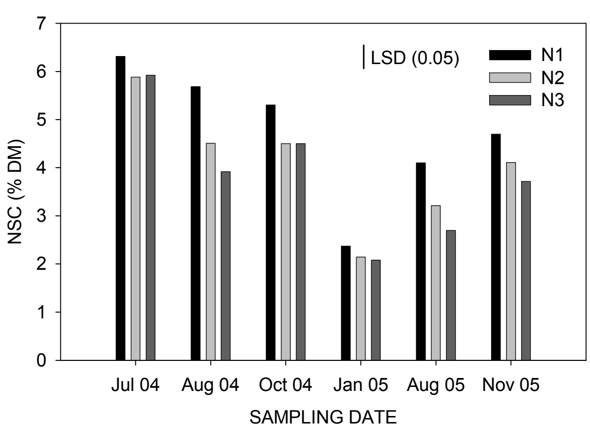


Figure 5.29. Fluctuations in NSC over time for three levels of applied N

NSC levels were lowest in mid-summer and peaked in mid-July, although variation between seasons was high (Figure 5.30).

Annual variation in the levels of herbage NSC was best described by the quadratic equation:

$$y = 2.2902 + 0.0260x - 0.0001x^2$$

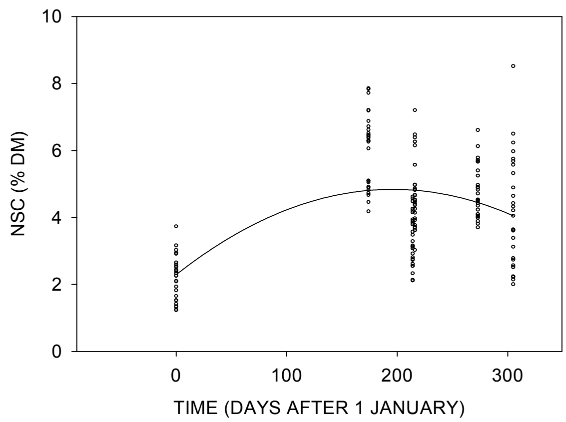


Figure 5.30. Annual variation in NSC content over all NxK treatments ($P<0.001$, $R^2=0.324$, $F_{2,159} = 38.1033$)

Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.14.

Table 5.14. The influence of sampling date and applied N and K on the NSC content of perennial ryegrass

	Herbage NSC content (% DM) - NxK experiment									
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	5.650	6.878	6.404	5.593	6.570	5.480	5.914	6.440	5.411	**6.038[e*]**
24-Aug-04	4.376	6.645	6.035	4.648	4.416	4.452	4.509	3.753	3.497	**4.703[cd]**
20-Oct-04	5.312	5.163	5.428	4.462	4.789	4.252	4.003	4.458	5.041	**4.767[d]**
20-Jan-05	2.284	2.546	2.283	2.405	2.164	1.874	1.995	2.194	2.059	**2.200[a]**
22-Aug-05	4.194	4.445	3.664	2.885	3.736	3.018	2.599	2.697	2.791	**3.336[b]**
21-Nov-05	3.914	5.009	5.171	4.607	3.681	4.032	3.917	3.656	3.571	**4.173[c]**
Mean	**4.288[ab*]**	**5.114[c]**	**4.831[bc]**	**4.100[a]**	**4.226[ab]**	**3.851[a]**	**3.823[a]**	**3.866[a]**	**3.728[a]**	

LSD (0.05) Date = 0.5892
LSD (0.05) Treatment = 0.7168
LSD (0.05) Date x Treatment = 1.7676 (NS)
CV = 23.4

	N1	**N2**	**N3**		**K1**	**K2**	**K3**
13-Jul-04	6.310	5.881	5.922		5.719	6.629	5.765
24-Aug-04	5.685	4.505	3.919		4.511	4.938	4.661
20-Oct-04	5.301	4.501	4.501		4.592	4.803	4.907
20-Jan-05	2.371	2.147	2.083		2.228	2.301	2.072
22-Aug-05	4.101	3.213	2.696		3.226	3.626	3.158
21-Nov-05	4.698	4.107	3.715		4.146	4.115	4.258
Mean	**4.744[b*]**	**4.059[a]**	**3.806[a]**		**4.070[a*]**	**4.402[a]**	**4.137[a]**

LSD (0.05) N = 0.4194
LSD (0.05) NxDate = 0.9332 (NS)
CV = 22.3
LSD (0.05) K = 0.5798 (NS)
LSD (0.05) KxDate = 0.9391 (NS)
CV = 22.6

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.2.1.3 *NxS*

Application of N resulted in a significant decrease in perennial ryegrass NSC content (P=0.006) in the NxS experiment. Sulphur applications did not affect the concentration of NSC in the herbage (Figure 5.31).

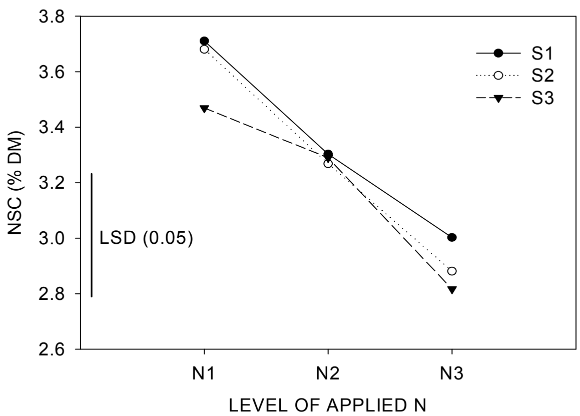


Figure 5.31. The effect of applied N and S on the NSC content of perennial ryegrass, averaged over all seasons.

Both date and level of applied N had a highly significant (P<0.001) effect on NSC concentration (Figure 5.32) at individual sampling dates. Higher levels of applied N resulted in decreased concentrations of NSC in the herbage, especially during the warmer months of the year (Figures 5.32 and 5.33). The seasonal response is reflected by a significant (P<0.001) date-N interaction. Sulphur application did not affect NSC levels at individual sampling dates in the NxS experiment.

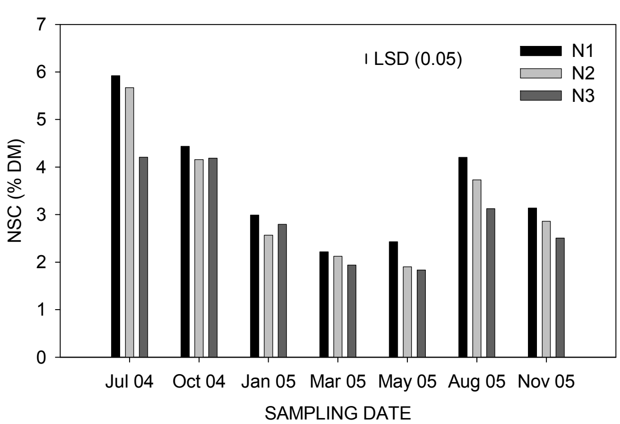


Figure 5.32. Fluctuations of NSC over time for three levels of applied N

Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.15.

Annual variation in the levels of herbage NSC in the NxS experiment was best described by the cubic equation:

$$y = 2.8131 - 0.0372x + 0.0004x^2 - 9.9361\exp{-7}x^3$$

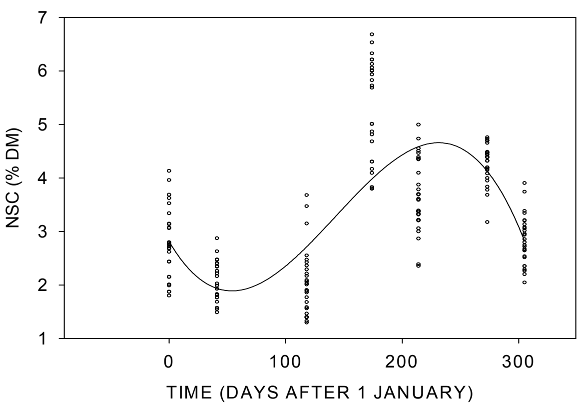


Figure 5.33. Annual variation in NSC content over all NxS treatments (P<0.001, R^2=0.496, $F_{3,183}$ = 60.0856)

Table 5.15. The influence of sampling date and applied N and S on the NSC content of perennial ryegrass

	Herbage NSC content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	6.181	5.852	5.736	6.014	5.412	5.582	4.617	3.783	3.976	**5.239[e*]**
20-Oct-04	4.522	4.502	4.284	3.684	4.571	4.218	4.220	4.057	4.349	**4.268[d]**
20-Jan-05	3.208	2.968	2.793	2.711	2.365	2.626	2.996	2.563	2.829	**2.784[b]**
01-Mar-05	2.354	2.215	2.084	2.073	2.236	2.067	1.704	2.178	1.939	**2.094[a]**
17-May-05	2.617	2.543	2.128	1.888	2.073	1.741	1.551	2.116	1.836	**2.055[a]**
22-Aug-05	3.924	4.222	4.472	3.799	3.387	3.998	3.509	3.008	2.858	**3.686[c]**
21-Nov-05	3.170	3.463	2.788	2.951	2.834	2.795	2.422	2.652	2.452	**2.836[b]**
Mean	**3.711[d*]**	**3.681[d]**	**3.469[cd]**	**3.303[bc]**	**3.268[bc]**	**3.289[bc]**	**3.002[ab]**	**2.908[a]**	**2.891[a]**	

LSD (0.05) Date = 0.3076
LSD (0.05) Treatment = 0.3239
LSD (0.05) Date x Treatment = 0.9227 (NS, P = 0.080)**
CV = 16.2

	N1	**N2**	**N3**		**S1**	**S2**	**S3**
13-Jul-04	5.923	5.669	4.194		5.604	5.140	5.098
20-Oct-04	4.436	4.158	4.172		4.142	4.377	4.227
20-Jan-05	2.990	2.567	2.796		2.972	2.632	2.749
01-Mar-05	2.218	2.125	1.940		2.044	2.210	2.030
17-May-05	2.429	1.901	1.834		2.019	2.244	1.902
22-Aug-05	4.206	3.728	3.125		3.744	3.539	3.776
21-Nov-05	3.140	2.860	2.509		2.847	2.983	2.678
Mean	**3.620[c*]**	**3.287[b]**	**2.939[a]**		**3.339[a]**	**3.304**	**3.209**

LSD (0.05) N = 0.1689
LSD (0.05) NxDate = 0.5093
CV = 15.6
LSD (0.05) S = 0.3364 (NS)
LSD (0.05) SxDate = 0.5822 (NS)
CV = 17.2

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.2.1.4 *PxS*

Neither P nor S application affected NSC content in herbage in the PxS experiment. As with the other experiments, there was a significant effect of sampling date on NSC values (P<0.001), which were lowest in the warmer months of the year (Figure 5.34). Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.16.

Annual variation in the levels of herbage NSC in the PxS experiment was best described by the cubic equation:

$$y = 3.5005 - 0.0514x + 0.0005x^2 - 1.1409\exp{-6x^3}$$

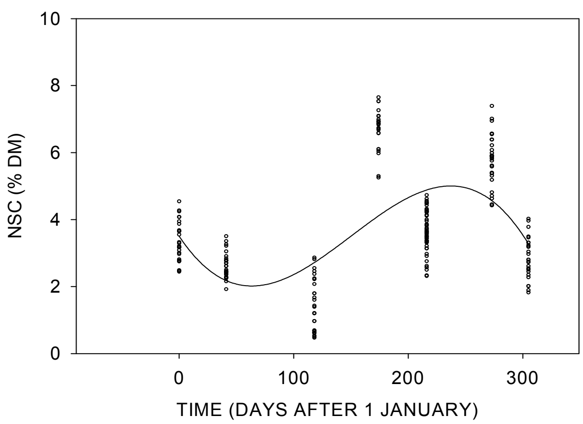


Figure 5.34. Annual variation in NSC content over all PxS treatments (P<0.001, R^2=0.305, $F_{3,212}$ = 31.0622)

Table 5.16. The influence of sampling date and applied P and S on the NSC content of perennial ryegrass

Herbage NSC content (% DM) - PxS experiment										
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	6.733	7.479	7.032	6.112	6.792	7.107	6.351	6.284	6.505	**6.711^{f*}**
24-Aug-04	3.915	4.021	4.292	3.882	3.963	4.067	3.967	3.807	4.157	**4.008^{d}**
20-Oct-04	5.810	5.333	6.529	5.856	5.215	5.363	6.171	5.713	6.048	**5.782^{e}**
20-Jan-05	3.121	3.368	4.351	2.934	3.201	3.463	3.742	2.999	2.967	**3.350^{c}**
01-Mar-05	2.452	2.562	2.492	2.284	2.818	3.004	2.486	2.842	2.792	**2.637^{b}**
17-May-05	1.508	1.175	1.423	1.487	1.676	1.174	1.794	1.645	1.121	**1.445^{a}**
22-Aug-05	3.190	3.429	3.188	3.270	3.077	4.122	2.823	3.742	3.071	**3.323^{c}**
21-Nov-05	3.359	3.034	2.555	2.643	3.684	2.782	2.703	2.471	2.444	**2.853^{b}**
Mean	**3.761^{abc*}**	**3.800^{abc}**	**3.983^{c}**	**3.558^{a}**	**3.803^{abc}**	**3.885^{bc}**	**3.755^{abc}**	**3.688^{ab}**	**3.638^{ab}**	

LSD (0.05) Date = 0.3476
LSD (0.05) Treatment = 0.2633 (NS, P = 0.099)**
LSD (0.05) Date x Treatment = 1.0429 (NS)
CV = 15.8

	P1	**P2**	**P3**		**S1**	**S2**	**S3**
13-Jul-04	7.082	6.670	6.380		6.398	6.852	6.881
24-Aug-04	4.076	3.971	3.977		3.921	3.931	4.172
20-Oct-04	5.891	5.478	5.977		5.946	5.420	5.980
20-Jan-05	3.614	3.199	3.236		3.266	3.189	3.594
01-Mar-05	2.502	2.702	2.706		2.407	2.740	2.763
17-May-05	1.369	1.446	1.520		1.597	1.499	1.239
22-Aug-05	3.269	3.490	3.212		3.094	3.416	3.460
21-Nov-05	2.983	3.036	2.539		2.902	3.063	2.594
Mean	**3.848^{a*}**	**3.749^{a}**	**3.694^{a}**		**3.691^{a*}**	**3.764^{a}**	**3.835^{a}**

LSD (0.05) P = 0.1678 (NS)
LSD (0.05) PxDate = 0.6044 (NS)
CV = 16.2
LSD (0.05) S = 0.1700 (NS)
LSD (0.05) SxDate = 0.5985 (NS)
CV = 16.0

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.2.1.5 *PxK*

There were no significant effects of either P or K on NSC concentration in the PxK experiment. NSC levels responded significantly to season (P<0.001), with levels increasing with cooler conditions (Figure 5.35). Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.17.

Annual variation in the levels of herbage NSC was best described by the cubic equation:

$$y = 3.1041 - 0.0361x + 0.0004x^2 - 8.8341\exp{-7}x^3$$

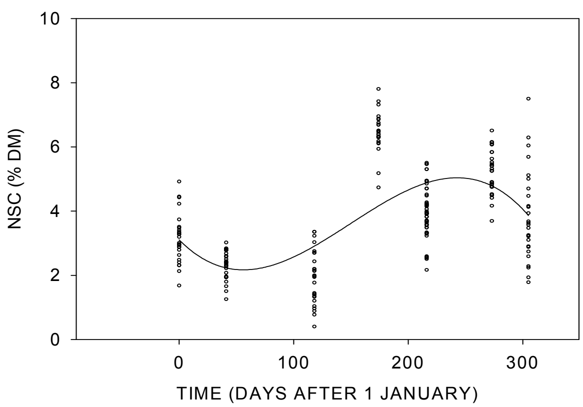


Figure 5.35. Annual variation in NSC content over all PxK treatments (P<0.001, R^2=0.349, $F_{3,212}$ = 37.9128)

Table 5.17. The influence of sampling date and applied P and K on the NSC content of perennial ryegrass

Herbage NSC content (% DM) - PxK experiment										
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	6.367	6.724	6.864	5.906	6.409	6.382	6.102	6.683	6.628	**6.452**[f*]
24-Aug-04	3.913	3.468	3.743	3.424	3.970	3.158	3.538	3.440	3.537	**3.577**[bc]
20-Oct-04	4.493	5.056	5.822	5.969	5.707	4.898	5.347	4.993	4.683	**5.219**[e]
20-Jan-05	2.732	3.906	2.756	3.287	3.339	2.717	3.608	2.585	3.379	**3.145**[b]
01-Mar-05	2.318	2.504	2.455	2.469	2.578	1.825	2.184	2.089	2.317	**2.304**[a]
17-May-05	1.786	1.935	2.001	1.825	1.412	2.394	1.789	2.249	1.977	**1.930**[a]
22-Aug-05	3.996	5.423	4.580	4.021	4.265	4.766	3.829	3.940	4.362	**4.354**[d]
21-Nov-05	3.679	3.397	2.893	3.343	3.761	5.671	4.011	4.052	3.701	**3.834**[c]
Mean	**3.660**[a*]	**4.052**[a]	**3.889**[a]	**3.780**[a]	**3.930**[a]	**3.976**[a]	**3.801**[a]	**3.754**[a]	**3.823**[a]	

LSD (0.05) Date = 0.4638
LSD (0.05) Treatment = 0.4047 (NS)
LSD (0.05) Date x Treatment = 1.3914 (NS)
CV = 20.7

	P1	**P2**	**P3**	**K1**	**K2**	**K3**
13-Jul-04	6.652	6.232	6.471	6.125	6.605	6.625
24-Aug-04	3.708	3.517	3.505	3.625	3.626	3.479
20-Oct-04	5.124	5.524	5.008	5.270	5.252	5.134
20-Jan-05	3.132	3.114	3.190	3.209	3.277	2.951
01-Mar-05	2.425	2.291	2.197	2.323	2.390	2.199
17-May-05	1.907	1.877	2.005	1.800	1.865	2.124
22-Aug-05	4.666	4.351	4.044	3.949	4.543	4.569
21-Nov-05	3.323	4.258	3.922	3.678	3.737	4.088
Mean	**3.867**[a*]	**3.896**[a]	**3.793**[a]	**3.747**[a*]	**3.912**[a]	**3.896**[a]

LSD (0.05) P = 0.2261 (NS)
LSD (0.05) PxDate = 0.8206 (NS)
CV = 20.9
LSD (0.05) K = 0.2165 (NS)
LSD (0.05) KxDate = 0.8341 (NS)
CV = 21.2

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.2.1.6 *KxS*

NSC content was unaffected by both applied K and S fertiliser in the KxS experiment. NSC levels were lowest in mid-summer and peaked with the onset of cooler conditions (Figure 5.36). Data reflecting the effects of treatment and sampling date on herbage NSC are summarised in Table 5.18.

Annual variation in the levels of herbage NSC was best described by the Gaussian 3-parameter equation:

$$y = 4.2875^{\left[-0.5\left(\frac{x-212.3980}{129.5904}\right)^2\right]}$$

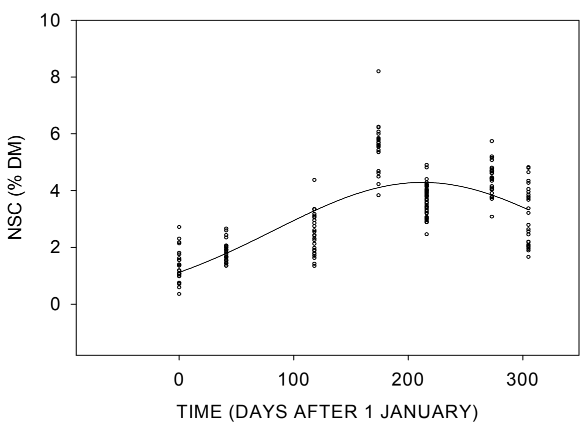


Figure 5.36. Annual variation in NSC content over all KxS treatments ($P<0.001$, $R^2=0.447$, $F_{2,213} = 140.2755$)

Table 5.18. The influence of sampling date and applied K and S on the NSC content of perennial ryegrass

	Herbage NSC content (% DM) - KxS experiment									
	Treatment									
Cut date	K1S1	K1S2	K1S3	K2S1	K2S2	K2S3	K3S1	K3S2	K3S3	Mean
13-Jul-04	5.325	5.849	4.695	5.721	6.671	5.565	4.994	5.172	5.717	5.523[g*]
24-Aug-04	3.609	3.613	3.231	3.818	4.006	3.613	3.815	3.432	3.718	3.650[e]
20-Oct-04	4.309	4.097	4.132	4.349	4.560	5.095	4.352	4.520	4.497	4.435[f]
20-Jan-05	1.605	1.339	1.239	1.073	1.586	1.746	0.932	1.324	1.121	1.330[a]
01-Mar-05	1.865	1.779	2.092	1.782	2.104	1.687	1.442	1.723	2.081	1.840[b]
17-May-05	3.252	2.450	2.082	2.169	2.753	1.911	2.519	2.330	3.012	2.498[c]
22-Aug-05	3.631	4.258	4.154	3.015	3.902	3.722	3.622	3.905	3.564	3.752[e]
21-Nov-05	3.027	2.902	3.260	2.675	2.680	3.748	3.545	2.668	3.171	3.075[d]
Mean	3.328[ab*]	3.286[ab]	3.111[a]	3.075[a]	3.533[b]	3.386[ab]	3.153[ab]	3.134[a]	3.360[ab]	

LSD (0.05) Date = 0.3770
LSD (0.05) Treatment = 0.3984 (NS)
LSD (0.05) Date x Treatment = 1.1311 (NS)
CV = 20.0

	K1	K2	K3	S1	S2	S3
13-Jul-04	5.290	5.986	5.294	5.347	5.898	5.326
24-Aug-04	3.484	3.812	3.655	3.747	3.683	3.520
20-Oct-04	4.179	4.668	4.457	4.337	4.392	4.575
20-Jan-05	1.394	1.468	1.126	1.203	1.416	1.369
01-Mar-05	1.912	1.858	1.749	1.697	1.869	1.953
17-May-05	2.595	2.278	2.620	2.647	2.511	2.335
22-Aug-05	4.014	3.546	3.697	3.423	4.022	3.813
21-Nov-05	3.063	3.034	3.128	3.082	2.750	3.393
Mean	3.242[a*]	3.331[a]	3.216[a]	3.186[a*]	3.318[a]	3.286[a]

LSD (0.05) K = 0.2421 (NS)
LSD (0.05) KxDate = 0.6402 (NS)
CV = 19.5
LSD (0.05) S = 0.2403 (NS)
LSD (0.05) SxDate = 0.6343 (NS)
CV = 19.6

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3 The effects of applied fertiliser N, P, K and S on acid detergent fibre and neutral detergent fibre in perennial ryegrass

5.1.3.1 *Acid detergent fibre*

5.1.3.1.1 *NxP*

Neither applied N nor P had any effect on ADF concentrations in herbage when averaged over the season or at individual sampling dates. Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.19.

Annual variation in the levels of herbage ADF was best described by the quadratic equation:

$$y = 33.5458 - 0.0945x + 0.0003x^2$$

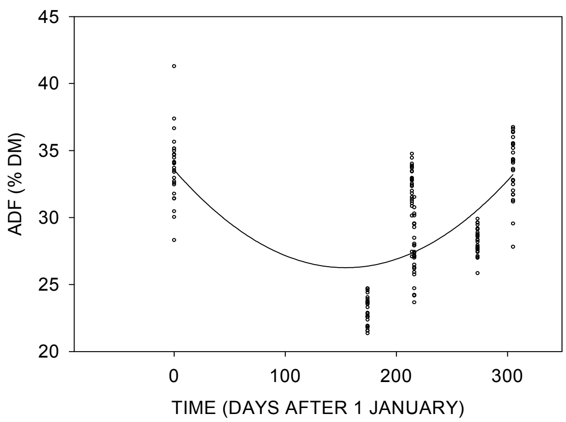


Figure 5.37. Annual variation in ADF content over all NxP treatments ($P<0.001$, $R^2=0.447$, $F_{2,159} = 64.3899$)

Table 5.19. The influence of sampling date and applied N and P on the ADF content of perennial ryegrass

	Herbage ADF content (% DM) - NxP experiment									
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	23.381	22.563	22.954	22.908	22.409	23.158	23.647	23.381	22.929	**23.037[a*]**
24-Aug-04	28.555	27.947	27.794	27.348	26.793	27.377	27.074	26.952	27.200	**27.449[b]**
20-Oct-04	28.072	28.475	27.498	28.714	27.777	29.177	28.676	27.892	27.986	**28.252[b]**
20-Jan-05	34.273	34.041	34.368	36.488	31.609	34.171	31.831	35.664	31.278	**33.747[d]**
22-Aug-05	29.585	32.926	31.193	33.375	32.492	31.293	33.020	33.233	33.326	**32.271[c]**
21-Nov-05	33.646	33.889	33.552	35.365	34.534	34.138	31.517	32.402	34.391	**33.715[d]**
Mean	**29.585[a*]**	**29.974[ab]**	**29.560[a]**	**30.700[b]**	**29.269[a]**	**29.886[ab]**	**29.294[a]**	**29.920[ab]**	**29.518[a]**	
LSD (0.05) Date = 1.009										
LSD (0.05) Treatment = 1.029 (NS)										
LSD (0.05) Date x Treatment = 3.027 (NS)										
CV = 5.8										
	N1	**N2**	**N3**		**P1**	**P2**	**P3**			
13-Jul-04	22.966	22.825	23.319		23.312	22.784	23.013			
24-Aug-04	28.099	27.173	27.075		27.659	27.231	27.457			
20-Oct-04	28.015	28.556	28.184		28.487	28.048	28.220			
20-Jan-05	34.227	34.089	32.924		34.197	33.771	33.273			
22-Aug-05	31.235	32.387	33.193		31.993	32.884	31.938			
21-Nov-05	33.696	34.679	32.770		33.509	33.609	34.027			
Mean	**29.706[a*]**	**29.952[a]**	**29.578[a]**		**29.860[a*]**	**29.721[a]**	**29.655[a]**			
LSD (0.05) N = 0.648 (NS)										
LSD (0.05) NxDate = 1.736 (NS)										
CV = 5.9										
LSD (0.05) P = 0.663 (NS)										
LSD (0.05) PxDate = 1.813 (NS)										
CV = 6.2										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.1.2 *NxK*

Neither N nor K affected ADF concentration when data were averaged over the entire season. Nitrogen did not affect the levels of ADF in the herbage at individual sampling dates. Effects of applied K on ADF were significant at the 10% level (P=0.054). ADF content increased as the pasture aged. Levels peaked in mid-summer and decreased slightly thereafter into the second season, but remained higher than first-season levels. Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.20.

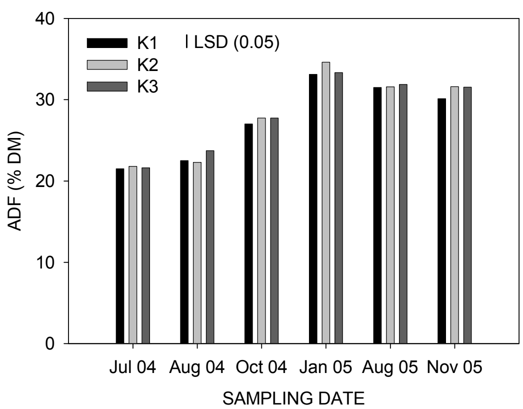


Figure 5.38. Fluctuations in ADF over time for three levels of applied K

Annual variation in the levels of herbage ADF was best described by the quadratic equation:

$$y = 33.4927 - 0.1101x + 0.0003x^2$$

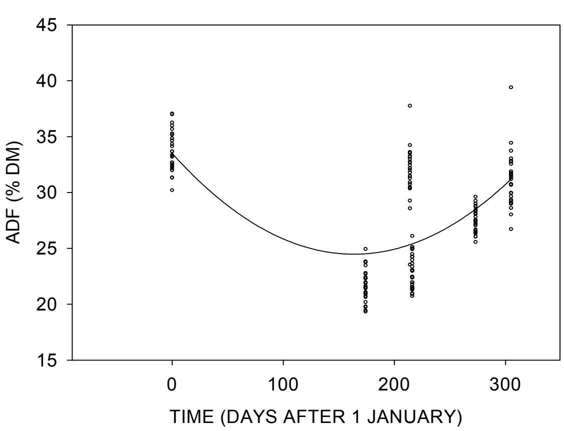


Figure 5.39. Annual variation in ADF content over all NxK treatments (P<0.001, R^2=0.469, $F_{2,159}$ = 70.0957)

Table 5.20. The influence of sampling date and applied N and K on the ADF content of perennial ryegrass

Herbage ADF content (% DM) - NxK experiment										
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	21.678	21.491	21.764	20.482	21.661	21.077	22.357	22.222	22.037	**21.641[a*]**
24-Aug-04	21.970	22.232	23.578	23.976	23.301	23.438	21.618	21.342	24.178	**22.848[b]**
20-Oct-04	27.528	27.087	27.378	26.416	27.709	27.257	27.100	28.440	28.619	**27.504[c]**
20-Jan-05	33.009	34.769	33.060	33.764	34.725	33.842	32.592	34.322	33.106	**33.688[e]**
22-Aug-05	31.396	30.906	31.245	29.752	31.365	31.292	33.377	32.525	33.078	**31.660[d]**
21-Nov-05	30.614	32.004	33.059	30.686	30.800	30.753	29.090	32.018	30.823	**31.094[d]**
Mean	**27.699[a*]**	**28.082[a]**	**28.347[a]**	**27.513[a]**	**28.260[a]**	**27.943[a]**	**27.689[a]**	**28.478[a]**	**28.640[a]**	
LSD (0.05) Date = 1.183										
LSD (0.05) Treatment = 1.187 (NS)										
LSD (0.05) Date x Treatment = 3.548 (NS)										
CV = 7.1										

	N1	**N2**	**N3**		**K1**	**K2**	**K3**
13-Jul-04	21.644	21.073	22.205		21.505	21.791	21.626
24-Aug-04	22.593	23.572	22.379		22.521	22.292	23.731
20-Oct-04	27.331	27.127	28.053		27.014	27.745	27.751
20-Jan-05	33.613	34.110	33.340		33.122	34.605	33.336
22-Aug-05	31.183	30.803	32.993		31.509	31.599	31.872
21-Nov-05	31.892	30.746	30.644		30.130	31.607	31.545
Mean	**28.043[a*]**	**27.905[a]**	**28.269[a]**		**27.634[a*]**	**28.273[b]**	**28.310[b]**
LSD (0.05) N = 0.677 (NS)							
LSD (0.05) NxDate = 1.859 (NS)							
CV = 6.6							
LSD (0.05) K = 0.610 (NS, P = 0.054)**							
LSD (0.05) KxDate = 1.919 (NS)							
CV = 6.8							

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.3.1.3 *NxS*

Acid detergent fibre showed no response to fertiliser N or S when data were averaged over the entire trial. Nitrogen application had no effect on ADF levels at individual sampling dates. Applied S appeared to have a slight effect on ADF levels in the second season (P=0.052), where intermediate and high levels of S fertiliser resulted in lower ADF content of herbage than did the low levels of applied S. By the end of the second season the ADF differences between levels of applied S had disappeared (Figure 5.40).

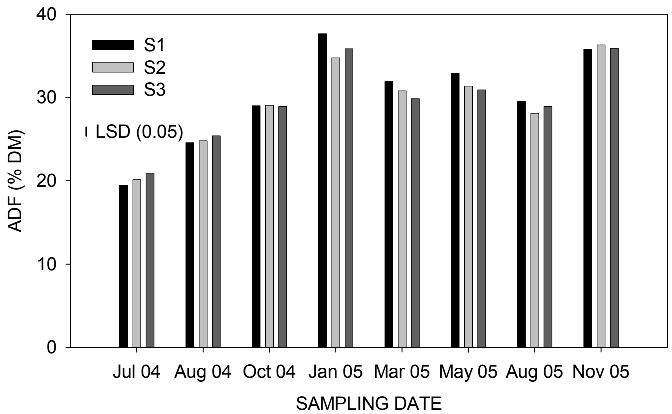


Figure 5.40. Fluctuations in ADF over time for three levels of applied S

Levels of ADF in the ryegrass were significantly affected by sampling date (P<0.001), with ADF increasing steadily into the second season and then leveling off to a large extent, with concentrations peaking in January and November. This is also reflected when data were pooled to show annual fluctuations in ADF content of herbage (Figure 5.41). Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.21.

Annual variation in the levels of herbage ADF was best described by the quadratic equation:

$$y = 37.0648 - 0.1442x + 0.0004x^2$$

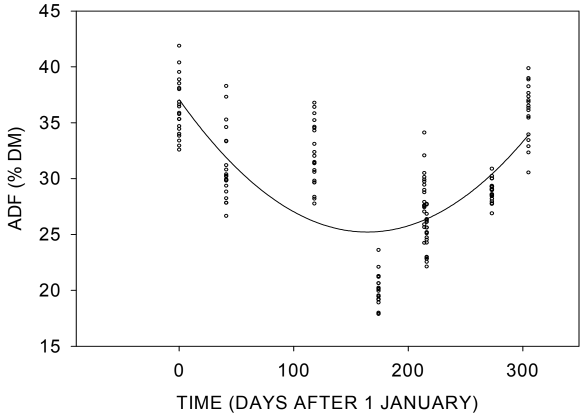


Figure 5.41. Annual variation in ADF content over all NxS treatments ($P<0.001$, $R^2=0.527$, $F_{2,159} = 88.5090$)

Table 5.21. The influence of sampling date and applied N and S on the ADF content of perennial ryegrass

	Herbage ADF content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	19.173	21.096	20.547	19.799	19.183	20.715	19.439	20.084	21.472	**20.171[a*]**
24-Aug-04	24.600	25.799	26.565	23.670	23.524	25.046	25.397	25.042	24.583	**24.914[b]**
20-Oct-04	29.009	29.290	29.200	29.219	28.240	28.620	28.764	29.643	28.843	**28.986[b]**
20-Jan-05	37.194	36.666	35.296	38.813	34.821	34.778	36.899	32.751	37.436	**36.073[d]**
01-Mar-05	32.492	31.451	30.095	31.441	31.206	29.281	31.781	29.693	30.154	**30.844[c]**
17-May-05	33.259	30.649	31.959	33.469	31.755	31.598	32.068	31.690	29.145	**31.732[c]**
22-Aug-05	28.828	29.029	28.773	29.749	26.762	28.379	30.054	28.477	29.593	**28.849[b]**
21-Nov-05	36.472	35.478	37.058	34.490	36.421	36.086	36.471	36.980	34.554	**36.001[d]**
Mean	**30.128[b*]**	**29.932[ab]**	**29.937[ab]**	**30.081[b]**	**28.989[a]**	**29.313[ab]**	**30.109[b]**	**29.295[ab]**	**29.500[ab]**	

LSD (0.05) Date = 1.595
LSD (0.05) Treatment = 1.047 (NS)
LSD (0.05) Date x Treatment = 4.784 (NS)
CV = 9.0

	N1	**N2**	**N3**		**S1**	**S2**	**S3**
13-Jul-04	20.272	19.899	20.310		19.470	20.126	20.911
24-Aug-04	25.654	24.080	25.007		24.556	24.788	25.398
20-Oct-04	29.166	28.693	29.080		28.997	29.058	28.893
20-Jan-05	36.386	36.137	35.696		37.635	34.746	35.837
01-Mar-05	31.346	30.643	30.542		31.904	30.783	29.843
17-May-05	31.956	32.274	30.968		32.932	31.364	30.901
22-Aug-05	28.877	28.297	29.375		29.544	28.089	28.915
21-Nov-05	36.336	35.666	36.002		35.811	36.293	35.899
Mean	**29.999[a*]**	**29.461[a]**	**29.622[a]**		**30.106[b*]**	**29.406[a]**	**29.575[ab]**

LSD (0.05) N = 0.623 (NS)
LSD (0.05) NxDate = 2.566 (NS)
CV = 8.5
LSD (0.05) S = 0.585 (NS, P = 0.052)**
LSD (0.05) SxDate = 2.499
CV = 8.3

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.3.1.4 *PxS*

Neither P nor S fertiliser affected ADF concentrations in the herbage either over the entire trial period or at individual sampling dates. As with results from the previous experiments, sampling date appeared to be the main factor affecting ADF levels (P<0.001), which steadily increased throughout the year (Figure 5.42).

Annual variation in the levels of herbage ADF was best described by the Gaussian 3-parameter equation:

$$y = 34.2604^{\left[-0.5\left(\frac{x-327.1084}{358.1319}\right)^2\right]}$$

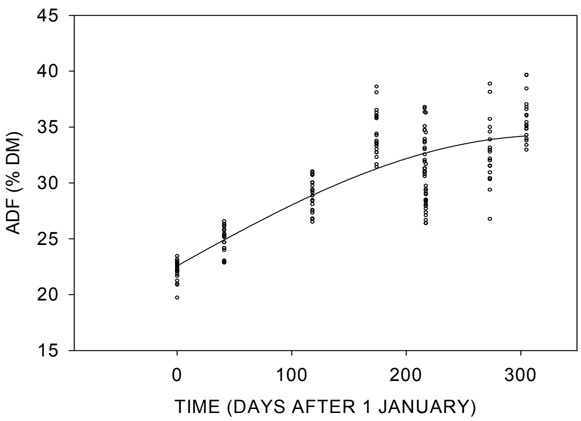


Figure 5.42. Annual variation in ADF content over all PxS treatments (P<0.001, R^2=0.696, $F_{2,159}$ = 181.9306)

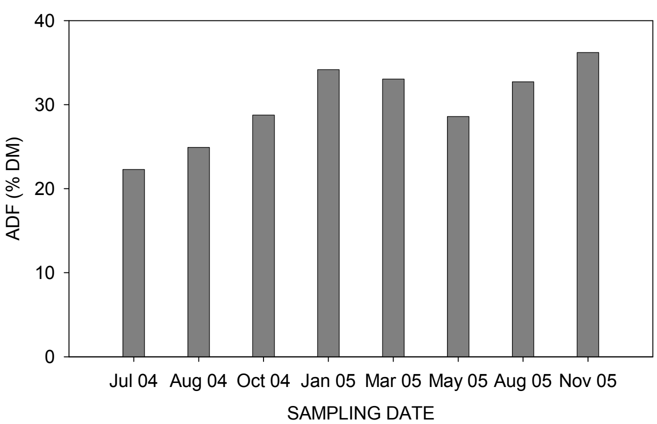


Figure 5.43. Fluctuations in ADF with sampling date

When averaged over all treatments, ADF values increased until mid-summer, then decreased into winter before climbing again. Second-season ADF values were much greater than those in the first season after planting (Figure 5.43). Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.22.

Table 5.22. The influence of sampling date and applied P and S on the ADF content of perennial ryegrass

Herbage ADF content (% DM) - PxS experiment										
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	22.172	21.220	22.661	22.070	22.950	22.444	21.654	22.851	22.630	**22.294[a*]**
24-Aug-04	24.260	25.133	24.831	24.619	25.184	25.490	24.496	25.288	24.997	**24.922[b]**
20-Oct-04	28.999	27.410	29.060	28.529	29.700	29.152	29.901	28.132	27.982	**28.763[c]**
20-Jan-05	35.386	32.797	35.282	33.225	36.880	33.572	34.014	34.433	31.881	**34.163[e]**
01-Mar-05	33.670	34.657	32.299	33.146	32.645	32.083	33.264	33.295	32.405	**33.052[d]**
17-May-05	27.809	30.411	27.524	29.180	30.767	28.496	28.577	26.934	27.579	**28.586[c]**
22-Aug-05	36.172	32.984	32.705	31.087	34.186	29.582	33.926	31.249	32.610	**32.722[d]**
21-Nov-05	36.144	35.065	35.333	35.863	37.259	35.019	35.402	36.926	38.809	**36.202[f]**
Mean	**30.576[ab*]**	**29.960[a]**	**29.962[a]**	**29.715[a]**	**31.196[b]**	**29.480[a]**	**30.154[ab]**	**29.888[a]**	**29.861[a]**	
LSD (0.05) Date = 1.103										
LSD (0.05) Treatment = 1.182 (NS)										
LSD (0.05) Date x Treatment = 3.309 (NS)										
CV = 6.4										
	P1	**P2**	**P3**		**S1**	**S2**	**S3**			
13-Jul-04	22.017	22.488	22.378		21.965	22.340	22.578			
24-Aug-04	24.742	25.098	24.927		24.458	25.202	25.106			
20-Oct-04	28.490	29.127	28.671		29.143	28.414	28.731			
20-Jan-05	34.488	34.559	33.443		34.209	34.703	33.578			
01-Mar-05	33.542	32.625	32.988		33.360	33.533	32.262			
17-May-05	28.581	29.481	27.697		28.522	29.371	27.867			
22-Aug-05	33.954	31.619	32.595		33.728	32.806	31.633			
21-Nov-05	35.514	36.047	37.046		35.803	36.417	36.387			
Mean	**30.166[a*]**	**30.130[a]**	**29.968[a]**		**30.149[a*]**	**30.348[a]**	**29.768[a]**			
LSD (0.05) P = 0.768 (NS)										
LSD (0.05) PxDate = 1.897 (NS)										
CV = 6.4										
LSD (0.05) S = 0.728 (NS)										
LSD (0.05) SxDate = 1.935 (NS)										
CV = 6.5										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.1.5 *PxK*

Applied fertiliser did not affect ADF levels at individual sampling dates in the PxK experiment. ADF concentration increased throughout the growing season and peaked in summer (Figure 5.44), although variation between seasons was high.

Annual variation in the levels of herbage ADF was best described by the Gaussian 3-parameter equation:

$$y = 34.0971^{\left[-0.5\left(\frac{x-277.5743}{328.8177}\right)^2\right]}$$

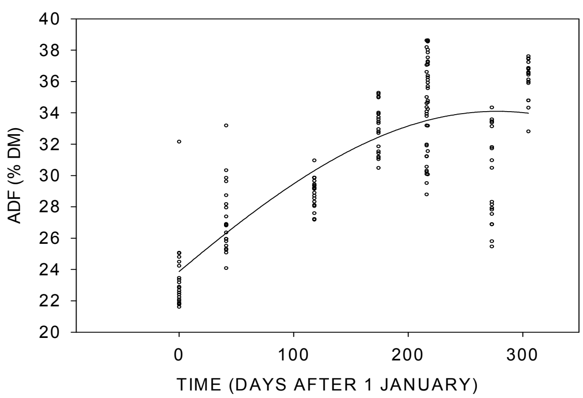


Figure 5.44. Annual variation in ADF content over all PxK treatments ($P<0.001$, $R^2=0.624$, $F_{2,159} = 131.9227$)

Date had a highly significant effect on ADF ($P<0.001$). In the PxK experiment, ADF levels increased into the second season and, although they decreased during winter, they never fell to the concentrations observed during the first growing season (Figure 5.45). Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.23.

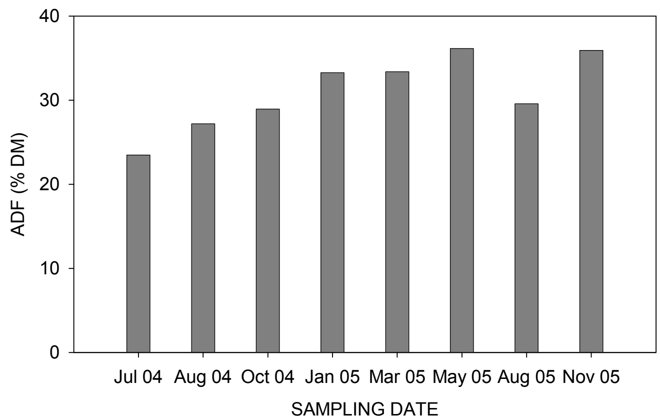


Figure 5.45. Fluctuations in ADF with sampling date

Table 5.23. The influence of sampling date and applied P and K on the ADF content of perennial ryegrass

Herbage ADF content (% DM) - PxK experiment										
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	23.084	23.337	23.319	22.659	22.830	26.339	22.585	23.066	24.050	**23.474[a*]**
24-Aug-04	26.941	26.911	28.499	26.232	26.894	29.424	25.749	26.111	28.067	**27.203[b]**
20-Oct-04	29.622	29.068	28.630	29.543	28.377	28.300	29.012	28.581	29.396	**28.948[c]**
20-Jan-05	32.159	33.916	33.984	33.335	32.615	32.942	32.260	34.240	34.074	**33.281[d]**
01-Mar-05	31.558	33.694	32.597	33.466	31.777	36.443	32.214	35.302	33.419	**33.386[d]**
17-May-05	37.786	35.245	35.271	36.700	36.839	33.906	35.975	37.534	36.136	**36.155[e]**
22-Aug-05	31.038	28.535	28.506	30.725	30.976	29.450	30.126	29.020	27.812	**29.576[c]**
21-Nov-05	36.381	37.092	36.849	35.001	35.115	36.586	34.992	34.567	36.672	**35.917[e]**
Mean	**31.071[ab*]**	**30.975[ab]**	**30.957[ab]**	**30.958[ab]**	**30.678[a]**	**31.674[b]**	**30.364[a]**	**31.053[ab]**	**31.203[ab]**	

LSD (0.05) Date = 1.274
LSD (0.05) Treatment = 0.929 (NS)
LSD (0.05) Date x Treatment = 3.821 (NS)
CV = 6.9

	P1	**P2**	**P3**	**K1**	**K2**	**K3**
13-Jul-04	23.247	23.943	23.234	22.776	23.077	24.569
24-Aug-04	27.450	27.517	26.643	26.307	26.639	28.663
20-Oct-04	29.107	28.740	28.996	29.392	28.675	28.775
20-Jan-05	33.353	32.964	33.525	32.585	33.590	33.667
01-Mar-05	32.616	33.896	33.645	32.413	33.591	34.153
17-May-05	36.101	35.815	36.548	36.820	36.539	35.105
22-Aug-05	29.360	30.384	28.986	30.630	29.511	28.589
21-Nov-05	36.774	35.567	35.410	35.458	35.591	36.702
Mean	**31.001[a*]**	**31.103[a]**	**30.873[a]**	**30.798[a*]**	**30.902[a]**	**31.278[a]**

LSD (0.05) P = 0.568 (NS)
LSD (0.05) PxDate = 2.147 (NS)
CV = 6.9
LSD (0.05) K = 0.532 (NS)
LSD (0.05) KxDate = 2.040 (NS)
CV = 6.6

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.1.6 *KxS*

Treatment did not affect ADF levels in the KxS experiment. ADF concentration in the herbage increased over the growing season, leveling off at the beginning of summer (Figure 5.46). Data reflecting the effects of treatment and sampling date on herbage ADF are summarised in Table 5.24. Annual variation in the levels of herbage ADF was best described by the Gaussian 3-parameter equation:

$$y = 33.6960^{\left[-0.5\left(\frac{x-277.0290}{311.4051}\right)^2\right]}$$

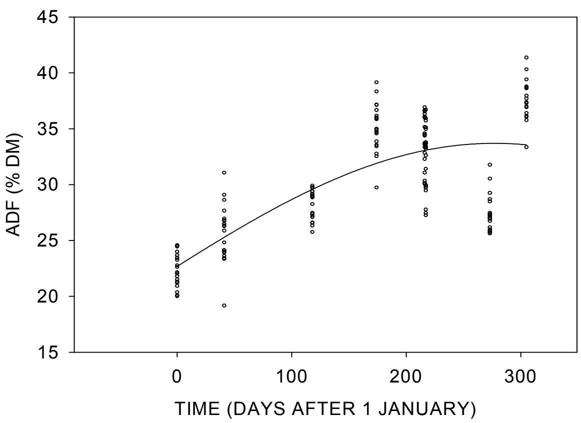


Figure 5.46. Annual variation in ADF content over all KxS treatments ($P<0.001$, $R^2=0.568$, $F_{2,159} = 104.5550$)

ADF responded similarly to sampling date as was observed in the previous experiments. ADF concentrations in the herbage increased from planting, peaking in mid-summer, when they declined although never to the same levels as occurred during the first season after planting (Figure 5.47).

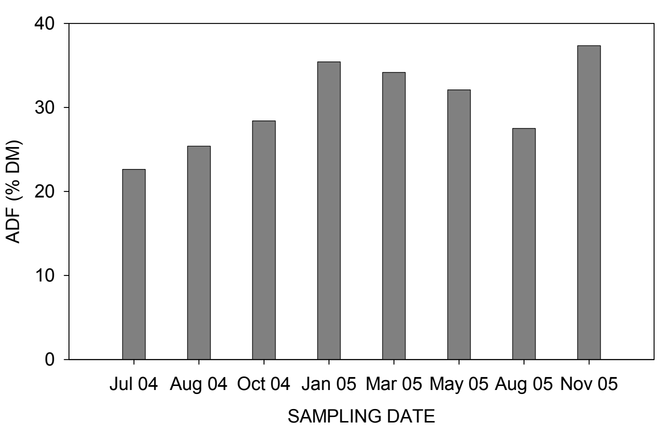


Figure 5.47. Fluctuations in ADF with sampling date

Table 5.24. The influence of sampling date and applied K and S on the ADF content of perennial ryegrass

Herbage ADF content (% DM) - KxS experiment										
	Treatment									
Cut date	K1S1	K1S2	K1S3	K2S1	K2S2	K2S3	K3S1	K3S2	K3S3	Mean
13-Jul-04	23.078	22.564	22.497	22.240	22.860	21.545	21.696	25.463	21.748	**22.632[a*]**
24-Aug-04	24.424	25.060	23.919	24.115	25.710	28.510	27.071	24.534	25.109	**25.384[b]**
20-Oct-04	28.100	28.480	28.165	29.586	28.108	27.522	28.033	28.246	29.329	**28.397[c]**
20-Jan-05	35.974	35.520	33.135	35.849	35.026	34.491	35.655	36.094	37.076	**35.424[f]**
01-Mar-05	33.321	34.392	32.362	34.527	34.513	35.252	33.677	35.387	34.172	**34.178[e]**
17-May-05	31.174	32.128	31.465	35.202	32.278	31.893	30.813	32.572	31.437	**32.107[d]**
22-Aug-05	28.254	28.955	27.231	26.862	27.274	26.467	26.998	27.943	27.543	**27.503[c]**
21-Nov-05	37.014	36.437	38.665	37.105	38.889	37.364	36.764	37.416	36.525	**37.353[g]**
Mean	**30.167[ab*]**	**30.442[ab]**	**29.680[a]**	**30.686[ab]**	**30.582[ab]**	**30.380[ab]**	**30.088[ab]**	**30.957[b]**	**30.367[ab]**	

LSD (0.05) Date = 1.236
LSD (0.05) Treatment = 1.077 (NS)
LSD (0.05) Date x Treatment = 3.622 (NS)
CV = 7.2

	K1	K2	K3	S1	S2	S3
13-Jul-04	22.713	22.215	22.969	22.338	23.629	21.930
24-Aug-04	24.468	26.112	25.571	25.203	25.101	25.846
20-Oct-04	28.248	28.405	28.536	28.573	28.278	28.338
20-Jan-05	34.876	35.122	36.275	35.826	35.546	34.901
01-Mar-05	33.358	34.764	34.412	33.842	34.764	33.929
17-May-05	31.589	33.124	31.607	32.396	32.326	31.598
22-Aug-05	28.147	26.867	27.494	27.371	28.057	27.080
21-Nov-05	37.372	37.786	36.902	36.961	37.580	37.518
Mean	**30.096[a*]**	**30.549[a]**	**30.471[a]**	**30.314[a*]**	**30.660[a]**	**30.143[a]**

LSD (0.05) K = 0.605 (NS)
LSD (0.05) KxDate = 20.063 (NS)
CV = 6.9
LSD (0.05) S = 0.598 (NS)
LSD (0.05) SxDate = 2.093 (NS)
CV = 7.0

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.2 *Neutral detergent fibre*

5.1.3.2.1 *NxP*

Applied N and P did not affect herbage concentrations of NDF, either at individual sampling dates or over the entire trial period. Neutral detergent fibre showed a highly significant response to sampling date (P<0.001), increasing over time and peaking in January before decreasing slightly in the second season. Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.25. Annual variation in the levels of herbage NDF (Figure 5.48) was best described by the quadratic equation:

$$y = 57.8680 - 0.1810x + 0.0006x^2$$

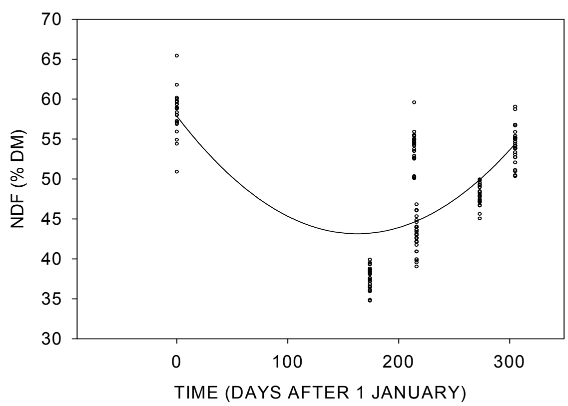


Figure 5.48. Annual variation in NDF content over all NxP treatments (P<0.001, R^2=0.551, $F_{2,159}$ = 97.7119)

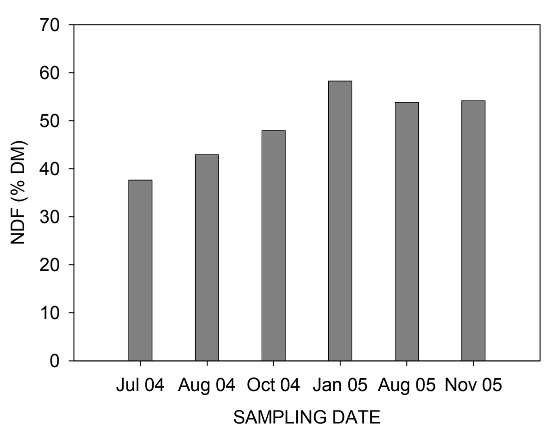


Figure 5.49. Fluctuations in NDF with sampling date

Table 5.25. The influence of sampling date and applied N and P on the NDF content of perennial ryegrass

	Herbage NDF content (% DM) - NxP experiment									
	Treatment									
Cut date	N1P1	N1P2	N1P3	N2P1	N2P2	N2P3	N3P1	N3P2	N3P3	Mean
13-Jul-04	37.979	38.313	37.404	37.040	37.584	37.227	38.759	37.009	37.254	**37.619[a*]**
24-Aug-04	42.643	43.205	43.838	43.404	41.417	44.521	40.689	42.934	43.640	**42.921[b]**
20-Oct-04	47.489	47.944	47.482	47.286	47.689	48.965	47.330	48.192	49.374	**47.972[c]**
20-Jan-05	57.803	60.298	58.488	57.242	57.648	57.388	59.385	61.877	54.176	**58.256[e]**
22-Aug-05	52.976	54.802	54.659	54.013	52.894	54.185	52.438	53.848	54.679	**53.833[d]**
21-Nov-05	53.866	53.954	54.556	55.645	55.787	53.859	52.650	53.190	54.128	**54.182[d]**
Mean	**48.792[a*]**	**49.752[a]**	**49.404[a]**	**49.105[a]**	**48.837[a]**	**49.358[a]**	**48.542[a]**	**49.508[a]**	**48.875[a]**	
LSD (0.05) Date = 1.126										
LSD (0.05) Treatment = 1.074 (NS)										
LSD (0.05) Date x Treatment = 3.378 (NS)										
CV = 4.0										
	N1	N2	N3		P1	P2	P3			
13-Jul-04	37.898	37.284	37.674		37.926	37.635	37.295			
24-Aug-04	43.229	43.114	42.421		42.245	42.519	44.000			
20-Oct-04	47.638	47.980	48.298		47.368	47.941	48.607			
20-Jan-05	58.863	57.426	58.479		58.144	59.941	56.684			
22-Aug-05	54.146	53.697	53.655		53.142	53.848	54.508			
21-Nov-05	54.125	55.097	53.323		54.054	54.310	54.181			
Mean	**49.316[a*]**	**49.100[a]**	**48.975[a]**		**48.813[a*]**	**49.366[a]**	**49.212[a]**			
LSD (0.05) N = 0.640 (NS)										
LSD (0.05) NxDate = 2.021 (NS)										
CV = 4.2										
LSD (0.05) P = 0.608 (NS)										
LSD (0.05) PxDate = 1.918 (NS**, P = 0.075)										
CV = 4.0										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.3.2.2 *NxK*

Neither N nor K had any effect on NDF levels in herbage, either over the entire trial period or at individual sampling dates. The response to sampling date was highly significant (P<0.001) and identical to that which occurred in the NxP experiment (Figure 5.50). Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.26.

Annual variation in the levels of herbage NDF was best described by the quadratic equation:

$$y = 59.448 - 0.2299x + 0.0007x^2$$

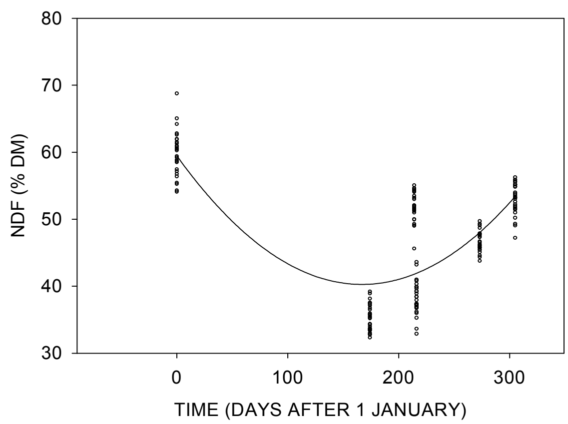


Figure 5.50. Annual variation in NDF content over all NxK treatments ($P<0.001$, $R^2=0.624$, $F_{2,159} = 132.1537$)

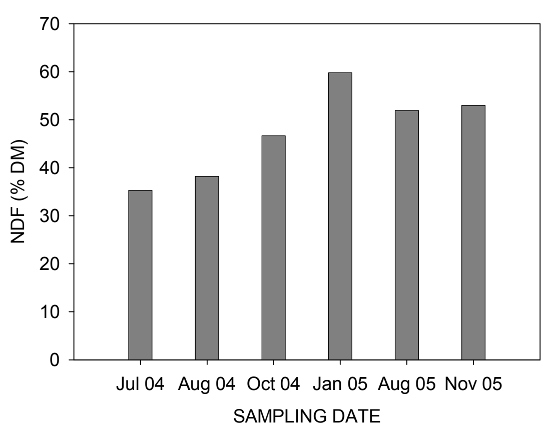


Figure 5.51. Fluctuations in NDF with sampling date

Table 5.26. The influence of sampling date and applied N and K on the NDF content of perennial ryegrass

Herbage NDF content (% DM) - NxK experiment										
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	36.180	35.381	34.831	34.564	34.385	35.586	35.892	34.416	36.612	**35.316[a*]**
24-Aug-04	37.821	37.519	41.667	38.355	38.066	38.295	38.138	36.063	37.855	**38.198[b]**
20-Oct-04	46.702	46.307	47.634	46.831	47.805	44.984	46.890	47.465	45.255	**46.653[c]**
20-Jan-05	58.058	57.960	59.533	61.362	59.374	61.751	60.073	62.695	57.380	**59.798[e]**
22-Aug-05	52.014	50.923	49.772	52.473	52.389	51.916	52.748	54.186	50.883	**51.923[d]**
21-Nov-05	53.453	54.694	54.631	51.188	54.455	54.040	50.603	51.582	52.391	**53.004[d]**
Mean	**47.371[a*]**	**47.130[a]**	**48.011[a]**	**47.462[a]**	**47.746[a]**	**47.762[a]**	**47.391[a]**	**47.735[a]**	**46.729[a]**	

LSD (0.05) Date = 1.289
LSD (0.05) Treatment = 2.145 (NS)
LSD (0.05) Date x Treatment = 3.868 (NS)
CV = 4.7

	N1	**N2**	**N3**	**K1**	**K2**	**K3**
13-Jul-04	35.464	34.845	35.640	35.545	34.727	35.676
24-Aug-04	39.002	38.239	37.352	38.104	37.216	39.272
20-Oct-04	46.881	46.540	46.537	46.808	47.193	45.958
20-Jan-05	58.517	60.829	60.049	59.831	60.010	59.555
22-Aug-05	50.903	52.259	52.606	52.412	52.499	50.857
21-Nov-05	54.259	53.228	51.526	51.748	53.577	53.687
Mean	**47.504[a*]**	**47.657[a]**	**47.285[a]**	**47.408[a*]**	**47.537[a]**	**47.501[a]**

LSD (0.05) N = 1.094 (NS)
LSD (0.05) NxDate = 2.216 (NS)
CV = 4.8
LSD (0.05) K = 1.105 (NS)
LSD (0.05) KxDate = 2.283 (NS)
CV = 4.9

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.2.3 *NxS*

Applied N and S fertiliser did not affect the levels of NDF in the herbage when data were averaged over the entire trial period. The response of NDF to N and S at individual sampling dates was not significant, although there was a N-time interaction (P=0.02), whereby applied N did appear to have a slight effect on NDF in the second season (P=0.058). High levels of N resulted in higher NDF values in herbage but only over the winter months. In general, NDF values peaked in summer and were at their lowest in the winter months, although seasonal variation was high (Figure 5.52). Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.27.

Annual variation in the levels of herbage NDF was best described by the quadratic equation:

$$y = 60.2397 - 0.2027x + 0.0006x^2$$

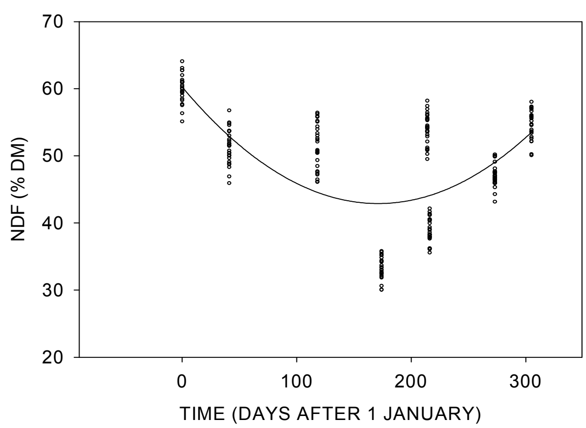


Figure 5.52. Annual variation in NDF content over all NxS treatments (P<0.001, R^2=0.464, $F_{2,211}$ = 91.3061)

Table 5.27. The influence of sampling date and applied N and S on the NDF content of perennial ryegrass

Herbage NDF content (% DM) - NxS experiment										
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	33.967	35.084	33.952	32.475	32.427	31.568	31.989	34.390	33.934	**33.310**^{a*}
24-Aug-04	40.165	40.854	40.083	36.596	37.431	38.611	39.863	39.078	37.856	**38.949**^{b}
20-Oct-04	48.233	47.618	45.866	47.343	45.739	47.818	47.113	46.478	46.676	**46.999**^{c}
20-Jan-05	61.715	61.255	59.865	60.525	59.798	61.529	60.130	56.984	59.365	**60.130**^{f}
01-Mar-05	53.477	52.370	51.013	51.818	51.308	50.188	51.598	49.324	51.350	**51.383**^{d}
17-May-05	51.333	49.172	50.542	50.623	50.264	50.113	54.541	50.978	54.422	**51.332**^{d}
22-Aug-05	53.354	51.499	53.606	54.697	53.754	53.630	56.151	53.915	55.191	**53.977**^{e}
21-Nov-05	55.506	54.720	55.964	54.046	54.470	56.712	53.881	56.153	51.614	**54.785**^{e}
Mean	**49.719**^{c*}	**49.071**^{abc}	**48.861**^{abc}	**48.515**^{ab}	**48.149**^{a}	**48.771**^{ab}	**49.408**^{bc}	**48.413**^{a}	**48.801**^{abc}	

LSD (0.05) Date = 1.350
LSD (0.05) Treatment = 0.931 (NS, P = 0.057)**
LSD (0.05) Date x Treatment = 4.050 (NS)
CV = 4.7

	N1	**N2**	**N3**		**S1**	**S2**	**S3**
13-Jul-04	34.335	32.157	33.240		32.811	33.810	33.151
24-Aug-04	40.367	37.546	38.932		38.875	39.121	38.850
20-Oct-04	47.239	46.967	46.766		47.563	46.612	46.801
20-Jan-05	60.945	60.618	58.827		60.790	59.346	60.253
01-Mar-05	52.286	51.105	50.758		52.298	51.001	50.850
17-May-05	50.349	50.333	53.314		52.166	50.138	51.692
22-Aug-05	52.820	54.027	55.085		54.734	53.056	54.142
21-Nov-05	55.397	55.076	53.883		54.478	55.114	54.763
Mean	**49.217**^{b*}	**48.479**^{a}	**48.850**^{ab}		**49.214**^{b*}	**48.525**^{a}	**48.841**^{ab}

LSD (0.05) N = 0.601 (NS, P = 0.058)**
LSD (0.05) NxDate = 2.245
CV = 4.5
LSD (0.05) S = 0.605 (NS, P = 0.086)**
LSD (0.05) SxDate = 2.398 (NS)
CV = 4.8

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.3.2.4 *PxS*

Neutral detergent fibre showed no response to applied fertiliser in the PxS experiment. Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.28.

Annual variation in the levels of herbage NDF (Figure 5.53) was best described by the quadratic equation:

$$y = 60.9912 - 0.2074x + 0.0006x^2$$

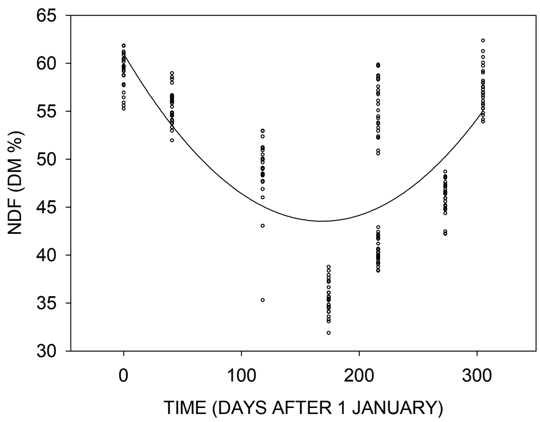


Figure 5.53. Annual variation in NDF content over all PxS treatments ($P<0.001$, $R^2=0.490$, $F_{2,213} = 102.1710$)

After planting, plant NDF values increased until they peaked in mid-summer (the beginning of the second season), after which they decreased slightly towards winter, rising again to peak the following summer (end of the second season, Figure 5.54).

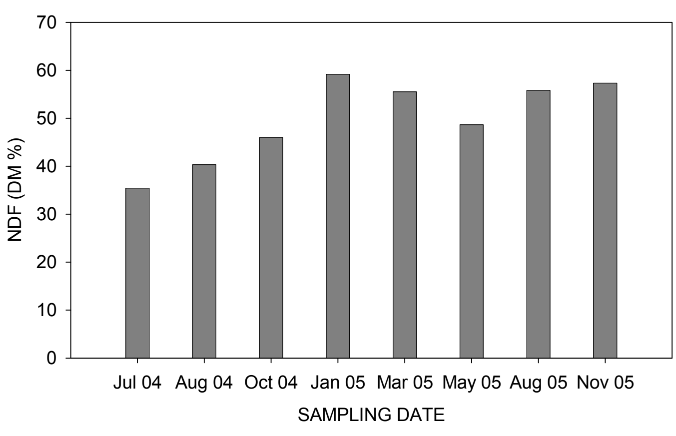


Figure 5.54. Fluctuations in NDF with sampling date

Table 5.28. The influence of sampling date and applied P and S on the NDF content of perennial ryegrass

	Herbage NDF content (% DM) - PxS experiment									
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	34.735	34.342	36.420	35.910	35.574	34.437	35.755	36.088	35.647	**35.434^{a*}**
24-Aug-04	39.819	40.692	40.209	39.722	40.761	40.829	40.441	40.332	40.453	**40.362^{b}**
20-Oct-04	45.413	46.415	46.788	45.048	46.795	45.972	46.861	44.403	46.424	**46.013^{c}**
20-Jan-05	59.749	57.583	58.667	57.619	59.370	59.818	60.399	59.690	59.485	**59.153^{g}**
01-Mar-05	56.556	56.199	53.893	55.689	56.198	56.417	55.165	55.147	54.474	**55.526^{e}**
17-May-05	44.217	50.411	49.589	50.156	49.913	47.404	49.185	49.049	48.093	**48.668^{d}**
22-Aug-05	58.686	54.021	56.414	53.715	57.430	53.908	58.294	55.127	54.922	**55.835^{e}**
21-Nov-05	59.628	55.638	56.456	56.521	59.437	57.615	55.564	57.113	58.073	**57.338^{f}**
Mean	**49.850^{ab*}**	**49.413^{a}**	**49.805^{ab}**	**49.297^{a}**	**50.685^{b}**	**49.550^{ab}**	**50.208^{ab}**	**49.619^{ab}**	**49.697^{ab}**	

LSD (0.05) Date = 1.337

LSD (0.05) Treatment = 1.262 (NS)

LSD (0.05) Date x Treatment = 4.010 (NS)

CV = 4.5

	P1	**P2**	**P3**		**S1**	**S2**	**S3**
13-Jul-04	35.166	35.307	35.830		35.467	35.335	35.501
24-Aug-04	40.240	40.438	40.409		39.994	40.595	40.497
20-Oct-04	46.205	45.938	45.896		45.774	45.871	46.395
20-Jan-05	58.666	58.936	59.858		59.256	58.881	59.324
01-Mar-05	55.549	56.101	54.929		55.803	55.848	54.928
17-May-05	48.072	49.157	48.776		47.853	49.791	48.362
22-Aug-05	56.374	55.018	56.114		56.898	55.526	55.082
21-Nov-05	57.241	57.858	56.917		57.237	57.396	57.381
Mean	**49.689^{a*}**	**49.844^{a}**	**49.841^{a}**		**49.785^{a*}**	**49.905^{a}**	**49.684^{a}**

LSD (0.05) P = 0.744 (NS)

LSD (0.05) PxDate = 2.353 (NS)

CV = 4.6

LSD (0.05) S = 0.742 (NS)

LSD (0.05) SxDate = 2.328 (NS)

CV = 4.5

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.2.5 *PxK*

Applied fertiliser did not affect NDF levels in the PxK experiment.

A seasonal growth curve showed NDF values peaking in summer (Figure 5.55) and decreasing with the onset of cooler weather. However, variation was high between seasons (Figure 5.56), with NDF values in the second season dropping in the cooler months, but as low as levels observed in the first season. Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.29.

Annual variation in the levels of herbage NDF was best described by the quadratic equation:

$$y = 62.0635 - 0.2054x - 0.0006x^2$$

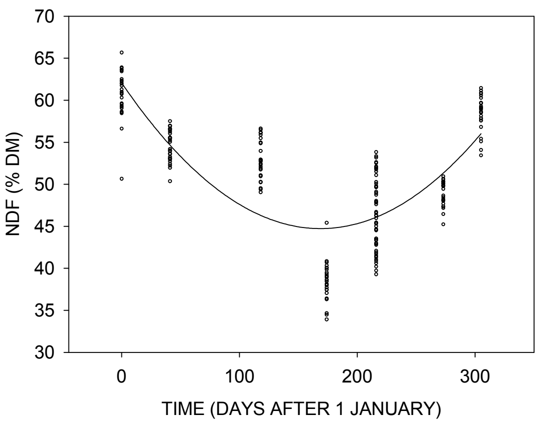


Figure 5.55. Annual variation in NDF content over all PxK treatments ($P<0.001$, $R^2=0.627$, $F_{2,213} = 179.1227$)

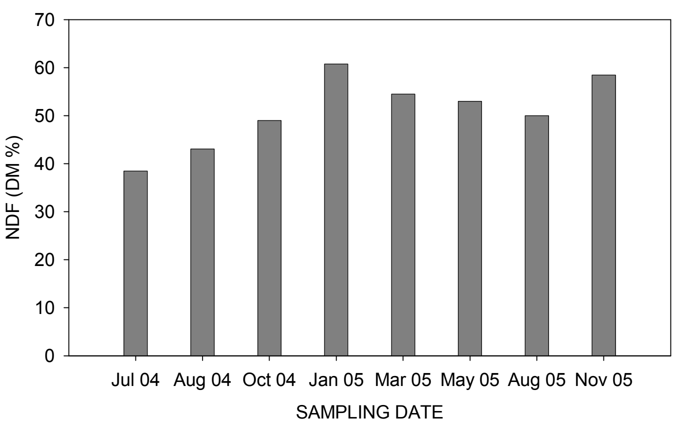


Figure 5.56. Fluctuations in NDF with sampling date

Table 5.29. The influence of sampling date and applied P and K on the NDF content of perennial ryegrass

	Herbage NDF content (% DM) - PxK experiment									
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	40.300	38.855	39.467	37.552	37.142	36.933	37.274	39.813	38.727	**38.452**[a*]
24-Aug-04	42.394	42.453	42.853	43.483	45.138	40.110	43.570	43.641	43.856	**43.055**[b]
20-Oct-04	49.165	48.769	47.904	50.202	49.200	48.789	48.137	49.149	49.704	**49.002**[c]
20-Jan-05	62.619	58.265	59.986	60.563	61.383	61.635	58.358	61.985	62.107	**60.767**[g]
01-Mar-05	52.680	53.857	54.856	53.547	55.009	56.699	55.006	54.432	54.425	**54.501**[e]
17-May-05	52.656	52.878	51.066	53.432	52.389	52.851	53.321	55.659	52.991	**53.027**[d]
22-Aug-05	51.632	49.683	47.060	50.877	51.538	49.225	51.017	49.661	49.288	**49.998**[c]
21-Nov-05	59.553	59.121	59.077	56.920	56.699	59.754	57.893	57.186	60.128	**58.481**[f]
Mean	**51.375**[ab*]	**50.485**[ab]	**50.284**[a]	**50.822**[ab]	**51.062**[ab]	**50.749**[ab]	**50.572**[ab]	**51.441**[b]	**51.403**[ab]	
LSD (0.05) Date = 1.297										
LSD (0.05) Treatment = 1.147 (NS)										
LSD (0.05) Date x Treatment = 3.891 (NS)										
CV = 4.5										
	P1	**P2**	**P3**		**K1**	**K2**	**K3**			
13-Jul-04	39.541	37.209	38.605		38.375	38.603	38.376			
24-Aug-04	42.567	42.910	43.689		43.149	43.744	42.273			
20-Oct-04	48.613	49.397	48.997		49.168	49.039	48.799			
20-Jan-05	60.290	61.194	60.817		60.513	60.544	61.243			
01-Mar-05	53.798	55.085	54.621		53.745	54.432	55.327			
17-May-05	52.200	52.891	53.990		53.136	53.642	52.303			
22-Aug-05	49.458	50.547	49.989		51.176	50.294	48.524			
21-Nov-05	59.250	57.791	58.402		58.122	57.668	59.653			
Mean	**50.715**[a*]	**50.878**[a]	**51.139**[a]		**50.923**[a*]	**50.996**[a]	**50.812**[a]			
LSD (0.05) P = 0.680 (NS)										
LSD (0.05) PxDate = 2.212 (NS)										
CV = 4.5										
LSD (0.05) K = 0.701 (NS)										
LSD (0.05) KxDate = 2.177 (NS)										
CV = 4.4										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.3.2.6 *KxS*

Neither K nor S affected NDF values in the KxS experiment. Sampling date had a highly significant effect (P<0.001), both in terms of seasonal effects (Figure 5.57) and time after planting (Figure 5.58). NDF increased in the herbage after planting, peaking in summer. Values decreased towards winter but remained higher than those observed in the first season. Data reflecting the effects of treatment and sampling date on herbage NDF are summarised in Table 5.30.

Annual variation in the levels of herbage NDF was best described by the quadratic equation:

$$y = 62.1246 - 0.2451x - 0.0008x^2$$

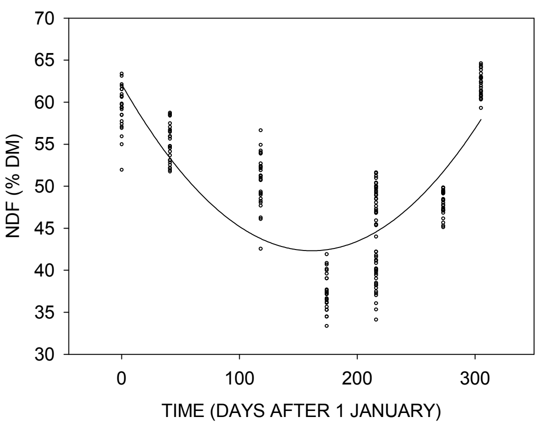


Figure 5.57. Annual variation in NDF content over all KxS treatments (P<0.001, R^2=0.658, $F_{2,213}$ = 205.1855)

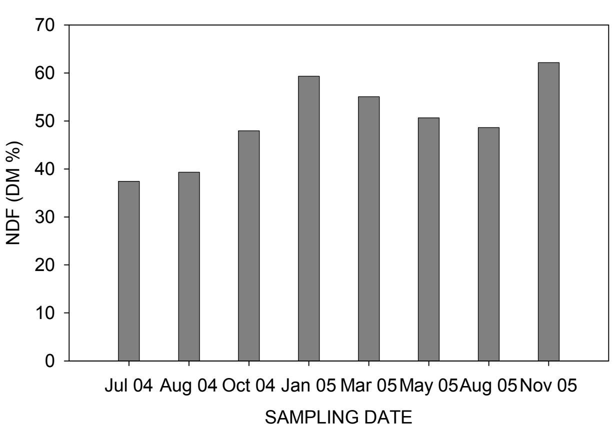


Figure 5.58. Fluctuations in NDF with sampling date

Table 5.30. The influence of sampling date and applied K and S on the NDF content of perennial ryegrass

	Herbage NDF content (% DM) - KxS experiment									
	Treatment									
Cut date	**K1S1**	**K1S2**	**K1S3**	**K2S1**	**K2S2**	**K2S3**	**K3S1**	**K3S2**	**K3S3**	**Mean**
13-Jul-04	37.516	38.263	37.970	35.749	37.950	38.175	37.536	37.706	35.960	**37.425[a*]**
24-Aug-04	39.056	38.461	38.698	39.857	39.909	41.518	39.096	37.884	39.422	**39.322[b]**
20-Oct-04	48.096	48.153	48.129	47.365	47.992	47.518	48.039	47.165	49.208	**47.963[c]**
20-Jan-05	57.829	61.193	59.137	60.171	59.922	58.347	60.036	58.950	58.265	**59.316[f]**
01-Mar-05	53.009	55.094	53.665	55.477	56.281	55.912	55.117	56.553	54.470	**55.064[e]**
17-May-05	50.128	50.714	49.464	52.328	49.622	52.289	51.863	51.071	48.550	**50.670[d]**
22-Aug-05	48.469	49.854	48.946	47.439	48.483	48.864	47.429	49.242	48.700	**48.603[c]**
21-Nov-05	60.952	63.300	63.107	61.946	62.915	60.027	62.132	63.030	61.899	**62.145[g]**
Mean	**49.382[a*]**	**50.629[a]**	**49.889[a]**	**50.041[a]**	**50.384[a]**	**50.331[a]**	**50.156[a]**	**50.200[a]**	**49.559[a]**	

LSD (0.05) Date = 1.362
LSD (0.05) Treatment = 1.359 (NS)
LSD (0.05) Date x Treatment = 4.085 (NS)
CV = 4.7

	K1	**K2**	**K3**		**S1**	**S2**	**S3**
13-Jul-04	37.916	37.291	37.067		36.934	37.973	37.368
24-Aug-04	38.739	40.428	38.801		39.336	38.751	39.879
20-Oct-04	48.126	47.625	48.137		47.834	47.770	48.285
20-Jan-05	59.386	59.480	59.083		59.345	60.021	58.583
01-Mar-05	53.923	55.890	55.380		54.534	55.976	54.682
17-May-05	50.102	51.413	50.495		51.440	50.469	50.101
22-Aug-05	49.090	48.262	48.457		47.779	49.193	48.837
21-Nov-05	62.453	61.629	62.354		61.677	63.082	61.678
Mean	**49.967[a*]**	**50.252[a]**	**49.972[a]**		**49.860[a*]**	**50.404[a]**	**49.927[a]**

LSD (0.05) K = 0.757 (NS)
LSD (0.05) KxDate = 2.184 (NS)
CV = 4.4
LSD (0.05) S = 0.725 (NS)
LSD (0.05) SxDate = 2.181 (NS)
CV = 4.4

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4 The effects of applied fertiliser N, P, K and S on phosphorus, calcium and sulphur in perennial ryegrass

5.1.4.1 *Phosphorus*

5.1.4.1.1 *NxP*

When averaged over all sampling dates, the concentration of P in perennial ryegrass showed a significant linear response to increasing levels of applied N and P (Figure 5.59). Highly significant decreases in herbage P accompanied N fertiliser additions (P<0.001), while increasing levels of P significantly increased the concentration of P in herbage (P=0.036). Herbage P responses over the year showed no N-P interactions.

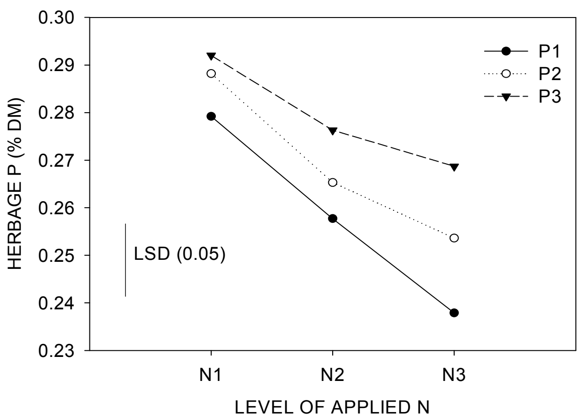


Figure 5.59. The effect of applied N and P on herbage P concentration averaged over all seasons.

Both date and level of applied N had a highly significant (P<0.001 and P=0.008 respectively) effect on herbage P concentration (Figure 5.60) at individual sampling dates. P concentration was lowest at high levels of applied N, particularly during the warmer spring and summer months. This response was evidenced by a significant (P=0.021) date-N interaction. Phosphorus application did not affect herbage P concentration at individual sampling dates in the NxP experiment. Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.31.

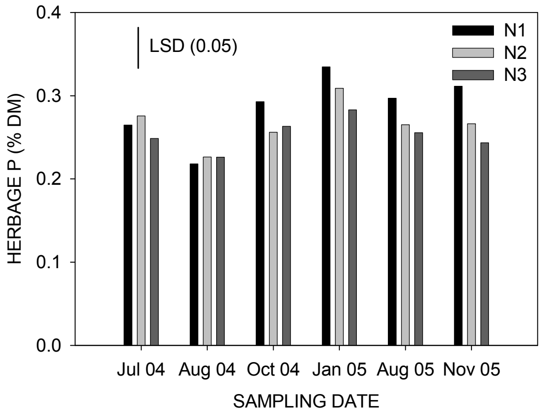


Figure 5.60. Fluctuations in herbage P over time for three levels of applied N

Annual variation in the levels of herbage P (Figure 5.61) was best described by the quadratic equation:

$$y = 0.3095 - 0.0006x + 0.000x^2$$

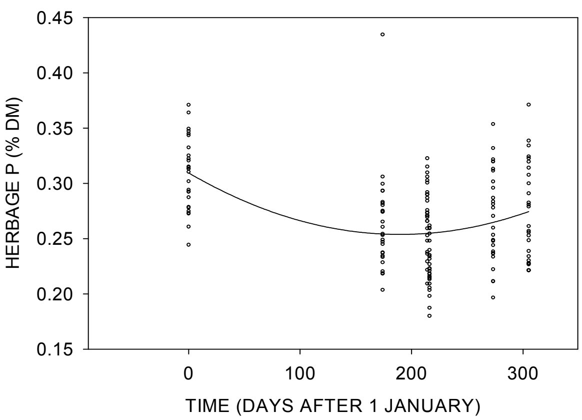


Figure 5.61. Annual variation in herbage P over all NxP treatments (P<0.001, R^2=0.203, $F_{2,159}$ = 20.267)

Table 5.31. The influence of sampling date and applied N and P on herbage P content of perennial ryegrass

	Herbage P content (% DM) - NxP experiment									
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	0.249	0.274	0.271	0.255	0.253	0.320	0.236	0.240	0.270	**0.263[b*]**
24-Aug-04	0.201	0.223	0.230	0.217	0.228	0.234	0.215	0.216	0.248	**0.224[a]**
20-Oct-04	0.317	0.271	0.291	0.274	0.276	0.219	0.232	0.298	0.260	**0.271[b]**
20-Jan-05	0.350	0.319	0.336	0.287	0.309	0.331	0.284	0.276	0.289	**0.309[c]**
22-Aug-05	0.282	0.310	0.299	0.263	0.261	0.272	0.236	0.256	0.275	**0.273[b]**
21-Nov-05	0.276	0.332	0.325	0.251	0.266	0.283	0.224	0.236	0.270	**0.274[b]**
Mean	**0.279[bc*]**	**0.288[bc]**	**0.292[c]**	**0.258[abc]**	**0.265[abc]**	**0.276[bc]**	**0.238[a]**	**0.254[ab]**	**0.269[abc]**	
LSD (0.05) Date = 0.01507										
LSD (0.05) Treatment = 0.03587 (NS, P=0.099)**										
LSD (0.05) Date x Treatment = 0.04520										
CV = 9.2										
	N1	**N2**	**N3**		**P1**	**P2**	**P3**			
13-Jul-04	0.265	0.276	0.249		0.247	0.256	0.287			
24-Aug-04	0.218	0.226	0.226		0.211	0.222	0.237			
20-Oct-04	0.293	0.256	0.263		0.274	0.282	0.257			
20-Jan-05	0.335	0.309	0.283		0.307	0.301	0.319			
22-Aug-05	0.297	0.265	0.256		0.260	0.276	0.282			
21-Nov-05	0.311	0.266	0.244		0.250	0.278	0.293			
Mean	**0.286[b*]**	**0.266[ab]**	**0.253[a]**		**0.258[a*]**	**0.269[a]**	**0.279[a]**			
LSD (0.05) N = 0.01985										
LSD (0.05) NxDate = 0.02939										
CV = 10.3										
LSD (0.05) P = 0.02296 (NS)										
LSD (0.05) PxDate = 0.02936 (NS)										
CV = 10.8										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.4.1.2 *NxK*

When data were averaged over the entire trial period, application of N resulted in a significant decline in herbage P (P=0.008), especially at high levels of applied N in the NxK experiment. Potassium applications did not affect concentrations of P in the herbage (Figure 5.62).

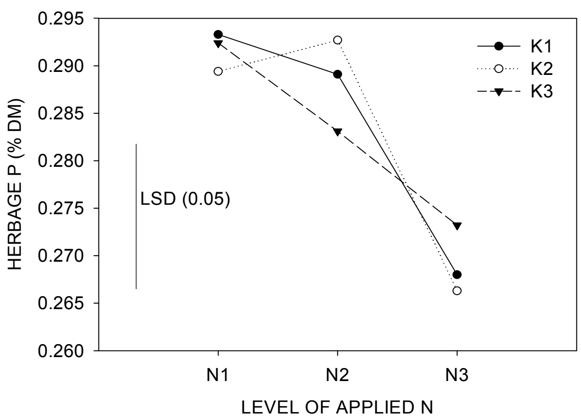


Figure 5.62. The effect of applied N and K on herbage P concentration averaged over all sampling dates.

Effects of both date and N application on herbage P concentration at individual sampling dates were highly significant (P<0.001). P in herbage was lower at high levels of N application, notably in the second year after the pasture had been established (Figure 5.63), although there was no significant date-N interaction. The level of applied K did not affect P concentration in the herbage.

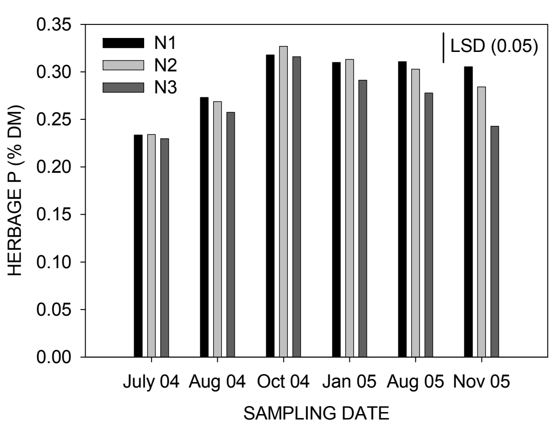


Figure 5.63. Fluctuations in herbage P over time for three levels of applied N

Annual variation in the levels of herbage P (Figure 5.64) was best described by the quadratic equation:

$$y = 0.3013 - 0.0005x + 0.000x^2$$

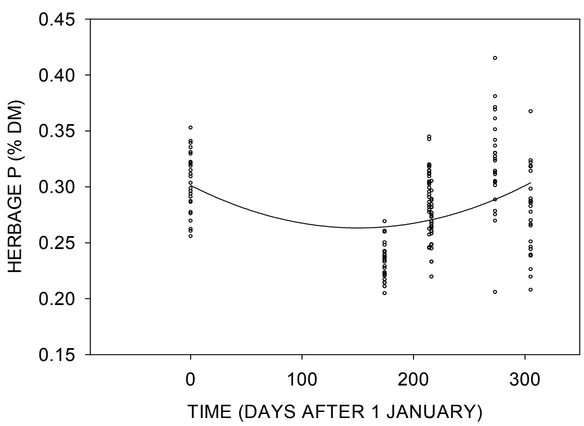


Figure 5.64. Annual variation in herbage P over all NxK treatments ($P<0.001$, $R^2=0.149$, $F_{2,159} = 13.946$)

Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.32.

Table 5.32. The influence of sampling date and applied N and K on herbage P content of perennial ryegrass

	Herbage P content (% DM) - NxK experiment									
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	0.2449	0.2235	0.2325	0.2405	0.2268	0.2354	0.2335	0.2265	0.2291	**0.233[a*]**
24-Aug-04	0.2697	0.281	0.2685	0.252	0.2851	0.2691	0.2531	0.2532	0.2661	**0.266[b]**
20-Oct-04	0.2843	0.3355	0.3335	0.3249	0.3423	0.313	0.3137	0.2973	0.3366	**0.320[d]**
20-Jan-05	0.3299	0.2884	0.3115	0.3186	0.3052	0.3154	0.2856	0.2894	0.299	**0.305[cd]**
22-Aug-05	0.3071	0.3071	0.3177	0.3048	0.3034	0.3001	0.2895	0.2742	0.2694	**0.297[c]**
21-Nov-05	0.3241	0.3012	0.2906	0.294	0.2933	0.2653	0.2324	0.2572	0.239	**0.277[b]**
Mean	**0.293[c*]**	**0.289[bc]**	**0.292[c]**	**0.289[bc]**	**0.293[c]**	**0.283[abc]**	**0.268[ab]**	**0.266[a]**	**0.273[abc]**	

LSD (0.05) Date = 0.01608
LSD (0.05) Treatment = 0.02127 (NS, P = 0.064)**
LSD (0.05) Date x Treatment = 0.04824 (NS)
CV = 9.6

	N1	**N2**	**N3**		**K1**	**K2**	**K3**
13-Jul-04	0.234	0.234	0.230		0.240	0.226	0.232
24-Aug-04	0.273	0.269	0.258		0.258	0.273	0.268
20-Oct-04	0.318	0.327	0.316		0.308	0.325	0.328
20-Jan-05	0.310	0.313	0.291		0.311	0.294	0.309
22-Aug-05	0.311	0.303	0.278		0.301	0.295	0.296
21-Nov-05	0.305	0.284	0.243		0.284	0.284	0.265
Mean	**0.292[b*]**	**0.288[b]**	**0.269[a]**		**0.283[a*]**	**0.283[a]**	**0.283[a]**

LSD (0.05) N = 0.01075
LSD (0.05) NxDate = 0.02715 (NS)
CV = 9.4
LSD (0.05) K = 0.01520 (NS)
LSD (0.05) KxDate = 0.02775 (NS)
CV = 9.6

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.4.1.3 *NxS*

There were no significant effects of either N or S fertiliser on herbage P concentration when data were averaged over all sampling dates. Sampling date had a highly significant effect ($P<0.001$) on P concentration and there was a significant date-N interaction ($P=0.005$) where higher levels of applied N depressed herbage P, especially in the second year (Figure 5.65). Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.33.

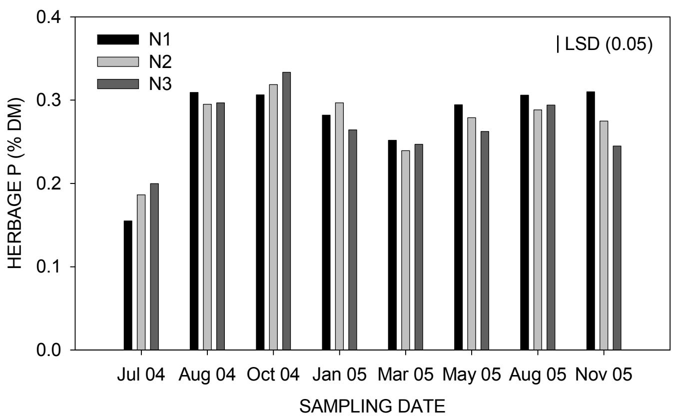


Figure 5.65. Fluctuations in herbage P over time for three levels of applied N

Annual variation in the levels of herbage P (Figure 5.66) in the NxS experiment was best described by the quadratic equation:

$$y = 0.2622 - 0.0002x + 0.000x^2$$

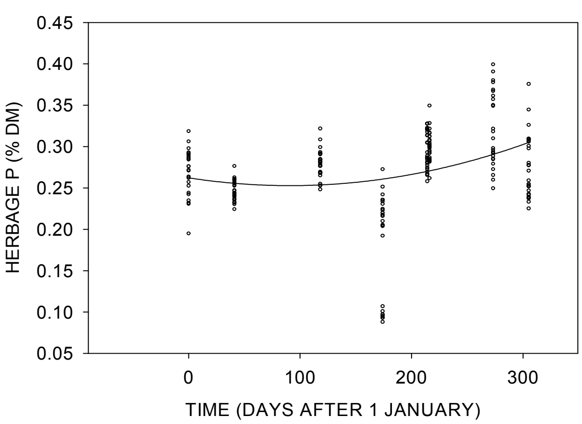


Figure 5.66. Annual variation in herbage P over all NxS treatments ($P<0.001$, $R^2=0.104$, $F_{2,210} = 12.241$)

Table 5.33. The influence of sampling date and applied N and S on herbage P content of perennial ryegrass

	Herbage P content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	0.164	0.132	0.169	0.187	0.176	0.196	0.184	0.238	0.191	**0.182[a*]**
24-Aug-04	0.301	0.306	0.321	0.294	0.290	0.301	0.298	0.292	0.301	**0.300[de]**
20-Oct-04	0.315	0.300	0.305	0.334	0.302	0.320	0.345	0.316	0.343	**0.320[e]**
20-Jan-05	0.286	0.271	0.289	0.352	0.265	0.274	0.257	0.282	0.254	**0.281[cd]**
01-Mar-05	0.238	0.266	0.252	0.238	0.237	0.243	0.249	0.248	0.244	**0.246[b]**
17-May-05	0.293	0.305	0.286	0.284	0.273	0.279	0.272	0.257	0.259	**0.279[cd]**
22-Aug-05	0.308	0.299	0.311	0.286	0.280	0.300	0.290	0.281	0.311	**0.296[cd]**
21-Nov-05	0.318	0.310	0.302	0.268	0.267	0.290	0.250	0.242	0.242	**0.277[c]**
Mean	**0.278[a*]**	**0.274[a]**	**0.279[a]**	**0.280[a]**	**0.261[a]**	**0.275[a]**	**0.268[a]**	**0.269[a]**	**0.268[a]**	
LSD (0.05) Date = 0.02196										
LSD (0.05) Treatment = 0.02166 (NS)										
LSD (0.05) Date x Treatment = 0.06039 (NS)										
CV = 12.3										
	N1	**N2**	**N3**		**S1**	**S2**	**S3**			
13-Jul-04	0.155	0.186	0.200		0.178	0.175	0.186			
24-Aug-04	0.309	0.295	0.297		0.297	0.296	0.308			
20-Oct-04	0.306	0.319	0.334		0.331	0.306	0.320			
20-Jan-05	0.282	0.264	0.264		0.265	0.273	0.272			
01-Mar-05	0.252	0.239	0.247		0.242	0.250	0.246			
17-May-05	0.295	0.279	0.262		0.283	0.278	0.275			
22-Aug-05	0.306	0.288	0.294		0.294	0.287	0.307			
21-Nov-05	0.310	0.275	0.245		0.279	0.273	0.278			
Mean	**0.277[a*]**	**0.268[a]**	**0.268[a]**		**0.271[a*]**	**0.267[a]**	**0.274[a]**			
LSD (0.05) N = 0.01112 (NS)										
LSD (0.05) NxDate = 0.03141										
CV = 11.3										
LSD (0.05) S = 0.01159 (NS)										
LSD (0.05) SxDate = 0.03602 (NS)										
CV = 12.5										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.1.4 *PxS*

Applied phosphorus had a significant linear (P=0.002) effect over the year on herbage P concentration (Figure 5.67) as well as on individual sampling dates (P<0.001, Figure 10). P concentration in herbage varied significantly with sampling date (P<0.001) but there was no date-applied P interaction. Sulphur applications did not affect the concentrations of P in the herbage. Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.34.

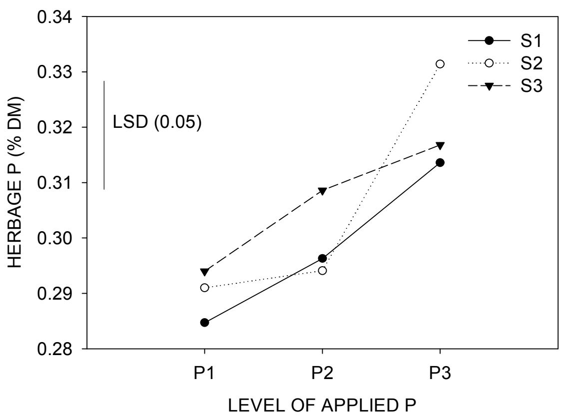


Figure 5.67. The effect of applied P and S on herbage P concentration averaged over all sampling dates.

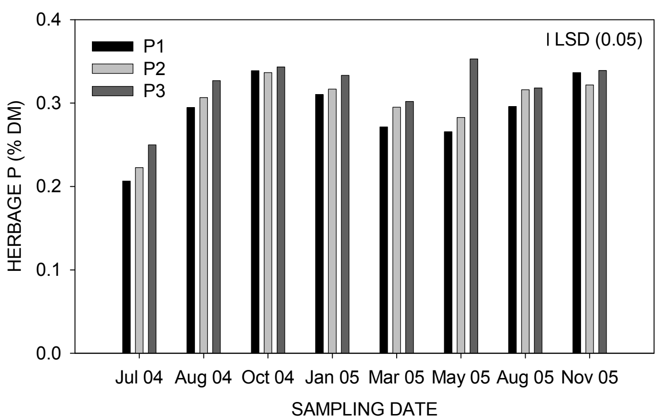


Figure 5.68. Fluctuations in herbage P over time for three levels of applied P

Annual variation in the levels of herbage P (Figure 5.69).was best described by the quadratic equation:

$$y = 0.3163 - 0.0007 + 0.000x^2$$

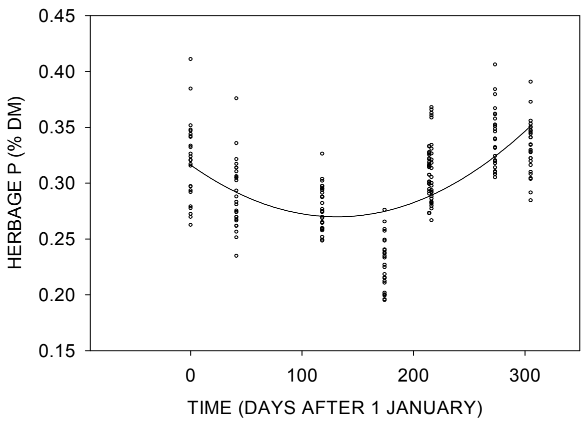


Figure 5.69. Annual variation in herbage P over all PxS treatments ($P<0.001$, $R^2=0.364$, $F_{2,210} = 60.957$)

Table 5.34. The influence of sampling date and applied P and S on herbage P content of perennial ryegrass

Herbage P content (% DM) - PxS experiment										
	Treatment									
Cut date	P1S1	P1S2	P1S3	P2S1	P2S2	P2S3	P3S1	P3S2	P3S3	Mean
13-Jul-04	0.199	0.205	0.216	0.220	0.215	0.233	0.246	0.253	0.251	**0.226^{a*}**
24-Aug-04	0.295	0.298	0.291	0.301	0.312	0.307	0.327	0.322	0.332	**0.309^{cd}**
20-Oct-04	0.339	0.336	0.341	0.327	0.340	0.343	0.337	0.329	0.363	**0.340^{f}**
20-Jan-05	0.289	0.315	0.327	0.322	0.294	0.335	0.352	0.315	0.333	**0.320^{de}**
01-Mar-05	0.266	0.260	0.289	0.292	0.274	0.319	0.291	0.304	0.311	**0.289^{b}**
17-May-05	0.265	0.271	0.261	0.285	0.277	0.287	0.294	0.485	0.279	**0.300^{bc}**
22-Aug-05	0.293	0.310	0.285	0.309	0.318	0.320	0.316	0.316	0.322	**0.310^{cd}**
21-Nov-05	0.333	0.333	0.343	0.315	0.323	0.326	0.345	0.328	0.344	**0.332^{ef}**
Mean	**0.285^{a*}**	**0.291^{ab}**	**0.294^{ab}**	**0.296^{b}**	**0.294^{ab}**	**0.309^{c}**	**0.314^{c}**	**0.331^{d}**	**0.317^{c}**	

LSD (0.05) Date = 0.01591
LSD (0.05) Treatment = 0.00978
LSD (0.05) Date x Treatment = 0.04773 (NS)
CV = 8.7

	P1	P2	P3		S1	S2	S3
13-Jul-04	0.206	0.223	0.250		0.222	0.224	0.233
24-Aug-04	0.295	0.307	0.327		0.308	0.311	0.310
20-Oct-04	0.339	0.336	0.343		0.334	0.335	0.349
20-Jan-05	0.310	0.317	0.333		0.321	0.308	0.331
01-Mar-05	0.271	0.295	0.302		0.283	0.279	0.306
17-May-05	0.266	0.283	0.288		0.281	0.279	0.276
22-Aug-05	0.296	0.316	0.318		0.306	0.315	0.309
21-Nov-05	0.337	0.322	0.339		0.331	0.328	0.338
Mean	**0.290^{a*}**	**0.300^{b}**	**0.313^{c}**		**0.298^{a*}**	**0.297^{a}**	**0.306^{a}**

LSD (0.05) P = 0.00711
LSD (0.05) PxDate = 0.02545 (NS)
CV = 8.1
LSD (0.05) S = 0.01144 (NS)
LSD (0.05) SxDate = 0.02561 (NS)
CV = 8.2

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.1.5 *PxK*

Herbage P concentration increased linearly (P=0.042) with increasing levels of applied P (Figure 5.70). A significant linear P-K interaction was evident (P=0.023), especially at higher levels of applied P, where high levels of applied K resulting in lower herbage P concentrations.

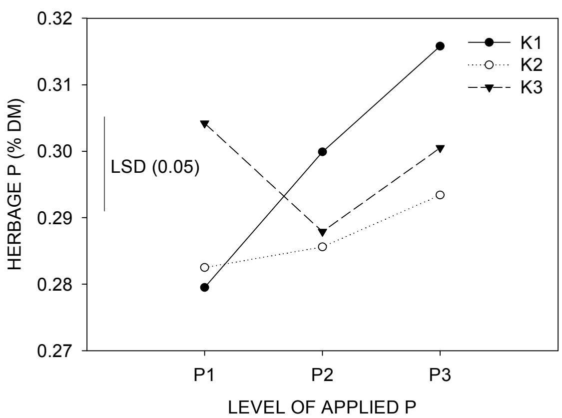


Figure 5.70. The effect of applied P and K on herbage P concentration averaged over all sampling dates.

Sampling date had a highly significant effect (P<0.001) on P concentration in the PxK experiment. A significant (P=0.005) response to applied P was evident at high levels of P (Figure 5.71). Applied K did not affect herbage P concentration. Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.35.

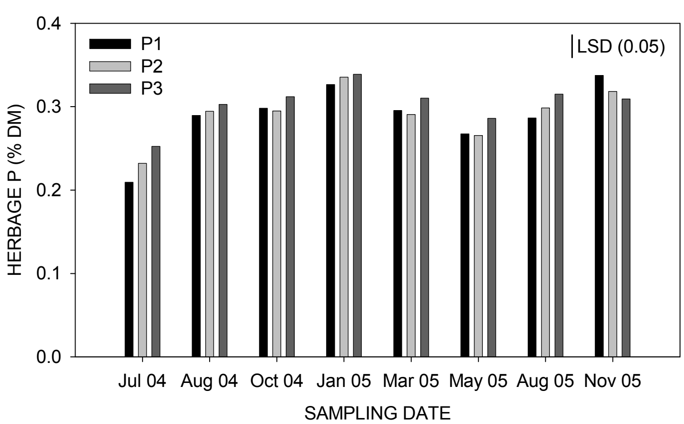


Figure 5.71. Fluctuations in herbage P over time for three levels of applied P

Annual variation in the levels of herbage P (Figure 5.72) in the PxK experiment was best described by the quadratic equation:

$$y = 0.3289 - 0.0008x + 0.0000x^2$$

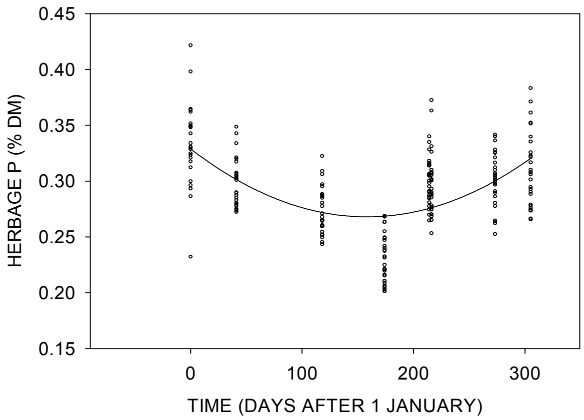


Figure 5.72. Annual variation in herbage P over all PxK treatments (P<0.001, R^2=0.325, $F_{2,210}$ = 51.345)

Table 5.35. The influence of sampling date and applied P and K on herbage P content of perennial ryegrass

	Herbage P content (% DM) - PxK experiment									
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	0.204	0.213	0.210	0.230	0.231	0.234	0.267	0.254	0.236	**0.231[a*]**
24-Aug-04	0.274	0.277	0.318	0.313	0.280	0.290	0.299	0.324	0.286	**0.296[c]**
20-Oct-04	0.288	0.308	0.298	0.302	0.275	0.308	0.323	0.295	0.318	**0.302[c]**
20-Jan-05	0.337	0.319	0.324	0.332	0.339	0.336	0.371	0.305	0.340	**0.334[d]**
01-Mar-05	0.300	0.295	0.291	0.299	0.293	0.279	0.306	0.306	0.319	**0.299[c]**
17-May-05	0.264	0.268	0.270	0.267	0.263	0.267	0.305	0.263	0.290	**0.273[b]**
22-Aug-05	0.279	0.284	0.297	0.316	0.290	0.289	0.319	0.303	0.323	**0.300[c]**
21-Nov-05	0.291	0.296	0.317	0.341	0.313	0.301	0.337	0.298	0.293	**0.310[c]**
Mean	**0.280[a*]**	**0.282[a]**	**0.291[ab]**	**0.300[bc]**	**0.286[ab]**	**0.288[ab]**	**0.316[c]**	**0.293[ab]**	**0.301[bc]**	

LSD (0.05) Date = 0.01383
LSD (0.05) Treatment = 0.01588
LSD (0.05) Date x Treatment = 0.04150 (NS)
CV = 7.8

	P1	**P2**	**P3**		**K1**	**K2**	**K3**
13-Jul-04	0.209	0.232	0.252		0.234	0.233	0.227
24-Aug-04	0.289	0.294	0.303		0.295	0.294	0.298
20-Oct-04	0.298	0.295	0.312		0.304	0.293	0.308
20-Jan-05	0.327	0.335	0.339		0.347	0.321	0.334
01-Mar-05	0.295	0.291	0.310		0.302	0.298	0.296
17-May-05	0.267	0.266	0.286		0.279	0.265	0.276
22-Aug-05	0.286	0.298	0.315		0.305	0.292	0.303
21-Nov-05	0.301	0.318	0.309		0.323	0.302	0.303
Mean	**0.284[a]**	**0.291[a]**	**0.303[b]**		**0.298[a]**	**0.287[a]**	**0.293[a]**

LSD (0.05) P = 0.01080
LSD (0.05) PxDate = 0.02315 (NS)
CV = 7.9
LSD (0.05) K = 0.01283 (NS)
LSD (0.05) KxDate = 0.02322 (NS)
CV = 8.0

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.1.6 *KxS*

Herbage P concentration was unaffected by both applied K and S fertiliser in the KxS experiment. Sampling date did, however, affect P concentration in the experiment (P<0.001). Data reflecting the effects of treatment and sampling date on herbage P are summarised in Table 5.36.

Annual variation in the levels of herbage P (Figure 5.73) was best described by the quadratic equation:

$$y = 0.3465 - 0.0012x + 0.000x^2$$

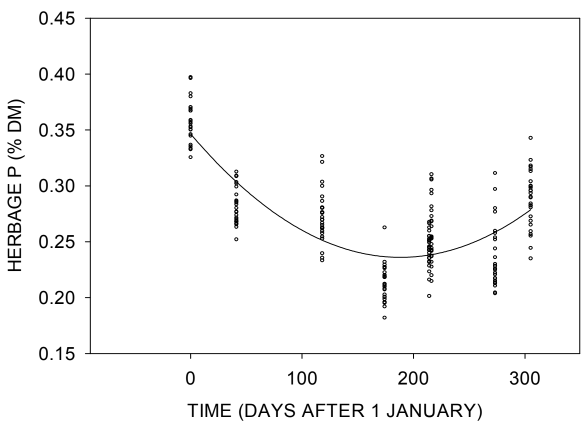


Figure 5.73. Annual variation in herbage P over all KxS treatments ($P<0.001$, $R^2=0.628$, $F_{2,210} = 179.850$)

Table 5.36. The influence of sampling date and applied K and S on herbage P content of perennial ryegrass

	Herbage P content (% DM) - KxS experiment									
	Treatment									
Cut date	**K1S1**	**K1S2**	**K1S3**	**K2S1**	**K2S2**	**K2S3**	**K3S1**	**K3S2**	**K3S3**	**Mean**
13-Jul-04	0.196	0.204	0.206	0.221	0.221	0.219	0.212	0.223	0.214	**0.213[a*]**
24-Aug-04	0.259	0.266	0.240	0.262	0.261	0.269	0.262	0.272	0.243	**0.259[c]**
20-Oct-04	0.237	0.223	0.225	0.241	0.246	0.247	0.246	0.248	0.216	**0.236[b]**
20-Jan-05	0.354	0.346	0.371	0.346	0.343	0.357	0.370	0.359	0.370	**0.357[f]**
01-Mar-05	0.286	0.277	0.282	0.279	0.282	0.276	0.280	0.286	0.291	**0.282[de]**
17-May-05	0.278	0.261	0.283	0.268	0.293	0.249	0.275	0.269	0.278	**0.273[d]**
22-Aug-05	0.255	0.238	0.235	0.248	0.258	0.240	0.245	0.228	0.243	**0.243[b]**
21-Nov-05	0.316	0.269	0.293	0.296	0.288	0.276	0.293	0.277	0.286	**0.288[e]**
Mean	**0.273[a*]**	**0.261[a]**	**0.267[a]**	**0.270[a]**	**0.274[a]**	**0.267[a]**	**0.273[a]**	**0.270[a]**	**0.268[a]**	

LSD (0.05) Date = 0.01342
LSD (0.05) Treatment = 0.01678 (NS)
LSD (0.05) Date x Treatment = 0.04026 (NS)
CV = 8.2

	K1	**K2**	**K3**		**S1**	**S2**	**S3**
13-Jul-04	0.202	0.220	0.216		0.210	0.216	0.213
24-Aug-04	0.255	0.264	0.259		0.261	0.266	0.251
20-Oct-04	0.228	0.244	0.237		0.241	0.239	0.229
20-Jan-05	0.357	0.348	0.366		0.356	0.349	0.366
01-Mar-05	0.281	0.279	0.286		0.282	0.282	0.283
17-May-05	0.274	0.270	0.274		0.274	0.274	0.270
22-Aug-05	0.243	0.249	0.239		0.249	0.241	0.239
21-Nov-05	0.293	0.287	0.285		0.302	0.278	0.285
Mean	**0.267[a]**	**0.270[a]**	**0.270[a]**		**0.272[a]**	**0.268[a]**	**0.267[a]**

LSD (0.05) K = 0.00895 (NS)
LSD (0.05) KxDate = 0.02202 (NS)
CV = 7.9
LSD (0.05) S = 0.00885 (NS)
LSD (0.05) SxDate = 0.02196 (NS)
CV = 7.8

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.2 *Calcium*

5.1.4.2.1 *NxP*

Neither applied N nor P affected overall herbage Ca in the NxP experiment. Nitrogen did, however, affect Ca content on a per cut basis (P=0.041), as did sampling date (P<0.001). There was a highly significant date-N interaction (P<0.001), which indicates that time of year affects the extent of the response to applied N (Figure 5.74). Phosphorus application did not affect herbage Ca concentrations at individual sampling dates. Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.37.

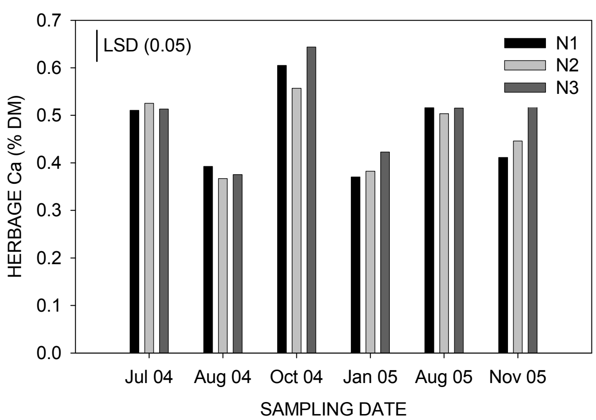


Figure 5.74. Fluctuations in herbage Ca over time for three levels of applied N

Annual variation in the levels of herbage Ca (Figure 5.75) was best described by the Gaussian 3-parameter equation:

$$y = 0.5174^{\left[-0.5\left(\frac{x-383.5122}{515.8642}\right)^2\right]}$$

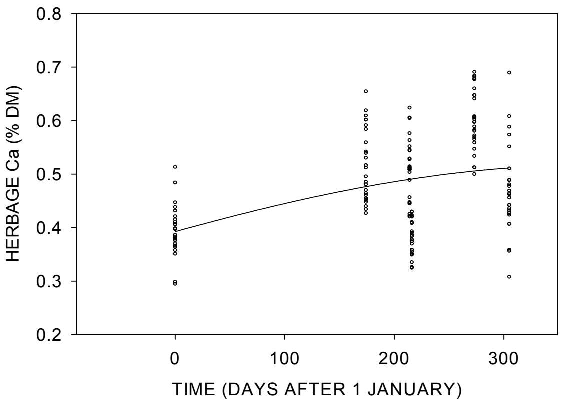


Figure 5.75. Annual variation in herbage Ca over all NxP treatments ($P<0.001$, $R^2=0.172$, $F_{2,159} = 16.568$)

Table 5.37. The influence of sampling date and applied N and P on herbage Ca content of perennial ryegrass

Herbage Ca content (% DM) - NxP experiment										
	Treatment									
Cut date	**N1P1**	**N1P2**	**N1P3**	**N2P1**	**N2P2**	**N2P3**	**N3P1**	**N3P2**	**N3P3**	**Mean**
13-Jul-04	0.442	0.535	0.554	0.507	0.526	0.544	0.501	0.489	0.549	**0.516**^{c*}
24-Aug-04	0.376	0.421	0.381	0.367	0.369	0.365	0.367	0.376	0.383	**0.378**^{a}
20-Oct-04	0.604	0.614	0.597	0.550	0.570	0.551	0.649	0.633	0.648	**0.602**^{d}
20-Jan-05	0.346	0.392	0.373	0.365	0.406	0.377	0.429	0.416	0.423	**0.392**^{a}
22-Aug-05	0.494	0.581	0.473	0.471	0.518	0.522	0.487	0.496	0.563	**0.512**^{c}
21-Nov-05	0.410	0.425	0.398	0.424	0.469	0.445	0.550	0.529	0.555	**0.467**^{b}
Mean	**0.445**^{a*}	**0.495**^{ab}	**0.463**^{ab}	**0.447**^{a}	**0.476**^{ab}	**0.467**^{ab}	**0.497**^{ab}	**0.490**^{ab}	**0.520**^{b}	
LSD (0.05) Date = 0.02449										
LSD (0.05) Treatment = 0.05795 (NS)										
LSD (0.05) Date x Treatment = 0.07346										
CV = 8.8										
	N1	**N2**	**N3**		**P1**	**P2**	**P3**			
13-Jul-04	0.511	0.526	0.513		0.483	0.517	0.549			
24-Aug-04	0.392	0.367	0.375		0.370	0.389	0.376			
20-Oct-04	0.605	0.557	0.644		0.601	0.606	0.599			
20-Jan-05	0.370	0.382	0.423		0.380	0.405	0.391			
22-Aug-05	0.516	0.504	0.515		0.484	0.532	0.519			
21-Nov-05	0.411	0.446	0.545		0.462	0.474	0.466			
Mean	**0.468**^{a*}	**0.464**^{a}	**0.502**^{b}		**0.463**^{a*}	**0.487**^{a}	**0.483**^{a}			
LSD (0.05) N = 0.03264										
LSD (0.05) NxDate = 0.04041										
CV = 8.6										
LSD (0.05) P = 0.03599 (NS)										
LSD (0.05) PxDate = 0.04817 (NS)										
CV = 10.0										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.2.2 *NxK*

Combined data over all cuts indicated a significant response of herbage Ca to applied N (P=0.004), Ca increasing as higher levels of fertiliser N was applied. K did not affect levels of Ca in herbage (Figure 5.76).

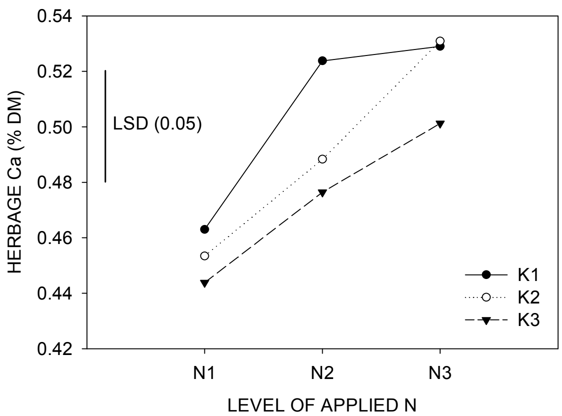


Figure 5.76. The effect of applied N and K on herbage Ca concentration averaged over all sampling dates.

In the NxK experiment, both date and applied N had highly significant effects (P<0.001) on herbage Ca (Figure 5.77) on a per cut basis. Low levels of applied N resulted in lower levels of herbage Ca, particularly in spring and summer. This linear date-N interaction was also highly significant (P<0.001). Herbage Ca did not show a significant response to applied K. Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.38.

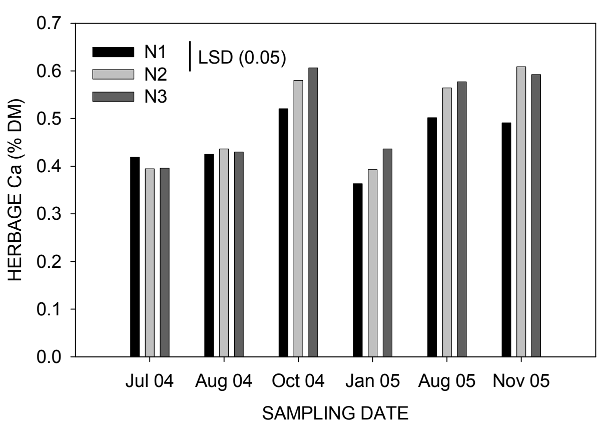


Figure 5.77. Fluctuations in herbage Ca over time for three levels of applied N

Annual variation in the levels of herbage Ca (Figure 5.78) in the NxK experiment was best described by the Gaussian 3-parameter equation:

$$y = 3.3532^{\left[-0.5\left(\frac{x-2779.2848}{1313.7204}\right)^2\right]}$$

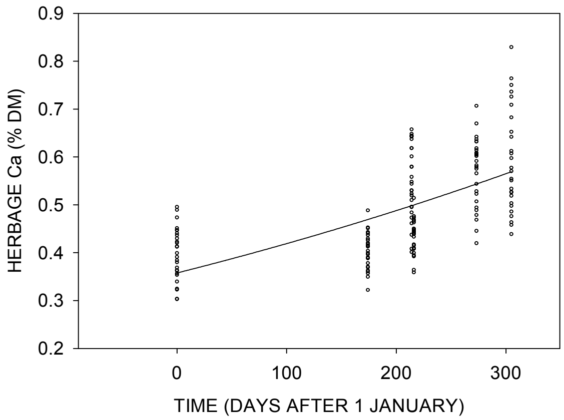


Figure 5.78. Annual variation in herbage Ca over all NxK treatments (P<0.001, R^2=0.379, $F_{2,159}$ = 48.461)

Table 5.38. The influence of sampling date and applied N and K on herbage Ca content of perennial ryegrass

Herbage Ca content (% DM) - NxK experiment										
	Treatment									
Cut date	**N1K1**	**N1K2**	**N1K3**	**N2K1**	**N2K2**	**N2K3**	**N3K1**	**N3K2**	**N3K3**	**Mean**
13-Jul-04	0.434	0.421	0.401	0.405	0.393	0.387	0.387	0.407	0.394	**0.403**^{ab*}
24-Aug-04	0.430	0.438	0.407	0.444	0.451	0.415	0.444	0.437	0.409	**0.430**^{b}
20-Oct-04	0.483	0.541	0.539	0.595	0.523	0.623	0.576	0.594	0.648	**0.569**^{cd}
20-Jan-05	0.371	0.355	0.365	0.433	0.384	0.362	0.437	0.448	0.423	**0.397**^{a}
22-Aug-05	0.533	0.493	0.479	0.608	0.600	0.485	0.635	0.596	0.501	**0.548**^{c}
21-Nov-05	0.527	0.473	0.473	0.659	0.580	0.587	0.696	0.702	0.633	**0.592**^{d}
Mean	**0.463**^{ab*}	**0.453**^{a}	**0.444**^{a}	**0.524**^{c}	**0.488**^{abc}	**0.476**^{ab}	**0.529**^{c}	**0.531**^{c}	**0.501**^{bc}	

LSD (0.05) Date = 0.03185
LSD (0.05) Treatment = 0.04458
LSD (0.05) Date x Treatment = 0.09555
CV = 11.0

	N1	**N2**	**N3**		**K1**	**K2**	**K3**
13-Jul-04	0.419	0.395	0.396		0.409	0.407	0.394
24-Aug-04	0.425	0.436	0.430		0.439	0.442	0.410
20-Oct-04	0.521	0.580	0.606		0.551	0.553	0.603
20-Jan-05	0.363	0.393	0.436		0.413	0.396	0.383
22-Aug-05	0.502	0.564	0.577		0.592	0.563	0.488
21-Nov-05	0.491	0.609	0.677		0.627	0.585	0.564
Mean	**0.453**^{a*}	**0.496**^{b}	**0.520**^{b}		**0.505**^{a*}	**0.491**^{a}	**0.474**^{a}

LSD (0.05) N = 0.02676
LSD (0.05) NxDate = 0.05601
CV = 11.2
LSD (0.05) K = 0.03768 (NS)
LSD (0.05) KxDate = 0.06206 (NS, P = 0.087)**
CV = 12.1

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.4.2.3 *NxS*

Applied N created a significant herbage Ca response over all sampling dates (P<0.001), with Ca content increasing with increasing levels of applied N (Figure 5.79). When averaged over all sampling dates, applied S did not produce a significant response.

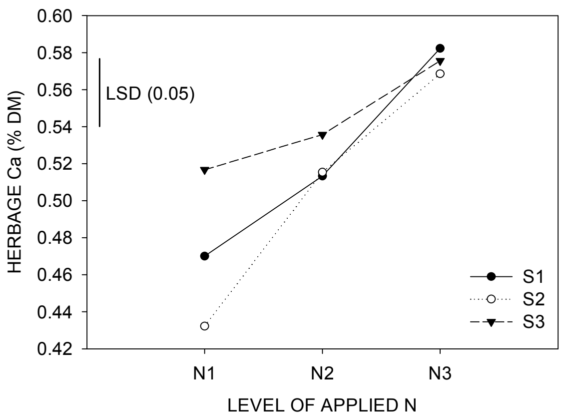


Figure 5.79. The effect of applied N and S on herbage Ca concentration averaged over all sampling dates.

Applied N affected Ca concentration at each sampling date, especially in the second season, where lower N rates resulted in significantly lower (P<0.001) herbage Ca. Calcium varied significantly with date (P<0.001), increasing during times of accelerated plant growth (Figure 5.80). The increasing difference in herbage Ca between N2 and N3 over time is evidence of a significant date-N interaction (P=0.016). There was no Ca response to applied S. Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.39.

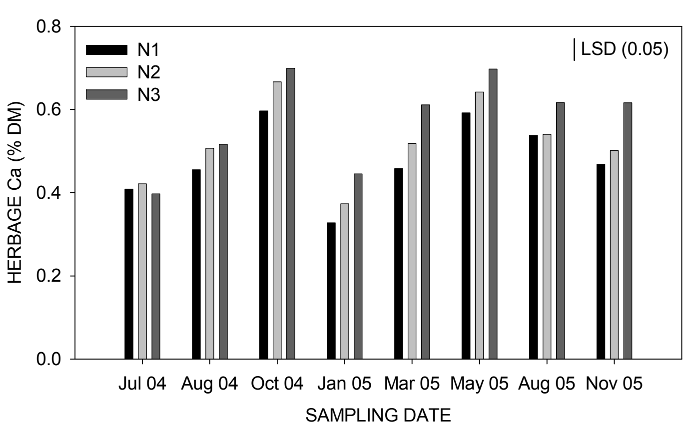


Figure 5.80. Fluctuations in herbage Ca over time for three levels of applied N

Annual variation in the levels of herbage Ca (Figure 5.81) was best described by the Gaussian 3-parameter equation:

$$y = 0.5581^{\left[-0.5\left(\frac{x-286.6606}{423.7831}\right)^2\right]}$$

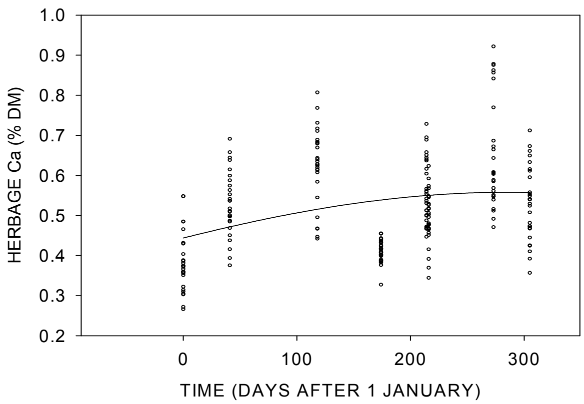


Figure 5.81. Annual variation in herbage Ca over all NxS treatments ($P<0.001$, $R^2=0.113$, $F_{2,211} = 13.479$)

Table 5.39. The influence of sampling date and applied N and S on herbage Ca content of perennial ryegrass

	Herbage Ca content (% DM) - NxS experiment									
	Treatment									
Cut date	N1S1	N1S2	N1S3	N2S1	N2S2	N2S3	N3S1	N3S2	N3S3	Mean
13-Jul-04	0.397	0.404	0.425	0.415	0.406	0.442	0.409	0.386	0.399	0.409^{a*}
24-Aug-04	0.438	0.447	0.480	0.485	0.515	0.530	0.570	0.473	0.507	0.494^{b}
20-Oct-04	0.628	0.569	0.592	0.671	0.685	0.643	0.685	0.671	0.767	0.657^{d}
20-Jan-05	0.343	0.299	0.340	0.350	0.343	0.427	0.437	0.488	0.410	0.382^{a}
01-Mar-05	0.460	0.416	0.499	0.524	0.512	0.519	0.600	0.584	0.649	0.529^{bc}
17-May-05	0.526	0.452	0.618	0.658	0.644	0.624	0.716	0.689	0.687	0.624^{d}
22-Aug-05	0.527	0.462	0.625	0.512	0.507	0.601	0.622	0.570	0.657	0.565^{c}
21-Nov-05	0.441	0.409	0.555	0.492	0.512	0.500	0.620	0.634	0.595	0.528^{bc}
Mean	0.470^{ab*}	0.432^{a}	0.517^{cd}	0.513^{bc}	0.515^{bc}	0.536^{cd}	0.582^{e}	0.562^{de}	0.584^{e}	

LSD (0.05) Date = 0.03951
LSD (0.05) Treatment = 0.04524
LSD (0.05) Date x Treatment = NS
CV = 12.7

	N1	N2	N3		S1	S2	S3
13-Jul-04	0.409	0.421	0.398		0.407	0.409	0.422
24-Aug-04	0.455	0.510	0.516		0.498	0.478	0.506
20-Oct-04	0.597	0.667	0.700		0.662	0.642	0.654
20-Jan-05	0.328	0.373	0.445		0.377	0.377	0.393
01-Mar-05	0.458	0.518	0.611		0.528	0.504	0.556
17-May-05	0.532	0.642	0.697		0.633	0.595	0.643
22-Aug-05	0.538	0.540	0.616		0.554	0.513	0.628
21-Nov-05	0.468	0.501	0.616		0.518	0.518	0.550
Mean	0.473^{a*}	0.522^{b}	0.575^{c}		0.522^{a*}	0.504^{a}	0.544^{a}

LSD (0.05) N = 0.03182
LSD (0.05) NxDate = 0.06485
CV = 12.3
LSD (0.05) S = 0.05280 (NS)
LSD (0.05) SxDate = 0.06852 (NS)
CV = 13.1

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.2.4 *PxS*

Neither data averaged over all sampling dates nor that analysed at individual sampling dates provided evidence of significant effects or interactions to applied fertilisers in the PxS experiment. Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.40.

Annual variation in the levels of herbage Ca (Figure 5.82) in the PxS experiment was best described by the Gaussian 3-parameter equation:

$$y = 1.0610^{\left[-0.5\left(\frac{x-1290.7758}{916.9140}\right)^2\right]}$$

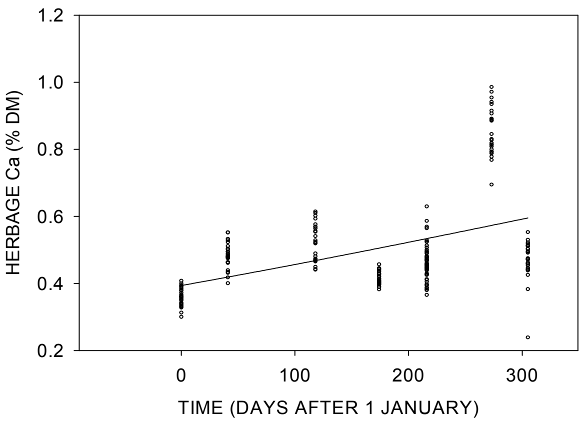


Figure 5.82. Annual variation in herbage Ca over all PxS treatments ($P<0.001$, $R^2=0.201$, $F_{2,213} = 26.797$)

Table 5.40. The influence of sampling date and applied P and S on herbage Ca content of perennial ryegrass

	Herbage Ca content (% DM) - PxS experiment									
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	0.405	0.423	0.407	0.411	0.403	0.412	0.410	0.428	0.438	**0.415^{b*}**
24-Aug-04	0.491	0.430	0.466	0.459	0.409	0.441	0.414	0.454	0.494	**0.451^{c}**
20-Oct-04	0.876	0.870	0.890	0.804	0.858	0.884	0.791	0.802	0.894	**0.852^{f}**
20-Jan-05	0.358	0.376	0.354	0.360	0.344	0.350	0.334	0.381	0.380	**0.360^{a}**
01-Mar-05	0.526	0.493	0.472	0.428	0.491	0.491	0.458	0.487	0.499	**0.483^{d}**
17-May-05	0.542	0.488	0.507	0.506	0.511	0.521	0.506	0.555	0.568	**0.523^{e}**
22-Aug-05	0.483	0.470	0.463	0.453	0.462	0.463	0.480	0.466	0.515	**0.473^{cd}**
21-Nov-05	0.426	0.463	0.474	0.468	0.477	0.492	0.493	0.414	0.523	**0.470^{cd}**
Mean	**0.513^{ab*}**	**0.501^{ab}**	**0.504^{ab}**	**0.486^{a}**	**0.495^{a}**	**0.507^{ab}**	**0.486^{a}**	**0.498^{a}**	**0.539^{b}**	
LSD (0.05) Date = 0.02906										
LSD (0.05) Treatment = 0.03905 (NS)										
LSD (0.05) Date x Treatment = 0.08717 (NS)										
CV = 9.6										
	P1	**P2**	**P3**		**S1**	**S2**	**S3**			
13-Jul-04	0.411	0.409	0.425		0.408	0.418	0.419			
24-Aug-04	0.462	0.436	0.454		0.454	0.431	0.467			
20-Oct-04	0.879	0.849	0.829		0.824	0.843	0.889			
20-Jan-05	0.363	0.352	0.365		0.351	0.367	0.361			
01-Mar-05	0.497	0.470	0.481		0.471	0.490	0.487			
17-May-05	0.512	0.513	0.543		0.518	0.518	0.532			
22-Aug-05	0.472	0.459	0.487		0.472	0.466	0.480			
21-Nov-05	0.454	0.479	0.477		0.462	0.451	0.496			
Mean	**0.506^{a*}**	**0.496^{a}**	**0.507^{a}**		**0.495^{a*}**	**0.498^{a}**	**0.516^{a}**			
LSD (0.05) P = 0.02433 (NS)										
LSD (0.05) PxDate = 0.04758 (NS)										
CV = 9.2										
LSD (0.05) S = 0.02280 (NS)										
LSD (0.05) SxDate = 0.04809 (NS)										
CV = 9.3										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.2.5 *PxK*

Herbage Ca showed an overall significant linear response to applied K (P=0.003) in the PxK experiment (Figure 5.83). Applied P did not affect Ca concentration in the herbage.

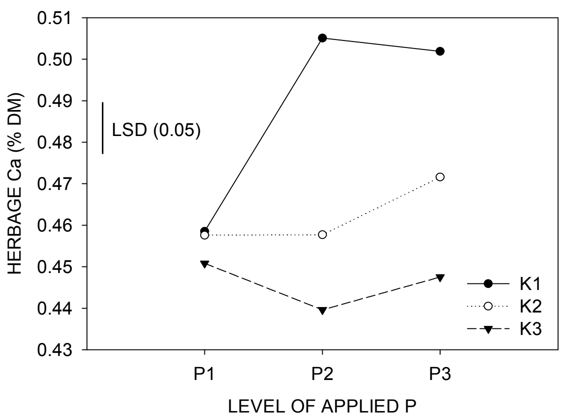


Figure 5.83. The effect of applied P and K on herbage Ca concentration averaged over all sampling dates.

Calcium response to applied K varied significantly at each sampling date (P<0.001). This response was affected by a slight date-K interaction (P=0.062), where high levels of K resulted in significantly lower levels of Ca in the plant, particularly in the second season (Figure 5.84).

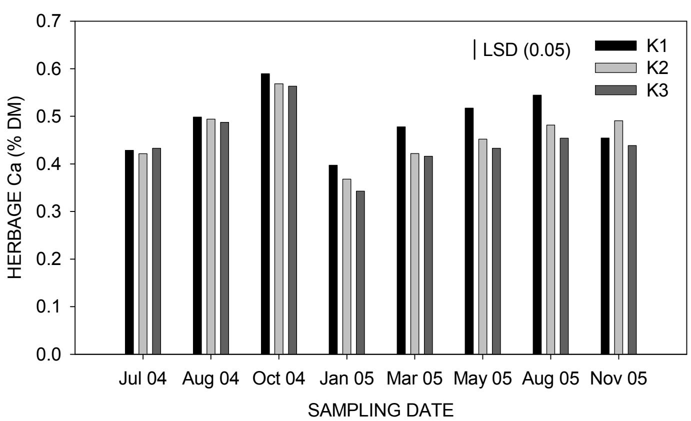


Figure 5.84. Fluctuations in herbage Ca over time for three levels of applied K

Annual variation in the levels of herbage Ca (Figure 5.85) was best described by the Gaussian 3-parameter equation:

$$y = 0.5046^{\left[-0.5\left(\frac{x-308.3928}{419.9395}\right)^2\right]}$$

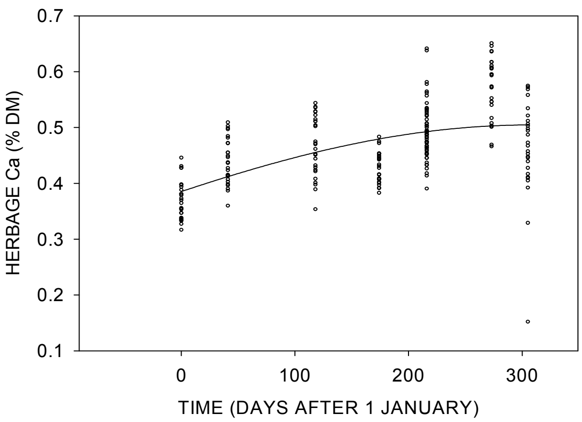


Figure 5.85. Annual variation in herbage Ca over all PxK treatments ($P<0.001$, $R^2=0.304$, $F_{2,213} = 46.485$)

Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.41.

Table 5.41. The influence of sampling date and applied P and K on herbage Ca content of perennial ryegrass

Herbage Ca content (% DM) - PxK experiment										
	Treatment									
Cut date	**P1K1**	**P1K2**	**P1K3**	**P2K1**	**P2K2**	**P2K3**	**P3K1**	**P3K2**	**P3K3**	**Mean**
13-Jul-04	0.398	0.402	0.412	0.446	0.417	0.440	0.442	0.445	0.446	**0.428[b*]**
24-Aug-04	0.487	0.477	0.481	0.520	0.485	0.501	0.488	0.521	0.480	**0.493[d]**
20-Oct-04	0.576	0.573	0.595	0.573	0.554	0.557	0.619	0.578	0.538	**0.574[e]**
20-Jan-05	0.367	0.362	0.346	0.407	0.394	0.348	0.418	0.348	0.334	**0.369[a]**
01-Mar-05	0.462	0.429	0.407	0.478	0.421	0.415	0.494	0.416	0.427	**0.439[bc]**
17-May-05	0.505	0.464	0.453	0.516	0.424	0.389	0.530	0.468	0.457	**0.467[cd]**
22-Aug-05	0.499	0.486	0.465	0.579	0.450	0.416	0.556	0.508	0.481	**0.493[d]**
21-Nov-05	0.374	0.467	0.448	0.522	0.517	0.450	0.468	0.488	0.418	**0.461[c]**
Mean	**0.459[ab*]**	**0.458[ab]**	**0.451[ab]**	**0.505[d]**	**0.458[ab]**	**0.440[a]**	**0.502[cd]**	**0.472[bc]**	**0.448[ab]**	
LSD (0.05) Date = 0.02902										
LSD (0.05) Treatment = 0.02963										
LSD (0.05) Date x Treatment = 0.08706 (NS)										
CV = 10.2										
	P1	**P2**	**P3**		**K1**	**K2**	**K3**			
13-Jul-04	0.404	0.434	0.444		0.429	0.421	0.433			
24-Aug-04	0.482	0.502	0.496		0.498	0.494	0.487			
20-Oct-04	0.581	0.561	0.578		0.589	0.568	0.563			
20-Jan-05	0.358	0.383	0.367		0.397	0.368	0.343			
01-Mar-05	0.433	0.438	0.446		0.478	0.422	0.416			
17-May-05	0.474	0.443	0.485		0.517	0.452	0.433			
22-Aug-05	0.483	0.482	0.515		0.545	0.482	0.454			
21-Nov-05	0.430	0.496	0.458		0.455	0.491	0.439			
Mean	**0.456[a*]**	**0.467[a]**	**0.474[a]**		**0.488[b*]**	**0.462[a]**	**0.446[a]**			
LSD (0.05) P = 0.02646 (NS)										
LSD (0.05) PxDate = 0.04866 (NS)										
CV = 10.2										
LSD (0.05) K = 0.02015										
LSD (0.05) KxDate = 0.04805 (NS, P = 0.062)**										
CV = 9.9										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

*** Means significantly different at the 10% level (0.1)*

5.1.4.2.6 *KxS*

Applied K and S had no significant effect on herbage Ca in the KxS experiment. There was a significant effect of date (P<0.001) on Ca content at individual sampling dates. Data reflecting the effects of treatment and sampling date on herbage Ca are summarised in Table 5.42.

Annual variation in the levels of herbage Ca (Figure 5.86) was best described by the Gaussian 3-parameter equation:

$$y = 0.4587^{\left[-0.5\left(\frac{x-297.6600}{455.8588}\right)^2\right]}$$

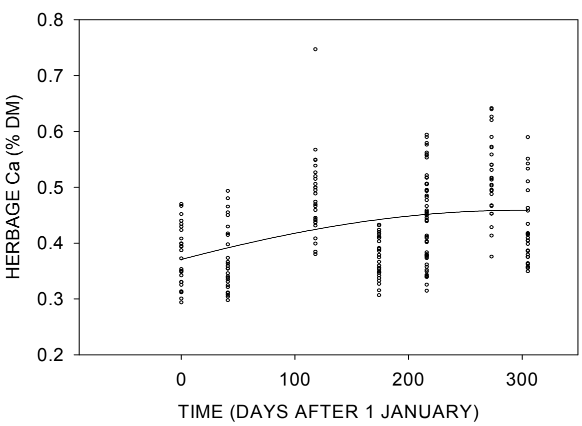


Figure 5.86. Annual variation in herbage Ca over all KxS treatments (P<0.001, R^2=0.141, $F_{2,213}$ = 17.466)

Table 5.42. The influence of sampling date and applied K and S on herbage Ca content of perennial ryegrass

Herbage Ca content (% DM) - KxS experiment										
	Treatment									
Cut date	K1S1	K1S2	K1S3	K2S1	K2S2	K2S3	K3S1	K3S2	K3S3	Mean
13-Jul-04	0.364	0.393	0.338	0.393	0.399	0.382	0.369	0.358	0.375	**0.375[ab*]**
24-Aug-04	0.515	0.519	0.502	0.424	0.534	0.494	0.471	0.499	0.494	**0.495[de]**
20-Oct-04	0.530	0.496	0.483	0.561	0.531	0.529	0.531	0.556	0.453	**0.519[e]**
20-Jan-05	0.393	0.391	0.330	0.359	0.425	0.343	0.376	0.393	0.367	**0.375[ab]**
01-Mar-05	0.391	0.417	0.390	0.345	0.433	0.351	0.347	0.334	0.337	**0.372[a]**
17-May-05	0.502	0.523	0.562	0.456	0.486	0.475	0.464	0.483	0.406	**0.484[d]**
22-Aug-05	0.486	0.430	0.381	0.397	0.451	0.384	0.385	0.350	0.371	**0.404[bc]**
21-Nov-05	0.483	0.478	0.393	0.426	0.449	0.370	0.410	0.393	0.427	**0.425[c]**
Mean	**0.458[a*]**	**0.456[a]**	**0.422[a]**	**0.420[a]**	**0.463[a]**	**0.416[a]**	**0.419[a]**	**0.421[a]**	**0.404[a]**	

LSD (0.05) Date = 0.03009
LSD (0.05) Treatment = 0.06597 (NS)
LSD (0.05) Date x Treatment = 0.09027 (NS)
CV = 11.5

	K1	K2	K3		S1	S2	S3
13-Jul-04	0.365	0.391	0.367		0.375	0.383	0.365
24-Aug-04	0.512	0.484	0.488		0.470	0.518	0.497
20-Oct-04	0.503	0.540	0.513		0.540	0.528	0.488
20-Jan-05	0.371	0.376	0.378		0.376	0.403	0.347
01-Mar-05	0.399	0.377	0.339		0.361	0.395	0.359
17-May-05	0.529	0.472	0.451		0.474	0.497	0.481
22-Aug-05	0.432	0.410	0.369		0.423	0.410	0.379
21-Nov-05	0.451	0.415	0.410		0.440	0.440	0.397
Mean	**0.445[a*]**	**0.433[a]**	**0.414[a]**		**0.432[a*]**	**0.447[a]**	**0.414[a]**

LSD (0.05) K = 0.03626 (NS)
LSD (0.05) KxDate = 0.05051 (NS)
CV = 11.4
LSD (0.05) S = 0.03602 (NS)
LSD (0.05) SxDate = 0.05161 (NS)
CV = 11.7

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.3 *Sulphur*

5.1.4.3.1 *NxS*

Sulphur levels in herbage showed no response to applied fertiliser in the NxS experiment when data were averaged over all sampling dates. There was also no S response evident at individual sampling dates, where applied N and S had no effect on the S content of herbage. Data reflecting the effects of treatment and sampling date on herbage S are summarised in Table 5.43.

Annual variation in the levels of herbage S (Figure 5.87) was best described by the cubic equation:

$$y = 0.2833 + 0.0018x - 8.785\exp^{-6} x^2 + 9.972\exp^{-9} x^3$$

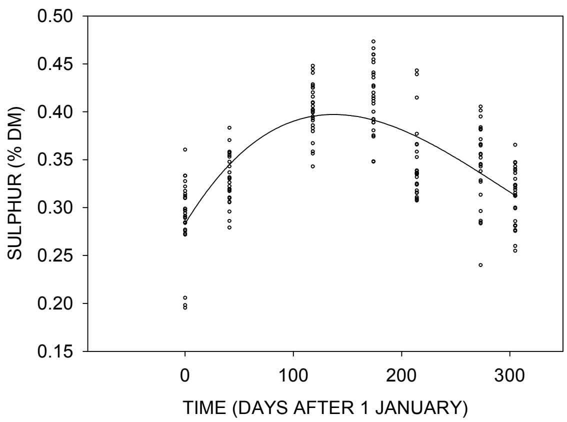


Figure 5.87. Annual variation in herbage S over all NxS treatments (P<0.001, R^2=0.520, $F_{3,183}$ = 66.1669)

Table 5.43. The influence of sampling date and applied N and S on herbage S content of perennial ryegrass

	Herbage S content (% DM) - NxS experiment									
	Treatment									
Cut date	**N1S1**	**N1S2**	**N1S3**	**N2S1**	**N2S2**	**N2S3**	**N3S1**	**N3S2**	**N3S3**	**Mean**
13-Jul-04	0.383	0.399	0.421	0.431	0.416	0.408	0.426	0.415	0.436	**0.415**^{d*}
20-Oct-04	0.321	0.331	0.326	0.368	0.350	0.345	0.374	0.336	0.369	**0.346**^{c}
20-Jan-05	0.312	0.258	0.323	0.252	0.277	0.300	0.286	0.315	0.295	**0.291**^{a}
01-Mar-05	0.305	0.343	0.324	0.322	0.323	0.355	0.326	0.332	0.328	**0.329**^{bc}
17-May-05	0.400	0.424	0.410	0.408	0.388	0.419	0.389	0.379	0.402	**0.402**^{d}
22-Aug-05	0.336	0.318	0.317	0.322	0.334	0.351	0.337	0.369	0.416	**0.344**^{c}
21-Nov-05	0.344	0.307	0.314	0.280	0.308	0.334	0.279	0.302	0.333	**0.311**^{b}
Mean	**0.343**^{ab*}	**0.340**^{a}	**0.348**^{ab}	**0.341**^{a}	**0.342**^{ab}	**0.359**^{ab}	**0.345**^{ab}	**0.347**^{ab}	**0.369**^{b}	

LSD (0.05) Date = 0.01832
LSD (0.05) Treatment = 0.02698 (NS)
LSD (0.05) Date x Treatment = 0.05495 (NS)
CV = 8.7

	N1	**N2**	**N3**		**S1**	**S2**	**S3**
13-Jul-04	0.401	0.418	0.426		0.413	0.409	0.422
20-Oct-04	0.326	0.354	0.361		0.354	0.339	0.344
20-Jan-05	0.298	0.277	0.299		0.284	0.284	0.306
01-Mar-05	0.324	0.333	0.329		0.318	0.333	0.336
17-May-05	0.411	0.405	0.390		0.399	0.397	0.410
22-Aug-05	0.323	0.336	0.374		0.332	0.340	0.361
21-Nov-05	0.321	0.307	0.305		0.301	0.306	0.327
Mean	**0.344**^{a*}	**0.347**^{a}	**0.355**^{a}		**0.343**^{a*}	**0.343**^{a}	**0.358**^{b}

LSD (0.05) N = 0.01547 (NS)
LSD (0.05) NxDate = 0.03132
CV = 8.8
LSD (0.05) S = 0.01423 (NS, P = 0.064)**
LSD (0.05) SxDate = 0.03314 (NS)
CV = 9.4

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

** *Means significantly different at the 10% level (0.1)*

5.1.4.3.2 *PxS*

There was no evidence of an S response to applied P and S, either when data were averaged over all sampling dates or when dates were analysed individually. Data reflecting the effects of treatment and sampling date on herbage S are summarised in Table 5.44.

Annual variation in the levels of herbage S (Figure 5.88) was best described by the cubic equation:

$$y = 0.3205 + 1.348\exp^{-1} x - 3.449\exp^{-6} x^2 - 3.697\exp^{-9} x^3$$

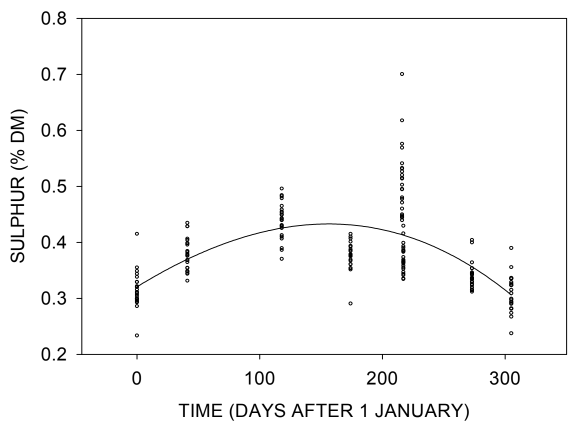


Figure 5.88. Annual variation in herbage S over all PxS treatments ($P<0.001$, $R^2=0.404$, $F_{3,185} = 41.7877$)

Table 5.44. The influence of sampling date and applied P and S on herbage S content of perennial ryegrass

Herbage S content (% DM) - PxS experiment										
	Treatment									
Cut date	**P1S1**	**P1S2**	**P1S3**	**P2S1**	**P2S2**	**P2S3**	**P3S1**	**P3S2**	**P3S3**	**Mean**
13-Jul-04	0.353	0.388	0.386	0.374	0.371	0.382	0.353	0.405	0.374	**0.376^{c*}**
24-Aug-04	0.475	0.487	0.539	0.484	0.540	0.493	0.512	0.537	0.494	**0.507^{e}**
20-Oct-04	0.324	0.343	0.337	0.327	0.361	0.338	0.353	0.337	0.338	**0.340^{b}**
20-Jan-05	0.296	0.302	0.344	0.315	0.304	0.325	0.336	0.300	0.328	**0.317^{a}**
01-Mar-05	0.365	0.356	0.405	0.369	0.363	0.393	0.392	0.380	0.399	**0.380^{c}**
17-May-05	0.401	0.449	0.468	0.403	0.451	0.424	0.455	0.457	0.450	**0.440^{d}**
22-Aug-05	0.362	0.354	0.388	0.378	0.388	0.355	0.379	0.362	0.387	**0.373^{c}**
21-Nov-05	0.279	0.311	0.335	0.313	0.309	0.310	0.306	0.308	0.306	**0.309^{a}**
Mean	**0.357^{a*}**	**0.374^{ab}**	**0.400^{b}**	**0.371^{ab}**	**0.386^{ab}**	**0.378^{ab}**	**0.386^{ab}**	**0.386^{ab}**	**0.384^{ab}**	

LSD (0.05) Date = 0.01971
LSD (0.05) Treatment = 0.02891 (NS)
LSD (0.05) Date x Treatment = 0.05913 (NS)
CV = 8.6

	P1	**P2**	**P3**		**S1**	**S2**	**S3**
13-Jul-04	0.376	0.376	0.377		0.360	0.388	0.381
24-Aug-04	0.500	0.505	0.514		0.490	0.521	0.509
20-Oct-04	0.335	0.342	0.342		0.335	0.347	0.338
20-Jan-05	0.314	0.315	0.321		0.316	0.302	0.332
01-Mar-05	0.375	0.375	0.390		0.375	0.366	0.399
17-May-05	0.439	0.426	0.454		0.420	0.452	0.447
22-Aug-05	0.368	0.374	0.376		0.373	0.368	0.377
21-Nov-05	0.309	0.311	0.307		0.300	0.309	0.317
Mean	**0.377^{a*}**	**0.378^{a}**	**0.385^{a}**		**0.371^{a*}**	**0.382^{a}**	**0.387^{a}**

LSD (0.05) P = 0.01829 (NS)
LSD (0.05) PxDate = 0.03180 (NS)
CV = 8.3
LSD (0.05) S = 0.01720 (NS)
LSD (0.05) SxDate = 0.03095 (NS)
CV = 8.0

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.1.4.3.3 *KxS*

There were no significant effects of either K or S fertiliser on herbage S concentration when data were averaged over all sampling dates. Applied K did have a significant effect (P=0.047) on herbage S at individual sampling dates (Figure 5.89). Data reflecting the effects of treatment and sampling date on herbage S are summarised in Table 5.45.

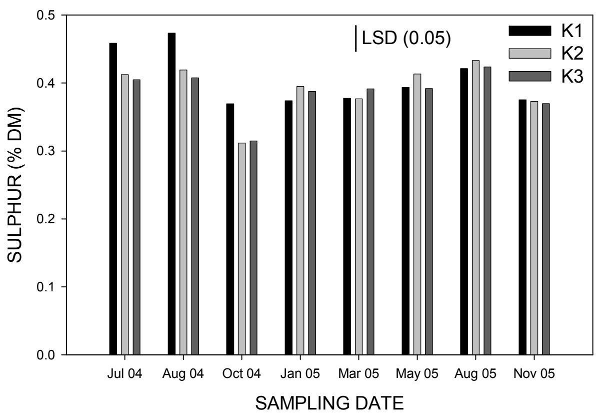


Figure 5.89. Fluctuations in herbage S over time for three levels of applied K

Annual variation in the levels of herbage S (Figure 5.90) was best described by the cubic equation:

$$y = 0.3820 - 1.087\exp^{-5} x + 3.595\exp^{-6} x^2 - 1.302\exp^{-8} x^3$$

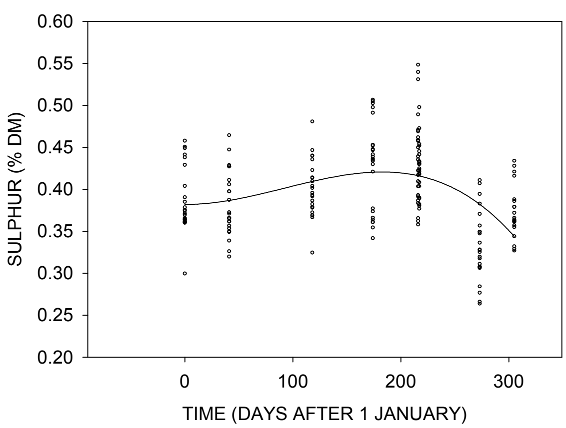


Figure 5.90. Annual variation in herbage S over all KxS treatments (P<0.001, R^2=0.210, $F_{3,185}$ = 16.4147)

Table 5.45. The influence of sampling date and applied K and S on herbage S content of perennial ryegrass

Herbage S content (% DM) - KxS experiment										
	Treatment									
Cut date	K1S1	K1S2	K1S3	K2S1	K2S2	K2S3	K3S1	K3S2	K3S3	Mean
13-Jul-04	0.477	0.441	0.458	0.410	0.393	0.435	0.414	0.386	0.414	0.425[d*]
24-Aug-04	0.471	0.467	0.482	0.422	0.420	0.416	0.394	0.430	0.399	0.433[d]
20-Oct-04	0.362	0.361	0.386	0.306	0.335	0.294	0.284	0.324	0.336	0.332[a]
20-Jan-05	0.380	0.353	0.388	0.405	0.390	0.391	0.396	0.390	0.377	0.385[bc]
01-Mar-05	0.370	0.378	0.385	0.371	0.383	0.376	0.397	0.391	0.386	0.382[bc]
17-May-05	0.390	0.399	0.391	0.381	0.439	0.420	0.372	0.406	0.397	0.400[c]
22-Aug-05	0.411	0.402	0.451	0.440	0.437	0.422	0.408	0.425	0.438	0.426[d]
21-Nov-05	0.404	0.341	0.381	0.359	0.371	0.389	0.364	0.374	0.372	0.373[b]
Mean	0.408[b*]	0.393[ab]	0.415[b]	0.387[ab]	0.396[ab]	0.393[ab]	0.379[a]	0.391[ab]	0.390[ab]	
LSD (0.05) Date = 0.02352										
LSD (0.05) Treatment = 0.02790 (NS)										
LSD (0.05) Date x Treatment = 0.07057 (NS)										
CV = 10.1										
	K1	K2	K3		S1	S2	S3			
13-Jul-04	0.458	0.412	0.405		0.434	0.407	0.435			
24-Aug-04	0.473	0.419	0.408		0.429	0.439	0.432			
20-Oct-04	0.370	0.312	0.315		0.317	0.340	0.339			
20-Jan-05	0.374	0.395	0.388		0.394	0.378	0.385			
01-Mar-05	0.377	0.377	0.391		0.379	0.384	0.382			
17-May-05	0.393	0.413	0.392		0.381	0.415	0.403			
22-Aug-05	0.421	0.433	0.424		0.420	0.421	0.437			
21-Nov-05	0.375	0.373	0.370		0.375	0.362	0.381			
Mean	0.405[b*]	0.392[ab]	0.386[a]		0.391[a*]	0.393[a]	0.399[a]			
LSD (0.05) K = 0.01523										
LSD (0.05) KxDate = 0.03717										
CV = 9.4										
LSD (0.05) S = 0.01710 (NS)										
LSD (0.05) SxDate = 0.04066 (NS)										
CV = 10.1										

* *Means in the same column or row for a specified treatment are not significantly different (P=0.05)*

5.2 Discussion

5.2.1 Crude protein and non-protein nitrogen

When assessing the nutritional value of herbage for livestock total herbage N is often expressed as crude protein (CP). CP is the concentration of total N in the herbage multiplied by 6.25, a factor derived from the average concentration of N in plant proteins (16%). It is one of the most important components of the diet of a dairy cow as it provides essential amino acids that cattle are unable to synthesise. However, the rumen has a limited capacity to convert ammonia to microbial protein and any excess NH_3 is converted to urea in the liver, most of which is excreted in urine. If there is too much ammonia for conversion to urea, ammonia toxicity (urea poisoning) will occur.

The significant CP response to increasing the rate of N application is consistent with the results of others. Van Vuuren *et al*. (1992) observed an increase of between 2.8 and 3.8 % CP (depending on season) when increasing N application from 275 to 500 kg N ha^{-1} $year^{-1}$. Wilkins *et al*. (2000) reported highly significant increases in mean N (and therefore CP) content of herbage of between 5 and 8 % N when increasing level of applied N from 100 to 600 kg N ha^{-1} $year^{-1}$. Excessive N fertilisation can lead to high herbage CP levels, which can cause metabolic problems in cows and result in a loss of production and lowered fertility (Ferguson *et al*., 1993). Vendramini *et al*. (2008) showed that increases in applied fertiliser N resulted in herbage CP with greater concentrations of rumen degradable protein and increased effective CP degradability, which resulted in increased ruminal ammonia concentrations. Moller *et al*. (1993) reported an apparent association between high blood/milk urea concentrations and reduced milk production and reproductive performance. For high yielding dairy cattle, Whitehead (1995) recommends the optimum CP concentration in the diet to be between 15 – 20 % of dry weight. This is supported by van der Merwe *et al*. (2001), who suggest herbage CP levels above 20 % DM to be sufficiently high as to consider monensin (an ionophore or antibiotic rumen manipulator) supplementation in order to reduce dairy cow blood and milk urea concentrations.

The current study elicited two points worth considering when formulating a fertiliser schedule. Firstly, CP response to applied fertiliser is strongly dependent on time of year. The range of CP values in this study (12.99 – 31.39 % DM) indicates periods of excessive and low N supply (Miles *et al*., 2000). Newly-established (immature) herbage and herbage that has experienced a period of slow growth (such as at the end of winter) appear to be particularly at risk of high CP concentrations. Low levels of N application (20 to 40 kg N ha^{-1}) were more than sufficient to maintain adequate herbage CP for livestock production during these periods. Secondly, even during periods of maximum herbage production, N applications of 80 kg N ha^{-1} resulted in average herbage CP concentrations above Whitehead's (1995) recommended optimum. This suggests that applications above 40 kg N ha^{-1}, regardless of time of the year, may increase the risk of high ruminal concentrations of NH_3 and the loss of production associated with such concentrations.

P, K and S did not affect CP concentration in herbage, except in the PxK experiment where increased levels of K lowered herbage CP. Whitehead (2000) reports similar findings in literature (Reith *et al*., 1964; Laughlin *et al*., 1973; Banwart and Pierre, 1975), suggesting that the application of K may reduce herbage N (and thus CP) if applied in conjunction with fertiliser N.

The nitrogen content of plant material can be divided into nitrogen fixed as protein (true protein, from which the CP content is derived) and non-protein nitrogen (NPN). The NPN fraction comprises amide-, ammonia- and nitrate-nitrogen. High NPN fractions in herbage can be toxic to ruminants. This occurs when the ammonia released from the NPN exceeds the rumen microbes' ability to convert it into protein. Rumen pH rises and, in severe cases, normal rumen function may eventually cease, causing muscular tremors, spasms, respiratory difficulty and death. In its milder form, ammonia toxicity depresses dry matter intake, restricts milk production and decreases reproductive ability.

Greenhill (1936) conducted one of the first experiments to determine the amounts and relative proportions of nitrogen compounds occurring in grass. He found that, while herbage concentrations of amide- and ammonium-nitrogen were unrelated to total N or to each other, higher proportions of NPN and nitrate-nitrogen were almost always associated with increases in total herbage N. Many authors have since reported similar results (Deinum and Dirven, 1974; Bartholomew and Chestnutt, 1977; Goh and Kee, 1978; Sibma and Alberda, 1980; Theron *et al*., 2002). Theron *et al*. (2002) described significant ($P<0.05$) increases in NPN as a percentage of total N with increasing levels of applied N, while Goh and Kee (1978) found consistent increases in NPN with each increment of N added. In fact, the increase in herbage N components is disproportionate, with the NPN fraction increasing to a greater extent than the true protein fraction with added fertiliser N (Hoffman and Brehm, 1999).

The NPN values in this study (range 0.22 – 1.92 and average 0.89 % DM) were similar to those reported in 1978 by Goh and Kee (0.19 to 1.53 % DM), but averaged considerably more than those (0.33 – 0.58 % DM) reported by Hunt (1973). Consistent with the results of the earlier studies mentioned above, increasing the level of applied N induced higher NPN concentrations in the herbage. NPN levels were, with exceptions, higher in immature herbage (the first analysis after establishment). This is probably due to an initial lack of true protein synthesis, which commences as the plant matures (Hoffman and Brehm, 1999).

Increased levels of NPN translate into increased herbage nitrate concentrations in the plant (Greenhill, 1936). Nitrate, a component of NPN, is frequently reported in the literature due to the animal health issues (particularly grass tetany) that may result from cattle grazing high-nitrate herbage. Nitrate in the plant is converted to nitrite in the rumen. At high rumen nitrite levels, the nitrite diffuses into the blood and competes with oxygen for uptake by haemoglobin. This results in a decrease in the oxygen carrying capacity of the blood.

Although the effects of excessive levels of NPN or NO_3-N are well known, there is no general agreement on critical or toxic levels (Hanway *et al*., 1963). Based on a literature review, Eckard (1990) reported that toxicity could occur beyond a NO_3-N limit of 0.21 – 0.35 % DM. Prins (1983), however, suggested that NO_3-N limits for grazed grass are 2 % or even greater and van Burg (1966) reported that a NO_3-N content of 0.3 – 0.6 % DM is required just in order to achieve maximum grass yields. Unlike annual ryegrass, perennial ryegrass is not known to accumulate potentially toxic levels of NPN. In addition, levels of NPN and NO_3-N tend to fall rapidly within a few days of peaking. Hunt (1973) observed that, even at the highest level of N application, the risk of nitrate poisoning was over by day 16.

Phosphorus and sulphur did not affect NPN concentration in this study. Goh and Kee (1978) reported that increasing inputs of S did not affect NPN concentrations in perennial ryegrass, except at high rates of N, when S lowered NPN levels. K decreased herbage NPN in the NxK experiment (at 10% level) and in the PxK experiment. NPN may accumulate when growth of plants well supplied with N is restricted by a factor such as a K (Griffith *et al*., 1964) or S (Goh and Kee, 1978) deficiency, or when excessive N is applied. Theron *et al*. (2002) suggested this is because the plant cannot assimilate the increased nitrogen to protein, resulting in a relative increase in NPN. K is involved in protein synthesis (Mengel and Kirkby, 2001). This may explain why, at high K levels, increasing levels of N did not affect herbage NPN. The increased availability of K facilitated the conversion of nitrate into protein, thus limiting NPN production.

5.2.2 Non-structural carbohydrates

Carbohydrates are the major source of energy in dairy cattle diets, providing energy for rumen microbes and the host animal (Nutrient requirements of dairy cattle). The carbohydrate component of forage is made up of non-structural (NSC) and fibrous or structural (SC) fractions. NSC's are found within the cells of plants and are more digestible than structural carbohydrates. The NSC fraction in plants comprises sugars, starches, organic acids and other reserve carbohydrates such as fructans. In grasses, this fraction is made up mostly of fructans and sucrose.

While high NSC concentrations in the plant are desirable, NSC's are often increased in the diet at the expense of fibre to meet the energy demands of lactating dairy cows. This may have health implications in terms of cellulose digestion. Increased levels of NSC result in decreased ruminal peptide, amino acid and NH_3 concentrations. NSC levels greater than 40% can result in the inhibition of cellulose digestion (el-Shazly, 1961, reported by Tillman and Sidhu, 1969; Griswold et al., 2003) as fibre-degrading (cellulolytic) bacteria require their entire N supply to come from NH_3. The end result is decreased digestion of NDF and ADF. The optimal concentration of NSC for lactating dairy cows is not well defined (National Research Council, 2001), although Nocek (1997) suggests that NSC levels should not exceed between 30 and 40 % of dry matter to avoid acidosis and other metabolic problems.

NSC values in this study ranged from 0.93 to 8.52 % DM. Reported concentrations of NSC in perennial ryegrass varies greatly: McKenzie *et al*. (1999b) recorded concentrations of 3.1 to 13.2 % DM while Miller et al. (2001) noted a range of NSC values from 6.5 to 35.9 % DM.

Increasing the rate of applied N reduced NSC concentration from an overall mean of 4.41 % DM at an application rate of 20 kg N ha^{-1} to 3.53 % DM at an N level of 80 kg ha^{-1}. This is consistent with other studies of N application responses (e.g. Hight *et al*., 1968, Moller *et al.*, 1996, Binnie *et al*., 2001, Tas, 2006), which observed that increases in crude protein are at the expense of NSC content. The inverse relationship that exists between CP and NSC appears to be related more to environmental factors than due to genetic effects. Research by Tas *et al*. (2006) showed that an increase in herbage NSC was not related with a decrease in CP content when comparing cultivars. Binnie *et al*. (2001) noted a 28.6 % reduction in NSC when N application level was increased from 0 to 90 kg N ha^{-1}. Garstang (1981) reported an initial increase in NSC when N rate was increased from 0 to 50 kg N ha^{-1}, but thereafter increasing rates of N depressed carbohydrate content. Low NSC concentrations in herbage may decrease the efficiency of protein utilisation during autumn and winter and have the potential to limit animal production even when the digestibility of herbage is high (Dove and Milne, 1994). Moller *et al.* (1993) observed that lower producing herds in the study had higher blood urea levels and higher pasture protein but lower pasture soluble carbohydrate concentrations compared with the higher producing herds.

On average, the NSC concentration of herbage in this study declined over winter, probably as a result of reduced solar radiation (Binnie *et al*., 2001). NSC content during August and September differed between experiments and years. The NxP and NxS experiments showed NSC increasing to peak in October, while in the NxK experiment it peaked in July and decreased thereafter. This would indicate that, on average, the energy intake of dairy animals need not be compromised if pasture management allows for a longer rotation period when days are cooler and shorter. Such practice is also beneficial for overall pasture health, as pasture will then have sufficient time to replace energy reserves depleted during grazing. This is supported by Moller *et al.* (1996), who suggested that, due to the practice of reducing rotation lengths in August - September (late winter - early spring) in order to allow grazing of highly digestible material, animals on New Zealand dairy farms were likely to be consuming less NSC by grazing N-fertilised pasture than non-fertilised pasture.

5.2.3 Acid detergent fibre and neutral detergent fibre

Acid detergent fibre (ADF) and neutral detergent fibre (NDF) are components of the structural carbohydrate fraction. NDF determination is considered the method that best separates structural from non-structural carbohydrates as it measures most of the chemical compounds considered to comprise fibre (cellulose, hemicellulose and lignin). ADF, however, remains a widely used measure of fibre and quantifies the cellulose and lignin concentration in plants (National Research Council, 2001).

Herbage concentrations of ADF (19.17 to 38.89 % DM) and NDF (31.57 to 63.3 % DM) in this study were comparable to those obtained for perennial ryegrass in other studies. Moller *et al*. (1996) and Binnie *et al*. (2001) recorded ADF concentrations of 22.0 to 33.5

and 25.9 to 37.2 % DM respectively. McKenzie *et al.* (1999b) obtained values for NDF concentrations of 44.0 to 58.2 % DM, while Moller *et al*. (1996) reported NDF values of 35.5 to 48.5 % DM.

Seasonal variation in both ADF and NDF was significant (P < 0.001) in all experiments, with fibre levels at their lowest in the cooler months and then increasing later in spring and peaking in summer when the pasture entered the reproductive phase. Many authors have reported increases in cell wall content and lignin as pasture matures (Taweel, 2006) and this was evident in November to January with pastures in the reproductive stage having increased ADF and NDF content.

Meissner *et al*. (1989) observed a strong correlation between % NDF and organic matter intake by lambs. Moller *et al*. (1996) reported significantly reduced concentrations of ADF and NDF with increasing rates of applied N. As performance is strongly dependent on intake in ruminants fed primarily forage diets (Waldo and Jorgensen, 1981), one would consider the strategic use of N fertiliser to be beneficial in delaying the increase in fibre that occurs as pasture matures. However, in this study neither ADF nor NDF were affected by N application, although responses did differ at different times during the season. These findings agree with those reported by Binnie *et al*. (2001), who observed no significant effect of either N application level or date on ADF concentration. In a review on the effect of N fertilisation on chemical composition, intake, digestion and nutritive value of fresh herbage, Peyraud and Astigarraga (1998) observed that studies on the response of structural carbohydrates to increasing levels of applied N are varied in their findings. They concluded that the composition of the NDF fraction is unaffected by the level of N fertilisation (Wilman *et al*., 1977) and that any increase in digestibility is due rather to an increase in leaf material that is low in cell wall content (Waite, 1970). Similarly, herbage NDF and ADF concentration was not affected by applied P, K or S. While these results are unsubstantiated in the literature with regard to perennial ryegrass, Malhi *et al*. (2004) reported that applied P, K and S had no effect on the fibre content of Timothy (*Phleum pretense* L.).

5.2.4 Phosphorus, calcium and sulphur

Herbage P in this study declined significantly with increasing levels of applied N. The lowest levels of herbage P were observed in the NxP experiment. In this experiment the highest herbage P concentration (0.279 % DM) was less than that of the lowest herbage P content in the PxS and PxK experiments (0.290 and 0.284 % DM respectively). Whitehead (2000) suggested this may be due to an increase in soil acidity caused by the application of N. Since uptake of P is influenced by soil pH, this may lead to herbage P deficiency. Gahoonia *et al.* (1992) found, however, that fertiliser N applied in an ammoniacal form (as was the case in this investigation) encourages P mobilisation and plant uptake through the acidification of the rhizosphere. They found that plants absorbed more P when supplied with ammonium than with nitrate or without N. The lowered P concentration observed when applied N was a factor could therefore have been due more to a dilution effect in higher yielding plots than to decreased uptake of P. In a study on the effect of fertilisers on grass species, Wolton *et al.* (1968) noted symptoms of phosphate deficiency where high levels of N were applied without fertiliser P. This indicates an increase in P demand at higher levels of N, which would enhance the dilution effect. The values indicate that P supply was adequate for grass growth. However, in many instances P concentration was below the concentration of 0.30 % DM recommended by the National Research Council (2001) for dairy cows yielding 10 kg of milk per day. Low herbage P concentrations occurred more frequently in the experiments where N was a factor, although even the highest herbage P value was below the 0.37 % recommended for dairy cows yielding 20 kg of milk per day. Phosphorus deficiency symptoms in livestock include reduced feed intake, impaired reproduction, decreased milk production, slower growth and lethargy (Whitehead, 2000).

Phosphorus applications significantly increased herbage P, which is in agreement with studies by Tainton *et al*. (1999), McKenzie *et al*. (1999b) and Bailey (1991). Increasing level of applied P from P1 to P3 resulted in approximately the same increase in herbage P regardless of the other fertiliser treatment in the experiment (NxP: 0.021; PxS: 0.023; PxK: 0.019 % DM). Applied K and S did not affect herbage P concentrations.

Sampling date had a significant effect on P concentration in herbage. Newly established herbage had the lowest P concentrations in winter. P levels increased to a maximum in mid-summer and dropped in autumn before peaking again in spring. Reay and Marsh (1976) reported a similar pattern, with P levels highest in spring, falling to about half that level in summer to their lowest in autumn. They found that P application had no immediate effect on P levels, so seasonal changes were unrelated to fertiliser application.

Calcium deficiency in terms of plant requirements is rare (Whitehead, 2000) and in this study all values were above the 0.2 – 0.3 % DM recommended by McNaught (1970) as the critical concentration for perennial ryegrass. In contrast, high-producing dairy cows find it difficult to meet their Ca requirements from dietary sources (Bredon & Dugmore, 1995). Lactating cows have a dietary Ca requirement of approximately 0.62 % DM (depending on breed and stage of lactation – National Research Council, 2001). Apart from a few higher values in October 2004, herbage Ca concentration was below this requirement, indicating a need for Ca supplementation. Decreased herbage Ca results in increased risk of animals displaying Ca deficiency symptoms, which include retarded growth in younger animals and osteomalacia in older animals. Lowered Ca content will also alter the herbage Ca:P ratio, which is recommended to be between 2:1 and 1:1 to avoid the negative effects of mineral imbalances (National Research Council, 2001).

In this study, the application of N fertiliser resulted in an increase in Ca content. This is in contrast with results presented by Hopkins *et al*. (1994), who showed that the application of fertiliser N was associated with a decrease in the herbage content of Ca. When soil Ca supplies are limited, the increase in growth caused by the application of N may reduce the concentration of Ca in herbage. However, when soil Ca is abundant, herbage Ca may increase due to the synergistic effect between nitrate and cation uptake (Whitehead, 2000). The pre-trial soil analysis (Table 3.1) indicated that Ca was the dominant cation in the soil (824 mg l^{-1}, as opposed to K: 157 mg l^{-1} and Mg: 224 mg l^{-1}). It would therefore have been extracted into the soil solution by the increase in nitrate ions when fertiliser N was applied.

Similarly, when applied with fertiliser N, the addition of fertiliser K resulted in a decrease in herbage Ca. This is consistent with results of studies by Dampney (1992) and Razmjoo and Kaneko (1993), which found that K applications decreased the Ca content of herbage. K is preferentially bonded to nitrate over Ca and Mg. Increasing the K content in the soil through K fertilisation would have encouraged the formation and uptake of potassium nitrate, which would have increased dry matter production and hence the dilution of the Ca concentration in the herbage. P and S application did not affect herbage Ca, which is consistent with other reports in literature (Whitehead, 2000).

Calcium content was significantly affected by sampling date in all but the PxS experiments. In the first season, Ca concentration increased from winter to peak in spring (October 2004), decreasing in summer until January 2005, when levels started to increase again. From the autumn of the second season (April 2005) onwards, Ca content varied only slightly with sampling date. Correspondingly, Crush *et al.* (1989) observed a spring peak in herbage Ca, which they attributed to a sharp reduction in ammonium levels that occurs then (ammonium and low soil temperatures inhibit Ca uptake in winter). Reay and Marsh (1976), however, reported Ca to be lowest in spring, rising to a maximum in early autumn before falling again in late autumn.

The critical level of S in herbage has been reported as 0.2 % (Jones *et al*., 1972), 0.23 % (Goh and Kee, 1978) and 0.28 % (Metson, 1973). Herbage S in this study ranged from 0.2 to 0.7 % DM (average 0.38, median 0.37 % DM), which indicates that in some instances, herbage S may have been approaching deficiency levels. The optimum level of sulphur in the ruminant diet is between 0.16 and 0.24 % S (Bredon & Dugmore, 1995), below which the risk of reduced protein synthesis and poor utilisation of dietary N is increased (National Research Council, 2001; Whitehead, 2000). Of greater concern is the increased risk of S toxicity, which has been reported at S concentrations as low as 0.5 % DM (National Research Council, 2001). Excessive dietary S can interfere with the absorption of other elements (particularly copper and selenium) and reduce feed intake and animal performance (National Research Council, 2001). McAllister *et al.* (1997) noted that a polioencephalomalacia-like syndrome could be induced with diets containing 0.5 % S. The National Research Council (2001) recommends that maximum dietary S not exceed 0.4 % DM. Almost 38 % of the samples analysed had S contents greater than 0.4 %, which is cause for concern, especially since the samples with high S values were not necessarily limited to those originating from plots receiving high levels of fertiliser S.

There was no herbage S response to applied fertiliser in this study. Whitehead (2000) reports that herbage S response to applied N is dependent on S supplies in the soil and fertiliser N may increase, decrease or have no effect on the concentration of S in grass. While responses to fertiliser S are reported to be common in countries such as Ireland (Murphy and Quirke, 1997) and New Zealand (Goh and Kee, 1978), crop responses to applied S, and cases of S deficiency, in South Africa are rare (Miles and Manson, 2000; Miles, 2006). Miles and Manson (2000) believe that, in soils that are tilled annually, there is little risk of S deficiency due to the mineralisation of organic S. In addition, many of these soils would have been fertilised with S-containing superphosphates, allowing the build-up of inorganic S and buffering the soil against an S deficiency.

5.3 Conclusions

Results from this study showed that the quality of perennial ryegrass herbage, especially in terms of feed value to dairy cows, can be significantly affected by applied fertiliser. The extent of the response was affected by sampling date (i.e. time of year) and this must be taken into account when planning a fertiliser management strategy.

Crude protein content of herbage increased with increasing levels of applied N. The extent of the response was influenced by season, as indicated by the range of CP values obtained over the trial period. The results provide evidence that supports changing N fertiliser management practice with season. It is recommended that application levels be decreased during late winter, as there is an increased risk of high herbage CP concentrations during periods of slow growth. Applications of 20 - 30 kg N ha^{-1} should be sufficient to maintain CP concentrations within acceptable limits and make allowance for the decreased N mineralisation that occurs during cold months. During periods of maximum herbage production, it is suggested that N applications of 40-50 kg N ha^{-1} are sufficient. P, K and S did not affect CP concentration in herbage, except in the PxK experiment where increased levels of K lowered herbage CP.

Applied N considerably increased the concentration of NPN in perennial ryegrass herbage. P and S did not affect NPN levels, while applied K decreased NPN content in the NxK and PxK experiments. While perennial ryegrass is not known to accumulate potentially toxic levels of NPN, there is no general agreement on what levels could be considered toxic. Sub-clinical toxicity may occur well before actual toxic levels are reached and this could affect animal performance and reproduction. It is therefore advisable to keep N applications to the minimum required to maintain production, especially over periods of slow pasture growth.

Non-structural carbohydrate content of herbage was decreased by applied N. Lowered NSC content often accompanies increases in herbage CP. A reduction in applied N at times of the year when there is an accumulation of herbage CP and NPN may be a method to manage not only excessive protein content, but also dropping levels of NSC. The practice of increasing rotation lengths in late winter may also assist in maintaining acceptable concentrations of NSC in perennial ryegrass. Short rotations, especially when pasture growth is slow, do not allow sufficient time for plant energy reserves to be replenished.

Neither NDF nor ADF was affected by applied fertiliser. Seasonal variation in NDF and ADF was significant in all experiments, with fibre levels peaking in summer when pasture entered the reproductive phase. Some authors have reported reduced concentrations of NDF and ADF with applied N (Moller *et al*., 1996). It appears, however, that N does not directly affect fibre content, but rather has a dilution effect by increasing the proportion of material that is low in cell wall content (Waite, 1970).

Herbage P in this study declined significantly with increasing levels of applied N. Although soil analysis indicated that P supply was adequate for grass production, in many instances herbage P concentration was below that recommended by the National Research Council (2001) for dairy cows yielding 10 kg of milk per day. Phosphorus deficiency can lead to decreased feed intake and reduced milk production, as well as impaired reproduction. Livestock systems utilising pastures that receive regular N applications may benefit from pasture herbage analysis to ensure sufficient herbage P levels for animal production.

Nitrogen application increased the Ca content of herbage, probably due to Ca being the dominant cation in the soil and a synergistic effect that exists between nitrate and cation uptake (Whitehead, 2000). In spite of the increased herbage Ca, the majority of herbage analyses indicated Ca content below that required to meet the dietary needs of high-producing dairy cows. In view of the negative effects of high applications of fertiliser N, increasing the quantity of N applied would not be considered a viable solution to low Ca content in herbage. The addition of fertiliser K resulted in lower herbage Ca values. Potassium is preferentially bonded to nitrate over Ca and Mg. This accounted for the decrease in herbage Ca noted when the amount of K applied was increased.

There was no herbage S response to applied fertiliser in this study. Previous soil management included the use of S-containing superphosphates, which would have allowed the build-up of inorganic S, buffered the soil against an S deficiency and reduced plant response to applied S.

CHAPTER 6

Factors affecting apparent intake

6.1 Results

6.1.1. NxP

Apparent intake in the NxP experiment was primarily affected by the amount of herbage available for grazing (Figure 6.1). The effects of copper (Cu) became important at low levels of herbage availability, while phosphorus content of herbage affected apparent intake in plots with higher average disc meter readings.

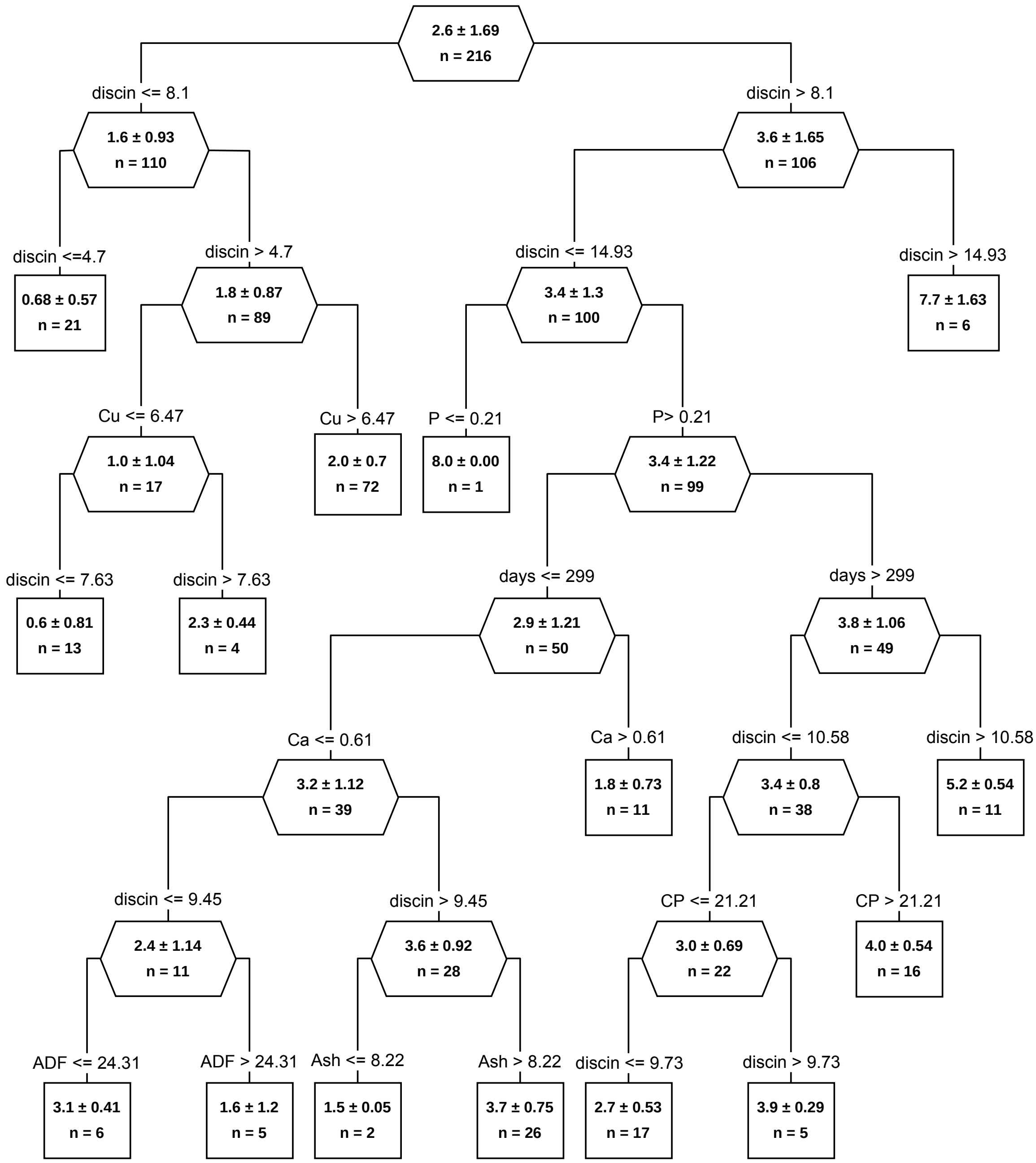


Figure 6.1. Regression trees for apparent intake of perennial ryegrass in the NxP experiment. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

The relationship between the amount of herbage pre-grazing (as measured by disc meter) and subsequent apparent intake (Figure 6.2) was highly significant. This disproved the assumption that the difference in quantity of available herbage between plots (particularly those with N as a factor) did not affect apparent intake.

The relationship between disc height and apparent intake was best described by the equation:

$$y = -1.1676 + 0.4582x$$

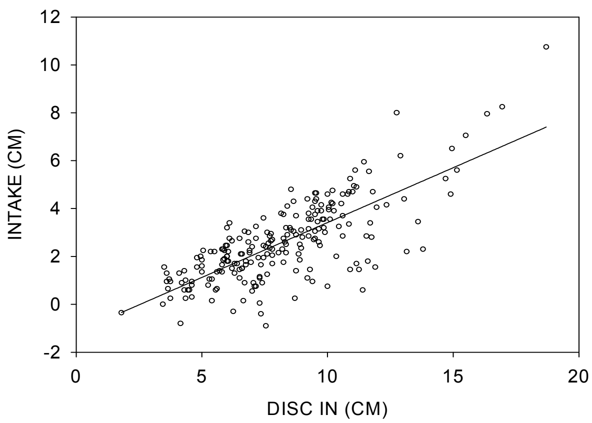


Figure 6.2. Relationship between herbage available prior to grazing and subsequent apparent intake in the NxP experiment (P<0.001, R^2 = 0.5768, $F_{1,214}$ = 291.720)

Applied fertiliser did not appear to affect apparent intake directly (Table 6.1), although herbage P and CP – which were affected by applied P and N in the experiment – featured in the top four most important factors influencing apparent intake.

Table 6.1. Relative importance of experimental and quality factors in determining apparent intake in the NxP experiment

	F	G
1	**Variable**	**Relative importance**
2	Discin	100.00
3	P	22.07
4	Ca	20.46
5	CP	17.64
6	Fe	16.82
7	Fat	14.22
8	Days	9.68
9	NDF	8.42
10	Ash	7.80
11	ADF	5.39
12	Cu	4.58
13	Mg	4.02
14	Zn	2.68
15	% dry matter	2.65
16	K	2.45
17	N level	1.98
18	Mn	1.54
19	NPN	0.98
20	NSC	0.32
21	Na	0.19
22	Replicate	0.00
23	Applied N	0.00
24	Applied P	0.00
25	P level	0.00

6.1.2. NxK

The amount of material available prior to grazing was the dominant factor influencing apparent intake in the NxK experiment (Figure 6.3). Crude protein affected apparent intake at higher levels of herbage availability.

A highly significant relationship existed between the amount of herbage pre-grazing (as measured by disc meter) and subsequent apparent intake (Figure 6.4) in the NxK experiment.

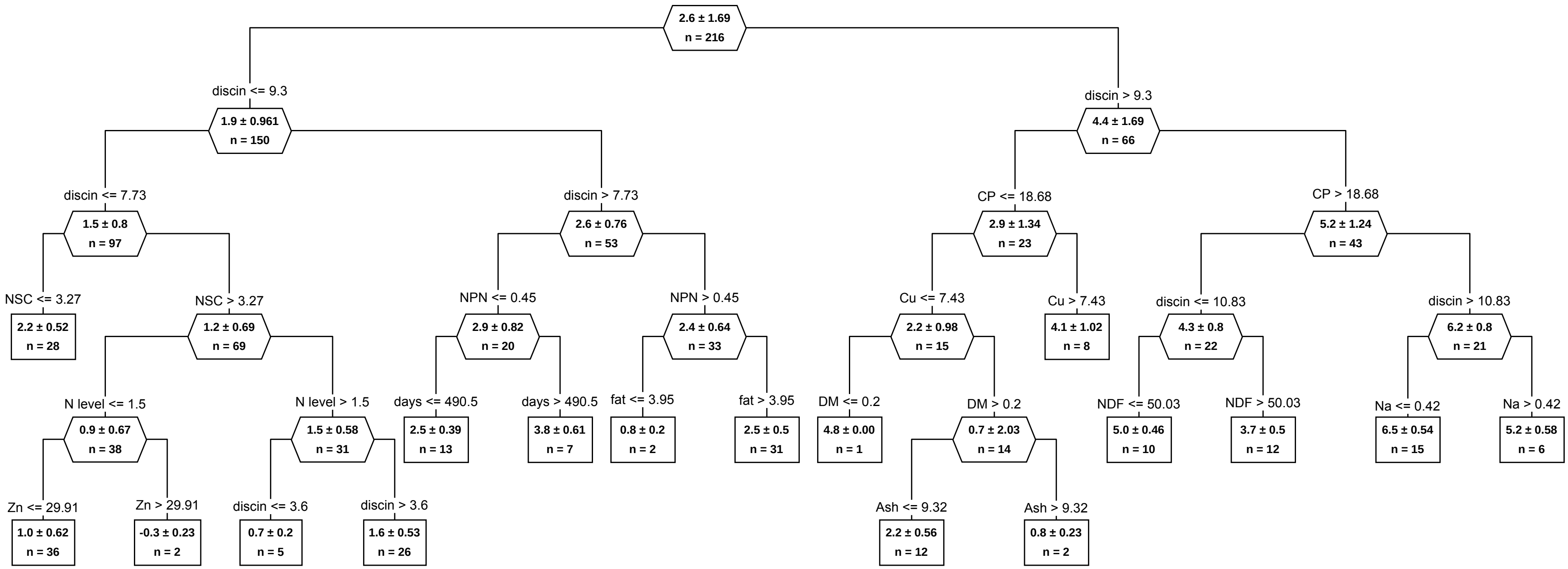


Figure 6.3. Regression trees for apparent intake of perennial ryegrass in the NxK experiment. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

The relationship between disc height and apparent intake was best described by the equation:

$$y = -0.7951 + 0.4192x$$

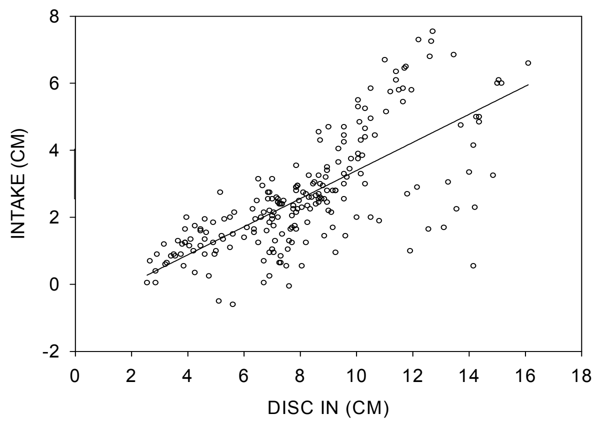


Figure 6.4. Relationship between herbage available prior to grazing and subsequent apparent intake in the NxK experiment (P<0.001, $R^2 = 0.5229$, $F_{1,214} = 234.5523$)

The number of days after planting had a high relative importance after the amount of grazing available (Table 6.2). Related to that, the fibre content of herbage influenced apparent intake to a greater extent than did the other components examined.

Table 6.2. Relative importance of experimental and quality factors in determining apparent intake in the NxK experiment

	G	H
1	**Variable**	**Relative importance**
2	Discin	100.00
3	Days	78.50
4	Mg	55.19
5	NDF	29.86
6	ADF	29.26
7	CP	26.71
8	P	22.92
9	K	20.63
10	Fat	17.18
11	Ash	13.02
12	% dry matter	8.99
13	Cu	7.45
14	NPN	4.35
15	NSC	4.11
16	Na	2.73
17	Fe	2.63
18	Zn	2.42
19	Ca	1.47
20	N level	1.27
21	Replicate	0.00
22	Applied K	0.00
23	Mn	0.00
24	Applied N	0.00
25	Potassium level	0.00

6.1.3 NxS

The amount of herbage available was the primary splitting criterion in the NxS experiment (Figure 6.5). At lower levels of herbage availability, % dry matter and the number of days after planting further split the data, while "discin" and "days" remained the chief criteria in plots with more available grazing.

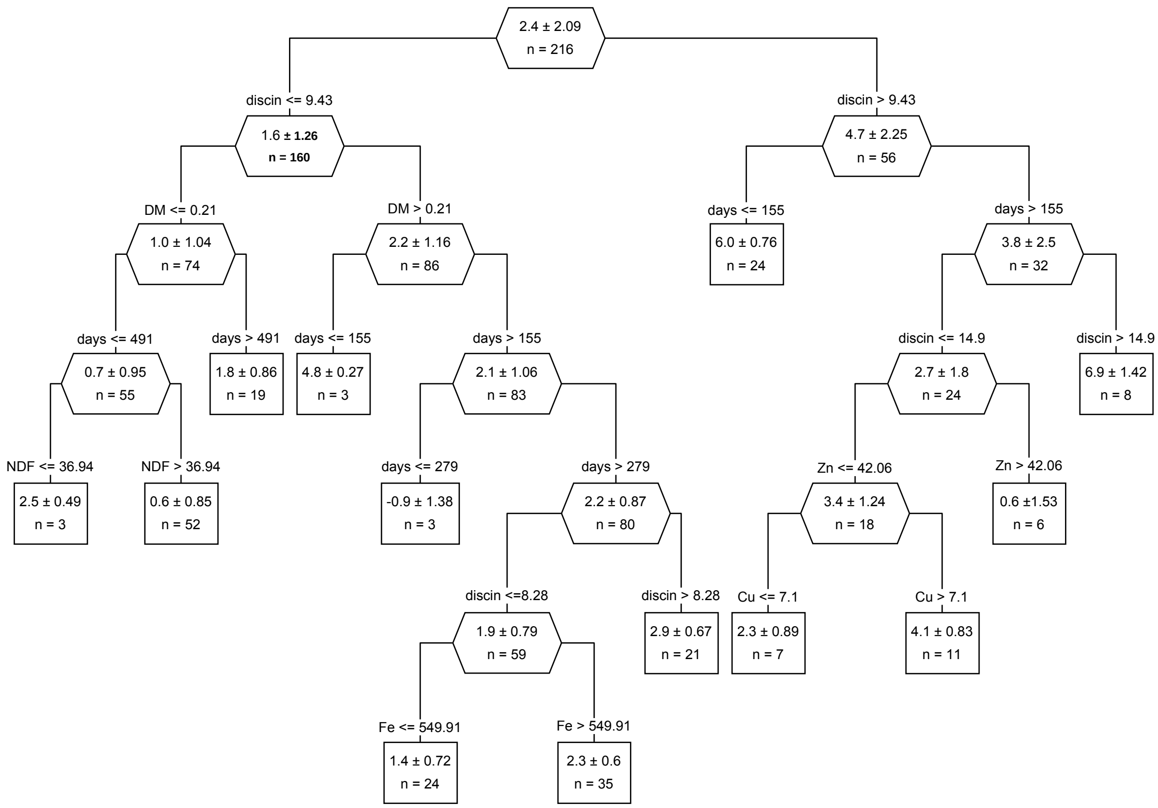


Figure 6.5. Regression trees for apparent intake of perennial ryegrass in the NxS experiment. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

A highly significant relationship existed between the amount herbage pre-grazing (as measured by disc meter) and subsequent apparent intake (Figure 6.6) in the NxS experiment.

The relationship between disc height and apparent intake was best described by the equation:

$$y = -0.9104 + 0.4085x$$

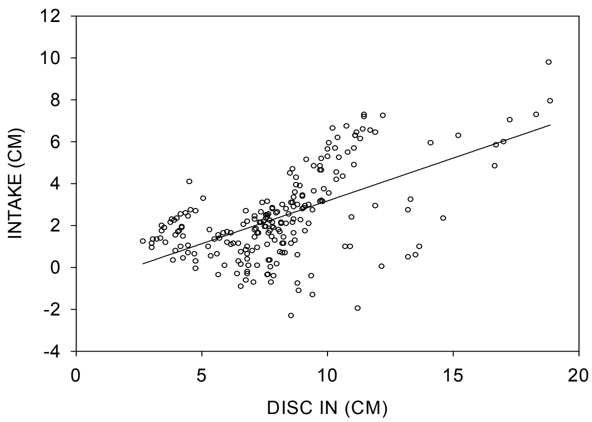


Figure 6.6. Relationship between herbage available prior to grazing and subsequent apparent intake in the NxS experiment ($P<0.001$, $R^2 = 0.3515$, $F_{1,214} = 115.9755$)

The amount of herbage available for grazing, the age of the pasture and consequently fibre content had the highest relative importance values in terms of factors influencing apparent intake (Table 6.3). Applied fertiliser and the levels thereof had no relative importance.

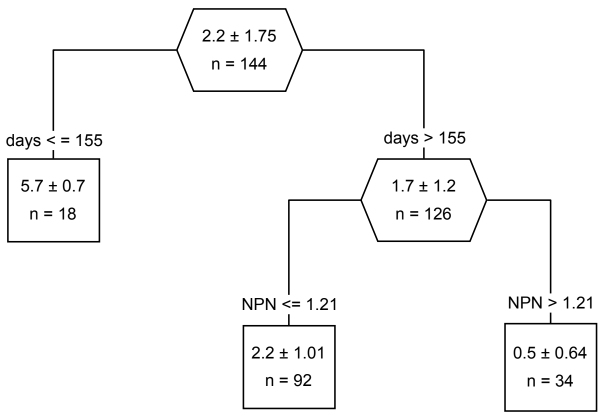
Table 6.3. Relative importance of experimental and quality factors in determining apparent intake in the NxS experiment

	F	G
1	**Variable**	**Relative importance**
2	Discin	100.00
3	Days	97.13
4	NDF	81.14
5	ADF	79.97
6	P	55.64
7	Mg	51.23
8	Ash	10.48
9	% dry matter	10.46
10	Ca	10.32
11	S	9.40
12	Zn	7.71
13	NPN	7.33
14	NSC	6.81
15	Na	6.30
16	CP	4.90
17	Fe	4.61
18	Fat	3.85
19	Cu	2.72
20	Mn	1.94
21	Replicate	0.00
22	Applied S	0.00
23	Nitrogen level	0.00
24	Sulphur level	0.00
25	K	0.00
26	Applied N	0.00

6.1.4 PxS

Without nitrogen as a treatment, herbage availability did not differ significantly between treatments in the PxS experiment (Figure 6.7a). Pasture age ("days") was the primary splitting criterion. When the pasture was older than 155 days, NPN became an important factor in determining apparent intake.

a) All variables



b) Quality-related variables

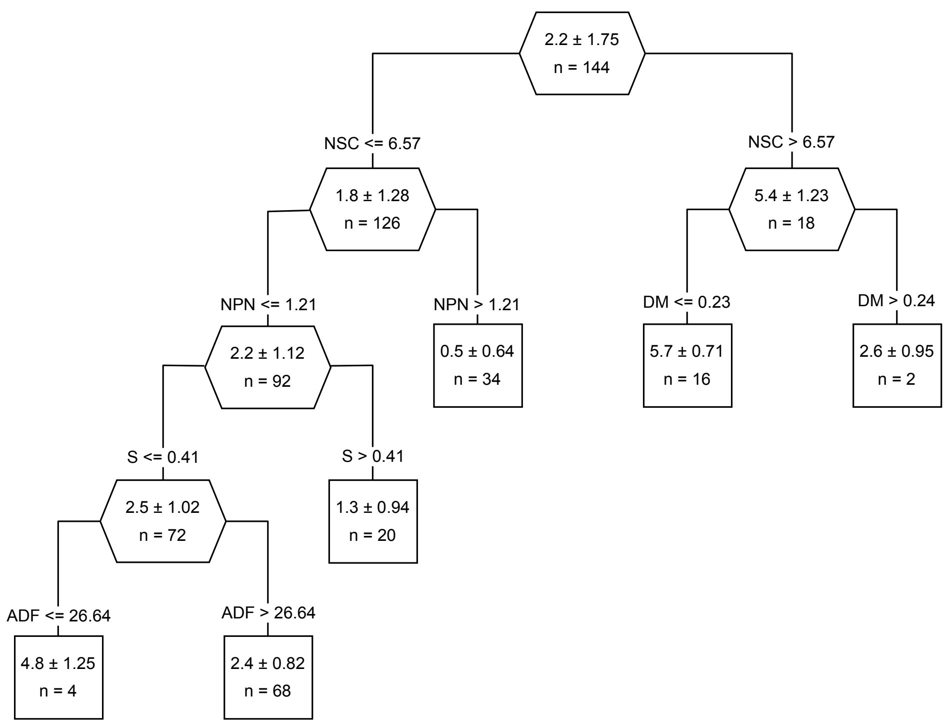


Figure 6.7. Regression trees for apparent intake of perennial ryegrass in the PxS experiment: a) all variables and b) quality-related variables only. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

When only quality-related variables were included in the analysis, sugar content (NSC) was the primary splitting criterion (Figure 6.7b). At lower levels of NSC, non-protein nitrogen, sulphur and fibre affected apparent intake, while at higher NSC levels only dry matter was selected to split the data.

In spite of the fact pasture availability was not a factor in determining apparent intake, a highly significant relationship continued to exist between the amount of herbage pre-grazing and subsequent apparent intake in the PxS experiment (Figure 6.8).

The relationship between disc height and apparent intake was best described by the equation:

$$y = -1.4169 + 0.4854x$$

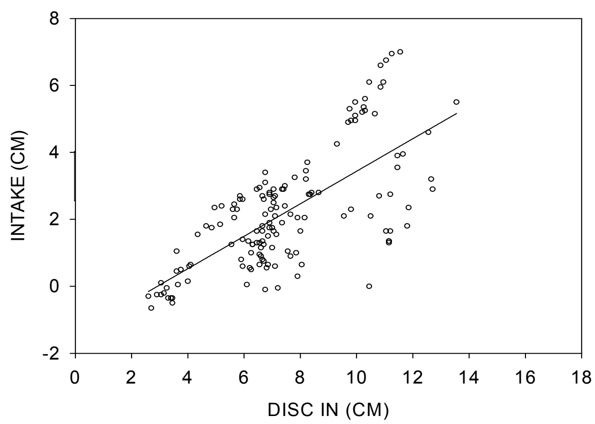


Figure 6.8. Relationship between herbage available prior to grazing and subsequent apparent intake in the PxS experiment (P<0.001, R^2 = 0.4831, $F_{1,142}$ = 132.7226)

In terms of relative importance in the PxS experiment (Table 6.4), pasture age was the most important variable in determining apparent intake, while fibre, sugar and magnesium content also influenced apparent intake but to a lesser extent.

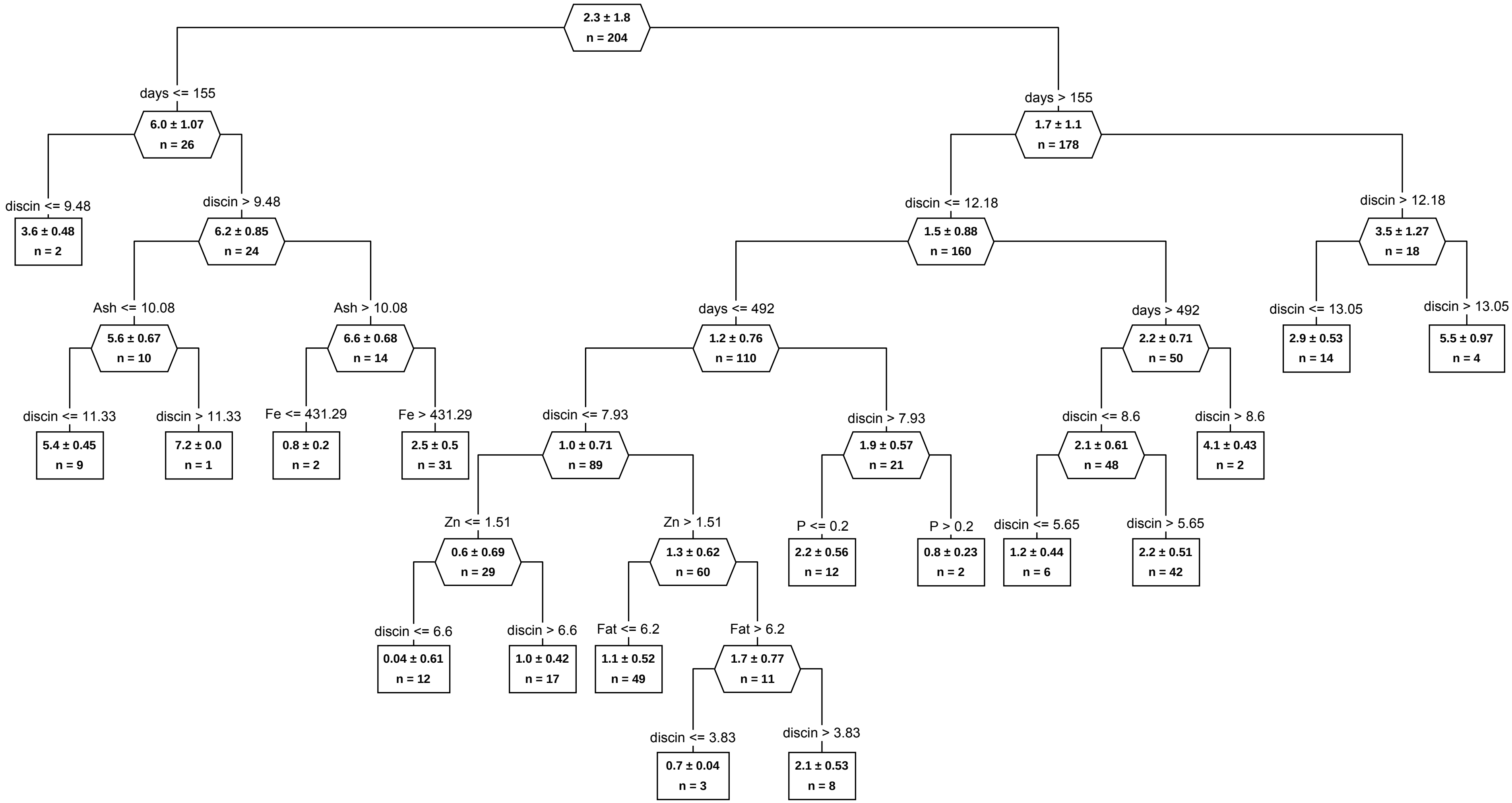
Table 6.4. Relative importance of experimental and quality factors in determining apparent intake in the PxS experiment

	F	G
1	**Variable**	**Relative importance**
2	Days	100.00
3	ADF	84.00
4	NSC	82.28
5	Mg	81.83
6	NDF	79.43
7	NPN	28.48
8	Discin	23.83
9	Fat	23.60
10	CP	10.05
11	% dry matter	4.67
12	Sulphur level	0.00
13	Phosphorus	0.00
14	Sulphur	0.00
15	Phosphorus level	0.00
16	Replicate	0.00
17	Ca	0.00
18	Ash	0.00
19	K	0.00
20	Na	0.00
21	P	0.00
22	S	0.00
23	Zn	0.00
24	Cu	0.00
25	Mn	0.00
26	Fe	0.00

6.1.5. PxK

The primary splitting criterion in the PxK experiment was pasture age, which, along with herbage availability, dominated the regression tree (Figure 6.9a). When only quality-related factors were included in the analysis (Figure 6.9b), ADF (fibre) was the primary splitting criterion. At higher levels of ADF, sugars and non-protein nitrogen were also included as factors that affected apparent intake.

a) All variables



b) Quality-related variables

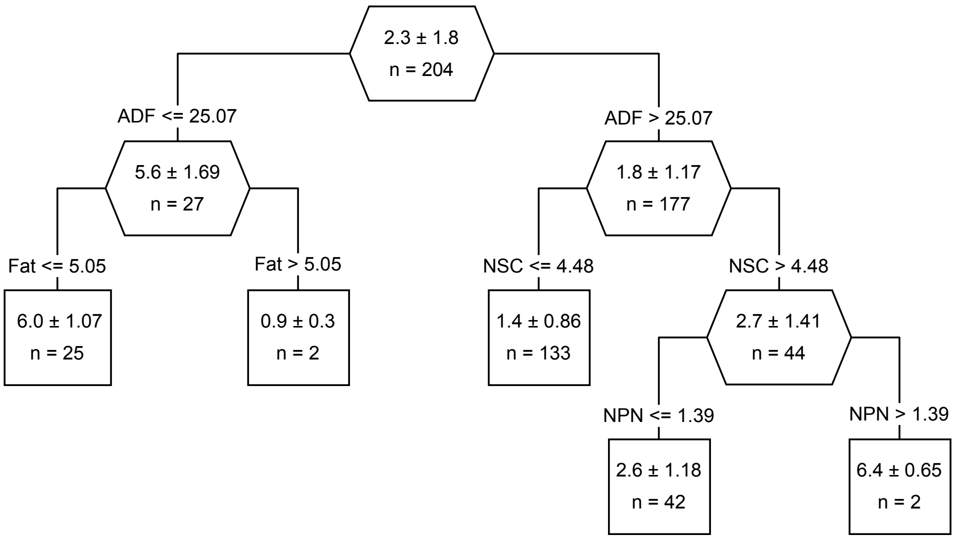


Figure 6.9. Regression trees for apparent intake of perennial ryegrass in the PxK experiment: a) all variables and b) quality-related variables only. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

A highly significant relationship existed between the amount herbage pre-grazing (as measured by disc meter) and subsequent apparent intake (Figure 6.10) in the PxK experiment.

The relationship between disc height and apparent intake was best described by the equation:

$$y = -1.4713 + 0.4740x$$

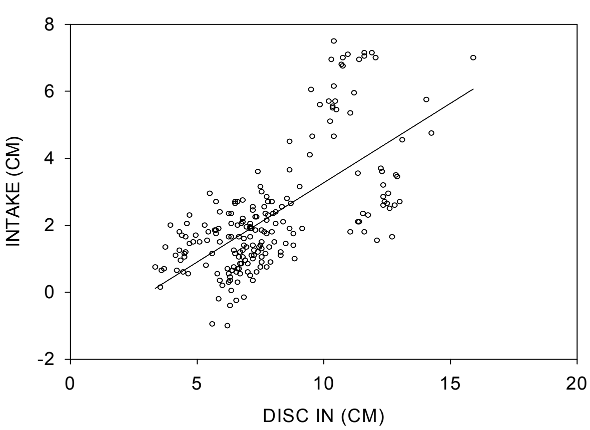


Figure 6.10. Relationship between herbage available prior to grazing and subsequent apparent intake in the PxK experiment (P<0.001, $R^2 = 0.4353$, $F_{1,202} = 155.7262$)

Pasture age had the greatest importance in determining apparent intake in the PxK experiment. Fibre and sugar content had a high relative importance, while applied fertiliser had no direct effect on apparent intake (Table 6.5).

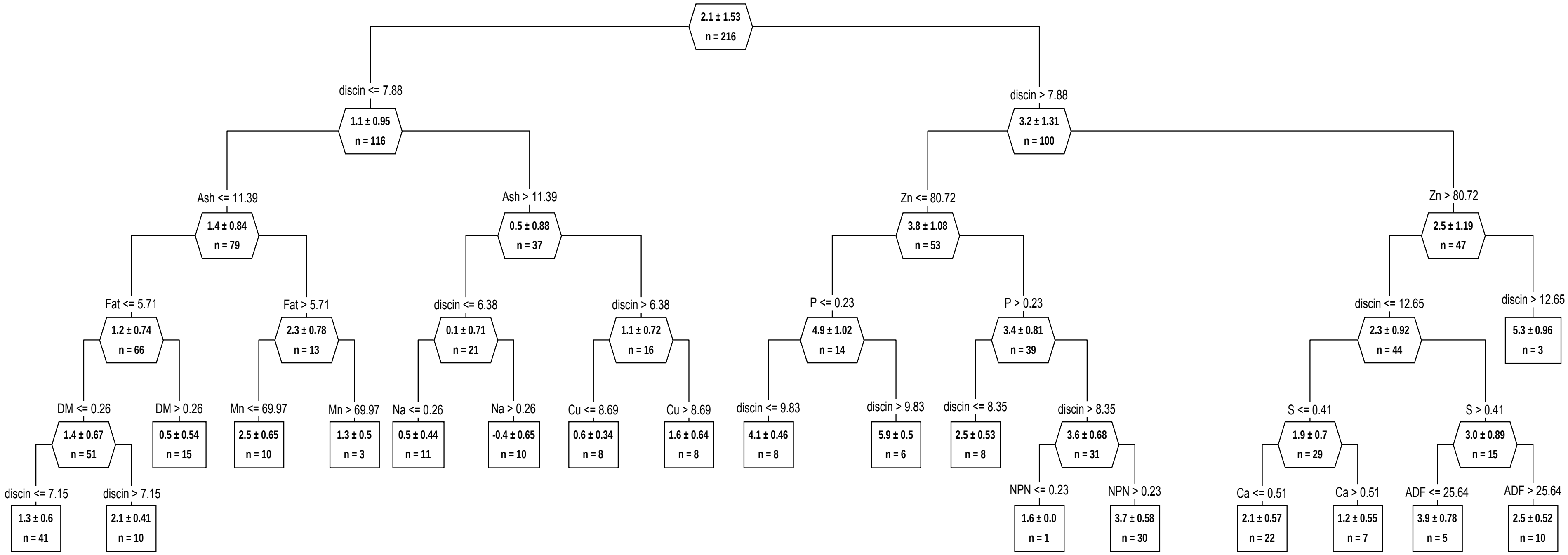
Table 6.5. Relative importance of experimental and quality factors in determining apparent intake in the PxK experiment

	F	G
1	**Variable**	**Relative importance**
2	Days	100.00
3	ADF	76.47
4	NSC	69.41
5	NDF	69.41
6	Mg	42.10
7	NPN	38.13
8	Discin	29.87
9	Zn	12.54
10	Fat	5.28
11	Na	3.84
12	Ash	3.44
13	CP	3.42
14	Ca	3.33
15	Cu	2.27
16	K	2.21
17	Fe	1.96
18	% dry matter	1.46
19	P	0.98
20	Mn	0.91
21	Potassium level	0.83
22	Replicate	0.74
23	Phosphorus level	0.66
24	Applied phosphorus	0.00
25	Applied potassium	0.00

6.1.6. KxS

The regression tree for the KxS experiment was extensive, indicating a complex apparent intake response (Figure 6.11). The primary splitting criterion was herbage availability, after which ash (in low herbage plots) and zinc (in high herbage plots) appeared to play a role in selection. Herbage availability was selected as a splitting criterion throughout the KxS regression tree.

a) All variables



b) Quality-related variables

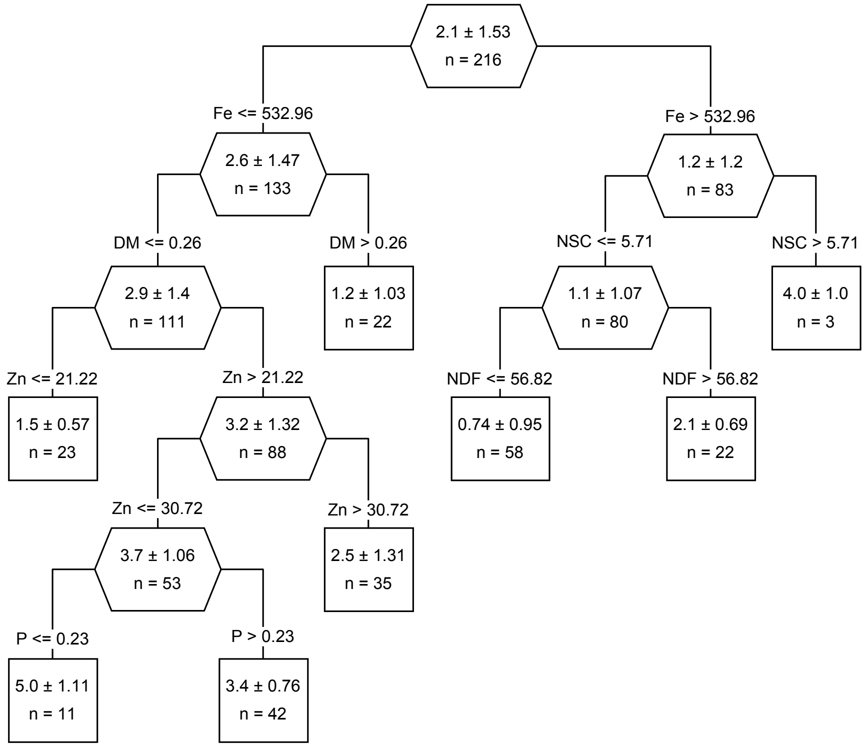


Figure 6.11. Regression trees for apparent intake of perennial ryegrass in the KxS experiment: a) all variables and b) quality-related variables only. The splitting criteria are presented at each dichotomy. Means (± se) and number of members for each group in the terminal (rectangle) and non-terminal (broken rectangle) leaf node are presented.

A highly significant relationship existed between the amount of herbage pre-grazing (as measured by disc meter) and subsequent apparent intake (Figure 6.12) in the KxS experiment.

The relationship between disc height and apparent intake was best described by the equation:

$$y = -1.5550 + 0.4775x$$

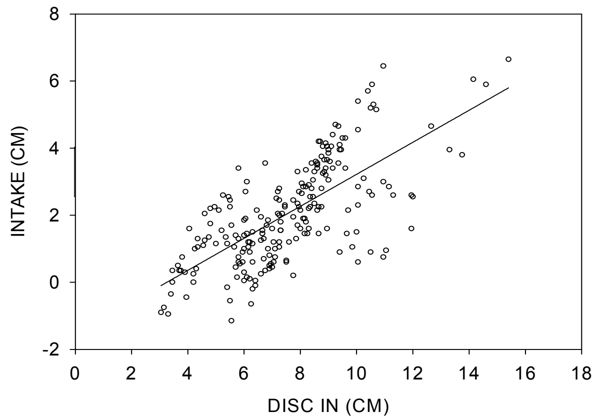


Figure 6.12. Relationship between herbage available prior to grazing and subsequent apparent intake in the KxS experiment (P<0.001, R^2 = 0.4951, $F_{1,202}$ = 209.8911)

Herbage availability displayed the highest relative importance in the KxS experiment (Table 6.6). Sugar content was the second most important variable, but was a comparatively poor determinant of apparent intake.

Table 6.6. Relative importance of experimental and quality factors in determining apparent intake in the KxS experiment

	F	G
1	**Variable**	**Relative importance**
2	Discin	100.00
3	NSC	44.69
4	Fe	35.94
5	NDF	29.67
6	K	26.32
7	Zn	19.25
8	Fat	14.38
9	P	12.76
10	Ash	12.16
11	Days	12.13
12	Mg	9.38
13	Mn	9.37
14	ADF	9.02
15	% dry matter	6.68
16	Ca	6.62
17	NPN	5.25
18	S	4.67
19	Cu	4.02
20	Na	3.14
21	Replicate	2.31
22	Sulphur level	1.06
23	CP	0.40
24	Potassium level	0.00
25	Potassium	0.00
26	Sulphur	0.00

6.2 Discussion

Milk production in dairy cows is largely controlled by the quantity and quality of feed consumed (Reeves *et al*., 1996) and the relationship between voluntary food intake and animal productivity is well recognized (Allden and Whittaker, 1970). Many studies have investigated ruminant intake (Soest, 1964; Allden and Whittaker, 1970; Dougherty *et al*., 1992; Faverdin, 1999; Astigarraga et al., 2002; Rutter *et al*., 2004; Smit *et al*., 2005) in an attempt to establish the factors that determine preference and intake. Intake appears to be a complicated issue, starting with the amount of material on offer and ease of forage prehension and then being confounded by effects relating to the physical and chemical properties of the sward (Barre *et al*., 2006).

Classification and regression tree (CART) analysis is a non-parametric technique that can select from among a large number of variables those that are most important in determining the dependent variable to be explained (Yohannes and Hoddinott, 1999). It is a binary decision tree that shows the explanatory variable at each dichotomy that best divides the data into relatively homogenous groups (Morris and Fynn, 2003). A weakness of using CART is that there is no probability level or confidence interval associated with predictions derived from using it (Yohannes and Hoddinott, 1999). However, it was deemed to be useful for this study in order to graphically represent the complex set of factors that may have influenced apparent intake.

Herbage allowance is one of the most important factors affecting intake by ruminants (Dougherty *et al*., 1992), especially in a homogenous pasture where selection between grass species is not a concern. CART analysis identified the primary determinant of apparent intake in the NxP, NxK and NxS experiments as the amount of material available to be grazed. This was particularly evident in the experiments where N was a factor as pre-grazing herbage mass was significantly different between treatments. Gibb *et al*. (1997) considered sward height below 7 cm to be limiting for cattle grazing, which suggests that herbage availability was probably a limiting factor in the low-N plots (see table 4.2 for pre-grazing sward heights). Allden and Whittaker (1970) reported that plant height is much more closely related to the intrinsic availability of herbage than is yield per hectare, while Taweel (2006) pointed out that herbage allowance can refer either to the area of land allocated for grazing or to the amount of grazing within a set area. It is for this reason that the term availability is used in preference to herbage allowance. Limited availability of herbage restricts intake by decreasing bite size and bite rate rather than through any form of selection process. In a study on morphological characteristics of perennial ryegrass leaves influencing intake, Barre *et al.* (2006) found blade length to be important, explaining 49 % of the variation in fresh matter intake rate. Thus, in conditions where herbage availability is limited, the ease with which pasture is obtained will be the overriding factor influencing intake. In the NxP experiment there were no variables of any significance in determining apparent intake other than pasture height.

In the NxK, NxS, PxK and PxS experiments apparent intake was greater on younger pasture (<= 155 days). ADF and NDF content negatively affected apparent intake in most experiments. This was expected as fibre increases with pasture maturity and is supported by Meissner *et al*. (1989) as well as in research by Smit *et al*. (2006), who reported negative effects on preference for NDF concentration. Fibre ferments and passes from the reticulorumen more slowly than the non-fibre constituents of feeds (Jung and Allen, 1995), consequently creating physical fill within the rumen. Although Waldo (1986) concluded that NDF is the best single chemical predictor of intake, there is variation in intake that is unexplained by NDF. Jung and Allen (1995) explain this is due to intake not being limited exclusively by physical fill and NDF not fully describing the filling effects of feeds.

That fibre is not the only explanatory variable is apparent in the results of this study, which show other variables to have scored equally in relative importance for determining apparent intake. Crude protein did not affect apparent intake to any great extent, but high levels of NPN did restrict apparent intake. NSC content positively affected apparent intake in the PxK, PxS and KxS experiments but was of low relative importance in the NxK and NxS experiments. Smit *et al*. (2006) conducted a study on preference among perennial ryegrass cultivars. They concluded that, of the four parameters determined to significantly affect apparent intake (ash, NDF, NSC and DOM), NSC concentration showed the highest correlation with preference.

Pre-grazing average pasture height was not significantly different in the experiments that did not contain N as a treatment. This provided a better assessment of which factors might restrict or encourage intake when herbage availability is not limiting. For this reason an analysis containing only quality parameters was included in the results for the PxK, PxS and KxS experiments. The analysis using quality variables indicated that overall, NSC, NPN and ADF are common determinants of apparent intake. That there are parameters that score consistently high on the scale of relative importance in determining apparent intake is interesting, especially in the light of research by Yeates *et al*. (2002), which indicated that cows do not regulate diet choice within the short-term time frame of a meal. Rather than selecting for nutritive value, one must then conclude that cows are making preference decisions based on immediate feedback. The indicator variables should therefore be able to be explained in terms of meeting short-term needs, which appears to amount to what is palatable and the time taken to feel full. In addition, it is important to note that preference for certain factors will not increase intake when dairy cows are fed a homogenous pasture. In a grazing experiment where four perennial ryegrass cultivars were fed alone to the same dairy cows, Smit *et al.* (2005) found no differences in DM intake between cultivars, whereas when the cultivars were offered together the cows selected among them.

6.3 Conclusions

In conditions where herbage availability is limited, the ease with which pasture is obtained is the overriding factor influencing apparent intake. Limited availability of herbage restricts apparent intake by decreasing bite size and bite rate rather than through any form of selection process. This was evident in the experiments where N was a factor and pre-grazing herbage mass was significantly different between treatments. It was confirmed by CART analysis, which identified the primary determinant of apparent intake in the NxP, NxK and NxS experiments as the amount of material available to be grazed.

Analysis of quality parameters indicated that NSC, NPN and ADF are common determinants of apparent intake. Research by Yeates *et al.* (2002) indicated that cows do not regulate diet choice within the short-term time frame of a meal. Thus intake is determined by short-term needs rather than by meeting long-term nutrient requirements. Fibre creates physical fill within the rumen, thus restricting intake. High NPN content is associated with high nitrate values. The reduction in intake of herbage with high nitrate content may be due to reduced palatability or to a negative feedback system limiting the intake of nitrate and ammonium. Increased NSC content is associated with increased intake, possibly through the effect of sugar on herbage palatability.

It is important to note that preference for certain quality parameters does not increase intake when dairy cows are fed a homogenous pasture. In that case, intake is determined by herbage availability and, if this is not limited, by physical gut fill.

CHAPTER 7

General conclusions

Current fertiliser recommendations for P, K and S are based on soil analysis and plant requirements. Application of these nutrients in increasing amounts did not affect perennial ryegrass yield. The lack of yield response confirmed that application rates based on soil analysis will not limit yield. Perennial ryegrass yield was not negatively affected by application rates in excess of those recommended, although with no yield benefit exceeding the recommended rates would constitute an unnecessary expense. Due to the sufficiency of P, K and S in the soil, there was no indication of whether or not fertiliser recommendations could be decreased without affecting yield.

Yield and quality responses to N differed depending on time of year, indicating a need for a flexible N fertiliser schedule. Rather than the current practice of 50 kg N ha^{-1} post-grazing, a revised model is recommended which incorporates seasonal responses to N. During periods of slow growth, such as during winter, or on newly-established (immature) herbage, low levels of N application (20 to 40 kg N ha^{-1}) were sufficient to maintain production and decreased the risk of high CP concentrations in herbage. The study provided no evidence for split applications greater than 40 to 50 kg N ha^{-1} at any time during the growing season. Even during periods of maximum herbage production, N applications of 80 kg N ha^{-1} resulted in average herbage CP concentrations above the recommended optimum for animal production. Lowered NSC content often accompanies increases in herbage CP. A reduction in applied N at times of the year when there is an accumulation of herbage CP and NPN may be a method to manage not only excessive protein content, but also dropping levels of NSC. The practice of increasing rotation lengths in late winter may also assist in maintaining acceptable concentrations of NSC in perennial ryegrass. Short rotations, especially when pasture growth is slow, do not allow sufficient time for plant energy reserves to be replenished.

Increasing levels of applied N decreased herbage P significantly. Although soil analysis indicated that P supply was adequate for grass production, in many instances herbage P concentration was below that recommended by the National Research Council (2001) for dairy cows yielding 10 kg of milk per day. Phosphorus deficiency can lead to decreased feed intake and reduced milk production, as well as impaired reproduction. Livestock systems utilising pastures that receive regular N applications may benefit from pasture herbage analysis to ensure sufficient herbage P levels for animal production. Similarly, herbage analyses indicated that the majority of samples had Ca content below that required to meet the dietary needs of high-producing dairy cows. While N application significantly raised Ca values, the increase was not sufficient to meet livestock requirements. In view of the negative effects of high applications of fertiliser N, increasing the quantity of N applied would not be considered a viable solution to low Ca content in herbage.

The primary determinant of apparent intake was identified as the amount of material available to be grazed. When herbage availability is not limiting, NSC, NPN and ADF are determinants of apparent intake. As cows do not regulate diet choice within the short-term time frame of a meal (Yeates *et al*., 2002), intake is determined by short-term needs rather than by meeting long-term nutrient requirements. Fibre creates physical fill within the rumen, thus restricting intake. High NPN content is associated with high nitrate values. The reduction in intake of herbage with high nitrate content may be due to reduced palatability or to a negative feedback system limiting the intake of nitrate and ammonium. Increased NSC content is associated with increased apparent intake, possibly through the effect of sugar on herbage palatability. It is important to note that preference for certain quality parameters does not increase intake when dairy cows are fed a homogenous pasture. In that case, apparent intake is determined by herbage availability and, if this is not limited, by physical gut fill.

A field study of this complexity presented with a number of challenges. The high number of laboratory analyses meant that it took four years before the analysis of the entire data set was complete. In the interim, samples and results were mislaid. It was also difficult to maintain continuity when writing over such a long period of time. Rather than analysing each individual sampling date it may have been better to group sampling dates into seasons, thus decreasing the number of analyses required. Deciding what to do with all the data can also be problematic, as the project tended to evolve as it progressed. Simplifying the analyses as much as possible did help in part. The trial was set up to limit the number of treatment interactions, which controlled the complexity of the trial. However, the decision to combine two seasons' data proved not to be the best method of analysis, especially for perennial ryegrass. Responses in the second year were very different to those in the first and by averaging data from the two seasons this information was lost. Trials involving animals can pose problems, especially if the animals are borrowed. The animals used in this trial were borrowed from the Animal Science section at the Cedara Research Station. The animals were only available until 14h00 each day, as the Animal Science schedule required them to be milked shortly thereafter. The trial was not too severly affected, fortunately, as by that time the cows had grazed sufficiently for them to be removed. Some animals were not willing participants in the grazing trial and were subsequently replaced with more compliant animals. It is suggested that, to avoid the time-wasting exercise of replacing animals and electric fences when the trial requires grazing, the animals to be used are subjected to a trial-run beforehand.

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