DYNAMICS OF NITROGEN IN THREE CONTRASTING PASTURES GRAZED BY SHEEP

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ABSTRACT

The dynamics of nitrogen (N) were studied during two years (March 1989 - May 1991) in three contrasting pastures grazed by sheep. The pastures were: ryegrass-white clover, herbal ley (a legume-based pasture of interest for "organic" agriculture) and pure ryegrass receiving 400 kg fertiliser N/ha/yr.

This study was undertaken on a recent alluvial soil at DSIR Grasslands, Palmerston North. Treatments were replicated as small paddocks, and periodically mob-grazed with sheep. Frequent soil measurements provided estimates for leaching and denitrification. Herbage yields and botanical composition were recorded, and symbiotic N_2 fixation was measured in swards of the two treatments containing forage legumes. Soil total N and carbon were measured annually, providing estimates of the partial mass balance for N.

The soil mineral N pool was dominated (especially in the systems receiving no fertiliser N) by the highly concentrated pulse of N returned in the excreta of grazing animals to a small proportion of the grazed area. In the pure grass sward the large inputs of fertiliser N had a significant effect in increasing the amount of mineral N available in the top 45 cm of soil. On average, in composite samples including urine-affected and non-affected areas, about 30 kg/ha-45 cm more mineral N was available throughout the year in the ryegrass fertilised with N than in the legume-based pastures. This consistently high level of soil mineral N in the ryegrass+N sward was responsible for the greatest annual herbage yield; however annual losses by leaching and denitrification were 5 to 6 times greater than in the legueme-based pastures.

A common feature of the three pastures was the small amount of N recovered in animal products, with most of the N that circulated through the plant to the sheep being returned to the soil in urine. This concentrated input was localised in about 10% of the area, which provided the major avenues for N

escape from the pastures receiving no fertiliser N. It was estimated that a little more than half the nitrate leached (total, about 6 kg NO_3-N/ha/yr) arose from this restricted area, but in the grass+N pasture the contribution of animal-induced losses was proportionally smaller than in the legume-based pastures. Fertiliser N, by increasing soil mineral N, offered more site opportunities for N leaching and denitrification, in addition to that from urine. Here, only one-quarter of leached nitrate (total, 41 kg NO_3-N/ha/yr) arose from urine patches. Denitrification accounted for 4-5 kg N/ha/yr from the legume-based pastures, but 20 kg N/ha/yr from swards receiving fertiliser N. Ammonia volatilisation, which was estimated using data from previous studies at this site, was enhanced by direct emission from the fertiliser N (urea) as it is hydrolysed on the soil surface.

Calculation of N inputs and outputs for these three pastures indicated that the two legume-based systems were more or less in balance, but in the pasture receiving fertiliser N some 180 kg N/ha/yr was unaccounted for. This difference may reflect incorporation of N into soil organic matter, as indicated by a small increase in soil total N during the second year.

Pasture production (average of two years) from the herbal ley was about 15 t DM/ha/yr, or about 90% of the yield from pasture receiving fertiliser N, and some 25-30% more than from ryegrass-clover. Symbiotic N_2 fixation, estimated by the acetylene reduction assay to have been 140-150 kg N/ha/yr, was similar in both systems based on forage legumes. The herbal ley utilised soil N more efficiently than the ryegrass-clover and ryegrass+N pastures, hence achieving an outstanding yield of herbage. It is argued that this apparently better exploitation of soil N was brought about largely by stimulation of microbial biomass in the rhizosphere around chicory roots, with the additional N that was scavenged by bacteria being made available to this herb after protozoan digestion of the bacteria. A herbal ley offers the possibility of sustaining a high level of forage production, but with reduced N emissions to the environment.

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DEDICATION

This work is dedicated to my immediate family, wife Isabel son Gonzalo and daughter Pilar. Also to my parents, especially to the memory of my late father, who died while I was studying in New Zealand.

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CHAPTER 1
GENERAL INTRODUCTION

Nitrogen (N) is generally considered the essential nutrient that most limits plant productivity. In contrast N occurs in a vast supply in the atmosphere, so much that each square metre of soil supports above it approximately 8000 kg of elemental N (Jarrel, 1990). But this N is present as an inert gas and cannot be used directly by higher forms of plant and animal life. The covalent triple bond of the N_2 molecule is highly stable and can be broken chemically only at elevated temperatures and pressures or by nitrogen-fixing microorganisms. The biochemical process of N_2 fixation constitutes the main source of N returned to the earth and is responsible for much of the fertility of agricultural soils (Stevenson, 1982).

In grassland soils of temperate regions N also accumulates in large quantities in the organic matter. Values varying widely, but commonly about 5000-7000 kg N/ha accumulate in the top 50 cm of soil (Walker *et al.*, 1959; Ball, 1979). However, this large amount of N does not necessarily imply a large supply to pasture plants, since the nutrient is substantially immobilised in organic compounds, and it is only after mineralisation that N is available to plants. Henzell and Ross (1973) pointed out that if it were not for this 'bottle neck' to N flow, highly productive pasture ecosystems could function on a small quantity of actively cycling N, with inputs being required only to balance losses.

Nitrogen is one of many elements involved in cyclic transformations, usually described as the Nitrogen Cycle where atmospheric N moves into the soil and is taken up by plants which in turn are consumed by grazing animals. Much of the N is then returned to the soil in animal wastes and plant residues, eventually cycling back to the atmosphere. In this cycle, N exists for varying times in 'pools' and moves within and between these pools (Gandar and Ball,

1982). The rates of exchange are governed by the rates and extent of several biological and chemical transformations and by the speed of transport of the various forms of N (Keeney and Gregg, 1982).

1.1 Nitrogen cycling in grassland soils

The role of N in grassland soils is probably similar to that in other systems. Also, the components, transformations and pathways are essentially the same and the most important have been described in several reviews (Paul and Juma, 1981; Keeney and Gregg, 1982; Stevenson, 1982). However, the distribution of the components and the rates of many processes in grasslands may differ significantly from those in other agricultural and forest ecosystems. The most marked differences are associated with the dominance of below-ground biomass, the large amount of internal N cycling and the important role of the larger herbivores. The studies of N cycling in grasslands during the last 10 years have provided a better understanding of the N fluxes, transformations and processes. Different approaches and scenarios have been used in these studies, and some of them have been summarised in the reviews of Woddmansee *et al.* (1981), Gandar (1982), Steele (1982), Ball and Field (1987), Steele and Vallis (1988).

From these investigations comes a general conclusion that relatively stable natural grasslands lose only small quantities of nutrients each year. Presumably, because the vegetation is well adapted to the site and all available niches are filled, such that most of the available N (also other nutrients) is taken up by plants before it can be lost. However, with human disturbance, the natural ecosystem becomes 'leaky' for a period of time before a new steady state is achieved. A classical example is during the artificial transformation of a forest ecosystem to grassland (for instance most of New Zealand and Western European pastures), giving rise to the so called 'managed grassland' (Wallwork, 1976).

Most of the pastures in temperate regions had this common origin, and now one of the most important characteristics is that they are maintained by the activities of grazing herbivores (Wallwork, 1976), otherwise such grassland would revert to scrub species or eventually to woodland (Scott *et al.*, 1985).

Thus, in the process of development and maintenance of managed grasslands a certain degree of intensification is required (soil cultivation, sowing, increase of grazing pressure, fertilisation etc.). Associated with this disturbance is a reduction of the synchrony between N availability and plant root absorption (Jarrell, 1990), leaving quantities of N in soil solution that are much more susceptible to loss by volatilisation, leaching and denitrification. This process occurring in the whole soil is aggravated by the localised losses of N in intensively grazed areas (Field and Ball, 1982; Ball and Ryden, 1984) but, as pointed out by O'Connor (1990), this is only a more extreme expression of the same mechanism of N loss which is centred on the changing C:N ratio resulting from animals processing herbage. Animals, by selecting grazing sites and through the patchiness of nutrient return, have been considered by O'Connor (1990) as a 'disturbance-related phenomena' and also as part of what has become known as patch dynamics (Pickett and White, 1985).

N loss in intensively managed pastures, as explained by Ball (1982), is governed by the C:N ratio in the area of soil affected by animal urine. In this regard O'Connor (1990) postulated that regardless of the mechanisms of C accumulation, there is a limit to C storage in soil which is set mainly by climate and this will determine the upper limit of N in organic matter, so that addition of N beyond that limit is likely to be followed by equivalent loss.

1.2 Major transformations and fluxes during N cycling in New Zealand pastures

The main features of N cycling in the soil-plant-animal complex related to New Zealand (NZ) pastures were examined by Ball and Field (1987) and are

summarised in Table 1.1. These authors also indicated a rating in terms of the importance of each process, the adequacy of present techniques for studying them, and the level of understanding acquired within NZ grasslands. (Some of the ratings in Table 1.1 were arbitrarily altered by the writer in view of more recent information available. The original rating is indicated in parentheses). Some of the transformations and processes of N cycling are discussed in the following paragraphs of this section.

The major transformations in the soil are the simultaneous processes of mineralisation-immobilisation, the result of which for practical reasons we normally refer to as net mineralisation or net immobilisation. In soils that have not received nitrogen fertiliser and in the absence of significant biological fixation, inorganic N is derived almost entirely from the decomposition of organic N. This includes the soil organic matter and recently added plant and animal residues. Several studies of N dynamics in grassland soil, including mineralisation and nitrification (Macduff and White, 1984; 1985), have concluded that N released by mineralisation constitutes an important fraction of the total annual flux of mineral N through the plant available pool and is also responsible for the seasonal changes of mineral N.

In grassland soil net mineralisation varies widely (Walker *et al.*, 1954; Edmeades and Goh, 1978, Ross and Bridger, 1977; Steele and Dawson, 1980); but there is consensus that net mineralisation rates in pasture soils are generally insufficient to provide available N to allow grass to express its full potential for growth (Field and Ball, 1978). Carran (1979), examining the annual pattern of N uptake in grass swards (which reflected a proportion of net mineralisation), concluded that only in mid spring could there be a flush of mineralisation while throughout the rest of the year this process operates at a consistently low rate.

The rate of mineralisation is controlled by climatic and management factors as well as the size and nature of the pool of soil organic matter. Soil type also

Table 1.1 Present understanding of fluxes, transformations and processes involving nitrogen in NZ pastures

SOILS							
	Immobilisation	Mineralisation	NH_4^+ fixation	Volatilisation	Nitrification	Leaching	Denitrification
Importance	**	***	*	***	***	***	**(*)
Techniques	*	*	*	*(**)	**	**	*(**)
Understanding	*	*	*	**	**	**	*

PLANTS							
	Symbiotic fixation	Uptake	Translocation	Assimilation	Partitioning	Senescence	
Importance	***	***	***	**	***	**	
Techniques	**	**	*	*	*	*	
Understanding	***	*	*	*	**	*	

ANIMALS							
	Intake	Production	Excretion	Species	Indirect Influences		
					Defoliation	Treading	
Importance	**	*	***	***	**	**	
Techniques	**	**	***	*	**	*	
Understanding	***	**	**	*	*	*	

(Adapted from Ball and Field, 1987) Rating: *** = Good, ** = Moderate, * = Poor (original rating where altered)

influences the rate of mineralisation, being higher in non-allophanic compared with allophanic soils (Jackman, 1964); hence the largest accumulation of organic matter in New Zealand is observed in the volcanic soils of the central North Island.

In general the rate of mineralisation is closely related to nitrification in 'managed pastures' and is also subject to substantial spatial and temporal variability (Macduff and White, 1984; White *et al.*, 1987). This imposes a complication in field measurements where soil mineral N is involved (plant availability, N losses by leaching and denitrification, volatilisation).

Other processes occurring in the soil which influence the ultimate fate of N are volatilisation, leaching and denitrification, which constitute the main pathways for N losses. These losses of N are considered the focal point for the impact of N in the wider environment and are exacerbated by the grassland system depending on continuous applications of fertiliser N. Losses of N and the effect of fertiliser N on the N cycle will be analysed in more detail in particular chapters of this thesis.

Another N transformation of major importance is the symbiotic fixation. In contrast with the chemical reduction of dinitrogen (an industrial process) which requires high temperature and pressure, the biological fixation lowers the activation energy of the N_2 molecule and mediates the reduction through the activity of the nitrogenase enzyme at ambient temperature and 1 bar pressure (Postgate, 1982). In the symbiosis the host plant receives reduced N substrate in exchange for providing carbon-based energy to the bacteria.

Symbiotic N fixation is the main source of N for NZ agriculture. Its importance in supplying the N required for pasture production and indirectly to other agricultural activities has been reviewed by Steele (1982), Crush and Lowther (1985) and Ball and Crush (1986).

In spite of the well recognised contribution of symbiotic N fixation, N deficiency remains as a limiting factor to pasture production in many parts of NZ. Soil N availability and climatic limitations for legume growth (Crush and Lowther, 1985) are considered the most important factors influencing symbiotic fixation. Management factors are also notable in maintaining an appropriate balance between legumes and grasses.

In the early stage of development of NZ pastures or in N-deficient soils, legumes dominate the sward once deficiencies of other nutrients have been corrected. Under these circumstances the input of N by biological fixation can be substantially high, exceeding 500 kg N/ha/yr (Sears *et al.*, 1965). But in well developed swards, with grass growth stimulated by a better level of N availability leading to a reduction in legume yield through competition for light, water and nutrients, legume yield and fixation per unit of growth progressively decline. Under this more productive grass-dominant sward N fixation is substantially lower than that recorded in the early stages of pasture development, with values fluctuating between 100-300 kg N/ha/yr (Hoglund *et al.*, 1979; Cowling, 1982; Crush *et al.*, 1983).

Symbiotic N fixation is subject to significant seasonal and annual variation, being especially influenced by climatic stress. Available results on the seasonal pattern for N fixation in temperate grasslands indicate that N fixation is severely limited when the soil temperature (measured at 0900 h, 10 cm depth) is below 8 °C, or when topsoil moisture is less than 70% of available water (Ball and Crush, 1986). On an annual basis, Crouchley (1979) reported biological fixation of 90 and 160 kg N/ha/yr during consecutive years with dry and moist summers under uniform pasture management.

It is generally accepted that grazed pastures are intermediate between natural (or semi-natural) ecosystems, characterised by well developed mechanisms for the conservation of N (and other nutrients), and arable agriculture in which a large proportion of the annual plant-N production is removed from the

system. The direct effect of animals on the N cycle is complex and the degree of influencing transformations and fluxes depends principally on the level of intensification of the system. The negative effect of animals on the N cycle is focused in the concentrated return of N in excreta, a theme which has been examined in reviews on intensively grazed pastures (Ball and Ryden, 1984; Ball and Field, 1987). The positive and negative effects of animals in a range of grazing intensities have been analysed by Floate (1981), who concluded that the direct effect of herbivores are important in the maintenance of soil fertility. The effect of animals in the N cycle is expressed through consumption of herbage, treading on soil and vegetation, return of nutrients in excreta and removal of N in animal products.

Summary of the present understanding of the N relationships in grassland soils

In the last decade several interrelationships operating the N cycle in grassland soil have been recognised. These can be summarised as follow:

i) Grassland productivity is severely limited by soil N availability and consequently herbage yield responds up to very high levels of N fertiliser (Ball and Field, 1982; Meer and Lohuyzen, 1986).

ii) In highly productive, well managed legume-based pastures symbiotic N fixation can rarely supply sufficient N to the whole system to achieve more than 70% of the potential pasture production (Ball and Field, 1982; Steele, 1982).

iii) When adequate N is supplied (principally as fertiliser N in intensive grassland systems) a substantial proportion of the N applied may be lost from the system. This loss of N is important not only because of its agricultural significance, but also because of its potential impact on the wider environment and human health (nitrate leaching to ground

water and emission of oxides of nitrogen to the atmosphere (Ryden *et al.*, 1984; Ryden, 1986)).

iv) Grassland management practices have also been reported to have an effect on N dynamics in pastures, thereby affecting productivity (Brock *et al.*, 1983) and the extent and forms of N losses (Field and Ball, 1982; Brock *et al.*, 1990).

1.3 Objectives of this thesis

To improve efficiency of N utilisation and reduce losses in grassland soils, a better understanding of N relationships is required. In particular the dynamics of the mineral N pool deserve further investigation, because of the relationship between the amount of N available for plant nutrition and for potential losses. Consideration of this complex topic as it relates to three contrasting grassland systems (legume-based pasture, representing the main NZ system; pure ryegrass fertilised with N, simulating the European grassland; and a herbal ley mixture of interest for the organic agriculture) on a well developed New Zealand soil constitutes the general objective of this thesis.

Four studies were carried out to address this purpose. The specific objectives were:

i) Comparison of the agronomic performance of the three contrasting pastures grazed by sheep. The assessment of pasture production, dynamics of botanical composition and N flux through the herbage yield were the specific objectives of this study. The aim was to improve the understanding of the efficiency of N utilisation from either fertiliser N or symbiotic N fixation.

ii) Regular measurement of soil mineral N to compare the size and changes of this pool in the three pastures. The aim of this study was

to gain more detailed information about the factors influencing the magnitude and changes in soil mineral N; for example, the effect of grazing animals on the conversion of herbage N to the soil available pool and the subsequent effect on plant N uptake during regrowth. The effect of urine from grazing animals on the mineral N pool and the proportion of area affected were also examined, as was the effect of animal urine patches on spatial variability of soil mineral N.

iii) The estimation of loss of NO_3-N by leaching in the three pasture systems and its relative impact on N supply to the pasture and the potential risk for the environment. The aim of this study was to improve understanding of the relationships between nitrate losses and the source and amount of external N inputs, pasture production, herbage N yield and the grazing pressure in different grassland systems under similar management and environmental conditions.

iv) The measurement of total denitrification to compare the rate of gas production as well as the annual losses of N in the three pasture systems. To determine the proportion of nitrous oxide in total denitrification was a specific objective of this study. The aim was to gain information on the relationship of the pattern of total denitrification to field characteristics such as soil moisture content, temperature and soil nitrate concentration.

CHAPTER 2
DESCRIPTION OF THE EXPERIMENTAL PROCEDURE

2.1 Experimental Site

This study was carried out at DSIR Grasslands, Palmerston North from January 1989 to May 1991. The soil, a Manawatu fine sandy loam (Cowie, 1978), comprised approximately 45 cm of fine sandy loam overlying medium and coarse sand, down to 2-2.5 m depth, then gravel. In terms of the Soil Taxonomy Classification (Soil Survey Staff USDA, 1975) this soil is a mixed mesic Dystric Eutrochrept (R.H. Wilde, pers. comm.). Some chemical and physical properties of soil from the experimental area at the beginning of the study are shown in Table 2.1.

Table 2.1 Chemical and physical properties of soil from the experimental area

	Depth (cm)			
	0-7.5	7.5-15	15-30	30-45
Total N (%)	0.20	0.12	0.08	0.06
Total C (%)	1.89	1.03	0.64	0.46
C/N ratio	9.4	8.6	8.0	7.7
pH (in water)	5.7	5.8	5.7	5.5
Bulk density (g/cm^3)	1.21	1.24	1.30	1.35
Field capacity (% vol.)	34.7	40.0	38.0	40.0
Olsen P (ppm)	7.0			
Ca (me./100g)	6.70			
Mg (me./100g)	1.14			
K (me./100g)	0.51			

The site was levelled, cultivated and resown to pasture in 1968-69 after a long period in dairy farming (Ball *et al.*, 1978), and has since been under pasture irregularly grazed by sheep. No fertiliser or lime application records have

been kept, but soil chemical analyses (Table 2.1) indicate that the fertility status is below the average for similar soils under pasture in the area.

2.2 Climate

The climatic information obtained from the DSIR Meteorological station (250 m from the experimental area) during the experimental period is presented in Table 2.2. The average annual rainfall is 974 mm (30 year-period) and class A pan evaporation is 1013 mm.

The area is normally summer-dry with pan evaporation exceeding rainfall by more than 25 mm per month in December, January and February (DSIR Meteorological records). During the experimental period, late spring (November 1989) and mid summer (February 1990) were significantly drier than the long term average. Therefore, to preclude sward failure through atypically dry conditions, supplemental irrigation was applied to bring the water received up to the average rainfall for each month. Water stress was not removed by this irrigation policy over the summer period. Winter conditions in 1989 (especially July) were cooler and drier than average and significantly affected pasture growth, as will be discussed later.

2.3 Description of the treatments

The experiment consisted of three treatments:

- Ryegrass-white clover pasture (conventional NZ system).
- Herbal ley pasture (modified 'Clifton Park' mixture, Foster,1988).
- Ryegrass+N400 pasture (pure ryegrass sward receiving 400 kg N/ha per year as urea split into 8 dressings).

Details of the pasture species, cultivar mixtures and sowing rates in each system are given in Table 2.3. The treatments were replicated four times and arranged in a randomised block design. The area of each plot was 200 m^2.

Table 2.2 Measured rainfall, evaporation and mean monthly air temperature during the experimental period January 1989 to May 1991

Months	J	F	M	A	M	J	J	A	S	O	N	D
1989												
Rainfall (mm)	92.4	75.3	89.2	43.8	111.1	88.2	53.4	53.1	25.3	123.1	23.0	58.8
Pan evap. (mm)	181.3	135.4	110.2	76.0	24.9	17.9	25.1	42.6	64.1	83.8	151.8	147.3
Mean air temp. (°C)	19.6	18.0	17.2	14.5	11.9	8.8	7.7	9.8	12.4	13.8	16.0	15.7
1990												
Rainfall (mm)	104.2	17.5	184.1	66.3	92.1	124.5	85.4	111.2	16.9	83.7	98.3	50.7
Pan evap. (mm)	115.2	157.2	109.2	67.9	39.9	20.1	22.2	35.8	64.1	103.0	115.2	160.1
Mean air temp. (°C)	17.6	20.1	17.9	14.4	11.9	9.4	9.0	10.1	10.1	13.4	14.9	16.4
1991												
Rainfall (mm)	120.2	131.5	28.7	102.7	80.3							
Pan evap. (mm)	163.0	106.0	113.3	50.0	34.7							
Mean air temp. (°C)	17.6	17.4	16.7	13.1	11.3							

Table 2.3 Pasture seed mixtures and rates of sowing for the Grass-clover, Herbal ley and Grass+N400 systems

Species/Cultivars	kg/ha	
(1) GRASS-CLOVER SYSTEM		
Trifolium repens L. 'Grasslands Huia'	3	(white clover)
Lolium perenne L. 'Grasslands Nui'	5	(perennial ryegrass)
Lolium perenne L. 'Yatsyn'	5	(perennial ryegrass)
Lolium (perenne x multiflorum) x perenne 'Grasslands Marsden'	10	(perennial ryegrass)
Total	23	
(2) HERBAL LEY SYSTEM (Modified Clifton Park mixture)		
Dactylis glomerata L. 'Grasslands Kara'	3	(cocksfoot)
Holcus lanatus L. 'Massey Basyn'	2	(yorkshire fog)
Festuca pratensis Huds. 'Aber S 53'	1	(meadow fescue)
Phalaris aquatica L. 'Grasslands Maru'	2	(phalaris)
Festuca arundinacea Schreb. 'Grasslands Roa'	3	(tall fescue)
Poa trivialis L.	0.5	(rough-s. meadow grass)
Bromus willdenowii Kunth. 'Grasslands Matua'	5	(prairie grass)
Lolium perenne x multiflorum 'Grasslands Manawa'	2	(short rot. ryegrass)
Lolium multiflorum Lam. 'Grasslands Tama'	2	(tetraploid ryegrass)
Cynosurus cristatus L.	1	(crested dogstail)
Phleum pratense L. 'Grasslands Kahu'	2	(timothy)
Trifolium repens L. 'Grasslands Tahora'	1	(white clover)
Trifolium repens L. 'Grasslands Huia'	1	(white clover)
Trifolium repens L. 'Grasslands Pitau'	1	(white clover
Trifolium pratense L. 'Grasslands Pawera'	1	(red clover)
Trifolium pratense L. 'Grasslands Hamua'	1	(red clover)
Lotus corniculatus L. 'G32'	1	
Lotus pedunculatus Cav. 'Grasslands Maku'	2	
Cornilla varia 'G34'	1	(crown vetch)
Medicago sativa L. 'Grasslands Oranga'	1	(lucerne)
Hedysarum coronarium 'Aokau'	2	(sulla)
Cichorium intybus 'Grasslands Puna'	1	(chicory)
Sanguisorba minor	3	(sheep's burnet)
Plantago lanceolata	1	(ribgrass)
Achillea millefolium	0.5	(yarrow)
Onobrychis viciifolia 'Fakir'	6	(sainfoin)
Total	47	
(3) GRASS + N400 SYSTEM		
Lolium perenne L. 'Grasslands Nui'	5	(perennial ryegrass)
Lolium perenne L. 'Yatsyn'	5	(perennial ryegrass)
Lolium (perenne x multiflorum) x perenne 'Grasslands Marsden'	10	(perennial ryegrass)
Total	20	

2.4 Establishment of the experimental swards

Before sowing, the residual vegetation was eliminated with herbicide ('Roundup' + 'Citowett'), applied twice with an interval of three weeks. During this period (January-February 1989), two light irrigations (sprinkler) were carried out to provide conditions for germination and regrowth of weeds before the second spraying. A basal fertilisation with 600 kg/ha of single superphosphate to remove deficiencies of phosphorus (P) and sulphur (S) was made at this stage.

The seedbed preparation started in the first week of March 1989. The aim was to work the top 2 cm of the soil using the following procedure: discs (three passes with very limited cut), tyne harrows (twice), levelling (once) then seed sowing by hand on the 21 March 1989. The seed was covered using chain harrows and a Cambridge roller. The seed of the two lotus species (*Lotus corniculatus* and *Lotus pedunculatus*) and sulla (*Hedysarum coronarium*) in the herbal ley sward was inoculated with the appropriate rhizobial strains. Five days after sowing a further rhizobium mixture was sprayed onto the soil surface of the herbal ley to reinforce the initial inoculation.

After the experimental swards were established, each plot was fenced and provided with a water trough. The area was surrounded by an enclosed race with pasture to keep additional sheep, in an attempt to minimise the tendency of sheep to camp on the periphery of the plots.

2.5 General management of the experimental trial

Once established, the Herbal ley pasture was managed following the guidelines of the New Zealand Biological Producers' Council (Anon. 1988) for organic agriculture. In practical terms this approach avoids the use of soluble fertilisers and prohibits the use chemical pesticides. The fertiliser policy (other than N for the Grass+N400 treatment) was similar for the whole experimental

area. Nutrient status was periodically checked using MAF soil 'Quick tests'. Potassium sulphate (100 kg/ha) was applied in August 1988, and rock phosphate (North Carolina) mixed with elemental S in May 1990, at a rate of 25 and 20 kg/ha of P and S, respectively.

During the early stage of pasture establishment, weeds were controlled (except in the Herbal ley) by herbicides. MCPA + Asulox was used in the Grass+N400 pasture to control weeds and some resident clovers, while MCPB + Asulox was used in the Grass-clover pasture as a specific herbicide mixture to avoid legume damage while removing dock (*Rumex obtusifolius*) and other broadleaf weed species.

The plots were grazed by sheep 9 times in 1989 and 10 times in 1990. The frequency of defoliation varied with seasonal changes in pasture growth rates following the guidelines for grazing management in this environment given by Brougham (1970). Stocking rates were determined in relation to an approximate estimation (visual and pasture probe) of the herbage yield before the start of grazing. The aim was that all plots were grazed at the same time for a period of 3 to 4 days. Thus the temporary stocking rate fluctuated between 380 to 600 stock units (s.u.)/ha according to the treatment and the period of the year. Toward the end of each grazing, sheep numbers were adjusted in an attempt to leave a stubble of approximately 1-2 cm height.

To minimise fertility transfer, replicate four (excluded from measurements) was used to pre-feed the sheep on pasture of appropriate treatments for 24 to 36 h immediately before each grazing. These plots were managed to ensure reasonably that the stock entered and left the experimental plots in a relatively empty state.

2.6 Analytical procedures

Analytical procedures for the measurements shown in Table 2.1 are presented

in this section. The methods for other analyses appear in the Materials and Methods of the following Chapters for each specific study.

2.6.1 Soil total nitrogen and total carbon

Soil total N and organic C were measured 3 times during the study period, in February 1989, 1990 and 1991, respectively, on soil samples obtained from 4 depths: 0-7.5, 7.5-15, 15-30 and 30-45 cm. Soil samples from each plot comprised 15 cores (2.5 cm internal diameter) taken at random in 1989 and 45 cores from each plot in the following years.

Soil total C was analysed by the combustion method (Tabatabai and Bremner, 1970). The instrument used was a Leco GC-90 Gravimetric Carbon Determinator and the sample was combusted at 1650 °C in disposable ceramic cups.

Soil total N was analysed by the Kjeldahl method (Bremner, 1965) and N determined colorimetrically on the diluted (1 N H_2SO_4) centrifuged digest by the hypochlorite-phenol reaction with ammonium.

2.5.2 Soil pH

Soil pH was measured in distilled water at soil:solution ratio of 1:2.5 after a 24 h equilibration period.

2.5.3 Phosphorus, calcium, magnesium and potassium

The status of these nutrients were estimated using the MAF soil 'Quick tests' (Cornforth and Sinclair, 1984).

2.5.4 Bulk density

Bulk density was measured according to the method described by Blake and Hartge (1986). Soil cores were removed using metal cylinders of 5 cm diameter and 7 cm height. The samples were dried at 105 °C and weighed. Bulk density was calculated as the oven dried mass divided by the field volume of the sample.

Twenty one cores were removed from the 0-7.5 cm depth and 7 from the following depths 7.5-15, 15-30 and 30-45 cm in the whole experimental area before the experiment was established (Table 2.1). Bulk density was measured again in the top soil, using 4 cores per plot (36 in the total area) in August 1990 and August 1991, to check if any significant changes occurred between treatments and between years.

2.5.5 Field capacity

Field capacity was estimated at the beginning of the experiment using the procedure described by Cassel and Nielsen (1986). In 3 sites of the experimental area the soil was wetted to below 50 cm depth. The area was covered with a polyethylene sheet and then sampled after 48 h to determine the water content of the soil layers 0-7.5, 7.5-15, 15-30 and 30-45 cm by the gravimetric method. This procedure was repeated 3 times during winter in 1989 after heavy rainfall to ensure that the profile was wetted below 50 cm. The average of these measurements multiplied by the appropriate bulk density (Table 2.1) gave the moisture content by volume (θ_v) for this arbitrary but useful parameter.

CHAPTER 3
FORAGE PRODUCTION

3.1 Introduction

Herbage production in developed grassland of temperate regions has largely followed two different approaches. One represents the age-old philosophy of grassland systems based on forage legumes (principally white clover) as the main source of the inputs of N required to sustain pasture production. The other reflects a relatively recent technology, now well established in European grasslands, where production is based largely on perennial grasses with large fertiliser applications, particularly of N, and intensive management practices to support high levels of livestock production.

In recent years, with the increasing interest in organic agriculture, the old practice of including forage herbs in pastures has emerged as an attractive variant to current approaches for the grassland systems of temperate regions.

3.1.1 Legume-based pastures

This type of pasture has been characteristic of New Zealand agriculture (also that in some areas of Australia, South America, and North America), in which intensive livestock production systems have been developed on swards comprising clovers grown in association with temperate grasses species (Sears, 1962; Levy, 1970). These mixed swards take advantage of the better cool-season activity of temperate grasses (Mitchell, 1956; Davison and Robson, 1986) and symbiotic N fixation of clovers in the spring-summer period (Hoglund *et al.*, 1979).

The major advantage attributed to legume-based pastures is their role in providing N through symbiotic N fixation. In New Zealand, the estimated rates

of symbiotic N fixation in permanent ryegrass-white clover swards are in the range 100-300 kg N/ha/yr (Hoglund *et al.*, 1979). Ball and Field (1985) estimated, from the cost of direct substitution of fertiliser N for fixed N, that approximately one million t N/yr fixed by forage legume provides an equivalent value for legumes of one billion NZ dollars per year. The contribution of the legume component to herbage quality, and its effect on animal nutrition, are other important characteristics of the mixed swards (Thomson, 1984).

In spite of mixed legume pastures being seen theoretically as an efficient, low-cost animal production system, for many reasons (small legume content, poor soil fertility, periods of drought, inefficient pasture management) N inputs by the symbiotic process appear to be a limiting factor to pasture production in relation to the potential imposed by climate and soil (Ball, 1979). In New Zealand it has been established that N deficiency limits annual production from some of the best pastures by 25 to 35% (Ball and Field, 1987).

In addition, herbage yield fluctuations (within and between years) and the difficulties associated with management of legume-based pastures have been considered as other factors that limit the extent to which this system has been adopted, especially in the Northern Hemisphere.

3.1.2 Grassland based on fertiliser nitrogen

Grasses require a good supply of all four major fertiliser nutrients, N, P, S and K. Of these, N most commonly limits yield. In Europe, fertiliser N has been used in increasing amounts in intensively managed livestock systems to enhance profitability, either by increasing forage yield or reducing production costs by substitution of concentrates (Lazenby, 1981; Robson *et al.*, 1989).

Grass responds to applications of fertiliser N in a characteristic way. The general form of the response curve to fertiliser N has been well established from trials (mostly cut trials) throughout NW Europe and is shown in Fig. 3.1.

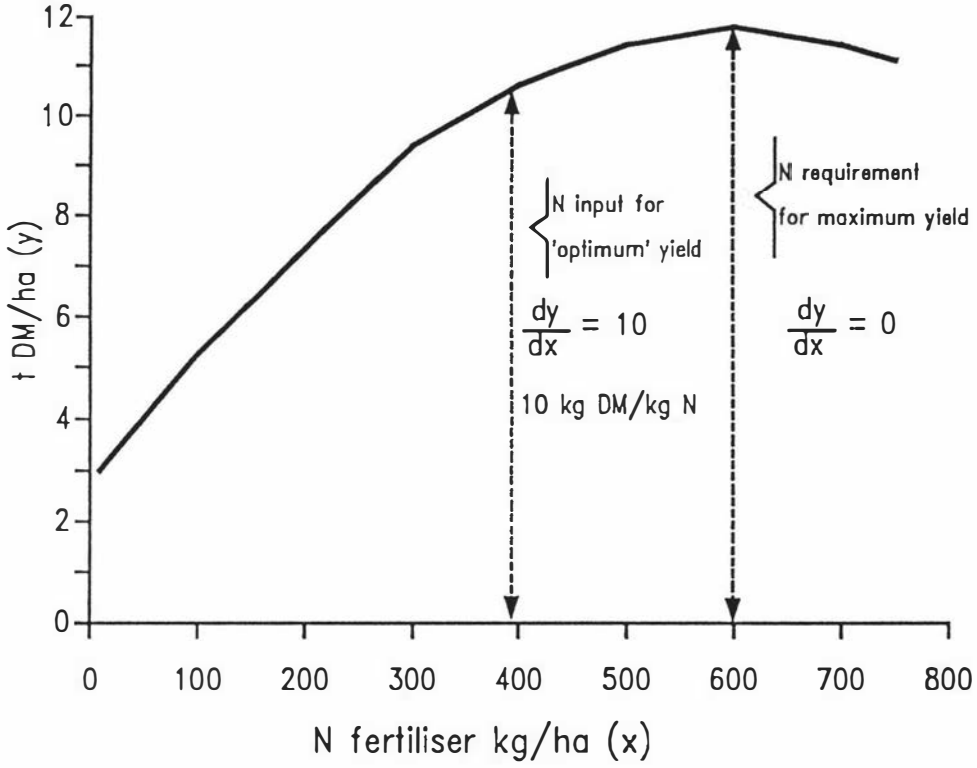

Figure 3.1 General form of the response curve to fertiliser N for grass swards in Europe (after Morrison et al. 1980)

This figure shows that the response in DM yield is almost linear up to a fertiliser application of about 300-400 kg N/ha/yr. Yield increases at a constant rate of about 20 kg DM per kg of additional N. Beyond 300-400 kg N/ha the response rate diminishes until a maximum yield is reached at about 600 kg N/ha. The point where the response drops to 10 kg DM of grass per kg of N is usually defined as the optimum fertiliser rate (Robson *et al.*, 1989). At this level 90% of the maximum yield is achieved with 60% of the N which would be needed to achieve the maximum yield. A large variation in the slope of the curve can be expected from site to site, or under grazed or cut systems (Baker, 1986).

In NW Europe, the use of fertiliser N under the current cost:price relationships is economically attractive. Also, larger yields, greater reliability of early-season growth and improved opportunity for forage conservation are all factors that farmers consider valuable and allow flexibility in grassland management (Holmes, 1989). However, much of the applied N may be lost, and this loss represents an economic cost as well as posing an environmental risk.

In cut swards 65-90% of the applied N can be recovered in herbage (Robson *et al.*, 1989). However, because of the small conversion of N to animal products (10-15%), much of the ingested N is returned to pastures in forms which are easily subject to loss (Ball and Ryden, 1984). The rapid decomposition of urea in urine releases ammonium ions which can be lost as NH_3 gas by volatilisation, and the ammonium which remains can be oxidised to nitrate that is vulnerable to leaching (Ryden *et al.*, 1984; Garwood and Ryden, 1986).

3.1.3 Herbal ley

The herbal or organic ley is still largely based on ideas developed about the turn of this century, especially by Robert Elliot. His 'Clifton Park' mixture later formed the basis of more formal research at the Welsh Plant Breeding Institute

from 1919 onwards (Foster, 1988). The philosophy behind the Herbal ley is to include certain herbs, which produce deep roots, as a specific part of the seeds mixture. These roots break up and aerate the soil and bring minerals to above-ground parts of the plant, where they may enrich the diet of grazing animals and the upper layers of soils.

Forage herbs may be defined as non-gramineous and generally non-leguminous species which are included in seed mixtures for the establishment of herbal pastures or leys. The most commonly used of these are: chicory (*Cichorium intybus*), ribgrass or ribwort (*Plantago lanceolata*), sheep's burnet or salad burnet (*Poterium sanguisorba*), sheep's parsley (*Petroselinum crispum*) and yarrow (*Achillea millefolium*). Other minor species are occasionally noted in the literature; e.g. caraway (*Carum carvi*), cat's ear (*Hypochoeris radicata*) and broad-leafed plantain (*Plantago major*).

The alleged benefit conferred by the herbs has not been scientifically demonstrated, and most of the evidence in support of any advantages to be derived from inclusion of herb species in pastures is anecdotal. The research into herbs ceased in the 1950s, possibly because the conclusions reached by previous studies, particularly in respect of the management (Milton, 1943) and on feeding value (Thomas *et al.*, 1956) were not promising.

It is likely that under modern intensive agricultural practices, when competing with more aggressive grasses and clovers, herbs do not survive long enough to be of significant agronomic or dietary value. According to Woodward and Foster (1987), the practice of frequent cutting for silage, and hard grazing would militate against the survival of herbs under intensive management. The same authors also suggest that without a major breeding programme for herbs to compete with modern varieties, there will be limited possibilities to put these ideas into practice.

In New Zealand, several herbs and other pasture species for specific

environments are currently being bred and evaluated at DSIR Grasslands. Among the herbs, the breeding programme for chicory has been completed and a cultivar released as 'Grasslands Puna' (Rumball, 1986). The breeding programmes for other forage herb species (plantain, yarrow, salad burnet, sheep's parsley) are still in progress (W. Rumball, pers. comm).

3.1.4 Objective of this study

In this study an attempt was made to establish different swards representing the three approaches outlined above. During two years, their agronomic performance (periodically mob-stocked by sheep) was assessed in terms of pasture production, dynamics of botanical composition, and N flux through the herbage yield. An effort was also made to achieve a better understanding of the efficiency of N utilisation by the three systems from either fertiliser N or symbiotic N fixation.

3.2 Materials and Methods

A detailed account of the experimental site, climate, establishment and management of the trial has been presented in Chapter 2.

Herbage yield was recorded (20 times from May 11 1989 to May 13 1991) under cage enclosures within the plots. Two cages, each 1.7 m^2, were located at random in each plot immediately before grazing. Sampling was approximately at the mid-point of each grazing period. Samples were harvested with an electric shearing handpiece, leaving a residual stubble similar to that left by the sheep. Herbage samples from within each plot were bulked and the fresh weight recorded in the field. Subsamples were drawn for the determination of dry matter and botanical composition, and chemical analyses.

3.2.1 Dry matter yield

Dry matter (DM) was determined in 100 g fresh herbage, dried overnight at 80 °C in a forced-air oven. Botanical composition was determined in fresh sub-samples (one per plot) by hand-dissection. For each plot, samples were then grouped as grasses, legumes and other species, dried overnight, ground and stored for chemical analyses.

Herbage accumulation rate was expressed as the total DM yield divided by the number of days between two measurements. When the results of herbage yield are presented as accumulation rates, the values are plotted at the mid-point of the period between successive measurements.

Fertiliser N (urea) on the Grass+N400 treatment was applied by hand soon after grazing, allowing some flexibility for the application to be undertaken just before or during a rainfall event. Rates of N application and the splits during 1989 and 1990 are shown in Table 3.1

Table 3.1 Rates of application and split of fertiliser N to Grass+N treatment during the experimental period

First year, 1989		Second year, 1990-91	
Date	Kg N/ha	Date	Kg N/ha
16 May	80	31 March	80
3 July	40	7 May	40
15 August	80	25 June	40
23 September	40	11 August	80
31 October	40	16 September	40
24 November	80	31 October	40
20 December	40	29 November	40
		3 January	40

3.2.2 Herbage chemical analyses

To determine total herbage N, 0.14 g sub-samples were digested by the Kjeldahl method with modification to include nitrate by the addition of salicylic acid (Bremner, 1965), following which NH_4-N was determined colorimetrically on a Technicon 'AutoAnalyser'.

Herbage nitrate levels were determined colorimetrically in water extracts (0.2 g plant material in 200 ml), following hydrazine reduction to nitrite by the sulphanilamide-naphthyl ethylene diamide method (Henzell *et al.*, 1968).

3.2.3 Symbiotic N fixation

The rate of symbiotic nitrogen fixation (sNf) was determined by a modification of the acetylene reduction assay used by Hardy *et al.* (1968). Details of the modifications are in Hoglund and Brock (1978), and this has been the standard technique adopted by DSIR Grasslands. Briefly, fourteen 25 mm diameter x 75 mm deep soil cores were selected randomly from each paddock, placed in a 1 litre incubation vessel and sealed. Approximately 10% of the volume of the air head space was replaced with 60 ml of acetylene, and the samples incubated for 1 h in a shaded area. A gas blank containing no soil cores was also incubated at each sampling. At the end of the incubation, gas samples were transferred to evacuated 5 ml vials using double-ended needles.

Gas samples were analysed for ethylene in a Pye Unicam series 204 gas chromatograph equipped with flame ionising detector. The 4 mm i.d. x 0.9 m glass column was packed with 'Poropak T' and maintained at 125 °C. The sample ethylene concentration was determined by comparing ethylene peak heights with standard curves prepared from known concentrations of ethylene. The gas blank ethylene was then subtracted. A ratio of 3:1, ethylene produced : N fixed was assumed (Hardy *et al.*, 1968). Time and area factors

were used for calculating kg N fixed/ha to 75 mm soil depth/day. One replication was additionally sampled from 75 to 150 mm to measure N fixed and, if necessary, added it to the value for the 0-7.5 cm depth.

3.3 Results and Discussion

3.3.1 Herbage yield and botanical composition

Total herbage DM yields, legume contents and total herbage N yields are presented on a seasonal basis in Tables 3.2 and 3.3 (1989-90 and 1990-91, respectively) for the three systems under comparison. The patterns of DM accumulation throughout the experimental period are presented in Fig. 3.2 and the contributions to yield of the main species appear in Fig. 3.3 for the Grass-clover and Herbal ley.

Herbage accumulation followed a typical pattern, reflecting high rates of growth in late winter and early spring (Fig. 3.2). Grass-clover and Herbal ley showed a similar accumulation rate, while Grass+N400 was characterised by a large increase in the daily accumulation rate from August to the end of October (from 20 up to 80 kg DM/ha/day). Late spring-early summer (particularly November) of 1989 was dry (see Table 2.2) and the soil moisture content dropped markedly (Fig. 3.4), affecting more severely the Herbal ley and Grass-clover than the Grass+N400 system. This probably reflects the sensitivity of the legume component to restriction in soil moisture (Hart, 1987), especially during the establishment year. In this period there is also usually a decline in grass production as a result of the physiological stage, 'post-flowering dormancy' (Robson *et al.*, 1989). When fertiliser N was withheld during the period January to March 1989 (Table 3.1), the yield of Grass+N400 pasture declined to a similar level as in the other two systems (Fig. 3.2), demonstrating the poor residual effect of fertiliser N, as well as the strong dependency of this intensive system on continuing inputs of fertiliser N.

Table 3.2 Total herbage and legume yields and total herbage N yield (1 June 1989 to 31 May 1990)

		Grass-clover	Herbal ley	Grass+N400	Lsd (0.05)
			Kg/ha		
Winter	Total herbage	1001	1429	2658	473
	Legume	33	266	-	*
	Total N yield	40	57	109	14
Spring	Total herbage	3742	4710	6476	1218
	Legume	831	1809	-	*
	Total N yield	91	139	198	32
Summer	Total herbage	3175	4643	4682	1048
	Legume	1206	2164	-	*
	Total N yield	101	147	135	29
Autumn	Total herbage	2966	3419	3699	401
	Legume	916	831	-	ns
	Total N yield	95	112	119	8
YEAR	**Total herbage**	**10884**	**14201**	**17515**	**1513**
	Legume	**2986**	**5070**	**-**	*
	Total N yield	**327**	**455**	**562**	**42**

(*) denotes significant differences at P≤0.05

Table 3.3 Total herbage and legume yields and total herbage N yield (1 June 1990 to 31 May 1991)

		Grass-clover	Herbal ley	Grass+N400	Lsd (0.05)
			Kg/ha		
Winter	Total herbage	2001	1981	3606	325
	Legume	308	545	-	*
	Total N yield	70	69	137	9
Spring	Total herbage	3873	5695	5381	406
	Legume	686	1422	-	*
	Total N yield	93	165	161	40
Summer	Total herbage	3523	5572	4083	1014
	Legume	704	1593	-	*
	Total N yield	109	178	118	31
Autumn	Total herbage	3100	3315	3359	751
	Legume	775	840	-	ns
	Total N yield	99	116	118	12
YEAR	**Total herbage**	**12497**	**16563**	**16429**	**1314**
	Legume	**2473**	**4400**	**-**	*
	Total N yield	**371**	**528**	**534**	**54**

(*) denotes significant differences at P≤0.05

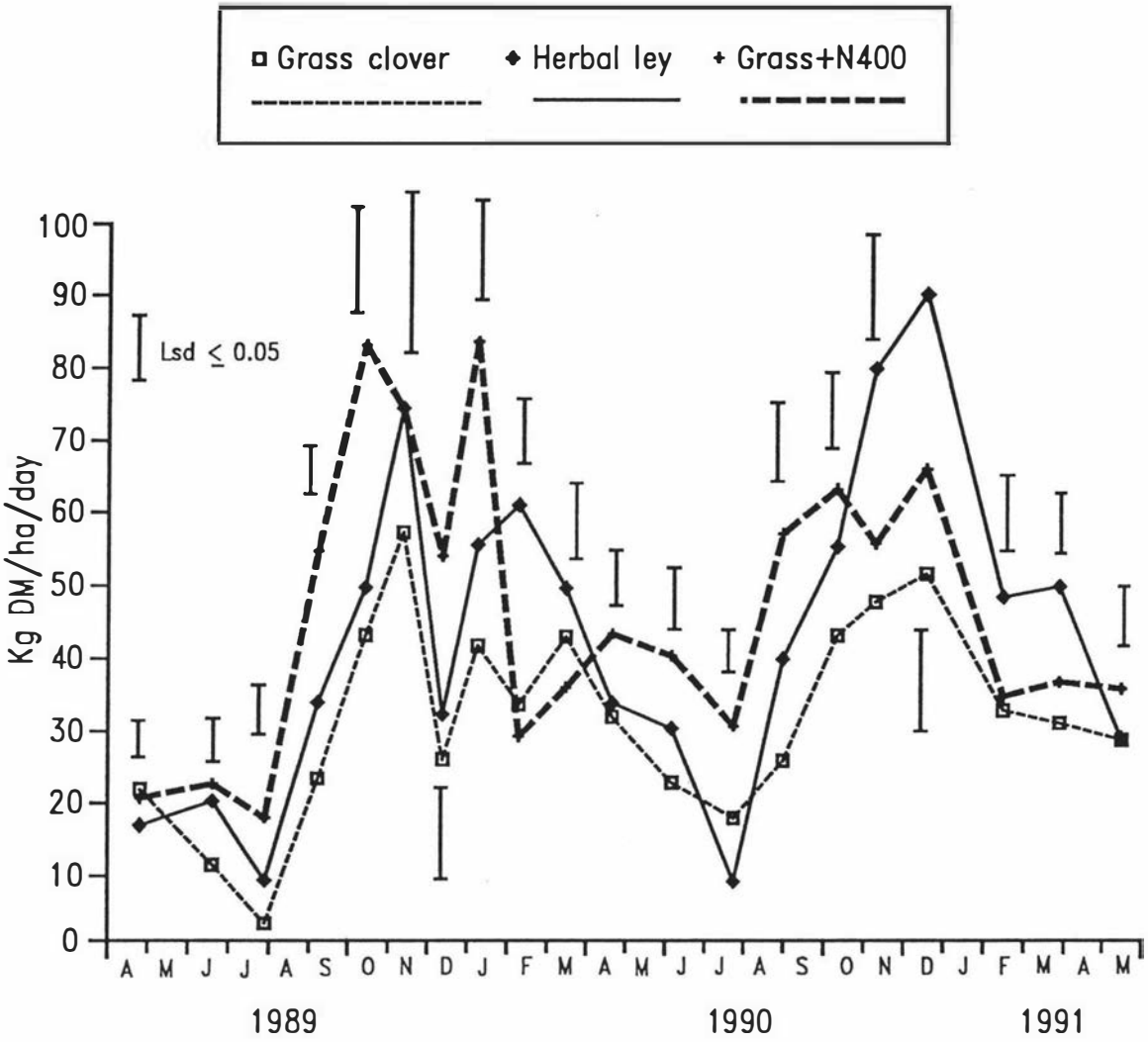

Figure 3.2 Herbage accumulation rate

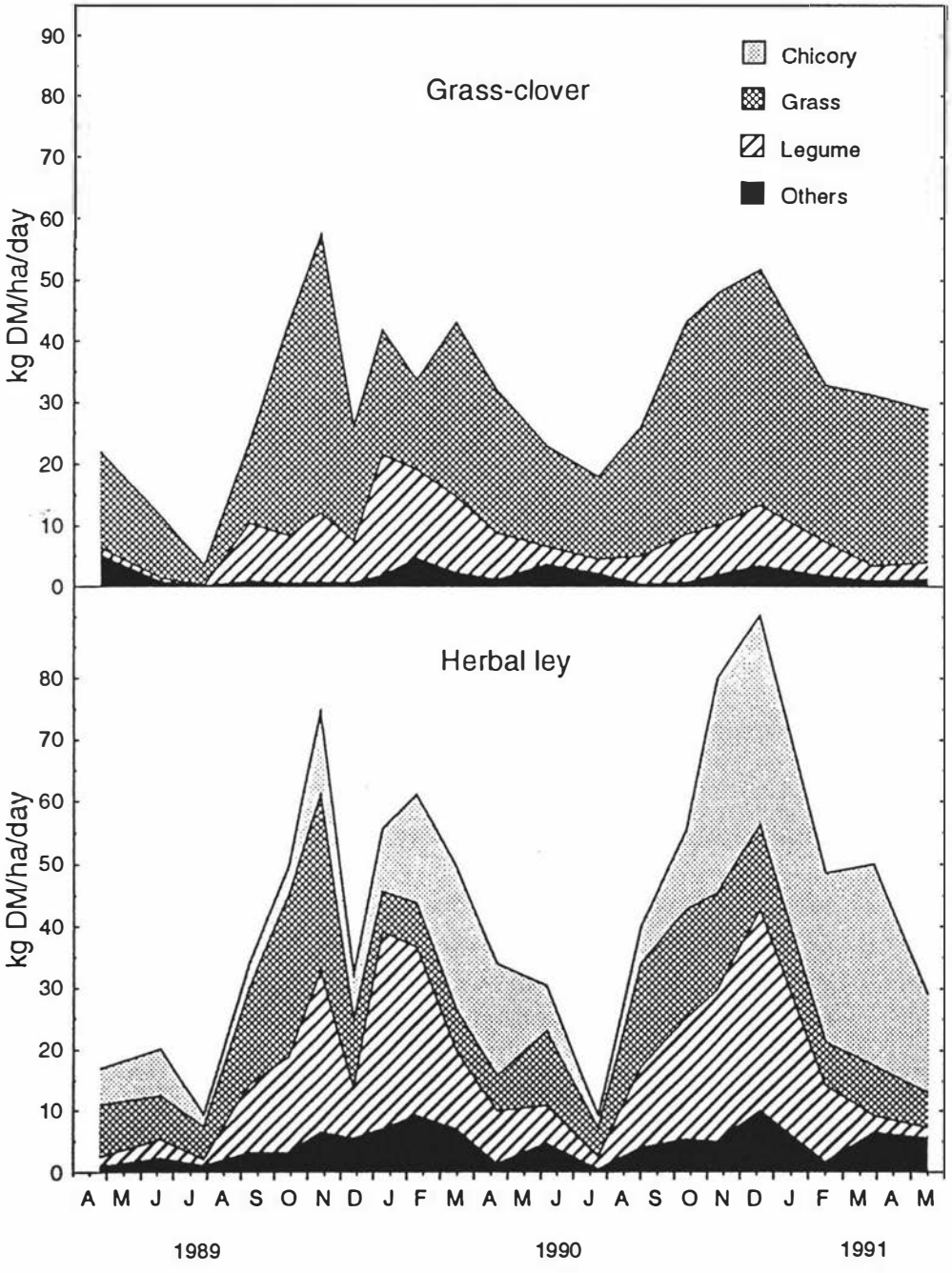

Figure 3.3 Dynamics of pasture composition

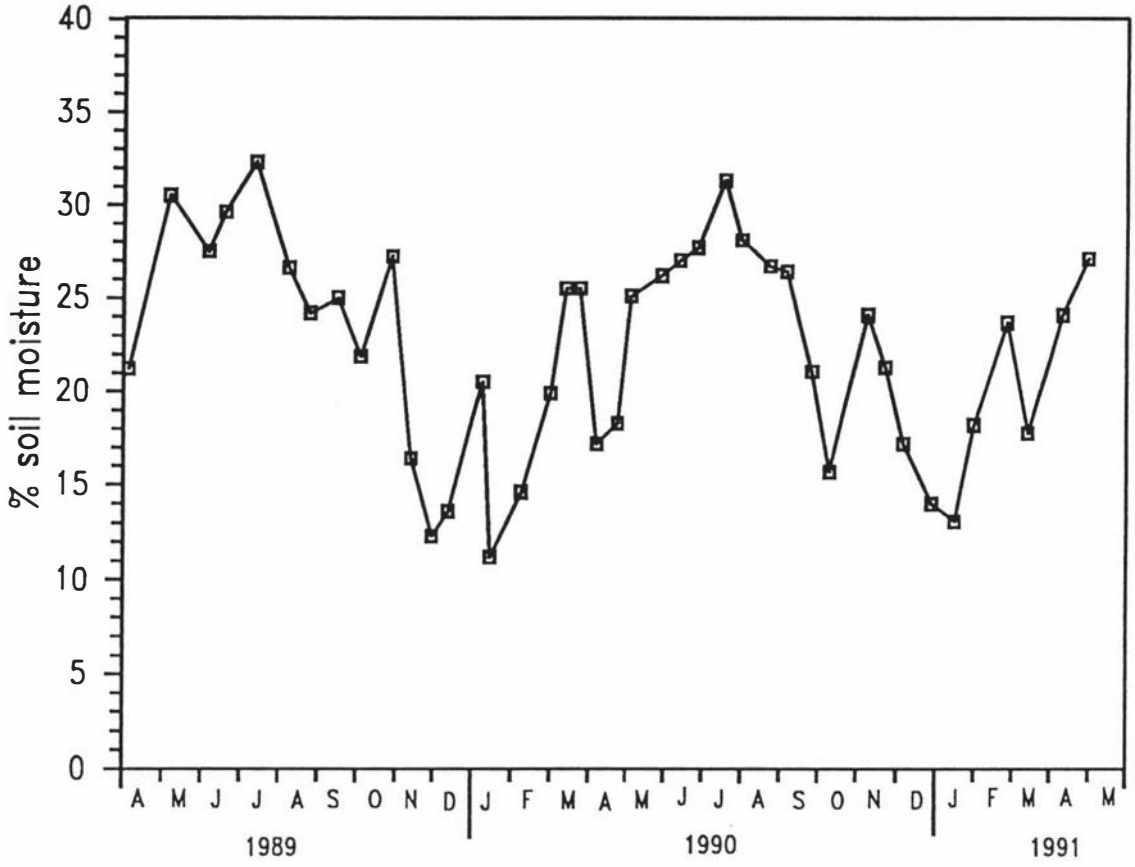

Figure 3.4 Gravimetric soil moisture (0–15 cm depth)

In the second year the pattern of growth of the Herbal ley differed from that of Grass-clover and Grass+N400 pastures. In winter, Herbal ley presented a low rate of herbage accumulation (about 10 kg DM/ha/day), not too dissimilar from that of the Grass-clover. But its production was at a maximum in the late spring-summer period, rivalling or even exceeding that of the Grass+N400 during the period October 1990 to April 1991. Most of this enhanced production was due to the high rate of growth of legumes (principally white and red clovers) and chicory (see Fig. 3.3 for botanical composition).

The major components of the Herbal ley were chicory and other herbs, white clover and red clover, and a mixture of grasses, all participating in variable proportions through the year. The contribution of each species to herbage DM yield at each grazing is given in Appendix 1. Among the herbs, chicory was the most important species, and in some periods of the year it also was the major contributor to total yield from the Herbal ley. The contribution of other herbs to the DM yield was small. Only plantain and yarrow were present with some quantitative interest at certain periods of the year, but always with a presence below 5% of the total yield. White clover and red clover were the main legumes permanently present in the Herbal ley sward. White clover predominated over red clover in winter and spring, while the proportion of red clover increased over the mid-summer. On a yearly basis white clover contributed about 20% and red clover 15% to the herbage DM yield. Lotus and lucerne were present only at very low proportions, contributing less than 1% to the total DM yield.

The annual herbage production (Tables 3.2 and 3.3) was about 17 t DM/ha for the Grass+N400 pasture during the two-year study. But the annual yield of Grass-clover increased from 10.9 to 12.5 t DM/ha in the second year, with an enhanced yield of grasses over legumes. The Herbal ley also showed a dramatic improvement from 14.2 to 16.6 t DM/ha in the second year and the annual yield was then very similar to that of the intensive system receiving 400 kg N per ha per year. The legume content of Herbal ley was 35% and 26%

for year one and two, respectively, with a seasonal emphasis in spring and summer. Therefore the increased production in the second year was largely due to an enhanced contribution from grasses and other non-leguminous species, principally chicory (Fig. 3.3). The legume content of the Grass-clover pasture fluctuated between 15 and 38% (except winter of the first year) according to the season and its contribution to total yield was generally lower than in the Herbal ley (Tables 3.2 and 3.3).

3.3.2 Herbage N yield

Total herbage N yields for the three systems during the two years are given in Tables 3.2 and 3.3. Nitrogen yield in the Grass-clover system was generally significantly lower than that from both the Grass+N400 and Herbal ley, during the two years of study, on both a seasonal (seasons comprise similar period in each year) and annual basis. Herbal ley provided a notable turnover of N through herbage (455 and 528 kg N/ha for year one and two, respectively), which was not much less than that recorded from the grass sward receiving substantial inputs of fertiliser N.

In general the values for the Grass-clover and Grass+N are lower than those found by Ball *et al.* (1978) in the same area; but the N yields from Grass-clover are higher than typical values for mixed pasture not receiving fertiliser N in NW Europe. For instance, in The Netherlands N yield fluctuates in most cases between 100 and 250 kg N/ha/yr (Meer and Lohuyzen, 1986), and in southern England it is about 295 kg N/ha/yr (Whitehead, 1970).

Cumulative N herbage yield was related to the corresponding cumulative herbage DM yield by a linear relation during the period from August 1989 to March 1991. This type of relationship was proposed by Sallete and Huché (1989) to evaluate the effect of different grass management treatments on the dry matter yield and on the N uptake dynamic in long term studies.
The linear, fitted model for the three systems can be presented as:

Grass-clover:	35.5 kg DM/kg N yield	r=0.99
Herbal ley:	31.5 kg DM/kg N yield	r=0.99
Grass+N400:	32.8 kg DM/kg N yield	r=0.99

The fact that data for the three pastures could be fitted to linear models indicates that each soil-pasture system exhibited a constant behaviour in terms of N uptake. The slope of these linear models gives the dimension of kg of DM produced per kg of N yield. According to Salette and Huché (1989), (who expressed the relationship in terms of kg of N required per tonne of DM produced) the slope of such models represents an "adjusted need" of the sward for the nutrient that is much more representative of the sward behaviour than any average N content.

3.3.3 Symbiotic N fixation

Seasonal patterns of sNf for the Grass-clover and Herbal ley pastures are presented in Fig. 3.5. The general pattern of sNf was similar to that found at this site by Hoglund and Brock (1978). Higher activity occurred in early spring, in response to the increase of temperature (Table 2.3), appropriate soil moisture content (Fig. 3.4) and a significant proportion of legumes in the sward. In summer the temperature and legume content were satisfactory, but soil moisture was too low to promote a more intense activity. Later, in autumn, soil moisture improved and temperature was still adequate, but the legume content of the sward declined markedly (see Fig. 3.3). Therefore, under the conditions of this experiment, only in two or three months was it possible to observe an intense fixation of atmospheric N.

Total sNf in the first year was similar for both the Grass-clover and Herbal ley at 150 and 156 kg N/ha respectively (Table 3.4). These values are within the range found by Hoglund and Brock (1978) in a developed Grass-clover pasture grazed by sheep. The similarity in the amount of N fixed by the two systems contrasts with the fixation efficiency (kg N fixed/tonne legume yield).

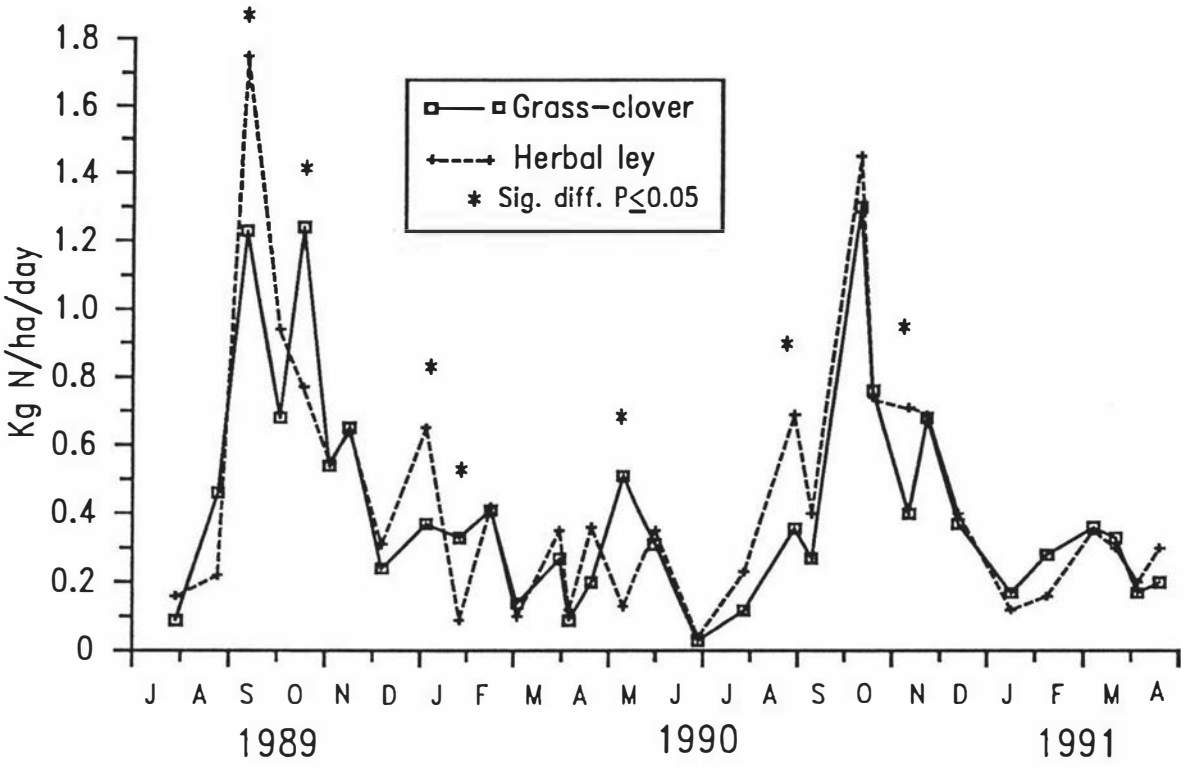

Figure 3.5 Changes in symbiotic N fixation (0–7.5 cm depth)

Table 3.4 Legume yield, symbiotic N fixation and fixation efficiency

	Legume yield (t/ha)	sNf (kg/ha)	Fixation efficiency (kg N fixed/t leg.)
		1989-1990	
Grass clover	3.0	150	50.0
Herbal ley	5.1	156	30.6
		1990-1991	
Grass clover	2.5	123	49.7
Herbal ley	4.4	144	32.7

Table 3.5 Nitrogen input and DM yield

	Herbage yield (t/ha)	Herbage N yield (kg/ha)	N input (kg/ha)	kg DM yield / kg N input
		1989-1990		
Grass-clover	10.9	327	150	72.7
Herbal ley	14.2	455	156	91.0
Grass+N400	17.5	562	420	41.7
		1990-1991		
Grass-clover	12.5	371	123	101.6
Herbal ley	16.6	528	144	115.0
Grass+N400	16.4	534	400	41.0

(N input = symbiotic N fixation or fertiliser N)

Grass-clover was much more efficient on this basis than the Herbal ley (Table 3.4). The legume content of the former varied between 3 and 2.5 t/ha for the first and second year respectively, fixing about 50 kg N per tonne of legume. The legume content of the Herbal ley was significantly higher (5.1 and 4.4 t DM/ha for the first and second year respectively) but the N fixed was similar to that of the Grass-clover, resulting in a lower efficiency of 30.6 and 32.7 kg of N fixed per tonne of legume for the first and second year, respectively. This difference could be because the Herbal ley had less aggressive grasses and more deep-rooting species (principally chicory) than the Grass-clover pasture, leaving more mineral N available to legumes, which could depress the fixation process by simple substitution of soil N uptake for symbiotic N fixation (Ball, 1979). This mechanism has been well illustrated in the literature (Eady, 1981; Hoglund and Brock, 1987).

Symbiotic N fixation represented 46% and 33% of total herbage N yield for year one and two, respectively, in the Grass-clover system, and 34 and 27%, respectively, in the Herbal ley. These results indicate that the Herbal ley was using more soil N than the Grass-clover pasture, while also fixing a similar amount of N. The final result was a significant difference in forage production and N yield in favour of the Herbal ley.

3.3.4 Nitrogen inputs and herbage yield

The three pasture systems showed different patterns of utilisation of external N inputs in relation to herbage DM and N yields. Some of these relationships are given in Table 3.5. Grass+N400 produced the highest yield the first year, but required more than double the input of N, in relation to the other two systems, to achieve this. Herbage DM production per kg of N input was higher in the Herbal ley than in Grass-clover and, more obviously, than in Grass+N (Table 3.5). During the second year the Herbal ley produced the same, substantial high herbage and N yields as Grass+N400 but required only about one third of the external input of N.

Another fascinating aspect of these results is the difference in apparent utilisation of soil N. Table 3.5 indicates that the Herbal ley and Grass-clover made greater utilisation of soil N than Grass+N400. But more intriguing is the difference between Grass-clover and Herbal ley. Both, with similar N inputs by sNf, showed a large difference (more than 120 kg N/ha/yr) in apparent utilisation of soil N. So the Herbal ley, while fixing a substantial amount of atmospheric N, somehow enhanced the utilisation of soil N such that a larger amount of N was taken up to yield more herbage than did Grass-clover.

This large difference in utilisation of soil N opens up an interesting question. Did the additional N come from better exploitation of the current soil mineral N or was there some sort of stimulation of mineralisation of organic N? If the first explanation were true, why did the intensive measurements of mineral N in soils not show that difference (see Chapter 4). The other possibility is that the different deep-rooting systems of some of the species in the Herbal ley promoted a more active mineralisation of organic N (a phenomenon that has been recently described by Clarholm, 1989 and Wheatley *et al.*,1990) and at the same time a rapid uptake of N, in this way avoiding accumulation of mineral N in the soil profile. This hypothesis may indirectly be supported by the mineral N values measured periodically during two years (which consistently were at the same low level under the Herbal ley as under Grass-clover), by the similar amount of symbiotic N fixation by the Grass-clover and Herbal ley (accumulation of mineral N could suppress the symbiotic process), and the low level of nitrate leaching (see Chapter 5).

3.3.5 Grazing animals and herbage intake

The grazing management followed the recommendations for a pasture rotationally grazed by sheep, with the practical aim to graze all plots at the same time during 3-4 days. The number of animals per plot was adjusted so as to start with a similar pasture allowance (kg DM per animal per day), and towards the end of grazing some adjustment in the number of animals was

necessary to leave each plot with a reasonably similar, medium to low post-grazing pasture mass.

The average of the animal grazing days per season and an estimation of DM intake per animal per day according to the measured herbage DM yield at the time of grazing are given in Table 3.6. These results indicate that under the management used in this experiment, animals did not have any difficulty in achieving an adequate daily intake, which was always in excess of the maintenance level for mature dry sheep.

The intake of DM was slightly greater in the Herbal ley than in Grass+N400 and a major difference was observed in relation to the Grass-clover pasture. In spite of intake of herbage being a very complex and variable process, influenced by a large number of factors, it seems likely that in this experiment the enhanced intake observed in the Herbal ley could be due to differences in sward structure and herbage mass, two non-nutritional factors which have a recognised effect in influencing the rate of intake (Poppi *et al.*, 1987).

Pasture composition affecting diet selection is another factor which influences DM intake (Arnold, 1987). An ancillary study about preference for species in the Herbal ley sward was conducted in winter-spring of 1990, in a collaborative study with Ms Jeaninie Geelhoed (Agricultural University, Wageningen, The Netherlands). Height changes in marked species during three grazing periods were measured periodically to indicate any pattern of preference for the main species of the Herbal ley pasture during defoliation. During the initial hours of grazing, there was a strong pressure on highly accessible grasses, but not chicory in spite of its similar height. After some decrease in the height of grasses, chicory disappeared most rapidly, compared with clovers, yarrow and plantain. The grazing pressure on chicory and plantain was not high, which is an important point in relation to the persistence of these herbs.

Accumulated animal grazing days (Table 3.6) give a total value for the year

Table 3.6 Animal grazing days and intake of dry matter per grazing day

	Grass-clover		Herbal ley		Grass+N400	
	Sheep grazing days	intake (kg DM/d)	Sheep grazing days	intake (kg DM/d)	Sheep grazing days	intake (kg DM/d)
			1989-1990			
Winter	834	1.20	1058	1.35	2060	1.29
Spring	2946	1.27	3294	1.43	4466	1.45
Summer	2835	1.12	3805	1.22	3417	1.37
Autumn	2579	1.15	2442	1.40	3082	1.20
YEAR	**9194**	**1.18**	**10599**	**1.35**	**13025**	**1.32**
SR	**18**	**a**	**20**	**b**	**25**	**b**
			1990-1991			
Winter	1170	1.13	1415	1.40	2521	1.43
Spring	3367	1.15	4485	1.27	4237	1.27
Summer	3202	1.10	4097	1.36	3460	1.18
Autumn	2627	1.18	2536	1.38	3057	1.21
YEAR	**10966**	**1.14**	**12533**	**1.35**	**13275**	**1.27**
SR	**21**	**a**	**24**	**b**	**25**	**b**

SR = average stocking rate/ha was calculated from the annual sheep grazing days divided by 365, and multiplied by 0.7 according to the M.A.F. conversion table (Cornforth and Sinclair, 1984).

(Different letters for DM intake denote significant differences at $P \leq 0.05$ for the annual values).

which can be expressed as an average stocking rate (number of stock units/ha/yr). During the two years, stocking rate for Grass+N400 was the same (25), representing well the carrying capacity of an intensively managed grassland system. The stocking rate on the Grass-clover and Herbal ley increased in the second year by 3 and 4 s.u. respectively. With this increment the Herbal ley was lower than Grass+N400 by only one s.u. (but with a greater apparent DM intake). These results indicate that the Herbal ley could be a low-input system, without dependence on fertiliser N, and could provide better herbage and animal production than the traditional Grass-clover system, and be very close to a high-producing system based on the heavy use of N fertiliser.

3.4 Conclusions

A Herbal ley pasture was established in a grazing study to compare it with a conventional Grass-clover pasture and a pure grass sward receiving 400 kg fertiliser N/ha/yr.

All species sown in the Herbal ley established well. But as the experiment progressed, many herb species disappeared under the prevailing management, involving periodic mob-stocking with sheep. Others were still present in low proportions, but chicory was most important among these herbs and has made a substantial contribution to total yield, followed by white clover and red clover.

The dynamics of the botanical composition in the Herbal ley reflected the general ecological principle that in a wide mixture of species under grazing, about 80% of the herbage yield is usually achieved by three or four species. Herbage yield was greater in the system receiving fertiliser N, especially during the establishment year. In the second year the Herbal ley improved its production to the same level as that from the intensive Grass+N400 system. Grass-clover production was about 25 % below that of the other two systems

in the second year.

Fertiliser N played a key role in determining herbage production in Grass+N400. The greater pasture production was almost entirely dependent on fertiliser N, and herbage yield was very sensitive to its being withheld. In Grass+N400 pasture, fertiliser N determined a lower apparent utilisation of soil N, and this system was also characterised by a less efficient conversion of N input DM yield.

Symbiotic N fixation was the major input of N in the Grass-clover and Herbal ley systems and was vital to sustaining herbage production. Legumes in the Grass-clover pasture were clearly more efficient at fixing N than legumes in Herbal ley, on the basis of legume forage yield.

Herbal ley produced more forage from legumes than Grass-clover, but symbiotic N fixation was similar in both systems. Herbal ley was efficient in terms of herbage production per unit of N input. Because of an effective combination of biological processes (a significant amount of symbiotic N fixation with an enhanced utilisation of soil N), this system had a high N turnover through the plants and hence produced more pasture.

CHAPTER 4
MINERAL NITROGEN

4.1 Introduction

The total amount of N in grassland soils is large, with values varying widely, but usually above 5000 kg/ha (Walker *et al.*, 1959). However, in spite of this abundance, biologically available N in mineral forms constitutes only a small fraction (and normally a transitory pool) of the total N content of soils (Haynes, 1986). Mineral N compounds detected in soils usually comprise six forms: exchangeable NH_4^+, non-exchangeable NH_4^+, NO_2^-, NO_3^-, N_2O and N_2 (Young and Aldag, 1982). Of these, ammonium and nitrate are the only major ionic forms of N available for direct uptake by plants, and usually account for less than 2% of the total N of agricultural soils (Melillo, 1981, Woodmansee *et al.*, 1981).

4.1.1 Origin of mineral N

Mineral N is produced when organisms ingest food materials that are higher in hydrolysable N than the organisms require or can utilise at that time and the surplus N is excreted in waste products (Jarrell, 1990). The initial conversion to ammonium is referred to as ammonification, and the subsequent oxidation of this compound to nitrate is termed nitrification. The general term mineralisation may include both the ammonification and nitrification processes. Therefore, the basic feature of these biological transformations centres on reduction and oxidation reactions. These forms of mineral N are utilised by microorganisms (immobilisation), particularly during the process of decomposition of organic residues with low N content; and that portion of mineral N that is not immobilised by soil microorganisms can be absorbed and assimilated by growing plants (Haynes, 1986).

The complexity of the N cycle in field soils has stimulated research on individual N transformations during mineralisation-immobilisation processes. Thus the majority of these fundamental aspects has been extensively studied within basic sciences like microbiology, biochemistry, and plant physiology. An important part of the relevant results for agricultural soils in this topic has been synthesised in excellent reviews, such as by Paul and Juma (1981), Jansson and Persson (1982), and Haynes (1986). However, studies of single transformations often provide knowledge that is too specialised to be informative for understanding the ecological unity of these transformations in the complete process of plant production and subsequent animal utilisation on grassland soils. In fact, studies of N transformations normally do not consider the notable role of grazing animals in influencing pathways and reactions in an ecological perspective. Grazing animals affect N transformations through consumption of herbage, treading on soil and vegetation, return of N in excreta and removal of N in animal products (Floate, 1981).

4.1.2 Mineral N: its role in agricultural production and environmental pollution

The supply of mineral N in agricultural soils is rarely sufficient to match the demand for the potential plant growth. In grassland soils in particular, forage production has been extensively reported to be severely limited by N availability (Ball and Field, 1982; Meer and Lohuyzen, 1986). But in the process of supplying adequate N (principally by fertiliser N) to meet the traditional challenge of maximising food production, a new problem has been created, because a substantial proportion of the N input may be lost from the system. This loss of N is a focus of concern not only because of its economic significance for the agriculture, but also because of its potential impact on the wider environment and human health (nitrate leaching to ground water and emission of nitrous oxide to the atmosphere: Ryden *et al.*, 1984; Ryden, 1986).

The origin of this incongruity can be illustrated in Fig. 4.1. The pool of mineral

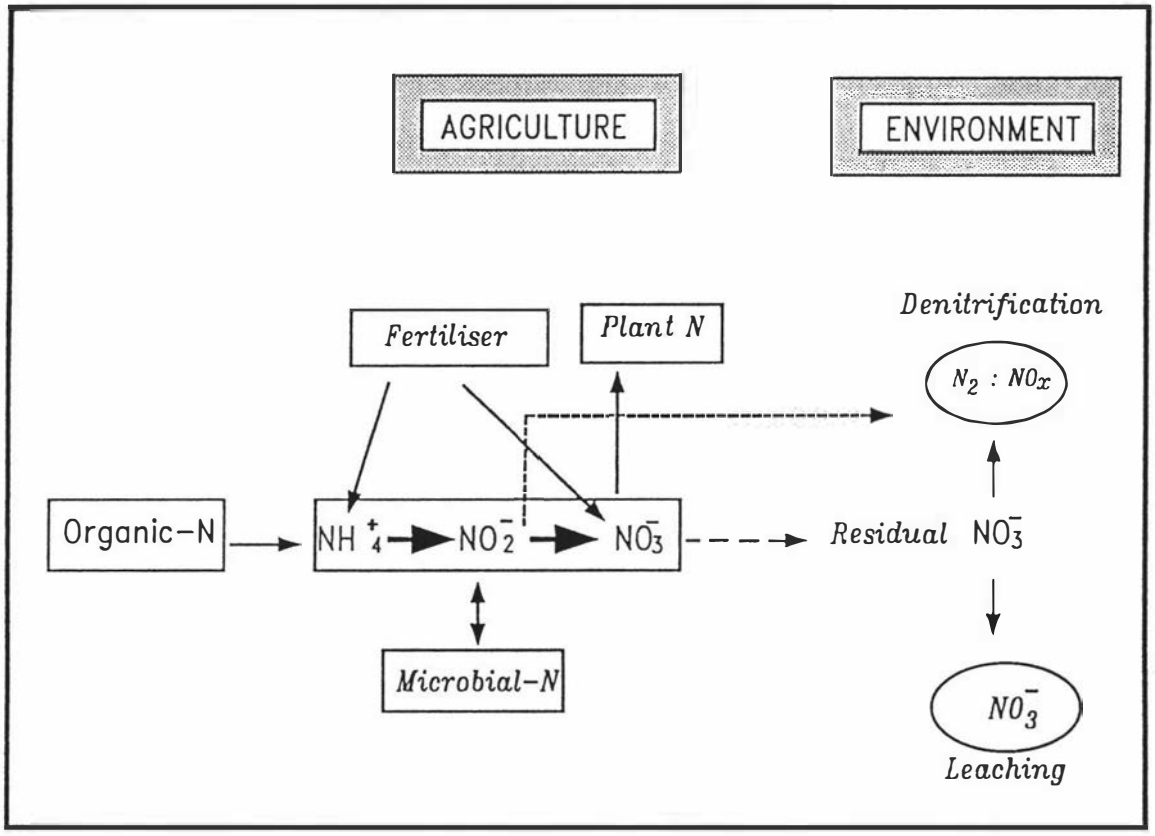

Figure 4.1 Relationships between some N transformations and their role in agricultural production and environmental pollution.

N, which is the net result of simultaneous inputs and outputs (governed by several competing processes), can be considerably increased by fertiliser application and eventually create an excess of mineral N, especially with large rates of application that abruptly interrupt the natural cycle. If this increased mineral N pool is in excess of the biological demand (plant uptake and soil microorganisms), and subsequently ammonium is subject to nitrification, the nitrate produced will constitute a potential risk because it is the substrate for both leaching and denitrification.

The key role of nitrification in facilitating N losses, and also its adverse effect in soil acidification, have received great attention for many years. The limiting of the rate of nitrification and the development of slow-release fertilisers has been the ambition of both agronomists, and more recently, environmentalists. However, it seems that the process of nitrification is inherent to agricultural development. In this regard Verstraete (1981) postulated that the capacity of nitrification reflects nutrient availability, and its rate is largely governed by the supply of substrate (NH_4-N) in most agricultural soils. In contrast, in natural ecosystems it is recognised that the low level of nitrification is vital for N conservation (Vitousek *et al.*, 1979).

Thus under the more usual soil conditions in intensive agriculture, where microbial development (immobilisation) is limited by available carbon (C) and energy (Schmidt, 1982), most of the remaining ammonium may be oxidised to nitrate. In other words, the availability of substrate would be the main factor for controlling the rate of nitrate accumulation, and then the potential N loss, with its detrimental effect both for agriculture and the environment. But although nitrification is an environmental disadvantage, in certain agricultural conditions it can still be an agronomically desirable process. For instance, in soils that are fertilised with ammoniacal fertiliser or urea, and in urine patches, nitrification may result in: (i) conversion of potentially phytotoxic ammonium to less toxic nitrate, thus creating better conditions for plant growth; (ii) decreased gaseous losses of N through ammonia volatilisation (Haynes, 1986); and (iii)

improved absorption of cations (especially K, Ca and Mg) most probably as co-ions in the uptake of nitrate. To improve the efficiency of N utilisation and reduce losses from grassland soils, a better understanding of N dynamics is required. In particular net mineralisation, because of its relationship with the amount of N available for plant nutrition, and nitrification, because it is a prelude to potential losses, need further study. In this regard, since Stanford and Smith (1972) proposed a model to predict the rate of mineralisation, a great effort has been dedicated to improving the mathematical modelling of this process (Molina *et al.*, 1980; Darrah *et al.*; 1983; Deans *et al.*, 1986; Torben and Rosswall, 1987; Barraclough, 1988). However, the estimation of net mineralisation in field conditions during plant growth is still very difficult.

The objective of this study was to measure the size of the mineral N pool in three contrasting grassland systems and how this pool changes with time. Concurrently, it was decided to determine the importance of grazing animals on the conversion of herbage N to the soil available pool, and the subsequent effect on plant N uptake during regrowth. Detailed studies of the dynamics of N and net N mineralisation were carried out between consecutive grazings under autumn and spring conditions.

4.2 Materials and Methods

The study of mineral N was carried out on the three grassland systems, Grass-clover, Herbal ley and Grass+N400. A detailed account of the experimental site, climate, establishment and management of the trial has been given in Chapter 2.

In this study several aspects of mineral N were examined through the following measurements.

4.2.1 Sample size for mineral N analysis

To determine the number of soil cores per plot, a preliminary study to estimate the variance of the population to be sampled was carried out according to Stein's two-stage sample procedure (Steel and Torrie, 1980). With the analysis of 20 individual cores (25.4 mm i.d.) for mineral N (NH_4-N and NO_3-N) at 4 depths (0-7.5, 7.5-15, 15-30 and 30-45 cm depth), the minimum difference between means that could be detected using different numbers of cores was calculated. This information was the criterion for precision. In addition, the practical possibility of sampling the experiment and extracting the soils with KCl during the same day was the basic factor considered for determining the number of cores per sample in each plot.

4.2.2 Routine measurements

From April 1989 to April 1991, mineral N (NH_4-N and NO_3-N) was measured regularly at a frequency of 2 samplings per regrowth period (approximately 10-12 days before and after each grazing).

Soil samples from each plot comprised 15 cores taken at random to 4 depths (0-7.5, 7.5-15, 15-30 and 30-45 cm depth). The 15 soil cores were bulked and mixed. On the same day of sampling, the soils were extracted with 2 M KCl solution (Douglas and Bremner, 1970) and NH_4-N and NO_3-N were determined colorimetrically by an automated method in a Carlo-Erba Flow Auto-Analyzer. Soil moisture content was determined in each sample by the gravimetric method (Gardner, 1965). The results were expressed as kg/ha of NH_4-N, NO_3-N and total mineral N using the bulk density values for each soil layer (Table 2.1). Nitrate in the soil solution was estimated as the concentration of NO_3-N in the water fraction of the field soil sample.

Analysis of variance was used to test for treatment differences at each measurement date, according to a randomised block design. When there was

a considerable difference between Grass+N400 and the treatments receiving no fertiliser N, an additional comparison between Grass-clover and Herbal ley only was made using a t-test.

4.2.3 Detailed measurements

4.2.3.1 Intensive sequential measurement of mineral N in the field

Mineral N (NH_4-N and NO_3-N) was periodically measured during 2 regrowth periods in autumn and spring 1990.

Soil samples from each plot comprised 15 cores (25.4 mm i.d.) taken at random to 2 depths (0-7.5 and 7.5-15 cm) at the following times:
Autumn: One day before grazing, then 1, 2, 4, 8, 11, 15, 18, 22 and 28 days after grazing.
Spring: One day before grazing, then 1, 7, 14, 21, 28 and 35 days after grazing.

Mineral N (NH_4-N and NO_3-N) was determined as for the routine measurement (section 4.2.2). Herbage regrowth was periodically measured by a standard technique, taking 3 quadrats of 0.5 m^2 per plot. Herbage samples were analysed for total N using the Kjeldahl digestion method with modification to include nitrate by addition of salicylic acid (Bremner, 1965). Total N was determined colorimetrically in a Carlo-Erba Flow Auto-Analyzer.

4.2.3.2 Net mineralisation measured in PVC tubes embedded into the soil

Net mineralisation under field conditions during 2 regrowth periods in autumn and spring (concurrent with the study of the previous section) was estimated by analysing soil samples from undisturbed soil cores contained in PVC tubes embedded to 15 cm depth in Grass-clover, Herbal ley and Grass+N400 pastures. The principles of this technique were described by Raison *et al.*

(1987), working in forest soils. This method avoids the effects of soil disturbance and altered environmental conditions in mineralisation studies of soil N.

Immediately after the area was grazed (27 March 1990 in autumn and 17 September 1990 in spring), each plot was allocated 20 tubes, inserted at random in 4 sets of 5 units. The tubes were polyvinyl chloride cylinders having an internal diameter of 5 cm and a capacity of approximately 147 cm^3 in each section of 7.5 cm (see Fig.4.2). At weekly intervals (for 5 weeks) 4 tubes (one from each set) were removed from each plot and individually analysed for mineral N in separate sections of 0-7.5 cm and 7.5-15 cm depth. Each tube protruded 4 cm above the soil surface. The herbage was cut at ground level and removed before setting the tubes into the soil. To prevent any nitrate leaching and plant uptake of N, each unit was adequately protected from rain and light effects with a reflective cover located 2 cm above the tube, and a plug of non-absorbent cotton wool was placed inside the exposed end of each cylinder.

Analysis of variance was used to test for treatment differences at each week. As the values of mineral N in individual cores were highly variable and non-normally distributed, a transformation was required as recommended by White *et al.* (1987) for any statistical procedure that assumes a normal distribution and constant variance. In this case a log normal transformation was used to stabilise the variances. However, for ease of interpretation of the dynamics of N in pastures (one of the objectives of this study), and for a better illustration of the impact of grazing animals through excreta deposition, the arithmetic means were presented in the results, with letters indicating significant differences obtained from the variance analyses performed on the corresponding transformed values.

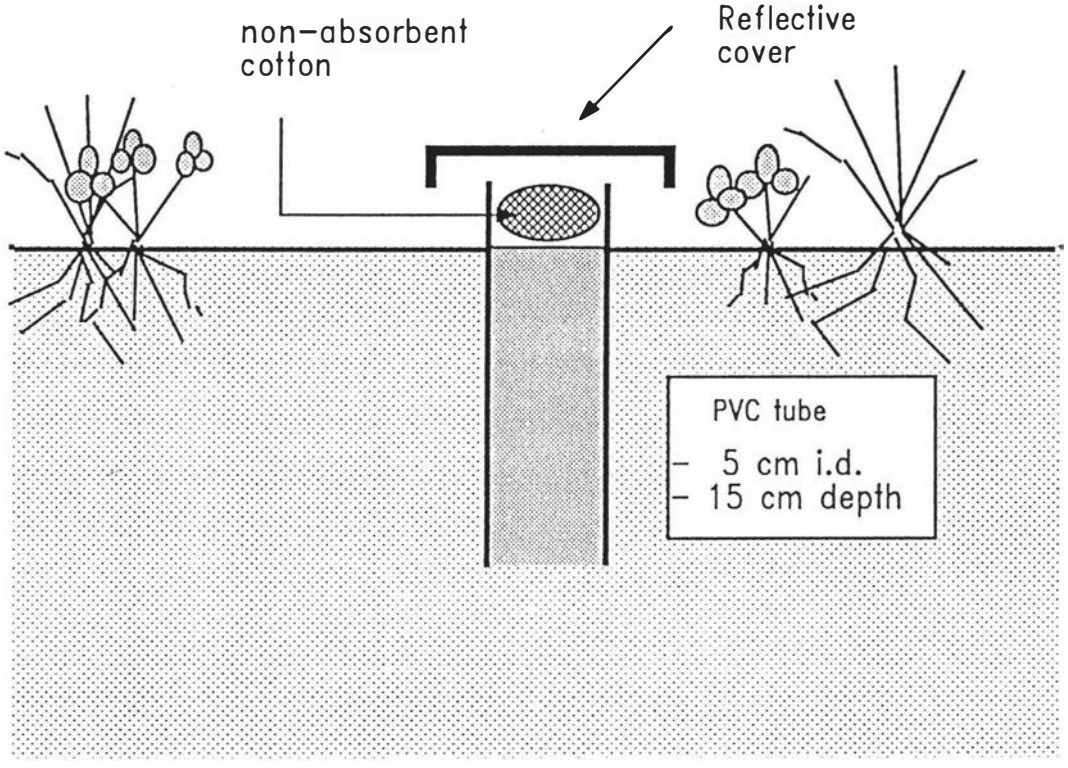

Figure 4.2 Diagrammatic representation of the incubation tubes embedded into the soil

4.3 Results and Discussion

4.3.1 Preliminary study for detecting sample variance

A preliminary study on soil sample variance was required to determine the optimum sample size for estimating mineral N. Table 4.1 shows the results of mineral N determined at 4 depths with the corresponding standard deviation of the sample calculated by analysing 20 cores per depth interval. Applying the Stein's two-stage method (Steel and Torrie, 1980), the minimum difference between means that could be detected as a significant at $P \leq 0.05$ was estimated using different numbers of cores per sample, according to the equation : $d = t \times s/\sqrt{n}$ (d=the half-width of the minimum difference to be detected; *t*=tabulated t for the desired confidence level and the degrees of freedom of the initial sample; s=standard deviation of the sample). This was done for each sampled layer and the results are given in Table 4.2. Taking 16 to 20 cores per sample would enable differences between means of ± 1-2 kg N/ha depth interval to be detected as significant at $P \leq 0.05$.

A compromise between precision and "effort" (based on the time spent in sampling and preparing the soil extract for mineral N measurement and the equipment available) was considered to decide the number of cores per soil sample. The combination of these two factors is graphically presented in Fig. 4.3. With this information it was decided that the sample should comprise 15 cores per plot. This sample size allowed the detection of differences between means as low as ± 6 kg of mineral N per ha-45 cm and it permitted sampling and soil extraction with KCl of the complete experiment in one day. Thus, transformations of N in soil samples due to external factors could be minimised (Robinson, 1975; quoted by Young and Aldag, 1982).

Table 4.1 Mean and standard deviation for mineral N in a sample of 20 soil cores at each depth

Depth	no. of cores	Mean	SD (sample)
(cm)		kg N/ha depth intervals	
0-7.5	20	12.02	4.04
7.5-15	20	8.81	2.28
15-30	20	13.69	4.94
30-45	20	9.21	4.90
Profile	20	43.71	11.11

Table 4.2 Differences (+ or -) between sample means that can be detected as significant at P≤0.05 for different numbers of soil cores per sample

	Number of cores							
Depth (cm)	4	8	12	16	20	30	40	60
	Kg N/ha depth intervals							
0-7.5	4.2	3.0	2.4	2.1	1.9	1.5	1.3	1.1
7.5-15	2.4	1.7	1.4	1.2	1.1	0.9	0.7	0.6
15-30	5.2	3.6	3.0	2.6	2.3	1.9	1.6	1.3
30-45	5.1	3.6	2.9	2.6	2.3	1.9	1.6	1.3
Profile	11.6	8.2	6.7	5.8	5.2	4.2	3.7	3.0

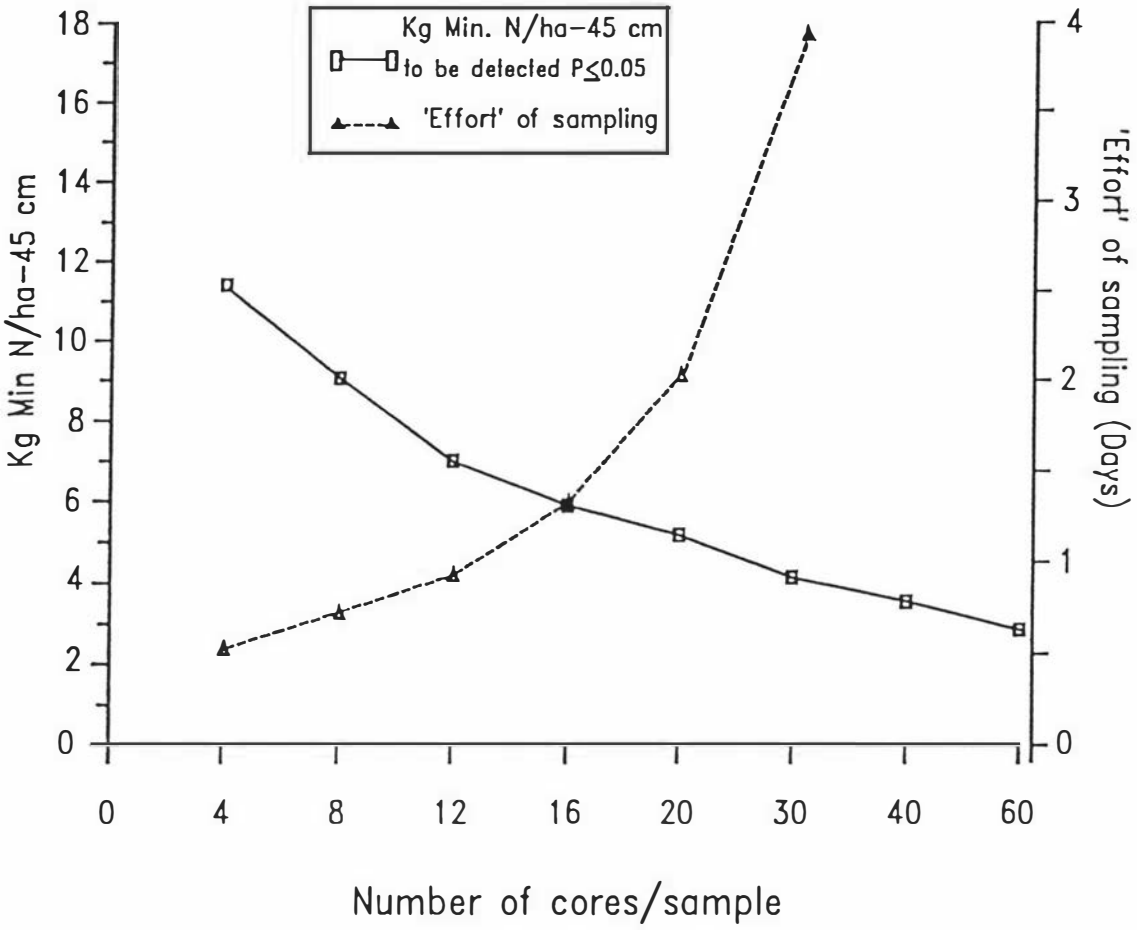

Figure 4.3 Precision/'effort' relationships in mineral N measurements

4.3.2 Changes in mineral N pool: long term measurements

4.3.2.1 Routine measurements

The variations in mineral N for the three pasture systems under study are presented in Fig. 4.4 (values are shown in Appendix 2). Grass-clover and Herbal ley followed approximately the same pattern of mineral N distribution during the course of this experiment. These two pastures were quite different from Grass+N400, which presented high values and considerable variability between sampling dates.

At the beginning of this study (April 1989), mineral N was at a high level in the three pasture systems. This result may reflect the enhanced mineralisation of organic N that could occur during the soil cultivation (Dowdell and Cannell, 1975) at the establishment phase of the experimental swards (March 1989). In this experiment a minimal cultivation of 2 cm depth was able to stimulate mineralisation, to raise the mineral N to levels similar to that achieved by Grass+N400 when 80 kg/ha of N fertiliser were applied (Fig.4.4).

In Grass+N400, the peaks of mineral N reflect recent fertilisation. The response in mineral N was variable in this aspect. For instance, in 2 of the 5 times that high rates (80 kg N/ha) of fertiliser N were applied, the level of mineral N measured a few days later accounted for similar values to the N input. In 3 other occasions (May 89, August 89 and 90) the soil analysis recovered only about 50% of the N applied. A variable effect was also observed with the lowest rate of fertilisation (40 kg N/ha).

The lack of a consistently significant increment in soil mineral N after application of fertiliser suggests that a considerable amount of fertiliser N could be immobilised in the soil microbial biomass (especially in periods when plant uptake of N was expected to be low) or lost from the system (probably volatilised as NH_3-N during urea hydrolysis). Microbial immobilisation after N

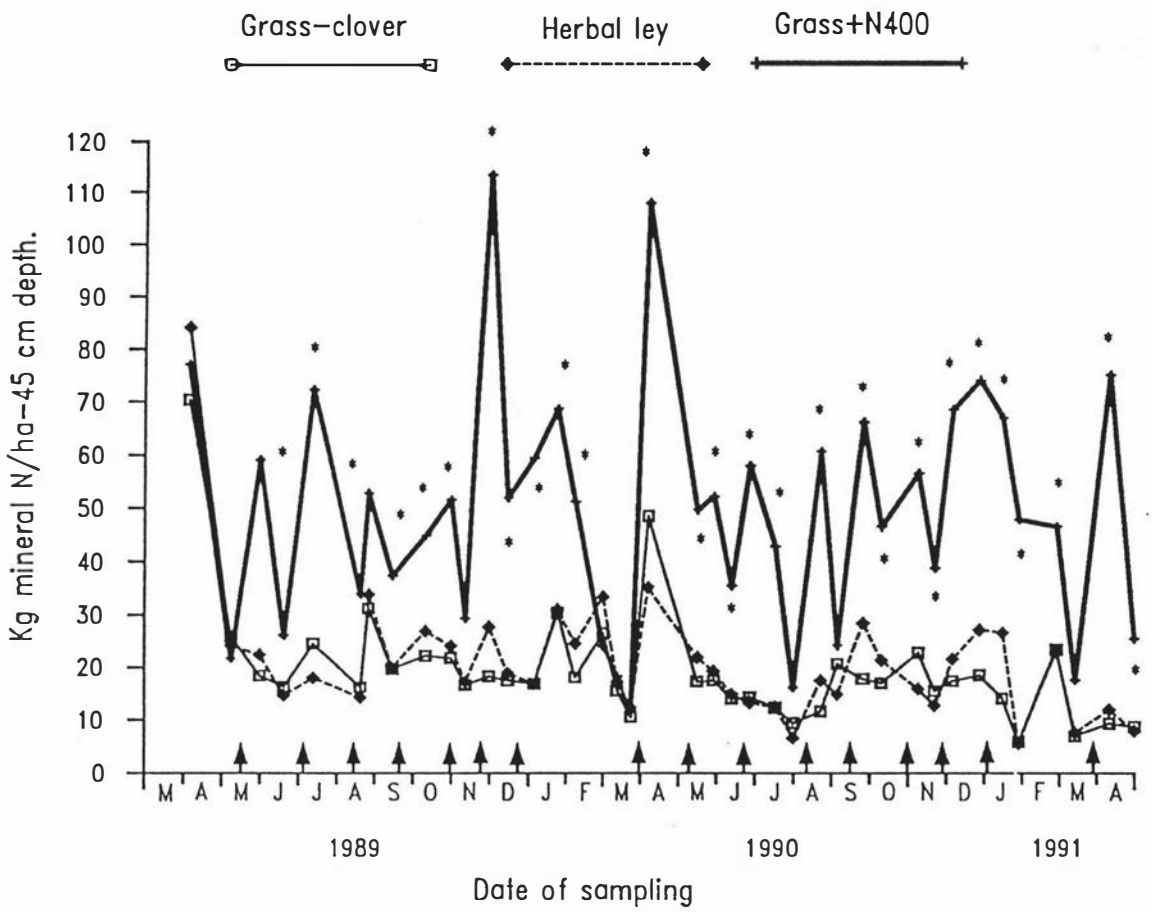

Figure 4.4 Total soil mineral N measured throughout the two year study of the three different pastures

(* Significant differences at 0.05 level)

(Arrows indicate fertiliser N application to Grass+N400 treatment)

fertilisation has been observed in grassland by Bristow *et al.* (1987) and Bristow and Jarvis (1991), Ledgard *et al.* (1988, 1989).

Mineral N measured in the Grass+N400 treatment was statistically different from that in Grass-clover and Herbal ley treatments throughout most of the period of this study. This effect was associated with the continuous application of fertiliser N. The greatest values were recorded in samplings closest to the date of N application. In contrast, the lower values always occurred in those measurement some weeks after N was applied. In summer, when the fertiliser N was stopped (January-March), the level of mineral N declined to the same low values observed in the systems receiving no fertiliser N. These results show the limited residual effect of fertiliser (urea) N in this experiment.

On the other hand, if it is assumed that grassland systems without fertiliser N are operating most of the time under severe limitation of N (Ball and Field, 1982), it is clear that the level of mineral N shown in this experiment (21.3 ± 11.5 and 22.6 ± 12.9 kg mineral N/ha average for the long term measurement for Grass-clover and Herbal ley, respectively) is representing only a portion of that required to reach the potential pasture growth at this site. In contrast, for grass+N400 to achieve maximum yield, supposedly without N limitations, mineral N measured in soil was on average 50 ± 20 kg mineral N/ha. Thus to be supplied with sufficient N, the pure grass sward had to grow in a soil medium that on average maintained about 30 kg mineral N/ha above the natural level observed under Grass-clover and Herbal ley. To achieve this objective, fertiliser N had to be applied regularly.

In real terms, the mineral N detected by soil analysis is the residual N after a number of transformations have taken place simultaneously. The paradox is that pastures receiving no fertiliser N exhibit N deficiency most of the time (Ball and Field, 1987), despite their growth in a soil medium that consistently maintains a residual of about 22 kg N/ha available for plant uptake. One of

the reasons is that most forage species can take up only a fraction of the available N because their roots are unable to explore and deplete the root zone completely (Onema *et al.*, 1989).

More dramatic are pure grass swards, which can not take advantage of the leguminous component for incorporating atmospheric N as an alternative source for N nutrition. Pure grass swards, being totally dependent on soil available N, require a large mineral N pool remaining as a residual to be able to take up N at the appropriate rate for achieving maximum yield. The residual mineral N in Grass+N400 (average 50 kg/ha) represented about 70-100% of the N taken up by herbage during each regrowth period (1700-2000 kg DM with 3-3.5% N approximately). Therefore this grassland system is quite inefficient in utilising soil mineral N. The implication of this aspect for N losses will be analysed in the following Chapters on leaching and denitrification.

4.3.2.2 Forms of mineral N

The total amount of mineral N is important for plant production, but the forms of mineral N are important not only for plant growth, but are also of special interest from an environmental viewpoint. The distribution of ammonium and nitrate in relation to total mineral N for Grass-clover and Herbal ley is presented in Fig. 4.5. Grass+N400 is shown in Fig. 4.6; but in this case because of the complicated pattern of the graph, total mineral N and nitrate only were drawn.

It is clear from both Figures 4.5 and 4.6 that the enhanced mineralisation of organic N at the beginning of this study as a consequence of soil cultivation also stimulated rapid nitrification. About 80% of total mineral N was in nitrate form. It may be inferred that this soil had a good capacity for nitrification providing the conditions were favourable, especially with abundant ammonium substrate.

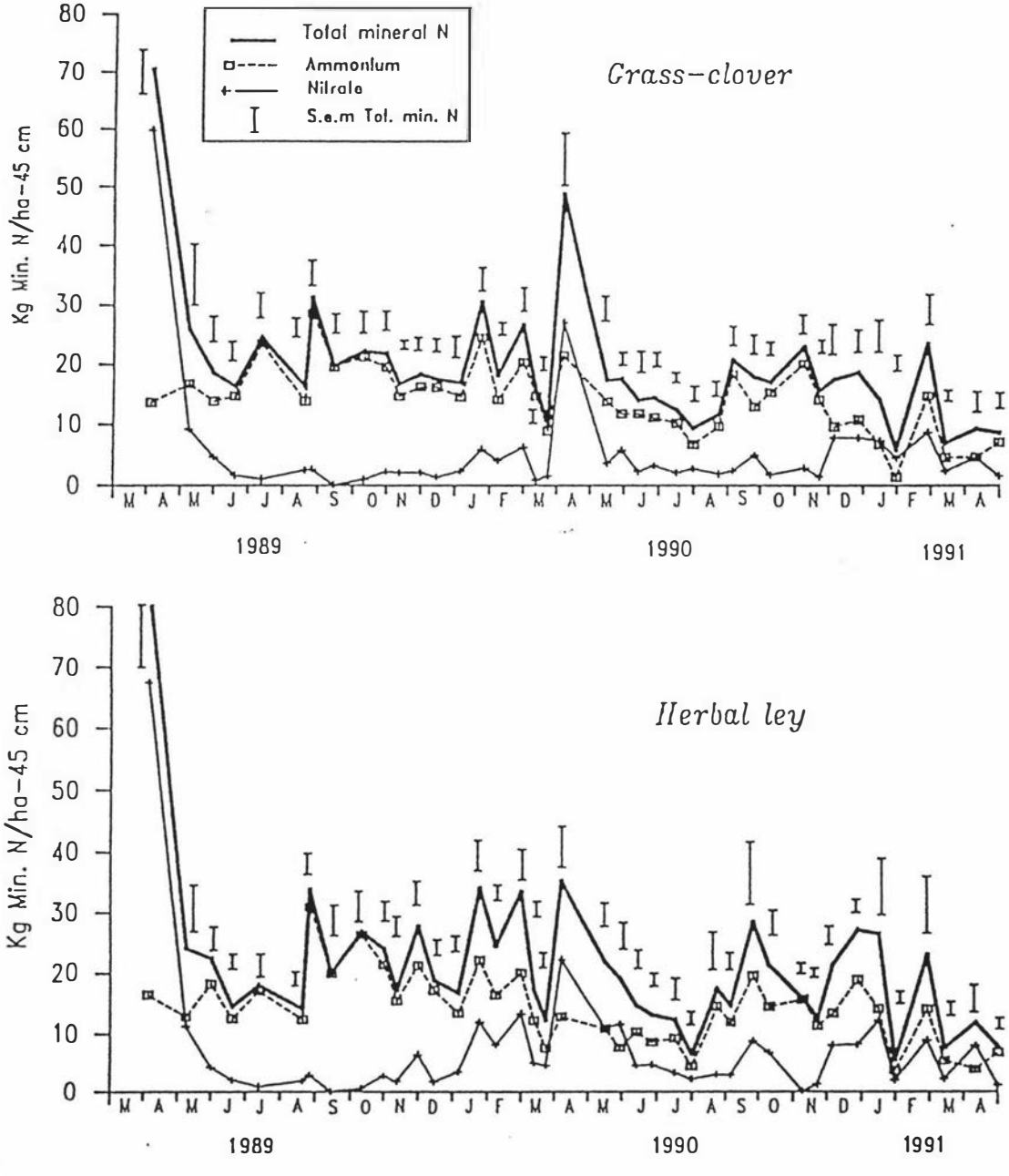

Figure 4.5 Forms of soil mineral N measured throughout the two year study in the legume–based pastures

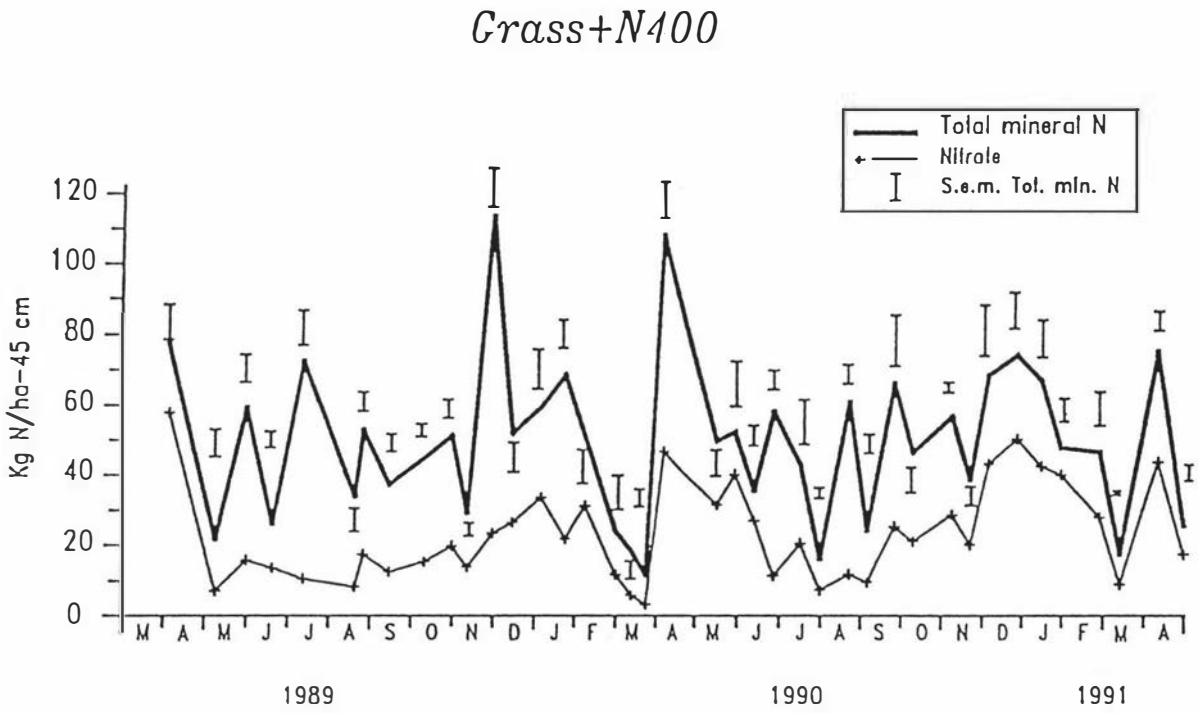

Figure 4.6 Forms of soil mineral N measured throughout the two year study in the pure grass sward receiving fertiliser N

Soil nitrate levels in Grass-clover and Herbal ley pastures were generally low. With few exceptions, nitrate was below 10 kg/ha during the study period. Grass-clover increased its nitrate in autumn up to 30 kg NO_3-N/ha for only a short period, while Herbal ley showed this tendency but extended it for a longer period through summer-autumn. The difference may be explained by the more active deep-rooting systems of some herb species (Chicory principally) in summer, that could stimulate nitrification possibly through the improvement of some physical and biological properties associated with the rhizosphere, as has recently been reported by Wheatley *et al.* (1990).

The enhanced nitrate accumulation observed in early April in Grass-clover and Herbal ley coincided with a rapid increment of total mineral N in response to the recovery of soil moisture content (Fig. 3.4, Chapter 3) and a relatively low rate of pasture growth in this period (Fig. 3.2, Chapter 3).

The low level of nitrate in the systems receiving no fertiliser N may be due to a combined effect of a relatively low rate of nitrification as a result of the limited availability of ammonium substrate, and a reasonably constant rate of disappearance through plant uptake, microbial immobilisation and losses. All of these can occur simultaneously but with different emphasis according to the climatic conditions during the year.

The level of nitrate in Grass+N400 (Fig. 4.6) was higher than in the other two pastures, varying between 10 and 45 kg NO_3-N/ha approximately. The highest values were also in summer, and in general there was a more dynamic pattern throughout the study period, with changes in nitrate closely associated with the changes in total mineral N.

The application of urea fertiliser and possibly the higher return of N in the urine of animals grazing pastures receiving fertiliser N (Jarvis *et al.*, 1989a) resulted in a considerable supply of ammonium substrate for nitrification. Hence in Grass+N400, not only was the absolute amount of nitrate larger than

in Grass-clover and Herbal ley, but also the ratio NH_4:NO_3 was considerably lower.

The nitrate accumulated over summer gradually declined from mid autumn until the end of winter, then began to accumulate again. The decline in winter may be the result of a lower rate of nitrification, determined by low temperature, combined with losses through leaching and denitrification. Further analysis of losses by the latter processes will be considered in Chapter 5 and 6, respectively.

Since the accumulation of nitrate is a prerequisite for leaching and denitrification, and in winter nitrification is reduced to a certain extent by temperature (Macduff and White, 1985), the dynamics of this process in summer-autumn, and the amount accumulated at the beginning of winter, are crucial in determining the potential risk of NO_3-N losses. Obviously other important processes like plant uptake can significantly moderate this adverse effect.

4.3.2.3 Distribution of mineral N in soil profiles

The mineral N distribution in soil profiles of a representative sampling each season for Grass-clover, Herbal ley and Grass+N400 is shown in Fig. 4.7. Mineral N was present in all soil layers of the three pastures, although to a much smaller extent below 15 cm.

In winter about 50% of total mineral N was in the top 15 cm for Grass-clover and Herbal ley. The same proportion but with larger values was observed in summer. Deeper layers accounted for the other half of mineral N, with about 20-30% of the total mineral N in the 15-30 and 30-45 cm depths, respectively. In spring, Herbal Ley maintained the same pattern, but Grass-clover showed only traces of mineral N below 15 cm depth. Ammonium always occurred in a larger proportion than nitrate.

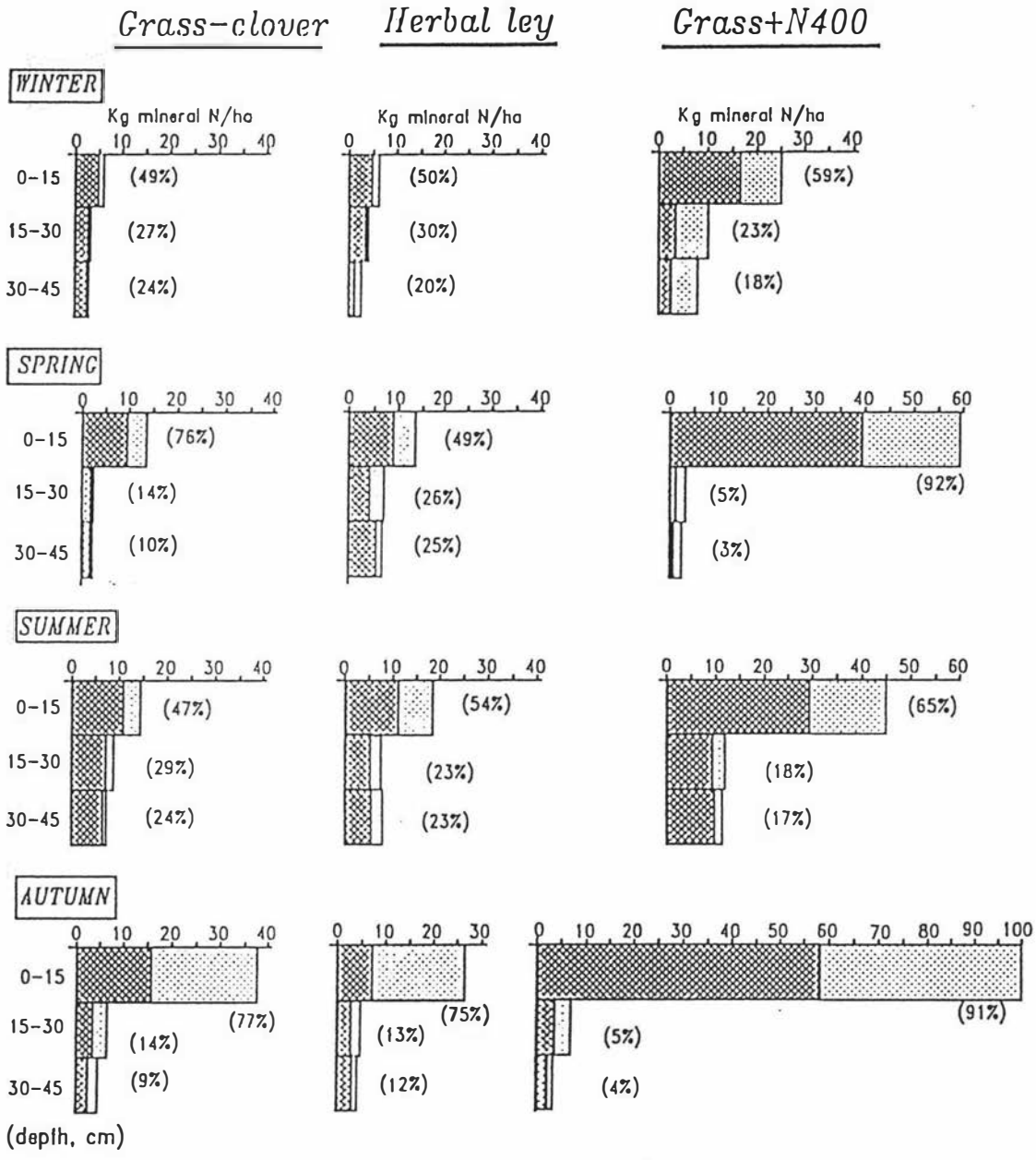

Figure 4.7 Representative profiles for the soil mineral N observed during each season

Ammonium Nitrate

(Figures in parentheses are % of the total soil mineral N in the profile)

In autumn it was different. Both Grass-clover and Herbal ley accumulated more mineral N in the top 15 cm which represented about 76% of total mineral N for the profile. Of this amount, the larger proportion was in the form of nitrate rather than ammonium.

Grass+N400 invariably had more mineral N than the other two pastures in the top layer, and this amount represented a much larger proportion of the total mineral N in the soil profile. Extreme cases were in spring and autumn, which reflected recent application of N fertiliser. Nitrate was higher than ammonium only in autumn, as in the other two pastures.

It is apparent from these results that the effect of N fertiliser was only concentrated in only the top 15 cm. Below 15 cm depth the distribution of mineral N followed a similar pattern, both in amount and forms of mineral N, to Grass-clover, especially in spring and autumn.

With the exception of Grass+N400 in winter, it seems that mineral N present below 15 cm in the sampled profile is a product of mineralisation of organic N rather than movement of N from the top layer; the reason for this assumption is that ammonium was the main form of mineral N. In summer, Grass-clover and Herbal ley data show that in the lower layers mineralisation was an active process; in spite of the amount of mineral N present at depth being small, it constitutes a significant proportion of total mineral N measured at any one time in the whole profile.

Mineralisation of N in deeper layers has not been studied in such detail as in the topsoil. However, one of the few studies (Hadas *et al.*, 1986) found that net mineralisation in deep layers (60 to 120 cm) could contribute up to about 30% of the N mineralised in the whole profile. The same authors reported a good correlation between N potentially mineralisable and total N weighted with respect to the soil layer. In this regard, soil mineral N at depth shown in Fig. 4.7 seems well related to the total N measured in each layer (Table 4.3).

Although this observation cannot be conclusive, it is an indication that the pattern of mineral N at depth followed some related soil characteristic; more obviously in the Grass-clover and Herbal ley soils; less so for Grass+N where the fertiliser N influences the mineral N in the top soil more than in the subsoil.

Table 4.3 Soil total N at different depths in three grassland systems sampled in February 1990 (mean ±SD)

Depth (cm)	Grass-clover	Herbal ley	Grass+N400
	Kg N/ha		
0-15	2932 ±183 (55%)	3088 ±331 (52%)	2960 ±246 (53%)
15-30	1365 ±195 (26%)	1560 ±195 (26%)	1501 ±113 (26%)
30-45	1012 ±202 (19%)	1275 ±421 (22%)	1215 ±537 (21%)

(Figures in parentheses are the percentage contribution to the total soil N in the profile 0-45 cm depth)

The lack of accumulation of mineral N in lower layers of the Grass+N400 pasture may be indicative that under the conditions of this experiment, the probability of significant leaching of nitrate was small, despite the heavy rate of fertiliser N applied. Alternatively, this feature indicates the possibility that N input by fertiliser could follow other pathways in the complex N cycle.

4.3.3 Detailed measurements

4.3.3.1 Field measurements (autumn)

The level of mineral N to a depth of 15 cm before grazing was 6.3, 5.7 and 5.5 kg N/ha in the Grass-clover, Herbal ley and Grass+N400, respectively (grazing started at time 0, 23 March 1990, Fig. 4.8). In the Grass+N400, fertilisation had been discontinued the previous January (because of poor

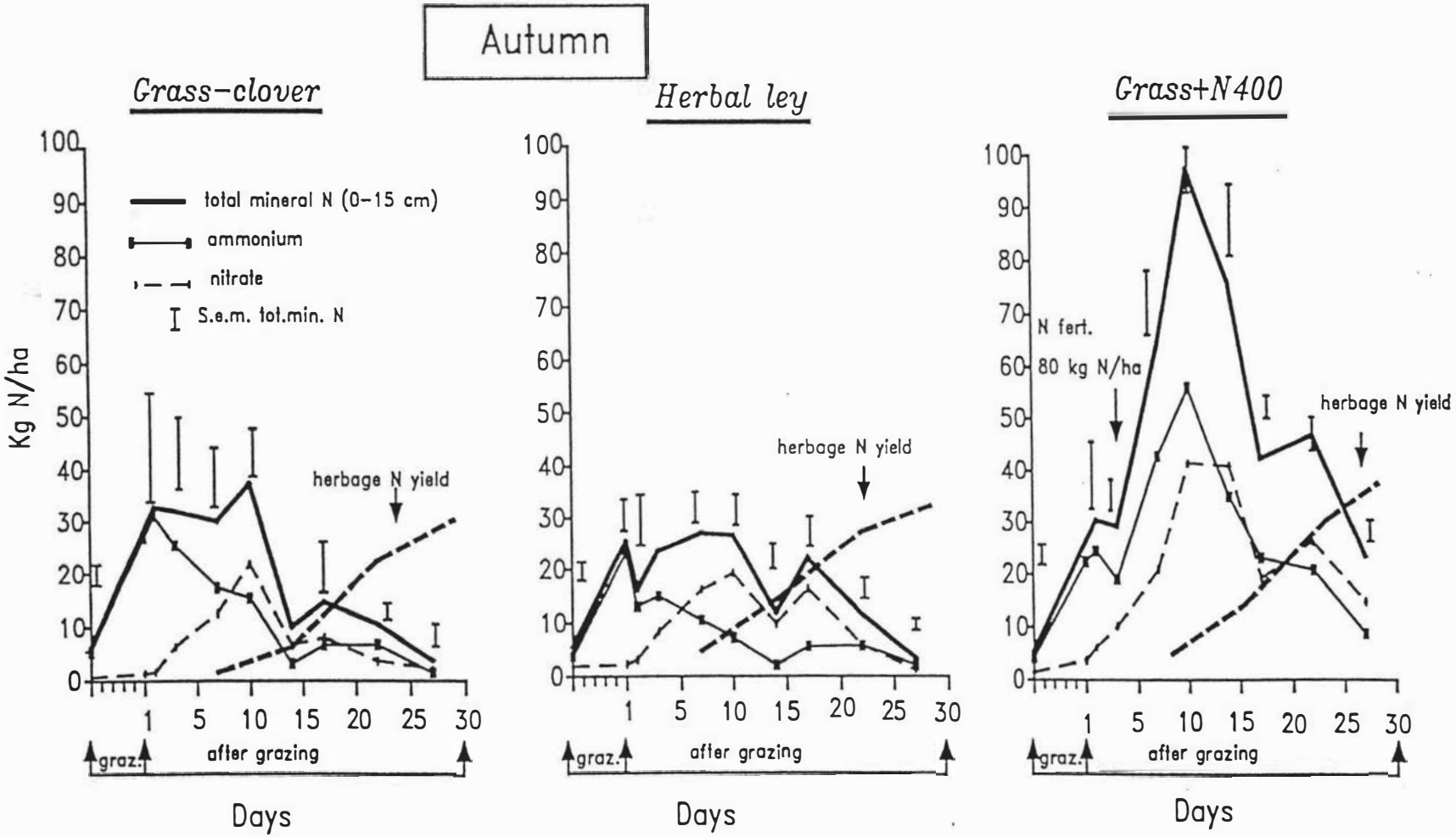

Figure 4.8 Dynamics of mineral N in soil, and yield of N by plants, during regrowth of pasture

responses in summer), after application of 400 kg N/ha during the previous year.

After 4 days of intensive grazing by sheep (up to 400 sheep/ha) soil mineral N in the three systems rose from about 5 to about 25-28 kg/ha -15 cm (measured one day after grazing ceased). Since the sampling was random these values should represent the average of soil mineral N in areas unaffected and affected by returns of animal excreta. The results indicate that much of the organic N held in the herbage mass before grazing was returned to the soil, where it was recoverable despite the possibility of concurrent losses by volatilisation of ammonia (Ball and Ryden, 1984; Jarvis *et al.*, 1989a, b). The effect of excreta of grazing animals in the soil after grazing is the most likely pathway for this transformation; but decomposition of herbage residues, herbage tissue damage or reduced uptake of N by the recently-defoliated sward could all be contributing to some extent. The net increment of soil mineral N immediately after grazing represented 51%, 39% and 68% of the amount of N in the herbage before grazing, for Grass-clover, Herbal ley and Grass+N400 respectively.

During the next 3 or 4 days after grazing the nitrification rate increased markedly and nitrate accumulated mainly because of limited demand for N for incorporation into other pools.

Nitrogen fertiliser (80 kg N/ha as urea) applied 4 days after the completion of grazing to the grass+N400 treatment (Fig. 4.8) brought soil mineral N from 29 kg/ha -15 cm (measured on day 4) to 98 kg/ha -15 cm at day 10. This increase represents 86% of the fertiliser N applied.

From 10 days after grazing soil total mineral N decreased in the three systems, most obviously as a result of vigorous plant growth and associated uptake of N by plants (Fig. 4.8). The N yield is an average value from plants growing in areas unaffected and affected by excretal return; detailed

comparisons of soil and plant behaviour in both types of conditions have been reported in Ball and Ryden (1984), Thomas *et al.* (1990) and Whitehead and Bristow (1990). At this period of the year symbiotic N fixation was minimal (see Fig. 3.5 in Chapter 3) so that soil available N was the main source for plant uptake. As plant growth proceeded, ammonium and nitrate were depleted more quickly, and at the end of the regrowth period the amount of mineral N in topsoil was similar to that present at the end of the previous regrowth period. However, other factors such as ammonia volatilisation, denitrification and immobilisation of N by soil microorganisms could also be responsible for some portion of the decline in mineral N in soil through the regrowth period.

4.3.3.2 Net mineralisation in tubes (autumn)

Results from the field incubation in autumn, with tubes embedded into the soil, appear in Fig. 4.9 and Table 4.4, and some data for soil and climatic conditions during the period of this study appear in Table 4.5. Without the effects of plant uptake and leaching, net mineralisation at the end of the 5 weeks' incubation resulted in the accumulation of 18.3 and 17.5 kg mineral N/ha-15 cm in Grass-clover and Herbal ley, respectively. The daily rate of mineralisation was about 1 kg/ha/day for the period of 5 weeks. But the results also indicate (Table 4.4) that a higher rate of mineralisation occurred between weeks 1 and 2 after grazing, when the rate was about 3.2 kg/ha/day for both Grass-clover and Herbal ley. The smaller amount of soil mineral N during the first week (Grass-clover and Herbal ley), in relation to that measured in the sward, could have been due to an enhanced microbial immobilisation inside the tubes, stimulated by the fresh carbonaceous materials originating from the roots of plants that were defoliated during the installation of tubes. However, such an explanation is only one possibility.

The results from the system receiving fertiliser N (Fig. 4.9 and Table 4.4) suggest that some net immobilisation of N may have occurred, because 80

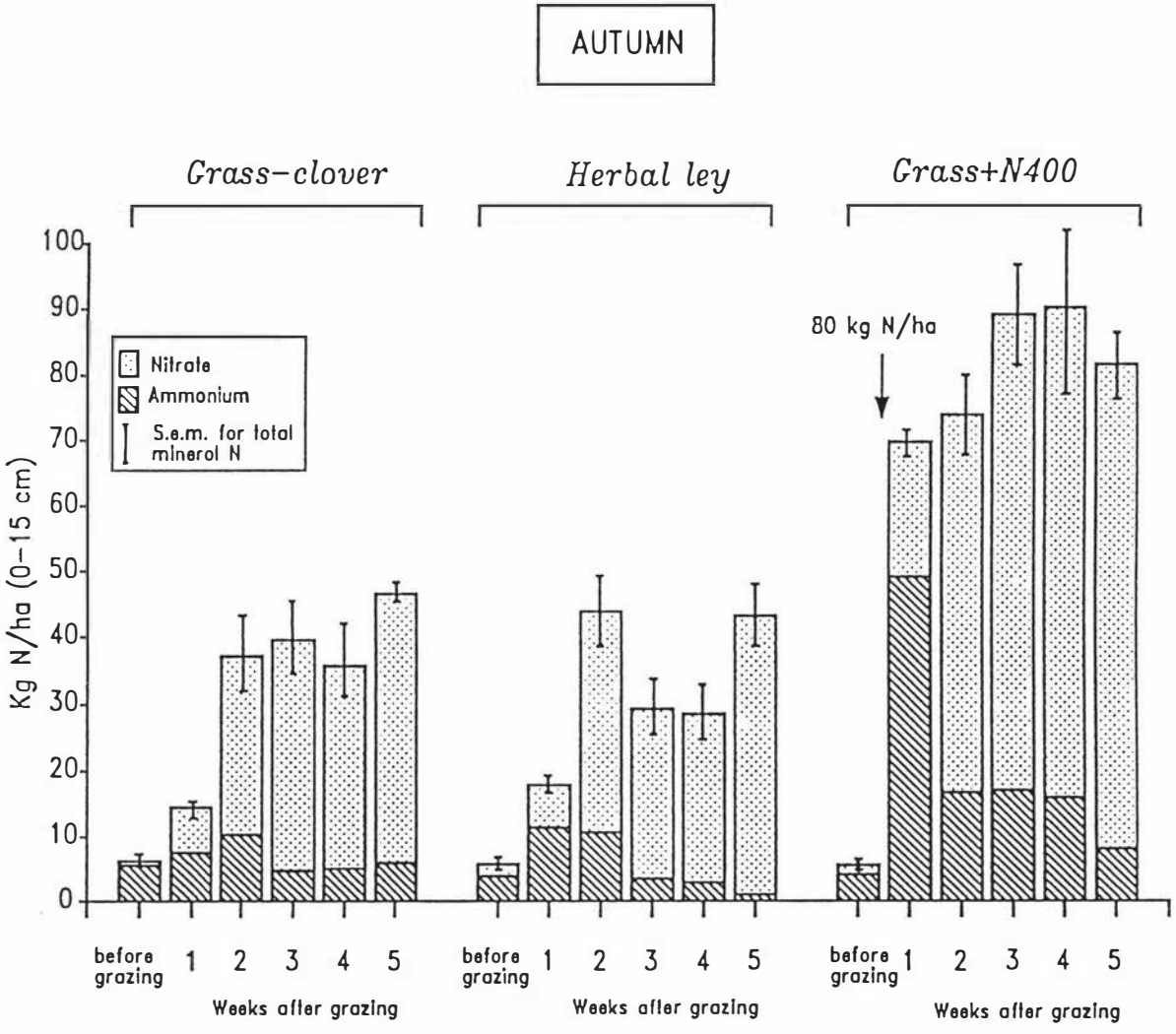

Figure 4.9 Recovery of mineral N from undisturbed soil cores contained in PVC tubes (15 cm deep) during the mineralisation study in autumn conditions

Table 4.4 Total soil mineral N for each week of field incubation under contrasting seasonal conditions

	Grass-clover	Herbal ley	Grass+N400
Sampling	Mineral N (Kg/ha-15 cm depth)		
	AUTUMN		
before grazing	6.3 a	5.7 a	5.5 a
after grazing	28.3 a	25.5 a	26.1 a
Week 1	14.6 a	17.9 a	69.7 b
Week 2	37.1 a	43.8 a	73.9 b
Week 3	39.5 a	29.3 a	89.1 b
Week 4	35.7 a	28.4 a	90.2 b
Week 5	46.6 a	43.1 a	81.6 b
	SPRING		
Before grazing	10.5 a	7.9 a	14.6 a
After grazing	21.9 a	27.6 a	31.0 a
Week 1	9.1 a	24.1 b	40.6 b
Week 2	64.3 a	98.7 a	80.7 a
Week 3	34.5 a	21.2 a	97.5 b
Week 4	53.6 a	28.3 b	61.5 a
Week 5	42.5 a	43.6 b	94.1 a

Values within rows followed by the same letter do not differ significantly at $P \leq 0.05$

Table 4.5 Soil and climatic conditions during incubation studies in the field: autumn and spring 1990

	AUTUMN 26 March - 29 April	SPRING 17 Sept. - 22 October
Rainfall (mm)	61.0	57.3
Pan evaporation (mm)	82.8	101.3
Soil temperature (C) daily mean 10 cm depth		
Week 1	16.5	12.0
Week 2	15.9	12.6
Week 3	15.9	13.2
Week 4	14.1	14.2
Week 5	15.3	15.8
Gravimetric soil moisture in incubation tubes (%)		
Start of incubation	21.8	22.1
End of incubation	21.6	22.0

kg/ha of fertiliser N was applied after grazing. In this treatment there is excellent concurrence between results obtained in the sward (Fig. 4.8) and from the tubes (Fig. 4.9) one week after grazing both in terms of total mineral N (70 kg N/ha) and NH_4-N:NO_3-N. However, in view of the 30 kg mineral N/ha -15 cm observed in the sward prior to application of the fertiliser, much of the added N remained unaccounted-for.

Nitrification became an active process in this field incubation, both with and without fertiliser N, so ammonium must have been quite freely available to nitrifiers. Because nitrifiers are known to be poor competitors for ammonium (Fisk and Fahey, 1990), we can infer that microbial immobilisation was low in this experiment (soil with low C:N ratio), but this provides no lead as to what happened to the "missing" N in the Grass+N400 system.

Nitrification occurred quickly under the prevailing environmental conditions. Most of the ammonium was oxidised to nitrate during the first two weeks of incubation. At the end of the incubation more than 80% of the mineral N was in nitrate form, both with and without fertiliser N.

4.3.3.3 Field measurements (spring)

In spring, the recovery of mineral N measured immediately after grazing was similar to that recorded in autumn for Herbal ley and Grass+N400 pastures (Figs 4.8 and 4.10). However, Grass-clover increased its mineral N pool by only 12 kg/ha by the effect of grazing, representing about 50% of the increased mineral N observed in autumn. This difference may be explained by the lower herbage yield at this grazing than in autumn, therefore less N was cycled through the grazing animals.

As in autumn, mineral N measured immediately after grazing comprised almost entirely ammonium in the three systems (Fig. 4.10). This result supports the hypothesis that most of the increased mineral N (average)

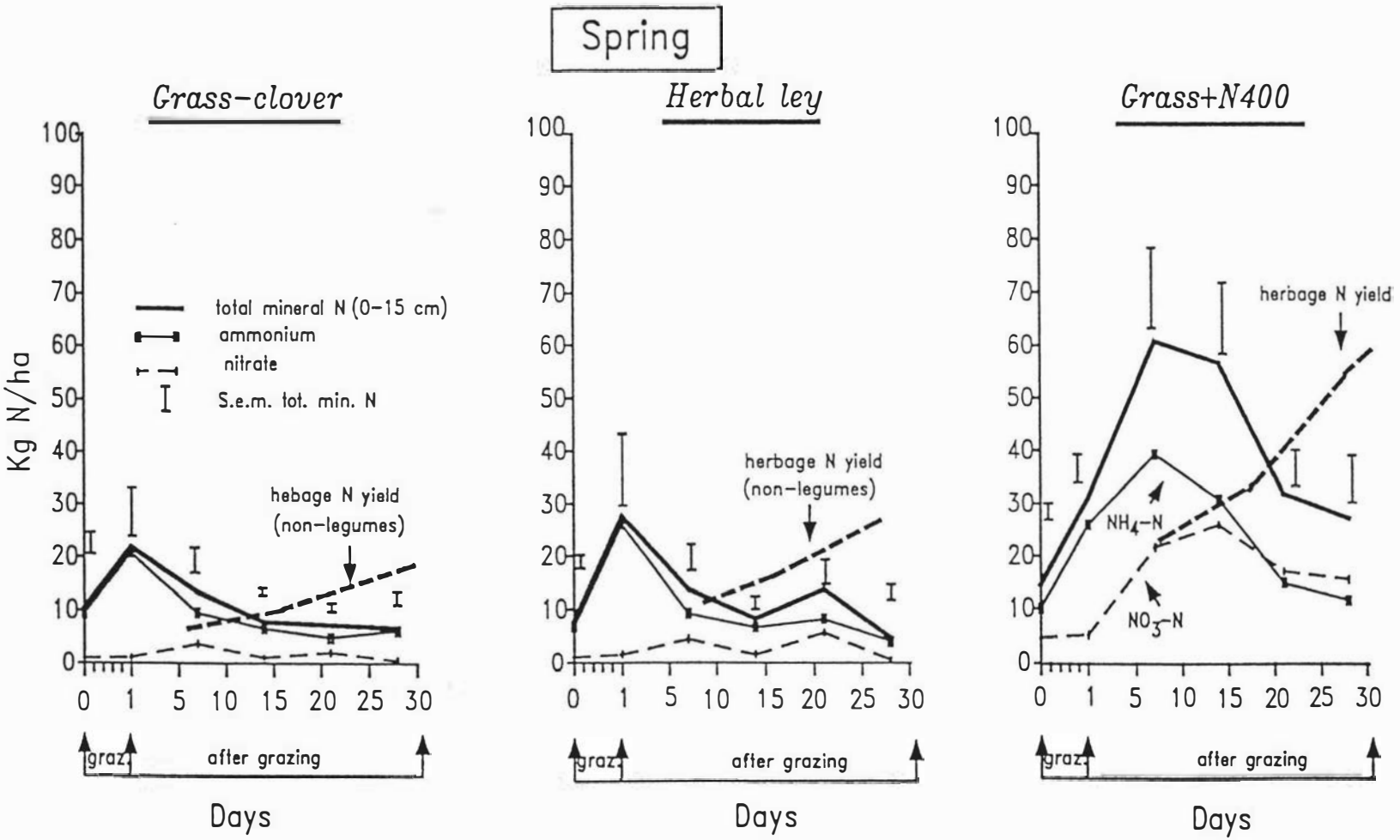

Figure 4.10 Dynamics of mineral N in soil, and yield of N by plants, during regrowth of pasture

measured in soil was derived from the urine of grazing animals.

The study in spring showed that the decline in mineral N in the systems receiving no fertiliser N was faster than in autumn, probably in response to a more rapid plant growth and hence uptake of N. In Fig. 4.10 it appears that the increased mineral N after grazing was sustained for only a few days. For instance, by the sampling one week after grazing the level of mineral N dropped to a similar level to that measured at the beginning of this cycle. Nitrification was also more limited in spring, most probably due to a limitation of the substrate ammonium.

In spring, plant growth was faster than in autumn. But of the total N yield only a fraction is accounted for by soil available N, because in this period there was a considerable input of N from symbiotic fixation by the legume components (0.6-0.8 kg N/ha/day). Hence the significant difference in the figure for herbage N yield in autumn and spring both for Grass-clover and Herbal ley. But when N yield of non-leguminous components (Fig. 4.10) was considered, the changes in soil mineral N are approximately in balance with the uptake of N by the non-legume components of the sward.

Grass+N400 increased mineral N from 34 (measured immediately after grazing) to 60 kg N/ha (measured one week later) following the application of 40 kg/ha of fertiliser N (urea). This increase represents 65% of the fertiliser N. In contrast with Grass-clover and Herbal ley, nitrification and accumulation of nitrate was much more important in this treatment. This result confirms the importance of substrate in the nitrification process, and accumulation of nitrate in soil.

By the end of the regrowth period, mineral N had declined dramatically to a similar level to that measured before grazing. In this case, where the pure grass sward was dependent on soil available N, plant N uptake (60 kg/ha, Fig.4.10) equated reasonably well with the 40 kg of fertiliser N plus 25 kg

increase in mineral N as a result of grazing. In contrast, under autumn conditions plant N uptake and residual mineral N at the end of the regrowth period accounted for about 55% of fertiliser N applied.

4.3.3.4 Net mineralisation of N in tubes (spring)

Net mineralisation of N measured in embedded tubes during a study in spring appears in Fig. 4.11 and Table 4.4. During the first week there was evidence of N immobilisation, probably due to the same reason as in autumn. Net mineralisation measured by the end of the incubation period (difference between week 5 and N measured after grazing, Table 4.4) was 20, 16.0 and 23.1 kg N/ha for Grass-clover, Herbal ley and Grass+N400 respectively.

In general, the results in spring were more variable than those observed in autumn (within sample variability, and probably more variation in the dynamic processes of mineralisation-immobilisation), hence it may be difficult to make the correct interpretation of the abrupt changes between one week and another. However, in the systems without fertiliser N it appears that the net mineralisation of N was enhanced between week 1 and 2 (8-18 kg N/ha/day) as it was to a lesser extent in autumn (3-3.6 kg N/ha/day, Table 4.4).

In spring, nitrification was again an important process, but not as intense as it was in autumn. There is no a clear explanation for this difference, which was also apparent in the field measurement (Fig. 4.10). Soil moisture content was similar during both periods of incubation (Table 4.5), but the lower soil temperature in spring during the first three weeks of study could be one factor influencing the delay of nitrification.

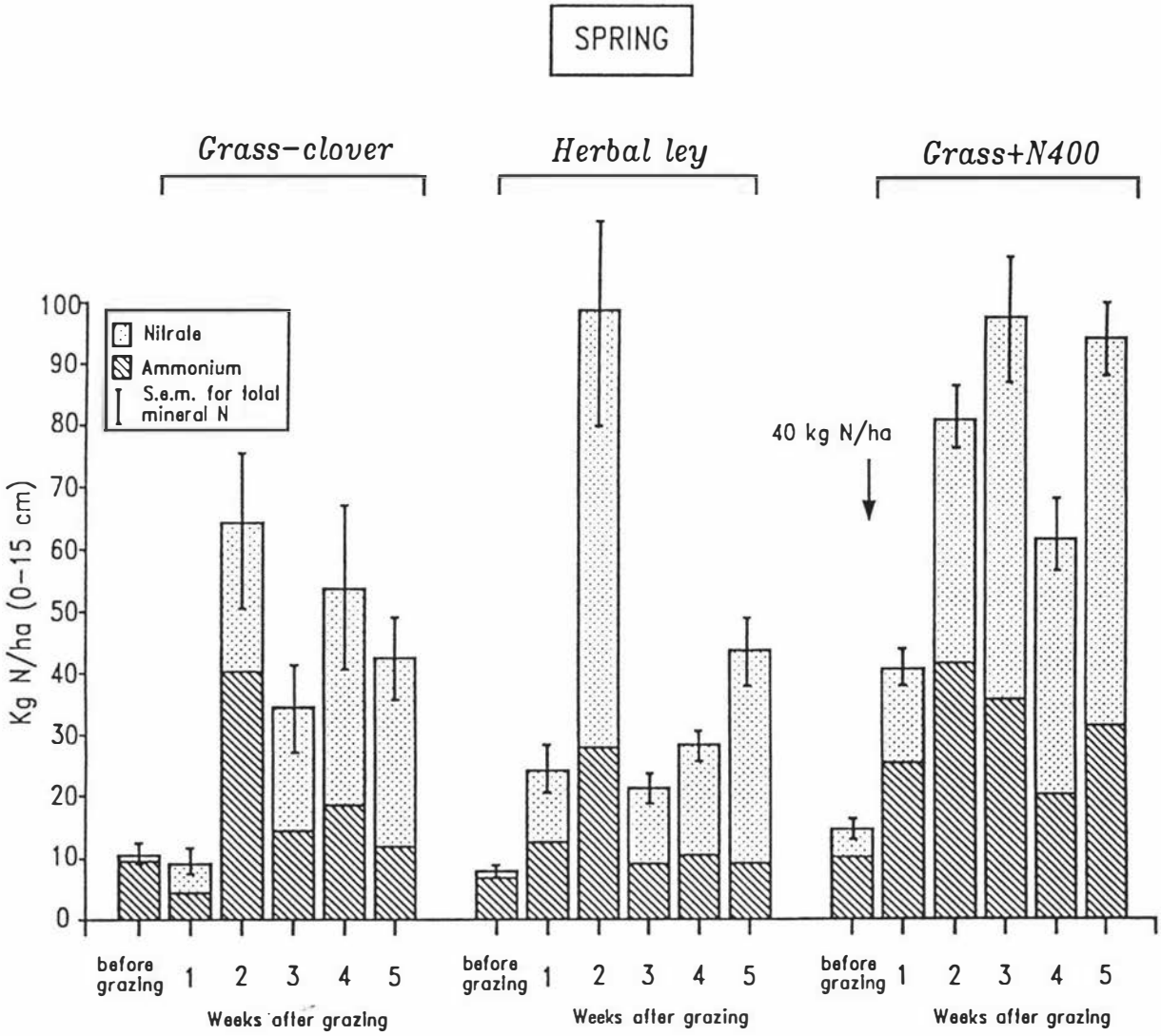

Figure 4.11 Recovery of mineral N from undisturbed soil cores contained in PVC tubes (15 cm deep) during the mineralisation study in spring conditions

4.3.3.5 Spatial variability in mineral N

The study and understanding of mineral N in pasture soils is bedevilled by spatial variability, stemming from the aggregation of excess dietary N into urine patches and dung pats by grazing ruminants (Thompson and Coup, 1940a, b). To illustrate this problem, mineral N data from the 60 individual cores (from the PVC tubes incubated immediately after grazing during the regrowth period of Grass-clover pasture in spring) are presented in a graph of frequency distribution (Fig. 4.12). The result shows a markedly skewed distribution of values for mineral N. Higher extreme values could presumably represent the soil samples affected by the aggregation of N in animal excreta, principally urine. To examine this possibility, the data were separated in two populations, which were statistically tested to be different at $P \leq 0.01$. In addition, the probability of finding a value smaller than the lowest class value (135) from the bigger population (black in Fig. 4.12) was 8.3%.

Although the results obtained by this procedure cannot be conclusive, since they are based on only one sample data set, they are at least an indication of the possible proportion of the samples (and also the area) that could be affected by animal excreta and the magnitude (kg mineral N/ha-15 cm) that this influence could have in a bulked soil sample. Under the conditions of this experiment, 10% of soil cores were displaying a possible effect of urine from an ostensibly uniform grazed sward. The arithmetic mean mineral N for 10% of the area was 223.1 kg N/ha-15 cm and for the rest of the soil (90% of the area) was 20.5 kg N/ha-15 cm.

Repeating this same exercise with the results from the Herbal ley and Grass+N400, the area presumably affected by animal excreta during a grazing period was also about 10% - the mineral N (kg N/ha-15 cm) was 211.1 (10% area) and 24.5 (90% area) for the Herbal ley, and 183.6 (10% area) and 62.8 (90% area) for Grass+N400. Using all the information obtained from the field incubation study both in autumn and spring, the area presumably affected by

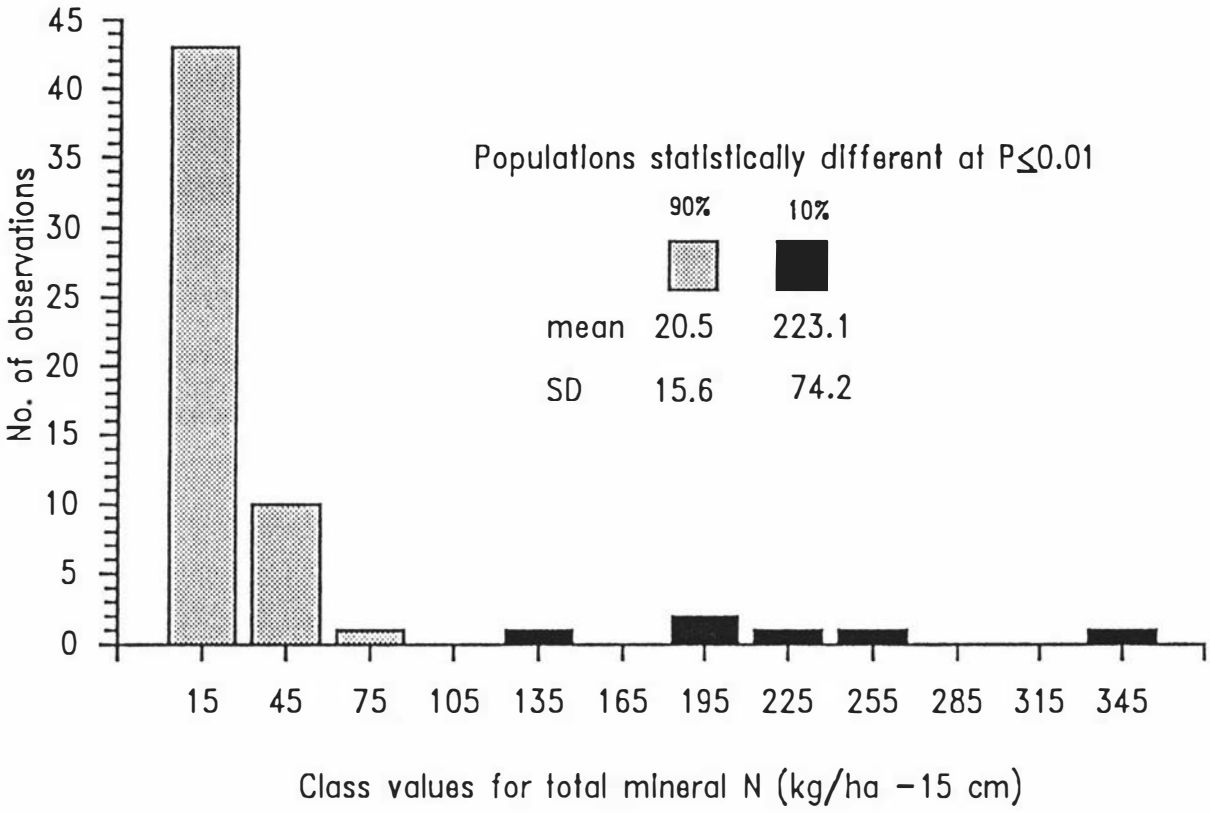

Figure 4.12 Frequency distribution for class values of total mineral N observed in enclosed soil samples in grass–clover pasture in spring (n=60)

animal excreta during a grazing period under the management condition of this experiment ranged between 6 and 14%. Similar estimates were obtained by applying the calculation of the area covered in one urination and the number of urinations per day indicated by Jackman (1960) in a similar environment to that of the present study. More recently, Thomas *et al.* (1990) reported that at any one time about 11% of a plot area (1500 m^2) was affected by the excreta of sheep in a comparative study with and without excretal return.

The data displayed in Fig 4.12 can be used to illustrate further the problem of spatial variability in the study of N in pastures. There are various way of representing the average value of mineral N in soils exhibiting pronounced spatial variability of mineral N and skewed frequency distributions for sample populations (all values in kg mineral N/ha-15 cm):

i) Use the simple arithmetic mean i.e.
$\sum x/n = 40.7$
where x is a single measured value and n is the number in each sample.

ii) Log transform the data to approximately normalise the sample distribution and calculate the best estimate of the arithmetic mean of the population from which the sample was drawn (by the maximum likelihood method) i.e.
$\chi_e = \exp(\xi + V/2) = 43.1$
(White *et al.*, 1987, where ξ and V are the arithmetic mean and variance of natural logarithms of x).

iii) Estimate the median value of the sample population, e.g. the geometric mean. White *et al.* (1987) found that the geometric mean consistently underestimated the arithmetic mean for highly skewed data sets which was also true for these observations i.e.
$\chi_g = \exp(\xi) = 18.4$

But if skewness is due to a relatively small proportion of outlying values e.g. 10% or less, then χ_g might be a reasonable estimate of true mean of the bulk of the values in the sample population (e.g. 90%).

iv) Postulate that there are really two sample populations, one urine affected, the other non-affected in the approximate proportion of 9:1, in which case the individual arithmetic means were 223.1 and 20.5. Note of course that the calculation of a weighted arithmetic mean for the whole population is be given by
0.1 x 223.1 + 0.9 x 20.5 = 40.7
which must be the same as the simple arithmetic mean.

A single analysis of a bulked sample of cores simulates the arithmetic mean calculated from individual cores and is therefore a weighted mean. In this regard, this form of analysis is useful for budget studies, since it is the best representation of the absolute amount of available N in a certain volume of soil.

On the other hand, if the environmentalist is more interested in the urine-affected areas, from which the bulk of the N escapes to the wider environment (Ball and Ryden, 1984), it might be better to concentrate on the outlying values arising from recent urine patches. Considerations like these would point to the need for compartmentalised research to understand better the N dynamics in those parts of the sward affected by recent urination.

4.4 Conclusions

The Grass-clover and Herbal ley pastures followed a similar pattern of change in soil mineral N during the study period. Both systems were different from Grass+N400 pasture which exhibited the highest values of soil mineral N.

The pure grass sward, being dependent on soil available N, required a large

amount of residual mineral N to be able to take up N at an appropriate rate to achieve maximum yield. This high level of mineral N was maintained with only periodic applications of fertiliser N. As soon as fertiliser N was stopped, mineral N declined to similar values recorded in pastures receiving no fertiliser N. This aspect emphasises the dependency of pure grass swards on N fertilisation.

Ammonium was the predominant form of mineral N. Nitrate was present at low levels during most of this study in the legume-based pastures, especially in the Grass-clover system. Nitrate accumulation was recorded for only a few weeks in autumn, a period in which mineralisation of soil N was stimulated by the recovery of soil moisture content, and when plant uptake of N was moderated by the declining pasture growth rate in this period of the year.

In the Grass+N400 system, nitrate was present at higher levels and for longer periods than it was in the two legume-based pastures. This difference was presumably due to a better availability of ammonium as a substrate for nitrification.

Nitrification in summer-autumn and the accumulation of nitrate by the beginning of the drainage season are crucial in determining the potential risk for nitrate losses. The detailed studies indicated that the flux of nitrate could also be stimulated by the "pulse" of ammonium detected immediately after grazing. For instance, the extent of leaching of nitrate from the topsoil will depend on exactly when a drainage event might occur in relation to the sequence of transformations: urea-ammonium-nitrate-organic N, that is the characteristic of the grazing-regrowth cycle of pastures.

Although immobilisation of N is a microbial process, this experiment indicates that most of the mineral N in soil (especially in systems receiving no fertiliser N) was apparently converted into a organic form through uptake by plants rather than microbial immobilisation, and then transformed, in part, back to

mineral N through the grazing animals.

Since nitrate formation is minimised by maximising the residence time of N in a plant (organic) form, different management options (set stocking, rotational grazing, frequency and intensity of defoliation, etc) may have important influences, not only on pasture utilisation and production, but also on the management of mineral N in the soil-plant-animal system.

In general, mineral N accumulated in the top 15 cm of the three grassland systems. But this was much more pronounced in Grass+N400, apparently as a result of fertiliser N application.

The presence of mineral N below 15 cm, especially as ammonium, suggests that mineralisation of organic N (rather than the movement of N) was the most probable determinant of the level of mineral N measured down the profile.

Use of tubes embedded in soil and incubated in the field provided some additional, useful perspectives. The mineral N regime in the soil was obviously dominated by a "pulse" input, observable soon after grazing, mainly as ammonium. The particular grazing management of this experiment focuses attention on the importance of the urine of grazing ruminants as a N substrate for pasture regrowth in the absence of fertiliser N. There was only limited evidence for effective net mineralisation of soil N throughout the period of regrowth.

The analysis of individual cores showed that approximately 10% of the samples (by area) for mineral N displayed extremely high values attributable to the uneven aggregation of N through the excreta of grazing animals. It was statistically detected that the extreme values could represent a different population from that of the main body of the soil. The large concentration of N in urine patches influenced the results of mineral N in such a way that any sort of single average could probably represent only an approximation to the

reality that occurs in the field. Moreover, bulking and mixing the soil from the subsamples provides a "weighted average", which in a strict sense is the best representation of the concentration of N in the "body" of the soil (an important consideration in budget studies). But this "body" may contain individuals from different populations, highly contrasting (variable) in the element that it is intended to measure to characterise a field plot. Compartmentalised research is suggested to understand better the N dynamics in those areas of the sward either affected or unaffected by recent excretion.

CHAPTER 5
LEACHING

5.1 Introduction

The term leaching describes the transport of chemicals in water soluble forms below a defined depth of soil. Here we are interested solely in the leaching of N which will be predominantly in the form of nitrate. In agricultural ecosystems the zone of leaching interest normally corresponds to the lower limit of the root zone. The rate of nitrate leaching depends of the volume rate of water flow across the bottom limit of the root zone and the concentration of nitrate in that water (White 1988).

The movement of nitrate below the root zone is of economic importance to agriculture because it represents a loss of this nutrient from the soil-plant system. But if that nitrate reaches aquifers that are used as a source of water supply for human consumption, the concentration of nitrate may eventually increase to a level unacceptable for human health. These health effects are reported to include methaemoglobinaemia, cancer, and possibly others (Follet and Walker, 1989). The public health standard for nitrate in drinking water has been set at 10 mg NO_3-N/litre in the USA, and 11.3 mg NO_3-N/litre by the World Health Organisation. This latter limit has been adopted in most European countries (Jury and Nielsen, 1989).

In recent years the increased level of nitrate in ground water has become a more important focus of concern than the simple economic effect for agriculture. However, modern agriculture is one of the activities that has been considered to have a large influence on the overload of the soil-water-plant system with N. This appears to have resulted in increased nitrate leaching and the contamination of water resources, principally in some regions of the USA (Follet and Walker, 1989) along with other developed countries in Europe (Juergens- Gschwind, 1989).

5.1.1 Mechanisms that govern the leaching of nitrate in the soil profile

The leaching of nitrate in soil is governed by the principles of solute movement which have been described and discussed by Wild and Cameron (1980), as well as Cameron and Haynes (1986) and Jury and Nielsen (1989). The understanding of these principles has served as the basis for the development of both methods for measurement and for predicting nitrate leaching in different agricultural ecosystems. However, it must be recognised that there still exists a number of difficulties in quantifying this complex process. Some of these concerns are outlined below.

Two mechanisms govern the movement of any non-reactive dissolved ion, such as nitrate, through soil during steady-state flow. Being non-reactive there is no interaction between the nitrate and the soil. The primary mechanism is often convection or mass flow of the ion with moving soil solution which itself moves in response to a hydraulic gradient in the soil water potential. Secondly there will be diffusion of the ion within the solution from areas of high concentration to areas of low concentration.

It has been observed in heterogeneous porous media where the water flow exhibits a range of pore water velocities, that the coupling of mass flow and solute diffusion leads to an apparent diffusion coefficient considerably greater than the molecular diffusion coefficient of the solute in bulk solution. Jury and Nielsen (1989) suggest that this effect arises because, although the convective flux of the solution can be treated as one dimensional steady state flow, on a microscopic soil pore scale, water can be moving in a more complex three-dimensional flow around the solid soil particles. Solute in this water is not only diffusing and being convected with the average water flux, but it is also being spread out as it moves with the water around the solid particles and this component of the convective flux is called "hydrodynamic dispersion".

5.1.2 Estimation of nitrate leaching under field conditions

In spite of the general principles of solute movement being reasonably well understood, it is still difficult to quantify nitrate leaching in the field , and even more difficult to predict it. According to White (1988) the measurement of the amount of water passing across a surface of interest and the nitrate concentration in that water presents conceptual and technical problems associated with the difficulty of access for sampling, and because of the spatial and temporal variability of both water flux and nitrate concentration. Because of these complications only a limited number of methods have been used to measure nitrate leaching losses, and all of them have limitations. Some of the methods most commonly used have been described, and their advantages and disadvantages discussed by Wild and Cameron (1980), Cameron and Haynes (1986), Cameron and Scotter (1987) and White (1988). Briefly, these methods can be grouped into the following categories:

Firstly there are lysimeter studies, where the measurements are limited to a defined and controlled volume of soil. The inputs and outputs of water and nitrate concentration can often be measured with a degree of precision. However lysimeters are expensive, and adequate replication is often not possible. Representivity is also a concern.

As well, there have been tile drainage and catchment studies. Samples of water taken from tile drainage can be used to calculate nitrogen losses from a defined area of soil. Catchment studies involve sampling and monitoring the total water drained from a confined catchment area. This can also provide an integrated measure of the leaching loss.

It is also possible to sample the soil solution using small cores which are extracted for the measurement of nitrate. Otherwise sampling the soil solution can be performed using porous cups. The nitrate concentration can be measured and when combined with a

measurement of water flux from a soil water balance, the flux of nitrate can be calculated.

In a similar manner, borehole sampling can be used to obtain deep cores from the unsaturated zone above the aquifer. This can be is used when the objective is to examine the profile distribution of nitrates, and can possibly be used to calculate the nitrate flux if some water flow information is available.

These methods all have characteristic limitations. It is important to consider these when intending to use them for a particular objective in a specific agricultural ecosystem. In this regard, Wild and Cameron (1980) concluded that as a general rule field measurements tend to be *ad hoc*, that is, they do not lead to generalisations on the rate or amount of leaching. Primarily this is due to the difficulty of accurately calculating the flux of water and ensuring the concentration of nitrate is that in water which is effectively moving.

5.1.3 Factors influencing leaching losses in grassland soils

Leaching is the result of the net water flux through the soil and the soil's nitrate content, and hence the nitrate concentration of the leaching water. Thus, a number of factors related to the climate (rainfall, evapotranspiration, temperature), soil properties (texture, structure, content of organic matter), fertiliser application (type, rate, form and time of application), type of land use and management conspire to interact in a complex way to determine the relative influence of the water drainage and the size of the nitrate pool. In general these factors apply to most agricultural ecosystems but with different impacts between situations. The enormous number of environments that can be created according to various combinations of the factors mentioned above is well illustrated in the reviews of Cameron and Scotter (1987) and White (1988). In this section attention will be focused on aspects of land use and management, and in particular their effect on leaching from pasture soils.

Leaching losses from extensively farmed pastoral soils are reported to be quite small (Kilmer 1974). The inferred reason for this is that under more extensive conditions, plant uptake of N leaves little mineral N in the soil profile (Huntjens, 1971a, b). Furthermore, nitrification is normally a limited process (Vitousek, 1979), so that nitrate available for leaching is minimal. In contrast, leaching losses can be relatively large on intensively managed pastures where high fertiliser N rates, and associated increases in DM yield and N content are combined with high stocking rates (Steele and Shannon, 1982; Ball and Ryden, 1984; Steele *et al.*, 1984; Parsons *et al.*, 1991). Nitrate leaching can also be increased by losses from unevenly distributed urine patches in which N is highly concentrated in space (O'Connor, 1974; Quin 1978; Ball *et al.*, 1979; Floate, 1981).

The N applied in urine (70 to 90% as urea) rapidly hydrolyses to ammonium carbonate which itself decomposes to release ammonium ions (White, 1988). The low C/N ratio in the urine-affected soil has been reported to retard microbial immobilisation of N (Carran *et al.,* 1982) and the concentration of mineral N in the urine patch might thus exceed the plant uptake capacity. Ammonium can be lost by volatilisation as ammonia, but subsequent nitrification of ammonium in the soil also produces a large amount of nitrate (Ball and Ryden, 1984) which by virtue of being an anion has the potential for leaching.

In recent years, since the deleterious environmental effects of intensive pastoral systems have been recognised, especially in some European countries, the focus of research has been concentrated on the search for, and definition of, sward systems that minimise nitrate leaching. This has led to an increased interest in legume-based pastures, because apart from the benefit of legumes in fixing N (Cowling, 1982) and on the quality of herbage (Thomson, 1984), there is evidence of a lower release of nitrogenous compounds to soil water and the atmosphere from grass-clover swards than from grass fertilised with N (Ryden and Garwood, 1984; Parsons *et al.*, 1991).

But as was recently discussed by Parsons *et al.* (1991), it remains to be shown whether grass-clover swards can be widely used under European conditions to establish a N cycle which has little adverse effect on the environment, whilst maintaining acceptable agricultural production.

In this study the objective was to estimate nitrate leaching from three different grassland systems. Losses are discussed in relation to the source of N, amount of external N input, pasture production, N yield and stocking rate.

5.2 Materials and Methods

The study of leaching of nitrate was carried out on three grassland systems, Grass-clover, Herbal ley and Grass+N400 (ryegrass sward receiving 400 kg N/ha/yr as urea). A detailed account of the experimental site, climate, establishment and management of the trial has been given in Chapter 2.

5.2.1 Water balance and nitrate in the soil solution

The nitrate leached was calculated as that contained in the water draining below 45 cm, using the concentration measured in the soil solution at 30-45 cm depth. More specifically, the cumulative drainage for every 10 days multiplied by the average concentration of nitrate in the soil solution gave the leaching loss for that period. It was assumed that drainage carried nitrate to below 45 cm principally by mass flow. The concentration of nitrate in the soil solution was measured during the period April 1989 to April 1991, at a frequency of two samplings per pasture regrowth period at approximately 10-12 days after and before each grazing. Determination of nitrate in the soil solution has been detailed in Chapter 4, section 4.2.2.

Drainage was estimated from a simple soil water balance for the top 45 cm of soil following the method described by Kerr and Clothier (1975), namely

$$D = R + I - E \pm \Delta S$$

where D is drainage, R is rainfall, I is irrigation, E is evapotranspiration and ΔS is the change in soil water storage.

E was calculated according to the procedure described by Field *et al.* (1985), based on Priestley and Taylor (1972). This required the mean daily temperature, whereas the incoming solar radiation was estimated from the bright sunshine hours by the method of de Lisle (1966). The weather data of rainfall, mean air temperature and sunshine hours were measured at a meteorological station (DSIR Grasslands) within 500 m of the experimental site. Irrigation was used occasionally during the establishment year of 1989 and was incorporated into the water balance accordingly.

The estimation of the soil water balance started each year on 1 April (before the drainage season) immediately after a measurement of soil moisture content in the top 45 cm. Drainage was assumed to occur when the soil moisture content of the top 450 mm exceeded 170 mm, the "field capacity" which was estimated from the information presented in Table 2.1.

Analysis of variance was used to test for treatment differences. As drainage was calculated as the average for the whole area, being the same for each plot, differences in N leached were due to the different concentrations of nitrate in the soil solution measured in each plot.

5.2.2 Nitrate in deep samples

In October 1990, when drainage below 45 cm had ceased, the soil below 45 cm was sampled with a Dutch auger (55 mm i.d.) in sections of 25 cm to a depth of 2 m. Two samples per plot in 3 replications (6 samples per pasture system) were taken and analysed for mineral N following the method described in section 4.2.2. Gravimetric soil moisture content was also determined for each sample.

The mean value for nitrate in the soil solution at different depths along with the

standard error of the mean (s.e.m.) was calculated. For the total mineral N (ammonium and nitrate) measured in the profile between 50-200 cm, an analysis of variance was used to test for treatment differences in the randomised block design. An additional comparison between Grass-clover and Herbal ley using a t-test was performed when these treatments were substantially smaller than the Grass+N400. Now the effect of this larger treatment in the pooled variance was removed, allowing for a more precise comparison between the treatments receiving no fertiliser N.

5.3 Results and Discussion

5.3.1 Soil water balance and drainage

The cumulative water balance was calculated every 10 days during 1989 and 1990 starting on 1 April each year. Soil moisture content, rainfall, evapotranspiration and the estimated drainage when water input exceeded 170 mm ("field capacity") in the top 45 cm are presented in Tables 5.1 and 5.2.

The drainage period commenced in May in both years. In 1989 irrigation, in parentheses in Table 5.1, contributed 24 mm to the soil moisture content before the first drainage event. Cumulative drainage in 1989 (Table 5.1) was 215 mm, an amount 55 mm smaller than in 1990 (Table 5.2), despite a contribution of an extra 57 mm from irrigation during 1989. The maximum rate of drainage occurred between 10-20 June in 1989 when 44 mm were recorded. In 1990 the largest event was measured between 9-19 August when 63 mm of rainfall coincided with an initially high soil moisture content. This resulted in 55 mm of drainage.

The period of drainage in 1990 was limited to 4 months between May and August. In the previous year (1989) it extended until October, but with long periods when drainage was not recorded. In general the drainage period

Table 5.1 Water balance for the 0-45 cm depth during the drainage season in 1989

Date	Soil water	Rain (irrig.)	Evapotransp.	Drainage
	(mm)	(mm)	(mm)	(mm)
1 April	117			
11 April	118	8 (12)	19	0
21 April	113	9	14	0
1 May	139	25 (12)	11	0
11 May	168	67	9	29
21 May	167	23	8	16
31 May	170	19	5	11
10 June	168	15	7	10
20 June	170	51	5	44
30 June	168	20	6	16
10 July	166	13	10	5
20 July	170	40	5	31
30 July	157	0	13	0
9 August	143	0	14	0
19 August	147	3 (12)	11	0
29 August	170	26 (12)	11	4
8 September	164	30	16	20
18 September	156	11	19	0
28 September	138	2	20	0
8 October	152	32	18	0
18 October	170	60	21	21
28 October	152	21	31	8
7 November	137	17	32	0
17 November	114	12	35	0
27 November	98	6	22	0
30 November	128	0 (36)	6	0
Total		594	368	215

(values in parentheses correspond to irrigation)

Table 5.2 Water balance for the 0-45 cm depth during the drainage season in 1990

Date	Soil water	Rainfall	Evapotransp.	Drainage
	(mm)	(mm)	(mm)	(mm)
1 April	100			
11 April	94	6	12	0
21 April	92	9	11	0
1 May	130	50	12	0
11 May	143	25	12	0
21 May	170	41	8	6
31 May	165	26	10	21
10 June	169	45	7	34
20 June	170	39	5	33
30 June	170	39	7	32
10 July	170	41	6	35
20 July	169	21	8	14
30 July	170	22	8	13
9 August	170	34	10	24
19 August	169	63	9	55
29 August	163	11	14	3
31 August	160	0	3	0
Total		472	142	270

during the two years followed a similar distribution to that observed for this soil in previous years (Field *et al.*,1985). However the amount recorded by these authors was larger than the values reported in the present study. Greater rainfall and a water balance calculated for only the top 30 cm in their study could in part account for the difference.

The method used in this study for the estimation of drainage assumed that water carried nitrate by piston flow through the top 45 cm depth. The possible effect of other phenomena, such as preferential flow of water through macropores (Scotter, 1978), might affect to some extent the validity of the leaching calculations. Some of the nitrate might be carried by preferential flow rapidly through the 30-45 cm zone and escape detection. The results of the deep sampling for nitrate (section 5.3.5) are relevant to this topic.

5.3.2 Nitrate concentration in the soil solution

Nitrate concentrations in the soil solution at the 30-45 cm depth during 1989 and 1990 for the three pastures under comparison are shown in Fig. 5.1 (and in Appendix 3). In this figure drainage is superimposed on the line of nitrate concentration in the soil solution to indicate when leaching occurred. If the drainage coincides with a nitrate concentration above the recommended level of 10 mg NO_3-N/l the potential exists for pollution of the environment.

Nitrate concentration in the soil solution was high in the three pasture systems at the beginning of this study. These raised values were associated with soil cultivation during the establishment of the experimental swards. However, this progressively decreased to levels below the critical concentration of 10 mg NO_3-N/l just as drainage commenced in 1989.

After the initial effect of soil cultivation disappeared, the concentration of nitrate in the soil solution was in general low, at least for Grass-clover and the Herbal ley. With only one exception for Herbal ley in January 1989, the concentration

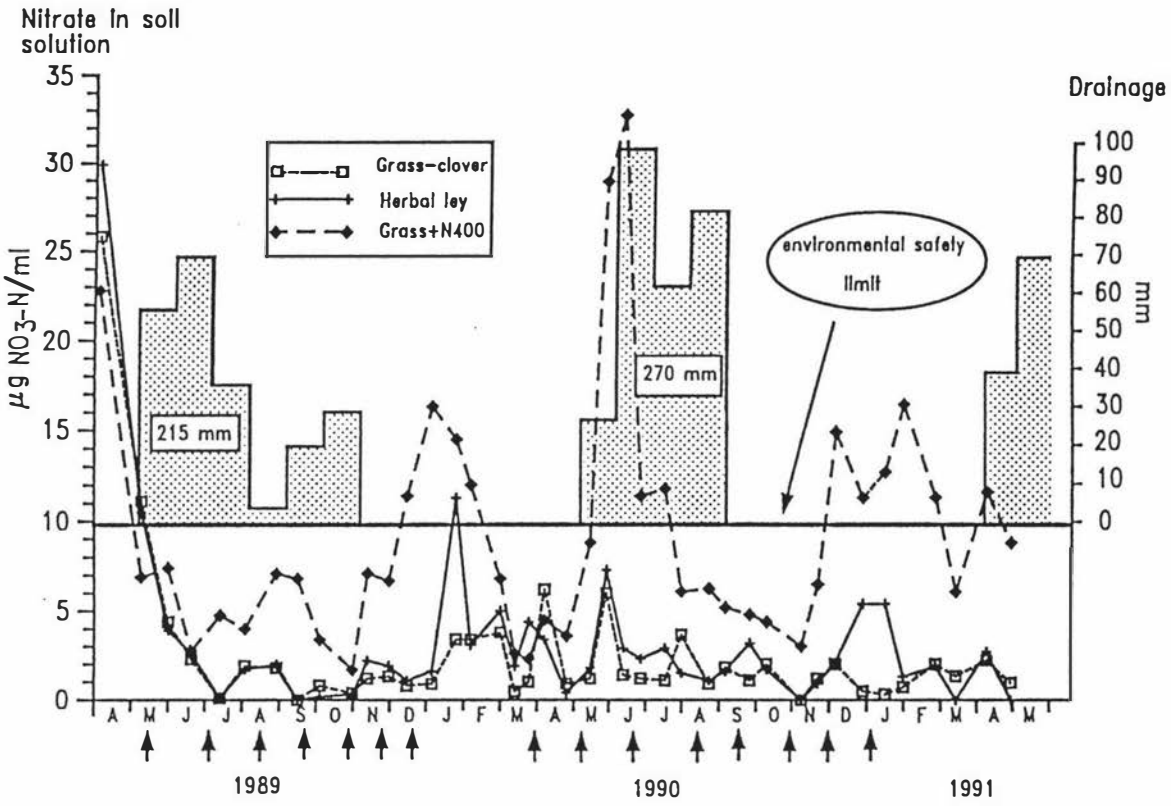

Figure 5.1 Nitrate in the soil solution at 30–45 cm depth and drainage below 45 cm depth during the two-year study

(Arrows indicate fertiliser N application to Grass+N treatment)

of nitrate in soil solution remained well below the "environmental safety limit".

The effect of the fertiliser N, application of which started in May 1989, (indicated by arrows in Fig. 5.1) was a rapid increase in the concentration of nitrate at 30-45 cm depth. As is shown in Fig. 5.1 the concentration of nitrate in the soil solution in Grass+N400 was more variable and clearly different from the concentration observed in the legume-based pastures. However, initially this heightened level was not sufficient to exceed the critical concentration of 10 mg NO_3-N/l. The first peak in nitrate concentration above this level was observed in December 1989 (but no drainage occurred in that period), following by others in May-June 1990 and summer 1990-91.

The peak of nitrate concentration in Grass+N400 at the beginning of the winter 1990 (30-33 mg NO_3-N/l) coincided with a large event of drainage. This caused the largest leaching of nitrate during the 2 year study.

The nitrate concentration in the soil solution for Grass+N400 during 1990 followed a pattern similar to that observed in some catchment studies (Wild and Cameron, 1980; Haigh and White, 1986) where the concentration of nitrate was initially high and gradually decreased through drainage season, and then increased again in spring.

5.3.3. Nitrogen losses by leaching

The amount of NO_3-N lost by leaching from the three pasture systems is shown in Tables 5.3 and 5.4 for years 1989 and 1990, respectively. In 1989 the largest leaching loss occurred in May for the three pastures under comparison. At that time, the Grass+N pasture had received only the first application of N fertiliser (see Fig. 5.1), so most of the nitrate leached in that period should correspond to the nitrate accumulated during the period of soil cultivation in March 1989. For the remainder of 1989 leaching losses were small for the three systems, the largest value being less than 3 kg of nitrate

Table 5.3 Drainage and losses of nitrate by leaching during 1989

		Grass-clover	Herbal ley	Grass+N400	
	Drainage (mm)	Leaching (kg NO_3-N/ha-45 cm)			Lsd (P≤0.05)
May	56	5.4	5.0	3.9	2.3
June	70	1.0	2.0	2.8	1.7
July	36	0.2	0.2	1.6	0.7
August	4	0.1	0.1	0.3	0.2
Sept.	20	0.1	0.1	1.4	0.2
October	29	0.1	0.1	0.7	0.3
YEAR	215	6.9	7.5	10.7	3.9
s.e.m.		0.9	0.4	1.6	

Table 5.4 Drainage and losses of nitrate by leaching during 1990

		Grass-clover	Herbal ley	Grass+N400	
	Drainage (mm)	Leaching (kg NO_3-N/ha-45 cm)			Lsd (P≤0.05)
May	27	1.1	1.4	5.6	1.6
June	99	2.0	3.3	23.4	10.6
July	62	0.9	1.5	7.0	3.4
August	82	1.8	1.1	5.1	2.6
YEAR	270	5.8	7.3	41.1	16.4
s.e.m		0.9	1.64	8.0	

per ha per month for Grass+N400. The total amount of N lost by leaching during 1989 was 6.9, 7.5 and 10.7 kg N/ha for Grass-clover, Herbal ley, and Grass+N400, respectively. These values did not differ statistically at $P \leq 0.05$.

During the second year (Table 5.4) the annual losses of N for Grass-clover and Herbal ley were of the same order as that estimated in 1989, although in this case leaching was limited to only 4 months. The values varied between 0.9 and 3.3 kg NO_3-N/ha per month. In contrast, the amount of N lost by Grass+N400 pasture was 4 times greater than that estimated for the previous year. The results in Table 5.4 show that the largest amount of leaching (23 kg NO_3-N/ha) occurred during June. This resulted from the high concentration of nitrate in soil solution being combined with some 99 mm of drainage estimated for that month (Fig 5.1). The total amount of N lost was 41.1 kg/ha, a value 5-6 times greater than the amount estimated in the systems based on legumes. The annual losses of the Grass+N400 were smaller than those reported by Field *et al.* (1985) on the same soil type from a grass-clover sward receiving 450 kg N/ha. However, they also found that the leaching from this sward was about 2.5 and 4 times greater than from a pasture receiving no fertiliser N during two consecutive years. In a more recent study, also on the same soil type, Brock *et al.* (1990) reported leaching losses of 17 kg N/ha/yr from a legume-based pasture (no fertiliser N was added in this case) rotationally grazed by sheep.

5.3.4 Nitrogen leaching in relation to N input, herbage N yield and animal grazing days

The N lost by leaching during 1990 for the three pasture systems was compared with N input, herbage N yield and animal grazing days (Table 5.5). This table was constructed with information given in Chapter 3 (Herbage Production) and the values were accumulated through the spring, summer, autumn and winter of 1989-1990.

Table 5.5 Leaching of nitrate (1990) in relation to N input, N yield and animal grazing days from September 1989 to August 1990

Pasture	Leaching	N input	N yield	Grazing days
	(kg NO_3-N/ha)	(kg N/ha)	(kg N/ha)	(x1000)
Grass-clover	5.8	144	357	9.5
Herbal ley	7.3	152	476	11.0
Grass+N400	41.1	400	589	13.5

(N inputs = symbiotic N fixation or fertiliser N)

N inputs: Leaching of nitrate for each treatment were 5.8 7.3 and 41.1 kg NO3-N/ha/yr (Table 5.5) which represent 4.0%, 4.8% and 10.3% of the N input for Grass-clover, Herbal ley and Grass+N400, respectively.

The identification of the exact rates of fertiliser N to recommend is difficult and will depend upon a number of factors. The available information, mostly from the European countries, indicates however that with a rate of 400 kg N/ha per year there are only limited possibilities for maintaining soil nitrate below the prescribed environmentally safe limit. Current recommendations to reach this objective suggest that a safe application of N fertiliser to grazed grass would be up to 200 kg N/ha/yr and for cut grass 250-300 kg N/ha/yr (t'Mannetje and Jarvis, 1990). Under the condition of this experiment, 400 kg N/ha did not produce the amount of leaching commonly reported in the literature from pastures receiving a similar fertiliser application, but with a longer history of N fertilisation. However, there is no guarantee that these relatively small values could be maintained if the fertilisation at that level continued for a longer period.

Herbage N yield and animal grazing days: The losses of N by leaching represented 1.6, 1.6 and 7% of the N circulating through the herbage, and hence through the animal, for Grass-clover, Herbal ley and Grass+N400 pastures. In Chapter 3 attention was drawn to the efficiency of Herbal ley

pasture in transforming N input into N yield and hence into forage production. Here it could be added that it was also efficient in avoiding N losses by leaching, in spite of the increased N fluxes through plant and animals. In relation to Grass-clover, 30% more N cycled through herbage in Herbal ley, but the leaching of N was maintained at the same low level as in Grass-clover. In contrast, N yield in Grass+N400 was 26% greater than in Herbal ley (23% more animal grazing days too). Nevertheless, leaching losses increased 460%. In relation to Grass-clover these proportions were 70% and 608% for the differences in N yield and leaching, respectively. These results suggest that if a low level of mineral N in the profile is maintained (as in Grass-clover and Herbal ley, Chapter 4) an increased amount of N can circulate through the herbage (and the animals) with less leaching loss than will occur when the enhanced production is associated with a large residual soil mineral N.

As herbage N yield is an indicator of the N ingested by animals these results indicate that leaching losses are less directly related to the animal in pastures heavily fertilised with N than in unfertilised systems. Similar attention was drawn by Field *et al.* (1985) who reported that for an increment in sheep grazing days of the order of 20% when 450 kg of N were applied, leaching estimates increased by up to 360%. A possible explanation is that under heavy fertilisation with N, proportionally more leaching occurs from the background soil mineral N level that has been raised by the fertiliser (see Fig. 4.4 Chapter 4) than from the areas affected with animal excreta. Testing of this hypothesis is obviously very difficult. Using the information available from Chapter 4 (section 4.3.3.5 Spatial variability) about the area presumably affected by recent urine patches and the concentration of mineral N in both affected and unaffected areas, it should be possible to establish some comparison between Grass-clover and Grass+N systems concerning the proportional contribution to leaching from both areas. This needs to assume that leaching is linearly related to the concentration of soil mineral N. In the Grass-clover system the concentration of mineral N (kg/ha-15 cm) in urine patches, comprising 10% of area, and in the rest of the field (90%) were in the

ratio of 11 : 1 (223 : 20 kg N/ha-15 cm). Thus we can make the following calculations:

- Urine patches :	0.1 area x 11 [N]	=	1.1
- Not affected :	0.9 area x 1 [N]	=	0.9
			2.0

Therefore the relative contribution to total N loss would be:
- Urine patches : 55%
- Non-affected areas : 45 %

So in this example when no fertiliser N has been added and the level of mineral N in the soil was small in relation to that in the areas affected by urine, 10% of the area made a greater contribution (55%) to the leaching than the rest (90%) of the soil. As the leaching estimated in Grass-clover pasture was 5.8 kg NO_3-N/ha one could speculate that 3.2 kg came from the urine patches, and was an animal associated loss, and some 2.6 kg escaped from the remaining soil.

Using the same procedure as above for the Grass+N400 pasture, it was found that the concentration of mineral N recoverable from the 10% of the area affected by urine patches (184 kg mineral N/ha) was only 3 times the mineral N measured in the areas non-affected (63 kg mineral N/ha) because fertiliser N had raised the background of the mineral N status of the soil (Chapter 4, section 4.3.3.5).

- Urine patches :	0.1 area x 3 [N]	=	0.3
- Not affected :	0.9 area x 1 [N]	=	0.9
			1.2

Thus the relative contribution to the total N loss would now be:
- Urine patches : 25%
- Non-affected areas : 75%

In this case most of the mineral N for leaching apparently originated from the area non-affected by urine patches. Proportionally the area non-affected by urine patches made a greater contribution to total leaching than in the Grass-clover pasture because of the much higher concentration of soil mineral N for the whole area. The final result was a disproportionate difference in leaching between both systems in relation to the differences observed in N yield and stocking rate. Making the same speculative calculation as for the Grass-clover, of the 41 kg N/ha lost by leaching in Grass+N400 system, 31 kg of N may have come from the soil non-affected by urine patches and 10 kg of N from urine patches.

5.3.5 Nitrate measured in deep samples

At the end of the drainage season in 1990 soil mineral N was measured in deep samples taken below the root zone, down to 2 m. The distribution of NO_3-N in the soil profile for Grass-clover, Herbal Ley and Grass+N400 pastures is shown in Fig. 5.2. Grass-clover and Herbal ley showed a similar pattern of nitrate in the soil solution, the levels increasing with depth from about 1 to 7.5 mg NO_3-N/l soil solution. Grass+N400 pasture in general showed higher values than Grass-clover and Herbal ley at all depths, particularly below 1 m where the concentration of nitrate increased up to 30 mg NO_3-N/l soil solution.

It is difficult to relate the nitrate measured at the end of the drainage season to the nitrate previously estimated to be lost by leaching. Making the assumption that the average bulk density of soil below 50 cm was similar to the value measured at the 30-45 cm depth, namely 1.35 g/cm^3, it is possible to express the results in terms of kg N/ha. The results for nitrate, ammonium and soil water appear in Table 5.6. The values for ammonium were large and highly variable in Grass+N400. In this treatment only a few samples from a single plot exhibited this high content of ammonium, which was found in layers of silty clay soil. There is no a clear explanation for the presence of these

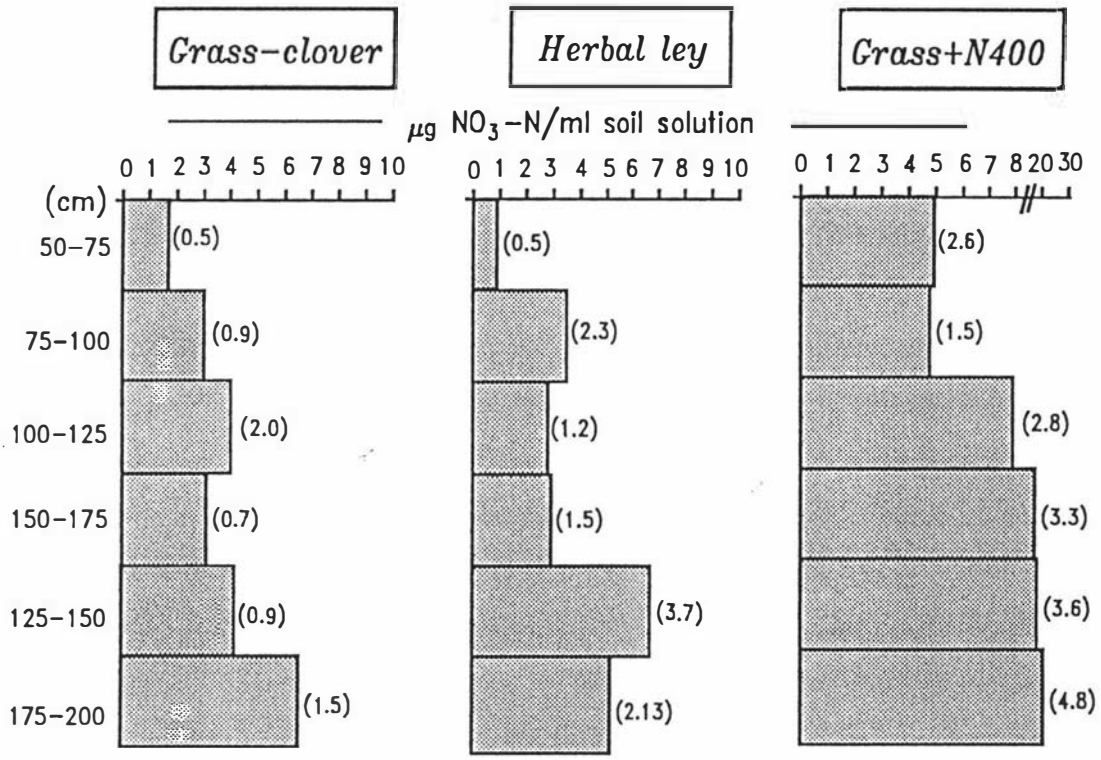

Figure 5.2 Nitrate in the soil solution measured in deep samples at the end of the drainage season (October 1990)

(Numbers in parentheses indicate S.e.m.)

Table 5.6 Mineral N and soil water (SW) below 50 cm depth measured in samples taken in October 1990

Depth	Grass-clover			Herbal ley			Grass+N400		
(cm)	NH_4-N	NO_3-N	SW	NH_4-N	NO_3-N	SW	NH_4-N	NO_3-N	SW
	--kg/ha--		mm	--kg/ha--		mm	--kg/ha--		mm
50-75	4.1	0.8	49	2.7	0.4	44	1.7	1.5	40
-100	5.4	1.2	44	2.0	1.1	53	3.0	1.1	53
-125	5.4	1.9	58	2.4	1.2	63	18.9	4.0	59
-150	5.1	2.2	73	2.0	2.4	93	6.1	8.6	84
-175	7.1	3.7	91	3.4	6.4	87	10.5	9.5	93
-200	4.7	5.9	89	0.7	4.4	85	27.3	12.4	94
Total	31.8	15.7	404	13.2	15.9	425	67.5	37.1	423
s.e.m	5.5	1.8	28	5.8	6.8	52	33.5	7.5	32
NH_4-N	b			c			a		
NO_3-N		b			b			a	
SW			a			a			a

(Different letters denote significant differences at P≤0.05, for total values)

Soil water (SW) in mm for each layer was calculated as follows:

$$\frac{\theta v \times 250 \text{ mm depth}}{100}$$

θ_v was calculated from the gravimetric moisture content (%) measured in each sample multiplied by an average bulk density (assumed to be 1.35 g/cm^3, the same as that measured at 30-45 cm depth; Table 2.1)

large quantities of ammonium at these depths, and in the absence of any previous sampling, it is not possible to indicate whether there was any change during the course of this study.

The soil water measured in the deep samples (expressed in mm of water for each layer in Table 5.6) represents approximately the water-filled pore space displaced by piston flow during drainage. This assumption should be valid because the soil in October did not show a severe water stress (moisture deficit in the top 45 cm was about 30%), so the water extraction by plants below 45 cm was presumably minimal. Thus the estimated drainage in 1990 of 270 mm below 45 cm depth would have moved the leaching front to a depth between 150 and 175 cm in the three pastures. Table 5.6 indicates that the NO_3-N recovered between 50 and 150-175 cm depth was in the vicinity of 8 kg N/ha in the legume-based pastures, a value which agrees reasonably well with the estimated nitrate leaching using the water balance model (Table 5.4 for 1990). However, for the system receiving fertiliser N the amount of nitrate accounted for between 50 cm and the depth of 150-175 cm was in the range 15-25 kg N/ha, representing only some 40-60% of the estimate for nitrate leaching (41 kg N/ha; Table 5.4) from the root zone. With the data available in this study it is not possible to offer a definitive explanation for this discrepancy, but the fact that only two deep cores were taken from each plot, and a great deal of variability was encountered between plots of any one treatment, suggests that spatial variability provides the most likely reason.

5.4 Conclusions

Nitrate concentration in the soil solution was maintained at a low level in the legume-based systems. In the pasture based on fertiliser N, the level of nitrate was in general much higher and showed a variable pattern. On some occasions the concentration increased above the limit considered safe for the environment.

As Grass-clover and Herbal ley both exhibited low concentrations of nitrate and the drainage did not differ substantially between the two years, nitrate losses by leaching were consequently small in both systems during the period of this study. Although Grass+N400 had the same amount of drainage, leaching of nitrates was 6-7 times greater in 1990 than it was in Grass-clover and Herbal ley. As the amount of drainage would be the same for all treatments, and the grazing intensity was approximately the same, the increment in nitrate concentration as a consequence of the N fertilisation was the reason for the increased leaching loss.

From the environmental point of view it is preferable to report along with the amount of nitrate leached, the corresponding drainage. According to the drainage recorded in 1989 (215 mm), losses of NO_3-N of 21.5 kg/ha or more would be required to generate a leachate concentration of 10 mg NO_3-N/l. In 1990, with a drainage of 270 mm, the critical level was 27 kg NO_3-N/ha, which was exceeded by the 41.1 kg/ha of NO_3-N leached in Grass+N400, but not by the 5.8 and 7.3 kg/ha calculated for Grass-clover and Herbal ley, respectively.

In relation to N inputs, apparently not only the amount, but also the form of N to enter the soil plant system is important in determining leaching. The abrupt increase in the mineral N pool when fertiliser N is applied seems to be a critical factor in determining the final result of leaching.

The Herbal ley was the more efficient pasture in terms of the large amount of N circulating through the herbage/animal system and the small losses of N by leaching. In contrast, the Grass+N400 pasture during 1990 exhibited a disproportionate increase in nitrate leaching relative to the other pastures and differences in N yield and animal grazing days. The explanation was that under heavily fertilisation with N, proportionally more of the nitrate was lost from the whole soil volume than from the urine patches receiving a more direct influence from the N circulating through the herbage/animal system. It was calculated that under the conditions of this experiment the urine patches could

contribute 25% of nitrate leaching in the Grass+N400 systems, but more than double that percentage (55%) in Grass-clover where the concentration of mineral N in the rest of the soil was much smaller.

The amount of nitrate deeper in the soil profile at the end of the drainage season agreed with that estimated using the water balance model and nitrate concentration in soil the solution for the legume-based pastures. But nitrate measured in the deep samples in the Grass+N400 pasture was only about half that predicted by the water balance model.

CHAPTER 6
DENITRIFICATION

6.1 Introduction

The production of gaseous nitrogen compounds is potentially one of the most important pathways of loss affecting the ultimate fate of N (Ryden, 1986). Nitrogen can be evolved to the atmosphere as a result of several physical, biological and chemical reactions. The major processes involved are ammonia volatilisation (Freney *et al.*, 1983); biological denitrification producing nitric oxide, nitrous oxide and dinitrogen (Fillery, 1983); chemodenitrification producing nitric oxide, nitrogen dioxide, nitrous oxide, dinitrogen and methyl nitrite (Chalk and Smith, 1983) and losses during nitrification as nitric oxide and nitrous oxide (Lipschultz *et al.* 1981). This chapter concentrates on the losses of N by biological denitrification.

The interest in biological denitrification arises from several concerns. For example on a global scale, denitrification is an important component of the nitrogen cycle, and one of the main pathways for replenishing atmospheric N previously fixed by biological, atmospheric and industrial processes (Lewis, 1986). From the agricultural point of view it is important because a significant amount of N can be lost from the soil-plant-system (Ryden, 1986; Myrold, 1988). Also environmental attention has been focused on nitrous oxide (a gas product of denitrification) because NO derived from atmospheric N_2O is an important factor that may alter the vertical profile of ozone in the stratosphere which can affect the penetration of UV radiation to the Earth's surface (Crutzen, 1981). The other role of nitrous oxide is as a greenhouse gas that can absorb longwave radiation emitted from the Earth's surface (250 times the effect of carbon dioxide mole for mole).

Denitrification is the process by which N oxides, principally nitrate, and nitrite

are reduced to dinitrogen gas. A general pathway for this process as suggested by Payne (1981) is represented as:

$$\overset{(+5)}{NO_3^-} \rightarrow \overset{(+3)}{NO_2^-} \rightarrow \overset{(+2)}{NO} \rightarrow \overset{(+1)}{N_2O} \rightarrow \overset{(0)}{N_2} \qquad (1)$$

Most denitrification is carried out by respiratory denitrifiers that gain energy by coupling N-oxide reduction to electron transport phosphorylation (Tiedje, 1988). Nearly all respiratory denitrifiers prefer to use O_2 as their electron acceptor and will reduce N-oxides only when O_2 is not available. The process is described as a dissimilatory reduction (originated by dissimilatory denitrifying bacteria) since the products of nitrate reduction, N_2O and N_2, are not assimilated but released to the atmosphere (Haynes and Sherlock, 1986).

6.1.1 The organisms involved in biological denitrification

Relatively few species of bacteria (about 23 genera - Firestone, 1982) are capable of anaerobic respiration, and hence denitrification. Most denitrifying bacteria are chemoheterotrophs, that is, they use chemical compounds as energy sources (not light) and they use organic carbon as an electron donor (reductant) and source of cellular carbon (Firestone, 1982).

These denitrifying organisms, although apparently limited to the bacteria, are biologically and taxonomically diverse (Knowles, 1981). This characteristic, plus the complex enzyme system required in the different steps of the reaction shown in equation (1), further complicates the understanding and the analysis of the entire denitrification process. In this regard, Knowles (1981) recognised that while most of the bacteria involved in the process of denitrification possess all the reductases necessary to reduce NO_3^- to N_2, some lack NO_3^- reductase and then are "NO_2^- dependent"; others lack N_2O reductase and thus yield N_2O as a terminal product. Yet others may possess the N_2O reductase but cannot reduce NO_2^- to N_2O.

6.1.2 Factors affecting biological denitrification

The general requirements for denitrification are: i) total or partial anaerobic conditions, ii) the presence of appropriate microorganisms with metabolic capacity, iii) electron donors (such as C, reduced S compounds or molecular H_2) and iv) N oxides to act as a terminal electron acceptors. This shows that denitrification can be affected by a wide range of physical and biological factors. Several reviews and discussions on denitrification including Firestone (1982), Knowles (1981) and Haynes and Sherlock (1986) describe the general effects on denitrification of a number of factors: aeration and soil moisture, organic C, nitrate supply, nitrifiable N, pH, temperature, plants, animals and tillage methods. Further complications arise since the importance of the controlling factors changes according to the scale and objective of the investigation. In this regard Groffman *et al.* (1988) postulate that although denitrification is primarily controlled by oxygen, nitrate and carbon (proximal factors), it is also affected by many physical and biological factors (distal), which in turn participate with different emphasis according to the scale of the study. The thesis of the same authors is that as the temporal and spatial scale of an investigation increases above the cellular level, distal factors become increasingly more significant as a focus of the study, as is outlined below :

Scale of study	Factor controlling denitrification
Organism	O_2, NO_3^- and C
Microsite	Organic matter, physical disruptions
Field	Water, nitrification, decomposition
Landscape	Soil type, land use
Regional	Soil type, land use, community structure, geography
Global	Biome type, climate

(Groffman *et al.* 1988)

From a number of studies at a field scale summarised in Colburn and Dowdell

(1984), Rolston *et al.* (1984), Linn and Doran (1984), Groffman and Tiedje (1991), it seems that in agricultural soils, moisture content appears to be the dominant factor controlling denitrification, especially in soils fertilised with N where the concentration of nitrate is usually high. In non-agricultural soils where plant competition for nitrate is high, the soil nitrate production may become an important limiting factor.

Soil moisture is a major factor in determining the soil air/water balance and hence the aerobic and anaerobic activities. Under field moisture regimes, aerobic microbial activity normally increases with soil water content until a point is reached where the water restricts the diffusion and availability of oxygen, and at the same time increases the volume of the anaerobic micro-environment in which denitrification may occur (Linn and Doran, 1984).

The role of soil carbon in denitrification has been less well studied than either nitrate or water. The effect of soil carbon (especially that supplied by the plant roots) in promoting denitrification on a field scale appears to be important in some cases (Woldendorp, 1962; Volz *et al.,* 1976; Smith and Tiedje, 1979). But this effect does not always occur in a positive correlation with denitrification (Guenzi *et al.*, 1978). Smith and Tiedje (1979) concluded from a study with and without plants that this lack of coincidence relates to the status of soil nitrate. They found that denitrification was greater in planted than unplanted soils if there was sufficient nitrate, but when nitrate availability was low the presence of plants decreased denitrifying activity, probably because of root competition for N. Thus, whether the presence of roots increases or decreases denitrification in field soils probably will also depend on the combination of a number of other soil parameters such as native soil carbon, oxygen diffusion (pore size and water content) and the rate of nitrate supply to the root zone.

6.1.3 Field measurement of denitrification by the acetylene technique

Of the biogeochemical processes, denitrification has perhaps been the most difficult to study in the field because of an inability to measure the product of the process and the complexities associated with spatial variability in denitrification rate in the field. A number of methods have been used to measure denitrification in the field (Hauck, 1986; Smith, 1988). However, during the last decade both acetylene and ^{15}N based methods have been the most common and widely used in a range of soil conditions. These methods have been implemented utilising incubation of undisturbed soil cores or soil cover (chambers) sampling approaches.

The methods based on the acetylene-inhibition technique have been widely used during the last decade in field studies of denitrification. This technique was proposed by Yoshinari *et al.* (1977), based on the blocking of the reduction of N_2O to N_2, and provided a rather simple quantitative means of assessing gaseous nitrogen losses. Since this technique was developed, the studies of denitrification in agricultural systems have increased notably, as has the understanding of the process. Ryden *et al.* (1979) were among the first of a number of workers to use this technique to quantify denitrification in different agricultural systems.

Laboratory studies have shown that nitrous oxide is the sole gaseous product of denitrification in soils incubated in atmospheres containing 0.1-10% v/v acetylene. Production of N_2O-N in the presence of acetylene is equivalent to the production of N_2O-N plus N_2-N in the absence of acetylene (Yoshinari *et al.*, 1977; Ryden *et al.*, 1979). As the N_2O produced during denitrification is easily measured against its background concentration in the atmosphere, the measurement of N_2O production in the presence of acetylene provides a basis for a versatile and widely applicable method to study total denitrification loss from soil.

A systematic comparison of the acetylene-inhibition methods with the most widely accepted ^{15}N methods to measure denitrification was carried out by Parkin *et al.* (1985). This study concluded that denitrification rates from the acetylene core-methods were not significantly different from those estimated from the ^{15}N method. Denitrification rates as measured by both methods were highly variable. More recently, Duxbury (1986) and Tiedje *et al.* (1989) have summarised the main advantages and disadvantages of the acetylene-inhibition methods as follows:

Advantages:

i) The relatively low cost compared to the ^{15}N methods.

ii) The improvement in the sensitivity over previous methods (detection limits 0.5 ng N/g soil/day).

iii) The use of the natural nitrate substrate pool.

iv) The large number of samples that may be assayed which reduces the problems related to the spatial and temporal variability.

v) The versatility of the method allowing laboratory, field and remote studies.

Disadvantages

i) Acetylene affects other processes such as nitrification. This could be a limitation in soils of very low nitrate concentration.

ii) Acetylene inhibition can fail because of reduced diffusion into the soil. This may be an important limitation in impermeable clay soils where it is also likely that the potential for denitrification is high.

iii) Contaminants in the acetylene may affect denitrification.

iv) The diffusion of the acetylene, the recovery of N_2O and the significant water solubility of N_2O are all important physical aspects that can lead to inaccurate results.

Of these potential problems the only one which has been reported that cannot be overcome with appropriate care and design is the acetylene inhibition of

nitrification. This is a problem in natural ecosystems which can be solved using the gas-phase recirculation core method (Tiedje *et al.*, 1989).

Several variations of the acetylene inhibition technique have been proposed, but most involve either i) *in situ* treatment of soil with acetylene using an enclosure placed over the soil surface followed by the determination of N_2O emission (e.g. Ryden *et al.,* 1979; Colburn *et al.,* 1984) or ii) incubation of soil cores with acetylene followed by the analysis of N_2O (e.g. Aulakh *et al.* 1982; Parkin *et al.* 1984). In a comprehensive comparison of cores and chambers, Ryden *et al.* (1987) found a strong relationship between denitrification rates in cores versus chambers over a wide range of denitrification rates (0.005 - 1.27 kg N/ha /day). An additional advantage of cores is that it is possible to run numerous core incubations, cheaply and quickly, while chamber measurement can be expensive and time consuming, limiting the number of replicates and/or sites that can be analysed.

In summary, when compared directly, cores and chambers provided comparable measurement of denitrification. From a practical point of view where management permits, for example in cropped areas and mown grassland (Ryden *et al.*, 1987) or when measurements of instantaneous flux of N gases are required, especially nitrous oxide for atmospheric chemistry objectives, chamber methods are preferred (Tiedje *et al.*, 1989). However, the incubation of cores permits a more accurate measurement of denitrification in grazed pastures where a large number of samples are required to reduce the problems of spatial variability resulting from the grazing and excretion pattern. Also cores appear to be more useful for the estimation of total N gas production for N budget studies (Tiedje *et al.*, 1989).

In this study the objective was to measure total denitrification from three different grassland systems to compare their rate of gas production, as well as the annual losses of N. An effort was made to find some relationship between the pattern of total denitrification and field parameters. The

proportion of nitrous oxide in total denitrification was also estimated.

6.2 Materials and Methods

The study of denitrification was carried out on three grassland systems, Grass-clover, Herbal ley and Grass+N400. A detailed account of the experimental site, climate, establishment and management of the trial has been given in Chapter 2.

The rate of denitrification was measured using the acetylene-inhibition technique (Yoshinari *et al.*, 1977) using the soil core incubation system under field conditions as described by Ryden *et al.* (1987).

The incubation of soil in the presence of C_2H_2 permits the assay of both N_2 fixation by reduction of C_2H_2 to C_2H_4 and denitrification by C_2H_2 inhibition of the N_2O reduction (Yoshinari *et al.*, 1977). Based on this principle the same incubation was used in this study for the determination of symbiotic N fixation (sampling after 1 h incubation and determining C_2H_4 in a gas chromatograph equipped with flame ionising detector, see Section 3.2.3) and denitrification by analysing gas samples after 24 h incubation in a gas chromatograph equipped with an electron capture detector.

6.2.1 Measurement of total denitrification

Denitrification rate was measured twice between grazings. Fourteen 25 mm diameter x 75 mm deep soil cores were collected randomly from each paddock, placed in a 1 litre incubation vessel, then sealed with a lid fitted with a rubber gasket and incorporating 1 terumo venoject rubber stopper. After all soil samples were taken, approximately 10% of the volume of the air head space was replaced with 60 ml of acetylene. Each vessel was incubated for 24 h in a hole of slightly larger dimensions in the ground in a shaded place adjacent to the study area. After 1 h a gas sample of 5 ml was obtained from

each vessel for symbiotic N fixation assay, see section 3.2.3 (the double-ended needle for sampling the gas was maintained for few a seconds to allow the system to come to pressure equilibrium again after the 5 ml of gas was withdrawn). At the end of the 24 h incubation, gas samples were transferred to evacuated 5 ml vials using double-ended needles, and analysed for N_2O in a Pye Unicam 204 gas chromatograph with a 1.5 m column packed with Poropack QS and an electron capture detector. Column and detection temperature were 50 °C and 350 °C, respectively.

6.2.2 Estimation of nitrous oxide

Three gas blanks containing soil cores from one complete replication (Grass-clover, Herbal ley and Grass+N400) were also incubated without acetylene to estimate the rate of N_2O emission from the soil. In this replicate it was possible to establish the proportion of N_2O to the total denitrification by comparing the results of the incubation with (total denitrification) and without (only N_2O) acetylene. It was assumed that the ratio (N_2O:N_2O+N_2) obtained from each treatment in one replication was similar for the corresponding treatments in the rest of the experiment. By applying this ratio obtained from one replication to the mean total denitrification measured in this experiment it was possible to establish an approximate pattern of N_2O emission at each sampling.

6.2.3 Preliminary estimation of the soil oxygen concentration

One of the reasons for incubating soil cores in a closed system is to improve the diffusion of the acetylene to all the denitrifying sites by exposing the entire outer surface of soil cores to the atmosphere in the incubation vessel. However, the aeration of the outer part of the core may become different to that *in situ* in the field. Smith (1989) considers this to be of major significance in soils with massive structures. The same author found that the denitrification rate increased sharply when the external oxygen concentration of soil cores

decreased below 10-12 %. In view of this observation, the oxygen concentration in the soil atmosphere was monitored at the time of sampling. The soil atmosphere was sampled from perforated probes fitted at the top with a terumo venoject rubber stopper and installed at two depths: 0-7.5 cm and 0-15 cm. A soil air sample was withdrawn with a 1 ml tuberculin syringe, then O_2 and CO_2 were analysed in a Shimadzu GC-8A gas chromatograph, equipped with a thermal conductivity detector.

The objective of this measurement was to determine whether it was necessary to modify the incubation environment of the core approximately to that existing in the field, by lowering the oxygen concentration in the vessel. During the first 8 measurements of denitrification, soil oxygen concentration ranged between 20.01 to 21.18%. Therefore it was concluded that the composition of the soil atmosphere was not an obstacle to using the soil core incubation technique without adjustments to the composition of the air in the vessel.

6.2.4 Calculations to convert ppm N_2O by volume to g/ha/day

e.g. Sample concentration of 2.0 μl/litre of N_2O, incubation temperature at sampling time, 8.5 °C; incubation time 24 h; volume of the vessel head space, 0.50 litre; number of cores per vessel, 14; area of the 14 cores 68.8 cm^2.

Temperature correction: Changes in volume due to temperature at sampling time were calculated from the equation:

$$V_2 = T_2 V_1/T_1$$

If the soil temperature is 8.5 °C, one mole of N_2O at 8.5 °C has a volume of (273 + 8.5) x 22.4/273 = 23.097 litres.

Conversion from volume to weight units (μl/litre to g/litre)
44 g N_2O occupies 23.097 l at 8.5 °C
i.e. Gas density = 44/23.097 x 10^{-6} g N_2O/μl = 1.095 x 10^{-6} g/μl

Therefore to convert from µl/l to g/l we have:
2 (µl/l) x 1.905×10^{-6} (g/µl) = 3.81×10^{-6} g N_2O/l

To convert from N_2O to N_2O-N

atomic weight of N_2O = 14 x 2 + 16 = 44

N fraction of N_2O = 14 x 2 / 44 = 0.636

therefore N_2O-N = 0.636 x N_2O

Area sampled with 14 cores = 68.8 cm^2, then the gas emission from 1 m^2 is:
10000/68.8 = 145.3 times the emission from 14 cores.

final calculation:

3.81×10^{-6} g N_2O/litre x 0.5 litre x 145.3 x 0.636 = 176.04×10^{-6} g/m^2/day
= 1.76 g N_2O-N/ha/day

6.2.5 Volume fraction of soil air

In April 1990 the total soil porosity was measured in each treatment in the top 7.5 cm (5 determinations per treatment) according to the following expression (White, 1987):

$$\text{Total porosity} = 1 - \frac{\text{bulk density}}{\text{particle density}} \times 100$$

Bulk density was measured in undisturbed soil cores according to the method of Blake and Hartge (1986) and particle density for this soil was assumed to be 2.65 g/cm^3 (Brent Clothier, pers. comm.). The results of these measurements are presented in Table 6.1.

Table 6.1 Bulk density and total soil porosity measured in the top 75 mm in April 1990

	Bulk density	Total soil porosity
Pastures	g/cm^3	% v/v
Grass-clover	1.29	51.3
Herbal ley	1.29	51.2
Grass+N400	1.30	50.9

It was assumed that the total porosity did not change significantly in this grassland soil under the different pasture treatments. Then at each soil sampling for denitrification, using the values of total porosity and the actual volumetric soil moisture content, the fractional air space, as an indicator of soil aeration as a whole, was estimated according to the following expression (White, 1987) :

$$\text{Air-filled porosity} = \frac{\text{volume of soil air}}{\text{total soil volume}}$$

$$= \text{Total porosity} - \theta_v$$

6.2.6 Statistical analysis

Variance analysis at each sampling date permitted a statistical comparison of the denitrification rate between treatments.

6.3 Results and Discussion

6.3.1 Rate of denitrification

The rate of denitrification for the period between 12 September 1989 and 31 May 1991 for Grass-clover, Herbal ley and Grass+N400 is shown in Fig. 6.1.

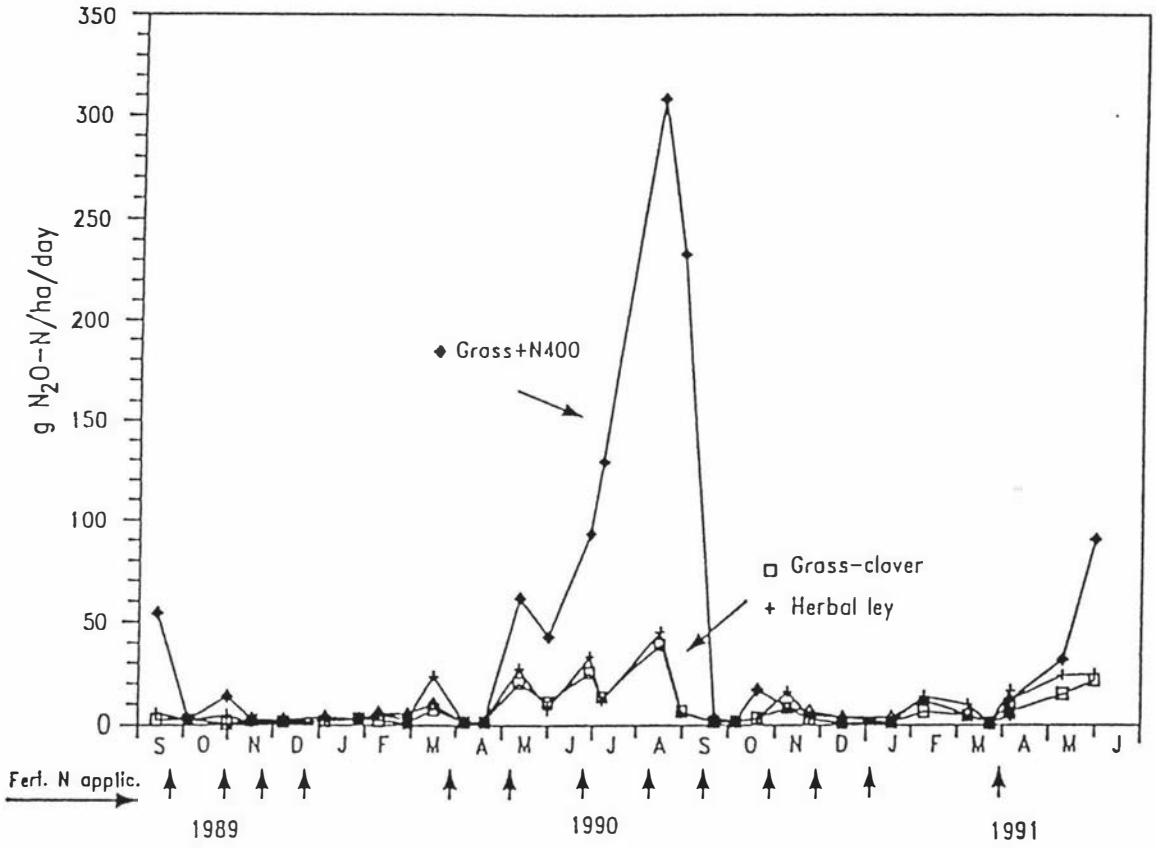

Figure 6.1 Seasonal variation of the rate of denitrification in the three grassland systems

Denitrification was characterised by a seasonal pattern with greater activity observed in late autumn and winter. In this period total denitrification rate was increased several times in the three grassland systems, particularly in Grass+N400 which was also significantly different from the two legume-based pastures.

During spring and summer the denitrification rate was in general limited, with values smaller than 20 g N/ha/day, and no significant differences between treatments were found. It is difficult to know if the N emission in this period was principally due to biological denitrification occurring from a small proportion of microsites still anaerobic, or the small emission arose from other processes such as chemodenitrification and nitrification, as has been reported by Bremner and Blackmer (1978) and Breitenbeck *et al.* (1980). In this regard the results of Denmead *et al.* (1979) indicate that nitrous oxide is always emitted from grassland soils even when conditions are not favourable to denitrification. Therefore it is quite possible that the low rate of N emission during most of the year corresponds to a "background" arising from several processes, and that the N escaped principally as nitrous oxide as is shown in figures 6.2 and 6.3.

This pattern of denitrification loss agrees with other field measurements reported from different environments. For example, Groffman and Tiedje (1989) working with nine soils of different texture and drainage showed that over 80% of the annual loss of N by denitrification occurred during brief periods (3-6 weeks) of high activity, but the loss of N in their experiments was concentrated in spring and fall. In another field study Ryden (1983) found that at most times during the year denitrification proceeded at a rate less than 10 g N/ha/day, and that the potential for denitrification was high in soils fertilised with N.

The rate of denitrification was highly variable as has been demonstrated in many field studies. It was difficult to establish any tendency in this respect.

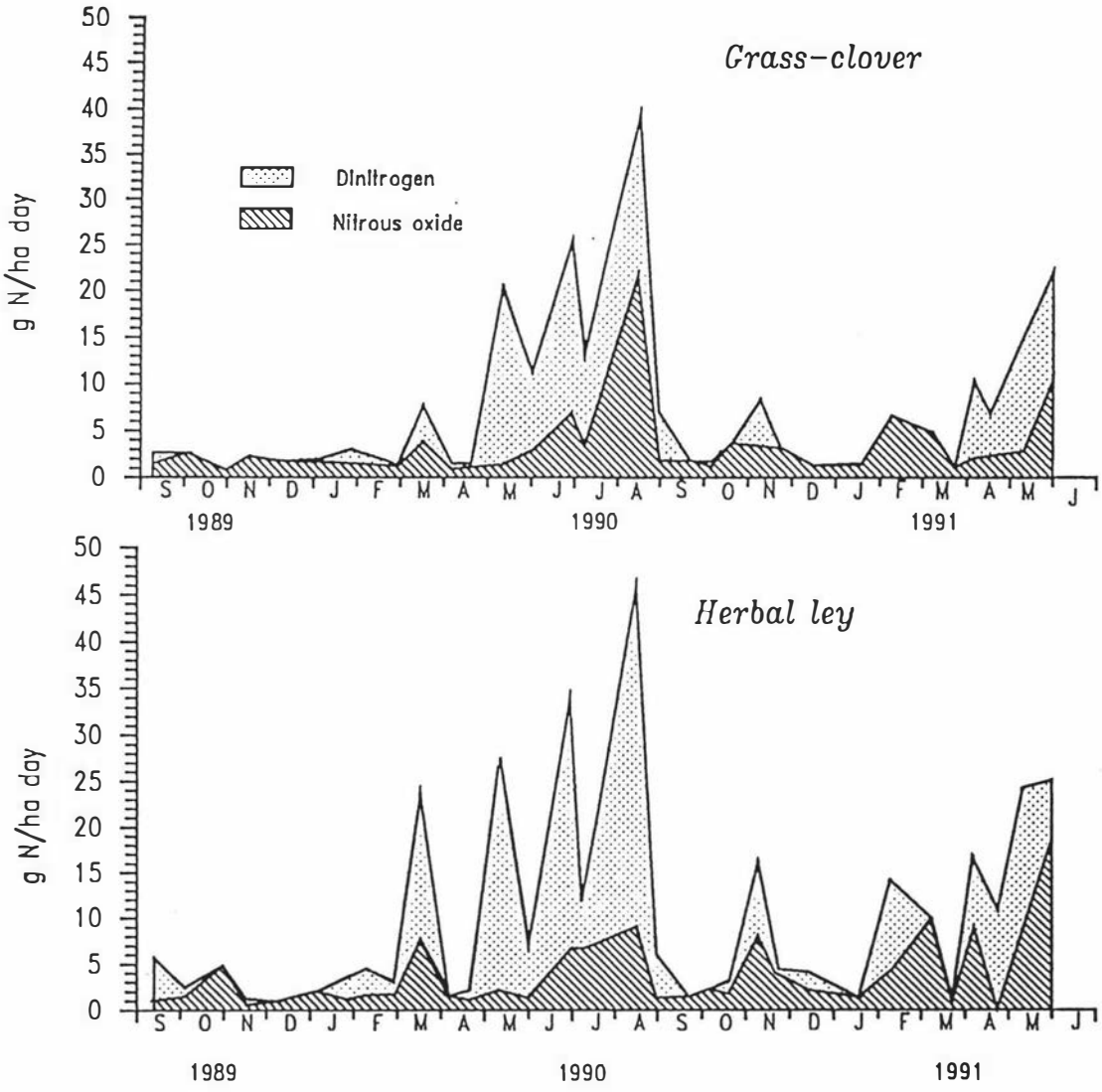

Figure 6.2 Rate of denitrification and proportion of product gases in Grass-clover and Herbal ley pastures

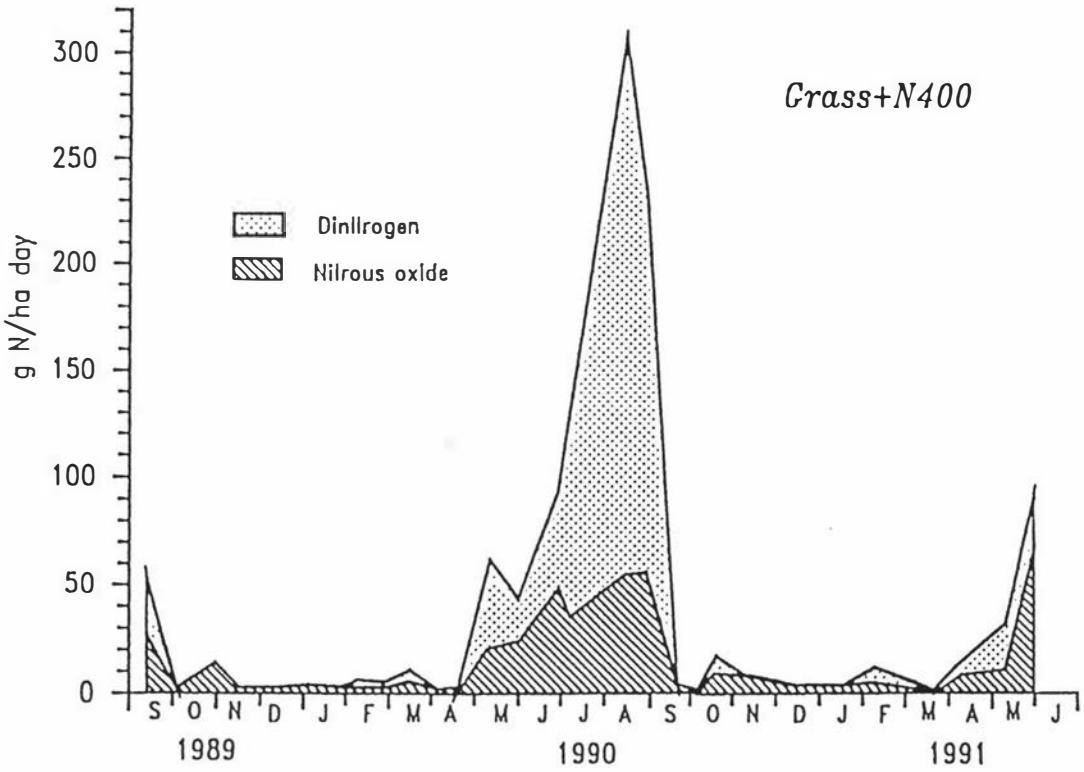

Figure 6.3 Rate of denitrification and proportion of product gases in Grass+N400

In Table 6.2 is shown the range in the coefficient of variation of the rate of denitrification observed through the year in the three grassland systems.

Table 6.2 Seasonal range in the coefficient of variation (%) of the denitrification rate in the three grassland systems

	Grass-clover	Herbal ley	Grass+N400
Summer	5-60	12-61	16-30
Autumn	6-70	24-66	4-78
Winter	13-72	11-70	7-39
Spring	28-50	26-76	20-41

The spatial variability in the rate of denitrification is large in most soils, due to the natural heterogeneity (Folorunso and Rolston, 1984). But often it is increased by the effect of animal grazing and excreta return, thus reducing the usefulness of the enclosure method. Therefore for regular routine measurement in grazed pastures the core incubation technique used in this study seems to be, at present, the most reasonable alternative. This conclusion is supported by Jarvis *et al.* (1991) from an extensive study on the rate of denitrification in a wide range of environments in England.

6.3.2 The effect of soil moisture on aeration and denitrification

The enhanced rate of denitrification occurred primarily when the soil moisture content was maintained for an extended period above the "field capacity". The changes in soil moisture content during the denitrification study are shown in Figure 6.4. Soil moisture content is an important factor influencing aeration, since with increasing soil moisture content, air in soil pores is displaced with water. Therefore the influence of soil moisture content on denitrification is due to a reduction in oxygen content which is essential before denitrification will occur.

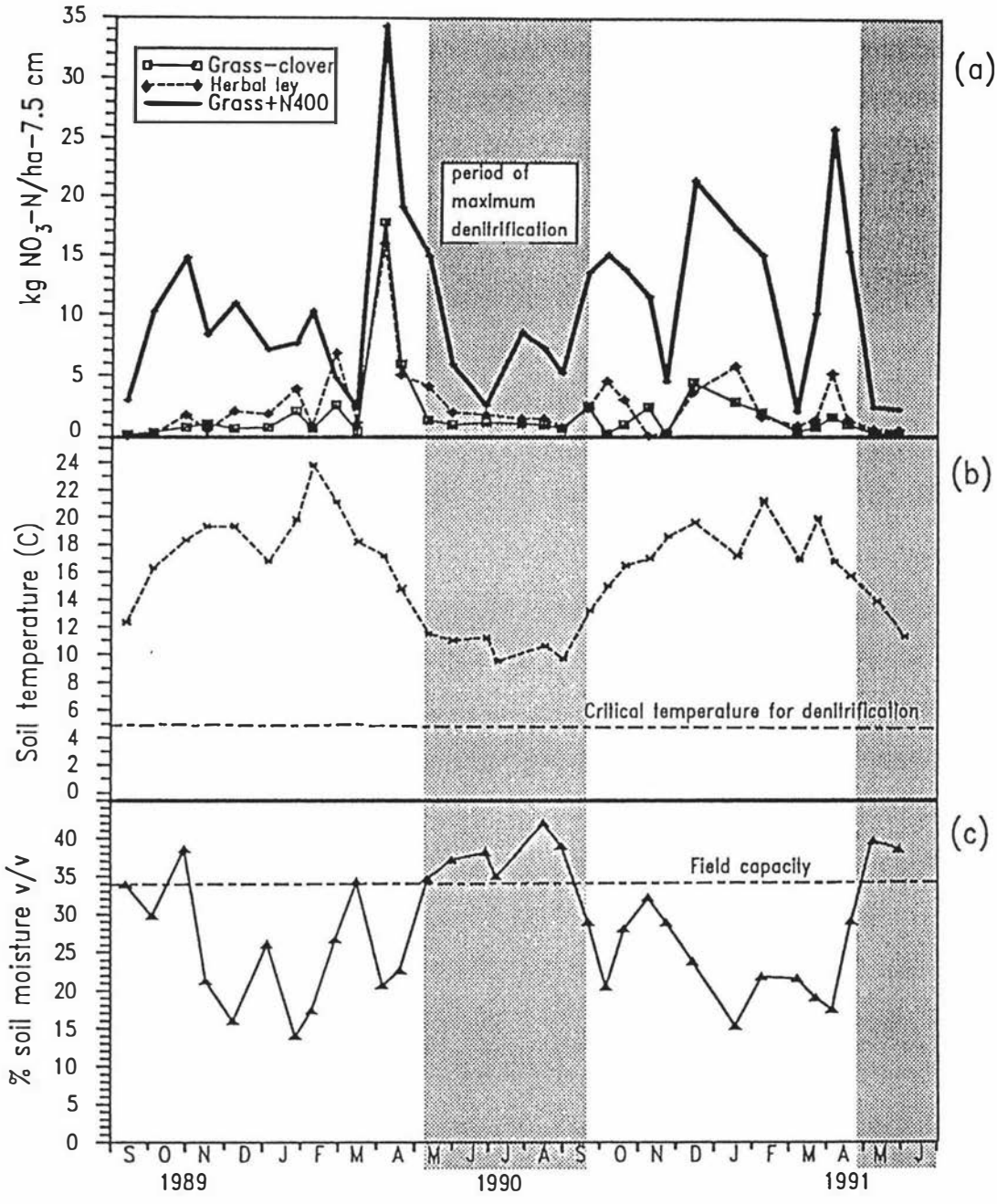

Figure 6.4 (a) Soil nitrate, (0–7.5 cm); (b) mean daily soil temperature (10 cm), and (c) soil moisture (0–7.5 cm) during denitrification study

In this experiment soil porosity ranged between 50.9 and 51.3 % v/v (Table 6.1) and when the soil moisture content in winter reached field capacity (about 34 % v/v), the air-filled porosity decreased below 17% of the soil volume. This value appears to be the critical level below which the onset of anaerobic conditions was conducive to denitrification (Figures 6.5 and 6.6), the rate of which will depend on other factors such as NO_3^- supply, temperature etc.

The results displayed in Figures 6.5 and 6.6 agree with the recent finding of Jordan (1989). His work in pastures indicated that denitrification was generally an active process when the air-filled porosity was smaller than 30% v/v and when the soil moisture content was greater than 25% in the top 10 cm of the profile of both a freely drained clay loam and a poorly drained silty clay receiving fertiliser N.

Further indication of the importance of soil moisture in controlling the anaerobic conditions and hence its association with denitrification is the abrupt fall in the emission rate (Fig.6.1) at the same time that the soil moisture content declined below the "field capacity" in late winter (Fig.6.4). This result indicates that this soil has the capacity to return quickly after rain to a relatively high soil moisture tension. Therefore the rate of denitrification in this freely drained grassland soil is likely to be high only when the rain occurs over prolonged periods. Those conditions normally occurred in winter and together with a low evapotranspiration rate, created a soil moisture regime, such as that displayed in Figure 6.4, which was favourable for the process of denitrification.

6.3.3 Effect of soil nitrate and urea fertiliser

During the period of maximum denitrification activity (mid-May to early September) a distinct separation of the fertiliser N and legume-based systems occurred. Total denitrification rate was 5-6 times higher in G+N400 pasture (Fig. 6.1). Two possible factors contributing to this effect are: the higher levels

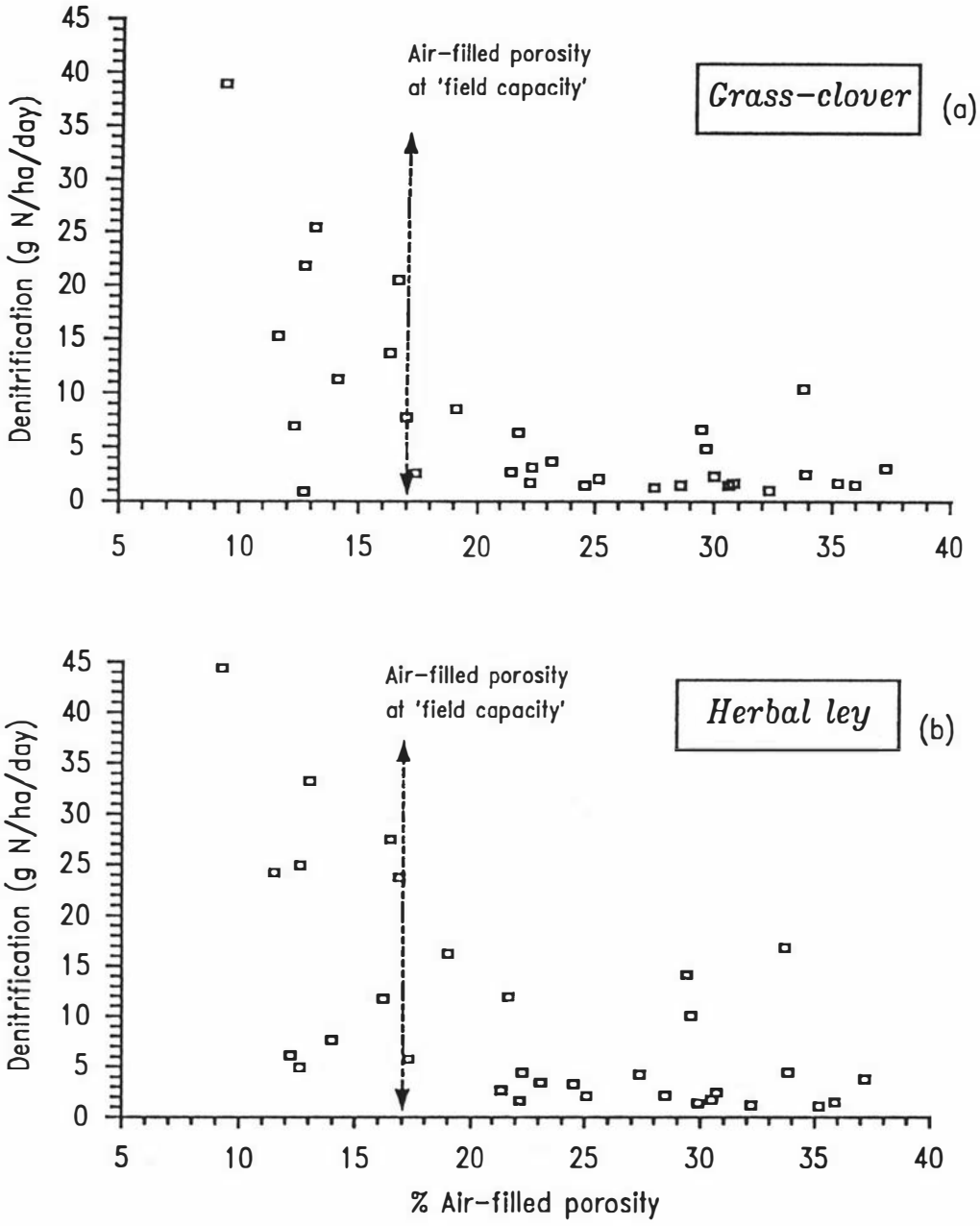

Figure 6.5 Relationship between denitrification and the the air–filled porosity

Air–filled porosity at 'field capacity' was estimated as:
total soil porosity (Table 6.1) – θv at 'field capacity' (Table 2.1)

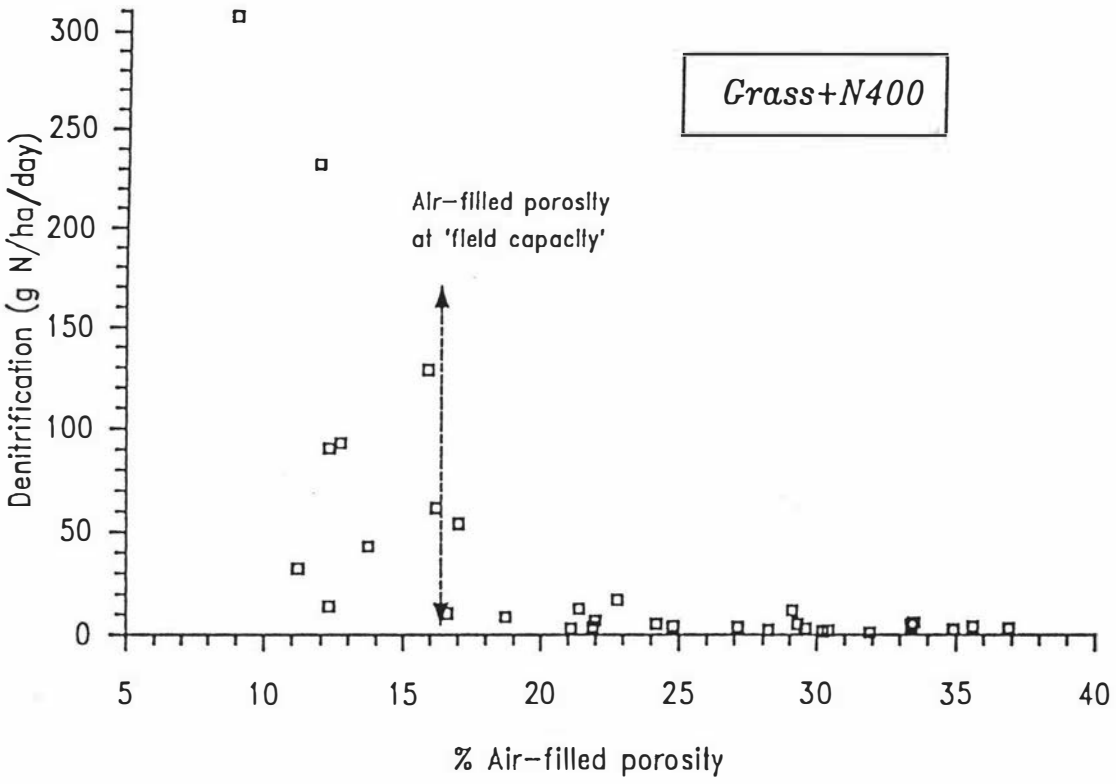

Figure 6.6 Relationship between denitrification and the air-filled porosity

of nitrate in the Grass+N400 system and the transformations of the urea fertiliser itself.

The most important of these factors was the production of substrate NO_2^- and NO_3^- when the conditions were favourable for the denitrification process. For instance, nitrate formed by nitrification of ammonium (aerobic zones) could have diffused to anaerobic sites within the soil profile to be denitrified. This mechanism has been well explained by Knowles (1978). He found that nitrification and denitrification could occur simultaneously, but in separate microsites on opposite sides of an aerobic-anaerobic interface. Similar evidence was obtained by Starr *et al.* (1974) and Koike and Hattori (1978). So the greater quantities of NO3-N in the Grass+N400 system (Fig. 6.4) allowed for more denitrification in this way than did either Grass-clover or Herbal ley.

Furthermore, in this experiment, nitrite, which can accumulate during the nitrification of urea (Christianson *et al.*, 1979), may have been an important source for denitrification too. More recently Magalhaes *et al.* (1987) found that the nitrous oxide lost during nitrification of urea in an acid soil was derived entirely from the fertiliser N. This possibility arises because the difference in soil nitrate between Grass+N400 and the legume-based pastures shown in Fig. 6.4 during the period of active denitrification seems relatively small to explain the large difference in denitrification. But in this experiment it is not possible to know if the enhanced denitrification originated from soil nitrate or from transient nitrite accumulation during the transformation of the urea fertiliser.

Denitrification studies in laboratory have found that the apparent Km values for denitrification (concentration required to give half the maximum velocity of denitrification) are normally in the range of 5 to 290 μM NO_3^- (approximately 0.013 to 0.77 μg NO_3-N/g soil). However, in the field the diffusion of nitrate to the microsites where denitrification occurs may become an important limiting factor (Phillips *et al.*, 1978). So Km values for nitrate in soils have been

reported (Bowman and Focht, 1974; Yoshinari *et al.*, 1977) to be much higher than those obtained in cultures and range from approximately 130 to 12,000 μM NO_3^- (approximately 0.34 to 32 μg NO_3-N/g soil).

In studies of denitrification in grassland soil the threshold value of nitrate for 'higher' rates of denitrification has been reported to be equivalent to 2-5 kg NO_3-N/ha in the top 7.5 cm (Ryden, 1986; Jordan, 1989). In the present study, the concentration of soil nitrate was an important factor limiting the rate of denitrification, principally in the legume-based systems (Fig. 6.4). In Grass+N400 it was apparent that soil nitrate, on average, was above the threshold level referred to in the literature for denitrification in the field. It remains questionable whether a greater nitrate concentration in winter would still increase denitrification to higher levels. In this regard, denitrification and leaching are competitive loss processes for available nitrate, and in this soil leaching losses were greater than denitrification; but under other conditions opposite results may occur, as was found by Jordan (1989) in a poorly drained soil. Also it is important to consider the possibility that some N_2O may leave the soil dissolved in drainage water. Losses of dissolved N_2O ranging from 0.25 to 4.4 kg N/ha have been measured in drainage from agricultural soils (Dowdell *et al.*, 1979), which were comparable with the gaseous losses of N_2O over the same period.

6.3.4 Indirect effect of animals on denitrification

The grassland systems receiving no N fertiliser (Grass-clover and Herbal ley) showed a special pattern of denitrification in winter (Fig. 6.2), which was closely associated with the stage of regrowth. Normally there were two measurements between grazings, and the higher level of denitrification was obtained in the measurement soon after grazing. This pattern was only evident in the legume-based pastures where soil nitrate was apparently a limiting factor for denitrification. In the Grass+N400, however, the whole area was affected by fertiliser N creating more "site-opportunities" for denitrification

to occur at a high rate.

It is suggested that the enhanced denitrification associated with grazing of the legume-based pastures resulted from the N returned in animal excreta. In this regard, Sherlock and Goh (1983) found a positive effect of sheep urine on N_2O release, even greater than from corresponding applications of ammonium sulphate and urea. If this is the case, we could expect (as it was also explained for leaching) that the small proportion of the area affected by urine patches (see section 4.3.3.5) had a greater denitrification activity and made a larger contribution to the total denitrification from each paddock than the non-affected areas. But because the urine patches were only 10% of the area, their impact on denitrification was small when compared with the effect of fertiliser N. In Grass+N400, the effect of the urine patches was overshadowed by the greater effect of the fertiliser N in the total N emission for the whole paddock.

The transitory effect of the grazing observed in Grass-clover and Herbal ley may have been due to a rapid decrease in the nitrate from the areas affected by animal excreta due to the effect of other competitive processes such as leaching, plant uptake and immobilisation.

In addition to the direct influence of the N returned in animal excreta, other factors associated with the grazing could also have influenced the denitrification rate. For example, immediately after grazing more soil nitrate may accumulate as a consequence of the limited plant uptake from the recently defoliated sward (see Fig. 4.8, Chapter 4). Also trampling and disruption of the structure at the soil surface could have created more anaerobic conditions (at least temporarily) which occurs to some extent during grazing with high stocking rate and high soil moisture content (Warren *et al.*, 1986; Tollner *et al.*, 1990). Fresh residues (with available C) could have been incorporated during grazing, or combinations of all of these effects could have occurred and been reflected in the characteristic pattern of denitrification (Fig.

6.2).

6.3.5 Effect of soil temperature

Denitrification has been reported to be markedly influenced by temperature. The lower limiting soil temperature for high denitrification has been estimated in the field to be in the range 4-6 °C (Ryden, 1986; Jordan, 1989). In the range 10-35 °C denitrification is considered to be a temperature-dependent process with a $Q_{10} = 2$ (Stanford *et al.*, 1975; Dawson and Murphy, 1972).

In this experiment the soil temperature (Fig. 6.4) was not a critical factor for denitrification, in spite of the lowest values occurring during the period conducive to denitrification (winter). These were at least 3 °C above the temperature usually cited as the critical temperature for the process. But soil temperature in this experiment could also be a factor controlling the rate of denitrification, especially in the Grass+N400 system where the substrate supply was apparently adequate. In this regard Bijay-Singh *et al.* (1989) found that in grassland soils at 20 °C the rate of denitrification was approximately three times higher than at 8 °C, particularly at low moisture tension.

The pattern of diurnal variation of soil temperature is shown in Fig.6.7. This figure illustrates that in July, which may be representative of the period when denitrification was active, diurnal variation was probably less important in controlling the rate of denitrification than it would be in other periods of the year. For example, the diurnal temperature variation was most marked in January; therefore the rate of denitrification should fluctuate more in a day in January than in July. But other conditions did not favour denitrification in this period. However, soil temperature might have a greater influence on denitrification in this soil under irrigation in summer.

Several workers have observed marked diurnal variability in the rate of nitrous oxide emission (Denmead *et al.*, 1979; Blackmer *et al.*, 1982), but most of these studies were carried out in the range of 10-30 °C where soil temperature

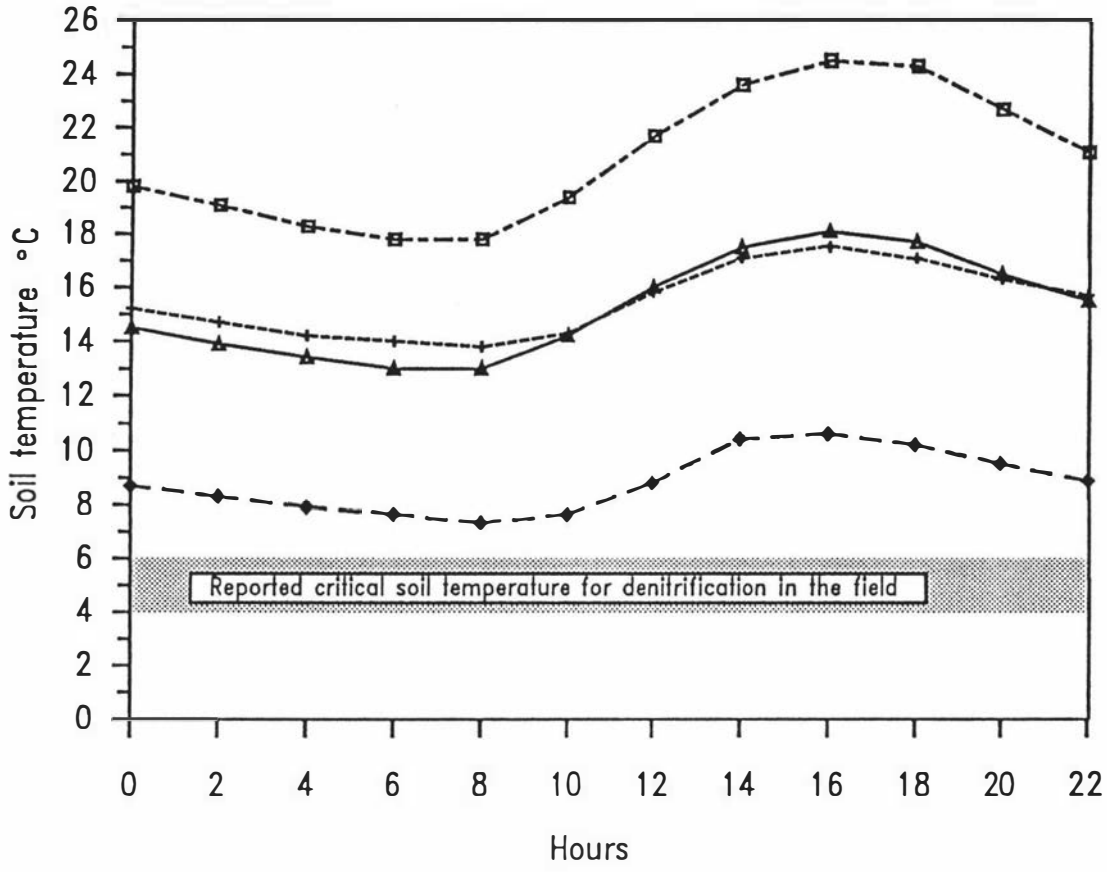

Figure 6.7 Diurnal variation of soil temperature at 10 cm depth. Monthly averages for January, April, July and October during 1990

may have a larger effect on the rate of emission by changing the solubility of nitrous oxide in soil water. Nevertheless, in this experiment we could expect the peak rate of denitrification to occur in the afternoon and the minimum near sunrise.

6.3.6 Product gases (N_2O and N_2) from denitrification

The proportion of nitrous oxide and dinitrogen produced during denitrification appears in Figs 6.2 and 6.3. When total denitrification increased in winter, N_2 was the most important product gas in the three grassland systems, but a significant emission of N_2O was also associated with these larger denitrification rates. Some workers have reported that the ratio N_2O:N_2 produced during denitrification is extremely variable (Ryden *et al.*, 1979; Rolston *et al.*, 1982). In general the amount of N_2 produced is greater than that of N_2O with the N_2O mole fraction in field experiments of the order of 0.12-0.18 (Ryden *et al.*, 1979) and 0.20-0.30 (Rolston *et al.*, 1982).

Nitrous oxide losses may also arise from nitrification in calcareous soils with high ammonium content (Bremner and Blackmer, 1978; Breitenbeck *et el.*, 1980), conditions which could occur for short periods during the hydrolysis of urea.

Under conditions providing relatively inactive denitrification during summer-early autumn, N_2O:N_2 ratio was at its highest (ca 1 or > 1) when most or all of the product gas was nitrous oxide, probably arising from nitrification. By contrast, when the overall rate increased several fold under wetter conditions in late autumn-winter, the N_2O:N_2 ratio fell (range ca 0.1 to 0.3), even though emissions of N_2O increased. Obviously, N_2 was the main product gas from this enhanced denitrification process. On an annual basis the ratio N_2O:N_2 was 0.38 0.30 and 0.27 for Grass-clover, Herbal ley and Grass+N400, respectively. In general it has been reported that N_2 is the dominant gaseous product in most soils, usually accounting for about 80 % of the gaseous N

evolved from the soil surface (Eichner, 1990).

However, the interest in determining the gas product ratio is only limited, because it is impossible to assume the denitrification rate from one of the components, and it is not credible to estimate nitrous oxide only from the total denitrification. Therefore if the objective of the study is to measure the emission of nitrous oxide from an environmental point of view, then it is preferable to measure directly the emission of this gas without blocking the reaction with acetylene. On the other hand, if the interest is in the total losses of N by denitrification, then the use of the acetylene method and measurement of the total gas emission is recommended.

6.3.7 Annual N losses by denitrification

In this experiment total N losses during 1990 (Fig. 6.8) were 3.4, 4.4 and 19.3 kg N/ha/yr for Grass-clover, Herbal ley and Grass+N400, respectively. For the Grass N system this value represents 4.8% of the fertiliser N applied. Values of the same order were reported by Ryden (1983) and Ball and Ryden (1984) from a cut sward. In the legume-based pastures the annual losses of N correspond approximately to 2-3 % of annual symbiotic N fixation. The annual loss from the Grass+N400 pasture system was similar to the annual loss found by Jarvis *et al.* (1991) in a grazed sward fertilised with 450 kg N/ha/yr on a freely drained soil. From the same study these authors reported higher losses of N (47.5 kg N/ha) from a poorly drained soil.

Annual emissions of nitrous oxide (Fig.6.8) were 1.3, 1.3 and 5.3 kg N_2O-N/ha/yr for Grass-clover, Herbal ley and Grass+N400, respectively. In the present study the annual emission of nitrous oxide represented 1.3% of fertiliser N and about 1% of the input by legume fixation. Both figures are in the range 0.5-1.5 % of N fertiliser emitted as N_2O quoted by McElroy and Woofsy (1985) and Ryden (1981). In relation to the legume-based pastures, there is less information available. The review of Eichner (1990) of a wide

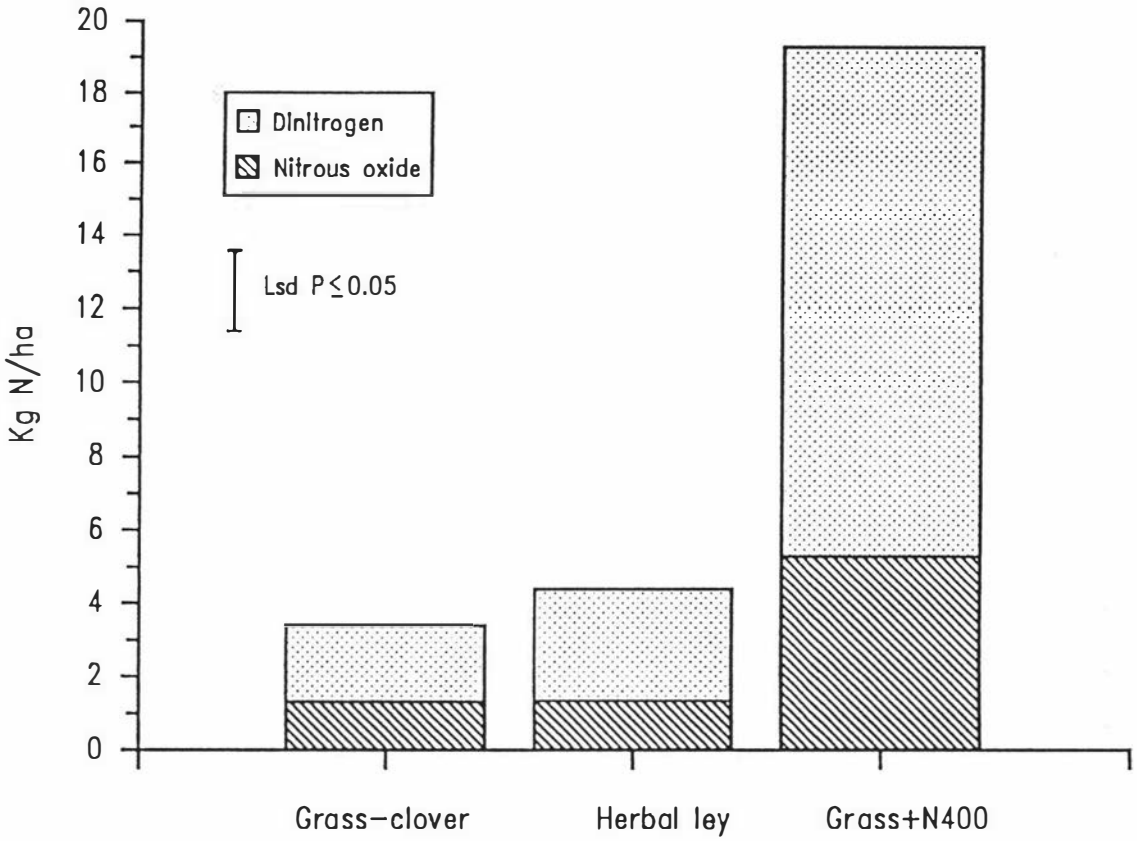

Figure 6.8 Total annual (1990) denitrification measured in the three grassland systems

range of agricultural systems showed that the emission of nitrous oxide from soils cropped with legumes ranged from 0.34 to 4.6 kg N_2O-N/ha/yr.

6.4 Conclusions

The higher losses of N by denitrification occurred in winter when the soil moisture was maintained for an extended period above field capacity. Under this soil moisture content, the air-filled porosity fell below 0.17 m^3/m^3 creating an environment conducive to the highest potential for field denitrification during the year.

When the anaerobic conditions were favourable, denitrification rate was controlled by the presence of substrate (NO_3^- and/or NO_2^-). This was principally realised by the periodic application of urea fertiliser in the Grass+N400 system and to a lesser extent by the excreta of animals in the legume-based pastures.

Soil temperature during the period conducive to denitrification was apparently above the critical level for the denitrification process. But information in the literature indicates that soil temperature higher than that measured in winter could increases the rate of denitrification two times (if $Q_{10} = 2$) for a 10 °C rise, especially when the substrate supply is not restricted.

When biological denitrification occurred, N_2 was the most important product gas. The ratio N_2O:N_2 was highest during summer-early autumn under relatively inactive denitrification. In this period much of the product gas probably arose from nitrification.

The annual loss of N in Grass+N400 was 5 to 6 times greater than in Grass-clover and Herbal ley. A similar difference was observed in losses of N by leaching. While in the latter case losses were clearly related to the concentration of soil nitrate, it seems that not only soil nitrate but other

transient compounds, such as NO_2^- formed during the transformation of urea fertiliser, may also have been a substrate for denitrification.

CHAPTER 7
GENERAL DISCUSSION

7.1 Introduction

The aim of this chapter is to examine and compare the changes in the components of the N cycle for the three grassland systems under study for two years. The time scale chosen for the discussion was the "regrowth period" which in pastures (especially under rotational grazing) constitutes a chain of events associated with perturbations of the natural cycle of N. These major perturbations may be seen as: i) the rate of plant uptake of N being abruptly reduced by the effect of defoliation and ii) most of the N accumulated in the herbage being returned to the soil in localised areas and in highly concentrated form through the animal excreta. Both mechanisms have a significant short term impact on the pool of soil mineral N (see Chapter 4). The grassland system relying on fertiliser N is subject to an additional perturbation by the large artificial pulse of N normally in amounts (40 to 80 kg N/ha) several times greater than the immediate requirement for plant growth, and possibly also in excess of that which can be rapidly assimilated by other soil biological processes.

Annual balances of N with the identification of the major inputs, transformations and outputs have been useful in providing quantitative information on the net changes of N in the systems and are important in long term studies. But a better understanding of the dynamics of N in grazed pastures seems to require more concentrated attention on the fate of N at those critical times when the herbage N is in part converted to valuable animal products (the aim of the farmer), but in the process may also be subject to significant losses (Ball, 1979), with detrimental effect on both the economics of the farm and the wider environment.

Particular attention is also required on the system receiving fertiliser N. A significant impact on the dynamics of N probably occurs not only through the increase in herbage yield and N content (and the subsequent inefficient utilisation of this N by animals), as has been profusely mentioned in the literature, but also during the interval since N was applied to pasture and recovered by the plant.

7.2 Format of the information used in this chapter

For this discussion, the data obtained during the two years in the different studies presented in the previous chapters were organised per grazing period. The accumulated values of the measured components of the N cycle as well as other values estimated from the available literature (details in the next section) are presented in Appendix 4a, 4b and 4c (for each grassland system). From this information several figures were prepared to compare the dynamics of N in Grass-clover, Herbal Ley and Grass+N400 pastures during the study period. With the objective of presenting a comparative annual budget of N in the three pastures, a year comprising nine grazings between 19 February 1990 and 15 February 1991 is displayed in detail in this chapter. Also some annual relationships between pools and transformations were obtained for this defined year.

7.3 Assumptions

7.3.1 Partition of N intake by animals

To partition N intake the values indicated by Henzell and Ross (1973) for mature sheep feeding herbage with about 3.5% N content were used.

- N retention in animal product 5%
- N excreted as urine 71%
- N excreted as dung 24%

Nitrogen ingested by animals was considered to be equal to N yield. Precautions were taken during herbage measurements to leave approximately the same herbage residue in both the grazed paddocks and the evaluation cages.

7.3.2 Nitrogen losses by volatilisation

It was assumed that N loss by volatilisation occurred from the urine of the animals and from urea fertiliser (in the case of Grass+N400). The losses from urine-affected areas were estimated from the results of an experiment done in the same area of this study and reported in Ball and Ryden (1984) for different periods of the year. Volatilisation loss as a percentage of N applied in urine and urea fertiliser was estimated as follows:

- 16% during the warm moist season (September, October, November and April)
- 66% during the warm dry season (December, January, February and March)
- 5% during the cool moist season (May, June, July and August).

The volatilisation losses from urea fertiliser broadcast on pasture were estimated as 5-30% of the N applied, from the study of Selvarajah *et al.* (1989). The lowest percentage was applied to the cool moist season, the highest to the warm dry season and 15-16% during the warm moist season, following similar criteria as for the volatilisation of N from urine.

7.3.3 Nitrogen inputs in rainfall

An annual N input in rainfall was estimated as 3 kg N/ha from Miller (1961). A proportion of this figure was calculated according to the number of days comprising each regrowth period.

7.3.4 Nitrogen input by non-symbiotic fixation

An annual input of 13 kg N/ha was estimated from the study of Grant and Lambert (1979). As for N input by rainfall, a proportion of this annual value was calculated for each regrowth period.

7.4 Comparison of N inputs, N harvested in yield and N outputs in the three grassland systems

7.4.1 Nitrogen inputs

The input of N during each regrowth period is shown in Fig. 7.1 for the three grassland systems. In Grass-clover and Herbal ley the major input of N was from symbiotic N fixation, while in Grass+N400 it was due to the periodic application of urea fertiliser. Other inputs, such as N in rainfall and asymbiotic fixation by free-living organisms, made a small "background" contribution to the total N supplied to each grassland system.

The pattern of N input in Grass-clover and Herbal ley was similar. The amount of N inputs at each regrowth to these systems varies according to the season and the extent of the regrowth period. Fig. 7.1 shows that the application of urea fertiliser imposed a pattern of N input markedly different from that observed for the natural process of symbiotic fixation.

The effect of two rates of fertiliser application (40 and 80 kg N/ha), and the suspension of the fertilisation for a period from January to March 1990, determine that the supply of N in Grass+N400 pasture occurred in artificially contrasting and sporadic events (fertiliser N was normally applied after each grazing period). This constitutes one of the main differences in relation to the legume-based pastures where N inputs by the symbiotic process occurred gradually through the regrowth period. But the symbiotic process supplied N directly to the legumes only, and this N had to be subsequently transferred to

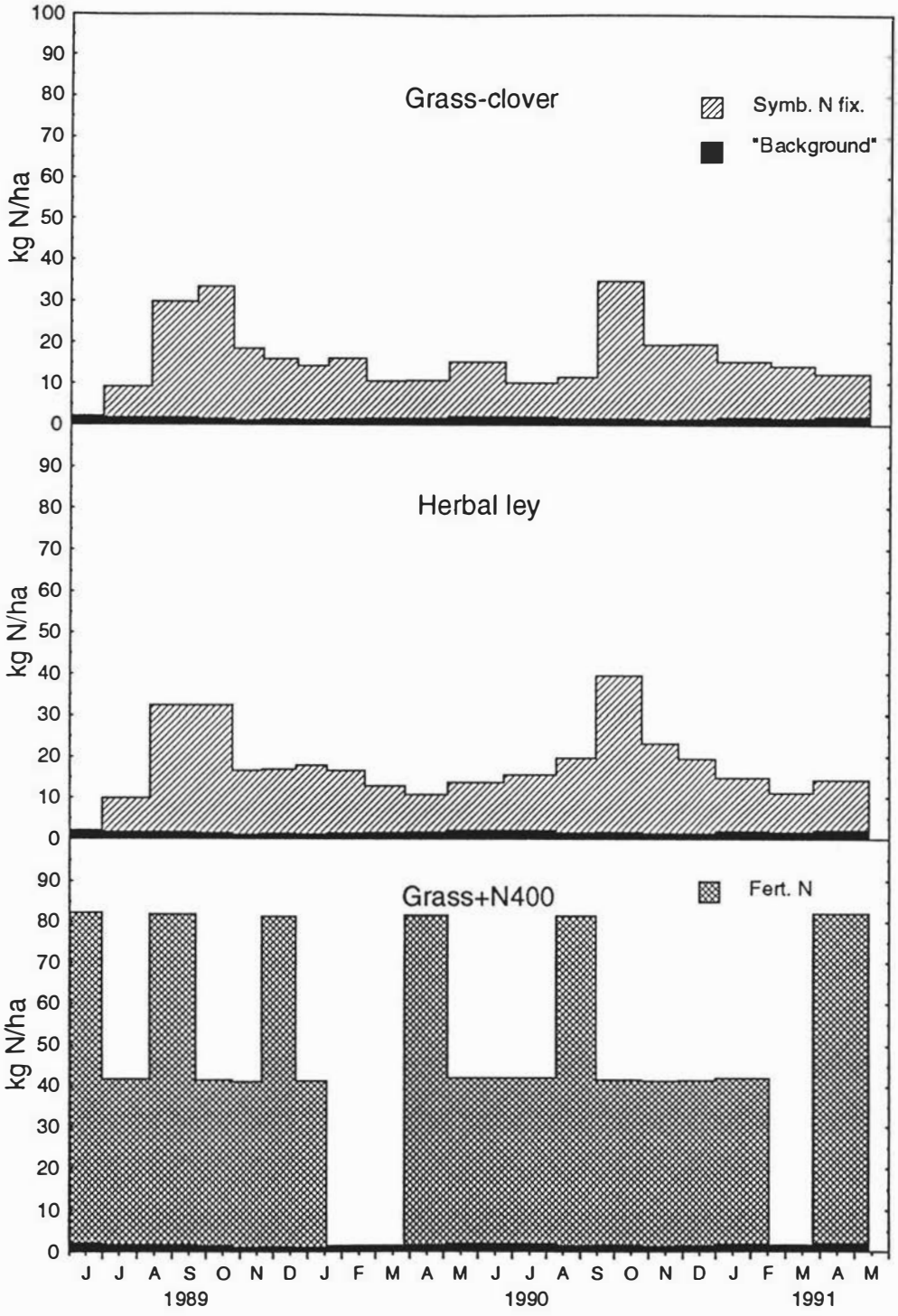

Figure 7.1 Nitrogen inputs during each regrowth period

the whole soil-plant system through recycling via plant residues (also a gradual process) and via the grazing animal (a less gradual process).

Given that the average herbage growth rate measured in this experiment was about 50 kg DM/ha/day (Fig. 3.2), with an N content of about 3.5%, the demand for N would be approximately 1.8 kg N/ha/day in the harvested herbage and probably no more than 2.9 kg N/ha/day in the whole plant. It becomes clear that the common practice of applying fertiliser N after each grazing at the rates mentioned before leaves, for a considerable period, an amount of available N several times greater than that required to match the immediate plant requirement. Several days would elapse before the active plant growth could recover a significant amount of the fertiliser N. Although the soil microbial biomass can temporarily immobilise part of the fertiliser N (Ledgard *et al.*, 1988; 1989), it is also unlikely that this and other soil processes could assimilate in a short time the excess N resulting from an application of fertiliser.

The different pattern of N inputs in the legume-based pastures and Grass+N400 influenced the dynamics of the pool of mineral N in the soil. These distinctions can be seen both in the long term tendency (Fig. 4.4) and in the more detailed studies carried out between grazing periods (Figs 4.8 and 4.10 for autumn and spring conditions, respectively). The large increment in the mineral N pool observed in the Grass+N400 (after each application of fertiliser N in particular) could constitute another focal point for the biological inefficiency in N utilisation in fertiliser-based swards, especially when compared with legume-based pastures.

Thus, while both legume-based and N-fertilised pastures are subject to similar inefficiency when N is converted to animal products (especially if the N content of the herbage does not differ significantly as in this experiment), the Grass+N400 system is more susceptible to loss of N because of the additional amount of soil mineral N resulting from the fertiliser application.

7.4.2 Nitrogen yield

The flow of N through herbage yield in the three grassland systems is presented in Fig. 7.2. For the legume-based pastures total N yield was presented as the sum of the N yield of non-legume and legume components of the sward.

The amount of N in harvested yield was closely associated with the DM production in the three systems (Chapter 3), but in Figure 7.2 it is possible to appreciate the fluctuations of N in herbage during each regrowth period at different times of the year.

Grass-clover pasture showed a more uniform pattern of N yield than the Herbal ley both in the legume and non-legume component. The greater variation in N yield observed in Herbal ley occurred principally during the period of spring and summer when this grassland system demonstrated the highest potential for pasture production (Fig. 3.2 in Chapter 3). The enhanced N yield (more apparent in 1990) in Herbal ley compared with Grass-clover affected both the legume and non-legume component. The difference in the legume component was presumably due in part to a small increase in the rate of Snf in the period August-November in 1990 (Figure 3.5), reflected also in a slightly greater accumulation of N input by this means in Herbal ley in relation to Grass-clover (Fig. 7.1). But because the legume component in Herbal ley was significantly greater than in Grass-clover (Fig 3.3 and Table 3.3), and hence there was less N fixed on a per legume basis (Table 3.4), we may infer that the legumes in Herbal ley had taken up more soil N than legumes in Grass-clover. This inference becomes more apparent when we compare the N yield of the non-legume components in Fig. 7.2. Because these species depended on soil mineral N, they must have used more soil N to account for the greater N yield observed in Herbal ley than in the Grass-clover system.

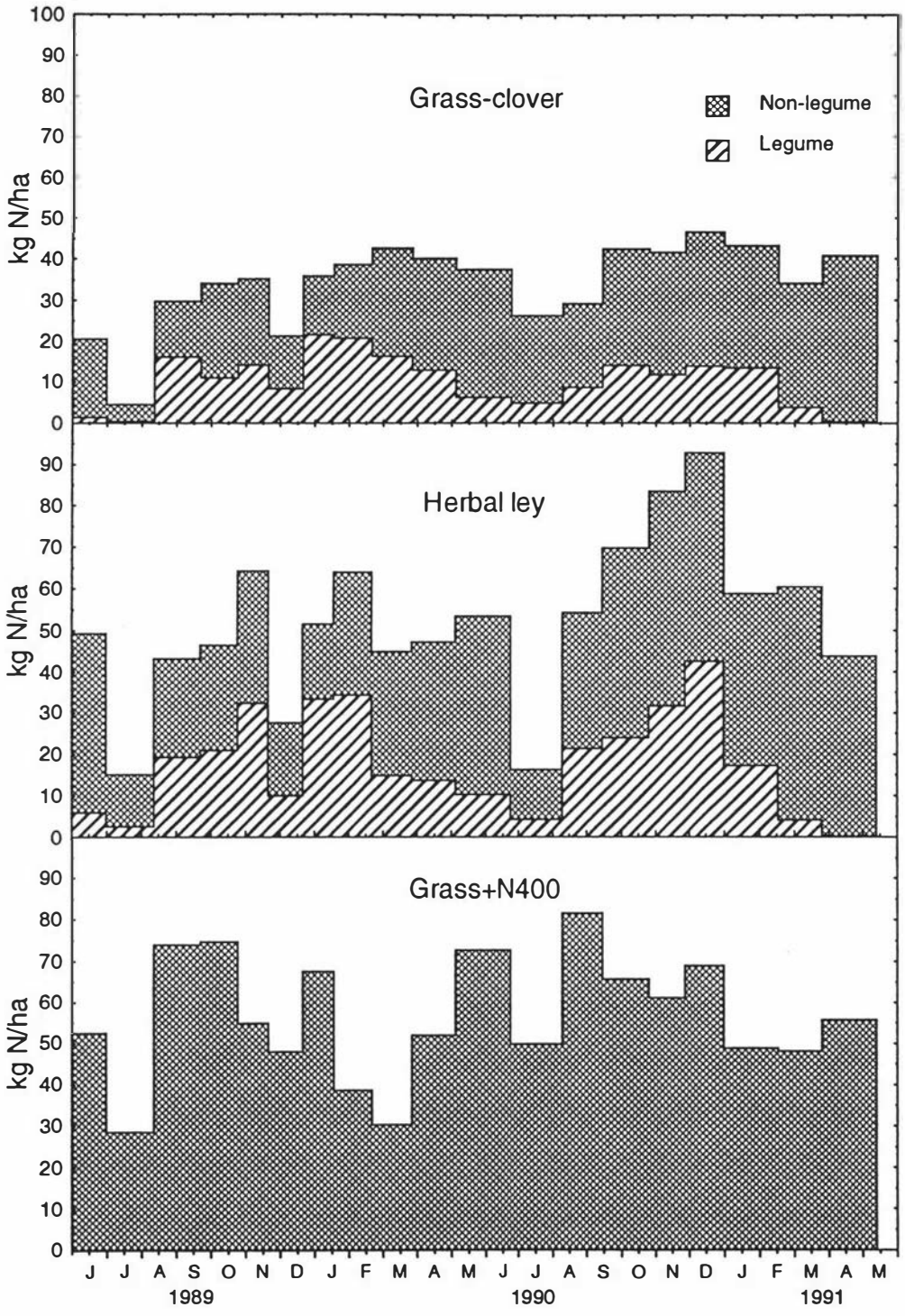

Figure 7.2 Herbage N yield in the three grassland systems

In Chapter 3, the difference in the apparent utilisation of soil N between Grass-clover and Herbal ley was attributed to a more active mineralisation of organic N by the species in the Herbal ley. Coupled with a greater potential for plant growth, as occurred with chicory in spring-summer (Rumball, 1986; Clark *et al.*; 1990), this could result in a rapid uptake of N, in which case the enhanced mineralisation of N would not be reflected in the measured soil mineral N (see Fig 4.4).

Several workers during the last 3 or 4 years have drawn attention to the significant effect of roots in inducing N mineralisation, which is also tightly coupled to root uptake. The explanation given is that carbon (C) released into the soil from roots could enhance the availability of inorganic N to plants by stimulating microbial activity in the rhizosphere zone (Robinson *et al.*, 1989). The same author pointed out that a greater amount of N mineralisation may expected when the substrate released from the root has a high C:N ratio than when it is relatively close. The mechanism governing this process is the result of a complex interaction between plant and microorganisms in the rhizosphere which has been explained in Clarholm (1985, 1989), and Kuikman and Van Veen (1989). Clarholm (1989) suggests that benefits to the plants from local N mineralisation are due principally to the associated increase in bacterial-protozoan interaction around the roots. This ecological interrelationship may be explained as follows:

i) The release of carbonaceous compounds from the root increases the microbial biomass in the rhizosphere compared with the bulk of the soil (Clarholm, 1985; Newman, 1985).

ii) The larger microbial biomass stimulated by the energy supplied by plant roots results in an increased breakdown of soil organic matter in support of their own growth (Clarholm, 1985; Robinson *et al.*, 1989). The effect is analogous to a positive priming action, as discussed by Jenkinson (1966). Nutrients such as N, P and S in mineral form in the soil, or released by the enhanced decomposition of soil organic matter,

are temporarily immobilised in the microbial biomass.

iii) Predation of the enhanced microbial biomass by protozoa stimulates the mineralisation and turnover of bacterial N. The mineralisation of soil organic N has also been shown to be promoted by protozoa (Kuikman and Van Veen, 1989).

iv) N released by this bacteria-protozoan interaction in the rhizosphere is readily taken up by the plant (Clarholm, 1989).

It is generally accepted that growing roots are a significant source of C for the microbial biomass, but not all studies have demonstrated such effect on stimulation of net mineralisation of organic N in the rhizosphere, as for example the work of Klemedstsson *et al.* (1987). Therefore it seems that differences in root exudation by different plant species (Rovira, 1969), and even cultivars of the same species (Liljeroth and Baath, 1988), may result in differential effects on the type of microorganisms in the rhizosphere (Martin, 1971; Parkinson *et al.*, 1963) and hence in the root-induced mineralisation process.

Thus, in this experiment the enhanced N yield observed in Herbal ley may have been the result, at least in part, of "root-induced" N mineralisation. If the driving force for this process is an exudate rich in soluble carbon, this may be the case in Herbal ley. Some of the most important species in the Herbal ley (chicory) have the particular characteristic of possessing a rooting system highly rich in soluble sugar content which increases the C content of the exudates. This characteristic of chicory was demonstrated by Douglas and Poll (1986). They measured a higher yield of roots, having also a soluble sugar content varying from 10.6 to 20.5%.

In addition, the enhanced uptake of N by Herbal ley in relation to the Grass-clover system was reflected in the pattern of herbage nitrate concentration shown in Fig. 7.3. If the nitrate concentration in herbage is considered to be an index of N nutrition (van Burg, 1966), then it is clear that species in Herbal

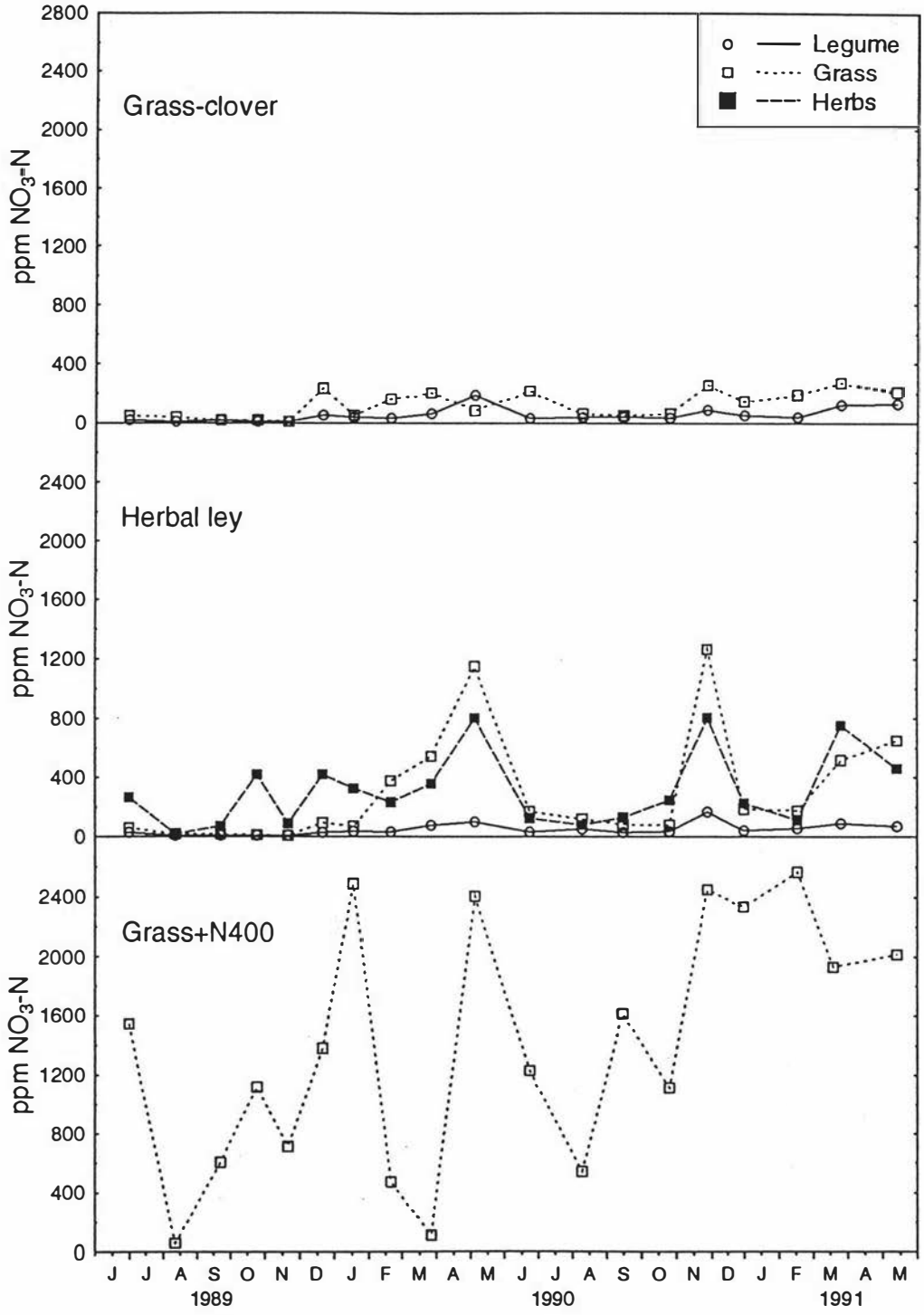

Figure 7.3 Nitrate concentration in herbage

ley, principally herbs and grass species (other than ryegrass, see Table 2.3), were able to take up more soil N than grasses in the Grass-clover system; hence the influence of N uptake on the large difference in pasture production between the two systems.

Herbage N yield in Grass+N400 (Fig. 7.2) was greater than Grass-clover most of the time, but not always when compared with Herbal ley. The higher levels in Grass+N400 were closely associated with the higher rate of fertiliser N application (Fig. 7.1) and conversely, the lowest N yield was measured in January-February 1990 during the longest period that fertiliser N was suspended. The decreased N yield in summer did not occur in the same proportion in the legume-based pastures, demonstrating the strong dependency of the pure grass sward on fertiliser N. This effect also appears in the herbage nitrate concentration of the Grass+N400 system (Fig. 7.3), which shows a lower value in February - March 1990, increasing rapidly in April after the fertiliser N was resumed. In general, nitrate concentration in herbage was higher and more variable in Grass+N400 than in the other two pastures, presumably as a consequence of the greater and more variable input of fertiliser N.

7.4.3 Nitrogen outputs

Nitrogen outputs from the three grassland systems during each regrowth period are shown in Fig. 7.4. Part of the N leaves the farm in animal products which may be considered as a "productive output". In contrast, N leaching, denitrification and volatilisation are undesirable outputs constituting losses of N from the system.

Figure 7.4 shows that the output of N in animal product corresponds to a small fraction only of the total N output from the three systems. In Grass-clover, N in animal product through the year was more uniform than in the other two pastures as a result of a less variable pattern in pasture production and

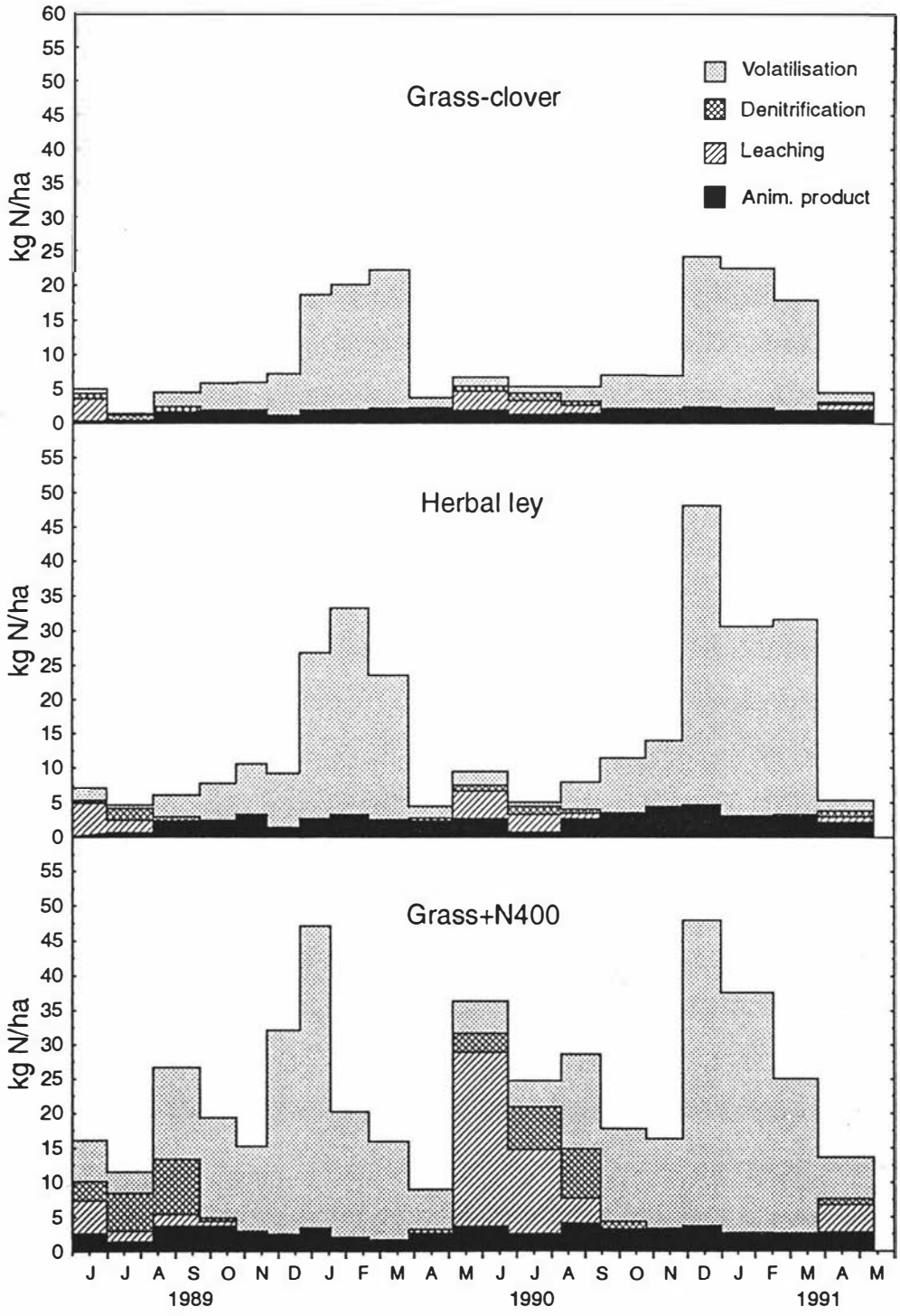

Figure 7.4 Nitrogen outputs during each regrowth period

herbage N yield, as was shown in Fig. 7.2

Because N in animal produce was also a small proportion of the N yield, most of the N ingested by animal was recycled to the soil, particularly as urine. This flow is an important component of the N cycle (Parsons *et al.*, 1991) and also influences the N loss processes (Ball *et al.*, 1979).

The greatest losses of N occurred presumably by volatilisation. As was explained in 7.3, volatilisation was not measured in this study but was calculated using a proportion of N volatilised from N excreted in urine, according to the available literature in New Zealand. In this way it was possible to offer an integrated view of the main losses processes. But it is necessary to recognise that most of the recent research (Jarvis *et al.*, 1989a, b; Jarvis *et al.*, 1990; Parsons *et al.*; 1991) on volatilisation of N in pastures shows annual values substantially lower than the result calculated for the presented study. This large discrepancy may be due to differences in the environmental conditions where the studies were carried out and/or the method used in each case. The obvious conclusion is that more research is required on this theme, especially in New Zealand, where information is scarce.

The losses of N other than by volatilisation were relatively small in the legume-based pastures, and these were concentrated principally in winter. For the rest of the year N was lost by volatilisation with the greatest values in summer when temperature and moisture conditions were favourable for this process. Because volatilisation was calculated from the urine excreted, the greater pasture production of Herbal ley in summer and larger N flow through animals increased potential for losses by volatilisation.

In general, the legume-based pastures showed a similar pattern of N losses, which was clearly different from Grass+N400. Volatilisation was greater in Grass+N400 not only because of the effect of animals but also because of the

direct emission from the urea fertiliser. For that reason volatilisation appears greater than in the Herbal ley in some periods of the year, in spite of the N yield (Fig. 7.2) being similar or even smaller in Grass+N400 than in Herbal ley.

Larger differences were observed in winter. Losses of N in Grass-clover and Herbal ley were reduced, but in Grass+400 substantial amounts of N escaped through leaching and denitrification. In winter, the climatic conditions were favourable for both processes in the three grassland system, but only Grass+N400 exhibited large values. Because the magnitude of leaching and denitrification is related to the availability of nitrate, in Grass+N400 in addition to the N recycled by the animals, the supply of fertiliser N (Fig.7.1), by increasing the level of soil mineral N (Fig. 4.4), was also another important cause of N losses (nitrate dependent) when conditions were appropriate.

7.5 Relationships of N inputs, herbage N yield and its conversion to animal product, and total losses of N during each regrowth period

The magnitude of the major components of N dynamics in the three grassland systems during each regrowth period is presented in Fig. 7.5. The objective of this figure is to put in a logical sequence the fate of N from a productive point of view, but also displaying the associated flux of N to the wider environment. It was also intended to show the dynamic balance between inputs and outputs of N during each regrowth period of the 2-year study.

The objective of the farmer for incorporating N into the soil (either by fertiliser N or the symbiotic process) is to improve pasture production, principally by increasing DM yield (of the above ground part of the plant). Subsequently plant N has to be converted to animal product which is the ultimate target of a grassland enterprise. But a significant amount of N may escape from the system which is a matter of concern for the scientists interested in the environment, but relatively ignored by the farmer and the rest of the community.

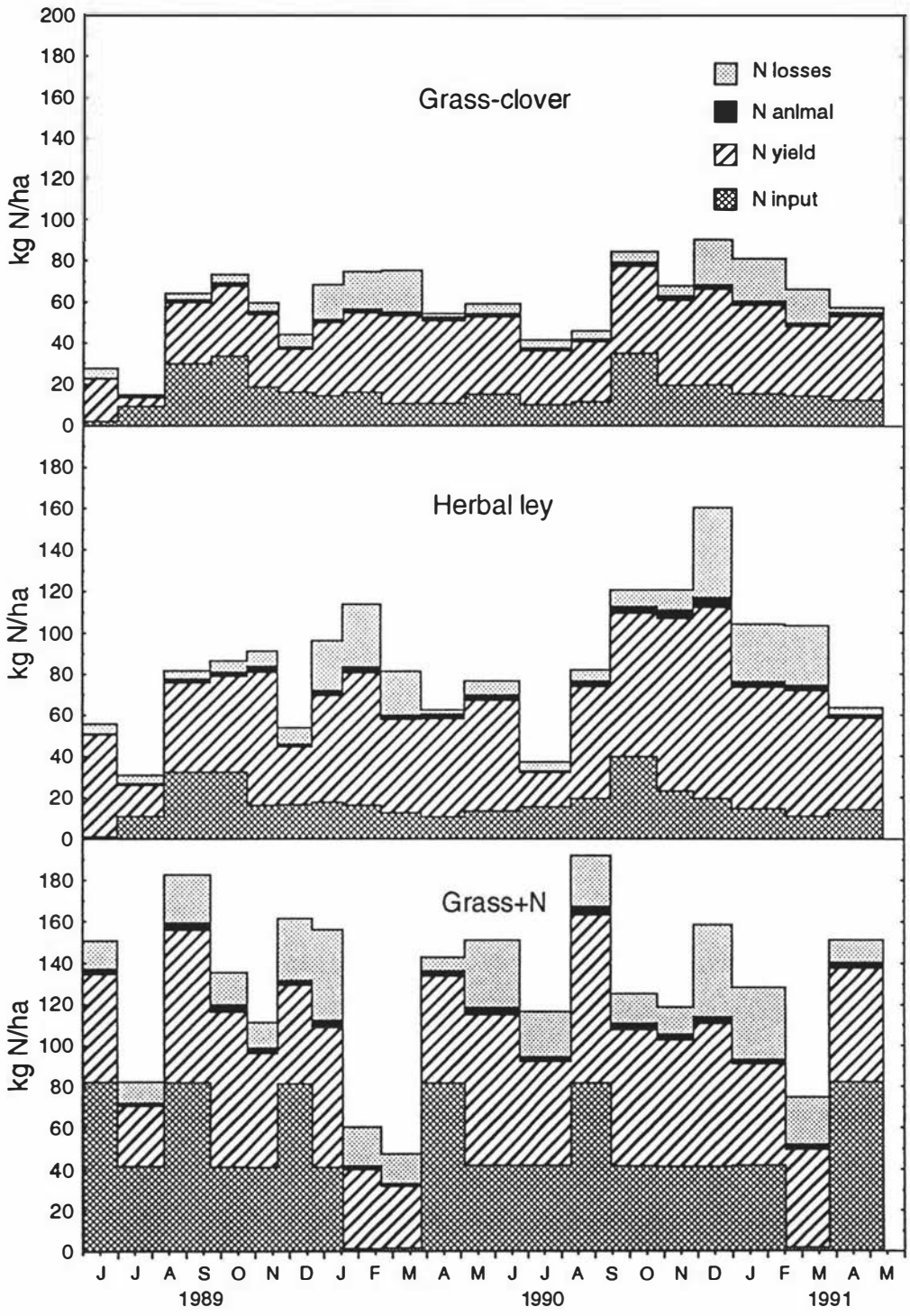

Figure 7.5 Nitrogen flux during each regrowth period

From the simple view point of the link between N inputs and herbage N yield, Fig. 7.5 shows that the best relationship was obtained in the Herbal ley pasture (more N yield per unit of N inputs). The apparent difference in N yield for similar N inputs for the two legume-based pasture is another way to visualise the differential utilisation of soil N by these two pastures over the whole period.

In both legume-based pastures during most of this study, herbage N yield was greater than the corresponding input of N for the period, indicating that the external input (principally symbiotic N fixation) was complemented by soil mineral N, much of which was recycled through the animal, in supplying the plant. If we consider that N in herbage is only a fraction of the demand of the whole plant, the importance of both external input and soil N in providing adequate N for high yields becomes more obvious.

In the Grass+N400, N input by fertiliser was less closely correlated with N yield than in the legume-based pastures. In several regrowth periods inputs of N exceeded N recovered in herbage (this comparison is based on net values and does not mean that N in the plant corresponds in real terms to the N applied). On the other hand in summer, fertiliser N was withheld in some regrowth periods and there was practically no input of N.

In general Fig. 7.5 indicates that the biological efficiency of N utilisation, expressed by the relationship N yield : N input, seems to be as follows: Herbal ley > Grass clover > Grass+N400. But because farmers normally respond to economic signals rather than to biological efficiency, a higher yield obtained with cheap fertiliser N may be their logical option, as has been the case for the last 40 years in most countries of western Europe.

In contrast, the biological efficiency of N utilisation in animal product was assumed to be only about 5% for the sheep farming system used in this experiment. However, although agriculture is governed mostly by biological

factors, it is also influenced by many other factors, so that the biological efficiency of a component process cannot be measured independently of the total system which integrates a number of other elements (Spedding, 1988). The conversion of plant N into animal product results in economic efficiency because the animals can process herbage as an intermediate product that has no direct value to humans. This point is critical when the land cannot be used for crops for direct consumption. In addition, animal products often result in economic efficiency due to a higher value per unit weight of the animal protein compared with plant protein, reflecting principally consumer preference and not necessarily how many people can be fed.

Losses of N during each regrowth period are also displayed in Fig. 7.5. The magnitude of the N losses was greater than the N exported from the system in animal products. A higher level of losses, representing a larger proportion of N yield, was observed in Grass+N400 than in the legume-based pastures. But losses of N appear well compensated for by the inputs of fertiliser N during most of the period. In Grass-clover and Herbal ley losses of N were sometimes greater than inputs, especially in summer, but are reasonably balanced by larger inputs from symbiotic fixation in other periods of the year. Annual budgets will be presented later to further explain the relationship between inputs and outputs.

Losses of N in a "non-scientific" perspective

The losses of N have recently attracted the attention of scientists (mostly in western Europe for grassland systems) because of the possible impact on the environment. Previously, some attention was directed toward controlling the losses of N because it was a valuable resource. But since agriculture is an economic activity, where the farmer has to pay for the fertiliser (inputs) and subsequently receives money for the production (part of the outputs), and if losses of N (the other part of the output) are of significant importance for human life, then it would be logical in a society largely governed by economic variables that these losses of N should be accounted for in some way too.

Until now N losses have been considered as a "neutral component" of the agricultural business.

This issue is complex, because if the importance of N emission is of concern for the environment, then the impact of agriculture has to be considered in a wider context with all the other sources of N emission. Scientific research has played a key role in discovering the existence of the problem but until now has contributed only a small amount of data (mostly point measurements). The available data also show large discrepancies in the measurements, for example of N leaching, volatilisation and denitrification. They therefore have limited utility where losses have to be controlled on a large scale. Therefore it seems that this problem is only in its early stage of diagnosis, and the solution in the future will require more knowledge, concurrently with political decisions.

7.6 Annual N budget and changes in soil total N

7.6.1 Annual N budget

The term N budget is defined as the application of mass conservation principles so that N is conserved in the various transformations and biological processes of the system (Legg and Meisinger, 1982). Thus N inputs and outputs must balance, after allowance for changes in N storage in the system. Ecosystems generally gain or lose organic N at a diminishing rate until an equilibrium N level is reached that is determined by climate-soil-plant-management variables. However, it is difficult to measure small changes in soil N which are likely to occur from year to year relative to the very large pool of total soil N. Therefore, due to the short time scale of this experiment (for measuring significant changes in soil N) the objective of this section is to compare in quantitative form, only as an approximation, the major inputs and outputs of N expressed on an annual basis in the three grassland systems. No statistical analysis was attempted as some parameters were estimated

from the literature and also because statistical treatment may create an impression of accuracy which is not intended here.

Annual N budgets (in kg N/ha) were calculated from the cumulative values after each regrowth period of the components of the N cycle during one year. The results are presented in Tables 7.1, 7.2 and 7.3 for Grass-clover, Herbal ley and Grass+N400, respectively. In addition a graphic comparison is displayed in Fig. 7.6.

Input of N was substantially greater in Grass+N400 than in Grass-clover and Herbal ley. This represents the difference between the magnitude of the fertiliser N application and the symbiotic fixation in the legume-based pastures. Annual inputs of N were 147, 170 and 416 kg N/ha/yr for Grass-clover, Herbal ley and Grass+N400, respectively. The amount of N input was different, but probably more important biologically was the way the N was incorporated into the system, as was pointed out in section 7.4.1. In general, fertiliser application, as with many other controls introduced by modern agriculture, is essentially achieved by the elimination (or reduction) of biological processes. In this case symbiotic fixation is replaced by artificial fertiliser N with the associated increase in energy consumption.

Outputs of N from the three systems were 104, 155 and 234 kg N/ha/yr for Grass-clover, Herbal ley and Grass+N400, respectively. Of the total output, N in animal product represented a small proportion, being about 17% in the legume-based pastures and 11% in the Grass+N400 pasture. The difference between total outputs and N retention in the animal represented losses in a non-productive form. Of the losses of N, the highest proportion was accounted for in N volatilisation, especially in Grass+N400 where in addition to the emission of ammonia from animal urine (96 kg N/ha) another important avenue was the volatilisation of N directly from the urea fertiliser (52 kg N/ha) during its hydrolysis on the soil surface.

Table 7.1 Components of the N budget during a year comprising nine grazings followed by regrowth of the Grass-clover system (19 February 1990 - 15 February 1991)

Date	Nr	Nns	Snf	HNY	LNY	Na	Nl	Nd	Nvu
	kg N/ha/regrowth period								
19 Feb.									
26 Mar.	0.3	1.3	9.0	42.6	16.2	2.1	0.0	0.1	20.0
4 May	0.3	1.4	9.0	40.0	12.8	2.0	0.0	0.3	1.4
22 June	0.4	1.7	13.0	37.4	6.2	1.9	2.7	0.8	1.3
9 August	0.4	1.7	8.0	26.0	4.8	1.3	2.0	1.1	0.9
14 Sept.	0.3	1.3	9.9	29.0	8.5	1.5	1.1	0.6	2.1
25 Oct.	0.3	1.4	33.2	42.3	13.9	2.1	0.0	0.1	4.8
26 Nov.	0.3	1.1	18.1	41.5	11.6	2.1	0.0	0.1	4.7
30 Dec.	0.3	1.2	18.1	46.5	13.8	2.3	0.0	0.1	21.8
15 Feb.	0.4	1.6	13.3	43.2	13.4	2.2	0.0	0.1	20.2
TOTAL	3.0	12.7	131.7	348.5	101.2	17.5	5.8	3.3	77.2

Nr = N in rain; Nns = N non-symbiotic; Snf = N symbiotic; HNY = herbage N yield; LNY = legume N yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvu = N lost by volatilisation from urine.

Annual Budget (kg N/ha/yr)

Inputs		Outputs	
Nr =	3.0	Na =	17.5
Nns =	12.7	Nl =	5.8
Nsnf =	131.7	Nd =	3.3
	147.4	Nvu =	77.2
			103.8

Outputs/inputs x 100 = 70.4 %
Apparent gain of N (kg/ha/yr) = 43.6

Soil mineral N (kg N/ha-45 cm)
Initial = 10.5
Final = 21.7
Δ Soil mineral N = + 11.2

Table 7.2 Components of the N budget during a year comprising nine grazings followed by the regrowth of the Herbal ley system (19 February 1990 - 15 February 1991)

Date	Nr	Nns	Snf	HNY	LNY	Na	Nl	Nd	Nvu
	kg N/ha/regrowth period								
19 Feb.									
26 Mar.	0.3	1.3	11.3	44.9	14.9	2.2	0.0	0.4	21.0
4 May	0.3	1.4	9.1	47.2	13.8	2.4	0.0	0.4	1.7
22 June	0.4	1.7	11.6	53.4	10.4	2.7	4.0	0.9	1.9
9 August	0.4	1.7	13.5	16.3	4.3	0.8	2.4	1.2	0.6
14 Sept.	0.3	1.3	18.0	54.3	21.4	2.7	0.8	0.6	3.9
25 Oct.	0.3	1.4	37.8	69.7	83.9	3.5	0.0	0.1	7.9
26 Nov.	0.3	1.1	21.8	83.5	31.7	4.2	0.0	0.3	9.5
30 Dec.	0.3	1.2	18.0	92.7	42.5	4.6	0.0	0.2	43.4
15 Feb.	0.4	1.6	12.8	58.7	17.2	2.9	0.0	0.3	27.5
TOTAL	3.0	12.7	153.9	520.7	240.1	26.0	7.3	4.4	117.4

Nr = N in rain; Nns = N non-symbiotic; Snf = N symbiotic; HNY = herbage N yield; LNY = legume N yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvu = N lost by volatilisation from urine.

Annual Budget (kg N/ha/yr)

Inputs			Outputs		
Nr	=	3.0	Na	=	26.0
Nns	=	12.7	Nl	=	7.3
Nsnf	=	153.9	Nd	=	4.4
		169.6	Nvu	=	117.4
					155.1

Outputs/inputs x 100 = 91.4 %
Apparent gain of N (kg/ha/yr) = 14.5

Soil mineral N (kg N/ha-45 cm)
Initial = 12.1
Final = 10.4
Δ Soil mineral N = -1.7

Table 7.3 Components of the N budget during a year comprising nine grazings followed by regrowth of the Grass+N400 system (19 February 1990 - 15 February 1991)

Date	Nr	Nns	Nf	HNY	Na	Nl	Nd	Nvf	Nvu
				kg N/ha/regrowth					
19 Feb.									
26 Mar.	0.3	1.3	0	30.3	1.5	0.0	0.2	0.0	14.2
4 May	0.3	1.4	80	52.0	2.6	0.0	0.6	4.0	1.8
22 June	0.4	1.7	40	72.7	3.6	25.3	2.8	2.0	2.6
9 August	0.4	1.7	40	49.8	2.5	12.2	6.2	2.0	1.8
14 Sept.	0.3	1.3	80	81.6	4.1	3.6	7.1	8.0	5.8
25 Oct.	0.3	1.4	40	65.8	3.3	0.0	1.0	6.0	7.5
26 Nov.	0.3	1.1	40	61.1	3.1	0.0	0.3	6.0	6.9
30 Dec.	0.3	1.2	40	69.0	3.5	0.0	0.2	12.0	32.3
15 Feb.	0.4	1.6	40	48.8	2.4	0.0	0.3	12.0	22.9
TOTAL	3.0	12.7	400	531.1	26.6	41.1	18.7	52.0	98.2

Nr = N in rain; Nns = N non-symbiotic; Nf = fertiliser N; HNY = herbage N yield; LNY = legume N yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvf = N lost by volatilisation from urea fertiliser; Nvu = N lost by volatilisation from urine.

Annual Budget (kg N/ha/yr)

Inputs			Outputs		
Nr	=	3.0	Na	=	26.6
Nns	=	12.7	Nl	=	41.1
Nf	=	400.0	Nd	=	18.7
		415.7	Nvu	=	95.8
			Nvf	=	52.0
					234.2

Outputs/inputs x 100 = 56.3 %
Apparent gain of N (kg/ha/yr) = 181.5

Soil mineral N (kg N/ha-45 cm)
Initial = 11.7
Final = 54.9
Δ Soil mineral N = + 43.2

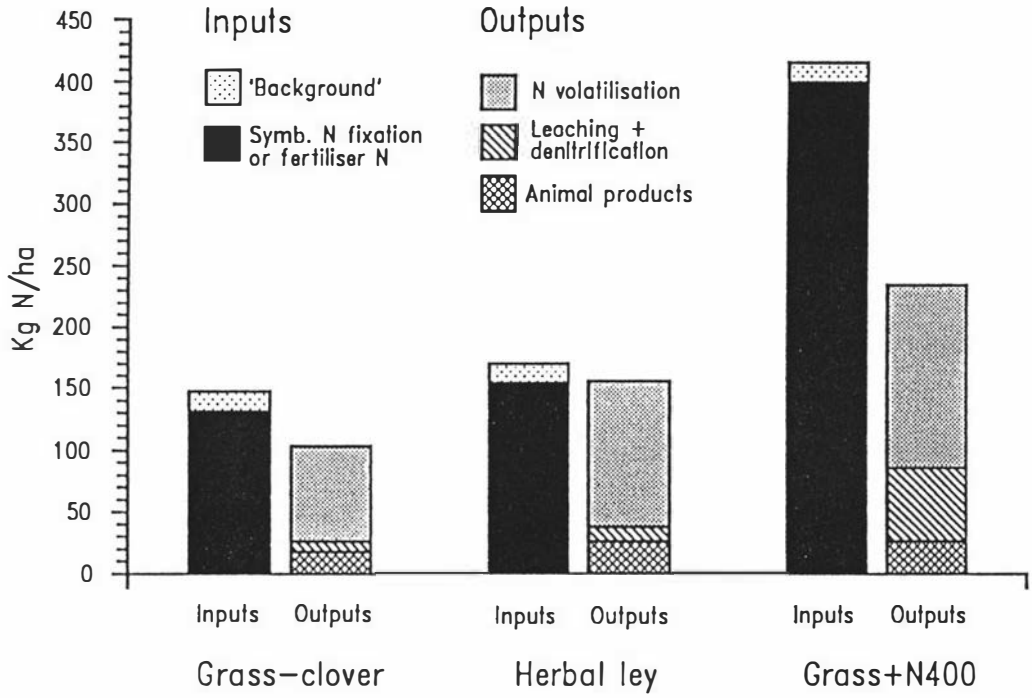

Figure 7.6 Annual N budget for the three grassland systems

(Background = N in rainfall + non-symbiotic N fixation)

In the three grassland systems outputs of N were smaller than inputs, but the ratio and the hypothetical net change in soil N were different. In Grass-clover, output of N represented 70.4% of the annual inputs, with an apparent net gain of 44 kg N/ha/yr. In Herbal ley, outputs and inputs of N were practically in balance. Outputs comprised 91.4% of N input with a small apparent gain of 14 kg N/ha/yr. The magnitude of the difference in N was greater in the Grass+N400 than in the other two pastures. The outputs of N were only 56.3% of the total inputs, and consequently there was an apparent gain of 182 kg N/ha/yr. This apparent gain was accounted for in the increase of total soil N (next section).

From the information used in the preparation of the annual budgets (Tables 7.1 to 7.3) another aspect of N dynamics can be calculated to illustrate some additional differences between the pasture systems under study. Table 7.4 shows the quantitative relationships between herbage N yield, the fate of N after being consumed by animals and total N losses from each pasture system. Animal N cycling accounted for the greater fraction of the intake in the three systems. Herbage N yield was similar (being similar in both N content and DM yield) in Herbal ley and Grass+N400, so that N in animal produce and recycled to the soil also coincided. In Grass-clover pasture the amount of N movement through this pathway was smaller. There was a consistent relationship between the level of production, the amount of N cycling through the animals and the total N losses in the legume-based pastures. In fact, N yield in Herbal ley was about 49% greater than in Grass-clover, and the same proportion was carried on through N cycled by animals and reflected in the differences in N losses. Thus, loss of N was closely associated with production in the proportion of about 5 kg of N loss per kg of N in animal produce. Table 7.4 shows that total N losses corresponded to 25% of the herbage N in both legume-based systems.

Losses of N in Grass+N400 were proportionally less related to the N cycling through animals than was the case in the legume-based pastures. Table 7.4

Table 7.4 Relationships between herbage N yield, animal N cycling and total N losses in a year comprising nine grazings (19 February 1990-15 February 1991)

	Grass-clover	Herbal ley	Grass+N400
		kg N/ha/year	
N yield	349	521	531
N animal produce	18	26	27
N cycling by animals	331	495	504
Total N losses	86	129	208
Losses as % of N yield	25	25	39

(Animal N cycling = N yield - N in animal produce)

shows that the total loss of N was about 61% (129 vs 208 kg N/ha) greater in Grass+N400 than in Herbal ley, in spite of the difference in N cycling through the animals being only 2% (495 vs 504 kg N/ha). This was because an important proportion (52 kg N/ha, Table 7.3) of the total N losses in Grass+N corresponded to the direct emission of ammonia from the urea fertiliser. Also, because of the higher level of soil mineral N maintained by the periodic application of fertiliser N, losses of N could be increased from the whole soil in addition to that associated with N cycled through the animals.

7.6.2 Soil total nitrogen and carbon

Total soil N and C were determined annually during the experimental period. The results for the three pasture systems appear in Tables 7.5 and 7.6, for N and C, respectively. In general, there was no significant difference in the changes of total soil N between years in the three pastures, with the exception being Grass+N400 at 0-75 cm depth between 1990-91. Despite the lack of statistical significance a consistent trend to increase with time was apparent, especially in the topsoil of the Grass+N400 from 1990 to 1991.

Monitoring total soil N is important to detect if any system is 'N-gaining' 'N-losing' or 'N-stable' (O'Connor, 1974). However, the studies of soil total N in permanent pastures are compounded by difficulties related to spatial variability (Vallis, 1973) and the large pool of N (Ball, 1979). The error associated with the sampling intensity and the laboratory analysis in the studies of soil N in pasture soils is well documented in Ball (1979). One of the major difficulties arises because some important agronomic aspect like, for example, the input of N by symbiotic fixation, or N losses such as leaching, represent only a small fraction of the total pool. It is therefore difficult, if not impossible, to detect changes in the total pool of that magnitude on an annual basis. Ball (1979) showed that a change of 100 kg N/ha in the top 15 cm of a pasture soil (white clover - ryegrass) represented a change of 0.006% in the total N content, which required a sampling intensity of between 140-170 soil cores per 250 m^2

Table 7.5 Changes in total soil N (%) at different depths in the three grassland systems

Pasture	depth (cm)	Date of sampling		
		Feb. 1989	Feb. 1990	Feb. 1991
Grass-clover		Total N (%)		
	0-7.5	0.20 a	0.20 a	0.22 a
	7.5-15	0.12 a	0.12 a	0.13 a
	15-30	0.08 a	0.07 a	0.09 a
	30-45	0.06 a	0.05 a	0.08 a
Herbal ley				
	0-7.5	0.21 a	0.21 a	0.23 a
	7.5-15	0.14 a	0.13 a	0.14 a
	15-30	0.09 a	0.08 a	0.09 a
	30-45	0.07 a	0.06 a	0.08 a
Grass+N400				
	0-7.5	0.20 a	0.21 a	0.23 b
	7.5-15	0.13 a	0.12 a	0.13 a
	15-30	0.08 a	0.08 a	0.08 a
	30-45	0.06 a	0.06 a	0.08 a

Different letters in the same row denote significant differences ($P \leq 0.05$) of total N between years

Table 7.6 Changes in total soil C (%) at different depths in the three grassland systems

Pasture	depth (cm)	Date of sampling		
		Feb. 1989	Feb. 1990	Feb. 1991
Grass-clover		Total C (%)		
	0-7.5	1.89 a	1.92 a	2.07 a
	7.5-15	1.03 a	1.13 b	1.23 c
	15-30	0.64 a	0.71 ab	0.83 b
	30-45	0.46 a	0.56 a	0.68 a
Herbal ley				
	0-7.5	1.91 a	1.96 a	2.13 a
	7.5-15	1.21 a	1.13 a	1.23 a
	15-30	0.74 a	0.74 a	0.82 a
	30-45	0.57 a	0.62 a	0.77 a
Grass+N400				
	0-7.5	1.89 a	2.07 a	2.13 a
	7.5-15	1.09 a	1.17 a	1.21 a
	15-30	0.69 a	0.72 a	0.81 a
	30-45	0.46 a	0.66 a	0.71 a

Different letters in the same row denote significant differences ($P \leq 0.05$) of total C between years

plot to be detectable.

In spite of the obstacles outlined above and the recognition that 2 years may not be a sufficient period to characterise the dynamics of total soil N in a grassland system, the general trends observed in the annual N budget (Fig.7.6) and soil total N content (Table 7.5) suggest that Grass-clover and Herbal ley may apparently be in equilibrium or with a small tendency to gain N. Grass+N400 showed a similar tendency in total soil N, but the N budget (Fig. 7.6) appears with a large difference between inputs and outputs, indicating that an important proportion of N was unaccounted for, in contrast to the legume-based pastures. One possible explanation for this discrepancy is that not all the losses (about 181 kg N/ha/year) were accounted for in the annual budget, or alternatively that the difference is truly accounted for in the significant increment of soil total N at 0-7.5 cm depth between February 1990 and February 1991 (Table 7.5). This would approximate 180 kg N/ha (0.02% increase in total N in the 0-7.5 cm depth, and a bulk density of 1.21 g/cm^3).

Total soil C showed a similar trend to soil N, with only a few values significantly different between years within depths in the Grass-clover pasture Table 7.6). The overall data for carbon exhibit a trend of increasing organic matter and hence the associated tendency for an increment in soil N too. Several authors have reported an increment in soil N, especially in topsoil of recently sown pastures. The period of gain to reach a stable condition is variable, and it has been reported to extend for a period of 5 to 10 years, especially in legume-based pastures (Sears and Evans, 1953; Jackman, 1966) and in swards receiving high rates of fertiliser N (Wolton, 1955).

In view of the considerable losses of N generally observed in intensively grazed pasture (Field and Ball, 1982), the inputs of N and the intensity of pasture utilisation are both vital for maintaining the balance of the system. In this regard the study of Hoglund (1985) established that C and N increased linearly with the length of the residual post-grazing yield, emphasising the

importance of the pasture litter pathway for raising the soil organic matter pool, thus ensuring a supply of C to the soil which in turn is the substrate for stabilising mobile N in organic compounds (immobilisation). Under the experimental conditions of Hoglund's study, a mean residual yield of about 600 kg DM/ha apparently was the critical level to maintain C and N in a steady-state in the top 10 cm of soil. He also observed an important practical aspect: maximum animal consumption per unit area was not coincident with lowest residuals, which highlighted the scope for good pasture management to surmount difficulties in maintaining the soil N balance without the need to reduce stocking rates.

In the present study residual yields were not systematically measured. But visual observation and some occasional measurements, either by the standard cutting technique or estimation using a pasture probe, suggested a residual DM fluctuating between 700 and 900 kg DM/ha. In a parallel study under a different grazing system, Brock and Fletcher (1992) recorded a residual yield of 600 and 1000 kg DM/ha in rotational and set-stocking systems, respectively, under a heavy stocking rate, and with soil N and C analyses indicating steady-state conditions (J.L. Brock, pers. comm.). So, as it was pointed out by Hoglund (1985), on average under normal farming conditions it may be possible to manage the soil-plant-animal system in an appropriate way such that C and N can be maintained in balance. Of course, extreme seasonal changes in herbage production can temporarily alter any pasture management system, generating conditions conducive to either a temporarily negative or positive shift in organic matter. Under farming conditions a negative shift may occur, for instance, when pasture growth is drastically reduced in a drought period, or conversely to a significant accumulation in those so-called "good years" when pasture growth is largely above the corresponding carrying capacity of the farm.

7.7 Major conclusions and suggestions for future research

i) Fertiliser N was an important factor in increasing herbage yield from the pure ryegrass sward, but also a determinant of the magnitude and type of N losses. The losses of N were disproportionately greater than in the legume-based pastures when they were compared on an N yield and stocking rate basis. The mechanism by which fertiliser N improved production but increased losses was centred on the increase in the size of the soil mineral N pool. This pool provides plant available N but also NO_3-N in the soil solution which is susceptible to leaching. Also the periodic application of urea fertiliser accentuated the gaseous losses creating more "site opportunities" initially for volatilisation, and subsequently for denitrification, than in the legume-based swards. In the pure grass sward fertilised with N, the importance of the animals in promoting N losses was overshadowed by the direct effect of fertiliser N.

ii) The impact of the animal on N losses was concentrated in approximately 10 % of the grazing area. The large concentration of mineral N in these areas aggravated the typical problem of spatial variability in soil mineral N in such a way that any single average value can represent only an approximation of the reality that occurs in the field. It was concluded that in studies focusing on the environmental aspect of N, compartmentalised investigations on areas affected by animal excreta should be more useful, especially when no fertiliser N is applied and the majority of losses are mediated by animals.

iii) The commonly accepted concept that conventional white clover-ryegrass pasture operates under continuous N deficiency and hence is incapable of a greater yield, compared with a pure sward fertilised with N, was confirmed in this study. However, Herbal ley pasture, based also on biological N fixation as the main source of external N, was able

to produce a similar yield to the pasture heavily fertilised with N. The explanation offered was that a combination of different species could also occupy different ecological niches in the soil. This was reflected in a more efficient utilisation of soil mineral N by some non-legume species, while the legume components were able to fix N symbiotically at a rate similar to the Grass-clover pasture.

These results open the opportunity to speculate in a field experiment on the relatively recent concepts of the ecological interactions between the rhizosphere of agricultural plants and the different trophic levels of the soil biota. Local mineralisation of organic N and subsequent utilisation by plants resulting from this interaction expand the possibilities to utilise soil N in a more effective manner, with obvious benefits for agriculture but also with less risk for polluting the environment.

Any progress in this field will enhance the utilisation of the appropriate combination of pasture species, highlighting at the same time the role that plant breeding programmes could play in the development of the suitable pasture mixtures.

Suggestions for future research:

Aspects of N in soil fertility

The possibility of accelerating the N cycling by stimulating localised mineralisation of N, and hence pasture production, requires a considerable amount of research to evaluate its real expression at the field scale. Some aspects are:

* The persistence of the Herbal ley pasture in the long term, under several grazing systems and environments.

* Studies of animal preferences of pasture species in the Herbal ley system.

* Changes in soil mineral N and changes in soil total N and C in the long term. This is important because of the potential for losses of N from the system when soil N is in the mineral form.

* The level of symbiotic N fixation to maintain the balance between a greater exploitation of soil N (and eventual increase of losses) and external inputs.

* Studies on the dynamics of the microbial biomass in the rhizosphere of the main pasture species, in comparison with the rest of the soil.

N losses

On the particular subject of N losses, two important aspects to be followed up are:

* The indirect effect of grazing animals in winter on the denitrification process, through their influence on soil structure and the supply of available C and NO_3^- to the denitrifier organisms.

* The study of the volatilisation of ammonia from pastures, integrating areas affected and unaffected by urine patches, which is especially important for budget studies.

CHAPTER 8
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APPENDICES

Appendix 1 Changes in botanical composition of the herbal ley pasture

24 October 1989	% Yield
G. Tama + G. Manawa	32.2
White clover	22.2
Chicory	9.1
Yorkshire fog	8.8
Red clover	7.9
Poa trivialis	2.8
Bromus	2.4
Plantain	2.3
Cocksfoot	1.6
Others	10.7

18 December 1989	% Yield
G. Tama + G. Manawa	27.5
Chicory	22.8
White clover	14.4
Red clover	11.2
Yorkshire fog	3.8
Crested dogstail	1.5
Cocksfoot	1.4
Plantain	1.3
Others	11.9
DM	4.2

19 February 1990	% Yield
Chicory	28.1
Red clover	24.2
White clover	20.1
Yorkshire fog	5.8
Plantain	5.6
Yarrow	3.8
Cocksfoot	1.9
Bromus	1.3
Lolium	1.2
Others	4.3
DM	3.7

4 May 1990	% Yield
Chicory	53.1
Red clover	7.7
White clover	10.4
Yarrow	4.5
Cocksfoot	3.7
Lolium	3.6
Yorkshire fog	2.7
Plantain	2.4
Poa	1.7
Bromus	2.5
Others	7.7

20 November 1989	% Yield
White clover	25.2
Lolium	22.5
Chicory	18.2
Red clover	10.9
Yorkshire fog	5.8
Poa	2.3
Plantain	2.1
Cocksfoot	1.4
Bromus	1.2
Phalaris	1.0
Others	7.5
DM	1.9

17 January 1990	% Yield
White clover	33.7
Red clover	24.2
Chicory	17.8
Yorkshire fog	4.0
Lolium	3.6
Cocksfoot	1.3
Others	11.3
DM	4.1

26 March 1990	% Yield
Chicory	46.6
Red clover	12.0
White clover	12.8
Yorkshire fog	5.6
Yarrow	3.7
Bromus	1.9
Lolium	1.2
Cocksfoot	1.6
Plantain	4.1
Others	6.6
DM	3.9

22 June 1990	% Yield
Chicory	24.0
White clover	15.0
Lolium	10.6
Yorkshire fog	9.4
Red clover	5.2
Timothy	3.7
Yarrow	3.6
Cocksfoot	7.2
Poa	9.2
Plantain	2.8
Others	7.3
DM	2.0

Appendix 1 continued

14 September 1990	% Yield
White clover	26.8
Chicory	14.9
Poa	14.7
Yorkshire fog	7.4
Lolium	6.4
Red clover	6.3
Crested dogstail	3.9
Cocksfoot	2.6
Plantain	0.9
Phalaris	0.7
Others	12.8
DM	1.6

26 November 1990	% Yield
Chicory	43.2
White clover	16.6
Red clover	14.4
Poa	5.4
Yorkshire fog	4.1
Lolium	3.5
Cocksfoot	2.5
Yarrow	1.7
Bromus	1.6
Crested dogstail	1.3
Plantain	1.2
Others	2.7
DM	1.8

15 February 1991	% Yield
Chicory	53.1
Red clover	23.7
White clover	7.0
Cocksfoot	6.9
Lolium	2.9
Plantain	1.5
Yorkshire fog	1.3
Others	1.8
DM	1.8

25 October 1990	% Yield
White clover	23.7
Chicory	22.8
Poa	12.2
Red clover	12.1
Yorkshire fog	7.5
Lolium	5.0
Plantain	2.6
Cocksfoot	2.2
Crested dogstail	1.5
Timothy	1.1
Phalaris	1.1
Others	6.3
DM	1.9

31 December 1990	% Yield
Chicory	37.7
Red clover	19.2
White clover	16.9
Plantain	3.9
Yorkshire fog	3.9
Lolium	3.6
Cocksfoot	3.0
Yarrow	2.6
Poa	1.9
Others	2.1
DM	5.2

26 March 1991	% Yield
Chicory	64.7
Cocksfoot	11.6
Red clover	4.2
Yarrow	3.9
White clover	2.0
Yorkshire fog	2.0
Poa	1.8
Plantain	1.0
Others	4.9
DM	3.9

Appendix 2 Soil mineral N in the top 45 cm depth

Date		Grass-clover			Herbal ley			Grass + N		
		NH_4	NO_3-N	Total	NH_4	NO_3-N	Total	NH_4	NO_3-N	To
		kg N/ha - 45 cm			kg N/ha - 45 cm			kg N/ha - 45 cr		
1989	04/04	13.6	59.8	73.4	16.5	67.5	84.0	19.4	57.8	77
	08/05	16.7	9.2	25.9	12.9	11.3	24.2	14.7	7.1	21
	01/06	13.8	4.7	18.5	18.3	4.2	22.5	43.4	15.7	59
	19/06	14.7	1.7	16.4	12.6	2.0	14.6	12.5	13.7	26
	12/07	23.4	1.1	24.5	17.1	0.9	18.0	61.9	10.5	72
	02/08	13.8	2.5	16.3	12.4	1.8	14.2	25.7	8.3	34
	26/08	28.6	2.6	31.2	31.0	2.9	33.9	35.3	17.5	52
	14/09	19.6	0.2	19.8	20.0	0.1	20.1	24.8	12.5	37
	03/10	21.2	1.0	22.2	26.3	0.6	26.9	29.5	15.3	44
	30/10	19.5	2.3	21.8	21.4	2.6	24.0	31.6	19.7	51
	13/11	14.6	2.1	16.7	15.5	1.8	17.3	15.4	13.9	29
	29/11	16.2	2.1	18.3	21.3	6.5	27.8	90.2	23.2	113
	14/12	16.1	1.4	17.5	17.2	1.7	18.9	25.6	26.4	52
1990	05/01	14.5	2.4	16.9	13.5	3.4	16.9	26.0	33.5	59
	24/01	24.5	5.9	30.4	22.1	12.0	34.1	46.9	21.7	68
	07/02	14.1	4.1	18.2	16.5	8.1	24.6	20.0	31.2	51
	01/03	20.2	6.3	26.5	20.0	13.4	33.4	12.3	11.7	24
	12/03	14.6	0.9	15.5	12.2	5.0	17.2	12.5	5.9	18
	23/03	8.9	1.6	10.5	7.6	4.6	12.2	8.5	3.2	11
	06/04	21.5	27.0	48.5	12.9	22.3	35.2	61.3	46.7	108
	14/05	13.7	3.6	17.3	10.9	11.0	21.9	18.4	31.3	49
	28/05	11.7	5.8	17.5	7.7	11.6	19.3	12.2	40.0	52
	12/06	11.7	2.3	14.0	10.3	4.5	14.8	8.6	26.9	35
	26/06	11.1	3.2	14.3	8.6	4.6	13.2	46.6	11.4	58
	16.07	10.2	2.1	12.3	9.2	3.2	12.4	22.5	20.4	42
	31/07	6.7	2.7	9.4	4.4	2.2	6.6	8.9	7.3	16
	22/08	9.7	1.9	11.6	14.6	2.9	17.5	48.9	11.8	60
	05/09	18.3	2.4	20.7	12.0	2.8	14.8	14.6	9.6	24
	25/09	12.9	4.9	17.8	19.6	8.8	28.4	41.0	25.1	66
	09/10	15.2	1.8	17.0	14.6	6.8	21.4	25.5	21.0	46
	08/11	20.0	2.8	22.8	15.8	0.1	15.9	28.1	28.5	56
	21/11	14.0	1.5	15.5	11.4	1.3	12.7	18.7	20.4	38
	05/12	9.6	7.8	17.4	13.5	8.1	21.6	25.4	43.0	68
	27/12	10.7	7.8	18.5	19.0	8.2	27.2	23.8	50.3	74
1991	15/01	6.7	7.3	14.0	14.3	12.3	26.6	24.6	42.4	67
	31/01	1.4	4.5	5.9	3.7	1.9	5.6	8.0	39.9	47
	26/02	14.7	8.7	23.4	14.2	8.9	23.1	18.7	27.8	46
	13/03	4.6	2.4	7.0	5.4	2.2	7.6	8.6	9.0	17
	10/04	4.7	4.6	9.3	3.9	8.0	11.9	31.5	43.6	75
	30/04	7.1	1.6	8.7	6.8	1.1	7.9	8.1	17.4	25

Appendix 3 Concentration of NO_3-N in the soil solution (µg/ml), 30-45 cm depth

Date		Grass-clover		Grass + N		Herbal ley	
1989	04/04	25.78	(2.6)	22.71	(6.0)	29.89	(10.8)
	08/05	11.05	(0.2)	6.88	(6.0)	10.51	(5.4)
	01/06	4.40	(1.5)	7.37	(1.8)	3.85	(1.0)
	19/06	2.28	(0.8)	2.75	(2.4)	2.65	(1.6)
	12/07	0.07	(0.1)	4.75	(2.7)	0.09	(0.0)
	02/08	1.86	(1.4)	4.02	(1.7)	1.69	(0.6)
	28/08	1.80	(0.2)	7.14	(2.2)	2.00	(0.1)
	14/09	0.00	(0.0)	6.77	(1.1)	0.00	(0.0)
	03/10	0.81	(0.5)	3.37	(0.6)	0.22	(0.1)
	30/10	0.40	(0.1)	1.67	(0.4)	0.31	(0.1)
	13/11	1.20	(0.5)	7.10	(1.9)	2.18	(0.4)
	29/11	1.31	(0.9)	6.67	(2.9)	1.88	(1.4)
	14/12	0.79	(0.2)	11.40	(3.0)	1.13	(0.4)
1990	05/01	0.87	(0.1)	16.33	(4.2)	1.64	(0.3)
	24/01	3.43	(0.4)	14.52	(6.1)	11.31	(6.9)
	07/02	3.39	(1.5)	12.01	(4.6)	3.13	(1.5)
	01/03	3.77	(1.7)	6.78	(4.1)	4.98	(2.4)
	12/03	0.47	(0.3)	2.61	(2.6)	1.87	(1.8)
	23/03	0.98	(0.4)	2.31	(0.3)	4.40	(3.4)
	06/04	6.20	(2.7)	4.51	(0.9)	3.37	(0.1)
	24/04	0.92	(0.6)	3.60	(1.4)	0.44	(0.3)
	14/05	1.16	(0.4)	8.78	(1.7)	1.79	(0.6)
	28/05	6.00	(1.4)	28.90	(10.0)	7.30	(4.6)
	12/06	1.38	(0.2)	32.71	(8.6)	2.89	(1.6)
	26/06	1.20	(0.2)	11.41	(2.3)	2.34	(0.4)
	16/07	1.08	(0.2)	11.82	(3.3)	2.90	(0.4)
	31/07	3.65	(3.1)	6.09	(1.5)	1.51	(0.1)
	22/08	0.88	(0.2)	6.24	(2.3)	1.09	(0.1)
	05/09	1.82	(0.1)	5.16	(1.0)	1.60	(0.1)
	25/09	1.09	(0.3)	4.08	(1.6)	3.18	(0.5)
	09/10	2.00	(0.6)	4.40	(1.1)	1.70	(0.7)
	08/11	0.00	(0.0)	3.03	(1.0)	0.00	(0.0)
	21/11	1.18	(0.3)	6.46	(2.3)	0.90	(0.1)
	05/12	2.00	(0.2)	14.91	(6.0)	2.12	(0.1)
	27/12	0.50	(0.4)	11.30	(0.1)	5.40	(2.8)
1991	15/01	0.30	(0.2)	12.70	(1.0)	5.40	(3.4)
	31/01	0.71	(0.0)	16.36	(2.2)	1.25	(0.1)
	26/02	1.99	(0.5)	11.32	(2.9)	1.87	(0.3)
	13/03	1.34	(0.2)	6.90	(0.8)	0.00	(0.0)
	10/04	2.22	(1.1)	11.58	(2.0)	2.67	(0.7)
	30/04	0.95	(0.9)	8.83	(1.0)	0.00	(0.0)

Values in parentheses are standard error of mean

Appendix 4a Components of the N budget during two years comprising 20 grazings followed by regrowth of the Grass-clover system (4 April 1990 - 13 May 1991)

Date	Nr	Nns	Snf	HNY	LNY	Na	Nl	Nd	Nvu
	kg N/ha/regrowth period								
4 April									
15 May	0.3	1.0	0.0	31.9	2.2	1.6	3.2	0.2	3.6
30 June	0.4	1.8	0.0	20.4	1.4	0.3	3.2	0.8	0.7
11 Aug.	0.3	1.5	7.3	4.5	0.4	0.2	0.2	0.8	0.2
21 Sept.	0.3	1.5	28.1	29.8	16.1	1.5	0.2	0.7	2.1
24 Oct.	0.3	1.2	32.0	34.1	10.9	1.7	0.1	0.1	3.9
20 Nov.	0.2	0.9	17.4	35.3	14.1	1.8	0.0	0.1	4.0
20 Dec.	0.2	1.1	14.6	21.1	8.3	1.1	0.0	0.1	6.0
17 Jan.	0.2	1.0	12.8	35.9	21.4	1.8	0.0	0.1	16.8
19 Feb.	0.3	1.2	14.5	38.6	20.5	1.9	0.0	0.1	18.1
26 Mar.	0.3	1.3	9.0	42.6	16.2	2.1	0.0	0.1	20.0
4 May	0.3	1.4	9.0	40.0	12.8	2.0	0.0	0.3	1.4
22 June	0.4	1.7	13.0	37.4	6.2	1.9	2.7	0.8	1.3
9 August	0.4	1.7	8.0	26.0	4.8	1.3	2.0	1.1	0.9
14 Sept.	0.3	1.3	9.9	29.0	8.5	1.5	1.1	0.6	2.1
25 Oct.	0.3	1.4	33.2	42.3	13.9	2.1	0.0	0.1	4.8
26 Nov.	0.3	1.1	18.1	41.5	11.6	2.1	0.0	0.1	4.7
30 Dec.	0.3	1.2	18.1	46.5	13.8	2.3	0.0	0.1	21.8
15 Feb.	0.4	1.6	13.3	43.2	13.4	2.2	0.0	0.1	20.2
25 Mar.	0.3	1.4	12.5	34.1	3.7	1.7	0.0	0.2	16.0
13 May	0.4	1.7	10.1	40.7	0.2	2.0	0.8	0.3	1.4

Nr = N in rain; Nns = N non-symbiotic; Snf = N symbiotic; HNY = herbage N yield; LNY = legume N yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvu = N lost by volatilisation from urine.

Appendix 4b Components of the N budget during two years comprising 20 grazings followed by regrowth of the Herbal ley system (4 April 1990 - 13 May 1991)

Date	Nr	Nns	Snf	HNY	LNY	Na	Nl	Nd	Nvu
				kg N/ha/regrowth period					
4 April									
15 May	0.3	1.0	0.0	-	-	-	3.0	0.4	-
6 June	0.2	0.9	0.0	49.3	6.0	2.5	2.4	0.5	1.7
11 Aug.	0.5	1.5	8.1	15.1	2.6	0.8	1.8	1.7	0.5
21 Sept.	0.3	1.5	30.6	43.2	19.2	2.2	0.2	0.6	3.1
24 Oct.	0.3	1.2	30.8	46.5	20.9	2.3	0.2	0.1	5.3
20 Nov.	0.2	0.9	15.4	64.2	32.4	3.2	0.0	0.1	7.3
20 Dec.	0.2	1.1	15.5	27.5	10.0	1.4	0.0	0.1	7.8
17 Jan.	0.2	1.0	16.7	51.6	33.3	2.6	0.0	0.1	24.2
19 Feb.	0.3	1.2	15.0	64.0	34.3	3.2	0.0	0.1	30.0
26 Mar.	0.3	1.3	11.3	44.9	14.9	2.2	0.0	0.4	21.0
4 May	0.3	1.4	9.1	47.2	13.8	2.4	0.0	0.4	1.7
22 June	0.4	1.7	11.6	53.4	10.4	2.7	4.0	0.9	1.9
9 August	0.4	1.7	13.5	16.3	4.3	0.8	2.5	1.2	0.6
14 Sept.	0.3	1.3	18.0	54.3	21.4	2.7	0.8	0.6	3.9
25 Oct.	0.3	1.4	37.8	69.7	83.9	3.5	0.0	0.1	7.9
26 Nov.	0.3	1.1	21.8	83.5	31.7	4.2	0.0	0.3	9.5
30 Dec.	0.3	1.2	18.0	92.7	42.5	4.6	0.0	0.2	43.4
15 Feb.	0.4	1.6	12.8	58.7	17.2	2.9	0.0	0.3	27.5
25 Mar.	0.3	1.4	9.5	60.5	4.2	3.0	0.0	0.3	28.4
13 May	0.4	1.7	12.2	43.8	0.2	2.2	0.8	0.8	1.6

Nr = N in rain; Nns = N non-symbiotic; Snf = N symbiotic; HNY = herbage N yield; LNY = legume N yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvu = N lost by volatilisation from urine.

Appendix 4c Components of the N budget during two years comprising 20 grazings followed by regrowth of the Grass+N400 system (4 April 1990 - 13 May 1991)

Date	Nr	Nns	Nf	HNY	Na	Nl	Nd	Nvf	Nvu
				kg N/ha/regrowth period					
4 April									
15 May	0.3	1.0	0	30.4	1.5	1.9	0.7	-	3.4
30 June	0.4	1.8	80	52.5	2.6	4.8	2.8	4.0	1.9
11 Aug.	0.3	1.5	40	28.7	1.4	1.6	5.5	2.0	1.0
21 Sept.	0.3	1.5	80	74.0	3.7	1.7	8.0	8.0	5.3
24 Oct.	0.3	1.2	40	74.7	3.7	0.7	0.5	6.0	8.5
20 Nov.	0.2	0.9	40	54.9	2.7	0.0	0.3	6.0	6.2
20 Dec.	0.2	1.1	80	48.0	2.4	0.0	0.1	16.0	13.6
17 Jan.	0.2	1.0	40	67.6	3.4	0.0	0.1	12.0	31.7
19 Feb.	0.3	1.2	0	38.7	1.9	0.0	0.2	0.0	18.1
26 Mar.	0.3	1.3	0	30.3	1.5	0.0	0.2	0.0	14.2
4 May	0.3	1.4	80	52.0	2.6	0.0	0.6	4.0	1.8
22 June	0.4	1.7	40	72.7	3.6	25.3	2.8	2.0	2.6
9 August	0.4	1.7	40	49.8	2.5	12.2	6.2	2.0	1.8
14 Sept.	0.3	1.3	80	81.6	4.1	3.6	7.1	8.0	5.8
25 Oct.	0.3	1.4	40	65.8	3.3	0.0	1.0	6.0	7.5
26 Nov.	0.3	1.1	40	61.1	3.1	0.0	0.3	6.0	6.9
30 Dec.	0.3	1.2	40	69.0	3.5	0.0	0.2	12.0	32.3
15 Feb.	0.4	1.6	40	48.8	2.4	0.0	0.3	12.0	22.9
25 Mar.	0.3	1.4	0	48.1	2.4	0.0	0.2	0.0	22.5
13 May	0.4	1.7	80	55.7	2.8	4.0	0.8	4.0	2.0

Nr = N in rain; Nns = N non-symbiotic; Nf = fertiliser; NHNY = herbage N yield; yield; Na = N in animal product; Nl = N lost by leaching; Nd = N lost by denitrification; Nvf = N volatilisation from urea fertiliser; Nvu = N lost by volatilisation from urine.