A STUDY OF SOME ASPECTS OF THE NITROGEN ECONOMY OF NEW ZEALAND GRASSLAND SOILS

by

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Submitted for the Degree of
Doctor of Philosophy
at the University of Waikato,
Hamilton, New Zealand.
August, 1977.

ACKNOWLEDGEMENTS

I wish to gratefully acknowledge the following people who have rendered assistance throughout the course of this study:

Professor A.T. Wilson and Dr. W.M.H. Saunders for helpful guidance and instruction throughout the course of study and in the preparation of this manuscript.

Mr C.B. Dyson for assistance with the statistical analyses.

Dr. U. Sarathchandra for supplying the *Nitrosomonas* culture.

Mr M. Brown for NH_4^+ and NO_3^- analyses reported in chapter 7.

The Plant Chemical Analysis Section, Ruakura Agricultural Research Centre for the herbage analyses reported in chapter 7.

Technical staff of the Ministry of Agriculture and Fisheries, Kaikohe, for field assistance.

Field staff of the Ministry of Agriculture and Fisheries for collection of soil samples.

The Waikato University Photographic Unit for preparation of figures 9.1 and 10.1.

The Waikato University Draftsmen for preparation of figures 3.1, 5.4 and 13.1.

Mr and Mrs A.W. Holland for their hours spent in proof reading this manuscript.

My wife Linda for typing this manuscript and all her encouragement throughout this study.

ABSTRACT

Nitrogen deficiency is widespread and is a major limitation to grassland productivity in New Zealand, indicating inadequate nitrogen supply, losses from the nitrogen cycle, or a combination of both. The intensive grassland farming system on which New Zealand agriculture is based is largely dependent on symbiotic fixation of atmospheric nitrogen by clovers, and many estimates indicate that the amount of nitrogen fixed annually should be adequate to maintain a high level of pasture production. It appears, therefore, that losses of nitrogen may be the more important factor contributing to nitrogen deficiency in New Zealand pastures. One transformation which will affect the conservation of nitrogen in soil, and which has received little attention in New Zealand, is nitrification. It was the objective of this thesis to investigate some aspects of nitrification in New Zealand grassland soils.

A technique which has recently been applied to studies on nitrogen transformations in soils involves the use of variations in the natural abundance of ^{15}N. The possible use of this technique for studying nitrogen transformations in New Zealand soils was investigated.

A perfusion technique suitable for the measurement of inherent oxidation activities of the indigenous nitrifying organisms in New Zealand grassland soils was developed. Soils were perfused with 0.005M $(NH_4)_2SO_4$ at 25°C, and the rate of oxidation of ammonium to nitrate measured over a sixteen hour period. Rates of nitrification measured over the initial sixteen hours of perfusion, referred to as the Initial Nitrification Activity (INA), ranged from <0.02 μg $N_{oxidised}$/g soil/hour to 5.70 μg $N_{oxidised}$/g soil/hour in sixty nine soils selected to provide a representative sample of agricultural soils from the major soil groups of New Zealand.

High rates of INA (>2 μg $N_{oxidised}$/g soil/hour) were found in yellow-

brown loams, some red and brown loams, and soils with a pH above 7.0. All other soils exhibited rates of nitrification <1 μg $N_{oxidised}$/g soil/hour.

Differences in INA were found to predict the rates of nitrification occurring in the field in two soils using an in-situ incubation technique. The mean rate of nett nitrification measured over a forty seven week period in the 0 - 7.5 cm depth of Wharekohe silt loam (INA = 0.07 μg $N_{oxidised}$/g soil/hour), was 0.68×10^{-2} μg $N_{oxidised}$/g soil/hour, and that in the 0 - 7.5 cm depth of Kiripaka silt loam (INA = 0.81 μg $N_{oxidised}$/g soil/hour) was 5.4×10^{-2} μg $N_{oxidised}$/g soil/hour. This difference resulted in a different ratio of ammonium to nitrate between the soils. In Wharekohe, the mean values of ammonium and nitrate nitrogen present in the 0 - 7.5 cm depth over the forty seven week experimental period were 15.7 ± 1.7 and 0.9 ± 0.4 kg N/ha respectively. In Kiripaka, the figures were 6.5 ± 1.3 and 6.6 ± 1.4 kg N/ha.

Rates of nett mineralisation of soil organic nitrogen were also determined in Wharekohe silt loam and Kiripaka silt loam by an in-situ incubation technique. In the former soil, the mean rate of nett mineralisation over a forty seven week period was 0.99 ± 0.08 kg N/ha (0 - 7.5 cm)/day, and in the latter soil 0.74 kg N/ha (0 - 7.5 cm)/day.

When soils were perfused with 0.005M $(NH_4)_2SO_4$ for up to twenty days, four general patterns of nitrification were observed.

1) Ammonium was rapidly oxidised to nitrate, the rate of oxidation being linear or near linear from the commencement of the perfusion.
2) Ammonium was oxidised only slowly to nitrate.
3) Ammonium was oxidised slowly to nitrate at the commencement of perfusion, but increased logarithmically with time until a steady rate of nitrification was observed.
4) Type 3 nitrification with a temporary accumulation of nitrite

during the initial stages of perfusion.

Type 1 nitrification was observed only in soils having a high INA, type 2 nitrification in soils of low pH (mean pH 5.5), and type 4 nitrification only in soils with a pH above 7.0, with the exception of two podzolic soils. Type 3 nitrification was observed in the majority of the soils studied.

Large populations of ammonium oxidisers (1.6×10^4 to 2.2×10^6/g soil) and nitrite oxidisers (5.4×10^4 to 1.9×10^7/g soil) were found in the 0 - 7.5 cm depth in New Zealand soils under improved grassland. In contrast to these high numbers, a low population of nitrifying organisms (555 ammonium oxidisers and 1434 nitrite oxidisers/g soil) was found in a native forest soil. Populations of nitrifying organisms were largest close to the soil surface and, in general, declined with depth.

Generation times of ammonium oxidisers were estimated to be in the order of three to five days and were dependent on pH. A variation in the rate of ammonium oxidation per ammonium oxidising cell was found between soils, therefore INA could not be considered as indicative of the size of the nitrifying population.

Multiple linear regression analysis showed both pH and percent total nitrogen to be significantly correlated with INA. A detailed study of the effect of pH on nitrification showed that soils containing allophane, an amorphous aluminium silicate, exhibited higher rates of nitrification at a given pH than other soils. Nitrification was also able to proceed at lower pH values in the former soils than in the latter. This difference may be partly rationalised by theoretical differences in surface pH of soil colloids.

A study was made of the fate of ^{15}N enriched $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ applied to two soils, one of medium (Waimate North clay loam) and the

other of low (Wharekohe silt loam) INA. Recovery of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ nitrogen in pasture herbage was 47.4 and 35.8 percent on the former soil, and 20.3 and 42.5 percent on the latter respectively. After soil nitrogen was taken into account there was still up to 66.8 percent of the nitrogen applied not accounted for. It was concluded that leaching was the major mechanism of loss, although some evidence of losses by denitrification from the Wharekohe soil was found.

The differences in rates of nitrification measured in New Zealand grassland soils are of agronomic significance since the rate of nitrification determines the form of inorganic nitrogen available for plant uptake. Ammonium, and not nitrate, is the major form of nitrogen available for assimilation by plants in several yellow-brown earths, and also in some podzolic soils, yellow-brown sands, recent soils from alluvium and yellow-brown pumice soils. Although differences in rates of nitrification do not appear to affect pasture production, they will, of necessity have to be considered if specialised crops which show a preference for assimilation of either ammonium or nitrate are grown on these soils.

Soils having a high rate of nitrification are associated with high concentrations of nitrate in groundwaters of the Waikato.

A low rate of nitrification in soils such as Wharekohe silt loam appears to be an important mechanism for the conservation of nitrogen. Any agricultural practice which will increase the rate of nitrification should be carefully considered and the overall effect on the nitrogen economy of the soil evaluated. If increasing the rate of nitrification results in a lower nitrogen status, then increased inputs of nitrogen either by symbiotic nitrogen fixation or fertiliser application will be required to maintain the same level of pasture production.

In general, it appears that the disadvantages of high rates of

nitrification outweigh the advantages, and agricultural practices should therefore be designed to minimise the rate of nitrification in soils.

The extent of nitrogen isotope discrimination in various reactions occurring in New Zealand soil-pasture systems was determined. Symbiotic fixation of atmospheric nitrogen by clover, assimilation of inorganic nitrogen by *Trifolium repens*, the oxidation of ammonium to nitrate by nitrifying organisms and the volatilisation of NH_3 from urine spots all showed discrimination in favour of ^{14}N. A previously unmeasured fractionation in animals was determined. In cattle, urine was depleted by about 2‰ and faeces enriched by about 2‰ relative to the animal feed.

$\delta^{15}N$ values were measured in the 0 - 7.5 cm depth of sixty one New Zealand grassland soils. The mean $\delta^{15}N$ value was 3.2‰ with a range of -1.1 to 6.8‰. The $\delta^{15}N$ values in two soil profiles showed that $\delta^{15}N$ values increased with depth reaching a maximum value at 20 cm in Wharekohe silt loam and 50 cm in Waimate North clay loam. New Zealand soils appear to be less enriched in ^{15}N than many American soils, and it is suggested that this reflects the large annual input of symbiotically fixed nitrogen which has a negative $\delta^{15}N$ value, although the mechanism of loss (i.e. via denitrification, leaching or volatilisation of NH_3) of nitrogen from different soils may be important.

$\delta^{15}N$ values were also determined for nitrate nitrogen in groundwater collected from a limestone cave under native forest in the Waitomo region, and also for nitrate nitrogen in the groundwater of shallow aquifiers in the Waikato.

The $\delta^{15}N$ values for two groundwater samples from the Waitomo area were - 1.6 and 3.8‰ while nine samples collected from the Waikato were in the range of 5.2 to 10.0‰. It is suggested that the isotopic composition of groundwater nitrogen in the Waikato is a result of a

major contribution of nitrogen derived from urine. This suggests that the grazing animal is an important contributor to the high nitrate concentration in groundwaters of the Waikato.

Because of the variability of $\delta^{15}N$ values that were found in grazed soil-pasture systems, and the complexity of fractionations which occur in such systems, small variations in the natural abundance of ^{15}N appear to be only of limited use for even qualitative studies of nitrogen transformations.

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CHAPTER 1

INTRODUCTION

The intensive grassland farming system on which New Zealand agriculture is based, is largely dependent on symbiotic fixation of atmospheric nitrogen by clovers for building and conserving the pool of soil organic nitrogen, from which plant available nitrogen is obtained through mineralisation. Such a system is possible largely because of New Zealand's temperate climate and adequate rainfall which is normally well dispersed throughout the year. However, in spite of estimates of nitrogen fixation by clover ranging up to 670 kg N/ha/year (Sears et al. 1965; Weeda, 1970), nitrogen is a major limiting factor to pasture production in many parts of New Zealand (O'Connor & Gregg, 1971; Steele, 1976b).

It cannot be claimed that nitrogen is any more essential than other elements required for plant production, but it is taken up in larger amounts than any other nutrient derived from soil or fertiliser (Viets, 1965). It can be calculated from data of McNaught (1969) that 10,000 kg of pasture herbage dry matter, consisting of sixty percent ryegrass and forty percent clover, will contain 388 kg nitrogen; 260 kg potassium; 6.1 kg calcium; 3.8 kg phosphorus; 2.6 kg sodium; 2.4 kg magnesium; 118 g manganese and 18 g boron. Because of the continual requirement for such large quantities of nitrogen to support intensive grassland production, conservation of nitrogen in a soil-plant system is extremely important. Any transformation of nitrogen occurring in soil which results in either an increase or decrease in conservation of nitrogen, becomes important to the overall economy of nitrogen cycling in a soil-plant system. Nitrification, although only a small part of the total nitrogen cycle in soils (figure 1.1), is one factor which may be expected to have a large influence on conservation of nitrogen, but has received little attention in New Zealand.

The term nitrification as officially approved by the Soil Science Society of America (1962) is defined as: "The biological oxidation of

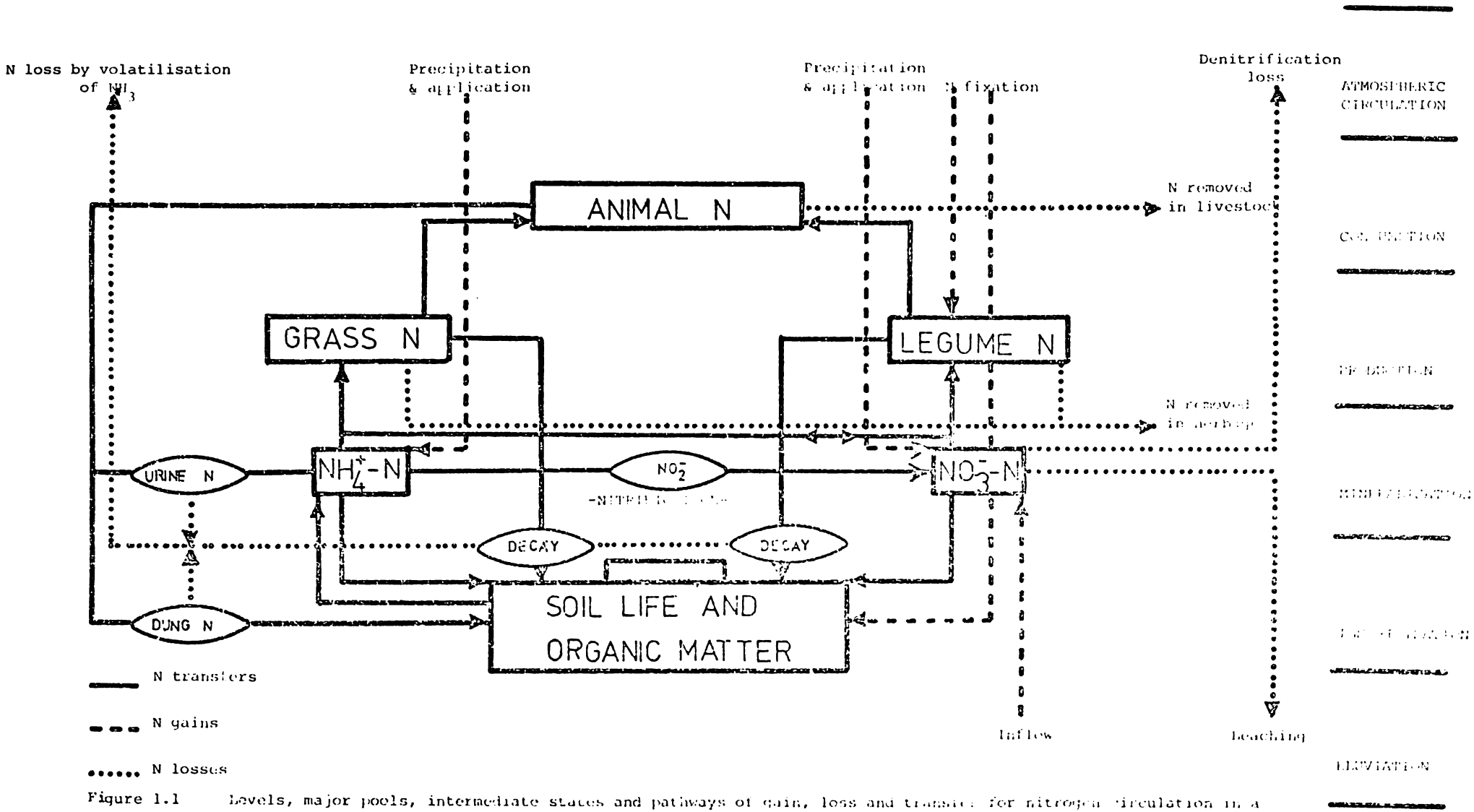

Figure 1.1 Levels, major pools, intermediate states and pathways of gain, loss and transfer for nitrogen circulation in a grazed legume-grass system (after O'Connor, 1974)

ammonium salts to nitrites and the further oxidation of nitrites to nitrates". This definition is inadequate, since, as it presupposes the identity of the initial substrate, it excludes heterotrophic oxidation of nitrogen compounds such as amines (Schmidt, 1954, 1960). An alternate definition was suggested by Alexander et al. (1960), namely: "The biological conversion of nitrogen in organic or inorganic compounds from a reduced to a more oxidised state". Such a definition requires only an increase in the oxidation state of nitrogen, but is again limited to biological oxidations, and therefore excludes chemical oxidation, a process which has been demonstrated in soils (Allison and Doetsch, 1951).

A further confusion in terminology often occurs in agronomic literature where nitrification is used to describe processes resulting in the formation of nitrates, thus combining ammonification and nitrification.

Despite its limitations, the official definition will be adhered to in this discussion, and any departure from this will be indicated.

The importance of nitrification in soils has been repeatedly emphasised over the years, and often a good nitrifying soil was considered as a productive and fertile soil. However, in more recent literature, some doubt has been associated with the value of nitrification, Alexander (1965) describing it as a "mixed blessing, and possibly, a frequent evil". The importance attached to nitrification is largely a matter of the emphasis placed on each of several individual effects of nitrification.

Agronomically, nitrification is considered of importance as it largely determines the form of nitrogen which is available to plants. It is commonly assumed or claimed that plants prefer or even require nitrate nitrogen, thus placing a special emphasis on the role of nitrification in soils. The experimental basis for this opinion is questionable, recent data (e.g. Ross and McNeilly, 1975b) suggesting

that in some environments plants may utilise largely, if not entirely, ammonium nitrogen.

Conversion of ammonium to nitrate changes a slowly leached cationic form of nitrogen to a rapidly leached anionic form. Data on loss of nitrogen by leaching are difficult to obtain, and published data often tends to be contradictory. However, it is generally considered that loss of nitrogen through leaching from grass clover associations is small (Allison, 1965; Whitehead, 1970), although recent data on nitrate concentrations in ground waters of the Waikato, New Zealand, have challanged this opinion (Baber, 1977). Conversion of a slowly mobile ion to a highly mobile ion may also be expected to effect the transport of nitrogen to the plant root.

The instability and reactivity of nitrates may lead to volatile losses of nitrogen from soils (Fuhr and Bremner, 1964). Nitrification is also an obligatory predecessor of denitrification (Hiltbold and Adams, 1960). Although nitrification may promote gaseous loss of nitrogen via chemo-denitrification or biological denitrification, reduction of nitrification activity of a soil has been shown to promote gaseous loss of urea nitrogen as ammonia (Bundy and Bremner, 1974).

Since the oxidation of ammonium ions brings about the formation of nitric acid, nitrification may increase soil acidity, especially if nitrates are lost by leaching.

Nitrification also has important environmental and health implications. Recently, Baber (1977) found that levels of nitrate in gound waters of the Waikato frequently exceeded 10 mg nitrogen/ℓ, and reported levels of nitrate up to 58 mg nitrogen/ℓ. High nitrate levels in water are undesirable, not only because of possible eutrophication of rivers and lakes, (Harmeson et al. 1971) but also because, if used for human consumption, may produce methaemoglobinaemia, a condition restricting

the transport of oxygen in the blood which may be caused through excessive intake of nitrate (West et al. 1968). Also, nitrite which may be formed during reduction of nitrate in the gut, can react with secondary amines under acidic conditions to form N-nitrosamines, many of which are reported as being carcinogenic, and possibly mutagenic and tetratogenic (Wolff and Wasserman, 1972).

High nitrate levels in soil may induce high nitrate contents in pasture (Nowakowski and Cunningham, 1966). High intake of nitrate by animals has been shown to induce methaemoglobenaemia in cattle (Campbell et al. 1954) and sheep (Holtenius, 1957); a decrease in thyroid function in rats and sheep (Bloomfield et al. 1960); decreased lactation rates in cows (Muhrer et al. 1956) and abortion in cattle (Simon et al. 1959).

There is no doubt that the rate of nitrification in a soil will have important effects on nitrogen cycling in that soil, and possibly also affect the health of humans and animals associated directly or indirectly with the particular system. The question as to whether nitrification is a "blessing or frequent evil" remains open, and is only likely to be answered by applied research in which the soil-plant system is considered as an integrated reaction system. The requirement for this type of research is endorsed by the observation that nitrification activity in a pure culture medium is often materially different from what takes place in soil. Because of the complex organic-inorganic inter-relationships which occur in soils, it may be expected that the kinetics, and possibly even the mechanism of nitrification will differ from that which takes place in pure culture mediums (Quastel and Scholefield, 1951).

A technique which has recently been applied to studies on nitrogen transformations in soils involves the use of variations in the natural abundance of ^{15}N. It is well documented that the natural abundance of ^{15}N in total and mineralisable soil nitrogen varies between soils (Cheng

et al. 1964: Bremner and Tabatabai, 1973; Feigin et al. 1974a,b), and is also often significantly different from that of fertiliser nitrogen (Kohl et al. 1971; Freyer and Aly, 1974; Edwards 1973; Shearer et al. 1974b; Rennie and Paul, 1975). This latter difference led Kohl et al. (1971) to postulate that the contribution of fertiliser nitrogen to nitrogen in surface water could be estimated by measurement of its isotopic composition. This approach has been severely criticised (Hauck et al. 1972), and it is generally considered that, because of the small difference in ^{15}N abundance between soil and fertiliser nitrogen, and its high variability, the use of natural variations in the abundance of ^{15}N can at best be semi-quantitative (Bremner and Tabatabai, 1973; Feigin et al. 1974a,b). In field studies, even qualitative estimates may be of doubtful validity (Black and Waring, 1977). There is, however, a complete lack of ^{15}N data available for New Zealand soils, and therefore no basis for a valid evaluation of the possible use of such a technique under New Zealand conditions.

It was the objective of the present thesis to:

1) Investigate nitrification activity in New Zealand soils in order to gain an understanding of the implications of differences in the rate of nitrification to New Zealand grassland farming.
2) Obtain some background data on variations in the natural abundance of ^{15}N in New Zealand agricultural systems, and to evaluate the use of such data as an aid to studying nitrification specifically, and nitrogen transformations in general.

Detailed objectives may be found following literature reviews in chapters 2 and 8.

SECTION A

A Study of Nitrification in New Zealand Grassland Soils

CHAPTER 2

LITERATURE REVIEW

Contents

2.1.0	Introduction
2.2.0	Historical aspects of nitrification
2.3.0	Autotrophic nitrifying organisms
2.4.0	Biochemistry of autotrophic nitrification
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2.1.0 INTRODUCTION

In the present chapter it is not intended to present an exhaustive review of the literature on nitrification, but to summarise the present state of knowledge to gain an appreciation of the mechanisms of nitrification, and factors which may be expected to affect nitrification under field conditions. From this it is intended to define areas in which knowledge is lacking, and thereby provide a valid basis for the research reported in this thesis.

2.2.0 HISTORICAL ASPECTS OF NITRIFICATION

Utilisation of nitrifying organisms can be traced back to at least the fourteenth century when saltpetre was collected for the production of gunpowder, and also for the preservation of meat. Although the production of nitrate was not known to be a biological process, saltpetre was known to accumulate naturally in places associated with animal excreta (Boerhaave, 1753), graveyards, and burial mounds (Niklewski, 1910). Pasteur (1862) suggested that the oxidation of ammonium may be of biological origin after he demonstrated the biological oxidation of alcohol to acetic acid. This suggestion was verified by Schloesing and Muntz (1877) who showed that the oxidation of ammonium in sewage was biological. The following year, Warrington (1878) showed that the oxidation of ammonium under soil conditions was also probably biological.

During the following years many workers attempted to isolate the nitrifying organisms from soil, and the first successful isolations of nitrite oxidising bacteria were reported by Winogradsky (1890); Warrington (1891) and Frankland and Frankland (1890). Winogradsky (1890, 1891) also successfully isolated the nitrite forming bacteria. From these results it was established that the oxidation of ammonium occurred in two stages, namely:

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

and that autotrophic bacteria were largely responsible for the oxidation. Isolation of the first heterotroph that could effect nitrification was reported by Nelson (1929). Other mechanisms which have been suggested are photochemical (Berthelot and Gaudechon, 1911) and chemical (Allison and Doetsch, 1951) nitrification. It is intended to examine each mechanism of nitrification separately.

2.3.0 AUTOTROPHIC NITRIFYING ORGANISMS

On the basis of the dominant nitrifiers in enrichments, and the frequency of isolation of specific bacteria in pure culture, it appears that only two autotrophic genera are prominent in soil nitrification in fertile soils, *Nitrosomonas* and *Nitrobacter* (Alexander, 1965). The former genera are ammonium oxidisers and the latter nitrite oxidisers. Nitrifying bacteria discussed here are gram-negative chemolithotrophic bacteria classified in the family *Nitrobacteraceae*. With the exception of *Nitrobacter winogradskyi*, all of the nitrifying bacteria are obligate chemolithotrophs (Watson, 1974).

2.3.1 Classification and Morphology of Ammonium Oxidising Bacteria

Four genera of ammonium oxidising bacteria are recognised in Bergey's manual of Determinate Bacteriology (Watson, 1974).

2.3.1(a) *Nitrosomonas europaea* (Winogradsky, 1892). Rods, 0.8 - 0.9 by 1.0 - 2.0 µm, occurring singly, rarely in chains. When motile, possess one or two subpolar flagella 3 - 4 times the length of the rod. Cells have cytomembranes which form flattened lamellae in the peripheral regions of the cytoplasm. Optimum growth temperature, 25 - 30°C. Optimum growth pH 7.5 - 8.0. Habitat: soils.

2.3.1(b) *Nitrosospira briensis* (Winogradsky and Winogradsky, 1933). Cells are tightly wound spirals with 3 - 20 turns. Width of spiral filament, 0.3 - 0.4 µm; amplitude of the spiral, 0.8 - 1.0 µm. Motile or non-motile; when motile, cells are propelled by one to six peritrichous flagella 3 - 5 µm in length. Optimum growth temperature 25 - 30°C. Optimum growth pH 7.5 - 8.0. Habitat: soils.

2.3.1(c) *Nitrosococcus nitrosus* (Buchanan, 1925). Large spheres, 1.5 - 1.7 µm in diameter, with thick cell membranes. Motility has not been demonstrated. Optimum temperature 20 - 25°C. Habitat: soils.

II *Nitrosococcus oceanus* (Watson, 1971). Cells are spherical to ellipsoidal, 1.8 - 2.2 µm in size. Occur singly, in pairs and occasionally as tetrads. Motile or non-motile. When motile, propelled by a single flagellum or a small tuft of peritrichous flagella. Optimum growth temperature 25 - 30°C. Optimum growth pH 7.5 - 8.0. Habitat: Atlantic and Pacific Oceans.

2.3.1(d) *Nitrosolobus multiformis* (Watson et al. 1971). Cells composed of multiple ellipsoidal units, randomly arranged, forming lobular pleomorphic cells which are internally compartmentalized, having one to five membrane-bound central areas surrounded by numerous smaller membrane-bounded areas rich in glycogen. Cells 1.0 - 1.5 µm by 1.0 - 2.5 µm. Motile by means of 1 - 20 flagella randomly arranged. Optimum growth temperature 25 - 30°C. Optimum growth pH 7.5 - 7.8. Habitat: soils.

2.3.1(e) *Nitrosovibrio tenuis* is a recently isolated genus from Hawaii (Harms et al. 1976) not yet recognised in Bergey's Manual. Cells are 1.1 - 3.0 µm by 0.3 - 0.4 µm. Motile by means of 1 - 4 subpolar to lateral flagella. Isolated vibrio is void of an extensive cytomembrane system.

2.3.2 Classification and Morphology of Nitrite Oxidising Bacteria

Three genera of nitrite oxidising bacteria are recognised in Bergey's Manual of Determinate Bacteriology (Watson, 1974).

2.3.2(a) *Nitrobacter winogradskyi* (Winslow et al. 1917). Cells short rods, often wedge or pear shaped, 0.6 - 0.8 by 1.0 - 2.0 µm. Cells possess a polar cap of cytomembranes arranged to form flattened vesicles. Usually non-motile but when grown in continuous culture motile cells with a single subterminal flagellum are produced. Optimum growth temperature 25 - 30°C. Optimum growth pH 7.5 - 8.0. Habitat: soil.

2.3.2(b) *Nitrospina gracilis* (Watson and Waterbury, 1971). Cells are long, slender rods, 0.30 - 0.40 by 2.7 - 6.5 µm. Spherical forms 1.35 - 1.45 µm diameter are found in old cultures. Occur singly or in pairs. Optimum growth temperature 25 - 30°C. Optimum growth pH 7.5 - 8.0. Habitat: South Pacific Ocean.

2.3.2(c) *Nitrococcus mobilis* (Watson and Waterbury, 1971). Cells spherical 1.5 - 1.8 µm in diameter following division, but elongating with dimensions of 1.8 by 3.5 µm just prior to division. Cells occur singly or in pairs. Motile by means of one or two flagella inserted subpolarly in elongated cells. Optimum temperature range 25 - 30°C. Optimum pH range, 7.5 - 8.0. Habitat: South Pacific Ocean.

2.3.3 Physiology of Autotrophic Nitrifying Bacteria

2.3.3(a) Nutrition. All of the organisms discussed above are obligate chemolithotrophs with the exception of *Nitrobacter winogradskyi*. Chemolithotrophic bacteria fullfil their energy requirements by the

oxidation of ammonium or nitrite and their carbon requirements by the fixation of carbon dioxide (Watson, 1974).

Essential elements for the growth of *Nitrosomonas* and *Nitrobacter* spp. are: phosphorus, magnesium and iron (Aleem and Alexander, 1960; Bomeke, 1950; Lees and Meiklejohn, 1948; Meiklejohn, 1953); copper and molybdenum (Kiesow, 1962; Zavarzin, 1958; Lees, 1948; Lees and Meiklejohn, 1948); potassium (Welch and Scott, 1960) and calcium (Kingma Boltjes, 1935), although there is no evidence of a requirement for significant amounts of calcium (Aleem and Alexander, 1960; Bomeke, 1950; Lees and Meiklejohn, 1948).

2.3.3(b) Aeration. Chemolithotrophic bacteria are strictly aerobic, nitrification being suppressed by a decrease in the oxygen partial pressure of their atmosphere (Amer and Bartholomew, 1951).

2.3.3(c) Acidity. Optimum pH for activity of *Nitrosomonas* and *Nitrobacter* spp. appears to be strain dependent. Winogradsky (1933) reported that the pH optima of six strains of *Nitrosomonas* ranged from 6.0 - 7.0, and seven strains of *Nitrobacter* from 6.3 - 9.4.

2.3.3(d) Light. Nitrification is slower in the light than in the dark (Fraps and Sterges, 1935; Waksman and Madhok, 1937), oxidation of nitrate being inhibited by light (Warrington, 1878).

2.3.3(e) Surface. Many workers have noted that the supernatant of nitrifying cultures were clear (Frankland and Frankland, 1890; Winogradsky, 1890) or that the sediment in liquid cultures of nitrifying bacteria was a better innoculant than the supernatant (Warrington, 1884). Lees and Quastel (1946b) failed to show nitrification activity in the perfusing

solution removed from a soil of high nitrification activity and concluded that nitrification was a surface phenomenon. However, although it appears that nitrifying bacteria prefer to be associated with a surface, Engel and Alexander (1958) showed that a surface was not a requirement for nitrification as they successfully propagated *Nitrosomonas europaea* in a medium entirely free from solid particles.

Readers requiring more detailed information on chemolithotrophic nitrifying bacteria are referred to reviews by Meiklejohn (1953) and Alexander (1965), and to papers by Watson (1971) and Watson and Mandel (1971).

2.4.0 BIOCHEMISTRY OF AUTOTROPHIC NITRIFICATION

Oxidation of ammonium to nitrate involves a change in the valence of the nitrogen atom from -3 to +5, corresponding to the loss of eight electrons.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad (2.1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (2.2)$$

Values for the change in free energy have been reported as -65.2 to -84.0 Kcal per mole for equation 2.1 and -17.5 to -20.0 Kcal per mole for equation 2.2 (Gibbs and Schiff, 1960).

Although there is an extensive literature on the biochemistry of nitrification, the intermediates and enzymes involved are still largely hypothetical. A discussion of these is considered to be outside the scope of the present review.

2.5.0 HETEROTROPHIC NITRIFYING ORGANISMS

Several heterotrophic organisms are able to increase the oxidation state of nitrogen, but unlike autotrophs, the heterotroph is unable to use this exothermic reaction as the sole source of energy for cell synthesis.

There is no evidence to suggest that the energy released by ammonium or nitrite oxidising heterotrophs is coupled with biosynthetic processes (Alexander et al. 1960), and most heterotrophs are able to grow well in conditions which preclude nitrification (Hirsch et al. 1961).

Eylar and Schmidt (1959) prepared nine hundred and seventy eight isolates of heterotrophs from twelve high nitrifying soils, of which sixty six formed NO_2^-, and sixteen formed NO_3^- when grown on glucose-peptone medium. Their data may be summarised as follows:

Isolate	Number	Number Forming NO_2^-	Number Forming NO_3^-
Actinomycetes	222	16	0
Bacteria	341	24	1
Fungi	415	26	15
Total	978	66	16

A summary of data from Alexander et al. (1960) also shows that a variety of soil heterotrophs are able to increase the oxidation state of nitrogen.

Genus	Number of Strains Tested	Number of Nitrifying Strains
Mycobacterium	4	1
Nocardia	20	3
Streptomyces	124	33
Micromonospora	2	2
Streptosporangium	1	1
Total	151	40

Hirsch et al. (1961) reported that forty eight out of one hundred and sixty one strains of actinomycetes tested produced NO_2^- from NH_4^+, and Odu and Adeoge (1970) found that ten out of the eleven isolates of heterotrophic organisms they studied were able to produce either NO_2^- or NO_3^- from nitrogenous organic compounds.

A summary of reported nitrogen-oxidising heterotrophic species is presented in table 2.1. Fungi appear to be the most common and most efficient NO_2^- and NO_3^- producers of the organisms listed (Odu and Adeoge, 1970; Eylar and Schmidt, 1959). The amount of NO_2^- and NO_3^- formed from NH_4^+ by heterotrophic organisms in culture media is seldom more than a few ppm, whereas autotrophic nitrifying bacteria can form in excess of 1,000 ppm NO_3^- (Alexander et al. 1960). However, heterotrophic organisms can utilise a wide range of organic nitrogenous compounds, and yields of NO_2^--N in excess of 190 ppm have been reported for heterotrophic cultures grown with organic substrates (Doxtader and Alexander, 1966).

Aspergillus flavus has attracted considerable attention because it is capable of producing reasonable quantities of nitrate (Eylar and Schmidt, 1959). Schmidt (1960) found that pure cultures of *Aspergillus flavus* in alfalfa and barnyard manure produced NO_3^-, but was unable to obtain evidence of NO_3^- production by *Aspergillus flavus* in mixed culture environments, even though the fungus was capable of colonising the soil in the presence of a mixed flora inoculm.

The actual importance of heterotrophic nitrifying organisms in soils remains obscure, and while there is no direct evidence for the contribution of heterotrophic organisms to nitrification in natural environments, in some situations it appears that the formation of nitrate cannot be accounted for on the basis of the known characteristics of chemolithotrophic bacteria (Alexander et al. 1960; Ishaque and Cornfield, 1974).

Table 2.1 Nitrogen oxidising heterotrophic micro-organisms, their substrates and products

Micro-organism	Substrate	Product	Reference
Fungi			
Aspergillus flavus	$(HN_4)SO_4$	nitrite	Eylar and Schmidt, 1959
	urea; protone casein; peptone	nitrate	
Penicillium spp.	peptone	nitrite nitrate	Eylar and Schmidt, 1959
Crlalosporium spp.	protone peptone	nitrate	Eylar and Schmidt, 1959
Aspergillus flavis	peptone	nitrate	Schmidt, 1954
Penicillium spp.	amino	N-formyl hydroxy-amino-acetic acid	Dulaney and Gray 1962
Fusarium spp.	pyruvic oxime	nitrite	Doxtader and Alexander 1966
Actinomycetes			
Nocardia spp.	ammonium	nitrite	Hirsch et al. 1961
Streptomyces	ammonium	nitrite	Hirsch et al. 1961
Micromonspora spp.	ammonium	nitrite	Hirsch et al. 1961
Streptosporangium	ammonium	nitrite	Hirsch et al. 1961
Nocardia petroleophila	ammonium	nitrite	Hirsch et al. 1961
Nocardia autotrophica	ammonium	nitrite	Hirsch et al. 1961
Streptomyces nitrificans	urethan	nitrite	Isenberg et al. 1954
Nocardia corollina	pyruvic acid	nitrite	Jensen, 1951
Bacteria			
Mycobacterium	ammonium	nitrite	Hirsch et al. 1961
Alcaligenes spp.	ammonium	nitrite	Jensen, 1951
Agrobacterium spp.	ammonium	nitrite	Jensen, 1951
Achromobacter	oxime	nitrite	Quastel et al. 1950
Corynebacterium	oxime	nitrite	Quastel et al. 1950
Corynebacterium simplex	nitrophenols	nitrite	Gundersen and Jensen, 1956
Azotobacter vinelandii	N_2; ammonium	bound hydroxyl-amine	Saris and Virtanen, 1957

2.6.0 BIOCHEMISTRY OF HETEROTROPHIC NITRIFYING ORGANISMS

Because of the large number of nitrogenous compounds which can be oxidised by heterotrophic organisms, and the numerous genera and species capable of oxidising nitrogenous compounds, the biochemistry of heterotrophic nitrification appears to be poorly understood, and is considered to be outside the scope of the present review.

2.7.0 ABUNDANCE OF CHEMOLITHOTROPHIC NITRIFYING BACTERIA IN SOILS

Relatively few studies have been reported on the size of the nitrifying population in different soils, possibly because accurate estimations are difficult to obtain due to limitations imposed by commonly used counting methods (e.g. Taylor, 1962).

Low numbers of chemolithotrophic bacteria (100 - 1,000 per gram soil) were found by Frederick (1957) in Clermont (pH 3.0) and Mallot (pH 7.8) soils and much higher numbers (10,000 - 50,000 per gram soil) in Genesse soil (pH 7.7). The low population in the Clermont soil was ascribed to low pH, and that in the Mallot soil to lack of substrate.

Counts varying from 200 - 8,000 chemolithotrophic bacteria per gram of soil were reported for 75 Australian soils by Sims and Collins (1960). The detection of over 1,000 bacteria per gram of soil collected from desert areas which had not received rain for nearly two years indicates that drought, high temperature and other extensive variations in environmental conditions have a relatively minor effect on the distribution of chemolithotrophic bacteria.

In a climosequence of New Zealand tussock grassland soils, Tan (1967) found that the number of ammonium oxidisers increased from 50 per gram of soil in an acid Carrick soil (pH 5.2) to 1,700 per gram of soil in Conroy soil (pH 6.4). Similar results for three Canadian soils were reported by Pang et al. (1975), who found that in an acid Keld soil

(pH 5.4) there were only 7 *Nitrosomonas* spp. and 2 *Nitrobacter* spp. per gram of soil, whereas in Wellwood soil (pH 6.6) there were 1,075 *Nitrosomonas* spp. and 313 *Nitrobacter* spp. per gram of soil. In a third soil, Marton (pH 8.2), 936 *Nitrosomonas* and 272 *Nitrobacter* spp. were found per gram of soil. Irrespective of the soil investigated, the number of *Nitrosomonas* spp. was approximately three and one half times that of the *Nitrobacter* spp.

Some indication of the effect of soil management on numbers of chemolithotrophic bacteria can be gained from work reported from Rothamsted summarised below (Ziemiecka, 1932):

Fertiliser History	Ammonium Oxidisers per gram soil	
	Broadbalk	Barnfield
Unmanured	252	384
P, K, No N	406	334
$(NH_4)_2SO_4$	1949	252
$NaNO_2$	-	987
Rape Cake	1336	3687
Farmyard Manure	2243	3198

Ammonium oxidisers appear to be most numerous in soils receiving organic nitrogen or $(NH_4)_2SO_4$. Similar results were gained more recently by Soriana and Walker (1973) which are summarised below:

Field	Long-Term Fertiliser Treatment	Number of Ammonium Oxidisers per gram soil
Broadbalk	Dung	18,000
"	No fertiliser	4,310
"	Castor meal	1,880
"	P, K, Na, Mg only	98

Field	Long-Term Fertiliser Treatment	Number of Ammonium Oxidisers per gram soil
Broadbalk	P, K, Na, Mg, NH_4	210
"	-	1,270
Barnfield	No fertiliser	40
"	P, K, Na, Mg Nitrochalk	853
"	Dung	11,160
"	P, K, Na, Mg	-
Parkgrass	$(NH_4)_2SO_4$ Lime	10
"	$(NH_4)_2SO_4$	-
"	No fertiliser Lime	172
"	No fertiliser	-
"	$(NH_4)_2SO_4$ Superphosphate Lime	1,550
"	$(NH_4)_2SO_4$ Superphosphate	-
"	Minerals but no N Lime	440
"	Minerals $(NH_4)_2SO_4$ Lime	2,575
"	Minerals $(NH_4)_2SO_4$	160
"	Dung guano lime	1,984
"	Dung guano	26

The highest numbers of ammonium oxidising organisms were found in soils which had received either an organic or an ammonium fertiliser and had a nearly neutral reaction. No nitrifiers were found in the acid soils of Parkgrass (pH about 4). Soriana and Walker (1973) also estimated the numbers of ammonium oxidising bacteria in soils outside Rothamsted.

Type of Soil	Number of Ammonium Oxidising Bacteria per gram soil
Sandy soil	3,136
Sandy soil	990
Chalky clay soil treated with compost	4,290
Organic soil	2,600
Highly organic soil	2,933

In all the soils studied by Soriana and Walker (1973), *Nitrosomonas* spp. were only isolated from soils receiving dung, whereas *Nitrosocystis* and *Nitrosospira* were isolated from the other soils. Sims and Collins (1960) detected *Nitrosomonas*, *Nitrosogloea* and *Nitrosococcus* spp. in the Australian soils they studied. Winogradsky and Winogradsky (1933) isoalted *Nitrosomonas* spp. from garden soils, *Nitrosocystis* mainly from forest soils, and *Nitrosospira* from virgin soils. *Nitrosocystis* and *Nitrosogloea* spp. are not recognised in Bergey's Manual of Determinative Bacteriology and are considered as *nomina dubia* (Watson, 1971).

2.8.0 CHEMICAL NITRIFICATION

Early literature on chemical nitrification was reviewed by Waksman and Madhok (1937), and more recent reviews have been presented by Mortland (1958) and Allison (1973). Allison (1973) concluded that there is no evidence to suggest that chemical oxidation of NH_4^+ to NO_2^- is of importance in soils, but recent literature has suggested that chemical oxidation of NO_2^- to NO_3^- may be of some importance. At pH values below 5, chemical oxidation of NO_2^- to NO_3^- can occur rapidly (Allison and Doetsch, 1951) a possible mechanism being as follows:

$$3HNO_2 \rightarrow HNO_3 + 2NO + H_2O \quad (2.5)$$

in the presence of air,

$$2NO + O_2 \rightarrow 2NO_2 \quad (2.6)$$

which may then react to form HNO_3

$$2NO_2 + H_2O \rightarrow HNO_3 + HNO_2 \quad (2.7)$$

This reaction may account for formation of NO_3^- in soils too acid for the growth of *Nitrobacter* (Allison and Doetsch, 1951).

Smith and Clark (1960) found that soil enhanced the tendency of NO_2^- to convert to NO_3^-. In solutions buffered at pH 4.0 - 4.5, thirty six percent of added NO_2^- (500 mg NO_2-N per 100 ml) was oxidised to NO_3^- in a helium-oxygen atmosphere in twenty four hours. When NO_2^- was added to Fort Collins loam (pH 4.2 - 4.7) fifty five percent of it was oxidised to NO_3^- in eighteen hours.

Oxidation of NO_2^- to NO_3^- may not only be confined to extremely acid soils since Reuss and Smith (1965) found that twenty four hours after addition of $NaNO_2$, twenty five percent of the added NO_2^- had been oxidised to nitrate in a soil at pH 4.6, and fourteen percent in a soil at pH 5.6. Steam sterilised and non-sterilised soils gave identical results. Using a cation exchange resin buffered at pH 5.2, it was found that forty four percent of added NO_2^- was oxidised to NO_3^- in twenty four hours, and seventy percent in ninety six hours. The presence of oxygen was essential for the formation of NO_3^-.

Another process occurring in soils which has been examined recently is the reaction of nitrous acid with organic matter, which at low pH values can lead to production of NO (Stevenson and Swaby, 1964; Fuhr and Bremner, 1964; Stevenson et al. 1970). Any NO present in aerobic soils is likely to be oxidised to NO_2^- and further to NO_3^- as discussed above.

Further quantitative data are required before a full assessment of the importance of chemical nitrification in soils can be made.

2.9.0 PHOTOCHEMICAL NITRIFICATION

Photochemical nitrification was first suggested by Berthelot and Gaudechon (1911) who noted oxidation of NH_4^+ to NO_2^- in solution. Considerable work, reviewed by Waksman and Madhok (1937), followed this report and several workers demonstrated photochemical oxidation of NH_4^+ to NO_2^- in solution, and a few workers (e.g. Dhar, 1935) postulated photochemical nitrification to occur in soils. Such claims were discounted by Waksman and Madhok (1937), whose view was supported by the inability of several workers to demonstrate the process as occurring in soils (Fraps and Sterges, 1935; Waksman et al. 1937).

There seems little doubt, given suitable conditions, that NH_4^+ can be oxidised to NO_2^- in solution photochemically (see for example Rigg et al. 1952), but there is no evidence to support the occurrence of photo-oxidation in soils. Even if photo-oxidation of NH_4^+ does occur in soils it is likely to be of little importance in temperate grasslands because of the high density plant cover.

2.10.0 THE SIGNIFICANCE OF AUTOTROPHIC, HETEROTROPHIC AND CHEMICAL NITRIFICATION IN SOILS

In most soils, nitrification appears to be primarily a biological process, since addition of biological inhibitors largely stops nitrification (see Goring 1962a,b). The frequency of isolation of chemolithotrophic bacteria, and the relatively large numbers of these organisms present in most soils, indicate that nitrification by autotrophic organisms is the major mechanism (see sections 2.3.0 and 2.7.0). It is often assumed that the responsible bacteria are strains of *Nitrosomonas* and *Nitrobacter*, and results of micro-organism counts by techniques such as the most probable number technique are often described as *Nitrosomonas* spp. and *Nitrobacter* spp. without any specific experimental support.

The large number of heterotrophic organisms which are able to oxidise nitrogen compounds (see section 2.5.0) and the abundance of these organisms in soils may suggest that heterotrophs play an important role in nitrification. However, when 2-chloro-6(trichloromethyl)pyridine, a compound which specifically inhibits autotrophic but not heterotrophic nitrifying organisms (Shattuck and Alexander, 1963; Laskowski et al. 1975), is added to soil, nitrification, by and large, ceases (Goring 1962a,b). Heterotrophs, unlike autotrophs, are not obligately associated with nitrification for development. Therefore the occurrence of heterotrophs in soils indicates a potential for activity rather than an actual activity.

Alexander et al. (1960) calculated that fewer *Nitrobacter* cells are required to oxidise a given amount of nitrogen than *Nitrosomonas* cells. However, numbers of *Nitrobacter* reported in soils often exceed the number of *Nitrosomonas* (e.g. Morrill and Dawson, 1967) which may support a suggestion that the amount of NO_2^- available as substrate exceeds that of NH_4^+. Many heterotrophs are able to form small amounts of NO_2^-, but only a few fungi, e.g. *Aspergillus flavus* are able to form NO_3^-.

There is little data to support the occurrence of chemical or photochemical oxidation of NH_4^+ in soils, and it is generally agreed that it is of little importance (Waksman and Madhok, 1937; Waksman, 1952). Chemical oxidation of NO_2^-, however, becomes possible in soils at about the pH level that autotrophic oxidation ceases. In solution studies the critical pH has been found to be about 5.2 (Allison and Doetsch, 1951). Local areas of acidity may allow chemical oxidation of NO_2^- when the average soil pH is above 5.2, since Reuss and Smith (1965) reported oxidation of NO_2^- in steam sterilised soils at pH 5.6.

Present data suggests that autotrophic bacteria are the major mechanism of nitrification in most soils, but lack of direct quantitative

data prevents a full assessment of heterotrophic and chemical nitrification.

2.11.0 ENVIRONMENTAL FACTORS AFFECTING NITRIFICATION

2.11.1 pH

The most favourable pH for growth of *Nitrosomonas* spp. in soils is above pH 7.6 while that for *Nitrobacter* spp. appears to be in the range of pH 6.2 to 7.0 (Morrill and Dawson, 1961).

Nitrosomonas and *Nitrobacter* show low sensitivity to high hydroxyl ion concentrations, and have been reported as functioning at pH 13 (Meek and Lipman, 1922). Although *Nitrobacter* survive at high pH, Millbank (1959) found that their rate of proliferation was greatly reduced at pH values above 7.5 - 7.7, and Martin et al. (1942) quoted the "threshold" value of pH 7.7 for NO_2^- oxidation, above which the *Nitrobacter* population builds up extremely slowly. The proliferation rate of *Nitrosomonas* spp. is unaffected by alkaline pH within a wide range (Millbank, 1959).

Nitrifying organisms appear to be more sensitive to acidity than most of the common soil organisms, and no accumulation of nitrate was recorded by Weber and Gainey (1962) in a liquid medium buffered at pH levels below approximately 6.0. It is apparent, however, that pH optima for bacteria in soil differs somewhat from those noted for bacteria in solution cultures (McLaren and Skujins, 1963).

Despite a voluminous literature relative to nitrification activity in acid soils, data tends to be extremely variable and therefore to some extent contradictory. This may be due, at least in part, to differing pH optima for different isolates (Winogradsky, 1933; Ulyanova, 1961), or differing soil characteristics. The lower limit of activity may therefore be expected to vary somewhat with the soil and the indigenous organisms

present. The pH range below which nitrifiers no longer function has been placed at between pH 3.9 and 4.5 (Gerretson, 1942), at pH 4.1 (Meek and Lipman, 1922) and about pH 4.5 (Broadbent et al. 1957). Numerous workers have noted NO_3^- accumulation at low pH values; Olsen (1929) at pH 3.7; Waksman (1923) and Humfield and Erdman (1927) at between pH 4.4 and 4.8; Boswell (1955) and Weber and Gainey (1962) at pH 4; or in soils of large lime requirement (Abbot et al. 1931; Fred and Graul, 1916; White, 1913).

There is some evidence that *Nitrosomonas* spp. may adapt to their environment. Ulyanova, (1961, 1962) found that the optimum pH for activity of *Nitrosomonas* spp. was often similar to that of the environment from which they were isolated, although this was not always the case.

2.11.2 Temperature

It is well established that nitrification, like other biological reactions, is temperature dependent. Temperature optima for nitrification appear to be dependent on the temperature regime of individual soils (Mahendrappa et al. 1966; Thiagalingham and Kanchiro, 1973). The optimum temperature for nitrification has been reported as 40°C (Ishaque and Cornfield, 1974) and 35°C (Myers, 1975) for tropical soils, and as around 25°C for soils of cooler regions (Sabey et al. 1956; Mahendrappa et al. 1966). Intermediate optima in the range of 27 - 35°C have been reported by Frederick (1956). Temperatures above optimum reduce the rate of nitrification, which ceases at 40 - 45°C (Warrington, 1879; Meiklejohn, 1954; Harmsen and Kolenbrander, 1965). Although nitrification is also reduced by sub-optimal temperatures, it has been reported as occurring at 3 - 5°C (Warrington, 1884; Broadbent et al. 1958) and at temperatures around 0°C (Gerretsen, 1942; Sabey et al. 1956).

Pure culture studies of fungi (Jensen, 1969; Jensen and Reynolds 1971; Smith, 1964) and bacteria (Howell et al. 1971) have shown greater

microbial growth rates at constant temperature than at fluctuating temperatures, particularly if the amplitude of fluctuation exceeds 10°C. Burgess and Griffin (1968), however, claimed that temperature fluctuation per se does not affect the rate of fungal growth, and Powers et al. (1965) reported a slower bacterial growth rate at a constant mean temperature of 16°C than under cyclical 5 - 27°C. Biederbeck and Campbell (1971) found that incubation of soil at low fluctuating temperatures caused a sharp decline in the viable count of micro-organisms, particularly non-spore forming bacteria, an effect which could be reduced by addition of ammonium sulphate or peptone prior to incubation (Campbell et al. 1973a).

The rate of nitrification has been reported to be significantly lower (Campbell et al. 1971; 1973a; 1973b; Cook, 1952) and higher (Frederick, 1956) with fluctuating rather than constant mean temperature.

Biederbeck and Campbell (1973b) found that when incubation temperature was shifted downward, microbial population levels decreased markedly whereas rates of ammonification and nitrification increased significantly, resulting in a temporary increase in the level of mineral nitrogen. This "kill" of microbial cells was much more extensive under fluctuating than under constant temperature. Considerable loss of viable cells occurred, not only when shifting to low temperatures (13°C - 2°C), but also when shifting from high to medium temperatures (18°C - 7°C). The validity of this laboratory data was supported by NO_3^- and temperature data from a four year field trial in which it was found that a sudden flush in NO_3^- occurred each year with the onset of the first cold spell every autumn, or with the occurrence of a cold spell in late spring.

Nitrification has been found to be slower when incubation temperature was shifted from sub-optimal to optimal than vice-versa (Chandra, 1962). The importance of preceeding temperature has also been stressed by Campbell and Biederbeck (1972).

The type of temperature shift also affects nitrification. Campbell et al. (1973a) showed that results obtained with a square-wave type diurnal temperature variation, can be quite different from those obtained with a sine-wave pattern, which is more aligned to what occurs under field conditions (Geiger, 1959).

In most soils the potential for nitrification in the intermediate temperature range (10 - 30°C) far exceeds that for ammonification, and NH_4^+ is unlikely to accumulate. At low temperatures, however, ammonification tends to be favoured more than nitrification, and small accumulations of NH_4^+ may occur (Campbell et al. 1970).

2.11.3 Soil Moisture

The effect of soil moisture on microbial activity has been the subject of many investigations, but comparisons between soils are often difficult, because the moisture content in many investigations has been expressed as a percentage of some soil constant (e.g. Greaves and Carter, 1920; Russell et al. 1925; Calder, 1957; Robinson, 1957). A few investigations, however, have expressed soil moisture in terms of tension, which allows a more valid comparison between soils.

The optimum moisture for nitrification in soils has been stated as equivalent to that held by soil between 0.15 and 0.50 bars tension (Miller and Johnson, 1964). Nitrification will proceed above 15 bars tension (Miller and Johnson, 1964; Reichman et al. 1966) with complete inhibition occurring at 115 bars tension (Justice and Smith, 1962). Nitrification has also been observed below 0.15 bars tension (Miller and Johnson, 1964; Parker and Larsen, 1962). It appears that nitrification is limited at high tension by moisture deficit, and at low tension by aeration (Miller and Johnson, 1964). Most soils examined by Miller and Johnson (1964) showed maximum nitrification at tensions corresponding to greater than

twenty percent air-filled pores. The only soil examined which showed nitrification activity at zero tension contained sixteen percent air-filled pores at that tension.

Ammonification will proceed faster than nitrification in soils of high or low moisture tension, and this may lead to accumulation of NH_4^+ (Miller and Johnson, 1964; Robinson, 1957).

Sabey (1969) combined studies on the effect of constant temperatures (Sabey et al. 1956; 1959; 1969) and constant moisture (Sabey, 1969) on the production of nitrate in the presence of non-limiting NH_4^+, and concluded it was possible to estimate NO_3^- accumulation for any period of time at all temperatures from 0 - 25°C, and at all soil moistures from 0.1 - 15 bars tension in medium textured soils of the midwestern United States. The importance of temperature-moisture content interaction in quantifying microbial mediated processes was stressed by Cameron and Kowalenko (1976) who found that the optimum moisture content for the activity of nitrifying bacteria was dependent on temperature.

Campbell et al. (1974) compared nitrification in field samples subjected to wetting and drying with nitrification in laboratory incubated samples with the same diurnal temperature variation as found in the field, and found that wetting and drying accounted for seventy to ninety percent of the NO_3^- produced in the 0 - 2.5 cm depth. They accredited the difference in nitrification to the lack of production of easily mineralisable nitrogen under constant moisture conditions, the production of which occurs naturally under conditions of frequent wetting and drying (Birch, 1960; Stevenson, 1956). In a similar study, Campbell et al. (1975) found wetting and drying to be the most important factor affecting NO_3^- changes in the top 2.5 cm of soil, temperature effects accounting for only seventeen percent of the NO_3^- produced by nitrification.

2.11.4 Salt Concentration

Salts which are not directly toxic to nitrifying bacteria may reduce nitrification in soils by exerting an osmotic suction. Johnson and Guenze (1963) found that osmotic tensions above 3.3 bars reduced NO_3^- production in soil in a linear way as the salt concentration in soil increased. Their data also suggested that the nitrifying population in a calcareous soil had a greater salt tolerance than organisms in a non-calcareous soil.

2.11.5 Aeration

The importance of oxygen on the conversion of NH_4^+ to NO_3^- was first realised by Schloesing (1873), before it was known that nitrification was a biological reaction. Although nitrification is an aerobic process, it can occur at extremely low partial pressures of oxygen (Amer and Bartholomew, 1951; Greenwood, 1962). Many studies on the effect of oxygen partial pressure on nitrification have been carried out by regulating the oxygen supply in the ambient or soil atmosphere (Amer and Bartholomew, 1951; Broadbent and Stojanovic, 1952; Nommik, 1956; Wijler and Delwiche, 1954). The partial pressure of oxygen in the gaseous phase however is related only indirectly to the rate of supply at sites of microbial activity where diffusion through soil solution may become rate limiting (Bremner and Shaw, 1958a,b; Wijler and Delwiche, 1954).

Lemon and Erickson (1952, 1955) measured oxygen diffusion rates in undisturbed soil with a platinum electrode. Oxygen diffusion rates were greater in larger aggregates with greater porosity, and declined with increasing depth below the surface. The thickness of the moisture film had a large effect on oxygen supply in soils of low porosity, a similar conclusion was reached by Brandat et al. (1964) in laboratory studies.

2.11.6 Nutrient Supply

There are few reports in the literature of nutrients other than NH_4^+ and NO_2^- limiting nitrification. Fraps and Sterges (1939) reported that phosphorus deficiency was sufficiently severe in many soils to restrict the rate of nitrification. It appeared that nitrite-oxidisers were more sensitive to phosphorus deficiency than ammonium-oxidisers. Molybdenum was implicated as limiting nitrification by Bertrand (1973) who found that ammonium molybdate solution sprayed on to soil (100 g/ha Mo) increased N fixation, ammonification and nitrification.

2.11.7 Season

Any effect which seasons may have on nitrification activity, or the population of nitrifiers in soil, is really only a reflection of environmental factors such as temperature, moisture, aeration or substrate, all of which show some variation with time of year. Maximum populations of nitrifying organisms have been recorded in spring and early summer in Iowa soils (Thorne and Brown, 1937), in spring and autumn in Japanese soils (Sakai, 1960) and in summer in New Zealand soils (Ross, 1960).

2.11.8 Depth

Variation of nitrification activity with depth has only received attention by a few investigators. Warrington (1884) observed that nitrification activity apparently decreased with depth in soil, activity seldom being present below 45 cm in Rothamsted soils. Studies on NO_3^- accumulation in soils during incubation normally show most rapid nitrification of applied $(NH_4)_2SO_4$ in samples collected close to the soil surface (Eno and Ford, 1958; Dancer and Peterson, 1974). Eno and Ford (1958) found that on incubation, nitrification of $(NH_4)_2SO_4$ was very slow in samples collected below 1.3 m, although appreciable numbers of ammonium

oxidising bacteria were reported as being present at depths greater than 2.4 m. Dancer and Peterson (1974) suggested a close association between rate of nitrification and soil organic matter content.

Presumably, populations of nitrifying organisms will accumulate largely where supplies of energy, substrate, nutrients and oxygen are maximal, providing that environmental conditions of moisture, temperature and pH are satisfactory. Such conditions are most likely to occur close to the soil surface.

2.12.0 EFFECT OF TYPE OF CLAY AND PARTICLE SIZE ON NITRIFICATION

Clay minerals are known to exert a marked influence on the activity of bacteria in pure culture (Stotzky and Rem, 1966), largely because clay minerals, especially those with high cation exchange capacities, are able to sustain the pH of the media at levels adequate for growth. It also appears, however, that stimulation of bacterial activity may occur by mechanisms other than buffering (Stotzky, 1966a). Stotzky (1966b) concluded that respiration of bacteria increased with an increase in the cation exchange capacity of clay minerals and also possibly with surface area of the particles, while particle size did not appear to be an important characteristic.

Lees and Quastel (1946b) were the first workers to draw attention to the importance of colloidal soil particles on nitrification activity. They postulated that *Nitrosomonas* spp. metabolised largely absorbed NH_4^+, and showed the rate of nitrification to be dependent on cation exchange capacity of the soil. Contrary to Lees and Quastel's (1946b) hypothesis, Goldberg and Gainey (1955) concluded that the availability of NH_4^+ to nitrifying bacteria in liquid cultures containing different soil minerals was directly related to the quantity of unabsorbed NH_4^+. They found that the addition of potassium-saturated soil minerals to a filtered medium

resulted in significant increases in NO_3^- accumulation for bentonite (9.1%), montmorillonite (6.7%) and muscovite (19.1%), and a significant decrease for kaolin (20.7%). When ammonium-saturated soil minerals were added, no significant alteration in the rate of NO_3^- accumulation occurred for bentonite, a small depression was noted for montmorillonite (7.0%) and a large depression for muscovite (70.3%) and kaolin (68.8%).

Working with one hundred percent calcium-saturated clays at pH 7, Kai and Harada (1969) found that montmorillonite stimulated nitrification more than halloysite which in turn stimulated nitrification more than allophane. When fifty percent calcium-saturated clay was added to solution cultures without pH adjustment, allophane supported the highest rate of nitrification and this was accredited to its high buffering capacity. Kai and Harada (1969) also measured the adsorption of ammonium and nitrite oxidising organisms on to montmorillonite, halloysite and allophane from solutions containing 14,500 organisms at pH 6.9 and 4.8, and found a high adsorption (>96.6%) in all cases.

Nishio and Furasaka (1971b) found that the rate of NO_2^- oxidation in perfused soil could be considerably increased by dispersion of the soil by shaking, and suggested this was due to the activation of NO_2^- oxidising organisms which were previously inactive. They found that in an undispersed soil, NO_2^- was oxidised exponentially at first and then in a cubic root relation later. Such a relationship was suggested to be due to the existance of bacteria in bacterial masses formed on open surfaces of soil or by partially filling pores. In a mass of bacteria, NO_2^- availability limited oxidative activity of cells inside a cell mass.

It is well known that clays such as illite, montmorillonite and vermiculite are able to fix comparatively large quantities of ammonium in a form that is not removed by leaching with KCl (Stanford and Pierre, 1946; Bower, 1950; Allison et al. 1951). This ammonium has only a low

availability to nitrifying organisms (Allison et al. 1951; Bower, 1951; Allison et al. 1953; Raju and Mukhopadhyay, 1974).

2.13.0 THE EFFECT OF PLANT ROOTS AND ORGANIC MATERIALS ON NITRIFICATION

Winogradsky and Omeliansky (1899) observed that small amounts of glucose, peptone, asparagine and sodium butyrate inhibited nitrification. Russell (1914) suggested that the lower NO_3^- content of cropped soil compared with uncropped soil, even after being corrected for crop uptake, was due to a diminished NO_3^- production in the presence of plants, and an inhibitory effect of grasses on nitrification was suggested by Lyon et al. (1923). A similar suggestion by Theron (1951) appeared to stimulate renewed interest in the effect of plants and organic matter on nitrification, and subsequently, inhibitory effects were postulated by many other workers (Eden, 1951; Stiven, 1952; Mills, 1953; Berlier et al. 1956; Greenland, 1958; Boughey et al. 1964; Rice, 1964; Munro, 1966a,b).

Three field plots representing two stages of old field succession and the climax of three vegetation types (oak-pine forest; post oak-blackjack oak forest; tall grass prairie) were studied by Rice and Pancholy (1972) who concluded that inhibition of nitrification increased during progression towards climax vegetation. Such a hypothesis, they argued, could also be supported on grounds of conservation of energy, since the reduction of NO_3^- to NH_4^+ in plants requires considerable expenditure of energy. Elimination of NO_3^- from the nitrogen cycle reduces loss of nitrogen by leaching and denitrification, therefore improving nitrogen conservation in the soil. The experimental evidence to support this hypothesis, however, was largely circumstantial.

Many workers have examined root or plant extracts for nitrification inhibitory effects without attempting to identify specific inhibitors. Root extracts of a tropical grass, *Hyparrhenia filipendula* were demonstrated

to inhibit enrichment cultures of nitrifying bacteria by Munro (1966a,b). Neal (1969) found that root extracts of *Agropyron dasystachyum*, *Agropyron smithii*, *Bromus pumpellianus*, *Festuca scabrella*, *Phleum pratense*, *Stipa comata* and *Stipa spartea* did not suppress nitrification by *Nitrosomonas europaea*, whereas significant suppression by root extracts from *Bouteloua gracilis* and *Taraxacum officinale*, and to a lesser extent by *Plantago purshii* and *Bromus tectorum* was noted. Washings of ryegrass, wheat, salad rape, lettuce and onion have also been reported to reduce nitrification activity (Moore and Waid, 1971). Odu and Akerele (1973) found that nitrification was in fact increased when soil was incubated with root extracts of four grasses (*Andropogon tectorum*, *Chloris gayana*, *Panicum maximum*, *Pennisetum purpureum*) and two legumes (*Calopogonium mucunoides* and *Stylosanthes gracilis*) possibly because of suppression of soil heterotrophic bacteria.

Contrary to the findings of Boughey et al. (1964) and Munro (1966a,b), Purchase (1974) reported that although washings of living roots of *Hyparrhenia filipendula* prolonged the lag period of NO_2^- oxidisers in solution cultures, daily additions of root washings to soil did not inhibit nitrification. Incubation of roots in soil with added $(NH_4)_2SO_4$ caused considerable immobilisation of mineral nitrogen with consequential depression of nitrate accumulation, but no inhibitory effect of nitrification of the surplus NH_4^+ was noted. Neither was any inhibitory effect noted when macerated roots were mixed with soil and perfused with $(NH_4)_2SO_4$. It was concluded that roots of *Hyparrhenia filipendula* were not inhibitory to nitrification, and this was supported by an increase in numbers of NH_4^+ oxidisers in the vicinity of *Hyparrhenia filipendula* plants following application of $(NH_4)_2SO_4$.

Inhibition of nitrification by glucose has been reported by many workers (Winogradsky and Omeliansky, 1899; Heubult, 1929; Nelson, 1931; Jensen, 1950a). Meiklejohn (1951) however, established that the toxicity

of glucose was due to formation of toxic decomposition products during heat sterilisation. If glucose was sterilised by filters without heat, no inhibition occurred.

A further conclusion reached by Meiklejohn (1951) was that physiological work done with impure cultures requires very cautious interpretation, since some substances may stimulate non-nitrifiers present in the culture which in turn may suppress nitrification. Such an effect was reported by Kaczmarek (1972a,b, 1973) who found that glucose, starch and gelatin were not toxic to nitrifying organisms, although they decreased the nitrification activity. It was postulated that the suppression of nitrification was due to either metabolites or competition from heterotrophs since addition of glucose, starch or gelatin caused intensive development of groups of heterotrophs, the period of maximal development of the heterotrophs being coincident with the period of highest inhibition of nitrification. Conversely, Engel (1934) found that addition of glucose to soil increased nitrification, not because of any direct effect on the nitrifiers, but because it increased numbers of nitrogen fixing bacteria which provided more substrate for the nitrifiers.

Peptone has also been found to be inhibitory to the growth of one or both of the nitrifiers (Winogradsky and Omeliansky, 1899; Beijerinck, 1914; Kingma Boltjes, 1935). Inhibition by peptone was found to be due to free amino acids (Kingma Boltjes, 1935). The mechanism of inhibition is not clear but Lees (1963) suggested that amino acids may either bind essential trace elements or specifically block some key reaction in the cells.

Inhibition of nitrification by urea (Winogradsky and Omeliansky, 1899; Meyerhof, 1916a,b, 1917) is apparently due to traces of cyanate in the urea (Dirnhuber and Schutz, 1948). Cyanate is known to be highly

toxic to *Nitrobacter* (Taussig, 1960).

Ammonia is toxic to nitrifying organisms, the level of toxicity increasing with increasing pH (Stojanovic and Alexander, 1958; Engel and Alexander, 1960). Kaczmarek (1972b, 1973) accredited the inhibitory effect of casein hydrolysate on nitrification to the formation of NH_3 during metabolism. The inhibition of nitrification in soil by nitrogen-rich organic compounds has also been discussed by Cornforth and Davies (1968), Hoflich (1968) and Smith (1964a, 1967).

Recently, considerable interest has been shown in tannins as possible inhibitors of nitrification. Basaraba (1964) reported that addition of 0.125 percent (w/w) of tannins had little effect on nitrification whereas additions of 0.5 to 2 percent (w/w) reduced but not inhibited nitrification over a two week laboratory incubation. Tannins produced by several species of plants were found to inhibit nitrification (Rice, 1965). Rice and Pancholy (1973) reported complete inhibition of nitrification by as little as 2 ppm of condensed or hydrolyzable tannins, or 0.17 ppm of tannic acid. Many plants (*Pinus echinata, Quereus stellata, Quereus marilandica, Aristida oligantha,* and several prairie grasses) are known to produce tannins (Rice and Pancholy, 1973). In the grasses, concentrations were found to be higher in shoots than in roots. Tannins have also been reported as occurring in soils. Blum and Rice, (1969) reported 600 - 800 ppm tannic acid in the top 5 cm of soil under a tall grass prairie in Oklahoma. Tannic acid was found to a depth of 75 cm with a zone of accumulation in the B horizon, indicating that tannic acid is sufficiently stable to leach through soil. Rice and Pancholy (1973) reported 20 ppm of tannins in the 0 - 15 cm depth under another tall grass prairie. They calculated that the amount of tannin added each year from plants was considerably below the amount found in the soil indicating an accumulation with time.

Several phenolic acids and phenolic glycosides which are inhibitors of nitrification have also been isolated from plants (Rice and Pancholy, 1974). Of the compounds isolated, ferulic acid was found to be the most potent inhibitor. This compound has been reported as being present in many soils (Guenzc and McCalla, 1966; Hennequin and Juste, 1967; Wang et al. 1967; Patrick, 1971; Chou and Muller, 1972).

Although much data has accumulated which suggest that plant roots or plant root extracts may inhibit nitrification, the exact effect of plant roots on nitrification remains unresolved. Observations by Purchase (1974) that inhibition of nitrification in solution cultures could not be duplicated in soil studies, indicates a requirement for some care in interpretation of possible inhibitory effects of plant materials. Recent data on specific compounds such as tannins has indicated that they may play a role in inhibiting nitrification in soils but more quantitative data is required to evaluate the role of such substances in soils. The presence of these compounds in soils is not considered sufficient evidence to support their role as "controllors" of nitrification. The fact remains, that large populations of nitrifying organisms are known to exist in soils supporting species of plants suggested to inhibit nitrification.

2.14.0 CHEMICAL NITRIFICATION INHIBITORS

Many agricultural chemicals which are used to control weeds, insects, parasites and diseases also affect biological nitrification. The inhibitory effect of a particular chemical is dependent on its chemical nature, the amount applied, soil properties and environmental factors (Bollen, 1961; Martin, 1963). In general, at normal rates of application, soil fumigants and fungicides exert a greater inhibitory affect on nitrifying bacteria (Stark et al. 1939; Kincaid and Volk, 1952; Wenseley, 1953) than insecticides and herbicides (Domsch and Paul, 1974). The

high nitrification inhibitory property of most fumigants is probably due to the large part of the soil mass which comes in contact with fumigants immediately after application.

A detailed discussion of all chemical inhibitors is outside the scope of the present review. However, to facilitate a comprehensive understanding of nitrifying organisms, some of the agricultural chemicals known to inhibit or retard nitrification are listed in section 2.14.1, specific nitrification inhibitors are discussed in section 2.14.2, followed by a discussion of some of the effects of inhibiting nitrification in soils in section 2.14.3. Readers requiring more detailed information are referred to reviews by Gasser, 1970; Hauck, 1972; Hauck and Koshino, 1971; and Prasad et al. 1971.

2.14.1 Inhibition of Nitrification by Fungicides, Fumigants, Herbicides and Pesticides

Many chemicals used to control soil and plant borne diseases and pests are known to inhibit or delay nitrification. Although a full discussion of fungicides, fumigants, herbicides and pesticides is outside the scope of the present review, it is considered pertinent to name some of the chemicals which are known to retard or inhibit nitrification. A glossary of chemical names is included in Appendix 1.

The following products have been found to either retard or inhibit oxidation of either ammonium or nitrite in soil or culture studies:

Product	Reference
Acetamides (CDEA & CDAA)	Otten et al. 1957; Teater et al. 1958
Aldrin	Garretson and San Clemente, 1968
Aminotriazole	Otten et al. 1957
Ammonium bromide	Smith and Weeraranata, 1975
Ammonium thiocyanate	Smith et al. 1945

Product	Reference
Anbam	Nishihara and Tsuneyoshi, 1964
Baygon	Garretson and San Clemente, 1968
Benomyl	Mazur and Hughes, 1975
Botran	Casely and Broadbent, 1968
Captan	Wainwright and Pugh, 1973
Chemagro	Casely and Broadbent, 1968
2-chlorobenzamide	Smith and Weeraranata, 1975
Chloropropham	Otten et al. 1957
Chloropicrin/proporgyl bromide	Good and Carter, 1965
Chlorophenates	Mikkelsen, 1965
Chloropropenes	Good and Carter, 1965
Cyanoguanidine	Smirnov, 1968
Cyanuric acid	Hauck and Stephenson, 1964
Dalapon	Otten et al. 1957
Dazomet	Smith and Weeraranata, 1975; Gasser and Peachey, 1964
DBCP	Koike, 1961
DD	Koike, 1961; Gasser and Peachey, 1964; Ross and McNeilly, 1975a
Di-allate	Chandra, 1964
Dicyanodiamide	Komatsu et al. 1965
Dieldrin	Chandra, 1967
Dithane	Harada, 1961
Dowfume W-85	Koike, 1961
Dyrene	Dubey and Rodriguez, 1970
EDB	Koike, 1961
Eptam	Winely and San Clemente, 1968
Fenitrothion	Ross, 1974

Product	Reference
Ferbam	Jacques et al. 1959
Heptachlor	Chandra, 1967; Winely and San Clemente, 1968
IMTD	Radwan, 1965
Lanstan	Caseley and Broadbent, 1968
Lindane	Garretson and San Clemente, 1968
Linuron	Domsch and Paul, 1974
Malathion	Garretson and San Clemente, 1968
Maneb	Jacques et al. 1959
Manzote	Jacques et al. 1959
Melamine	Hauck and Stephensen, 1964
Metham-sodium	Gasser and Peachey, 1964
Methyl bromide	Gasser and Peachey, 1964; Good and Carter, 1965; Ross and McNeilly, 1975a
Methyl isothiocyanate	Gasser and Peachey, 1964
2-methyl-mercapto-4,6-bis-isopropyl-amino-striazine	Szabo, 1964
Monzet	Hirabayashi et al. 1967
Monolinuron	Domsch and Paul, 1974
Monuron	Caseley and Luckwell, 1965
Mylone	Chandra and Bollen, 1961
Nabam	Chandra and Bollen, 1961
Nemagon	Koike, 1961
Parathion	Garretson and San Clemente, 1968
PCMB	Vlassak, 1962
Phenylacetic acid (2,3,6-TBA)	Chandra, 1964
Phenoxybutyric acid (2,4-DB)	Chandra, 1964
PRDG-10	Koike, 1961

Product	Reference
Simazine	Caseley and Luckwell, 1965
Sodium arsenite	Quastel and Scholefield, 1951
Sodium chlorate	Smith et al. 1945
TDE	Winely and San Clemente, 1968; Garretson and San Clemente, 1968
Telodrin	Srivastava, 1966
Telone	Koike, 1961
Thiourea	Reddy, 1964; Lees, 1963
Thiram	Wainwright and Pugh, 1973
TMTD	Radwan, 1965
Tributyl-3-chlorobenzyl	Smith and Weeraranata, 1975
Trichloroacetic acid and TCA	Otten et al. 1957
Vapam	Koike, 1961
Verdasan	Wainwright and Pugh, 1973
Zineb	Jacques et al. 1959

It appears as though alkylamine triazines inhibit *Nitrobacter* spp. preferentially (Dubey and Rodreguez, 1970).

Whether or not the above compounds will actually inhibit nitrification under field conditions will depend on the extent to which they come in contact with the soil. In solution tests, Mazur and Hughes (1975) found Benomyl, Dyrene and Moneb were all inhibitory to nitrification, but none of these products showed inhibitory effects under field conditions. This was suggested to be due to the lack of movement of the compounds from the turf into the soil as all compounds are of low water solubility.

2.14.2 Specific Chemical Nitrification Inhibitors

Much of the biochemistry of nitrifying organisms has evolved from

studies of specific inhibitors. Agriculturally, inhibition of nitrification in cropped or grassland soils may improve the utilisation of nitrogenous fertilisers by reducing losses of nitrogen by leaching and denitrification. Many of the chemicals used for control of soil and plant pests are phytotoxic, and therefore considerable interest has been shown in recent years in finding specific nitrification inhibitors for agricultural use. Just how sensitive nitrifying organisms are to some compounds is illustrated by data of Powlson and Jenkinson (1971) which showed that carbon disulphide liberated from rubber bungs during incubation experiments reduced rates of nitrification.

As with fungicides, fumagents, herbicides and pesticides, the number of inhibitors is large, but some of the known inhibitors are listed below:

Inhibitor	Reference
allylthiourea	Lees, 1946; 1952
amines	Meyerhof, 1916
2-amino-pyridine	Ansorge et al. 1967; Jauert et al. 1968
4-amino-1,2,4-triazole	Bundy and Bremner, 1973
1-amino-2-thiourea	Bundy and Bremner, 1973
2-amino-4-chloro-6-methyl-pyrimidine	Bundry and Bremner, 1973
ammonia	Aleem and Alexander, 1960
p-aminosalicylic acid	Quastel and Scholefield, 1951
arginine	Lees, 1952
arsenite	Butt and Lees, 1960a
asparagine	Fred and Davenport, 1921
carbon disulfide	Powlson and Jenkinson, 1971

Inhibitor	Reference
catechol	Lees and Quastel, 1946a
chloride	Hahn et al. 1942
2-chloro-6-(trichloromethyl) pyridine	Goring, 1962a,b
5-chloro-2-trichloromethyl pyridine	Andreeva and Shcheglova, 1967
2-chlorophenyl isothiocyanates	Harada et al. 1964
3-chlorophenyl isothiocyanates	Harada et al. 1964
4-chlorophenyl isothiocyanate	Harada et al. 1964
O-3-chloroaniline	Ansorge et al. 1967; Janert et al. 1968
m-3-chloroaniline	Ansorge et al. 1967; Janert et al. 1968
m-chloroacetanilide	Andreeva and Shcheglova, 1967
p-chloroacetanilide	Andreeva and Shcheglova, 1968
chlorate	Lees and Quastel, 1945; Meiklejohn, 1952
cyanide	Engel and Alexander, 1960
cysteine	Quastel and Scholefield, 1949
3,5-dichloropyridine	Andreeva and Shcheglova, 1968
2,4-diamino-6-trichloro-methyl-s-triazine	Bundy and Bremner, 1973
2,5-dichloroaniline	Bundy and Bremner, 1973
dicyandiamide	Allison et al. 1925; Bundy and Bremner, 1973
dimethyl disulfide	Bremner and Bundy, 1974
dimethyl sulfide	Bremner and Bundy, 1974
dinitrophenol	Engel and Alexander, 1960

Inhibitor	Reference
ethylurethane	Lees and Quastel, 1946a; Lees, 1952
fluoride	Lundgren and Krikszems, 1959
quanidine	Lees and Quastel, 1946c
histidine	Lees, 1952
hydrazine	Lees and Quastel, 1946a; Engel and Alexander, 1960
hydroxylamine	Lees and Quastel, 1946a; Lundgren and Krikszems, 1959
8-hydroxyquinoline	Lundgren and Krikszems, 1959
hydrogen sulphide	Bremner and Bundy, 1974
iodoacetate	Lundgren and Krikszems, 1959
methionine	Quastel and Scholefield, 1949; 1951
methylamines	Lees and Quastel, 1946a
mercapto compounds	Brown et al. 1954
3-mercapto-1,2,4-triazole	Bundy and Bremner, 1973
methyl mercaptan	Bremner and Bundy, 1974
monochlorophenylisothiocyanates	Harada et al. 1964
nitro compounds	Meyerhof, 1916b
o-nitroanilines	Ansorge et al. 1967; Janert et al. 1968
m-nitroanilines	Ansorge et al. 1967; Janert et al. 1968
nitrophenols	Butt and Lees, 1960a
p-nitroaniline	Butt and Lees, 1960a
peptone	Fred and Davenport, 1921; Buswell et al. 1954
phenylmercuric acetate	Bundy and Bremner, 1973
potassium azide	Bundy and Bremner, 1973

Inhibitors	Reference
quinhydrone	Lees and Quastel, 1946a
quinine	Zavarzin, 1958
quinacrine	Zavarzin, 1958
rivanol	Zavarzin, 1958
salicylaldoxime	Lees, 1946
sodium azide	Bundy and Bremner, 1973
sodium diethyldithiocarbamate	Bundy and Bremner, 1973
sulfathiazole	Bundy and Bremner, 1973
sulfoxide	Quastel and Scholefield, 1951
thiocyanate	Gleen, 1951
thiourea	Quastel and Scholefield, 1951; Jensen and Sornsen, 1952

Some appreciation of the relative effectiveness of some of the chemical inhibitors may be gained from a study by Bundy and Bremner (1973), who tested a range of twenty four proposed inhibitors in a range of three soils and found that the effectiveness of the most potent nitrification inhibitors decreased in the order: 2-chloro-6-(trichloromethyl) pyridine> 4-amino-1,2,4-triazole>sodium or potassium azide>2,4-diamino-6-trichloro-methyl-S-triazine>dicyandiamide>3-chloroacetanilide>1-amidino-2-thiourea> 2,5-dichloroaniline>phenylmercuric acetate>3-mercapto-1,2,4-triazole or 2-amino-4-chloro-6-methyl-pryimidine>sulfathiazole>sodium diethyldithio-carbamate.

Several chemicals have now been registered as commercial nitrification inhibitors, 2-chloro-6-(trichloromethyl)pyridine under the trade name "N-Serve" by Dow Chemical Company; 2-amino-4-chloro-6-methylpyrimidine under the trade name "AM" by Toyo Koalsu Company, and Sulfathiazole ("ST") which is obtainable from Sigma Chemical Company.

Reports on "AM" and "ST" in the literature have been few and varied in the success of nitrification inhibition (see Patrick et al. 1968; Prasad and Lakhdive, 1969; Bundy and Bremner, 1973.

"N-Serve" has been widely tested and has showed some degree of success in pot and field studies. Goring (1962a,b) investigated the control of nitrification of $(NH_4)_2SO_4$; NH_4NO_3; $(NH_4)_2HPO_4$; $NaNO_2$ and urea by "N-Serve" in eighty seven soils in laboratory studies. He found that the minimum concentration required to delay conversion of ammonium to nitrite for at least six weeks ranged from 0.05 ppm to 20 ppm depending on the soil. The chemical was highly sorbed by organic matter but not appreciably sorbed by the clay fraction of soil. Although highly toxic to ammonia-oxidisers, "N-Serve" appeared to have low toxicity to nitrite oxidisers, fungi, bacteria and seedling plants. Laskowski et al. (1975) also found "N-Serve" to be of low toxicity to several soil micro-organisms.

"N-Serve" has also been found to be more effective when placed in bands in the soil than when broadcast (Goring, 1962b; Gasser, 1965).

The original observations by Goring (1962a,b) have been confirmed by many workers, e.g. Turner et al. (1962); Erh et al. (1967); Sabey (1968); Janert et al. (1968); Hoflich (1968); Ansorge et al. (1967); Patrick et al. (1968); Prasad and Lakhdive (1969).

The effectiveness of "N-Serve" appears to be dependent to some extent on soil type. Bundy and Bremner (1973) reported that nitrification inhibitors were more effective in sandy than in clay soils. The effectiveness and period of inhibition of "N-Serve" is reduced by high organic carbon contents (Lewis and Stefanson, 1975; Redeman et al. 1964). Herlihy and Quirke (1975) found that as well as being dependent on soil type, the half-life of "N-Serve" was also dependent on temperature, varying from forty three to seventy seven days at $10^{o}C$, and from nine to sixteen days at $20^{o}C$. The Q_{10} values were found to be highest in

coarse textured soils.

Coating of solid fertilisers with "N-Serve" is difficult. Briggs (1975) found that "N-Serve" was lost during removal of acetone solvent using a rotary evaporator, the final concentration of "N-Serve" being 0.9 percent of fertiliser N instead of 2 percent. Using ether as the solvent, approximately 25 percent of the added inhibitor was lost. If treated fertiliser was left standing in a dish under a plastic sheet, 25 percent was lost overnight, 41 percent after four days and 60 percent after ten days. Following application of treated fertilisers to the surface of soils, 82 percent of the added inhibitor was lost within twenty four hours of application when 5 mm of water was applied following application. In a dry soil, only 8 percent of the inhibitor remained after three days. When placed in the soil vapour losses from "N-Serve" were small and half-life was calculated to be twenty eight days in a normal soil, and fifty days in a peat treated soil. Movement of ammonium from bands of "N-Serve" treated $(NH_4)_2SO_4$ placed in the soil was found to be faster than that of "N-Serve".

2.14.3 Inhibition of Nitrification and Uptake of Fertiliser Nitrogen by Grasslands

Although many crop studies using nitrification inhibitors have been reported in the literature, only a limited number of studies have been conducted on grasslands. Application of "AM" with urea (128 kg N/ha) in one, two or three dressings to Pangola grass in Trinidad greatly increased yields when all the urea was applied in one dressing, but not when dressings were divided (Weir and Davidson, 1968). Treating $(NH_4)_2SO_4$ with "N-Serve" did not increase yields from an established grass/clover sward in the United Kingdom over that of untreated pastures (Gasser and Penny, 1965). In further field experiments, Gasser (1965) measured the

nitrification of $(NH_4)_2SO_4$, alone and treated with "N-Serve", applied to pasture on a sandy-loam and clay-loam soil in autumn. Placing fertiliser in rows decreased its nitrification and "N-Serve" enhanced this effect. In broadcast applications, "N-Serve" was less effective.

In a pot experiment with Italian Ryegrass (*Lolium multiflorum*) Nielsen and Cunningham (1964) found that the yields of tops of plants receiving NO_3^- were greatest at 100 ppm N while plants receiving NH_4^+ treated with "N-Serve" showed maximum growth at 200 ppm N. Increasing NO_3^- application above 100 ppm N greatly decreased yields of tops whereas increasing NH_4^+ application above 200 ppm N did not. Although root weights were similar for plants receiving both forms of nitrogen, an interesting difference in distribution was noted. Where plants received NH_4^+ the roots were well distributed throughout the soil, but when plants received NO_3^-, the roots were mainly concentrated in the top of the soil. In another experiment (Cunningham and Nielsen, 1965) Italian ryegrass yielded more herbage with NO_3^- than NH_4^+ up to 200 ppm N, but the reverse occurred at concentrations above 200 ppm N.

Gasser et al. (1967) reported that adding "N-Serve" to fertilised grassland decreased growth and nitrogen uptake with $(NH_4)_2SO_4$ but increased them with $Ca(NO_3)_2$. Such an effect was postulated to be due to an alteration of the NH_4:NO_3^- balance in the soil. Subsequently, Spratt and Gasser (1970) showed that initially ryegrass grew better and took up more nitrogen from $(NH_4)_2SO_4$ treated with "N-Serve" than with $Ca(NO_3)_2$, but final yields of dry matter were identical for both forms.

Inhibiting nitrification in a pot experiment was found to decrease the NO_3^- concentration in ryegrass herbage (Nowakowski and Cunningham, 1967b) and a similar effect has been reported for field experiments (Nowakowski, 1968).

Application of "N-Serve" to keep fertiliser nitrogen in the NH_4^+ form

may decrease denitrification (Mitsui et al. 1964) and leaching (Hoflich, 1968; Janert et al. 1968) of nitrogen, and as a result retain fertiliser nitrogen in the rooting zone for a longer period of time (Carter et al. 1967).

The form of nitrogen available to a plant will influence its elemental composition through its effect on the cation-anion balance within a plant. Increasing rates of application of NO_3^- have been found to decrease the concentrations of phosphorus, chloride and sulphur while NH_4^+ decreased the concentration of calcium (Nielsen and Cunningham, 1964). When the form of nitrogen absorbed by Italian ryegrass is largely NH_4^+, a clear antagonism exists between nitrogen uptake and other cations (sodium, potassium, calcium, magnesium) (Cunningham and Nielsen, 1965). Italian ryegrass receiving NO_3^- had a higher amount of total nitrogen, total soluble nitrogen and NO_3^- and a lower amount of protein nitrogen, amide nitrogen and L-amino nitrogen than that receiving NH_4^+. These differences were found to increase with increasing amounts of nitrogen applied (Nowakowski and Cunningham, 1966). A higher percent nitrogen in ryegrass receiving NO_3^- than those receiving NH_4^+ was also reported by Nielsen and Cunningham, 1964).

2.15.0 DESCRIPTION OF NITRIFICATION ACTIVITY BY MATHEMATICAL MODELS

Mathematical models for three methods of studying nitrification activity in soils will be considered:

1) Perfusion
2) Continuous leaching
3) Incubation

In addition to these, the effect of competition between two nitrifying species will also be considered.

2.15.1 Perfusion Technique

The perfusion technique for studying nitrification was introduced by Lees and Quastel (1946a). It consisted in perfusing a column of soil with oxygenated liquid by a circulatory process. The perfusion solution, which normally contained ammonium salts, was continuously circulated from a reservoir, where mixing of the solution took place, through the soil and back into the reservoir. The rate of perfusion was adjusted so that no water-logging occurred.

When the amount of NO_3^- formed was plotted against time of perfusion, typically a sigmoid type curve was found which could be described by:

$$\log \frac{y}{A-y} = K(t-t_1) \qquad (2.8)$$

where y is the NO_3-N produced (µg N/g soil); A the asymptotic value approached by y; t the time from commencement of perfusion in days; t_1 the time when $y = \frac{1}{2}A$; and K a constant (Lees and Quastel, 1946a).

2.15.2 Continuous Leaching Studies

McLaren (1969a) described a model for consecutive reactions in an idealised soil column subject to continuous flow, and solved for concentrations of NH_4^+, NO_2^- and NO_3^- as functions of both time and depth within a column which had a maximum population of nitrifying organisms. The concentration of NH_4^+ at any distance from the surface, given time of flow through a column of uniform catalytic content, was given by

$$\left[NH_4^+\right] = \left[\overline{NH_4^+}\right]_0 e^{-K_1 X} \qquad (2.9)$$

where $K_1 = k_1/\varepsilon k_o$, εk_o being the flow rate in the column in cm/day and k_1 the specific reaction rate of $NH_4^+ \rightarrow NO_2^-$; and X the depth from the surface.

Similarly, the rate of change of $\left[NO_2^-\right]$ was given by:

$$d\left[NO_2^-\right]/dX = K_1 \left[NH_4^+\right] - k_2 \left[NO_2^-\right] \qquad (2.10)$$

where k_2 is the specific reaction rate of $NO_2^- \rightarrow NO_3^-$. The $[NO_3^-]$ at any distance X was found by subtraction.

Four major assumptions were made in this approach:

1) There was a maximal stable nitrifying population.

2) Aeration was sufficient to hinder any denitrification.

3) Any mixing of ions by diffusion taking place within the flowing masses was ignored.

4) The material in the column had zero exchange capacity.

McLaren (1969b) expanded the above approach to include growth of nitrifying organisms making essentially the same assumptions as noted above. His model, summarised by McLaren (1970), was based on a relationship of the form:

$$-\frac{d[s]}{dt} = A\frac{dm}{dt} + \alpha m \frac{k''\beta m[s]}{Km+S} \qquad (2.11)$$

where $[s]$ is the substrate concentration

m is biomass

t is time

Km is a saturation constant

A, α, β are proportionality constants

k'' is a specific rate constant.

The amount of enzyme per unit of m involved in waste metabolism is given by β. The first term relates the disappearance of substrate to microbial growth, the second is the Pirt term for maintainence, and the third represents waste oxidation. The rate of downward movement of the solution in cm/day within the soil is given by $dX/dt = \varepsilon k_o$, where k_o is the rate of penetration into the soil and ε is an expansion factor.

The mathematical description was again expanded by McLaren (1971) to describe populations of nitrifying organisms as a function of depth of column being leached with nutrients and nutrient concentration profiles in an idealised soil. A further expansion was presented by Cho (1971).

Solution of the equations outlined by McLaren (1969b, 1971) required integration over both time and distance, and some procedural errors were included in his original derivations which were pointed out by Saunders and Bazin (1973) and subsequently corrected by McLaren (1973a).

A more general treatment of McLaren's model was presented by Saunders and Bazin (1973). The assumptions basic to Saunders and Bazin's (1973) model were:

1) The material under study had no exchange capacity.
2) The initial population of *Nitrosomonas* and *Nitrobacter* was uniformly distributed throughout the column.
3) There was neither dispersion nor hydrodynamic diffusion.
4) Oxygen and other metabolites were present in excess.
5) Wastes were disposed of sufficiently well so as not to poison the organisms.
6) The transformation of NH_4^+ to NO_2^- by *Nitrosomonas* and the oxidation of NO_2^- to NO_3^- by *Nitrobacter* were taken as first order with respect to NH_4^+ and NO_2^- concentrations respectively.
7) The specific rates of the reactions were proportional to the biomass densities of the relevant organisms.

Two models were derived by Saunders and Bazin (1973). In one model it was assumed that the specific growth rates of the organisms were independent of the nutrient concentrations, and in the second model that the specific growth rates of the organisms were dependent on the nutrient concentration.

Both McLaren's (1969a, 1973a) and Saunders and Bazin's (1973) models are incomplete in that the outcomes of actual experiments cannot be unequivocally predicted. However, both models have substantially contributed to understanding the systems they have set out to describe.

The models of McLaren (1970) were tested in laboratory experiments using continuous flow through a column packed with ninety percent sand

and ten percent soil by Ardakani et al. (1975). Experiments showed that urea hydrolysis obeyed first order and Michaelis-Menton kinetics, and rates of NH_4^+ and NO_2^- oxidation fell between zero and first order. It was found that models approximated concentration profiles of the nitrogen-species in solution when tested under appropriate conditions. Reasonable agreement between model predictions and experimental results had previously been reported by Kirda et al. (1974).

Mathematical models have been used to calculate rate constants per organism for oxidation of NO_2^- to NO_3^- (Ardakani et al. 1973); NH_4^+ to NO_2^- (Ardakani et al. 1974a,b) and also apparent rate constants for NH_4^+ oxidation and NO_3^- reduction, but not on a per cell basis (Starr et al. 1974; Misra et al. 1974a,b,c).

2.15.3 Incubation Studies

Seifert (1972) found that the dependence of nitrification in incubation studies on time could be expressed as

$$\Sigma N = \frac{C}{m+1} .t^{m+1} \qquad (2.12)$$

where N is the amount of nitrate nitrogen, t is the time and c and m are constants, since its logarithmic form:

$$\log \Sigma N = q+K.\log t \qquad (2.13)$$

suggested the possibility of a linear relationship between log ΣN and log t which was verified experimentally. It was also shown that the equations for integration and rate curves were of the same form, differing only in the constants.

2.15.4 Competition Between Species During Nitrification

When soil is perfused with ammonium salts, the nitrifying population multiplies as described by equation 2.8 (Chase et al. 1967); Morrill and Dawson, 1967) until a maximum population is obtained (Lees

and Quastel, 1946a). Nishio and Furusaka (1971a), however, found that when fresh substrate was added to a perfusion system, the populations of nitrifiers increased, even though the rate of oxidation was constant. If nitrifiers multiply in numbers beyond the carrying capacity of the soil surface, excess cells could be circulated in the perfusing solution and entrapped by a soil filtering action during re-perfusion. In a continuous leaching system, the excess population of organisms could be permanently removed from the system and the soil may eventually exhibit a maximum population.

In soil, the nitrifying population has to compete with other organisms for the surface available for growth. McLaren and Ardakani (1972) have considered the effect of competition between two species for the same surface area and nutrient substrate.

If two chemoautrophic species, a and b, oxidise NH_4NO_2 in a column of soil continuously infiltrated with the substrate, with sufficient concentrations of other metabolites such that γ (the maximum specific growth constant in the absence of any kind of inhibition of growth) is truly a constant, the equation for the rate of growth of nitrifiers, dN/dt, during nitrification of the initial addition of substrate:

$$dN/dt = \gamma N(1-\delta N_{max}) \qquad (2.14)$$

may be modified to read, for each species:

$$dN_a/dt = \gamma_a N_a \left(1 - \frac{N_a}{N_{maxa}} - \frac{\phi N_b}{N_{maxa}}\right) \qquad (2.15)$$

and

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{N_b}{N_{maxb}} - \frac{\theta N_a}{N_{maxb}}\right) \qquad (2.16)$$

where N is the population of NH_4^+ oxidisers at time t after the addition of NH_4^+, and N_{max} is the maximum population realizable in 1 cc of soil. The term $(1-N/N_{max})$ indicates that the rate of growth of a population declines as the useable space between cells is reduced. In equation

2.15 and 2.16 N_{maxa} and N_{maxb} are the maximum populations to be found if species a and b are grown separately. ϕ measures the inhibitory effect of an individual species b on the growth of species a, and θ is the corresponding effect of adding an individual of species a on the growth of species b. It is assumed that one organism of a covers θ times the area covered by one cell of b, and one cell of b can cover ϕ times the area coverable by one of a. Hence $\theta.\phi = 1$. For the surface area in 1 cc of soil

$$N_{maxa} = \phi\ N_{maxb} \tag{2.17}$$

Substituting equation 2.17 into 2.15 gives:

$$dN_a/dt = \gamma_a N_a \left(1 - \frac{N_b}{N_{maxb}} - \frac{N_a}{\phi N_{maxb}}\right) \tag{2.18}$$

and the equivalent substitution into equation 2.16 gives:

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{N_b}{N_{maxb}} - \frac{N_a}{\phi N_{maxb}}\right) \tag{2.19}$$

dividing equation 2.18 by 2.19 gives:

$$dN_a/N_a = \gamma_a dN_b/\gamma_b N_b \tag{2.20}$$

which for initial populations of N_a and N_b can be integrated to give

$$N_a = N_{oa} N_b^{\gamma_a/\gamma_b} / N_{ob}^{\gamma_a/\gamma_b} = N_b^{\gamma_a/\gamma_b} e^c \tag{2.21}$$

where c is a constant for some specified initial populations of a and b. This gives the population of N_a at any time t in terms of the population of N_b. Substituting equation 2.21 into equation 2.19:

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{e^c N_b^{\gamma/a\gamma b}}{\phi N_{maxb}} - \frac{N_b}{N_{maxb}}\right) \tag{2.22}$$

with only N_b as the dependent variable.

With competitive growth, the maximum populations of nitrifying organisms are approached at lower rates than with simple logistic growth. The increase in numbers of nitrifying organisms with time does not plot as a straight line in semilog plot for competitive growth. If a straight line is found experimentally, it suggests that only one species is

present, or is present in overwhelming numbers.

If the specific death rate k of a cell is dependent upon N, equation 2.14 may be modified to read:

$$dN/_{dt} = \gamma N(1-\frac{N}{N_{max}}) - kN(1+\frac{\sigma N}{N_{max}}) \qquad (2.23)$$

Expansion of equation 2.23 to take into account competition between two species gives:

$$dN_a/dt = \psi_a N_a \left(1-\psi_a \frac{N_a}{N_{maxa}} - \frac{\phi N_b}{N_{maxa}}\right) \qquad (2.24)$$

where $\psi'a = (\gamma a+ka)/\psi a$ (2.25

and:

$$dN_b/dt = \psi_b N_b \left(1-\psi'_b \frac{N_b}{N_{maxb}} - \frac{\theta N_a}{N_{maxb}}\right) \qquad (2.26)$$

where

$$\psi'b = (\gamma_b+k_b)/\psi_b \qquad (2.27)$$

Although the effective population $N = N_a+\phi N_b$ is applicable to either equation, substitution into both 2.25 and 2.26 does not reduce them to simultaneous equations that can be explicitly solved.

The value of a model such as the one presented by McLaren and Ardakani depends on whether or not existing data can be utilsed to predict the outcome of future experiments. It presents a valuable basis upon which to examine growth of mixed populations in soils.

2.16.0 NITRIFICATION STUDIES ON NEW ZEALAND SOILS

Although only a few direct studies have been conducted on nitrification in New Zealand soils, some interest has been shown in the mineralisation of soil nitrogen. White (1959) incubated ten soils from Canterbury and North Otago for twenty eight days at fifty percent water holding capacity and 30°C. $Ca(OH)_2$, equivalent of 13.6 tonnes $CaCO_3$ per ha, was added to four soils prior to incubation. White's results

may be summarised as follows:

Soil	pH	NH_4-N (ppm)	NO_3-N (ppm)
Waiareka	7.2	Trace	86
Waiareka	6.8	Trace	96
Waiareka	6.7	Trace	113
Waiareka	6.3	Trace	91
Temuka	7.2	4	173
Templeton	6.0	2	93
Cass - Lime	5.3	54	25
+ Lime		113	25
Howai - Lime	5.3	81	59
+ Lime		9	162
Hurunui - Lime	5.3	117	4
+ Lime		163	8
Oxford - Lime	5.3	127	25
+ Lime		116	109

The six soils which possesssed a pH of 6.0 or greater all nitrified most of the mineralised nitrogen during the incubation period, but the four soils of pH 5.3 nitrified only a small proportion of the available NH_4^+. Addition of lime increased the amount of nitrification in the Oxford and Howai soils but not in the Cass or Hurunui soils. In a more recent study, Ross and McNeilly (1975b) also found considerable differences in the nitrification activity of a climosequence of nine South Island tussock soils incubated for fifty two days at sixty percent water holding capacity and 24°C. Four yellow-brown earths (Tawhiti, Lammerlaw, Carrick, McKerrow) produced appreciable amounts of NH_4^+ and only negligible amounts of NO_3^-. Obelick fine sandy loam, an alpine yellow-brown earth

formed appreciable amounts of NO_3^-, and Conroy shallow sandy loam produced mainly NO_3^-. Two other soils (Ouden and Tima) produced little mineral nitrogen. Harihari loamy sand accumulated NH_4^+ initially but this was largely oxidised to NO_3^- on incubation. NO_3^- was thus produced in the driest soils and also in the oldest soil of the climosequence but not by the more developed members. It was concluded that differences in nitrifying activity could be partly explained by soil acidity, but other factors were probably involved. The four yellow-brown earths which did not nitrify ranged in pH from 4.4 to 5.1, whereas the pH of the alpine yellow-brown earth (Obelick) which did not nitrify was 5.0, and the pH of the Conroy soils which produced mainly NO_3^- was 6.4. A significant negative correlation coefficient between moisture, organic carbon, total nitrogen, carbon:nitrogen ratio and the change in NO_3^- on incubation was reported. A positive correlation was found between pH and the change in NO_3^- on incubation. It appears, therefore, that NH_4^+ would be the major form of nitrogen available to plants in upland and high country yellow-brown earths.

A study of nitrification in tussock grassland soils was conducted by Ross (1958, 1960). Examination of nitrification activity close to *Festuca* tussock plants (tussock zone) and between well spaced *Festuca* plants (intertussock zone) at four sites on three soils (Taupo, Cass, Tekoa) showed no consistant differences between the tussock and inter-tussock zones. It was concluded that *Festuca* tussock plants do not generally stimulate the activity of nitrifying organisms. Considerable variation in nitrifying activity between individual samples of the same soil were apparent which indicated irregular distribution of nitrifying organisms in these soils. Nitrifying activity in the Cass soil, which was very low, did not appear to be influenced by season. In the Taupo soil, however, NH_4^+ was oxidised more rapidly in summer than

in spring, but no seasonal differences were found in the oxidation of NO_2^- (Ross, 1960).

Following cropping, nitrification activity of both the Cass and Taupo soils increased (Ross, 1960). When introduced pastures were sampled, a higher nitrification activity was noted in samples collected from tussock grassland.

Robinson (1963) found the nitrifying population of a low-fertility New Zealand grassland soil (Craigieburn) to be very small and the soil did not accumulate NO_3^- when perfused in the laboratory with $(NH_4)_2SO_4$. The population of nitrifying bacteria and the ability of the soil to nitrify on incubation could be increased with field treatment with urea. The increased population developed in the presence of urea appeared to persist longer if lime was also applied.

Tan (1967) demonstrated the effect of increasing acidity and decreasing temperature on the numbers of nitrifying bacteria in a climosequence of soils in central Otago. His results may be summarised as follows:

Soil	Soil Group	pH	Ammonium Oxidisers per g/soil
Conroy	B.G.E.	6.4	1,700
Omakau	B.G.E.	6.1	1,000
Pigburn	B.G.E. - Y.G.E.	6.3	1,500
Blackstone	Y.G.E.	5.8	600
Tawhiti	Upland Y.G.E.	5.5	120
Carrick	High country YBE	5.2	50

In incubation studies, most of the mineralised nitrogen in the brown-grey earths (B.G.E.) was oxidised to NO_3^- whereas in the yellow-brown earths (Y.B.E.) most of the mineralised N accumulated as NH_4^+.

In other studies involving incubation of soils at sixty percent water holding capacity and 24°C, most of the mineralised nitrogen in Motupiko silt loam (Ross, 1974) and Kokatau silt loam (Ross and McNeilly, 1975a) was oxidised to NO_3^-.

The recovery of urea nitrogen by pasture growing on two soils of different nitrification activities was investigated by Steele (1976b). Following application of nitrogen to Wharekohe silt loam, little of the applied nitrogen appeared in the soil as NO_3^-, and the apparent recovery by pasture was fifty five percent of an August application and fifty six percent of a September application. Following an August application of urea to Kiripaka silt loam, little of the applied fertiliser nitrogen appeared in the soil as NO_3^- and the apparent recovery by pasture was fifty six percent. However, following a September application, more of the applied nitrogen was nitrified and apparent plant recovery fell to thirty three percent. The increased rate of nitrification in the Kiripaka soil following a September application was accredited to a lower soil moisture content and higher soil temperature. It was suggested that the lower recovery of fertiliser nitrogen on the Kiripaka soil following a September application of urea was due to the increased rate of nitrification which resulted in a higher loss of nitrogen.

2.17.0 RESEARCH REQUIREMENTS

In the present section it is intended to summarise some of the areas in which knowledge is lacking and indicate possible topics for further research.

1) The present state of knowledge suggests that in most soils, nitrification is the result of the activity of chemolithotrophic bacteria. On the basis of the dominant nitrifiers in enrichments,

and the frequency of isolation of specific bacteria in pure culture, it appears that *Nitrosomonas* and *Nitrobacter* spp. are the prominent chemolithotrophic bacteria in fertile soils, but other chemolithotrophs have been implicated in non-fertile soils. More data is required on the distribution of various species of chemolithotrophic nitrifying bacteria in soils throughout the world. The growth of bacteria in natural habitats tends to be much slower than under optimum laboratory conditions. In order to understand the rates of reactions of elements undergoing oxidation in soil, one needs to know the numbers of each kind of relevant organism in a unit volume, as well as the rate of metabolism per microbe. Only when such data is available can real values be given to parameters in mathematical models (McLaren, 1973b). As far as the author is aware, at the commencement of the present study, there had been no reported isolation of nitrifying bacteria from New Zealand soils. Reports of numbers of organisms in New Zealand soils are also limited and relate only to some low fertility and tussock grassland soils. Collection of data on populations of nitrifying organisms and their activity in a range of New Zealand soils would appear to be a fundamental requirement to understanding nitrification in New Zealand soils.

2) The biochemistry of chemolithotrophic nitrifying bacteria remains unresolved.

3) Chemolithotrophic bacteria have been isolated from environments varying from desert soils in Australia and perma-frost soils in Canada, to highly fertile soils in Britain and the United States of America. They are therefore able to survive in extremely different environments. Data available suggests that some adaptation

of chemolithotrophic bacteria to a particular environment occurs, as pH and temperature optima for isolates from different soils and geographical locations are often different, and frequently show a relationship to the environment from which the organisms were isolated. Few quantitative data are available on the effects of soil factors on nitrification in New Zealand soils.

4) Organic matter has been implicated in the chemical reduction of NO_2^- in soils, and because of the high organic matter content of New Zealand soils, and their acidic nature, the importance of this reaction requires investigation.

5) Although it has been shown that a large number of heterotrophic organisms can increase the oxidation state of a number of nitrogenous compounds, quantitative data is required on the contribution of heterotrophic organisms to nitrification in soil systems.

6) The effect of clay on nitrification activity has received some attention, but quantitative data on specific interactions between nitrifying bacteria and surface is limited. Allophane, an important clay mineral in many New Zealand volcanic soils has been little investigated with respect to nitrification.

7) Plant roots and organic materials have been implicated as affecting rates of nitrification, but quantitative data under field conditions is not sufficient to support hypothesises on this subject.

8) Incubation studies on New Zealand soils have suggested some variation in their nitrification activity. More quantitative data is required to substantiate that a difference in nitrification activity between soils does occur, and to determine if such a difference is related to soil properties.

9) Many mathematical models of nitrification have been presented, but

at present these are unable to unequivocally predict the outcome of actual experiments. This is due, at least in part, to lack of sufficient quantitative data on various aspects of nitrification on which to base a model. This indicates a lack of understanding on many aspects of nitrification.

10) In order to answer the question as to whether nitrification is a "blessing or mixed evil", more information is required from complete soil-plant-environmental systems, where the rate of nitrification is evaluated.

The objective of the present study was to determine if differences in nitrification activity occur between New Zealand grassland soils, and if so, to investigate possible causative factors. It was also proposed to investigate the effect of differences in the rate of nitrification on soil inorganic nitrogen under field conditions. At the same time it was hoped to reach some conclusion on the effect of nitrification on nitrogen cycling in soils, by investigating the fate of nitrogen fertilisers in soils of differing nitrification activity. Because it is known that nitrification activity in a pure culture medium is often materially different from what takes place in soil, the emphasis in the present study was placed on examination of soil systems rather than culture systems.

CHAPTER 3

MEASUREMENT OF THE RATE OF NITRIFICATION IN A SELECTION OF GRASSLAND SOILS

3.1.0 INTRODUCTION

In the previous chapter it was established that there is a lack of information on the nitrification activity of New Zealand soils. Research reported in the present chapter examined:

a) The nitrification activity of sixty nine samples of grassland soils from the major New Zealand soil groups, using a perfusion technique.

b) The numbers of chemolithotrophic nitrifying bacteria in some of these soils using the most probable number dilution technique.

c) The relationship of some soil chemical properties with rates of nitrification.

3.2.0 EXPERIMENTAL

3.2.1 Development of the Perfusion Technique

A technique which consisted of perfusing a column of soil with aerated solution by a continuous circulatory process was first described by Lees and Quastel (1944). The technique has proved useful for many types of studies including soil sterilisation (Lees and Quastel, 1944), nitrification (Lees and Quastel, 1946a,b,c), sulphur metabolism (Andus, 1946), degradation studies of specific chemicals in soil (Weeraratne, 1975) and elective culture of micro-organisms (Sharp and Taylor, 1969). Because the technique has been successfully used by Lees and Quastel (1946a) for studies on nitrification, it was decided to examine the technique for use in the present study.

Although many perfusion systems have been developed (Andus, 1946; Lees, 1947; Collins and Sims, 1956; Gundersen, 1960; Morrill and Dawson, 1964; Singh and Hanna, 1965; Traxler, 1965; Sharp and Taylor,

1969; Goswami and Green, 1971; Weeraratne, 1975), most have proved unsatisfactory for routine laboratory use, either because they were very complex, often consisting of numerous parts, or were based on a series of glass tubes and bottles joined together by rubber tubing and rubber bungs. Removal of rubber components from perfusion apparatus is desirable both for ease of cleaning, and also because rubber has been found to release sufficient quantities of carbon disulphide to reduce the activity of nitrifying organisms (Powlson and Jenkinson, 1971).

A modified perfusion apparatus recently developed in this laboratory (Baber, 1977) was considered satisfactory for use in the present study. Each perfusion unit (figure 3.1) consisted of three parts, a soil reservoir, a solution reservoir, and a bubble lift pump assembly. All parts were inter-changeable between perfusion units, which had the advantage of requiring a minimum of spare parts to be kept. Assembly of each unit was simple as the soil and solution reservoirs were connected using a ground glass cone and socket joint, and the bubble lift pump assembly was connected to the solution reservoir by a ground glass ball and socket joint, held together by a spring clamp. The complete unit was constructed of glass which facilitated easy cleaning.

The bubble lift pump was operated by a stream of air from a small acquarium aerating pump which lifted alternate bubbles of perfusing solution and air from the solution reservoir to the top of the soil column. The perfusion solution then percolated through the soil and drained back into the solution reservoir. The rate of perfusion was easily controlled either by control of the voltage to the aerating pump or by constricting the air supply. The perfusion unit described in figure 3.1 was designed to operate with ten to one hundred grams of

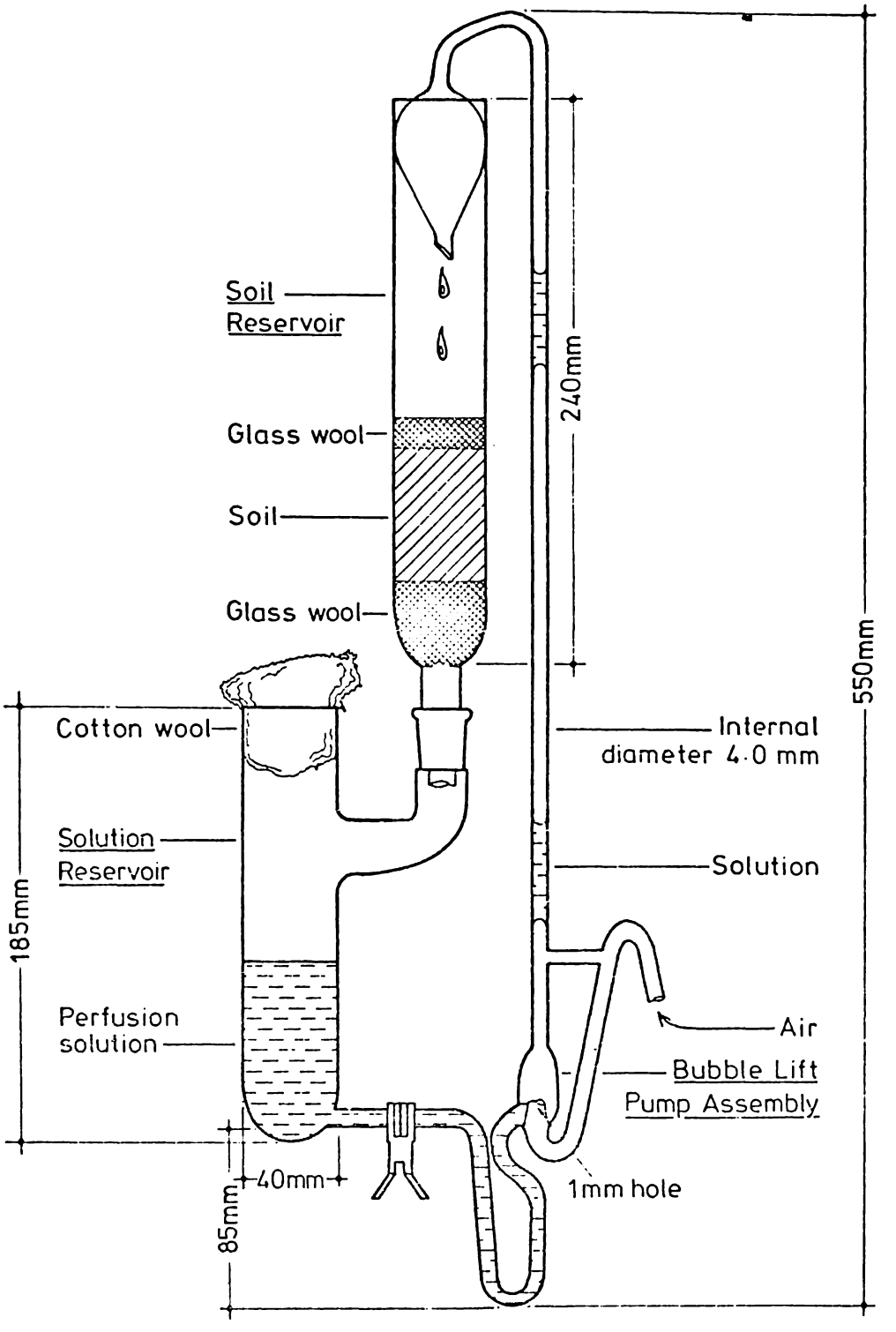

Figure 3.1 Soil perfusion unit

soil, and one hundred mls of perfusion solution. Using ten grams of soil and one hundred mls of perfusion solution, and using the analytical techniques outlined in section 3.2, sampling of the perfusion solution at twenty four hour intervals allowed accurate measurement of rates of nitrification down to 0.02 µg N_{ox}/g soil/hr. Lower rates of nitrification could be measured by increasing the amount of soil in the perfusion unit.

Prior to use, each perfusion unit was thoroughly washed with "ProtoSol" (a cleanser marketed by Biological Laboratories Limited, Auckland, New Zealand), rinsed with tap and distilled water, and autoclaved for fifteen minutes at 103 kN/m^2.

Only field moist soil samples collected less than seventy two hours prior to commencement of perfusion were used. Moist soil was passed through a 4 mm aperture sieve to remove root and plant material, and the equivalent of 10 g oven dry soil (105^{o}C) was mixed with 20 g acid washed autoclaved quartz sand and placed between two plugs of glass wool, also previously acid washed and autoclaved, in the soil reservoir. The sand was added to improve percolation, a problem with some of the poorly structured soils studied.

Several variables known to affect the rate of nitrification in perfusion experiments were investigated in preliminary experiments.

a) Concentration of substrate in perfusion solution

The objective of perfusion experiments outlined in this chapter was to specifically study nitrification. It was desirable, therefore, to remove differences in substrate concentrations between soils which arose because of differences in ammonifying capacities. This was accomplished by addition of NH_4^+ as $(NH_4)_2SO_4$ to the perfusion solution. Preliminary investigations showed that maximum rates of nitrification during perfusion were obtained with 0.005M

$(NH_4)_2SO_4$, therefore 0.005M $(NH_4)_2SO_4$ was adopted as the standard perfusion solution for all experiments. The concentration of NH_4^+ in the perfusion solution was maintained approximately constant by daily additions of 0.005M $(NH_4)_2SO_4$ as required.

b) pH of perfusion solution

If soil was perfused with 0.005M $(NH_4)_2SO_4$ for several days, the pH of the perfusion solution declined with a consequent decrease in the rate of nitrification (see section 5.3.). To prevent this, saturated $Ca(OH)_2$ was added to the perfusion solution as required to maintain the pH of the perfusion solution at the pH of the soil, as measured potentiometrically with a soil:water ratio of 1:2.5.

All perfusion experiments were duplicated and conducted in a dark room to prevent growth of photosynthetic micro-organisms. Light is also known to inhibit oxidation of NO_2^- (Warrington, 1878). Temperature was maintained at 25±1°C. This temperature was chosen because although the optimum temperature for nitrification is dependent on the site of isolation of the organism (Tandon and Dhar, 1934), in temperate regions it is approximately 25°C (Quastel and Scholefield, 1951).

The methodology for the perfusion experiments was as follows:

1) The perfusion apparatus was washed and autoclaved as described above.

2) A soil sample at field moisture was passed through a 4 mm aperture sieve to remove root and plant material. The equivalent of 10 g oven dry soil (105°C) was mixed with 20 g acid washed autoclaved quartz sand and placed between two plugs of glass wool, previously acid washed and autoclaved, in the soil reservoir.

3) The perfusion unit was then assembled with the ball and socket joint of the bubble lift pump assembly being lightly greased before clamping with a clip.

4) 100 ml of 0.005M $(NH_4)_2SO_4$, previously adjusted to the pH of the soil, was added to the solution reservoir.

5) Perfusion was commenced and sixty minutes later a sample (2 ml) of perfusing solution was withdrawn from the reservoir using a pipette which had been sterilised by autoclaving. The concentration of nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were determined as outlined in section 3.2.3. This provided an estimate of the NO_2^- and NO_3^- present in the soil at commencement of perfusion and provided a base line upon which to calculate NO_3^- accumulation.

6) A second sample was withdrawn after a further sixteen hours followed by subsequent sampling at twenty four or forty eight hour intervals.

7) The amount of NH_4^+ oxidised each day was added to each perfusion unit by addition of 0.05M $(NH_4)_2SO_4$ as required to maintain an approximately constant concentration of NH_4^+.

8) The pH of the perfusion solution was maintained at the pH of the soil when collected by manual additions of saturated $Ca(OH)_2$ as required.

9) One hour before sampling, each perfusion unit was weighed and solution lost by evaporation replaced with distilled autoclaved water.

When calculating the rate of nitrification it was necessary to include the NO_3^- removed in previous samples, and subtract the amount of NO_2^- and NO_3^- present at the first sampling one hour after commencement of perfusion.

The reproducibility of the technique is illustrated by the following data which reports the mean rate of oxidation of NH_4^+ to NO_3^- in six replicate samples of two soils.

	µg N oxidised per g soil per hour	
	Soil 1	Soil 2
	0.14	2.38
	0.12	2.54
	0.17	2.91
	0.14	2.61
	0.14	2.73
	0.15	2.80
mean	0.14	2.66
SD	0.02	0.19

Further advantages of the technique have been outlined by Lees and Quastel (1946a).

3.2.2 Selection, Collection and Storage of Soil Samples for Perfusion Experiments

Soils were selected to provide a representative sample of important agricultural soils from the major soil groups of New Zealand. Sampling sites, which varied in geographical location from Northland (latitude 35 7S) to Southland (latitude 46 36S) were chosen on high producing farms whose pastures could be considered as representative of the highest producing pastures on each soil type.

At each sampling site, twenty soil cores (0 - 7.5 cm) were collected using a 2.5 cm diameter sampler, previously sterilised by inflaming after washing with alcohol. Samples were transported to the laboratory in insulated boxes containing ice packs.

Samples from districts outside the Waikato were collected by field staff of the Soil and Field Research Organisation, Ministry of Agriculture and Fisheries.

3.2.3 Analytical Procedures

3.2.3(a) Ammonium, Nitrite and Nitrate Nitrogen

Samples for the determination of NH_4-N and NO_2-N plus NO_3-N were prepared by the steam distillation procedures of Bremner and Keeney (1965). Ammonium-N was distilled in the presence of MgO and collected in H_3BO_3 solution (6 g H_3BO_3/ℓ). A lower concentration of H_3BO_3 than recommended was used as the amount of nitrogen distilled was subsequently determined by Nessler's reagent. Nitrite and NO_3-N were subsequently distilled after reduction to NH_4^+ using Devarda's alloy (45% Al, 50% Cu, 5% Zn; < 0.149 mm particle size). The amount of distilled NH_4-N was determined using Nessler's reagent (Thompson and Morrison, 1951), colour development being measured exactly thirty minutes after addition of reagent. The NO_3-N was corrected for inclusion of NO_2-N.

Nitrite-N was determined by the sulphanilamide:n-(1-naphthyl)-ethylenediamine hydrochloride procedure as outlined by Hesse (1971).

All spectrophotometric measurements were made on an Unicam SP1800 Spectrophotometer.

3.2.3(b) Determination of Total Nitrogen

Air dry soil samples were ground to pass a 0.125 mm aperture sieve, and samples containing approximately 1 mg N were boiled with 0.3 g reduced iron (Kock-Light Laboratories, "ex iron carbonyl") in 4 ml of 1:3 concentrated H_2SO_4 (Analar):H_2O to reduce NO_3-N to NH_4-N (after Goh, 1972). After forty five minutes, samples were cooled and approximately 2 g of catalyst mixture (100 g K_2SO_4:10 g $CuSO_4$:1 g Se) and 5 mls concentrated H_2SO_4 added. The H_2SO_4 storage containers were kept sealed to prevent sorption of NH_3 from the laboratory atmosphere. Samples were then heated to 33°C and digestion continued for two and one half hours

after the mixture cleared.

The reduced iron method for inclusion of NO_3-N in total N determinations was selected after comparing it with the salicylic acid/ sodium thiosulphate method of Cope (1916). Total nitrogen in 0.2 g of Marua soil containing no NO_3-N was determined without any pretreatment, and following addition of 100 µg NO_3-N by the two methods. Recovery data presented in table 3.1 shows that recovery of NO_3-N was superior by the reduced-iron method.

The NH_4-N in the digestion mixture was determined by titration with 0.0025M H_2SO_4 after steam distillation by the semi-micro method of Bremner and Keeney (1965) following addition of 10M NaOH. The end point of titration was determined potentiometrically.

3.2.3(c) Determination of Total Organic Carbon

Total oxidisable carbon was determined by the chromic acid method outlined by Blackemore et al. (1972).

3.2.3(d) Measurement of pH

pH was measured potentiometrically at a soil:water ratio of 1:2.5 after overnight equilibration.

All analytical results are expressed on an oven-dry soil basis ($105^{o}C$).

3.2.4 Microbiological Procedures

3.2.4(a) Estimation of the Most Probable Number of Nitrifying Organisms

The most probable number of chemolithotrophic NH_4^+ and NO_2^- oxidisers was determined by the serial tenfold dilution method outlined by Alexander and Clark (1965). Each culture tube contained an inoculum

Table 3.1 Recovery of nitrate-nitrogen during total nitrogen determinations by Kjeldahl digestion following pretreatment of the soil using the salicylic acid/ sodium thiosulphate and reduced iron methods

	µg N in 0.2 g Marua soil		
	Control (no added NO_3-N)	Salicylic acid/ sodium thiosulphate method (100 µg NO_3-N added)	Reduced iron method (100 µg NO_3-N added)
	660	699	750
	662	701	757
	649	694	755
	664	689	763
	665	695	763
$\bar{x}$	660	696	758
mean % recovery of added NO_3-N	-	36	98

of 1 ml and 3 ml growth medium.

The growth medium for counting contained the following salts per litre:

Ammonium Oxidisers:

0.5 g $(NH_4)_2SO_4$

1.0 g K_2HPO_4

0.03 g $FeSO_4.7H_2O$

0.3 g NaCl

0.3 g $MgSO_4.7H_2O$

7.5 g $CaCO_3$

Nitrite Oxidisers:

0.006 g KNO_2

1.0 g K_2HPO_4

0.3 g NaCl

0.1 g $MgSO_4.7H_2O$

0.03 g $FeSO_4.H_2O$

1.0 g $CaCO_3$

0.3 g $CaCl_2$

The presence or absence of nitrite in the culture tubes after thirty days incubation at 25°C was determined visually using the sulphanilamide: n-(1-naphthyl)-ethylenediamine hydrochloride procedure outlined by Hesse (1971). When testing for the presence of NH_4^+ -oxidisers, if a negative test was obtained, a small amount of a mixture of zinc, manganese dioxide and copper metal (1:1:0.1) was added. This was necessary as sufficient NO_2^- -oxidising organisms were sometimes present in the inoculum to convert the produced NO_2^- to NO_3^-. If NO_3^- was present in the culture tube, the solutions immediately became pink on addition of the zinc, manganese dioxide, copper mixture.

Nitrifying organisms will be referred to as ammonium and nitrite oxidisers in preference to the more conventional terminology of *Nitrosomonas* and *Nitrobacter* spp., as the method of counting is specific only to autotrophic NH_4^+ and NO_2^- oxidisers and not to any particular species.

3.2.4(b) Estimation of the Generation Times of Ammonium Oxidising Organisms

Estimated generation times of NH_4^+ oxidisers were calculated according to the theory of Buswell et al. (1954).

According to Buswell et al. (1954) the logarithmic growth of bacteria can be represented by the following equation:

$$K_p = \frac{2.3}{t} \log \frac{P}{P_o} \qquad (3.1)$$

where P_o is the initial population; P the population at time t; and K_p the growth rate constant.

The rate of production of a chemical product which is the direct result of growth metabolism can be used as a measure of the rate of production of cellular protoplasm. Such measurements are accurate only under conditions in which the metabolic activity per unit of protoplasm is constant (Gunsalus, 1951). If this condition is met during the growth of NH_4^+ oxidisers, equation (3.1) can be written:

$$K_n = \frac{2.3}{t} \log \frac{N}{N_o} \qquad (3.2)$$

where N_o is the initial $[NO_2^-]$; N the $[NO_2^-]$ at time t; and K_n the NO_2^- rate constant. This relationship assumes that the population is actively growing and that the initial $[NO_2^-]$ is proportional to the initial population.

If the ratio K_n/K_p is constant, or in other words if the number of NH_4^+ oxidising cells formed at the expense of the oxidation of a given

amount of NH_4^+ is constant, then it is possible to estimate the population of NH_4^+ oxidisers by determining the amount of metabolic NO_2^- present.

The relationship between P and N in the exponential phase can be obtained when the equation for NO_2^- formation

$$dN = K_n N dt \tag{3.3}$$

and the equation for cell formation

$$dP = K_p P dt \tag{3.4}$$

are divided and integrated. The resulting expression is:

$$P = \frac{P_o}{N_o^{K_p/K_n}} N^{K_p/K_n} \tag{3.5}$$

When the equation is plotted log N vs log P, a straight line is obtained from which P can be determined from known values of $\left[NO_2^-\right]$. The validity of the equation depends on the constancy of the ratios K_p/K_n and P_o/N_o. As will be shown later, the ratio K_p/K_n varies between different populations of NH_4^+ oxidisers. However, in the present study, the ratio K_p/K_n was considered constant in any given population, and the generation time of NH_4^+ oxidisers during the logarithmic growth phase was estimated by plotting $\left[NO_2^- + NO_3^-\right]$ against time on a semi-logarithmic plot and determining the time taken for the $\left[NO_2^- + NO_3^-\right]$ to increase by a factor of 2.

3.2.5 Terminology

The term Initial Nitrification Activity (INA), which will be used to describe the nitrification activity of a soil, is defined as:

"the mean rate of oxidation of NH_4^+ from one hour to seventeen hours after commencement of perfusion, expressed as µg N oxidised per gram of soil per hour".

The mean rate of oxidation is used, not because the rate of oxidation is

considered to be constant, but to allow a comparison of rates of nitrification with different times of perfusion.

3.3.0 RESULTS AND DISCUSSION

3.3.1 Initial Nitrification Activity

A concept similar to Initial Nitrification Activity (INA) was used by Drs J. Parle and W.M.H. Saunders in an attempt to derive a nitrogen soil test. They proposed that the size of the nitrifying population in a soil should be dependent on the amount of substrate available, and therefore on the rate of mineralisation. Assuming that the nitrification activity was dependent on the population of nitrifying organisms, it should be possible to estimate the availability of nitrogen in a soil by measurement of nitrification activity over a short time period.

In the present study, the concept of INA was proposed to provide a means for the comparison of the "inherent" oxidation activities of the indigenous population of nitrifying organisms present in different soils at the time of sampling. The time over which oxidation activity was measured was restricted to sixteen hours to prevent extensive proliferation of the nitrifying organisms. As will be shown later, the generation time of ammonium oxidisers in the soils studied is dependent on pH, but even under optimum conditions, sixteen hours is equivalent to less than half of a generation time.

INA, pH, total N, organic C and C:N ratio data for a selection of grassland soils from the major New Zealand soil groups is presented in table 3.2. Soils are arranged according to soil group.

The highest INA values (generally >2.0 μg $N_{oxidised}$/g soil/hour) were found in the yellow-brown loams. These are light textured (sandy

Table 3.2 Initial nitrification activity (INA); pH; total N; total C; and C:N for a selection of New Zealand grassland soils

Soil	INA μg N_{ox}/g soil/hr	pH	Total N %	Total C %	C:N
Brown-grey earths					
Linnburn silt loam	0.41	6.0	0.238	3.31	13.9
Linnburn silt loam	0.51	6.1	0.213	2.74	12.9
Yellow-grey earths					
Claremont silt loam	0.18	6.6	0.263	3.06	11.6
Kaweku silt loam	0.54	6.5	0.276	3.75	13.6
Opuha silt loam	0.52	6.5	0.297	3.60	12.1
Timaru silt loam	0.51	6.5	0.188	2.46	13.1
Te Houka silt loam	0.76	6.3	0.290	4.99	17.2
Yellow-grey to yellow-brown earth intergrades					
Aparima silt loam	0.91	5.9	0.355	4.67	13.2
Atua silt loam, hillsoil	2.11	6.7	0.560	6.27	11.2
Oreti stoney loam	0.34	6.2	0.315	4.97	15.8
Southern and central yellow-brown earths					
Ahaura silt loam	0.06	6.0	0.385	4.06	10.5
Chaslands silt loam	0.79	5.9	0.391	4.86	12.4
Kokotau silt loam	0.08	5.6	0.275	3.59	13.1
Kokotau silt loam	0.20	6.2	0.260	4.53	17.4
Kourarau silt loam	0.49	6.0	0.475	5.04	10.6
Rosedale silt loam	<0.02	5.6	0.269	4.42	16.4
Rosedale silt loam	0.11	5.6	0.279	4.88	17.5
Waikiwi silt loam	0.91	6.3	0.398	4.85	12.2
Wehenga silt loam	<0.02	5.8	0.380	5.38	14.2
Wehenga silt loam	0.07	6.1	0.382	6.05	15.8
High country yellow-brown earths					
Lynwood fine sandy loam	0.78	5.92	0.463	6.71	14.5
Organic soils					
Rukuhia peat	0.62	5.0	1.909	43.48	22.8
Rukuhia peat	0.76	5.0	1.260	28.19	22.4

Table 3.2 continued...

Soil	INA µg N_{ox}/g soil/hr	pH	Total N %	Total C %	C:N
Rukuhia peat	0.11	5.1	2.240	44.52	19.9
Rukuhia peat	0.34	5.5	1.765	46.60	26.4
Rukuhia peat	0.93	5.5	2.179	47.32	21.7
Otonga peaty loam	0.31	5.2	2.463	31.08	12.6
Rukaka peat	0.67	4.5	1.539	26.94	17.5
Gley and gley-recent soils					
Harihari sandy loam	0.28	6.4	0.215	2.59	12.1
Hauraki clay	2.21	5.6	0.704	10.38	14.7
Koau sandy loam	0.02	5.9	0.332	3.93	11.8
Recent soils from alluvium					
Hokitika silt loam	0.06	6.2	0.321	3.80	11.8
Hokitika shallow sandy loam	0.34	6.5	0.271	3.52	13.4
Red-brown loams					
Kiripaka clay loam	0.18	5.4	0.429	5.14	11.9
Okaihau friable clay	1.57	5.7	0.671	6.49	9.7
Waimate North clay loam	2.27	6.1	0.752	6.52	8.7
Waiotu friable clay	1.68	5.8	0.548	6.76	12.3
Yellow-brown pumice soils					
Matahina gravel	0.38	5.3	0.263	3.26	12.4
Oruanui sand	0.40	5.4	0.360	5.09	14.1
Taupo silty sand	0.19	5.7	0.533	7.30	13.7
Tokoroa sandy silt	<0.02	5.3	0.652	8.17	12.5
Tokoroa sandy silt	0.29	5.5	0.726	8.41	11.7
Tokoroa sandy silt	0.78	6.0	0.669	7.65	11.4
Recent soils from volcanic ash					
Rotomahana sandy loam	0.52	6.02	0.265	3.06	11.6
Northern yellow-brown earths					
Marua clay loam	<0.02	5.5	0.426	6.70	15.8
Omanaia sandy clay loam	0.06	5.2	0.455	7.09	15.6
Tangitikei sand	0.47	6.0	0.119	2.31	19.4
Podzols					
Addison sandy loam	0.25	5.4	0.680	12.33	18.1
Hukerenui silt loam	0.53	6.3	0.263	3.92	14.9
Ohai silt loam	0.06	5.0	0.311	12.10	38.9

Table 3.2 continued...

Soil	INA µg N_{ox}/g soil/hr	pH	Total N %	Total C %	C:N
Okarito loam	0.16	5.6	0.898	17.23	19.2
Tinui silt loam	0.20	5.6	0.379	5.38	14.2
Wharekohe silt loam	0.07	5.5	0.331	4.87	14.7
Wharekohe silt loam	0.16	6.1	0.339	6.18	18.2
Yellow-brown sands					
Foxton black sand	0.07	6.4	0.160	2.23	13.9
Himatangi sand	0.84	5.8	0.365	4.57	12.5
Pukepuke brown sandy loam	5.70	7.4	0.270	2.98	11.0
Waitarere-Hokio Association	1.54	6.7	0.483	5.41	11.2
Rendzina					
Arapohue clay	2.88	7.4	0.504	5.96	11.8
Brown-granular loams and clays					
Awarua clay	0.74	5.4	0.418	6.32	15.1
Rangiuru clay	1.03	5.7	0.414	5.43	13.1
Waitakere clay	0.43	5.3	0.207	5.10	24.6
Yellow-brown loams					
Horotiu sandy loam	2.26	6.0	0.600	5.82	9.7
New Plymouth brown loam	2.29	6.1	0.932	11.63	12.5
Opuatia clay loam	2.18	5.6	0.194	12.39	13.6
Stratford sandy loam	1.75	5.4	0.893	9.15	10.3
Stratford sandy loam	2.12	6.6	0.919	9.48	10.3
Patua sandy loam	2.06	5.6	1.243	13.91	11.2
Te Kowhai	2.01	5.5	0.513	5.86	11.4

loams or silt loams) well structured soils, the top soil having a soft crumb or granular structure. They are typically derived from andesitic or fine rhyolitic ash, or sometimes alluvial mixtures of the two. The soils are well drained and aerated, possess a high cation exchange capacity, high organic matter content, and in their natural state a low percent base saturation, often accompanied by, especially in the immature and semimature soils from andesitic ash, a high level of total exchangeable bases (8 - 16 me%). Exchangeable potassium is generally high (> 1.0 me%), but the soils have a high phosphorus retention (N.Z. Soil Bulletin 1968).

The primary minerals of both andesitic and rhyolitic ashes have some similarities in that they contain considerable amounts of volcanic glasses and feldspars and smaller amounts of augite, hypersthene, hornblende and magnetite. These primary minerals weather to form the clay secondary mineral which is largely allophane, an amorphous aluminium silicate, and free hydrous iron hydroxides (Fieldes, 1971).

Allophane has characteristically a large specific surface area of the order of 500 square meters per gram (Milestone, 1972; Greenland and Quirk, 1962; Gradwell and Birrell, 1954; Aomine and Otsuka, 1968); a surface charge which changes almost linearly with pH, cation absorption increasing with increasing pH and anion absorption increasing with decreasing pH (Fieldes and Schofield, 1960); and an isoelectric point near pH 6, a consequence of the surface aluminium (Cloos et al. 1969).

INA values of the red-brown loams were variable with one soil having an INA above 2 μg N_{ox}/g soil/hr, two soils having INA values between 1 and 2 μg N_{ox}/g soil/hr and one soil below 1 μg N_{ox}/g soil/hr. Nitrification activity of red-brown loams was generally less than that of the yellow-brown loams. Red-brown loams are formed from basalt or related rocks such as basonite or dolerite. Basalt weathers more readily than most rocks, and consequently it tends to produce heavier

textured soils than those produced from other rocks under the same climate (Swindale, 1971). The clay fraction of the immature red-brown loams (e.g. Kiripaka) consists of some amorphous material with much gibbsite and kaolin. In the more mature soils (e.g. Okaihau friable clay), either gibbsite or kaolin may be dominant according to the degree of weathering. Large numbers of iron oxide nodules are also present in the Okaihau soils, which are known locally as ironstone soils.

Red-brown loams are well structured, but some tend to have restricted drainage during the winter months.

Excluding the yellow-brown loams, red-brown loams and soils of near neutral pH, all other soils, with the exception of Hauraki clay, had an INA value < 1.0 µg N_{ox}/g soil/hr. Some soils of near neutral pH had a high INA, and it will be shown later that a relationship exists between INA and pH. Atua hill soil (pH 6.7), Pukepuke brown sandy loam (pH 7.4), Waitarere-Hokio association (pH 6.7) and Arapohue (pH 7.4) had INA values of 2.11; 5.70; 1.54 and 2.88 µg N_{ox}/g soil/hr respectively.

Considerable variation of the INA's within a soil group is apparent. For example, in the southern and central yellow-brown earths, INA varied from 0 in Wehenga soil to 0.91 µg N_{ox}/g soil/hr in Waikiwi soil. Not all of this difference can be explained by differences in pH.

Brown-grey and yellow-grey earths all showed low INA values as did yellow-grey to yellow-brown earth intergrades, with the exception of the Atua hill soil. Atua hill soil differed from the other soils examined in that it possessed a high pH (6.7) accompanied by a high total nitrogen content (0.560%).

Some of the yellow-brown earths, especially the more mature members, such as Marua and Omanaia, had very low INA values.

All podzolic soils examined had low (0.06 - 0.53 µg N_{ox}/g soil/hr) INA values. This may be because soils such as Wharekohe are structureless

and will therefore provide a poor habitat for aerobic autotrophs such as nitrifying organisms.

INA values of yellow-brown sands were extremely variable. Foxton black sand, a sand dune soil of low organic matter content, which is prone to drought in summer, had a low INA. In contrast, Pukepuke brown sandy loam, a sand plain soil with a good moisture status and high pH had an extremely high INA. It is likely that the high pH (7.4) was the major factor contributing to the high INA. The high INA of Arapohue (a rendzina) may also be attributed to its high pH 7.4).

Peats generally had low INA values. One surprising feature associated with peats was the ability of some peats to nitrify at low pH. Ruakaka peat, for example, with a pH of 4.5, nitrified at an initial rate of 0.67 µg N_{ox}/g soil/hr.

Gley and gley-recent soils and recent soils from alluvium had low INA values with the exception of Hauraki clay which contained a high content of total nitrogen (0.704%).

Yellow-brown pumice soils also exhibited low INA values.

No accumulation of NO_2^- occurred in the initial seventeen hours of perfusion in any of the soils studied. It appears therefore that it is the oxidation of NH_4^+ which is the rate limiting step of nitrification in New Zealand grassland soils.

3.3.2 Most Probable Number of Nitrifying Organisms

The most probable number (MPN) of nitrifying organisms was determined in some of the soils examined for INA (table 3.3) in order to test the hypothesis that the low initial nitrification activity measured in some soils, was due to a small population of nitrifying organisms. However, contrary to the hypothesis, all soils examined contained relatively large numbers of NH_4^+ and NO_2^- oxidisers. The numbers

Table 3.3 The most probable number of chemolithotrophic nitrifying organisms in a selection of grassland soils (0-7.5 cm)

Soil	pH	Most Probable Number		INA	$\mu g\ N_{ox}$/ hr/cell
		ammonium oxidisers /g soil	nitrite oxidisers /g soil		
Horotiu	5.5	1.4×10^6	3.6×10^5	2.70	1.93×10^{-6}
Linnburn	6.7	1.6×10^6	5.4×10^5	1.05	0.66×10^{-6}
Linnburn	6.6	3.5×10^5	1.3×10^5	0.27	0.77×10^{-6}
Linnburn	5.8	5.4×10^5	2.4×10^5	0.12	0.22×10^{-6}
Linnburn	5.8	1.7×10^5	0.33×10^5	<0.02	-
Marua	5.5	1.6×10^4	2.4×10^6	<0.02	-
Tokoroa	5.9	6.0×10^5	8.6×10^5	0.36	0.60×10^{-6}
Tokoroa	5.3	3.3×10^5	7.9×10^4	0.14	0.42×10^{-6}
Waimate North	6.1	1.6×10^5	5.4×10^4	2.27	1.42×10^{-6}
Wharekohe	6.1	1.6×10^4	9.0×10^5	<0.02	-
Wharekohe	6.1	3.5×10^5	1.9×10^7	0.16	0.46×10^{-6}
Okaihau	5.7	2.2×10^6	1.7×10^6	1.57	0.71×10^{-6}
Stratford	6.6	1.1×10^5	6.4×10^6	2.12	1.93×10^{-6}

of nitrifying organisms determined are considerably higher than numbers generally reported in the literature (see section 2.7.0).

This is in contrast to the report of Morrill and Dawson (1967) that soils which did not nitrify contained few nitrifiers. They found that at the completion of perfusion, soils which did not nitrify contained an average of 1.3×10^3 NH_4^+ oxidisers and 1.9×10^2 NO_2^- oxidisers per gram of soil. Marua, a soil in the present study which did not nitrify appreciable amounts of NH_4^+, contained 1.6×10^4 and 2.4×10^6 NH_4^+ and NO_2^- oxidisers per gram of soil respectively prior to perfusion.

Pang et al. (1975) reported that the number of NH_4^+ oxidisers was approximately 3.5 times that of the NO_2^- oxidisers irrespective of the soil under investigation. Graphs presented by Morrill and Dawson (1967) also showed that in some of the soils they studied, the number of NH_4^+ oxidisers was greater than the number of NO_2^- oxidisers. In the present study, either NH_4^+ or NO_2^- oxidisers could be present in larger numbers depending on the soil, a situation for which no reason is apparent.

A soil under native forest in the Waitomo region, just south of the Waikato, was examined and found to have only 554 NH_4^+ and 1434 NO_2^- oxidisers per gram of soil. This contrasts the high number of nitrifying organisms which is characteristic for grassland soils.

Not only was there variation in the numbers of NH_4^+ oxidisers (1.6×10^4 - 2.2×10^6) and NO_2^- oxidisers (5.4×10^4 - 1.9×10^7) in the present samples, but there was also a large variation in the oxidation activity per NH_4^+ oxidising cell. In general the soils with high INA values contained a larger number of NH_4^+ oxidisers and also appeared to have a higher oxidation rate per cell (table 3.3). Differences in strains of bacteria may be important since it has been shown that bacteria isolated from different soils have different nitrifying capacities (Fraps and Sterges, 1939; Pikous Kaya, 1940). For these reasons, INA

values cannot be considered indicative of the size of the nitrifying population.

3.3.3 Variation in the Most Probable Number of Nitrifying Organisms with Depth in the Profile

Two soils of contrasting INA values and profile characteristics were chosen to study changes in the nitrifying population with depth in the profile.

1) Wharekohe silt loam (figure 3.2) is a podzolic soil of low INA (0.07 µg N_{ox}/g soil/hr) formed from strongly weathered banded sandstone and mudstone. The top soil is structureless.
2) Waimate North clay loam (figure 3.3) is a well structured free draining red-brown loam of high INA (2.27 µg N_{ox}/g soil/hr) formed from basaltic scoria.

Both soils occur under similar climatic conditions which are discussed in chapter 7. Profile discriptions for both soils may be found in Appendix 2.

In the Wharekohe soil, both NH_4^+ and NO_2^- oxidisers remained relatively constant in numbers to a depth of 15 cm below the surface and then declined in numbers till none were present at 20 cm, the depth of the silica pan (table 3.4).

The largest population of NH_4^+ and NO_2^- oxidisers in the Waimate North soil was found in the 0 - 2.5 cm depth (table 3.4). Populations of both species then decreased in numbers to a depth of 10 cm. An increase in the population of NH_4^+ oxidisers was noted between 10 and 40 cm, followed by a decline in the numbers of both NH_4^+ and NO_2^- oxidising organisms.

Some difference in the ratio of NH_4^+:NO_2^- oxidising species may be noted in the two soils. In the Wharekohe soil, the number of NO_2^-

Figure 3.2 Wharekohe silt loam profile.

Figure 3.3 Waimate North clay loam profile.

Table 3.4 Variation of the most probable number of chemolithotrophic nitrifying organisms with depth in profiles of Wharekohe silt loam and Waimate North clay loam

Depth from soil surface (cm)	Wharekohe silt loam		Waimate North clay loam	
	ammonium oxidisers /g soil	nitrite oxidisers /g soil	ammonium oxidisers /g soil	nitrite oxidisers /g soil
0 - 2.5	11.6×10^3	11.6×10^3	54.6×10^3	21.5×10^3
2.5 - 5	5.3×10^3	20.7×10^3	37.4×10^3	12.3×10^3
5 - 7.5	19.5×10^3	26.9×10^3	6.9×10^3	0.74×10^3
7.5 - 10	11.8×10^3	15.7×10^3	4.7×10^3	0.70×10^3
10 - 15	15.1×10^3	47.9×10^3	10.1×10^3	2.02×10^3
15 - 20	0.6×10^3	3.1×10^3	18.9×10^3	0.33×10^3
20 - 30	0.0	0.0	18.7×10^3	0.34×10^3
30 - 40	n.d.	n.d.	17.5×10^3	0.32×10^3
40 - 50	n.d.	n.d.	3.4×10^3	0.64×10^3
50 - 60	n.d.	n.d.	2.4×10^3	0.41×10^3
60 - 70	n.d.	n.d.	0.029×10^3	0.01×10^3
70 - 80	n.d.	n.d.	0.024×10^3	0.008×10^3

n.d. = not determined.

oxidisers present at any given depth always equalled or exceeded the number of NH_4^+ oxidisers, whereas the reverse was true in the Waimate North soil. Table 3.3 shows that although the ratio of NH_4^+:NO_2^- oxidisers varies widely in different soils, the population of NH_4^+ oxidisers is normally but not invariably larger than that of NO_2^- oxidisers. In fact in the Waimate North sample included in table 3.3, the NO_2^- oxidising population exceeds the NH_4^+ oxidising population. It is not clear what factors determine the ratio of the two types of organisms in any given soil, although if a different oxidative capacity per cell exists for NH_4^+ oxidisers from different soils, a similar effect may occur for NO_2^- oxidisers. Since Alexander et al. (1960) calculated that a greater number of NH_4^+ than NO_2^- oxidisers is required to oxidise a given amount of NH_4^+, an alternative explanation may be that a greater amount of NO_2^- substrate than NH_4^+ substrate exists in the soil, indicating a possible production of NO_2^- by mechanisms other than chemolithotrophic bacteria. Although the latter suggestion does not agree with the currently held conception of nitrification, it is however, one which should be considered.

These results agree in general with reports in the literature that the largest populations of nitrifying organisms occur close to the surface, but in well drained soils substantial populations may occur to depths of around 1 m.

3.3.4 Patterns of Nitrification Activity with Perfusion

It was reported by Lees and Quastel (1946a) that when a soil was continuously perfused with an ammonium salt solution, the rate of nitrification increased until the reaction proceeded at a constant rate. This was interpreted as indicating proliferation and saturation of the soil surface by *Nitrosomonas* spp. Evidence in the literature appears

to support such a hypothesis (Chase et al. 1967; Morrill and Dawson, 1967). Bacterial saturation of a soil, however, will only occur if all other requirements of the bacteria are satisfied.

Perfusion of all soils examined for INA was continued for periods ranging from ten to twenty days, and four general patterns of nitrification were observed (figure 3.4):

Type 1: NH_4^+ was rapidly oxidised to NO_3^-, the rate of oxidation being linear or near linear from the commencement of perfusion.

Type 2: NH_4^+ was oxidised only slowly to NO_3^-.

Type 3: NH_4^+ was oxidised slowly to NO_3^- at the commencement of perfusion, but increased logarithmically with time until a steady rate of nitrification was obtained.

Type 4: Type 3 nitrification with a temporary accumulation of NO_2^- during the initial stages of perfusion.

Type 1 nitrification was observed only in yellow-brown and red-brown loams, i.e. it occurred only in soils with a high INA (table 3.5). This type of nitrification does not plot as a straight line on a semi-logarithmic graph. Two explanations are apparent for such a pattern of nitrification. Firstly, the nitrifying population may be near saturation at the commencement of perfusion, thus showing only a small increase in numbers during perfusion; or secondly, it may be due to competition between nitrifying organisms as discussed in section 2.15.4. To test these two hypothesises, Horotiu sandy loam, a yellow-brown loam exhibiting type 1 nitrification, was perfused for twenty days with 0.005M $(NH_4)_2SO_4$, and NH_4^+ and NO_2^- oxidising organisms estimated zero, five, ten and twenty days following commencement of perfusion. Results are presented in figure 3.5. No log phase was exhibited during accumulation of NO_3^- over the twenty day period, therefore confirming

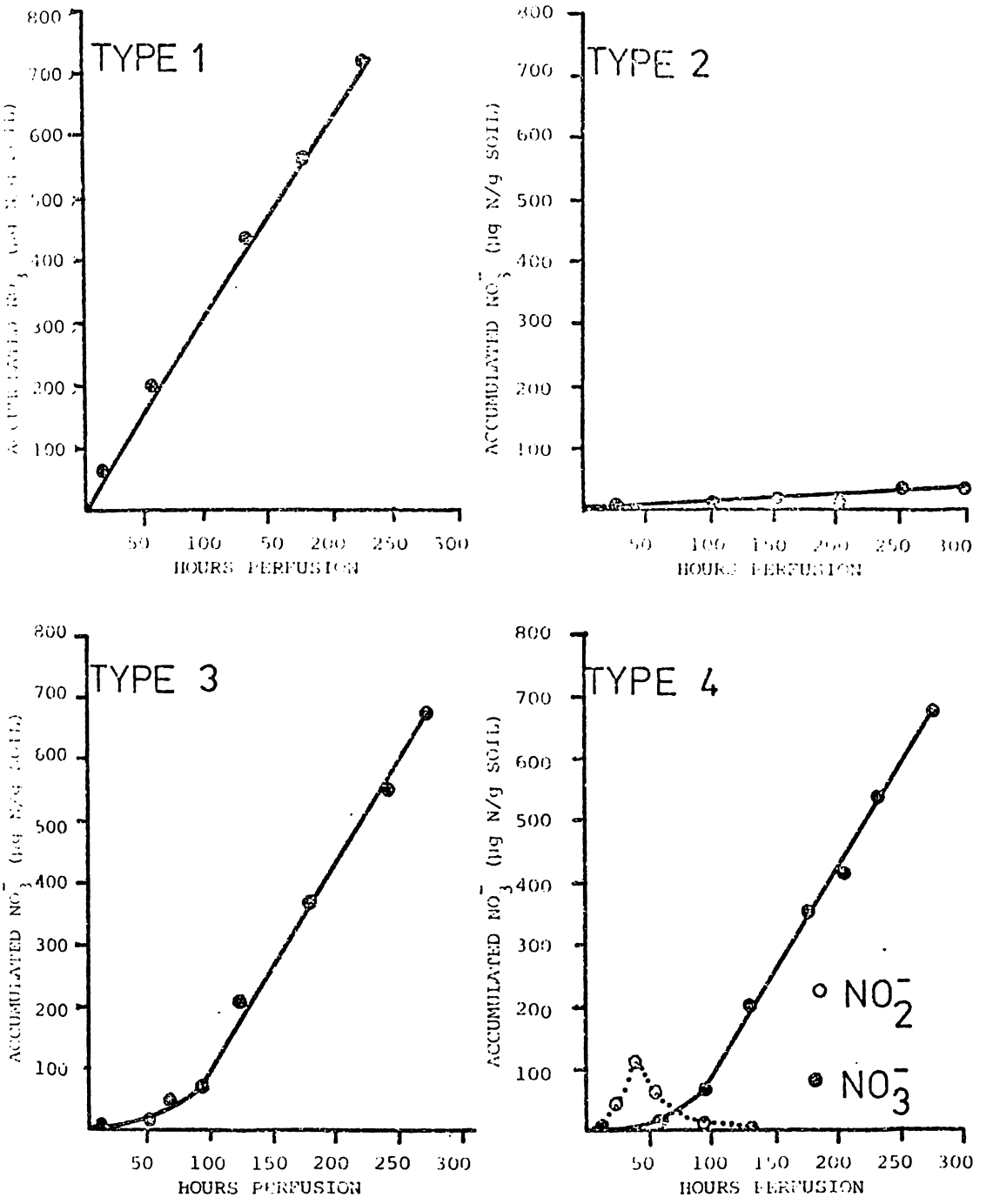

Figure 3.4 Typical patterns of nitrification observed when soils are perfused with 0.005M $(NH_4)_2SO_4$

Table 3.5 Type of nitrification pattern exhibited by a selection of New Zealand grassland soils when perfused with 0.005M $(NH_4)_2SO_4$

Soil Group	Nitrification Pattern				Total Soils Examined
	1	2	3	4	
Brown-grey earths	-	-	2	-	2
Yellow-grey earths	-	-	5	-	5
Yellow-grey to yellow-brown intergrades	-	-	3	-	3
Southern and central yellow-brown earths	-	3	7	-	10
High country yellow-brown earths	-	-	1	-	1
Northern yellow-brown earths	-	2	1	-	3
Podzols	-	-	5	2	7
Yellow-brown sands	-	-	3	1	4
Rendzina	-	-	-	1	1
Brown granular loams and clays	-	-	3	-	3
Yellow-brown loams	4	-	3	-	7
Organic soils	-	-	7	-	7
Gley and gley recent soils	-	-	3	-	3
Recent soils from alluvium	-	-	2	-	2
Red-brown loams	2	-	2	-	4
Yellow-brown pumice soils	-	1	5	-	6
Recent soils from volcanic ash	-	-	1	-	1
	6	6	53	4	69

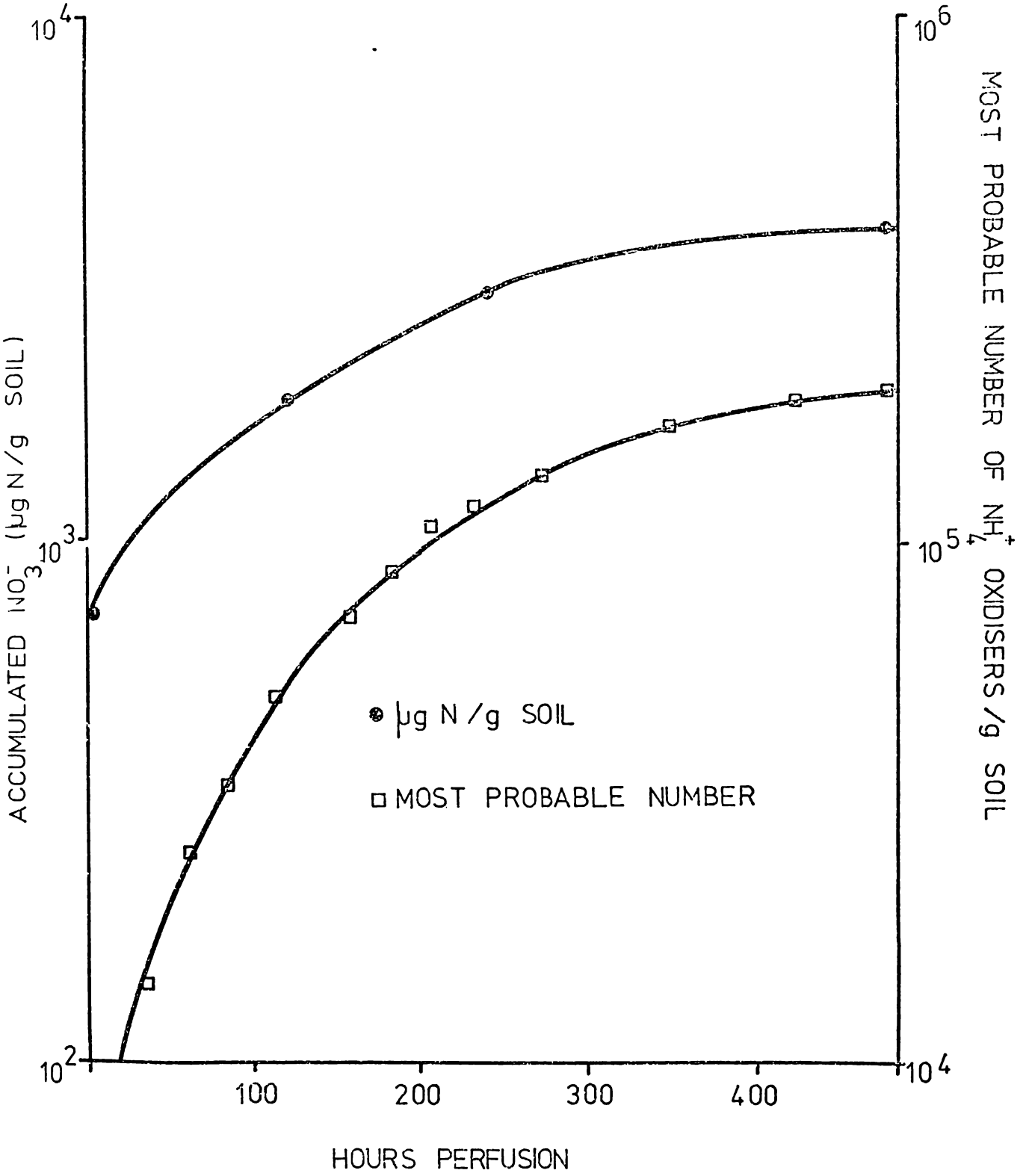

Figure 3.5 Changes in accumulated nitrate-nitrogen and the most probable number of nitrifying organisms when Dorotiu sandy loam was perfused with 0.005M $(NH_4)_2SO_4$

a type 1 nitrification pattern. Ammonium oxidisers increased nearly six fold (representing 2.5 generations) throughout the experiment but did not exhibit a log phase of growth. It would appear possible therefore that nitrification could be the result of more than one organism. Further support of such a hypothesis may be found in section 10.5.

Only six of the soils examined exhibited a type 2 nitrification pattern, five yellow-brown earths (Omonaia, Marua, Kokotau, Wehenga, Rosedale) and one yellow-brown pumice soil (Oruanui).

Marua clay, when perfused for 568 hours, produced only 70 µg NO_3^--N/g soil. the low oxidation rate was not due to an absence of nitrifying bacteria as 1.6 x 10^4 NH_4^+ oxidisers were present at the commencement of perfusion. Perfusion of Marua soil with a complete nutrient solution (as used in section 10.5), did not significantly increase the rate of nitrification over a twenty day perfusion, indicating that it was not a nutritional deficiency limiting nitrification.

The average pH of the seven soils exhibiting type 2 nitrification pattern was pH 5.5, and it is this factor which is considered to limit nitrification in these soils. Two additional experiments support this conclusion:

1) When the pH of Marua soil is increased by adding $CaCO_3$, nitrification occurs without delay (see section 5.2.3).
2) Although Wehenga soil perfused at pH 5.8 exhibited type 2 nitrification pattern, another sample of Wehenga perfused at pH 6.1 exhibited type 3 nitrification pattern. A similar phenomenon was noted for Kokotau soil in that when perfused at pH 5.6 a type 2 nitrification pattern was observed, whereas in a sample perfused at pH 6.2 type 3 nitrification was observed.

The type 2 nitrification pattern exhibited by yellow-brown earths in the present study is supported by incubation studies by Tan (1967) and Ross and McNeilly (1975b) who reported little nitrification of accumulated NH_4^+ in some acidic South Island yellow-brown earths.

The majority of soils examined exhibited type 3 nitrification pattern (table 3.5). That this type of nitrification passed through a log phase and plotted as a straight line on a semi-logarithmic scale, suggests that the nitrification activity was largely the result of one organism (see section 2.15.4). An increase in the oxidation rate on perfusion suggests that some factor which is limiting under field conditions is corrected in the perfusion unit, the most probable factor being substrate.

Type 4 nitrification pattern was observed only in soils which had a high pH with the exception of two podzolic soils of low INA. In the two latter soils it appeared as though some NO_2^- must accumulate in order to stimulate growth of the NO_2^- oxidising bacteria. Accumulation of NO_2^- at high pH is consistant with the conclusion of Harmsen and Kolenbrander (1965) that at pH values of 7.5 - 8.0 the rate of NO_2^- production exceeds that of NO_3^- production.

Nitrification of $(NH_4)_2SO_4$ in one hundred and sixteen soils of different pH values was studied by Morrill and Dawson (1967). They arranged the soils in four groups on the basis of the rate at which nitrification took place and the kind of nitrification produced. In the first group (average pH 7.85), NH_4^+ was rapidly converted to NO_2^- with a delay before being converted to NO_3^-, i.e. a similar pattern to that of type 4 nitrification. The second group (average pH 6.38) may be equated with type 3 nitrification, NH_4^+ being oxidised rapidly to NO_3^- without accumulation of NO_2^-. The third group (average pH 5.39)

oxidised NH_4^+ to NO_3^- only slowly without accumulation of NO_2^- and may be equated with type 2 nitrification. It is pertinent to note that the average pH of type 2 soils (pH 5.5) is similar to that reported by Morrill and Dawson (1967) for their third group (pH 5.39). The fourth group reported by Morrill and Dawson (average pH 5.12) did not produce measurable amounts of NO_2^- or NO_3^-, no such soils being found in the present study. Morrill and Dawson (1967) did not find an equivalent of type 1 nitrification pattern reported in the present study.

It is interesting to compare the most probable number of nitrifying organisms reported by Morrill and Dawson (1967) with those in the present study. In the most rapid nitrifying soils studied by Morrill and Dawson, on average 1.8×10^4 NH_4^+ oxidisers and 2.7×10^4 NO_2^- oxidisers per gram of soil were present at the conclusion of perfusion. In the present study, two type 1 soils, Horotiu and Okaihau, contained 7.3×10^4 and 2.2×10^5 NH_4^+ oxidisers and 3.6×10^5 and 1.7×10^6 NO_2^- oxidisers per g soil respectively at the commencement of perfusion, while Marua, a soil which did not nitrify rapidly, contained 1.6×10^4 NH_4^+ oxidisers and 2.4×10^6 NO_2^- oxidisers.

3.3.5 Generation Times of Ammonium Oxidisers

Generation times of NH_4^+ oxidisers were estimated from semi-logarithmic plots of NO_3^- accumulation against time of perfusion for soils exhibiting type 3 nitrification pattern. In general, the generation times of these organisms in soils are in the order of two to five days, and correlated with pH ($r=0.90$, significant 5%).

Soil	pH	Estimated Generation Time (Hours)
Awarua	5.42	110
Kokotau.	5.65	91
Taupo	5.69	64
Tokoroa	5.98	48
Wharekohe	6.12	54

These generation times are shorter than those reported by Morrill and Dawson (1967).

Average Soil pH	Average Generation Time For Ammonium Oxidisers (Hours)
7.85	40 ± 4
6.38	83 ± 4
5.39	146 ± 14

The pH dependence of generation time is also illustrated by Morrill and Dawson's (1967) data.

3.3.6 Relationship Between Initial Nitrification Activity, pH, Total Nitrogen, Total Organic Carbon and Carbon:Nitrogen Ratio

Multiple linear regression analysis was carried out using a selective regression programme on an ICL 2903 computer. Pukepuke brown sandy loam and Arapohue clay were removed for this analysis since, because they possessed a pH above 7, they were considered atypical of New Zealand soils in general.

When the pooled data for all soils was examined, only pH and percent total N were found to have an affect on INA, pH being the

most important. For all soils:

$$INA = -1.642 + 0.358\ pH + 0.423\ \%\ total\ N \quad (3.6)$$

$R^2 = 0.12$*. The standard deviation for pH was 0.179 and that for percent total N 0.284. Taken individually, pH was just significant at the 5 percent level, and percent total N non-significant.

Removal of organic soils increased R^2 to 0.26, and again only pH and percent total N had an affect on INA.

$$INA = -2.503 + 0.458^*\ pH + 1.136\ \%\ total\ N \quad (3.7)$$

The standard deviation for pH was 0.180 and that for percent total N 0.362.

When both organic soils and yellow-brown loams were excluded from the analysis, R^2 increased to 0.33.

$$INA = -3.122 + 0.513\ pH + 1.566\ \%\ total\ N \quad (3.8)$$

The standard deviation was 0.204 for pH and 0.427 for percent total N. However, a relationship with all four factors was also found ($R^2 = 0.44$):

$$INA = -4.109 + 0.418\ pH + 5.860^{**}\ \%\ total\ N - 0.279\ \%\ organic\ C + 0.097^*\ C{:}N \quad (3.9)$$

Standard deviations were 0.230 for pH, 1.706 for percent total N; 0.110 for percent total C, and 0.037 for C:N. The fact that a relationship with all four factors was found may be due to a high correlation between percent total N and percent total C ($r = 0.82$) once the organic soils and yellow-brown loams had been removed.

Considering the yellow-brown loams alone, pH gave the best relationship but was non-significant.

$$INA = 0.694 + 0.240\ pH \quad (3.10)$$

* significant at 5%

** significant at 1%

The regression coefficient for percent total N was -0.144.

Considering the organic soils alone, percent total N was best but correlation was poor

$$INA = 1.12 - 0.306 \% \text{ total N} \quad (3.11)$$

The regression coefficient for pH was 0.106.

The regression coefficient for pH in the organic soils and yellow-brown loams (0.173 ± 0.225) was not significantly different from the regression coefficient for pH for all soils combined minus the organic soils and yellow-brown loams (0.513 ± 0.204). However, the regression coefficient for percent total N in the organic soils and yellow-brown loams (-0.225 ± 0.204) was significantly different from that of all the other soils considered together (1.566 ± 0.427). Therefore, organic soils and yellow-brown loams are significantly different from the other soils considered, in the effect of percent total N on INA, and, although not significant in the present study, possibly also pH. An increase in percent total N in organic soils and yellow-brown loams has a smaller effect on INA than an equivalent increase in other soils.

3.4.0 GENERAL DISCUSSION AND CONCLUSIONS

Data presented show that large differences exist between soils in their rate of nitrification when perfused with $(NH_4)_2SO_4$. Mean differences between the major soil groups are summarised in the following table:

Soil Group	Number of Soils Examined	Mean pH	Mean INA ($\mu g\ N_{ox}/g$ soil/hr)
Yellow-brown loams	7	5.84	2.09
Red-brown loams	4	5.76	1.40
Organic soils	7	5.14	0.53
Yellow-grey earths	5	6.50	0.50
Yellow-brown pumice	6	5.54	0.34
Yellow-brown earths	14	5.85	0.29
Podzols	7	5.65	0.20

At a given pH, the INA of yellow-brown loams was higher than that of other soil groups. For example, although the average pH of the yellow-brown loams and yellow-brown earths examined were similar, the average INA of the former was higher than that of the latter. The reason for such a difference is not immediately apparent.

An increase in the percent total N in the yellow-brown loams and organic soils has a smaller effect on INA than in all other soils. This suggests either that percent total N is sufficiently high so that any further increase does not influence nitrification, a distinct possibility in view of the high percent total N in these soils, or some other factor exerts a larger influence than percent total N. One possibility is that the clay mineral allophane, which is dominant in these soils, has some effect on the nitrifying organisms, which supports increased nitrification at low pH. Allophane has been shown to stimulate the growth of E. coli due to its high buffering capacity (Cooper, 1977). If such an effect is important in nitrification, then the question arises as to why yellow-brown pumice soils in which allophane is also the major clay mineral, do not exhibit

the same high INA. The content of allophane, however, is much lower in pumice soils than in yellow-brown loams, and it is possible that in the former soils the exchange complex of the allophane is largely saturated by organic materials. The rate of mineralisation of organic matter is also known to be different in the yellow-brown pumice soils from that in other allophanic soils (Jackman, 1964).

Red and brown loams also showed a higher INA than would be expected from examination of other soils. Although some of the immature red loams contain allophane as their major clay constituent, halloysite and gibbsite are also present. In the more leached and weathered members of the group, allophane content is low while halloysite and gibbsite levels are higher (Fields, 1968; Gibbs et al. 1968; Ward, 1967). Brown loams are not generally considered to be allophanic soils but the presence of allophane is suspected in some soils. These soils contain a high percent clay which contains large amounts of free Fe_2O_3 and a form of poorly ordered gibbsite (Soil Bureau, 1968). Such clay minerals may be expected to exhibit properties similar to those of allophane. The possible role of allophane in nitrifiation activity in the yellow-brown loams is further discussed in section 5.5.3.

The large differences in nitrification activity observed between soil groups has several important implications in New Zealand agriculture. In high nitrifying soils the possible production of large amounts of NO_3^- may result in large losses of nitrogen by denitrification and leaching. The demand for NO_3^- by soil micro-organisms is apparently small, most appearing to prefer NH_4^+ (Jansson, 1958). Therefore, once converted to NO_3^- an important source of competition which may help to retain nitrogen in the soil is removed.

In the study of Baber (1977) many of the soils in the areas of high ground-water NO_3^- in the Waikato are yellow-brown loams which have a high rate of nitrification. In contrast to this, the soils in an area of low NO_3^- ground water were largely yellow-brown pumice soils or recent soils from volcanic material, both of which possess low rates of nitrification. The suggestion by Baber (1977) that NO_3^- in the shallow aquifiers of the Waikato is mainly recharged over the winter months is consistant with the observation of Holland and During (1977) that leaching of nitrogen from urine in the Waikato occurs largely in the autumn-winter period. This suggests that nitrification in urine patches may be an important predecessor of loss of nitrogen from many soils.

The low nitrification activity found in several yellow-brown earths of low pH suggests that NH_4^+ is the major source of nitrogen for plants in these soils. This suggestion is supported by incubation data of Tan (1967) and Ross and McNeilly (1975). Nitrogen economy in these soils requires careful evaluation before the application of lime, since an increase in the pH of these soils may be expected to be accompanied by an increase in nitrification which will provide larger amounts of NO_3^- which is susceptible to loss by denitrification and leaching. Such effects are likely to be most important in the high rainfall areas of Northland and the South Island. The mean INA (0.43 µg N_{ox}/g soil/hr) of the yellow-brown earths exhibiting type 3 nitrification pattern (average pH 6.0) was 14 times that of the yellow-brown earths exhibiting type 2 nitrification pattern (0.03 µg N_{ox}/g soil/hr; average pH 5.5). Ammonium nitrogen may also be expected to be the major source of plant nitrogen in some podzols, yellow-brown sands, recent soils from alluvium and yellow-brown pumice soils.

The following conclusions are drawn from the present study:

1) Large differences in the rate of nitrification in New Zealand grassland soils were measured by laboratory perfusion with $(NH_4)_2SO_4$.

2) Considerable variation of INA both within and between soil groups is apparent.

3) Yellow-brown loams have INA values significantly higher than those of other soils examined.

4) Medium-high INA values were found in the red and brown loams.

5) With the exception of yellow-brown loams, red and brown loams and soils of near neutral pH, all other soils examined had INA values < 1.0 µg N_{ox}/g soil/hr.

6) Large populations of nitrifying organisms (1.6×10^4 - 2.2×10^6 NH_4^+ oxidisers and 5.4×10^4 - 1.9×10^7 NO_2^- oxidisers per g soil) are present in the 0 - 7.5 cm depth in New Zealand soils under improved grassland.

7) Populations of nitrifying organisms are largest close to the soil surface, and in general decline with depth.

8) Some differences in the ratio of NH_4^+:NO_2^- oxidising organisms were noted. Although the population of NH_4^+ oxidisers is normally larger than that of NO_2^- oxidisers, this is not invariably the case. It is not clear what factors determine the ratio of the two types of organisms in any given soil.

9) INA values cannot be considered indicative of the size of the nitrifying population in a soil since a variation in the oxidative capacity per NH_4^+ oxidising cell was noted.

10) A native forest soil was found to possess a low population of nitrifying organisms (555 NH_4^+ and 1434 NO_2^- oxidisers per g soil).

11) On perfusion of soil with $(NH_4)_2SO_4$, four general patterns of

nitrification were observed:

Type 1: NH_4^+ was rapidly oxidised to NO_3^-, the rate of oxidation being linear or near linear from the commencement of perfusion.

Type 2: NH_4^+ was oxidised only slowly to NO_3^-.

Type 3: NH_4^+ was oxidised slowly to NO_3^- at the commencement of perfusion, but increased logarithmically with time until a steady rate of nitrification was observed.

Type 4: Type 3 nitrification with a temporary accumulation of NO_2^- during the initial stages of perfusion.

12) Type 1 nitrification was observed only in yellow-brown loams and red and brown loams, i.e. it occurred only in soils of high INA.

13) Type 2 nitrification was observed only in five yellow-brown earths and one yellow-brown pumice soil. It was concluded that type 2 nitrification pattern was the result of low pH, the mean pH of the soils exhibiting this type of nitrification being pH 5.5.

14) Type 3 nitrification occurred in most of the soils studied and was considered to be due to the removal of substrate limitations on perfusion.

15) Only soils of high pH and two podzolic soils exhibited type 4 nitrification. In the former soils this type of nitrification was considered the result of high pH, whereas in the latter it appeared that some accumulation of NO_2^- was required to stimulate growth of NO_2^- oxidising organisms.

16) Generation times of NH_4^+ oxidising organisms in soils were estimated to be in the order of two to five days and were found

to be related to pH.

17) pH, percent total N, percent total C and C:N ratio accounted for, at best, only 44 percent of the variation in INA when all soils except organic soils and yellow-brown loams were considered.

18) The effect of percent total N on INA is significantly different in organic soils and yellow-brown loams from that in other New Zealand soils.

CHAPTER 4

A STUDY OF THE AMOUNTS OF INORGANIC NITROGEN, RATES OF NITRIFICATION, AND NETT MINERALISATION OF SOIL NITROGEN IN TWO SOILS OF DIFFERENT INITIAL NITRIFICATION ACTIVITY

4.1.0 INTRODUCTION

In the previous chapter, assessment of nitrification activity using a laboratory perfusion technique was discussed. It was found that large differences in nitrification activity occur between soils, and some of the factors responsible for such differences were outlined. The object of research reported in the present chapter was to confirm that laboratory assessment of differences in nitrification activity reflect true differences which occur under field conditions. It was also proposed to estimate rates of nitrification under field conditions using an in-situ incubation technique, and also to make an estimation of nett mineralisation of soil nitrogen.

4.2.0 EXPERIMENTAL DESIGN, MATERIALS AND METHODS

4.2.1 Experimental Design

Experiments were conducted on two soils of contrasting INA situated at the D.S.I.R. Grasslands Substation at Kaikohe. The pastures were dominant ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), and had been previously grazed by sheep. These were removed during the experimental period.

Soil NH_4^+ and NO_3^- were determined at seven or fourteen day intervals for a period of forty seven weeks, and rates of nitrification and nett mineralisation under field conditions estimated using an in-situ incubation technique.

4.2.2 Soils

The two soils selected were Wharekohe silt loam, a soil of low INA (0.07 µg N_{ox}/g soil/hr) described in section 3.3.3; and Kiripaka clay loam, a soil of medium INA (0.81 µg N_{ox}/g soil/hr). The latter soil is

a moderately leached brown loam formed from weathered basaltic scoria and ash, is well structured but has restricted drainage. Some chemical and physical characteristics of the two soils are presented in table 4.1, and profile descriptions are included in Appendix 2.

4.2.3 Collection of Samples for the Estimation of Soil Inorganic Nitrogen

Eight random samples (0 - 7.5 cm) from each soil were collected at each sampling date, using a sampler of 5.1 cm diameter. The samples were chilled to 1 - 2^{o}C within one hour of collection and stored at this temperature till extracted.

4.2.4 Estimation of Rates of Nitrification and Nett Mineralisation of Soil Nitrogen

At each soil site, eight micro plots were prepared as follows: A P.V.C. micro plot tube (50.8 mm internal diameter; 85 mm long) was driven into the soil until the top of the tube was flush with the soil surface. The tube and sample were withdrawn and 10 mm of soil removed from the base of the tube. A disc of Whatman No. 44 filter paper was firmly pressed against the lower soil surface and 10 g of cation/anion exchange resin (Amberlite lRA 40) packed against the filter paper (figure 4.1). Another disc of No. 44 filter paper was placed below the resin. Glass wool was firmly pressed into the base of the tube and the complete micro-plot inserted into a prepared hole in the soil so that the top of the tube was flush with the soil surface (figure 4.1). Herbage was removed from the micro-plots by hand clipping to ground level, and paraquat (1.68 kg a.i./ha) applied to prevent regrowth of herbage acting as a sink for mineralised nitrogen. Paraquat at normal rates of application has been shown to have no effect on nitrification (Tu and Bollen, 1965).

Table 4.1 Some chemical and physical properties of the Wharekohe and Kiripaka soils (0 - 7.5 cm)

Soil Property	Wharekohe Soil	Kiripaka Soil
Quick test*		
pH	5.1	5.9
Ca	6	5
K	4	11
Mg	12	17
P	6	10
Total N (%)	0.31	0.48
Organic C(%)	7.2	8.6
Bulk density (g/cc)	0.91	0.76
Moisture at field capacity (%)	50.5	51

* Quick test values, Ministry of Agriculture and Fisheries

pH 1:2.5 soil:water ratio

Ca pp 40,000 in NH_4OAc extract at pH 4.7

K pp 250,000 in NH_4OAc extract at pH 4.7

Mg ppm in NH_4OAc extract at pH 4.7

P pp 50 m in .002N H_2SO_4 extract buffered to pH 3 with $(NH_4)_2SO_4$

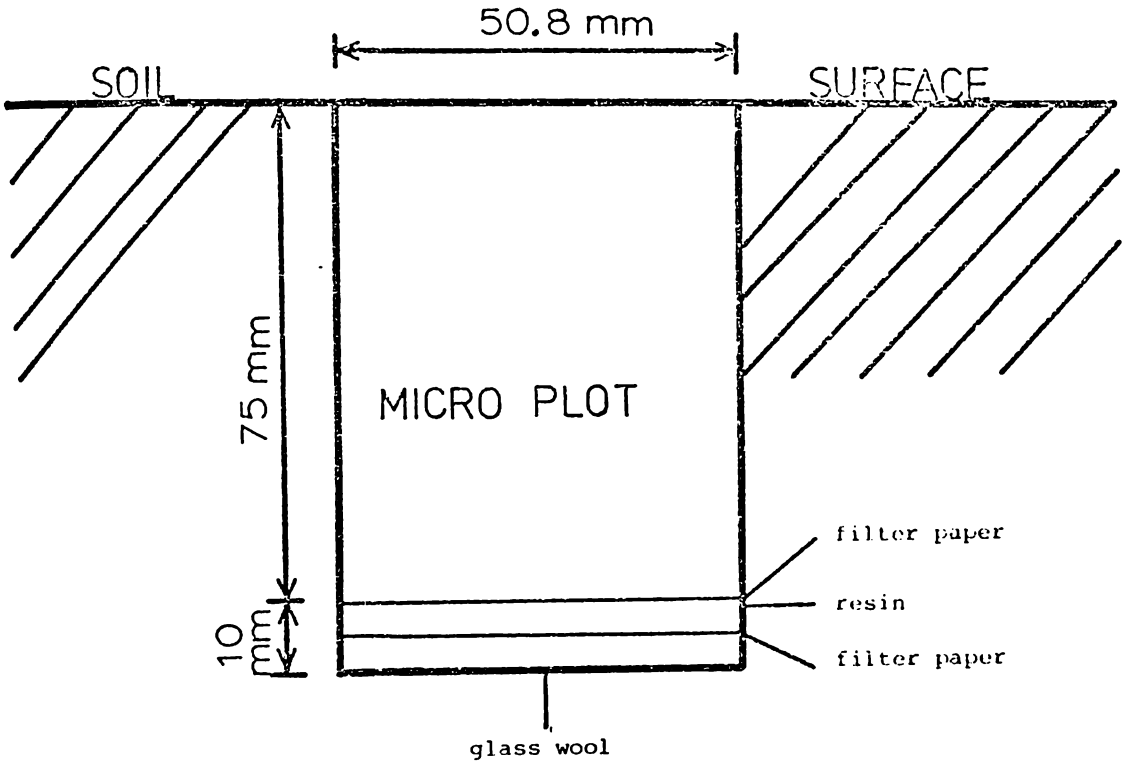

Figure 4.1 Micro-plot used for measurement of the rate of nitrification using an in situ incubation technique

Micro-plots were incubated in-situ for seven to fourteen days, withdrawn and NH_4^+ and NO_3^- determined in the soil and resin.

The amount of nett nitrification during incubation was estimated by subtraction of the NO_3^- present at commencement of incubation (determined on non-incubated samples collected at the commencement of each incubation period) from the NO_3^- contained in the soil and resin at completion of incubation. This also allowed calculation of an average rate of nitrification per unit weight of soil.

To estimate the nett amount of soil nitrogen mineralised, NH_4-N and NO_3-N determined in non-incubated samples collected at the commencement of each incubation period were subtracted from the amount determined in soil and resin following incubation. The result is referred to as nett mineralised nitrogen as the method makes no account of immobilisation processes in the soil. The incubations were replicated eight times.

Samples for inorganic nitrogen determinations were chilled to 1 - 2°C and transported to the laboratory in insulated boxes containing ice packs, stored at 2°C and extracted within forty eight hours of collection.

4.2.5 Analytical Techniques

4.2.5(a) Extraction of Soil Inorganic Nitrogen

Soil inorganic nitrogen was extracted by shaking of soil with 2M KCl (soil:extractant ratio 1:10) for one hour. Extracts were prepared for analysis by filtering.

4.2.5(b) Extraction of Inorganic Nitrogen from Resin

Resin was placed in a 2 cm diameter leaching tube on top of a small glass wool plug. The resin was leached three times with 50 ml aliquots of 1M NaCl, and NH_4^+ and NO_3^- determined in each leachate.

4.2.5(c) Determination of Ammonium and Nitrate in Extracts

NH_4^+ and NO_3^- were determined using an autoanalyser (Brown, 1973; Kamphake et al. 1967).

4.2.5(d) Recording of Climatic Data

Detailed procedures used for recording climatic data may be found in section 7.4.5.

4.3.0 RESULTS AND DISCUSSION

4.3.1 Inorganic Soil Nitrogen

The amounts of NH_4-N and NO_3-N determined in the two soils at each sampling date are presented in figures 4.2 and 4.3. The difference in the relative proportions of NH_4-N and NO_3-N in the two soils is apparent. This can be illustrated by considering the mean values of NH_4-N and NO_3-N prior to incubation:

	kg N/ha (0 - 7.5 cm)		
	NH_4-N	NO_3-N	Total
Kiripaka	6.5 ± 1.3	6.6 ± 1.4	13.1
Wharekohe	15.7 ± 1.7	0.9 ± 0.4	16.6

Although the mean total inorganic nitrogen present in each soil is of the same order of magnitude, the Kiripaka soil contained equal quantities of NH_4^+ and NO_3^- whereas the Wharekohe soil contained largely NH_4^+. Such a difference supports the difference in nitrification activity between the two soils measured in the laboratory.

The occurrence of NO_3^- in the Wharekohe soil was largely restricted to spring, although some NO_3^- was present in the autumn.

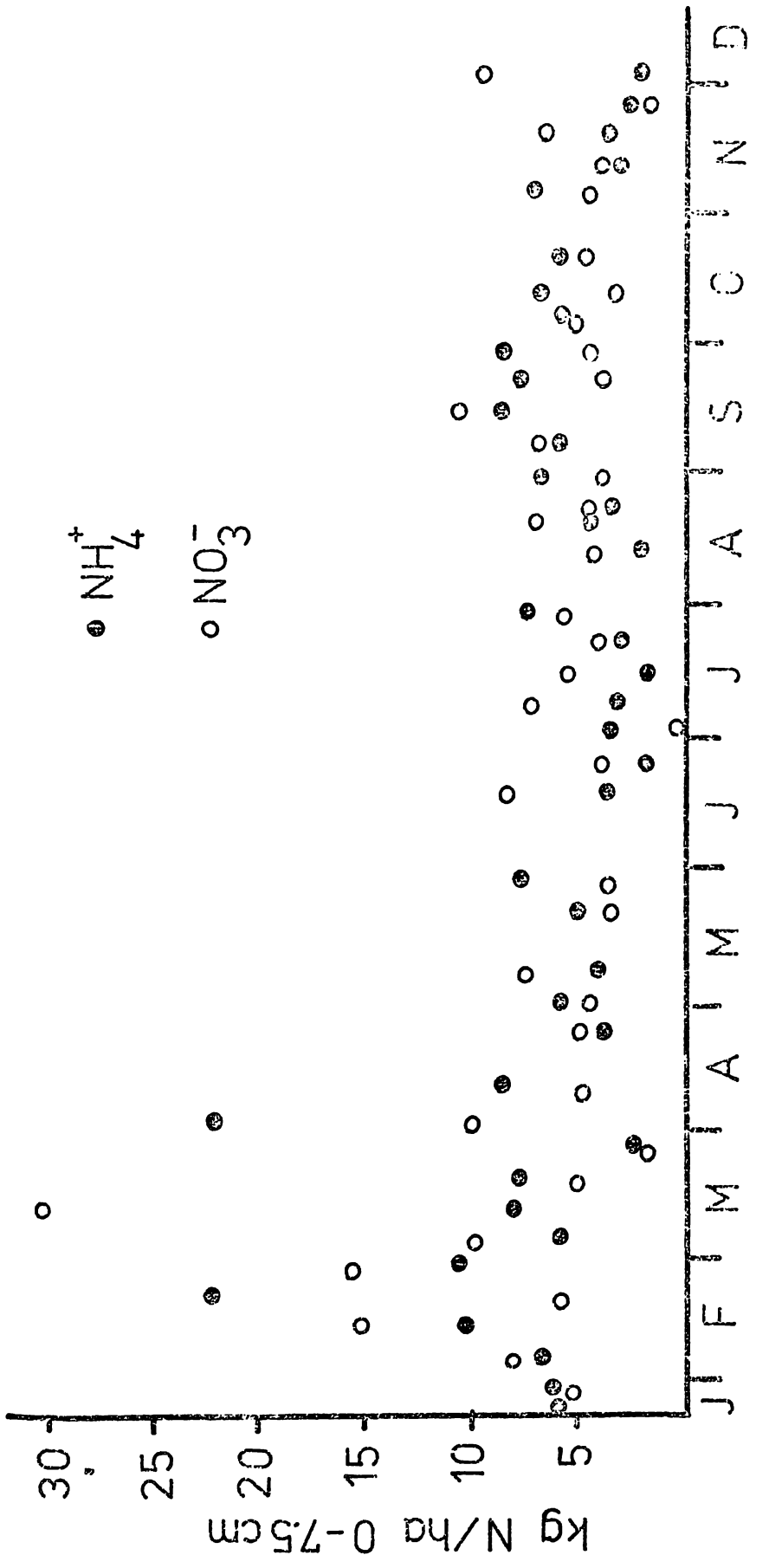

Figure 4.2 Amounts of ammonium- and nitrate-nitrogen in the 0 - 7.5 cm depth of Kiripaka silt loam during a forty-seven week period

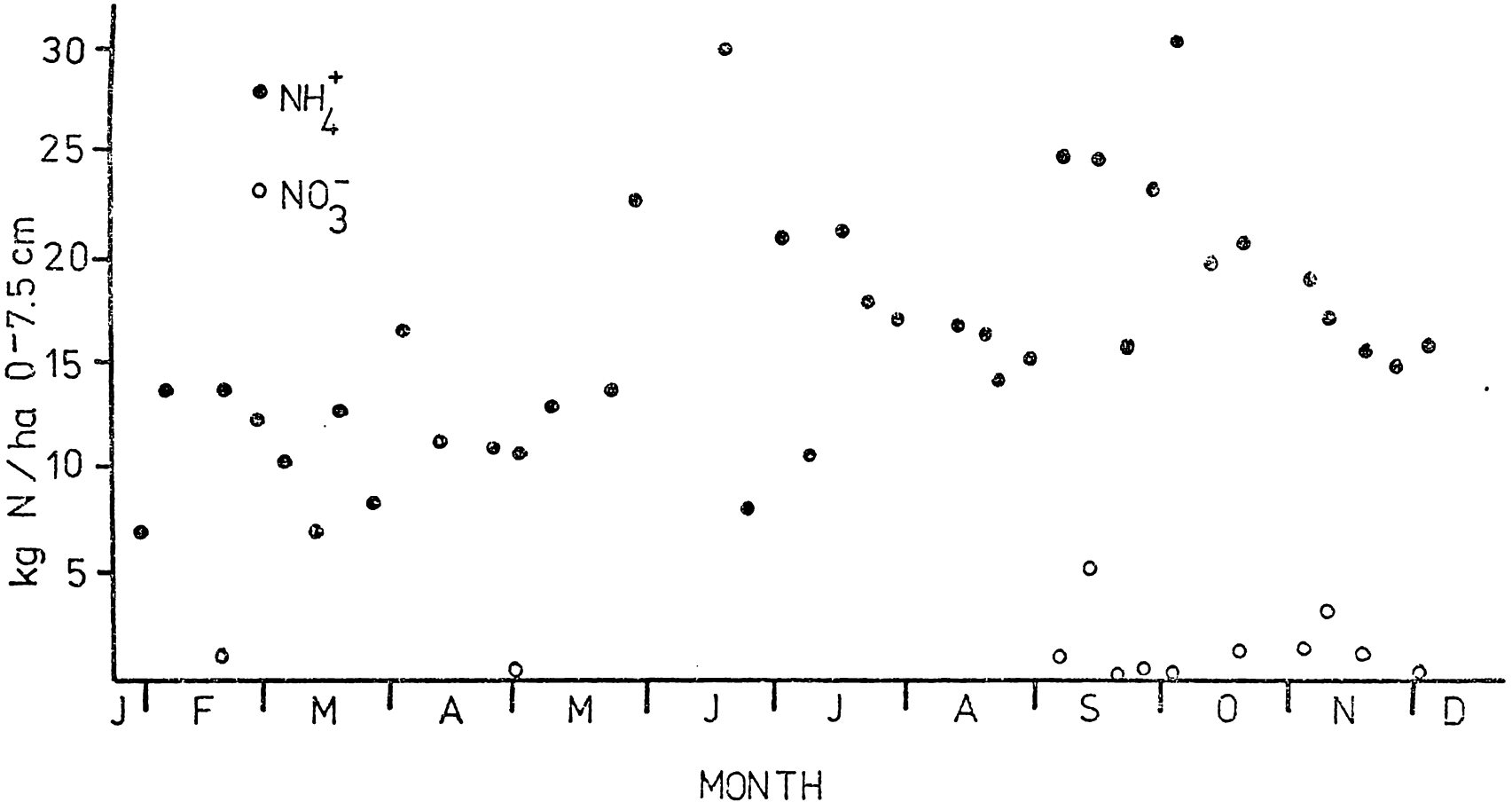

Figure 4.3 Amoumts of ammonium- and nitrate-nitrogen in the 0 - 7.5 cm depth of Wharekohe silt loam during a forty-seven week period

4.3.2 Rate of Nitrification and Nett Mineralisation

An indication of the amount of mineralised NH_4^+ which is likely to be oxidised to NO_3^- under field conditions is shown by the ratio of NH_4^+ and NO_3^- present in the soil and resin at the conclusion of each incubation (table 4.2 and 4.3). In the Wharekohe soil, little nitrification of mineralised NH_4^+ occurred, with the exception of the September/January period, when a maximum of 17.2 percent of the inorganic nitrogen was present as NO_3^-. In the Kiripaka soil, a greater proportion of the inorganic nitrogen was present as NO_3^-, the percent oxidation being generally high during the March-July period, lower during August, September and October, and increasing again in November/December. At no time during the year was more than 83.8 percent of the total inorganic nitrogen present as NO_3^-, which indicates that the population of nitrifiers was unable to completely oxidise the NH_4^+ substrate. When a plant is introduced into the system, the nitrifying organisms will have to compete with an additional competitor for NH_4^+ substrate, so the percent transformations reported are likely to be lower rather than higher under pasture.

The nett nitrification for each incubation period (NO_3^- present at the end of incubation -NO_3^- present at commencement of incubation) and the mean rate of nitrification per hour for both soils are presented in table 4.4. The mean rate of nitrification during the experimental period was significantly lower ($t = 3.8$; significant 1%) in the Wharekohe soil (0.68×10^{-2} µg N_{ox}/g soil/hr) than in the Kiripaka soil (5.3×10^{-2} µg N_{ox}/g soil/hr). Peak rates of nitrification were recorded in the Wharekohe soil during October/November/December, whereas in the Kiripaka soil peak rates occurred in the autumn. The negative values for nitrification during winter and early spring in the Kiripaka soil may indicate that some loss of inorganic nitrogen occurred by mechanisms such as immobilisation or denitrification.

Table 4.2 Variation in the ratio of ammonium to nitrate in the Wharekohe soil at the conclusion of incubations

Date of Incubation	Ammonium (kg N/ha)	Nitrate (kg N/ha)	Total (kg N/ha)	Nitrate as % of Total
14/1 - 21/1	7.87	1.38	9.25	14.9
21/1 - 28/1	12.22	0.69	12.91	5.3
28/1 - 4/2	9.91	0.94	10.85	8.7
4/2 - 11/2	15.99	0.98	16.97	5.8
11/2 - 18/2	15.99	0.94	16.98	5.5
18/2 - 25/2	19.89	0.00	19.89	0.0
25/2 - 4/3	19.13	0.08	19.21	0.4
4/3 - 11/3	13.47	0.20	13.67	1.5
11/3 - 18/3	17.21	0.14	17.35	0.8
18/3 - 25/3	11.59	0.00	11.59	0.0
25/3 - 1/4	16.47	0.69	17.16	4.0
1/4 - 8/4	21.07	0.74	21.81	3.4
8/4 - 22/4	15.55	0.17	15.72	1.1
22/4 - 29/4	15.98	0.00	15.98	0.0
29/4 - 6/5	16.60	0.00	16.60	0.0
6/5 20/5	40.20	0.68	40.88	1.7
20/5 27/5	20.22	0.78	21.00	3.7
27/5 - 17/6	20.35	0.77	21.12	3.6
17/6 - 24/6	17.19	0.77	17.96	4.3
24/6 - 1/7	15.62	0.68	16.30	4.2
1/7 - 8/7	16.79	0.74	17.53	4.2
8/7 - 15/7	16.48	0.38	16.86	2.3
15/7 - 22/7	17.94	0.31	18.25	1.7
22/7 - 29/7	8.80	0.83	9.63	8.6
29/7 - 12/8	31.93	0.21	32.14	0.7
12/8 - 19/8	23.33	0.26	23.59	1.1
19/8 - 23/8	16.93	0.04	16.97	0.2
23/8 - 30/8	18.33	0.41	18.74	2.2
30/8 - 7/9	26.73	1.37	28.11	4.9
7/9 - 15/9	35.22	2.47	37.69	6.6
15/9 - 21/9	23.62	3.92	27.54	14.2
21/9 - 28/9	25.10	0.00	25.10	0.0
28/9 - 5/10	41.47	0.60	42.07	1.4
5/10 - 12/10	48.16	0.77	48.93	1.6
12/10 - 19/10	36.52	1.71	38.23	4.5
19/10 - 3/11	54.82	11.35	66.17	17.2
3/11 - 10/11	28.68	4.35	33.03	13.2
10/11 - 17/11	34.11	0.77	34.88	2.2
17/11 - 24/11	27.92	5.14	33.06	15.5
24/11 - 1/12	31.90	4.65	36.55	12.7
1/12 - 8/12	35.43	4.21	39.64	10.6

Table 4.3 Variation in the ratio of ammonium to nitrate in the Kiripaka soil at the conclusion of incubations

Date of Incubation	Ammonium (kg N/ha)	Nitrate (kg N/ha)	Total (kg N/ha)	Nitrate as % of Total
14/1 - 21/1	10.61	4.17	14.78	28.2
21/1 - 28/1	6.92	22.90	29.82	76.8
28/1 - 4/2	10.02	13.20	23.22	56.8
4/2 - 11/2	12.90	8.25	21.15	39.0
11/2 - 18/2	23.47	20.82	44.27	47.0
18/2 - 25/2	16.62	25.22	41.84	60.6
25/2 - 4/3	9.71	27.46	37.17	73.9
4/3 - 11/3	4.79	18.36	23.15	79.3
11/3 - 18/3	10.69	38.43	49.12	78.2
18/3 - 25/3	5.98	8.24	14.22	57.9
25/3 - 1/4	8.69	9.98	18.67	53.5
1/4 - 8/4	12.24	51.77	64.01	80.9
8/4 - 22/4	4.45	22.98	27.43	83.8
22/4 - 29/4	6.96	7.91	14.87	53.2
29/4 - 6/5	3.47	10.05	13.52	74.3
6/5 - 20/5	5.37	13.83	19.20	72.0
20/5 - 27/5	8.16	9.57	17.73	54.0
27/5 - 17/6	3.07	8.31	11.38	73.0
17/6 - 24/6	1.82	6.90	8.72	79.1
24/6 - 1/7	4.52	2.68	7.20	37.2
1/7 - 8/7	3.23	8.30	11.53	72.0
8/7 - 15/7	2.78	5.97	8.75	68.2
15/7 - 22/7	2.86	7.60	10.46	72.6
22/7 - 29/7	8.59	7.15	15.74	45.4
29/7 - 12/8	4.41	12.56	16.97	74.0
12/8 - 19/8	6.36	5.98	12.34	48.5
19/8 - 23/8	5.34	4.12	9.46	43.6
23/8 - 30/8	7.07	5.15	12.22	42.1
30/8 - 7/9	5.75	8.40	14.15	59.4
7/9 - 15/9	8.13	8.40	16.53	50.8
15/9 - 21/9	5.15	8.10	13.25	61.1
21/9 - 28/9	11.97	4.40	16.37	26.9
28/9 - 5/10	7.52	12.31	19.83	62.1
5/10 - 12/10	6.34	5.70	12.04	47.3
12/10 - 19/10	11.66	5.66	17.32	32.7
19/10 - 3/11	7.04	7.91	14.95	52.9
3/11 - 10/11	3.39	7.46	10.85	68.8
10/11 - 17/11	3.56	10.32	13.88	74.4
17/11 - 24/11	4.03	7.20	11.23	64.1
24/11 - 1/12	2.67	5.31	7.98	66.5
1/12 - 8/12	2.94	9.72	12.66	76.8

Table 4.4 Estimation of the rate of nitrification in soil samples incubated in situ in Wharekohe silt loam and Kiripaka silt loam

Date of Incubation	Days Incubation	Wharekohe Total Nitrification (kg N/ha)	Wharekohe Mean rate of Nitrification ($\mu g\ N_{ox}/g$ soil/hr)	Kiripaka Total Nitrification (kg N/ha)	Kiripaka Mean rate of Nitrification ($\mu g\ N_{ox}/g$ soil/hr)
			$\times 10^{-2}$		$\times 10^{-2}$
14/1 - 21/1	7	0.53	0.46	1.60	1.67
21/1 - 28/1	7	0.00	0.00	18.06	18.86
28/1 - 4/2	7	0.26	0.23	7.36	7.69
4/2 - 11/2	7	0.60	0.52	1.48	1.55
11/2 - 18/2	7	0.60	0.52	5.64	5.89
18/2 - 25/2	7	0.00	0.00	19.16	20.01
25/2 - 4/3	7	0.08	0.07	11.71	12.23
4/3 - 11/3	7	0.20	0.17	8.23	8.59
11/3 - 18/3	7	0.14	0.12	8.01	8.36
18/3 - 25/3	7	0.00	0.00	2.90	3.03
25/3 - 1/4	7	0.69	0.60	7.84	8.19
1/4 - 8/4	7	0.04	0.03	41.58	43.42
8/4 - 22/4	14	0.00	0.00	17.85	9.32
22/4 - 29/4	7	0.00	0.00	6.96	7.27
29/4 - 6/5	7	0.00	0.00	5.21	5.44
6/5 - 20/5	14	0.68	0.29	-6.13	-
20/5 - 27/5	7	0.00	0.00	5.87	6.13
27/5 - 17/6	21	0.17	0.05	4.32	1.50
17/6 - 24/6	7	0.09	0.08	-1.58	-
24/6 - 1/7	7	0.00	0.00	-1.38	-
1/7 - 8/7	7	0.74	0.65	7.33	7.65
8/7 - 15/7	7	0.21	0.18	-1.44	-
15/7 - 22/7	7	0.21	0.18	1.76	1.84
22/7 - 29/7	7	0.87	0.76	2.95	3.08
29/7 - 12/8	14	0.21	0.09	6.58	3.44
12/8 - 19/8	7	0.09	0.08	1.35	1.41
19/8 - 23/8	4	0.00	0.00	-3.22	-
23/8 - 30/8	7	0.41	0.36	1.66	1.73
30/8 - 7/9	8	1.28	0.98	4.20	3.84
7/9 - 15/9	8	0.76	0.59	1.28	1.17
15/9 - 21/9	7	0.00	0.00	-2.80	-
21/9 - 28/9	7	0.00	0.00	0.41	0.42
28/9 - 5/10	7	0.00	0.00	7.54	7.87
5/10 - 12/10	7	0.00	0.00	0.29	0.30
12/10 - 19/10	7	1.20	1.05	2.24	2.34
19/10 - 3/11	15	9.30	7.57	2.99	1.46
3/11 - 10/11	7	2.30	2.01	2.76	2.89
10/11 - 17/11	7	0.00	0.00	6.54	6.83
17/11 - 24/11	7	3.43	2.99	0.36	0.38
24/11 - 1/12	7	4.48	3.91	2.96	3.09
1/12 - 8/12	7	3.74	3.26	0.24	0.25
		$\bar{x}$	0.68	$\bar{x}$	5.35
		S.D.	1.43	S.D.	7.72

The rates of nitrification reported here are nett rates only and assume that loss of NO_3^- by denitrification or leaching did not occur. Loss of NO_3^- by the former mechanism is likely, but loss by the latter would not occur under the conditions of the present study.

The nett amount of nitrogen mineralised in each soil is presented in figures 4.4 and 4.5. Nett mineralisation in the Wharekohe soil (figure 4.4) tended to show some seasonal variation whereas in the Kiripaka soil, although nett mineralisation declined during January/March period, no increase in the rate was apparent in the spring. Both soils showed a nett negative mineralisation during some incubation periods in the winter.

The mean rate of nett mineralisation in the Wharekohe soil (0.99 ± 0.08 kg N/ha 0 - 7.5 cm/day) was significantly higher than that in the Kiripaka soil (0.74 ± 0.06 kg N/ha 0 - 7.5/day).

Nett mineralisation for each seven day period was correlated with climatic factors for each period (table 4.5). Correlations were not very satisfactory and at most explained fifty percent of the variation in the Wharekohe soil, and twenty five percent of the variation in the Kiripaka soil. The highest correlation was found between nett mineralisation in the Wharekohe soil and mean recorded solar radiation ($r = 0.73$; significant 1%). Such a correlation was not unexpected since mean recorded solar radiation reflects cyclic changes in the climatic factors.

Although the original objective of the incubation technique was to measure amounts and rates of nett nitrification, it also provided estimates of nett mineralisation which appear to be of the correct order of magnitude. In the Wharekohe soil, nett mineralisation was 361 kg N/ha (0 - 7.5 cm depth)/year or 17.1 percent of the total soil nitrogen. The comparable figures for the Kiripaka soil are 270 kg N/ha (0 - 7.5 cm depth)/year and 9.9 percent. Field incubation in open tubes provides an environment close to natural conditions since measurement of moisture

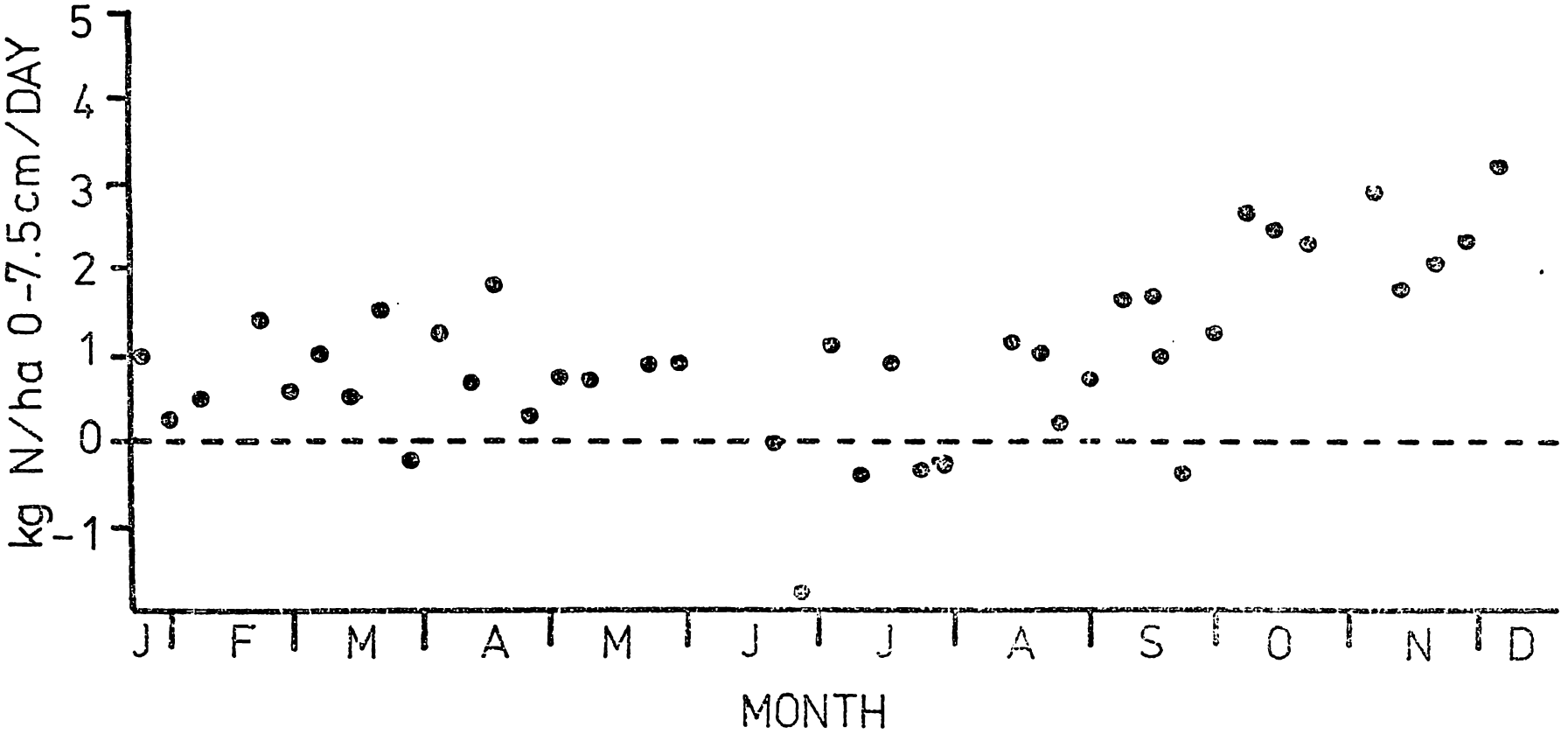

Figure 4.4 Nett mineralisation of soil organic nitrogen in the 0 - 7.5 cm depth of Wharekohe silt loam during a forty-seven week period

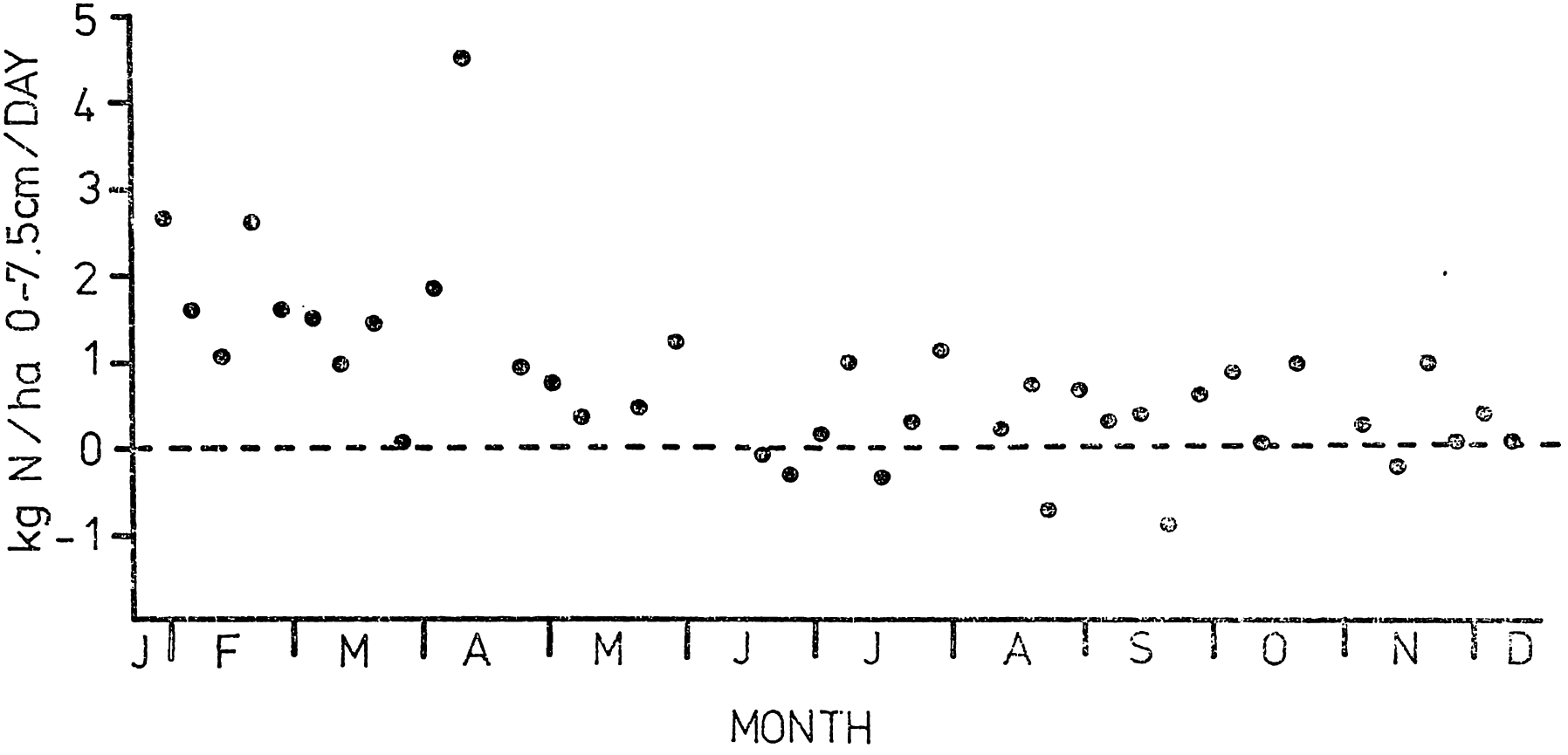

Figure 4.5 Nett mineralisation of soil organic nitrogen in the 0 - 7.5 cm depth of Kiripaka silt loam during a forty-seven week period

Table 4.5 Correlation of some climatic factors with nett mineralisation of soil nitrogen in the Wharekohe and Kiripaka soils

	Correlation coefficient of climatic factor with nett mineralisation	
	Wharekohe	Kiripaka
Mean recorded solar radiation	0.73**	-0.13
Extra atmospheric radiation	0.59**	0.25
Mean rainfall	0.05	-
2 cm minimum soil temperature	-	0.5**
2 cm maximum soil temperature	-	0.49**
Mean 10 cm 9 a.m. soil temperature	0.27	-

** significant at 1%

levels showed them to be comparable inside and outside the incubation tubes despite the break in the soil profile by insertion of the exchange resin.

Estimation of rates of mineralisation vary widely with soil type. Broadbent et al. (1964) calculated the annual mineralisation of nitrogen in Egmont black loam to be 661 kg N/ha (0 - 30.5 cm) or 4.2 percent of the total nitrogen, and that for Hamilton clay loam to be 774 kg N/ha (0 - 30.5 cm) or 9.9 percent of total nitrogen. Jackman (1964) also made estimates of mineralisation of nitrogen in New Zealand soils based on the relationship

$$t_{\frac{1}{2}} = \frac{0.693}{K_1}$$

where $t_{\frac{1}{2}}$ is the half-life for changes in the level of soil organic nitrogen and K_1 is the fraction of soil nitrogen which is mineralised each year. Jackman's estimates were:

Soil	K_1 %
Matapiro	12.1 ± 8.5
Tokomaru	11.5 ± 4.9
Hamilton	12.8 ± 6.5
Taupo	4.9 ± 3.5
Oropi	2.6 ± 2.0
Waiotu	4.9 ± 3.0
New Plymouth	7.0 ± 6.0

The mean K_1 value for the three non-allophanic soils (Matapiro, Tokomaru, Hamilton) was 12.1 ± 4.4 and that for the allophanic soils 4.62 ± 2.0. The lower percentage of total soil nitrogen mineralised each year in allophanic soils may partially explain the lower percentage of total

nitrogen mineralised in the Kiripaka soil in the present study.

Considering the mild climate of Northland (mean annual temperature 14°C), nett mineralisation in the order of the values reported in the present study appear realistic, but are high when compared with estimates of Walker et al. (1954) who calculated that each 0.1 percent nitrogen in the top 15 - 23 cm released, on average, 11.3 kg of mineral nitrogen per year. This figure represents approximately 1.25 percent of the total nitrogen content of the soil.

Estimates of nett mineralisation in the present study are of the same order of magnitude as estimates of potentially mineralisable nitrogen for short term incubation studies (15 - 17.9 percent of indigenous nitrogen) in two surface soils studied by Chichester et al. (1975).

4.4.0 GENERAL DISCUSSION AND CONCLUSIONS

The prediction from laboratory perfusion experiments that a difference existed between Wharekohe silt loam and Kiripaka clay loam in their nitrification activities was substantiated by field data. In Wharekohe silt loam NH_4^+ was the major form of inorganic nitrogen present, whereas in Kiripaka silt loam approximately equal amounts of NH_4^+ and NO_3^- were present.

Incubation of undisturbed soil samples "in-situ" under field conditions showed that on average in the Wharekohe soil only 4.7 percent of the mineralised nitrogen was oxidised to NO_3^- whereas in the Kiripaka soil on average 60.2 percent of the mineralised nitrogen was oxidised to NO_3^-.

A difference between the nitrification activity of Wharekohe silt loam and Kiripaka clay loam was further supported by the determination of a lower mean rate of nett nitrification (0.68×10^{-2} µg N_{ox}/g soil/hr) in the former than in the latter (5.35×10^{-2} µg N_{ox}/g soil/hr). Of

significance is the apparently slow rate of nitrification which occurs under field conditions.

Estimation of nett mineralisation indicates a faster rate of turnover of organic nitrogen in the Wharekohe soil than in the Kiripaka soil.

It is therefore concluded that the differences in nitrification activity measured in the laboratory using the perfusion technique are true differences which also occur under field conditions.

CHAPTER 5

A STUDY OF THE RELATIONSHIP BETWEEN pH AND THE RATE OF NITRIFICATION

5.1.0 INTRODUCTION

It has been established (chapter 3) that pH has an affect on both the rate of nitrification and generation time of nitrifying organisms in soils. It was also noted that yellow-brown loams showed a substantially higher rate of nitrification activity at a given pH than many other soils. It was the objective of the present study to examine the relationship between pH and rate of nitrification in more detail.

5.2.0 PERFUSION OF SOILS SATURATED WITH $CaCO_3$

5.2.1 Introduction

In an attempt to establish if the type 2 nitrification pattern exhibited by Marua clay (chapter 3) was due to limitations imposed by the pH, $CaCO_3$ was added to a sample of Marua clay prior to perfusion, and the subsequent nitrification compared to that of other soils perfused in the presence of $CaCO_3$.

5.2.2 Experimental

Six soils were selcted for perfusion with $CaCO_3$ to provide a range of nitrification characteristics:

Soil	Soil Group	INA ($\mu g\ N_{ox}$/g soil/hr)	Type of Nitrification Pattern
Marua	Yellow-brown earth	<0.02	2
Wharekohe	Podzol	0.07	4
Ruakaka peat	Organic	0.67	3
Tokoroa	Yellow-brown pumice	<0.02	3
Stratford	Yellow-brown loam	2.12	1
Pukepuke brown sandy loam	Yellow-brown sand	5.70	3

Five grams of $CaCO_3$ and 10 g of soil were mixed and perfused as outlined in section 3.2.2. All sampling and analytical techniques were as described in section 3.2.3. Following perfusion, the soil in the perfusion unit was washed with 200 ml of distilled water to remove non-absorbed nitrifying organisms in the perfusion solution, and the most probable number of nitrifying organisms estimated (section 3.2.4). All experiments were duplicated.

5.2.3 Results and Discussion

The pH of the perfusing solution of the Wharekohe, Stratford and Marua soils was pH 7.6; Ruakaka pH 7.4; Pukepuke pH 7.2; and Tokoroa pH 7.1.

Addition of $CaCO_3$ to soils rapidly increased their rate of nitrification. Marua, a soil of type 2 nitrification pattern when perfused in the absence of $CaCO_3$, showed a rapid increase in the rate of nitrification when saturated with $CaCO_3$ (figure 5.1). Nitrite occurred in the perfusion solution of all soils, and this was expected since the rate of proliferation of *Nitrobacter* spp. is greatly reduced at pH values above 7.5 - 7.7, whereas the proliferation rate of *Nitrosomonas* spp. is unaffected by alkaline pH within a wide range (Millbank, 1959).

All the soils studied reached a rate of oxidation of NH_4^+ which was constant with time after 664 hours of perfusion. The constant rate of oxidation with time will be referred to as Potential Nitrification Activity (PNA) which is defined as:

"the mean rate of oxidation of NH_4^+ when the rate of oxidation is constant with time of perfusion, expressed as µg N oxidised per gram of soil per hour".

Nitrite was still present in the perfusing solution of all soils after 664 hours. Because the rate of oxidation of NH_4^+ was constant at this time,

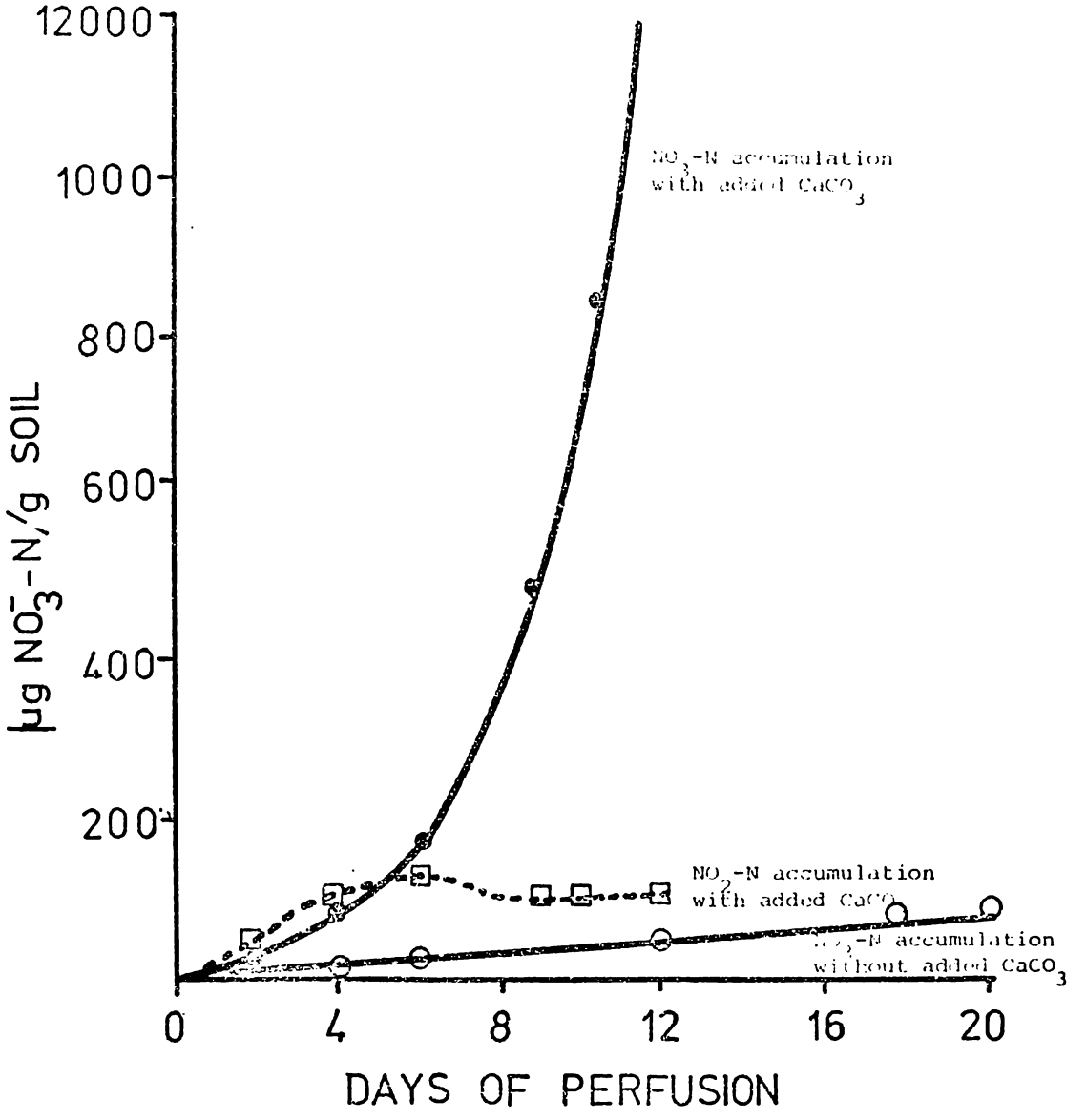

Figure 5.1 Accumulation of nitrite- and nitrate-nitrogen when Marua clay loam was perfused with 0.005M $(NH_4)_2SO_4$ with and without added $CaCO_3$

it appears that the system was saturated with NH_4^+ oxidising organisms but insufficient NO_2^- oxidisers were present to convert all the NO_2^- to NO_3^-.

Some care must be taken in interpreting results of perfusion experiments conducted above pH 7. In the initial survey of nitrification activity of New Zealand grassland soils (chapter 3), a selection of perfusion solutions was examined for the presence of nitrifying organisms at the conclusion of perfusion using the Most Probable Number technique. Very few organisms were found in any of the solutions examined, and this supports the hypothesis of Lees and Quastel (1946a) that nitrification is largely a surface phenomenon. However, in all cases, the perfusing solutions of the soils saturated with $CaCO_3$, contained both NH_4^+ and NO_2^- oxidisers in solution. It may be, therefore, that the rate of oxidation reached in the perfusion units containing $CaCO_3$ was a result of both soil and solution factors. Such an observation must place some doubt on the validity of perfusion studies of soils saturated with $CaCO_3$.

The potential nitrification activities, Most Probable Number of nitrifying organisms present at the conclusion of perfusion, and the rate of NH_4^+ oxidation per NH_4^+ oxidising cell are presented in table 5.1. It is possible that the higher rate of oxidation per cell reported for the Wharekohe and Marua soils was due to the presence of a large number of organisms in solution. All soils reached a similar population of NH_4^+ oxidisers with the exception of Marua which possessed a lower value.

The absence of a lag phase when Marua soil was perfused with $CaCO_3$ substantiates the presence of a large nitrifying population as found with the Most Probable Number technique (table 3.3).

Generation times of NH_4^+ oxidising organisms in the perfusion units were estimated from semilogarithmic plots of accumulated $NO_3^- + NO_2^-$ against time (figure 5.2). Estimated generation times were:

Table 5.1 Potential nitrification activities and the Most Probable Number of nitrifying organisms present in soils perfused while saturated with $CaCO_3$

Soil	PNA	Most Probable Number of Nitrifying Organisms/g soil		μg N_{ox}/ammonium Oxidising Cell/hour
		Ammonium Oxidisers	Nitrite Oxidisers	
Ruakaka	57.08	1.5×10^{8}	3.1×10^{7}	0.38×10^{-6}
Tokoroa	54.14	7.3×10^{8}	4.0×10^{7}	0.07×10^{-6}
Pukepuke	40.36	1.0×10^{8}	3.8×10^{8}	0.38×10^{-6}
Stratford	39.36	2.5×10^{8}	2.4×10^{8}	0.15×10^{-6}
Wharekohe	36.33	3.5×10^{8}	3.5×10^{7}	1.04×10^{-6}
Marua	22.45	2.2×10^{-7}	1.5×10^{8}	1.02×10^{-6}

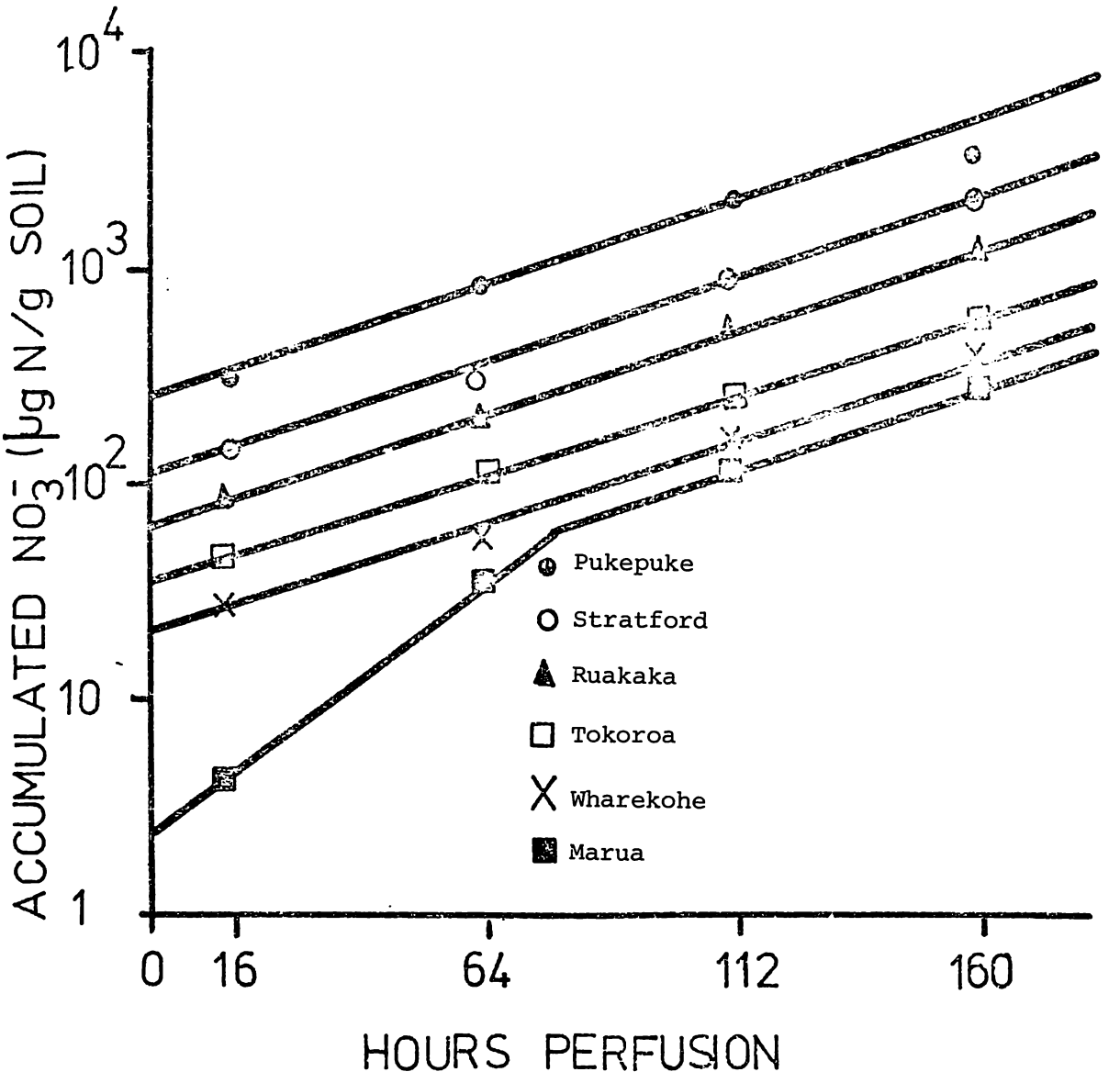

Figure 5.2 Semilogarithmic plot of accumulated nitrite- and nitrate-nitrogen against time of perfusion for six soils perfused with 0.005M $(NH_4)_2SO_4$ in the presence of $CaCO_3$

Pukepuke	36 hours
Stratford	37 hours
Tokoroa	38 hours
Wharekohe	38 hours
Ruakaka	40 hours

These generation times are still substantially longer than what may be expected in pure cultures of *Nitrosomonas* spp. (Engel and Alexander, 1958b).

Two logarithmic oxidation phases were observed in Marua soil (figure 5.2). The first phase (estimated generation time seventeen hours) lasted approximately forty hours and was followed by a second phase during which the estimated generation time was similar to that found in other soils. A similar occurrence was found in Tokoroa soil when perfused without $CaCO_3$. During phase 1 oxidation, (which also lasted approximately forty hours), a semilogarithmic plot gave an estimated generation time of NH_4^+ oxidisers of seven hours (figure 5.3). Such a generation time is unlikely since it is less than that reported for pure culture (Engel and Alexander, 1958b).

The Most Probable Number of nitrifying organisms was determined at intervals throughout perfusion of Tokoroa soil in the absence of $CaCO_3$ to determine if the apparent sudden change in oxidation rate of NH_4^+ was reflected in the number of nitrifying organisms present. It was found that the growth of NH_4^+ oxidisers plotted as a straight line on a semi-logarithmic plot (figure 5.3), therefore exhibiting a normal exponential growth pattern.

Because accumulation of $NO_2^- + NO_3^-$ during perfusion with $CaCO_3$ plots as a straight line on semilogarithmic plot against time, the nitrification is most likely due to proliferation of one organism (McLaren and Ardakani, 1972).

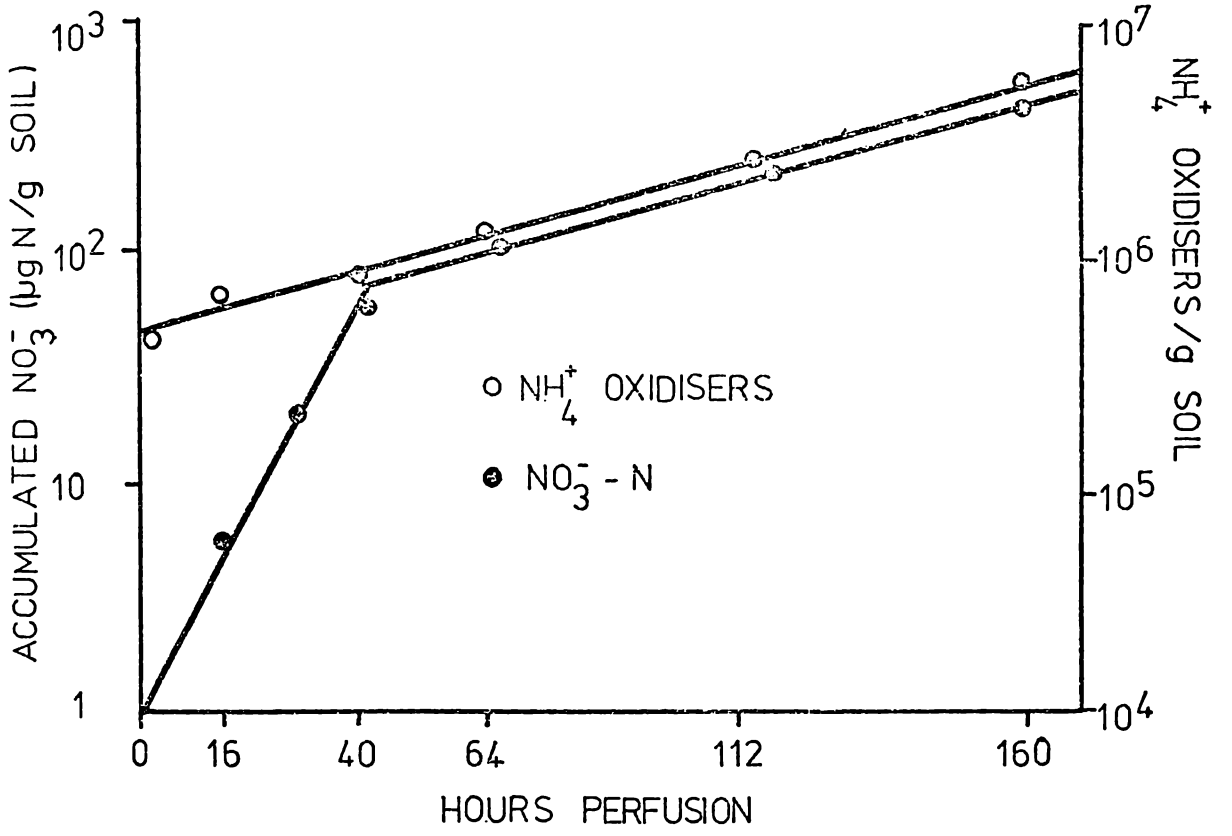

Figure 5.3 Semilogarithmic plot of accumulated nitrate-nitrogen and the most probable number of ammonium oxidisers against time of perfusion for Tokoroa sandy silt perfused with 0.005M $(NH_4)_2SO_4$

5.3.0 COMPARISON OF THE RATE OF NITRIFICATION IN A PERFUSION UNIT WITH AND WITHOUT CONTROL OF pH

5.3.1 Introduction

Oxidation of NH_4^+ to NO_3^- is an acidifying process, and the pH in a perfusion unit will decrease unless alkali is added to neutralise the acid formed. The rate of decrease in pH for a given soil will depend on the rate of nitrification and the buffering capacity of the soil.

In the present section, the decline in pH of a rapidly nitrifying soil was investigated and compared with the rate of nitrification in a soil of controlled pH.

5.3.2 Experimental

A sample of Horotiu sandy loam was perfused with 0.005M $(NH_4)_2SO_4$ in a modified perfusion unit (figure 5.4) connected to a pH-stat (Radiometer PHM 64 research pH meter; TTT60 titrator; ABU11 autoburette) which maintained the pH of the perfusing solution at the pH of the original soil (pH 5.5) by titration with saturated $Ca(OH)_2$. By placing combination electrodes in the perfusion solution reservoir and micro-reservoir, it was possible to measure the change in pH of the perfusion solution as it passed through the soil. The rate at which the perfusion solution passed through the soil was regulated so that the drop in pH did not exceed 0.1.

In an accompanying experiment a second sample was perfused for 1144 hours without control of pH.

All sampling and analytical procedures were as outlined in section 3.2.3.

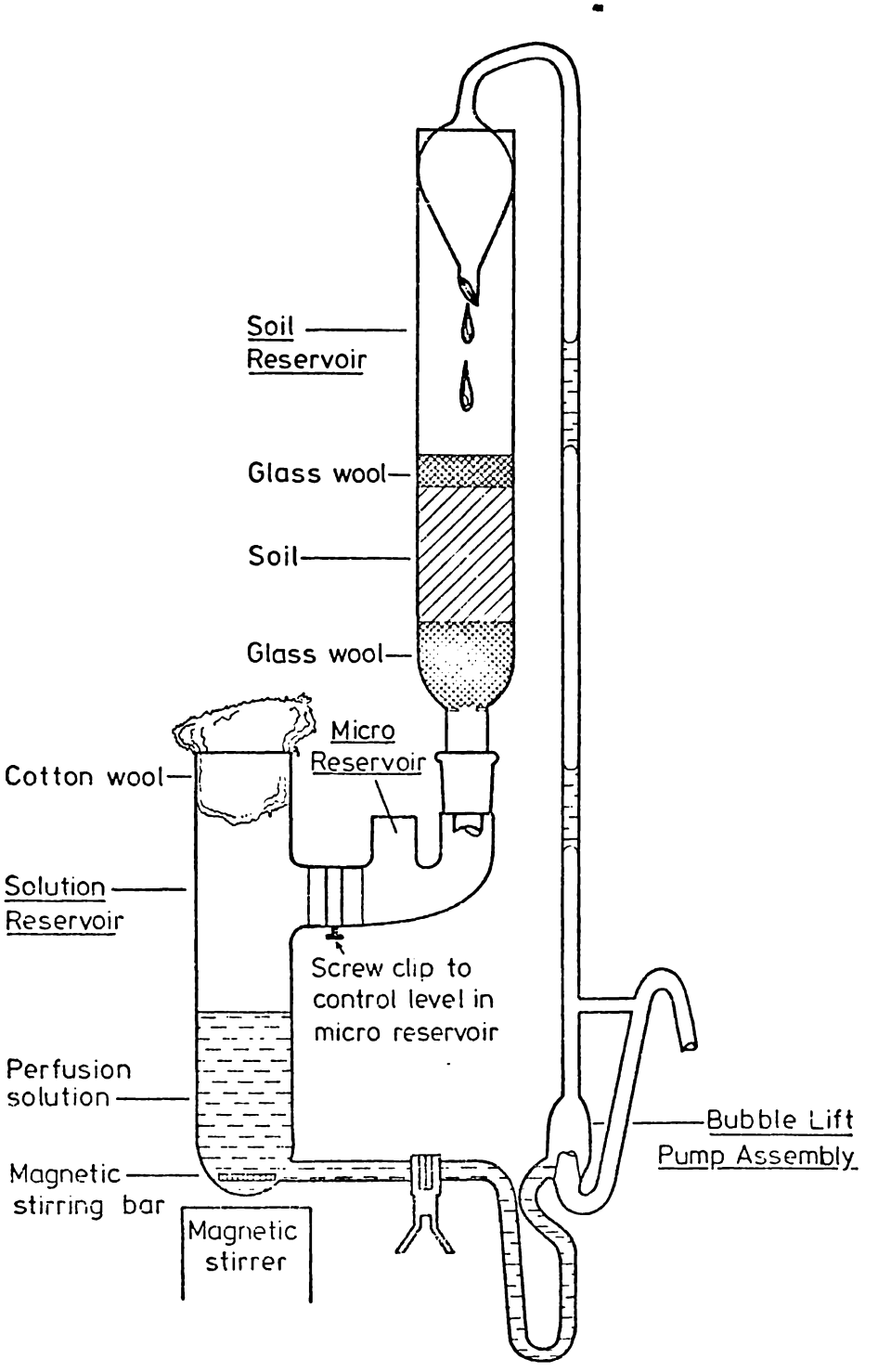

Figure 5.4 Modified perfusion unit

5.3.3 Results and Discussion

The accumulation of NO_3^- during 480 hours perfusion of Horotiu sandy loam with and without control of pH is presented in figure 5.5, and the decline in pH and rate of nitrification in Horotiu sandy loam perfused for 1144 hours without control of pH is shown in table 5.2.

The decline in pH of any soil with perfusion will be dependent on the buffering capacity of the soil. The increasing difference with time between the accumulation of NO_3^- in soils with and without control of pH demonstrates the importance of pH control in nitrification experiments.

No explanation is apparent for the ability of Horotiu sandy loam to nitrify at rapid rates even when the pH of the bulk solution was 3.7. However, reports of nitrification at low pH are not uncommon in the literature, Olsen (1929) noting accumulation of NO_3^- at pH 3.7, Boswell (1955) and Weber and Gainey (1962) at pH 4. They are, however, difficult to rationalise with pure culture studies where nitrification seldom occurs below pH 6.0 (Weber and Gainey, 1962).

5.4.0 DETERMINATION OF THE RATE OF NITRIFICATION AND MOST PROBABLE NUMBER OF NITRIFYING ORGANISMS IN A SOIL PERFUSED AT DIFFERENT pH'S

5.4.1 Introduction

The present section investigates the change in rate of nitrification and numbers of nitrifying organisms when the same soil is perfused at different pH values.

5.4.2 Experimental

Ten grams of Stratford sandy loam were perfused in the modified perfusion unit (figure 5.4) connected to a pH stat (Radiometer PHM64

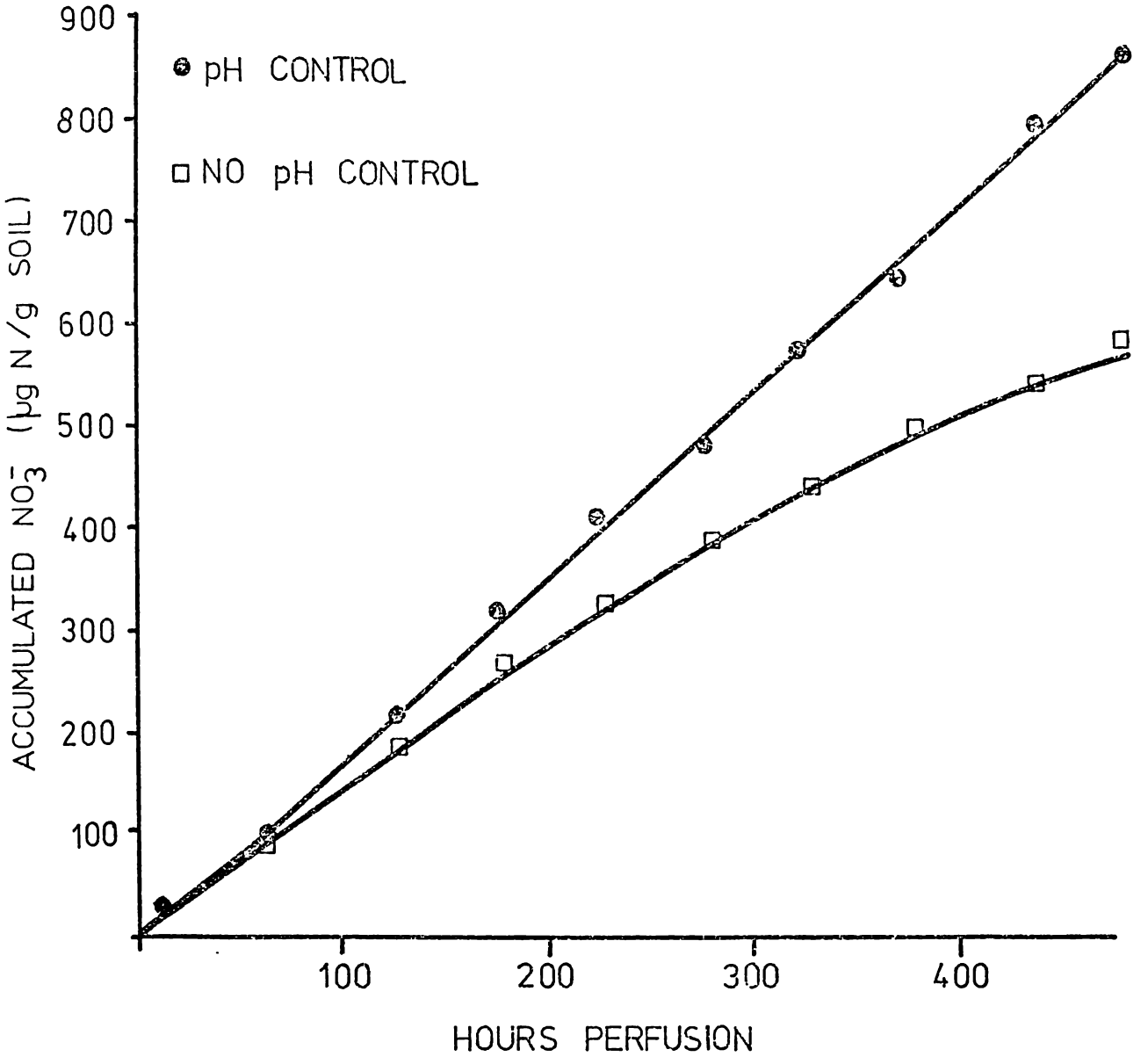

Figure 5.5 Accumulation of nitrate-nitrogen with time of perfusion when Horotiu sandy loam was perfused with 0.005M $(NH_4)_2SO_4$ with and without control of pH

Table 5.2 Change in the pH of the perfusion solution and rate of nitrification when Horotiu sandy loam is perfused without control of pH

Hours of Perfusion	pH of Perfusion Solution	µg N_{ox} /g soil /hour	Hours of Perfusion	pH of Perfusion Solution	µg N_{ox} /g soil /hour
16	5.50	1.13	616	4.24	0.99
64	5.02	1.55	664	4.01	0.25
136	4.98	1.28	712	4.14	0.89
184	4.95	1.91	760	4.05	0.73
232	4.81	1.10	808	3.99	1.16
280	4.75	1.20	856	3.91	1.10
328	4.65	1.28	904	3.86	0.51
376	4.55	1.12	952	3.86	1.12
424	4.50	0.96	1000	3.85	0.30
472	4.44	1.10	1048	3.85	0.79
520	4.32	0.63	1096	3.74	0.58
568	4.28	0.93	1144	3.72	0.88

research pH meter; TTT60 titrator; ABU11 autoburette). The perfusion solution and soil were adjusted to pH 5.5 by titration with 0.025M H_2SO_4 until an equilibrium state was approached. The pH was then maintained constant by titration with saturated $Ca(OH)_2$, and perfusion continued until the rate of nitrification was constant with time, when the Most Probable Numbers of nitrifying organisms were estimated.

The experiment was repeated at pH 6.5 commencing with a fresh soil sample.

5.4.3 Results and Discussion

The PNA and Most Probable Number of nitrifying organisms in Stratford sandy loam perfused at pH 5.5 and pH 6.5 were as follows:

pH	PNA ($\mu g\ N_{ox}/g$ soil/hr)	*Most Probable Number		μg N oxidised/NH_4^+ oxidising cell/hr
		NH_4^+ oxidisers	NO_2^- oxidisers	
5.5	2.85	1.4×10^6	5.2×10^6	2.04×10^{-6}
6.5	8.60	3.5×10^6	2.4×10^7	2.45×10^{-6}

*Estimated by Dr U. Sarathchandra, Ruakura Agricultural Research Centre, Hamilton, New Zealand.

An increase in pH from 5.5 to 6.5 increased the potential nitrification activity by a factor of three, largely because of an increase in the population of nitrifying organisms. An increase in both numbers and activity of nitrifying organisms may be expected as the pH of the soil becomes more favourable.

5.5.0 DETERMINATION OF THE RELATIVE NITRIFICATION RATES OF FOUR SOILS PERFUSED AT pH VALUES RANGING FROM 7.0 TO 4.0

5.5.1 Introduction

In chapter 3 it was found that the regression coefficient for pH with INA for yellow-brown loams was different, although not statistically significant, from that of other soils. It was also found that INA was higher in yellow-brown loams than in other soils of similar pH. These phenomena are further investigated in the present section.

5.5.2 Experimental

Four soils of different nitrification characteristics were selected for this study:

Soil	Soil Group	INA ($\mu g\ N_{ox}/g$ soil/hr)	Type of Nitrification Pattern
Pukepuke brown sandy loam	Yellow-brown sand	5.70	3
Marua clay	Yellow-brown earth	<0.02	2
Te Kuiti	Yellow-brown loam	4.16	1
Stratford sandy loam	Yellow-brown loam	2.12	1

These soils were perfused in the modified perfusion unit (figure 5.4) connected to a pH stat (Radiometer PHM 64 Research pH meter; TTT60 titrator; ABU11 autoburette).

The pH of the perfusing solution (0.005M $(NH_4)_2SO_4$) was adjusted to pH 7.0 with saturated $Ca(OH)_2$ and the soils perfused for twenty days, maintaining a constant pH in the perfusion unit by titration with saturated $Ca(OH)_2$. After twenty days of perfusion the rate of

nitrification was constant with time and the soil was considered to be "bacterially saturated". The rate of nitrification was then measured over a six hour period.

Following measurement of the nitrification rate, the perfusion solution was titrated down by 0.5 pH units with 0.025M H_2SO_4 until an equilibrium situation between the soil and perfusion solution was approached. Equilibrium was considered to be approached when the change in pH of the perfusion solution was less than 0.05 units during passage through the soil. The system was then allowed to equilibrate for sixteen hours and then the rate of nitrification was measured over a six hour period, pH being maintained constant during the measurement period by titration with saturated $Ca(OH)_2$. This process was repeated and the nitrification activity measured at 0.5 pH unit intervals down to pH 4.0. All experiments were duplicated.

All sampling and analytical techniques were as described in section 3.2.3.

5.5.3 Results and Discussion

Relative rates of nitrification for the four soils studied at pH values of 7.0 to 4.0 are shown in figure 5.6. The rate of nitrification in Marua soil was more affected by low pH than the other three soils studied.

The oxidation of NO_2^- by *Nitrobacter* in liquid culture media, in soil and absorbed on negatively charged resins was compared as a function of hydrogen ion concentration by McLaren and Skujins (1963). They found the pH for half maximum nitrification rate was about 0.5 pH units higher with soil than in liquid suspension culture. It was concluded that such a difference was to be expected since NO_3^- is repelled from and H^+ attracted to negatively charged surfaces of soil or resin. Therefore, to achieve a

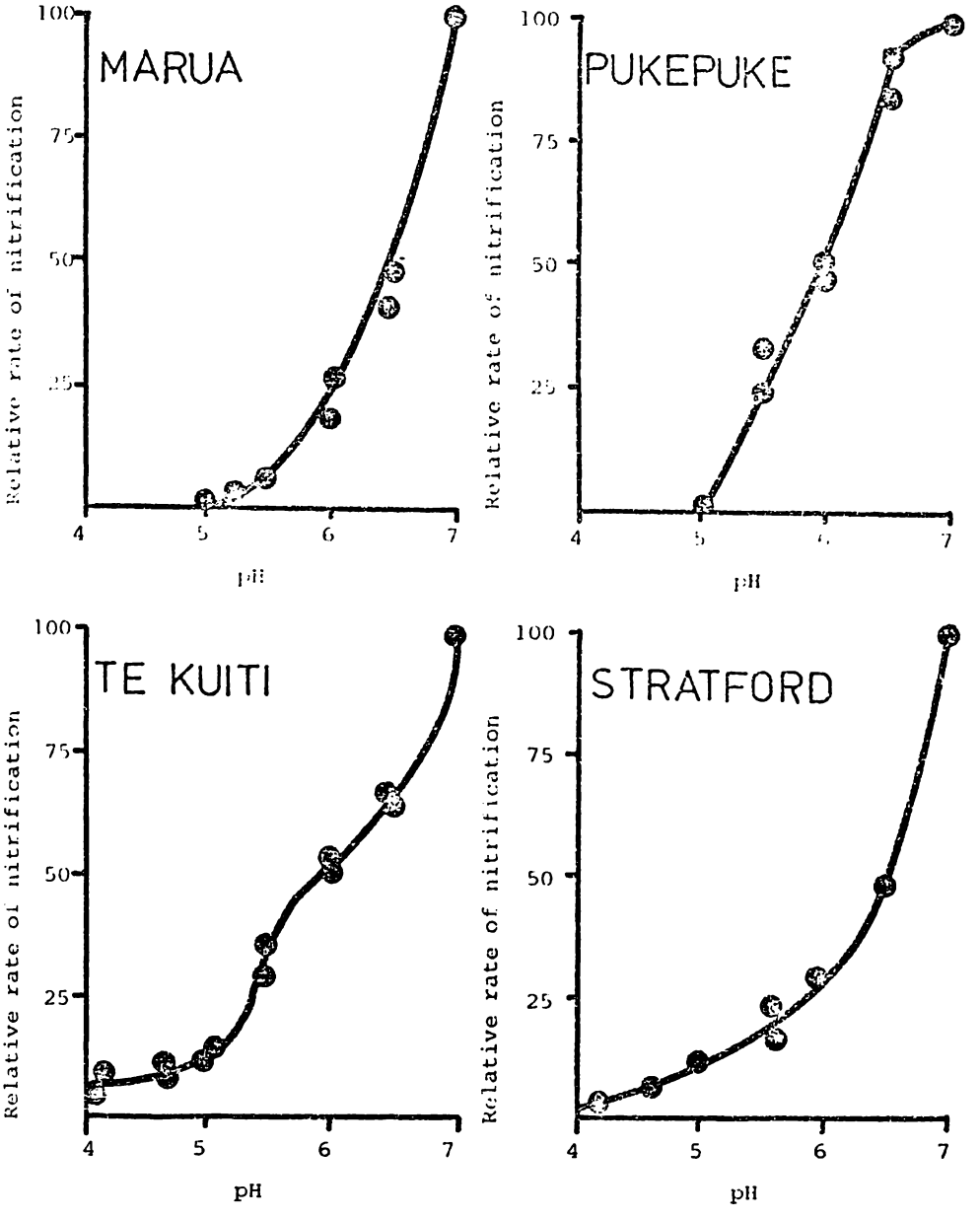

Figure 5.6 Relative rates of nitrification for four soils perfused with 0.005M $(NH_4^+)SO_4$ at pH values of 7.0 to 4.0

H^+ concentration at such a surface equal to that observed in suspension, one must use a liquid phase of a higher pH for the soil or resin. The clay mineral fraction of the soil studied by McLaren and Skujins (1963) was largely crystalline.

Kaolinite and 2:1 type clay minerals possess a nett negative charge over the pH range 3 - 10 (Buchanan and Oppenheim, 1968; Fields and Schofield, 1960; Schofield, 1949; Swartzen-Allen and Matijevic, 1974; 1975). Allophane, however, has a point of zero charge which has been reported to occur in the pH range of 5.5 - 6 (Fields and Schofield, 1960), pH 4.9 (Birrell, 1961) and at pH 7.0 (Wada and Ataka, 1958). Similarly the isolectric points for New Zealand allophanes have been reported in the range of pH 4.6 - 6.0 (Milestone, 1972) and at pH 5.8 for a Japanese allophane (Horikawa, 1975). A distinction between the point of zero charge and iso-electric point of allophane is necessary, since although these essentially occur at the same pH where non-specific adsorption takes place, this is not the case where specific adsorption is involved (Wright and Hunter, 1973). Allophane differs from the crystalline clay minerals therefore, in that it commonly exhibits a nett positive charge at normal soil pH values.

The pH measured in bulk solution may not necessarily be that encountered by the nitrifying organism. An equation for the difference between $[H^+]$ at a charged surface $[H^+]_s$ and that in a bulk solution $[H^+]_b$ was derived by Hartley and Roe (1940). The local concentration of the ion is determined by the electrostatic potential (Ψ) at the surface:

$$[H^+]_s = [H^+]_b \exp(-F\Psi/RT) \qquad (5.1)$$

where F is the faraday, R the gas constant and T the absolute temperature.

Therefore:

$$pH_s = pH_b + (F/RT) \qquad (5.2)$$

where pH_s is the pH of a charged surface and pH_b that in the bulk solution. In the case of a small particle, the potential can be replaced by the zeta potential (ζ) At 25°C, equation 2 approximates:

$$pH_s = pH_b \ (\zeta/60) \qquad (5.3)$$

where pH_s is the pH at the shear plane.

Measurement of electrophoretic mobility of allophane therefore permits calculation of the surface pH at the plane of shear using equation 5.3.

Surface pH's for allophane over a range of bulk solution pH's are presented in table 5.3.

In the present study, the pH of the bulk solution at which the rate of nitrification was twenty five percent of that at pH 7 was determined from figure 5.6 as:

Soil	pH
Marua	6.18
Te Kuiti	5.43
Stratford	5.40
Pukepuke	5.56

Therefore, twenty five percent relative nitrification occurred in the Te Kuiti, Stratford and Pukepuke soils when the pH of the bulk solution was 0.75, 0.78 and 0.62 pH units lower than that of Marua.

The major clay components of Marua soil are crystalline, and the pH at the soil surface may be expected to be approximately 0.5 pH units lower than that in bulk solution from consideration of McLaren's and Skujin's (1963) work. Allophane is the major clay mineral present in Te Kuiti and Stratford soils, and at pH 5.4, in the bulk solution, the

Table 5.3 Estimation of the difference in solution pH and the pH on the surface of Ohaupo allophane in 10^{-3} M NaCl

pH of Bulk Solution	*Electrophoretic Mobility (microns/sec per volt/cm)	Zeta Potential (millivolts)	Calculated pH on Surface of Allophane	$pH_b - pH_s$
7.22	-0.57	-7.39	7.10	-0.12
7.10	-0.85	-11.02	6.92	-0.18
7.00	-0.88	-11.41	6.81	-0.19
6.70	-0.77	-9.98	6.53	-0.17
6.30	0.00	0.00	0.00	0.00
6.08	0.27	3.5	6.14	0.06
5.30	1.40	18.15	5.60	0.30
5.00	1.60	20.74	5.35	0.35
4.40	1.80	23.33	4.79	0.39
4.20	1.80	23.33	4.59	0.39

* Data from D.H. Taylor, School of Science, University of Waikato, Hamilton, New Zealand.

pH of the allophane will be approximately 0.3 pH units higher. This provides an estimated difference between the clay fraction of Marua soil and Te Kuiti and Stratford soils of approximately 0.8 pH units, or similar to that measured when the rate of nitrification was twenty five percent of that at pH 7.0. Pukepuke is not an allophanic soil but contains a clay fraction which is largely amorphous to X-rays (Steele 1976a) and which may exhibit properties similar to those of allophane.

The difference in nitrification activity exhibited by allophanic soils in the pH range of 4.5 - 6.0 can be rationalised by theoretical considerations of surface pH, but such considerations do not include the effect of organic materials on the surface pH. Differences in surface pH cannot be used to explain nitrification below pH 4.5 in these soils.

It appears that death of nitrifying organisms occurred at low pH because at the completion of the experiment with Stratford sandy loam, increasing the pH from 4.0 to 7.0, increased the relative nitrification from 2.5 percent to only 5.6 percent of the original rate of nitrification at pH 7.0.

5.6.0 THE EFFECT OF LIME AND NITROGEN FERTILISERS ON NITRIFICATION UNDER FIELD CONDITIONS

5.6.1 Introduction

Perfusion of soil with an NH_4^+ substrate, or an increase in the pH in a perfusion unit, results in an increase in the population of nitrifying organisms (see section 3.3.4 and 5.4.3). In the present section, soil samples from a field trial were examined to determine if addition of substrate as nitrogen fertiliser, or an increase in soil pH following application of lime, resulted in changes of nitrification activity or population of nitrifying organisms.

5.6.2 Experimental

Duplicate soil samples were collected from a Ministry of Agriculture and Fisheries trial (F.D. 109) located at Hindon Field Research Area, Hindon. The trial was commenced in July 1974, and the following treatments sampled in November 1976:

1) Control (no lime:no nitrogen).

2) No lime:300 kg N/ha/year applied as $(NH_4)_2SO_4$ in five two-monthly applications.

3) 5,000 kg lime/ha one only:no nitrogen.

4) 5,000 kg lime/ha one only:300 kg N/ha/year applied as $(NH_4)_2SO_4$ in five two-monthly applications.

The soil on the experimental area was Wehenga silt loam and the trial area was grazed by sheep.

The INA and PNA of the soil samples were determined by perfusion with 0.005M $(NH_4)_2SO_4$ as outlined in section 3.2.2. The Most Probable Number of NH_4^+ and NO_3^- oxidisers was determined as outlined in section 3.2.4.

5.6.3 Results and Discussion

Both lime and nitrogen increased INA, PNA, and the MPN of nitrifying organisms, but lime and nitrogen together had the largest effect (table 5.4). Lime also increased the rate of oxidation/NH_4^+ oxidising cell/hour from 0.14×10^{-6} without lime to 0.78×10^{-6} with lime.

Application of lime increased soil pH from pH 5.8 to pH 6.7. Such an increase may be expected to increase the INA of the soil but indications are that substrate was limiting, as lime and nitrogen together provided the largest increase.

Table 5.4 Initial Nitrification Activity (INA), Potential Nitrification Activity (PNA) and the Most Probable Number of nitrifying organisms in samples of Wehenga silt loam collected from a lime by nitrogen experiment at Hindon

Treatment		pH	INA± SE*	PNA± SE*	Most Probable Number of Nitrifying Organisms Prior to Perfusion		µg N_{ox}/ Ammonium Oxidising Cell/ Hour
					Ammonium Oxidisers	Nitrite Oxidisers	
+L**	+N***	6.7	1.05±	2.76±	1.6×10^6	5.4×10^5	0.66×10^{-6}
+L	-N	6.5	0.27±	2.98±	3.5×10^5	1.3×10^5	0.79×10^{-6}
-L	+N	5.8	0.12±	1.04±	5.4×10^5	2.4×10^5	0.22×10^{-6}
-L	-N	5.8	0.01±	1.05±	1.7×10^5	0.33×10^5	0.06×10^{-6}

* Standard Error

** Lime

*** Nitrogen

5.7.0 GENERAL DISCUSSION AND CONCLUSIONS

Addition of $CaCO_3$ to acidic soils increases the pH of the soil bringing it closer to the optimum pH range for biological oxidation of NH_4^+. Therefore the large increase in the rate of nitrification and population of nitrifying organisms when soil was perfused in the presence of $CaCO_3$ was not unexpected. Field data also showed that both nitrification activity and numbers of nitrifying organisms had increased in the two years following an application of lime.

The generation times of NH_4^+ oxidisers were reduced to thirty six to forty hours when soils were perfused with $CaCO_3$. The presence of nitrifying organisms in the perfusing solution of soils containing $CaCO_3$ casts doubt on the validity of such experiments, since the final rate of nitrification reached by such a system may not be dependent on soil factors alone.

No reason is apparent for the two logarithmic phases shown by Marua soil when perfused with $CaCO_3$ and by Tokoroa soil when perfused in the absence of $CaCO_3$. In the latter soil, the initial logarithmic phase could not be ascribed to a rapid increase in the numbers of NH_4^+ oxidisers, firstly because the MPN of NH_4^+ oxidisers increased in a logarithmic manner similar to the accumulation of NO_3^- during the second logarithmic phase; and secondly the estimated generation time of NH_4^+ oxidisers during the initial phase was shorter than what would be expected even in a pure culture system. One possibility is that there were a number of inactive nitrifying organisms present when the soil was collected and these became active during the initial stages of perfusion. Nishio and Furusaka (1971a,b) reported that dispersion of a soil considerably increased the rate of NO_2^- oxidation and suggested that this was due to activation of NO_2^- oxidising organisms which were previously inactive. The reverse situation, i.e. organisms becoming inactive due to occlusion

by other organisms during perfusion, has also been suggested for NO_2^- oxidising bacteria by Nishio and Furusaka (1971a,b).

The dependence of rate of nitrification on pH was demonstrated by perfusion of soils ranging from pH 7.0 to pH 4.0. The curve for the relative rate of nitrification in Marua soil at pH values from pH 5.0 to 7.0 is similar in shape to that of McLaren and Skukins (1963) curve for the Yolo silt loam. The curves for the other three soils studied (Pukepuke, Te Kuiti, Stratford) are different, higher rates of nitrification being observed at low pH. The increased rate of nitrification at pH values around pH 4.5 to 5.5 can be rationalised by differences between surface pH and the pH of the perfusing solution. However, such differences cannot be used to rationalise nitrification at pH values below 4.5.

It appears that changes in soil pH stimulates different changes in the nitrification activity in allophanic soils than in non-allophanic soils. It is likely that such differences arise because the presence of allophane, which is positively charged at the pH of most soils, may in some way modify the habitat of the surface living nitrifying organisms in such a way that they are able to nitrify when the bulk solution pH is as low as pH 3.7. Stimulation of nitrification by addition of fifty percent calcium-saturated allophane has been reported by Kai and Harada (1969). There is a paucity of information on surface reactions between nitrifying organisms and the surface of allophane. This field requires further investigation.

Further field evidence was obtained to support that changes in nitrification activity observed in the laboratory may also be expected to occur under field conditions.

CHAPTER 6

THE EFFECT OF UREA ON NITRIFICATION IN SOILS

6.1.0 INTRODUCTION

All the soils studied in the present investigation have been collected from intensively grazed grasslands, and have been found to contain large numbers of NH_4^+ and NO_2^- oxidising organisms. The grazing animal is an important part of the nitrogen cycle in that it ingests largely organically bound nitrogen and excretes a large proportion as urea. The latter is rapidly hydrolysed to NH_4^+ in most soils, providing large amounts of substrate for nitrifying organisms.

Beef cattle excrete about ninety five percent of the nitrogen ingested and dairy cattle about seventy five percent (Walker et al. 1954). It appears that both cattle and sheep excrete about 0.8 g of nitrogen in their faeces for every 100 g dry matter consumed, the remainder being excreted in the urine (Barrow and Lambourne, 1962; Barrow, 1967). Similar estimates have been made by other workers. Sears et al. (1948) and Sears (1950) reported that sheep grazing grass/clover swards in New Zealand returned seventy to seventy five percent of the excreted nitrogen in urine. It can therefore be estimated that if dairy cows ingest 12,000 kg dry matter/ha/year on average containing 3.8 percent total nitrogen, the return of nitrogen in excreta will be the equivalent of about 342 kg N/ha. Of this, it may be expected that approximately 96 kg will be returned in faeces and 246 kg in urine.

A major result of cycling nitrogen through animals is the redistribution of that nitrogen when returned to the soil. The nitrogen content of urine is very variable, the range reported for cattle being 2.5 - 13 g N/ℓ with a mean of about 8 g N/ℓ, and that for sheep from 5.7 - 14.7 g N/ℓ with a mean of about 9 g N/ℓ (Whitehead, 1970). Whitehead (1970) calculated that the average amount of nitrogen applied to an area covered by a cattle urination of 2 ℓ containing 8 g N/ℓ distributed over 2787 cm^2 is equivalent to 576 kg N/ha; and for a sheep

urination of 150 mls containing 9 g N/ℓ and covering 290 cm^2, 423 kg N/ha.

In addition to providing a large amount of substrate to nitrifying organisms, hydrolysis of urea in urine spots results in an increase in soil pH which will effectively shorten the generation time of the organisms in the volume of soil affected by the urine. The pH increase following hydrolysis of urea will be dependent on several factors including the rate of hydrolysis, concentration of urea and the buffering capacity of the soil. An indication of the increase in pH which may be expected can be gained from Doak (1952) who reported that urine applied at two litres per 9290 cm^2 raised the pH of the soil surface from 5.3 to 7.8 within four hours.

The object of the investigation reported in the present chapter was to examine the changes in nitrification activity and populations of nitrifying organisms following application of urea to soils in a laboratory incubation experiment.

6.2.0 EXPERIMENTAL MATERIALS AND METHODS

Eighty five gram sub-samples of freshly collected Tokoroa sandy silt (INA <0.02 μg N_{ox}/g soil/hr) were compressed into polythene cylinders (7.0 cm diameter) to a depth of 5 cm to provide a bulk density of 0.57 g/cc which may be expected under field conditions.
Three treatments were applied:

1) Control.
2) 0.4596 g urea in 25 ml distilled water per container.
3) 0.8816 g $(NH_4)_2SO_4$ in 25 ml distilled water per container.

The above treatments are equivalent to an application of 600 kg N/ha or approximately the amount which occurs in urine (Whitehead, 1970). The containers were adjusted to sixty percent water holding capacity and incubated at 25°C. Two containers were removed from incubation, mixed,

and pH, NH_4^+, NO_3^- and the most probable number of nitrifying organisms (MPN) determined (as outlined in sections 3.2.3 and 3.2.4). The experiment was repeated at zero, three, five, seven, ten, fifteen, twenty, thirty, sixty and ninety days after commencement of incubation.

6.3.0 RESULTS AND DISCUSSION

Following application of urea the soil pH increased from pH 5.0 to above pH 7.0, where it remained for ten days. Application of $(NH_4)_2SO_4$ slightly depressed soil pH, while the pH of the control decreased during incubation (table 6.1).

Changes in the NH_4-N and NO_3^--N during incubation are presented in table 6.2. In the control, 253.9 g N/g soil was mineralised during the ninety day incubation period. Little nitrification occurred during the initial five days of incubation, but at the completion of the experimental period most of the mineralised nitrogen had been nitrified. In the urea treatment little nitrification was apparent until after five days of incubation, the most rapid nitrification occurring between ten and twenty days incubation following which the rate of nitrification decreased. Little nitrification occurred after thirty days by which time the pH had decreased to 4.6. No nitrification of $(NH_4)_2SO_4$ occurred during incubation possibly because of inhibition by low pH. However, it appears as if pH was not the only factor involved since some nitrification occurred in the control treatment at pH values similar to that of the $(NH_4)_2SO_4$ treatment. It is possible that a salt effect inhibited nitrification in the $(NH_4)_2SO_4$ treatment and initiated the decline in numbers of nitrifying organisms.

Both INA and MPN increased during incubation of the control, followed by a decrease in MPN between sixty and ninety days (tables 6.3 and 6.4). These changes demonstrate that soil incubation is not a satisfactory

Table 6.1 Changes in the pH of Tokoroa sandy silt during incubation with and without added nitrogen

Days Incubation	Treatments		
	Control	Urea N	$(NH_4)_2SO_4$
0	5.0	-	-
3	5.0	7.8	4.6
5	5.0	7.8	4.8
7	5.1	7.6	5.0
10	5.0	7.6	4.9
15	4.9	5.6	4.9
20	4.6	4.7	4.9
30	4.6	4.6	4.9
60	4.6	4.6	4.9
90	4.6	4.5	4.9

Table 6.2 Changes in the NH_4^+ and NO_3^- nitrogen in Tokoroa sandy silt during incubation with and without added nitrogen

Days Incubation	Treatments: Control NH_4-N (µg N/g soil)	Control NO_3-N (µg N/g soil)	Urea N NH_4-N (µg N/g soil)	Urea N NO_3-N (µg N/g soil)	$(NH_4)_2SO_4$ NH_4-N (µg N/g soil)	$(NH_4)_2SO_4$ NO_3-N (µg N/g soil)
0	74	33	-	-	-	-
5	152	46	1461	50	2761	46
10	313	183	1532	188	2761	48
20	150	202	734	649	2481	47
30	189	201	629	848	2511	59
60	4	329	469	987	2481	44
90	5	356	458	1016	2510	59

Table 6.3 Changes in the Initial Nitrification Activity (INA) of Tokoroa sandy silt during incubation with and without addition of nitrogen

Days Incubation	Treatments		
	Control (µg N/g soil)	Urea N (µg N/g soil)	$(NH_4)_2SO_4$ (µg N/g soil)
0	<0.02	n.d.	n.d.
5	<0.02	0.15	<0.02
10	0.07	1.82	<0.02
20	0.13	2.64	<0.02
30	0.10	2.66	<0.02
60	0.29	0.89	<0.02

n.d. = not determined.

Table 6.4 Changes in the Most Probable Number of nitrifying organisms present in Tokoroa sandy silt during incubation with and without added nitrogen

Days Incubation	Treatments: Control		Urea		$(NH_4)_2SO_4$	
	Ammonium Oxidisers g soil^{-1}	Nitrite Oxidisers g soil^{-1}	Ammonium Oxidisers g soil^{-1}	Nitrite Oxidisers g soil^{-1}	Ammonium Oxidisers g soil^{-1}	Nitrite Oxidisers g soil^{-1}
0	1.6×10^4	7.2×10^4	-	-	-	-
5	3.8×10^4	1.0×10^5	2.8×10^5	5.7×10^4	2.1×10^4	4.6×10^4
10	8.0×10^4	7.4×10^5	2.2×10^6	4.0×10^6	6.1×10^4	9.8×10^4
20	1.4×10^5	2.4×10^5	8.0×10^5	3.9×10^6	4.2×10^3	8.6×10^4
30	8.7×10^4	2.3×10^5	4.4×10^5	5.2×10^5	8.6×10^2	2.3×10^4
60	4.1×10^4	2.2×10^5	1.9×10^4	5.7×10^4	3.7×10^2	5.6×10^4
90	1.3×10^3	3.7×10^4	3.6×10^3	2.9×10^4	-	-

technique for studying nitrification in soil since the nitrification observed may be the result of changes in the population of nitrifying organisms during incubation, rather than a reflection of the inherent nitrifying population.

Following application of urea, both INA and MPN increased. INA increased from 0 µg N_{ox}/g soil/hr to a maximum of 2.66 µg N_{ox}/g soil/hr thirty days after application of urea, following which it decreased. The MPN of NH_4^+ oxidisers increased by a factor of 138 to reach a maximum population of 2.2×10^6/g soil.

Application of $(NH_4)_2SO_4$ did not increase INA, and this is consistent with the lack of nitrification during incubation. The MPN of nitrifying organisms decreased during incubation reaching a population of zero after ninety days.

It appears, therefore, that in New Zealand grazed grasslands urine may be an important factor in maintaining the large nitrifying population close to the soil surface. Two reasons are apparent for this. Firstly, the pH rise which accompanies hydrolysis of urea provides a pH favourable for multiplication of nitrifying organisms. At pH 7.10 the generation time of NH_4^+ oxidising organisms in Tokoroa sandy silt is approximately thirty eight hours in conditions where substrate is non-limiting (see section 5.2.3). If a generation time of thirty eight hours is assumed, 6.3 generations could occur in ten days. The change in the MPN of NH_4^+ oxidisers indicates that seven generations occurred during the first ten days in incubation suggesting that the NH_4^+ oxidisers were growing logarithmically during that period.

Secondly, urine provides adequate substrate for the nitrifying population. The fact that no nitrification occurred in the presence of $(NH_4)_2SO_4$ suggests that addition of substrate alone to Tokoroa sandy silt will not stimulate nitrification unless accompanied by an increase in pH.

An intensive sampling of grazed areas for determination of the variation in the population of nitrifying organisms relative to urine spots is required in order to determine the exact dependance of the size of the nitrifying population on the return of urine to soil. Any such affect may result in different populations, for example, under sheep as opposed to cattle grazing.

It appears that nitrification in Tokoroa sandy silt will not occur below approximately pH 4.6, since nitrification in the urea treatment essentially stopped after sixty days when the soil pH had fallen to 4.6, even though adequate substrate was still present. That cessation of nitrification was due to limiting pH was shown by perfusing remaining samples of urea treated soil at the conclusion of the ninety day incubation. When the pH of the perfusion solution was maintained at pH 4.5, no nitrification occurred. However, if the pH of the perfusion solution was increased to pH 7.0, nitrification proceeded until oxidation of the remaining substrate was complete. This pH below which no nitrification occurs is in the range which is normally reported in the literature for soils (e.g. Gerretson, 1942; Meek and Lipman, 1922).

CHAPTER 7

A STUDY OF THE FATE OF AMMONIUM AND NITRATE FERTILISER IN TWO SOILS OF DIFFERENT INITIAL NITRIFICATION ACTIVITIES

7.1.0 INTRODUCTION

Evidence was presented in chapters 3 and 4 that New Zealand grassland soils differ widely in their "initial" and "potential" rates of nitrification, and that this produces differences in the form of nitrogen available for plant uptake. No field evidence is available as to whether such differences will affect rate of pasture production, although it has been suggested (Steele, 1976b) that differences in nitrification activity affect the recovery of nitrogen fertiliser by pasture.

The objective of the present chapter was to follow the fate of nitrogen applied as ammonium and nitrate to two soils, one having a high and the other a low rate of nitrification. To do this, two experiments were conducted adjacent to each other on two soils, one using ^{15}N as a tracer, and the other a non-tracer experiment. It was hoped that the results of this experiment would provide some understanding of the role of nitrification in New Zealand grassland systems.

The study was conducted in Northland, principally because soils with very different rates of nitrification occur in close proximity to each other, and nitrogen is considered to be a major limitation to pasture production (Steele 1976b).

The two soils chosen for this study, Wharekohe silt loam and Waimate North clay loam, have previously been described (section 3.3.3). Profile descriptions for both soils are included in Appendix 2. The pasture on both soils was predominantly ryegrass *Lolium perenne* and white clover *Trifolium repens*.

7.2.0 PATTERN OF PASTURE GROWTH ON WHAREKOHE SILT LOAM AND WAIMATE NORTH CLAY LOAM

The seasonal pattern of pasture growth in Northland differs from

that of most other parts of New Zealand in that a relatively even rate of pasture growth occurs throughout the year (figure 7.1). There are no accentuated peaks of pasture growth as are apparent in the pasture growth curves from Rukuhia and Winchmore. The locations of the pasture sites described in figure 7.1 are shown in figure 7.2.

There is normally no early spring flush of pasture growth in Northland, and the rate of pasture growth in late winter and early spring is lower than in the Waikato (During, 1972).

Rate of pasture growth and percentage clover in the pasture on a D.S.I.R. trial on Wharekohe silt loam at the Grasslands sub-station, Kaikohe (P.J. Rumball, unpublished data), are presented in figures 7.3, 7.4 and 7.5. The pasture on the trial area was sown to Ariki ryegrass, cocksfoot and white clover in autumn 1969 and received an annual fertiliser dressing of 500 kg potassic super per ha (7% P; 14% K), and 10 kg sulphur per ha. Pasture production was estimated using the rate of growth technique (Lynch, 1966). The figures presented are the mean of estimates from twelve 50 x 20 cm cages distributed in three blocks over a total area of 1.8 ha.

The rate of growth curves presented illustrate a relatively high rate of pasture production (up to 42 kg DM/ha/day) during winter, with an increasing rate of pasture growth during September, October, November, and December. In 1973/74 and 1974/75 pasture production during February was limited by rainfall.

The contribution of clover herbage to total pasture production showed a similar pattern for the three years. The clover content was highest during January, February and March, a consequence of the higher temperature optima for clover growth (24^{o}C) than for ryegrass growth (18 - 21^{o}C; Mitchell, 1956), declined during winter and remained low during the spring growth, possibly because clover was the poorer

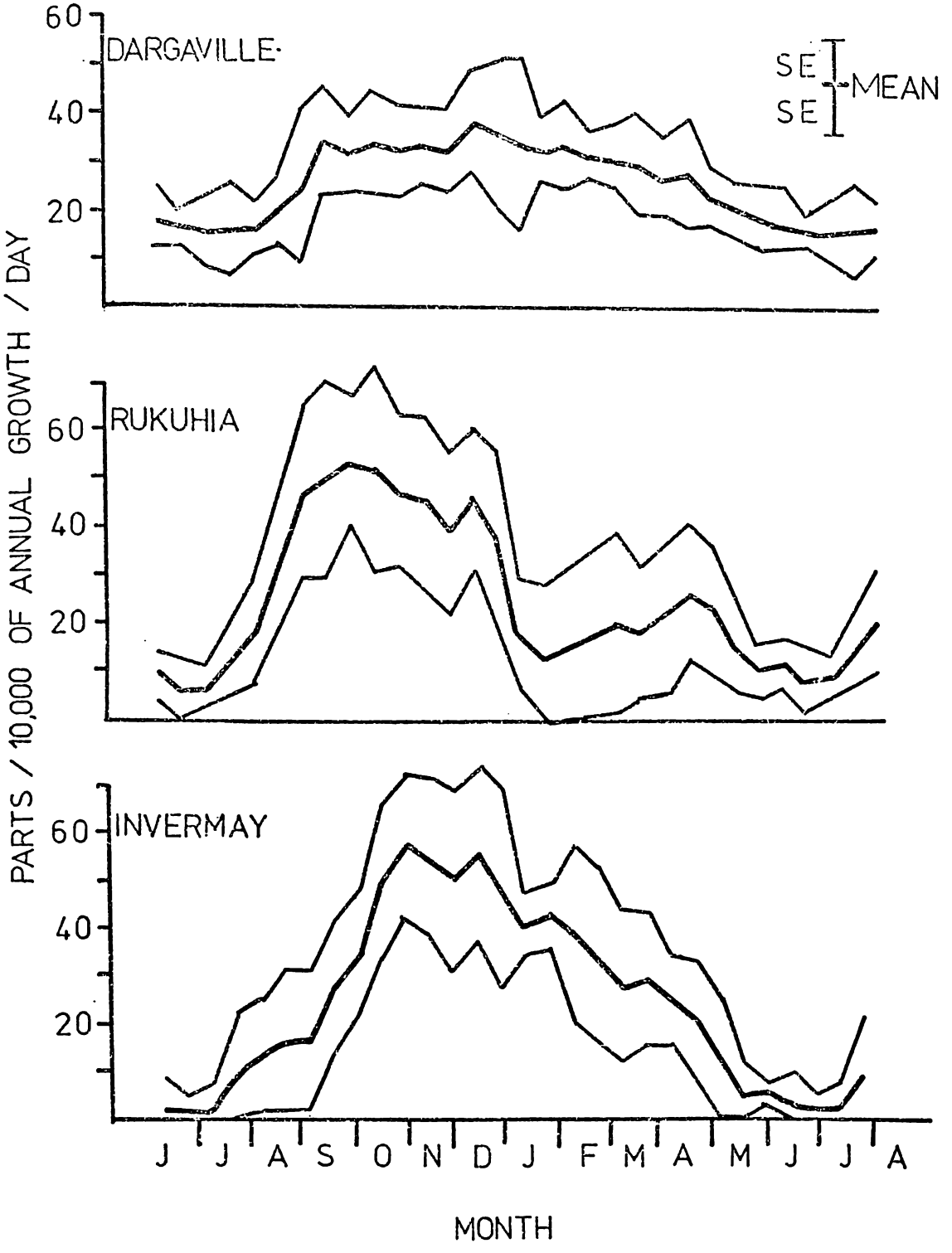

Figure 7.1 Seasonal pattern of pasture growth at Dargaville, Rukuhia and Winchmore

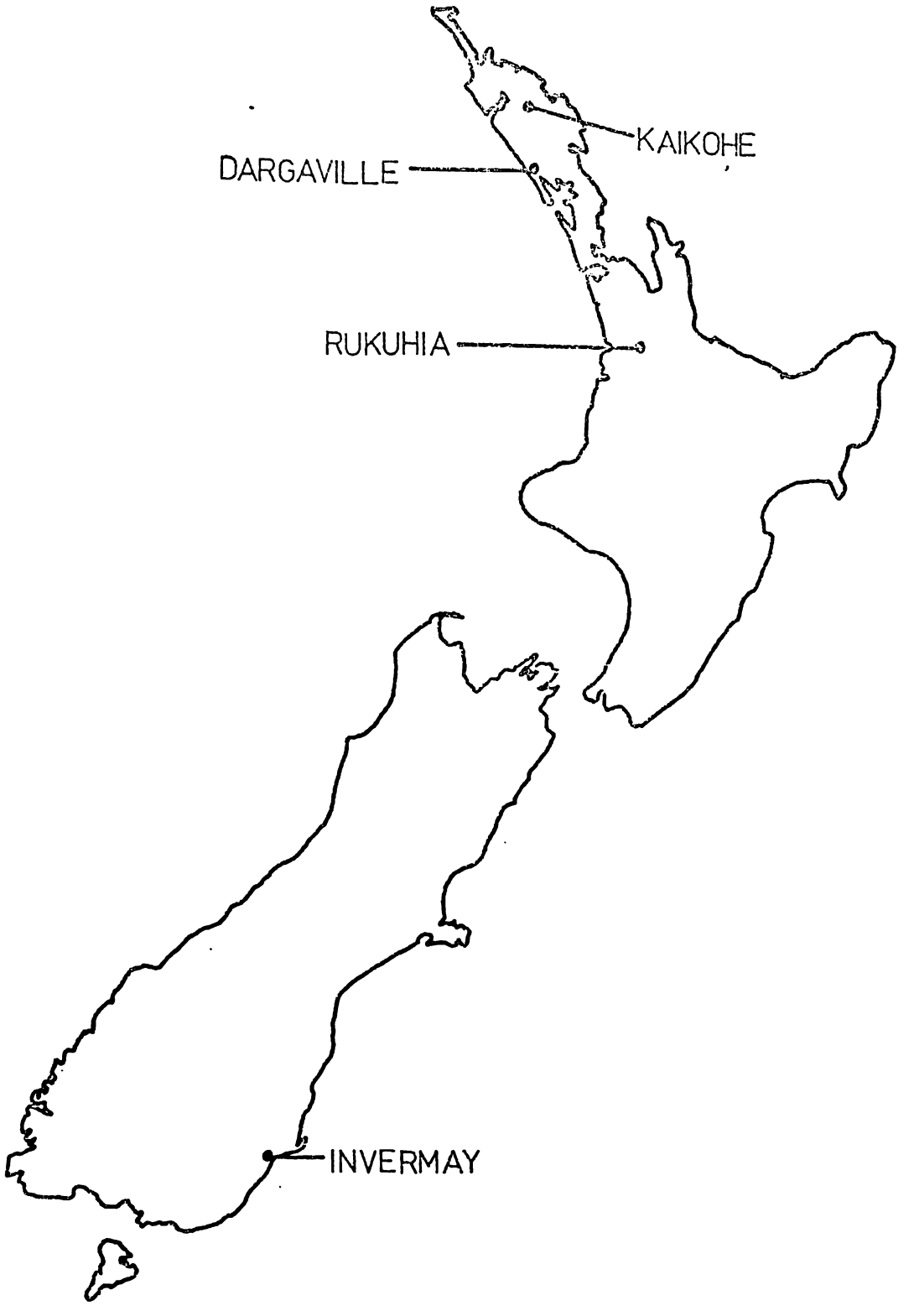

Figure 7.2 Location of experimental sites

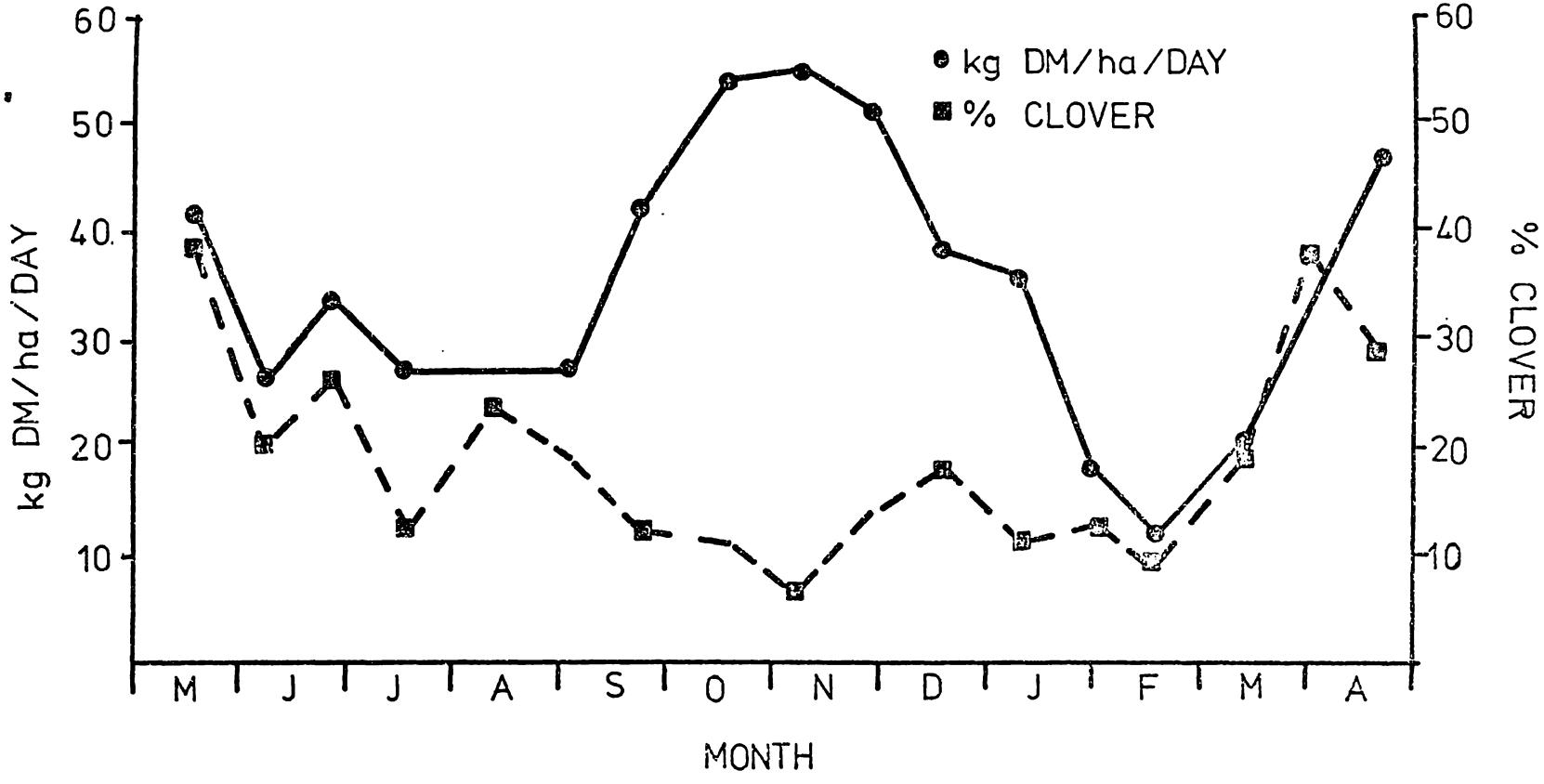

Figure 7.3 Rate of pasture growth and percentage clover in the pasture on the experimental site on Wharekohe silt loam (1973 - 1974)

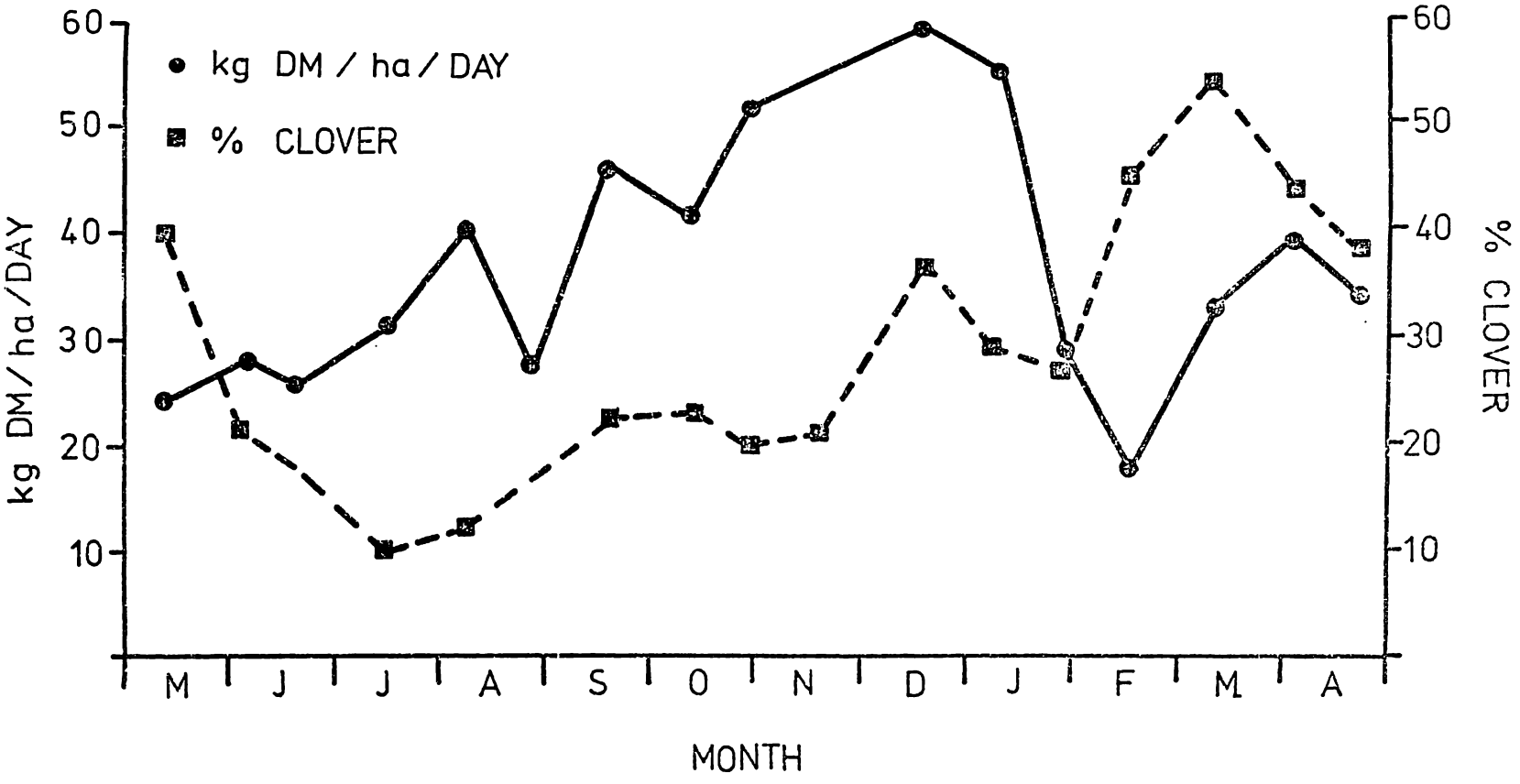

Figure 7.4 Rate of pasture growth and percentage clover in the pasture on the experimental site on Wharekohe silt loam (1974 - 1975)

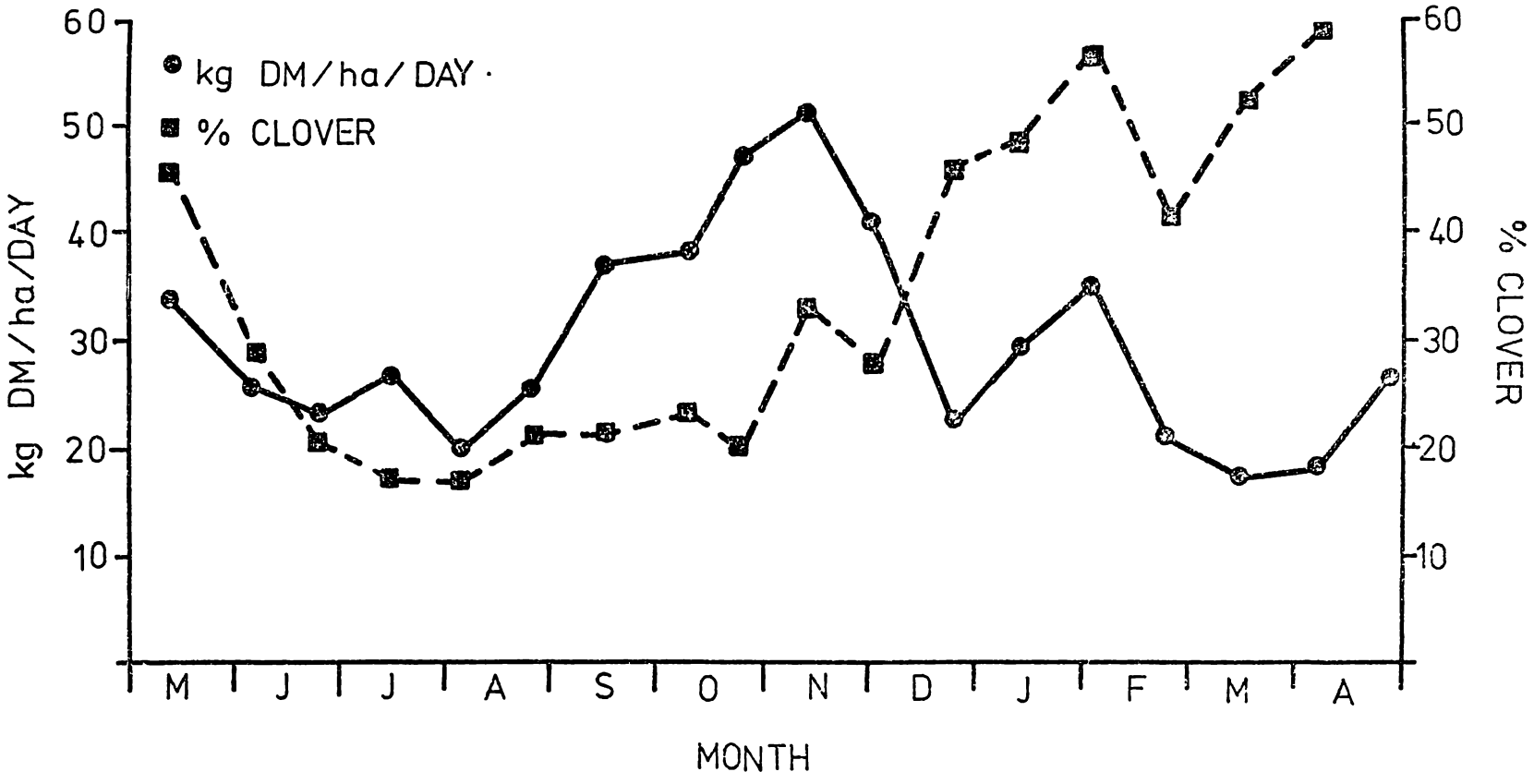

Figure 7.5 Rate of pasture growth and percentage clover in the pasture on the experimental site on Wharekohe silt loam (1975 - 1976)

competitor for light (Langer, 1973).

Rate of pasture growth and response to nitrogen fertiliser had beeen measured over a three year period on Waimate North clay loam, in the same field as used for the fertiliser study reported in the current chapter (figures 7.6, 7.7, 7.8; K.W. Steele and M.B. O'Connor, unpublished data). Nitrogen, as nitrolime (21% N; 13% Ca), was applied to one half of a 1.62 ha area, 309 kg nitrolime/ha (63.5 kg N/ha) being applied every two months. The pasture, predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) was managed under rotational grazing by dairy cows. Pasture production was measured at approximately twenty one day intervals by the rate of growth technique (Lynch and Mountier, 1954; Lynch, 1966). The figures presented are means from three cages (1.65 x 2.29 m) randomly distributed over each treatment.

There were considerable differences in the pattern of pasture production for the three years (table 7.1). In 1973/74 (figure 7.6) a severe nitrogen deficiency occurred in September, October and November, and large responses in pasture growth to applied nitrogen were obtained. Pasture growth over summer was severely restricted by a moisture deficit and very little response to applied nitrogen was recorded. Autumn and winter pasture growth was good and a response to added nitrogen was measured from March to August.

The pasture growth pattern for 1974/75 (figure 7.7) is probably more characteristic of the district (see figure 7.1), the growth pattern being relatively flat throughout the year. Added nitrogen increased the rate of pasture production in all months with the exception of November and December.

In 1975/76 (figure 7.8) the growth pattern was distinguished by a high rate of growth in September and a low rate of growth in February and March. A response to added nitrogen was recorded throughout the year

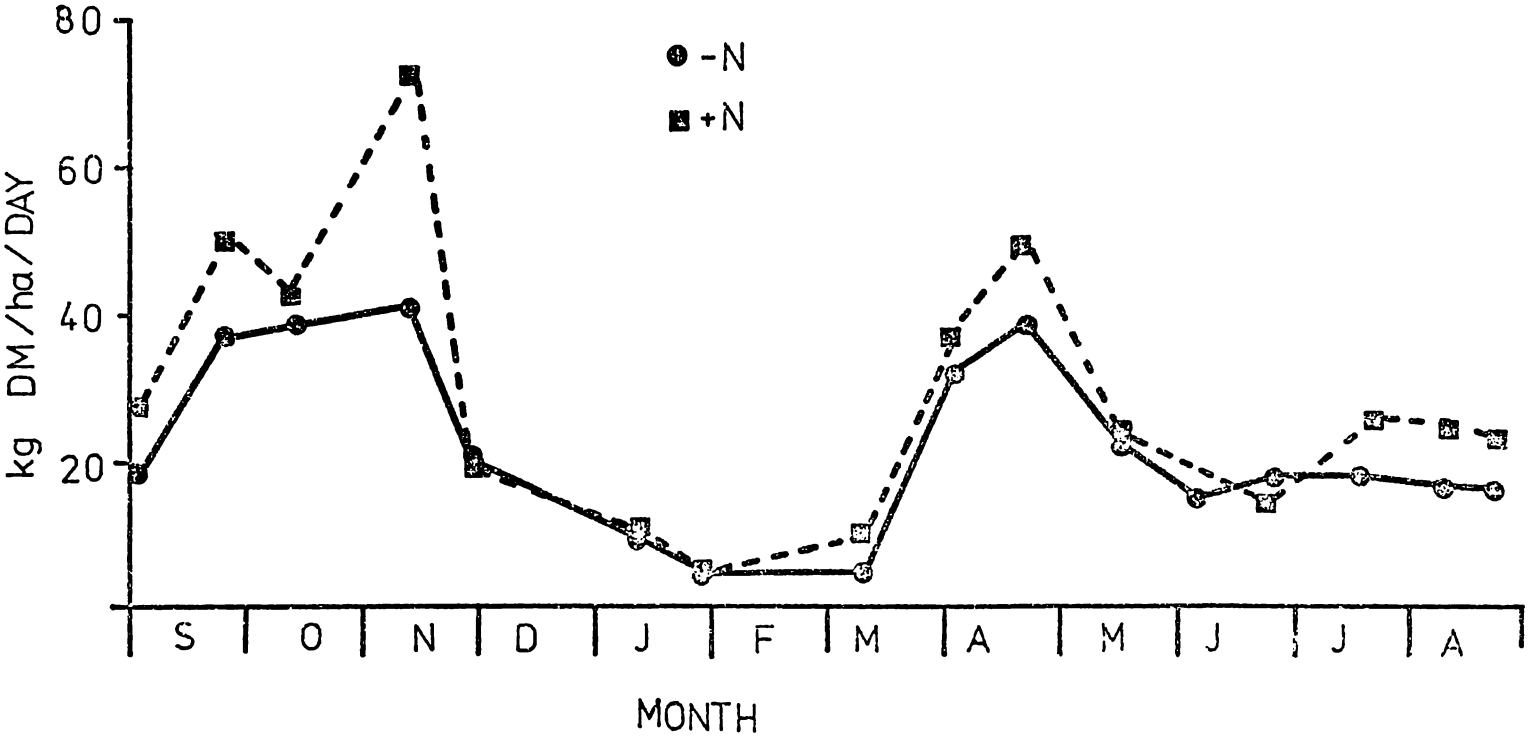

Figure 7.6 Rate of pasture growth with and without added nitrogen on the experimental site on Waimate North clay loam (1973 - 1974)

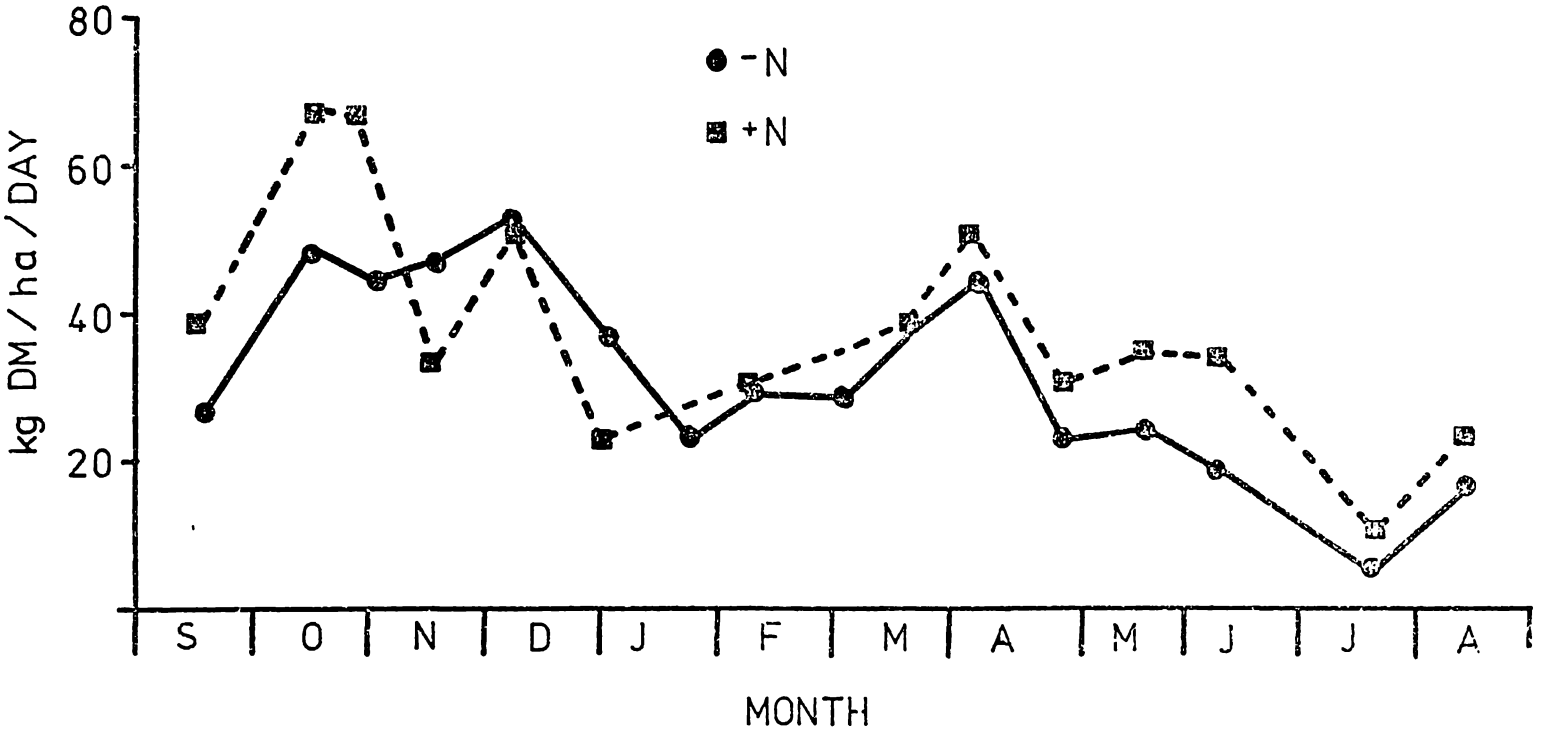

Figure 7.7 Rate of pasture growth with and without added nitrogen on the experimental site on Waimate North clay loam (1974 - 1975)

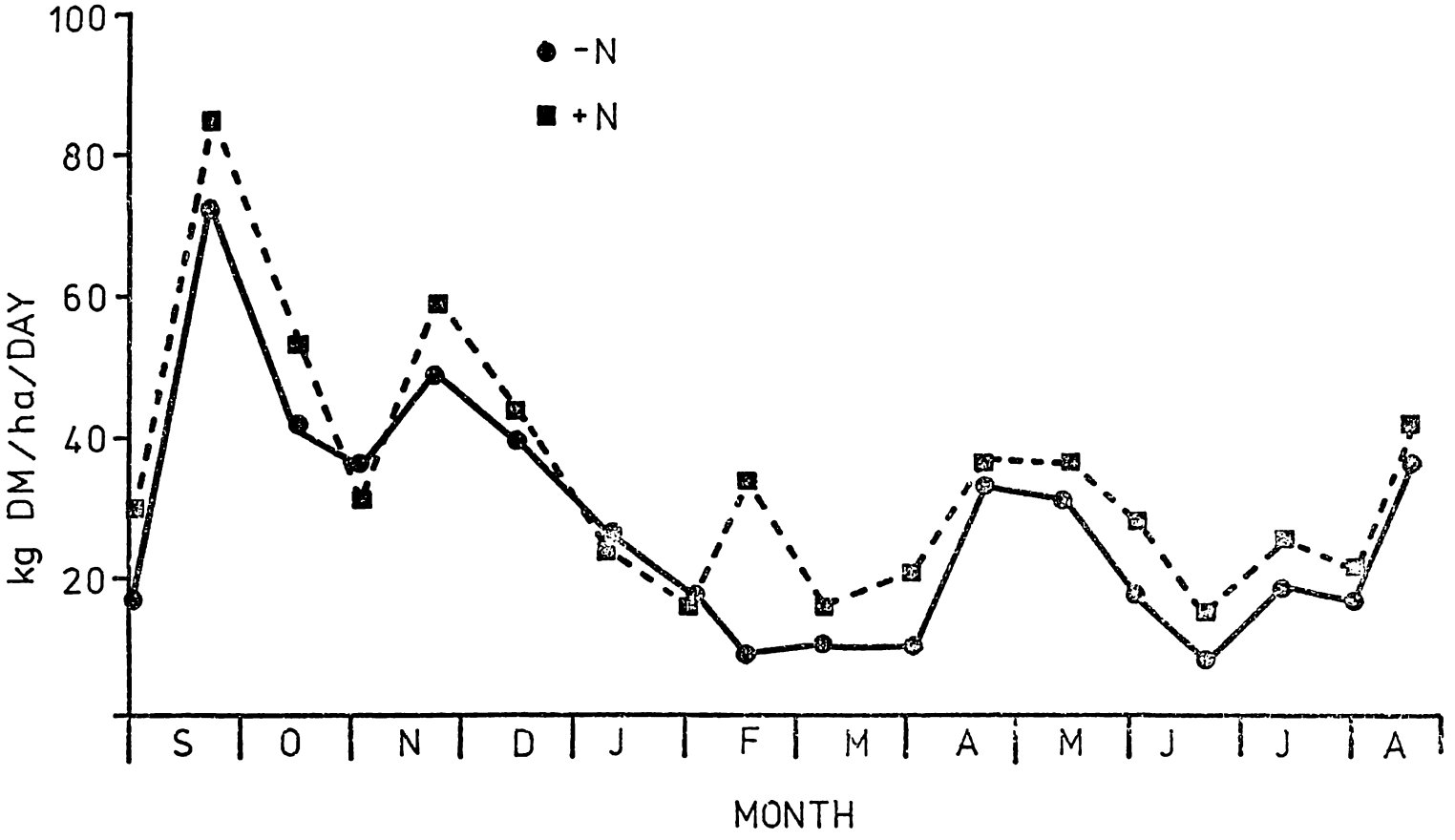

Figure 7.8 Rate of pasture growth with and without added nitrogen on the experimental site on Waimate North clay loam (1975 - 1976)

Table 7.1 Seasonal and yearly totals of pasture production on Waimate North clay loam, with and without added nitrogen

Year	Treatment	Spring kg DM/ha	Summer kg DM/ha	Autumn kg DM/ha	Winter kg DM/ha	Total kg DM/ha
1973/74	-Nitrogen	3030	710	2300	1680	10530
	+Nitrogen	4160	850	2780	2080	12460
1974/75	-Nitrogen	3970	2570	2720	1800	10860
	+Nitrogen	4790	2810	3430	2140	13060
1975/76	-Nitrogen	4380	1960	1960	1890	
	+Nitrogen	5120	2370	2650	2430	
1976	-Nitrogen	1330*				
	+Nitrogen	1630				

*Part spring only.
DM Dry Matter.

Summary		kg DM/ha	Percentage Response to Nitrogen %	Percentage Response to Nitrogen kg DM/ha
Total all Springs	-N	12710		
	+N	15690	23.4	2980
Total all Summers	-N	5240		
	+N	6030	15.1	790
Total all Autumns	-N	6980		
	+N	8860	26.9	1880
Total all Winters	-N	5680		
	+N	8850	55.8	3170
Total all years	-N	30610		
	+N	39430	28.8	8820

with the exception of January.

The largest response during the three years, both in amount of dry matter increase and percentage increase to applied nitrogen, occurred in winter (table 7.1). A large response to applied nitrogen was also recorded in spring and autumn with only a small increase in pasture production over summer. The small response to fertiliser nitrogen over summer was most likely due to the moisture deficit which occurred in two of the three years reported as well as a higher relative soil nitrogen status. The overall response in pasture dry matter to applied nitrogen fertilisers from July 25, 1973 to October 27, 1976 was 28.8 percent (8,820 kg DM/ha) representing an increase of 7.7 kg DM/kg N applied. The efficiency of nitrogen fertiliser during the year may be calculated as follows:

	kg DM/kg N applied
Autumn	6.6
Winter	11.1
Spring	10.5
Summer	2.8

indicating that availability of nitrogen is a major limitation to pasture production during autumn, winter and spring. The increases in pasture dry matter per kg nitrogen applied are comparable to the range reported by O'Connor and Gregg (1971) of 8 - 12 kg DM/kg N applied for North Auckland trials, and the 7.8 - 12.7 kg DM/kg N applied reported for a spring application of nitrogen fertilisers in Northland by Steele (1976b).

7.3.0 A NON-TRACER STUDY OF NITROGEN FERTILISER TRANSFORMATIONS IN SOIL:EXPERIMENTAL PROCEDURE

7.3.1 Trial Design

A randomised block design trial (11 treatments by 4 replications) was laid down at both trial sites. Plot size was 3 x 2 m with a 1 m buffer strip between each plot. Basal fertiliser, 600 kg superphosphate (10% p)/ha was applied to each site in late March 1976, and an application of 1,500 kg lime/ha was applied to the Waimate North soil in April 1976. A further basal application of fertiliser was applied to both trial areas at the end of July 1976 as follows:

50 kg P/ha as $Ca(H_2PO_4)_2$

80 kg K/ha as KCl

58 kg S/ha as S

1.2 kg Cu/ha as $CuSO_4.5H_2O$

0.07 kg Mo/ha as $Na_2MoO_4.2H_2O$

0.85 kg B/ha as $Na_2B_4O_7.10H_2O$.

The pastures on both soils were predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The trial areas were closed from grazing and trimmed with a rotary mower to a height of 4.5 cm on August 11 for the Waimate North trial and August 13 for the Wharekohe trial. All clippings were removed. On September 1, both trial areas were retrimmed to a height of 4.5 cm, clippings removed and the following treatments applied:

1) Control

2) 50 kg N/ha as $Ca(NO_3)_2$

3) 100 kg N/ha as $Ca(NO_3)_2$

4) 200 kg N/ha as $Ca(NO_3)_2$

5) 50 kg N/ha as $(NH_4)_2SO_4$

6) 100 kg N/ha as $(NH_4)_2SO_4$

7) 200 kg N/ha as $(NH_4)_2SO_4$

8) 50 kg N/ha as $(NH_4)_2SO_4$ + "N-Serve"; 1.0 kg a.i./ha

9) 100 kg N/ha as $(NH_4)_2SO_4$ + "N-Serve"; 2.0 kg a.i./ha

10) 200 kg N/ha as $(NH_4)_2SO_4$ + "N Serve"; 4.0 kg a.i./ha

11) "N-Serve" only; 2.0 kg a.i./ha.

The "N-Serve" was supplied by Dow Chemical Co. as M-2728, nitrogen stabiliser concentrate for experimental purposes only. The analysis supplied was:

Active ingredients: 239.65 g/ℓ	
Nitropyrin 2-chlor-6-(trichloromethyl)pyridine	22.2%
Related chlorinated pyridines	2.5%
Inert ingredients	75.3%

The above analysis was not considered satisfactory for a compound supplied for experimental purposes.

The required amount of "N-Serve" was diluted with 50 ml acetone, mixed with the fertiliser and allowed to dry prior to field application. A discussion on this type of preparation is included in section 7.4.1.

All fertilisers were applied dry to plots by hand, while the "N-Serve" only treatment was sprayed on to the plots in 2 ℓ of water after being emulsified with teepol.

7.3.2 Sampling Procedures

Soil samples for determination of NH_4^+ and NO_3^- were collected, using a 2.5 cm diameter soil sampler, on a per plot basis for treatments one, three, six, nine and eleven. Depth of sampling was 0 - 5 and 5 - 15 cm on the Wharekohe soil and 0 -5, 5 - 15 and 15 - 30 cm on the Waimate North soil. Samples were collected one, three, five, seven, fourteen, twenty one and twenty eight days after application of fertiliser, ten

samples being collected per plot. All soil samples were frozen immediately after collection and stored frozen until analysed.

Dry matter production was measured twenty one, forty two, sixty three and eighty four days after application of treatments by cutting two strips from each plot to a height of 4.5 cm using a rotary motor mower (area harvested = 1/3,645 ha). Pasture harvested was weighed on a top-loading balance and the dry matter percentage determined by drying a 100 g subsample for twelve hours at 85°C.

Botanical composition was determined by dissecting a 50 g subsample, cut from the centre strip of each plot using hand shears, into grasses, clovers and weeds. Dissections were dried at 85°C for twelve hours, weighed for calculation of botanical composition, and stored for chemical analysis.

7.3.3 Chemical Analysis

7.3.3(a) Soil Inorganic Nitrogen

Soil inorganic nitrogen was extracted by shaking 10 g soil with 100 ml 2M KCl for sixty minutes. NH_4^+-N and NO_3^--N were determined on a Technicon autoanalyser (Brown, 1973; Kamphake et al. 1967) by Mr M.W. Brown, Ruakura Agricultural Research Centre, Hamilton.

7.3.3(b) Determination of Plant Chemical Analysis

Plant chemical analysis was determined by Ruakura Agricultural Research Centre, Hamilton. Magnesium was determined by the method of Allan (1958), potassium and calcium by the method of Clinton (1967), nitrogen by the method of Gehrke et al. (1972), and phosphorus by the method of Jackson (1958).

7.3.4 Recording of Climatic Data

A Climatological station controlled by Grasslands Division, D.S.I.R., Kaikohe, was situated adjacent to the Wharekohe trial area. All climatological observations were recorded at 0900 hours New Zealand standard time.

Rainfall was collected in a rain gauge of 12.70 cm diameter at the collecting rim, 30 cm above ground.

Relative humidity was calculated from means of dry and wet-bulb temperatures from thermometers exposed in a Stevenson screen. Maximum and minimum air temperature was recorded on a thermometer housed in a Stevenson screen.

Mean daily earth temperature (10 cm, 20 cm) at the observation time, was read from bent-stem thermometers with the bulb sunk into bare soil. Soil temperature (2 cm, 10 cm) was also recorded continuously on the trial site using a Japanese OTA -15 to +15^{o}C temperature recorder.

Total bright sunshine was measured with a Campbell-Stokes pattern sunshine recorder.

Wind speed was derived from daily reading of a rotary cup anemometer mounted 6 m above ground.

Evaporation was measured from a raised pan evaporimeter identical to the United States Weather Bureau Class A evaporimeter. The pan was a cylindrical tank 120.65 cm internal diameter and 25.4 cm deep on a stand 20.3 cm above ground.

At the Waimate North site, soil temperature was recorded at 2 cm and 10 cm continuously using a Japanese OTA -15 to +50^{o}C temperature recorder. Rainfall was measured daily using a rain gauge of 9.16 cm diameter at the collecting rim 10 cm above ground.

Climatological data for the duration of the experimental period are included in Appendix 3.

7.4.0 A NON-TRACER STUDY OF NITROGEN FERTILISER TRANSFORMATIONS: RESULTS AND DISCUSSION

7.4.1 Pasture Dry Matter Production

Pasture dry matter yield (tables 7.2 and 7.3) on both soils was increased by application of nitrogen in cuts one and two, was variable in cut three, while all treatments showed a depression in yield at cut four. A visual indication of yield response at the first cut on the Waimate North soil is presented in figure 7.9.

Application of $Ca(NO_3)_2$ caused severe leaf burn within twenty four hours of application. The degree of leaf burn was assessed visually on a scale of 0 - 3, results of which are reported in tables 7.4 and 7.5. A score of 0 indicates no leaf burn, and a score of 3 severe leaf burn. $(NH_4)_2SO_4$ also caused some minor leaf burn on the Waimate North trial at high rates of application and a more severe leaf burn on the Wharekohe trial. All symptoms of leaf burn had disappeared by the first dry matter harvest, so although leaf burn may adversely affect yields at the first cut, it should have had little effect on subsequent cuts. Leaf burn probably occurred because fertiliser was applied to moist herbage and the first rain of any consequence did not occur until two days after fertiliser application.

The increase in dry matter yield following application of NO_3^--N to the Wharekohe soil was small, only the 200 kg N/ha treatment showing a yield significantly greater than (P = 5%) the control. Neither of the 50 kg NH_4^+-N/ha treatments produced more than the control treatment in total yield but both showed a significant yield increase in cut one and a significant yield depression in cut three. The total yield of the 100 kg NH_4^+-N/ha treatments was not significantly better than the 50 kg N/ha treatments, but the 200 kg N/ha treatments were (P = 1%). Treatment

Table 7.2 Pasture dry matter yields following the application of nitrogen fertiliser to Wharekohe silt loam

Treatment	Cut 1 kg DM/ha	Cut 2 kg DM/ha	Cut 3 kg DM/ha	Cut 4 kg DM/ha	Total Yield kg DM/ha	Relative Yields
1	562.4 f* U	960.9 d S	860.2 a PQ	1008.3 a P	3391.8 cd RS	100
2	846.7 bc PQR	1023.3 cd S	741.9 c QR	917.5 ab PQ	3529.4 bcd RS	104
3	651.7 e T	983.8 d S	774.1 bc QR	873.3 ab PQ	3282.9 d S	97
4	928.0 a P	1318.3 b QR	727.9 c R	797.4 c R	3771.6 b QR	111
5	789.3 cd QR	1095.2 cd S	760.1 c QR	912.4 ab PQ	3557.0 bcd RS	105
6	810.6 bcd PQR	1351.1 b Q	725.6 c R	760.5 b Q	3647.8 bc RS	108
7	862.2 ab PQ	2021.8 a P	682.5 c R	793.4 b Q	4359.9 a P	129
8	821.6 bcd PQR	1143.3 c RS	720.8 c R	879.6 ab PQ	3565.3 bcd RS	105
9	684.8 e ST	1329.2 b QR	752.2 c QR	785.0 b Q	3551.2 bcd RS	105
10	754.1 d RS	1887.3 a P	706.8 c R	779.8 b Q	4128.0 a PQ	122
11	538.8 f U	946.1 d S	921.7 a P	984.8 a PQ	3391.4 cd RS	100

*Duncan's Multiple Range Test. Values followed by different lower case letters are significantly different at the 5% level of probability, and values followed by different upper case letters are significantly different at the 1% level of probability.

Table 7.3 Pasture dry matter yields following the application of nitrogen fertiliser to Waimate North clay loam

Treatment	Cut 1 kg DM/ha	Cut 2 kg DM/ha	Cut 3 kg DM/ha	Cut 4 kg DM/ha	Total Yield kg DM/ha	Relative Yields
1	640.4 e T	732.7 f T	1097.7 def RST	960.8 a P	3431.6 f V	100
2	935.7 c RS	1038.3 cde RS	1092.7 def RST	811.7 bc RS	3878.4 e TU	113
3	710.4 de ST	1061.7 cd RS	1272.0 ab PQ	860.9 b QR	3905.0 de STU	114
4	842.3 cd ST	1441.8 b Q	1340.3 a P	934.6 a PQ	4559.0 b QR	133
5	1155.6 b PQR	953.5 e S	1024.6 ef ST	821.5 bc RS	3955.2 de ST	155
6	1180.2 b PQR	1145.5 c R	1165.8 bcd QRS	780.6 cd RS	4272.1 c RS	124
7	1399.0 a PQ	1708.4 a P	1220.8 bc PQR	730.7 d S	5058.9 a P	147
8	1142.7 b QR	956.5 de S	1001.5 f T	818.9 bc RS	3919.6 de STU	114
9	1247.0 ab PQ	1071.3 c RS	1080.1 def RST	777.6 cd RS	4176.0 cd ST	121
10	1408.7 a P	1531.6 b Q	1165.5 bcd QRS	740.8 d S	4846.6 a PQ	141
11	687.2 de ST	780.3 f T	1127.5 cde QRST	950.4 a PQ	3545.4 f UV	103

*Duncan's Multiple Range Test. Values followed by different lower case letters are significantly different at the 5% level of probability, and values followed by different upper case letters are significantly different at the 1% level of probability.

Table 7.4 Visual assessment of leaf burn twenty four hours after application of nitrogen fertiliser to Wharekohe silt loam

Treatment	Burn Index *				
	Replication 1	Replication 2	Replication 3	Replication 4	$\bar{X}$
1	0	0	0	0	0
2	2	2	3	1	2
3	3	3	1	3	2.5
4	1	2	2	4	2.25
5	1	0	0	0	0.25
6	2	2	1	1	1.5
7	3	2	3	2	2.5
8	0	0	0	0	0
9	2	2	2	1	1.75
10	3	3	3	2	2.75
11	0	0	0	0	0

* 0 No visual symptoms of leaf burn.
3 Severe leaf burn.

Table 7.5 Visual assessment of leaf burn twenty four hours after application of nitrogen fertiliser to Waimate North clay loam

Treatment	Burn Index *				
	Replication 1	Replication 2	Replication 3	Replication 4	$\bar{X}$
1	0	0	0	0	0
2	2	2	3	3	2.5
3	3	3	1	3	2.5
4	3	3	2	2	2.5
5	0	0	0	0	0
6	0	0	2	1	0.75
7	0	1	0	1	0.5
8	0	0	0	0	0
9	1	0	0	0	0.25
10	1	1	1	0	0.75
11	0	0	0	0	0

* 0 No visual symptoms of leaf burn.
3 Severe leaf burn.

Figure 7.9 Waimate North trial area twenty-one days after application of treatments. Plot a lower left is treatment 1 (control) and plot at lower right is treatment 7 (200 kg N/ha as $(NH_4)_2SO_4$).

of $(NH_4)_2SO_4$ with "N-Serve" had no significant effect on yield.

Total yield response to $Ca(NO_3)_2$ was dependent on the rate of application to the Waimate North soil, the 200 kg N/ha application being significantly better than 100 kg N/ha which was not significantly better than the 50 kg N/ha treatment, but the latter two treatments were significantly better than the control. The dry matter response to application of $(NH_4)_2SO_4$ was directly dependent on the rate of application, each application rate being significantly different from all the other application rates. Treatment of $(NH_4)_2SO_4$ with "N-Serve" had no affect on dry matter production.

It is likely that a large proportion of the "N-Serve" was lost by volatilisation both during preparation of the fertiliser and following application of fertiliser to the soil. Briggs (1975) found that when nitrogen fertiliser was coated with "N-Serve", twenty five percent of the "N-Serve" was lost overnight when the fertiliser was left standing in a dish under a plastic sheet. Following surface application to a dry soil only eight percent remained three days after application. Because of the low water solubility and volatile nature of "N-Serve" it would appear to have little application in New Zealand grassland farming where application of nitrogen fertilisers is normally made to the soil surface. Movement of NH_4^+ from bands of "N-Serve" treated $(NH_4)_2SO_4$ is more rapid than the movement of "N-Serve" (Briggs, 1975), therefore, NH_4^+ may be expected to rapidly move outside the inhibitory influence of "N-Serve" as it is washed into the soil. A further problem with the use of "N-Serve" in New Zealand grassland soils is that its effectiveness is reduced by high organic carbon contents (Lewis and Stefanson, 1975; Redeman et al. 1964). However, further evaluation of the product appears warranted in cropping systems where fertiliser is placed in the soil. Some inhibition of nitrification by "N-Serve" in

New Zealand soil was reported by Goh and Young (1975) in a pot experiment with wheat, although inhibition was incomplete.

When discussing dry matter responses of pasture to nitrogen fertiliser, some consideration should be given to variation in seasonal climate between years. Data from table 7.1 suggests that for spring applications of nitrogen, the dry matter response decreases as pasture production in the absence of nitrogen increases. This is illustrated by the following data from table 7.1:

Year	Total Pasture Production Without Added Nitrogen (kg DM/ha)	Extra Pasture Production With Added Nitrogen (kg DM/ha)
1973	3030	1130
1974	3970	820
1975	4380	740

A direct comparison between rates of pasture growth during the trial period and previous years is difficult on the Wharekohe soil since the data for previous years comes from an adjacent area which is likely to be higher in fertility than the trial area. However, the pattern of pasture growth appears similar (figure 7.10). Direct comparison is possible on the Waimate North soil where the rate of pasture growth in the trial period appears low during the September/early October period, and high during the later October/November period.

Climate data summarised in table 7.6 (figures 7.11 and 7.12) shows that October had more rain days than normal and 70 mm more rain than the average for 1922-1969. Maximum and minimum air temperatures were also low. During October, rainfall was nearer average but maximum and minimum temperatures were low. November was wetter than average and

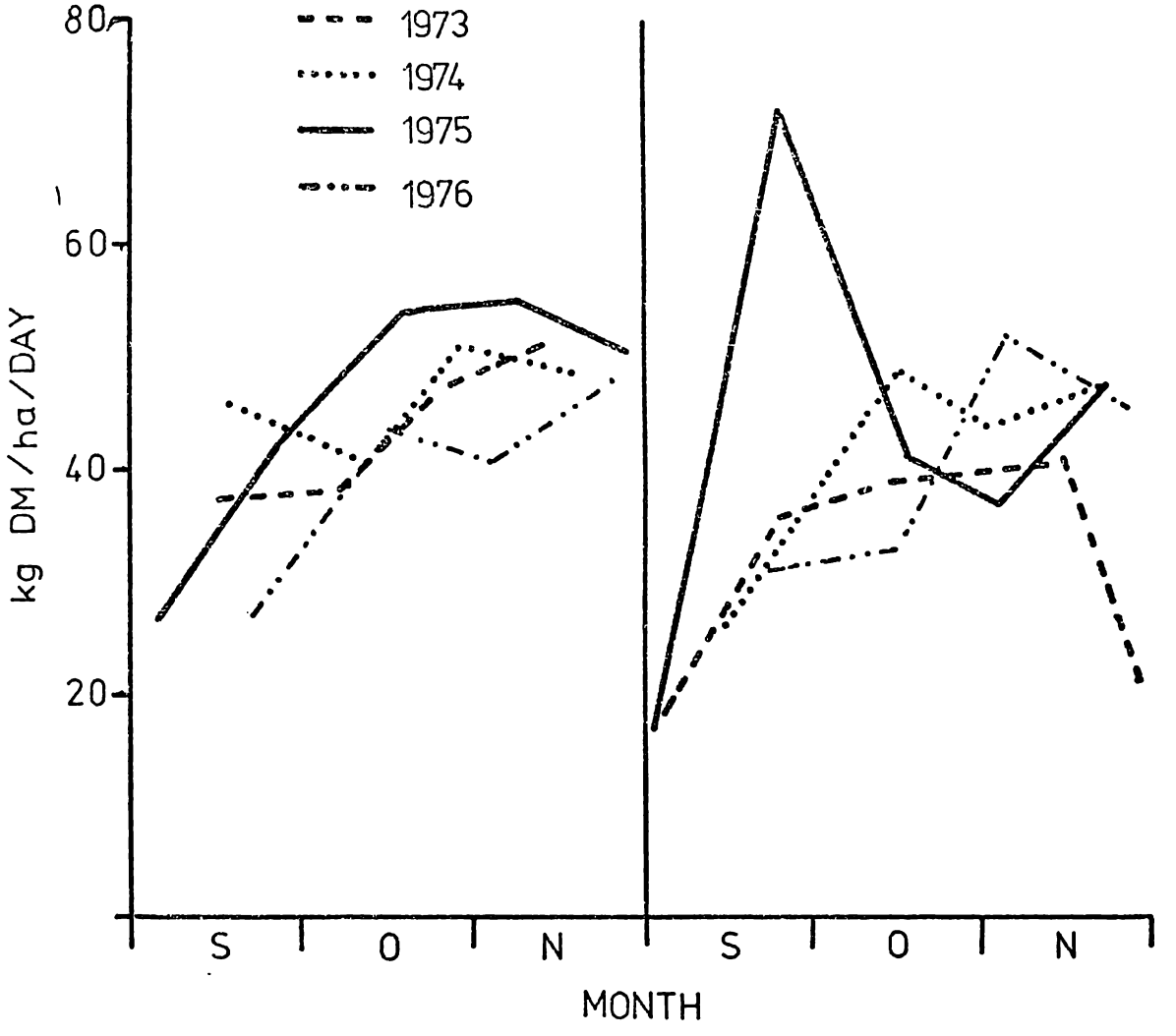

Figure 7.10 Rate of pasture growth on Wharekohe silt loam and Waimate North clay loam during four consecutive spring periods

Table 7.6 Summary of climate data for September, October and November over a 4 year period at the Wharekohe trial site

Month	Year	Highest Rain (mm)	Rain Days 0.1 mm	Wet Days 1.0 mm	Total Rainfall	Mean Temp Max.	Mean Temp Min.	Total Sunshine	Total Wind km	Total Evaporation
Sep	1973	53.4	17	13	271.4	16.0	8.3	112.3	11028	137.5
	1974	21.9	20	13	96.2	16.2	9.1	152.4	5926	79.1
	1975	16.9	20	16	122.9	15.9	9.6	124.0	6576	57.6
	1976	41.0	24	19	204.0	14.8	7.9	130.3	11340	70.0
	Mean	33.3	20	15	173.6	15.7	8.7	129.8	8718	86.1
Oct	1973	38.7	21	19	277.7	17.5	11.1	109.9	11311	95.2
	1974	28.1	21	11	98.7	17.3	10.0	159.0	6971	106.2
	1975	17.2	12	8	63.4	17.3	10.4	163.1	6630	114.8
	1976	37.8	19	17	145.6	16.4	9.4	171.3	11474	109.4
	Mean	30.5	18	14	146.4	17.1	10.2	150.8	7528	425.6
Nov	1973	32.7	11	8	119.6	19.5	11.5	168.2	9003	128.7
	1974	25.3	15	10	120.8	19.7	12.2	165.9	7561	121.0
	1975	18.5	15	9	61.3	20.5	12.5	152.8	10764	123.5
	1976	41.5	22	8	191.0	17.4	10.4	188.3	11163	118.8
	Mean	29.5	16	9	123.2	19.3	11.7	161.3	9623	123.0
Mean 1922 - 1969										
Sep					134					
Oct					125					
Nov					99					

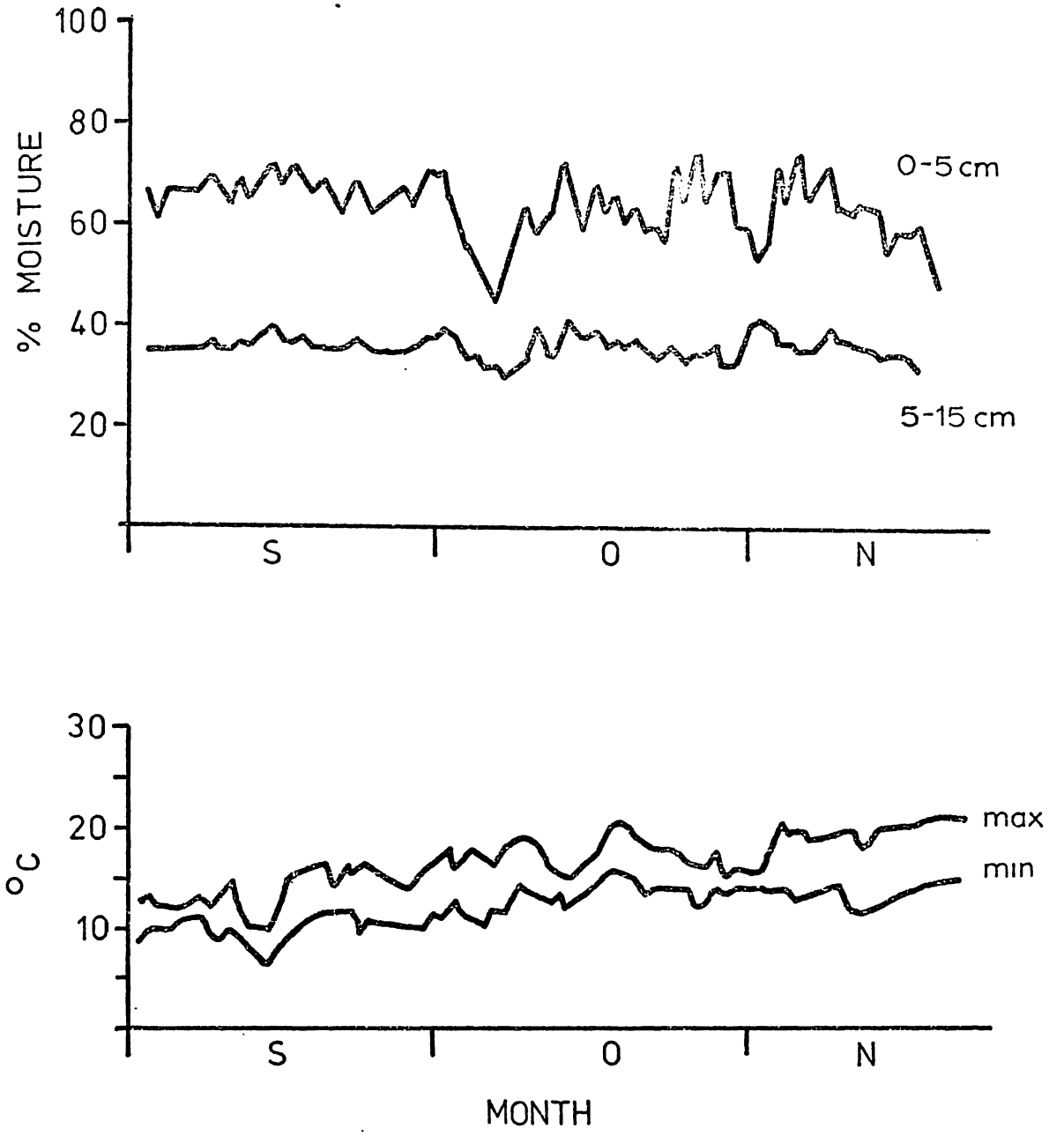

Figure 7.11 Soil moisture (0 - 5 cm; 5 - 15 cm) and maximum and minimum soil temperatures (2.5 cm depth) for Wharekohe silt loam

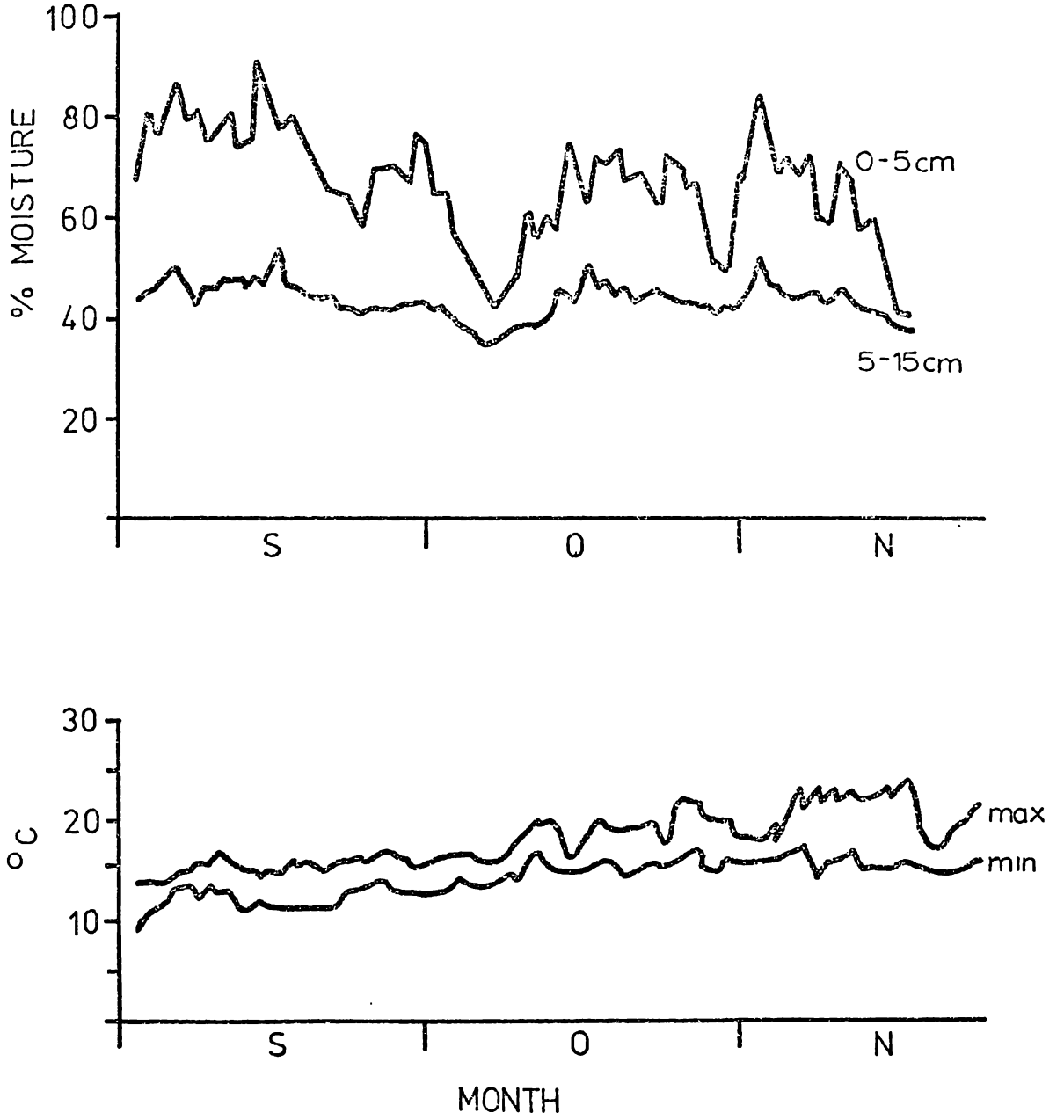

Figure 7.12 Soil moisture (0 - 5 cm; 5 - 15 cm) and maximum and minimum soil temperatures (2.5 cm depth) for Waimate North clay loam

temperatures were below average.

The lower rate of pasture growth during early spring in the trial period relative to other years may be a consequence of the lower temperatures and higher rainfall. The total rainfall of 540.6 mm over the experimental period was 182.6 mm (51 percent) above the 1922-1969 average.

Evapotranspiration losses can be calculated from measured evaporation from an open water surface and subtracted from precipitation data to give excess precipitation which is available for movement of nitrogen through the soil (Fitzgerald and Richard, 1960). Calculated excess precipitation was 162, 103.6, and 119.8 $\frac{mm}{h}$ for October, November and December respectively.

7.4.2 Plant Recovery of Applied Nitrogen

The apparent recovery of applied nitrogen in pasture herbage was calculated as follows:

$$\text{Apparent \% Recovery} = \frac{\text{N content of treated herbage} - \text{N content of control herbage}}{\text{N Applied}} \times 100$$

The term apparent is used because the technique measures only the nett change in nitrogen uptake by plants and gives no assessment of the complex transformations occurring in the soil which result in the nett change observed. In future discussions, this method of estimating the apparent recovery of fertiliser nitrogen will be referred to as the difference technique.

Calculation of recovery of fertiliser nitrogen by the above method assumes that addition of nitrogen to soils has no affect on the organic pool of nitrogen. Estimates of plant recovery of fertiliser nitrogen by the difference technique are normally higher than equivalent estimates

based on tracer techniques (Janson, 1971). This difference is known as the priming effect for which three explanations have been proposed.

1) Addition of fertiliser nitrogen increases the mineralisation of soil organic nitrogen (Broadbent, 1965; Andreeva and Shcheglova, 1968; Legg and Standford, 1967; Sapozhnikov et al. 1968), possibly because of osmotic effects of the added fertiliser (e.g. Broadbent and Nakashima, 1971).
2) The difference is a feature of the mineralisation-immobilisation turnover in the soil and as such is more apparent than real (Jansson, 1958; Stewart et al. 1963; Nommik, 1968).
3) Fertiliser nitrogen stimulates plants to penetrate an increased soil volume, and therefore reach more non-tagged soil nitrogen (Aleksic et al. 1968).

Jansson (1971) concluded that present evidence supports the second possibility as the most likely explanation, firstly because no well definied theoretical or experimental evidence has been presented to support the first possibility, and secondly, the priming effect is normally larger with ammonium than nitrate fertilisers. This latter observation supports the second hypothesis (loc. cit) since NH_4^+ as opposed to NO_3^-, is preferentially drawn into the organic phase of the turnover system and may be replaced by the mineralisation outflow of non-tagged nitrogen which becomes available for crop uptake (Jansson, 1971).

The third suggestion requires further investigation since Sapozhnikov et al. (1968) demonstrated, using a split root technique, that fertilised plants increased their utilisation of soil nitrogen even when there was no contact between fertiliser and soil.

It is important to stress that no matter which of the three hypotheses is correct, the overall effect of an application of fertiliser

nitrogen on plant uptake can only be estimated by the non-tracer difference technique. For this reason, control plots not receiving fertiliser nitrogen should be included in tracer experiments.

The use of the difference technique where non-leguminous crops are concerned is dependent largely on the one assumption that addition of nitrogen has no effect on the organic pool of soil nitrogen. Utilisation of this technique in systems which include leguminous species requires an additional assumption, that application of fertiliser nitrogen has no effect on the fixation of nitrogen by legumes. Such an assumption is unlikely to hold when nitrogen fertiliser is added to New Zealand pastures which normally contain a large percentage of white clover, especially if a reduction in the clover content of the sward is induced. In these circumstances, although the overall effect of nitrogen fertiliser on plant uptake can again only be estimated by the difference technique, tracer techniques may provide a better estimation of actual plant uptake of fertiliser nitrogen.

All treatments on the Wharekohe soil showed an increased nitrogen uptake in cuts one and two with the exception of treatment three in the latter cut (table 7.8). All fertiliser treatments showed a depressed nitrogen uptake in the third and fourth cuts. A similar pattern was observed on the Waimate North soil (table 7.9), all treatments showing increased nitrogen uptake in cuts one and two, and treatments three, four and seven showing an increased nitrogen uptake in cut three. All treatments showed a depressed nitrogen uptake in the final cut. The total effect on nitrogen uptake over the twelve week experimental period was that treatments two and three on the Wharekohe soil showed a depression in nitrogen uptake, while all treatments on the Waimate North soil showed an increased nitrogen uptake. The apparent recovery of fertiliser nitrogen was higher on the Waimate North soil for all treatments.

Table 7.7 Apparent recovery of nitrogen fertiliser by pasture on Waimate North clay loam

Treatment	Percentage of Applied Fertiliser in Pasture Herbage				
	Cut 1	Cut 2	Cut 3	Cut 4	Total
2	18.5	19.1	-10.5	-19.4	7.7
3	4.0	17.0	8.5	- 9.9	19.6
4	4.9	17.2	3.5	- 1.8	23.8
5	29.9	22.1	-15.1	-18.0	18.9
6	29.4	18.0	- 2.9	-11.9	32.6
7	19.5	28.3	1.0	- 8.2	40.6
8	40.6	11.4	-19.6	-17.9	14.5
9	30.3	17.4	- 7.3	-13.7	26.7
10	23.6	18.7	- 0.1	- 8.7	33.5

Table 7.8 Apparent recovery of nitrogen fertiliser by pasture on Wharekohe silt loam

Treatment	Percentage of Applied Fertiliser in Pasture Herbage				
	Cut 1	Cut 2	Cut 3	Cut 4	Total
2	13.6	2.6	-18.9	- 0.5	- 3.2
3	4.3	- 8.6	- 8.1	- 6.5	-18.9
4	10.6	9.7	- 6.0	- 4.2	10.1
5	11.2	13.5	-14.1	- 0.1	10.5
6	5.9	25.8	-12.4	-11.0	8.3
7	4.6	38.3	-6.2	- 6.9	29.8
8	29.3	0.3	-16.6	- 3.6	9.4
9	11.3	16.3	- 9.1	-10.9	7.6
10	4.7	33.7	- 5.9	- 7.1	25.4

Some insight into the pattern of nitrogen uptake by the pasture may be gained by considering nitrogen uptake of legume and non-legume components separately. On the Wharekohe soil, uptake of nitrogen by non-legumes was higher on fertiliser treatments than on the control for cuts one to three, with the exception of treatment eight which showed similar nitrogen uptake to the control in the third cut. In cut four, uptake of nitrogen by non-legumes on all fertiliser treatments was lower than on the control (table 7.9). On the Waimate North soil, uptake of nitrogen by treatments two, three, four, six, seven, nine and ten was greater than that of the control in all four cuts, the remaining treatments showing a small depression in yield in the fourth cut (table 7.10).

Application of fertiliser nitrogen depressed the total nitrogen yield of legumes over the twelve week period on both soils (table 7.9; 7.10). As will be discussed in section 7.5.4, the lower uptake of nitrogen by clovers can be largely attributed to a change in botanical composition, both depression of nitrogen uptake and reduction of clover yield being least in the 50 kg N/ha treatments.

It may be assumed that non-legume nitrogen is derived entirely from soil nitrogen, whereas legume nitrogen may be obtained from symbiotic nitrogen fixation as well as by uptake of inorganic soil nitrogen. The reduced contribution of clover nitrogen to the total nitrogen harvested, therefore indicates a reduced input of fixed nitrogen. The overall depression of nitrogen uptake found in some treatments is therefore likely to be a reflection of a reduction in nitrogen fixation in the pasture rather than indicating uptake of nitrogen fertiliser.

Estimations of apparent recovery of fertiliser nitrogen by grasses using the difference technique normally lie in the range of twenty five to eighty five percent (Reid and Castle, 1965; Cowling and Lockyer, 1967) whereas similar estimations for mixed swards are generally lower, and

Table 7.9 Total amount of nitrogen in legume and non-legume pasture herbage on Wharekohe silt loam

Treatment	Cut 1		Cut 2	
	Legume N	Non-Legume N	Legume N	Non-Legume N
1	9.75	14.91	24.90	20.07
2	4.18	27.29	13.64	32.63
3	5.79	23.12	9.50	26.83
4	14.94	30.94	13.07	51.37
5	11.08	19.19	14.36	37.29
6	6.40	24.17	11.86	58.87
7	3.57	30.30	8.63	113.07
8	12.16	27.17	14.27	30.85
9	5.68	30.32	13.76	36.81
10	4.44	29.61	3.43	108.94
11	9.09	11.17	22.74	19.16

Treatment	Cut 3		Cut 4	
	Legume N	Non-Legume N	Legume N	Non-Legume N
1	21.33	15.97	18.95	18.48
2	9.23	18.60	21.71	15.49
3	9.32	19.83	14.28	16.70
4	3.42	21.79	13.72	15.32
5	11.82	18.44	22.47	14.89
6	5.85	19.02	11.36	15.12
7	1.83	22.99	6.08	17.58
8	13.98	15.04	22.09	13.56
9	9.31	18.90	11.68	14.80
10	2.58	22.86	5.57	17.61
11	20.96	19.11	25.55	15.29

Treatment	Total	
	Legume N	Non-Legume N
1	74.93	69.43
2	48.76	94.01
3	38.89	86.48
4	45.15	119.42
5	59.73	89.81
6	35.47	117.18
7	20.11	183.94
8	62.50	86.62
9	40.43	100.83
10	16.02	179.02
11	78.34	64.73

Table 7.10 Total amount of nitrogen in legume and non-legume pasture herbage on Waimate North clay loam

Treatment	Cut 1		Cut 2	
	Legume N	Non-Legume N	Legume N	Non-Legume N
1	11.95	16.99	14.99	13.95
2	12.82	25.65	9.11	29.36
3	3.81	42.20	10.45	35.56
4	5.98	57.33	11.97	51.34
5	13.78	26.21	14.88	25.11
6	8.49	38.51	8.80	38.20
7	5.97	79.62	11.75	73.84
8	17.40	17.27	12.27	22.40
9	8.29	25.01	6.40	39.97
10	4.97	61.40	7.48	58.89
11	16.74	15.50	14.64	17.60

Treatment	Cut 3		Cut 4	
	Legume N	Non-Legume N	Legume N	Non-Legume N
1	22.80	22.00	25.71	17.61
2	14.62	24.95	15.64	17.95
3	7.28	46.01	8.46	24.94
4	10.90	41.03	10.62	29.05
5	14.47	22.78	17.29	17.02
6	10.79	31.14	12.86	18.51
7	4.14	42.75	6.83	20.04
8	10.67	24.32	18.63	15.73
9	6.70	30.80	10.71	18.94
10	7.14	37.55	7.77	18.09
11	23.68	23.32	19.53	20.40

Treatment	Total	
	Legume N	Non-Legume N
1	75.45	70.55
2	52.19	97.91
3	30.00	148.71
4	39.47	178.75
5	60.42	91.12
6	40.94	126.36
7	28.69	216.25
8	58.97	79.72
9	32.10	114.72
10	27.36	176.17
11	74.59	76.82

sometimes negative. Cowling (1961) applied four rates of nitrogen fertiliser (0, 39, 118, 235 kg N/ha) to grasses alone and to mixed grass/clover pasture. The apparent recovery of nitrogen by grass alone ranged from fifty four percent at the lowest level of nitrogen to eighty percent at the highest. Where nitrogen was added to mixed swards, the apparent recovery was negative for the low and medium rates of application and nineteen percent at the highest level. When the apparent recovery of nitrogen by a mixed sward was calculated on the nitrogen yield of the grass fraction alone, the recovery of fertiliser nitrogen was forty percent, forty eight percent and fifty nine percent for the increasing levels of nitrogen. Chestnutt (1972) calculated the average apparent recovery of five annual applications of 201 kg N/ha to grass or grass/ clover swards was eighty one percent and twenty four percent respectively.

It appears, therefore, that following application of nitrogen to a mixed grass/clover sward, although the difference technique will accurately assess the overall effect of an application of fertiliser nitrogen on plant uptake, it provides no estimate of the uptake of fertiliser nitrogen. It is possible, however, that calculation of the nitrogen yield of the grass fraction alone may give a reasonable estimate of fertiliser nitrogen uptake. As outlined above, Cowling (1961) estimated the apparent recovery of fertiliser nitrogen by a mixed grass/clover sward by measuring the nitrogen uptake of both grass and clover or grass alone. Using the former calculation, recovery of nitrogen fertiliser was low or negative whereas with the latter calculation recoveries were higher but still fourteen to twenty one percent below the recoveries by grass grown alone. If it is assumed that losses from grass pastures and grass/ clover pastures are similar, then similar recoveries may be expected in both systems. Therefore it appears that calculations based on nitrogen uptake by grass only in a grass/clover sward do not reflect uptake of fertiliser nitrogen.

Apparent recovery of nitrogen by grass/clover (method 1) is compared with that of grass alone (method 2) in table 7.11. As with Cowling's data, recoveries by the latter method are considerably higher than the former. Whether or not either of these methods reflect uptake of nitrogen fertiliser by the pasture will be discussed in section 7.6.1 in comparison with ^{15}N uptake data. If the latter method is to accurately estimate the uptake of fertiliser nitrogen, uptake of fertiliser nitrogen by clover must be small relative to the assimilation by the associated grasses. Such a condition may hold under some conditions since Walker et al. (1956) found that when grass and white clover were grown together in pots, the clover absorbed only five to six percent of applied nitrogen over a wide range of application rates. Also, if application of nitrogen severely depresses clover yield, as occurred in the present study, the yield of nitrogen in clover herbage will represent only a small fraction of the total nitrogen yield of herbage.

The difference technique will only accurately estimate fertiliser recovery in the herbage of a mixed grass/clover pasture, when the clover component of the pasture is small, or when there is no change in botanical composition following fertiliser application.

7.4.3 Major Nutrients in Plant Herbage

All levels of major nutrients in ryegrass and clover herbage were adequate for healthy pasture growth (McNaught, 1969, tables 7.12, 7.13, 7.14, 7.15).

As nitrogen is taken up by plants in larger amounts than any other element, the form in which it is taken up, i.e. NH_4^+ or NO_3^-, may be expected to have some effect on the overall cation-anion balance within the plant. If both NH_4^+ and NO_3^- are available for plant uptake, a relationship between percentage nitrogen and anion content in ryegrass

Table 7.11 Apparent recovery of fertiliser nitrogen by pasture, calculated from nitrogen uptake by grass and clover (method 1) and by grass alone (method 2)

Wharekohe	Percentage Recovery of Applied Fertiliser	
Treatment	Method 1	Method 2
2	-3.2	49.2
3	-18.9	17.1
4	10.1	25.0
5	10.5	40.8
6	8.3	47.8
7	29.8	57.3
8	9.4	34.4
9	7.6	30.6
10	25.4	54.8
Waimate		
Treatment	Method 1	Method 2
2	7.7	54.7
3	19.6	78.2
4	23.8	54.1
5	18.9	41.1
6	32.6	55.8
7	40.6	75.7
8	14.5	18.3
9	26.7	44.2
10	33.5	52.8

Table 7.12 Chemical composition of ryegrass *(Lolium perenne)* herbage grown on Wharekohe silt loam with and without added nitrogen

Treatment	Cut 1						Cut 2					
	%N	%P	%Mg	%Ca	%Na	%K	%N	%P	%Mg	%Ca	%Na	%K
1	3.78	0.617	0.176	0.47	0.07	3.60	4.08	0.577	0.154	0.40	0.13	3.37
2	3.45	0.600	0.185	0.47	0.10	3.79	4.38	0.606	0.181	0.49	0.13	3.69
3	4.21	0.546	0.178	0.49	0.13	3.70	3.31	0.549	0.156	0.43	0.06	3.84
4	4.25	0.569	0.150	0.40	0.06	4.00	4.70	0.581	0.175	0.47	0.11	3.89
5	3.20	0.599	0.176	0.45	0.10	3.65	4.40	0.634	0.183	0.45	0.13	3.78
6	3.48	0.623	0.154	0.38	0.07	3.61	5.12	0.661	0.180	0.39	0.13	3.69
7	3.82	0.603	0.146	0.36	0.08	3.84	6.02	0.665	0.182	0.34	0.09	3.80
8	4.59	0.647	0.171	0.44	0.11	3.76	3.50	0.647	0.157	0.43	0.08	3.68
9	5.18	0.619	0.170	0.32	0.11	3.42	4.42	0.540	0.152	0.47	0.19	2.93
10	4.42	0.616	0.156	0.36	0.08	3.74	6.00	0.699	0.191	0.43	0.11	3.80
11	3.12	0.630	0.165	0.42	0.07	3.86	3.62	0.609	0.151	0.43	0.13	3.33
	Cut 3						Cut 4					
1	3.69	0.584	0.190	0.52	0.10	3.46	3.00	0.480	0.170	0.54	0.09	3.15
2	3.38	0.567	0.177	0.49	0.09	3.22	3.15	0.495	0.185	0.58	0.09	3.09
3	3.28	0.582	0.184	0.53	0.09	3.48	2.80	0.501	0.173	0.55	0.07	3.00
4	3.31	0.566	0.170	0.49	0.10	3.41	2.83	0.484	0.157	0.51	0.08	2.92
5	3.52	0.572	0.181	0.53	0.09	3.29	3.00	0.451	0.169	0.52	0.09	2.85
6	3.15	0.630	0.161	0.48	0.11	3.51	2.80	0.501	0.170	0.49	0.08	3.09
7	3.55	0.624	0.159	0.49	0.11	3.66	2.62	0.451	0.165	0.49	0.09	3.18
8	3.41	0.604	0.162	0.48	0.09	3.29	3.00	0.582	0.180	0.55	0.10	3.12
9	3.39	0.580	0.156	0.48	0.10	3.25	2.67	0.493	0.163	0.48	0.10	2.90
10	3.50	0.575	0.148	0.47	0.13	3.33	2.63	0.506	0.169	0.54	0.11	2.96
11	3.75	0.630	0.159	0.48	0.08	3.47	3.08	0.531	0.169	0.49	0.07	3.05

Table 7.13 Chemical composition of white clover *(Trifolium repens)* herbage grown on Wharekohe silt loam with and without added nitrogen

Treatment	Cut 1						Cut 2					
	%N	%P	%Mg	%Ca	%Na	%K	%N	%P	%Mg	%Ca	%Na	%K
1	5.40	0.470	0.188	0.99	0.21	2.67	5.32	0.500	0.170	0.90	0.15	2.72
2	5.88	0.560	0.185	1.07	0.21	3.02	4.90	0.462	0.173	1.12	0.31	2.68
3	5.52	0.448	0.186	1.03	0.20	2.91	5.49	0.577	0.185	1.10	0.15	3.19
4	5.28	0.496	0.170	0.23	0.13	2.92	5.80	0.602	0.179	0.19	0.13	3.41
5	5.22	0.498	0.171	0.91	0.14	2.65	5.80	0.597	0.191	0.97	0.14	3.26
6	5.53	0.557	0.178	0.99	0.13	2.83	5.89	0.625	0.188	1.09	0.13	3.32
7	5.18	0.580	0.170	0.79	0.18	3.15	6.01	0.684	0.171	1.13	0.13	3.14
8	5.40	0.491	0.177	1.06	0.13	2.75	5.45	0.602	0.187	1.12	0.13	3.27
9	5.72	0.556	0.174	0.91	0.02	2.99	5.45	0.609	0.169	1.09	0.19	2.96
10	5.40	0.713	0.186	1.12	0.13	3.15	4.78	0.531				
11	4.92	0.453	0.183	1.02	0.15	2.75						
	Cut 3						Cut 4					
1	4.99	0.454	0.201	1.08	0.17	2.59	4.82	0.417	0.219	1.16	0.14	2.67
2	4.82	0.447	0.188	1.22	0.18	2.70	5.10	0.393	0.199	1.13	0.17	2.54
3	5.50	0.420	0.197	1.00	0.11	2.62	5.16	0.370	0.192	1.03	0.12	2.67
4	4.90	0.453	0.193	1.00	0.12	2.78	5.36	0.375	0.186	1.09	0.12	2.88
5	5.00	0.487	0.192	1.00	0.11	2.63	5.40	0.377	0.203	1.09	0.13	2.66
6	4.80	0.540	0.179	1.02	0.10	2.48	5.15	0.434	0.186	1.14	0.15	2.39
7	5.27	0.556	0.188	0.86	0.10	2.76	4.98	0.388	0.170	1.07	0.17	2.37
8	5.05	0.552	0.179	0.98	0.10	2.57	5.36	0.400	0.183	1.14	0.14	2.36
9	4.78	0.508	0.176	1.00	0.19	2.51	5.06	0.390	0.190	1.17	0.13	2.49
10	4.80	0.491	0.162	0.87	0.11	2.50	5.07	0.393	0.196	1.11	0.16	2.42
11	5.11	0.416	0.182	0.97	0.13	2.48	5.23	0.380	0.189	1.09	0.15	2.51

Table 7.14 Chemical composition of perennial ryegrass (*Lolium perenne*) herbage grown on Waimate North clay loam with and without added nitrogen

Treatment	%N	%P	%Mg	%Ca	%Na	%K	%N	%P	%Mg	%Ca	%Na	%K
1	3.62	0.517	0.147	0.47	0.22	2.94	3.20	0.611	0.146	0.39	0.07	3.75
2	3.48	0.410	0.160	0.50	0.37	2.90	3.50	0.483	0.151	0.47	0.29	3.21
3	4.35	0.446	0.199	0.64	0.48	2.61	4.08	0.474	0.156	0.52	0.27	3.93
4	4.25	0.416	0.159	0.47	0.37	3.08	4.22	0.476	0.146	0.51	0.18	3.09
5	3.19	0.473	0.164	0.50	0.29	2.73	3.70	0.528	0.131	0.39	0.18	2.88
6	4.72	0.467	0.151	0.48	0.26	3.08	3.90	0.450	0.159	0.47	0.41	2.78
7	4.25	0.394	0.202	0.56	0.39	2.96	4.98	0.471	0.169	0.48	0.19	3.30
8	3.51	0.490	0.150	0.46	0.19	2.81	3.09	0.496	0.170	0.47	0.28	2.64
9	3.50	0.406	0.156	0.46	0.34	2.47	4.22	0.452	0.144	0.41	0.28	2.85
10	5.30	0.512	0.155	0.47	0.27	2.74	4.22	0.410	0.189	0.56	0.42	2.43
11	3.78	0.483	0.148	0.46	0.17	3.08	3.49	0.529	0.156	0.49	0.20	2.83
			Cut 3						Cut 4			
1	3.28	0.522	0.168	0.50	0.18	2.84	3.60	0.459	0.200	0.62	0.32	2.59
2	3.00	0.440	0.146	0.42	0.21	2.75	3.34	0.440	0.188	0.56	0.33	2.52
3	4.05	0.410	0.187	0.48	0.39	2.31	3.52	0.399	0.189	0.59	0.32	2.45
4	3.61	0.403	0.163	0.50	0.31	2.35	3.90	0.399	0.173	0.54	0.21	2.84
5	3.02	0.502	0.163	0.48	0.29	2.45	3.28	0.470	0.190	0.57	0.25	2.43
6	3.23	0.445	0.146	0.43	0.23	2.88	3.33	0.444	0.197	0.62	0.37	2.41
7	3.71	0.390	0.171	0.52	0.21	2.76	3.31	0.400	0.199	0.63	0.37	2.38
8	3.08	0.472	0.158	0.46	0.27	2.31	3.29	0.486	0.197	0.62	0.22	2.58
9	3.23	0.424	0.154	0.46	0.24	2.63	3.23	0.441	0.176	0.56	0.24	2.61
10	3.52	0.390	0.178	0.56	0.41	2.28	3.00	0.430	0.194	0.60	0.39	2.13
11	3.50	0.488	0.150	0.43	0.22	2.70	3.38	0.444	0.192	0.56	0.31	2.61

Table 7.15 Chemical composition of white clover (*Trifolium repens*) herbage grown on Waimate North clay loam with and without added nitrogen

Treatment	Cut 1						Cut 2					
	%N	%P	%Mg	%Ca	%Na	%K	%N	%P	%Mg	%Ca	%Na	%K
1	5.52	0.450	0.199	1.24	0.17	2.75	5.05	0.467	0.164	1.07	0.17	3.78
2	5.25	0.467	0.187	1.09	0.19	2.98	4.57	0.563	0.182	1.24	0.17	3.18
3	5.59	0.498	0.188	1.19	0.17	3.63	5.50	0.527	0.173	1.29	0.16	2.87
4	5.92	0.507	0.204	1.17	0.13	3.15	5.32	0.552	0.172	1.21	0.10	3.02
5	5.42	0.431	0.189	1.19	0.13	2.68	5.42	0.704	0.190	1.27	0.13	2.78
6	5.62	0.519	0.182	1.22	0.17	2.69	5.30	0.485	0.169	1.09	0.17	2.54
7	5.08	0.500	0.220	1.18	0.14	2.82	5.21	0.517	0.192	1.33	0.13	2.83
8	6.32	0.460	0.205	1.21	0.16	2.65	5.30	0.530	0.178	0.99	0.13	2.77
9	5.88	0.550	0.176	1.14	0.15	2.87	5.15	0.504	0.193	1.11	0.17	2.62
10	5.60	0.570	0.186	0.96	0.15	2.73	5.49	0.543	0.180	1.10	0.12	2.80
11	5.69	0.518	0.183	1.09	0.19	2.82	5.30	0.434	0.174	1.05	0.15	2.84
			Cut 3						Cut 4			
1	5.34	0.462	0.216	1.08	0.15	2.82	5.45	0.438	0.219	1.30	0.24	2.33
2	5.60	0.446	0.197	1.19	0.17	2.73	5.70	0.413	0.193	1.28	0.25	2.30
3	5.35	0.463	0.198	1.18	0.18	2.55	5.55	0.397	0.191	1.25	0.22	2.23
4	5.35	0.487	0.193	1.22	0.16	2.51	5.60	0.404	0.220	1.49	0.14	2.65
5	5.35	0.438	0.205	1.29	0.19	2.27	5.72	0.402	0.228	1.35	0.20	2.01
6	5.35	0.417	0.191	1.18	0.15	2.58	5.72	0.407	0.196	1.21	0.22	1.77
7	5.06	0.418	0.191	1.02	0.09	2.40	5.43	0.381	0.219	1.46	0.19	1.97
8	5.58	0.437	0.202	1.28	0.20	2.00	5.47	0.388	0.219	1.36	0.17	1.37
9	5.30	0.422	0.210	1.26	0.16	2.64	5.60	0.397	0.229	1.38	0.25	2.31
10	5.15	0.491	0.193	1.00	0.13	2.60	5.64	0.398	0.221	1.34	0.20	2.17
11	5.77	0.492	0.193	1.30	0.28	2.27	5.63	0.398	0.210	1.28	0.26	2.16

herbage would not be expected since uptake of nitrogen would be accompanied by uptake of both cations and anions. However, if nitrogen is available for uptake largely as NH_4^+, such as would occur in a soil of low nitrification activity, a relationship between percentage nitrogen and the anion content of the herbage may occur, e.g. N/P ratio. Herbage chemical analysis data for ryegrass from the Waimate North trial showed no correlation between nitrogen and phosphorus (figure 7.13; $r = 0.05$), but a significant relationship was found in ryegrass herbage from the Wharekohe trial ($F = 19.29$, significant 1%, figure 7.14). The line of best fit was found to be a cubic regression of the form:

$$y = \alpha_0 + \alpha_1(x_{1i} - \bar{x}) + \alpha_2(x_{2i} - \bar{x}) + \alpha_3(\bar{x}_{3i} - \bar{x}_3) + \Sigma i$$

giving the estimated regression line of y on x as:

$$y = 0.232 + 0.088\ x + 0.012\ x^2 - 0.002\ x^3$$

The estimated regression line of x on y is:

$$x = -30.37 + 200y - 406.5y^2 + 280y^3$$

Sixty one percent of the variation in percent phosphorus could be accounted for by the variation in percent nitrogen ($R^2 = 0.61$). The dependence of the uptake of phosphorus on nitrogen uptake suggests that nitrogen was largely taken up as a cation and is consistant with the low rate of nitrification in that soil.

7.4.4 Pasture Botanical Composition

All nitrogen fertiliser treatments severely affected botanical composition of the pasture.

On the Wharekohe trial, nitrogen fertiliser reduced the contribution of clover to the dry matter yield, the degree of depression of clover being directly related to the rate of nitrogen application (table 7.16), the only exception being in cut one of the $Ca(NO_3)_2$ treatments. Depression of clover yield was greatest in cuts two and three for the

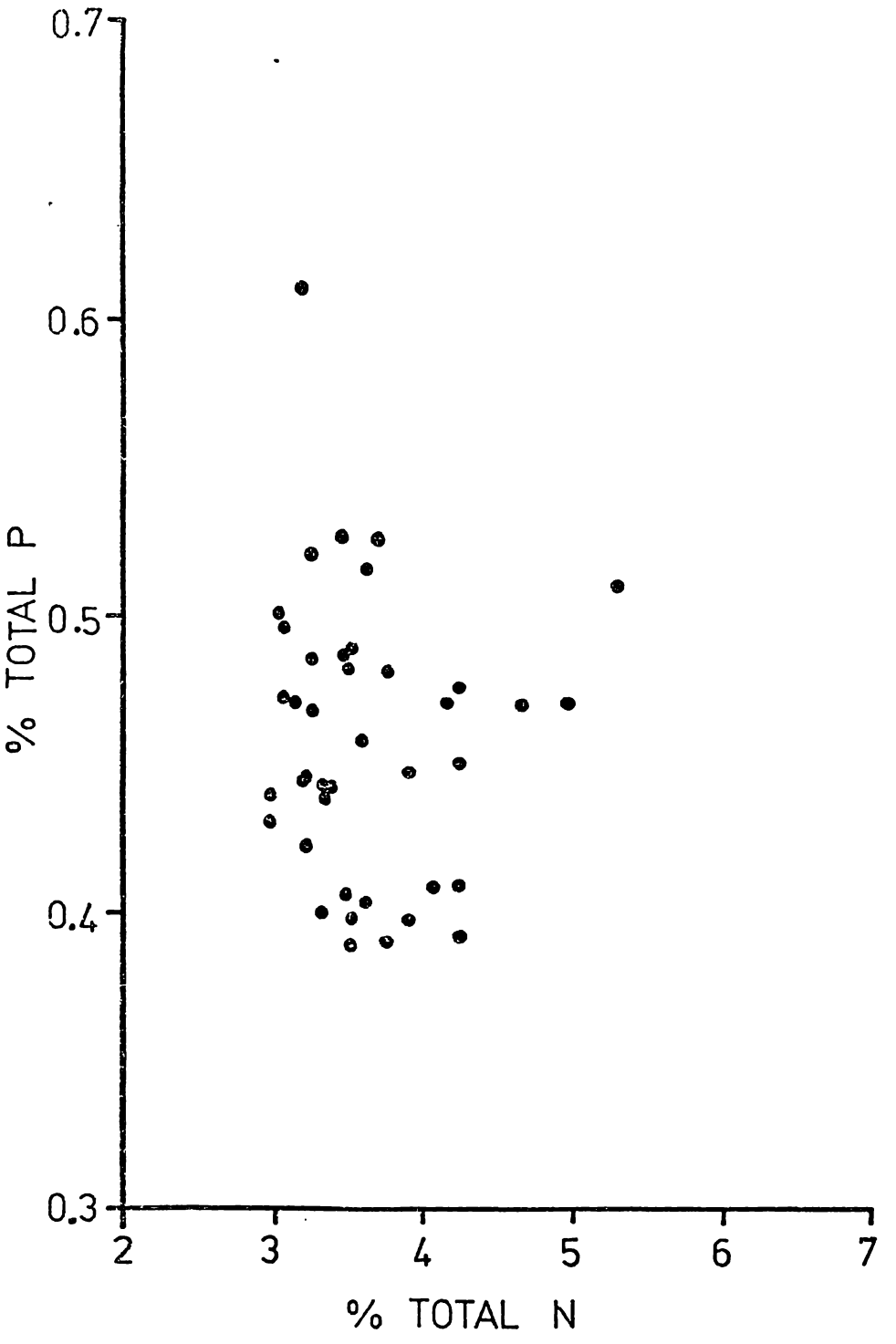

Figure 7.13 Total nitrogen and phosphorus in ryegrass herbage from the Waimate North trial

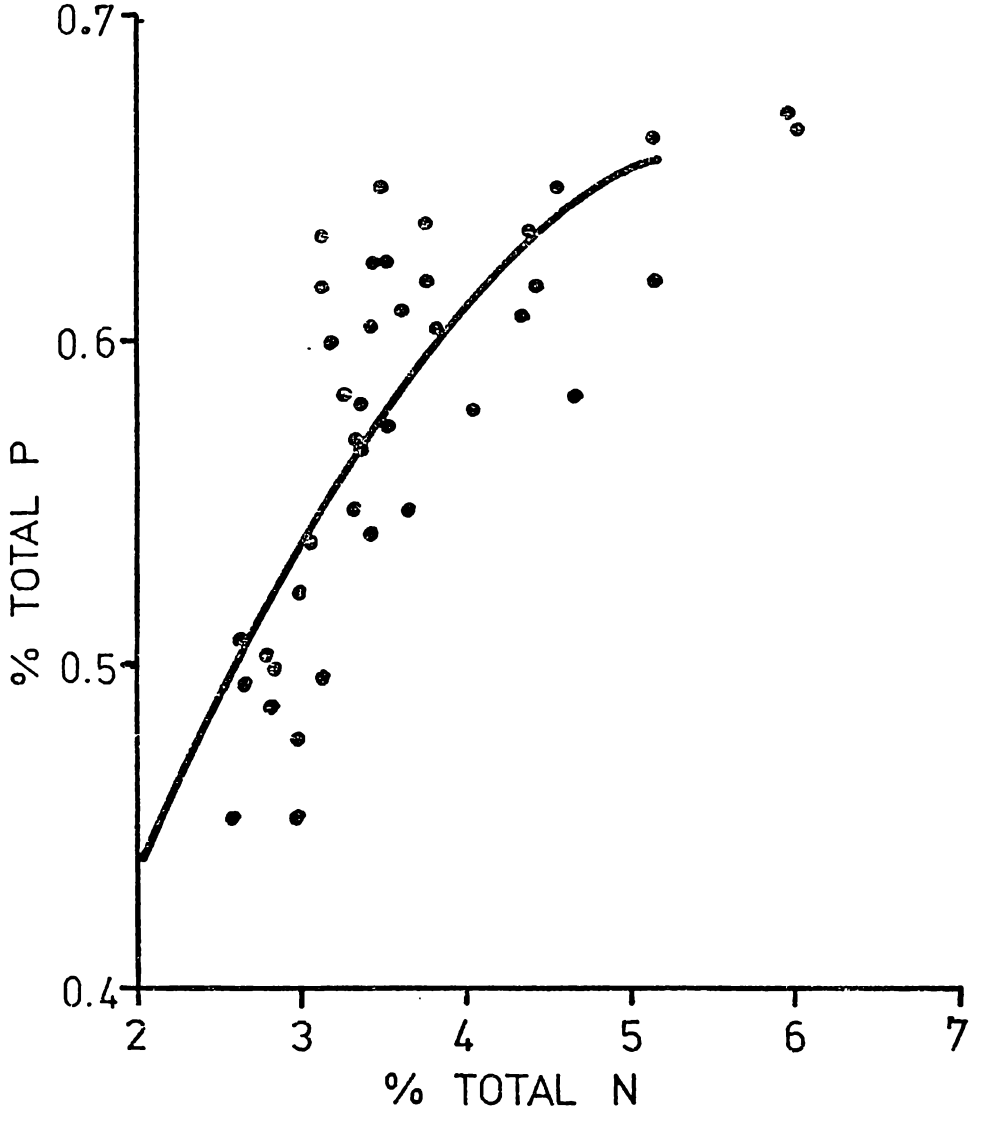

Figure 7.14 The relationship between total nitrogen and total phosphorus in ryegrass herbage from the Wharekohe trial

Table 7.16 Changes in the botanical composition of pasture following the addition of nitrogen fertiliser to Wharekohe silt loam

Treatment	Cut 1			Cut 2			Cut 3			Cut 4		
	Grass	Clover	Weeds	Grass	Clover	Weeds	Grass	Clover	Weeds	Grass	Clover	Weeds
		%			%			%			%	
1	61.9	32.1	6.0	48.0	48.8	3.2	45.1	49.7	5.2	52.5	39.0	8.5
2	86.7	8.4	4.9	71.6	27.2	1.2	70.8	25.8	3.4	46.7	46.4	6.9
3	81.3	16.1	2.6	82.1	17.6	0.3	77.2	21.9	0.9	67.8	31.7	0.5
4	77.3	20.5	2.2	81.0	17.1	1.9	89.3	9.6	1.1	66.2	32.1	1.7
5	69.5	26.9	3.6	74.2	22.6	3.2	65.1	31.1	3.8	48.5	45.6	5.9
6	83.7	14.3	2.0	84.7	14.9	0.4	82.6	16.8	0.6	70.4	29.0	0.2
7	89.7	8.0	2.3	91.6	7.1	1.3	94.6	5.1	0.3	84.4	15.4	0.2
8	69.3	27.4	3.3	70.3	22.9	6.8	57.2	38.4	4.4	44.3	48.6	7.1
9	84.1	14.5	1.4	80.6	19.0	0.4	72.7	25.9	1.4	69.4	29.4	1.2
10	87.9	10.9	1.2	95.6	3.8	0.6	91.8	7.6	0.6	85.3	14.1	0.6
11	63.5	34.3	2.2	52.3	44.6	3.1	52.3	44.5	3.2	46.8	49.6	3.6

200 kg N/ha treatments where clover yield was reduced from 48.8 percent in the control to 7 percent in treatment seven and 3.8 percent in treatment ten in the second cut, and from 49.7 percent in the control to 5.1 and 7.6 percent in treatments seven and ten respectively in cut three. A visual example of the change in botanical composition on the Wharekohe trial forty two days after application of fertiliser is provided in figures 7.15, 7.16, 7.17 and 7.18.

In the final cut, the percentage contribution of clover to total dry matter yield on the 50 kg N/ha treatments on the Wharekohe soil had returned to a level similar to the control plots but some suppression of clover was still evident at the higher rates of nitrogen application.

Nitrogen fertiliser also reduced the contribution of clover to the dry matter yield on the Waimate North trial, the degree of depression again being directly related to the rate of fertiliser nitrogen. Some depression in percentage clover was still evident in all treatments at the conclusion of the trial (table 7.17).

It is thought that the reduction in legume growth following nitrogen application to grass/legume pastures is due to increased competition from grasses (Dilz and Mulder, 1962; Donald, 1963; MacLeod, 1965; Mouat and Walker, 1959; Stern and Donald, 1962a,b; Wilson, 1962), light being an important factor (Langer, 1973). Blackman (1938), for example, concluded that the suppression of clover in mixed swards following the addition of nitrogen was caused partly by shading of the clover through the increased growth of the grasses, and partly by the added nitrogen reducing nitrogen fixation by the clover and increasing competition for nitrogen between grass and clover. Competition for light has also been suggested as an important competitive factor in studies by Robinson and Sprague (1947), Sprague and Garber (1950) and Holmes and MacLusky (1955).

Changes in botanical composition of pastures following application of

Figure 7.15 Control plot on Wharekohe silt loam forty two days after commencement of trial. Forty eight percent of dry matter yield was clover herbage.

Figure 7.16 Experimental plot on Wharekohe silt loam forty two days after application of 50 kg N/ha as $(NH_4)_2SO_4$. Clover contributed twenty two percent of the herbage dry matter yield.

Figure 7.17 Experimental plot on Wharekohe silt loam forty two days after application of 100 kg N/ha as $(NH_4)_2SO_4$. Clover contributed fourteen percent of the herbage dry matter yield.

Figure 7.18 Experimental plot on Wharekohe silt loam forty two days after application of 200 kg N/ha as $(NH_4)_2SO_4$. Clover contributed seven percent of the herbage dry matter yield.

Table 7.17 Changes in the botanical composition of pasture following the addition of nitrogen fertiliser to Waimate North clay loam

Treatment	Cut 1			Cut 2			Cut 3			Cut 4		
	Grass	Clover	Weeds	Grass	Clover	Weeds	Grass	Clover	Weeds	Grass	Clover	Weeds
		%			%			%			%	
1	54.0	33.8	12.2	54.8	40.5	4.7	57.3	38.9	3.8	48.3	49.1	2.6
2	72.0	26.1	1.9	78.6	19.2	2.2	72.2	23.9	3.9	61.9	33.8	4.3
3	89.9	9.6	0.5	80.0	17.9	2.1	86.8	10.7	2.5	80.3	17.7	2.0
4	87.4	12.2	0.6	81.1	15.6	3.3	79.9	15.2	4.9	78.9	20.3	0.8
5	76.7	22.0	1.3	69.6	28.8	1.6	69.8	26.4	3.8	60.5	36.8	2.7
6	85.6	12.8	1.6	80.4	14.5	5.1	81.7	17.3	1.0	70.7	28.8	0.5
7	91.5	8.4	0.1	86.7	13.2	0.1	92.0	6.7	1.3	82.2	17.1	0.7
8	73.3	24.1	2.6	73.3	24.2	2.5	77.4	19.1	3.5	54.9	41.6	3.5
9	87.7	11.3	1.0	86.7	11.6	1.7	87.0	11.7	1.3	74.0	24.6	1.4
10	93.6	6.3	0.1	89.9	8.9	1.2	87.2	11.9	0.9	80.9	18.6	0.5
11	54.9	42.8	2.3	60.1	35.4	4.5	59.1	36.4	4.5	59.9	36.5	3.6

nitrogen fertiliser are not always consistant even when pasture management is the same. In a previous study on Wharekohe silt loam (Steele, 1976b), application of 100 kg N/ha as urea had little effect on botanical composition. This may be due to the fact that the percentage of clover in the pasture was low (eight percent) and also that different forms of nitrogen are known to have different effects on botanical composition (Whitehead, 1970). The stage of cutting also has an important effect on botanical composition, the more advanced the growth stage before cutting, the greater the depression of clover (Hughes and Evans, 1951; Walker et al. 1952, 1953).

Frequent defoliation of pasture tends to offset the effect of applied nitrogen on white clover by reducing the shading caused by grass (Blackman and Templeman, 1938; Holliday and Wilman, 1965; Robinson and Sprague, 1947; Sprague and Garber, 1950). However, since the dry matter response to an application of nitrogen is dependent on the time interval between nitrogen application and harvest (Wilman, 1965), some sort of balance must be reached in order to optimise yield response without excessive detrimental effect on botanical composition which may cause a post response slump in pasture production.

In the present study, application of 200 kg N/ha reduced the contribution of clover to dry matter yield even twelve weeks after application, whereas in the 50 kg N/ha treatments the percentage clover had returned to that of the control on the Wharekohe soil but not on the Waimate North soil.

7.4.5 Soil Inorganic Nitrogen

In the control plots on Wharekohe silt loam, NO_3-N was absent at all samplings but NH_4-N was maintained at relatively high levels (20 to 50 ppm) throughout the experimental period (table 7.18). When nitrogen

Table 7.18 Inorganic soil nitrogen ± standard deviation present in Wharekohe silt loam following the addition of nitrogen fertiliser

Treatment	Depth of Sample	1 Day After Application of N		3 Days After Application of N		5 Days After Application of N	
		NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)
1	0 - 5	28.2 ± 4.4	0	20.8 ± 5.3	0	50.3 ± 10.8	0
	5 - 15	10.4 ± 2.3	0	7.5 ± 1.7	0	16.7 ± 1.6	0
3	0 - 5	21.3 ± 3.2	177.1 ± 15.9[3]	25.9 ± 4.7	154.9 ± 15.9[3]	53.6 ± 3.4	34.5 ± 1.7[3]
	5 - 15	8.2 ± 2.5	5.5 ± 3.4[3]	10.0 ± 1.6	5.0 ± 2.2[3]	18.2 ± 2.8	13.9 ± 4.7[3]
6	0 - 5	155.4 ± 19.7[3]	0	141.9 ± 20.3[3]	1.6 ± 0.9	123.5 ± 16.6[3]	0
	5 - 15	15.8 ± 1.8[3]	1.9 ± 1.6[2]	11.7 ± 6.8	0	18.8 ± 2.0	0
9	0 - 5	167.1 ± 2.3[3]	1.2 ± 1.6	145.6 ± 23.8[3]	0.8 ± 0.9	159.2 ± 18.4[3]	0
	5 - 15	14.9 ± 3.5	1.1 ± 1.4	14.9 ± 4.2[3]	0	41.4 ± 15.2[3]	0
11	0 - 5	27.6 ± 8.3	0	25.7 ± 2.9	0	49.7 ± 12.6	0
	5 - 15	10.2 ± 1.3	0	7.6 ± 2.7	0	16.6 ± 1.7	0
		7 Days After Application of N		14 Days After Application of N		21 Days After Application of N	
1	0 - 5	25.7 ± 9.0	0	26.9 ± 6.7	0	39.6 ± 9.9	0
	5 - 15	16.5 ± 2.7	0	13.9 ± 1.8	0	17.6 ± 1.5	0
3	0 - 5	33.0 ± 6.8	13.3 ± 2.2[3]	29.4 ± 2.7	0.9 ± 1.0	44.0 ± 0.5	0
	5 - 15	16.8 ± 3.1	4.8 ± 5.3	14.4 ± 1.2	1.6 ± 0.3[3]	20.3 ± 3.0	
6	0 - 5	101.8 ± 13.2[3]	0	35.8 ± 5.4[1]	0	41.7 ± 14.3	0
	5 - 15	29.3 ± 6.2[3]	0	19.8 ± 2.0[3]	0	33.6 ± 14.5[1]	0
9	0 - 5	68.9 ± 8.5[3]	0	40.6 ± 6.1[2]	0	45.2 ± 10.7	0
	5 - 15	19.4 ± 2.1	0	21.2 ± 2.9[3]	0	26.6 ± 7.5[1]	0
11	0 - 5	29.6 ± 3.1	0	25.6 ± 1.7	0	41.7 ± 6.2	0
	5 - 15	13.6 ± 2.0	0	12.4 ± 6.5	0	19.9 ± 1.1	0

Table 7.18 continued.

Treatment	Depth of Sample	28 Days After Application of N	
		NH_4-N (ppm)	NO_3-N (ppm)
1	0 - 5	33.3 ± 5.9	0
	5 - 15	19.8 ± 2.3	0
3	0 - 5	39.0 ± 4.0	0
	5 - 15	19.3 ± 2.8	0
6	0 - 5	33.7 ± 3.7	0
	5 - 15	18.8 ± 2.9	0
9	0 - 5	28.3 ± 3.4	0
	5 - 15	16.2 ± 2.8	0
11	0 - 5	26.1 ± 0.3	0
	5 - 15	13.5 ± 1.2	0

[1] Significantly different from control at P = 0.1.

[2] Significantly different from control at P = 0.05.

[3] Significantly different from control at P = 0.01.

was applied, soil inorganic nitrogen showed a large increase the day after application, but then declined rapidly to reach a level similar to the control plots after twenty one days. Soil inorganic nitrogen declined more rapidly following application of $Ca(NO_3)_2$ than $(NH_4)_2SO_4$.

For easier interpretation of soil inorganic nitrogen levels, the amounts of fertiliser nitrogen recovered from the Wharekohe soil are reported in table 7.19. Most of the applied nitrogen appeared to stay in the 0 - 5 cm depth although some fertiliser nitrogen did apparently move into the 5 - 18 cm depth. There is also some evidence of nitrification of applied NH_4^+ in treatments six and nine, although the amount of nitrification appears to be very small.

In the control plots on Waimate North clay loam, NO_3-N levels were low (2.7 to 7.3 ppm) at all times of sampling but NH_4-N levels were high (26 to 49 ppm). When inorganic nitrogen was applied, soil inorganic nitrogen showed a large increase the day after application (table 7.20), but then declined rapidly. In contrast to the Wharekohe soil, a statistically significant amount of fertiliser was still present in the Waimate North soil twenty eight days after application, most of the NH_4^+ fertiliser having been oxidised to NO_3^-.

Nitrate-nitrogen moved down the profile, substantial amounts being present in the 5 - 15 cm depth twenty four hours after application, and in the 15 - 30 cm depth seven days after application (table 7.21). Although NH_4-N remained largely in the 0 - 5 cm depth some did move down the profile. However, it appears that the major movement of nitrogen from $(NH_4)_2SO_4$ down the profile occurred after oxidation to NO_3^-.

Application of "N-Serve" did not appear to retard nitrification.

Table 7.19 kg N/ha fertiliser nitrogen present as NH_4^+ and NO_3^-

Wharekohe		1		3		5	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
3	0 - 5	0	79.6[3]	0	69.6[3]	0	15.5[3]
	5 - 18	0	5.8[3]	0	5.3[3]	0	14.7[3]
6	0 - 5	57.2[3]	0	54.4[3]	0.7	32.9[3]	0
	5 - 18	5.5[3]	2.0[2]	4.4	0	2.2	0
9	0 - 5	62.4[3]	1.2	56.0[3]	0	48.9[3]	0
	5 - 18	4.8	1.1	7.8[3]	0	26.2[3]	0

		7		14		21	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
3	0 - 5	0	6.0	0	0.4	0	0
	5 - 18	0	5.7	0	1.7[3]	0	0
6	0 - 5	34.2[3]	0	4.0[1]	0	0.9	0
	5 - 18	13.6[3]	0	6.2[3]	0	16.9[1]	0
9	0 - 5	19.4[3]	0	6.2[2]	0	2.5	0
	5 - 18	3.1	0	7.7[3]	0	9.5[1]	0

		28	
		NH_4	NO_3
3	0 - 5	0	0
	5 - 18	0	0
6	0 - 5	0	0
	5 - 18	0	0
9	0 - 5	0	0
	5 - 18	0	0

[1]Significantly different from control at P = 0.1.

[2]Significantly different from control at P = 0.05.

[3]Significantly different from control at P = 0.01.

Table 7.20 Inorganic soil nitrogen ± standard deviation present in Waimate North clay loam following the addition of fertiliser nitrogen

Treatment	Depth of Sample	1 Day After Application of N		3 Days After Application of N		5 Days After Application of N	
		NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)
1	0 - 5	41.7 ± 5.5	7.2 ± 1.9	40.9 ± 5.5	4.9 ± 0.5	37.8 ± 5.3	4.5 ± 1.1
	5 - 15	12.6 ± 1.3	3.9 ± 0.9	15.4 ± 6.3	4.7 ± 1.5	10.0 ± 2.4	1.6 ± 0.0
3	0 - 5	41.0 ± 5.7	180.7 ± 16.5[3]	45.7 ± 6.9	169.5 ± 16.1[3]	24.8 ± 4.5	30.0 ± 9.5[3]
	5 - 15	11.8 ± 0.9	23.4 ± 4.1[3]	9.4 ± 3.1	8.0 ± 1.4[2]	8.7 ± 1.8	56.7 ± 4.6[3]
6	0 - 5	185.1 ± 27.7[3]	12.1 ± 3.6	172.3 ± 15.9[3]	22.1 ± 4.5[3]	135.6 ± 8.5[3]	26.5 ± 4.4[3]
	5 - 15	19.3 ± 1.3[3]	5.9 ± 2.0	24.0 ± 4.4[1]	7.7 ± 1.0[3]	11.6 ± 3.2	11.4 ± 3.8[3]
9	0 - 5	183.7 ± 20.1[3]	10.1 ± 4.7	175.4 ± 11.6[3]	20.9 ± 1.9[3]	143.7 ± 15.3[3]	17.4 ± 2.7[3]
	5 - 15	15.0 ± 0.5[3]	3.4 ± 1.1	8.4 ± 0.9	4.6 ± 1.9	14.9 ± 2.3[3]	11.1 ± 0.5[3]
11	0 - 5	41.7 ± 8.3	6.9 ± 2.5	32.9 ± 6.1	5.3 ± 0.3	43.7 ± 4.2	5.2 ± 1.8
	5 - 15	12.6 ± 1.8	7.0 ± 2.1	8.9 ± 1.3	2.9 ± 0.1	11.7 ± 3.9	3.8 ± 1.8
		7 Days After Application of N		14 Days After Application of N		21 Days After Application of N	
1	0 - 5	35.2 ± 1.7	4.6 ± 0.9	41.4 ± 5.2	3.5 ± 0.5	49.5 ± 8.9	7.3 ± 3.8
	5 - 15	16.1 ± 1.9	3.5 ± 0.8	17.8 ± 2.0	2.6 ± 1.4	16.1 ± 3.7	3.7 ± 0.8
	15 - 30	12.1 ± 1.3	5.3 ± 0.9	16.0 ± 2.9	1.9 ± 0.7	8.3 ± 4.6	1.8 ± 0.7
3	0 - 5	35.1 ± 5.6	18.0 ± 3.2[2]	42.9 ± 0.9	23.7 ± 3.5[3]	52.6 ± 7.4	12.6 ± 4.7
	5 - 15	16.6 ± 3.8	13.1 ± 5.1[3]	23.1 ± 6.9	11.9 ± 2.6[3]	16.2 ± 2.3	7.6 ± 0.4[3]
	15 - 30	12.5 ± 1.8	41.4 ± 11.5[3]	21.6 ± 7.2	37.1 ± 2.8[3]	17.2 ± 4.3	21.3 ± 4.9[3]
6	0 - 5	93.2 ± 5.6[3]	22.6 ± 2.3[3]	55.6 ± 6.4[3]	23.9 ± 9.4[3]	61.3 ± 13.6	22.0 ± 7.3[3]
	5 - 15	15.2 ± 1.3	11.7 ± 1.6[3]	24.3 ± 7.2	10.4 ± 3.0[3]	14.3 ± 4.5	10.9 ± 1.0[3]
	15 - 30	15.4 ± 4.4	10.1 ± 5.5	23.0 ± 3.9[3]	7.3 ± 0.1[3]	16.0 ± 1.1[3]	12.7 ± 6.5[3]

Table 7.20 continued.

Treatment	Depth of Sample	7 Days After Application of N		14 Days After Application of N		21 Days After Application of N	
		NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	NO_3-N (ppm)
9	0 - 5	110.6 ± 22.9[3]	17.3 ± 3.1[3]	67.2 ± 7.4[3]	18.5 ± 6.8[3]	53.6 ± 8.9	12.3 ± 3.1[1]
	5 - 15	16.2 ± 1.6	12.4 ± 2.1[3]	24.4 ± 8.6	15.2 ± 4.5[3]	15.0 ± 4.6	6.3 ± 1.4[2]
	15 - 30	11.3 ± 1.1	11.7 ± 2.9[3]	29.9 ± 9.3[3]	13.8 ± 3.0[3]	10.6 ± 3.4	4.7 ± 0.7[3]
11	0 - 5	34.8 ± 0.8	4.0 ± 0.9	41.7 ± 7.8	3.5 ± 0.6	47.1 ± 5.4	10.4 ± 4.9
	5 - 15	12.8 ± 2.2	2.3 ± 1.9	17.7 ± 5.6	3.0 ± 1.2	15.1 ± 4.6	4.1 ± 0.7
	15 - 30	11.2 ± 2.3	1.6 ± 2.1	10.3 ± 2.2	- -	- -	- -

		28 Days After Application of N	
1	0 - 5	26.8 ± 2.7	2.7 ± 0.6
	5 - 15	9.1 ± 1.2	2.3 ± 1.0
	15 - 30	6.6 ± 1.4	1.8 ± 0.7
3	0 - 5	28.6 ± 1.4	7.6 ± 0.9[3]
	5 - 15	9.1 ± 2.1	4.7 ± 1.8[1]
	15 - 30	6.8 ± 0.8	21.2 ± 5.3[3]
6	0 - 5	35.6 ± 5.2[2]	10.9 ± 3.0[3]
	5 - 15	9.2 ± 1.3	4.4 ± 1.7[1]
	15 - 30	7.6 ± 2.1	6.5 ± 3.5[2]
9	0 - 5	39.7 ± 3.7[3]	7.9 ± 0.9[3]
	5 - 15	7.7 ± 0.7	3.3 ± 1.4
	15 - 30	6.1 ± 1.2	6.3 ± 0.6[3]
11	0 - 5	36.5 ± 2.3	4.9 ± 2.0
	5 - 15	11.3 ± 1.0	2.9 ± 1.1
	15 - 30	- -	- -

[1] Significantly different from control at P = 0.1

[2] Significantly different from control at P = 0.05

[3] Significantly different from control at P = 0.01

Table 7.21 kg N/ha fertiliser nitrogen present as NH_4^+ and NO_3^-

Waimate North		1		2		5	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
3	0 - 5	0	66.9[3]	0	63.5[3]	0	9.8[3]
	5 - 15	0	18.8[3]	0	3.1[2]	0	50.4[3]
6	0 - 5	55.3[3]	1.9	50.7[3]	6.6[3]	37.8[3]	8.5[3]
	5 - 15	6.5[3]	1.9	8.3[1]	2.9[3]	1.5	9.5[3]
9	0 - 5	54.8[3]	1.1	51.9[3]	6.2[3]	40.9[3]	12.5[3]
	5 - 15	2.3[3]	0	0	0	4.7[3]	9.2[3]

		7		14		21	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
3	0 - 5	0	5.2[2]	0	7.8[3]	0	2.0
	5 - 15	0	9.2[3]	0	9.0[3]	0	3.8[3]
	15 - 30	0	36.6[3]	0	35.6[3]	0	19.8[3]
6	0 - 5	22.4[3]	6.9[3]	5.5[3]	7.9[3]	4.6	5.7[3]
	5 - 15	0	7.9[3]	6.3	7.5[3]	0	7.3[3]
	15 - 30	3.3	4.9	2.7[3]	5.5[3]	7.8[3]	11.0[3]
9	0 - 5	29.1[3]	4.9[3]	10.0[3]	5.8[3]	1.6	1.9[1]
	5 - 15	0	7.5[3]	6.4	12.2[3]	0	2.5[2]
	15 - 30	0	6.5[3]	14.1[3]	12.1[3]	2.3	2.9[3]

		28	
		NH_4	NO_3
3	0 - 5	0	1.9[3]
	5 - 15	0	2.3[1]
	15 - 30	0	19.6[3]
6	0 - 5	3.4[2]	3.2[3]
	5 - 15	0	2.0[1]
	15 - 30	1.0	4.8[2]
9	0 - 5	5.0[2]	2.0[3]
	5 - 15	0	1.0
	15 - 30	0	4.6[3]

[1]Significantly different from control at P = 0.1.

[2]Significantly different from control at P = 0.05.

[3]Significantly different from control at P = 0.01.

7.5.0 A TRACER STUDY OF NITROGEN FERTILISER TRANSFORMATIONS IN SOIL : EXPERIMENTAL PROCEDURES

7.5.1 Trial Design

A randomised block design (three treatments x four replications) trial was laid down at each site. Each plot (26 cm x 26 cm) was defined by a galvanised steel frame (6.5 mm thick) driven into the Wharekohe soil to a depth of 18 cm and the Waimate North soils to a depth of 34 cm. A 2 cm lip on each plot was left above the soil surface. Basal fertiliser dressings were applied to both trial areas as outlined in section 7.2.2.

Pasture on the micro plots was trimmed with hand shears to a height of approximately 5 cm and the following treatments applied on September 1, 1976:

1) Control
2) 100 kg N/ha as $(NH_4)_2SO_4$; 11.6724 atom % ^{15}N excess
3) 100 kg N/ha as $Ca(NO_3)_2$; 11.6724 atom % ^{15}N excess

Both fertiliser treatments were applied in 111 ml distilled water per plot.

Following application of treatments, one micro-plot from each treatment on both soils was covered with a perspex glasshouse which sealed onto the top of the metal micro-plot frame with a rubber seal. The glasshouse was connected into a gas-sorption line as shown in figure 7.19. An air flow of 500 cm^3/minute was maintained through each glasshouse. The outside of each glasshouse was wound with bare concordin wire (0.71 mm diameter) at 10 mm intervals through which an electric current was passed so that the unit acted as a demister, preventing condensation of moisture on the internal surfaces of the glasshouse. Any moisture condensing inside the glasshouse may redissolve any NH_3, NO

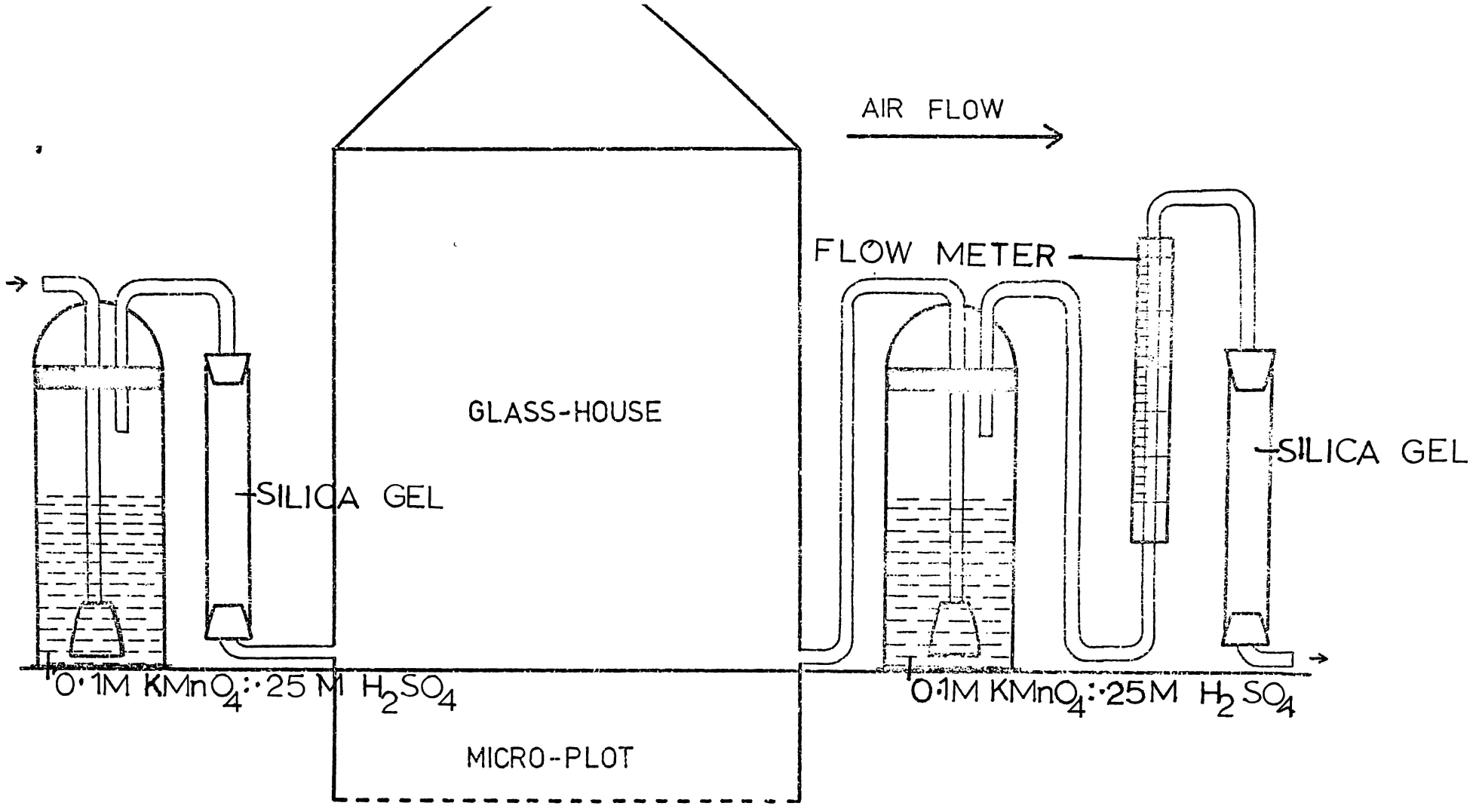

Figure 7.19 Gas sorption line

and NO_2 liberated. The current passing through the concordin wire was controlled with a Variac adjustable transformer. To aid in temperature control inside the glasshouse, fifty percent shading mesh was placed over each unit. The field layout of the glasshouses is shown in figures 7.20 and 7.21.

The sorption solution used in the gas line was 0.1M $KMnO_4$:.25M H_2SO_4 (Bundy and Bremner, 1973b). This solution will absorb NH_3 because of its acidic nature, and NO and NO_2 is retained in the solution by oxidation to nitrate. The objective of the glasshouse was to determine if any NH_3 was evolved following application of $(NH_4)_2SO_4$ to a slightly acidic soil in which little nitrification occurred. Unfortunately, because of the remoteness of the experimental area from the laboratory, no estimation of N_2O evolution could be made.

Daily rainfall was added to each glasshouse as distilled water.

Soil temperature (2 cm) was recorded continuously inside and outside a glasshouse and a manometer was also placed inside and outside a glasshouse. This monitered differences in moisture and temperature between the inside and outside of the glasshouse. A maximum difference in temperature of $3^{o}C$ was measured while soil moistures were not significantly different.

The micro-plots not covered with glasshouses were connected to a reservoir below ground level to facilitate collection of surface runoff water (figure 7.22). Mercuric chloride (40 mg Hg) was added to each runoff water collection bottle to prevent micro-organism growth in water samples.

7.5.2 Micro-Plot Sampling Procedures

The gas-sorption bottles for each glasshouse were changed daily up to eleven days following commencement of the experiment, and then

Figure 7.20 Micro-plot experimental area on the Wharekohe silt loam.

Figure 7.21 Micro-plot experimental area on the Waimate North clay loam soil.

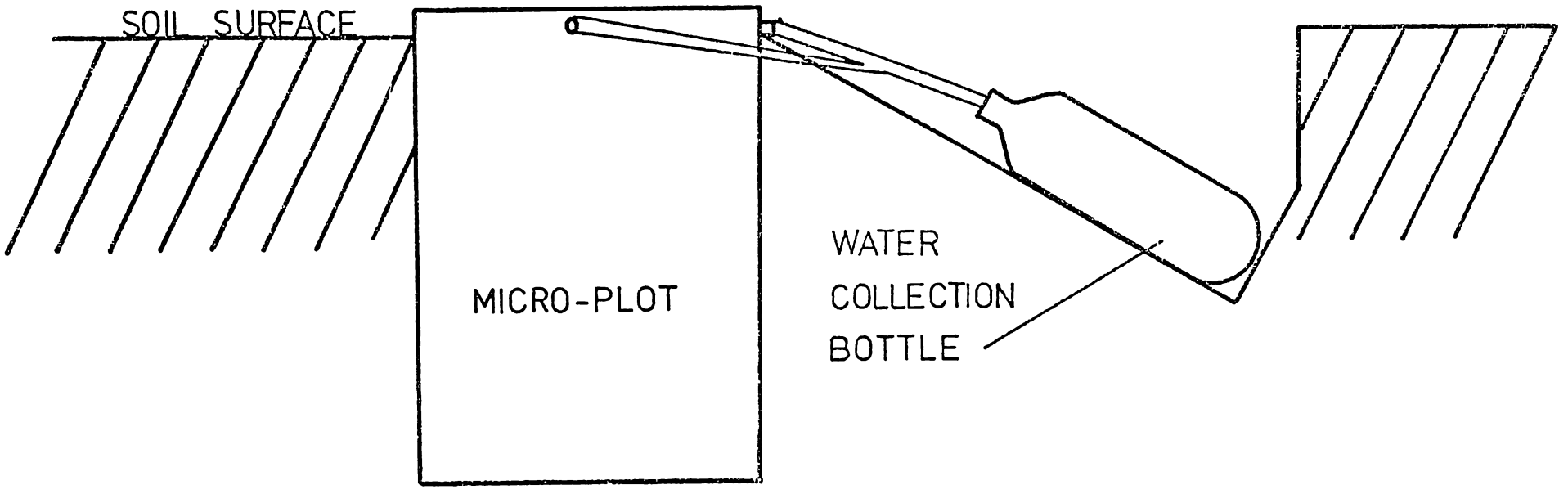

Figure 7.22 Design of open micro-plot to allow collection of surface runoff water

again at fifteen and twenty one days, after which gas sorption was discontinued. Silica gel in the sorption lines was changed daily.

A closed steel cylinder (5 cm diameter) was placed in one plot of each treatment and left for twenty four hours (figure 7.23). An air sample was collected from each cylinder into a previously evacuated vacu-container using a double ended needle. The cylinders were then shifted to a new location. This sampling was conducted for five days following fertiliser application, the objective being to determine the isotopic composition of the air sample to gain an indication if any denitrification occurred.

Twenty one days after application of treatments, two micro-plots from each treatment on each soil were removed and dismantled for analysis. Herbage was hand clipped to ground level, dried at 85^{o}C for twelve hours and stored for analysis. The soil was divided into 0 - 5 and 5 - 18 cm depths on the Wharekohe soil, and 0 - 5, 5 - 15, 15 - 30 and 30 - 34 cm depths on the Waimate North soil. Each soil section was weighed on a top-loading balance, sub-sampled for determination of root nitrogen, and the remaining soil dried for forty eight hours at 35^{o}C, ground to pass a 2 mm seive, and stored in sealed plastic bags. Sub-samples for inorganic and root nitrogen were stored frozen until analysis.

Herbage was clipped with hand-shears on the remaining plots twenty one, forty two, sixty three and eighty four days after application of treatments, following which the two remaining micro-plots on each soil treatment were dismantled as outlined above.

All herbage samples were stored frozen until drying.

7.5.3 Chemical Analysis

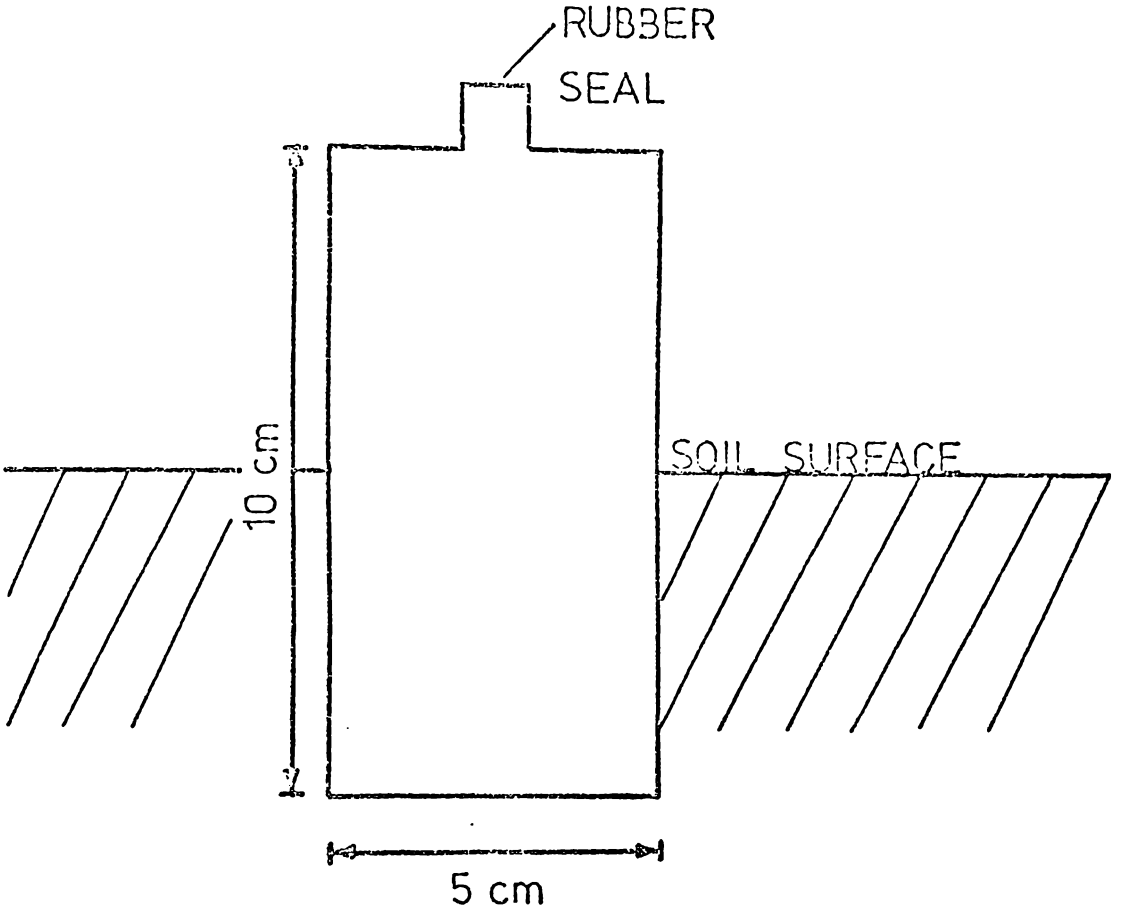

Figure 7.23 Steel cylinder used for collection of atmospheric samples above experimental plots

7.5.3(a) Determination of Total Soil Nitrogen

Total soil nitrogen and its isotopic composition was determined as in section 9.2.1.

7.5.3(b) Determination of Total Plant Nitrogen

Plant herbage was dried at 85°C and ground using a Thomas-Wiley intermediate series 3383 mill fitted with a 60 mesh sieve.

Roots were extracted from frozen soil samples by gentle agitation in distilled water followed by wet sieving employing a jet of water. Extracted roots were washed in distilled water, dried at 85°C and ground using a Thomas-Wiley intermediate series 3383 mill fitted with a 60 mesh sieve.

Total nitrogen of root and herbage samples and their isotopic composition were determined as outlined in section 9.2.2.

7.5.3(c) Determination of NH_3, NO and NO_2 Sorbed by the Acid Permanganate Solution

NH_3, NO and NO_2 sorbed by the acid permanganate solution were determined as described by Bundy and Bremner, (1973b).

7.5.3(d) Determination of Inorganic Nitrogen in Runoff Water Samples

NH_4^+ and NO_3^- in water samples were determined using the micro-distillation method of Bremner and Keeney (1965), the distilled NH_3 being sorbed in boric acid solution (6 g H_3BO_3/ℓ) and the amount present determined with Nessler's reagent (see section 3.2.3).

7.5.4 Estimation of Soil Contamination of Herbage and Root Samples Used for Isotope Analysis

Soil contamination of root and herbage samples used for isotope

analysis was determined using a method based on X-ray fluorescence. The instrument used was an Ortec tube excited Fluorescence Analyser model 6100 based on an energy dispersive system. The multichannel analyser of the X-ray fluorescence system was connected to a DEP PDP 11/05 computer, in which Ortec programmes, written in ORACL, were used to compute results. The multichannel analyser contained a region of interest facility which allowed the analysis of only specified elements by the computer.

Plant and soil samples for analysis were ground in a Thomas-Wiley intermediate series 3383 mill fitted with a 60 mesh sieve, placed in XRF sample cups and stored in an evacuated desiccator to bring all samples to a similar moisture content.

Standards were prepared by mixing uncontaminated herbage grown in pots in the glasshouse with known amounts of soil on a weight/weight basis. In the Waimate North soil it was found that analysis of either iron or titanium provided a reasonable estimate of soil contamination, whereas in the Wharekohe soil only titanium could be used. As only relative, as opposed to absolute, concentrations of iron and titanium were required to compute percentage soil contamination, the iron and titanium content of the one hundred percent soil standard was assigned a value of one hundred, and that of the pure plant sample zero. All intermediate samples were assigned the appropriate value based on their weight/weight composition.

X-ray fluorescence was carried out with the specimen chamber evacuated, all samples being counted for one hundred seconds.

Quadratic fit concentration was used to compute the soil contamination as this gave the best relationship in analysis of standards as illustrated by the results of a series of standards for the Waimate North soil:

Standard Number	Element	Intensity Counts/Second	Theoretical Concentration (%)	Linear Fit Concentration (%)	Quadratic Fit Concentration (%)
1	Ti	0.0000	0	2.6	0.5
	Fe	49.2867	0	2.3	0.3
2	Ti	20.4870	10	9.1	9.1
	Fe	533.0689	10	11.4	10.8
3	Ti	48.7955	20	17.9	20.4
	Fe	888.7019	20	21.5	19.5
4	Ti	312.8155	100	100.4	100.0
	Fe	3632.6081	100	99.5	100.0

The reproducibility of the method is shown by the following analysis of ten subsamples from one bulk sample.

Sub-Sample Number	Titanium Quadratic Concentration %	Iron Quadratic Concentration %
1	27.2	26.8
2	28.7	28.2
3	27.7	26.6
4	27.9	26.6
5	24.8	27.0
6	26.1	28.2
7	25.7	27.9
8	24.7	26.8
9	24.5	26.8
10	30.0	27.9
$\bar{x}$	26.7	27.3
SD	1.9	0.7

7.6.0 A TRACER STUDY OF NITROGEN FERTILISER TRANSFORMATIONS: RESULTS AND DISCUSSION

7.6.1 Plant Recovery of Applied Nitrogen

Plant recoveries of fertiliser nitrogen calculated from the isotopic composition of herbage are presented in table 7.22. Dry matter yields on each micro-plot were similar to the yields reported for the non-tracer trial.

Following application of nitrogen fertiliser, most of the fertiliser recovered by plants appeared in the pasture herbage within the first six weeks following application. Recovery of nitrogen from $Ca(NO_3)_2$ applied to the Waimate North soil was an exception, major amounts of fertiliser nitrogen being recovered in the third and fourth cuts. A possible explanation for this is that the plants were able to recover NO_3^- which had moved down the profile.

Total recovery of NO_3^--N in pasture herbage from the Wharekohe soil was considerably lower than that of NH_4^+-N. This is consistant with the differences in dry matter production found between the two types of fertiliser (table 7.2), and the rapid disappearance of nitrate from the Wharekohe soil (table 7.18).

On the Waimate North soil, although the average recovery of NO_3-N was 47.4 percent, recovery of NH_4-N was only 35.8 percent, or 11.6 kg N/ha less than the recovery of NO_3-N. This difference is consistent with the extra total nitrogen yield of 11.4 kg/ha in pasture herbage following application of $Ca(NO_3)_2$ than $(NH_4)_2SO_4$ (table 7.10), despite a significantly lower dry matter yield ($P = 5\%$) following application of the former.

Comparison of the present recovery values with those in table 7.11, calculated by the difference technique, shows that neither the

Table 7.22 Recovery of tagged nitrogen fertiliser by pasture on Wharekohe silt loam and Waimate North clay loam

Treatment	% of Fertiliser in Plant Herbage				
	Cut 1	Cut 2	Cut 3	Cut 4	Total
Wharekohe silt loam					
NH_4-N	23.9	13.1	1.6	2.9	41.5
"	20.7	14.2	3.7	4.9	43.5
NO_3-N	11.2	5.9	2.6	2.0	21.7
"	10.7	4.6	1.0	2.6	18.9
Waimate North clay loam					
NH_4-N	15.7	9.2	7.8	3.6	36.3
"	14.3	9.9	7.6	3.5	35.3
NO_3-N	6.5	13.9	14.0	11.2	45.6
"	19.2	3.9	16.4	9.6	49.1

calculation based on total nitrogen yield, nor the calculation based on the nitrogen yield of grass alone, provides an accurate estimate of plant receovery of fertiliser nitrogen.

7.6.2. Soil Nitrogen

Three weeks after application of treatments to Wharekohe silt loam, the majority of the $(NH_4)_2SO_4$ nitrogen remaining in the soil was in the 0 - 5 cm depth, only an average of 6.6 percent being present in the 5 - 18 cm region (table 7.23). A similar pattern of distribution for $Ca(NO_3)_2$ was found, although the amounts recovered were much lower (an average of 15.1 percent 0 - 5 cm; 1.7 percent 5 - 18 cm). Some of the NH_4^+ and NO_3^- fertiliser had leached below the micro-plots by the time of the three week sampling, as soil samples collected immediately below the NH_4^+ and NO_3^- plots had $\delta^{15}N$ values of total soil nitrogen of 112 ‰ and 188‰ respectively.

Twelve weeks after application, an average of 16.8 percent of the $(NH_4)_2SO_4$ nitrogen was found in the 0 - 5 cm depth and 3.6 percent in the 5 - 15 cm depth. Little of the $Ca(NO_3)_2$ nitrogen remained in the soil, only 8.5 percent in the 0 - 5 cm depth and 2.0 percent in the 5 - 18 cm depth.

Three weeks after application of treatments to Waimate North clay loam 42.3 percent of the applied $(NH_4)_2SO_4$ nitrogen remained in the 0 - 5 cm region, only 2.5 percent and 1 percent being in the 5 - 15 and 15 - 30 cm depths respectively (table 7.24). Less of the $Ca(NO_3)_2$ nitrogen remained in the soil and more was found in the 5 - 15 and 15 - 30 cm depths than for $(NH_4)_2SO_4$. Some of the applied $Ca(NO_3)_2$ nitrogen had leached below 34 cm at twenty one days as soil immediately below the $Ca(NO_3)_2$ plots showed an enrichment ($\delta^{15}N$ = 128‰) of total soil nitrogen. No such enrichment was found below the $(NH_4)_2SO_4$ plots.

Table 7.23 Distribution of tagged nitrogen fertiliser three and twelve weeks after application to Wharekohe silt loam

Treatment	% of Fertiliser in Plant Herbage	% of Fertiliser in Plant Roots	% of Fertiliser in Soil (0 - 5 cm)	% of Fertiliser in Soil (5 - 18 cm)	% of Fertiliser in Runoff Water	Total % Recovery of Fertiliser
3 Weeks After Application						
NH_4-N	24.6	7.4	33.7	8.5	3.5	77.7
"	23.9	-	-	-	-	-
"	20.9	-	-	-	-	-
"	22.7	11.4	44.9	4.6	0	83.6
NO_3-N	16.5	6.2	16.6	2.5	0.5	42.3
"	11.2	-	-	-	-	-
"	10.7	-	-	-	-	-
"	8.5	7.3	13.6	0.9	0	30.3
12 Weeks After Application						
NH_4-N	41.5	4.0	16.4	3.4	1.9	67.2
"	43.5	6.4	17.1	3.8	1.2	72.0
NO_3-N	21.7	2.0	8.1	2.4	0.5	34.7
"	18.9	2.0	8.9	1.5	0.4	31.7

Table 7.24 Distribution of tagged nitrogen fertiliser three and twelve weeks after application to Waimate North clay loam

Treatment	% of Fert. in Plant Herbage	% of Fert. in Plant Roots	% of Fert. in Soil 0 - 5 cm	5 - 15 cm	15 - 30 cm	30 - 34 cm	% of Fert. in Runoff Water	Total % Recovery of Fert.
Three weeks after application								
NH_4-N	26.3	12.8	41.8	0	2.0	0	0.1	83.0
"	15.7	-	-	-	-	-	-	-
"	14.3	-	-	-	-	-	-	-
"	26.1	7.5	42.8	5.1	0	0	0.1	81.6
NO_3-N	25.3	4.2	28.1	3.4	6.4	2.6	0.1	70.0
"	6.5	-	-	-	-	-	-	-
"	19.2	-	-	-	-	-	-	-
"	28.4	6.9	17.6	7.8	10.2	4.6	0.1	75.6
Twelve weeks after application								
NH_4-N	36.3	16.9	11.6	1.7	0.3	0.5	0.1	67.3
"	35.3	8.8	20.2	2.9	0	0	0.1	67.3
NO_3-N	45.6	8.8	5.8	0.1	0	0	0.1	60.4
"	49.1	13.2	4.1	0.8	0.8	0	0.1	68.1

Twelve weeks after application, 15.9 percent of the applied $(NH_4)_2SO_4$ nitrogen remained in the top 5 cm of soil, 2.3 percent in the 5 - 15 cm depth, 0.2 percent in the 15 - 30 cm depth and 0.3 percent in the 30 - 40 cm depth. Of the $Ca(NO_3)_2$ nitrogen, 5.0 percent, 0.4 percent and 0.4 percent remained in the 0 - 5, 5 - 15 and 15 - 30 cm depths respectively. Enrichment of soil nitrogen in ^{15}N immediately below both $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ plots indicates that fertiliser nitrogen had leached below the plots.

7.6.3 Gaseous Losses of Nitrogen

No evidence of loss of NH_3, NO or NO_2 from either soil was found.

Air samples collected in the steel tubes varied in isotopic composition and ranged from +0.2‰ to -1.7‰ relative to atmospheric nitrogen. This may be indicative of some denitrification since biological denitrification depletes ^{15}N in the evolved gas and enriches ^{15}N in the residual substrate.

7.6.4 Nitrogen in Runoff Water

Only relatively small amounts of inorganic nitrogen were lost in surface runoff water from the Wharekohe soil, and losses from the Waimate North soil were negligible (table 7.25).

The greater loss of ammonium than nitrate from the Wharekohe soil is consistent with the higher retention of ammonium fertiliser in the top 5 cm of soil. It is pertinent to note that some nitrogen in the form of ammonium was lost from the control plots. During wet weather, a film of water remains on the surface of the Wharekohe soil, and it is likely that an equilibrium situation is approached between the nitrogen in soil solution and the surface water. When rain next occurs, the existing layer of surface water is removed, taking with it the nitrogen in

Table 7.25 Inorganic nitrogen content of surface runoff water from Wharekohe silt loam and Waimate North clay loam

Treatment	Days Collection	NH_4-N (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	Total Inorganic Nitrogen (kg N ha^{-1})
Wharekohe silt loam				
NH_4-N	21	3.470	0.004	3.474
Control	21	0.078	0	0.078
NO_3-N	21	0.357	0.114	0.471
Control	84	0.601	0	0.601
NO_3-N	84	0.328	0.176	0.504
NH_4-N	84	1.865	0	1.865
NO_3-N	84	0.235	0.126	1.170
NH_4-N	84	1.150	0.202	1.170
Control	84	0.181	0	0.181
Waimate North clay loam				
NH_4-N	21	0.025	0.040	0.065
Control	21	0.021	0.112	0.027
NO_3-N	21	0.008	0.007	0.017
Control	84	0.037	0.039	0.076
NO_3-N	84	0.035	0.103	0.148
NH_4-N	84	0.066	0.073	0.139
NO_3-N	84	0.029	0.053	0.082
NH_4-N	84	0.029	0.083	0.112
Control	84	0.019	0.110	0.129

solution.

7.6.5 Total Recovery of Applied Nitrogen Fertiliser

Mean total recovery of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ nitrogen in pasture herbage, roots and soil three weeks after application to the Wharekohe soil was 80.5 percent and 36.3 percent respectively, representing losses of 19.5 percent and 63.7 percent of the applied fertiliser nitrogen (table 7.23). Twelve weeks after application the mean total recoveries were 69.6 percent and 33.2 percent representing an additional loss of 10.9 percent of the applied $(NH_4)_2SO_4$ nitrogen but no further loss of $Ca(NO_3)_2$ nitrogen. Losses of nitrogen, therefore, were greatest in the initial twenty one days following fertiliser application when levels of inorganic soil nitrogen were high.

Three weeks after fertiliser application to the Waimate North soil, the mean total recovery of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ nitrogen in pasture herbage, roots, soil and runoff water was 82.3 percent and 72.8 percent respectively, representing losses of 17.7 percent and 27.2 percent of the applied fertiliser nitrogen (table 7.24). Twelve weeks after application, the mean total recoveries were 67.3 percent and 64.3 percent representing an additional loss of 15 percent and 8.5 percent of the applied $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ nitrogen respectively. Although the largest losses occurred in the initial three weeks following fertiliser application, substantial losses from both forms of nitrogen occurred between three and twelve weeks. Although no evidence of isotopic enrichment of nitrogen below the $(NH_4)_2SO_4$ plots was found three weeks after application of fertiliser, it is likely that some leaching did occur since considerable amounts of fertiliser nitrogen were found in the 15 - 30 cm depth in the non-tracer trial.

Some evidence for the ability of pasture to recover nitrogen from the

15 - 34 cm depth on the Waimate North soil is gained from consideration of the amount of fertiliser nitrogen present in these depths at three and twelve weeks. Three weeks after application of fertiliser an average of 11.9 percent fertiliser nitrogen was present in the 15 - 34 cm depth, whereas at twelve weeks only 0.4 percent remained even though an additional total loss of 8.5 percent of the $Ca(NO_3)_2$ nitrogen had occurred.

7.7.0 GENERAL DISCUSSION AND CONCLUSIONS

Pasture production in Northland during autumn, winter and spring is limited by the availability of nitrogen. Spring application of nitrogen fertiliser produces a large increase in dry matter production but this may be followed by a post response depression attributable to a decrease in the proportion of clover in the pasture and therefore a reduction in the input of symbiotically fixed nitrogen. Yield responses by pasture on Wharekohe silt loam to $Ca(NO_3)_2$ were poor, and isotopic data showed that 63.7 percent of a 100 kg N/ha application was lost from the soil within three weeks of application. Enrichment of ^{15}N in soil nitrogen below the isotope micro-plots indicated that some of the nitrogen was lost by leaching. This is somewhat surprising since the Wharekohe soil contains a pan approximately 6 to 16 cm below the soil surface, and in all cases except one, the micro-plots were imbedded in the pan. Examination of undisturbed pan in an area adjacent to the trial showed that the pan contained several large cracks through which water may pass. It is also possible that insertion of the micro-plots caused some fragmentation of the pan. During winter, the pan in the Wharekohe soil is saturated with water and it is possible that some movement of NH_4^+ and NO_3^- through the pan could occur.

Loss of nitrogen following application of $(NH_4)_2SO_4$ to Wharekohe silt

silt loam was 30.4 percent of a 100 kg N/ha application, or half the loss from an equivalent $Ca(NO_3)_2$ application. Inorganic soil nitrogen data indicated that nitrification of applied NH_4^+ was negligible, but the presence of only small amounts of NO_3^- is not sufficient evidence to say that more nitrification did not occur.

It appears that much of the fertiliser nitrogen was lost by leaching, but losses by denitrification must be considered. The high level of soil moisture and relatively warm soil temperatures, when considered in conjunction with the structureless nature of the top soil of Wharekohe silt loam, provide conditions suitable for rapid rates of denitrification (Stefanson, 1972; Bailey and Beauchamp, 1973). Collection of isotopically light N_2 gas from above the plots suggests that such losses do occur, and quantitative determination of the extent of such losses is required. If the micro-plots were not defined by a steel casing, lateral movement through the soil would be expected, such losses have been reported from the Wharekohe soil by During (1972).

Losses of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ nitrogen from Waimate North clay loam were similar over the twelve week experimental period. Rapid nitrification of $(NH_4)_2SO_4$ occurred following application and up to 30 percent of the applied $(NH_4)_2SO_4$ was present in the soil as NO_3^- at one time.

For a complete evaluation of the effect of nitrification in Waimate North clay loam, cycling of nitrogen fertiliser with a complete inhibition of nitrification would have to be compared with cycling of fertiliser nitrogen without inhibition of nitrification. Such a condition was not met in the present study.

"N-Serve" had no effect on either pasture dry matter production or the nitrification of $(NH_4)_2SO_4$. For reasons outlined above (section 7.4.1) it appears that "N-Serve" has little application in New Zealand grassland

farming where nitrogen fertiliser is normally applied to the soil surface.

Deficits in the ^{15}N balances reported in the present study for the $(NH_4)_2SO_4$ treatments on Wharekohe silt loam and both treatments on Waimate North clay loam are of similar magnitude to deficits reported in the literature. Henzell (1971) reported deficits of nitrogen recovery which were dependent on fertiliser rate increasing from 4.8 percent for an annual application of 64 kg N/ha/year applied to Rhodes grass to 42.7 percent for an annual application of 456 kg N/ha/year. Other deficits reported include 14 - 25 percent from field lysimeters (Brown and Volk, 1966), 0 - 63 percent from cores of soil plus pasture in pots sunk into the ground (Dilz and Woldendorp, 1960) and 21.1 to 23.4 percent from open field micro-plots (Vallis et al. 1973). The loss of nitrogen from the $Ca(NO_3)_2$ treatment on the Wharekohe soil was large when compared with the average deficit quoted above, but not outside the range of deficits reported.

The large nitrogen deficit found following application of $Ca(NO_3)_2$ to Wharekohe silt loam suggests that the type of nitrogen fertiliser applied and the method of application should be aimed at minimising nitrification of applied fertilisers. For example it may be preferable to use nitrogen fertilisers which promote acidic conditions (e.g. $(NH_4)_2SO_4$) rather than fertilisers such as urea which produce an increase in the surrounding pH on hydrolysis. However, any long term use of $(NH_4)_2SO_4$ may lead to a general reduction in soil pH (Henzell, 1971) and this may necessitate correction of soil pH with lime.

Following application of nitrogen fertiliser, pasture management should be modified to minimise any change in botanical composition of the pasture since a decrease in the clover content will reduce the nitrogen input into the system which may have long term detrimental

effects.

The use of small defined plots for ^{15}N tracer studies associated with large scale nitrogen trials appears to be a useful method for studying nitrogen transformations in soils. If ^{15}N is applied to non-defined micro-plots three major problems arise.

1) A high dilution of enriched fertiliser occurs because of possible lateral movement in the soil.
2) Undefined micro-plots are normally sampled using a soil corer which introduces a variability into the sampling.
3) It is not known without doubt that unrecovered ^{15}N is actually lost from the soil and not merely redistributed in the soil.

To facilitate accuracy in ^{15}N field experiments it would therefore appear beneficial to enclose micro-plots to define their limits. Definition of the limits of micro-plots prevents lateral dispersion of nitrogen and enables collection of surface runoff water as reported in the present study. Sampling errors can also be minimised by withdrawing the complete micro-plot and mixing before sub-sampling for analysis. The close agreement found between replicates in the present study indicates that for most experiments two replicates are adequate.

The rate of fertiliser application and its enrichment of ^{15}N will be dependent on the background nitrogen in the soil and the dilution factor which it is required to determine.

Because ^{15}N techniques will not accurately estimate the overall effect of nitrogen fertiliser on plant uptake, control plots not receiving nitrogen applications should be included in tracer experiments. Conversely, the difference technique will accurately estimate the overall effect of fertiliser nitrogen on plant uptake but will provide no estimation of the recovery of fertiliser nitrogen by mixed grass/clover pastures.

The major disadvantage of defined micro-plots is that since they restrict lateral movement, they may force water deeper into the profile, and if a pan is present as in the Wharekohe soil, water may be held on top of the pan.

In conclusion, large deficits of applied nitrogen were found on both soils studied and the mechanism of such losses requires investigation. If lysimeter plots were fitted to the base of field micro-plots the determination of actual leaching losses should be possible.

SECTION B

A STUDY OF THE NATURAL ABUNDANCE OF ^{15}N IN NEW ZEALAND GRASSLAND SOILS, AND THE FRACTIONATION OF ^{15}N AND ^{14}N DURING NITROGEN TRANSFORMATIONS IN GRAZED GRASSLAND SYSTEMS

CHAPTER 8

LITERATURE REVIEW

8.1.0 INTRODUCTION

The abundance of the stable nitrogen isotopes (^{14}N, ^{15}N) has been found to be relatively constant in the atmosphere (Dole et al. 1954), a variation of only 1 part in 7,000 being found by Junk and Svec (1958) in air samples collected from various sites and altitudes. The absolute abundance of ^{15}N in the atmosphere is 0.366 atom percent (Nier, 1950).

There are differences between the $^{15}N/^{14}N$ ratios of some naturally occurring substances (table 8.1), this being the result of one or more of the following factors.

i) The isotopic ratio of the source material.

ii) Chemical equilibrium fractionation which results in the concentration of an isotope in one species of a chemical equilibrium reaction. For example, Urey (1947) calculated the isotope equilibrium constant K for the reaction

$$^{14}NH_{4\,(aq)}^{+} + {}^{15}NH_{3\,(g)} \rightleftharpoons {}^{15}NH_{4\,(aq)}^{+} + {}^{14}NH_{3\,(g)}$$

$$\text{as } K = \frac{[\text{products}]}{[\text{reactants}]}$$

$$= \frac{\left[{}^{15}NH_4^+\right]_{(aq)}\left[{}^{14}NH_3\right]_{(g)}}{\left[{}^{14}NH_4^+\right]_{(aq)}\left[{}^{15}NH_3\right]_{(g)}}$$

$$= \frac{\left[{}^{15}NH_4^+ / {}^{14}NH_4^+\right]_{(aq)}}{\left[{}^{15}NH_3 / {}^{14}NH_3\right]_{(g)}}$$

$$= 1.035$$

The isotope equilibrium constant for this reaction has been experimentally determined as 1.034 ± 0.002 (Kirshenbaum et al. 1947).

iii) Chemical kinetic fractionation can occur in non-equilibrium systems, ^{14}N usually being concentrated in the products and ^{15}N in the unreacted substrate when a reaction does not go to completion.

Table 8.1 Variation in ^{15}N abundance of some naturally occurring substances

Sample	$\delta^{15}N‰$	Reference
Methane gas	- 13.0	Hoering, 1955
NH_3 from coke oven gas	- 9.0	Bokhoven and Theeuwen, 1966
Coal (Wyoming)	- 5.0	Smith and Hudson, 1951
Pyridine coke oven gas	0.0	Bokhoven and Theeuwen, 1966
Atmosphere	0	Junk and Svec, 1958
Coal (Netherlands)	+ 2.0	Bokhoven and Theeuwen, 1966
Seaweed	+ 8.1	Hoering, 1955
Coal (Oklahoma)	+ 11.4	Smith and Hudson, 1951
Sol ammoniac (Mexico)	+ 13.0	Hoering, 1955
Natural gas (Netherlands)	+ 18.0	Bokhoven and Theeuwen, 1966
Pitchblende (Colorado)	+719.0	White and Yagoda, 1950
Uraninite (Canada)	+953.0	White and Yagoda, 1950

Two methods of expressing the proportion of stable nitrogen isotopes occurring in nature have been used in the literature. Kohl et al. (1971) defined $\delta^{15}N$ as the per mil enrichment in ^{15}N compared to a standard, normally atmospheric nitrogen.

$$\delta^{15}N = \frac{\left[\frac{^{15}N}{^{14}N + ^{15}N}\right]_{sample} - \left[\frac{^{15}N}{^{14}N + ^{15}N}\right]_{atmos.\ N}}{\left[\frac{^{15}N}{^{14}N + ^{15}N}\right]_{atmos.\ N}} \times 1000$$

Several other workers (Riga et al. 1971; Edwards, 1973; Bremner and Tabatabai, 1973; Rennie et al. 1976) have calculated $\delta^{15}N$ in the same manner.

Hauck (1973) and Freyer and Aly (1974) defined $\delta^{15}N$ not as a function of the ^{15}N abundance, but as a function of the $^{15}N{:}^{14}N$ ratios.

$$\delta^{15}N = \frac{\left(^{15}N/^{14}N\right)_{sample} - \left(^{15}N/^{14}N\right)_{atmos.\ N}}{\left(^{15}N/^{14}N\right)_{atmos.\ N}} \times 1000$$

This latter definiton is the one which is normally used in the stable isotope geochemistry of carbon and oxygen (see for example Craig, 1957), and to maintain uniformity in stable isotope research, it is the preferable definition for ^{15}N studies.

Because of the low natural abundance of ^{15}N, essentially the same results are obtained by both methods of calculation, and accordingly comparison of data calculated by either method is possible.

8.2.0 $\delta^{15}N$ VALUES OF SOIL NITROGEN

Determination of $\delta^{15}N$ values for total soil nitrogen show that most soils, with the possible exception of some organic and forest soils, have positive $\delta^{15}N$ values (table 8.2). Differences in the $\delta^{15}N$ in the profile

Table 8.2 Variation in the ^{15}N abundance of total soil nitrogen

Soil	$\delta^{15}N‰$	Reference
Forest soil, Ao horizon	-7.0	Riga et al. 1971
Peat	-2.8	Hoering, 1955
Clarion sandy loam (Iowa)	+3.0	Cheng, Bremner and Edwards, 1964
Hayden sandy loam (Iowa)	+7.0	Cheng, Bremner and Edwards, 1964
Grundy sandy clay loam (Iowa)	+16.0	Cheng, Bremner and Edwards, 1964
Peat, Eire	+1.9	Hoering, 1955
Edina silt loam (virgin)	+2.0	Cheng, Bremner and Edwards, 1964
Edina silt loam (cultivated)	+1.0	Cheng, Bremner and Edwards, 1964

are also known to occur. Delwiche and Steyn (1970) found the ^{15}N enrichment in two profiles of Yolo fine sandy loam to be greatest at 20 cm, while in two further profiles where some disturbance in the soil had probably taken place during the past century, ^{15}N enrichments were greatest at approximately two metres depth. In each of the soils examined, there appeared to be some correlation of isotopic composition with total nitrogen, although this was not universally true. Rennie et al. (1976) found that ^{15}N enrichment was generally greatest at a depth of approximately 45 cm in both a chernozemic and Luvisolic profile.

An increase of the $\delta^{15}N$ values with an increase in the total organic matter in soil has been theoretically predicted by Shearer et al. (1974a), because immobilisation reactions are favoured in soils of high organic matter content.

Cheng et al. (1964) found variations in the ^{15}N enrichment of various forms of soil nitrogen (table 8.3). The $\delta^{15}N$ values for nitrate nitrogen derived from soils that were incubated in the laboratory were consistently one-third to one-half as large as those for total soil nitrogen. It is likely, however, that the nearer to completion of mineralisation, the higher would be the $\delta^{15}N$ value gained (Kohl et al. 1972). A similar conclusion was reached by Bremner and Tabatabai (1973) who found from nitrogen isotope analysis of soils and soil derived nitrate that there was a considerable range in the ^{15}N enrichment of the nitrate nitrogen produced by aerobic incubation of different soils, and that the $\delta^{15}N$ value of this nitrate nitrogen depended upon the time of incubation and could differ markedly from that of the total soil nitrogen. Variation in the ^{15}N enrichment of several fractions of soil nitrogen has also been reported by Bremner et al. (1966).

Feigin et al. (1974a) obtained constant and presumably characteristic $\delta^{15}N$ values for soil derived nitrate nitrogen after five weeks of

Table 8.3 The $\delta^{15}N$ values of various forms of nitrogen in soils (from Cheng, Bremner and Edwards, 1964)

Form of Nitrogen	Soil Type				
	Grundy	Hayden	Austin	Clarion	Glencoe
Total	+16	+ 7	+ 5	+ 3	+ 2
Hydrolyzable:					
Total	+18	+10	+ 7	+ 5	+ 4
Ammonium	+ 7	+ 7	+ 3	+ 6	+ 5
Hexosamine	+25	+ 8	+ 0	+ 2	+ 2
Amino acid	+16	+14	+12	+ 5	+ 8
Hydroxyamino acid	+19	+11	+ 8	+ 7	+ 3
Nonhydrolyzable	- 3	- 2	- 1	0	- 4
N-mineralised	+ 6	+ 2	+ 1	+ 1	+ 1
Fixed ammonium	+ 6	+ 6	+ 4	+ 2	0

incubation, and Rennie et al. (1976) found that $\delta^{15}N$ values for soil derived nitrate nitrogen after twenty one days incubation were only slightly lower than those from field samples for two Chernozemic soils. Similar results were reported by Freyer and Aly (1975). The results of Feigin et al. (1974a), Rennie et al. (1976) and Freyer and Aly (1975) are contrary to data reported by Delwiche and Steyn, (1970), Bremner and Tabalabai (1973) and Edwards (1973).

Similarities between the $\delta^{15}N$ values of total soil nitrogen and nitrate nitrogen extracted from non-incubated samples in the data of Rennie et al. (1976); $\delta^{15}N$ values obtained by Feigen et al. (1974a,b) for samples which had been incubated for twenty nine weeks and a number of the samples reported by Bremner and Tabatabai (1973) are demonstrated by the fact that they fall essentially on the same regression line (Rennie et al. 1976). This suggests that under field conditions, the $\delta^{15}N$ values of nitrate nitrogen are similar to those of total nitrogen, and $\delta^{15}N$ values may only be lower under short term incubation studies when the rate of mineralisation may be expected to be higher than what occurs under field conditions.

The data reported by Rennie et al. (1976) for soils of the Great Plains differed markedly from data for soils of the Midwest in that the $\delta^{15}N$ values of the nitrate nitrogen mineralised under field conditions not only approximated those of total soil nitrogen, but also did not differ appreciably from those produced during a twenty one day incubation period. Rennie et al. (1976) considered this lack of agreement to be significant in view of the intensive vs extensive soil management practices (i.e. high vs very low fertiliser nitrogen inputs) in the Midwest and Great Plains regions respectively.

8.3.0 ISOTOPE FRACTIONATIONS DURING CHEMICAL, PHYSICAL AND BIOLOGICAL REACTIONS

8.3.1 Isotope Effects During the Fixation of Nitrogen

Hoering and Ford (1959) studied nitrogen fixation by four species of *Azotobacter* and found no consistent isotope effect. They concluded that the rate determining step in the mechanism did not therefore involve the bonding of nitrogen. Delwiche and Steyn, (1970) working with *Azotobacter vinelandii* found an isotope discrimination effect giving a β of 1.004. β is defined as R_a/R_f with $R_a = {}^{15}N/{}^{14}N$ in the atmosphere and $R_f = {}^{15}N/{}^{14}N$ in the nitrogen fixed. This compares with a β of 1.0022 reported by Hoering and Ford (1959). Delwiche and Steyn (1970) stated that it was probable that differing results could be obtained with any species depending upon the conditions of observation, and it was difficult to define what might be the rate limiting factor under natural conditions. The fixation of ${}^{14}N$ appeared to be favoured slightly under the conditions studied.

Measurement of the isotopic composition of clover, grass and soil nitrogen collected from the same site showed that the $\delta^{15}N$ value of the clover was less than that of the grass or total soil nitrogen (Delwiche and Steyn, 1970). The suggestion was made that much of the nitrogen in the clover had its origin in atmospheric nitrogen whereas that of the grass came primarily from the soil.

Rennie et al. (1976) compared the $\delta^{15}N$ value of nitrogen in faba beans and that in barley to estimate the amount of nitrogen in the faba bean that was derived from symbiotic nitrogen fixation. They assumed that if the faba bean derived its nitrogen from two sources, namely the air and the soil, it was possible to estimate the amount of nitrogen derived from each source. This theory also assumed equal discrimination

in the uptake of ions by both crops, an assumption which was neither substantiated nor disproved.

8.3.2 Isotope Effects During Denitrification

Wellman et al. (1968) and Delwich and Steyn (1970) examined isotope fractionation during denitrification by *Pseudomonas denitrificans*, and reported β values of 1.02 and 1.017 respectively. Both of these values are smaller than the theoretical value for simple bond rupture. For the breaking of a simple diatomic N-O bond the ratio of isotopic partition functions for $^{15}NO:^{14}NO$ is 1.0659 at 298.1 ^{o}K (Urey, 1947). If this value is multiplied by the inverse of the square root of the isotopic reduced mass ratios (Bigeleisen, 1949), a theoretical value of 1.09 for the maximum kinetic isotope effect in N-O rupture is found. Wellman et al. (1968) concluded that the N-O scission step was not totally rate controlling, and this conclusion is consistant with their observation that NO_3^- was exhausted at sixty percent production of N_2.

Wellman et al. (1968) also reported that preferential reduction of $^{14}NO_3^-$ and $^{14}NO_2^-$ has been demonstrated with other denitrifiers (a *Bacillus* and an *Alcaligenes* isolated from soils). The nett result of denitrification would therefore be a net retention of ^{15}N in the soil system in excess of ^{14}N when compared with atmospheric nitrogen.

8.3.3 Isotope Discrimination During Nitrification

Delwich and Steyn (1970) examined the nitrification reaction for discrimination using *Nitrosomonas europaea* cultures. Calculations for isotope discrimination where the substrate is limited have been presented by Tong and Yankwich (1957). Four measurable quantities are: the isotope ratio of the starting material, R_{ao} (which is the same as that of the accumulated product at complete reaction $R_{b\infty}$); the isotope ratio of

the residual reactant at time t, R_{at}; the isotope ratio of the product accumulated to time t, R_{bt}; and the reaction co-ordinate at time t, f. If we define a quantity g as

$$g = (A'')_t/(A'')_o = e^{-k''\int Fdt}$$

then $(A')_t/(A')_o = e^{-k''\int Fdt} = g^r$.

The above four input data can then be expressed as

$$R_{ao} = R_{b\infty} = \frac{(A'')_o}{(A')_o}$$

$$R_{at} = \frac{(A'')_t}{(A')_t} = \frac{R_{ao}}{g^{r-1}}$$

$$R_{bt} = \frac{(B'')_t}{(B')_{t'}} = \frac{R_{ao}(1-g)}{(1-g^r)}$$

$$f = \frac{(B')_t + (B'')_t}{(A')_o + (A'')_o} = \frac{(1-g) + R_{ao}(1-g)}{1+R_{ao}}$$

When R_{at}, R_{bt} and f are determined

$$\beta = \frac{\ln\left[\frac{1}{(1-f)} - \frac{f(R_{bt} - R_{at})}{(1-f)\ (R_{bt} + 1)}\right]}{\ln\left[\frac{1}{(1-f)} + \frac{f(R_{bt} - R_{at})}{(1-f)\ R_{at}\ (R_{bt} + 1)}\right]}$$

β for the nitrification reaction has been reported as 1.026 (Delwiche and Steyn, 1970) and 1.0169 (Freyer and Aly, 1974). Therefore, in a reaction which does not go to completion, ^{15}N would tend to accumulate in the reduced nitrogen fraction and the nitrate formed would be depleted of the heavy isotope, the extent of isotope depletion realised would be a function of the completeness of the reaction.

To date, no published data has appeared on the isotope discrimination during oxidation of nitrite by *Nitrobacter* spp. but this may be expected to be of importance only under certain circumstances, as nitrite does not normally occur in soils.

8.3.4 Isotope Fractionation During Ammonia Volatilisation

Most soils in New Zealand are acidic, and because of this, any NH_3 which enters the soil is rapidly converted to NH_4^+.

$$NH_3 + H \rightleftharpoons NH_4^+ \quad K = 9.34$$

However, under urine patches where a high concentration of urea occurs, the hydrolysis of urea increases the pH of the urine spot to such an extent that NH_3 may be lost from the system as a gas. If any gaseous ammonia is lost, an equilibrium isotope fractionation can occur.

$$^{15}NH_{3\,(gas)} + {}^{14}NH_{4\,(aq)}^{+} \rightleftharpoons {}^{14}NH_{3\,(gas)} + {}^{15}NH_{4\,(aq)}^{+}$$

$$\text{Isotopic equilibrium constant} = \frac{\left[{}^{15}N/{}^{14}NH_4^+\right]_{(aq)}}{\left[{}^{15}N/{}^{14}NH_3\right]_{(g)}}$$

$$= 1.034$$

As a result of this, NH_3 depleted in ^{15}N will be lost.

8.3.5 Isotope Discrimination During Nitrogen Assimilation

Azotobacter vinelandii, *Hansenula californica*, *Pichia terricola* and *Schwanniomyces alluvius* all preferentially utilised $^{14}NH_4^+$, concentrating ^{15}N in the unassimilated ammonium, provided assimilation was not complete (Delwiche and Steyn, 1970). The kinetic isotope effect was greater in the bacterium than in the yeasts, and also greater during assimilation than during fixation by *A. vinelandii*.

No studies have been reported on isotopic fractionation during assimilation of nitrogen by plants.

8.3.6 Other Possible Mechanisms For Isotope Fractionation in Soils

The chemical properties of ^{14}N and ^{15}N are identical, and the

separation of the nitrogen isotopes resulting in the concentration of either depends on their differences in mass, and therefore physical properties (Hauck, 1973). Spedding et al. (1955) accomplished an almost complete separation of ^{14}N and ^{15}N in ammonia by means of ion-exchange resins. It is possible that the soil colloid could also serve as a fractionating medium with the chromatographic fractionation of both cationic and anionic isotopic species. β values of 0.9993 for retention of NH_4Cl on Dowex 5 resin, 1.0021 for retention of KNO_3 on Dowex 1 resin and 0.992 for retention of NH_4Cl on Ione clay (a kaolinitic clay soil) were determined by Delwiche and Steyn (1970). The fractionation factors obtained were small in comparison with values for isotope fractionation in biological reactions. It is possible, however, that the chromatographic process with downward movement of ions in soil could introduce significant separation of isotopes.

8.4.0 THE USE OF SMALL VARIATIONS IN ^{15}N ABUNDANCE FOR TRACER STUDIES

Any naturally occurring substance with a positive or negative $\delta^{15}N$ value can be considered as a tracer. Kohl et al. (1971) attempted to estimate the fractional contribution of soil organic nitrogen and fertiliser nitrogen to nitrate levels of the Sangamon river and Lake Decatur, Illinois. Their approach was based on the fact that a $\delta^{15}N$ difference of 10‰ existed between fertiliser nitrogen ($\delta^{15}N = +3‰$) and soil nitrogen ($\delta^{15}N = +13‰$). It was assumed that if all the nitrate in the waters was derived from fertiliser, the $\delta^{15}N$ value of nitrate would be the same as that of the fertiliser (i.e. $\delta^{15}N = +3‰$). If fertiliser nitrogen did not contribute to the nitrate in the waters, then the $\delta^{15}N$ value would be the same as soil derived nitrate (i.e. $\delta^{15}N = +13‰$). Intermediate values were assumed to reflect the fractional contribution.

The approach of Kohl et al. (1971) was severely criticised by Hauck et al. (1972) on the following basis:

1) The value of $\delta^{15}N = +3‰$ used by Kohl et al., presupposes that fertiliser nitrogen enters into and is released from the soil organic complex without a change in its isotopic identity, or else that all fertiliser-derived nitrate is formed from the fertiliser directly.

2) That the $\delta^{15}N = +13‰$ for soil derived nitrate was not representative of the total area of the study, 2.331 square kilometers, because of inherent variation in the isotope composition of the soils and also because the $\delta^{15}N$ value of mineralised nitrogen is dependent upon the time of incubation.

3) The effect of biological isotope fractionation could not be evaluated in the study.

4) Other sources of nitrate input (e.g. rainfall and symbiotic nitrogen fixation) were not properly considered.

A comparison was made of the use of ^{15}N depleted, unenriched, and ^{15}N enriched fertilisers in determining the contribution of inorganic ammonium fertilisers to the nitrate nitrogen extracted from Webster soil following a 30°C aerobic incubation for two to five weeks by Edwards (1973). The natural abundance approach was considered to be unsatisfactory largely due to replicate variability, the fluctuations in $\delta^{15}N$ values of the nitrate from replicated samples of each fertiliser treatment being of the same order of magnitude as the mean difference between the $\delta^{15}N$ values of natural fertiliser nitrogen and the soil derived nitrate nitrogen Fertiliser ammonium in soil, therefore, largely loses its isotopic identity. Bremner and Tabatabai (1973) concluded that it was difficult to characterise $\delta^{15}N$ values of soil derived nitrate since they depended upon the time of incubation and often differed significantly from those

of total soil nitrogen. An analogous study was carried out by Meints et al. (1975). *Sorghum vulgare* var. *sudanense Hitche* was grown on three Illinois soils which were either unfertilised or fertilised with unenriched or ^{15}N enriched $(NH_4)_2SO_4$. In this experiment, the $\delta^{15}N$ values of the total nitrogen of plant tissue samples from plots receiving unenriched N were significantly lower than those of unfertilised samples. The conclusions drawn from this experiment were diametrically opposite to those of Edwards (1973). Edward's experiment was essentially repeated by Feigin et al. (1974c) using soil from central Illinois. Under the conditions of this experiment, the difference between the two sources of nitrate was maintained as the nitrogen atoms underwent various transformations in the soil. Estimates of percent fertiliser derived nitrate based on natural variation of ^{15}N were either significantly lower than or not significantly different from estimates based on ^{15}N enriched fertiliser. It was concluded that, with certain soils and under certain conditions, variations in the natural abundance of ^{15}N allow the detection of fertiliser nitrogen in nitrates produced by soils, although estimates based on such variations were neither very precise nor very accurate in comparison with estimates using artifically enriched materials.

In a recent study, Black and Waring (1977) investigated the ^{15}N abundance in components from a 100 ha catchment of uniform soil, and concluded that because of the large variability, even qualitative estimates of the relative contribution of various nitrogen sources to nitrogen in soil and water using variations in the natural abundance of ^{15}N seemed very doubtful. They based their conclusion on several factors:

i) $\delta^{15}N$ values for nitrogen fertilisers ranged from -4.1 to +1.0‰.

ii) Total soil nitrogen showed $\delta^{15}N$ values ranging from 2.9 to 6.7‰.

iii) The $\delta^{15}N$ value for nitrate in one of the major forms of nitrogen fertiliser sources was within the range of values for the $\delta^{15}N$ nitrogen released by mineralisation.

iv) The $\delta^{15}N$ of nitrate released by mineralisation from three sites ranged from 0.8 to 4.0‰ and no consistant relationship existed between the $\delta^{15}N$ of total nitrogen and nitrate released by mineralisation.

It appears that variations in the natural abundance of ^{15}N in agricultural systems are of little use at present in studying nitrogen transformations under field conditions because of the inherent variability in $\delta^{15}N$ values.

8.5.0 VARIATION IN THE ISOTOPIC COMPOSITION OF FERTILISER NITROGEN

Freyer and Aly (1974) reported that the most commonly used ammonium and nitrate fertilisers have different $\delta^{15}N$ values (table 8.4), and in general, ammonium nitrogen is depleted in ^{15}N relative to atmospheric nitrogen because the lighter ^{14}N isotope is kinetically favoured in the ammonia synthesis. Nitrate nitrogen was reported to have higher $\delta^{15}N$ values than ammonium nitrogen and atmospheric nitrogen. This is the result of isotopic exchange reactions occurring after the oxidation of ammonia in the production of nitric acid (Mahenc, 1965; Spindel, 1954). Freyer and Aly (1974) stated that the different $\delta^{15}N$ values found depend on temperature, concentration, and pressure in the production process.

Edwards (1973) and Shearer et al. (1974b) also reported negative $\delta^{15}N$ values for fertiliser ammonium. Rennie et al. (1976), however, reported that although $\delta^{15}N$ values of ammonium nitrogen in a selected number of commercial fertilisers were consistantly lower than for nitrate nitrogen, in no instance were highly negative values found. These data

Table 8.4 $\delta^{15}N$ values of some commercial fertilisers

Fertiliser	$\delta^{15}N$ NO_3^--N	$\delta^{15}N$ NH_4^+-N	$\delta^{15}N$ Reduced N	Reference
Anhydrous NH_3		+3.6		Shearer et al. 1974
Anhydrous NH_3		-2.7		Shearer et al. 1974
$(NH_4)_2HPO_4$		+1.0		Shearer et al. 1974
$(NH_4)_2HPO_4$		-0.4		Shearer et al. 1974
$(NH_4)_2HPO_4$		-1.6		Shearer et al. 1974
$(NH_4)_2SO_4$		-1.1		Shearer et al. 1974
$(NH_4)_2SO_4$		-1.9		Shearer et al. 1974
$(NH_4)_2SO_4$		+0.8		Shearer et al. 1974
$(NH_4)_2SO_4$		-4.1		Black and Waring, 1977
NH_4NO_3	+1.0	-2.5		Shearer et al. 1974
NH_4NO_3	+4.4	-2.7		Shearer et al. 1974
NH_4NO_3	+1.6	-0.7		Shearer et al. 1974
NH_4NO_3	+1.6	-1.8		Shearer et al. 1974
NH_4NO_3	+1.8	-0.6		Shearer et al. 1974
NH_4NO_3	+1.6	-4.6		Shearer et al. 1974
NH_4NO_3	+1.9	-1.1		Black and Waring, 1977
NH_4NO_3	+2.1	-1.2		Freyer and Aly, 1974
Urea			+0.9	Shearer et al. 1974
Urea			+1.9	Shearer et al. 1974
Urea			-1.7	Shearer et al. 1974
Urea			-2.1	Black and Waring, 1977

conflict with Kohl's et al. (1971) who reported a $\delta^{15}N$ value for anhydrous ammonia ($\delta^{15}N = +3.7‰$) greater than that for fertiliser nitrate. The $\delta^{15}N$ values of Australian nitrogen fertiliser reported by Black and Waring agree with those reported above.

It is apparent that the $\delta^{15}N$ values for both ammonium and nitrate fertilisers can be expected to vary widely and this places a severe restriction on the possibility of relating variation in ^{15}N abundance of inorganic or organic soil nitrogen to past fertiliser nitrogen use. It may be, however, that some of the differences in $\delta^{15}N$ values reported arise from variations in analytical techniques between laboratories.

8.6.0 RESEARCH REQUIREMENTS

No data is available on variations in the natural abundance of ^{15}N in New Zealand agricultural systems. Because of this, it was decided to collect some basic ^{15}N data for New Zealand grassland systems in order to establish whether or not such data may be used for studies of nitrogen transformations.

CHAPTER 9

METHOD OF ISOTOPE ANALYSIS

9.1.0 INTRODUCTION

To determine the isotopic composition of stable elements by mass spectrometery, it is necessary that the element under analysis be converted to a suitable gas. In the case of nitrogen, the most suitable gas is molecular nitrogen.

Preparation of samples for isotopic analysis of nitrogen involves three steps:

a) Conversion of the nitrogen to ammonium nitrogen.

b) Separation of the ammonium nitrogen from the reagents used to prepare the ammonium nitrogen.

c) Conversion of the ammonium nitrogen to molecular nitrogen.

For accurate isotope analysis, all procedures employed must be quantitative, in order to avoid possible isotope fractionation.

9.2.0 SAMPLE PREPARATION AND THE CONVERSION OF NITROGEN TO AMMONIUM NITROGEN

9.2.1 Total Soil Nitrogen

Soil samples were air dried and ground in a ring mill to pass a 120 mesh sieve. Total nitrogen in a soil sample containing approximately 1 mg nitrogen was converted to ammonium nitrogen by Kjeldahl digestion following pretreatment with reduced iron to convert nitrite and nitrate nitrogen to ammonium nitrogen, as outlined in section 3.2.3. Digestion was carried out in 24 x 200 mm test tubes, fitted with tear drop stoppers to prevent excessive loss of acid during digestion, seated in an aluminium block heated by an electric element. The temperature of digestion was carefully controlled so as to prevent loss of nitrogen by overheating (Bremner, 1965) accompanied by isotopic fractionation. The correct ratio of catalyst mixture (K_2SO_4:$CuSO_4.5H_2O$:Se;10:1:1 mixture) to concentrated

H_2SO_4 will maintain a digestion temperature of 320-330^{o}C, but thermometers were included in some digestion tubes as a check during digestion.

Separation of the ammonium from the digestion mixture was accomplished by steam distillation of the total digestion mixture following addition of 20 ml of 40 percent NaOH. The distillate was collected in boric acid solution (20 ml boric acid per litre), the amount of ammonium nitrogen being determined potentiometrically by titration with 0.0025M H_2SO_4. The distillation unit was fitted with a silver condenser to prevent ammonium absorption. Between samples the distillation unit was cleaned by back-flushing the condenser and still with distilled water followed by steaming for three minutes. When enriched ^{15}N samples were steam distilled, 1 mg of unenriched NH_4^+ was steam distilled between each sample followed by steaming for three minutes after backflushing with distilled water.

Following titration, the distillate was adjusted to pH 3.0 with 0.1M H_2SO_4 and concentrated to 2 - 3 ml on a hot plate at 80 - 90^{o}C. The concentrated distillate was then washed into a 10 ml screw-top vial using distilled water and again concentrated to 2 - 3 ml on a hot plate, followed by evaporation to dryness in an oven at 100^{o}C. When dried, the vial was sealed and stored at -20^{o}C until isotopic analysis.

All glassware used for preparation of isotope samples was washed with two percent aqueous hydrofluoric acid to remove contamination.

9.2.2 Total Plant Nitrogen

Plant samples were prepared for isotopic analysis by Kjeldahl digestion after drying at 85^{o}C and grinding in a Thomas-Wiley intermediate series 3383 mill fitted with a 60 mesh sieve.

9.2.3 Nitrate in Water

Ammonium in water samples was removed by steam distillation in the presence of MgO. Nitrate nitrogen was then distilled after reduction to NH_4^+ using Devarda's alloy (ground to pass a 100 mesh sieve, 50 Cu:5 Al:5 Zn).

Where it was necessary to concentrate a sample prior to steam distillation, this was accomplished by removal of water by vacuum distillation at 50°C in a rotary distillation unit. $HgCl_2$ (40 mg Hg/ℓ) was added to all samples prior to distillation to prevent any microbial activity, and the pH of the sample was adjusted to approximately 6.0 to prevent loss of NO_3^- from acid samples.

9.2.4 Total Blood Nitrogen

Blood samples were prepared for isotope analysis by Kjeldahl digestion as outlined in section 3.2.3.

9.2.5 Urine Samples

Urine samples were prepared for isotope analysis by Kjeldahl digestion as outlined in section 3.2.3.

9.2.6 TOTAL FAECAL NITROGEN

Faecal samples were freeze dried and ground to pass a 100 mesh sieve. Samples were then prepared by Kjeldahl digestion as outlined in section 3.2.3.

9.3.0 CONVERSION OF AMMONIUM NITROGEN TO MOLECULAR NITROGEN

Conversion of ammonium nitrogen to molecular nitrogen was carried out in a modified conversion apparatus designed by Ross and Martin (1970), (figure 9.1).

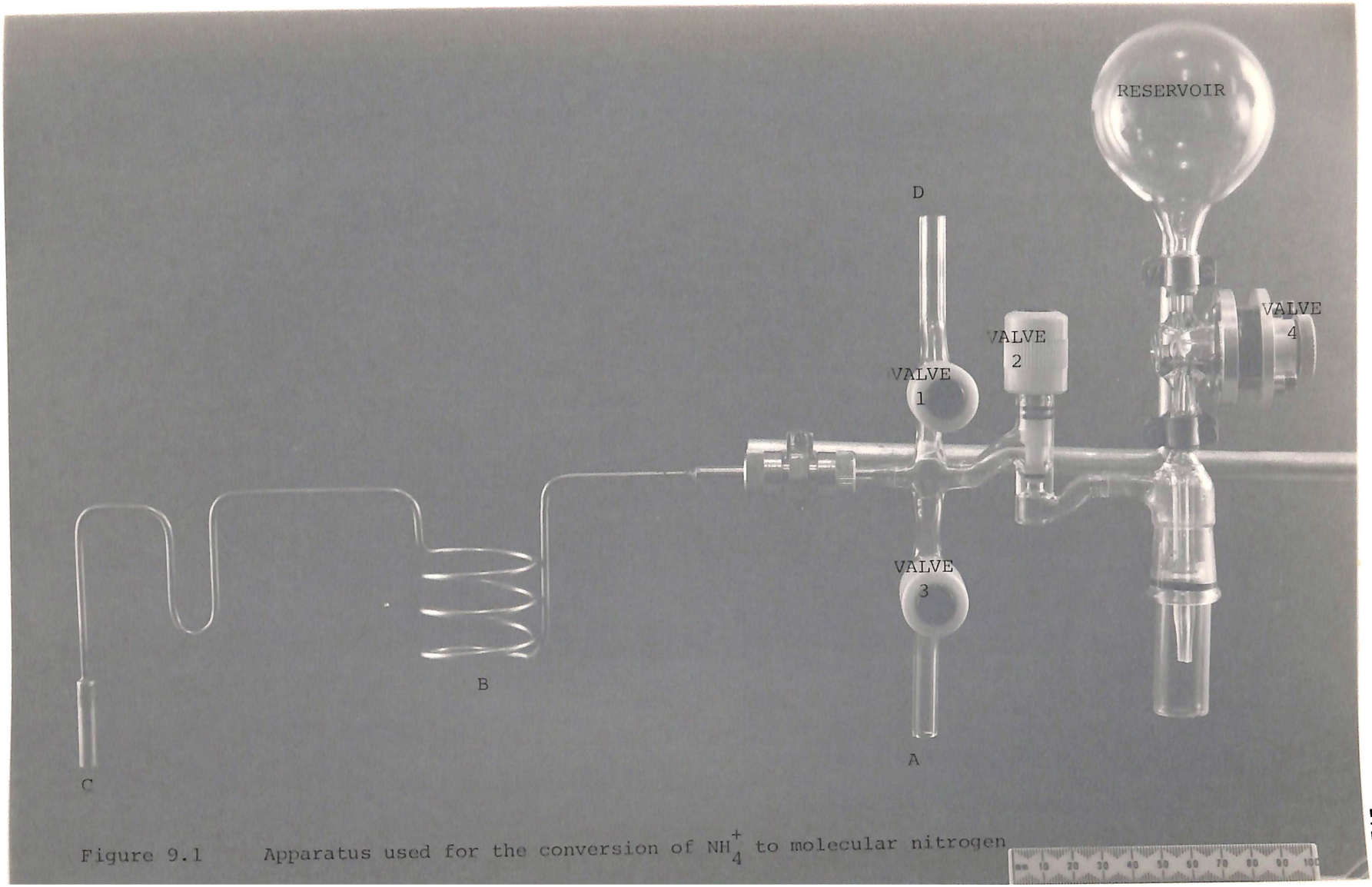

Figure 9.1 Apparatus used for the conversion of NH_4^+ to molecular nitrogen

The 120 ml reservoir contained air-free hypobromite solution under an atmosphere of helium. A was connected to a rotary vacuum pump, C to the mass spectrometer inlet system, and D was open to the atmosphere.

Lithium hypobromite was used in preference to sodium hypobromite as the former is more stable. Lithium hypobromite solution was prepared by adding 4 ml of analytical reagent grade bromine to 120 ml of cold (0 - 5^{o}C) 10 percent w/v solution of analytical reagent grade lithium hydroxide and stirring until the bromine dissolved. This solution was degassed after addition to the reservoir and placed under an atmosphere of helium. One millilitre of this solution will oxidise approximately 5 mg of nitrogen. The reaction may be represented as:

$$2NH_3 + 3LiOBr = 3LiBr + 3H_2O + N_2$$

An 'O' ring was placed over the top of a 10 ml sample vial and the sample vial attached to the 'U' socket by closing valve 1 and opening valves 2 and 3. The 'U' socket was lightly greased so as to provide a vacuum tight seal. After the conversion apparatus was evacuated the rotary vacuum pump was closed off by closing valve 3 and the inlet valve to the mass spectrometer opened so that the conversion apparatus became part of the vacuum system of the mass spectrometer.

All isotopic measurements were carried out on a Micromass 602C instrument equipped with a twin inlet system and dual collector plates. The programmed power supply unit of the instrument supplied the accelerating potential and can be operated in either a manual or scan mode. The programmed power supply was used in the manual position when the instrument was operated in the ratio mode as this provided better stability. However, the scan position was used to check for vacuum leaks in the conversion assembly. To do this the mass spectrometer was focused on the peak at mass 28 using the scan mode. If there was no appreciable rise in the mass 28 peak when the mass spectrometer was

connected to the conversion assembly, the vacuum was considered to be satisfactory. If a serious vacuum leak was present it would also be detectable on the Pirani gauge.

To convert ammonium nitrogen to molecular nitrogen, the mass spectrometer inlet capillary was shut off from the conversion assembly but the variable reservoir of the mass spectrometer inlet system was left connected. Valves 1, 2 and 3 were closed and the sample vial immersed to a depth of approximately 10 mm in a dry-ice acetone freezing mixture. At the same time the stainless steel helix (B) was immersed in liquid nitrogen. After a few seconds, valve 4 was opened and approximately 1 ml of hypobromite solution admitted to the reaction chamber. When all the hypobromite solution was frozen, valve 2 was opened to admit the nitrogen to the mass spectrometer inlet system. Valve 2 was then closed, the hypobromite solution thawed to release the remaining nitrogen, refrozen, and valve 2 opened again to allow the remaining gas to enter the mass spectrometer inlet system. If this latter procedure was not carried out, it was found that some isotopic fractionation occurred, especially in highly enriched samples. The dry-ice acetone cold trap had two functions. Firstly it reduced the vapour pressure of ammonia to a very small value hence preventing contamination of the reaction chamber by retention of ammonia. This precaution was necessary as when alkaline hypobromite solution is added to ammonium salts under vacuum, ammonia will be produced unless sufficient hypobromite is present to oxidise it before it can escape from solution. Secondly, by freezing the hypobromite completely it prevented the possibility of carrying solution over into the liquid nitrogen cold trap causing possible blocking.

When all the nitrogen gas was in the mass spectrometer inlet system, the conversion assembly was closed off from the mass spectrometer inlet

system and valve 1 opened to release the vacuum. The sample vial could now be removed. The inside and outside of the capillary tube connecting the reservoir to the reaction chamber was rinsed with distilled water after each sample, the liquid nitrogen cold trap removed from the helix, and the helix heated with a hot air gun while the helix was under vacuum. This was gained by closing valves 1 and 2 and opening valve 3. It is essential to remove all traces of water from the cold trap between samples as there is a possibility of memory effects caused by the adsorption of nitrogen gas on ice particles in the trap.

A new sample vial was normally attached to the conversion apparatus while the isotopic composition of the previous sample was being determined. This allowed a minium of ten minutes for degassing the conversion assembly. Sample vials were normally discarded after use. If vials were to be reused, they were washed in 2 percent aqueous hydrofluoric acid to remove contamination.

The helix of the nitrogen trap was made of stainless steel tube of 2 mm external diameter. The length of tubing in the helix was 300 mm. This provided a highly efficient liquid nitrogen trap with a small heat capacity. The liquid nitrogen trap prevented water from entering the mass spectrometer and removed nitrous oxide from the sample. Nitrous oxide is produced during the oxidation of ammonium salts with hypobromite and interferes in the measurement of mass 30 peak, which was used for measurement of high enrichments. When measuring low enrichments, only mass 28 and 29 peaks were used, and nitrous oxide will not therefore cause contamination problems.

Following reaction of $(NH_4)_2SO_4$ with alkaline hypobromite, nitrogen ions of $(^{14}N^{14}N)^+$, $(^{14}N^{15}N)^+$ and $(^{15}N^{15}N)^+$, mass 28, 29 and 30, appear in the mass spectrum of N_2 gas. The relative number of ions of each

type approaches the ideal statistical values given by:

$$(p + q)^2 = p^2 + 2pq + q^2 \qquad (9.1)$$

where p is the atom fraction of ^{14}N, q the atom fraction of ^{15}N, and p plus q is equal to unity (Hauck et al. 1958). It is therefore necessary only to measure mass 28 and mass 29. Any effect of small differences in ionization probabilities of the various isotopic molecules is removed by referring all mass ratio values to those obtained with standard samples measured on the same instrument.

9.4.0 NITROGEN STANDARDS FOR MASS SPECTROMETRY

The internationally accepted standard for nitrogen isotopic analysis is atmospheric nitrogen, the isotopic composition of which remains relatively constant (Dole et al. 1954), and has been determined precisely (Nier, 1950). In the present study a working standard of ammonium sulphate (analytical reagent, Ajax chemicals) was used. One ml aliquots of a 1000 ppm N solution in 0.1M H_2SO_4 were pipetted into 10 ml sample vials and stored frozen. Sufficient sample vials were prepared to last the entire study. When required, the standards were evaporated to dryness and the ammonium nitrogen converted to molecular nitrogen in the normal manner (see section 9.3).

The working standard was calibrated against atmospheric nitrogen prepared for isotopic analysis by exposure of air to Fiesers solution to remove oxygen (Soloway, 1951). Whenever a working standard was introduced into the mass spectrometer, considerable care was taken to ensure that no atmospheric contamination of the standard occurred. This was done by scanning for mass 32 (oxygen) and mass 40 (argon) peaks. The presence of oxygen alone cannot be used as a measure of atmospheric contamination as some oxygen may originate from decomposition of the hypobromite solution. The presence of argon, however, can be

used to determine whether or not the standard has been contaminated by the atmosphere. If an argon peak was detected, the standard was discarded and another prepared. Finally, before the reference was used, it was compared to another reference sample. This was done to ensure that no isotopic fractionation had occurred during the preparation of the standard.

All isotopic compositions are reported as per mil enrichment of ^{15}N relative to atmospheric nitrogen. The following procedure was used to change from the working standard to atmospheric nitrogen (after Hulston, 1962).

By definition:

$$\delta N^{15} = \frac{\left[\frac{^{15}N}{^{14}N}\right]_x - \left[\frac{^{15}N}{^{14}N}\right]_{std.}}{\left[\frac{^{15}N}{^{14}N}\right]_{std.}} \times 1000 \qquad (9.2)$$

Let x represent the unknown,

w the working standard,

s the international standard (atmospheric nitrogen),

w.r.t. with respect to.

From equation 9.2

$$\left[\frac{^{15}N}{^{14}N}\right]_x = \left[\frac{^{15}N}{^{14}N}\right]_w \cdot 1 + \frac{^{15}N\ (x\ w.r.t.\ w)}{1000} \qquad (9.3)$$

Substituting for $\left[^{15}N/^{14}N\right]_x$ and $\left[^{15}N/^{14}N\right]_w$ by means of equation 9.2 it is found that:

$$\left[\frac{^{15}N}{^{14}N}\right]_s \cdot 1 + \frac{^{15}N\ (x\ w.r.t.\ w)}{1000} = \left[\frac{^{15}N}{^{14}N}\right]_s \cdot 1 + \frac{^{15}N\ (x\ w.r.t.\ s)}{1000}$$

$$\cdot\ 1 + \frac{^{15}N\ (x\ w.r.t.\ w)}{1000} \qquad (9.4)$$

Hence:

$$\delta^{15}N \text{ (x w.r.t. s)} = \delta^{15}N \text{ (w w.r.t. s)} + \delta^{15}N \text{ (x w.r.t. w)} + \frac{\delta^{15}N \text{ (w w.r.t. s)} \cdot \delta^{15}N \text{ (x w.r.t. w)}}{1000} \quad (9.5)$$

or:

$$\delta^{15}N \text{ (x w.r.t. s)} = 1 + \frac{\delta^{15}N \text{ (w w.r.t. s)}}{1000} \cdot \delta^{15}N \text{ (x w.r.t. w)} + \delta^{15}N \text{ (w w.r.t. s)} \quad (9.6)$$

The composition of the working standard relative to atmospheric nitrogen was +0.22‰ which is similar to the $\delta^{15}N$ for $(NH_4)_2SO_4$ reported by Shearer et al.(1974).

9.5.0 CALCULATION OF MEASURED UNCORRECTED δ VALUES

If a mass spectrometer were completely stable and no ion beam fluctuation or instrumental drift occurred with time, then δ, the per mil difference between the isotope ratio of a measured sample (R_S) and that of a reference of known isotope ratio (R_R), could be estimated from a single measurement of the difference $R_S - R_R$. However, because of fluctuations in ion beams and instrumental drift, several independent determinations of $R_S - R_R$ are required for a precise estimation of δ. This is normally accomplished by making several alternative determinations of R_S and R_R and thereby several interpolated estimates of $R_S - R_R$.

For estimation of a δ value, the coarse isotopic ratio (R_C) is preset on the mass spectrometer for near balance of the major and minor ion beams, and the remaining imbalance for the reference (r) and sample (s) determined from a chart recording or by using a digital integrator. Therefore $R_R = R_C + r$ and $R_S = R_C + s$.

A close examination of the method for calculation of $\delta^{15}N$ values and its associated standard error outlined in the Micromass manual,

(Appendix 4), a method which is widely accepted (A.T. Wilson, pers. comm.), showed it to be statistically incorrect (C. Dyson, pers. comm.) for several reasons:

1) $\bar{r}$ as the mean of the r's suffered in being on the average half a cycle later than s and hence $\bar{s}$. Therefore the assumption that $\bar{X}$, the mean difference between s and r, and $\bar{r}$ are quite compatible is incorrect. ($\bar{X}$ is symmetric in r and s with respect to time).

2) Ten estimates of X were generated from 6 r and 6 s determinations and assumed independent.

3) The estimates of X were also "smoothed" and this was not taken into account in the calculations.

Hence a new method for calculation of measured uncorrected δ values for mass spectrometric analysis was derived.

For a series of consecutive determinations of r and s, commencing with a determination of r and ending with one of s:

$$r_1, s_1, r_2, s_2 \ldots\ldots\ldots\ldots r_{n-1}, s_{n-1}, r_n, s_n$$

it is desired to use the greatest possible number of largely independent estimations of s-r in an unbiased fashion so that δ has the smallest correctly determined stable confidence interval estimate it is possible to obtain. Since s and r are not determined simultaneously but alternately, and it is assumed, equally spaced in time, the difference in time must be considered in calculation of s-r values.

Let

$$x_1 = s_1 - \left[\frac{r_1 + r_2}{2}\right] \qquad y_1 = \left[\frac{s_1 + s_2}{2}\right] - r_2$$

$$x_2 = s_2 - \left[\frac{r_2 + r_3}{2}\right] y_2 \left[\frac{s_2 + s_3}{2}\right] - r_3$$

.....

$$x_{n-1} = s_{n-1} - \left[\frac{r_{n-1} + r_n}{2}\right] y_{n-1} \left[\frac{s_{n-1} + s_n}{2}\right] - r_n \qquad (9.7)$$

therefore providing alternative sets of (n-1) largely independent estimations of (s-r).

$$\text{The mean value of } x, \bar{x} = \frac{\sum_{i=1}^{n-1} x}{n-1} \qquad (9.8)$$

Also, the mean value of r in the x column,

$$\bar{r} = \frac{\frac{1}{2}r_1 + r_2 + \ldots\ldots\ldots\ldots + r_{n-1} + \frac{1}{2}r_n}{n-1} \qquad (9.9)$$

By definition:

$$\delta \text{ (in } ‰) = \frac{R_S - R_R}{R_R} \times 1000 \qquad (9.10)$$

$$\therefore \delta \text{ (in } ‰) = \frac{\bar{x}}{R_C + \bar{r}} \times 1000 \qquad (9.11)$$

If the mass spectrometer has a ratio offset facility to change the coarse ratio of the sample by a given amount, thus enabling gases of high or low relative enrichment to be measured against a given standard, the amount of offset (R_O) can be simply included in equation (10.11) to give:

$$\delta ‰ = \frac{R_O + \bar{x}}{R_C + \bar{r}} \times 1000 \qquad (9.12)$$

$$\text{The variance of } x, \sigma_a^2 = \sum_{i=1}^{n-1} \frac{(x_i - \bar{x})^2}{n-2} \qquad (9.13)$$

Since each value of x is somewhat smoothed, σ_a^2 is artifically low and

a correction factor must be applied. The true variance of s-r, σ_o^2, is estimated by:

$$\sigma_o^2 = \frac{4\sigma_a^2}{3 - \frac{1}{n-1}} \qquad (9.14)$$

(J.E. Waller, pers. comm.). The standard error of δ, σ_m, is given by:

$$\sigma_m = \frac{\sqrt{\frac{\sigma_o^2}{n-1}}}{R_C + \bar{r}} \times 1000 \qquad (9.15)$$

and the confidence half-interval C_{n-1} by

$$C_{n-1} = t_{n-2}\,\sigma_m \qquad (9.16)$$

t_{n-2} being the value of the t-statistic with (n-2) degrees of freedom at the appropriate significance level.

Although the above method of calculation will correct for linear drift, it does not correct for consistent curvilinear drift. Nor, for low degrees of freedom, is the value obtained for σ_m very stable. Using the y sequence of (s-r) values in (9.7) $\bar{y}$ replaces $\bar{x}$ in (9.8) above and σ_b^2 replaces σ_a^2 in (9.13). Now set $\bar{z} = \frac{1}{2}(\bar{x} + \bar{y})$ and $\sigma_c^2 = \frac{1}{2}(\sigma_a^2 + \sigma_b^2)$. Replacing $\bar{x}$ by $\bar{z}$ in (9.12), σ_a^2 by σ_c^2 in (9.14), and the number of degrees of freedom in (9.16) above 2n-4, we obtain a modified $\bar{z}$ corrected for curvilinear drift and an associated σ_c^2 stabilised by benefiting from twice the number of degrees of freedom. Note that $\bar{y}$ is highly dependent on $\bar{x}$ and should not be regarded as providing any further information apart from the correction for curvilinear drift. The individual y values are, however, somewhat independent of the x's and they do, therefore, provide valid further information on the variation of δ. Hence there is a change in the term involving n in (9.16) but not in (9.15). This modification does not correct for possible bias in $\bar{r}$ (in whose expression (9.9) $\frac{1}{2}r_1$ is changed to $\frac{1}{4}r_1$ and $\frac{1}{2}r_n$ to $\frac{3}{4}r_n$), but any effect is of small order and may usually be ignored.

Alternatively, if a sequence of s and r determination is begun with r, and completed with r_n, the combining of the two alternative determinations is much more complicated, there being different numbers of x's and y's. In this case means for $\bar{z}$ and σ_c^2 weighted according to the number of contributions must be used rather than simple means. This leads also to changes in formulae (9.8), (9.13), (9.14), (9.15) and (9.16) where the n-1 of the x analysis becomes n-2 in the y version, and $n^{-3/2}$ in the z version.

The arithmetic for $s_1, \ldots, r_n$ and $s_1, \ldots, s_n$ sequences corresponds to that presented here with r and s interchanged in equation (9.7) and then the signs of all x's and y's in (9.7) changed. Thereafter the procedure is precisely as given above.

It is also possible to include a test to determine if both s and r values are affected by drift to the same extent. Such a test would be useful in detection of contaminated samples which may produce a drift in s but not r. If the drift for s and r values were different, then δ becomes a function of time and not a constant. Differential drift between s and r may by tested by regressing linearly one set of the (s-r) pairs on the time at which each pair was determined. If s and r were affected differently by drift, a regression coefficient significantly different from zero would be obtained and the results from that determination rejected.

The number of s and r values required for the estimation of δ will depend to a large extent on the size of confidence interval sought. The product of the run length and the varying factor $\left(\frac{t_{2n-4}}{\sqrt{n-1}}\right)$ which multiplies σ_o towards obtaining the confidence interval shows a minimum value for a run length of about ten values. The expected ratio for Cn-1 for run lengths of eight, twelve and sixteen values (n-1 = 3, 5 and 7

resp) is 1.95 : 1.25 : 1 respectively. This demonstrates the superiority of a run length of twelve over that of eight, with only a moderate expected decrease in C_{n-1} by increasing the run length from twelve to sixteen. If it were standard practice to estimate σ values from six s and six r values (i.e. a run length of twelve), the 95% confidence interval of the isotopic enrichment would be expressed as:

$$(R_o + \bar{z} \pm \frac{2.306}{\sqrt{5}} \sqrt{\frac{4}{3 - 1/5}}\,\sigma_c) \times \frac{1000}{R_C \pm \bar{r}} \text{ ‰} \qquad (9.17)$$

using equations (9.12) and (9.15) to (9.17) in the Z version

$$\text{i.e. } (R_o + \bar{z} \pm 1.233\ \sigma_c) \times \frac{1000}{R_C \pm \bar{r}} \text{ ‰} \qquad (9.18)$$

where $\bar{z} = \frac{\bar{x} + \bar{y}}{2}$ and $\sigma_c^2 = \frac{\sigma_a^2 + \sigma_b^2}{2}$

9.6.0 CORRECTIONS APPLIED TO CALCULATED UNCORRECTED δ VALUES

To convert δ^1, the measured uncorrected value, to δ, the following equation was used:

$$\delta = \delta^1 (C_T \times C_M \times C_V) + C_E$$

where C_T is the tail contribution correction factor

C_M is the memory correction factor

C_V is the value mixing correction factor

C_E is the zero enrichment correction factor.

a) Tail Contribution, C_T

In some mass spectrometers, the tail of the large mass 28 peak may contribute to the relatively small mass 29 peak. If this occurs, the δ value will be underestimated.

If I_A = collector current by N_2 ions of mass 29, i the tail

contribution to mass 29 peak, and

$x = \frac{i}{I_{A_s} + i}$ = fractional tail contribution measured on the

standard, the corrected δ value is deduced from

$$R' = \frac{I_A + i}{I_B} \quad \text{and} \quad S' = \frac{I_{As} + i}{I_{Bs}} \tag{9.20}$$

Since $I_B = I_{Bs}$ as sample and standard are always measured at the same ion current,

$$R = R' - XS' \text{ and } S = S' - XS \tag{9.21}$$

Hence the corrected δ value can be calculated

$$\delta = \delta^1/(1-X) \tag{9.22}$$

On the Micromass 602C used, the tail contribution of mass 28 to mass 29 was undetectable, therefore no correction factor was required.

b) Zero Enrichment, C_E

Background peaks at masses 28, 29 and 30 are present in most mass spectrometers. Because of the relatively larger hydrocarbon contribution at mass 29 and the higher natural abundance of carbon isotopes which contribute to peaks arising from $C^{12}O^+$ and $C^{13}O^+$, the I_{28}/I_{29} background ratio may be greater than that for atmospheric nitrogen. Instrument factors may also lead to a small zero enrichment value. This can be measured by equilibrating the same sample between the two sides of the inlet system, and if required, providing the background peaks are stable and independent of sample pressure, a simple correction factor can be applied. For the instrument used, C_E was less than 0.02‰ and therefore no correction was necessary.

c) Memory Effect, C_M

Memory effects in the conversion assembly due to adsorption of nitrogen gas on ice particles, or ammonium adsorption on to glass surfaces have already been discussed (section 9.3). Memory effects may also occur due to hold up of nitrogen in the mass spectrometer inlet system. Such problems may be minimised by allowing sufficient pumping out time between samples, and by determining the isotopic composition of samples in batches of samples of similar isotopic composition.

d) Valve Mixing, C_V

In any mass spectrometer containing a change over valve, a certain amount of mixing of the sample and reference gases will occur in the change over valve due to seat leakage, but once again the mixing of samples was small and no correction was required.

9.7.0 OTHER POSSIBLE SOURCES OF ERROR

9.7.1 Errors Involved in Chemical Preparation of Samples

Errors may be introduced at any stage in the preparation which is not quantitative. Errors may be included in both the Kjeldahl digestion and the steam distillation step. Both are possible sources of isotopic fractionation.

For accuracy in ^{15}N measurements, care should be exercised during the following steps in sample preparation:

1) Cleanliness of glassware by washing in 2% aqueous hydrofluoric acid to remove contamination.
2) Careful temperature control during Kjeldahl digestion.
3) Sufficient distillation time for complete distillation.

4) If Devarda's alloy is used in the distillation step it must be finely ground to ensure a complete reduction of NO_3^-.

5) A silver condenser to prevent absorption of NH_4^+ on the condenser walls.

6) Thorough washing of still between samples.

7) Prevention of overheating samples during concentration following distillation.

8) Complete conversion of ammonium salt to N_2 gas.

It is preferable that the ammonium salt prepared for conversion to molecular nitrogen is ammonium sulphate, as this can be safely evaporated to dryness without loss provided the evaporating solution is below pH 3. Ammonium chloride, however, is volatile and extensive losses can occur during the evaporation stage of sample preparation.

9.7.2 Atmospheric Contamination of Samples

The contribution of atmospheric nitrogen to the sample was minimised by checking for a rise in the mass 28 peak following connection of the conversion assembly to the mass spectrometer inlet system and prior to the conversion of the sample to molecular nitrogen.

9.8.0 PRECISION AND ACCURACY OF ^{15}N DETERMINATIONS

Precision may be defined as the degree to which analysis of identical samples can be reproduced; and accuracy as the deviation of the observed value from the absolute value.

The reproducibility of measurements gives a criterion of the precision of a single analysis. Several replicates of a selection of samples were analysed and it was found that the standard deviation varied with the type of sample (table 9.1). This variation was probably, at least in part, related to the difficulty of obtaining a representative

Table 9.1 Determination of $\delta^{15}N$ values of six replicates of a soil, pasture, urine and faeces sample

	$\delta^{15}N$ Relative to Atmospheric Nitrogen			
	Soil	Pasture	Urine	Faeces
	+2.90	+0.63	-1.49	+2.74
	+3.16	+0.77	-1.37	+3.32
	+2.91	+0.89	-1.72	+3.09
	+2.66	+0.69	-1.18	+3.38
	+2.92	+0.83	-1.49	+3.13
	+2.74	+0.77	-1.67	+3.01
$\bar{x}$	+2.88	+0.76	-1.49	3.11
S.D.	0.17	0.09	0.20	0.23

Table 9.2 Reproducibility of isotope analysis of a standard on different dates

	+16.17
	+16.04
	+15.91
	+15.99
	+15.67
	+16.20
$\bar{x}$	+16.00
S.D.	0.19

sub-sample of the material as well as errors introduced during chemical preparation of samples.

During analysis, a slightly enriched sample of ammonium sulphate was determined on several different days to provide an estimate of day to day variation in determination of $\delta^{15}N$ values (table 9.2).

To avoid accidental errors, all isotopic analyses were determined in duplicate.

Because of possible isotopic fractionation in the analyser, and unknown exact values of input resistances, the determined isotopic composition of an unknown sample will deviate from the true isotopic ratio in an unknown manner. For this reason it is difficult to determine absolute abundance ratios with high precision. However, as errors in determination will be equal for sample and standard, it is possible to determine the difference in isotopic composition between a standard and sample with a high degree of precision. For this reason, accuracy in stable isotope mass spectrometry is only relative.

9.9.0 REPRODUCIBILITY OF $\delta^{15}N$ VALUES IN THE PRESENT STUDY

As can be seen from examples quoted above (e.g. tables 9.1 and 9.2), the total error involved in $\delta^{15}N$ determinations is less than $\pm$ 0.5‰. The standard error for the micromass measurement was in the range of 0.05‰.

CHAPTER 10

STUDIES OF THE FRACTIONATION OF ^{15}N AND ^{14}N DURING NITROGEN TRANSFORMATIONS WHICH OCCUR IN GRAZED GRASSLAND SYSTEMS

10.1.0 ISOTOPE EFFECTS IN THE SYMBIOTIC FIXATION OF ATMOSPHERIC NITROGEN BY CLOVERS

10.1.1 Introduction

A major problem in nitrogen balance studies in mixed clover/ryegrass grassland systems is the absence of a reliable quantitative method for measuring symbiotic nitrogen fixation. The use of ^{15}N tracers for measurement of symbiotic nitrogen fixation by ladino clover was reported by McAuliffe et al. (1958) and more recent investigations have been reported by Vallis et al. (1967), Henzell et al. (1968), Broeshart (1974) and Fried and Broeshart (1975). The ^{15}N method assumes that whereas clover has two possible sources from which to obtain its nitrogen, i.e. atmospheric or soil nitrogen, the associated grasses are entirely dependent on soil nitrogen. If the soil nitrogen is enriched in ^{15}N relative to atmospheric nitrogen, and assuming that the ^{15}N enrichment of soil nitrogen available to clover and associated grasses is the same, measurement of ^{15}N enrichments of clover and ryegrass herbage will allow calculation of the proportion of nitrogen in the clover herbage derived from atmospheric nitrogen.

Because of improvement in ^{15}N measuring techniques over recent years, it was considered that it may now be possible to develope a technique similar to the ^{15}N tracer method, but which relies solely on variations in the natural abundance of ^{15}N. Such a technique would have obvious benefits in nitrogen balance studies in mixed clover/ryegrass pastures.

Delwiche and Steyn (1970) compared the ^{15}N enrichment of clover and grass collected from a lawn under irrigation and found that the ^{15}N enrichment of clover was significantly less than that of grass or total soil nitrogen. They concluded that much of the nitrogen in clover had its origin in atmospheric nitrogen whereas that of the grass came primarily

from soil. That the difference in isotopic composition represented a distinction between atmospheric and soil nitrogen was supported by the results of another study which showed that the isotopic composition of nitrogen of soybean plants is a function of the level of cobalt in the nutrient medium provided, cobalt being required for fixation of atmospheric nitrogen.

The objective of the present study was to determine if it is possible to determine the amount of nitrogen in clover herbage derived from atmospheric nitrogen by measurement of the $\delta^{15}N$ values of the clover and ryegrass herbage.

10.2.0 DETERMINATION OF THE FRACTIONATION OF ATMOSPHERIC NITROGEN DURING SYMBIOTIC FIXATION : EXPERIMENTAL

White clover (*Grasslands Huia*) was grown in sand (0.2 - 2.0 mm particle size) culture in a glasshouse under various nitrogen treatments. Prior to use the quartz sand was soaked in thirty percent concentrated HCl for forty eight hours, continuously washed with tap water for twenty four hours, rinsed with distilled water, autoclaved and dried in an oven at 105°C for forty eight hours. Fourteen cm diameter pots were used, each pot containing 1.5 kg sand.

Thirty two clover seeds per pot were planted on May 18, 1976, and the following treatments applied:

1) Complete nutrient solution minus nitrogen.
2) Complete nutrient solution minus nitrogen until 6 weeks after germination, followed by complete nutrient solution containing 285 ppm N as NH_4NO_3.
3) Complete nutrient solution minus nitrogen until 4 weeks after germination, followed by complete nutrient solution containing 285 ppm N as NH_4NO_3.

4) Complete nutrient solution minus nitrogen until 2 weeks after germination, followed by complete nutrient solution containing 285 ppm N as NH_4NO_2.

5) Complete nutrient solution containing 285 ppm N as NH_4NO_2.

All treatments were replicated five times and innoculated with Rhizobia strain TAI on May 2, 1976.

The nutrient solution used was devised (K.R. Middleton, pers. comm.) to produce a clover plant with what could be considered as a balanced mineral content. The composition of the nutrient solution was:

1.405 g/ℓ K_2HPO_4

2.7447 g/ℓ KH_2PO_4

1.9916 g/ℓ K_2SO_4

2.9578 g/ℓ $MgSO_4.7H_2O$

0.2338 g/ℓ NaCl

1.029 g/ℓ $CaCl_2.2H_2O$

Trace elements were added according to Middleton and Toxopeus (1973). The pH of the nutrient solution was pH 6.5.

On average, 50 ml of the required nutrient solution was applied to pots twice weekly. Each pot was weighed daily and distilled water added as required to maintain the pots at seventy five percent of their water holding capacity.

The $\delta^{15}N$ of the NH_4^+ and NO_3^- nitrogen was adjusted to +10.52‰ and +10.59‰ respectively by addition of ^{15}N enriched $(NH_4)_2SO_4$ and $Ca(NO_3)_2$.

Clover seed germinated on May 22, 1976 and the seedlings were thinned to sixteen per pot on May 26, and to fourteen per pot on June 3. Clover herbage was harvested on August 2, 1976 by hand clipping to the sand surface. Herbage was dried for twelve hours at 85°C and weighed. Following removal of herbage the entire contents of the pot (sand + roots)

were emptied into a 2ℓ breaker and 1ℓ distilled water added. Roots were extracted by gentle agitation. KCl was then added to make an approximately 2M KCl solution and the mixture was stirred for one hour. A solution sample was then withdrawn for analysis of NH_4^+ and NO_3^- as outlined in section 3.2.3. Total nitrogen and $\delta^{15}N$ values for total nitrogen were determined on the plant herbage and root samples as outlined in chapter 9, and $\delta^{15}N$ values of NH_4^+ and NO_3^- determined as outlined in chapter 9.

10.2.1 Determination of $\delta^{15}N$ of Clover and Associated Ryegrass Herbage in a Field Trial

Herbage samples were provided by the Nutrition Centre, Ruakura Agricultural Research Centre, Hamilton. Samples of mixed pasture (white clover, ryegrass and paspallum) were hand clipped, dissected into the appropriate species, frozen in liquid nitrogen and freeze dried. All samples were collected from a single non-grazed plot. Total nitrogen and $\delta^{15}N$ for total nitrogen was determined on ryegrass and clover herbage as outlined in section 9.2.2.

10.2.2 Determination of the Fractionation of Atmospheric Nitrogen During Symbiotic Fixation : Results and Discussion

Dry matter yields of herbage, roots, and herbage + roots were dependent on the amount of nitrogen applied (table 10.1 , figure 10.1).

Since treatment 1 received no addition of nitrogen, the nitrogen in herbage and roots, after correction for seed nitrogen, can be assumed to be derived entirely from atmospheric nitrogen. Therefore measurement of $\delta^{15}N$ values of total plant nitrogen will provide an estimate of nitrogen isotopic fractionation during symbiotic fixation. To determine the variation between plants in herbage dry matter production and its

Table 10.1 Dry matter yield of clover herbage ± standard deviation and roots from a glasshouse experiment with plants receiving different amounts of applied nitrogen

Treatment	Herbage DM Yield (g/pot)	Root DM Yield (g/pot)	Total DM Yield (g/pot)
1	1.32 ± 1.10	0.59 ± 0.02	1.91 ± 0.09
2	1.50 ± 0.16	0.61 ± 0.05	2.11 ± 0.10
3	2.05 ± 0.12	0.73 ± 0.06	2.78 ± 0.17
4	2.37 ± 0.06	1.13 ± 0.11	3.50 ± 0.20
5	2.87 ± 0.16	1.14 ± 0.04	4.01 ± 0.40

DM = Dry Matter.

Figure 10.1 Clover plants grown with different amounts of fertiliser nitrogen. Treatments from left to right 1, 2, 3, 4, 5, 5. (For full description of treatments refer to text).

isotopic composition, the herbage of individual plants was harvested separately in one replicate of treatment 1 (table 10.2). From this data, the mean isotopic fractionation during symbiotic nitrogen fixation, as reflected by the isotopic composition of herbage nitrogen, appears small, i.e. -0.82‰, corresponding to a fractionation factor of 1.0008. This is in agreement with Hoering and Ford (1960) and Delwiche and Styne (1970) who reported small fractionation factors (β = 1.002 and 1.004 respectively) for nitrogen fixation by *Azobacter*. The small fractionation observed is not unexpected since the rate limiting step in nitrogen fixation does not involve the bonding of nitrogen.

Total nitrogen balances were calculated for each pot, and therefore it was possible to estimate the amount of nitrogen in clover tops and roots which was derived from atmospheric nitrogen (table 10.3). No nodules were present on the roots of plants in treatment five at harvest so it can be assumed that all the plant nitrogen was derived from fertiliser nitrogen as indicated by the mass balance. On average, 2.5 percent of the nitrogen applied to treatment five was not recovered at the conclusion of the experiment and this was considered to be within the experimental error of the technique used.

Some variation occurred between $\delta^{15}N$ values of herbage and root nitrogen (table 10.4). Although such differences were not consistant between treatments, there was a general trend for the $\delta^{15}N$ value of herbage to increase relative to the $\delta^{15}N$ value of roots as the proportion of plant nitrogen derived from fertiliser increased. The percentage of plant nitrogen derived from the atmosphere was calculated from herbage $\delta^{15}N$ values making the assumption that plant herbage containing only atmospheric or fertiliser nitrogen would have a $\delta^{15}N$ value of -0.8‰ and +9.1‰ respectively (table 10.4). The proportion

Table 10.2 Herbage dry matter yield and $\delta^{15}N$ values for individual clover plants grown in the glasshouse in the absence of soil nitrogen

Plant Number	Herbage DM (g/plant)	^{15}N Herbage N
1	0.12	-0.73
2	0.12	-0.90
3	0.05	-0.71
4	0.08	-0.85
5	0.10	-0.96
6	0.03	-0.31
7	0.13	-0.84
8	0.15	-0.97
9	0.09	-0.48
10	0.14	-1.14
11	0.05	-0.85
12	0.09	-0.84
13	0.03	-1.19
14	0.01	-0.75
$\bar{x}$	0.085	-0.82
SD	0.045	0.23
SE	0.012	0.06

Table 10.3 Estimation of nitrogen fixation by mass balance for clovers grown in a sand culture and receiving different amounts of applied nitrogen

Treatment	Residual Nitrogen (mg) A	Total Fertiliser Seed Nitrogen (mg) B	Total Plant Nitrogen (mg) C	Fixed Nitrogen C-(B-A) D	% of Plant Nitrogen Fixed
1	0	0.48	65.11	64.63	99.3
2	19.09	43.17	85.19	61.11	71.7
3	24.38	100.09	109.24	33.53	30.7
4	25.16	157.01	134.17	2.32	1.7
5	79.10	249.51	167.91	-2.50	-

Table 10.4 Mean $\delta^{15}N$ values for herbage and root samples of clover grown in sand culture receiving different amounts of fertiliser nitrogen

Treatment	Roots ^{15}N	Herbage ^{15}N	^{15}N Roots − ^{15}N Herbage
1	0.45	-0.82	1.27
2	3.46	2.00	1.46
3	5.90	6.24	-0.34
4	7.74	8.46	-0.72
5	7.94	9.11	-1.17

of plant nitrogen derived from atmospheric nitrogen calculated from $\delta^{15}N$ values are in good agreement, within experimental error, with those calculated from nitrogen mass balances (table 10.5). It appears, therefore, that under the conditions of the present experiment, the proportion of clover nitrogen derived from atmospheric nitrogen can be calculated from the $\delta^{15}N$ value of clover herbage, providing the fertiliser source available to the plant has a different $^{15}N/^{14}N$ ratio to that of atmospheric nitrogen.

The present experiment was not designed to calculate a fractionation factor for assimilation of nitrogen by clover, but some evidence was gained to support a preference for uptake of ^{14}N. This is derived from a comparison of $\delta^{15}N$ values of fertiliser nitrogen, residual nitrogen in the sand at the completion of the experiment, and plant nitrogen for treatment five. The following differences were found:

	$\delta^{15}N$
NH_4^+ applied	10.6
NO_3^- applied	10.5
Residual NH_4^+ in sand	12.7
Residual NO_3^- in sand	11.5
Total root nitrogen	7.9
Total herbage nitrogen	9.1

It appears that ^{14}N was assimilated by the plant in preference to ^{15}N. It would also appear that any denitrification loss was minimal since the $\delta^{15}N$ value for residual NO_3^- was less than that for NH_4^+, and if denitrification was of importance, an increase in the $\delta^{15}N$ of residual NO_3^- would be expected.

At the conclusion of the experiment, samples of sand from each plot were tested using the Most Probable Number technique, as outlined in section 3.2.4, for the presence of nitrifying organisms. All tests

Table 10.5 Estimation of the percentage of atmospheric derived nitrogen in clover herbage estimated by nitrogen mass balance or by δN-15 values of herbage

Treatment	Atmospheric Derived Nitrogen in Clover	
	Calculated by Nitrogen Mass Balance	Calculated From Herbage δN-15 Values
1	99.3	100
2	71.7	71.6
3	30.7	28.9
4	1.7	6.5
5	0	0

were negative.

In treatment five where relatively large amounts of nitrogen were present in the sand at the conclusion of the experiment, the clover plant showed some preference towards the uptake of NH_4^+, since the ratio of residual NH_4^+ to NO_3^- was 1:4.2. This would also explain the higher $\delta^{15}N$ of residual NH_4^+ since in a kinetic isotope fractionation, the nearer to completeness the reaction, the higher the $\delta^{15}N$ content in the unreacted substrate.

10.2.3 Determination of $\delta^{15}N$ of Clover and Associated Ryegrass Herbage in a Field Trial : Results and Discussion

Examination of $\delta^{15}N$ values of clover and associated ryegrass herbage (table 10.6, figure 10.1) shows that several problems adversely affect the use of the difference between the $\delta^{15}N$ value of clover and ryegrass herbage to estimate the contribution of atmospheric derived nitrogen to the total nitrogen in clover.

On seven occasions, the $\delta^{15}N$ of clover herbage was less than that determined for clover growing in the glasshouse in the absence of soil nitrogen, indicating a larger fractionation than that measured in the previous section. Factors which control the size of the isotopic fractionation during symbiotic nitrogen fixation therefore require investigation.

A further complicating factor is the change in $\delta^{15}N$ of ryegrass herbage with time of sampling. In mid summer, virtually no difference existed between the $\delta^{15}N$ value of ryegrass and clover herbage, therefore making estimation of the percentage contribution of atmospheric nitrogen to nitrogen in clover herbage impossible.

The negative $\delta^{15}N$ values for clover herbage indicate that under

Table 10.6 Variation in the $\delta^{15}N$ value of clover and ryegrass between August and April

Date of Harvest	Weeks Regrowth	$\delta^{15}N$ Clover	$\delta^{15}N$ Ryegrass
August 20, 1974	2	-0.24	1.98
September 3, 1974	4	-0.44	1.98
September 17, 1974	6	-1.16	2.31
October 1, 1974	8	-1.65	2.22
October 29, 1974	12	-1.14	0.34
November 5, 1974	3	-0.89	0.46
November 19, 1974	5	-1.26	0.04
November 26, 1974	6	-1.55	0.94
January 7, 1975	1	-0.65	-1.14
January 21, 1975	3	-1.33	-0.85
January 28, 1975	4	-0.72	0.54
February 12, 1975	6	-0.45	0.39
April 1, 1975	1	-0.44	0.29
April 8, 1975	2	-0.56	-0.17
April 15, 1975	3	0.21	0.98
April 29, 1975	5	-0.40	1.82

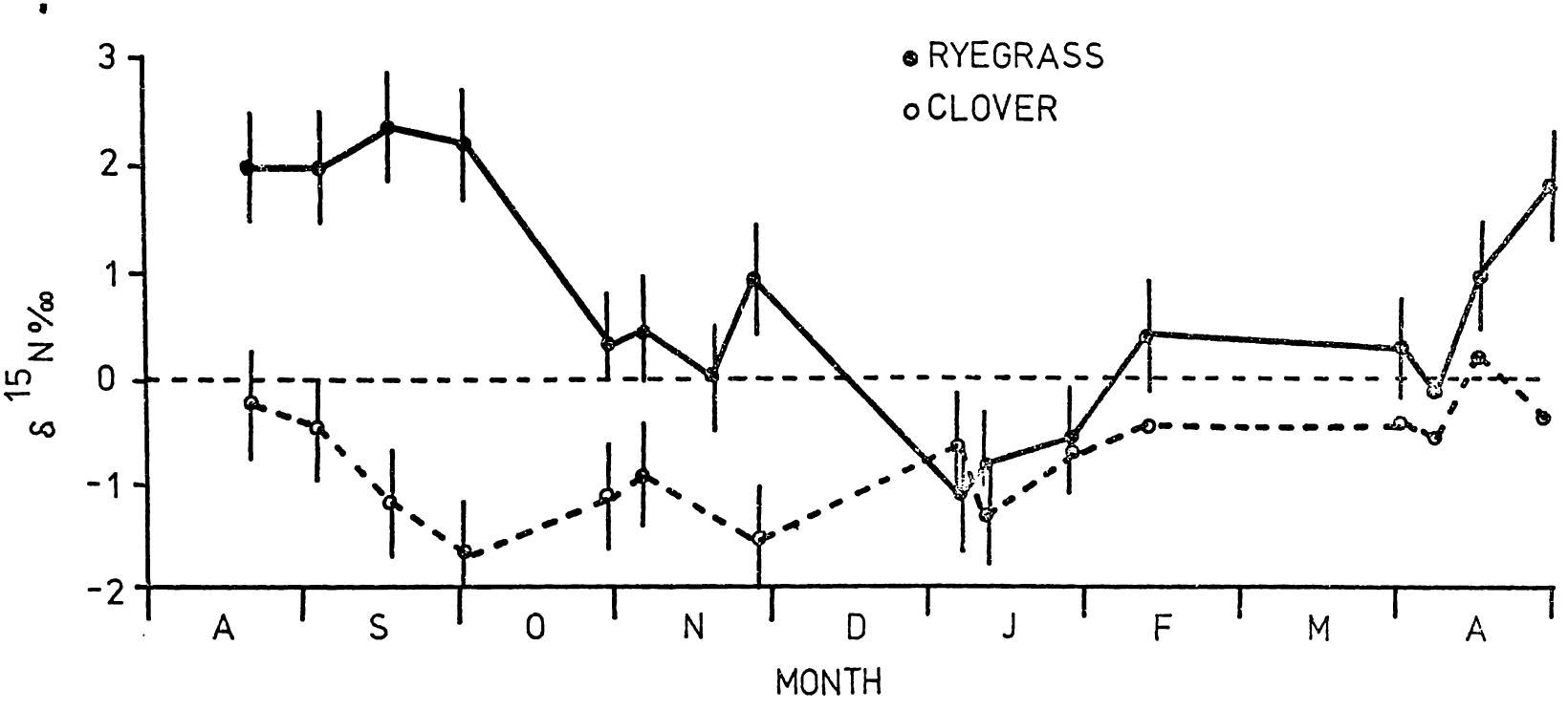

Figure 10.2 Variation in the $\delta^{15}N$ value of clover and ryegrass herbage during the year

the experimental conditions imposed, a relatively large proportion of the clover nitrogen was derived from atmospheric nitrogen.

The change in $\delta^{15}N$ of ryegrass herbage with time is difficult to interpret since no similar published data is available. It is possible that the $\delta^{15}N$ of soil mineralised nitrogen available for plant uptake changes with time of year. This is quite probable since it has been shown that the $\delta^{15}N$ of soil nitrate in incubation studies is dependent on the time of incubation (Cheng et al. 1964; Kohl et al. 1972; Bremner and Tabatabi, 1973). Alternatively, the low $\delta^{15}N$ of ryegrass herbage in mid summer may indicate a direct transfer of nitrogen from clovers to the associated grasses. Any nitrogen originating directly from clovers would be expected to have a low $\delta^{15}N$ value if derived from atmospheric nitrogen.

Results of the glasshouse experiment show that the $\delta^{15}N$ value of clover herbage accurately reflects the proportion of nitrogen derived from the atmosphere, providing the fractionation factor for symbiotic fixation is known, as well as the isotope composition of other sources of nitrogen available to the clover, and any fractionation which occurs during assimilation of that nitrogen. Further investigation of the fractionation involved during symbiotic fixation is required.

Several problems are assocated with the use of $\delta^{15}N$ values of grass herbage grown in association with clover to determine the isotopic composition of available soil nitrogen. A basic assumption is that any fractionation which occurs during assimilation of soil nitrogen is the same in clovers and the associated grasses. A further assumption which is basic to the method is that soil nitrogen available to the clover is identical in isotopic composition to that available to the grasses. Although the first assumption could be relatively easily checked, the

latter assumption would be difficult to prove. If differences in ^{15}N enrichment of total soil nitrogen which occur with depth (see section 11.3.0) are considered to be indicative of possible differences in the isotopic composition of inorganic soil nitrogen, then a difference in the distribution of clover and grass roots in soil may provide a difference in the isotopic composition of soil nitrogen available for assimilation.

If direct transfer of clover nitrogen of low $\delta^{15}N$ value to associated grasses occurs, the use of $\delta^{15}N$ value of the grass from which to calculate the isotopic composition of available soil nitrogen will underestimate the contribution of atmospheric nitrogen to the nitrogen in clover.

There are, therefore, several questions to be answered before differences in the isotopic composition of clover and associated grasses can be used to estimate symbiotic nitrogen fixation.

Herbage of individual clover plants and associated ryegrass was collected from an area which had been intensively grazed by dairy cattle fourteen days prior to sampling. The following differences in $\delta^{15}N$ values of herbage were found:

$\delta^{15}N$ Total Nitrogen

Clover	Ryegrass
3.46	3.26
-0.65	2.27
0.72	2.48
-1.48	2.54
3.24	3.21

Such a variation in the $\delta^{15}N$ value of total nitrogen in clover herbage indicates that large differences exist between individual plants in

the amount of symbiotic fixation. This is possibly due, at least in part, to differences in the availability of soil nitrogen.

10.3.0 FRACTIONATION OF NITROGEN ISOTOPES BY ANIMALS

10.3.1 Introduction

No investigations have been reported on the effect of grazing animals on the isotope composition of nitrogen. Because of the importance of grazing animals in the overall cycling of nitrogen in grazed pasture systems, any change in the isotopic composition of nitrogen which is a result of animals, may be expected to be important when using variations in the natural abundance of ^{15}N to investigate nitrogen transformations. For this reason, the effect of animals on the isotopic composition of nitrogen was investigated.

10.3.2 Experimental

All samples for this study were collected from the Nutrition Centre, Ruakura Agricultural Research Centre, Hamilton.

Animals housed in indoor stalls were fed on a fixed diet for twenty one days with complete collection of urine, faeces and milk over the final seven days. The initial fourteen days were allowed for equilibration of animals on the diet. Blood samples were collected from the jugular veins of two animals at the conclusion of the seven day period.

Four types of diet were compared. Ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture which had been stored frozen for six months; hay and silage made from this pasture; and cake made from macerated pasture from which the juice had been removed from the fibrous residue by means of a belt press. This latter process removed approximately thirty five percent of the protein.

$\delta^{15}N$ values of total nitrogen were determined on fresh milk, urine, and blood samples, and freeze dried feed and faeces samples as outlined in section 9.2.6.

10.3.3 Results and Discussion

The animals were in approximate nitrogen balance during the seven day period under study (table 10.7). Data presented in table 10.8 shows significant differences in the $\delta^{15}N$ of feed, urine and faeces of the animals examined. Faeces were enriched by about 2‰ and urine depleted by about 2‰ relative to feed. Both milk and blood $\delta^{15}N$ values were considerably higher than feed. The $\delta^{15}N$ of urine was found to vary throughout the day, the depletion of $\delta^{15}N$ being greatest during the middle of the day, presumably when the animals were feeding (table 10.9).

A single urine and faeces sample was collected from two pigs whose diet consisted of maize meal in order to determine if a similar fractionation occurred in non-ruminants. Results were similar to those reported above for cows and steers.

Animal	Feed $\delta^{15}N$	Urine $\delta^{15}N$	Faeces $\delta^{15}N$
Pig 1	3.1	1.8	4.4
Pig 2	3.1	0.8	4.0

It us unlikely, therefore, that rumen microflora are the sole source of the observed fractionation in ruminants.

One feature of the reported data is the relative consistancy of the faeces enrichment in ^{15}N over that of the feed. If faeces enrichment is constant for a given feed, it may be possible to use $\delta^{15}N$ values of

Table 10.7 Seven day nitrogen balance estimates for four Jersey cows

Animal	Diet	Total Nitrogen Intake (kg/7 days)	Total Nitrogen Losses * (kg/7 days)	^{15}N Intake (g/7 days)	^{15}N Losses * (g/7 days)
Jersey cow 1	Pasture	2.72	2.67	10.72	10.52
2	Pasture	2.61	2.37	10.28	9.34
3	Cake	2.26	2.25	8.91	8.86
4	Cake	2.22	1.99	8.75	7.84

*Calculated from values for faeces, urine and milk.

Table 10.8 Variations in $\delta^{15}N$ in feed, faeces, blood and milk of animals fed on different diets

Animal	Diet	$\delta^{15}N$ Feed	Urine	Faeces	Milk	Blood
Angus Steer 1	Hay	0.6	-0.8	2.5	-	-
Angus Steer 2	Hay	0.6	-0.6	2.7	-	-
Angus Steer 3	Hay	0.6	-0.6	2.4	-	-
Angus Steer 4	Hay	0.6	-1.0	1.8	-	-
Angus Steer 5	Silage	0.6	-2.1	2.5	-	4.7
Angus Steer 6	Silage	0.6	-2.8	2.1	-	4.9
Jersey Cow 1	Pasture	0.6	-1.9	2.3	-	-
Jersey Cow 2	Pasture	0.6	-1.7	2.6	4.3	-
Jersey Cow 3	Pasture	0.6	-1.5	2.6	4.2	-
Jersey Cow 4	Pasture	0.6	-2.3	2.3	-	-
Jersey Cow 5	Cake	0.7	-1.4	2.3	4.3	-
Jersey Cow 6	Cake	0.7	-2.4	3.1	4.3	-
Jersey Cow 7	Cake	0.7	-2.3	1.8	-	-
Jersey Cow 8	Cake	0.7	-2.5	2.3	-	-

Table 10.9 Diurnal variation in ^{15}N abundance in the urine of steers

Collection Period	$\delta^{15}N$	
	Angus Steer	Angus Steer
1200 - 1500	-3.2	-3.2
1500 - 1800	-2.0	-1.6
1800 - 0900	-1.1	-1.0
0900 - 1200	-3.2	-3.5

faeces to determine the proportional intake of two feeds which differ in nitrogen isotopic composition.

This reported isotopic fractionation by animals further complicates the use of variations in the natural abundance of ^{15}N for studying nitrogen transformations in grazed pasture systems.

10.4.0 ISOTOPIC FRACTIONATION DURING AMMONIA VOLATILISATION

10.4.1 Introduction

Ammonia volatilisation from urine applied to soil is difficult to quantify since the amount of loss is a function of many variables such as air speed, soil moisture, pH and temperature (Volk, 1959; Stewart, 1970; Watkins et al. 1972). Losses of nitrogen can be high, Watson and Lapins (1969) reporting losses in excess of eighty percent from urine applied to Western Australian soils.

The equilibrium isotope reaction

$$^{15}NH_{3(g)} + {}^{14}NH_{4(aq)} \rightleftharpoons {}^{14}NH_{3(g)} + {}^{15}NH_{4(aq)}$$

has a fractionation factor of 1.034 (Kirshenbaum et al. 1947), that is, gaseous ammonia should be 34‰ lighter than aqueous ammonia. Such a fractionation will result in the loss of isotopically light ammonia gas leaving an isotopically heavy residue of NH_4^+ in the soil.

10.4.2 Experimental

Two soils, Wharekohe silt loam and Waimate North clay loam, were used in this experiment. Both soils have been previously described (section 3.3.3). Profile descriptions are included in Appendix 2.

A soil sample (25 cm diameter, 10 cm deep) was placed in a desiccator connected to a gas sorption line (figure 10.3) with a flow rate of 50 cm^3/

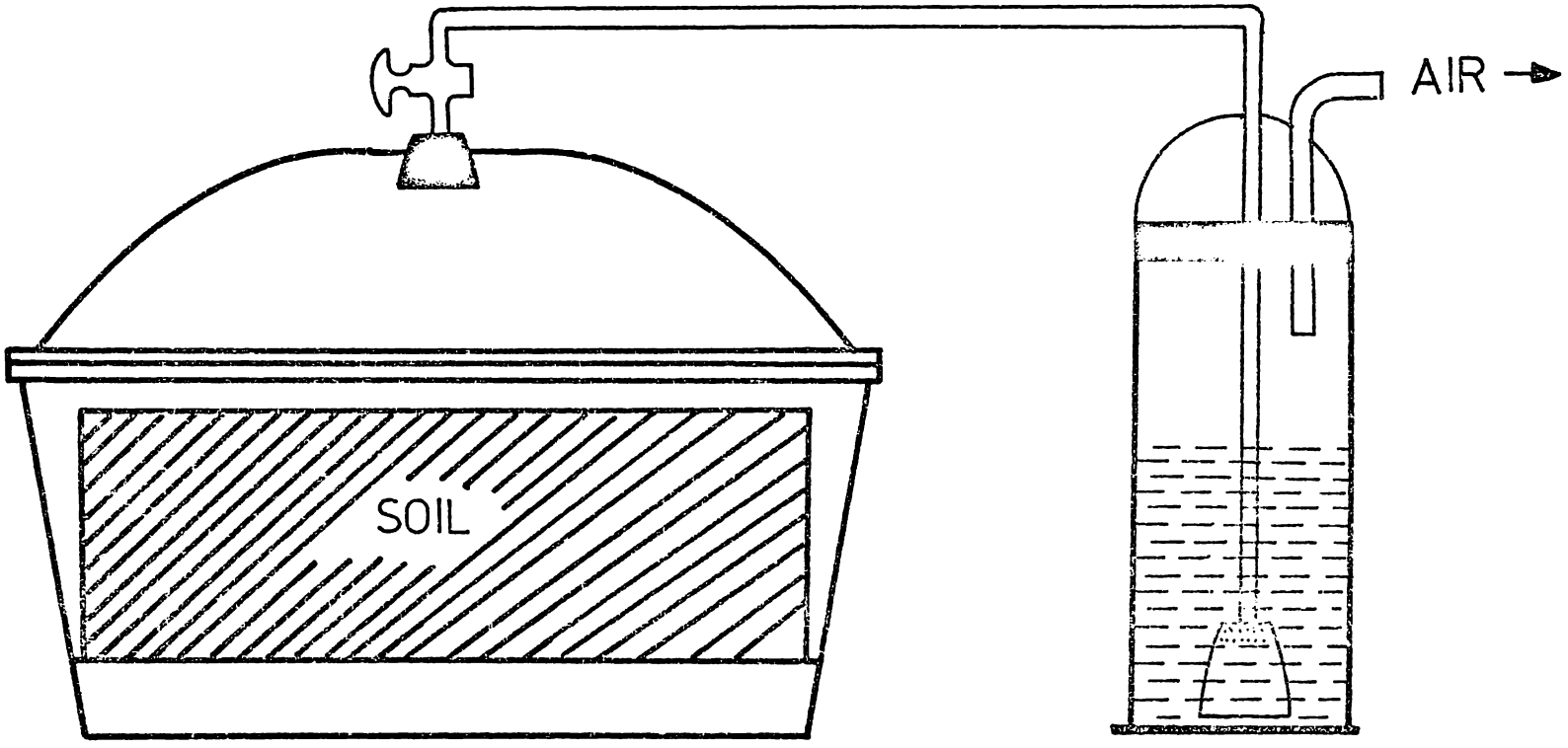

Figure 10.3 Experimental equipment for collection of volatilised NH_3 following application of urea to soils

minute. The sorption solution was 0.1M H_2SO_4.

The main concentration of nitrogen in urine of fourteen cattle studied in the previous section was 8.21 g N/ℓ which agrees closely with the mean of 8 g N/ℓ quoted by Whitehead (1970). The former concentration of nitrogen was applied as urea (Analar) at a rate of 654 kg N/ha and NH_3 volatilised from the soil collected for either twenty four or seventy two hours following application. At the completion of the experimental period, subsamples of the soil were extracted with 2M KCl and $\delta^{15}N$ values determined on the extracted NH_4^+ + NO_3^-, and NH_3 as outlined in chapter 9. All experiments were conducted at 25 ± 1°C.

10.4.3 Results and Discussion

The ammonia gas collected was consistantly isotopically depleted in ^{15}N, and the average isotopic difference between the volatilised ammonia and the soil ammonium + nitrate was 31.5‰.

Soil	Hours Collection	NH_3 $\delta^{15}N$	$NH_4^+ + NO_3^-$ $\delta^{15}N$	
Wharekohe	24	-25.1	7.9	33.0
Wharekohe	72	-13.0	17.1	30.1
Waimate North	24	-19.7	12.8	32.5
Waimate North	24	-17.0	13.2	30.2

That the mean difference between $\delta^{15}N$ for NH_3 volatilised and the soil NH_4^+ and NO_3^- of 31.5‰ was less than the theoretical value of 34‰ indicates the presence of inorganic nitrogen in the soil which was not participating in the equilibrium fractionation reaction.

Ammonia volatilisation appears to be the largest possible

fractionation which occurs under agricultural systems. Because an equilibrium fractionation is not dependent on the proportion of the substrate reacted, as are kinetic isotope fractionations, large losses of NH_3 from urine patches will leave residual NH_4^+ of high ^{15}N enrichment.

10.5.0 ISOTOPIC FRACTIONATION DURING NITRIFICATION

10.5.1 Introduction

A kinetic fractionation factor for conversion of NH_4^+ to NO_3^- by *Nitrosomonas europeae* of 1.026 was reported by Delwiche and Steyn (1970) who assumed that isotope fractionation was a first order reaction. A lower fractionation factor, 1.0169, was reported by Freyer and Aly (1975) for a study of nitrification in soil.

In order to characterise nitrification in New Zealand grassland soils, isotope fractionation during nitrification by a mixed soil isolate and by a pure *Nitrosomonas* spp. culture was investigated.

10.5.2 Experimental

Two isolates of nitrifying organisms were used for the experiment. The first isolate was a mixed culture isolate obtained by suspending 20 g of Horotiu sandy loan in 100 ml of distilled water, and withdrawing 1 ml of the suspension to use as an innoculum. The second was a pure culture of *Nitrosomonas* spp. isolated from the same soil by Dr. U. Sarathchandra, Ruakura Agricultural Research Centre, Hamilton.

Experiments were conducted in the modified perfusion unit (figure 5.4). Each innoculate was placed in 100 g autoclaved quartz sand and perfused with 100 ml of the following nutrient solution:

0.6405 g/ℓ $(NH_4)_2SO_4$	2 µg/ℓ $CoCl_2.6H_2O$
1.0 g/ℓ K_2HPO_4	100 µg/ℓ $ZnSO_4.7H_2O$
0.03 g/ℓ $FeSO_4.7H_2O$	20 µg/ℓ $CuSO_4.5H_2O$
0.3 g/ℓ $MgSO_4.7H_2O$	200 µg/ℓ $MnCl_2.4H_2O$
0.3 g/ℓ $CaCl_2$	100 µg/ℓ $Na_2MoO_4.2H_2O$

The pH of the perfusion solution was maintained at pH 7.0 by automatic titration with saturated $Ca(OH)_2$. All experiments were conducted at 25 ± 1°C.

Perfusion was continued for fourteen days prior to measurements being taken to allow proliferation of the micro-organisms.

For the soil isolate, nitrification was allowed to proceed until a predetermined percentage of the NH_4^+ substrate had been oxidised and then the $\delta^{15}N$ of the NH_4^+ and NO_2^- + NO_3^- determined. The sand and perfusion units were then thoroughly washed with distilled water (pH 7.0) and nutrient solution, and another experiment started using the same nitrifying culture which remained on the sand.

When the pure isolate of *Nitrosomonas* spp. was used, the oxidation of NH_4^+ was allowed to proceed till approximately fifty percent completion, and then the $\delta^{15}N$ of NH_4^+ and NO_2^- determined.

10.5.3 Results and Discussion

The dependence of the $\delta^{15}N$ values of NH_4^+ and NO_2^- + NO_3^- on the percentage of NH_4^+ oxidised is illustrated in figure 10.4. The $\delta^{15}N$ of unreacted NH_4^+ plots as a straight line on a semilogarithmic plot (figure 10.5) and this supports the use of equations such as those outlined by Tong and Yankwich (1957) for calculation of fractionation factors. The mean determined fractionation factor by the method of Tong and Yankwich (1957) (see section 8.3.3) was 1.011 which is considerably less than the

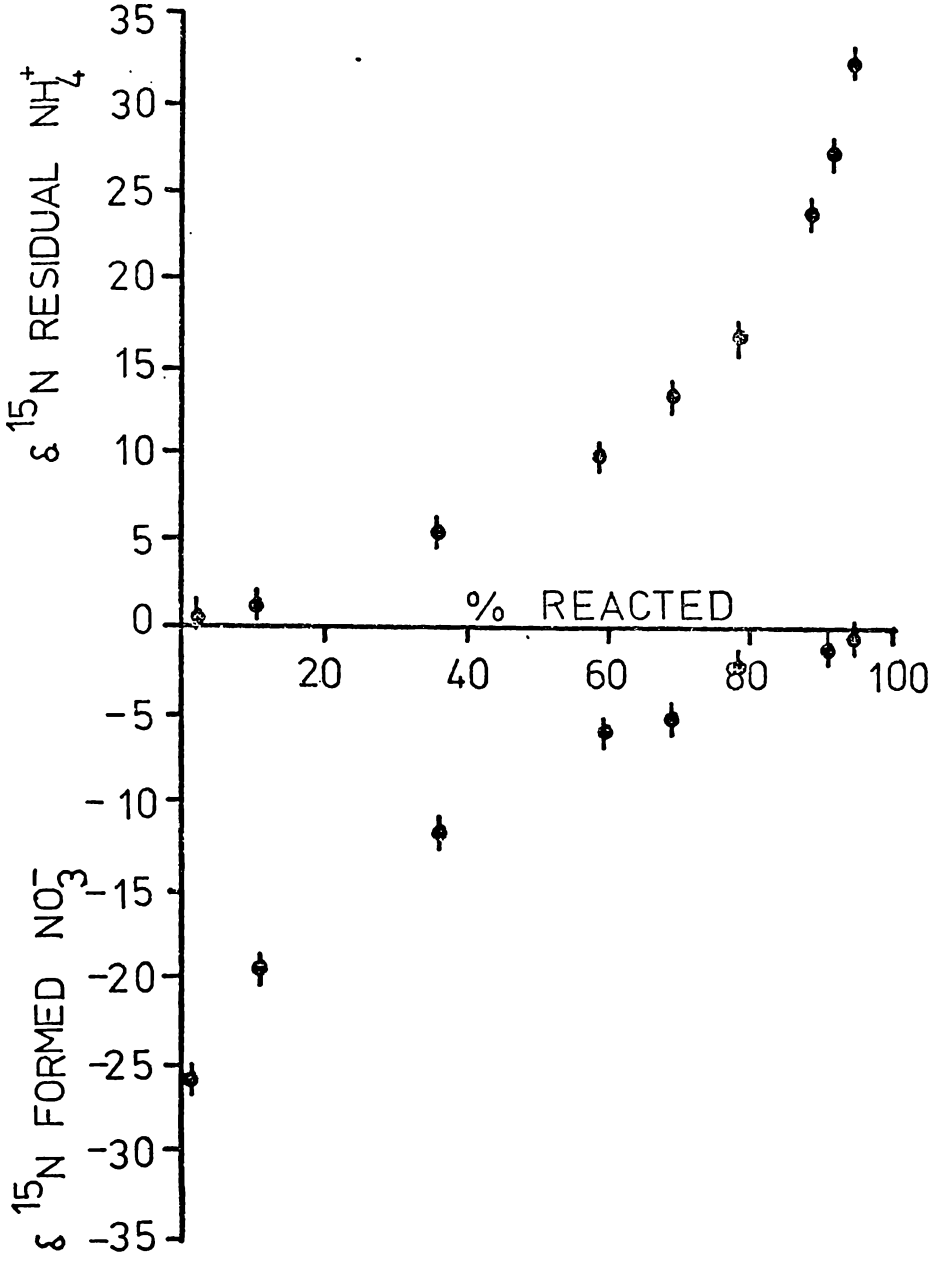

Figure 10.4 The relationship between $\delta^{15}N$ values of NH_4^+ and NO_3^-, and the percentage of substrate (NH_4^+) oxidised during nitrification by a mixed culture isolated from Horotiu sandy loam

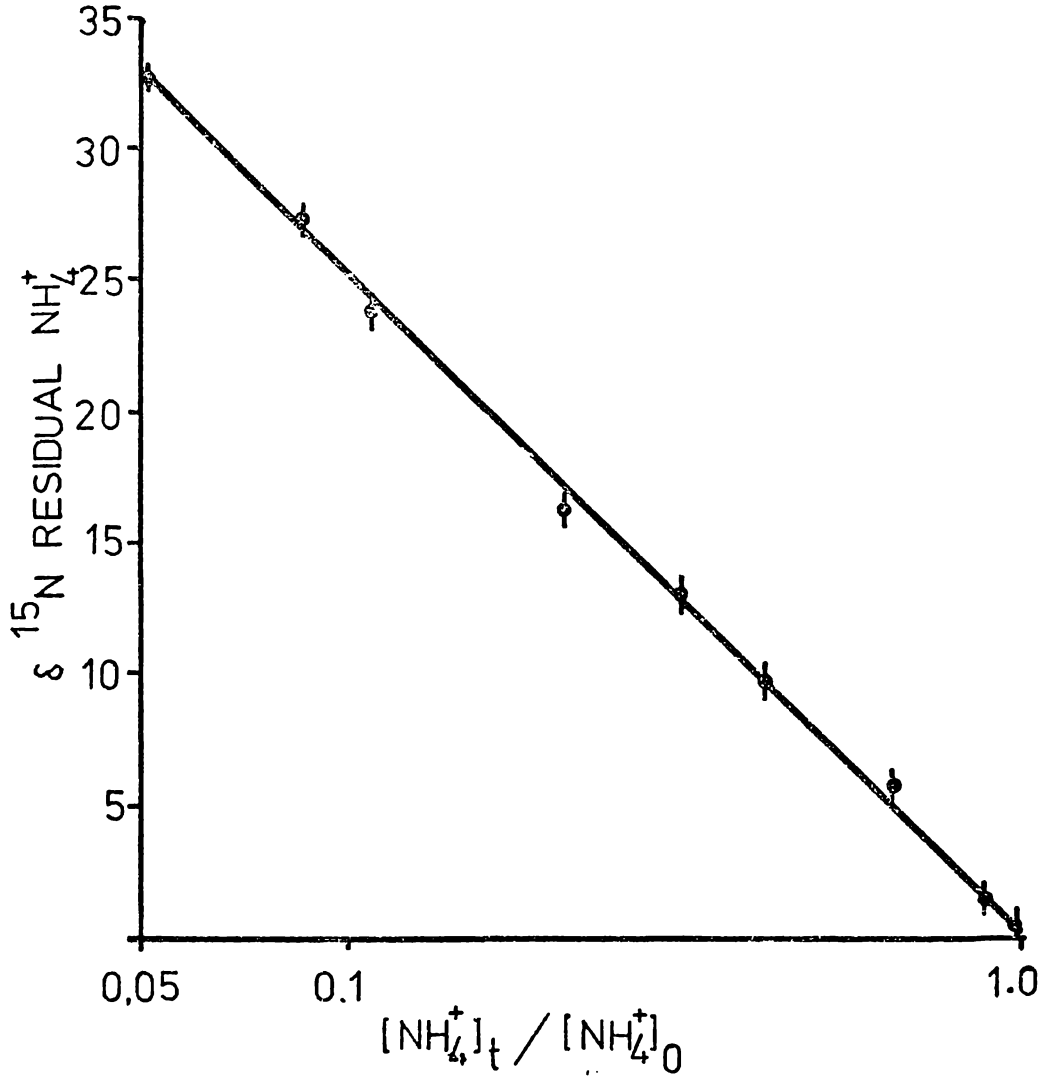

Figure 10.5 Semilogarithmic plot of $\delta^{15}N$ values of residual NH_4^+ against $NH_{4,t}^+ / NH_{4,0}^+$ during nitrification by a mixed culture isolated from Horotiu sandy loam

fractionation factors of 1.026 and 1.017 reported by Delwich and Steyn (1970) and Freyer and Aly (1975) respectively.

The mean determined fractionation factor for the pure *Nitrosomonas* spp. isolate was 1.022 which is intermediate to the fractionation factors of Delwich and Steyn (1970) and Freyer and Aly (1975).

The observed difference in the fractionation factors may indicate that the micro-organism principally responsible for nitrification in soil is not the *Nitrosomonas* spp. isolated, or alternatively competition for NH_4^+ substrate in a mixed culture may in some way modify the isotopic fractionation. That more than one organism is responsible for nitrification in Horotiu soil is supported by the lack of a logarithmic phase on perfusion of Horotiu soil (see section 3.3.4).

10.6.0 GENERAL DISCUSSION AND CONCLUSIONS

A necessary condition for the valid use of methods based on differences in the natural isotopic composition of soil, fertilisers and atmospheric nitrogen to determine the contribution of fertiliser and soil nitrogen to nitrogen in rivers, lakes and plants is that each source of nitrogen must maintain its isotopic identity throughout all transformations. Consideration of the results presented in this chapter suggest that this condition is unlikely to be met, particularly in grazed clover/grass pasture systems.

In the pasture system studied in section 10.2.5, herbage from an area not receiving nitrogen fertiliser could vary in $\delta^{15}N$ value from -1.6 to +2.3‰ depending on the type of herbage and time of year. Fractionation by grazing animals could increase the $\delta^{15}N$ range of total excreted nitrogen from -3.6 to +4.3‰. This range of $\delta^{15}N$ values is very similar to that which would be expected for fertiliser nitrogen (-4.1 to +1.9‰, Black and Waring, 1976).

The largest fractionation in soils appears to occur because of an equilibrium fractionation during volatilisation of NH_3 from urine patches, the effect of the fractionation on the residual NH_4^+ in soil being dependent on the extent of NH_3 loss.

Because of the inherent variability in $\delta^{15}N$ values and possible fractionations which may occur in grazed pasture systems, it is concluded that use of variations in the natural abundance of ^{15}N to study fertiliser transformations in such systems is of doubtful validity. Such a conclusion is in agreement with that of Hauck et al. (1972) and Black and Waring (1976).

CHAPTER 11

VARIATION IN THE $\delta^{15}N$ VALUES OF TOTAL NITROGEN IN NEW ZEALAND GRASSLAND SOILS

11.1.0 INTRODUCTION

It is well established that although the abundance of ^{14}N and ^{15}N in the atmosphere is relatively constant (Dole et al. 1954) some variation in the ^{15}N abundance in soils does occur (see e.g. Hoering, 1955; Cheng et al. 1964; Riga et al. 1971). At present it is not known why different soils have different $\delta^{15}N$ values, but collection of data from soils of different localities and origins may help to elucidate the reasons for the observed differences. As no $\delta^{15}N$ data was available for New Zealand soils, it was decided to determine the $\delta^{15}N$ of soils used in the nitrification survey of chapter 3 in order to provide some background data on New Zealand soils, and determine if any correlation existed between $\delta^{15}N$ for total soil nitrogen, and the amount of total soil nitrogen or Initial Nitrification Activity.

11.2.0 EXPERIMENTAL

$\delta^{15}N$ values of total soil nitrogen were determined for samples used in the nitrification survey reported in chapter 3. All experimental procedures were as outlined in chapter 9.

11.2.1 Variation in Percentage Total Nitrogen and the $\delta^{15}N$ Values of Total Nitrogen for a Number of Samples Collected From a Small Area

In order to gain an estimate of the variability of percent total nitrogen and the $\delta^{15}N$ values of total nitrogen in a given area, sixteen 2.5 cm diameter (0 - 7.5 cm depth) soil cores were collected by random sampling an area of approximately 0.5 ha. The area was one half of a ryegrass/white clover paddock on a heavily stocked dairy farm. Each soil core was treated as an individual sample and total nitrogen and the $\delta^{15}N$

value of total nitrogen determined (table 11.1). The range of values determined for both percent total nitrogen and the $\delta^{15}N$ value of total nitrogen was approximately forty percent of the mean.

11.3.0 RESULTS AND DISCUSSION

The distribution of $\delta^{15}N$ values for total soil nitrogen (0 - 7.5 cm) showed a peak between +3 and +3.5‰ with no results outside the range of -1.1 to +6.8‰ being recorded (table 11.2, figure 11.1). These values are comparable in magnitude to those of three Australian soils (0 - 10cm) reported by Black and Waring (1976) of 2.9, 5.0 and 6.7‰, and to that reported by Vallis et al. (1967) for a red podzolic soil in South-East Queensland. Other $\delta^{15}N$ values for total soil nitrogen reported in the literature are:

Author	Country	Number of Samples	Range $\delta^{15}N$‰	Mean $\delta^{15}N$‰
Bremner and Tabatabai (1973)	U.S.A.	16	-4.0 to 3.0	-0.2
Cheng et al. (1964)	U.S.A.	28	-1.0 to 17.0	6.3
Rennie and Paul (1975)	Canada	5	6.1 to 12.5	9.8
Riga et al. (1971)	Belgium	69	-7.0 to 6.0	2.6
Bremner et al. (1966)	U.S.A.	40	-3.0 to 18.0	6.2
Delwich and Steyn (1970)	U.S.A.	39	2.0 to 11.0	5.4
Present study	N.Z.	61	-1.1 to 6.8	3.2

Direct comparison of the above results is difficult because samples are often collected from different depths, which sometimes are not specified, and from soils which have been subjected to many different soil management practices. In general, it appears that organic and forest soils tend to have $\delta^{15}N$ values close to zero or slightly negative,

Table 11.1 Percent total nitrogen and $\delta^{15}N$ values of total nitrogen for sixteen soil cores (2.5 cm diameter, 0 - 7.5 cm depth) collected at random from a 0.5 ha area

Sample No.	% Total N	$\delta^{15}N‰$
1	1.888	2.96
2	0.978	2.97
3	1.150	3.25
4	1.999	2.56
5	1.417	3.11
6	1.201	2.72
7	1.128	3.15
8	1.133	2.64
9	1.282	3.82
10	0.954	3.16
11	1.184	3.45
12	1.318	3.52
13	1.244	2.94
14	1.165	3.26
15	1.143	3.34
16	1.220	3.67
Range	0.954 - 1.417	2.64 - 3.82
Mean	1.182	3.16
SD	0.113	0.35
SE	0.028	0.09

Table 11.2 Variation in the $\delta^{15}N$ values of total nitrogen in a selection of New Zealand grassland soils (0 - 7.5 cm depth)

Addison sandy loam	1.7	Patua sandy loam	4.2
Ahaura silt loam	3.2	Pukepuke brown sandy loam	2.9
Aparima silt loam	4.7	Rangiuru clay	2.5
Arapohue clay	3.2	Rosedale silt loam	2.3
Awarua clay	4.8	Rosedale silt loam	1.8
Chaslands silt loam	5.8	Rotomahana sandy loam	1.6
Foxton black sand	0.4	Rukuhia peat	-0.3
Harihari sandy loam	-1.1	Rukuhia peat	2.4
Hauraki clay	5.9	Rukuhia peat	1.0
Hokitika silt loam	2.9	Rukuhia peat	-0.5
Hokitika shallow sandy loam	1.9	Rukuhia peat	2.3
Horotiu sandy loam	3.9	Stratford sandy loam	3.1
Hukerenui silt loam	3.7	Stratford sandy loam	3.6
Kaweku silt loam	4.6	Taupo silty sand	2.4
Kiripaka silt loam	5.2	Te Houka silt loam	2.7
Koau sandy loam	3.8	Te Kowhai	4.2
Kokotau silt loam	3.0	Timaru silt loam	6.3
Kokotau silt loam	3.4	Tinui silt loam	3.7
Linnburn silt loam	6.0	Tinui silt loam	3.4
Linnburn silt loam	6.8	Tokoroa sandy silt	2.7
Lynwood fine sandy loam	3.7	Tokoroa sandy silt	2.6
Matahina gravel	2.9	Tokoroa sandy silt	2.5
New Plymouth brown loam	2.2	Waikiwi silt loam	4.1
Ohia silt loam	3.0	Waictu friable clay	3.7
Okaihau friable clay	4.1	Waitakere clay	5.6
Okaihau friable clay	4.4	Waitakere clay	3.9
Okarito loam	2.3	Waitarere-Hokio assocation	2.2
Opua silt loam	3.0	Wehenga silt loam	3.4
Oreti stoney loam	2.3	Wehenga silt loam	3.8
Oruani sand	3.3	Wharekohe silt loam	1.6
Otonga peaty loam	1.8		

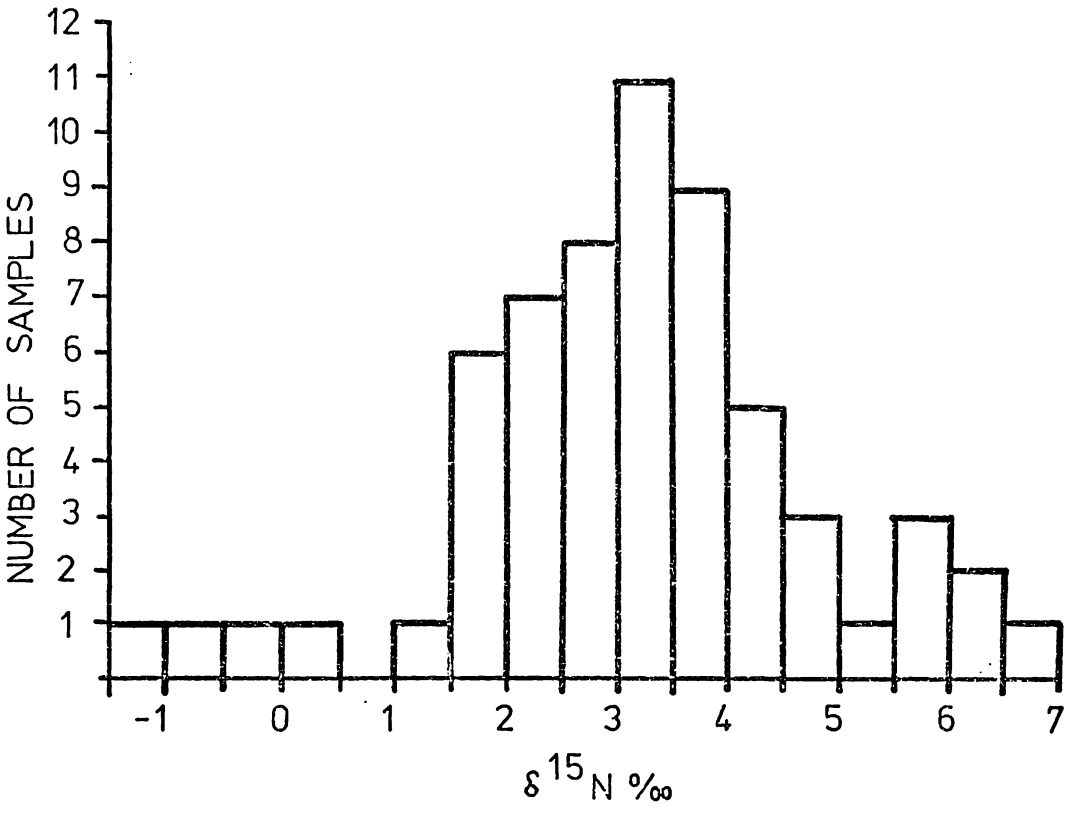

Figure 11.1 $\delta^{15}N$ values for total soil nitrogen in sixty one New Zealand grassland soils (0 - 7.5 cm)

whereas most agricultural soils have positive $\delta^{15}N$ values. This is illustrated in Bremner and Tabatabai's (1973) data where virgin soils were reported as having lower $\delta^{15}N$ values than their cultivated counterparts. Freyer and Aly (1975) also reported higher $\delta^{15}N$ values for total nitrogen in agricultural than in forest soils.

It appears that, in general, the 0 - 7.5 cm depth of New Zealand grassland soils is less enriched in ^{15}N than many of the American and Canadian soils, since $\delta^{15}N$ values up to +18‰ have been reported (Bremner et al. 1966). Since it appears that most forest and virgin soils have low or negative $\delta^{15}N$ values for total nitrogen, it is likely that positive $\delta^{15}N$ values are, at least in part, the result of agriculture. Further, many New Zealand grassland soils have not reached an equilibrium organic matter content, and losses of nitrogen are likely to be low from a grassland/soil system while organic matter is still accumulating. This, to some extent, will restrict the enrichment of ^{15}N which may take place.

The ultimate $\delta^{15}N$ value of total soil nitrogen, if in fact a stable $\delta^{15}N$ value is obtained, may be indicative of an approach to equilibrium in a soil/pasture system. Such a $\delta^{15}N$ value would therefore be the result of a balance between nitrogen inputs and losses of such a system. Since the major input of nitrogen into New Zealand pasture systems, i.e. symbiotic nitrogen fixation, has a negative $\delta^{15}N$ value, the positive $\delta^{15}N$ values normally found in agricultural soils are indicative of losses occurring from the system which favours the retention of ^{15}N over ^{14}N. Information is required on the time taken for a soil/animal/grassland system to approach an isotopic equilibrium situation, and if in fact such a condition is ever reached.

Rennie and Paul reported a relatively good correlation between total soil nitrogen content and its $\delta^{15}N$ value for nineteen of the twenty four soils they examined. No such relationship was found in the present study

(r = 0.10).

The variation in total nitrogen and $\delta^{15}N$ of total nitrogen with depth in the profile was investigated in Wharekohe silt loam and Waimate North clay loam (figure 11.2). These two soils have been previously described (section 3.3.3). Profile descriptions are included in Appendix 2. Total nitrogen decreased with depth in both soils. In the Wharekohe soil $\delta^{15}N$ values of total nitrogen increased from 2‰ in the 0 - 2 cm depth to a maximum of 7.6‰ in the 15 to 20 cm depth. In the Waimate North soil $\delta^{15}N$ for total soil nitrogen increased from 3.6‰ in the 0 - 2 cm depth to a maximum of 9.2‰ in the 45 to 50 cm depth, then decreased to 7.0‰ in the 90 - 100 cm region.

Variation of $\delta^{15}N$ value of total soil nitrogen with depth is apparently dependent on the soil. In a profile of Yolo fine sandy loam to a depth of one hundred and forty cm, Delwiche and Steyn (1970) reported that the ^{15}N enrichment was greatest at a depth of approximately twenty cm. In two further profiles of Yolo soil sampled to five and nine meters, the ^{15}N enrichment was greatest at a depth of approximately two meters. In each of the sites examined there appeared to be some correlation of isotopic composition with total nitrogen but this was not universally true. The ^{15}N abundance in a brown chernozemic and grey luvisolic profile reported by Rennie and Paul was generally greatest at a depth of approximately forty five cm. In another profile of a chernozemic soil, however, the ^{15}N enrichment was greatest at a depth of about fifteen cm, and then decreased to an approximately constant value showing a small increase at two hundred and seventy cm.

The low ^{15}N enrichment close to the soil surface in New Zealand grassland soils is most likely a reflection of the continual input of large amounts of symbiotically fixed nitrogen which is depleted in ^{15}N. The mechanism of loss of nitrogen (i.e. denitrification, leaching,

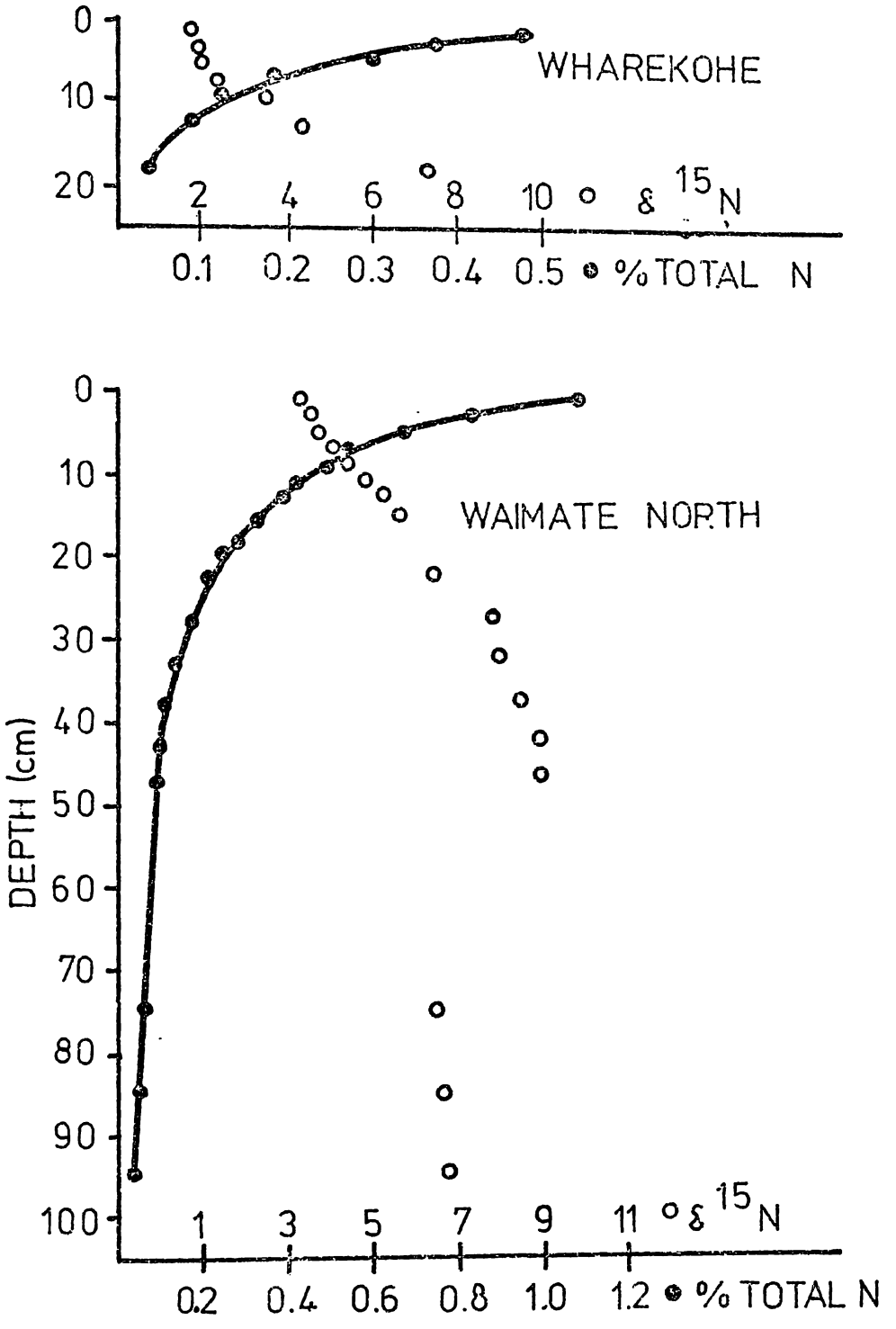

Figure 11.2 Change of $\delta^{15}N$ values for total soil nitrogen with depth from the soil surface in Wharekohe silt loam and Waimate North clay loam

volatilisation of NH_3), however, will also be expected to influence the enrichment of ^{15}N in soil nitrogen, since the extent and type of isotope fractionation differ between mechanisms.

CHAPTER 12

DETERMINATION OF $\delta^{15}N$ VALUES OF NITRATE NITROGEN IN SOME GROUND WATERS OF THE WAIKATO

12.1.0 INTRODUCTION

Recently Baber and Wilson (1972) and Baber (1977) reported high concentrations of nitrate in shallow aquifiers of the Waikato, several being considerably higher than the 10 mg N/ℓ suggested by the World Health Organisation (1971) for potable waters. Nitrate concentrations in ground waters are of interest both because of possible deleterious effects on human and stock health (e.g. Campbell et al. 1954, West et al. 1968) and also as a possible contributor to eutrophication of rivers and lakes (e.g. Harmeson et al. 1971). For this reason, some of the wells and bores originally sampled by Baber (1977) were resampled and $\delta^{15}N$ values determined on the nitrate to provide some background information on $\delta^{15}N$ values of nitrate in shallow aquifiers of the Waikato.

12.2.0 EXPERIMENTAL

A selection of wells providing a range of nitrate concentrations, originally sampled by Baber (1977), were sampled and $\delta^{15}N$ values for nitrate nitrogen determined as outlined in chapter 9. Water samples were collected into two litre containers containing 80 mg Hg as $HgCl_2$ to prevent microbial depletion of nitrate. Samples were collected from a source as close as possible to the bore or well, and in all cases taps were allowed to run for approximately ten minutes prior to collection. All samples were prepared for isotope analysis within twelve hours of collection. Samples were stored at $5^{o}C$.

The Waipuna cave samples were collected from water entering a limestone cave under native forest. These samples provided samples not influenced by agriculture.

12.3.0 RESULTS AND DISCUSSION

Concentrations of nitrate nitrogen determined in the present study

were of the same order of magnitude as those reported by Baber (1977), table 12.1). The $\delta^{15}N$ values for nitrate nitrogen from ground waters of agricultural areas ranged from 5.2 to 10.0‰, and the two samples collected from Waipuna cave were 3.8 and -1.6‰. No relationship was found between nitrate content and its $\delta^{15}N$ value as reported by Kohl et al. (1971) for drain tile effluent from an Illinois corn belt watershed, and by Freyer and Aly (1975) for shallow wells in a specific region of Germany. In both of the latter studies, nitrate in ground water was believed to be of fertiliser origin, a negative correlation between nitrate and its $\delta^{15}N$ value being reported in both studies.

With the exception of the Waipuna cave samples, all the $\delta^{15}N$ values for nitrate were in the range of 5 to 10‰. According to reports in the literature, $\delta^{15}N$ values for soil nitrate may be equal to or less than the $\delta^{15}N$ of the total soil nitrogen (Kohl et al. 1972; Bremner and Tabatabai, 1973; Rennie et al. 1976; Freyer and Aly, 1975; Feigin et al. 1974a; Delwiche and Steyn, 1970; Edwards, 1973). If an isotopic fractionation occurs during mineralisation of soil organic nitrogen, it appears as if the NH_4^+ will be depleted in ^{15}N relative to the total soil nitrogen, and nitrification again favours ^{14}N. Therefore, that the ^{15}N enrichment of soil nitrate will be equal to or less than that of total soil nitrogen has a theoretical basis also.

Data presented in section 10.2.2 suggested that some preference for ^{14}N during assimilation of both NH_4^+ and NO_3^- occurs, and the effect of such a selection on the residual soil nitrate available for leaching requires evaluation. However, it would be expected that any effect under field conditions would be small since maximum plant production is limited by nitrogen availability on most New Zealand soils. Such an argument indicates that nitrate in ground water under agricultural land is not derived directly from soil nitrogen. Such a hypothesis is also supported

Table 12.1 Nitrate nitrogen concentrations and the $\delta^{15}N$ of nitrate nitrogen for a selection of ground waters from the Waikato region

Location*	mg NO_3-N/ℓ Reported By Baber (1977)		Present Study		
	Range	Mean	Date Collected	mg N/ℓ	$\delta^{15}N‰$
T.J. Bear	11.7 - 46.3	24.2	10.3.77	38.8	5.7
A. Berkers	11.0 - 58.0	29.4	10.3.77	34.2	7.4
R.M. Hooker	29.0 - 53.0	38.6	11.3.77	30.2	10.0
R.E. Mitchell	-	13.5	11.3.77	13.2	7.5
F. Fyers	-	0.5	11.3.77	5.7	7.8
F. Malcolm	-	5.0	11.3.77	5.2	9.3
F.G. Dixon	-	6.3	11.3.77	3.9	6.4
N.A. Owens	-	23.1	11.3.77	3.2	7.6
D. Llewellyn	-	0.2	25.8.76	0.2	5.2
Waipuna Cave	-	-	22.8.76	0.9	3.8
Waipuna Cave	-	-	22.8.76	0.2	-1.6

* Exact locations may be found in Baber (1977).

by $\delta^{15}N$ values reported for ryegrass herbage in section 10.2.3 if they are assumed to reflect the $\delta^{15}N$ of inorganic nitrogen available for plant uptake in the absence of grazing animals.

Introducing animals to a pasture system also introduces a major possible source of nitrogen isotope fractionation via equilibrium isotope fractionation during ammonia volatilisation. Such a fractionation is not dependent on the percentage of substrate reacted as is, for example the isotopic fractionation during nitrification. Isotope fractionation during ammonia volatilisation leads to loss of NH_3 gas depleted in ^{15}N, and concentration of ^{15}N in the residual NH_4^+ which is available for nitrification, therefore producing nitrate enriched in ^{15}N. Soil $NH_4^+ + NO_3^-$ with $\delta^{15}N$ values of up to 17.1‰ were reported in section 10.4.3 as a direct result of isotope fractionation due to ammonia volatilisation. Loss of ammonia from urine spots therefore appears to be a major mechanism for enrichment of ^{15}N in soil inorganic nitrogen.

Kreitler (1975) compared $\delta^{15}N$ values of nitrate from the decomposition of animal waste with nitrate derived from the mineralisation of organic nitrogen, and found $\delta^{15}N$ values in the range of 10 to 22 °/oo and 2 to 8 °/oo for nitrate derived from the former and latter sources respectively. He concluded that the isotopic ratio of nitrate from decomposition of animal waste is controlled by the volatilisation of ^{15}N depleted ammonia gas during the decomposition of urea in urine.

Since all the $\delta^{15}N$ values for nitrate in ground water under agricultural land in the present study are above the average $\delta^{15}N$ value for total soil nitrogen (table 11.2), and many are above the upper limit recorded for soil nitrogen, it appears that nitrate in ground water is not derived directly from mineralisation, but much of it is derived from urine nitrogen. The actual isotopic composition of nitrate in ground water will be determined largely by the amount of ammonia gas lost through

volatilisation from urine patches, and the amount of dilution of nitrate derived from urine with nitrogen from other sources.

The lower $\delta^{15}N$ values of the nitrate in water collected from the Waipuna cave indicates that this nitrate is most likely derived from mineralisation of soil organic matter as opposed to animal waste. The reported $\delta^{15}N$ values are consistant with $\delta^{15}N$ values for nitrate in ground water in forests reported by Freyer and Aly (1975) and the expected range of $\delta^{15}N$ values for total soil nitrogen in forest soils reported by Riga et al. (1971).

The $\delta^{15}N$ values of ground waters in the Waikato also provide further evidence that significant losses do occur via volatilisation of ammonia from urine patches.

SECTION C

CHAPTER 13

CONCLUSIONS

13.1.0 STUDY OF NITRIFICATION IN NEW ZEALAND GRASSLAND SOILS

Rates of nitrification were measured, using a perfusion technique, in samples of sixty nine high producing grassland soils which were considered representative of the major soil groups in New Zealand.

It was concluded that:

1) Measurement of the rate of nitrification over a short time of perfusion (sixteen hours) provides a mean for the comparison of the inherent oxidation activities of the indigenous population of nitrifying organisms present in different grassland soils. The rate of nitrification over the first sixteen hours of perfusion is referred to as the Initial Nitrification Activity (INA) expressed as μg $N_{oxidised}$/g soil/hr.

2) Large differences in the Initial Nitrification Activity (<0.02 to 5.70 μg N_{ox}/g soil/hr) occur between New Zealand grassland soils.

3) Yellow-brown loams possess Initial Nitrification Activities (generally >2 μg N_{ox}/g soil/hr) significantly higher than those of other soils examined with the exception of soils of near neutral pH.

4) Red and brown loams possess medium to high Initial Nitrification Activities (0.18 to 2.27 μg N_{ox}/g soil/hr).

5) With the exception of yellow-brown loams, red and brown loams and soils of near neutral pH, all other soils have Initial Nitrification Activities <1.0 μg N_{ox}/g soil/hr.

6) Four general patterns of nitrification occur when New Zealand grassland soils are perfused with $(NH_4)_2SO_4$.

   Type 1: NH_4^+ is rapidly oxidised to NO_3^-, the rate of oxidation being linear from the commencement of perfusion.

   Type 2: NH_4^+ is oxidised only slowly to NO_3^-.

   Type 3: NH_4^+ is oxidised slowly to NO_3^- at the commencement of

perfusion, but increases logarithmically with time until a steady rate of nitrification is observed.

Type 4: Type 3 nitrification with a temporary accumulation of NO_2^- during the initial stages of perfusion.

7) Type 1 nitrification occurs only in yellow-brown loams and red and brown loams, i.e. only in soils of high Initial Nitrification Activity.

8) Type 2 nitrification occurs only in some yellow-brown earths and yellow-brown pumice soils. It is concluded that this type of nitrification pattern is the result of low pH, the mean pH of the soils exhibiting this type of nitrification pattern being pH 5.5.

9) Type 3 nitrification occurs in most New Zealand grassland soils and is considered to be due to the removal of substrate limitations on perfusion.

10) Only soils of high pH, with the exception of some podzolic soils, exhibited type 4 nitrification. In the former soils this type of nitrification is considered the result of high pH, whereas in the latter it appears that some accumulation of NO_2^- is required to stimulate the growth of NO_2^- oxidising organisms.

11) pH, percent total N, percent organic C and C:N ratio account for, at best, only forty four percent of the variation in Initial Nitrification Activity when all soils except organic soils and yellow-brown loams are considered.

12) The effect of percent total N on Initial Nitrification Activity is significantly different in organic soils and yellow-brown loams than in other New Zealand grassland soils.

The number of nitrifying organisms in some New Zealand soils was investigated using the most probable number technique.

It was concluded that:

13) Large populations of nitrifying organisms (1.6×10^4 - 2.2×10^6 NH_4^+ oxidisers and 5.4×10^4 - 1.9×10^7 NO_2^- oxidisers per g soil) are present in the 0 - 7.5 cm depth in New Zealand improved grassland soils.

14) Populations of nitrifying organisms are largest close to the soil surface, and in general decline with depth.

15) Differences in the ratio of NH_4^+ : NO_2^- oxidising organisms occur between soils. Although the population of NH_4^+ oxidisers normally exceeds that of NO_2^- oxidisers, this is not invariably the case. It is not clear what factors determine the ratio of the two types of organisms in any given soil.

16) INA values do not indicate the size of the nitrifying population since there is variation in the oxidative capacity per NH_4^+ oxidising cell.

17) A native forest soil in the Waitomo region contains a low population of nitrifying organisms (555 NH_4^+ and 1434 NO_2^- oxidisers per g soil).

18) Generation times of NH_4^+ oxidising organisms in soils are in the order of two to five days and are dependent on pH.

The amounts of inorganic nitrogen, rates of nitrification and rate of nett mineralisation of soil organic nitrogen in two soils of low and medium INA was investigated using an in situ incubation technique.

It was concluded that:

19) Differences in rates of nitrification measured in the laboratory using the perfusion technique reflect differences occurring under field conditions.

20) Differences in rates of nitrification produce differences in the ratio of NH_4^+ : NO_3^- under field conditions. In Wharekohe silt loam

(INA = 0.07 μg N_{ox}/g soil/hr) the mean value of NH_4^+ and NO_3^- present in the 0-7.5 cm depth over a forty seven week sampling period was 15.7 ± 1.7 and 0.9 ± 1.4 kg N/ha respectively. The equivalent figures for Kiripaka silt loam (INA = 0.81 μg N_{ox}/g soil/hr) were 6.5 ± 1.3 and 6.6 ± 1.4 kg N/ha.

21) Rates of nitrification under field conditions are slow. When grazing animals were absent, the mean rate of nett nitrification determined over a forty seven week period in the 0 - 7.5 cm depth of Wharekohe silt loam and Kiripaka silt loam was estimated to be 0.68×10^{-2} μg N_{ox}/g soil/hr, and 5.4×10^{-2} μg N_{ox}/g soil/hr respectively.

22) The average rate of nett mineralisation of organic nitrogen in Wharekohe silt loam (0.99 ± 0.08 kg N/ha 0 - 7.5 cm/day) is significantly greater than that of Kiripaka silt loam (0.74 ± 0.06 kg N/ha 0 - 7.5 cm/day). This represents a nett annual mineralisation of 17.1 percent of total soil nitrogen in Wharekohe silt loam and 9.9 percent of total soil nitrogen in Kiripaka silt loam.

The following conclusions were made from a detailed study of the relationship between pH and rate of nitrification:

23) Soils perfused in the presence of $CaCO_3$ show a high rate of nitrification (up to 57.08 μg N_{ox}/g soil/hr).

24) Large populations of NH_4^+ and NO_2^- oxidising organisms (10^8/g soil) develop when soils are perfused in the presence of $CaCO_3$.

25) When soils are perfused in the pH range of 7.1 to 7.6, the generation time of NH_4^+ oxidisers is in the range of 36 to 40 hours.

26) The absence of a lag phase when Marua clay loam (a soil exhibiting type 2 nitrification pattern when perfused without $CaCO_3$) is perfused in the presence of $CaCO_3$ substantiates the presence of a

large population of nitrifying organisms as found using the most probable number technique.

27) The rate of nitrification in perfusion units containing $CaCO_3$ cannot be considered the result of soil factors alone since nitrifying organisms exist in the perfusion solution in such experiments.

28) Horotiu sandy loam is able to nitrify when the pH of the perfusion solution is 3.7.

29) A change in the pH of the perfusion solution results in changes in the population of nitrifying organsisms, in the rate of nitrification, and the rate of nitrification per NH_4^+ oxidising cell.

30) When soils are acidified, the decline in the rate of nitrification is more rapid in non-allophanic than in allophanic soils as pH is decreased from 7.0 to 4.0. In the latter soils, nitrification still proceeds at pH 4.0, whereas in the former soils, nitrification ceases at approximately pH 5.0.

31) The rates of nitrification relative to that at pH 7 are higher in allophanic soils than non-allophanic soils between pH 4.0 and 6.0.

32) Differences between soils in rates of nitrification at low pH can, at least in part, be rationalised by theoretical consideration of surface pH of soil colloids.

33) Application of lime and or ammonium fertiliser in the field increase both the Initial Nitrification Activity and the most probable number of nitrifying organisms.

Urea was applied to Tokoroa sandy silt and the effects followed in a laboratory experiment.

It was concluded that:

34) Application of urea in amounts similar to that found in cattle urine produces a large increase in the population of NH_4^+ oxidisers. This is due partly to a more favourable pH for nitrifying organisms following hydrolysis of urea, and partly because of the increased supply of substrate.

35) Incubation of soil is not a satisfactory technique for studying nitrification since both the Initial Nitrification Activity and the population of nitrifying organisms may change during incubation.

The fate of nitrogen applied as ammonium sulphate and calcium nitrate was studied in two soils, one of high and the other of low nitrification activity.

It was concluded that:

36) Severe nitrogen deficiency occurs during spring in Northland.

37) The difference between the nitrogen contents of herbage on control and nitrogen treated plots cannot be used to estimate the recovery nitrogen by a mixed ryegrass/white clover pasture if a change in botanical composition occurs.

38) When the nitrogen assimilated by ryegrass is largely in the form of NH_4^+, a positive cubic relationship will exist between the percentage of herbage nitrogen and phosphorus.

39) Differences in rates of nitrification in soils produce differences in the proportion of applied NH_4^+ which is nitrified.

40) "N-Serve" is not an effective nitrification inhibitor when NH_4^+ fertiliser treated with "N-Serve" is applied to the soil surface. Because of its low water solubility and volatile nature, "N-Serve" appears to have little application in New Zealand grassland farming where application of nitrogen fertilisers is normally to the soil

surface.

41) Nitrate fertilisers should not be applied to podzolic soils in Northland because large losses of nitrogen can occur from these soils following application of nitrate nitrogen.

42) Large amounts of nitrogen are lost from soils (30 to 69 percent) when $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ is applied to the soil surface in spring.

43) Only small amounts of fertiliser nitrogen are removed in surface runoff water.

44) The major mechanism for nitrogen loss from Northland soils appears to be by leaching, but some loss does occur by denitrification from podzolic soils.

45) The technique described in the present study in which ^{15}N enriched fertiliser is applied to micro-plots associated with normal field experiments is a useful method for studying the fate of nitrogen fertiliser applied to soil. It could be improved by placing lysimeter plates below the micro-plots.

13.2.0 STUDY OF VARIATIONS IN THE NATURAL ABUNDANCE OF ^{15}N IN NEW ZEALAND GRAZED SOIL PASTURE SYSTEMS

The natural variations of ^{15}N in New Zealand grassland soils and some ground waters of the Waikato, and the extent of nitrogen isotope discrimination in various reactions occurring in New Zealand grassland systems, was determined.

It was concluded that:

1) No satisfactory published statistical method exists for the calculation of uncorrected δ values and their associated instrumental errors. A satisfactory method was derived.

2) The isotopic fractionation associated with symbiotic fixation of atmospheric nitrogen by *Trifolium repens* is small, a slight

preference for ^{14}N existing.

3) The $\delta^{15}N$ value of total nitrogen in clover herbage is dependent on the proportion of the nitrogen derived from the atmosphere by symbiotic fixation, providing that a significant difference exists between the $\delta^{15}N$ values of atmospheric and soil nitrogen.

4) *Trifolium repens* shows a preference for assimilation of ^{14}N.

5) The $\delta^{15}N$ of nitrogen in ryegrass herbage grown under field conditions in the absence of grazing animals varies with the time of year. It is suggested that this may represent different contributions of inorganic nitrogen derived from clover roots and nodules, and total soil organic nitrogen.

6) Cattle fractionate ^{14}N and ^{15}N. Faeces nitrogen is enriched by about 2‰ and urine nitrogen depleted by about 2‰ relative to feed. Rumen microflora are unlikely to be the sole source of the observed fractionation since a similar fractionation is also observed in pigs.

7) Volatilisation of NH_3 from urine patches introduces the largest isotopic fractionation of any single nitrogen transformation occurring in soils. Ammonia volatilisation results in concentration of ^{15}N in NH_4^+ remaining in the soil, the actual enrichment being dependent on the amount of ammonia volatilised.

8) A fractionation factor of 1.022 was determined for nitrification by a pure culture of *Nitrosomonas* spp.

9) A fractionation factor of 1.011 was determined for nitrification by a mixed isolate from the same soil from which the pure *Nitrosomonas* isolate was obtained. This may indicate that the *Nitrosomonas* spp. isolated is not the only organism responsible for nitrification in that soil.

10) The mean enrichment of ^{15}N in sixty one grassland soils (0 - 7.5 cm) is +3.2‰. Although the range of $\delta^{15}N$ values measured for New Zealand soils is similar to the few soils reported from Australia (2.9, 5.9, 6.7‰, Black and Waring, 1976; 6.5‰, Vallis et al. 1967), it is much lower than many American and Canadian soils where enrichments up to 18‰ have been reported (Bremner et al. 1966).

Several factors may contribute to the apparently lower ^{15}N enrichment of New Zealand grassland soils. The major input of nitrogen into pasture systems in New Zealand is from symbiotic fixation of atmospheric nitrogen by clovers, fixed nitrogen having a negative $\delta^{15}N$ value. The high amounts of nitrogen reportedly fixed annually in New Zealand pastures means that there is a large annual input of nitrogen depleted in ^{15}N. This would be expected to be reflected in the $\delta^{15}N$ of total nitrogen in the top soil. Cheng et al. (1964) and Riga et al. (1970) observed that soils fertilised with nitrogen tended to have lower $\delta^{15}N$ concentrations, i.e. $\delta^{15}N$ values closer to that of fertiliser and atmospheric nitrogen in the upper soil profile than the respective unfertilised soils, although the results were not consistent.

Many New Zealand soils are still accumulating nitrogen, and losses will therefore be less than would occur under an equilibrium situation. This will restrict the amount of enrichment in ^{15}N which can occur. The mechanism of nitrogen loss (i.e. denitrification, leaching or volatilisation of NH_3) will also affect the extent of ^{15}N enrichment in soil since a different fractionation factor is associated with each mechanism.

It appears that most forest soils have negative $\delta^{15}N$ values (Riga

et al. (1970) and in many cases virgin soils have a lower $\delta^{15}N$ value than their agricultural counterparts (Bremner and Tabatabi, 1973). The positive $\delta^{15}N$ values of New Zealand grassland soils therefore appears to be a direct result of pastoral farming. Two major fractionations occurring in grazed pasture systems are considered important in the accumulation of ^{15}N. Firstly, in the example quoted in section 6.1.1, it was estimated that if dairy stock ingested 12,000 kg DM/ha/year, approximately 96 kg N/ha/year would be returned to the soil as dung. This dung is enriched in ^{15}N by about 2‰ relative to the intake, and because the nitrogen is organically bound, direct losses of nitrogen from dung may be expected to be low. Secondly, volatilisation of ammonia from urine patches results in the concentration of ^{15}N in the residual NH_4^+, some of which will be assimilated by plants, therefore increasing the ^{15}N content of nitrogen re-entering the nitrogen cycle. If the amount of denitrification under agricultural systems is higher than that under forest, it will also contribute to an increase in N^{15} content. The equilibrium $\delta^{15}N$ value of a given soil will be a result of the respective nitrogen inputs and losses from that soil.

11) $\delta^{15}N$ values of total nitrogen increase below the 0 - 7.5 cm depth. This may be a result of a small contribution from recently fixed atmospheric nitrogen.

12) Nitrate nitrogen in ground water in the Waikato has $\delta^{15}N$ values in the range of 5.2 to 10.0‰. The enrichment of ^{15}N indicates that NO_3^- derived from urine is a major contributor to NO_3^- in ground water of the Waikato.

13) It is now possible to postulate general changes in $\delta^{15}N$ values of nitrogen as it flows through a grazed grassland system in the Waikato (figure 13.1). Variability in each $\delta^{15}N$ value was estimated

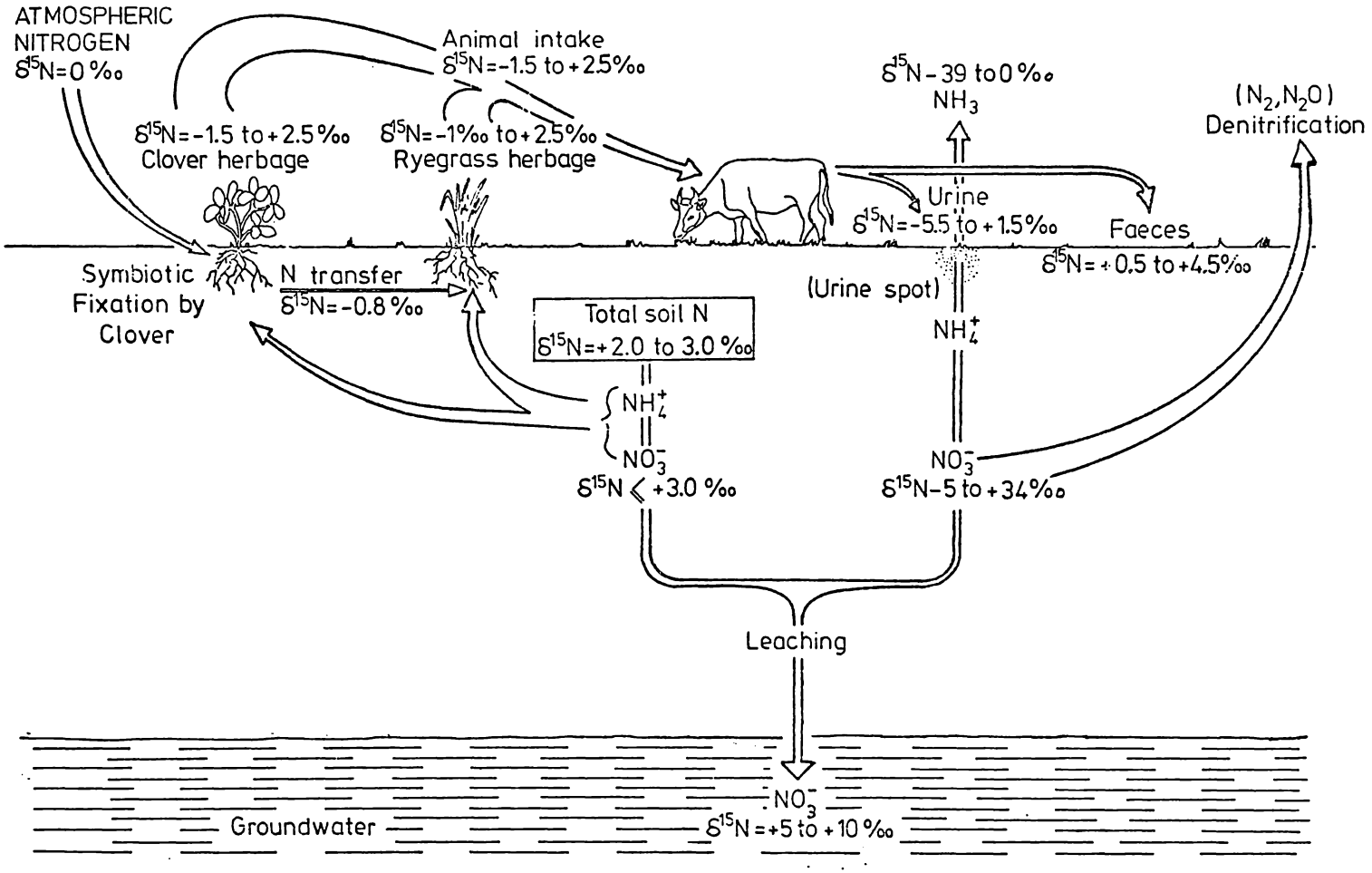

Figure 13.1 Changes of the $\delta^{15}N$ value of nitrogen as it flows through a grazed grassland system in the Waikato

from data presented in chapters 10, 11 and 12.

14) $\delta^{15}N$ values of components in a grazed grassland system cannot be used to provide quantitative information on short term transformations such as fertiliser uptake by plants, or the contribution of fertiliser nitrogen to nitrogen in groundwater. This is because of the inherent variability of $\delta^{15}N$ values, a result of the complexity of isotope fractionations which take place in such systems. Variations in the natural abundance of ^{15}N are useful, however, for the study of two specific components of the nitrogen cycle:

i) Symbiotic fixation of atmospheric nitrogen by clovers. Field measurements, using variations in the natural abundance of ^{15}N, will be possible if a method is developed to characterise the $\delta^{15}N$ value of soil inorganic nitrogen.

ii) To determine if urine nitrogen contributes to nitrogen in ground water. This is possible because of the large fractionation which takes place during NH_3 volatilistion.

It is important to remember, as pointed out by Hauck (1973), that nitrogen isotope ratios reflect cummulative effects of nitrogen cycle processes and thus permit one to speculate on a broad environmental scale using as a basis primary data. More data from all parts of the world are required to permit a valid examination of particular uses of $\delta^{15}N$ values in studying nitrogen transformations and balances on both a global and agricultural scale.

13.3.0 IMPLICATIONS OF THE RESEARCH REPORTED IN THIS THESIS TO NEW ZEALAND AGRICULTURAL PRACTICES

The conclusions reported above have several implications to New Zealand agriculture.

1) Agronomically, the difference in the ratio of NH_4^+ : NO_3^- in soils is of importance as it determines the form of nitrogen which is available for plant uptake. This difference does not appear to influence the rate of pasture production, but will of necessity have to be considered if specialised crops which show a preference for assimilation of either NH_4^+ or NO_3^- are grown on these soils. It is apparent that in many southern and northern yellow-brown earths, some podzolic soils, yellow-brown sands, recent soils from alluvium and yellow-brown pumice soils, NH_4^+ and not NO_3^- is the major form of nitrogen available for assimilation by plants.

2) The low rate of nitrification in soils such as Wharekohe silt loam appears to be an important mechanism for conservation of nitrogen. Once NH_4^+ is oxidised to NO_3^-, the nitrogen is largely excluded from the nitrogen cycle in soils, unless assimilated by plants. This occurs largely because most soil micro-organisms show a preference for NH_4^+ (Janson, 1958). Also NO_3^- is more likely to be removed from soil by leaching than NH_4^+, and nitrification must take place before denitrification can occur. In view of this, any agricultural practice, e.g. application of lime, which will result in increasing the rate of nitrification in these and related soils should be carefully considered, and the overall effect on the nitrogen economy of the soil evaluated. If increasing the rate of nitrification results in a decline in the nitrogen economy, then increased inputs of nitrogen, either by symbiotic nitrogen fixation or fertiliser application, will be required to maintain the same level of production.

Application of lime is one factor which will increase the rate of nitrification in a soil. For example, the mean INA of yellow-brown earths with a mean pH of 5.5 was 0.03 µg N_{ox}/g soil/hr, whereas the

mean INA of yellow-brown earths with a mean pH of 6.0 was 0.43 µg N_{ox}/g soil/hr. This represents an increase in the rate of nitrification by a factor of fourteen for a shift in pH of 0.5 units.

3) Baber (1977) reported high nitrate concentrations (up to 58 mg N/ℓ) in shallow aquifiers of the Waikato in areas where yellow-brown loams (soils having a high rate of nitrification) are prevalent. In contrast to this, low nitrate concentrations were reported in ground water in the Rotorua district by Grinstead and Wilson (1977), an area where soils possess a low rate of nitrification.

If soils which have a high rate of nitrification are directly responsible for nitrate in ground water, the agricultural practices on these soils should be critically examined and altered to reduce the rate of nitrification. It may be that in the future, public pressure will necessitate this action. The grazing animal, for example, is an important mechanism for the maintenance of high populations of nitrifying organisms in New Zealand grassland soils. Urine nitrogen is also a major contributor to nitrogen in shallow aquifiers in the Waikato. Agricultural practices in specific catchments, particularly those from which water is collected for human consumption, may have to be modified even to the extent of complete removal of the grazing animal.

4) The disadvantages of nitrification in New Zealand grassland soils appear to outweigh the advantages, and therefore, agricultural practices should, where possible, be designed to minimise the rate of nitrification.

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APPENDICES

APPENDIX 1

GLOSSARY OF CHEMICAL NAMES

Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4,-endo-5,8-dimethanonapthalene.
AM	2-amino-4-chloro-6-methyl-pyrimidine.
Baygon	o-isopropoxyphenylmethylcarbamate.
Botran	2,6-dichloro-4-nitroaniline.
Chemagro 2653	isometric mixture of 80% 1,2,5,-trichloro-4,6,-dinitrobenzene and 20% 1,2,3,-trichloro-4,6-dinitrobenzene.
Chloropicrin	trichloronitromethane.
CIPC	isopropyl N-(3-chlorophenyl)carbamate.
Dazomet	85% tetrahydro-3, 5-dimethyl-2H-1,3,5-thiadiazine-2-thione as dust.
DBCP	1,2 dibromo-3-chloropropane 97% plus 2% related compounds.
DD	Mixture of 60% 1,3 dichloropropene and 40% other related chlorinated hydrocarbons.
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-eno-5,8-dimethano-napthalene.
Dowfume W-85	see EDB.
Dyrene	2,4-dichloro-6-(0-chloroanilino)-s-triazine.
EDB	ethylene dibromide 83%.
Eptam	5-ethyl-di-N,N propylthiocarbamate.
Fenitrothion	dimethyl 3-methyl-4-nitrophenol phosphorothionite.
Heptachlor	1,4,5,6,7,10,10-heptachloro-4,7,8,9-tetrahydro-4,7-endo-methyleneidene.
Lanstan	1-chloro-2-nitropropane.
Lindane	γ-1,2,3,4,5,6-hexachloro cyclohexane.
Malathion	S-(1,2-di(ethoxycarbonyl)ethyl) dimethyl phosphorothiolothionate.
Maneb	manganese ethylenevisdithio-carbomate.
Metham sodium	sodium N-methyldithiocarbamate 31%.
Monuron	N'-(4-chlorophenyl)-N,N-dimethylurea.
Neburon	N'-butyl-N-(3,4 dichlorophenyl)-N-methylurea.
Nemagon	see DBCP.
N-Serve	2-chloro-6-(trichloromethyl)-pyridine.
Parathion	diethyl-4-nitrophenylphosphorothionate.
PCNB	1,2,3,4,5,-pentachloro-nitrobenzene.
PRD G	10-3,4 dichlorotetrahydrothiophen 1:1 dioxide 10%.
Propargil bromide	3 bromo-propyne.
Simazine	2-chloro-4,6-bisethylamino-1,3,5-triazine.
TDE	1,1 dichloro-2,2-di-(4-chlorophenyl)ethane.
Telodrin	1,3,4,5,6,7,8,8 octachloro-3a-4 tetrahydro-4,7 methanonaphthalan.
Telone	95% 1,3-dichloropropene and 5% related C_3 compounds.
Vapam	see metham sodium.

APPENDIX 2A

PROFILE DESCRIPTION

Soil:	Wharekohe silt loam.
Classification:	Podzol.
Location:	D.S.I.R. Substation, Cumbers Road, Kaikohe. N15/335328.
Topography:	Rolling hill country.
Vegetation:	Rolling hill country.
Native Vegetation:	Kauri forest.
Parent Material:	Strongly weathered banded sandstone and mudstone.
Rainfall:	1500 mm.
Land Use:	Sheep grazing.
Profile:	Described moist.
A 0 - 6 cm	Dark brown (10YR 4/4) silt; compact, very weakly developed fine nutty structure, grading to massive; numerous grass roots; distinct wavy boundary.
A 0 - 16 cm	Light grey (10YR 8/1) silt; massive; no roots; distinct wavy boundary.
A 16 cm +	Mottled bright yellowish borwn (10YR 6/8) and dark brown (10YR 7/3).

APPENDIX 2B

PROFILE DESCRIPTION

Soil:	Waimate North clay loam.
Classification:	Immature red loam from basaltic scoria.
Location:	Rochell and Patterson, Waikaramu Road, Waimate North. Profile in corner of silage pit in north-east corner of paddock opposite cowshed N15/408449.
Topography:	Rolling relief. On northern side of slope, Slope 8°.
Drainage:	Well drained.
Vegetation:	Ryegrass, white clover, brown top.
Native Vegetation:	Broadleaf forest (Purui).
Parent Material:	Basaltic scoria.
Rainfall:	1250-1500 mm.
Land Use:	High density dairy farming.
Profile:	Described moist.
A1 0 - 11 cm	Dark brown (10YR 3/2) clay loam; friable; moderately developed fine and very fine granular structure; many earth worms; numerous well developed grass roots; indistinct boundary.
A2 11 - 17 cm	Greyish brown (10YR 4/2) silt loam; very friable; moderately developed fine and very fine nutty structure; numerous grass roots, indistinct boundary.
AB 17 - 23 cm	Dull reddish brown (5YR 5/4) silt loam; weakly developed fine nutty structure; numerous grass roots; indistinct boundary.
B 23 - 67 cm	Reddish brown (5YR 4/8) silt loam; weakly developed blocky structure; compact; numerous grass roots; indistinct boundary.
BC 67 cm ↓	Dark reddish brown (5YR 3/4) containing soft basaltic scoria with brownish grey matrix (5YR 4/1) mottled with bright reddish brown (5YR 5/8); pale yellow (2.5Y 8/3) and reddish black (7.5R 2/1); compact; very few grass roots.

APPENDIX 2C

PROFILE DESCRIPTION*

Soil:	Kiripaka clay loam.
Classification:	a) Moderately leached brown loam. b) Moderately enleached suboxadic soil.
Location:	Pakaraka, North Auckland. South side main north highway, opposite Pakaraka School. N15/468409. Slope: flat. Altitude: 270ft.
Vegetation:	Cocksfoot, tall fescue roadside pasture. 8-10 years ago site was part of a highly topdressed pasture.
Parent Material:	Weathered basaltic scoria and ash. (Scoriaceous basalt surface below approximately 20,000 years old).
Native Vegetation:	Broadleaved forest.
Rainfall:	60 - 65 in.
Land Use:	Mainly pastoral land for dairying and fat lamb raising. Some market gardening.
A_{1p} 0 - 10 cm	Very dark greyish brown (10YR 3/2) clay loam, friable; moderately developed fine and very fine nutty and fine granular structures; peds well separated in place; many earthworms; numerous grass roots; distinct smooth boundary.
A_{12} 10 - 23 cm	Dark brown (7.5YR 3/2) silt loam; ;very friable; appears massive but has weakly developed fine and very fine nutty and very fine granular structures; peds powder on light pressure; many pores; contains 2 - 4% of scoriaceous basalt ($1/8$ - $1/2$ in); numerous grass roots; indistinct boundary.
B_1 23 - 41 cm	Dark brown (7.5YR 4/3-4/4) clay loam; friable; moderately developed medium and fine nutty, which breaks to very fine nutty structure; peds are coated and slightly finer - centres are (4/4) and outer surfaces (3/2); many pores; contains 2% of softened scoriaceous basalt ($1/4$ - $1/2$ in); numerous grass roots; indistinct boundary.
B_2 41 - 61 cm	Dark brown (7.5YR 3/2-4/4) clay loam; friable; more tightly packed and harder to dig; moderately developed medium nutty, which breaks to fine nutty and very fine blocky structures; peds more strongly coated surfaces (3/2) grading inward to (4/2) with centres (4/4); numerous very fine grass roots; indistinct boundary.

*From N.Z. Soil Bureau Bulletin 26.

BC	61 - 84 cm	Dark brown (7.5YR 4/2) silty clay; friable; very tightly packed in place; strongly developed medium and fine nutty, which breaks to very fine blocky structure; fewer pores; contains 2-3% of softened scoriaceous basalt ($1/8 - 1/2$ in); numerous very fine grass roots; indistinct boundary.
C	84 - 107 cm	Dark brown (7.5YR 4/2) silty clay loam; extremely tightly packed in place but peds separate readily when disturbed; strongly developed fine and very fine blocky structures; few pores; contains 5% of softened scoriaceous basalt ($1/4 - 3/4$ in), 2-3% of pin-point alumina; few very fine grass roots; distinct regular boundary,
	on	matrix of dark brown (7.5YR 4/2) clay and basalt stones; friable but tightly packed; moderately developed fine nutty and fine blocky structures; contains 50% of scoriaceous basalt and 2-3% of pin-point alumina. The soil to 33 in contains pin-head white moulds.

APPENDIX 3

METEROLOGICAL DATA

Date	Rainfall mm	Relative Humidity	Air Temp Max.	Air Temp Min.	Earth 10 cm	Earth 20 cm	Sunshine hours	Wind Km	Evaporation 0.1 mm
September									
1/9	0.8	94	14.1	5.4	7.4	8.6	2.5	152	0.4
2	-	97	14.9	4.6	6.6	8.6	4.8	134	1.4
3	11.2	83	13.3	6.6	9.0	9.6	1.0	274	1.2
4	15.5	82	13.4	7.3	9.2	9.5	0.7	682	3.4
5	41.0	91	12.9	9.1	10.3	10.4	-	586	4.5
6	38.3	91	14.4	10.4	11.8	11.3	0.1	561	-
7	21.7	97	14.2	9.8	10.6	11.1	0.2	407	0.5
8	25.1	96	14.8	10.0	11.6	11.5	0.1	353	1.5
9	1.0	82	15.6	8.6	10.2	10.9	4.2	250	2.0
10	4.5	81	16.5	10.6	12.1	12.2	9.4	434	-
11	4.6	79	15.7	9.4	11.5	11.9	6.2	712	3.9
12	2.3	67	15.0	9.5	11.2	11.4	5.6	860	4.8
13	6.9	70	14.6	8.3	9.5	10.2	5.1	734	4.9
14	2.5	75	13.7	8.0	9.1	9.8	6.9	634	2.4
15	3.4	88	13.9	8.6	9.8	10.2	6.2	409	3.0
16	0.1	80	14.2	7.4	8.9	9.9	7.2	345	2.5
17	0.6	80	14.3	6.1	9.1	9.7	5.6	285	2.6
18	2.0	71	13.3	6.3	9.0	9.5	6.4	264	2.3
19	-	79	14.9	6.5	8.3	9.1	6.4	136	2.6
20	-	74	14.7	5.6	7.6	8.9	7.7	196	2.8
21	3.3	85	14.6	6.4	8.6	9.2	4.4	117	0.7
22	1.1	88	14.1	5.9	9.6	9.9	2.9	188	2.2
23	0.3	93	15.9	7.6	11.3	10.9	3.7	301	2.1
24	1.5	70	14.4	8.6	10.6	10.9	1.4	429	1.9
25	-	81	17.8	10.1	11.3	11.2	7.8	375	3.6
26	4.0	85	15.4	9.3	10.9	11.6	4.8	533	2.4
27	12.1	90	16.0	9.3	11.4	11.4	3.0	168	3.9
28	-	83	13.9	6.9	11.0	11.0	1.4	233	1.4
29	0.2	86	16.1	8.0	10.3	10.5	5.3	241	1.2
30	-	80	17.2	6.2	10.0	10.6	9.3	347	3.9
October									
1/10	-	71	16.0	7.3	10.0	11.9	11.0	371	5.9
2	-	74	16.0	7.3	9.5	11.7	7.4	360	1.8
3	-	72	14.8	9.5	12.0	12.5	3.7	309	4.0
4	-	74	13.9	6.1	9.4	11.7	10.9	412	5.1
5	-	64	13.9	4.0	8.2	11.2	10.6	344	4.6
6	1.0	74	13.4	6.8	9.6	11.3	0.2	274	1.8
7	7.1	87	16.4	9.8	11.8	11.9	1.2	526	1.5
8	12.1	94	19.0	12.4	14.1	13.2	3.5	477	4.2
9	-	86	18.9	14.3	15.6	14.4	9.8	258	4.0
10	8.9	70	19.6	9.2	12.7	13.7	11.0	381	6.1
11	4.3	76	18.8	13.5	15.6	14.9	7.3	387	3.9
12	2.3	84	16.6	10.3	-	14.2	6.2	358	2.6
13	37.8	94	14.1	9.0	-	13.4	-	333	4.9
14	-	66	17.0	9.9	-	13.2	9.9	304	4.5

Date	Rainfall mm	Relative Humidity	Air Temp Max.	Air Temp Min.	Earth 10 cm	Earth 20 cm	Sunshine hours	Wind Km	Evaporation 0.1 mm
15	0.5	79	16.7	7.0	-	12.8	2 5	257	2.5
16	7.8	95	16.7	10.5	-	13.4		385	-
17	8.9	84	20.3	12.3	-	13.9	5.8	240	8.6
18	-	94	19.9	14.4	-	15.3	5.3	294	3.5
19	15.7	81	19.1	12.0	-	15.5	9.2	300	5.3
20	8.4	92	18.7	11.9	-	15.0	2.0	367	2.4
21	-	88	15.7	11.0	13.7	15.5	3.8	362	3.8
22	8.1	59	15.3	8.0	11.4	13.4	3.5	338	4.0
23	1.2	79	15.9	8.5	12.5	13.4	8.4	661	4.9
24	-	71	15.5	10.2	12.3	13.5	4.9	337	3.5
25	3.3	78	15.2	8.7	11.9	13.3	1.3	513	4.2
26	-	63	13.9	7.4	10.5	12.9	10.9	616	5.6
27	0.2	70	15.0	7.9	10.2	12.2	9.8	268	4.1
28	-	71	15.4	6.2	10.7	12.2	1.5	150	2.9
29	5.1	61	16.7	5.2	9.9	12.2	9.7	435	3.1
30	6.8	89	14.5	10.9	12.5	13.3	-	420	1.1
31	6.1	93	14.6	10.6	12.2	13.3	-	437	-
November									
1/11	16.1	97	14.6	10.9	12.4	12.8	-	* -	0.2
2	41.5	96	14.9	11.9	13.5	13.5	-	-	2.3
3	-	97	20.4	13.1	14.0	14.0	1.4	-	2.2
4	0.4	82	20.7	12.6	14.5	14.6	4.3	-	3.7
5	7.7	91	18.7	13.4	14.7	15.3	5.0	-	5.1
6	5.6	72	16.9	12.0	13.6	14.2	6.8	-	5.1
7	1.0	84	13.3	9.2	12.2	13.4	8.3	-	3.3
8	2.6	83	15.1	6.5	10.0	11.4	10.0	-	4.5
9	13.0	69	17.4	10.0	11.9	12.5	7.8	-	5.2
10	0.5	94	16.1	10.9	12.9	13.3	9.3	-	5.9
11	1.7	67	13.7	8.9	11.4	12.7	7.2	-	5.0
12	3.5	72	15.1	6.6	10.0	11.3	9.2	-	4.1
13	-	75	16.7	6.3	9.9	11.4	11.8	-	5.4
14	-	73	16.1	6.5	10.3	11.6	8.0	-	4.5
15	-	75	17.5	8.4	10.9	12.0	11.7	-	4.9
16	-	80	18.7	7.9	10.7	12.2	11.0	-	4.4
17	8.1	76	15.6	8.5	12.3	13.1	-	-	2.7
18	31.9	94	13.0	11.6	13.1	13.5	-	-	2.8
19	1.8	91	16.1	10.1	12.0	12.5	2.1	-	1.8
20	6.1	77	17.3	10.2	12.1	12.8	7.5	-	3.2
21	-	84	18.6	9.6	12.3	13.2	8.9	-	3.7
22	-	83	18.6	8.6	11.5	12.7	11.7	-	6.5
23	13.4	83	16.4	9.4	12.4	13.4	2.9	-	2.8
24	0.8	98	21.9	13.3	14.5	14.5	7.3	-	4.5

* Not determined.

APPENDIX 4

CALCULATIONS FROM MICROMASS 602C MANUAL

The results may be set out in the form shown below:-

Sample gas	-XYZ
Reference gas	-ABC (laboratory standard)
Ratio measured	-29/28
Peak height	-Reference major = Sample major 4.00×10^{-9} amps (10^{-8} amps full scale)
Ratio settings	-R(0.398 for example)
Ratio offset setting	-Ro (+0.01 for example)
Current ranges	-Ma = 10^{-8} A Mi = 10^{-10} (for example)

SAMPLE s	REFERENCE r	DIFFERENCE s - r	MEAN OF CONSECUTIVE READINGS	DEVIATION FROM MEAN	SQUARE OF DEVIATION FROM MEAN
s1					
	r1	s1 - r1 = A1			
		s2 - r1 = A2	$\frac{A1 + A2}{2} = X1$	$X1 - \bar{X}$	$(X1 - \bar{X})^2$
s2					
		s2 - r2 = A3	$\frac{A2 + A3}{2} = X2$	$X2 - \bar{X}$	$(X2 - \bar{X})^2$
	r2				
		s3 - r2 = A4	$\frac{A3 + A4}{2} = X3$	$X3 - \bar{X}$	$(X3 - \bar{X})^2$
s3					
		s3 - r3 = A5	$\frac{A4 + A5}{2} = X4$	$X4 - \bar{X}$	$(X4 - \bar{X})^2$
	r3				
		s4 - r3 = A6	$\frac{A5 + A6}{2} = X5$	$X5 - \bar{X}$	$(X5 - \bar{X})^2$
s4					
		s4 - r4 = A7	$\frac{A6 + A7}{2} = X6$	$X6 - \bar{X}$	$(X6 - \bar{X})^2$
	r4				
		s5 - r4 = A8	$\frac{A7 + A8}{2} = X7$	$X7 - \bar{X}$	$(X7 - \bar{X})^2$
s5					
		s5 - r5 = A9	$\frac{A8 + A9}{2} = X8$	$X8 - \bar{X}$	$(X8 - \bar{X})^2$
	r5				
		s6 - r5 = A10	$\frac{A9 + A10}{2} = X9$	$X9 - \bar{X}$	$(X9 - \bar{X})^2$
s6					
		s6 - r6 = A11	$\frac{A10 + A11}{2} = X10$	$X10 - \bar{X}$	$(X10 - \bar{X})^2$
	r6				
	$\frac{\Sigma r_n}{6} = \bar{r}$		$\frac{\Sigma X_n}{6} = \bar{X}$		$\Sigma (X_n - \bar{X})^2$

From these figures the following can be calculated:-

$$\delta = \frac{R_s - R_r}{R_r} \times 1000$$

$$\delta = \frac{(R + \bar{r} + Ro + \bar{X}) - (R + \bar{r})}{R + \bar{r}} \times \frac{(Mi/Ma)}{(Mi/Ma)} \times 1000$$

$$\delta = \frac{Ro + \bar{X}}{R + \bar{r}} \times 1000$$

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Numerical definition of σ :-

$$\sigma_1 = \sqrt{\frac{\Sigma (X_n - \bar{X})^2}{(n - 1)}}$$

$$\sigma_n = \sqrt{\frac{\Sigma (X_n - \bar{X})^2}{n\ (n - 1)}}$$

$$2\sigma = \frac{2\sigma_1}{\sqrt{n}}$$

σ expressed in $^{o}/_{oo}$ relative to reference $(R + \bar{r})$:-

$$\sigma_1 (^{o}/_{oo}) = \frac{\sqrt{\frac{\Sigma (X_n - \bar{X})^2}{(n-1)}} \times 10^3}{R + \bar{r}}$$

$2\sigma_n$ expressed in $^{o}/_{oo}$ relative to reference $(R + \bar{r})$:-

$$2\sigma_n (^{o}/_{oo}) = \frac{\sqrt{\frac{\Sigma (X_n - \bar{X})^2}{n(n-1)}} \times 10^3}{R + \bar{r}} = \frac{2\sigma_1 (^{o}/_{oo})}{\sqrt{n}}$$