THE INFLUENCE OF CULTURAL PRACTICES ON SOIL ORGANIC MATTER, SOIL BIOMASS SIZE AND NITROGEN LEACHING

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ABSTRACT

The effects of bare fallow, cultivation and nitrogen application on soil organic matter, soil biomass and nitrogen leaching were compared against the backgrounds of permanent grass pasture and grass/clover pasture. Cultivated plots were dug annually to a depth of 15cm. All plots received an annual dressing of 300kg ha^{-1} of Potassic Super. The Nitrogen treatment was applied as two equal applications of 100kg ha^{-1} Calcium Ammonium Nitrate in November and December each year.

The results indicated that both bare fallowing and cultivation reduced soil organic matter and soil microbial biomass. The use of N-fertilizer did not promote either the soil organic matter or soil microbial biomass; this is contrary to the general finding.

Legume nitrogen was found to leach as readily as the applied nitrogen and hence posed an environmental threat to groundwater quality. In all cultural practices the largest concentration of nitrate nitrogen (NO_3-N) was observed at the 50 - 100m depth, below the root zone. Vertical movement of groundwater was estimated at 1m $year^{-1}$. This confirmed the concern of possible groundwater pollution by nitrate nitrogen from agricultural activities.

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CHAPTER 1

1 General Introduction

1.1 Introduction

Agriculture manipulates energy fluxes, nutrient dynamics and hydrological cycles. Such manipulation may involve clearing and burning to remove woodland, ploughing, fertilizer application and seeding to create crops and pastures and intensive grazing by domestic animals. Increasingly, farmers, agricultural scientist and environmentalists are turning their attention to considerations of the chemical and biological integrity of the cropping systems. The reasons for this are obvious. When man converts natural ecosystems into agroecosystems, he modifies many specific features of their structure and dynamics. These modifications affect two basic ecosystem characteristics. They tend to reduce the importance of detritus food chains and to increase the importance of nutrient and energy exports from the system (Cox and Atkins, 1979). In particular the nitrogen cycle has captured the interest of both environmentalists and agriculturalists. The environmental concerns stem from the fact that potable water pollution by NO_3^- in run-off or groundwater has been linked to carcinogenic nitrosamines from NO_2^- and the depletion of ozone layer by soil evolved N_2O (Crutzen, 1981 and Byrnes, 1990). From the agricultural point of view nitrogen cycling is of paramount importance in that it is one of the most limiting nutrients in crop yields. Furthermore, in economic terms N fertilizer constitutes a large monetary cost of crop production.

For more than a century now mankind has always been keen to know more about the impact of his agricultural activities on the environment since this has a bearing on the long-term sustainability of agricultural systems. Jenkinson (1991) reported on British experiments as old as 150 years in which inorganic nutrients, in various combinations, were compared with farmyard manure - the traditional source of fertility. These experiments provided a wealth of information with regard to long-term effects of inorganic fertilisers and organic manures on soil organic matter levels. Over the years agricultural research has broaden its focus. Todate several other issues have been incorporated into this historic research thrust. These include research on changes in soil pH over time and nitrogen cycling in these agroecosystems. This kind of research is no longer limited to more conventional cropping systems such as cereals but has been extended to pastoral systems as well. For example in New Zealand there is currently a strong interest in cycling efficiencies of many plant nutrients in grazed pastures. It is believed that the grazing animals aggregate many plant nutrients into dung and urine excretions. These excretions contain concentrated forms of many plant nutrients, with N and potassium (K) levels being potentially high (Hogg, 1981). Once urine has been applied onto the soil much of the organic forms of N are rapidly converted to ammonium (NH_4^+) then nitrate (NO_3^-) ions which are susceptible to losses through leaching and other soil processes such as volatilisation and denitrification.

Managed agroecosystems generally tend to have greater inputs of N than unmanaged systems. Because of this, greater N losses are incurred under intensively managed systems. The actual extent or severity of the losses of N in such systems will depend on several factors. Allison (1966) and Campbell and Paul (1978) pointed out that the amount of N loss is a function of timing and rate of application, cropping system and moisture regime. With good management, losses could be minimised. However, increased exports of N from disturbed and unmanaged natural ecosystems such as forests have long been appreciated. Likens *et al.*, (1970) noted that clear felling of trees alters the N cycle within the ecosystem, greatly increasing the N leakage from the watersheds and resulting in increases in nitrate levels in river waters. Bormann *et al.*, (1968) reported and stressed that the nutrient cycle in a forest is closely geared to all the components of the ecosystem and the balance between decomposition and the nutrient uptake influences the conservation of nutrients within the ecosystem. In the absence of forest vegetation the bulk of mineralized nutrients, and mainly N, are rapidly flushed out from the watershed-ecosystems.

1.2 Nitrate problem in the Environment

The impact of nitrogen on the environment is now well appreciated and a more responsible attitude to the problem has been adopted. Research in crop production is aiming to provide a basis for acceptable compromise in conflicting goals of maximum yield, maximum profit and zero environmental pollution. Peterson and Russel (1991) gave some of the reasons for increasing studies in nitrogen cycling in agricultural production systems as:

(a) depletion of the atmosphere's protective ozone layer.

(b) high demands of energy required in the production of N fertilizers.

(c) accumulating scientific evidence showing that some agricultural practices deposit N in both surface water and groundwater supplies.

The protection of groundwater quality from which both public and private wells draw drinking water is a high priority in most countries. In New Zealand the concentration of NO_3^- in bore and well waters in many areas exceeds the upper limit for potable water of 10mg per litre suggested by the World Health Organisation (Steele and Judd 1984). This problem of nitrate pollution is not being experienced in this country alone. Other countries, for example, the United Kingdom (Wild and Cameron, 1980a) and U.S.A. (Magette and Shirmohammadi, 1989) are facing a similar problem.

Nitrate leaching is a particular problem on cultivated agricultural lands and it is often the most important channel of N losses from field soils. Wild and Cameron (1980b) reported that such losses range from 2 to 100kg ha^{-1} $year^{-1}$ in the UK. This N originates from mineralisation of soil organic matter and fertilizer N not used by crop plants. Addition of N which is essential for obtaining high crop yields commonly increases leaching losses. When high fertiliser rates are combined with heavy irrigation regimes on light-textured soil, leaching losses of nitrate nitrogen (NO_3^--N) can be large (Weil *et al.*, 1990). The processes involved in NO_3^- leaching and factors influencing losses have been studied extensively because of their economic and environmental significance (Cameron and Haynes 1986). However, data is needed on specific cultural practices and their impact on N leaching from the soils. Such studies could help develop best management practices for agricultural land to protect groundwater. It has been found that nitrate moves downward through the soil profile at a rate of about 1 to 2m per year depending on soil type and the underlying rock (Young *et al.*, 1979). This kind of information may not only make it possible to predict when problems in drinking water may arise but may also help identify which cultural practices pose the greatest risk of N pollution to ground water.

The current rising levels of nitrate in groundwater is attributed to application of N-fertilisers by farmers. Because of this, calls for the control, or in some cases a ban, on the use of N-fertilisers by farmers have been put forward, particularly in Europe. Work by Powlson *et al.*, (1986) strongly points to the residual N from previous seasons and from the soil organic N. The work suggests that organically bound N can be a major source of nitrate pollution when it is finally re-mobilised by soil microbes. This microbially produced N is subject to leaching if the re-mobilisation rate is not matched with the plant uptake.

In order to investigate more closely the complexity of this issue, a six-year experiment was set up at DSIR Grassland, Palmerston North The objective was to assess how cultural practices affect soil's N mineralisation potential and N leaching. In particular, the effect of cultivation, bare fallowing and legume N fixation was investigated. Additional investigation was to assess the impact of these cultural practices on soil microbial populations since the N mineralisation and soil biomass are closely linked. The soil biomass derives its energy and nutrient supplies through the decomposition process. The size of the biomass pool, therefore, reflect the amount of material available for decomposition (Carran 1983) and hence should reflect the long-term amount of C input to the soil (McGill *et al.*, 1986). Because of this close association between biomass and organic matter, biomass assessment can offer an alternative method of assessing the impacts of cultural practices on soil-plant systems. Past scientific work has established that the decline in soil biomass is far more sensitive to measuring effects of cultural practices on any change in organic inputs than is the measuring the total organic matter (Powlson *et al.*, 1987 and Powlson and Jenkinson, 1981). The measurement of microbial biomass could thus be a valuable tool for understanding and predicting the long-term effects of changes in soil conditions.

CHAPTER 2

2 Literature Review

2.1 Introduction

Nitrate is the principal pollutant associated with agriculture and the rising contaminations of nitrate in groundwater sources are widely attributed to the effect of past and present agricultural practices. Horne, (1982) indicates that despite this common knowledge it is very difficult to try to control this form of pollution. The fact that at least a proportion of N now found in water samples must derive from agricultural soils presents a problem to resource managers which, because it does not appear from a point source, has no potential cure. The cumulative effects of management practices on nitrate nitrogen (NO_3^--N) leaching and groundwater quality are frequently difficult to document because of the time required for expression and the diversity of interacting processes involved. The information required to correlate cultural and management practices with groundwater quality is frequently limited because of the time lag between activities at the soil surface and the ultimate impact on the aquifer (Schepers *et al.*, 1991). In spite of this generalisation, these researchers found a correlation between producers who exceeded fertiliser N recommendations and the highest groundwater NO_3^--N concentrations.

2.2 The Nitrogen Cycle

The pattern of movement of N through an ecosystem (figure 2.1) differs in several ways from that of other elements. First, the main reservoir of N is the atmosphere, which is 79% dinitrogen (N_2). This translates into 8×10^7kg of N above each hectare of earth's surface and this is the ultimate source and sink for all the N present in ecosystem (Gandar and Ball, 1982). Atmospheric N is not available to plants and must be fixed by certain soil micro-organisms or by chemical manufacturing processes before it can be used. Second, there is a distinct biotic sub-cycle for N that involves the

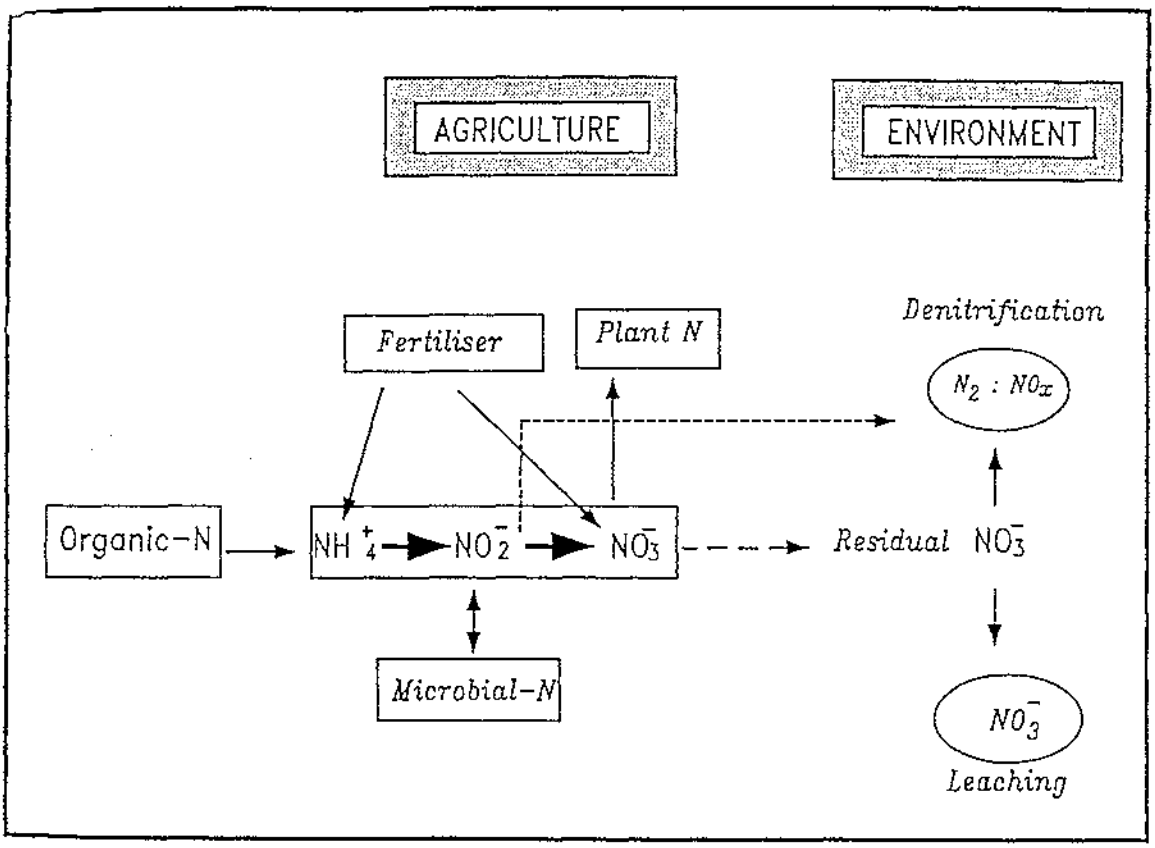

Figure 2.1: The relationship between some N transformations and their role in agricultural production and environmental pollution.

biological breakdown, in several steps, of organic forms. Even in the soil most nitrogen is unavailable for plant uptake as it is part of soil organic matter fraction. Hood (1976) estimated that soils, depending on level of fertility, may contain reserves of nitrogen varying from 1-5t ha^{-1} in top 15cm. Ball (1984) suggested an even greater proportion of 5 to 15t N ha^{-1}. Of these estimates Cameron and Haynes (1986) reported that about 90% is in organic form and that the actual available-N pools in most soils are very small relative to plant uptake. The implication of this is that N is taken up by plants from decaying organic matter before it enters the exchange complex. Johnson, (1992) reported that the N pools are replenished several times per year by the process of mineralisation or N_2 fixation or both.

2.2.1 Biological Fixation

Certain micro-organisms have the unique ability to convert atmospheric N_2 gas into plant available N. This conversion, called N-fixation, involves the reduction of molecular N_2 and the production of organic N compounds. The amount of fixation occurring locally depends on environmental conditions such as temperature, oxygen, moisture and nutrient availability (Mulder, 1975 and McClean and Cameron, 1990). Two systems of biological N-fixation operate. These are:

(a) fixation by free-living micro-organisms.

(b) fixation by micro-organisms which live in symbiosis with higher plants.

2.2.1.1 Fixation by Free-living microorganisms

The ability to fix N is present in a wide range of organisms. Nevertheless only a very small proportion of species is able to do so and about 87 species in 2 genera of archaebacteria, 38 genera of bacteria and 20 genera of cyanobacteria have been identified as diazotrophs, or organisms that can fix nitrogen (Dixon and Wheeler, 1986). The wide variety of diozotrophs ensures that most ecological niches will contain one or two representatives and that lost N can be replaced. The actual amounts fixed are variable (McClean and Cameron, 1990) and the contributions to agricultural soils are indirect since most non-symbiotic bacteria do not excrete fixed N and must be decomposed before the N can be released.

2.2.1.2 Symbiotic N-fixation

Among the various biological systems which are able to fix atmospheric N, the symbiosis of leguminoseae with Rhizobium seem to contribute most of N to the ecosystems and food production. Nitrogen fixation by legume accounts for 20% of the estimated biological N_2 fixed each year on earth, far more than that of the free-living microorganisms with the figures similar to those of all the N fixed chemically by industry (Quispel, 1974). Legumes have been used in crop production since ancient times. However, during the past two decades interest in legumes and their role in cropping systems has increased in response to the energy dependence of inorganic fertilizers, concern over soil erosion, and leaching of nutrients and associated groundwater contamination (Walters *et al.*, 1992). Today leguminous plants represent the only known crop that can be self sufficient in N nutrition and that may leave fixed N in the soil (France 1978). Their contribution in N_2-fixation is also important in natural uncultivated systems.

2.2.2 Mineralization and immobilization

Microbial decomposition constitutes the main process by which N held in the plant residues is released into the soil for reuse by plants. During the decomposition carbon is returned to atmosphere as CO_2 while N is converted into ammonium (NH_4^+) and nitrate (NO_3^-) forms of nitrogen through a process of mineralization. The microbes involved in the decomposition process also assimilate a part of the N, a phenomenon known as N-immobilization. Mineralization and immobilization of N, therefore constitute two major biological processes controlling availability of N to plants. These two processes proceed continuously and simultaneously. Depending on their relative dominance, there is either a net increase or a net decrease in the soil inorganic N concentration, which is known as mineralization-immobilization turnover. The chemical composition of the plants residues affects the overall mineralization rate. Of particular significance is the C:N ratio. This can be higher than that of the microbial biomass feeding on it (Houot *et al.*, 1989). With C:N ratio of 25:1 to 30:1 there may be net immobilization in the first few days or weeks of decomposition after which there will be net mineralization (Parnas, 1975 and Whitehead, 1986). Herman *et al.*, (1977) also found that the relative proportions of lignin and carbohydrates in plant residues is important.

2.3 The Fate of Nitrogen in the soil.

The management of the N transformations and its fate in the soil is important to insure that there is sufficient available-N for plant growth but with no NO_3^- in excess of the plant needs. Any excess of NO_3^- in the soil would be lost through leaching into the aquifers thereby posing health hazard. The first product of mineralization is NH_4^+. This is the hub of N transformation in the soil because its pool lies at the crossroads of the three major N processes ie ammonification nitrification and immobilization (Boyle and Paul, 1989). Since immobilisation has already been described above, we now concern ourselves with the first two.

When NH_4^+ is generated during the mineralisation process it is normally transformed into NO_3^- which is available for plant uptake. Any excess to plant needs is lost due to leaching process. Leaching is described as the movements of soil solute in soil solution down the soil profile. In the case of plant nutrients such as NO_3^-, the nutrient is considered lost if it is leached to depths beyond plant roots. Leaching is not the only way through which N can be lost from the soil. N can be lost through the denitrification process. This is a process whereby nitrate nitrogen is converted into gaseous forms: molecular nitrogen (N_2) and nitrous oxide (N_2O). The reaction involved may be purely chemical (chemodenitrification) or brought about by microorganisms (biodenitrification). Chemodenitrification is restricted to highly acidic soils and is generally less significant compared to biodenitrification (Loehr, 1979). In the absence of oxygen (O_2) some soil bacteria can use O_2 that is chemically bound to NO_3^- as the electron acceptor in their respiration. This results in the emission of nitrogen gases. Since this process is reductive, its rate will generally increase with development of anaerobic microsites that are frequently the result of increased soil moisture (Burford and Stefanson, 1973 and Mosier *et al.*, 1986). Increased soil moisture is not the only way through which biodenitrification can be initiated in the soil. The decrease in O_2 availability as a result of microbial activity in decaying organic matter can also create anaerobic microsites (Parkin, 1987). It is noteworthy remembering that the loss of NO_3^- due to denitrification has not only an economic implication but also an environmental one (Crutzen, 1981 and Byrnes, 1990).

The other crucial fate of NH_4^+ pool in the soil is loss due to ammonia (NH_3)

volatilisation. Much of the NH_4^+ in the soil can be lost trough this process and losses are high in soils with a pH greater than 7.0 (Alexander, 1961). Normally NH_3 exists in equilibrium with NH_4^+ but at high pH values the later predominates and may escape into the atmosphere in significant quantities.

2.4 Cultural practices and implication on soil N dynamics

The main difference between the nutrient dynamics of plants in the natural ecosystems and that of crop plants in agricultural systems is that the later involve the removal of nutrients in harvested material. The quantities may be substantial where foods are produced for direct human consumption or where industrial raw materials are harvested (Spedding *et al.*, 1981). The amount actually removed in animal production system are usually less depending on their intensity and whether excreta are recycled or not. The established view (Ball, 1979; Ball and Keeney, 1983 and Hoglund, 1985), is that grazing animals, by aggregating excess dietary N into urine patches, cause substantial N loss from intensively managed pastoral ecosystems. By contrast natural systems of vegetation do not leak their resources. Well established grasslands or forests lose very little nitrate to the rest of the environment. There is continuous annual cycle of growth, senescence and decay which includes the production of nitrate from dead leaves and roots but this nitrate can be repossessed by roots of grass or trees before it escapes into the ground water. This phenomenon implies a steady state situation in which inputs are always balanced with outputs. Measurements of nitrate in water drainage from natural grassland or forest usually show low nitrate contaminations (Addiscott *et al.*, 1991).

Arable farming is far from being a natural system. Unlike other systems it leaves soil bare of vegetation (fallow) and during this period there are no growing plants to capture any nitrate mineralised. The length of time for which the soil is bare depends greatly on the sequence of crops. These disturbances to vegetation cover are known to result in enhanced concentrations of inorganic N in soil and stream water, presumably because of a reduction in plant uptake of NH_4^+ and NO_3^- ions (Vitousek *et al.*, 1982).

In general, any activity that stimulates mineralization while concurrently failing to stimulate plant uptake should increase inorganic N in soil water. Seastedt and Hayes (1988) reported that the presence of vegetation can reduce N leaching losses about 5-fold. Trudgill *et al.*, (1991) ranked arable > grassland > woodland for nitrate losses from catchments.

Nitrogen demand by today's high yielding crops frequently exceeds the supply of this essential plant nutrient from the soil and sustained production of non leguminous crops usually requires the input of fertilizer N on an annual basis (Walters and Malzer, 1990). Leaching of N from the crop root zone can be considerable and contributes to the accumulation of NO_3^- in groundwater and surface water.

2.5 Nitrogen fertilizers in New Zealand Agriculture

Gow (1965) indicates that increased fertilizer use is cornerstone of land development in New Zealand. However, over the years there has been a shift in the way agriculturalists in New Zealand think about the place of chemical fertilizers in their agriculture and in particular N-fertilisers. Increased awareness of the economic importance of losses of nitrogen from New Zealand agricultural lands, together with the recognition that nitrate pollution is potentially a problem have stimulated research on nitrate leaching (Ryden *et al.*, 1984). There are two themes in N research in New Zealand. On one hand there is research which is aimed at decreasing N limitations in order to increase crop yield. On the other, there is research which is aimed at minimising the impact of N on environmental quality and in particular, upon fresh waters (Gandar and Ball, 1982). It is clear that these objectives may conflict, increasing production by elimination of N deficiency is likely to have adverse effects on environmental quality. An important problem for research in New Zealand and elsewhere is to specify the extent to which these objectives are compatible and to find alternative cultural practices that are more friendly to the environment.

Over the past 50 years New Zealand scientists and farmers have developed pastoral system based on association between clover and grass species consumed in situ by grazing animals (Ball and Crush 1985). Sears (1960) and Brougham (1973) ascribed the success of this technology to its low cost inputs, minimal forage conservation and high potential output. In comparison with other intensive grassland production in other developed countries, New Zealand agriculture is uniquely characterised by almost complete dependence on clover for the large inputs of N required to sustain productive pastures. Very little fertilizer N has been used (Ball *et al.*, 1979). Over the years, however, the use of fertilizer N has been on the increase in both pasture and cropping situations. This trend has a negative effect on groundwater quality.

2.6 Indexing Soil N Availability and leaching Potential

In humid temperate regions most of the available soil N taken up by the plants during the growing season comes from the potentially mineralizable organic N rather than the inorganic N present at the start of the season. The proportion will vary with soil type, weather and management practice. The implication of this generality is that any method used to predict soil N supply must take into account both the inorganic and the potentially mineralizable fractions. These two could be measured either separately or together (Whitehead, 1986). The inorganic N which is usually present in small amounts is very easy to measure and is available for plant uptake. The potentially mineralizable N is more difficult to measure, particularly in the field, and is only partially available because of several factors limiting mineralisation and these include temperature and water status of the soil. More often than not these are less than optimum. In spite of these limitations, the N mineralisation potential in laboratory is still considered a reasonable method to measure total soil N supply to plants (Stanford *et al.*, 1973).

There are several methods that have been devised to index soil N. These include the following:

(a) Biological methods
(i) Aerobic incubation
(ii) Anaerobic incubation

(iii) Bioassay or Exhaustive cropping
(b) Chemical methods

2.6.1 Biological Methods

The incubation methods for indexing soil N were first introduced by Waring and Bremner (1964). Since the time of their inception, these methods have been subjected to various modifications in an effort to make them more rapid and accurate. Keeney and Bremner (1967) pointed out that the incubation methods should be simple if they are to be used routinely in soil testing laboratories. Some of the modifications in these methods are variations in incubation time, temperature and use of amendments.

2.6.1.1 Aerobic Incubation

The aerobic method involves determination of the total mineral N produced when 10g of soil mixed with 30g of sand are treated with 6ml of water and incubated at 30°c for 14 days under aerobic conditions without loss of water (Keeney and Bremner, 1967). According to these two authors, this method is a simple and precise evaluation that provides a good index of nitrogen availability.

2.6.1.2 Anaerobic Incubation

The another version of incubation method is anaerobic approach. This method involves estimation of the ammonium N produced on incubation of soils under waterlogged conditions at 40°c for 7 days (Keeney and Bremner, 1966). This incubation method like the aerobic one can be used on both air-dried and field-moist soils.

2.6.2 Bioassay or Exhaustive Cropping

This method involves growing plants on the given soil sample and then do a yield analysis and/or sometimes tissue analysis on the percentage of N in the herbage.

The chemical analysis on plant tissue could be done on roots as well. While this method is time consuming, it more reliable than other approaches. Carlyle and Malcolm (1986) pointed out that this method is better than other methods in that it takes into account the ability of the plants to compete with the soil microbial population for the available N.

2.6.3 Chemical methods

A chemical method for the laboratory index of soil N availability is more convenient than the biological approaches discussed above in that it is usually rapid and more precise than biological methods (Keeney and Bremner, 1966). However, this method has one major weakness in that no chemical treatment of soil is likely to closely mimic the microbial processes responsible for mineralisation of N in the soil.

2.7 Soil Microbial Biomass

Soils are inhabited by a vast array of microbes which are responsible for the breakdown of organic matter and the mobilization of nutrients. Different methods are used to estimate the magnitude of the pool of these various soil microbial biomass components. The techniques used include the direct counting of the microbes (Soderstrom, 1977; Rosser, 1980 and Paul and Johnson, 1977), analysis of specific cellular components such as ATP or muramic acid (King and White, 1977; Fazio *et al.*, 1979 and West *et al.*, 1986), measurement of respiration rates (Anderson and Domsch, 1973), quantification of specific microbial processes such as N mineralization (Alef *et al.*, 1988), direct analysis of cellular components solubilized by fumigation (Brookes *et al.*, 1982 and Tate *et al.*, 1988), or the CO_2 produced by respiration of these products, fumigation-incubation method (Jenkinson, 1976 and Jenkinson and Powlson, 1980).

The common procedure currently employed appears to be the fumigation-incubation method (Parkinson and Paul, 1989). With this technique, soil samples are fumigated to kill existing microbes, re-inoculated with native soils and incubated. The CO_2 produced during the incubation period is measured directly by infrared or gas chromatographic analysis or it is collected in NaOH and titrated. To correct for respiration not associated with the fumigation of the microbial biomass, CO_2 produced in unfumigated controls is subtracted from that yielded in the fumigated samples. Although there are some problems with the use of this method, the method appears to be reasonably universally applicable. Its limitations primarily stem from the effects of exogenously supplied carbonaceous nutrients on microbial respiration and with extremely acidic soils (Martens and Cameron, 1985 and Vance *et al.*, 1987).

2.8 Soil Organic Matter

Another major change observed in managed soil-plant systems is the loss of soil organic matter through a combination of factors. Such factors include reduced carbon input and accelerated decomposition and erosion rates. Changes in edaphic environment, such as cultivation of virgin lands or altering the cultural practices have profound impacts on the rate of addition and decomposition of organic matter in a soil (Brady, 1984). Changes in organic matter content in a soil are of utmost importance in agriculture. The amount of soil organic matter influences the dynamics of microbial biomass in the soil.

CHAPTER 3

3 Experimental Design

3.1 Experimental Site

The experiment was conducted at the AgResearch Grasslands in Palmerston North, New Zealand. The site has an annual rainfall of about 974mm, mean of 30 year-period, (Jerez 1991). The soil type of the site is a sandy loam soil with roughly 45 cm of fine sandy loam overlying medium and coarse sand down to 2 m (P.R.Ball pers comm). Soils had a pH of 5.75 at the start of the experiment (1986). The site is fairly flat.

3.2 Treatments

The experiment consisted of six treatments. These were:

(a) Bare fallow (BF)
(b) Permanent Grass/Clover sward (PGC)
(c) Permanent Grass sward (PG)
(d) Six years cultivation with no N application (C_6-N)
(e) Four years cultivation with no N application (C_4-N)
(f) Six years cultivation with N application (C_6+N)

The plots that received BF treatment were bare fallowed on the 13.8.86 and maintained under bare fallow conditions throughout the entire period of the experiment using herbicidal sprays.

Plots that received the cultivation treatments were cultivated annually to a depth of 15cm and sown to Tama ryegrass (*Lolium multiflorum*) at 40Kg ha^{-1}. This species was chosen for two reasons:

(a) Because of its strong annual characteristic it mimicked closely an annual cereal crop.

(b) Because it is faster to establish than an other New Zealand ryegrass. Plots receiving the C_6-N and C_6+N treatments were brought under cultivation on the 2.10.86. Treatment C_4-N was brought under cultivation on the 13.10.88. This means plots receiving this treatment were two years longer under a grass/clover state.

The treatment with N application (C_6+N) had N applied annually for each subsequent grass crop. The N-fertilised plots received a total of 200kg ha^{-1} annually in two split applications. The first application was done in November and the second and last application in December. The N was applied in the form of Calcium Ammonium Nitrate.

The PGC and PG plots were established in winter 1985 and kept permanently undisturbed throughout the experimentation period.

3.3 Management of the Experiment

ALL experimental plots received an annual dressing of 250kg ha^{-1} of Potassic Super. On September 28, 1988 minor elements were applied; namely copper sulphate, zinc sulphate and boric acid. These were applied at 5kg ha^{-1}. Sodium molybdate was applied at a rates of 0.14kg ha^{-1}.

During the growing season the cropped plots were hand weeded to keep them weed free. The grass crop was cut about twice in the growing season using a rotary mower. The clips were taken away from the plots.

The BF plots were kept bare by spraying herbicides. The plots were sprayed soon as there were weeds coming up. Two herbicides were used interchangeably. One was a mixture of two herbicides: Roundup + Buster. The other one was Paraquat. Roundup and Buster were mixed in the following proportions:

Water	100 ml
Roundup	1.11 ml
Buster	1.67 ml
Wetting agent	1.0 ml

3.4 Experimental Design

The experiment was in a randomised block design with plots of 1m X 2m. The plots were demarcated using concrete buried to a depth of about 20cm. There were three blocks. However, the experiment was not balanced. Treatments BF and GF did not appear in one of blocks. Although these two treatments were not equally replicated, these two treatments appeared in pairs in the other two Blocks. Thus the treatment values used in the analysis are averages of the two plots that received the same treatment in the same block. The General Linear Model procedure from SAS package (SAS Institute Inc., 1990) was used to analyze the data as this procedure is specifically designed for the analysis of unbalanced designs.

3.5 Data Collection

At the end of a six-year period data was collected on the following:

(a) Available and potentially available soil nitrogen.
(b) Bioassay or Exhaustive cropping with plot soils in greenhouse experiments.
(c) Microbial biomass populations.
(d) Nitrogen leaching down the soil profiles.

The details on methods and materials used in these four major areas of focus in this study are given in respective chapters.

Soil organic matter, pH and soil bulk density were measured as part of the site description. The analyzed results of these soil properties are shown in Table 3.1 and the respective analysis of variance is given in Appendix 1. Soil samples were taken using metal corer of 2.5cm diameter and 15cm depth. 15 subsamples taken per plot, bulked and dried at 105°c over night and weighed.

Table 3.1: pH, organic matter and bulk density measured within the top 15cm of the soil.

TREATMENTS	pH	OM %	BUIK DENSITY
BF	4.52 c	5.50 c	1.51 a
PGC	4.97 a	6.75 a	1.29 b
PG	4.77 b	6.17 b	1.25 b
C_4-N	4.62 bc	5.70 c	1.23 b
C_6-N	4.77 b	5.33 c	1.29 b
C_6+N	4.60 bc	5.47 c	1.25 b
% CV	1.99	3.66	3.15
SE	0.009	0.04	0.002
L.S.D	0.19	0.43	0.08
P	**	**	**

ns : not significant
*, **, *** : significant at $P < 0.05$, 0.01, 0.001, respectively

The soil organic matter was determined by the dry combustion method. Samples of 20g were taken in sets of 5. The samples put in ceramic cups and then heated in a furnace at 900°c for three hours (Allison *et al.*, 1969). Prior to this heating, the soils had been oven dried over night at 105°c.

Soil pH was determined using the method described by Blakemore *et al.*, (1987).

Solution used was the 0.01 M $CaCl_2$. This was mixed with the soil in the ratio 1:2.5 (soil:solution).

Bulk density was assessed according to the method described by Blake and Hartage (1986). The mass of the dried divided by the field volume of the composite plot sample.

CHAPTER 4

4 Indexing Soil Mineral N: Incubation Technique

4.1 Introduction

Most studies on soil N-mineralisation have been short-term and motivated primarily by the need for rapid and reliable methods of assessing soil N availability. In these experiments incubation time was usually limited to a practical minimum period (7 to 14 days). Stanford *et al.*, (1974) recommended a two-week incubation. Although only a small proportion of the potentially mineralizable N (ie the quantity of soil organic N that is susceptible to mineralization according to first order-kinetics) is released during short-term incubations, results often appeared to reflect relative N-supplying capacities of the soils (Bremner 1965, Harmsen and Kolenbrander, 1965 and Hassink and Van Schreven, 1955). In this study the incubation was for a short-term period and as such the result reported here cannot be used to estimate long-term N-supplying capacities of the soils studied. For estimating long-term N-supplying potentials of the soils Stanford and Hanway (1955) proposed measurement of nitrate production that involved a procedure consisting of a series of incubations with the same set of soil samples. Before incubating the soil all the free or available nitrates are first leached out. The samples are then incubated and the leaching process repeated again following each incubation. Three consecutive 2-week incubations may be required. Legg *et al.*, (1971) suggested 2-week intervals with intermittent leaching over a period of 36 weeks.

4.2 Objectives

The study reported here was conducted to assess the capacities to supply N of five soils from plots that had received different cultural practices over a six-year period.

4.3 Methods and Materials

A short-term incubation method was used (Keeney and Bremner, 1966; Stanford and Haway, 1955). On the 25.8.92 soil samples were taken from the plots that had received a six-year cultural treatments. See 3.2 for details of treatments.

The field-moist bulk samples were sieved (2mm screen) and then separated into three sets of 5 subsamples for nitrogen determination. Each subsample was an equivalent of 10g dry soil. One set was analyzed unincubated. The other 2 sets were for incubation; one under aerobic conditions and the other under anaerobic conditions. For both the aerobic and anaerobic incubations each subsample of the field-moist was mixed with 30g of acid washed sand. The samples for aerobic incubation were added with 3ml of water to bring soils to estimated field capacity. For the anaerobic incubation 6ml of water was added to make them saturated. The samples were then incubated at 40°c for 14 days (Keeney and Bremner, 1966 and Ryan *et al.*, 1971).

Nitrate was extracted with 100ml of 2 M KCL on all samples. After the addition of KCL, the samples were shaken regularly for few hours before allowed to stand so that the soil-sand mixture settled and the supernatant liquid cleared up before filtering. The supernatant liquid was filtered under vacuum.

4.4 Mineral N Determination in Samples.

Samples were analyzed for N using the auto-analyzer. The calculation of NH_4^+-N and NO_3^--N concentrations in the soils from the auto-analyzer chart were then performed using the following equation:

$$Y = X\left(\frac{a}{b}\right)$$

Where: Y = NH_4^+-N or NO_3^--N in μgN/g dry soil.

X = ppm value of NH_4^+-N or NO_3^--N calculated from the standard curve (µg N/ml).

a = volume of 2 M KCL used for extraction.

b = dry weight of soil sample (g).

To get the actual quantity of N mineralised in each incubation, the initially available N was subtracted from the total N measured after incubation.

4.5 Results and Discussion

4.5.1 Available Soil N

Table 4.1 shows the size of the available inorganic N pools at the time of sampling. The respective table of the analysis of variance is given in the Appendices 2a and 2b. All treatments were significantly different from each other. The results showed that increasing years of cultivation tended to reduce the soil's available-N pools.

Table 4.1: Initially available soil nitrogen (mg N Kg^{-1} dry soil)

	SOIL NITROGEN LEVELS		
TREATMENTS	NH_4^+ -N	NO_3^- -N	TOTAL N
BF	5.42 b	1.38 b	6.80 b
PGC	9.93 a	2.50 a	12.42 a
C_4-N	4.03 c	1.58 b	5.61 c
C_6-N	2.17 d	1.34 b	3.51 d
PG	0.87 e	0.10 c	0.97 e
% CV	11.81	16.18	8.63
SE	0.30	0.13	0.33
Lsd	1.03	0.46	0.10
P	**	**	**

ns : not significant
*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

The differences between the GC, C_4-N and C_6-N, which represent different durations of cultivation of 0, 4, and 6 years respectively, highlighted this trend in decline in availability of N with increasing years of cultivation. Among the disturbed plots (ie both the cultivated and the bare fallow) available N pools were greater in soil from the BF plots. This observation seems to suggest that the absence of growing plants on the BF plots promoted the accumulation of inorganic N. The large pools of available-N observed in the BF plots in comparison to C_4-N, C_6-N and PG could not have been due to the fact that these plots had the greatest power to mineralize N following the cultural degradation of the soils. This conclusion was clearly attested by the fact that BF plots had the lowest mineral N flushes following incubation tests (Tables 4.2 and 4.3). Therefore, the large pools of available-N observed in BF pools at the time of sampling must have been a plain reflection of the absence of plant N-uptake.

The differences between the grass-only pasture and the cultivated plots was expected. According to Addiscott *et al.*, (1991) grassland differs from arable farming in that cereals are annual crops whereas grass is a perennial crop grown for its vegetation which is cut or grazed. Because grass absorb nitrogen whenever mineralization is occurring, grassland is potentially less leaky. Compared to annual crop grass is always present and hence can intercept most of the N that is mineralized and in particular in autumn early spring months. Cereals on the other hand die before harvest and do not establish as fast as grass with the on set of the growing season and so do not absorb much of the mineralized N. Grass grown for a single summer, as was the case in this study, behaves like an annual crop and dose not contribute to the build up of soil organic N as most of it will be leaching away. Similar findings have been reported by Adams *et al.*, (1979).

The difference between the PGC and the PG could have been due to high mineralization rate relative to that of plant and microbial immobilization in the PGC as a result of the high quality soil organic matter it is likely to have. Refer to the previous discussion on the influence of C:N ratio on net mineralization (section 2.2.2).

Data on available N show that ammonium nitrogen (NH_4^+-N) dominated nitrate-nitrogen (NO_3^--N) in both the cultivated and the uncultivated plots. This was rather unusual. In most cases NH_4^+-N constitutes a negligible portion of the total accumulated soil inorganic N in cultivated lands. With adequate soil aeration (as is the case with tilled lands) over a broad range of temperature and soil water contents, soil-derived NH_4^+-N is oxidized to NO_3^--N rapidly enough so that NH_4^+-N does not accumulate (Stanford and Epstein 1974).

The observed accumulation of NH_4^+ in cultivated plots could be explained by the pH. It is noteworthy noting that the pH of the soils were rather low across all treatments. All the study plots had pH lower than 5.0 (Table 3.1). The process of mineralization (ammonification + nitrification) that converts organic N to inorganic forms (ie NH_4^+ and NO_3^- respectively) is a biological one and is pH sensitive. Many microorganism convert organic N into NH_4^+. The NH_4^+ may then be oxidized to NO_2^- by bacteria of the genus Nitrosomonas. The NO_2^- may further be oxidized to NO_3^- by the bacteria of the genus Nitrobacter. Soil pH is apparently a principal factor responsible for the flushes of both NH_4^+ and NO_3^- in the soil. Alexander (1961) indicates that nitrification greatly decreases below pH of 6.0 and becomes negligible at 5.0 with complete cessation at pH 4.5. In general, there more genera of bacteria converting organic N to NH_4^+ ions (ammonification) than there are those converting the resultant NH_4^+ to NO_3^- (nitrification). In fact there only two known genera of bacteria that have species that carry out nitrification process. This suggests that at very low pH values nitrification is more severely limited with the result that NH_4^+ accumulates in the soil.

4.5.2 Incubations

The results of nitrogen analysis of the soils after incubation are given in Tables 4.2, and 4.3. The data show that incubation led to a marked increase in the amount of extractable N. In both aerobic and anaerobic incubation total potentially mineralizable soil N was greater than the initially available N (Table 4.1). The values were found to be typically highest, both in aerobic and anaerobic conditions, in soils sampled from uncultivated plots. This is in accordance with reports of Fleige and Baerumer (1974) and Blevins *et al.*, (1977). Working with no-tillage cropping systems, these workers found that decomposition rates were generally slower in no-tillage cropping systems. This maintained a high organic matter content in the upper soil strata, which in turn conserves soil N. This could help explain why similar results were observed in this study between cultivated plots and the headland or the permanent grass/clover swards.

Table 4.2: Potentially available soil nitrogen levels under aerobic incubation (mg N Kg^{-1} dry soil)

	SOIL NITROGEN LEVELS		
TREATMENT	NH_4^+ -N	NO_3^- -N	TOTAL N
BF	3.87	1.12 c	4.99 c
PGC	17.10	8.80 a	25.90 ab
PG	23.28	8.83 a	32.11 a
C_4-N	10.47	5.21 b	15.68 bc
C_6-N	7.22	4.03 bc	11.25 bc
% CV	57.93	26.15	40.18
SE	4.64	0.94	4.68
Lsd	ns	3.26	16.16
P	ns	***	*

ns : not significant
*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

The observed effects of cultivation were also in agreement with Dalal and Mayer (1986) who reported that cultivation and cropping of soils affected their chemical, physical and biological characteristics. They noted that cultivation of soil previously supporting native vegetation or pasture generally lead to reduced soil organic matter, organic carbon, and nitrogen. They ascribed this trend primarily to changes in temperature, moisture fluxes and aeration, to exposure of new soil surface resulting from aggregate disruption, to reduced addition of organic matter and frequently to increased soil erosion. The PGC and PG treatments appeared to conserve more organic N with no significant difference between them. In view of the above discussion, one can conclude that because of limited aeration found in uncultivated soil, the breakdown of organic matter over the past 6 years (Table 3.1) in these two treatments was relatively slow and hence N tended to accumulate in the organic form. This N accumulation was reflected by high flushes of mineral N upon the incubation of the soils.

Table 4.3: Potentially available soil nitrogen levels under anaerobic incubation (mg N Kg^{-1} dry soil)

	SOIL NITROGEN LEVELS		
TREATMENT	NH_4^+-N	NO_3^--N	TOTAL N
BF	27.07 b	0.23	20.51 b
PGC	58.25 a	0.39	46.22 a
PG	39.93 b	0.26	38.87 a
C_4-N	30.48 b	0.42	25.288 b
C_6-N	27.87 b	0.45	24.81 b
% CV	17.32	59.38	20.05
SE	3.92	0.13	3.90
Lsd	13.57	0.46	13.51
P	**	ns	*

ns : not significant
*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

There was no significant difference between C_6-N and C_4-N in terms of mineral N mineralised under aerobic incubation. This suggests that most of the N is mineralised during the early years of cultivation. In this study it appears that most of the observed soil degradation occurred within the first four years of cultivation.

By taking the mean of the cultivated plots and compare it to the values observed for the PGC and PG treatments, it was found that the N mineralized aerobically in cultivated plots was approximately half of that PGC and PG (52% and 40% respectively). The implication is that cultivated plots had lost the capacity to supply N. These figures further suggests that under the undisturbed conditions the PGC would be more leaky than the PG. Further evidence attesting this comes from significant difference (Table 4.1) observed between available N levels of the PGC (12.4mg N Kg^{-1}) and the PG (0.97mg N Kg^{-1}).

There was no difference between BF and all the cultivated plots. This seemed to reflect the equally low organic matter observed among them (Table 3.1). There was no difference in organic matter levels between them. This suggests that the residue incorporation and decomposition under cultivation can match those of the bare fallow. This is likely to depend on the productivity of the cropped system.

Anaerobic incubation results showed a similar trend to those of the aerobic incubation. However, when the two sets of data were compared one sees that the values of total N potentially available under aerobic conditions were, on average, about half (54%) that of the anaerobic conditions. The respective values of percent N of aerobic to anaerobic were as follows:

BF	24.3 %
PGC	56.0 %
PG	82.6 %
C_6-N	45.3 %
C_4-N	62.2 %

These data suggest that aerobic incubation methods tends to underestimate the soils' capacities to supply N. This insensitivity appears to be more pronounced the lower the organic matter content of the soil is. To see this compare the percentage mean of BF, C_4-N and C_6-N (45 %) and that of the PGC and PG (69 %) since these two groups differed significantly in their organic matter levels (Table 3.1).

When the amount of net N mineralized under aerobic conditions was regressed against the soil organic matter levels, the correlation was not significant. By contrast, however, correlation of the this soil property with the N mineralised under anaerobic conditions was significant at 5% significance. Its regression equation is given below.

$$OM = 4.32 + 0.0504N/g \qquad r = 0.956$$

These results indicate that the anaerobic method is better than the aerobic method in predicting soils's full potential to supply N since the nutrient supply is strongly linked with the amount of soil organic matter.

4.6 Conclusion

The cultivation and bare fallowing equally lower soil supplies of N. This has strong implication long-term land productivity. On the one hand the accumulation of organic matter in undisturbed lands could lead to groundwater contamination by N leachate should the lands be turned to arable. The initial cultivation may promote too rapid N mineralisation (as indicated by the incubation N flushes) which may be unmatched by that of plant uptake and thereby causing N leaching into aquifers.

From the results of this experiment on incubations it was concluded that the aerobic method is not reliable in predicting plant-available N in field soils nor is it good for indexing soils for N supply capacities. However, the high correlations of anaerobic results with those of both soil organic matter and soil pH suggest that where one needs to identify soils with a great potential to mineralise N, the anaerobic method can be used with greater reliability.

CHAPTER 5

5 Exhaustive Cropping

5.1 Introduction

Mineralisation values from incubation experiments may not always be a good estimate of plant available N. Mineral N measured by incubation methods presents a rough way of estimating the N pool of the soil. The estimates from the incubation tests are likely to over estimate the soil available N. This is because the incubation methods never accurately mimic the field conditions for N dynamics. In the field situation not all the mineralised N is available for plant uptake as some of it is subject to losses through leaching and denitrification. However, since nutrient N has the greatest impact on plant growth (Sahrawat, 1983), the study of plant growth and N uptake in experiments such as exhaustive cropping can provide a more reliable means of assessing long-term impacts of different cultural practices on soil's capacity to supply N. Being able to assess accurately the agricultural soils's potential to supply N is of paramount importance. Such potential has a profound impact on the amount of N that is subsequently available not only for plant uptake but also available for leaching losses and hence groundwater pollution problem. Therefore being able to predict accurately the N supply potential of the soils may be of utmost importance to N management in agroecosystems.

5.2 Objectives of the study.

The objective was to assess the extent of the soil-degradation impacts of the cultural practices in terms of the soil's capacity to supply N to the plants.

5.3 Methods and Materials

Soils from respective plots were sampled to a depth of 15 cm and then air dried and stored for six months before used. This study was initiated on February 4, 1993. Planting pots of 8 x 10 cm size were filled with 350g of plot soils. After filling the pots basal fertilizers were applied. Each pot received P in the form of Di-Sodium Hydrogen Phosphate (Na_2HPO_4) at a rate of 50kg ha^{-1} and K in form of Potassium Sulphate at a rate of 250kg ha^{-1}. No N was applied. The pots were then laid on a bench in the greenhouse in 3 replications.

5.3.1 Watering

An automatic watering system was set to irrigate the bench every eight hours for a duration of 15 minutes. The irrigation system was left to run for 7 days before planting to allow it stabilise before planting the ryegrass. Pots absorbed the water through capillary action. The top of the bench was covered on top with a spongy material lined on top with a perforated plastic sheet.

5.3.2 Planting

On February 11, 1993 the grass seeds were germinated on a germination paper. They were then sown 30 seeds per pot on February 13, 1993. After emergence the seedlings were thinned to 17 plants per pot on February 24, 1993.

5.3.3 Pest Control

The plants were sprayed with an aphicide as required.

5.3.4 Harvesting

Successive harvests were made at an interval of five weeks starting on March 16, 1993; the second cut was on April 26, the third on June 4 and the fourth and final harvest was on the 10th of July. Cuttings were made 2 cm above the soil surface. Each cutting was dried at 60°c and weighed. At the final harvest the roots were separately collected for dry weight analysis as well.

5.3.5 Herbage Chemical Analysis

To determine total N uptake in the harvested plant material the four cuts were bulked together. Composite samples were then finally ground using an sample grinder. For the determination of total N in the ryegrass plant material, duplicate sub-samples of 0.1g were digested by the Kjeldahl method. The roots were excluded in this tissue analysis. The method was modified by the addition of salicylic acid (Bremner 1965) so that the N analysis included the NH_4^+. The N analysis was done colorimetrically using the Technicon Auto-analyses.

5.4 Results and Discussion

Table 5.1 shows data from the four cuts of the ryegrass shoots from the greenhouse experiment. Its respective analysis of variance is shown in Appendices 5a and 5b. Included Table 5.1 is data on root dry matter (RDM) and total dry matter (TDM). TDM is the total of shoot dry matter (SDM) plus RDM. Treatment effects on both TDM and RDM analyses were equally highly significant.

Yields from pots that had soils from uncultivated plots (PGC and PG) were significantly greater, consistently in all cuts, compared to those of pots that had soils from the cultivated plots and the bare fallow. Cultivation and bare fallow effects were similar. Similarly there was no difference between the 4 year cultivation and the 6 year cultivation. However, these results indicated a trend of decreasing plant yield with increase in the duration of cultivation. This trend was in line with the results on soil organic matter content. In fact there was a high correlation between TDM and soil organic matter content ($r = 0.971$). This was significant at probability of 0.008. RDM correlated rather poorly with soil organic matter content with an r value of 0.8 ($P = 0.054$). The lack of significant differences among BF, C_6-N and C_4-N (all of which were disturbed systems) while there was significant difference between each of these three

Table 5.1: Data from the four successive cuts, root dry matter (RDM) and total dry matter (TDM) of the ryegrass plants in the greenhouse experiment (g^{-1} pot)

TREATMENTS	DRY MATTER YIELDS					
	FIRST CUT	SECOND CUT	THIRD CUT	FOURTH CUT	RDM	TDM
BF	0.21 bc	0.25 c	0.19 b	0.14 b	0.75 cb	1.55 c
PGC	0.41 a	0.42 a	0.35 a	0.19 a	1.22 a	2.58 a
PG	0.34 a	0.35 b	0.33 a	0.16 ab	0.97 b	2.16 b
C_4-N	0.23 b	0.27 c	0.19 b	0.14 b	0.82 bc	1.66 c
C_6-N	0.21 bc	0.26 c	0.21 b	0.13 bc	0.71 c	1.52 c
C_6+N	0.14 c	0.21 c	0.17 b	0.10 c	0.44 d	1.06 d
% C.V.	16.24	11.33	18	15	16.01	11.34
S.E.	0.0017	0.001	0.025	0.0046	0.0172	0.11
L.S.D	0.076	0.061	0.079	0.0391	0.239	0.36
P	**	*	*	***	***	***

ns : not significant at 5%

*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

and the C_6+N (a disturbed system too) underpins two important points:

(a) That the bare fallowing effect equalled those of cultivation in terms of soil degradation.

(b) That the application of N was more damaging than plain cultivation to the soil's long-term capacity to mineralise and supply plants with N.

The results on plant N uptake were not different in trend from those on TDM. Table 5.2 gives amounts of total N taken up per pot by the ryegrass plants. N uptake was also expressed in terms of uptake per kilogram of dry pot soil (mg N Kg^{-1} soil). The results on soils' N supply capacities based on total plant uptake of N were far greater than those determined by the two incubation methods. This is contrary to general scientific finding (section 5.1). These reversed differences observed between values from

Table 5.2: Nitrogen taken up by ryegrass expressed both in terms of total N uptake per pot (mg) and N uptake per kilogram of pot soil (mg).

TREATMENTS	TOTAL N UPTAKE PER POT	N UPTAKE PER KILOGRAM POT SOIL
BF	12.3 c	35.2 c
PGC	20.3 a	58.1 a
PG	17.0 b	48.6 b
C_4-N	13.0 c	37.1 c
C_6-N	12.0 cd	34.3 c
C_6+N	9.3 d	26.7 d
% C.V.	10.99	11.00
L.S.D	2.8	8.00
S.E.	2.8	2.5
P	***	***

ns : not significant
*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

exhaustive cropping data and those from incubations must have been due to later not fully measuring the potentially mineralizable N as a result of the restricted time. The 14-day incubation period appears to be inadequate. Values of N uptake were also greater than those of the initially available nitrogen. This is so because the former reflects cumulative net mineralization while the later reflects net mineralization at a specific point in time. This highlights the fact that given ideal soil conditions (ie temperature and water) such as those found in controlled environments like the greenhouse, with time the soil will mineralise more and more N until exhaustion point.

The plant performance in terms of N uptake and dry matter yield strongly reflated the organic matter levels of the soils. It is important to note the significance of the presence of legumes in grasslands. The PGC-treatment soils had a greater potential to supply the plants with N than the Headland soils. There are two possible reasons to explain this. Firstly, the presence of the legume improved the productivity of these ecosystems. This resulted in greater organic matter build up in PGC plots. The difference, however, was not big. The PGC plots had 6.8% organic matter while the PG plots was 6.2%. Secondly, the presence of a legume may not only have contributed to high quantity of organic matter in the PGC plots but may also have improved the quality of the soil organic matter. The plant performance on PG soil was found to be 84%, 84% and 80 % of those on PGC soil in terms of N uptake, TDM and RDM respectively. From these data it can be seen that the average performance of plants on PG soil was 83% of those on the PGC soil. This simple statistic clearly points out that the PGC has a greater potential to mineralise N of about 17% over and above that which the grass pasture is capable of mineralising upon the advent of cultivation; more so in the first year of cultivation.

Regression between initially available N and amount of N taken up per kilogram of pot soil was not significant. With regard to the incubation methods, only the anaerobic method showed a significant regression equation with N uptake. This equation was significant at $P = 0.003$. The correlation between the two was very strong ($r=0.982$). It was also observed that only the anaerobic method had significant regression equations against the RDM and TDM. These two equations were significant at probability 0.011 and 0.003 respectively. The corresponding r values of these two correlations were very high too (0.955 and 0.991 respectively). These observations reconfirms previous conclusion that of all the methods used to assess the soil N supply capacity, only the anaerobic incubation method is reliable.

5.5 Conclusion

Cultivation and bare fallow equally lower the soil's capacity to supply N. The application of N-fertilizer has an even stronger negative long-term effect on the soil's capacity to supply plants with N. This suggests that once a soil has been subjected to fertilizer application its productivity is undermined making it impossible to maintain, sustainably, plant yields without continuous reliance on N application. From the lack of significant difference between the durations of cultivation (4 years and 6 years) it can be concluded that when a grass or grass/clover pasture is subsequently turned into arable it can lose most of its organic N in the early years of cultivation. This rapid soil degradation implies that the release of the bound organic N may be so much rapid too to the extent that it may have a repercussion on groundwater quality.

The use of plant dry matter yields and N uptake as a method of determining soil's capacity to supply N is more reliable than the short-term incubation method. Both incubation methods underestimate the soils' potential to supply plant with N. Similar limitation is observed with the levels of initially available N. Furthermore, judging from the relative sizes of the coefficients of variation (% CVs) observed in these experiments it seems that both the TDM and N uptake are more reliable methods of determining a soil's capacity to supply N than the aerobic and anaerobic incubations. Both TDM and N uptake had a CV of 11 while that of the aerobic and anaerobic incubations were 40.2 and 20.5 respectively. Further evidence attesting this conclusion is the fact that of all the methods that were used to assess the potentials of the studied soils to mineralise N only the N uptake was sensitive enough to detect a difference between the PGC and PG treatments.

CHAPTER 6

6 Soil Microbial Biomass Assessment

6.1 Introduction

Soil microbial biomass constitutes the active fraction of soil organic matter (Paul and Voroney 1984) whose fast turnover makes it makes it an important source of nutrients; particularly nitrogen (Sparling, 1985). Bolton *et al.*, (1985) indicate that cropping practices and fertilisation affect the growth and activity of this biomass.

The fumigation-incubation method was used to assess the soil microbial populations. This method was described Jenkinson and Powlson (1976a). The procedure is based on the assumption that the extra CO_2-C released when a soil is fumigated, the fumigant removed and the soil incubated aerobically, comes from the cells killed by the fumigant and decomposed by subsequent recolonizing population of the microbial inoculum (Jenkinson and Powlson, 1976a and Shen *et al.*, 1984). Jenkinson and Powlson (1976a) and Jenkinson *et al.*, (1976) proposed that the flushes could be directly used as an approximate measure of the soil microbial biomass.

6.2 Objective

The objective was to assess the responsiveness of soil biomass size to different cultural practices.

6.3 Methods and Materials

Soil samples were collected on the 6th of May 1993. They were stored in sealed plastic bags at 25°c until May 17, 1993 when the experiment was set up. About 15 samples were collected to a depth of 7.5cm from each experimental plot to make one composite plot sample.

On the 17th of May, 1993 soils were sieved (2mm sieve) and moisture contents and water holding capacities (WHC) of the plot samples were estimated. To estimate the WHC about 20g of soil were taken from each sample and put in a funnel chocked with lint. The soils were saturated by placing on a metal stand in a dish of water ensuring that the level of water in the dish was at the same level with the soil surface. After an hour the samples were removed from water to allow all the water to drain away for 30 minutes before taking weights. The WHC was then calculated gravimetrically.

Duplicate subsamples of 100g dry soil were taken from each composite plot-sample and fumigated.The fumigation was done in a large desiccator (30.5 cm i.d). Each desiccator contained a beaker with 50ml of a fumigant (carbon disulphide) and 10g of anti-bumping granules. The air in desiccator was then evacuated (using a vacuum pump) until the fumigant stated to boil vigorously. The tap was then closed, pump stopped and the desiccator left at room temperature for 24 hours. The beaker of carbon disulphide was then removed and the fumigant vapour removed from the soil by repeated evacuation using the pump. About six 3-minute evacuations were adequate to remove the smell of the fumigant from the soil.

Carbon disulphide was used as the fumigant in place of the commonly used chloroform because it was more convenient to use. Carbon disulphide is alcohol free and has a distinct smell which makes it possible to detect any residues left in the soil following the aeration and evacuation of the fumigant. The success of this methodology requires that no traces of the fumigant should be present in the soil when samples are taken for incubation. Incomplete removal will interfere with the activity of the microbes introduced when the inoculum is added and therefore would alter the effectiveness of the introduced microbial population (Jenkinson and Powlson 1976b)

After fumigation each sample was placed in a glass jar of 8 x 18 cm in size and brought to 55% of its WHC before inoculated with 5g of unfumigated field moist soil to reintroduce soil microbes. The soils were then before placed in an incubator at 25°c for 10 days. To trap the CO_2 evolved during the incubation period, a small beaker containing 50ml of 1 M N_aOH was placed inside each sample-jar. To adjust the results for CO_2 that was in the atmospheric air in the incubation jars at the start of the incubation, blank jars were set up and this CO_2 in blank samples subtracted from the sample results. The CO_2 flushes evolved during 10 - 20 days was taken as the controls ie the endogenous respiration CO_2 (Merckx *et al.*, 1985).

After ten and twenty of incubation the samples were taken out of the incubator and to each added 2ml of barium chloride ($BaCL_2$) solution to precipitate the CO_2. This reagent was made up by dissolving 15g of this reagent in 75ml of distilled water. Each sample was then filtered through a 12.5cm (diameter) Whatman filter paper, No 42. Before filtering the filter papers were individually weighted after oven drying for an hour. After filtration the filter paper and the precipitate were oven dried over night. The weight of CO_2 precipitate was the calculated by subtracting weight of the filter paper from the total weight of filter paper plus filtrate.

6.3.1 Calculations

The calculation of the weight of evolved CO_2 in the precipitate and the microbial biomass for each sample were worked out using the equations (1) and (2) respectively.

$$C_w = Pw\frac{\frac{12}{197.3}}{Sw} \qquad (1)$$

Where:
C_w = weight of Carbon evolved (mg CO_2-C/g dry soil)
P_w = weight of precipitate in grams
S_w = dry weight of soil sample used.
12 = molecular weight of C
197.3 = molecular weight of B_aCO_3.

$$B_c = \frac{x-y}{0.42} \qquad (2)$$

Where:
B_c = microbial biomass in mg C/g dry soil.
x = CO_2 flush in 0 - 10 days.

y = CO_2 flush in 10 - 20 days.

0.42 = The fraction of biomass C mineralised to CO_2 over the prescribed incubation period. This fraction is usually assumed to be 0.42 or 0.45 with 0.45 commonly applied for calculations involving heavy textured soils.

Note: Both x and y are estimated using equation (1). Note also that the results in the above equations can be multiplied by a factor of 10^3 to express the results in μg C/g dry soil.

6.4 Results and Discussion

The microbial biomass results were rather confusing. They did not correspond to the expected outcome and were therefore not reported. These results on biomass data are given in Appendix 4a: Method One. They show that the PGC and PG treatments which had the greatest organic matter content had the least biomass values. Schnurer *et al.*, (1985) indicate that the general expectation is higher microbial biomass population in soils with high organic matter and this is in line with the report by Swift *et al.*, (1979) in which they stressed the importance of availability of energy in form of labial carbon compounds as the primary nutritional factor limiting microbial growth and activity in the soil. When an alternative method suggested by Vance *et al.*, (1987) was used in calculating the soil microbial biomass, the results (Appendix 4a: Method Two) were rather meaningful but treatment effects were not significantly different. The alternative method employed the following equation, a modification of equation (1) in section 6.3.1.

$$B_c = \frac{X}{0.45}$$

Where: B_c, X and 0.45 are as previously defined under methods and materials. Note: Here 0.42 is raised to 0.45.

It is very difficult to tell why the results of the calculated biomass were so insensitive in ranking the treatments. A possible explanation for this could have been the low soil pH of the soils used in the study. Although the technique of chloroform fumigation appears to be reasonably universally applicable, there are some problems associated with it especially with extremely acidic soils (Vance *et al.*, 1987). When soils are too acidic the assumption that the endogenous respiration rates in the samples corresponds to those of the controls (or second phase of incubation as the case may be) becomes invalid. Generally the two rates are assumed to be roughly equal. Because of this limitation Vance at al., (1987) proposed that a more accurate estimate of the soil biomass could be gained by not correcting for endogenous respiration and hence simply use the CO_2-C flush data in evaluating effects of management systems on size of soil biomass. One way in which the low soil pH is expected to affect these two rates is through delaying the recolonisation of the fumigated soil by the microbes from the inoculum. This has the problem of producing very high second readings and more so in soils rich in organic matter.

Table 6.1 shows data on CO_2-C flushes following the fumigation and incubation of the treatments soil. The first flush results (10 days after incubation) were not significantly different while that of the second flushes (20 day after incubation) were highly significantly different.

When the two CO_2-C-flushes were added up, the cumulative flush values (Table 6.1), the treatment effects were significant. The uncultivated plots (PGC and PG) now had the greatest biomass. This is in agreement with other researchers like Jenkinson and Powlson (1976a) whose work on soil biomass produced the following results. Woodland > grassland > arable > fallow. Nevertheless, the use of flush-totals in interpreting biomass results has some controversy in that the method assumes a zero endogenous respiration. This means that the results on CO_2-C-flushes based on the cumulative flush-readings would be confounded with the endogenously evolved CO_2-C. In other words this approach assumes that all the measured CO_2-C flush was evolved from the decomposition of the fumigant-killed biomass.

Table 6.1: CO_2-C flushes following the inoculation and incubation of the fumigated soil samples (μg C g^{-1} dry soil).

TREATMENTS	FIRST CO_2 FLUSH (10 days)	SECOND CO_2 FLUSHES (20 days)	CUMULATIVE FLUSHES READING
BF	248	60 b	307 cd
PGC	281	234 a	515 a
PG	267	254 a	508 a
C_4-N	275	103 b	378 b
C_6-N	235	116 b	351 cb
C_6+N	224	53 b	277 d
% CV	6.097	16.952	12.0
LSD	39.9	62.5	61.2
SE	0.241	0.59	14.96
P	ns	***	*

ns : not significant

*, **, *** : significant at $P < 0.05$, 0.01, 0.001, respectively

There was significant difference between 4-year cultivation and 6-year cultivation indicating that much of the loss in soil biomass as a result of cultivation occurred within 4 years of cultivation. The lack of difference between the two durations of cultivation with no N application while there was significant difference between both of these and the 6-year cultivation with N application clearly indicated that fertiliser N application had a negative impact on biomass size. The effect of fertilizer N on microbial biomass was unusual. In fact the C_6+N treatment had the lowest biomass content. Generally, one expects to see N-fertiliser positively influencing soil microbial biomass through increased crop production and greater return of organic C to the soil (Coote and Ramsey, 1983). In this study the N application did not promote soil biomass.

Since there was no significant difference between PGC and PG treatments (both uncultivated), the mean of the totals of these two was used as the reference point from which to compare cultivated and undisturbed treatments. In this comparison the BF, C_4-N C_6-N, and C_6+N supported, respectively, an active microbial biomass of 40, 31, 26 and 46% less than the uncultivated plots. On average the annual cropping systems supported about 34% less biomass than grasslands. By comparing this 34% with 40% (the value for BF), it is clear that BF supported less biomass than annual cropping situations. This observation is in line with that of Collins *et al.*, (1992) in which they found that the wheat-fallow rotation and annual cropping supported 70 % and 50 %, respectively, less biomass than the grass pasture. Similar results were reported by Patra *et al.*, (1990) where microbial C represented 4.3, 2.8 and 2.2 % of the total soil C present in the grass pasture, annual cropping and wheat-fallow rotation respectively. The BF treatment, as with fallow in crop rotations reported by Biederbeck *et al.*, (1984) and Jenkinson and Powlson, (1976a), tended to accelerate the loss of soil biomass when compared with the annual cropping situations. This loss could be due to decline soil organic matter and/or poor soil/water relationship. The bareness of the fallowed plots may have a bearing on water infiltration. High water losses due run-off are a possibility.

6.5 Conclusion

This study has shown that there is a strong decline in soil microbial biomass with decline in soil organic matter levels. It has also demonstrated that inorganic fertiliser, in particular nitrogen application, does not necessarily promote soil microbial biomass. Its influence on biomass could be pH dependent. Given the very low soil pH of our soils in the study, soil acidity suppressed the favourable influence of fertilizer-N.

CHAPTER 7

7 Nitrate Leaching

7.1 Introduction

The term leaching describes the transport of chemicals in soil solution below a defined depth of soil, and usually below root zone. This leaching of nutrients is a harmful phenomenon which causes financial losses to agriculture and may cause environmental pollution (Jaakkola, 1984). In this study we are concerned only with the leaching of N which is usually in the form of nitrate. Nitrate is particularly susceptible to leaching because it is not absorbed by soil. Ground water pollution by N from agricultural activities in developed countries, including New Zealand, has invoked a serious concern to stop any further degradation in ground water quality. However, preventing any further ground water contamination by nitrate calls for more knowledge on how different cultural practices impact on the soil/plant system.

The studies of nitrate profiles in agricultural soils may help to assess the extent of fertilizer loss. This loss is usually found in the range of 40-80 % of the fertiliser applied (Tomlinson, 1971). Examination of soil profiles can accurately reflect the recent history of land use (Sprent 1987). For example, when permanent grassland is ploughed, mineralization occurs and the additional nitrate produces a front in the nitrate profile. In fact, if leaching is predominantly by piston displacement (ie there was little by-pass or preferential flow), it is possible to determine the average annual displacement down the profile.

Scientific evidence shows that permanent grassland can use most of the nitrate applied in a moderate dose of fertiliser, giving few problems with groundwater quality. However, when such lands are cultivated there is a surge of mineralization, with the resultant increase in nitrate levels in the soil. Following this, the growing of crops with normal cultivation and moderate N-fertilisers poses a threat to groundwater quality. This is partly due to leaching of applied fertiliser and partly from the continued high rate of mineralization of residues after cultivation.

There are two approaches that can be employed in the study of nitrate leaching. Some studies use porous cup lysimeters to evaluate the NO_3^--N leaching losses; for example work by Goh *et al.*, (1979), Cameron and Hayness, (1986), Seastedt and Hayes, (1988) and Monaghan *et al.*, (1989). Lysimeters have an advantage of allowing quantitative measurements from a more define soil volume and they generally avoid the large variations associated with the field studies. However, compared with the other method given below, this method is more resource intensive and therefore not suitable for a researcher with limited resource. Moreover, different soil types show varying degrees of suitability for lysimeter studies. Heavy clay and peat soils tend to shrink upon drying and this results in an unacceptable water flows along the casing walls (Bergstrom and Johansson, 1991).

Other workers like Ryden *et al.*, (1984) and Cameron and Wild, (1984) use the deep core sampling method. This method involves taking soil samples at specified depth intervals down to 2m or greater to get a nitrogen profile of the soil. From these data amounts of N leached can then be quantified using the drainage component of the soil water balance.

7.2 Objectives

The aim of the deep core sampling was to appraise the N leaching potential of the studied cultural practices using the NO_3^--N levels in the soil profile and an estimate of the drainage component of the soil water balance.

7.3 Methods and Materials

In November, 1992 we took samples from deep cores using an auger 8.8 cm in diameter. One core was taken per plot up to a depth of 2m. Each core was sampled at four sampling depths. A depth interval of 50 cm was used. For the analysis of nitrate duplicate samples were taken and the mean of the two used for statistical analysis. The actual determination of nitrate levels was carried out using the same materials and procedure already outlined in Section 4.4.

7.3.1 Monthly Drainage Estimation

Soil water balance was calculated from the monthly rainfall and pan evaporation data gathered over a period of two years prior to the sampling date. From these data the 1992 drainage was estimated as 351mm. Monthly drainage was calculated using the following equations:

$$D = W_A - 75 \qquad (1)$$

$$W_A = W_p + P - 0.82E_p \qquad (2)$$

Where: D = Monthly drainage (mm).

W_A = Soil water storage for the target month (mm).

W_p = Soil water storage for the previous month (mm).

P = Precipitation for the target month (mm).

E_p = Pan evaporation during the target month (mm).

75 = Plant-available soil water (mm).

0.82 = A conversion factor to change pan evaporation to evapotranspiration. The 0.82 figure was taken from the work by Scotter *et al.*, (1979)

Note: The above calculation assumed that drainage (D) will occur in any particular month only if W_A is greater than that amount needed for plant growth (75mm). Ball, P.R., (1994 pers comm) suggest the 75mm is ideal for cropping situations while 50mm would be appropriate for pasture.

7.3.2 Soil Water in the Deep Cores at Sampling Time

The following equation was used to determine the equivalent depth of soil water present in respective horizons of the deep cores given the gravimetric soil water content (g H_2O/g dry soil).

$$W_d = \theta Z_d \times 10$$

Where: W_d = Water in a given soil core horizon (mm).
θ = Volumetric soil water content (cm^3 H_2O/cm^3 dry soil).
Z_d = Given soil horizon (cm).

Note: $\theta = w\,[\alpha/\beta]$
Where: w = Gravimetric soil water content (g H_2O/g dry soil).
α = Soil bulk density at respective core sections (g/cm^3).
β = water bulk density (g/cm^3).

7.3.3 Calculation of NO_3^--N leached per m^2

The amounts of NO_3^--N leached per m^2 through each 50cm core horizon was calculated using the equation given below. The total leached nitrate nitrogen due to 1992 drainage was estimated by adding the nitrate leached into the second and third core horizons. The exact proportion of nitrate added from the third horizon depended on the size of the total soil water depth between the two soil depths in question relative to the annual drainage.

$$N_L = (\alpha N_c)5 \times 10^2$$

Where: N_L = Amount of NO_3^--N leached in grams per m^2.
α = Soil bulk density (g/cm^3).
N_c = Soil N content (mg NO_3^--N/Kg).

Appendices 5a - 5e show the data from these calculations.

7.4 Results and Discussion

The NO_3^--N concentrations of the cores are given in Table 7.1 and their respective analysis of variance is shown in Appendix 5f. The levels were notably highest at depth of 0.5 to 1m for all treatments. In lower parts of the soil profile the amounts of NO_3^--N were lowest at the 1.5 to 2m depth. When nitrate levels were averaged across all depths for each core (Table 7.2) and treatments ranked according to these average treatment effects, the results were as follows:

C_6+N > PGC > BF > C_4-N > C_6-N > PG.

Table 7.1: Soil nitrogen levels down the soil profile in the sampled cores (mg NO_3^--N Kg^{-1} dry soil).

TREATMENTS	SOIL DEPTHS			
	0 - 50 cm	50 -100 cm	100 -150 cm	150 - 200 cm
BF	3.75 bc	5.05 bc	2.35 bc	1.05 b
PGC	5.25 a	7.75 b	4.35 ab	3.50 a
PG	0.97 e	2.27 c	0.63 c	0.43 b
C_4-N	2.77 cd	2.76 c	1.90 c	1.03 b
C_6-N	1.39 de	2.83 bc	2.40 bc	0.93 b
C_6+N	4.37 ab	13.17 a	4.63 a	2.73 a
L.S.D	1.07	4.96	2.19	1.06
SE	0.325	1.38	0.328	0.323
P	***	***	*	***

ns : not significant
*, **, *** : significant at $P < 0.05$, 0.01, 0.001, respectively

The data show that the C_6+N and PGC treatments had the greatest concentrations and both differed significantly from rest of the treatments. The C_6-N, C_4-N and BF treatments were not different. Of these only the BF treatment showed a significant difference from the PG treatment. The existence of a significant difference between the BF and the PG while there was none between it (BF) and the annually cropped but unfertilised treatments (C_4-N and C_6-N), suggests that the periods of bareness in these cultivated plots between seasons enhanced leaching making these treatments rather more comparable with the bare fallow (BF) than with the continuously grassed plots (PG).

Although there was no significant difference between BF and both the C_4-N and C_6-N), the tendency by the BF to surpass these cropped systems (Tables 7.2 and 7.3) strongly points to the importance of N uptake by growing plants with regard to the concern about groundwater pollution through NO_3^--N leachate.

Table 7.2: Nitrate levels in deep cores averaged across depths and expressed as mg NO_3^--N Kg^{-1} of dry soil and mg NO_3^--N L^{-1} of soil solution.

	NITROGEN LEVELS	
TREATMENTS	DRY SOIL	SOIL SOLUTION
BF	3.05 b	9.43 b
PGC	5.21 a	17.38 a
PG	0.83 c	2.66 c
C_4-N	2.12 bc	6.78 b
C_6-N	2.03 bc	6.86 b
C_6+N	6.23 a	19.38 a
L.S.D	1.52	3.99
SE	0.05	1.22
P	***	**

ns : not significant
*, **, *** : significant at $P < 0.05, 0.01, 0.001$, respectively

Table 7.3: Amounts of nitrate nitrogen leached into the 50 - 200cm soil depth (g NO_3^--N/m^2).

TREATMENTS	SOIL NITROGEN LEVELS
	NO_3^--N
BF	5.14 b
PGC	9.38 a
PG	1.48 b
C_4-N	2.98 b
C_6-N	4.46 b
C_6+N	12.67 a
L.S.D	4.05
SE	1.24
P	***

ns : not significant
*, **, *** : significant at $P < 0.05$, 0.01, 0.001, respectively

The presence of plants was intercepting the leaching N by immobilising it into organic forms. This was the reason why less amounts of NO_3^--N had been leached from the cropped systems relative to the BF.

Table 7.3 gives estimates of NO_3^--N leached beyond the root depth in the preceding year prior to the sampling. The respective analysis of variance is in Appendix 5g. These amounts of NO_3^--N leached were estimated at soil depths lower than 50cm. Ball, P.R., 1993 (pers comm) suggests this is a reasonable average root zone. Our simple model assumed, therefore, that any nitrate leached beyond this depth cannot be retrieved by plants and is therefore going to cause groundwater pollution problem. There were no differences in treatment effects among the C_4-N, C_6-N, BF and PG treatments. While there was a significant difference between the BF and PG in terms of mineral N levels (Table 7.2), the two soil/plant systems showed no apparent difference in terms of the estimated leaching losses. This suggests that the accumulating mineral N in bare fallow plots might have been subjected to some other pathways of N loss other than leaching; for example denitrification.

The C_6+N and PGC treatments were quite distinct from the rest. No significant difference, however, was observed between these two. The absence of significant difference between C_6+N and PGC shows that legume nitrogen can leach as readily as the applied fertilizer-N. This is contrary to the general belief that legume N does not leach and therefore pose no threat to groundwater quality. In fact this has been widely cited as one of the advantages of including a legume crop in crop rotations of low-input sustainable agricultural systems (Hauck, 1990). These results are in agreement with the findings of Field *et al.* (1985) in which they observed that the effect of a legume on N leaching equalled those of fertiliser N application. They found that the removal of clovers from a pasture decreased, while application of fertiliser N increased, the N loss with same amounts of N of 60 - 80kg N ha^{-1} $year^{-1}$.

Of all the sampled soil depths, the greatest concentrations of NO_3^--N were at depth of 0.5 to 1m. This confirms the widely expressed concern of nitrate enrichment of groundwater over and above the concentrations reckoned safe with regards to standards of drinking-water supplies. Since most of the agricultural crops have the bulk of their roots above the soil depth of a metre, any nitrate leached beyond this depth may pose a serious environmental threat to groundwater quality since the soil-plant system may not be able to retrieve this N. These amounts of nitrate in lower parts of the soil profile reflect nitrate leached below the root zone in past years up to that in which the cores were taken. In terms of N concentration per litre of soil solution (Table 7.2) only the PGC and C_6+N treatments had concentrations greater than the drinking water quality standard of 10mg NO_3^--N L^{-1} recommended by the World Health Organisation (Singh and Sekhon, 1979) or 11.3mg NO_3^--N L^{-1} in the European Community Countries (Whitemore *et al.*, 1992). Total amounts of nitrate nitrogen leached from these two treatments were found to be 84kg ha^{-1} and 117kg ha^{-1} respectively. These values were calculated using data in Table 7.3. The values assume 10kg ha^{-1} as the value at zero time

(Cameron and Wild, 1984). However these values are quite high. Most likely the true value at zero time involved here is far more than the 10kg ha^{-1} assumed. By employing the zero value we are assuming that leaching does not completely wash away all the N from a soil. This implies that whenever we are analysing soils for leachate quantities moved from a higher soil horizon to a lower one over a specified period, part of the measured quantity will always be attributed to the zero value.

Our results seemed to indicate that most (about 70%) of the 117kg ha^{-1} of N leached from the plots that had received N application could have come from the 200kg ha^{-1} N that had been applied in summer 1991. The 6-year cultivation without application lost only 35kg ha^{-1} of N. This means that about 82kg out of the 117kg leached in N-fertilized plots could have come from the residual N with mineralization contributing only 35kg. This proposition is in line with the results on exhaustive cropping. These results did not show the soils from the C_6-N treatment to have any significant capacity to supply mineral N microbially. Thus there is reason to believe that a considerable amount of N was left in the soil after the final crop in summer 1991. This N was subsequently leached out by the large drainage volumes estimated during winter of 1992 (Appendix 5a) preceding deep core sampling.

The problem of residual N posing threat to groundwater quality has been reported in the past. For example, a report by Bergstrom and Johansson (1991) emphasised that any crop that suffers significantly from drought stress during its growing season will leave a lot of residual N from the applied fertilizer-N. The fact that the summer of 1991 had low rainfall makes it is easy to appreciate why the last crop prior to sampling could have left a lot of residual N. According to Campbell and Paul (1978) a crop under dry land farming can leave between 28 - 57% of the fertilizer-N. This N is subsequently lost through leaching in winter if there is no growing crop to utilize this N. Given all these facts, it seemed reasonable, therefore, to assume that the high leaching losses observed in the N-fertilized plots 10 months after the final N application was from the residual fertilizer-N and not from the microbial re-mobilization of organic N suggested by P.R.Ball (1994 pers comm).

7.5 Conclusion

The trend in average nitrate levels down the soil profile or in the amounts of N leached from the studied soil/plant systems reflected strongly on N inputs. The N inputs into a soil/plant system through a legume can match those from moderate N-fertiliser application. As more N is added to ecosystem (through whatever means), the excess becomes subject to leaching losses.

Absence of plant uptake of mineral N, be it due to plot bareness or poor plant growth due to drought, was found to have a tremendous impact on the extent of nitrate leaching losses.

CHAPTER 8

8 General Discussion

In order to facilitate evaluating the final respective impacts on soil and environment quality of the cultural treatments under the study and therefore merits of the cultural practices, the treatments were broadly grouped into 5 categories of cultural practices.

(a) Bare fallow (BF)
(b) Cropping without Nitrogen Application (C - N)
(c) Cropping with Nitrogen Application (C + N)
(d) Permanent grass (PG)
(e) Permanent grass/clover pasture (PGC)

In this grouping the effects of cropping without N application were pooled over the 4 and 6 years of cultivation. Our general observation among the these cultural practices showed the following trend in terms of environmental problems (ie soil degradation and/or N leaching losses):

$$C + N = BF > C - N > PGC > PG.$$

The permanent grass pasture system (PG) ranked the best followed by the permanent grass/clover pasture (PGC) in our simple cultural-practices ranking. The reason why the PG and PGC treatments ranked topmost was because they tended to maintain high organic matter and soil pH (see site description section) and hence tended to conserve greater amounts of N (in a labile organic form) as indicated by the high flushes in mineral N following incubation tests. The pasture systems (both PG and PGC) scored very favourably too on soil biomass. This suggests the systems have greater capacity to maintain soil fertility. The high biomass must have been due to their high soil organic matter levels observed in these soils. PG was better than the PGC on the whole. This was because the later tended to leach a lot of N posing a big threat to groundwater pollution by nitrate nitrogen as a result of high N inputs from legume-N fixation. However, in general these two were very similar.

Nitrate levels down the soil profile indicated the cultural practices that rely on legume N can pose just as much an environmental threat to groundwater quality as those that apply fertiliser-N. Legume pastures may lose N through leaching about 8 to 10 times more than grass pasture (Kilmer *et al.*, 1974). In pasture swards managed to maintain a high proportion of a legume content, N inputs through legume fixation have been found to be in the range of 74 - 280kg ha^{-1} $year^{-1}$ in the UK and 85 - 342kg ha^{-1} $year^{-1}$ in New Zealand (Ball and Ryden 1984). These biological N inputs match normal N-fertiliser application rates.

Bare fallowing and cultivation with N application ranked lowest. In general, the practices that disturbed the soil: ie bare fallow and cultivation with or without N application tended to be very degrading to the soils. The soils under these practices had lost their organic matter and consequently the capacity to supply plants with N. Cultivation with N application appeared to be the worst. From the exhaustive cropping experiment it was observed that cultivation reduced the soil's natural capacity to supply mineral N. This was in agreement with the reports by Thompson *et al.* (1970) and Kandirgramathaiyah and MacKenzie (1970). These workers have reported that with cultivation the mineraliszable N fraction of the soil disappears. In this study it appeared that N application increased the rate at which the labile N forms were being depleted from the soil. This loss of the mineralizable N forms in the soil was found to have some repercussion not only on plant growth but on soil biomass as well.

The bare fallow and cultivation with N application also were found to pose nitrate problems with regard to groundwater quality. The absence of the growing plants to take up the mineralized N encouraged N loss due to leaching. Low and Armitage (1970) and Nielson and Jensen (1990) showed that in absence of growing plants the amounts of N leaching down the soil profile can be significant. Depending on the rooting depth of the subsequent crop concerned, the amount of rainfall and soil type, the leached N may never be retrieved by the plants. Data on the initially available N suggested that nitrate tends to accumulate in bare soils. This N, with no growing plants to take it up, will subsequently leach away if not lost due to denitrification.

Bare fallow was found to have the highest soil bulk density. Bulk density is one of the measures of soil physical conditions commonly assessed in soil studies. It is of paramount importance in agricultural soils as it influences root growth and penetration as well as water infiltration and soil aeration. This property is known to be influenced by cultivation and is also associated with organic matter. Nevertheless, this was not apparent in this study. There was no difference in soil bulk density between arable soils and pasture soils despite their significant differences in soil organic matter or tillage effects. Soils with plants growing on them, be they cultivated or not, had lower bulk density (on average $1.3 g/cm^3$) while that of the of BF soils was $1.5 g/cm^3$ (Table 3.1). The absence of growing plants for a prolonged period (6 years) on BF plots promoted soil compaction. Nevertheless, the mechanism through which the plants influenced soil bulk density was not clear.

In conclusion the three most important facts revealed by this study are:

(a) Shifting from reliance on N-fertilizers to legumes for the supply of N is not guarantee against N leaching into groundwaters.

(b) The importance of the presence of growing plants in intercepting leaching N.

(c) The application of N appeared to accelerate soil degradation. This needs further research to investigate the mechanisisms involved.

BIBLIOGRAPHY

Adams, J.A.; Campbell, A.S.; McKeengan, W.A.; McPherson, J.R. and Tokin, P.J. 1979. Nitrate and chloride in groundwater, surface water and deep soil profiles in Central Canterbury, New Zealand. Progress in Water Technology 11: 351-360

Addiscott, A.T.; Whitemore, A.P. and Powlson, D.S. 1991. Farming, Fertilizers and the Nitrate Problem. C.A.B. International. Wallingford, U.K.

Alef, K.; Beck, T.; Zelles, L. and Kleiner, D. 1988. A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Biology and Biochemistry 20: 561-565.

Alexander, M. 1961. Introduction to Soil Microbiology. John Wiley and Sons. New York

Allison, F.E. 1966. The fate of nitrogen applied to soils. Advances in Agronomy 18: 219-258

Allison, F.E.; Bollen, W.B. and Kleiner, C.D. 1969. Total carbon by dry combustion. In: Blake, C.A., Ed. Methods of Soil Analysis. Part 2. Chemical and Microbial Properties. Wisconsin. American Society of Agronomy. p 47-58.

Anderson, J.P.E. and Domsch, K.H. 1973. Quantification of bacterial and faunal contribution to soil respiration. Arch Mikrobiology 93: 113-127.

Ball, P.R. 1979. Nitrogen relationships in grazed and cut grass/clover systems. PhD. Thesis. Massey University. p 217

Ball, P.R. and Ryden, J.C. 1984. Nitrogen relationships in intensively managed temperate grasslands. Plant Soil 76: 23-33.

Ball, P.R. and Crush, J.R. 1985. Prospects for increasing symbiotic nitrogen fixation in temperate grasslands. Proceedings of the 15th IGC. 56-61.

Ball, P.R.; Brougham, R.W. and Brock, J.L. 1979. Nitrogen fixation in pasture. 1. Introduction and general methods. New Zealand Journal of Experimental Agriculture 7: 1-15.

Ball, P.R. and Keeney, D.R. 1983. Nitrogen losses from urine-affected areas of New Zealand pasture under contrasting seasonal conditions. Proceedings of 14th International Grassland Congress. Lexington. p 342-344.

Ball, P.R. and Keeney, D.R. 1979. Nitrogen fixation in pasture. I. Introduction and general methods. New Zealand Journal of Experimental Agriculture 7: 1-15.

Ball, P.R. 1984. Nitrogen relationships in intensively managed temperate grasslands. Plant Soil 76: 23-33.

Bergstrom, L. and Johansson, R. 1991. Leaching of nitrate from monolith lysimeters of different types of agricultural soils. Journal of Environmental Quality 20: 801-807.

Biederbeck, V.O.; Campbell, C.A. and Zentner, R.P. 1984. Effect of crop rotation and fertilisation on some biological properties of a loam in southwestern Saskatchewan. Canadian Journal of Soil Science 64: 355-367.

Blake, G.R. and Hartage, K.H. 1986. Bulk Density. In: Klute, A., Ed. Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. Madison, Wisconsin: Agronomy. p 363-376.

Blakemore, L.C.; Searle, P.L. and Daly, B.K. 1987. Methods For Chemical Analysis of Soils: Scientific report 80. New Zealand Soil Bureau. Lower Hutt, New Zealand.

Blevins, R.L.; Thomas, W.G. and Cornelius, P.L. 1977. Influence of no-tillage and nitrogen fertilization on certain soil properties after 5 years of continuous corn. Agronomy Journal 69: 383-386.

Bolton, H.Jr.; Elliot, L.F. and Papendick, R.I. 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biology and Biochemistry 17: 297-302.

Bormann, F.H.; Likens, G.E; Fisher, D.W. and Pierce, R.S. 1968. Nutrient loss accelerated by clearing of a forest ecosystem. Science 159: 882-884.

Boyle, M.; Paul, E.A. 1989. Nitrogen transformation in soil previously amended with sewage sludge. Soil Science American Journal 53: 740-744.

Brady, N.C. 1984. The Nature and Properties of Soils. Macmillan Publishing Company. New York. p 639

Bremner, J.M. 1965. Nitrogen availability indices. In: Black, C.A., Ed. Methods of Soil Analysis, Part 2 Agronomy 9: 1324-1345.

Brookes, P.C.; Powlson,D.S. and Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14: 319-329.

Brougham, R.W. 1973. Pasture management and production. Proceedings of the Ruakura Farms Conference. p 169-184.

Burford, J.R. and Stefanson, R.C. 1973. Measurement of gaseous losses of nitrogen from soils. Soil Biology and Biochemistry 5: 133-141.

Byrnes, B.H. 1990. Environmental effects of N-fertiliser use - An overview. Fertiliser Research 26: 209-215.

Cameron, K.C. and Wild, A. 1984. Potential aquifer pollution from nitrate leaching following the plowing of temporary grassland. Journal of Environmental Quality 13 (2): 274-278.

Cameron, K.C. and Haynes, R.J. 1986. Retention and movement of nitrogen in soils. In: Haynes, R.J.; Cameron, K.C.; Goh, K.M.; Sherlock, R., Eds. Mineral Nitrogen in the Plant/Soil System. Academic Press. New York. p 166-241.

Campbel, C.A. and Paul, E.A. 1978. Effects of fertilizer N and soil moisture on mineralization, N recovery and A - values under spring wheat in small lysimeters. Canadian Journal of Soil Science 58: 39-59.

Carlyle, J.C. and Malcolm, D.C. 1986. Nitrogen availability beneath pure spruce and mixed larch plus spruce stand growing on a deep peat. II. A comparison of N availability as measured by plant uptake and long-term laboratory incubation. Plant and Soil 39: 115-122.

Carran, R.A. 1983. Changes in soil nitrogen during pasture-crop sequences - A Review. Proceedings Agronomy Society of New Zealand 13: 29-31.

Collins, H.P.; Rasmussen, P.E. and Douglas Jr, C.L. 1992. Residue management effects on soil carbon and microbial dynamics. Soil Science American Journal 56: 783-788.

Coote, D.R. and Ramsey, J.F. 1983. Quantification of the effects of over 35 years of intensive cultivation of 4 years. Canadian Journal of Soil Science 63: 1-14.

Cox, G.W. and Atkins, M.D. 1979. Agricultural Ecology: Analysis of World Food Production Systems. W.H. Freeman Publishing Company. San Fransisco. U.S.A

Crutzen, P.J. 1981. Atmospheric chemical process of oxides. In: Delwiche, C.C., Ed.

Denitrification, Nitrification and Atmospheric Nitrous Oxides. John Willey and Sons. New York. p 17-44.

Dalal, R.C. and Mayer, R.J. 1990. Long-term trends infertility of soils under continuous cultivation and cereal cropping in Southern Queensland. VIII Available N indicices and their relationships to crop yield and N uptake. Australian Journal of Soil Research 28: 563-575.

Dalal, R.C. and Mayer, R.J. 1986. Long-term trends in fertilisation of soil under continuous cultivation and cereal cropping in Southern Queensland. II. Total organic carbon and its rate of loss from soil profile. Australian Journal of Soil Research 24: 281-292.

Dixon, R.O.D. and Wheeler, C.T. 1986. Nitrogen Fixation in Plants. Chapman and Hall Publishers. New York.

Fazio, S.D.; Mayberry, W.R. and White, D.C. 1979. Muramic acid assay in sediments. Applied Environmental Microbiology. 38: 349-350.

Field, T.R.O.; Ball, P.R. and Theobald, P.R. 1985. Leaching of nitrate from sheep-grazed pastures. Proceedings of the New Zealand Grassland Association 46: 209-214

Fleige, H. and Baeumer, K. 1974. Effects of zero tillage on organic carbon and total nitrogen and their distribution in different N-fractions in loessial soils. Agro-Ecosystems 1: 19-29.

France, A.A. 1978. Contribution of the legume-rhizobium symbiosis to the ecosystems and food production. In: Dobereiner, J., Ed. Limitation and Potentials for Biological Nitrogen Fixation in Tropics. New York: Plenum Press. p 65-74.

Gandar, P.W. and Ball, P.R. 1982. Nitrogen: An overview. In: Nitrogen Balance in New

Zealand Ecosystems. DSIR. New Zealand. p 13-31.

Goh, K.M.; Edmeades, D.C. and Hart, P.B.S. 1979. Direct field measurements of leaching losses of nitrogen in pasture and cropping soils using tension lysimeters. New Zealand Journal of Agricultural Research 22: 133-142.

Gow, N.G. 1965. Economic principles of fertilizer use. New Zealand Fertiliser Journal. June issue (2).

Harmsen, G.W. and Kolenbrander, G.J. 1965. Soil inorganic nitrogen. Agronomy 10: 43-92.

Hassink, J. and Van Schreven, D.A. 1955. Mineralization of inorganic nitrogen in soil. Advances in Agronomy 7: 299-398.

Hauck, R.D. 1990. Agronomic and public aspects of soil nitrogen research. Soil and Water Management 6 (2): 66-76.

Herman, W.A.; McGill, W.B. and Dormaar, J.F. 1977. Effects of initial chemical composition on decomposition of roots of three grass species. Canadian Journal of Soil Science 57: 205-215.

Hogg, D.E. 1981. A lysimeter study of nutrients losses from dung and urine application on pastures. New Zealand Journal of Experimental Agriculture 9: 39-46.

Hoglund, J.H. 1985. Grazing intensity and soil nitrogen accumulation. Proceedings of the New Zealand Grassland Association 46: 65-69.

Hood, A.E.M. 1976. Nitrogen, grassland and water quality in the United Kingdom. Outlook on Agriculture 8: 320-327.

Horne, B. 1982. The effects of fertilisers and soil management on quality of water supplies. Journal of Agricultural Education Association 57: 26-34.

Houot, S.; Moline, T.A.E.; Caseinate, R. and Clamp, C.E. 1989. Simulation by NCSOIL of net mineralisation in soil from the Deherian 36 Parcelles plots at Grigno. Soil Science American Journal 53: 451-455.

Jaakkola, A. 1984. Leaching losses of nitrogen from a soil under grass and crops in Finland. Plant and Soil 76: 59-66.

Jenkinson, D.S. and Powlson, D.S. 1976b. The effects of biocidal treatments on metabolism in soil. I. Fumigation with chloroform. Soil Biology and Biochemistry 8: 167-177.

Jenkinson, D.S.; Powlson, D.S. and Wedderburn, R.W.M. 1976. The effects of biocidal treatments on metabolism in soil. III The relationship between soil biovolume, measured by optical microscopy, and by fumigation. Soil Biology and Biochemistry 8: 189-202.

Jenkinson, D.S. and Powlson, D.S. 1976a. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biology and Biochemistry 8: 209-213.

Jenkinson, D.S. 1976. The effects of biocidal treatments on metabolism in soil. IV The decomposition of fumigated organisms in soil. Soil Biology and Biochemistry 8: 202-208.

Jenkinson, D.S. and Powlson, D.S. 1980. Measurement of microbial biomass in intact soil cores and in sieved soil. Soil Biology and Biochemistry 12: 579-581.

Jenkinson, D.S. 1991. The Rothamsted long-term experiments: Are they still of use?

Agronomy Journal 83: 2-10.

Jerez, B.E.R. 1991. Dynamics of nitrogen in three contrasting pastures grazed by sheep. PhD Thesis. Massey University. p 12

Johnson, D.W. 1992. Nitrogen retention in forest soil. Journal of Environmental Quality 21 (1): 1-12.

Kandirgamathaiyah, S. and MacKenzie, A.F. 1970. A study of soil nitrogen organic fractions and correlation with yield response of Sudan-sorghum hybrid grass on Quebec soils. Plant and Soil 33: 120-128.

Keeney, D.R. and Bremner, J.M. 1967. Determination and isotope-ratio analysis of different forms of nitrogen in soils. VI Mineralisable nitrogen. Soil Science American Journal 31 (1): 34-39.

Keeney, D.R. and Bremner, J.M. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agronomy Journal 58: 498-503.

Kilmer, V.J. 1974. Nutrient loss from grassland through leaching and run-off. In: Mays, D.A., Ed. Forage Fertilisation. Madison, Wisconsin: American Society of Agronomy. p 341-362.

King, J.D. and White, D.C. 1977. Muramic acid as a measure of microbial biomass in estuarine and marine samples. Applied Environmental Microbiology 33: 763-777.

Legg, J.O.; Chichester, F.W.; Stanford,G. and De Mar, W.H. 1971. Incorporation of ^{15}N-tagged mineral nitrogen into stable forms of soil organic nitrogen. Soil Science Society America Proceedings 35: 273-276.

Likens, G.E; Bormann, F.H.; Johson, N.M.; Fisher, D.W. and Pierce, R.S. 1970. Effects of forest cutting in the marine intertidal on nutrient budgets in the Hubbard Brook Watershed ecosystems. Ecological Monographs 40 (1): 23-47.

Loehr, R.C. 1979. Land Application of Wastes: Vol II. Van Nostrand Reinhold Company. New York.

Low, A.J. and Armitage, E.R. 1970. The composition of the leachate through cropped and uncropped soils in lysimeters compared with that of rain. Plant Soil 33: 393-411.

Maggette, W.L. and Shirmohammadi, A.S. 1989. Nitrate in shallow unconfined ground water beneath agricultural fields. In Dodd and Grace (Eds) Land and Water Use, Balkema, Rotterdam. p 297-304

Martens, R. and Cameron, K.C. 1985. Limitation in application of the fumigation technique for biomass estimates in amended soils. Soil Biology and Biochemistry 17: 57-63.

McClean, R.G. and Cameron, K.C. 1990. An introduction to the properties and management of New Zealand soils. In: Soil Science. Auckland, New Zealand. Oxford University Press.

McGill, W.B.; Cannon, K.R.; Robertson, J.A. and Cook, F.D. 1986. Dynamics of soil microbial biomass and water soluble organic C in Breton after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66: 1-19.

Merckx, R.; Hartog, A.D. and Van Veen, J.A. 1985. Turnover of root derived material and related microbial biomass formation in soils of different textures. Soil Biology and Biochemistry 17: 565-569.

Monaghan, R.M.; Cameron, K.C. and Mclay, C.D. 1989. Leaching losses of nitrogen from sheep urine patches. New Zealand Journal of Agricultural Research 32: 237-244.

Mosier, A.R.; Guenzi, W.D. and Schweizer, E.E. 1986. Soil losses of dinitrogen and nitrous oxide from irrigated crops in northeastern Colorado. Soil Science American Journal 50: 344-348.

Mulder, E.G. 1975. Physiology and ecology of free-living nitrogen fixation bacteria. In: Stewart, W.D.P., Ed. Nitrogen Fixation by Free-living Microorganisms. Cambridge: University of Cambridge. p 3-28.

Neilsen, N.E. and Jensen, H.E. 1990. Nitrate leaching from loam soils as affected by crop rotation and nitrogen fertiliser application. Fertiliser Research 26: 179-207.

Parkin, T.B. 1987. Soil microsites as a source of denitrification variability. Soil Science Society American Journal 51: 1194-1199.

Parkinson, D. and Paul, E.A. 1989. Microbial biomass. In: Page, A.L., Ed. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Madison, Wisconsin.: American Society of Agronomy: 821-830.

Parnas, H. 1975. Model for decomposition of organic material by microorganisims. Soil Biology and Biochemistry 7:161-169

Patra, D.D.; Brookes, P.C.; Coleman, K. and Jenkinson, D.S. 1990. Seasonal changes in soil microbial biomass in an arable and a grassland soil which have been under cultivation for many years. Soil Biology and Biochemistry 22: 737-742.

Paul, E.A. and Voroney, R.P. 1984. Field interpretation of microbial biomass activity measurements. In: Klug, M.J.; Reddy, C.A., Eds. Current Perspective in Microbial Ecology. 3rd ed. Washington: American Society for Microbiology. p

509-514.

Paul, E.A. and Johnson, R.L. 1977. Microscopic counting and adenosine 5'-triphosphate measurement in determining microbial growth in soils. Applied Environmental Microbiology 34: 262-269.

Peterson, T.A. and Russele, M.P. 1991. Afalfa and the nitrogen cycle in the Corn Belt. Journal of Soil and Water Conservation 46 (3): 231-235.

Powlson, D.S.; Pruden, G.; Johston, H.E. and Jenkinson, D.S. 1986. The nitrogen cycle in the Broadbalk wheat experiment: recovery and losses of ^{15}N-labelled fertilizer applied in spring and inputs of nitrogen from the atmosphere. Journal of Agricultural Science 107: 591-609

Powlson, D.S.; Brookes, P.C. and Christensen, B.T. 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biology and Biochemistry; 1987; 19: 159-164.

Powlson, D.S. and Jenkinson, D.S. 1981. A comparison of the organic matter, biomass, adenosine triphosphate and mineralizable nitrogen contents of the plowed and direct drilled soils. Journal of Agricultural Science 97: 713-721.

Quispel, A. 1974. Biology of Nitrogen Fixation. North Holland Publishing Company. Amsterdam. p 1

Rosser, D.J. 1980. Ethidium bromide a general purpose fluorescent stain for nucleic acid in bacteria and eukaryotes and its use in microbial ecology studies. Soil Biology and Biochemistry 12: 329-336.

Ryan, J.A.; Sims, J.L. and Peaslee, D.E. 1971. Laboratory methods of estimating plant available nitrogen in soil. Agronomy Journal 63: 48-51.

Ryden, J.C.; Ball, P.R. and Garwood, E.A. 1984. Nitrate leaching from grasslands. Nature. 311 (5981): 50-53.

Sahrawat, K.L. 1983. Correlation between index of soil nitrogen availability, percent in plant, nitrogen uptake, and dry matter yield of rice grown in greenhouse. Plant and Soil 74: 223-228.

SAS. 1990. SAS/STAT User's Guide. Vol 2, GLM-Varcomp. Cary, N.C USA, SAS Institute. p 891-996.

Schepers, J.S.; Moravek, M.G.; Alberts, E.E. and Frank, K.D. 1991. Maize production impacts on groundwater quality. Journal of Environmental Quality 20: 12-16.

Schnurer, J.; Carholm, M. and Rosswall, T. 1985. Microbial biomass and activity in agricultural soil with different organic matter contents. Soil Biology and Biochemistry 17: 611-618.

Scotter, D.R.; Clothier, B.E. and Turner, M.A. 1979. The soil water balance in a fragiaqualf and its effects on pasture growth in Central New Zealand. Australian Journal of Soil Research 17: 455-65.

Sears, P.D. 1960. Grass/Clover relationships in New Zealand. Proceedings of the 8th International Grassland Congress. p 130-133.

Seastedt, T.R. and Hayes, D.C. 1988. Factors influencing nitrogen concentration in soil water in a North American tallgrass prairie. Soil Biology and Biochemistry 20 (5): 725-729.

Shen, S.M.; Pruden, G. and Jenkinson, D.S. 1984. Mineralization and immobilization of N in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biology and Biochemistry 16: 437-444.

Singh, B. and Sekhon, G.G. 1979. Nitrate pollution of ground water from use of nitrogen fertilisers: A Review. Agriculture and Environment 4: 207-225.

Soderstrom, B.E. 1977. Vital staining of fungi in pure culture and soil with fluprescein diacetate. Soil Biology and Biochemistry 9: 59-63.

Sparling, G.P. 1985. The soil biomass. In: Vaughan, D.; Malcolm, R.E., Eds. Soil Organic Matter and Biological Activity. Dordrecht: Nijhoff. p 223-262.

Spedding, C.R.W.; Walsingham, J.M. and Hoxey, A.M. 1981. Biological Efficiency in Agriculture. Academic Press, London. p 52

Sprent, J.I. 1987. The Ecology of Nitrogen Cycle. University Press. New York.

Stanford, G. and Hanway, J. 1955. Predicting nitrogen fertiliser needs of Iowa soils. II A simplified technique for determining relative nitrate production in soils. Soil Science Society America Proceedings 19: 74-77.

Stanford, G.; Carter, J.N. and Smith, J.S. 1974. Estimation of potentially mineralizable soil nitrogen based on short-term incubation. Soil Science Society of America Proceedings 38: 99-102

Stanford, G. and Epstein, E. 1974. Nitrogen mineralisation - Water relations in soils[1]. Soil Science Society of America proceedings 38: 103-107.

Stanford, G.; Legg, J.O.; Smith, J.S. 1973. Soil nitrogen availability evaluation based on N mineralisation potentials of soils and uptake of labelled and unlabelled N by plants. Plant and Soil 39: 113-124.

Steele, K.W. and Judd, M.J. L1984. Leaching of nitrate and other nutrients from a grazed pasture. New Zealand Journal of Agricultural Research; 27: 3-11.

Swift, M.J.; Heal, O.W. and Anderson, J.M. 1979. Decomposition in terrestrial ecosystems. Blackwall Scientific Publications. Oxford.

Tate, K.R.; Ross, D.T. and Feltham, C.W. 1988. A direct extraction method of to estimate soil microbial C: Effects of experimental variables and some different calibrations procedures. Soil Biology and Biochemistry 20: 329-335.

Thompson, L.M.; Black, C.A. and Zoellner, J.A. 1970. Occurrence and mineralization of organic phosphorus and in soils with particular reference to associations with nitrogen ,carbon and pH. Soil Science 77: 185-196.

Tomlinson, T.E. 1971. Nutrient losses from agricultural land. Outlook on Agriculture 6: 272-278.

Trudgill, S.T.; Burt, T.P.; Heathwaite, A.L. and Arkell, B.P. 1991. Soil nitrate sources and leaching losses. Slapton, South Devon. Soil Use and Management 7 (4): 200-206.

Vance, E.D.; Brookes, P.C. and Jenkinson, D.S. 1987. Microbial biomass in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biology and Biochemistry 19: 697-702.

Vitousek, P.M.; Gosz, J.R.; Grier, C.C.; Melillo, J.M. and Reiners, W.E. 1982. A comparison analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecological Monographs 52: 155-177.

Walters, D.T. and Malzer, G.L. 1990. Nitrogen management and nitrification effects on nitrogen-15 Urea. 1. Yield and fertilizer use efficiency. Soil Science Society American Journal 54:115-122.

Walters, D.T.; Aulakh, M.S. and Doran, J.W. 1992. Effects of soil aeration, legume residues and soil texture on transformations of macro and macronutrients in soils. Soil Science 153 (2): 100-107.

Waring, S.A. and Bremner, J.M. 1964. Ammonium production in soils under waterlogged conditions as an index of nitrogen availability. Nature 201: 951-952

Weil, R.R.; Weismiller, R.A. and Turner, R.S. 1990. Nitrate contamination of groundwater under irrigated coastal plain soils. Journal of Environmental Quality 19: 441-448.

West, A.W.; Sparling, G.P. and Grant, W.D. 1986. Correlation between four methods to estimate total microbial biomass in stored air-dried and glucose-amended soil. Soil Biology and Biochemistry 18: 569-576.

Whitehead, D.C. 1986. Source and transformations of nitrogen in intensively managed grassland. In: Van Der Meer, H.G.; Ryden, J.C.; Ennik, G.C., Eds. Developments in Plant and Soil Science: Nitrogen Fluxes in intensive grassland systems.: Martinus Nijhoff Publishers. p 47-58.

Whitemore, A.P.; Bradbury, P.A. and Johnson, P.A. 1992. Potential contribution of ploughed grassland to nitrate leaching. Bent, J.B.; Jones,J.W., eds. Agriculture Ecosystems and Environment 39: 221-233.

Wild, A. and Cameron, K.C. 1980b. Soil nitrogen and nitrate leaching. In: Tinker, P.B., Ed. Soils and Agriculture. Oxford 2: 35-70.

Wild, A. and Cameron, K.C. 1980a. Nitrate leaching through soils and environmental considerations: With special reference to recent work in the U.K. In: Soil Nitrogen as Fertiliser or Pollutant. Proc. I.A.E.A./F.A.O. Symposium. Piracicaba, Brazil. p 289-307.

Young, C.P.; Oakes, D.B. and Wilkinson, W.B. 1979. The impact of agricultural practices on the nitrate content of groundwater in the principal United Kingdom aquifers. In: International Institute for Applied Systems. Conference on Environmental Management of Agricultural Watersheds. Smolenie, Czchoslovakia.

APPENDICES

Appendix 1: Analysis of variance for the soil physical properties.

SOURCE	DF	MS		
		pH	ORGANIC MATTER	BULK DENSITY
Blocks	2	0.00231 (ns)	0.03375 (ns)	0.00234 (ns)
Treatment	5	0.05847 (**)	3.21083 (**)	0.20415 (**)
Error	8	0.0089	0.3975	0.00151

Appendix 2a: Analysis of variance for the initially available NH_4-N and NO_3-N and that potentially available under incubation conditions.

SOURCE	DF	MS					
		FIELD MOIST SOIL AVAILABLE N		AEROBIC		ANAEROBIC	
		NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
Blocks	2	1.204 (*)	0.151 (ns)	240.5 (***)	0.895 (ns)	25.54 (ns)	0.041 (ns)
Treatment	4	26.29 (**)	1.817 (**)	192.71 (ns)	25.225 (***)	3521.58 (**)	0.027 (ns)
Error	6	0.222	0.048	53.97	2.216	34.46	0.045

Appendix 2b: Analysis of variance for total N (NH_4-N + NO_3-N) in field moist soil and the incubated soils

SOURCE	DF	MS		
		FIELD MOIST SOIL	AEROBIC INCUBATION	ANAEROBIC INCUBATION
Blocks	2	0.61 (ns)	270.5 (ns)	24.1 (ns)
Treatment	4	40.24 (**)	346.0 (*)	265.1 (*)
Error	6	0.21	54.52	38.11

Appendix 3a: Analysis of variance for the ryegrass cuts from the greenhouse experiment.

SOURCE	DF	MS					
		FIRST CUT	SECOND CUT	THIRD CUT	FOURTH CUT	ROOT DM	TDM
Blocks	2	0.0003 (ns)	0.0042 (ns)	0.0059 (ns)	0.0035 (**)	0.0857 (***)	0.0395 (ns)
Treatments	5	0.0292 (***)	0.0172 (***)	0.0184 (***)	0.0030 (***)	0.2072 (***)	0.8658 (***)
Error	10	0.0017	0.0011	0.0019	0.0005	0.0172	0.3953

Appendix 3b: Analysis of variance for the N uptake by ryegrass plants.

SOURCE	DF	MS	
		TOTAL N UPTAKE PER POT	N UPTAKE PER Kg POT SOIL
Blocks	2	8.17 (ns)	66.33 (ns)
Treatment	5	47.20 (***)	385.55 (***)
Error	10	2.37	19.34

Appendix 4a: Microbial biomass size of the studied plots (μg C g^{-1} dry soil)

TREATMENTS	METHOD ONE	METHOD TWO
BF	447.62 a	550 abc
GC	111.90 c	627 a
C_6-N	284.52 b	522 bc
C_4-N	408.34 ab	610 a
C_6+N	408.33 ab	489 c
H	32.03 c	593 ab
C.V.	16.95	5.89
L.S.D.	115.71	90
S.E	31.83	20
P	***	ns

Appendix 4b: Analysis of variance for the microbial biomass.

SOURCE	DF	MS				
		FIRST FLUSH	SECOND FLUSH	MEAN FLUSH	BIOMASS	
					Method one	Method Two
Blocks	1	0.00004 (ns)	0.00145 (ns)	0.00069 (ns)	3015.3 (ns)	0.00034 (ns)
Treatments	5	001035 (ns)	0.01394 (***)	0.00426 (***)	6076.9 (***)	0.00527 (ns)
Error	5	0.00024	0.00031	0.00037	2676.9	0.00111

Appendix 5a: Monthly rainfall and drainage data for the study site

1990	**P**	**E_p**	**E_t**	**W_A**	**P - E_t**	**D**
August	111.2	35.8	29.4	75	81.8	81.8
September	16.9	64.1	52.6	39.3	-35.7	0.0
October	83.7	103.0	84.5	38.5	-0.8	0.0
November	98.3	115.2	94.5	42.3	3.8	0.0
December	50.7	160.1	131.3	0.0	-80.6	0.0
TOTALS	366.8	478.2	392.3		-35.5	81.8
1991						
January	120.2	163.0	133.7	0.0	-13.5	0.0
February	131.5	106.7	87.5	44.0	44.0	0.0
March	28.7	113.3	92.9	0.0	-64.2	0.0
April	162.7	50.0	41.0	75	121.7	46.7
May	80.3	34.7	28.5	75	51.8	51.8
June	83.1	22.0	18.0	75	65.1	65.1
July	93.4	23.5	19.3	75	74.1	74.1
August	98.7	43.7	35.8	75	62.9	62.9
September	65.1	65.3	53.5	75	11.6	11.6
October	80.9	91.7	75.2	75	5.7	5.7
November	81.0	108.9	89.3	66.7	-8.3	0.0
December	81.2	108.8	89.2	58.7	-8.0	0.0
TOTALS	1106.8	931.6	763.9		342.9	317.9
1992						
January	77.2	140.3	115.0	20.9	-37.8	0.0
February	155.2	128.8	105.6	70.5	49.6	0.0
March	88.9	92.2	75.6	75	13.3	8.8
April	44.4	46.8	38.4	75	6.0	6.0
May	27.6	31.6	25.9	75	1.7	1.7
June	74.8	22.0	18.0	75	56.8	56.8
July	141.6	24.0	19.7	75	121.9	121.9
August	110.2	31.1	25.7	75	84.5	84.5
September	87.7	46.4	38.0	75	49.7	49.7
October	85.7	77.7	63.7	75	22.0	22.0
November	60.8	96.3	79.0	56.8	-18.2	0.0
TOTALS	954.1	737.4	604.6		349.5	351.4

Appendix 5b: Soil nitrate levels across treatments and blocks in mg NO_3-N Kg^{-1} dry soil.

		SOIL DEPTH (cm)			
BLOCK	TREATMENT	0 - 50	50-100	100-150	150-200
I	BF	3.5	5.0	2.7	1.3
	GC	5.2	6.3	6.3	3.3
	C_6-N	1.3	3.5	2.3	0.9
	C_4-N	2.5	3.5	1.4	0.9
	C_6+N	3.3	10.6	3.8	1.8
	H	0.9	2.4	0.9	0.1
II	C_6-N	1.4	3.4	4.0	1.8
	C_4-N	2.1	1.5	2.7	0.9
	C_6+N	4.7	10.2	6.2	3.8
	H	0.6	1.1	0.4	1.1
III	BF	3.8	5.1	2.0	0.8
	GC	5.3	9.2	2.4	3.7
	C_6-N	2.1	3.3	1.6	1.3
	C_4-N	2.6	1.6	0.9	0.1
	C_6+N	4.4	18.7	3.9	2.6
	H	1.0	0.3	0.6	0.1

Appendix 5c: Amounts of NO_3-N Leached per m^2 through the 50cm core section (in grams)

		SOIL DEPTH (cm)			
BLOCK	TREATMENT	0 - 50	50-100	100-150	150-200
I	BF	2.49	3.55	2.01	0.96
	GC	3.69	4.85	4.77	2.31
	C_6-N	0.86	2.80	1.73	0.68
	C_4-N	1.71	2.68	1.71	0.61
	C_6+N	2.36	7.42	2.87	1.15
	H	0.63	1.92	0.68	0.07
II	C_6-N	0.96	2.65	2.92	1.48
	C_4-N	1.41	1.12	2.01	0.68
	C_6+N	3.20	7.80	4.43	3.12
	H	0.36	0.90	0.30	0.88
III	BF	2.94	3.85	1.42	0.58
	GC	3.90	7.41	1.64	2.78
	C_6-N	2.66	2.39	1.07	1.08
	C_4-N	2.06	1.28	0.59	0.08
	C_6+N	3.37	14.49	2.77	2.13
	H	0.97	0.24	0.43	0.08

Appendix 5d: Estimated soil water depths for the core sections (mm)

		SOIL DEPTH (cm)			
BLOCK	TREATMENT	0 - 50	50-100	100-150	150-200
I	BF	183	145	212	215
	GC	195	144	179	186
	C_6-N	152	112	212	225
	C_4-N	160	126	205	189
	C_6+N	166	140	153	171
	H	190	152	219	192
II	C_6-N	176	144	219	205
	C_4-N	156	183	212	230
	C_6+N	156	211	218	181
	H	170	119	215	223
III	BF	171	211	214	210
	GC	174	171	214	212
	C_6-N	175	169	198	232
	C_4-N	201	184	197	220
	C_6+N	182	188	205	213
	H	173	170	198	218

Appendix 5e: Total NO_3-N leached per m^2 in 1992 (g)

BLOCK	TREATMENT	NITRATE LEACHED
I	BF	5.50
	GC	9.97
	C_6-N	4.61
	C_4-N	4.45
	C_6+N	10.68
	H	2.54
II	C_6-N	5.41
	C_4-N	2.70
	C_6+N	10.64
	H	1.27
III	BF	4.78
	GC	8.79
	C_6-N	3.37
	C_4-N	1.78
	C_6+N	16.69
	H	0.63

Appendix 5f: Analysis of variance for the NO_3-N levels in the deep cores.

SOURCE	DF	MS	
		NO_3-N/Kg Dry Soil	mg NO3-N/L Soil Sollution
Blocks	2	0.5853 (ns)	9.62 (ns)
Treatment	5	24.55 (***)	247.44 (***)
Depths	3	41.47 (***)	748.00 (***)
Error	53	6.15	63.09

Appendix 5g: Analysis of variance for the estimated leached NO_3-N.

SOURCE	DF	MS
		NO_3-N
Blocks	2	0.58 (ns)
Treatment	5	50.1 (***)
Error	8	3.96