AN ABSTRACT OF THE THESIS OF

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Dairy manures are often applied to cropland for disposal and as a source of N fertilizer. Recent trends in the dairy industry toward larger herd sizes, and increases in land costs resulting in less land available to dairy farmers for disposal of manure result in the possibility of excessively high manure application rates. Nitrogen in excess of crop needs may leach as NO_3^- into groundwater or be lost by denitrification to the atmosphere. A high level of NO_3^- in groundwater is hazardous to human and animal health. Denitrification losses can also have negative environmental impacts, because N_2O, one of the denitrification products, is a radiatively active trace gas that contributes to global warming. Additionally, N_2O in the stratosphere is oxidized to NO, which catalyses the destruction of O_3.

A field study was conducted to measure two components of N cycling in manure fertilized pastures: denitrification losses and net N mineralization. Plots were established in three soil types representing a range of drainage classes. Plots received 0 to 450 kg manure-N ha^{-1} y^{-1} in five to seven split applications.

Denitrification, soil respiration, moisture content, and temperature were measured periodically for 15 months. Denitrification was measured by the C_2H_2 inhibition method on intact soil cores.

Nitrogen mineralization rates were measured by a soil core-ion exchange resin method. Three successive incubations were summed to produce an estimate for annual net N mineralization.

Annual denitrification losses were increased by manure applications and varied by soil type. Highest losses (108 kg N ha^{-1} y^{-1} at the highest manure rate) were in the well drained Quillamook soil. Lowest losses (33 kg N ha^{-1} y^{-1} at the highest manure rate) were in the poorly drained Waldo soil. Denitrification losses ranged from 5 to 16% of applied N.

Denitrification was a significant component of the N budget in these manure fertilized pastures. Denitrification in the fall and early winter removed NO_3^- which would likely have leached from the soils. In the poorly drained Waldo soil, denitrification rates did not significantly increase beyond the lowest manure rate, and were probably limited by NO_3^-. Restricted nitrification or plant or microbial competition for NO_3^- kept NO_3^- concentrations below 3 mg NO_3^--N kg^{-1} soil. In the well drained Quillamook soil, denitrification rates increased linearly with each increment in manure rate.

Net N mineralization as measured by the soil core-IER method were several times higher than either of two alternate methods (plant N uptake and loss of soil organic N). It appears likely that the method positively biases net N mineralization through the effect of the exclusion of plant roots on soil water relations.

Nitrogen Dynamics in Western Oregon Perennial Grass Pastures Fertilized with Dairy Manure

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INTRODUCTION

Dairy manures are often applied to soils for disposal or as a fertilizer source for crops. Although manure is a valuable source of plant nutrients, particularly N, application of excessive amounts of manure or application at inappropriate times, can lead to low recovery of N in crops and unacceptably large losses of N to the environment. Recent trends in the dairy industry toward larger herd sizes, and increases in land costs resulting in less land available to dairy farmers for disposal of manure result in the possibility of excessively high manure application rates.

Nitrogen losses from land spread manure to the environment are primarily through three routes: NH_3 volatilization, denitrification, and NO_3^- leaching. Most volatilization loss occurs directly from the NH_4^+ in manure in the first few days after spreading. Volatilization losses are mostly affected by the inorganic N content, method of application and weather at the time of spreading and are therefore managed separately from denitrification and leaching losses. In contrast, denitrification and leaching losses occur only after mineralization of organic N and nitrification of NH_4^+ to NO_3^-. Information on the relative portion of NO_3^- leached or denitrified is important when either groundwater quality or trace gas emissions are of concern. The product of denitrification is mostly N_2 gas, but some N_2O is also produced. N_2O is a radiatively active gas involved in global climate change and in a

series of reactions catalyzing the destruction of O_3 in the stratosphere. High levels of NO_3^- in groundwater are hazardous to human and animal health.

Denitrification losses are affected by soil drainage and amount and type of nitrogen fertilization. Lack of aeration, sufficiently large NO_3^- concentrations and a and high levels of carbon availability increase denitrification rates. The N and C components of manure interact with inherent soil characteristics such as drainage and organic C content to determine denitrification losses.

Loss of N by leaching can be measured directly, by extracting and analyzing soil water at different depths for NO_3^- content. It may also be estimated by measuring net N mineralization, subtracting known outputs, and assigning the difference to leaching. Net N mineralization is affected by amount and timing of manure application, climate and soil characteristics such as drainage class.

CHAPTER 1

LITERATURE REVIEW

NITROGEN IN THE ENVIRONMENT

Nitrogen is an essential plant nutrient, and large amounts of N are applied as a fertilizer to a variety of crops worldwide. Nitrogen exists in the atmosphere in the form of the very stable N_2 gas. Nitrogen gas must be biologically or industrially "fixed" before it is available to plants. Projections made in 1982 suggest that biological and industrial N_2 fixation will need to at least double from 138 x 10^6 tons by the year 2000 in order to feed world populations (Keeney, 1982). A 1985 global N budget lists annual N_2 fixation (both biological and industrial) as 214 x 10^6 tons. Mid-range projections for human population increase indicate that N fertilization will need to increase 1.7 times to produce sufficient food for the increased population (Jenkinson, 1990).

Because recovery of fertilizer N by crops is imperfect, rarely exceeding 70% and averaging about 50% (Keeney, 1982), most of the N not taken up by plants will be leached or denitrified. As Jenkinson (1990) eloquently states:

> "Increasing quantities of nitrogen are being cycled every year and it is important that this nitrogen be used as efficiently as possible *and* that leakage of combined nitrogen into water and atmosphere be minimized. Only by understanding how and when these losses occur can we hope to control them, so that as much nitrogen as possible leaves the soil/plant/animal/sewage *cleanly*, as N_2 gas."

Currently, fertilized agricultural land is estimated to account for a small portion, about 6%, of the global N_2O flux, although a great deal of uncertainty exists and the contribution could be as small as 3% or as large as 21% (Davidson, 1990). Therefore, as agricultural and waste management practices are developed and refined, the potential for adverse environmental impacts should be monitored.

Fertilizer N in managed grasslands

Grasslands receive a range of N fertilizer rates, from little or nothing to very high rates. The dairy industry tends to use land intensively, with high fertilization rates and high animal densities. There are several reasons for this situation. First, the economies of scale push dairy farmers to increase their herd sizes to the maximum allowable. Second, maximum milk production is achieved by feeding cows the maximum metabolizable energy and N fertilization of pastures increases dry matter production. And thirdly, dairies tend to be located near population centers, where land is likely expensive and not easily available. These conditions set up the possibility of very high rates of manure-N application, and resulting degradation of groundwater quality.

Manure as an N source

Animal manures contain a mixture of inorganic and organic N. In a fresh dairy manure, most of the inorganic N is in the form of NH_4^+. The remaining organic N is a combination of undigested feed, microbial and digestive products.

Although the NH_4^+ is immediately available to a crop, the organic N must first be mineralized. The timing and rate of mineralization vary with environmental conditions and the C/N ratio of the material. Manure N availability was reviewed by Smith and Peterson (1982). The N available in the first year varied from 20 to 90% of the total N, depending on the type of manure. The importance of climate was illustrated by contrasting two studies. Under central California conditions, for a fresh bovine waste containing 3.5% N (dry weight basis), 75% was expected to be available in the first year. However in the Pacific Northwest, only 42% was predicted to be available. Beauchamp and Paul (1989), proposed and discussed a simple model for manure-N availability. Given a starting material with roughly equal proportions organic and inorganic N, they suggest that 20% of the organic N will be mineralized and available in the first year. Assuming a volatilization loss of 25% of the NH_4^+-N, 75% of the NH_4^+-N will be plant available as well. Under this model roughly half of the manure-N is available in the first year to a crop.

Application of manure to established grassland is typically either through surface application or injection. Because the primary factors limiting recovery of manure-N are NH_3 volatilization and denitrification, the application method has a large impact on the amount and route of N loss. When manure slurries are surface applied, volatilization dominates, whereas injection favors denitrification (Thompson et al., 1987). Volatilization losses can be reduced by acidification of the slurry (Thompson and Pain, 1989), and denitrification losses were reduced by nitrification inhibitors (Thompson et al., 1987). The season of application is also

important. Spring volatilization losses were higher than winter, while winter denitrification losses were higher than spring (Thompson et al., 1987).

N LOSSES: DENITRIFICATION AND NITRATE LEACHING

Denitrification and leaching in managed grasslands

Denitrification and leaching are alternate fates for NO_3^-, which have environmental impacts in either atmospheric chemistry or water quality. The relative importance of each pathway could potentially be manipulated by management decisions regarding timing, placement, type, and amount of N fertilization. Therefore the ability to predict the relative portion of NO_3^- in each pathway would be valuable information when management decisions regarding farming practice are made.

Garwood and Ryden (1986) point out the interdependence of leaching and denitrification with the observation that high leaching rates are often associated with low denitrification rates and vice versa. Addiscott and Powlson (1992) took a unique approach to leaching and denitrification losses in comparing 13 separate ^{15}N fertilized winter wheat experiments which took place over several different growing seasons and soil types. On the assumption that fertilizer N not recovered from the soil or plant is either leached or denitrified, they used a model to calculate leaching losses and calculated denitrification losses by difference. Their conclusion was that on average denitrification accounted for almost twice as much of N as leaching, or

about 10 kg N $ha^{-1}y^{-1}$ denitrified from 150 kg N $ha^{-1}y^{-1}$ applied as fertilizer. The range of denitrification losses was 0 to 22 kg N $ha^{-1}y^{-1}$.

Factors affecting denitrification

Denitrification is the use by microbes of NO_3^- or other N oxides as an electron acceptor in respiration (Firestone, 1982). The end product is mostly N_2 gas, but variable amounts of N in intermediate states of reduction are also released.

Denitrification is a major route of loss of N from both N poor desert ecosystems (Peterjohn and Schlesinger, 1991), and N rich agricultural ecosystems. Denitrification has been estimated to account for from 10% (von Rheinbaben, 1990) to 30% (Payne, 1983) of the N fertilizer applied to crops. Perennial grassland systems are more N efficient; losses from cut swards are expected to be about 5 to 10% of fertilizer N (Ryden, 1986). However, losses from grazed pasture, or losses from animal manures applied to pastures as fertilizer are higher (Ryden et al., 1984). Manure applications are expected to increase denitrification rates by providing both a C substrate (Paul and Beauchamp, 1989) and increasing soil NO_3^- concentrations.

Denitrification rates are primarily affected by three factors: C availability, soil NO_3^- concentration and O_2 tension. These factors are not independent of each other. For example, a high respiration rate may cause an anaerobic condition to develop where O_2 diffusion is only slightly impeded (Parkin, 1987). Nitrification is inhibited under anaerobic conditions (Schmidt and Belser, 1982) so NO_3^- may

become limiting sooner under continuous anaerobic conditions than under alternately aerobic and anaerobic conditions.

Because denitrification is a respiratory activity, it is directly dependent on the availability of C. Several studies have found that denitrification rates are closely correlated to the organic C content of soils (Webster and Goulding, 1989; Burford and Bremner, 1975), or to the amount or organic substrate added to a soil (Aulakh and Rennie, 1987).

Although denitrification rates are well correlated with the total organic C content of soil, many attempts have been made to measure a pool of available C in soils and correlate it to denitrification rates, generally with little success. For example, CO_2 production is one measure of C availability. In field studies, CO_2 production is often measured along with denitrification rates. Groffman and Tiedje (1991) attempted to use regression analysis to correlate CO_2 production and denitrification rates, but they were unable to explain more than 19% of the variability in denitrification rates and concluded that "our understanding of the regulation of denitrification and CO_2 production under field conditions is incomplete". Myrold (1988) found that respiration explained only 13% of the variability of denitrification rates.

It appears that the NO_3^- contents typically seen in agricultural soils are sufficient to support denitrification in the presence of a C source, and thus that C availability rather than NO_3^- availability is usually the driving force in denitrification. However, in some instances, NO_3^- content has been shown to be a

significant factor in determining denitrification rates, in particular where C is highly available (Elliott et al., 1991).

Denitrification rates are strongly affected by O_2 tension and distribution in soils (Firestone, 1982). Soil wetness and degree of aggregation are probably the main factors affecting O_2 tension. The distribution of O_2 was shown to vary within soil aggregates (Sexstone et al., 1985), but not all aggregates with anaerobic centers denitrified. In an unaggregated soil, O_2 inhibition rather than substrate limitation was the major factor limiting denitrification (Sexstone et al., 1988)

Leaching losses

Leaching losses are closely related to water movement through the soil profile, particularly where soil NO_3^- content is high (Pratt et al., 1976). Grasslands had previously been thought to have relatively low NO_3^- leaching losses because their dense rooting system effectively intercepts and absorbs NO_3^- (Legg and Meisinger, 1982). Recent work (Ryden et al., 1984) shows that this is not the case when grasslands are grazed rather than cut; NO_3^- concentrations beneath a grazed sward were 5.6 times greater than beneath a cut sward. These losses are partially a result of the low N efficiency of ruminants; much of the N consumed in grazing is returned as urine and feces. This causes as uneven distribution of N which enhances denitrification and leaching losses (Goulding and Webster, 1989).

In a study conducted in the Chino-Corona basin of southern California (Pratt et al., 1976), an area of intensive dairy production, irrigation water contained such

high amounts of NO_3^- that the control (un-manured) plots received almost 100 kg N $ha^{-1}y^{-1}$ through irrigation alone. Water leaching from the control plots contained from 10-20 mg NO_3^--N L^{-1}, whereas water leaching from plots receiving about 700 kg manure N $ha^{-1}y^{-1}$ (equivalent to 25 cows ha^{-1}) contained 51 mg NO_3^--N L^{-1}.

In the Netherlands, the current N fertilization rate recommendation for intensively managed grasslands is 400 kg N ha^{-1}. In a study of 14 intensive dairy farms (van der Meer and van Uum-van Lohuyzen, 1986), an average input of 383 kg fertilizer N ha^{-1} was added to the N in purchased feed supplements and compared to N outputs in milk and meat. The difference between known inputs and outputs was 440 kg N $ha^{-1}y^{-1}$ and the disposition of the excess N was unknown.

The groundwater NO_3^- concentration must be below 10 mg NO_3^--N L^{-1} in order to be considered safe for drinking by EPA (Environmental Protection Agency) standards. In Oregon, high levels of NO_3^- in groundwater due to agricultural usage are found in localized areas of the Willamette valley, and in Malheur county, in the eastern part of the state (Pettit and Thomas, 1986).

Leaching losses may be approached directly, through measurement of soil and groundwater NO_3^- content, or indirectly, by measuring other relevant N cycle processes and calculating leaching losses by difference. Net N mineralization is the difference between the opposing processes of N mineralization and immobilization (Jansson and Persson, 1982) and measures the input to the inorganic N pool. If the outputs (plant uptake and denitrification) are also measured, then leaching losses can be assessed.

This method is dependent on an accurate measurement of net N mineralization. Nitrogen mineralization assays can be divided into two groups: laboratory extractions and incubations (Stanford, 1982) and *in situ* incubations (Hart et al., in press). The laboratory methods have typically been developed to predict fertilizer response in agronomic crops and require calibration to the field to accurately predict net N mineralization. The *in situ* methods have been most frequently applied in forests and are designed to mimic natural conditions (soil temperature and soil water) as closely as possible. Soil in *in situ* incubations may be sieved to reduce spacial variability, or kept intact, to avoid the stimulation of N mineralization that is often associated with aggregate disruption. It has been repeatedly shown that disturbances such as sieving alter N mineralization rates, as does changing the soil water content (Myers et al., 1982) and temperature (Kladivko and Keeney, 1987) or aeration status (Linn and Doran, 1984).

In situ methods have been reviewed by Hart et al. (in press). They can be generally divided into those which are exposed to precipitation inputs (in which case leaching ions are usually captured by ion exchange resins), open but loosely covered, or enclosed in polyethylene bags. These methods vary in the extent to which they follow seasonal changes in soil moisture. All these methods depend on the exclusion of plant roots to prevent plant N uptake, so transpirational losses cannot occur and soil water content in the incubated soil during periods of plant water uptake is unlikely to be the same as soil water content outside the incubation

system. These methods have been fairly widely used in forests with apparent success.

However, it has not yet been possible to devise a method which does not alter the soil environment to some degree, with unpredictable results. Adams et al. (1989) observed that all *in situ* incubation methods developed to date alter the soil environment through

> "(i) the cessation of the carbon input from decomposing litter, and from fine-root turnover; (ii) increased carbon inputs from severed roots; (iii) modification of the moisture and temperature regimes relative to bulk soil; and (iv) accumulation of inorganic-N."

Because of the wide range in the ability of soils to mineralize N, which is partially an effect of recent organic matter additions, and partially due to inherent soil characteristics, it is unlikely that soils will respond the same to perturbations such as changes in temperature and moisture contents, and changes in the rate or kind of organic matter addition. Further research is needed to develop reliable and widely applicable N mineralization assays.

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CHAPTER 2

DENITRIFICATION LOSSES

ABSTRACT

Dairy farms generate large amounts of manure that must be disposed of periodically. Most of this manure is applied to croplands as a source of plant nutrients. Monitoring the fate of applied N is important because N applied in excess of crop needs may be lost by leaching into groundwater or to the atmosphere by volatilization or denitrification, with potentially harmful effects. Nitrate contamination of groundwater is hazardous to human and animal health and nitrous oxide (N_2O) produced during denitrification is a radiatively active gas, which is of increasing importance in global warming. Volatilized NH_3 contributes to acid precipitation after it is oxidized to NO_2^- or NO_3^-. and to eutrophication when it dissolves in rainwater and is deposited as NH_4^+.

Nitrogen loss by denitrification was measured as one component of a N budget for perennial grass pastures. Three soil types with a range of drainage classes were chosen to represent soils commonly used for pasture in the Pacific northwest. Plots received 0 to 450 kg manure-N ha^{-1} y^{-1} in five to seven split applications. Denitrification and respiration rates, soil moisture content, and soil temperature were measured.

Annual denitrification losses were affected by manure applications and soil type. Denitrification rates increased with increasing manure application rates. The

highest rates occurred in the well-drained Quillamook soil. The lowest rates were in the poorly-drained Waldo soil, probably because of limited availability of NO_3^-. Losses ranged from 5 to 16% of applied N. These results indicate that denitrification can be a significant pathway for N loss from manured pastures.

INTRODUCTION

Animal manures are a valuable source of plant nutrients, in particular N. Manure can replace or supplement inorganic fertilizers in cropping systems, however effective use of the manure N by farmers is limited by several factors. In modern, specialized farming operations, production of manure is concentrated in dairy and feedlot operations. Hauling costs for manure are high relative to inorganic fertilizer sources of N. Additionally, the timing of N availability is critical to optimum crop yields. Nitrogen in manure exists in organic and inorganic forms, and the exact timing of N mineralization from organic forms is dependent on a number of factors and is difficult to predict.

For these reasons, most manure produced by dairy farmers is applied where it is produced, typically on land used for pasture, hay or silage crops. The high cost of farmland and economic pressure to increase dairy herd sizes, with concommitant increases in the volume of manure produced, has resulted in the possibility of excessively high rates of manure being applied to land.

Nitrogen applied in excess of plant uptake can become a hazard in the environment (Keeney, 1982). Nitrate not used by plants can leach into groundwater and localized high levels of NO_3^- have been found in groundwater in Oregon (Pettit and Thomas, 1986). Denitrification is an alternate fate for unused NO_3^-. In this microbial process, NO_3^- is converted to N_2 gas and a small and variable portion of N_2O and NO (Firestone, 1982). Nitrous oxide as a radiatively active gas is directly involved in greenhouse warming. NO is directly involved in ozone destruction and indirectly in global warming.

This study is part of a larger effort to develop a N budget for grass pastures receiving dairy manure. The objectives of this portion of the study were to measure denitrification losses from dairy manure surface applied to grass pastures in three soil types and relate those losses to soil variables.

MATERIALS AND METHODS

Soil and Site description

Plots were established in the fall of 1989 on mixed perennial ryegrass (*Lolium perenne)* and orchardgrass *(Dactylis glomerata*) pastures in three soil types representing a range of drainage classes. The Amity silt loam (fine, mixed, mesic Argiaquic Xeric Argialboll) and Waldo silty clay loam (fine, mixed, mesic Fluvaquentic Haplaquoll) are in the Willamette Valley of western Oregon (44.30° N, 123.20°W). The Willamette Valley has a Mediterranean climate of hot, dry summers and cool, moist winters. The Quillamook silt loam (medial, isomesic Alic

Pachic Melandudand) is in Tillamook County, on the Pacific coast of Oregon (45.50°N, 123.90°W), in a maritime climate (Table 2.1).

Experimental Design

The experimental design was a randomized complete block. Manure treatments were completely randomized within each soil. Three replicate plots were used in the Amity and Quillamook site. The Waldo site has two replicate plots for manure treatments and four replicates for the control (no manure) treatment.

Manure Applications

Fresh dairy manure containing a mixture of urine and feces was used (1.6 g organic N kg^{-1} manure, 1.1 g NH_4^+-N kg^{-1} manure, J.A. Moore, personal communication) was applied at 0, 150, 300, and 450 kg manure-N ha^{-1} y^{-1} for two growing seasons. In order to maximize plant N uptake manure applications were split into five to seven parts (Table 2.2). After each application, regrowth of the plots was monitored. When the grass reached 20 to 25 cm, the plots were harvested, grass removed, and manure was applied again.

Denitrification measurements.

At each sampling date, three soil cores were taken from randomly selected points in each plot and soil temperatures at 10 cm were recorded. Denitrification rates were measured on intact soil cores by the acetylene (C_2H_2) inhibition

technique. With minor variations, the protocol described by Tiedje et al. (1989) was followed. Soil cores were taken with a steel corer, which extracts a relatively intact soil core about 15 cm long and 2 cm in diameter, encased in a 20-cm-long polyacrylic tube. To facilitate diffusion of C_2H_2 throughout the soil, the tube is slightly larger in diameter than the soil core. The tube was stoppered at both ends and 6 mL of air was added to the tube. The headspace was mixed with a 30-mL syringe. A 5.5-mL gas sample was put into a nominal 3-mL draw vacutainer (Becton-Dickson, Rutherford, NJ) for later analysis. Six mL of C_2H_2, producing a partial pressure of at least 100 kPa, was injected into the tube and the headspace was mixed again. Acetylene was made in the field by mixing water with calcium carbide in a sealed serum bottle. Cores were incubated at ambient soil temperature for 24 hours. At the end of the incubation, the tube headspace was mixed and a final 5.5-mL gas sample was withdrawn from the tube and put into a vacutainer. A sample of the soil was dried at 105°C to determine gravimetric water content.

Gas samples were analyzed by gas chromatography (Varian 3700 equipped with a ^{63}Ni electron capture detector) using a Porapak Q column. The carrier gas was 95% argon and 5% methane at a flow rate of 40 mL min^{-1}. The column was maintained at 35°C. The electron capture detector was operated at 350°C. Denitrification and respiration rates were calculated by multiplying the increase in concentration of N_2O and CO_2 in the headspace over the incubation period by the total void volume of the tube. Dissolved N_2O was calculated from the Ostwald coefficient (Wilhelm et al., 1977). Total void volume in the tube was calculated by

subtracting the volume occupied by water and the volume occupied by soil from the total volume of the tube.

Annual losses were calculated from the area under the curve defined by rate measurements for each replicate plot.

Statistical Analysis.

Treatment effects within a sampling date for each soil were determined on log transformed denitrification and respiration rates by standard analysis of variance (ANOVA) using SAS statistical software package (SAS Institute, Cary, NC) for a completely randomized design with three replicates and three subsamples (four or two replicates and four or six subsamples in the Waldo soil) and Tukey's HSD procedure to compare means at the p=0.10 level. Significance of treatment effects for annual loss were determined by ANOVA using the annual loss calculated for each replicate plot.

Statistical significance for soil NO_3^- concentrations was determined by F-protected east significant difference (FPLSD).

RESULTS AND DISCUSSION

Seasonal Patterns in Denitrification Rates

The two Willamette Valley sites are on different soil series but are located within 500 m of each other. They had similar temporal patterns of denitrification (Fig. 2.1a and 2.1b). In the Amity and Waldo soils, 77 and 83% of the annual loss

occurred in the four months from mid-October to mid-February. There were two peaks in denitrification rates: following the first fall rains and following a five-day freeze.

The Quillamook site is about 150 km northwest of the Willamette Valley sites. Denitrification rates peaked a month later there and only one peak was observed, however at this site a four-month time period (late October to late February) accounted for a similar portion of the annual loss (73%).

Sources of variability for denitrification rates

Because the factors that drive denitrification (denitrifier populations, soil C availability, restricted aeration, and availability of NO_3^-) are strongly affected by climatic events such as rainfall, freeze/thaw cycles, etc., denitrification rates are highly variable in time and can change by an order of magnitude in a week (Ball and Ryden, 1984). Denitrification rates also respond rapidly to perturbations such as fertilization with NO_3^- (Jarvis et al., 1991), irrigation (Rolston et al., 1982) or manure applications (Thompson et al., 1987). Denitrification rates increased within a day following injection of fermentation residues (Rice et al., 1988).

In this study, rapid changes in denitrification rates followed two climatic events: the first fall rains and a winter freeze/thaw cycle. For example, at the Willamette valley sites, rates increased 10 to 100 times (depending on manure treatment) in the two weeks following the first significant rainfall of the fall. During this time the respiration rate doubled (Fig 2.1a and 2.1b). Wetting of dry

soil (Patten et al., 1980) has been shown to stimulate denitrification rates, most likely by increasing the availability of soil C to denitrifying microorganisms. In both Willamette Valley sites, there was an approximately three-fold increase in denitrification following a freeze/thaw cycle. Freeze/thaw cycles have been shown to stimulate denitrification rates (Edwards and Kilham, 1986). Thompson (1989) suggests that high denitrification rates following thawing of frozen soil may be due to enhanced breakdown rates of organic matter. In the Amity soil, respiration rates increased following the thaw, but in the Waldo soil they decreased. Decreased heterotrophic respiration in the Waldo may be attributed to near saturated conditions.

Treatment effects on denitrification rates were significant only when rates were high. For the Waldo soil, this was the period from late October to early January. For the Quillamook, the June sampling date, and the four sampling dates from late October to early December, had significant treatment effects. In the Amity soil, spatial variability was higher, probably because of damage to the pasture by gophers. This obscured separation of manure rate effects, but the manured plots, considered as a group, had significantly ($p \leq 0.10$) higher denitrification rates than the control plots on five dates (Fig. 2.1b).

Manure had no effect on soil respiration rates. The average respiration rates were 18 for the Amity, 32 for the Waldo, and 28 mg CO_2-C g^{-1} soil d^{-1} for the Quillamook soil.

Soil temperature at 10-cm depth varied between a low of 6°C in December and a high of 16°C in June in the Willamette Valley sites. The mean soil temperature over the active denitrification period was 9°C. The Quillamook soil was slightly warmer, reaching a low of 10°C in the winter and a high of 20°C in July. Soil temperature was not included in the correlation analysis because the data set is incomplete and might yield biased results since not all sampling dates would be represented. Myrold (1988) found a weak negative correlation between denitrification rate and soil temperature. This is counter-intuitive, because one would expect microbial activity to be higher in warmer soils. This relationship is probably a result of a negative correlation between soil temperature and soil water content and a positive relationship between microbial activity and soil water content. In the Mediterranean climate of western Oregon, most rainfall occurs in the fall, winter, and spring, when soils are cooler.

Soil inorganic N content was measured four times (Fig 2.2a, b, and c). In the Willamette Valley sites, soil NO_3^- was always less than 4 mg NO_3^--N kg^{-1} soil. In the Quillamook soil, NO_3^- reached a high of 29 mg NO_3^--N kg^{-1} soil in the fall and manure treatment effects on soil NO_3^- concentration were significant ($p \leq 0.05$). These NO_3^- concentrations are below the 5 mg NO_3^--N kg^{-1} soil which Ryden (1986) suggests as a minimum requirement for significant denitrification to occur. However it must be noted that these values represent only bulk soil concentrations and that microsite concentrations could be much higher.

Spatial variability of denitrification rates was high. The distribution was positively skewed and resembled a log-normal distribution. The coefficient of variation (CV) was generally between 100 and 200% (before log transformation). The CV is a normalized measure of variability defined as the sample standard deviation divided by the sample mean. This variability was comparable to the variability commonly encountered in studies of denitrification. A highly skewed distribution can be produced under a multiplicative model, where the effect (denitrification rate) is the product, rather than the sum, of several other variables. A stochastic, multiplicative model using as variables respiration rates and denitrification enzyme activity was able to successfully model measured denitrification rates (Parkin and Robinson, 1989).

Although denitrification requires NO_3^-, low O_2 tension, and denitrifying organisms in addition to a source of available C, many investigators have concluded that the availability of C is the primary factor driving denitrification rates (Groffman and Tiedje, 1991). One rationale behind this is the observation that very high rates of O_2 consumption can create anaerobic conditions even where the diffusion of O_2 is not impeded, and even in fully aerobic atmospheres. Parkin (1987), dissected several soil cores in an attempt to localize the denitrifying activity. In the most extreme case an 80 mg leaf fragment was identified which had a specific denitrification rate (under fully aerobic conditions) more than 20,000 times the median denitrification rate of the bulk soil. He calculated that a water film on the decaying leaf, combined with a very high rate of oxygen consumption,

could create an anaerobic condition at the leaf surface. In light of these findings, factors that increase the spatial variability of organic C might be expected to increase the variability of denitrification rates. Goulding and Webster (1989) did find that applications of farm yard manure as contrasted with inorganic fertilizers increased both the magnitude and the variability of denitrification losses. Thompson (1989) found that with N held constant, denitrification decreased five times when the applied cattle slurry was diluted by one-half.

In other investigations into the source of variability in denitrification, Parkin et al. (1987) measured denitrification rates and phase I denitrification enzyme assays (PDA) on a large number of soil samples. Coefficients of variation for the denitrification rate measurements varied between 200 and 300. In this assay, a C source and NO_3^- are supplied in excess and only the distribution and activity of denitrifying organisms is measured. Even with two sources of variability removed, the CV was still around 40%.

In light of the complexity of the factors driving denitrification, it is no surprise that attempts to correlate measured variables with denitrification rate have met with only limited success. Correlation analysis by Burton and Beauchamp (1986) of 13 variables on three dates against denitrification rates detected only five significant relationships and no variable explained more than 35% of the denitrification rate variability. Myrold (1988) correlated denitrification with three variables (soil water content, respiration, and temperature). No single variable explained more than 29% of the variability, but a multiple regression including all

variables was able to explain 43% of the variability. Elliot et al. (1991) measured the denitrification rates of bulk soil and earthworm castings, along with respiration rate, moisture content, and inorganic N content, under a range of fertilizer and drainage regimes. For bulk soil, no correlations were significant. However for the earthworm castings, NH_4^+, moisture content and NO_3^- explained 25, 25, and 36% of the variability, respectively.

Parsons et al. (1991), measured six variables and incorporated them into a multiple regression with denitrification rates as the dependent variable. By using mean values by sampling date they were able to improve the r^2 of the multiple regression from 0.27 to 0.74. In this experiment, using mean values by date (n=13) improved correlations (Table 2.3). However, more variables were significantly correlated when spatial variability was incorporated by using individual data points ($n \approx 400$). Soil water content was the best predictor of denitrification in the Willamette valley soils. In contrast, respiration was best in the Quillamook soil. For each soil, correlations were further strengthened by restricting the dates used to the periods of most active denitrification (Table 2.3). This suggests that the measured variables are not sufficient to predict which soil conditions will produce high or low denitrification rates. They are better able to predict relative denitrification rate when soils are actively denitrifying.

Annual Losses

Manure applications increased denitrification losses in all soils (Table 2.4) however kinetics of the response to increasing manure rate varied. In the Quillamook soil, annual denitrification losses increased linearly with increasing manure rates but in the Willamette Valley sites, denitrification response was hyperbolic, approaching saturation at 300 kg manure-N $ha^{-1}y^{-1}$. These kinetics suggest that some factor related to the availability of one of the critical components (C, NO_3^-, or anaerobiosis) is limiting the denitrification rate in the Willamette Valley soils.

Because these three soils differ in both drainage class and climate, separating the contribution of climate and drainage to denitrification losses is not possible. However, some observations can be made. Past comparisons of the effects of soil drainage on denitrification losses have given contradictory results. Installation of mole drains in a poorly drained clay soil receiving NO_3^- fertilizer decreased denitrification losses by almost one-half (Colbourn and Harper, 1987). But a well-drained loam receiving cattle slurry had losses five times greater than a poorly drained silty clay loam (Thompson and Pain, 1989). These differences are probably because of the necessity for mineralization and nitrification of organic N before the N is available for denitrification. In a study directly comparing NO_3^- fertilizer with cattle slurry, imperfectly drained soils receiving NO_3^- directly had much greater denitrification losses than soils receiving the same amount of N as cattle slurry (Egginton and Smith, 1986). Where an organic N source is involved,

either the mineralization or the nitrification step may be rate limiting. The restricted aeration of the poorly drained Waldo soil, although providing the anaerobic volume necessary to denitrification, could also slow both mineralization of applied manure and nitrification of NH_4^+.

There is indirect evidence for slower mineralization of manure N in the Waldo soil. Plant N uptake was highly correlated with denitrification loss in the Waldo soil (R^2=0.89), but not in any other soil. This suggests that both denitrification and plant N uptake may have been N-limited in the Waldo soil because well-established grass swards are effective N scavengers. Also, the NO_3^- concentration of the Waldo soil was always the lowest of the three soils (Fig. 2.2a, b, and c).

Although there was a tendency towards increasing plant N uptake with manure treatments, manure treatment effects were not significant in the first year although there were significant differences in yield between soils ($p \leq 0.001$). For annual denitrification losses, however, both soil and manure effects were significant. This implies that manure applications affected denitrification rates in some way other than through N availability, probably through the stimulating effect of the available C in manure on the soil microbial populations.

SUMMARY AND CONCLUSIONS

Denitrification can be a significant route for N loss from fertilized pastures. From 5 to 16% of the applied N was lost through denitrification. In the Willamette

Valley soils, percent loss decreased with increasing fertilization rate, whereas in the Quillamook soil, the percent lost to denitrification increased with increasing manure rate. This indicates that denitrification in less well drained soils under Mediterranean climates cannot be relied upon to remove more than 20 to 30 kg N ha^{-1} y^{-1}, even under high loading rates. The capacity of the Quillamook soil to remove N by denitrification was much higher. The denitrification response was linear even up to the highest rate applied (586 kg manure-N $ha^{-1}y^{-1}$). It is likely that the more moderate climate, increased rainfall of the Pacific coast and good drainage of the Quillamook soil act in concert increase the rate of N cycling, so these results should not be extrapolated to the poorly drained soils also found along the Pacific coast.

Table 2.1. Selected characteristics of the soils.

Soil	MAP†	Drainage class	Organic C	Total N	Bulk density	pH‡
	mm		——g kg^{-1}——		Mg m^{-3}	
Amity	1070	somewhat poor	33.9	2.5	1.2	5.9
Waldo	1070	poor	32.9	2.3	1.2	6.1
Quillamook	2350	well	90.0	7.0	0.7	5.5

† Mean Annual Precipitation
‡ 2:1 H_2O:soil

Table 2.2. Manure application dates.

	Amity	Waldo	Quillamook
1990	10/15/89	10/15/89	03/22/90
	04/02/90	04/02/90	04/26/90
	05/01/90	05/01/90	05/24/90
	06/01/90	06/01/90	06/21/90
	07/05/90	08/02/90	07/30/90
	11/01/90	11/01/90	09/14/90
			11/20/90
Annual total† (kg N ha^{-1})	157	157	195
1991	04/22/91	04/22/91	03/22/91
	05/28/91	05/28/91	05/01/91
	07/10/91	07/10/91	06/12/91
	08/20/91	08/20/91	07/15/91
	10/17/91	10/17/91	08/12/91
			10/09/91
Annual total (kg N ha^{-1})	129	129	170

† Amount applied at the 150 kg N ha^{-1} rate; amounts for the 300 and 450 kg N ha^{-1} treatments are proportional to these actual rates.

Table 2.3. Correlation coefficients (r) for denitrification. Denitrification and respiration rates were log transformed. Values in table were significant at $\alpha = 0.10$.

		Soil		
	Variable	Amity	Waldo	Quillamook
all data	Soil water content	.56	.35	.19
(n=400)	Respiration	.09	.18	.41
mean (by date)	Soil water content	.53	.67	
(n=13)	Respiration			.70
means (active period†)	Soil water content	.75	.77	
	Respiration			.89

† Amity (n=6), Waldo (n=7), Quillamook (n=8).

Table 2.4. Annual denitrification losses by manure treatment. The figure in parentheses is the percent of applied manure N lost, after subtraction of the control. Means followed by the same letter within a column are not significantly different (Tukey's HSD, p=0.10).

	Soil		
Treatment	Waldo†	Amity‡	Quillamook‡
	kg N ha^{-1}		
control	11a	21a	12a
150 kg	24ab (8%)	36a (10%)	38ab (13%)
300 kg	31b (6%)	44a (7%)	58b (12%)
450 kg	33b (5%0	47a (5%)	108c (16%)

† n=4 for control treatment, n=2 for manure treatments
‡ n=3

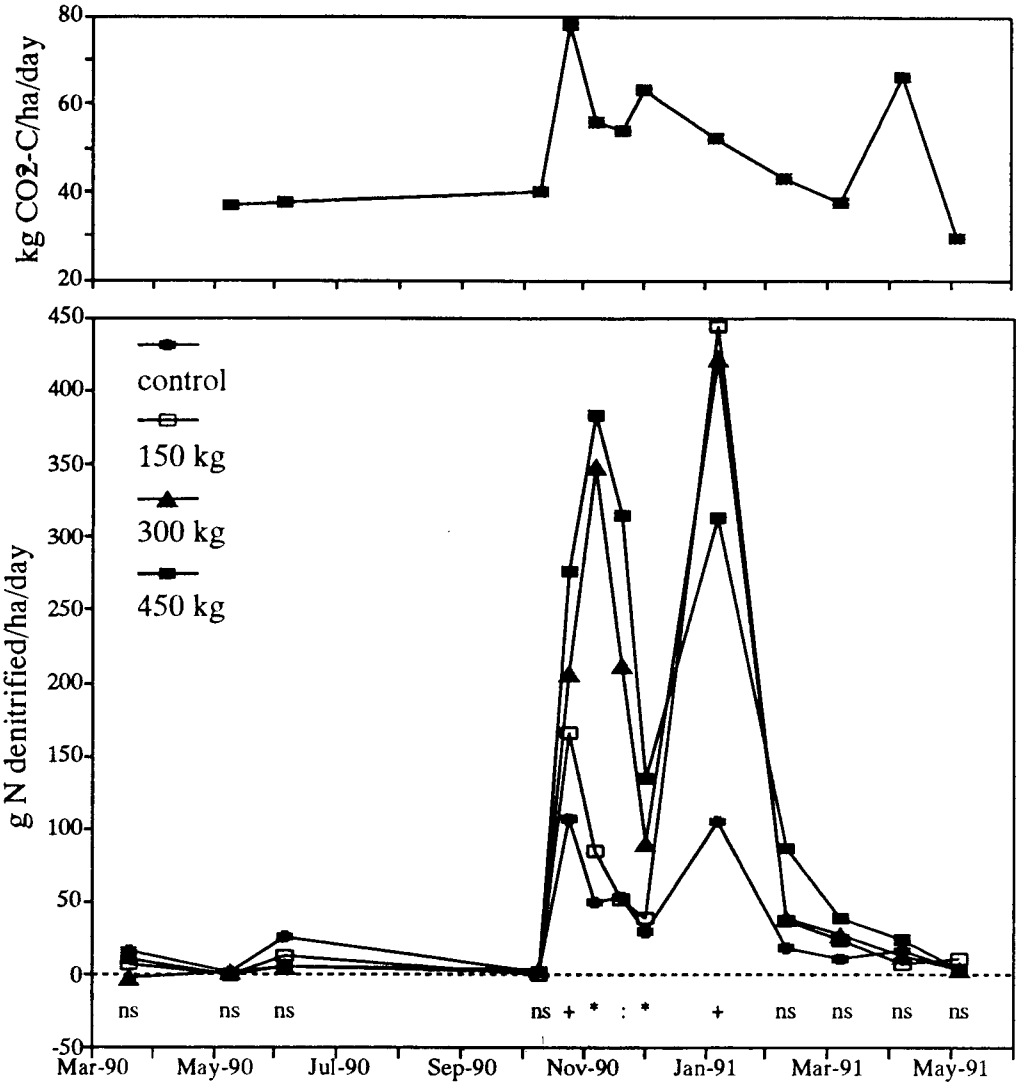

Figure 2.1a. Denitrification and respiration rates in the Waldo soil. Statistical significance is indicated by the following symbols: "+" for $0.05 < p \leq 0.10$,"*" for $0.01 < p \leq 0.05$, and ":" for $p \leq 0.01$. Respiration rates are averaged over all treatments.

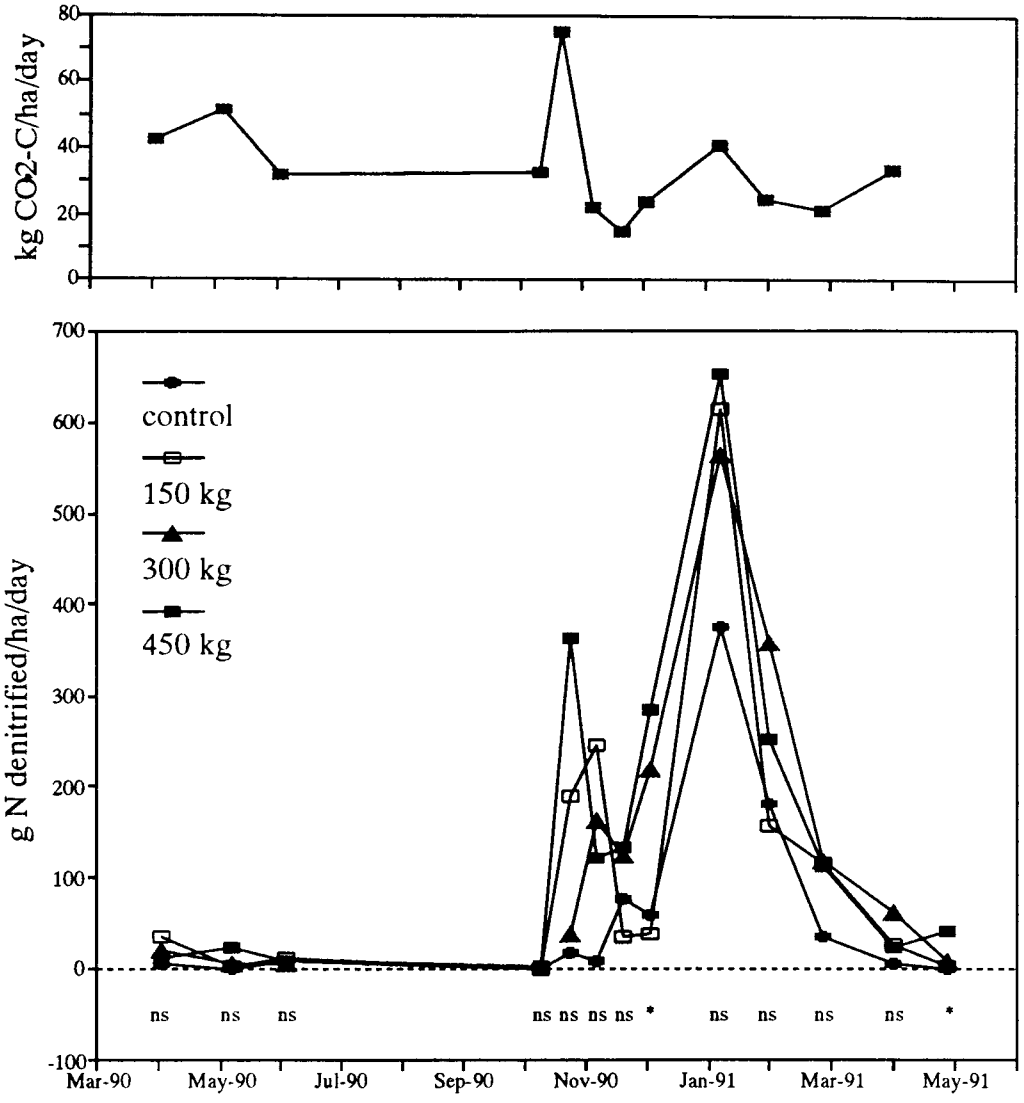

Figure 2.1b. Denitrification and respiration rates in Amity soil. Statistical significance is indicated by the following symbols: "+" for $0.05 < p \leq 0.10$,"*" for $0.01 < p \leq 0.05$, and ":" for $p \leq 0.01$. Respiration rates are averaged over all treatments.

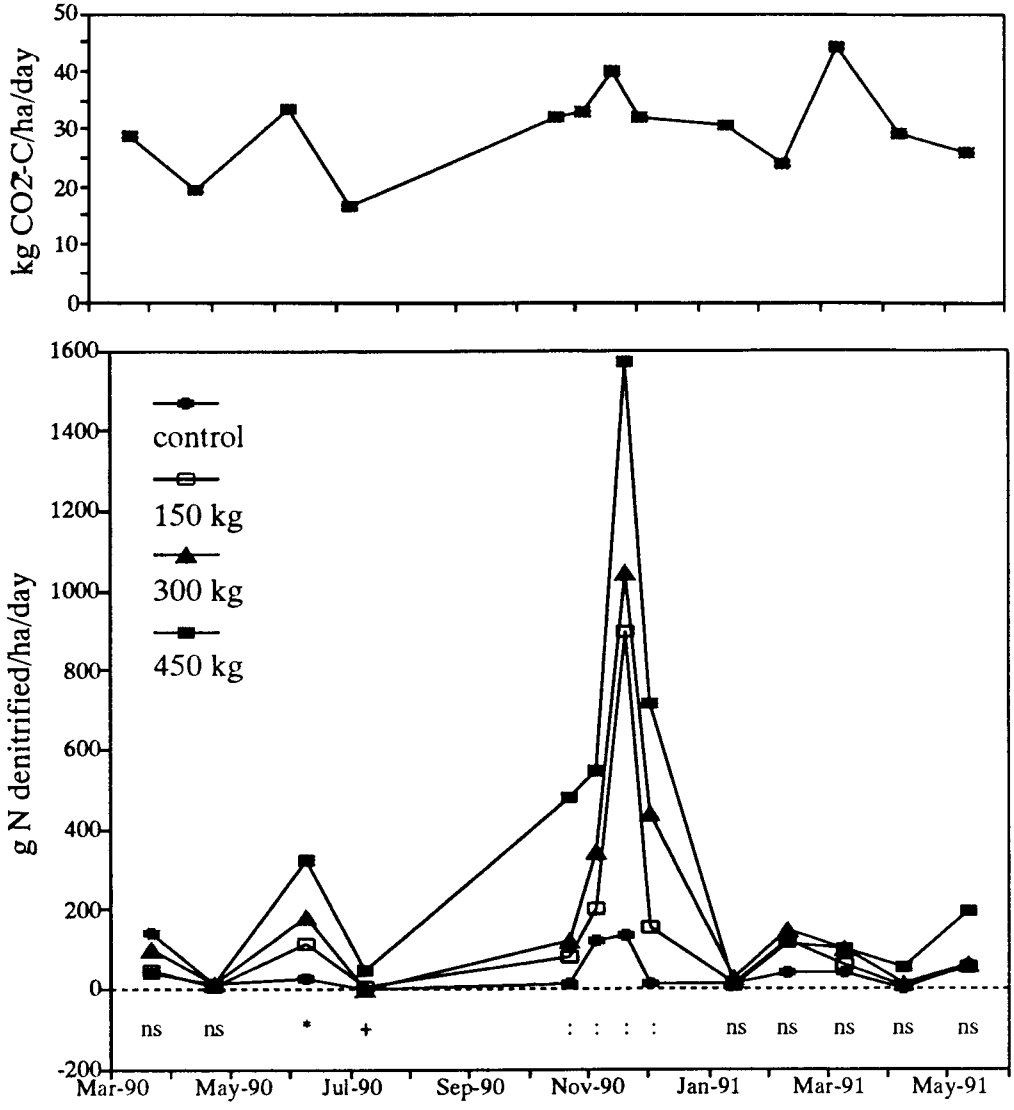

Figure 2.1c. Denitrification and respiration rates in Quillamook soil. Statistical significance is indicated by the following symbols: "+" for $0.05 < p \leq 0.10$,"*" for $0.01 < p \leq 0.05$, and ":" for $p \leq 0.01$. Resp[iration rates are averaged over all treatments.

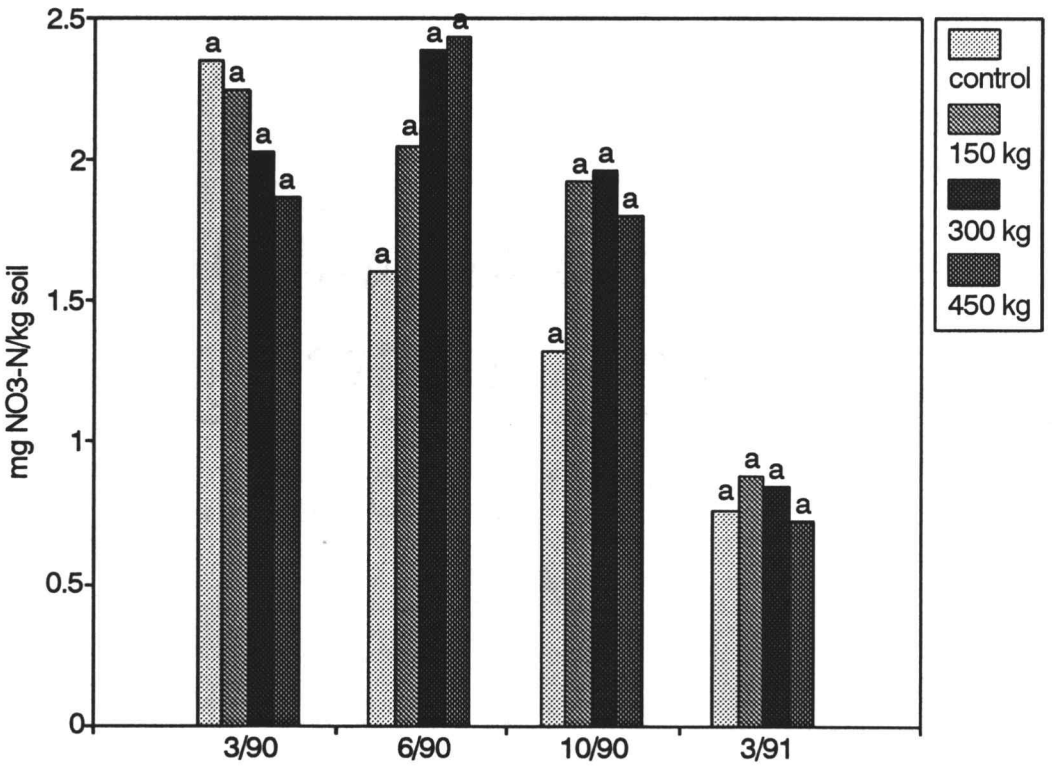

Figure 2.2a. Soil NO_3^- concentration in the Waldo soil. Means followed by the same letter are not significantly different (FPLSD, α = 0.05). However, in the 6/90 and 10/90 samplings the control treatment was significantly lower than the manure treatments (p=0.09 and 0.08 respectively).

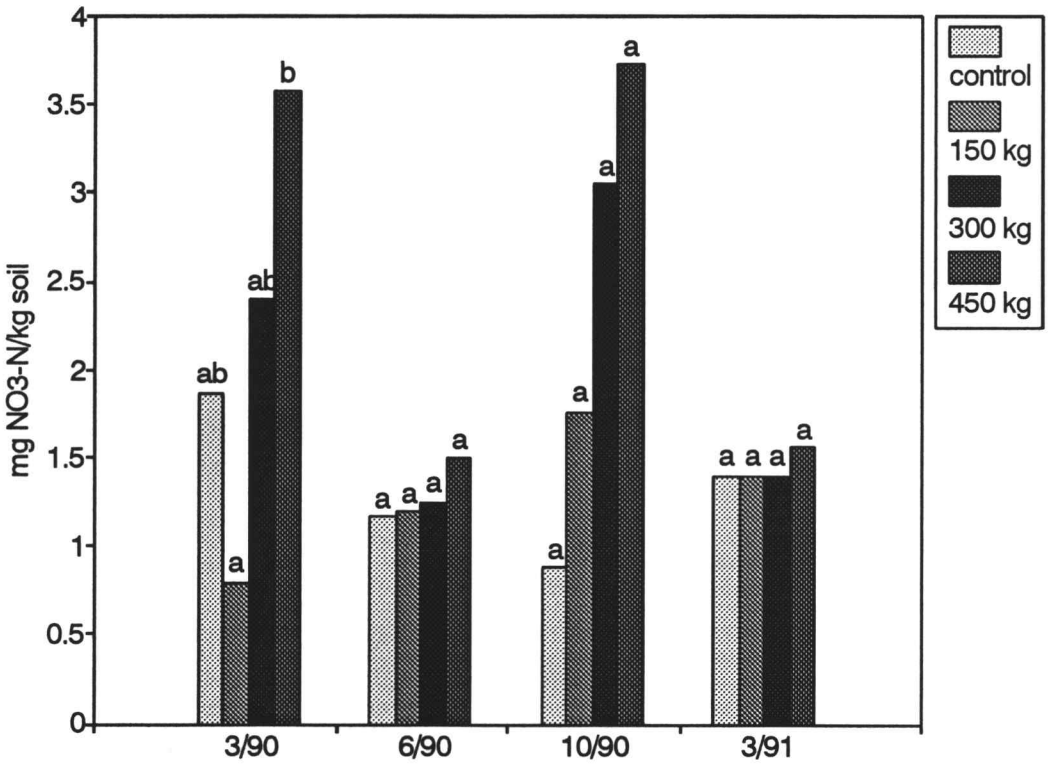

Figure 2.2b. Soil NO_3^- concentration in the Amity soil. Means followed by the same letter are not significantly different (FPLSD, $\alpha = 0.05$).

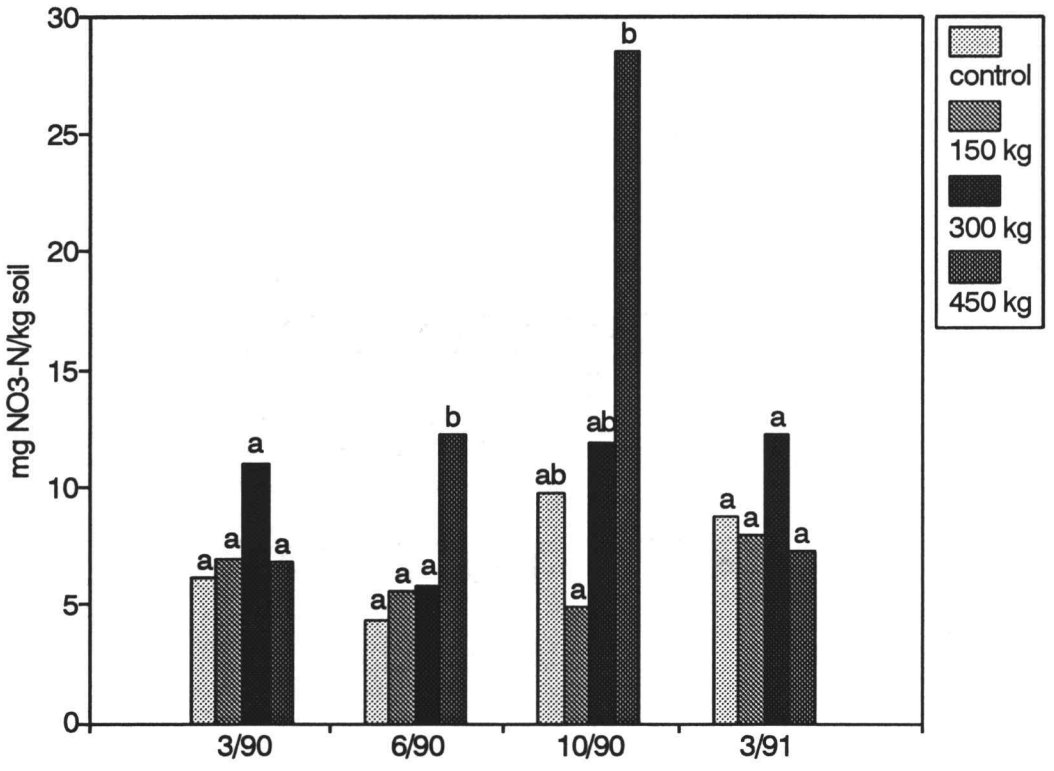

Figure 2.2c. Soil NO_3^- concentration in the Quillamook soil. Means followed by the same letter are not significantly different (FPLSD, $\alpha = 0.05$).

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CHAPTER 3

NITROGEN MINERALIZATION

ABSTRACT

Nitrate contamination of groundwater is a serious problem in some parts of the United States. Nitrogen fertilization in excess of plant demand can result in NO_3^- leaching. Dairy manures are often applied to crops as fertilizer and for disposal. The land-intensive nature of modern dairy farming creates the possibility of manure application rates in excess of what is environmentally safe, and the need to study the dynamics of manure-N.

As part of a N budget study in three soil types with a range of drainage classes, four rates of manure (0 to 450 kg manure-N ha^{-1} y^{-1}) were applied to permanent grass pasture. Nitrogen mineralization was estimated by three methods: plant N uptake, change in soil organic N content, and a soil core-ion exchange resin (core-IER) method. In the core-IER method, PVC tubes (5 cm diameter by 50 cm length) were driven into the ground to extract an intact soil core. Nylon mesh bags of ion exchange resins were placed at the bottom of the core to capture leached NO_3^-. Cores were replaced in the ground and incubated for a three- or six-month period. At the end of the incubation period, inorganic N in soil and resin bags was extracted and net N mineralization was determined.

Net N mineralization by the core-IER method was several times greater than net mineralization by plant uptake. High manure rates tended to have higher net mineralization, however high spatial variability prevented treatment effects from being statistically separable.

The soil core-IER method seemed to enhance net N mineralization. Well-established pastures are known to contain a large reservoir of readily mineralizable organic material and higher water content in the incubated soil could accelerate mineralization of this pool. Measurements of the soil organic N pool gave generally reasonable estimates of net N mineralization, however large confidence intervals make that method less useful for measuring net N mineralization. The plant uptake method had the smallest error and is probably the best measure of net N mineralization.

INTRODUCTION

Nitrate contamination of groundwater is a serious problem in some parts of the United States. Because NO_3^- is highly mobile, when N is supplied to soils in excess of crop needs, the potential for NO_3^- leaching exists. Dairies produce large amounts of manure, most of which is applied *in situ*. To protect groundwater quality, appropriate manure application rates must be determined. The results of this study and a separately funded study will be combined to construct a complete N budget. Manure application rates range from an unfertilized control to 450 kg N ha^{-1} y^{-1}, which is expected to be in excess of plant demand. The sites were chosen

to represent soils commonly used for dairy production. Two sites are in the Willamette Valley of western Oregon, and one site is in coastal Tillamook county, an area with a high density of dairies. Plant uptake, NH_3 volatilization, denitrification, leaching, surface runoff and soil organic N content were measured. Results of plant uptake (Table A1), soil organic N measurements, and an N mineralization assay will be reported here. Plant uptake, NH_3 volatilization, leaching losses and surface runoff have been independently reported (Moore et al., 1992.). Denitrification results are reported in Chapter 2.

Net N mineralization was measured in three ways: the core-IER method, plant uptake, and change in soil organic N. The three methods were compared and reasons for any differences were discussed. Seasonal effects on net N mineralization were discussed and an estimate of leaching losses was made.

MATERIALS AND METHODS

Experimental Design

The experimental design was a randomized complete block. Treatments were completely randomized within each soil. Three replicate plots with four subsamples were used at the Amity and Quillamook site. The Waldo site has two replicate plots for manure treatments and four replicate plots for the control (no manure) treatment. Six subsamples were used in the manure treatments and four in the control plots.

Soil and Site description

Plots were established on mixed perennial ryegrass (*Lolium perenne)* and orchardgrass *(Dactylis glomerata*) pastures in three soil types representing a range of drainage classes (Table 2.1). The Amity silt loam (fine, mixed, mesic Argiaquic Xeric Argialboll) and Waldo silty clay loam (fine, mixed, mesic Fluvaquentic Haplaquoll) are in the Willamette Valley of western Oregon. The Willamette Valley has a Mediterranean climate of hot, dry summers and cool, moist winters. The Quillamook silt loam (medial, isomesic Alic Pachic Melandudand) is in Tillamook County, on the Pacific coast of Oregon, in a maritime climate.

Manure Applications

Fresh dairy manure containing a mixture of urine and feces was used (1.6 g organic N kg^{-1} manure, 1.1 g NH_4^+-N kg^{-1} manure, J.A. Moore, personal communication) was applied at 0, 150, 300, and 450 kg manure-N ha^{-1} y^{-1} for two consecutive growing seasons (Table 2.2). In order to maximize plant N uptake, manure applications were split into five to seven parts. Manure was applied to the main plots with a modified fertilizer spreader. After each application, re-growth of the plots was monitored. When the grass reached 20 to 25 cm, the plots were harvested, grass removed, and manure was applied.

Soil core-ion exchange resin (core-IER) method.

For each of three seasons (spring, summer, and fall/winter), a set of PVC tubes (5 cm in diameter and 50 cm long, 25 cm long for the fall/winter set) were installed at random locations in the manure treated plots described above. A metal cap was placed on the tubes and a sledgehammer was used to drive the sharpened tubes into the ground. Compaction was minimal on most tubes. The tubes were removed from the ground, extracting a soil core containing about 1 kg soil. Approximately 2 cm of soil was removed from the bottom of the soil core so that a nylon mesh bag containing 10 g (dry weight) mixed bed ion exchange resin (Rexyn 300, Fisher Scientific) could be placed there. The resin bag was held in place by a piece of cheesecloth or fiberglass screening while the tube was reinserted into its hole.

Manure applications (after the first manure application) were made individually to the soil cores, at first with a modified syringe using diluted manure and later with frozen manure pellets, at or close to the same time as applications were made to the whole plots.

At the end of the incubation period, the soil cores and resin bags were removed from the ground. The resin bags were extracted in 100 mL of 2N KCl solution. Soil from the tube was mixed and a subsample was extracted in 2N KCl. Extracts were colorimetrically analyzed for NH_4^+ by the salicylate/nitroprusside method and NO_3^- by Cd reduction and diazotization (Alpkem, Clackamas, OR).

At the beginning and end of each incubation period, bulk soil was sampled to the appropriate depth (50 or 25 cm) and the NH_4^+ and NO_3^- content determined.

Modifications and improvements.

The method was modified after the observation was made at the end of the first (spring) set that annual grasses had invaded some tubes and in others, captured ryegrass crowns were growing. In the second (summer) set, living crowns were uprooted and replaced in the tube. That was moderately effective at controlling grass regrowth, but not completely. In the third (fall/winter) set, Simazine, a residual herbicide, was used. This was effective; no living vegetation was observed in the tubes.

Additional problems were encountered with the fall/winter set. Since the spring and summer sets were installed into moderately moist soils, it was possible to drive them with a cap and sledgehammer to a depth of 50 cm. Some compaction was noted, but it was not judged to be a serious problem. However, the fall/winter set was installed at the end of summer, into hard, dry soils. The 50-cm-long tubes broke while being installed. However, shorter tubes were installable, so 25-cm tubes were used for the fall/winter set.

Measurement of total soil N.

Total Kjeldahl N (TKN) was determined on soil samples taken in March, 1990, prior to beginning manure application and October, 1991, following two

growing seasons of manure applications. Soil was sampled to a depth of 15 cm. Soils were digested for TKN as described by Bremner and Mulvaney (1982). Ammonium from the TKN digests was determined as above. Inorganic N present at the final sampling time was not separately determined and is therefore included in the estimate of organic N.

Statistics

Annual net N mineralization rates were determined by summing the plot means for each of the three seasons together. Treatment effects were determined by standard analysis of variance (ANOVA) using SAS statistical package (SAS Institute, Cary, NC) for a randomized complete block design. Plant uptake data were analyzed by ANOVA.

A paired t-test was used to detect significant changes in soil organic N. If the 95% confidence interval of the difference (final TKN - initial TKN) for each treatment did not include zero, then there was a significant net accumulation or loss of organic N. Analysis of variance on the net accumulation or depletion was also run and 95% confidence intervals were constructed from the ANOVA results.

RESULTS AND DISCUSSION

Net N mineralization

Net N mineralization from soil organic matter (SOM) was estimated by three different methods: the soil core-IER method described above, plant N uptake

in unfertilized control plots, and direct measurement of the soil organic N (SON) pool. The methods did not agree well (Table 3.1). However, the plant uptake and core-IER methods ranked the soils similarly. In all soils, the core-IER estimates were higher than any other method.

Plant N uptake

Plant N uptake in unfertilized plots is often used to measure net N mineralization from soil (Legg and Meisinger, 1982). The amount of N mineralized varies considerably. Perennial ryegrass monocultures removed an average of 46 kg N ha^{-1} y^{-1} over the first three years after establishment (Harkess and Frame, 1986). Rangeley and Newbould (1986) estimated soil derived N as 91 kg N ha^{-1} y^{-1} in the second year of perennial ryegrass. Plant uptake in annual and perennial grasslands in a Mediterranean climate was about 100 kg N ha^{-1} y^{-1} in annual and perennial grasslands (Joffre, 1990). Hatch et al. (1991) measured net N mineralization of 263 kg N ha^{-1} method in unfertilized perennial grass swards.

Estimating N mineralization by plant uptake has the advantage of sampling a much larger volume of soil than incubation methods so consequently spatial variability is lower. However, there are disadvantages as well. Leaching or gaseous losses will not be included, and an increase in belowground (root) biomass N will not be measured. Whitehead et al. (1990) has shown that grass swards can continue to accrete belowground biomass at least to 15 years in age. Also, if plant uptake is limited by a factor other than N, it will not accurately reflect N mineralization.

Among the three soils studied (Waldo, Amity, and Quillamook), there were almost four-fold differences in plant N uptake in the unfertilized control plots (Table 3.1). The effects of manure treatment were not significant in the first year within a soil, however the soils were significantly different from each other ($p \leq 0.0001$). In the second year of treatment, N yields from the control plots dropped, whereas N yields from the manured plots increased. Second year N yields from the control plots were significantly lower ($p \leq 0.01$) than the manured plots in the Waldo and Quillamook, but not the Amity. Although the difference in N yield between the average of the manured plots and control plots was similar for all soils (about 50 kg N), variability in the Amity soil was higher and treatment effects were not significant (Table A1). The increased variability was probably due to gopher damage to the Amity plots. The soil by treatment interaction was not significant in either year, indicating that manure had a similar effect on N yield within each soil. The error associated with plant uptake was much smaller than the error associated with either the core-IER or the SON method, providing more sensitive detection of treatment effects.

Total N method.

Direct measurement of the SON pool by Kjeldahl digestion of soil has been used to measure net N mineralization. However, because of the large size of the total N pool small errors in measurement produce a large uncertainty in the estimated net loss or gain of soil N. Net N mineralization in the control plots was

53, 94, and 25 kg N ha^{-1} y^{-1} for the Waldo, Amity, and Quillamook soils (Table 3.1). These figures are surprisingly close to the N mineralization rates calculated by plant uptake for the Amity and Waldo soils, although the associated error was much higher.

These N mineralization rates are equivalent to 1.3, 2.1, and 0.3 % of the initial SON content (Table 3.2). Again, the Waldo and Amity turnover rates are similar to other estimates for the rate of turnover of organic N in grassland soils. Whitehead (1984) (as discussed in Whitehead, 1986) in a greenhouse study of 21 UK soils, found that from 1.5 to 4% of the total soil N was mineralized over a year. Jenkinson (1990) suggests 2.8 and 2.9% per year as typical for turnover of organic N in pasture and grassland (again in the UK). Differences in the absolute amount of N mineralized and the portion of the total N pool mineralized have been related to the management history of the site, particularly additions of organic materials and tillage (Johnston et al., 1989).

Core-IER method.

Incubation methods involving varying degrees of soil disturbance have been used to measure or predict net N mineralization in agricultural soils (Stanford, 1982). The core-IER method involves *in situ* incubation of an intact (undisturbed) soil core contained in a PVC tube, open at the top and bottom. A nylon mesh bag of mixed bed anionic/cationic resin beads traps ions leaching out the bottom. The method has been successfully applied in both forest and pasture soils.

The core-IER estimates for net N mineralization were much higher than either of the other methods, particularly for the Quillamook soil. Additionally, although a trend towards higher net N mineralization in the manure treatments can be seen (Table 3.3), because of the high spatial variability, the differences were not significant, except in the Waldo soil, where net N mineralization in the control plot was lower than the manured plots ($p \leq 0.05$).

Possible sources of bias in the core-IER method.

Where both plant uptake and an incubation method have been used simultaneously, agreement has varied from good to poor. The reason for the variability of agreement is presumably the effect of incubation conditions and amount and C/N ratio of mineralizable organic material in the soil cores on the opposing processes of N mineralization and immobilization.

Some investigators have found good agreement between incubation and plant uptake methods (Joffre, 1990; Hatch, 1991), however the methods do not always agree. For example, Rees (1989) in a study using ^{15}N, measured net N mineralization rates in incubated soil that were less than 11% of plant soil-derived N uptake. He suggested that microbial immobilization of N was in part responsible for the underestimate; increases in microbial biomass in the incubated soil accounted for 56% of the deficit. Hart and Firestone (1988) measured net N mineralization by a buried bag and core-IER methods. The two methods agreed well for a young forest. However, in an old growth forest net N mineralization by

the core-IER method was twice that of the buried bag method. The authors suggest that the NO_3^- adsorbed on the ion exchange resins may be protected against re-immobilization, thus reducing gross immobilization. Although this may have occurred in the current study, this would not explain the overestimation of net N mineralization in this experiment since on an annual basis, most (83, 76, and 73% for the Waldo, Amity, and Quillamook respectively) of the mineralized N was recovered from the soil, not the resin bags (Table 3.4). Only in the fall/winter set was any substantial amount of N leached to the resin bags.

The enhanced moisture status of the soil cores relative to the bulk soil may be a more important source of bias. Net N mineralization is most rapid at a water content close to field capacity, and falls off sharply as the soil approaches saturation (Myers et al., 1982). Linn and Doran (1984) confirmed the many previous reports that maximum microbial activity in soil occurs at approximately 60% of water holding capacity (WHC). (Water holding capacity is defined here as the total pore volume per cm^3 soil, synonymous with total porosity. Total porosity was calculated for each soil from bulk density and particle density.) Because the core-IER method depends on excluding plant roots to prevent plant N uptake, a side effect is that there are no transpirational water losses. Soil water content in the cores increased over the incubation period, even while the bulk soil was drying from evapo-transpirational losses (Fig 3.1a, b, and c). This effect was particularly strong in the spring and summer sets. At the end of these incubations the incubated soil had a water content between 50 and 70% of WHC, while the bulk soil had

water contents from 30 to 50% WHC. At the end of the fall/winter incubation both the bulk and incubated soils were much wetter, and the difference between them was less than five percentage points.

Direct evidence that increased soil water content increases net N mineralization is seen in Fig 3.2. For the fall/winter set for the Waldo soil in the unfertilized control treatment (Fig.3.2), soil water content varied between 68 and 92% WHC and net N mineralization and soil water content were positively correlated ($r=0.54$, $p \leq 0.0001$). A similar, but weaker, pattern was seen in the Quillamook soil ($r=0.36$, $p=0.05$).

There was a positive correlation between soil water content on net N mineralization in other seasons as well. Unfortunately, control of grasses was not as good, so it is possible that unmeasured plant N uptake, as well as transpirational water losses, occurred.

Established pastures have long been known to contain a large amount of organic material which is stable if undisturbed, but labile when the pasture is tilled. This pool increases with age and with utilization by grazing (manure input) rather than cutting. Whitehead (1990) measured the N content of stubble, litter, root, and soil macro-organic matter fractions of 8 and 15-year-old perennial ryegrass swards. The fractions contained, on average, 68, 12, 249, and 240 kg N ha^{-1} for a total of 569 kg N ha^{-1}. He suggested that 364 kg N ha^{-1}, or 64 % of this N is labile or readily mineralizable under disturbance such as tillage. The core-IER method as applied here does not involve a disturbance such as mixing, which would mimic

tillage. However, no roots or intact plant parts were observed in the cores after the three or six-month incubation. It should also be noted that, whatever the size of the potentially mineralizable organic matter fractions in these soils, this material is given three opportunities to contribute to the annual mineralization rate: once for each of three incubation periods.

Although the cores were wetter than the bulk soil, nitrification was not inhibited (Table 3.5). Net nitrification was about 74% over all soils and seasons. Least nitrification occurred in the Waldo soil in the fall/winter set. This is consistent with the seasonal high water table which occurs in this soil.

Soil effects

Net N mineralization by either the core-IER method or by plant N uptake was three to four times greater in the Quillamook than in the Amity or Waldo soils. This difference may be due to two factors. First, the total N content is almost twice that of the Willamette Valley sites. Second, the Quillamook site, because of its close proximity to a heifer barn, has probably received much greater recent additions of manure. In contrast, the Amity and Waldo sites have historically not been intensively managed. Estimated past N fertilization rates are 40 to 50 kg N ha^{-1} y^{-1} for the Waldo and 70 to 80 kg N ha^{-1} y^{-1} for the Amity (M.J. Gamroth, personal communication). They have been infrequently grazed. The different field histories of the sites would have effects on N availability lasting at least several years into the current treatment regime. Thus the treatment effects on N availability

may reflect past management as well as current management of the soil. Also, the spatial variability of almost every measured parameter was higher in the Quillamook site. Pasture utilization by grazing causes uneven distribution of N in manure which would be expected to increase spatial variability.

Estimation of leaching losses

Plant uptake can be considered a minimum estimate for net N mineralization since there will always be some denitrification and leaching losses. Denitrification losses were 10, 20, and 10 kg N ha^{-1} y^{-1} in unfertilized control plots for the Waldo, Amity and Quillamook soils and most of the loss occurred in the winter months (Chapter 2).

The core-IER method was intended to produce an independent estimate of leaching losses by placing an IER bag below the rooting zone and measuring inorganic N which moved below the rooting zone. In the spring and summer sets, although a large amount of N was mineralized, it remained in the soil above the resin bag and little N was leached to the resin bag. In contrast, in the fall/winter sets, most (63 to 85%) of the mineralized N was recovered from the resin bags. The 6 month period (October through March) accounted for 12 and 16% of annual net N mineralization in the Waldo and Amity soils, and 21% in the Quillamook soil (Table 3.2), or 32, 54 and 224 kg N ha^{-1} y^{-1} respectively. If those mineralization rates represent the upper end of net N mineralization, then plant uptake in that period can account for about 50% of mineralized N for the Waldo and Amity soils

and leaching losses in the 0 to 20 kg N ha^{-1} range would be predicted. These compare well with measured losses of about 7 kg N ha^{-1} (Moore et al., 1992) and would probably be considered acceptable losses from the standpoint of groundwater protection.

The status of the Quillamook soil with respect to N mineralization rates and potential leaching losses is somewhat more difficult to discern. Plant uptake can account for only about 13% of the N mineralized in the six-month fall/winter period and leaching losses could be as high as 196 kg N ha^{-1}. Of the 224 kg N ha^{-1} mineralized in the cores during that period, 190 kg N ha^{-1} was recovered from the resin bag (at 20 cm depth) and 34 kg N ha^{-1} was recovered from the soil while plant uptake for that period was 28 kg N ha^{-1}.

CONCLUSIONS

Plant uptake is probably superior to the core-IER incubation method for measurement of net N mineralization, at least in unfertilized well-established swards, where extensive root systems allow relatively little leaching. At high rates of fertilizer, plant uptake will not reflect net N mineralization as well and other methods must be brought into play. More replication in the core-IER method could reduce error and improve detection of treatment effects, and the method could be treated at least as an index of mineralization. More frequent sampling would minimize differences between bulk soil water content and incubated soil water content, and also further reduce variability.

Table 3.1. Measurement of net N mineralization rates in unfertilized controls by three different methods for the 1990 growing season (mean and 95% confidence interval).

	Method		
Soil	core-IER	plant N uptake†	SON‡
	kg N ha^{-1} y^{-1}		
Amity	338 ± 111	118 ± 18	94 ± 102
Waldo	237 ± 88	58 ± 18	53 ± 139
Quillamook	1213 ± 373	196 ± 16	25 ± 284

† Data courtesy of J.A. Moore (Table A1).
‡ Half of the net loss over two growing seasons. The error was also halved to provide an estimated confidence interval.

Table 3.2. Initial (March, 1990) and final (October, 1991) soil organic N.

Soil	Treatment	Total Kjeldahl N		
		Initial	Final	net change †
		kg N ha^{-1}		
Amity	control	4,615	4,327	-288 ± 203
	150 kg N	4,485	4,442	-43 ± 203
	300 kg N	4,342	4,530	188 ± 203
	450 kg N	4,718	4,740	22 ± 203
Waldo	control	4,172	4,066	-105 ± 278
	150 kg N	4,208	4,434	226 ± 393
	300 kg N	4,090	4,236	147 ± 393
	450 kg N	3,912	3,956	44 ± 393
Quillamook	control	7,117	7,068	-49 ± 568
	150 kg N	7,310	7,454	144 ± 568
	300 kg N	7,582	7,597	16 ± 568
	450 kg N	7,417	7,537	324 ± 695
All soils	control	-	-	-143 ± 174
	150 kg N	-	-	94 ± 195
	300 kg N	-	-	113 ± 195
	450 kg N	-	-	114 ± 209

† Mean and 95% confidence interval.

Table 3.3. Net N Mineralization by the core-IER method. Data for seasonal periods are means. Annual estimates are means and 90% confidence intervals.

	Waldo			
	Spring	Summer	Fall/winter	Annual
	kg N ha^{-1}			
Control§	110	142	32	264 ± 95
150 kg N†	73	173	50	296 ± 134
300 kg N†	87	296	42	425 ± 134
450 kg N†	141	265	63	469 ± 134
	Amity			
	Spring	Summer	Fall/winter	Annual
	kg N ha^{-1}			
Control†	194	91	54	338 ± 111
150 kg N‡	87	115	64	252 ± 91
300 kg N‡	185	96	62	352 ± 91
450 kg N‡	200	150	64	414 ± 91
	Quillamook			
	Spring	Summer	Fall/winter	Annual
	kg N ha^{-1}			
Control‡	370	454	224	1048 ± 360
150 kg N‡	310	316	257	923 ± 360
300 kg N‡	246	558	211	1012 ± 360
450 kg N‡	397	738	256	1391 ± 360

† n=2
‡ n=3
§ n=4

Table 3.4. NO_3^- and NH_4^+ collected on the resin bags as a percent of total net mineralized N.

Season	Soil		
	Waldo	Amity	Quillamook
	%		
Spring	5	11	20
Summer	14	23	4
Fall/winter	63	73	85

Table 3.5. Percent of mineralized N nitrified, averaged over all treatments.

Season	Soil		
	Waldo	Amity	Quillamook
	%		
Spring	87	75	71
Summer	70	62	81
Fall/winter	58	71	88

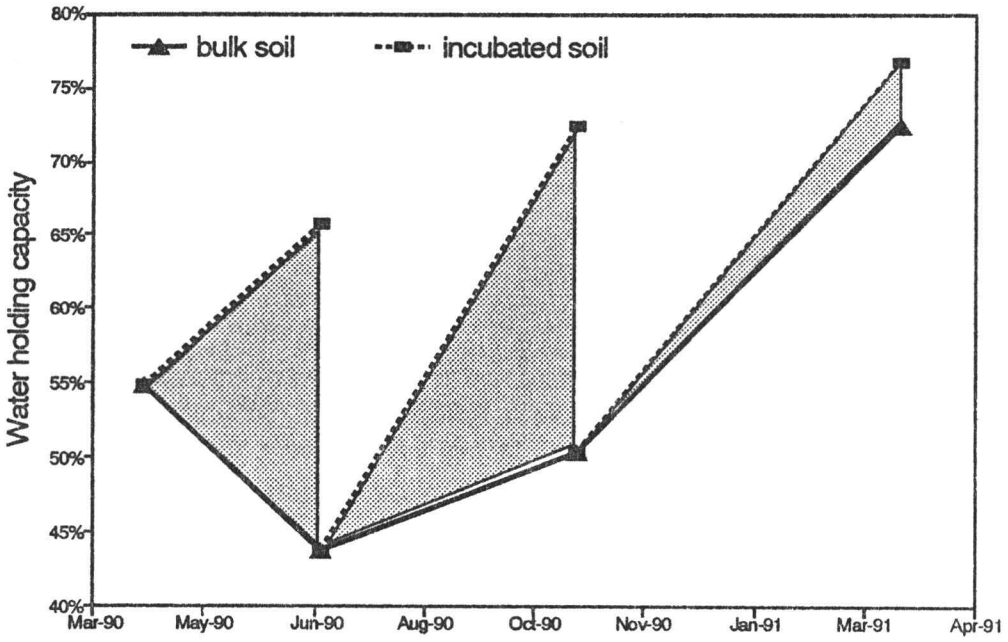

Figure 3.1a. Difference between bulk soil water content and water content of incubated soil for the Waldo soil. Water contents are expressed as percent of water holding capacity.

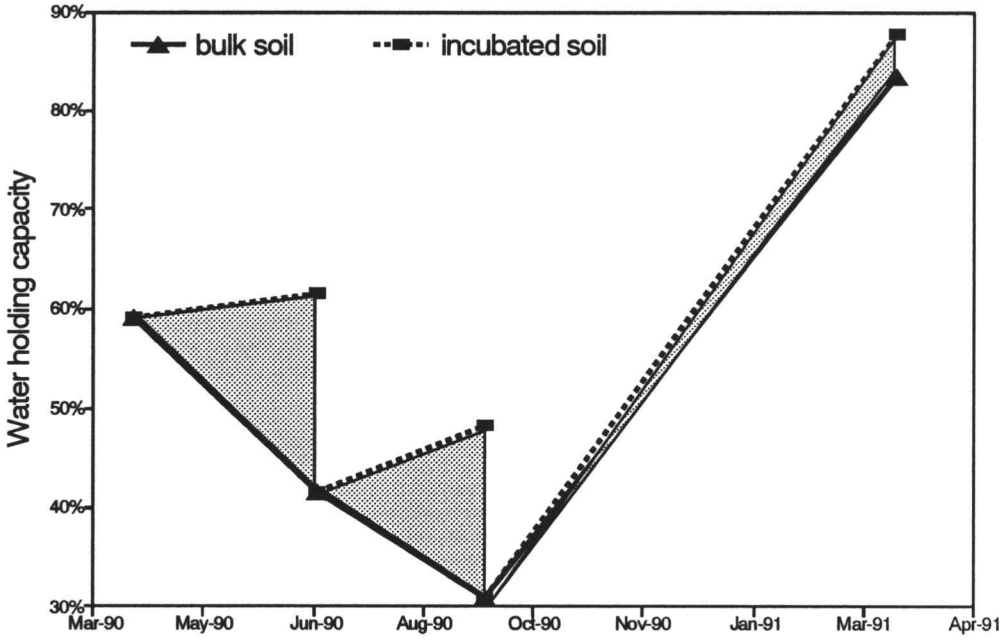

Figure 3.1b. Difference between bulk soil water content and water content of incubated soil for the Amity soil. Water contents are expressed as percent of water holding capacity.

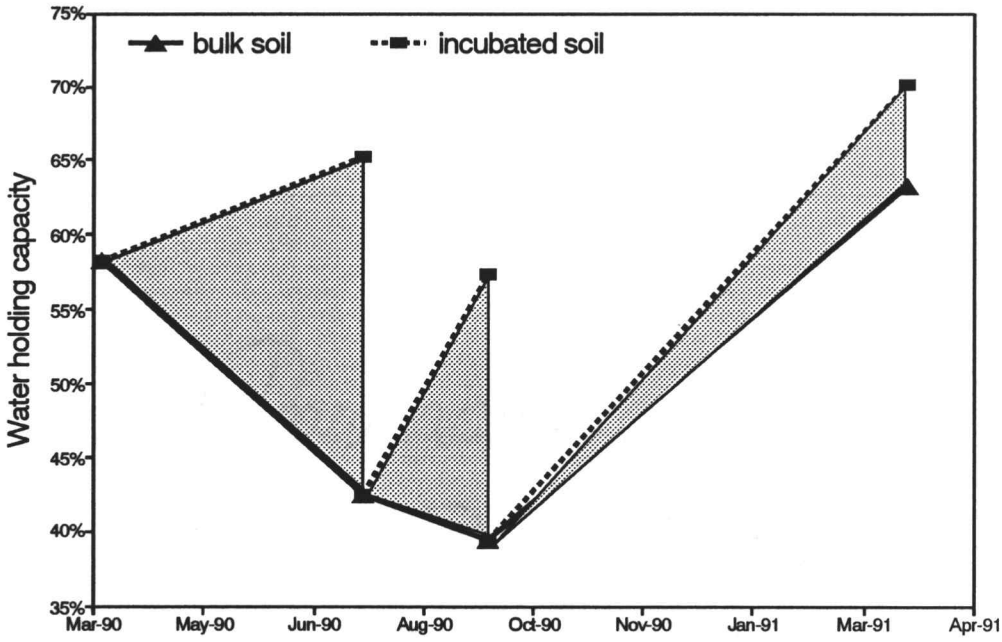

Figure 3.1c. Difference between bulk soil water content and water content of incubated soil for the Quillamook soil. Water contents are expressed as percent of water holding capacity.

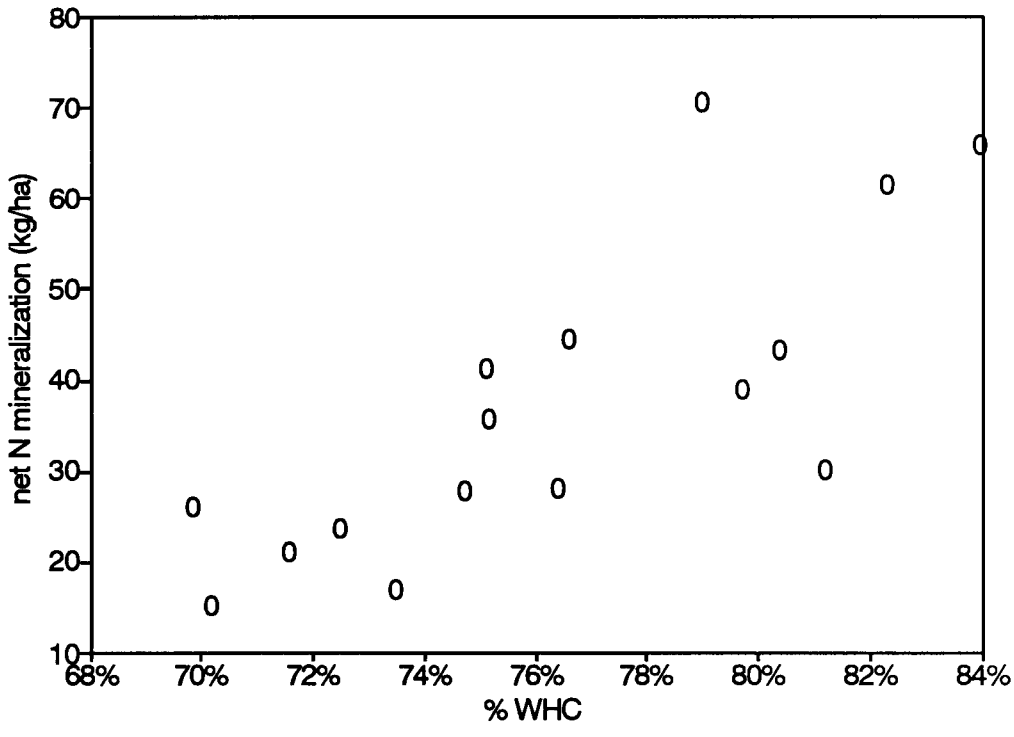

Figure 3.2. Net N mineralization rates and water content expressed as percent of water holding capacity for individual cores (Waldo soil, fall/winter set, control treatment). n=16.

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APPENDIX

Table A1. N in harvested biomass. Mean ± standard deviation; n=3 for Amity and Quillamook, n=2 for Waldo n=4 for control). Data courtesy of J.A. Moore and M.G. Gamroth

Soil	Treatment	Year	
		1990	1991
		——kgN/ha——	
Amity	control	123 ± 8	148 ± 13
	150 kg N	116 ± 4	176 ± 36
	300 kg N	139 ± 15	181 ± 19
	450 kg N	141 ± 13	220 ± 76
Waldo	control	58 ± 15	53 ± 17
	150 kg N	71 ± 18	86 ± 11
	300 kg N	75 ± 10	104 ± 8
	450 kg N	70 ± 9	129 ± 20
Quillamook	control	196 ± 11	176 ± 11
	150 kg N	216 ± 12	206 ± 11
	300 kg N	209 ± 16	246 ± 16
	450 kg N	202 ± 4	239 ± 18