6. INCUBATION TRIALS TO EXAMINE NITRIFICATION IN RELATION TO SOIL ACIDIFICATION

6.1 Introduction

In the paired-site study (Chapter 4) significantly more nitrate was found in surface (0-20 cm) fertilized, improved pasture paddock soils compared with soils from native pasture reserves. Ammonium-N and nitrate-N concentrations in the paddocks were similar over all depths. For the reserve soils, ammonium was proportionally much higher than nitrate particularly in the surface 0-20 cm. These observations suggest that differences in nitrogen cycling occurred between the paddock and reserve areas. Conclusions about the role of nitrification in causing these differences were difficult to draw because nitrate-N or ammonium-N concentrations, measured at a point in time, resulted from interacting processes. In the study of seasonal variation of nitrate and ammonium concentrations at *Newholme* (Chapter 5), a consistent nitrate to ammonium ratio of 0.8 occurred in the surface (0-5 cm) soils. At times, relatively high nitrate-N concentrations (> 20 mg/kg) accumulated, but ammonium-N concentrations never exceeded 10 mg/kg. Controlled laboratory incubation experiments in this chapter were designed to investigate nitrogen mineralization and nitrification in pasture soils of the NSW Northern Tablelands.

In each experiment, soil samples were mixed with acid-washed sand, amended with various rates of ammonium sulfate and incubated over time. At the completion of each incubation, the samples were extracted, and analysed for nitrate-N and ammonium-N. Soil samples used for the incubations were from adjacent reserve and paddock areas of six of the paired sites used in Chapter 4. As for that study, the term *reserve* refers to a roadside reserve area of native pasture. The term *paddock* applies to a fertilized, exotic pasture paddock that was regularly grazed.

6.1.1 Background

A key process in the nitrogen cycle, that has the potential to affect soil acidification, is nitrification by which ammonium, mineralized from legumes and other organic residues, is oxidized to nitrate. Nitrification produces a pool of nitrate, which if leached can cause soil acidification. This pool of nitrate is also available for plant uptake. It is commonly believed that pasture soils of the Northern Tablelands are dominated by ammonium rather than nitrate,

and that this ammonium domination is the result of inhibition of nitrification within the soil. It has also been suggested that ammonium domination of the region's soils preclude nitrate leaching (Chen *et al.* 1999). Alternatively, nitrate concentrations in the soil could be reduced through efficient pasture uptake. Crocker and Holford (1991) concluded that nitrate was recycled because it was taken up by pasture plants before it could be leached. In the Wagga Wagga area of southern NSW, it has been shown that nitrification of ammonium added to surface soil is stratified with depth (Young *et al.* 1995, 2002). This is important because if nitrate, produced near the surface, leaches down the profile before it can be used by plants, then the result could be acidifying at the surface relative to the depth of uptake. Also in southern NSW, nitrogen mineralization gradients through surface soil were found to influence soil pH_{KCl} (Paul *et al.* 2001). In addition, it is well established that nitrification rates are strongly dependant on seasonal changes in soil temperature and moisture (Anderson and Boswell 1964).

These studies suggest that an understanding of the processes of mineralization and nitrification of Northern Tablelands soils would be of assistance in interpreting nitrate and ammonium data from Chapters 4 and 5.

6.1.2 Objectives

- Experiment 1

  To determine the optimum rate of ammonium-N addition to measure the rate of nitrification and to make a preliminary assessment of differences in nitrification between two pasture management systems.

- Experiment 2

  To examine the influence of soil pH_{Ca}, pasture management and soil depth on mineralization and nitrification of Northern Tablelands soils.

- Experiment 3

  To investigate the effects of pasture management, temperature and soil moisture on nitrification for a Northern Tablelands pasture soil.

Three incubation experiments, undertaken to address these objectives, are discussed in this chapter. In the sections that follow, the experimental design, sample preparation, incubation procedure, laboratory analyses, statistical tests, results and summary are given for each experiment. The final part of the chapter draws conclusions from the three experiments.

6.2 Experiment 1: Optimum Incubation Conditions Determined

This experiment measured the rate of nitrification in soil samples amended with different concentrations of ammonium-N. The objective was to determine the optimum level of ammonium amendment for Experiments 2 and 3. Soil samples from both paddock and adjacent reserve areas were amended with six different rates of ammonium sulfate and incubated for between 4 and 28 days at 25°C. Only one soil type was used with samples taken from the 0-10 cm depth.

6.2.1 Site Details

Soil samples were collected from one site (Site 41) that had previously been used in the paired-site survey. The selected site lies 50 km northeast of Armidale. The paddock has a long superphosphate history (on average 125 kg superphosphate per hectare every second year for more than 40 years), an exotic pasture of cocksfoot (*Dactylis glomerata*), ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), and is regularly grazed with beef cattle. Native pasture on the reserve was predominately tussocky poa (*Poa sieberiana*). Soil at the site was classified as a Brown Kandosol and the soil chemical properties are summarized in Table 6.1.

Table 6.1 Soil chemical properties by depth of samples from reserve and paddock management areas Site 41 - Incubation 1

Soil Depth (cm)	pH_{Ca}	pH_W	$EC_{1:5}$ dS/m	Organic C (%)	Bray-P (mg/kg)	KCl-S (mg/kg)	NH_4^+-N (mg/kg)	NO_3^-N (mg/kg)	Total N (%)	ECEC ($cmol_c$/kg)
Reserve										
0-5	4.6	5.6	0.04	2.0	3.0	1.7	3	1.1	0.12	3.6
5-10	4.6	5.6	0.04	1.5	2.5	1.3	2	1.0	0.11	3.0
10-20	4.7	5.8	0.03	1.0	2.5	1.3	4	2.8	0.07	2.7
20-30	4.9	6.4	0.01	0.4	2.5	1.0	1	1.0	0.08	2.1
30-40	5.2	6.8	0.02	0.3	2.5	1.0	1	1.0	0.10	6.2
40-50	5.2	6.4	0.02	0.3	2.5	1.0	1	1.0	0.08	7.5
Paddock										
0-5	4.7	5.6	0.08	2.7	14.0	7.0	7	7.6	0.20	5.3
5-10	4.6	5.3	0.10	1.7	5.0	11.0	4	1.3	0.17	4.0
10-20	4.7	5.6	0.06	1.3	2.5	5.1	3	1.3	0.11	3.5
20-30	4.9	6.1	0.02	0.5	2.5	2.1	1	1.0	0.05	2.1
30-40	5.2	5.9	0.02	0.3	2.5	1.5	1	1.0	0.03	1.9
40-50	4.7	6.0	0.01	0.3	2.5	1.7	1	1.0	0.03	2.1

6.2.2 Soil Sampling Protocol

Four sample sets, each set comprising six soil cores, were collected along transects from both the paddock and reserve area in a spatial arrangement of three cores within each of four transects (Figure 6.1). The samples from each transect within each area, reserve or paddock, were bulked to produce a set with four replicates from each side of the fence. Soil was sampled from the 0-10 cm depth.

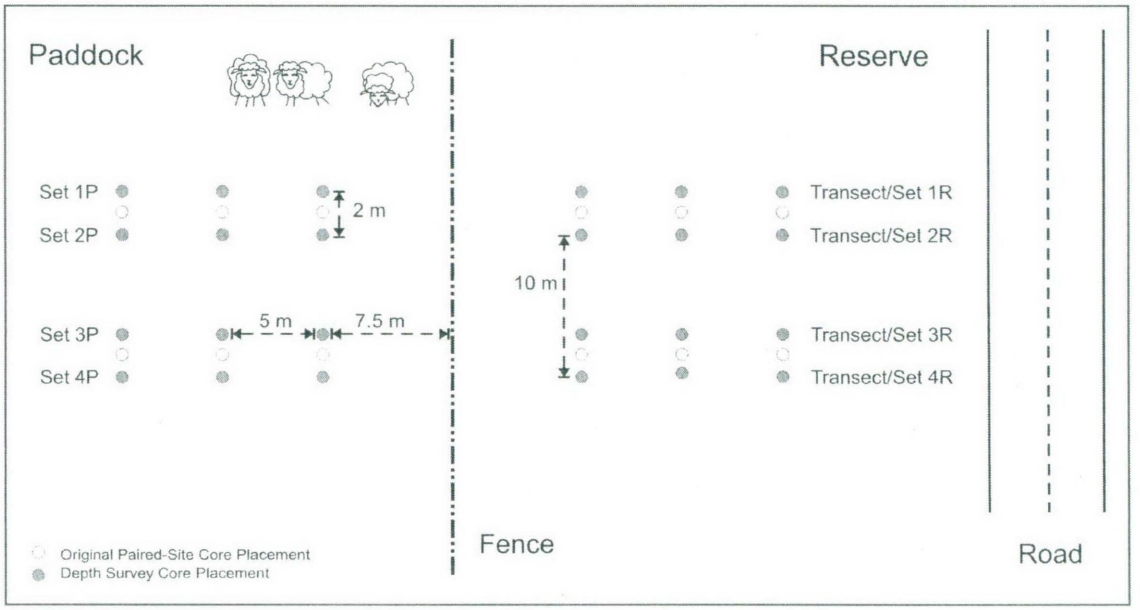

Figure 6.1 Soil core placement for soil sampling - Incubations 1 and 3

6.2.3 Experimental Design

A constant-temperature unit, normally used as a growth chamber for plants, was used for the incubation chamber. The temperature of the unit was set at 25°C, a temperature considered ideal for promoting nitrification. An optimal incubation temperature of 35°C has often been reported, but temperatures between 25°C and 35°C have been used for incubations (Bremner 1965). Nitrification is very slow in temperatures below 4°C and above 40°C (Russell 1950). Six amendment rates comprising deionized water only and 40, 80, 120, 160, 200 mg NH_4-N/kg (Rates-0, 40, 80, 120, 160, 200) were used for each incubation. These levels were selected as having an even distribution either side of 100 mg NH_4-N/kg, a rate often selected for incubation experiments, for example, Young *et al.* (2002). Between concentrations of about 150 and 200 mg NH_4-N / kg, a toxic salt effect has been observed (pers.comm. Mark Conyers 2003). Thus, an upper limit of 200 mg NH_4-N/kg was selected for the experiment to obtain data above the inferred nontoxic range. Two replicates (bulked samples from Transects 1 and 3) were used. Samples from each of the four replicates (Transects 2 and 4

in addition to Transects 1 and 3) were used for the control to provide a sound data baseline for the statistical analysis. Samples were incubated for periods of 4, 8, 12, 16, 20, 24 and 28 days before being analysed for nitrate-N and ammonium-N. Table 6.2 outlines the experimental design.

Table 6.2 Summary of experimental design - Incubation 1

	1	SOIL	From one site
×	2	MANAGEMENT TREATMENTS	Paddock and Reserve
×	6	AMENDMENTS	0, 40, 80, 120 160 and 200 mg NH_4-N/kg (Rates-0, 40, 80, 120, 160 and 200)
×	7	INCUBATION PERIODS	4, 8, 12, 16, 20, 24 and 28 days
×	2	REPLICATES	Transects 1 and 3
	168	*POTS*	
plus	28	POTS FOR CONTROL	Extra pots to include Transects 2 and 4 for base-line measure. Amended with Rate-0
plus	28	POTS FOR BLANKS	Sand and soil only. No amendment applied. Dry incubaation
	224	*POTS*	

6.2.4 Sample Preparation

Ten grams of each soil sample, air dried and sieved (< 2 mm), was mixed with 30 g acid-washed sand (0.25-0.5 mm diameter) and placed in an incubation jar. The jar was a 50 mL propyethelene sample jar (40 mm in diameter and 50 mm high) with a propyethelene screw top lid. Acid-washed sand was added to the soil sample to ensure adequate aeration of the sample (Bremner 1965). Immediately before incubation, the samples were amended with 6 mL of each rate of amendment (Figure 6.2). Bremner (1965) found that maximal aerobic mineralization of nitrogen for different soils during incubation could be obtained using 6 mL of water per 10 g of soil mixed with 30 g acid-washed sand. At the start of the experiment the weight of the incubation jar plus the soil-sand mixture and amendment was noted. This was so a check for evaporation could be made and moisture added during the incubations.

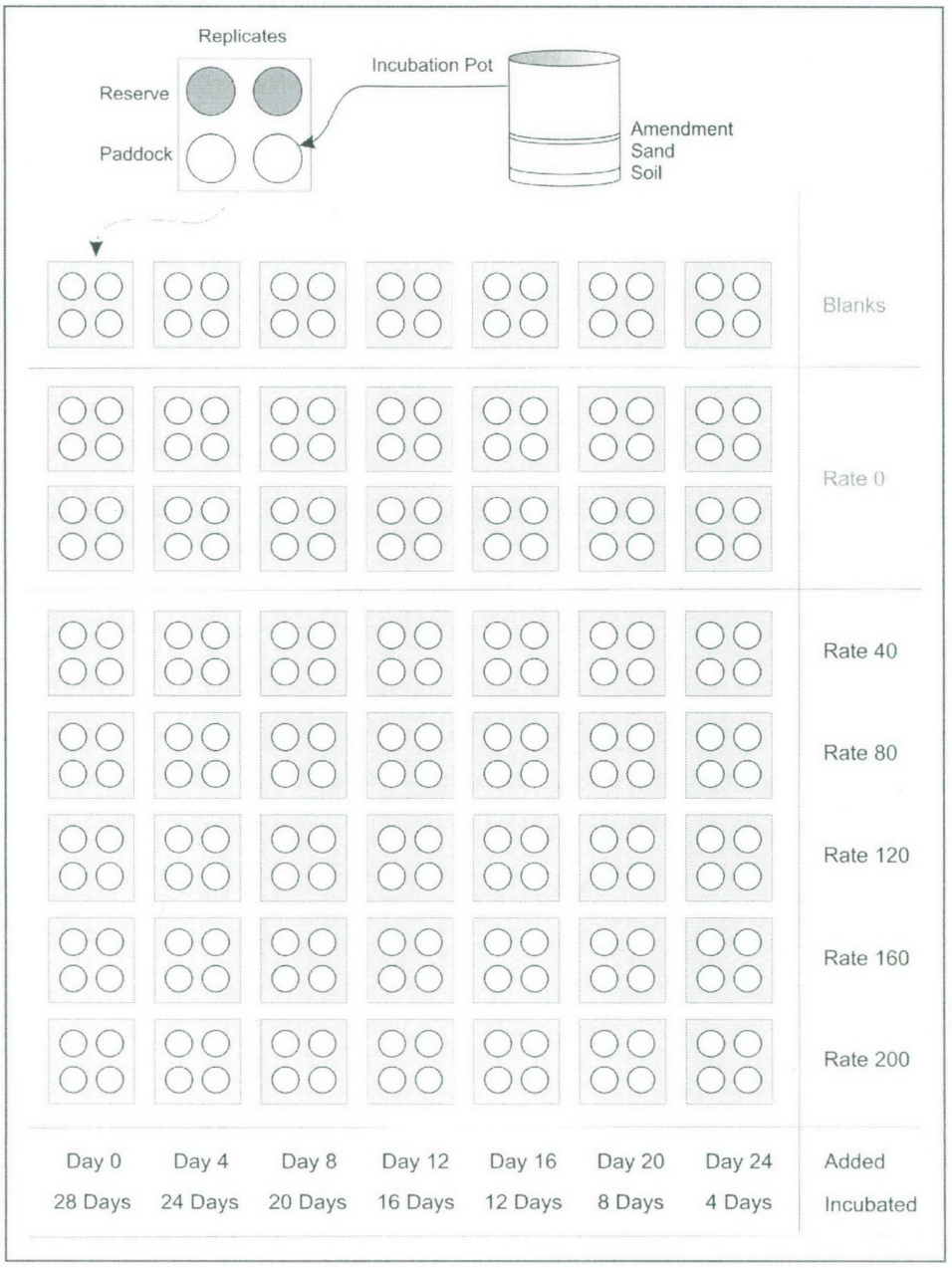

Figure 6.2 Schematic of pot numbers, soil management groups, amendment rates and incubation times - Incubation 1

6.2.5 Incubation Procedure

Samples for each set were amended at four day intervals, starting with the 28-day set, and the incubation pots, with lids closed, were placed in a constant-temperature cabinet set at 25°C. Every four days, for those sets running for more than four days, lids of the pots were removed for 20 minutes to aerate the sample, similarly to the procedures adopted by Paul *et al.* (2001 and Young *et al* (2002). This was to allow air and gas exchange and assist aerobic mineralization in the absence of a permeable membrane as specified in the procedure of

Bremner (1965). Every eight days the pots were weighed to check the moisture level. Deionized water was added if necessary to replace that lost by evaporation. Cabinet temperature was routinely monitored and did not fluctuate. At the end of the 28-day period, incubation of all pots stopped and the samples were immediately extracted for nitrate-N and ammonium-N.

6.2.6 Laboratory Analyses

Each sample was extracted with 30 mL of 2M KCl and the jars were tumbled end-over-end (15 revs/minute) for 1 hour. The contents were filtered using *Whatman* No. 42 filter paper and the filtrate read by a *Technicon* auto analyser using a dual-channel system. Ammonium ions were measured using the Adamsen *et al.* (1985) indophenol blue method with nitrate-N being reduced to ammonium-N via a cadmium column.

6.2.7 Statistical Tests

An average standard error was calculated for the data set to check similarity of the transects in each area. No other statistical analyses were undertaken for these data. Optimal incubation conditions were determined from inspection of the data tables and graphs.

6.2.8 Results and Discussion

A full set of results from the incubation, including measurements for each transect, is provided in Appendix 6.1. It is assumed that nitrification was the only mechanism by which nitrate was produced in the incubated soil, and that loss of nitrate by other pathways was negligible.

Average concentrations of ammonium-N and nitrate-N following incubation, as functions of ammonium-amendment rates and incubation times, are depicted for the paddock and reserve soils (Figure 6.3).

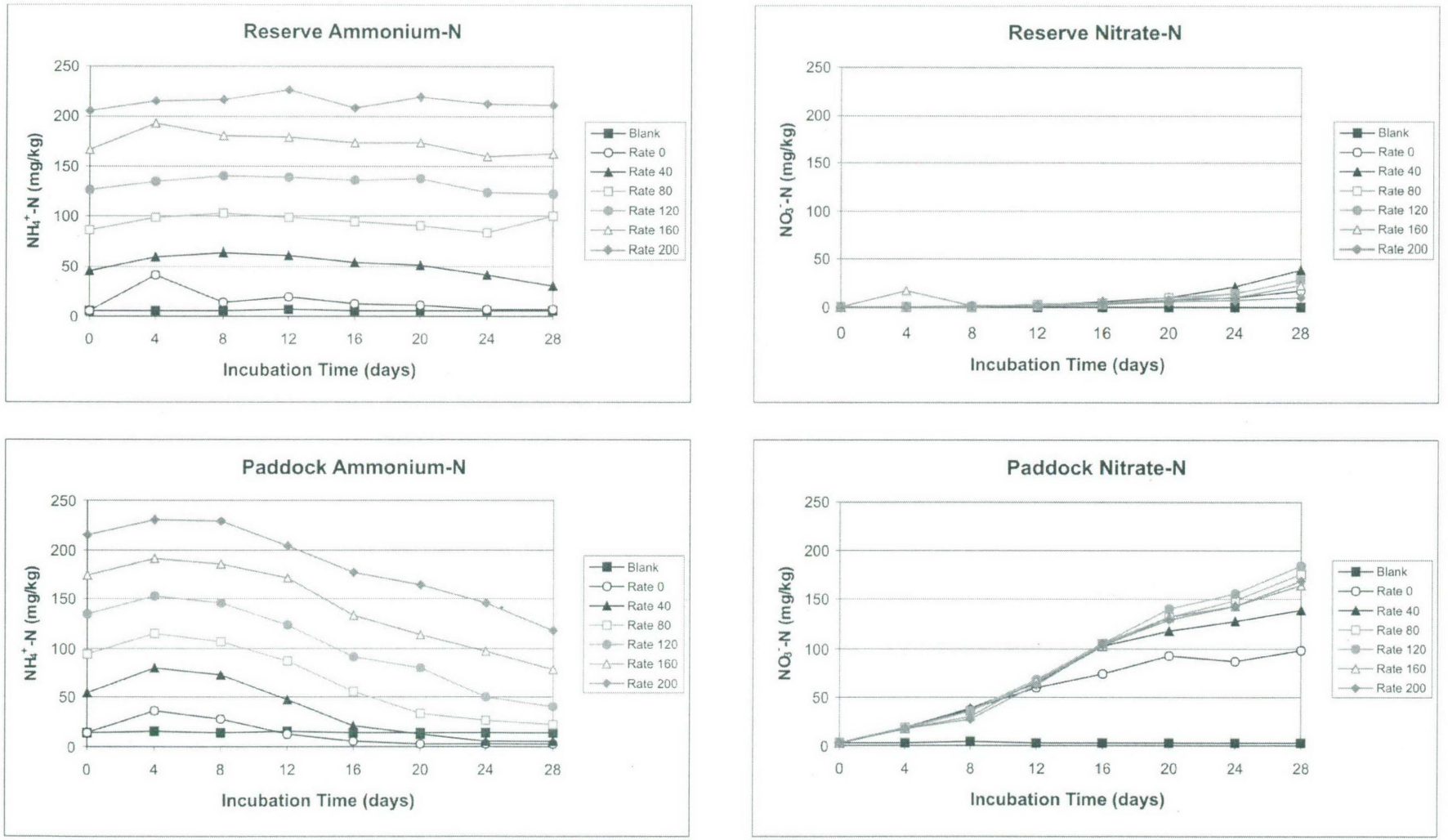

Figure 6.3 **Average ammonium-N and nitrate-N concentrations over time in paddock and reserve soil samples incubated with ammonium sulfate - Incubation 1**

It is apparent from these graphs that marked differences occur between the two differently managed areas, paddock and reserve. Very little ammonium-N was lost and very little nitrate-N accumulated in soil samples from the reserve. This indicated nitrification was very slow in the reserve. However, in samples from the paddock for each amendment rate, nitrification did occur and at a much faster rate than for the reserve. These differences will be discussed in the next experiment where soils from five depths from each of the paddock and reserve areas of six sites were incubated and statistical differences reported.

For the reserve soil (Figure 6.3) nitrification, as determined by a gain in nitrate-N, did not occur until near the end of the incubation. This suggests a lag phase during which an initially small population of nitrifying microorganisms multiplied in response to the availability of ammonium. By the second half of the incubation, population numbers of nitrifying bacteria were sufficient to produce measurable amounts of nitrate, although the concentration remained low. Had the incubation run for a longer period, it is presumed that nitrification would have continued to increase, possibly at an exponential rate.

In soil samples from the paddock (Figure 6.3), nitrification, as a gain in nitrate-N, commenced within the first four days of incubation. Initially, the rate was about 3.5 mg/kg/day of nitrate-N but for the duration of the incubation it averaged about 6.0 mg/kg/day for Rate-80 and above. In the Rate-0 samples, initially the nitrification rate was the same as the other treatments but declined after twelve days. The reason for this can be seen from the Rate-0 ammonium-N concentrations that approached zero by the middle of the incubation period. Rate-0 nitrate accumulation slowed because very little, or nil, ammonium remained available to nitrify. After sixteen days, a decline in the rate of nitrification was also seen for the Rate-40 samples as the ammonium supply was depleted in that treatment. Nitrification for Rates-80, 120, 160 and 200, are very similar. This suggests that the nitrate mineralized during incubation was independent of those ammonium amendment rates. That the amendment did not affect the nitrification rate as determined from nitrate accumulation suggests zero-order kinetics, where the rate of reaction is independent of the ammonium concentration, and this is consistent with other studies (Rochester 1989).

Interpretation of changes in ammonium-N concentrations is more complicated than for nitrate-N. This is because ammonium was being produced by net mineralization of soil organic nitrogen at the same time as it was being lost by nitrification. The change in

ammonium over time is the difference between the amount produced by mineralization of organic nitrogen and the amount lost by nitrification. Mineralization can be assessed by looking at changes in inorganic or mineral-N (nitrate-N plus ammonium-N) over time. In the absence of other gains or losses of inorganic-N in or out of the incubation jar, the change of inorganic-N over time is a measure of net mineralization. By subtracting the initial ammonium amendment, net mineralization for the different rates could be compared more easily (Figure 6.4). With ammonium addition a possible priming effect on mineralization could have produced additional ammonium during the incubation. In both the paddock and reserve soils, the ammonium amendments apparently did not affect mineralization (Figure 6.4). This indicates an absence of the priming effect that has been observed when nitrogen fertilizer is added to some soils (Westerman and Kurtz 1973; Broadbent 1965).

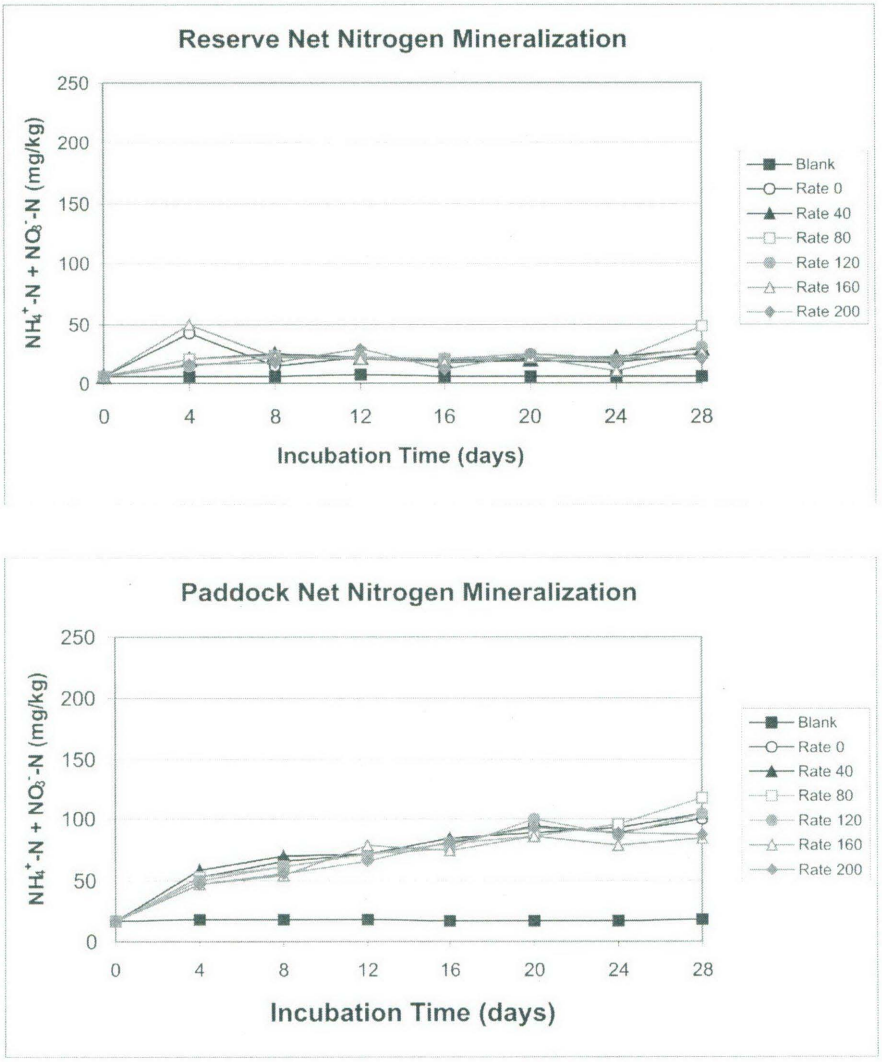

Figure 6.4 **Net nitrogen mineralization over time in paddock and reserve soil samples incubated with ammonium sulfate - Incubation 1**

Mineralization was greater for the paddock soil and an initial flush of mineralization of about 8 mgN/kg/per day over the first four days was observed. Labile organic matter or that which is readily mineralized with a low carbon to nitrogen ratio may be produced when a soil is air dried. Rapid mineralization upon wetting a dry soil has been termed the Birch effect (Birch 1959) and this could be a reason for the elevated values for the Day 4 data.

The potential for ammonium toxicity for this soil was considered. No evidence for this was discerned from these data within this incubation period. The rate of nitrate accumulation was unaffected by an increase in the ammonium amendment rate. A decrease in the rate of nitrification with increasing ammonium-N concentration could have signalled a toxic effect.

6.2.9 Incubation Conditions Determined

Although a trend was apparent in the soil samples from the reserve, very little ammonium was nitrified during the incubations and this was only towards the end of the incubation period. For the paddock soils, for ammonium-N, Rate-0 decreased until the twentieth day of incubation when it levelled out. More than twice the ammonium-N for Rate-0 was recorded after four days compared with the blank. After twelve days of incubation, Rate-0 was equal to the blank, then dropped to below the blank with subsequent incubation days. This indicated that all the available ammonium had nitrified. Rates-40 to 200 showed a steady decline that continued for the 28 days of incubation. Figure 6.3 shows that nitrification had not run to completion for these amendments although Rate-40 at 94% nitrification of the applied ammonium and Rate-80, at 91%, came close.

The rate of 100 mg NH_4^+-N/kg has been commonly used as an amendment for incubation experiments, yet this rate appears to be based on convention (pers.comm. Mark Conyers 2004). After assessing data from this experiment, a rate of 100 mg NH_4^+-N/kg and an incubation period of 24 days were selected as an ideal amendment rate for Experiment 2. Surplus ammonium would ensure nitrification would continue, yet not go to completion, within a 24-day incubation. It was assumed that nitrification for soil depths 0-2.5 cm and 2.5-5 cm (Experiment 2) would be at a much faster rate than the 0-10 cm depth of this experiment. Thus to compare nitrification rates over various depths, an ammonium amendment at Rate-100 would cater for faster nitrification in the surface soil.

The rate of 100 mg NH_4-N/kg was also accepted because it was used by Young *et al.* (2002)

and data from this experiment could be compared with their results. For Experiment 3, a rate of 20 mg NH_4^+/kg was selected. Reasons for this are given with that experiment.

Standard error between replicates were calculated for the data set. For nitrate-N, the average error was 6mg/kg and ammonium-N, 5 mg/kg. Errors were not plotted in Figure 6.3

6.2.10 Net Ammonium-Nitrogen and Net Nitrate-Nitrogen

Ammonium concentrations, measured in incubations such as these, reflect native ammonium already present in the soil, plus ammonium mineralized from organic matter in the sample, plus any ammonium added as an amendment, and less any ammonium nitrified or lost by immobilization during the incubation. Because the rate of mineralization is independent of the initial ammonium amendments, the influence of mineralization on changes in ammonium-N concentrations can be removed by subtracting the corresponding Rate-0 and applied ammonium-N concentrations. This also effectively removes the initial, before incubation, ammonium-N concentrations. The product of this calculation is defined here as *net ammonium-N.*

Nitrate concentrations reflect native nitrate already present in the soil, plus nitrate oxidized from ammonium during incubation. *Net nitrate-N* is calculated in a similar way to net ammonium-N.

Using Rate-100 as an example, net ammonium-N (mg N / kg) incubated is calculated using the following equation:

$$[\text{Net } NH_4^+\text{-N}_{(\text{Rate-100})}] = [NH_4^+\text{-N}_{(\text{Rate-100})}] - [NH_4^+\text{-N}_{(\text{Rate-0})}] - [NH_4^+ \text{ Applied}_{(\text{Rate-100})}]. \quad (1)$$

Net nitrate-N incubated was calculated, again using Rate 100 as an example:

$$[\text{Net } NO_3^-\text{-N}_{(\text{Rate-100})}] = [NO_3^-\text{-N}_{(\text{Rate-100})}] - [NO_3^-\text{-N}_{(\text{Rate-0})}]. \quad (2)$$

Net ammonium-N and net nitrate-N are defined in this way so that each can provide a measure of the ammonium-N amendment that has been nitrified during incubation. This can be seen in the graph for nitrate-N in the paddock soil (Figure 6.3). For the first twelve days, the initial ammonium in the soil plus the ammonium produced by mineralization were being nitrified, so that the nitrate-N concentration for Rate-0 remained the same as for the ammonium-amended samples. After day twelve, concentrations of nitrate-N in the Rate-0

incubation jars fell below those jars amended with ammonium as the initial and mineralized ammonium-N had all been oxidized. For Rate-40, the rate of nitrate production started to fall after sixteen days because most of the amendment had been nitrified. The gap in nitrate-N between amended and the Rate-0 jars (net nitrate-N) also increased. Nitrate-N for the other rates increased over the remainder of the incubation as the ammonium amendment was progressively nitrified. These equations therefore give relative nitrification values with which to compare one incubation result with another, or one incubated soil with another.

This method of assessing nitrification was proposed by Young *et al.* (2002) to demonstrate decreases in nitrification of added ammonium with depth (0-10 cm) in pasture soils from southern NSW. As discussed previously, southern NSW pasture soils where winter rainfall prevails, contrast with pasture soils from the Northern Tablelands with respect to soil acidification and acidification processes. To facilitate comparison of nitrification processes between the two areas, the method of Young *et al.* (2002) was adopted for the second incubation experiment in this chapter.

6.3 Experiment 2: Incubation Experiment to Investigate Management and Depth Influences on Nitrification

This incubation experiment examined the effects of depth and paddock management on nitrate and ammonium concentrations and related this to nitrification. Soil pH_{Ca} as a possible influencing factor was also investigated. Six soils, five depths and two management areas were used. Two ammonium amendments were applied, and samples were incubated at 25°C for 24 days.

6.3.1 Details of Sites

Six sites were selected from sites previously used in the earlier paired-site survey (Chapter 4). At each site, soil was sampled from the grazed, fertilized, exotic pasture paddock and adjacent unfertilized roadside reserve of native pasture. Table 6.3 gives a summary of chemical properties of the surface soils at the sites.

Table 6.3 Soil chemical properties for surface (0-5 cm) soils - Incubation 2
Soil pH for 5-10 cm soil depth shown in brackets

Site	pH_{Ca}	pH_W	$EC_{1:5}$ dS/m	Organic C (%)	Bray-P (mg/kg)	KCL-S (mg/kg)	NH_4^+-N (mg/kg)	NO_3^-N (mg/kg)	Total N (%)	ECEC ($cmol_c$/kg)	C:N Ratio
Reserve											
40	4.5 (4.6)	5.5 (5.7)	0.05	2.6	2.5	2.5	5	1	0.23	4	11.2
41	4.6 (4.6)	5.6 (5.6)	0.04	2	3	1.7	3	1.1	0.12	3.6	16.4
61	4.9 (4.9)	5.9 (6.1)	0.04	1.6	2.5	1.6	4	1	0.09	4.4	17
63	4.7 (4.6)	5.9 (5.9)	0.04	1.9	3	2.8	14	1	0.14	3.3	13.9
201	5.2 (5.1)	6.0 (6.1)	0.07	3	2.5	3.9	3	8.4	0.17	10.7	17.2
205	5.4 (5.0)	6.3 (6.3)	0.07	3.5	5	2.1	8	2	0.16	8.4	21.3
Paddock											
40	4.6 (4.4)	5.6 (5.5)	0.05	2.8	15	7.6	4	1	0.25	4.5	11.1
41	4.7 (4.6)	5.6 (5.3)	0.08	2.7	14	7	7	7.6	0.2	5.3	13.4
61	5.0 (4.6)	5.7 (5.7)	0.05	2.4	12	7.2	4	1	0.16	5.7	15
63	4.6 (4.5)	5.6 (5.6)	0.05	2	10	6.6	17	6.8	0.16	4.3	12.8
201	5.0 (4.7)	5.6 (5.6)	0.1	2.9	13	12	29	23	0.22	7	13.3
205	4.9 (4.6)	5.6 (5.7)	0.1	4.4	24	12	33	29	0.23	5.5	19.5

Sites for this experiment needed at least three times the level of phosphorus (BrayP) in the paddock as compared with the reserve, as evidence the paddock had been fertilized. Soil pH_{Ca} for the 5-10 cm depth of the paddock had to be less than, or equal to, that of the reserve. This depth was selected as it had the largest difference between paddock and reserve pH_{Ca}.

Ammonium concentrations in the paddock soil were considered; three sites had higher levels of ammonium-N in the paddock compared with other sites. Similarly ammonium-N concentrations in the paddock soil generally had to be higher than, or equal to, those in the reserve. Comparing paddock and reserve soils, four sites had higher concentrations of nitrate-N in the paddock soils and two sites (40 and 61) had similar concentrations. Such differences in ammonium and nitrate were considered representative of lighter-textured soils sampled in the paired-paddock study. Soil type between the six sites had to be similar in order to compare data. Soils from three sites, of one texture class (sandy soils), were classified as a Brown Kandosol (Site 41), Yellow Kandosol (Site 61) and a Bleached-Orthic Tenosol (Site 63). The other group (silty soils) comprised two Brown Sodosols (Sites 40 and 201) and a Yellow Chromosol (Site 205). Two sites (40 and 41) lie 50 km northeast of Armidale, two (61 and 63) are 40 km east of Armidale and two (201 and 205) are southwest of Walcha. Figure 4.1 shows the site localities.

6.3.2 Soil Sampling Protocol

Two sample sets, each comprising six cores 20 cm deep, were collected from paddock and

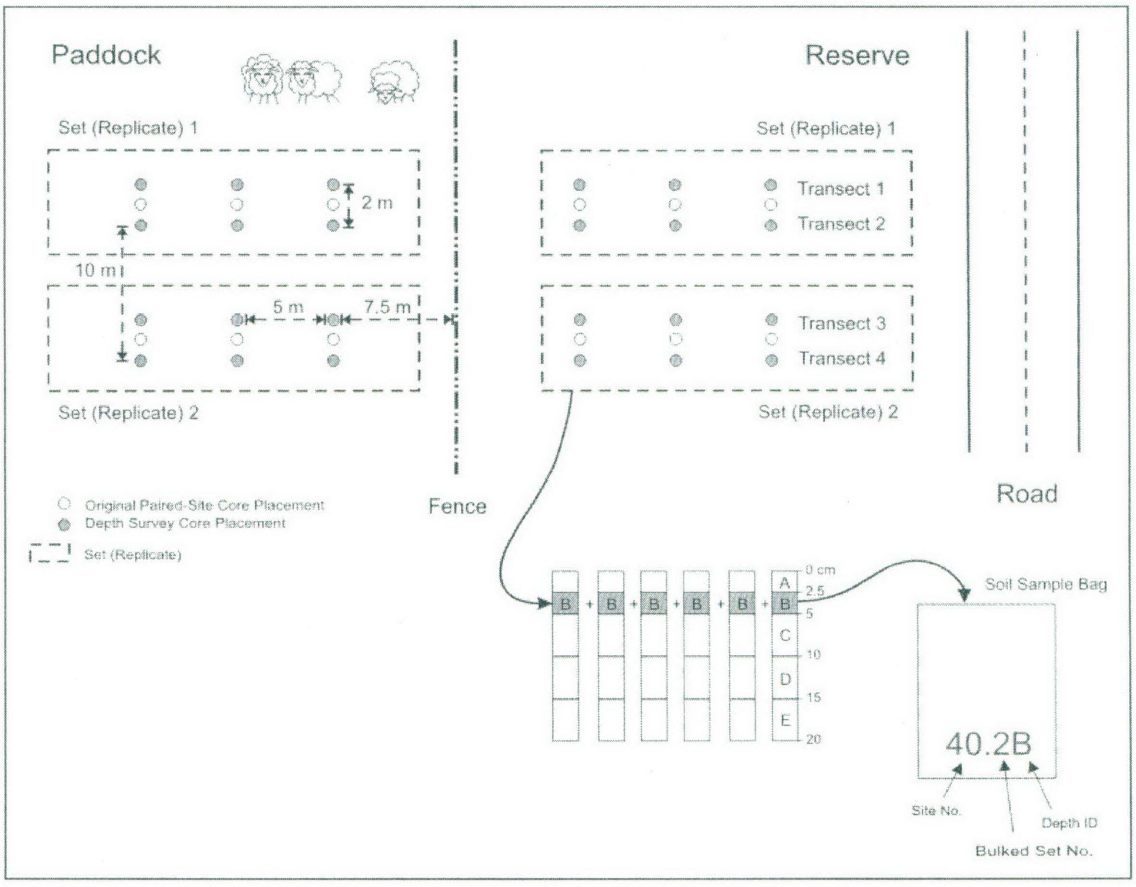

Figure 6.5 Soil core placement, sampling into depth intervals, bulking and labelling of sample - Incubation 2

reserve areas, with three cores from each transect in each area. Two within-area replicates were formed by grouping transects 1 with 2, and 3 with 4. Cores were split into depth increments (0-2.5, 2.5-5, 5-10, 10-15 and 15-20 cm) and the resulting samples were bulked (Figure 6.5).

6.3.3 Experimental Design

Soils from five depths, from two management areas, from each of the six sites were used in this experiment. Two ammonium amendments were applied to the soil samples to assess the rate of nitrification. A rate of 100 mg NH_4-N/kg, using $(NH_4)_2SO_4$ stock solution (Rate-100), was applied to half the samples to stimulate nitrification. The other half of the samples were amended with deionized water (Rate-0). Incubation of the samples proceeded at 25°C for 24 days. The design of the experiment is given in Table 6.4.

Table 6.4 Summary of experimental design - Incubation 2

	6	SOILS	From six sites
×	2	MANAGEMENT TREATMENTS	Paddock and Reserve
×	2	NH_4^+ AMENDMENTS	0 and 100 mg NH_4-N (Rate-0 and Rate-100)
×	1	INCUBATION PERIOD	24 days
×	2	REPLICATES	Transects 1+2 and 3+4
×	5	DEPTHS	0-2.5, 2.5-5, 5-10, 10-15 and 15-20 cm
×	1	TEMPERATURE	25°C
	240	*POTS*	

6.3.4 Sample Preparation, Incubation Procedure, Laboratory Analyses

Soil samples were prepared, amended and incubated as for Experiment 1 (Sections 6.2.4 and 6.2.5). Samples were extracted and tested for nitrate-N and ammonium-N as per Section 6.2.6. Soil pH_{Ca} (1:5 soil:0.01 M $CaCl_2$ suspension) was also measured. Appendix 6.2.1 lists the full set of results.

6.3.5 Derived Calculations

Net ammonium-N and net nitrate-N were calculated as previously described (Section 6.2.9) so that relative results could be compared.

6.3.6 Statistical Tests

Variables statistically tested for significance were management type, depth and soil texture class; three sites were included in each class (Table 6.5).

Table 6.5 Variables for statistical analysis - Incubation 2

Sites	6 sites grouped into two texture classes
Management Type	Paddock and Reserve
Depths	5 depths 0-2.5, 2.5-5, 5-10, 10-15, 15-20 cm
Texture 1 (sandy soil)	Sites 41, 61 and 63
Texture 2 (silty soil)	Sites 40, 201 and 205
Soil pH_{Ca}	Measured on 60 samples
Rate	2 rates 0 and 100 (deionized water and $(NH_4)_2SO_4$)

The tests were completed with and without using pH_{Ca} as a covariate for each variate, net ammonium-N and net nitrate-N. By including soil pH_{Ca} as a covariate, all treatment effects were effectively adjusted to what they would have been had soil pH_{Ca} been measured at the constant value of its mean. Depth data were not independent and the 5-10 cm sample came from that part of the sample between the 2.5-5 cm and 10-20 cm depth intervals. Here, depth of measurement was not random as each depth sample came from the one core. Thus, spatial serial correlation existed between data taken from successive depths of the same core. These repeated measures were tested using the REML directive in *GenStat* and a power correlation structure was specified, which would diminish in a power law with increasing distance between measurements. Discussion for these results focusses on the REML analysis on the variables using pH_{Ca} as a covariate.

6.3.7 Results

Correlation over depth, with and without the covariate soil pH_{Ca}, was considered in the REML analysis (Table 6.6 and Appendix 6.2.2). Nitrification in this experiment was determined from net ammonium-N or net nitrate-N calculated as previously described in Incubation (Section 6.2.10). Soil pH_{Ca} data for the individual sites are provided in Table 6.7. Average soil pH_{Ca} for the sites over depth is shown in Figure 6.6. The depth effect was significant ($P<0.001$) as was the management-by-depth interaction ($P=0.025$). Thus, soil pH_{Ca} changes over depth are dependant on management, differing between paddock and reserve areas.

Table 6.6 REML analysis - Incubation 2

Significant differences in bold; dash denotes not applicable

Factors\Variates	pH_{Ca}	Net NH_4^+-N		Net NO_3^-N	
With pH Covariate (Y/N)	-	N	Y	N	Y
pH Covariate	-	-	**<0.001**	-	**<0.001**
Texture	0.395	0.651	0.168	0.642	0.108
ManagementType	0.152	**0.026**	**<0.001**	**0.011**	**<0.001**
Depth	**<0.001**	0.842	0.072	0.133	0.160
ManagementType.Depth	**0.025**	0.539	0.422	**0.021**	0.130
ManagementType.Texture	0.251	0.221	0.663	**0.022**	0.124
Depth.Texture	0.375	0.502	0.627	0.122	0.149
ManagementType.Depth.Texture	0.590	0.629	0.875	0.086	0.167

Table 6.7 Soil pH_{Ca} by texture, site, depth and management area - Incubation 2

Texture Class 1 (Sandy Soils)				Texture Class 2 (Silty Soils)			
Site	Depth (cm)	pH_{Ca} Reserve	pH_{Ca} Paddock	Site	Depth (cm)	pH_{Ca} Reserve	pH_{Ca} Paddock
41	0-2.5	4.65	4.97	40	0-2.5	4.44	4.67
	2.5-5	4.51	4.71		2.5-5	4.31	4.31
	5-10	4.57	4.70		5-10	4.38	4.28
	10-15	4.61	4.71		10-15	4.42	4.34
	15-20	4.71	4.69		15-20	4.49	4.38
61	0-2.5	4.84	4.74	41	0-2.5	5.07	5.08
	2.5-5	4.76	4.58		2.5-5	5.01	4.69
	5-10	4.74	4.57		5-10	5.16	4.75
	10-15	4.75	4.62		10-15	5.23	4.82
	15-20	4.80	4.66		15-20	5.46	4.99
63	0-2.5	4.58	4.49	61	0-2.5	5.17	4.50
	2.5-5	4.44	4.32		2.5-5	5.08	4.44
	5-10	4.42	4.34		5-10	5.05	4.58
	10-15	4.46	4.43		10-15	5.08	4.75
	15-20	4.56	4.47		15-20	5.08	4.99

The results are assessed firstly by the variates net ammonium-N and net nitrate-N. The results are then examined by the variables texture, management and depth on the variates soil pH_{Ca}, net ammonium-N and net nitrate-N.

Net Ammonium-N and Net Nitrate-N

For net ammonium-N, the adjustment for pH_{Ca} as a co-variate was significant ($P<0.001$) in the REML analysis. Apart from that, the only other significant effect was a management effect on net ammonium-N ($P<0.001$). For net nitrate-N, again the pH_{Ca} co-variate adjustment was significant ($P<0.001$) and the only significant effect was management on net nitrate-N ($P<0.001$). Where net nitrate-N was not adjusted for pH_{Ca}, the effect of management was significant ($P=0.011$) and management-by-depth ($P=0.021$) and management-by-texture ($P=0.022$) interactions were significant. This showed those management effects that were dependent on soil pH_{Ca} changed with depth and changed with texture.

Ammonium added to soils from the reserve areas (Figure 6.7) generally did not nitrify with incubation apart from Site 201 (Figure 6.9). Site 201 (Table 6.8) had a net 10% loss of ammonium in the 0-2.5 cm layer, and a 30% loss over the 5-10, 10-15 and 15-20 cm depths. This corresponded with net accumulation of nitrate-N (Table 6.9) that averaged 30% over all depths. Site 201 had a higher concentration of nitrate-N from the soil test data for the reserve area (Table 6.3) compared with the other sites. Net ammonium-N gains in the reserve areas were evident for Sites 40 and 61 (Figures 6.8 and 6.9).

For the paddock soils (Figures 6.8 and 6.9), considerable variation between sites existed and the rate of nitrification was much higher than for soils from the reserves. Nitrification of ammonium occurred for all sites and net ammonium-N loss tended to equal net nitrate-N gain except for Site 205 (Tables 6.9 and 6.10). Nitrification of soil amended with Rate-0 also occurred for the paddock soils of all sites (Table 6.9). The soil from Site 63 showed a small loss of ammonium-N and a low nitrate-N gain (Tables 6.8 and 6.9). Site 205 had much higher ammonium-N in the paddock, with a 47% net gain of ammonium-N for the 0-2.5 cm depth (Table 6.8) and a net loss of nitrate-N of up to 22% for the 2.5-5 and 5-10 cm depths (Table 6.9). A small net loss of nitrate-N occurred for this site. For all sites, paddock soils showed a decrease of nitrate-N with depth for both Rate-0 and Rate-100 (Table 6.9). Net nitrate-N did not decrease with depth for the sites overall (Figure 6.7).

Table 6.8 Ammonium-N and net ammonium-N concentrations as functions of management area, amendment rate, site and depth - Incubation 2

[Net NH_4^+-N] mg/kg = [NH_4^+-N(Rate-100) - NH_4^+-N(Rate-0) - (Rate-100)]
[Net NH_4^+-N]% = [Net NH_4^+-N(Rate-100) /100 × 100]

Texture Group 1 (sandy soils): Sites 41, 61 and 63
Texture Group 2 (silty soils): Sites 40, 201 and 205

		Reserve				Paddock			
Site	Soil Depth (cm)	NH_4^+-N Rate-0 (mg/kg)	NH_4^+-N Rate-100 (mg/kg)	Net NH_4^+-N (mg/kg)	Net NH_4^+-N (%)	NH_4^+-N Rate-0 (mg/kg)	NH_4^+-N Rate-100 (mg/kg)	Net NH_4^+-N (mg/kg)	Net NH_4^+-N (%)
41	0-2.5	9	115	6	6	14	65	-50	-50
	2.5-5	13	120	7	7	1	36	-66	-66
	5-10	13	114	1	1	2	66	-36	-36
	10-15	16	111	-5	-5	1	50	-51	-51
	15-20	11	105	-6	-6	2	75	-27	-27
61	0-2.5	5	120	15	15	1	59	-42	-42
	2.5-5	16	125	9	9	6	59	-47	-47
	5-10	17	123	5	5	6	57	-48	-48
	10-15	23	123	0	0	3	51	-52	-52
	15-20	20	118	-2	-2	3	69	-33	-33
63	0-2.5	27	128	1	1	5	98	-7	-7
	2.5-5	14	121	7	7	6	101	-5	-5
	5-10	15	122	7	7	8	103	-5	-5
	10-15	13	118	4	4	8	104	-5	-5
	15-20	21	116	-6	-6	9	101	-8	-8
40	0-2.5	39	157	18	18	1	35	-66	-66
	2.5-5	30	144	14	14	2	77	-26	-26
	5-10	21	139	18	18	23	129	6	6
	10-15	23	128	5	5	28	139	11	11
	15-20	20	122	2	2	22	122	0	0
201	0-2.5	3	92	-10	-10	25	83	-42	-42
	2.5-5	7	102	-5	-5	13	107	-6	-6
	5-10	5	71	-34	-34	13	87	-25	-25
	10-15	3	69	-34	-34	10	70	-40	-40
	15-20	3	72	-31	-31	11	75	-37	-37
205	0-2.5	11	115	4	4	96	243	48	48
	2.5-5	6	110	4	4	32	156	24	24
	5-10	12	109	-3	-3	7	109	1	1
	10-15	16	123	7	7	10	103	-7	-7
	15-20	13	109	-5	-5	5	103	-1	-1

Table 6.9 Nitrate-N and net nitrate-N concentrations as functions of management area, amendment rate, site and depth - Incubation 2

[Net NO_3^--N] mg/kg = [NO_3^--N(Rate-100) - NO_3^--N(Rate-0)]
[Net NO_3^--N]% = [Net NO_3^--N(Rate-100) / 100 × 100]

Texture Group 1 (sandy soils): Sites 41, 61 and 63
Texture Group 2 (silty soils): Sites 40, 201 and 205

		Reserve				Paddock			
Site	Soil Depth (cm)	NO_3^--N Rate-0 (mg/kg)	NO_3^--N Rate-100 (mg/kg)	Net NO_3^--N (mg/kg)	Net NO_3^--N (%)	NO_3^--N Rate-0 (mg/kg)	NO_3^--N Rate-100 (mg/kg)	Net NO_3^--N (mg/kg)	Net NO_3^--N (%)
41	0-2.5	9.9	13.4	3.6	4	242.4	291.4	49.0	49
	2.5-5	8.7	11.4	2.7	3	99.1	163.2	64.2	64
	5-10	11.7	16.5	4.8	5	63.5	102.4	38.9	39
	10-15	9.1	16.7	7.6	8	51.9	112.4	60.5	60
	15-20	10.2	16.8	6.6	7	34.3	64.2	29.9	30
61	0-2.5	32.8	18.4	-14.4	-14	105.9	159.5	53.6	54
	2.5-5	17.5	3.6	-13.9	-14	31.3	75.2	44.0	44
	5-10	3.0	2.4	-0.6	-1	22.9	68.7	45.9	46
	10-15	2.1	2.3	0.2	0	24.8	76.0	51.2	51
	15-20	1.1	0.6	-0.5	0	19.6	51.2	31.6	32
63	0-2.5	28.6	30.1	1.5	1	68.2	79.3	11.2	11
	2.5-5	12.1	13.4	1.3	1	20.9	34.3	13.4	13
	5-10	3.1	3.6	0.5	0	19.0	25.1	6.0	6
	10-15	2.3	1.4	-0.9	-1	10.4	14.2	3.8	4
	15-20	2.01	1.01	-1.00	-1	5.9	13.0	7.1	7
40	0-2.5	10.9	12.7	1.8	2	123.8	184.3	60.5	61
	2.5-5	1.3	1.2	-0.1	0	89.3	92.4	3.1	3
	5-10	0.1	0.1	0.0	0	65.4	41.0	-24.4	-24
	10-15	0.0	0.0	0.0	0	5.3	3.0	-2.3	-2
	15-20	0.0	0.0	0.0	0	2.0	2.3	0.3	0
201	0-2.5	96.9	125.1	28.2	28	197.3	233.7	36.4	36
	2.5-5	63.5	80.5	17.0	17	86.3	100.6	14.3	14
	5-10	38.4	73.3	34.9	35	63.9	87.8	24.0	24
	10-15	24.4	51.5	27.1	27	39.4	76.4	37.0	37
	15-20	18.6	53.9	35.3	35	24.3	62.7	38.4	38
205	0-2.5	23.0	16.5	-6.5	-7	102.0	92.4	-9.7	-10
	2.5-5	15.8	26.5	10.7	11	99.5	77.2	-22.3	-22
	5-10	20.5	19.2	-1.3	-1	79.2	56.0	-23.3	-23
	10-15	6.3	5.4	-0.9	-1	14.8	22.7	7.9	8
	15-20	0.1	0.4	0.3	0	8.0	10.1	2.1	2

Effects of Texture

The only effect involving texture was a management effect on net nitrate-N that varied between the two texture classes and was affected by soil pH_{Ca}. Clay and sand content in the soil can significantly affect mineralization and nitrification, yet silt does not affect these processes (Strong *et al.* 1999). Mineralization in sandy soils is usually faster compared with soils with a high clay content resulting in higher nitrate-N concentrations (Mengel 1996). In soils with a high clay content that had been dried and then moistened again, a flush of mineralization can occur with incubation (Strong *et al.* 1999) but any effect would have been cancelled in the calculation of net ammonium-N. Both texture classes had low clay contents. The sandy-textured soils had an average clay content of 11%, with 15% silt and 74% sand, while the silty soils comprised 14% clay, 32% silt and 54% sand. The sandy soils had higher net nitrate-N (Table 6.9) the concentrations in the paddocks. However, net nitrate-N concentrations for the silty soils varied, with no discernible pattern between paddock and reserve areas and this could have caused the significant result. For these soils, differences between the paddocks and reserves were apparent for nitrate-N in both amended and unamended samples. This observation invalidates the effect of texture on net nitrate-N.

Management Effects

Management effects on both net ammonium-N and net nitrate-N were independent of soil pH_{Ca} and as such could have been produced by another factor, or factors. Management effects were also consistent with those effects observed in Incubation 1, with more nitrification occurring in samples from the paddock compared with those from the reserve. Considerable variation between the sites was evident that could have been confounded by initial nitrate-N and ammonium-N concentrations present in the sample before incubation. For example, the paddock soil of Site 63 showed only minimal nitrification compared with the other sandy soil sites (41 and 61); this paddock had a higher ammonium-N concentration from an earlier survey over 0-5 cm. For the silty soils, Sites 201 and 205 had high ammonium-N concentrations in the surface (0-5 cm) soil. Results for the reserve soil for Site 201 were similar to that from the paddock; this site had higher nitrate-N concentrations compared with the other sites. The problem of initial native ammonium-N and nitrate-N in the soil samples will be considered in Section 6.3.9.

The pattern of soil pH_{Ca} by management over depth (Figure 6.6) is similar to that of the paired-site study in Chapter 4 (Figure 4.22) except that a depth-by-management interaction

did not occur there. Soil pH_{Ca} for the paddock soil decreased between the depths 0-2.5 cm and 2.5-5 cm. Acidifying processes in these depths could have caused this drop in soil pH_{Ca}, but below 5 cm, soil pH_{Ca} increased over the remaining depths. The decrease in the paddock was larger than for the reserve over the depths 0-2.5 cm and 2.5-5 cm, but the increases were constant for both management areas.

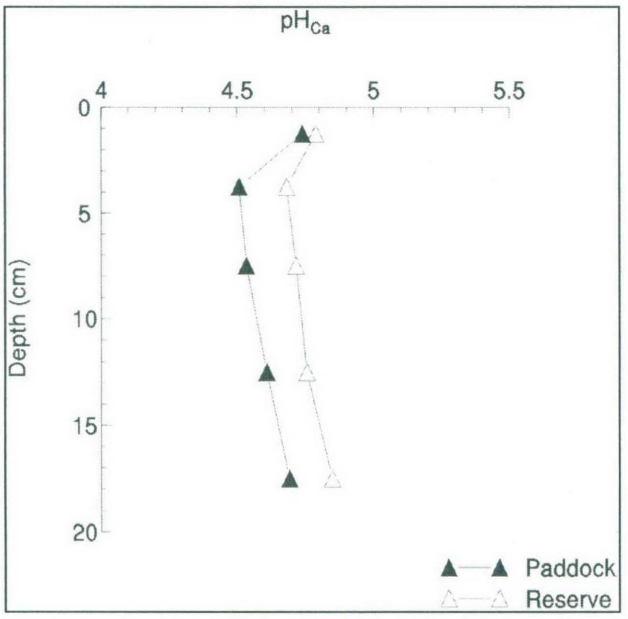

Figure 6.6 Soil pH_{Ca} by management area

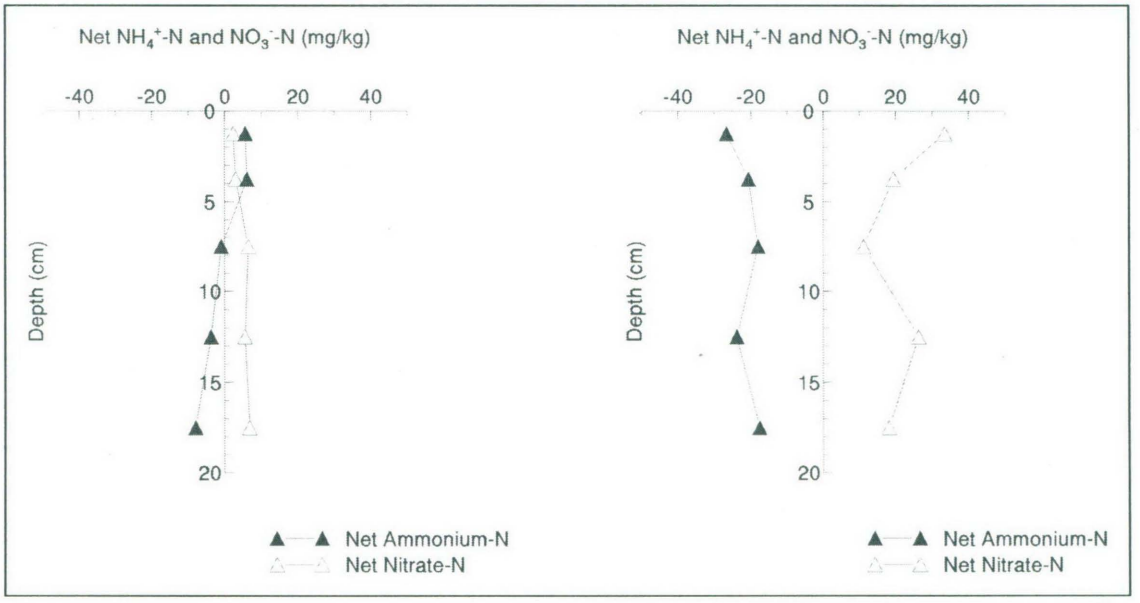

Figure 6.7 Nitrification of sites as a function of loss of ammonium-N and gain of nitrate-N for reserve (left) and paddock (right) soils

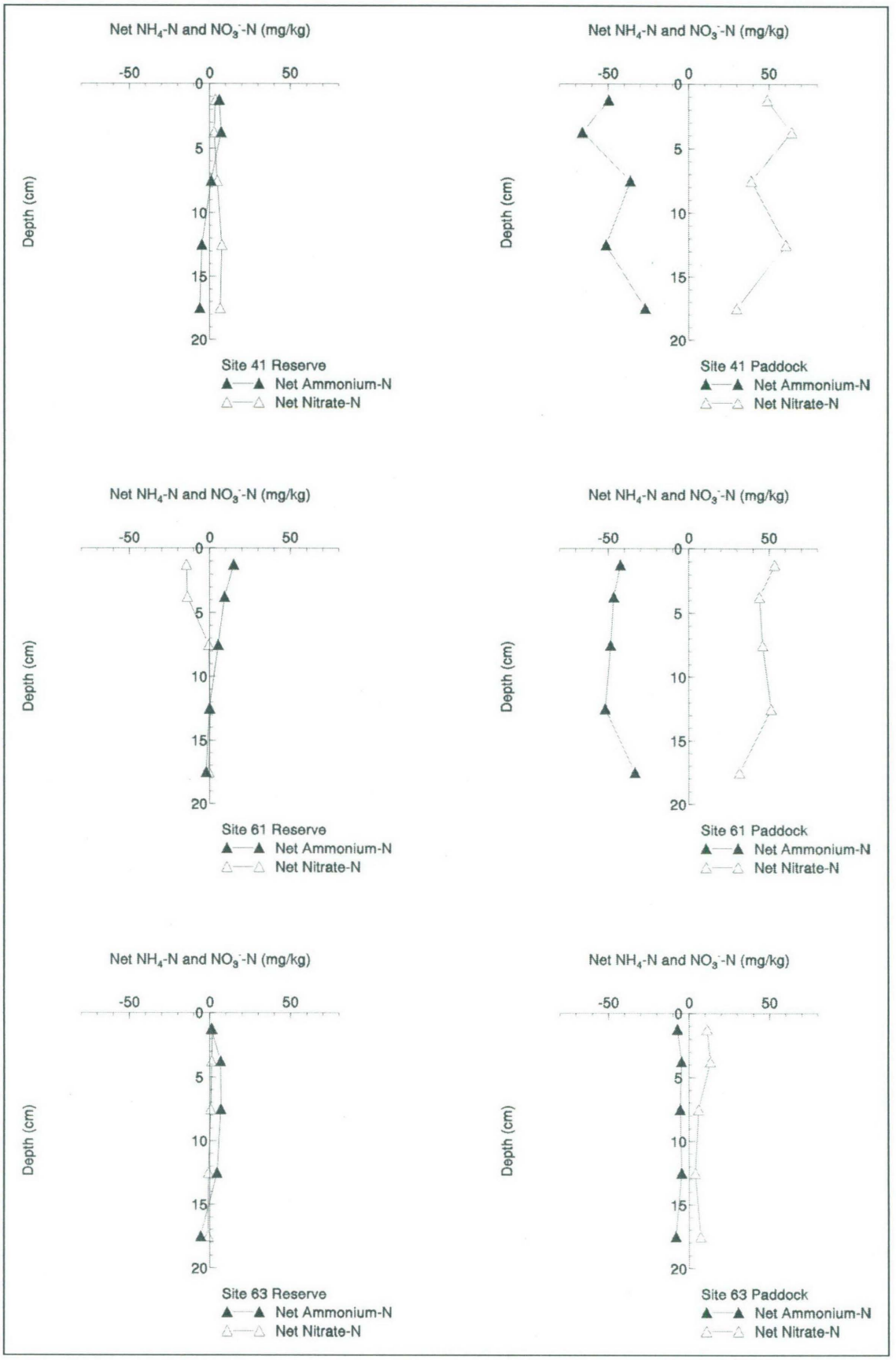

Figure 6.8 **Net ammonium-N and nitrate-N by management type and depth for each sandy soil site**

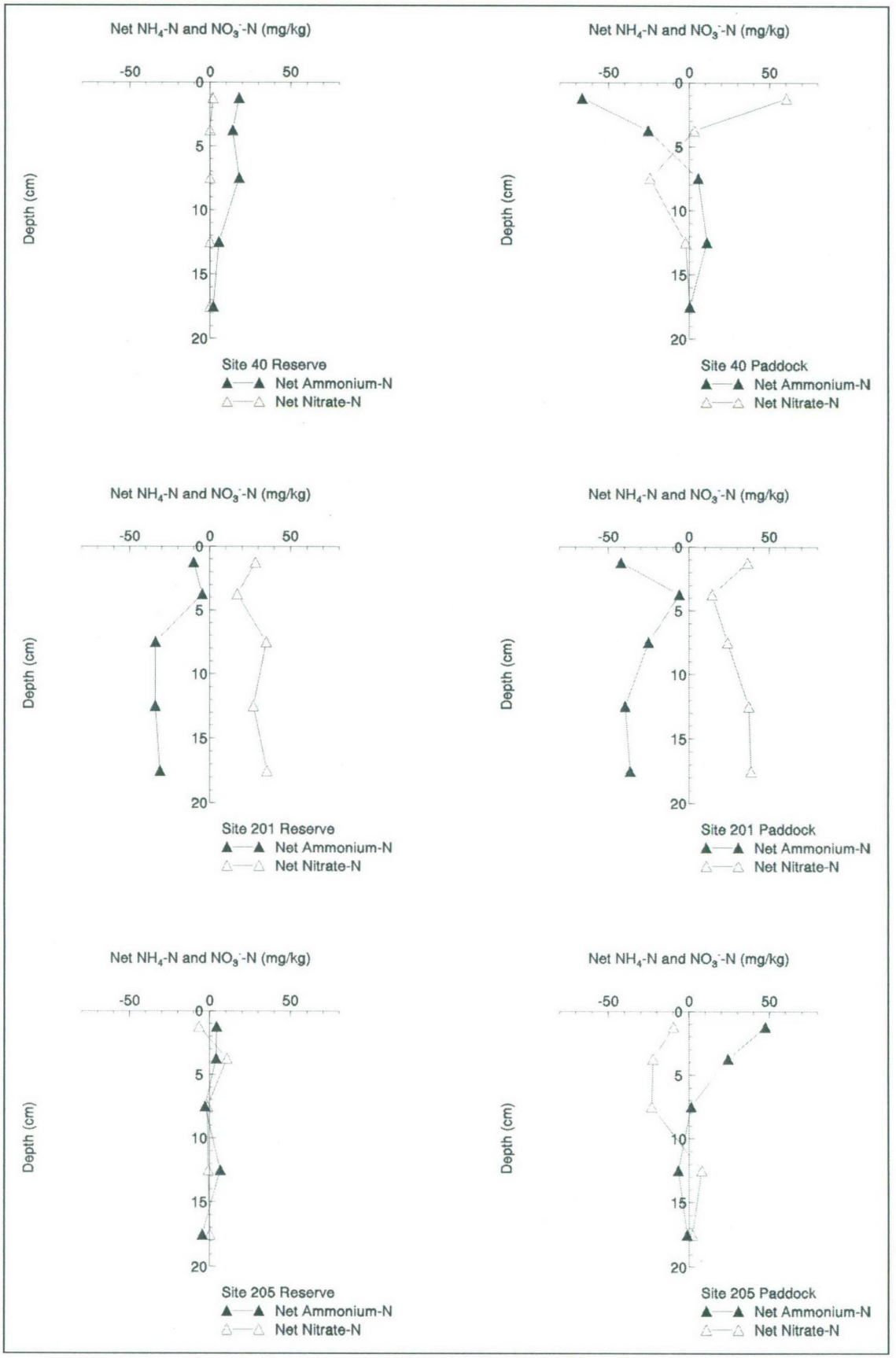

Figure 6.9 **Net ammonium-N and nitrate-N by management type and depth for each silty soil site**

6.3.8 Discussion

In the paired-sites survey (Chapter 4), the results suggested that nitrate leaching was not prevalent and also suggested differences in nitrification between the reserve areas and the paddocks. The *Newholme* repeated-measures study (Chapter 5) showed that some nitrate leaching may have occurred but no direct evidence of this could be given. This incubation experiment was under controlled conditions with no effects of plant uptake, denitrification or leaching, to produce data to make some conclusions about the nitrification process.

Very little oxidation of the ammonium amendment to nitrate occurred during the incubation for the soil samples from the reserve areas. A higher rate of nitrification of added ammonium was observed for the paddock soils. These results are consistent with those of Incubation 1 in this chapter. The results are also consistent with the observations from the paired-sites study (Chapter 4) where higher concentrations of nitrate were found in the paddock soils compared with those from the reserve. The effect can be understood if it considered that sowing legumes and fertilizing with superphosphate increases nitrogen fixation in the paddock areas. Higher total nitrogen concentrations in the paddock soils (Table 6.3) implies that more nitrogen would be available for mineralization. In addition, the carbon to nitrogen ratios (Table 6.3) for the six soils used in this incubation were all lower in the paddocks compared with those of the reserves. These ratios suggest that organic matter in the paddock soils would be more readily mineralized than in the reserves. Legume residues with a low carbon to nitrogen ratio can result in net nitrogen mineralization can result (Peoples *et al.* 2004). Because autotrophic nitrifiers are a small group of specialized bacteria, the nitrifying ability of a soil will depend on the exposure of that soil to ammonium ions. Thus the paddock soils with high rates of nitrogen mineralization would be expected to develop large active populations of nitrifying bacteria in the absence of other inhibiting factors such as low pH. That nitrification is greater in the paddock soils, is probably the result of increased nitrogen cycling and ultimately more vigorous nitrogen fixation. The only reserve soil to show appreciable nitrification of the added ammonium was Site 201 (Figure 6.9). Of the six sites, that reserve soil also contained the highest nitrate concentration (8.4 mg/kg NO_3^-N) in the 0-5 cm layer as sampled during the paired-sites study (Table 6.3). This suggests that a more active population of nitrifiers existed in that reserve.

Soil pH_{Ca} in this experiment varied significantly with depth. However, the changes were less than those recorded by Young *et al.* (2002) for pasture soils from southern NSW. In that

study, they measured a rapid decline of between 0.2 and 1.4 pH units between the 0-2 cm layer and the 8-10 cm layer for four out of five sites. In contrast, for the Northern Tablelands soils, the observed decrease of soil pH_{Ca} was only over the 0-5 cm and 0-10 cm depths. The difference between pH_{Ca} between 0-2.5 cm and the 5-10 cm layers was between 0.2 and 0.4 lower for five sites and 0.1 higher for one site (Table 6.7). Between 10 and 20 cm the pH increased. In the Young *et al.* (2002) study, the sites had a clay content between 10 and 40%, were examined. Soils in the experiment reported here had clay contents between 10 and 17%.

Nitrification rates can be restricted by soil pH with nitrification decreasing as soil acidity increases (Schmidt 1982) and has been found in work in southeast Australia (Young *et al.* 1995; 2002). A relationship between depth and the nitrification of ammonium to nitrate in either the paddock or reserve area was not evident from the results of the study reported here. For all sites, the paddock soils showed a marked decrease of nitrate-N with depth for both application rates, yet the accumulation of nitrate, as measured by net nitrate-N, only showed a slight decrease with depth for the paddock.

The results from this experiment contrasts with that of Young *et al.* (2002) who observed a decrease in nitrification of added ammonium with increased depth and decreased soil pH_{Ca}. They argued that an acidic subsurface layer within the top 10 cm could have restricted nitrification. A smaller depth interval was used by Young *et al.* (2002) with soil sampled in 2 cm intervals to a depth of 10 cm, whereas the data from the Northern Tablelands study are based on depths over 0-20 cm. A clear decrease in net average nitrate-N is apparent for the 0-10 cm depths for the paddock soil and this tends to support the previous assumption. In addition, soil pH_{Ca} decreased then increased over the 0-10 cm depths for this experiment. Thus, the perceived decrease in net nitrate-N over these depths is not related to soil pH_{Ca} in this study.

A number of process have been identified as causing changes to pH in sown, fertilized pasture soils in Australia (Helyar and Porter 1989) and these can result in changes to the distribution of pH with depth. Helyar and Porter (1989) considered nitrate leaching, humus accumulation, and transfers of alkalinity in plant and animal material to be the three main causes of acidification in pasture soils that had been sown with legumes and fertilized with phosphorus. As mineralization of organic matter generally occurs close to the surface of the soil, most of the nitrate produced by nitrification of mineralized ammonium could be expected to be in this

area. It follows that leaching of nitrate could be stronger near the soil surface. If this surface-produced nitrate is leached further down the profile and taken up by roots, a decrease in soil pH would occur at the site of nitrification relative to the lower depth of uptake. Plant litter deposited on the surface of the soil is a source of alkalinity. However, if an increase in plant residues results in increasing the humus pool, then this alkalinity could be offset to a greater or lesser degree by acidification of the dissociation of humic acids. Another consideration is that humus close to the soil surface will increase the pH_{BC}, which in turn would improve the resistance of the soil to pH changes.

In view of these possible mechanisms, it can be hypothesised that the lesser decline in soil pH_{Ca} with depth results from less nitrate leaching below 10 cm in these Northern Tablelands soils compared with those from southern NSW. In both environments, the return of alkalinity to the soil surface as plant litter maintains a relatively high soil pH in the top 2 or 2.5 cm, but below that layer the dominant process affecting pH is nitrate leaching. This occurs to a lesser degree on the Northern Tablelands. In Figure 6.6 the minimum soil pH_{Ca} was in the 2.5-5cm layer and increased below that. Using the soils in this study as an example, this pattern is consistent with some leaching of nitrate from the 2.5-5cm depth, with the nitrate being taken up by roots in the 5-20 cm layer. It is expected that in southern NSW, soil pH_{Ca} would decline less with depth in unfertilized, native pastures in a similar matter to the reserve sites in the Northern Tablelands study. However, such a comparison is not possible from the study of Young *et al.* (2002).

6.3.9 Evaluation of Method

Net ammonium-N was used to assess the relative rates of ammonium-N that were nitrified from the added ammonium. Similarly, net nitrate-N obtained relative rates of net nitrate-N gained through nitrification. This method was undertaken specifically to compare data from southern NSW, in particular that of Young *et al.* (2002). The method produced convincing and explicable patterns when presented by Young *et al.* (2002). However, anomalies are apparent in the results of the experiment reported here. For example, Table 6.9 shows that for the paddock soils considerable concentrations of nitrate-N were present in the incubation jars for the unamended Rate-0 and amended Rate-100 surface samples. The nitrate-N concentration at the end of the incubation is assumed to be the sum of the initial nitrate-N present in the soil, the nitrate-N produced from mineralized ammonium, and for Rate-100, the nitrate-N produced from the added ammonium. Initial nitrate-N and ammonium-N

concentrations were not required for this method and were not measured. Soil analyses presented elsewhere in this thesis indicate that initial nitrate-N concentrations would have made only a small contribution to the total nitrate-N present in the jar at the end of the incubation. This suggests that a substantial decrease in the total amount of nitrification with depth occurred in both the unamended and amended samples. It is also apparent (Table 6.9) that considerable nitrification occurred in both the amended and unamended surface samples. This would be consistent with nitrification being related to levels of organic matter, which decline with depth.

Thus presenting the data as nitrification of added ammonium-N is giving a skewed result. Nitrate-N concentrations decreased with increased depth for the paddock soils for both Rate-0 and Rate-100, yet net accumulation of nitrate did not decrease with depth. In addition, assuming that initial nitrate-N concentrations were not abnormally high, nitrate-N concentrations in the paddock are much higher close to the surface. Net nitrate-N results did not reflect this.

A further aspect that needs to be considered is the variation that was apparent between the sites in this experiment. This could have been due to the activity of soil nitrifying organisms, or the native nitrate and ammonium components in the soil. It is possible that a longer incubation time may have reduced the differences if nitrification of the available ammonium had not peaked. Optimal incubation conditions were determined from one soil (Site 41) only, and the other soils could have reacted differently.

6.4 Experiment 3: Incubation Experiment to Study the Influence of Management, Soil Moisture and Temperature on Nitrification

The objective of this experiment was to examine the seasonal effects of temperature and soil moisture on nitrification for a Northern Tablelands soil in relation to nitrification in southern NSW. Management differences were also considered. Incubation conditions imposed three temperatures: cold, warm and hot. Within each temperature group, three moisture levels were used to simulate dry (drought), moist and wet conditions. This was considered a suitable design to compare the speed of reaction of two different ammonium amendments, for different moisture levels, at a particular temperature, for two management regimes. Only one soil, from two adjacent differently-managed areas, was used.

6.4.1 Site and Soil

The same soil collected for Experiment 1 was used for this experiment, except that samples from transects 2 and 4 were used. As before, soil samples were from two adjacent management areas, an exotic pasture paddock and native pasture reserve. Site details are set down in Section 6.2.1 and the sampling design in Section 6.2.2.

6.4.2 Experimental Design

Temperatures of the constant-temperature cabinets were set at 7°C, 13°C and 19°C. These were at temperature intervals of 6°C with the warmest temperature 6°C lower than that of Experiment 1. Temperatures were based on the average of the mean maximum and minimum January and July temperatures for Armidale and Guyra (BoM 2004)[7]. The design of the experiment is provided in Table 6.10. In comparison, the average January temperature for Wagga Wagga is 24°C and 7°C in July (BoM 2004).

Moisture was applied to the pots at the rates of 0.6 mL, 3 mL and 6 mL. These levels were designed to simulate dry, moist and wet conditions and were based on the soil moisture content of the samples at pressures of 1 500, 130 and 10 kPa respectively (Appendix 6.3). The field capacity of the soil was 30%. The rate of 6 mL also coincided with the rate formulated by Bremner (1965), which was used in Experiment 1. All samples were incubated for 24 days. An amendment of 20 mg NH_4-N/kg using $(NH_4)_2SO_4$ stock solution was made

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[7] Armidale (Radio Station 2AD) 056013 January (13.4 - 27.1°C) July (0.3 - 12.2°C)
Guyra (Ag. Research Station) 056002 January (13.3 - 25.2°C) July (0.7 - 12.4°C)

up to the three different moisture levels. The rate of 20 mg NH_4-N/ kg differed from the optimum rate determined in Experiment 1. As very little moisture was to be applied to the incubation pots, a toxic salt effect could have occurred if a higher concentration was used. For low moisture amendments, the ratio of NH_4-N to moisture would have been high and effectively increased the concentration of NH_4-N that initially came into contact with the soil sample.

Table 6.10 Summary of experimental design - Incubation 3

	1	SOIL	From one site (Site 41)
×	2	MANAGEMENT TREATMENTS	Paddock and Reserve
×	2	NH_4^+ AMENDMENTS	0 and 20 mg NH_4-N / kg (Rate-0 and Rate-20)
×	1	INCUBATION PERIOD	24 days
×	2	REPLICATES	Transects 2 and 4
×	3	MOISTURE LEVELS	0.6 mL, 3 mL and 6 mL
×	3	TEMPERATURES	7°C, 13°C and 19°C
	72	*POTS*	

6.4.3 Sample Preparation

As for the first incubation experiment, 10 g of each soil sample was mixed with 30 g acid-washed sand and placed in an incubation jar. Half the samples were amended with 0.6 mL, 3 mL or 6 mL of $(NH_4)_2SO_4$ at Rate-20 and half with 0.6 mL, 3 mL or 6 mL deionized water (Rate-0).

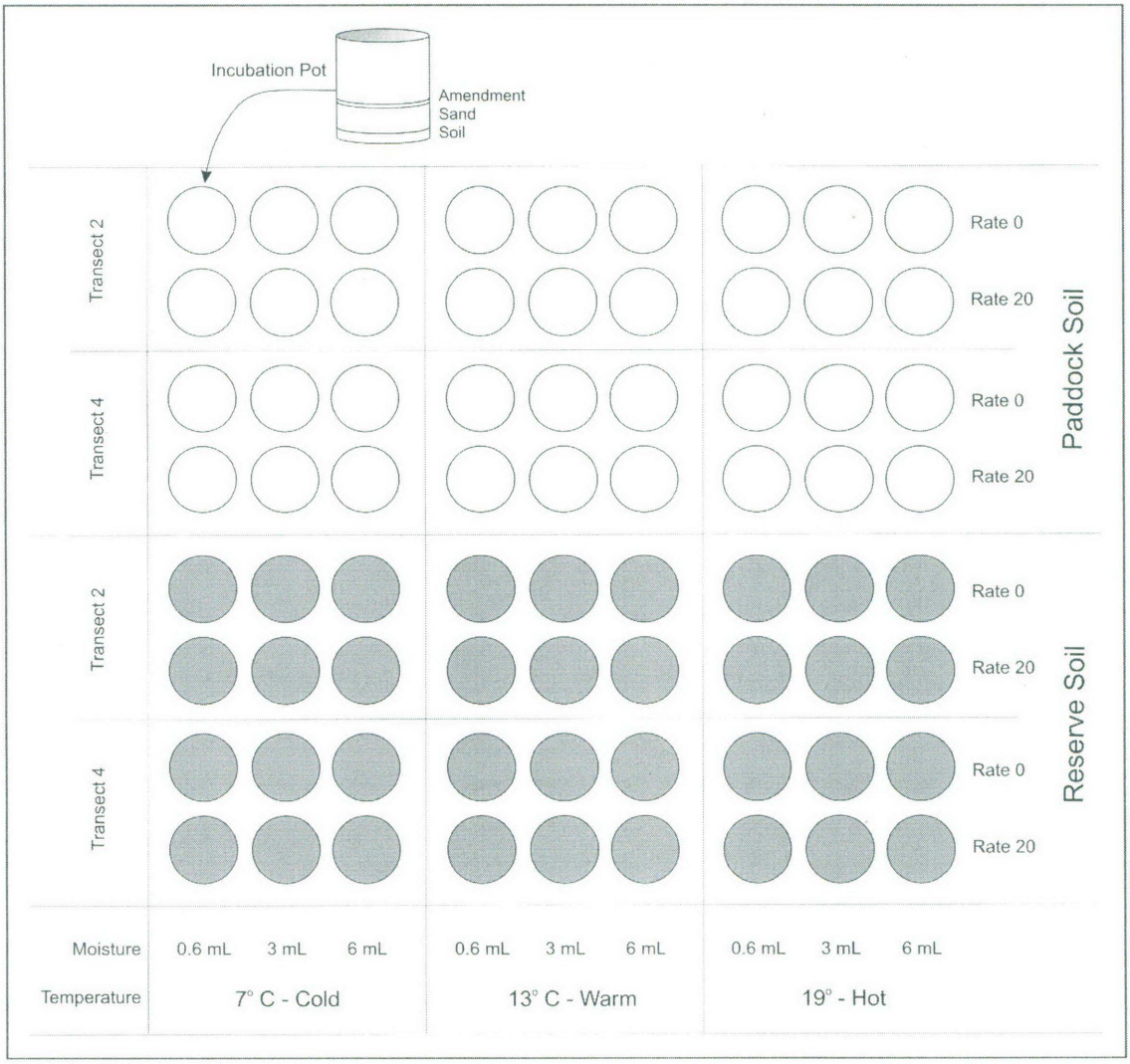

Figure 6.10 Schematic of pot numbers, soil management groups, moisture levels, temperatures, amendment rates and incubation times - Incubation 3

6.4.4 Incubation Procedure and Laboratory Analysis

Pot sets were each placed in controlled temperature cabinets, one set each at 7°C, 13°C and 19°C (Figure 6.10). Every four days the jars were aerated, and every eight days the moisture level was checked. Extra deionized water was needed to replace that lost by evaporation only for those samples incubating at 19°C. Incubation was for 24 days. At the end of the incubation period, the samples were extracted and analysed for nitrate-N and ammonium-N as described in Section 6.2.6.

6.4.5 Statistical Tests

An analysis of variance was completed for the variates ammonium-N and nitrate-N. For this analysis, treatment factors and levels are given in Table 6.11. Replicates were from the sampling scheme, by way of two transects from which soil samples were taken. For each variate, plots of residuals and diagnostic plots were generated to show whether further statistical analysis interpretation could be attempted (Appendix 6.3.2).

Table 6.11 Variables for statistical analysis - Incubation 3

Temperature	Cold, Warm and Hot (7°C, 13°C and 19°C)
Moisture	Dry, Moist and Wet (0.6 mL, 3 mL and 6 mL)
Management Type	Paddock and Reserve
Rate	0 and 20 (deionized water and $(NH_4)_2SO_4$)

6.4.6 Results

Nitrate-N and ammonium-N results are fully tabulated in Appendix 6.3.1 and averages are presented in Table 6.13. The analysis of variance summary (Table 6.12) is given and detailed statistical test results are provided (Appendix 6.3.2). The results are assessed by the variates, ammonium-N and nitrate-N, and by the effects of moisture, temperature and management.

Table 6.12 Analysis of variance - Incubation 3

Significant differences in bold

Factors\Variates	NH_4^+-N	NO_3^--N
Temperature	0.419	0.059
Management Type (MgtType)	0.452	**<0.001**
Rate	**<0.001**	0.169
Moisture	0.442	**<0.001**
Temperature.MgtType	0.428	0.296
Temperature.Rate	0.244	0.456
MgtType.Rate	0.097	**0.025**
Temperature.Moisture	**<0.001**	**0.018**
MgtType.Moisture	**<0.001**	**<0.001**
Rate.Moisture	0.496	0.258
Temperature.MgtType.Rate	**0.021**	0.462
Temperature.MgtType.Moisture	**<0.001**	0.566
Temperature.Rate.Moisture	0.728	0.660
MgtType.Rate.Moisture	0.108	0.246
Temperature.MgtType.Rate.Moisture	0.106	0.736

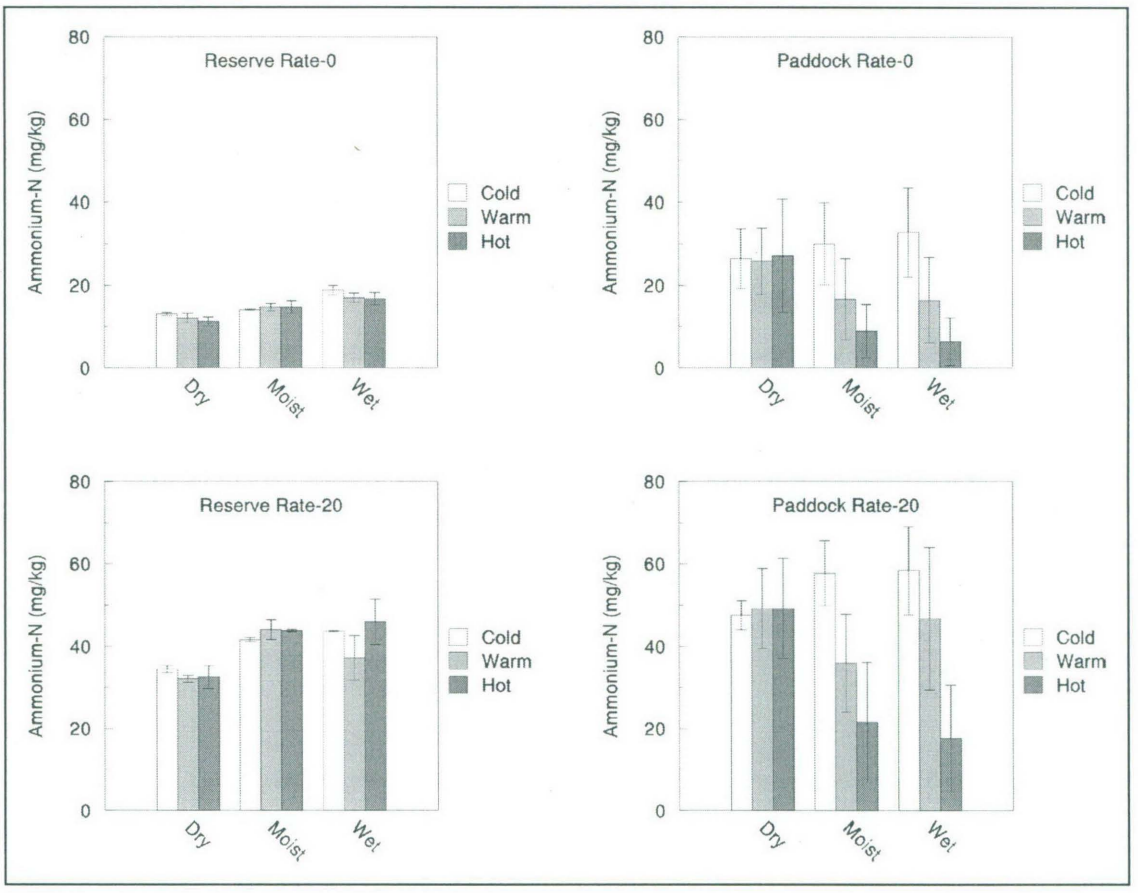

Figure 6.11 Rate-0 and Rate-20 ammonium-N concentrations as a function of moisture and temperature by management area

Ammonium-N

Significant differences ($P<0.001$) were evident for the interaction of temperature, moisture and management type for ammonium-N (Table 6.12). Although differences for the three moisture treatments were not significant in this analysis, nor were the differences as a result of temperature (Table 6.12), differences for the temperature-by-moisture interaction were significant ($P<0.001$). This implies that as both temperature and moisture increased, nitrification of ammonium increased. Significant differences ($P<0.001$) also occurred with moisture and management and indicates that an increase in moisture increased the rate of nitrification in the paddock (Tables 6.12 and 6.13). So for the paddock soil, as temperature increased in moist and wet conditions, the rate of ammonium nitrifying increased. Temperature did not affect nitrification (loss of ammonium) under dry conditions. Nitrification of ammonium was negligible in the reserve.

Significant differences for the temperature, rate and management interaction (P=0.02) were also apparent (Table 6.12). This implies that temperature and management effects changed with different rates of amendment application. The combination of temperature and management was not significant for ammonium-N, nor was that of rate and management (Table 6.12). The reason for this can be seen by looking Figure 6.11. Temperature affected both rates in the paddock (Table 6.13): as temperature increased, ammonium-N concentrations decreased faster for Rate-20 than they did for Rate-0.

Table 6.13 Ammonium-N and nitrate-N concentrations by management area and rate, as a function of temperature and moisture - Incubation 3

		Reserve				Paddock			
		NH_4^+-N Rate-0	NH_4^+-N Rate-20	NO_3^--N Rate-0	NO_3^--N Rate-20	NH_4^+-N Rate-0	NH_4^+-N Rate-20	NO_3^--N Rate-0	NO_3^--N Rate-20
Temperature	Moisture	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
1	28	13	35	1.0	0.9	26	48	5.1	3.8
	Moist	14	41	1.2	0.7	30	58	10.9	10.6
	Wet	19	44	1.2	0.7	33	58	12.8	11.9
2	28	12	32	1.2	0.8	26	49	9.8	9.6
	Moist	15	42	1.4	1.0	17	36	20.0	22.6
	Wet	17	32	1.4	1.1	16	47	23.3	36.9
3	28	11	33	1.5	0.7	27	49	18.5	16.6
	Moist	15	44	2.4	2.5	9	22	37.6	37.1
	Wet	17	40	3.7	3.0	6	18	48.3	68.0

Nitrate-N

The diagnostic plot (Appendix 6.3) suggested that the mean was proportional to variance for nitrate-N. As variance should be constant, a $\log_e$ transformation of the nitrate-N data was carried out. In the resulting analysis of variance summary (Table 6.12), management effects were significant (P<0.001) with less nitrification occurring in soils from the reserve. Moisture was also significant (P<0.001) and nitrate-N concentrations increased as moisture increased. These single variable data statistics differ from those of ammonium-N where only rate was significantly different. Moisture effects varied with management (P<0.001) and suggested that nitrate-N concentrations increased at a faster rate in the paddock with an increase in moisture (Tables 6.12 and 6.13). Management and rate effects were significant (P=0.025). Table 6.13 shows that nitrate-N concentrations increased with rate and were higher in the paddock compared with the reserve, where differences were not as obvious.

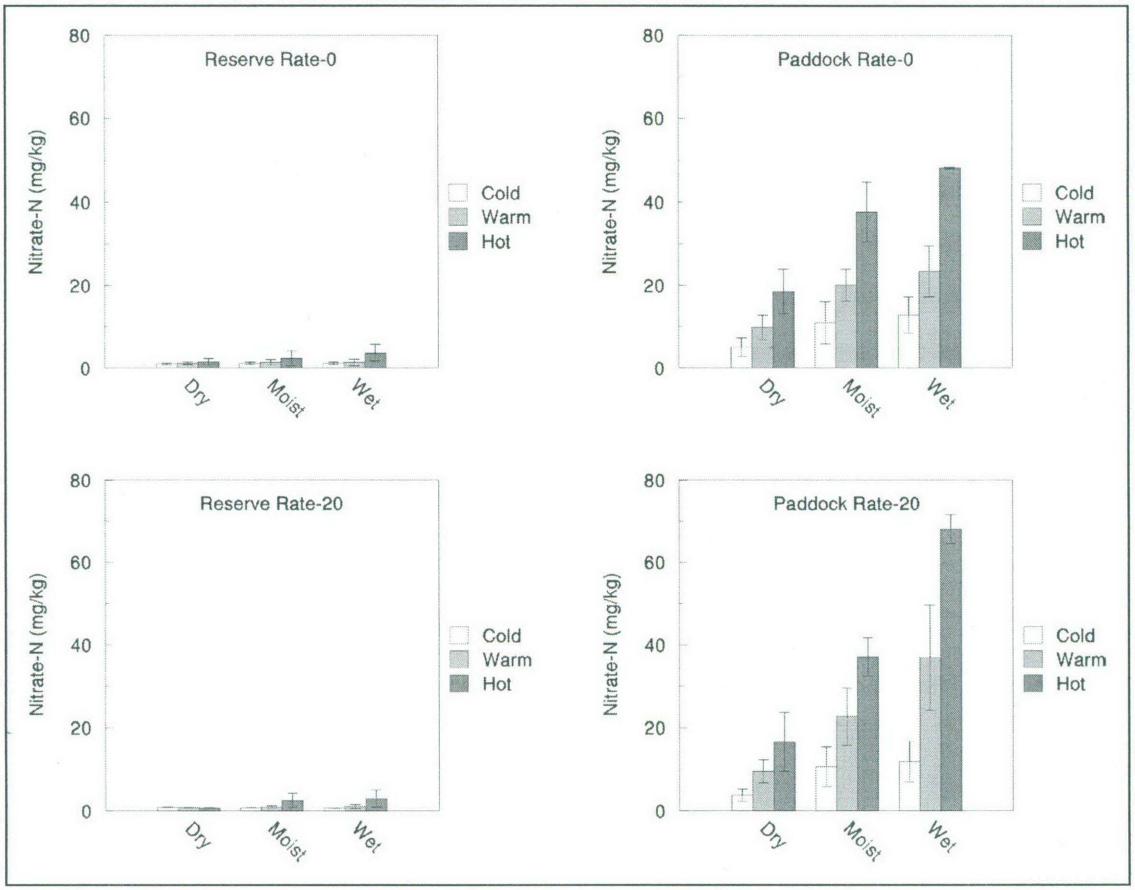

Figure 6.12 Rate-0 and Rate-20 nitrate-N concentrations as a function of moisture and temperature by management area

The analysis of variance summary (Table 6.12) implies that moisture effects on nitrate-N were dependant on temperature (P=0.018). That is, an interaction existed between moisture and temperature on nitrate-N. This is shown graphically in Figure 6.12 where it is clear that as moisture increased, nitrate-N concentrations increased at an increasing rate with increased temperature. An interaction of moisture and rate did not exist (Table 6.12). The reason for this is clear in Table 6.13. Very similar Rate-0 and Rate-20 concentrations show that the increase of nitrate-N with moisture was independent of rate under wet conditions and warm and hot temperatures. Nitrate-N concentrations for the reserve soils were not as affected by moisture, rate or temperature (Table 6.13 and Figure 6.12).

Temperature and Moisture Effects

Temperature alone had no direct, or main order, effect on ammonium-N or nitrate-N. For this incubation the temperature was the same for each management type, paddock and reserve. Within a particular temperature range 7°C (cold), 13°C (warm) or 19°C (hot), samples were

made up from each of the paddock and reserve areas. Thus temperature effects should be considered together with moisture effects. Nitrate-N concentrations were affected by moisture with incubation, but ammonium-N concentrations were not. Management affected nitrate-N with less nitrification occurring in samples from the reserve area compared with those from the paddock. Ammonium-N concentrations decreased as both temperature and moisture increased. Nitrate-N concentrations were also affected by the combined effects of temperature and moisture. However, because a moisture effect on nitrate-N existed, this interaction differed from that of ammonium-N. Moisture effects on nitrate-N concentrations changed with different temperatures; nitrate-N concentrations increased in moist and wet conditions at a faster rate in warm and hot temperatures.

Temperature and moisture affected the nitrification of ammonium to nitrate. Nitrification was limited by low moisture. That is, the impact of temperature on nitrate-N within the wet environment is greater than if the soil were dry or moist. As temperature and moisture increased, the rate of nitrification increased. The paddock soil had a higher and faster rate of nitrification compared with the reserve. Nitrification of the paddock soil proceeded with warmer temperatures under moist and wet conditions and was negligible under cold conditions. For the reserve soil nitrification was minimal across all temperatures and moisture levels.

Management Effects

Both ammonium-N and nitrate-N were affected by the interaction between management and moisture. Ammonium-N concentrations decreased with increased moisture in soils from the paddock areas. For paddock soil samples under dry conditions and for samples from the reserve area, ammonium-N concentrations did not decrease. The moisture effect on nitrate-N concentrations differed between paddock and reserve areas. Nitrate-N concentrations increased with an increase in moisture at a faster rate for the paddock soils. For the paddock soil, as temperature increased, the rate of nitrification, as a function of the loss of ammonium-N, increased but only under moist and wet conditions. Nitrification of ammonium was negligible in the reserve. This three-way interaction was not observed for nitrate-N. Here, moisture effects for nitrate-N changed with temperature and management effects differed with the different moisture levels. Nitrate-N concentrations increased with moisture and temperature in soils from the paddock area. Increases were apparent with increased moisture and temperature in the reserve area, but the values were all very low.

6.4.7 Discussion

For this experiment, a change of approach to the assessment of nitrification was made following concerns in Incubation 2 about what was being measured by net nitrate-N and net ammonium-N measurements. In this incubation, nitrate-N measured at the end of the incubation was taken as the measure of the total nitrification that had occurred. Nitrate concentrations in the samples at the start of the incubation were low. Field nitrate-N concentrations (0-10 cm) at the time of sampling were 0.2 mg/kg in the reserve and 3.4 mg/kg in the paddock. Ammonium-N concentrations at the end of the incubation were assumed to have been affected by gains from mineralization and losses from nitrification during the incubation. Field ammonium-N concentrations for these samples were 6.0 mg/kg in the reserve and 14.5 mg/kg in the paddock. The low concentration of ammonium amendment (20 mgN/kg) was made in anticipation that nitrification would be rapid enough to exhaust the initial ammonium plus mineralized ammonium in some treatments. In addition, the low concentration was to avoid the possibility of high ammonium to solution concentrations leading to ammonium toxicity in the dry (0.6 mL) treatment.

As with other microbial-driven soil processes, nitrification is influenced by temperature and moisture. Nitrification has been reported to occur in soil temperatures of between about 5°C and 40°C (Russell 1950; Tisdale *et al.* 1993). The rate of nitrification increases as temperature increases, except for very high temperatures. The optimum temperature for nitrification can be between 25°C and 35°C (Tisdale *et al.* 1993) although temperatures as high as 40°C and even 60°C have been reported (Schmidt 1982). An increase in moisture will also promote an increase nitrification until the soil approaches saturation point and the rate of nitrification declines. Nitrifying bacteria will still function at moisture levels below the plant-wilting point at matric potential of 1500 kPa until about 3 kPa (Tisdale *et al.* 1993). The right combination of an ideal temperature and moist conditions are needed to provide optimal conditions for nitrification. That is, although optimum moisture is present, nitrification would be retarded under very cold temperatures. Conversely, nitrification would proceed slowly in ideal warm temperatures where moisture is limited, such as during a drought episode. Other factors, such soil pH, aeration, the health and vigour of nitrifying organisms, population numbers or the supply of ammonium also influence nitrification (Tisdale *et al.* 1993).

The differences in nitrate concentrations shown in Figure 6.12 are indicators of the differences

in nitrification. The striking contrast between the reserve and paddock samples was expected from the results of the previous incubations. However, the current incubation showed that even for incubations designed to match hot, wet summer conditions, nitrification rates were still very low for the reserve. Thus the reserve area can be characterized as having low nitrification rates in general under all seasonal conditions. In contrast, nitrification in the paddock was higher, and faster, than that in the reserve. Seasonal effects were apparent for the paddock samples. Under cold (winter) conditions, nitrification rates were low but did increase with an increase in moisture. Nitrification then increased with moisture at a faster rate under warm conditions. Under hot conditions, nitrification was much higher with a much faster increase with moisture. These results are showing strong seasonal effects.

The results from this experiment are in line with those of Paul *et al.* (1999) who assessed the effect of moist-dry cycles on soil pH_{Ca} and on processes involving H^+ transformations by incubating surface soils (0-2 cm) from southern NSW. Samples were incubated at 80% field capacity for 7 days at 20°C. The samples were then split into three treatments (air dried, maintained at 80% field capacity or a combination of the two) and incubated for 28 days at 30°C. Paul *et al.* (1999) found for the dry soil some of the ammonium produced by net mineralization was not subsequently nitrified. Acidification in the moist soil was mostly attributed to nitrification. For the other soils, acidification was suppressed due to the decrease of nitrification on exposure to moist-dry cycles. The results from this incubation experiment infer that increased moisture and temperature increase the activity of nitrifying bacteria, particularly in samples from the paddock area. Conyers *et al.* (1995) proposed that changes in temperature and moisture affected changes in microbiological activity as the result a series of incubation experiments in which soil samples (0-10 cm) were incubated in artificial summer conditions (hot days, cool nights and dry) and 30 days of artificial autumn (warm, constant temperature and moist). The incubations were on closed systems without plants to determine and measure processes that could be responsible for changes in soil pH_{Ca} in acidic, mineral soils of the southwestern slopes of NSW Conyers *et al.* (1995).

Soil from only one site (Site 41) was used and because of this, only one insight to paddock or reserve effects. The responses measured may have been different had an alternative site been selected. Even though these results can only be inferred for Site 41, information can still be gained about the nitrification process on the NSW Northern Tablelands and can be related to the effects of seasonal moisture and temperature changes on processes in the field.

6.5 Conclusion

An obvious management effect on nitrogen cycling was apparent in all three incubation experiments. In Incubation 1 it was found that the rate of nitrogen mineralization was independent of the rate of ammonium application and this suggested an absence of a priming effect. No toxic effects were observed. The rate of nitrification was negligible in soil samples from the reserve until towards the end of the incubation period, when some nitrification was observed by way of nitrate-N gain. Nitrification, as a function of the loss of ammonium-N and nitrate-N gain, however, was quite marked in the soils from the paddock. In addition, nitrification was independent of the rate of ammonium application. The rate of application of ammonium amendment of 100 mg/kg and an incubation period of 24 days were selected as the optimum conditions for incubation.

Incubation 2 showed that nitrate-N concentrations generally decreased with increased depth for the paddock soils for both Rate-100 and Rate-0, yet accumulation of nitrate, expressed as net nitrate-N, did not decrease with depth. Soil pH_{Ca} did not affect nitrification in the reserve areas but some effect on nitrification over depth was observed for the paddock. Low nitrification of soils from the reserve suggested very little microbial activity from nitrifying bacteria. Nitrification in the paddock was higher and probably results from increased microbial activity with higher fertility in this area from fertilizer amendments and pasture management using legumes.

Nitrification was limited by low moisture, cool temperatures and management in Incubation 3. Nitrification of a soil from a fertilized, pastured paddock proceeded with warmer temperatures under moist and wet conditions. Nitrification of the paddock soil was negligible for the cold temperature but a small increase was noted with increased moisture. Nitrification was negligible for all temperatures and moisture for the reserve soil. Again, low nitrification of soils from the reserve suggested very little microbial activity and higher nitrification in the paddock from increased microbial activity.

In the NSW Northern Tablelands, paddock pastures undergo senescence in early autumn. A lag effect means the soil is still warm enough for mineralization of this plant organic-N to ammonium-N and subsequent nitrification to nitrate-N. However, at this stage plants are not taking up nitrate-N and so the pool of nitrate-N increases. Nitrification of ammonium to

nitrate is very slow during winter with the cold, dry weather. Plants are dormant and so do not take up nitrate. Nitrate cycling has virtually slowed to a standstill. Thus, the pool of nitrate-N remains static. Then during spring with increasing warmer days and rainfall, pasture plants start actively growing again and take up some of the pooled nitrate-N before it can be leached. This process continues through summer when pasture plants are growing vigorously and the pool of nitrate continues to be used by plants preventing leaching with rainfall. For soils from the reserve areas, a similar process would ensue except that the pool of nitrate would not increase to the extent that it would under the improved paddock pastures.

A different sequence occurs in southern NSW. In that area, dry conditions during summer lead to an accumulation of nitrate. Although plants stop taking up water and nitrate at the permanent wilting point, ammonium mineralizing bacteria and nitrifying bacteria still function, even if slowly. During senescence of annual grasses in late spring and in summer, these plants would not be taking up nitrate. This leads to an increase in the nitrate pool in autumn, which can be leached during autumn and winter rainfall before plants can take up the nitrate during winter and spring growth.