Nitrification in pastures on the Northern Tablelands of NSW

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Abstract
An incubation experiment assessed the effects of pasture management and soil depth on nitrification in grazing land on the Northern Tablelands of NSW. Soils from six paired sites, each comprising a paddock of exotic pasture amended with superphosphate and an adjacent roadside reserve of unfertilized pasture, were used in the experiment. Soil samples to a depth of 20 cm were amended with 6 mL of ammonium sulphate ($(NH_4)_2SO_4$) at the rate of 100mg of NH_4^+-N / kg or 6 mL of deionized water and incubated for 24 days at 25ºC. Results from one of the sites are reported. It was found that nitrification in the paddock was significantly higher than the reserve and consistent with pasture management that provided ideal conditions for nitrifying bacteria and greater microbial activity. Nitrate concentrations decreased with increased depth for the paddock, yet nitrification, net accumulation of nitrate, did not decrease with depth.

Key Words
Soil acidification, acid soil, soil nitrification, incubation, soil depth, grazing management

Introduction

Soil acidification in pastures can result in soil degradation and production losses from aluminium and manganese toxicity, molybdenum, calcium and magnesium deficiencies, and reduced nitrogen fixation. Soils may slowly acidify over time under natural conditions, but agriculture and pasture management can accelerate acidification (Helyar and Porter 1989; Fenton *et al*. 1996; Lockwood *et al*. 2003). Agricultural management practices that can affect soil acidification processes include growing legume pasture species, applying nitrogenous fertilizer and product removal. These practices influence those parts of the nitrogen and carbon cycles that are the major components of acidification processes in the soil. Soil acidification processes in exotic, fertilized pastures are principally loss of nitrate produced by nitrification in leaching or runoff; nitrification of ammonium fertilizers; production of organic acids from a build up of soil organic matter; and export of organic anions with removal of product (Helyar and Porter 1989).

Considerable research into the processes of soil acidification has been undertaken in southern NSW where winter rainfall tends to prevail and agriculturally induced soil acidification is widespread. Processes in the north of the State, with spring or summer-dominated rainfall are less well understood, but it is expected that they will vary from those of the south because of differences in climate and pasture and grazing management. A key process in the nitrogen cycle that has the potential to affect soil acidification is nitrification by which ammonium, mineralized from legume and other organic residues, is oxidized to nitrate. Young *et al.* (2002) observed a decrease in nitrification of added ammonium with increased depth in five surface (0-10 cm) soils collected from sites in southern NSW.

As part of the first full-scale project to assess acidity and acidification in northern NSW, a set of incubation experiments was undertaken. Preliminary results from one of these experiments are reported in this paper. The objective of this experiment was to assess the effects of pasture management and soil depth on nitrification in grazing land on the Northern Tablelands of NSW.

Methods

The Study Area
The Northern Tablelands extend from the Tenterfield area (29ºS, 151º50'E) in the north and south to Nundle (30º25'S, 151º10'E), and are bounded by an escarpment along the eastern boundary and a steep erosion scarp in the west (Harrington 1977; Walker 1977). Altitudes of the tablelands typically range between 700 m and 1 300 m with gentle rolling country and shallow open valleys (Walker 1977). Summers are usually warm and moist, and winters are dry and cool.

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Site Selection and Description

For this experiment six paired sites were selected from a possible 41 sites that had been used in an earlier paired-site survey. Each site comprised a paddock of grazing land of exotic pasture amended with superphosphate and an adjacent unfertilized roadside reserve of native pasture. Soil chemical parameters from the earlier survey were assessed. From this analysis, sites for the experiment were selected if the pasture paddock had at least four times the available phosphorus (Bray) and the pH was less than the reserve. Experimental data from one of these sites are reported in this paper. The site lies 50 km northeast of Armidale and is typical of other sites in this experiment that have granitic soil parent material. Soil at the site is a Mottled Mesonatric Grey Chromosol (Isbell 1996). The site has a long and reliable superphosphate history, an exotic pasture of cocksfoot (*Dactylis glomerata*), ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), and is regularly grazed with beef cattle. Soil chemical data are given in Table 1.

Table 1. Soil chemical properties

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 ($mmol_c/kg$)
Reserve										
0-5	4.6	5.6	0.04	2.0	3.0	1.7	2.8	1.1	0.12	36
5-10	4.6	5.6	0.04	1.5	2.5	1.3	2.4	1.0	0.11	30
10-20	4.7	5.8	0.03	1.0	2.5	1.3	4.3	2.8	0.07	27
20-30	4.9	6.4	0.01	0.4	2.5	1.0	0.9	1.0	0.08	21
30-40	5.2	6.8	0.02	0.3	2.5	1.0	0.5	1.0	0.10	62
40-50	5.2	6.4	0.02	0.3	2.5	1.0	0.7	1.0	0.08	75
Paddock										
0-5	4.7	5.6	0.08	2.7	14.0	7.0	6.8	7.6	0.20	53
5-10	4.6	5.3	0.10	1.7	5.0	11.0	3.9	1.3	0.17	40
10-20	4.7	5.6	0.06	1.3	2.5	5.1	3.1	1.3	0.11	35
20-30	4.9	6.1	0.02	0.5	2.5	2.1	1.1	1.0	0.05	21
30-40	4.8	5.9	0.02	0.3	2.5	1.5	0.8	1.0	0.03	19
40-50	4.7	6.0	0.01	0.3	2.5	1.7	0.8	1.0	0.03	21

Data are from the earlier paired-site survey.

Soil Sampling

Two sample sets, each comprising six cores 20 cm deep, were collected from both the paddock and reserve area in a spatial arrangement of three cores within each of four transects. Two within-area replicates were formed by grouping transects 1 with 2, and 3 with 4. Each core was divided into depth increments (0-2.5, 2.5-5, 5-10, 10-15 and 15-20 cm) and the sub samples bulked by depth interval within each set.

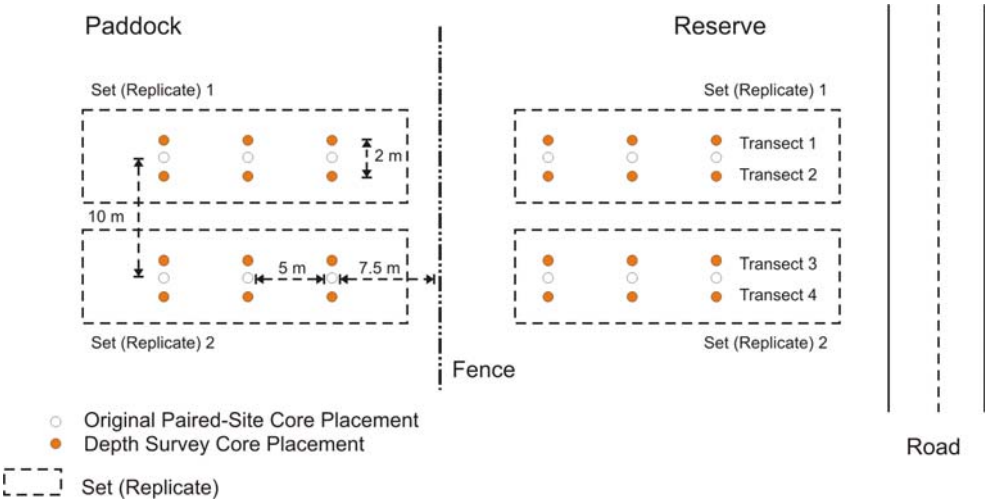

Figure 1. Soil core placement for soil sampling in paired site.

Incubation
Ten grams of each sample, air dried and sieved (< 2 mm), was mixed with 30 g acid-washed sand (0.25-0.5 mm diameter or 30-60 mesh) and placed in an incubation jar. A preliminary incubation was carried out to determine the optimum amendment rate and conditions for nitrification, and these were used in the experiment. The samples were amended with either 6 mL $(NH_4)_2SO_4$ at a rate of 100 mg NH_4-N / kg (Rate-100), or 6 mL of deionized water (Rate-0), and incubated in a constant temperature cabinet at 25ºC for 24 days. Ammonium was added to stimulate nitrification. Every four days the jars were aerated, and every eight days the moisture level checked and deionized water added to replace that lost by evaporation. At the end of the incubation period, the samples were analysed for nitrate-N and ammonium-N to obtain an estimate of the difference in nitrification between paddock and reserve soils at different depths.

Laboratory Methods
Each sample was extracted with 30 mL of 2M KCl and 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 being reduced to ammonium via a cadmium column.

Statistical Analyses
Response variables for nitrate and ammonium concentrations each consisted of 40 data observations, and were classified by two areas (paddock and reserve), two transect replicates, five depths and two rates (Rate-0 and Rate-100) of ammonium applied. This data structure was best analysed using linear mixed models (Verbyla *et al*. 1999) and fitting cubic smoothing splines to the correlated longitudinal data formed by taking repeated measurements over non-independent soil depths. Significance of fixed effects (areas, linear responses over depth, rates, and their interactions) was determined using the Wald statistic. The significance of random terms (factors determining the split plot nature of the sampling protocol, as well as spline components for departures from linearity over depth) was examined by testing for changes in log-likelihood of successive models. A recent application of this statistical approach is given by Lodge *et al*. (2003). It is acknowledged that replication as defined above may be considered "within site" pseudo-replication, and as such, may result in conservative estimates of variability of treatment effects. Consequently, interpretation of results is confined to this one site, and is not extended to any other sites or areas.

Results and Discussion
Ammonium-N concentrations (Table 2) reflect native ammonium already present in the soil; plus net ammonium mineralized from organic matter in the sample; plus any ammonium added as an amendment (Rate-100); and less any ammonium nitrified during the incubation. The net loss of ammonium mineralized to nitrate with incubation was calculated as Rate-100 minus Rate-0 minus 100 amendment adjustment. Positive values for depths 0-10 cm in the reserve (Table 2) show a net gain of ammonium probably mineralized from organic matter. As some ammonium remained in samples from both the paddock and reserve, not all had been nitrified during the incubation.

Table 2. Average concentrations of ammonium-N by rate, area and depth.

	Paddock			Reserve		
Soil Depth (cm)	Rate-0 (mg/kg)	Rate-100 (mg/kg)	Net Gain/Loss (mg/kg)	Rate-0 (mg/kg)	Rate-100 (mg/kg)	Net Gain/Loss (mg/kg)
0-2.5	15.1	62.4	-52.7	7.9	115.0	7.1
2.5-5	6.2	32.8	-73.4	9.2	119.3	10.1
5-10	6.4	63.8	-42.6	9.4	113.3	3.9
10-15	6.3	47.3	-59.0	14.8	110.6	-4.2
15-20	6.4	73.3	-33.1	8.3	103.9	-4.4

Nitrate-N concentrations (Table 3) reflect native nitrate already present in the soil, plus nitrate oxidized from ammonium during incubation. Nitrification for this incubation was calculated from the net accumulation (Rate-100 minus Rate-0) of nitrate. Very little nitrate (< 10 mg/kg) was produced for all depths in samples from the reserve (Table 3), and this was reflected by the high ammonium values for Rate-100 for the same samples (Table 2). For the paddock soil (Table 3; Figure 2), more nitrate was produced than in the reserve.

Table 3. Average concentrations of nitrate-N by rate, area and depth.

	Paddock			Reserve		
Soil Depth (cm)	Rate-0 (mg/kg)	Rate-100 (mg/kg)	Net Gain/Loss (mg/kg)	Rate-0 (mg/kg)	Rate-100 (mg/kg)	Net Gain/Loss (mg/kg)
0-2.5	251.3	299.6	48.3	9.6	13.2	3.6
2.5-5	99.4	168.6	69.2	8.5	11.3	2.8
5-10	62.6	103.2	40.6	11.5	16.4	4.9
10-15	50.9	113.6	62.7	8.9	16.5	7.6
15-20	34.1	63.5	29.4	10.3	16.7	6.4

The figures for the net gain of nitrate-N in Table 3 give a measure of the nitrification induced by the addition of 100mg of NH_4^+-N / kg to the soil sample. For the most part they are broadly similar to the corresponding estimates of nitrification from the loss of ammonium (Table 2). The exception is for the top 10 cm of soil from the reserve which showed net gains of ammonium. These gains suggest that the addition of ammonium had a stimulatory or priming effect on nitrogen mineralization in this soil, producing additional ammonium during the course of the incubation.

Nitrate-N concentrations for Rate-0 and Rate-100 were similar for soil from the reserve, but were significantly different for the paddock soil (Figure 2; Table 4). Nitrate concentration effects changed with depth in a constant manner for the reserve but in a non-linear manner for the paddock (Figure 2). However, the net accumulation of nitrate (Table 3) did not show any consistent trend with depth indicating that nitrification was not affected by soil depth.

Ammonium-N concentrations (Figure 2, Table 4) showed no effect over depth, or with any other factors interacting with depth, for soils from both the paddock and reserve. The effect of rate (Rate-0 versus Rate-100) for ammonium was statistically different for both the reserve and paddock. Rate-0 was the same for both areas and Rate-100 was higher in the reserve than the paddock.

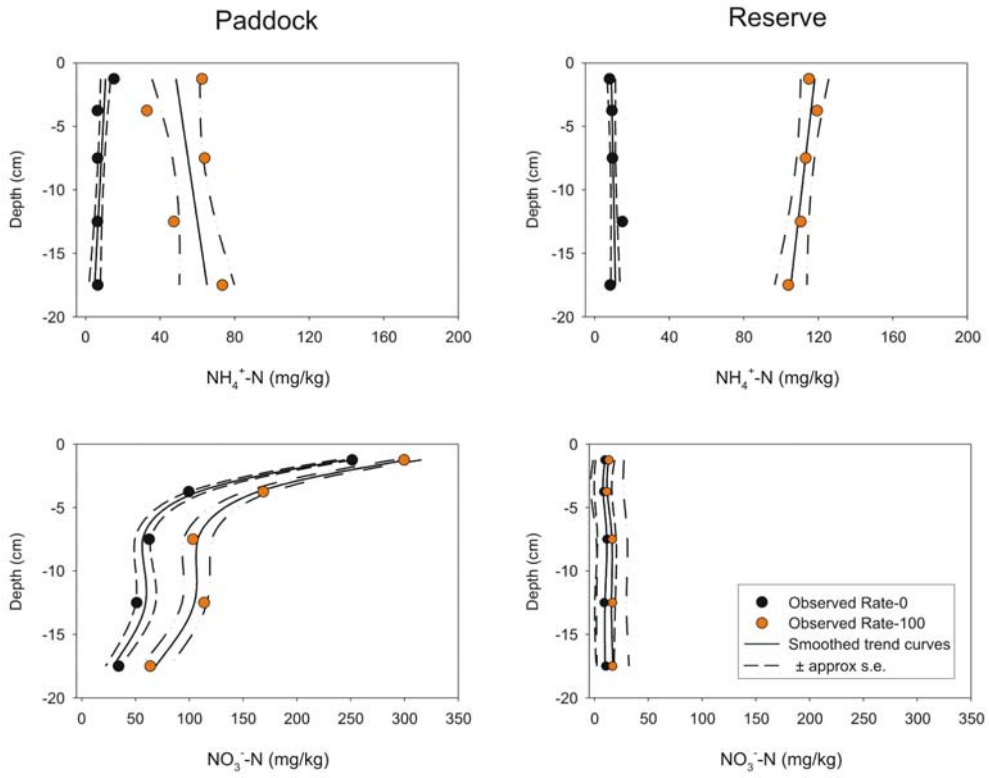

Figure 2. Soil core placement for soil sampling in paired site.

Table 4. Results from analysis of ammonium-N and nitrate-N concentrations

	Ammonium-N		Nitrate-N	
Term	Wald-statistic	*P*-value	Wald-statistic	*P*-value
Area	**18.2**	<0.001	**78.1**	<0.001
Depth	0.0	0.99	**379.4**	<0.001
Depth x Area	0.5	0.47	**402.3**	<0.001
Rate	**364.7**	<0.001	**67.6**	<0.001
Rate x Depth	0.1	0.73	0.7	0.42
Rate x Area	**48.1**	<0.001	**45.2**	<0.001
Rate x Depth x Area	3.0	0.09	1.6	0.21

Treatments with significant (P<0.05) effects are shown in bold font.
Depth effects represent linear responses over the range of soil depths examined.

The data imply that very little oxidization of ammonium to nitrate occurred during incubation for the reserve. For the paddock ammonium oxidation was higher, and nitrification occurred for both Rate-100 and Rate-0. This suggests that nitrate was being produced from both native ammonium and added ammonium, and that microbial activity by nitrifying bacteria was much higher in the paddock than the reserve.

Results from this experiment were consistent with results from the preliminary experiment for the same site that found obvious differences between paddock and reserve probably due to microbial activity. These earlier results indicate that nitrifying bacteria in the samples from the paddock reacted immediately to added ammonium, but it took about 28 days for this to start for the reserve. This suggested a greater population of these bacteria existed in the paddock.

Conclusion

Nitrification in the soils from the reserve was very low and indicative of very little microbial activity and probably a smaller population of nitrifying bacteria. Nitrification in the paddock was higher than the reserve and probably results from higher fertility in the paddock from fertilizer amendments and legumes. Similar results were found for soils incubated from the other sites and these will be reported elsewhere.

Pasture management that includes the growing of legumes would therefore seem to provide better conditions for nitrifying bacteria and greater microbial activity. Nitrate concentrations decreased with increased depth for the paddock soils. Yet nitrification, as calculated from the net accumulation of nitrate, did not decrease with depth. This result differs from that of Young *et al.* (2002). Reasons for this difference are to be investigated and further statistical analyses are to be carried out.

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References

Adamsen FJ, Bigelow DS, Scott GR (1985) Automated methods for ammonium, nitrate and nitrite in 2M KCl-phenylmercuric acetate extracts of soil. *Communications in Soil Science and Plant Analysis* **16**, 883-898.

Fenton G, Helyar K, Abbott T, Orchard P (1996) Soil acidity and liming. Agfact AC.19 (2nd edn). NSW Department of Agriculture, Wagga Wagga.

Harrington HJ (1977) Geology. In 'An Atlas of New England'. (Eds DAM Lea, JJJ. Pigram and L Greenwood) pp.25-46. (Department of Geography, University of New England: Armidale).

Helyar KR, Porter WM (1989) Soil acidification, its measurement and the processes involved. In 'Soil Acidity and Plant Growth'. (Ed. AD Robson) pp.61-101. (Academic Press: Sydney).

Isbell RF (1996) 'The Australian Soil Classification.' (CSIRO Publishing: Collingwood).

Lockwood PV, Wilson BR, Daniel H, Jones MJ (2003) 'Soil acidification and natural resource management. Directions for the future.' (NSW Department of Agriculture: Wagga Wagga).

Lodge GM, Murphy SR, Harden S (2003) Effects of continuous and seasonal grazing strategies on the herbage mass, persistence, animal productivity and soil water content of a Sirosa phalaris – subterranean clover pasture, North-West Slopes, New South Wales. *Australian Journal of Experimental Agriculture*, **43**, 539-552.

Verbyla AP, Cullis BR, Kenward, MG, Welham SJ (1999) The analysis of designed experiments and longitudinal data by using smoothing splines. *Applied Statistics*, **48**, 269-311.

Walker GT (1977) Relief. In 'An Atlas of New England'. (Eds DAM Lea, JJJ. Pigram and L Greenwood) pp.25-46. (Department of Geography, University of New England: Armidale).

Young SR, Black AS, Conyers MK (2002) Distribution of nitrification within surface soils under pasture. *Communications in Soil Science and Plant Analysis* **33**, 1507-1518.