CSIRO Publishing

Australian *Journal* of Soil Research

VOLUME 40, 2002

An international journal for the publication of original research into all aspects of soil science

All enquiries and manuscripts should be directed to:

Australian Journal of Soil Research
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7628
Fax: +61 3 9662 7611
Email: publishing.sr@csiro.au

CSIRO PUBLISHING

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/ajsr

Aust. J. Soil Res., 2002, **40**, 727–735

Nitrification in a Vertisol subsoil and its relationship to the accumulation of ammonium-nitrogen at depth

K. L. Page[A], *R. C. Dalal*[B], *N. W. Menzies*[A], *W. M. Strong*[C]

[A]School of Land and Food Sciences, The University of Queensland, St Lucia, Qld 4072, Australia.
[B]Department of Natural Resources and Mines, Indooroopilly, Qld 4068, Australia.
[C]Department of Primary Industries, Leslie Research Centre, Qld 4350, Australia.

Abstract

Unusually high concentrations of ammonium have been observed in a Vertisol below 1 m depth in south-east Queensland. This study investigated the possibility that an absence of nitrification is allowing this ammonium to accumulate and persist over time, and examined the soil environmental characteristics that may be responsible for limiting nitrifying organisms. The possibility that anaerobiosis, soil acidity, soil salinity, low organic carbon concentrations, and/or an absence of active nitrifying microorganisms were responsible for limiting nitrification was examined in laboratory and field studies. The presence/absence of anaerobic conditions was determined qualitatively using a field test to give an indication of electron lability. In addition, an incubation study was conducted and soil environmental conditions were improved for nitrifying organisms by adjusting the pH from 4.4 to 7, adjusting the electrical conductivity from 1.6 to 0.5 dS/m, amending with a soluble carbon substrate at a rate of 500 mg/kg, and using microorganisms from the surface horizon to inoculate to the subsoil. Over a 180-day period no nitrification was detected in the control samples from the incubation study, indicating that an extremely low rate of nitrification is likely to be responsible for allowing ammonium to accumulate in this soil. Analysis of the effect of soil environmental conditions on nitrification revealed that anaerobic conditions did not exist at depth and that pH, EC, organic carbon, and inoculation treatments added in isolation had no effect on nitrification. However, when inoculum was added to the soil in combination with pH, a significant increase in nitrification was observed, and the greatest amount of nitrification was observed when inoculum, pH, and EC treatments were added in combination. It was concluded that the reason for the low rate of nitrification in this soil is primarily the absence of a significant population of active nitrifying microorganisms, which may have been unable to colonise the subsoil environment due to its acidic, and to a lesser extent, its saline environment.

Introduction

Nitrogen is often one of the most limiting nutrients for plant growth, and consequently, obtaining and maintaining sources of nitrogen in cropping systems is extremely important. On a Vertisol in south-east Queensland, high concentrations of exchangeable ammonium have been observed at depths below 1.2 m. Between 1.2 and 3 m, total concentrations of ammonium-nitrogen in the order of 200 kg/ha have been observed (Hossain *et al.* 1996). If this large reservoir of ammonium can be accessed, for example through the incorporation of deep-rooted species into cropping rotations, it would provide a valuable source of nitrogen for landholders.

In order to be able to successfully access the subsoil ammonium at this site and identify other sites where similar accumulations may exist, it is important to understand how this ammonium has formed. The ammonium at the site is not present under areas of adjacent native vegetation (K. Page, unpubl. data), and so would seem to have formed during the last 60–70 years since the site has been cleared for cropping. However, the original source of the ammonium is unknown, and why the ammonium has been able to build up and persist

 10.1071/SR01087 0004-9573/02/050727

over time, given that nitrification occurs at a rapid rate in most agricultural soils, is also yet to be determined.

The fact that such high concentrations of ammonium are present in this subsoil indicates that nitrifying organisms are likely to be either absent or inhibited by soil environmental conditions. It should be noted that some authors have also speculated that periodic fixation of ammonium in clay interlayers may reduce nitrification rates by protecting ammonium from nitrifying organisms (Corbeels *et al.* 1999). However, it is known that the study soil contains low concentrations of fixed ammonium (<20 mg/kg at 1.5 m; K. Page, unpublished data). Consequently, it was considered that this mechanism would only play a minor role in limiting nitrification, and that either absent or inhibited nitrifying organisms were more likely to be responsible for the ammonium accumulation at this site. Three of the most common environmental conditions found to inhibit autotrophic nitrification are anaerobiosis, soil acidity, and high soil salinity.

Anaerobic conditions most commonly develop in soil environments due to waterlogging. Because oxygen is directly involved in the conversion of ammonium to nitrate, low oxygen or anaerobic conditions will inhibit nitrifying activity. Nitrification has been found to be inhibited at oxygen concentrations below about 0.3 μg/mL (Tate 2000).

Similarly, acidity has also been found to limit nitrifying organisms (Whitehouse and Leslie 1973; Sarathchandra 1978; Bohn *et al.* 1979; Chung and Zasoski 1993; Persson and Wiren 1995). The reason why nitrifiers are inhibited by acidic conditions may be due to direct H^+ or exchangeable Al^{3+} toxicity, or because of deficiencies in elements essential for microbial metabolism (Acea and Carballas 1985). The pH at which nitrification will be inhibited in soils is largely a function of the acid tolerance of the nitrifying population. Where acid-tolerant nitrifying bacteria exist, nitrification can occur at a soil pH as low as 3.4 (Pennington and Ellis 1993). However, in culture environments, nitrifiers not tolerant to acidity are generally found to be completely inhibited below a pH of 5.0–5.5 (Tate 2000).

A further environmental condition that will limit nitrification is high soil salinity (Johnson and Guenzi 1963; Agarwal *et al.* 1971; McClung and Frankenberger 1987; Murase *et al.* 1994; Inubushi *et al.* 1999; Rysgaard *et al.* 1999). All microbial populations are adversely affected by high salt concentrations; however, nitrifiers have been recorded to be especially sensitive to the osmotic stress that saline conditions place on the microbial cell (Johnson and Guenzi 1963; Inubushi *et al.* 1999). This is especially the case where nitrifiers from a naturally low salt environment are exposed to saline conditions (Inubushi *et al.* 1999).

The soil environment of the study site is characterised by an alkaline surface soil horizon (pH 8.6) that is underlain by an acidic subsoil (pH 4.9 at 1.2 m depth) (Dalal *et al.* 1995). The electrical conductivity (EC) of the soil also increases with depth and ranges from 0.24 dS/m at the surface to >1 dS/m at 1.2 m depth. These acidic and relatively saline conditions present in the subsoil have the potential to inhibit nitrification. Low oxygen conditions because of waterlogging at depth, low numbers of autotrophic nitrifying bacteria due to the absence of a consistent supply of ammonium, or low numbers of heterotrophic nitrifiers due to insufficient carbon substrate may also be responsible for limiting nitrification in this subsoil environment.

The objectives of the current study were thus to determine if the ammonium at this site had been able to persist over time due to the absence of nitrification and, if nitrification was negligible, to determine the soil environmental characteristic/s limiting nitrifying organisms.

Materials and methods

Study site

The study site was located at a property near Warra in south-east Queensland (26°47′S, 150°53′E). The site, which was originally under Brigalow (*Acacia harpophylla*) vegetation, was cleared during the mid 1930s and has been used for dryland agriculture, predominantly wheat cropping, ever since. The soil has been classified as a thermic, Typic Chromustert (Dalal *et al.* 1995; Hossain *et al.* 1996). Relevant site characteristics are summarised in Tables 1 and 2. Regular application of nitrogen fertiliser has not occurred at this site.

Soil sampling and processing

Soil sampling was conducted using a hydraulic soil corer (42 mm in diameter to 1.5 m depth and 39 mm in diameter to 3.0 m depth) at 5 different sites evenly spaced along a 100-m transect. Soil cores were collected in 0.3-m depths to 3 m. Sampling was undertaken in an area of native vegetation directly adjacent to the area of cultivation in which the accumulation of ammonium was observed. Soil from the area of native vegetation was used because soil ammonium concentrations were minimal in this area (Table 2), allowing easier control of experimental conditions. All soil samples were stored at 4°C until needed for analysis. For incubation studies, soil was thoroughly mixed by passing through a 5-mm sieve while still in the field-moist state. Soil moisture was determined by drying soil at 105°C for 24 h.

Soil redox capacity

To determine whether the subsoil was sufficiently oxidised to allow nitrification to occur, a field test for redox capacity was conducted at 3 locations in the area of cultivation and native vegetation. At each location

Table 1. Soil profile characteristics of the study site, reproduced from Dalal *et al.* (1995)

Depth (m)	Bulk density (Mg/m^3)	pH	Sand (%)	Silt (%)	Clay (%)
0–0.1	1.24	8.6	27	17	56
0.1–0.2	1.27	8.9	27	16	57
0.2–0.3	1.28	9.0	28	15	57
0.3–0.6	1.36	9.0	25	16	59
0.6–0.9	1.42	7.7	20	17	63
0.9–1.2	1.43	5.3	19	16	65
1.2–1.5	1.45	4.9	19	15	66

Table 2. Summary of various environmental characteristics for areas of cropping and native vegetation

Depth	NH_4 (mg N/kg)		Organic-C (%)		Organic-N (%)		pH		EC (dS/m)	
(m)	Native	Crop	Native	Crop	Native	Crop	Native	Crop	Native	Crop
0–0.3	1.72	2.5	0.9	0.38	0.10	0.05	7.9	8.8	0.4	0.2
0.3–0.6	0.46	0.3	0.33	0.23	0.04	0.03	8.4	8.6	1.2	0.5
0.6–0.9	0.29	0.4	0.25	0.16	0.03	0.02	7.7	7.9	1.5	0.5
0.9–1.2	0.6	2.7	0.25	0.18	0.02	0.02	4.9	5.6	1.5	0.8
1.2–1.5	0.5	14.2	0.18	0.18	0.02	0.01	4.5	4.9	1.5	1.0
1.5–1.8	0.5	18.9	0.13	0.15	0.01	0.01	4.4	4.5	1.6	1.1
1.8–2.1	0.9	16.1	0.11	0.15	0.01	0.01	4.3	4.4	1.6	1.3
2.1–2.4	0.5	11.4	0.14	0.11	0.01	0.01	4.3	4.5	1.7	1.6
2.4–2.7	0.5	8.9	0.12	0.11	0.01	0.01	4.3	4.4	1.7	1.6
2.7–3.0	0.9	4.9	0.09	0.12	0.01	0.01	4.2	4.3	1.7	1.7

soil cores were taken to 1.80 m and a portion of soil between 1.75 and 1.80 m immediately tested for redox capacity using a field test developed by Bartlett and James (1995). This is a qualitative test that places soils into 6 redox categories (ranging from very high to very low electron lability) based on the activity of key oxidising or reducing agents.

Incubation study

Soil between 1.2 and 3.0 m was used for this experiment because it is at this depth that the highest concentrations of exchangeable ammonium are observed in the area of cultivation. Once soil was thoroughly homogenised, 50 g of oven-dry weight equivalent soil was weighed out into 1-L plastic containers. The effects of pH, EC, and soil inoculum on nitrification were examined by applying a series of treatments in a 2-level (i.e. present/absent) factorial design. The pH was increased from 4.4 to 7.0 (±0.5) by using $CaCO_3$, the subsoil was inoculated with surface soil organisms by adding 0.5 g of surface soil to every 50 g of subsoil, and the EC of the soil was reduced from 1.6 to 0.5 dS/m (±0.05 dS/m) by leaching with deionised water. An organic carbon treatment, amended as sucrose at a rate of 500 mg C/kg, was also applied in an attempt to determine if heterotrophic nitrification would occur in this soil. The carbon treatment was not included in the factorial design with pH, EC, and inoculum. All treatments were applied to individual samples except for the EC treatments, where leaching was conducted on bulk soil and then separated out into individual samples. Each treatment was replicated 5 times, with soil from each sampling location representing a replication.

An ammonium solution was added to all samples to bring total ammonium concentration in the soil to 50 mg N/kg, and soil moisture was increased to field capacity [initial soil ammonium concentrations were <1 mg/kg (Table 2)]. The tops of the incubation jars were covered with plastic film and pierced several times with a needle to allow gas exchange. Soil was then incubated in the dark at 22°C and 70% humidity and checked 2 times each week to replace any moisture loss.

Destructive samplings were conducted at 20, 60, and 180 days and nitrate was measured. Nitrate concentrations were negligible at the commencement of the experiment in all samples.

Analytical methods

Exchangeable ammonium and nitrate were extracted by shaking soil (moist) for 1 h in a 2 M KCl solution [1 : 4 soil : solution ratio (Buresh *et al.* 1982)]. Ammonium was analysed using a colorimetric method based on the indo-phenol blue technique (Henzell *et al.* 1968), and nitrate-nitrogen was reduced to nitrite with hydrazine and a copper catalyst and the total nitrite produced measured using a procedure based on the Greiss-Ilosvay reaction as described by Bremner (Bremner 1965). Both ammonium and nitrate analyses were conducted using an automated analysis system. Electrical conductivity and pH were measured from a 1 : 5 soil : water extract (1 h shaking period) using a conductivity meter (Radiometer Model CDM83) and a glass/reference combination electrode (Radiometer GK2401C), respectively. Organic carbon was measured using the Walkley-Black procedure (Walkley and Black 1934) with a colorimetric finish (Sims and Haby 1971). Total nitrogen was extracted using a Kjeldahl digestion and the ammonium produced measured colorimetrically (Crooke and Simpson 1971). All results are reported on an oven-dry weight equivalent basis.

Statistical analyses

A 2 × 2 × 2 factorial analysis (pH × inoculation × EC) was used to test for interactions between treatments in the incubation experiment. One-way ANOVA analysis was used to test for changes in the nitrate concentration of control samples between the 20, 60, and 180 day time periods and for any difference between control samples and those amended with organic carbon. Analyses were conducted using the SAS statistical package (SAS 1999) and differences considered significant if $P < 0.05$.

Results and discussion

Redox capacity

Results from the redox capacity testing are summarised in Table 3. Results from samples analysed from both the area of cultivation and native vegetation were identical. The blue/green colour that was produced when the soil was exposed to a tetramethylbenzidine (TMB) solution is indicative of a 1-electron oxidisation of TMB, usually by Mn oxides (Bartlett and James 1995). A negative test for chromium oxidisation was observed, and

Table 3. Results of analyses conducted during field tests for redox capacity at 1.8 m depth

Test	Result
Tetramethylbenzidine (TMB) oxidation	Positive (blue/green colour)
Chromium oxidation	Negative
Ferrous iron test	Negative
Easily reducible iron	Positive
Reduced odours	Negative
pH	4.5

indicates that the soil did not contain components that were capable of oxidising the added Cr(III) to Cr(VI). However, soil acidity can interfere with the oxidisation of Cr(III) (Bartlett and James 1995). The presence of ferrous iron, reduced odours, and a neutral pH is diagnostic of soils that are highly reduced, and the absence of these conditions indicates that highly reduced conditions were not present in this soil. The positive test observed for easily reducible iron indicates that the soil did have some reducing potential; however, the ability of the soil to oxidise the TMB solution indicates that the oxidising propensity of the soil was greater than the reducing propensity (Bartlett and James 1995).

Considering the above results, the Warra subsoil at 1.8 m depth can be classified as a 'suboxic' soil. Suboxic soils are defined as those that have a medium level of electron lability and whose 'reducing tendency is balanced against oxidising propensity'. They are also soils that can be described as '…highly oxidised (with) nitrates present …' (Bartlett and James 1995). This result indicates that at the time of sampling, it is unlikely that there would have been any major barrier for nitrification because of the presence of anaerobic conditions.

It should be noted that while the oxidation status of the subsoil at the time of sampling is unlikely to have limited nitrification, it is possible that this oxidation status may decrease during periods of profile saturation. It is also possible that saturated aggregates could exist within the profile, and that nitrification rates in these areas may be inhibited due to low oxygen concentrations. One study examining nitrification at depth in another Vertisol soil, for example, proposed that aggregate saturation caused by soil shrinkage slowed nitrification rates and allowed ammonium accumulation to occur (Corbeels *et al.* 1999). However, while it is possible that low oxygen concentrations may sometimes operate to limit nitrification in this soil, the results discussed below, combined with the fact that the soil is not permanently reduced, would suggest that this is not the primary mechanism inhibiting nitrification at this site.

Incubation experiment

Analysis revealed that there was no significant difference ($P > 0.05$) between the nitrate concentration of control samples at the 20, 60, and 180 day time periods, and that the nitrate concentrations of these samples were always <1 mg/kg. This provides strong evidence that the rate of nitrification in this soil is extremely low, and provides some explanation as to why the ammonium at this site has been able to accumulate. At the 180-day sampling period, analysis of treatment effects on nitrate concentration revealed that, in isolation, inoculum, pH, EC, and organic carbon treatments had no significant effect ($P > 0.05$) on the production of nitrate. However, factorial analysis revealed that in combination, pH and inoculum treatments did significantly increase nitrate concentration, and when pH, EC, and

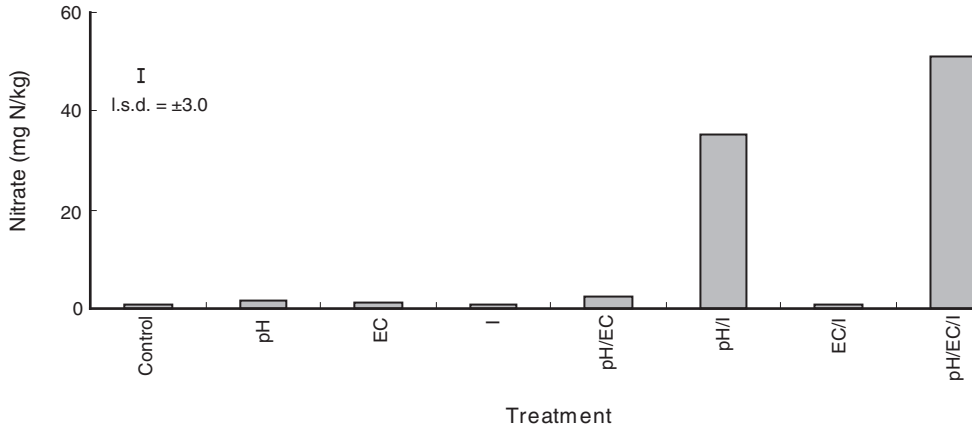

Fig. 1. Nitrate concentrations (mg N/kg) for pH, electrical conductivity (EC), inoculation (I) treatments and their combinations at the 180-day time period. Height of the bar for l.s.d. shows the significant value at $P = 0.05$.

inoculum treatments were added in combination, a further significant increase in nitrate production was observed (Fig. 1).

The lack of any significant effect on nitrate concentration when pH, EC, organic carbon, and inoculum treatments were added to the soil in isolation indicates that no single environmental effect is responsible for limiting nitrification in this soil. The lack of any organic carbon effect also indicates that it is unlikely that a significant population of active heterotrophic nitrifiers is present. Significant populations of heterotrophic nitrifiers have been observed to function in some acidic soils (Adams 1986; Vasilenko *et al.* 1993; Brierley *et al.* 2001), and in culture environments some heterotrophs have been observed to conduct nitrification via an inorganic pathway under acidic conditions (Vasilenko *et al.* 1993). Therefore, the possibility that heterotrophic nitrifiers are present at this site, but that they have been unable to oxidise the accumulated ammonium due to low concentrations of organic carbon, cannot be discounted (Table 2). However, the fact that no significant increase in nitrate concentration was observed when organic carbon and ammonium were added in combination would indicate that a large heterotrophic population capable of oxidising ammonium are unlikely to be present in this subsoil (Fig. 1).

The significant increases in nitrate concentration that were observed when inoculum/pH, or inoculum/pH/EC treatments were added to the soil indicate 3 things (Fig. 1). Firstly, the fact that nitrification was absent unless the soil was amended with inoculum indicates that an active autotrophic nitrifying population must be largely absent from the subsoil. Secondly, the fact that nitrification was only observed when inoculum was added to soil where the pH had been increased from 4.4 to 7.0 indicates that the nitrifying organisms contained within the inoculum used were inhibited by acidic conditions. Thirdly, the fact that a greater increase in nitrate concentration was observed when inoculum was added in combination with pH and EC treatments, rather than with pH in isolation (Fig. 1), indicates that the microorganisms used as inoculum were also somewhat inhibited by the saline conditions at depth. The failure to observe any nitrification when inoculum/EC treatments were added without increasing pH, however, indicates that the inoculum was primarily limited by the acidity of the subsoil.

The effect that subsoil pH and salinity had on nitrifying activity can be attributed to the fact that the organisms used to inoculate the subsoil were obtained from the top 0–5 cm of the soil profile. This section of the soil profile is characterised by a pH of 6.5 and an EC of ~0.4 dS/m. Given the numerous reports of nitrifier inhibition due to low pH (Whitehouse and Leslie 1973; Sarathchandra 1978; Bohn *et al.* 1979; Chung and Zasoski 1993; Persson and Wiren 1995), and soil salinity (Johnson and Guenzi 1963; Agarwal *et al.* 1971; McClung and Frankenberger 1987; Murase *et al.* 1994; Inubushi *et al.* 1999; Rysgaard *et al.* 1999), it is not surprising that, when microorganisms from this horizon were exposed to a pH of 4.4 and an EC >1.5 dS/m, nitrifying activity was inhibited until pH and EC conditions were amended to more closely represent those observed in the surface soil.

While the inhibition of autotrophic nitrifying organisms due to adverse pH and EC conditions is commonly recorded, a complete lack of active nitrifying organisms in a soil is less frequently observed. The fact that a subsoil was used in this study should not in itself be a reason for an absence of nitrifying activity. Active nitrification has been observed in subsoils at depths of 50 cm (Persson and Wiren 1995), 115 cm (Weier and MacRae 1993), and even at 230 cm (Swensen and Bakken 1998). However, although unusual, the complete absence of nitrifying organisms in soil has been reported. In some native soils in Spain, for example, microbiological analysis revealed a complete absence of autotrophic nitrifiers, despite the presence of an active overall microbiological population (Acea and Carballas 1985).

Indeed the reason for the apparent absence of active autotrophic nitrifying microorganisms in this subsoil may be related to the neutral to alkaline pH and relatively low EC of the surface soil horizon. Given the obvious intolerance of the surface soil nitrifiers to acidity and salinity, it is possible that these microorganisms have been unable to colonise the subsoil because of the environmental conditions present in this area. Although autotrophic nitrifiers tolerant to acidity (Pennington and Ellis 1993) and salinity (McClung and Frankenberger 1987) have been observed to develop in naturally acid and saline environments, it may have been difficult for such populations to develop in a subsoil overlain by a neutral to alkaline and low EC surface soil. This difficulty would have been further accentuated by the fact that elevated concentrations of subsoil ammonium do not occur under areas of adjacent native vegetation (Table 2). This indicates that it is only since the site has been cleared (i.e. within the last 60–70 years) that the ammonium at this site has formed, and consequently, it is only within this time period that excess ammonium would have been present to provide an incentive for nitrifiers to move into the subsoil. It should be noted that it is possible that diversification of the nitrifying population may have begun to occur in response to the extra ammonium source under the area of cultivation, but that this would not have been detected in the current study because only soil from the area of native vegetation was used during experiments. However, the current high concentrations of ammonium present in the profile under the area of cultivation (Table 2) would suggest that this diversification has not yet occurred to any great extent.

Conclusions

The results of this study indicate that subsoil ammonium has been able to accumulate and persist over time at this site because of an absence of any significant rate of nitrification. The lack of nitrification is attributed to an absence of an active nitrifying population. Such a population may have been unable to establish in the subsoil due to inhibition from subsoil acidity, and to a lesser extent, subsoil salinity. This absence of nitrification indicates that the accumulation of ammonium at this site is likely to be relatively stable and to persist in

the short to medium term. However, the actual source of the ammonium is yet to be identified. Resolution of this issue is highly desirable as it will allow easier identification of other sites that are likely to contain subsoil ammonium and will give an indication of whether the accumulation of ammonium at this site is likely to remain stable or increase over time.

References

Acea MJ, Carballas T (1985) First results of a study of the microbial population of humid zone granitic soils. *Anales de Edafafologia y Agrobiologia* **44**, 395–411.

Adams JA (1986) Identification of heterotrophic nitrification in strongly acid larch humus. *Soil Biology and Biochemistry* **18**, 339–341.

Agarwal AS, Singh BR, Kanehiro Y (1971) Ionic effects of salts on mineral nitrogen release in an allophanic soil. *Soil Science Society of America Proceedings* **35**, 454–457.

Bartlett RJ, James BR (1995) System for categorising soil redox status by chemical field testing. *Geoderma* **68**, 211–218.

Bohn HL, McNeal BL, O'Connor GA (1979) 'Soil chemistry.' 1st edn (John Wiley & Sons, Inc.: New York)

Bremner JM (1965) Inorganic forms of nitrogen. In 'Methods of soil analysis'. (Ed. CA Black) (American Society of Agronomy: Madison, WI)

Brierley EDR, Wood M, Shaw PJA (2001) Influence of tree species and ground vegetation on nitrification in an acid forest soil. *Plant and Soil* **229**, 97–104.

Buresh RJ, Austin ER, Craswell ET (1982) Analytical methods in ^{15}N research. *Fertilizer Research* **3**, 37–62.

Chung JG, Zasoski RJ (1993) Effect of high ammonium levels on nitrification, soil acidification, and exchangeable cation dynamics. *Communications in Soil Science and Plant Analysis* **24**, 2123–2135.

Corbeels M, Hofman G, Van Cleemput O (1999) Soil mineral nitrogen dynamics under bare fallow and wheat in vertisols of semi-arid Mediterranean Morocco. *Biology and Fertility of Soils* **28**, 321–328.

Crooke WM, Simpson WE (1971) Determination of ammonium in Kjeldahl digests of crops by an automated procedure. *Journal of the Science of Food and Agriculture* **22**, 9–10.

Dalal RC, Strong WM, Weston EJ, Cooper JE, Lehane KJ, King AJ, Chicken CJ (1995) Sustaining productivity of a Vertisol at Warra, Queensland, with fertilisers, no-tillage, or legumes 1. Organic matter status. *Australian Journal of Experimental Agriculture* **35**, 903–913.

Henzell EF, Vallis I, Lindquist JE (1968) Automatic colorimetric methods for the determination of nitrogen in digests and extracts of soil. *Transactions, 9th International Congress of Soil Science* **3**, 513–520.

Hossain SA, Dalal RC, Waring SA, Strong WM, Weston EJ (1996) Comparison of legume-based cropping systems at Warra, Queensland. I. Soil nitrogen and organic carbon accretion and potentially mineralisable nitrogen. *Australian Journal of Soil Research* **34**, 273–287.

Inubushi K, Barahona MA, Yamakawa K (1999) Effects of salts and moisture content on N_2O emission and nitrogen dynamics in Yellow Soil and Andosol in model experiments. *Biology and Fertility of Soils* **29**, 401–407.

Johnson DD, Guenzi WD (1963) Influence of salts on ammonium oxidation and carbon dioxide evolution from soil. *Soil Science Society of America Proceedings* **67**, 663–666.

McClung G, Frankenberger WT (1987) Nitrogen mineralization rates in saline *vs*. salt-amended soils. *Plant and Soil* **104**, 13–21.

Murase J, Topark-Ngarm B, Adachi T, Losirikul M, Kimura M (1994) Studies on saline soils in Khon Kaen region north-east Thailand V. Nitrification and nitrifying bacteria. *Soil Science and Plant Nutrition* **40**, 173–177.

Pennington PP, Ellis RC (1993) Autotrophic and heterotrophic nitrification in acidic forest and native grassland soils. *Soil Biology and Biochemistry* **25**, 1399–1408.

Persson T, Wiren A (1995) Nitrogen mineralization and potential nitrification at different depths in acid forest soils. *Plant and Soil* **168–169**, 55–65.

Rysgaard S, Thastum P, Dalsgaard T, Christensen PB, Sloth NP (1999) Effects of salinity on NH_4 adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* **22**, 21–30.

Sarathchandra SU (1978) Nitrification activities of some New Zealand soils and the effect of some clay types on nitrification. *New Zealand Journal of Agricultural Research* **21**, 615–621.

SAS (1999) 'SAS Version 8.' (SAS Institute Inc.: Cary, NC)

Sims JR, Haby VA (1971) Simplified colorimetric determination of soil organic matter. *Soil Science* **112**, 137–141.

Swensen B, Bakken LR (1998) Nitrification potential and urease activity in a mineral subsoil. *Soil Biology and Biochemistry* **30**, 1333–1341.

Tate RL (2000) 'Soil Microbiology.' 2nd edn (John Wiley & Sons: New York)

Vasilenko YS, Il'ina TK, Dokuchayev VV (1993) Heterotrophic nitrification during decomposition of ^{15}N-labeled fulvic acids in sod-podzolic soils. *Eurasian Soil Science* **25**, 71–78.

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37**, 251–264.

Weier KL, MacRae IC (1993) Net mineralization, net nitrification and potentially available nitrogen in the subsoil beneath a cultivated crop and a permanent pasture. *Journal of Soil Science* **44**, 451–458.

Whitehouse MJ, Leslie JK (1973) Movement, pH effect and nitrification of band-applied anhydrous ammonia, urea and sulphate of ammonia in an alkaline black earth. *Queensland Journal of Agricultural and Animal Sciences* **30**, 301–310.

Manuscript received 19 September 2001, accepted 31 January 2002