

# Controls on inorganic N species transformations and potential leaching in freely drained sub-soils of heavily N-impacted acid grassland

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**Abstract** In N-polluted, minimally managed soils, recent research has shown that ammonium-N, as well as nitrate-N, may move down through soil profiles. There is a need, therefore, to reassess what controls N species transformations below the rooting depth in such soils. The changes with depth down to 1 m in extractable ammonium-N and nitrate-N concentrations have been studied for two heavily N-impacted acid grassland soils near York, UK, for fresh soils and for soils incubated after either deionised water (d.w.) or ammonium-N spiking. Although in close proximity, the profiles showed marked differences in their relationships of ammonium- and nitrate-N to soil pH, C%, N% and C:N ratio. One profile was slightly more acidic at the surface, which seems to have changed the distribution of organic matter throughout the soil profile. Trends in ammonium production with depth were clearer when ammonium-N concentrations were recalculated on the basis of ammonium-N per unit mass of soil organic C. This then allowed a consistent trend with soil pH to be discerned for both profiles. Ammonium-N spiking showed that ammonium

substrate availability was limiting potential net nitrification rate at 20–60 cm depth for both profiles. Potential mineral-N production was considerable at depth, which would facilitate transport of N to surface waters and/or groundwaters.

**Keywords** Ammonium · Mineralization · Nitrate · Nitrification · Soil depth · Soil nitrogen

## Introduction

It is widely accepted that anthropogenic activities such as fossil fuel utilization, industrial N fixation for commercial fertilizer production and increased managed biological nitrogen fixation have altered the N cycle, doubling the amount of reactive N in the atmosphere (Galloway et al. 1995; Vitousek et al. 1997). Anthropogenic N deposition is of particular concern for low fertility status, semi-natural ecosystems due to its potential threat to their long-term stability and floristic diversity (Phoenix et al. 2003).

Nitrogen deposition can modify soil processes, including mineralization and nitrification dynamics, and may result in decreased soil pH, reduced buffer capacity, increased leaching of base cations, increased availability of  $\text{Al}^{3+}$  and  $\text{Fe}^{2+}$  and increased concentrations of other toxic metals (Fog 1988; Goulding et al. 1998; Aerts and Bobbink 1999). According to Hornung and Langan (1999), soil

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nitrification and N-immobilization are the key biochemical pathways dictating ecosystem responses to elevated atmospheric N inputs. As a net result of how these two processes respond to N additions, extra reactive N may be mobilized through the environment, potentially causing acidification in both terrestrial and aquatic ecosystems and eutrophication in freshwaters where P is not limiting (Goulding et al. 1998; Metcalfe et al. 1999; Edwards et al. 2000; Edwards and Withers 2007). Correlations between N deposition, N nutrition and N leaching have been well documented for a wide range of sites and nitrogen deposition rates (Jussy et al. 2004).

In spite of a general conclusions that N saturation occurs in upper soil horizons since the concept was introduced (Aber et al. 1989), scant attention has been paid to processes in subsoils. Soil organic matter (SOM) and its associated organic N has the potential to mineralize throughout agricultural and forest soil profiles down to depths of 1 m or even more, however (Cassman and Munns 1980; Powers 1980; Stevenson 1982; Hadas et al. 1986; David et al. 1996; Krug and Winstanley 2002; Vestgarden and Kjønaas 2003; Cresser et al. 2008; Riaz et al. 2008). Krug and Winstanley (2002) comprehensively reviewed soil N cycling and stressed the need for better understanding of vertical variations of N transformations in soil profiles as the extensive N mineralized below 30 cm depth is available for plant uptake and/or leaching into aquatic ecosystems. Limited study of N transformations in subsoils is perhaps surprising, as subsoils in many areas are now receiving higher N inputs throughout the year than they did in the past, for both agricultural and minimally managed ecosystems (Cresser et al. 2008; Riaz et al. 2008).

Soil physico-chemical properties can markedly affect soil N transformation dynamics at any depth in the profile. For example soil pH is widely considered as the most important factor governing nutrient bioavailability, plant primary productivity, and a range of soil microbial processes, and nitrification is highly pH sensitive (Kemmitt et al. 2006). Nitrification rates in soils usually are very variable however, depending upon nitrifying population and environmental factors including pH, but also soil moisture content, temperature, substrate concentration and oxygen availability (Krave et al. 2002). Both soil C and N and microbial biomass C and N may be effective predictors for mineral-N production rate

(Booth et al. 2005). The C:N ratio of surface soil organic matter may have potential as an indicator of nitrogen saturation and subsequent leaching from some systems, but not all (Curtis et al. 2003). There is considerable evidence that net mineralization and nitrification rates (and hence inorganic N leaching) are strongly influenced by soil N and/or C:N ratio (Wilson and Emmet 1999).

Many studies have emphasized the potential for increased inorganic N leaching as a consequence of N deposition, but the detailed mechanisms have not yet been fully explained. Cresser et al. (2004) postulated that in heavily N-polluted areas of the UK, especially those with acidic, peaty organic soils, ammonium-N transformation rates are slow enough for ammonium inputs to reach equilibrium with cation exchange sites. Under this scenario, ammonium concentrations in soil solutions and drainage water will be similar to those in precipitation, with modification due only to changes in the mobile anion concentrations. If leaching of nitrate and ammonium out of soils to streams occurs, then N species translocation to depth in soil profiles is also probable, narrowing the C:N ratio at depth which could be indicative of soil microbial immobilization of these species (Bengtsson et al. 2003).

The patterns of surface water nitrate-N concentrations in areas not affected by agricultural runoff mostly reflect total atmospheric N deposition, with highest concentrations in surface waters being coupled with elevated N deposition concentrations (INDITE 1994). Chapman and Edwards (1999) noted that elevated concentrations of nitrate have been found in both streams and lakes of northern Europe and the northern United States that strongly correlate with increased N deposition from the atmosphere. When the system is unable to cope with increased N from the atmosphere, soil N saturation and N leaching occurs (Aber et al. 1989). Nitrate leaching has been reported in laboratory experiments with N-treated soils from the Chronic N Amendment Study (Venterea et al. 2004). Little attention seems to have been paid to the potential contribution that ammonium leaching down soil profiles may make to nitrification at depth and nitrate leaching however, except by Riaz et al. (2008).

The present study, therefore, aimed to investigate, for a supposedly low nutrient status acid grassland soil, the hypotheses that:

1. N mineralization can occur at depth (below 60–100 cm) in soils from N impacted acid grasslands and result in higher N mobility in freely drained acid soils below the rooting zone.
2. Nitrate-N and/or ammonium-N can be produced in situ at depth, but also can potentially be mobilized to deeper soil layers from overlying soil horizons.
3. Soil physico-chemical properties can alter the N species transformation dynamics.
4. Bearing in mind that substrate availability is key factor, then changes in ammonification with depth will depend upon distribution of soil organic matter with depth as well as on the soil physico-chemical properties.

## Materials and methods

### Description of site

Soil profiles studied were from freely draining acid grassland at Hob Moor, near York, UK (53°57'30"N and 1°4'48"W, see Fig. 1), a Local Nature Reserve with 36.4 ha of predominantly unimproved grassland. The soils on the reserve vary between slowly permeable clay loams and more freely draining (and more acidic) very fine sandy loams and loamy sands. Moorsome and Redbrow are the dominant soil series (Bendelow and Carroll 1985). Redbrow soil series was sampled for this study. The site is dominated by seasonally wet pastures on the more heavily textured soils and a smaller area of freely draining acid grassland, with small peripheral patches of deciduous woodland. Management of the reserve attempts to maintain the soils at low nutrient status and retain high biodiversity of flora and fauna, birds and small mammals. The pasture is grazed by cattle imported for fattening for approximately 6 months every year in an attempt to reduce the nutrient status of the soil.

### Nutrient status history of site

The site has been affected by anthropogenic N and S deposition over recent decades (NEGTA 2005; UK Emissions Inventory Team 2008). No synthetic fertilizer additions have been made, so N status down soil profiles should reflect any disturbance of the natural balance of N cycling in response to high levels of N deposition in this part of the UK (Hornung

et al. 1995; NAEI 2007). The total N deposition was around 25 kg N/ha/year in the late 1990s, consisting of 4.6–6.2 kg of nitrate-N and 5.5–7.5 kg of ammonium-N in precipitation, 2.1–4.2 kg of N as NO<sub>2</sub>, and 2–4 kg of dry deposited ammonia-N (NEGTA 2005). A stagno-gley argillic brown earth profile from Hob Moor has been shown to contain 12.5 tonnes of N per hectare to 35 cm depth (Crowe et al. 2004), and the C:N mass ratio in that profile was always <10.0 over the depth intervals studied (0–35 cm).

The Holgate stream runs along two sides of the site, and the management strategy should reduce the risk of nitrate leaching into the surface water. However, over at least the past 8 years the authors have observed that the stream has been susceptible to eutrophication except where heavily shaded, supporting extensive aquatic plant growth that potentially can hinder the local drainage system. Nitrate-N concentrations in the stream sampled, only in February each year since 2001, ranged from 0.2 to 7.8 mg l<sup>-1</sup> (Riaz et al. 2008).

### Climate

The climate is changeable, with sunny summer months (June–September) with a mean temperature of 18–21°C. November to January are the coldest and wettest months. The precipitation pattern is also seasonally variable. Of the 639 mm annual rainfall, October–November receives 34.7%. The variation in temperature is also prominent around a mean monthly temperature of 13.5°C. June and July are warmest, with an monthly average temperature of 21°C. The mean monthly relative humidity is 80.3% (The Meteorological Office 2006).

### Soil sampling and preparation

The soils were sampled from the permanent grassland at two points close to soil profile 2 previously described by Riaz et al. (2008). Two soil profiles (A and B) were selected from the freely drained acid grassland area. When sampled down to 100 cm, these profiles provided substantial variation in soil physical and chemical properties. This was necessary to be able to assess how soil properties might be influencing N transformation dynamics over a range of soil depths.



**Fig. 1** A simplified location map and map of Hob Moor, near York, the UK. Ordnance survey<sup>©</sup> Crown copyright 2008. The profiles were in the N.E. corner of the moor

In May, 2007, surface vegetation and litter were removed from an untrampled face with a sharp knife with minimal disturbance to underlying surface soil layers. Two soil pits were dug to a depth of 1 m and soil was sampled below the litter with a pointed trowel in duplicate from five sampling depths at 20 cm increments, i.e. from 0 to 20, 20 to 40, 40 to 60, 60 to 80 and 80 to 100 cm from each soil profile. The soil samples were stored in sealed pre-labeled clean polythene bags to minimize loss of moisture, and brought immediately back to the laboratory.

Stones and roots were removed rapidly from each soil sample by careful hand picking using rubber gloves to minimize contamination. Each sample was then thoroughly mixed and immediately divided into two halves. One half was used to measure soil pH, moisture content, and initial KCl-extractable  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N, and hence total mineral-N, on the same day. The oven dried sub-samples after measurement of moisture content were then used to measure C%,

N% and C:N ratio. The second half of each sample was used for spiking and incubation experiments to study N species transformations of native soil N and ammonium-N spikes over the incubation period under controlled conditions. Whenever not needed immediately, soil samples were stored in a refrigerator at  $<4^\circ\text{C}$ .

#### Soil physico-chemical analyses

Soil pH was measured in duplicate for each field-moist soil sample in 0.5 M KCl at a 1:2 m:v (soil:solution) ratio after periodic agitation and equilibration of the suspension for 30 min at room temperature. This is more concentrated than the KCl or  $\text{CaCl}_2$  usually employed, as it was deemed important to ensure the soil pH measurement reflected differences between horizon acidities with minimal variation caused by mobile anion effects. The pH was measured with a glass/calomel electrode

and pre-calibrated Thermo Orion pH meter (Model 420).

Soil moisture content was measured in duplicate gravimetrically using a pre-heated oven to dry soil samples at 105°C overnight and moisture contents were expressed as a percent of oven-dry mass.

Soil organic C%, N% and C:N ratio of each sample were determined in duplicate using ball-milled, oven-dried soil samples with an Elementar Vario Macro, automated C and N Analyser calibrated with glutamic acid.

A fresh sub-sample of each field-moist soil was used to find initial concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. The 10.0 g soils were extracted in duplicate with 100 ml of 0.5 M KCl by shaking at 150 rpm for 1 h and filtering the extracts through Whatman No. 42 filter papers. The extracts were analyzed for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N on a Bran & Luebbe Auto-Analyser-3 using a standard manifold and matrix-matched standards. The results were corrected for reagent blanks, and final results were calculated on an oven dry soil basis as  $\text{mg N (kg soil)}^{-1}$ .

### Spiking experiments

An incubation experiment was designed to study the potential soil N transformation processes with depth down to 100 cm in the two soil profiles. The design involved two treatments, i.e. spiking with deionised water (d.w.) or with a known amount of ammonium-N in the form of  $(\text{NH}_4)_2\text{SO}_4$  in the same amount of water. Spiking with d.w. aimed simply to study the N species changes under controlled conditions, while spiking with ammonium-N was conducted primarily to investigate responses of the native N cycle to receiving artificial input of reduced nitrogen and whether nitrification was substrate limited. On the day of soil sampling, 10.0 g field-moist sub-samples were weighed in duplicate into two sets of 20 cleaned and labeled 125-ml plastic bottles. To each soil sample of the first set, 2.0 ml deionised water was added with a pre-calibrated Gilson pipette, while the second set of soil samples was spiked with 2.0 ml of  $(\text{NH}_4)_2\text{SO}_4$  solution containing 25  $\mu\text{g NH}_4\text{-N/ml}$  so each soil sample received 50  $\mu\text{g NH}_4\text{-N}$ . The samples were incubated for 1 week in loosely capped bottles to keep them well-aerated to facilitate aerobic microbial N transformations. The bottles plus samples were weighed to  $\pm 0.01$  g at the beginning of the

experiment and re-weighed periodically to check for any significant loss of moisture. Lost moisture was replenished by adding deionised water to restore initial mass of soil samples to maintain constant moisture conditions during incubation.

After incubation, the soils were extracted with 100 ml of 0.5 M KCl using an orbital shaker at 150 rpm for 1 h. The suspensions were filtered through Whatman No. 42 filter papers into clean, pre-labeled plastic bottles. The extracts were kept in a refrigerator at  $<4.0^\circ\text{C}$  until analyzed for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. The values for mineral N were calculated from the sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. Potential net N mineralization was calculated from the difference between post and pre-incubated mineral-N for d.w. spiked soils. Net nitrification was estimated from the difference between post and pre-incubated  $\text{NO}_3^-$ -N values (Satti et al. 2003). The net N mineralization rate was estimated by dividing net potential N mineralisation by incubation period (7 days) and net nitrification rate was calculated from dividing net nitrification by incubation duration (Hart et al. 1994).

### Statistical analysis

One-way ANOVA was used to test the significance of chemical differences between the sampling depths and between treatment effects. The Tukey HSD post-hoc test ( $\alpha = 0.05$ ) was used for the Multiple Mean Comparison technique. An independent sample *t*-test was used to test for the significance of differences in net nitrogen mineralization and net nitrogen nitrification for d.w.-spiked and ammonium-N-spiked (nitrification only) soil samples at selected depths, without assuming equal variance as Leven's test for equality of variance was significant at  $p < 0.05$ . Pearson correlation was used to study the nature of relationships between soil physico-chemical properties and N species concentrations.

All statistical analysis was performed using SPSS 15.0 for Windows software.

## Results

### Physical and chemical characteristics of soils

There were significant differences in pH, moisture content, and C and N content with depth for each



soil profile and between soil profiles (Table 1). Soil profile B was more acidic at 0–40 cm than profile A, and its pH changed more steeply with depth. Profile A was more acidic below 40 cm, and remained very acidic even at 100 cm depth with a pH value of 4.04. Systemic multiple comparison using Tukey's HSD post-hoc technique ( $\alpha = 0.05$ ) showed that soils from different sampling depths differ significantly in pH for both profiles except for 60–80 and 80–100 cm in profile A (Table 1).

Soils from profile A had slightly higher water contents at corresponding depths than those from profile B, and were the wettest at 60–100 cm; profile A showed no significant difference in soil moisture content over 0–60 cm (Table 1). The variation in moisture content with depth was more pronounced in soil profile A.

As expected, there were decreases in organic C%, N% and C:N ratio with depth for both soil profiles. In profile B, C%, N% and C:N ratio declined more sharply with depth than in profile A. The decline in C% with depth was significant down to 80 cm in both profiles (Table 1). Soil N% showed a similar trend, but the decline was significant down to 100 cm in profile B. Organic matter was more evenly distributed over depth in profile A.

There was a high degree of co-linearity between many of the variables and depth in the profile in the current study (data not shown). This imposed constraints upon the use of regression relationships

for establishing regulatory parameters controlling mineral N production at depth in the profiles.

#### Changes in nitrate-N content with depth and treatment

The initial KCl-extractable nitrate-N did not show marked variations with depth in either profile (Figs. 2a, 3a) but there was a relatively higher content in soil from 80 to 100 cm in profile A.

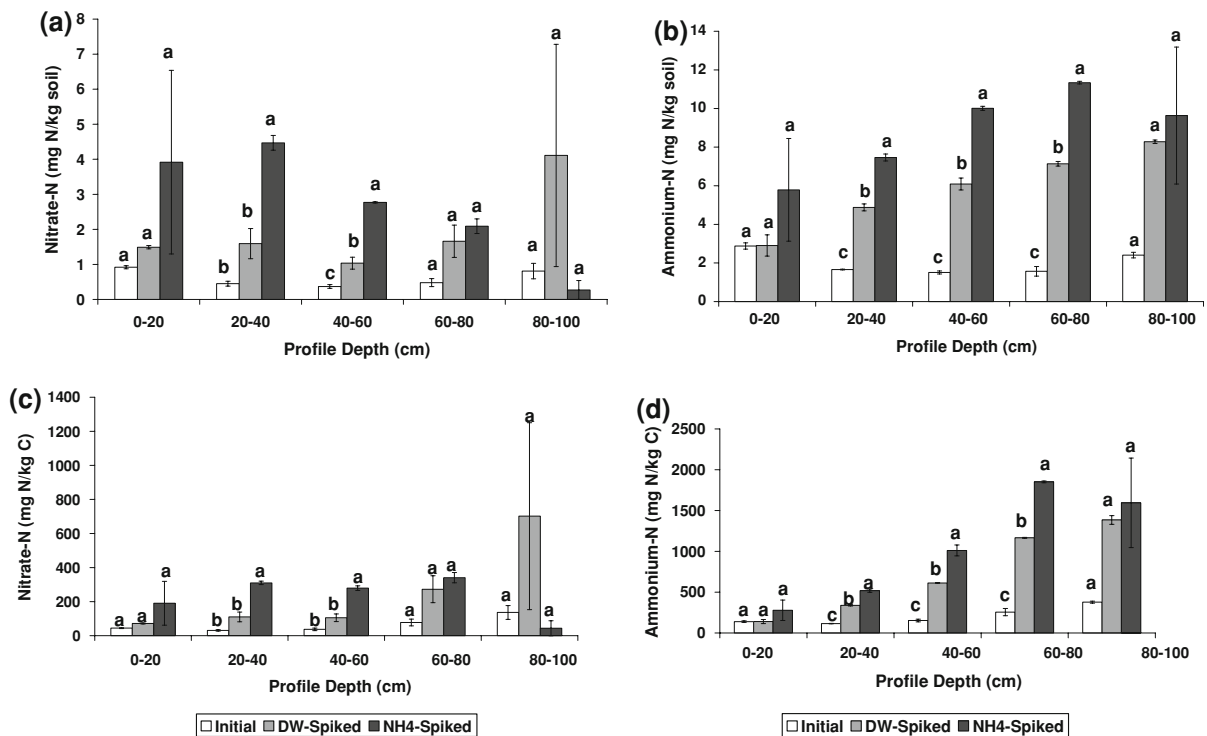
Incubation with d.w. showed statistically significant potential for nitrate-N production in soils from profile A only at 40–60 cm (Fig. 2a). Increases at all other depths were not statistically significant compared to initial values. Incubation of soils from profile B spiked only with d.w. resulted in significantly higher nitrate-N content in soils from 0 to 20 and 20 to 40 cm (Fig. 3a). This corresponds to potential in situ production, suggesting that ammonium supply was not limiting at these depths.

The ammonium-spiking treatment resulted in significantly higher nitrate-N content compared with initial nitrate-N content in soils from 20 to 40 and 40 to 60 cm of profile A (Fig. 2a). At 80–100 cm, nitrate-N content was apparently reduced as a result of ammonium-spiking (Fig. 2a), but the effect was not significant. For profile B (Fig. 3a), ammonium-spiking prior to incubation significantly enhanced nitrate-N content compared with both initial and post d.w.-incubation nitrate-N contents in soils from 0 to 20, 20 to 40, 40 to 60 and 80 to 100 cm.

**Table 1** Characteristics of soils from the study site

Profile	Depth (cm)	pH (KCl)	Moisture content (%)	C (%)	N (%)	C:N ratio
A ( <i>n</i> = 10)	0–20	3.58d* (0.01)	18.29b* (0.05)	2.07a* (0.02)	0.176a* (0.00)	11.79a (0.24)
	20–40	3.81c* (0.01)	18.89b (0.20)	1.44b* (0.02)	0.131b* (0.00)	10.98ab (0.48)
	40–60	3.92b* (0.01)	18.51b (0.06)	0.99c (0.06)	0.098c (0.00)	10.14abc (0.52)
	60–80	3.99a* (0.01)	22.13a (0.68)	0.61d* (0.01)	0.072d* (0.00)	8.53c (0.18)
	80–100	4.04a* (0.01)	21.45a* (0.20)	0.60d* (0.02)	0.066d* (0.00)	9.10bc* (0.19)
B ( <i>n</i> = 10)	0–20	3.38e (0.00)	17.49b (0.03)	2.95a (0.03)	0.218a (0.00)	13.54a (0.03)
	20–40	3.59d (0.01)	17.96b (0.09)	2.23b (0.01)	0.192b (0.00)	11.62b (0.06)
	40–60	4.08c (0.00)	16.84c (0.24)	1.03c (0.07)	0.102c (0.00)	10.13c (0.54)
	60–80	4.17b (0.01)	19.71a (0.24)	0.29d (0.00)	0.040d (0.00)	7.16d (0.04)
	80–100	4.23a (0.02)	17.35bc (0.14)	0.17d (0.01)	0.030e (0.00)	5.63e (0.14)

All values are means for duplicate soil samples. Standard errors of means are enclosed in parentheses. Means sharing different letters in a column differ significantly from each other at  $p < 0.05$  (Tukey HSD multiple comparison using one-way ANOVA with depth as factor). Values with asterisk (\*) in each column at specified depth for profile A differ significantly from those of profile B at same depth at  $p < 0.05$  (independent sample *t*-test)



**Fig. 2** Changes in **a** nitrate-N (mg N/kg soil), **b** ammonium-N (mg N/kg soil), **c** nitrate-N (mg N/kg C) and **d** ammonium-N (mg N/kg C) with depth in soil profile A at five sampling

#### Changes in ammonium-N content with depth and treatment

The initial extractable ammonium-N contents in soils from profile A were higher in soils from 0 to 20 and 80 to 100 cm than in those from 20 to 80 cm (Fig. 2b). Incubation with d.w. increased ammonium-N concentrations in all soils from profile A, but the increase was only significant at 20–40, 40–60 and 60–80 cm. Spiking with ammonium-N further increased extractable ammonium-N contents in soils from all depths for profile A, but not significantly for soils from 0 to 20 or 80 to 100 cm.

In soils from profile B, the initial extractable ammonium-N content decreased gradually but consistently with depth (Fig. 3b). Incubation with d.w. increased ammonium-N content for profile B for all sampling depths, but not significantly in soils from 20 to 40, 60 to 80 or 80 to 100 cm compared with initial values. The surface layer (0–20 cm) with the highest amounts of organic matter showed highest potential for in situ ammonium-N production, in contrast to profile

depths. All values are means of two replicates. Error bars show standard errors of means. Bars with different letters differ significantly from each other at  $p < 0.05$  at specified depth

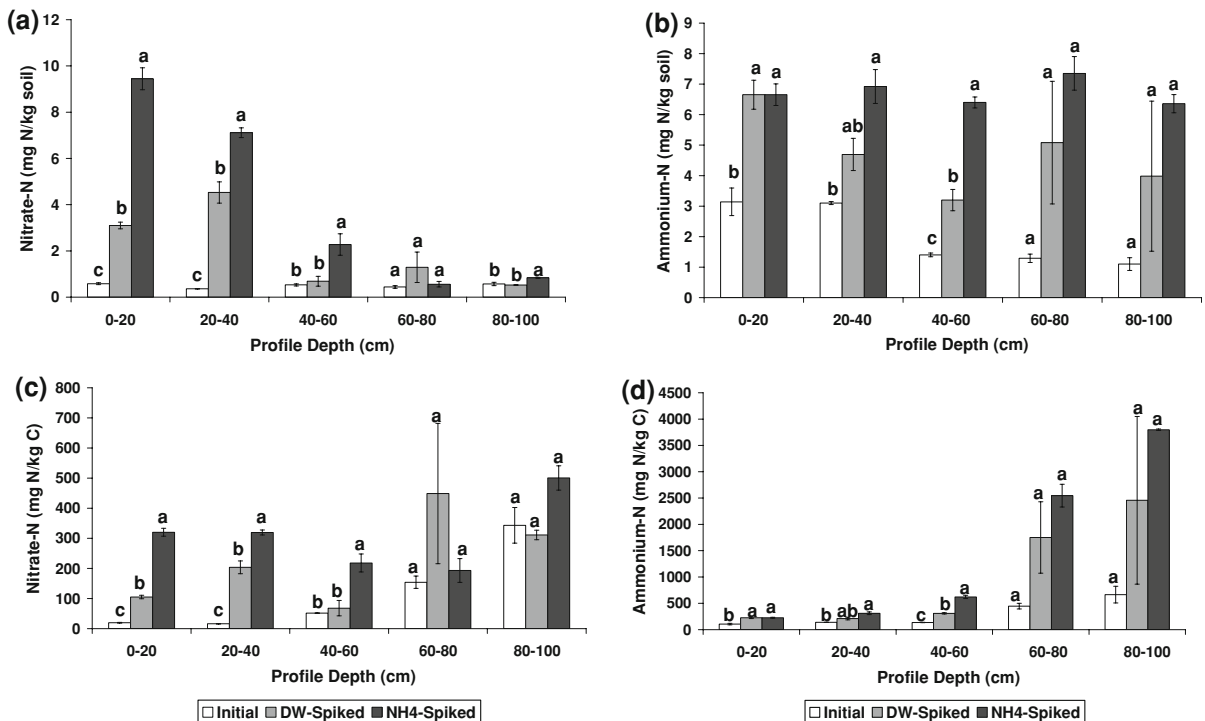
A. There were higher ammonium-N contents after spiking with ammonium-N compared with d.w. after 7-day incubation at 20–100 cm depth, but the increase was only significant in soil from 40 to 60 cm (Fig. 3b).

#### Changes in net mineralization with depth

Net mineralization apparently increased with sampling depth for soil profile A during d.w. incubation for 7-days but there were no significant differences between any of the selected depths ( $df = 4$ ,  $F = 3.957$ ,  $p = 0.082$ ; data not shown). Differences in d.w. spiked net mineralization for soil profile B were also non-significant between sampling depths ( $df = 4$ ,  $F = 2.024$ ,  $p = 0.229$ ; data not shown).

#### Changes in net nitrification with depth and treatment

Net nitrification in d.w. spiked soils from profile A did not differ significantly between any of the five sampling depths (Fig. 4). Treating these soils with



**Fig. 3** Changes in **a** nitrate-N (mg N/kg soil), **b** ammonium-N (mg N/kg soil), **c** nitrate-N (mg N/kg C) and **d** ammonium-N (mg N/kg C) with depth in soil profile B at five sampling

ammonium-N resulted in significantly higher net nitrification at 60–80 and 80–100 cm depth than at 0–20 or 20–40 cm, indicating potential of this soil profile to nitrify below the rooting zone. Soils from 20 to 40 cm also gave significantly higher net nitrification than the more acidic soil from 0 to 20 cm (Fig. 4). Figure 4 also shows that ammonium spiking (compared with d.w. spiking) significantly enhanced net nitrification rate in soils from 40 to 60 and 60 to 80 cm.

Soils below 40 cm depth from profile B, when compared to corresponding soils from profile A, showed lower net nitrification for d.w. spiked and ammonium-N spiked treatments (Fig. 4), in spite of the higher pH compared with profile A, but higher nitrification in near-surface soil. There were no significant differences in net nitrification between any ammonium-spiked soil samples at any sampling depth for profile B. The low net nitrification below 40 cm tied in well with low initial nitrate-N concentration below 40 cm in profile B (Fig. 3a). Figure 4 shows that spiking with ammonium-N compared with d.w. resulted in higher net nitrification at all depths in

depths. All values are means of two replicates. Error bars show standard errors of means. Bars with different letters differ significantly from each other at  $p < 0.05$  at specified depth

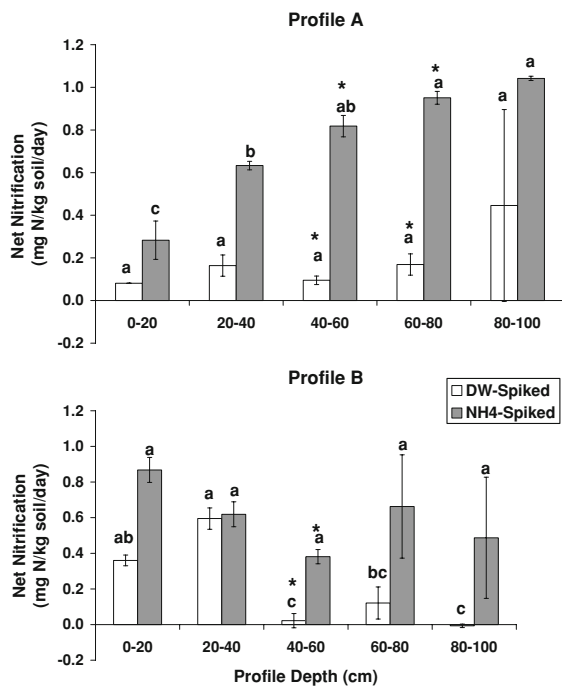
soils from profile B, but the increase in response to spiking was only significant in soil from 40 to 60 cm.

#### Changes in mineral-N contents with depth relative to soil C%

It is reasonable to assume that organic matter N mineralization rate, especially ammonification rate, might depend upon soil sample organic matter content rather than simply the total mass of soil. Therefore, in Figs. 2c, d and 3c, d the nitrate-N and ammonium-N contents for each depth increment were expressed per kg of soil C for profiles A and B, respectively.

For profile A, the initial ammonium-N per unit mass of soil C increased consistently with depth down to 100 cm (Fig. 2d), in marked contrast to the trend when results were expressed on per unit mass of soil (Fig. 2b). This trend was also seen in the d.w. incubated soils (Fig. 2d). Following ammonium-N spiking, however, some ammonium was being immobilised in soil from 80 to 100 cm (Fig. 2d) but in this case the increase was also consistent for results on a per kg of soil basis (Fig. 2b). Also for profile A,





**Fig. 4** Changes in net nitrification (mg N/kg soil/day) in soil profile A and soil profile B at specified depth increments. Values are means of two replicates. Error bars show standard errors of means. Bars sharing asterisk (\*) differ significantly from one another at specified depth at  $p < 0.05$  (independent sample *t*-test). Lower case letters on bars indicate for each treatment type significant differences between depths at  $p < 0.05$

initial nitrate-N and post d.w. incubation nitrate-N contents per unit mass of soil C increase steadily with depth (Fig. 2c). The trend after ammonium spiking in nitrate-N per unit mass of soil C with depth shows an increase in soil from 0 to 80 cm, but a decline in soil from greater depth (Fig. 2c), possibly reflecting the ammonium retention discussed above.

For profile B, when the ammonium-N concentration results were expressed per kg of soil C (Fig. 3d), the spatial trend down the soil profile was much more similar to that for profile A (Fig. 2d) than when results were expressed per kg of soil (compare Figs. 2b, 3b), with consistent steady increases with depth over the entire 0–100 cm range for both initial and post d.w. incubated ammonium N (Fig. 3d). In profile B this steady increase was down to 100 cm after ammonium spiking, compared to 80 cm for profile A (compare Figs. 3d, 2d). Trends for initial nitrate-N contents were also similar for both profiles when results were expressed on a per unit mass of soil

C basis (compare Figs. 3c, 2c). However, clearly factors other than organic C content were more important to nitrate production following incubation after either d.w. or ammonium-N spiking.

#### N species transformations in relation to soil physico-chemical properties

Correlations between N species concentrations and soil physico-chemical properties differed markedly between the two profiles (Table 2). The nitrate-N concentrations for d.w.-spiked and ammonium-spiked soils did not correlate significantly with any variable for profile A except soil N% for ammonium-spiked soils. For profile B, however, nitrate-N concentrations in d.w.-spiked and ammonium-spiked soils showed significant negative correlation with pH and positive correlations with organic C%, N% and C:N ratio (Table 2).

For profile A, initial ammonium-N contents, like initial nitrate-N contents, showed no significant correlation with any measured soil property. Incubation after d.w.-spiking resulted in significant negative relationships with C%, N% and C:N ratio and positive correlations with pH and moisture content (Table 2). The ammonium-N concentrations in ammonium-spiked soil samples followed similar trends only for pH, C% and N%. Initial ammonium-N for profile B, in contrast to profile A, correlated negatively with soil pH and positively with C%, N% and C:N ratio. In d.w. spiked or ammonium-spiked soils, ammonium-N correlated (positively) only with soil moisture content after ammonium spiking and incubation. When the data for the two profiles were considered together, the trends for nitrate in  $\text{NH}_4$ -spiked soils and initial ammonium-N were very similar to those for profile B data alone (Table 2).

Table 3 summarizes the correlations between net nitrogen mineralization and net potential nitrification rates for d.w. spiked and ammonium-spiked (for nitrification only) soil samples from profiles A and B and soil physico-chemical characteristics. Net mineralization and net nitrification rates differed between the soil profiles in their correlations with soil physical and chemical properties. The net nitrogen mineralization rate in d.w. spiked soil samples from profile A correlated positively with pH and moisture content, and negatively with C%, N% and C:N ratio. Net potential nitrification for profile A correlated

**Table 2** Correlation coefficients between soil physico-chemical properties and N species transformation parameters

Soil profile	Physico-chemical properties	Nitrate-N (mg N/kg soil)			Ammonium-N (mg N/kg soil)		
		Initial	d.w.-spiked	NH <sub>4</sub> -spiked	Initial	d.w.-spiked	NH <sub>4</sub> -spiked
A ( <i>n</i> = 10)	pH (KCl)	NS	NS	NS	NS	0.959**	0.635*
	C (%)	NS	NS	NS	NS	−0.959**	−0.649**
	N (%)	NS	NS	0.649*	NS	−0.977**	−0.658**
	C:N ratio	NS	NS	NS	NS	−0.846**	NS
	Moisture content (%)	NS	NS	NS	NS	0.781**	NS
B ( <i>n</i> = 10)	pH (KCl)	NS	−0.832**	−0.993**	−0.953**	NS	NS
	C (%)	NS	0.789**	0.990**	0.933**	NS	NS
	N (%)	NS	0.820**	0.978**	0.938**	NS	NS
	C:N Ratio	NS	0.700*	0.923**	0.859**	NS	NS
	Moisture Content (%)	NS	NS	NS	NS	NS	0.660*
A + B ( <i>n</i> = 20)	pH (KCl)	NS	NS	−0.875**	−0.871**	NS	NS
	C (%)	NS	NS	0.906**	0.846**	NS	NS
	N (%)	NS	NS	0.895**	0.847**	NS	NS
	C:N ratio	NS	NS	0.801**	0.762**	NS	NS
	Moisture content (%)	NS	NS	NS	NS	0.588**	0.601**

\*\*, \* Correlations are significant at  $P < 0.01$  and  $P < 0.05$ , respectively (two-tailed)

NS non-significant results

negatively with soil C%, N% and C:N ratio but positively with pH and moisture content for the ammonium-spiked soils. Net mineralization and net nitrification for the other treatments showed no correlations with soil parameters for this profile (Table 3). There was, however, significant positive correlation between net potential nitrification and net nitrogen mineralization in d.w. spiked soils from profile A. For profile B, net nitrification rate in d.w. spiked soil samples correlated negatively with pH, and positively with C%, N% and C:N ratio. Trends disappeared when data from both profiles were considered together.

## Discussion

### Links to soil physico-chemical characteristics

The changes in physico-chemical characteristics of the two soil profiles with depth looked superficially similar. However, there were several statistically significant differences between profiles at any given depth increment (Table 1). There were more distinct and obvious differences, however, between the profiles in N mineralization relationships to the selected characteristics, both with and without

ammonium-N spiking (Tables 2, 3). Initially that seemed surprising, bearing in mind the superficial similarity of the profiles. The responses of the two profiles to N additions therefore need firstly to be discussed separately.

The consistent trends seen in Figs. 2c, d and 3c, d when compared with Figs. 2a, b and 3a, b are very encouraging. However, even although expressing results on a soil C basis seemed intuitively sensible, it must be recognized that apparent soil C% influences might also be embracing soil N% effects, soil C:N ratio effects and soil pH effects. In spite of this it was decided to look at the link between ammonium-N production per unit mass of soil C and soil pH. The results are shown collectively for both profiles in Fig. 5 for the initial and post incubation ammonium-N data. This suggested that ammonium N per kg of soil organic C increased exponentially with pH over the range studied, and that these two parameters together may explain much of the variation in ammonium-N production. However, it could not be ruled out that the trend seen could be at least partially reflecting C:N ratio effects too, as pH and C:N ratio are strongly correlated. It should be remembered though that correlations between ammonium-N and C:N ratio differed markedly between the two profiles.

**Table 3** Correlation coefficients between net mineralization, net nitrification and soil physico-chemical characteristics

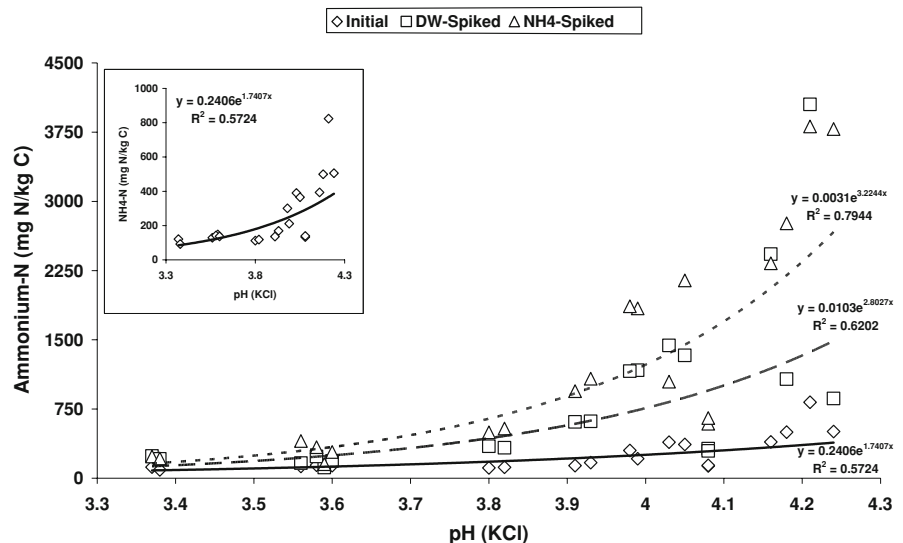
Soil profile	Parameters	Net nitrogen mineralization (mg N/kg soil/day)	Net nitrogen nitrification (mg N/kg soil/day)	
		d.w.-spiked	d.w.-spiked	NH <sub>4</sub> -spiked
A ( <i>n</i> = 10)	pH (KCl)	0.830**	NS	0.973**
	C (%)	−0.835**	NS	−0.969**
	N (%)	−0.848**	NS	−0.982**
	C:N ratio	−0.747*	NS	−0.846**
	Moisture content (%)	0.689*	NS	0.753*
	Net nitrogen mineralization	NA	0.773*	NA
B ( <i>n</i> = 10)	pH (KCl)	−0.640*	−0.814**	NS
	C (%)	NS	0.770**	NS
	N (%)	NS	0.805**	NS
	C:N ratio	NS	0.684*	NS
	Moisture content (%)	NS	NS	NS
	Net nitrogen mineralization	NA	NS	NA
A + B ( <i>n</i> = 20)	pH (KCl)	NS	NS	NS
	C (%)	NS	NS	NS
	N (%)	NS	NS	NS
	C:N ratio	NS	NS	NS
	Moisture content (%)	0.568**	NS	0.568**
	Net nitrogen mineralization	NA	0.675**	NA

\*\*, \* Correlations are significant at  $P < 0.01$  and  $P < 0.05$ , respectively (two-tailed)

NS non-significant results

NA results not applicable

**Fig. 5** Exponential relationships between ammonium-N (mg N/kg C) and pH (KCl) for both soil profiles (*n* = 20). *R*-squared values for initial, d.w.-spiked and NH<sub>4</sub><sup>+</sup>-spike treatments are placed near to *trend lines* in the figures. *Inset* The exponential relationship between initial ammonium-N (mg N/kg C) and pH (KCl) in a clearer way



The decreases in soil C:N ratio with depth (Table 1) could be due to mobility of N down these two soil profiles as a consequence of N deposition in

the past (Pilkington et al. 2005) and at the present time (Riaz et al. 2008). However it was also possible that, on relative basis, C declined more sharply than

N with depth. It is impractical to ignore the relationships between N additions on N dynamics and the influences of physical and chemical properties of soil on N transformations (Pérez et al. 1998), and between N input effects and effects of C losses via microbial respiration. For example, the sharper decline in soil C% with depth in profile B (Table 1) could partially explain why this profile showed such a sharp fall in C:N ratio compared with that in profile A.

#### Origins of ammonium found at different depths

Since organic matter in the soils over the depth range from 20 to 80 cm for profile A could be ammonified during d.w. incubation (Fig. 2b), the initial extractable ammonium-N concentrations in these soils were at least in part probably due to in situ ammonium production. However, in soil from 0 to 20 cm from profile A, a substantial part of the initial ammonium-N could have been due to leaching from the surface litter layer as in situ production was apparently not significant (Fig. 2b). The potential for in situ ammonium-N production during d.w. incubation showed considerable vertical variation, with relatively higher ammonium-N production in deeper soil layers where, although organic matter might be expected to be more recalcitrant, pH is higher and C:N ratio lower. Organic matter was more evenly distributed over depth in profile A than in profile B (Table 1), so more organic matter would be available for mineralization at depth in this profile.

Following d.w. incubation, soil from profile A, which was slightly less acidic near the surface than soil from profile B, displayed no significant mineral-N production in soil from 0 to 20 cm (Fig. 2a, b) and significant negative correlations between mineral-N production and C%, N% and C:N ratio (Table 3), which reflected the strong correlations for ammonium-N (Table 2). The fact that ammonium-N production decreased with increasing organic C content suggested that quality of organic matter, rather than quantity, effected N turnover in profile A soils (Fog 1988; Holub et al. 2005; Khalil et al. 2005; Pilkington et al. 2005). In profile A, more ammonium-N was produced during d.w. incubation as soil depth increased (Fig. 2b) and organic matter content fell. This was because ammonium-N production per unit mass of soil C increased sharply with depth (Fig. 2d), which demonstrated the value of

expressing ammonium-N on the basis of per unit mass of soil C when interpreting data. Net nitrification followed a similar trend, with a strong negative correlation with C% and N% only after ammonium-spiking (Table 3); this probably reflected the effects of limited natural ammonium-N production.

Profile B, on the other hand, showed higher mineral-N production in surface soil layers than lower in the profile, with significant increases in both ammonium-N and nitrate-N at 0–20 cm and in nitrate-N at 20–40 cm following d.w. incubation (Fig. 3a, b). This could be due to availability of a higher concentration of readily degradable organic matter at or near the surface than at depth (Table 1). The systematic behavior in mineral N production was much clearer when results were expressed per kg of soil C (Fig. 3c, d). The consistency between the two profiles became clear when Figs. 2d and 3d were compared. Kjønaas et al. (1998) and Aber et al. (1995) showed that availability of organic N may be one of the factors controlling mineral-N production in surface soils. This initial mineral-N was dominated by ammonium-N (Fig. 3a, b), suggesting that nitrification was relatively slower than ammonification in the field, or nitrate produced in the field was being taken up by plants. Once plant uptake effects were removed in the incubation experiment, nitrate-N too started to correlate with C%, N% and C:N ratio, regardless of whether extra ammonium-N was added (Table 2).

#### What else might ammonium production per unit mass of C tell us?

The more uniform distribution of organic matter throughout profile A compared with profile B was a striking difference (Table 1). Possibly the very low surface pH for profile B reduces mixing by soil fauna, so that organic matter at depth in profile B was older and therefore might be regarded as now more recalcitrant. Ammonium production per unit mass of C was, however, greater in soil from 80 to 100 cm from profile B (Fig. 3d) than in soil from the corresponding depth for profile A (Fig. 2d). If more of the C had been lost too, this could explain the very low soil C:N ratio at depth in profile B. This very low C:N ratio in profile B (Table 1) could at least partially explain why the residual organic matter is, in practice, very biodegradable in this profile.

### Observations on net nitrification

The profiles varied in their net nitrification rate relationships to soil characteristics for diverse treatments (Table 3). For profile A, net nitrification in ammonium-spiked soils showed a significant negative correlation with C:N ratio, as did net mineralization in d.w. spiked soils (Table 3), which was in line with findings of Gundersen and Rasmussen (1990) and Van Miegroet et al. (1990) who claimed that net nitrification increased with decrease in soil C:N ratio. However, no such relationships were seen for soil profile B. Possibly they were masked by more dominant influence from pH and soil organic matter content. Net nitrification correlated positively with soil moisture content for ammonium-

spiked soils from profile A, in agreement with previous studies (Tietema et al. 1992b; Evans et al. 1998).

Profile A showed a significant positive correlation between net nitrification and net N-mineralization for d.w. spiked soil samples, which could suggest that substrate availability was limiting net nitrification (Malý et al. 2002).

### Importance of N transformations at depth

The earlier observation that rates of mineral N production did not vary significantly with depth indicates that the inclusion of deeper soil layers in N response studies is important (Cassman and Munns 1980; Krug and Winstanley 2002). Both profiles

**Table 4** Changes with depth in mineral N (ammonium-N and nitrate-N) both as mg N/kg soil and mg N/kg C for all treatments in two soil profiles

Profile	Sampling depth (cm)	Nitrate-N (mg N/kg soil)			Ammonium-N (mg N/kg soil)		
		Initial	d.w.-spiked	NH <sub>4</sub> -spiked	Initial	d.w.-spiked	NH <sub>4</sub> -spiked
A	0–20	0.93 (0.05)a	1.49 (0.05)a	3.92 (2.62)a	2.88 (0.17)a	2.91 (0.55)d	5.79 (2.67)a
	20–40	0.45 (0.07)a	1.60 (0.44)a	4.47 (0.21)a	1.66 (0.20)a	4.88 (0.19)c	7.47 (0.19)a
	40–60	0.37 (0.06)a	1.04 (0.17)a	2.77 (0.02)a	1.51 (0.08)a	6.09 (0.31)bc	10.0 (0.11)a
	60–80	0.48 (0.12)a	1.67 (0.47)a	2.09 (0.21)a	1.57 (0.26)a	7.14 (0.12)ab	11.3 (0.07)a
	80–100	0.81 (0.22)a	4.11 (3.17)a	0.27 (0.27)a	2.26 (0.01)a	8.28 (0.09)a	9.64 (3.55)a
B	0–20	0.58 (0.04)a	3.10 (0.14)ab	9.45 (0.48)a	3.14 (0.46)a	6.65 (0.50)a	6.66 (0.36)a
	20–40	0.36 (0.01)a	4.53 (0.46)a	7.11 (0.21)b	3.10 (0.05)a	4.70 (0.54)a	6.93 (0.56)a
	40–60	0.54 (0.05)a	0.69 (0.22)c	2.28 (0.47)c	1.41 (0.07)b	3.20 (0.35)a	6.40 (0.13)a
	60–80	0.45 (0.06)a	1.29 (0.66)bc	0.56 (0.12)c	1.29 (0.14)b	5.08 (2.01)a	7.35 (0.55)a
	80–100	0.57 (0.07)a	0.52 (0.00)c	0.84 (0.03)c	1.10 (0.21)b	3.98 (2.46)a	6.36 (0.30)a
Profile	Sampling depth (cm)	Nitrate-N (mg N/kg C)			Ammonium-N (mg N/kg C)		
		Initial	d.w.-spiked	NH <sub>4</sub> -spiked	Initial	d.w.-spiked	NH <sub>4</sub> -spiked
A	0–20	45 (3)a	72 (3)a	191 (128)a	139 (10)bc	140 (25)e	278 (125)b
	20–40	31 (4)a	110 (29)a	310 (10)a	115 (3)c	338 (8)d	518 (20)ab
	40–60	37 (8)a	105 (23)a	279 (14)a	153 (17)bc	613 (3)c	1,011 (68)ab
	60–80	78 (20)a	273 (79)a	341 (30)a	256 (45)ab	1,165 (5)b	1,852 (11)a
	80–100	136 (41)a	701 (549)a	44 (44)a	378 (12)a	1,384 (53)a	1,595 (548)ab
B	0–20	20 (1)b	105 (6)a	320 (13)b	106 (14)b	225 (15)a	226 (10)c
	20–40	16 (1)b	204 (21)a	320 (8)b	139 (3)b	211 (25)a	311 (26)c
	40–60	52 (1)b	68 (26)a	218 (30)b	136 (3)b	309 (12)a	622 (31)c
	60–80	154 (21)b	449 (233)a	193 (40)b	447 (53)ab	1,751 (677)a	2,546 (217)b
	80–100	343 (59)a	311 (16)a	501 (40)a	665 (159)a	2,457 (1,593)a	3,798 (14)a

All values are mean of duplicate samples. Standard error of means is enclosed in parenthesis. Means with different letters in each column for specified soil profile for specified parameter differ significantly from each other at  $p < 0.05$  (ANOVA, Tukey HSD post hoc test)

showed significant potential to produce mineral-N in soils from 20 to 60 cm depth during incubations following d.w. and ammonium-N spiking. This observation could not be attributed to any single factor at these depths, as variations in soil moisture content, soil organic matter quality and quantity, and pH could all have influenced the relationship between N availability and N turn over in these soils (Vestgarden and Kjønaas 2003).

When nitrate-N and ammonium-N production were expressed relative to soil C content, as in Table 4, production of ammonium changed much more markedly with depth in the profiles and some new or additional significant trends emerged. This possibly reflected the effect of pH becoming more significant when the soil organic matter content was also taken into account.

After addition of ammonium-N, soil profile A showed significantly enhanced nitrate-N production at 20–40 and 40–60 cm, compared with 0–60 and 80–100 cm for profile B (Figs. 2a, 3a). Thus potentially ammonium being mobilized down the profiles would be at least partially nitrified at depth, contributing to nitrate leaching problems. Profile A apparently showed net immobilization of nitrate-N at 80–100 cm depth, however, indicating that micro-organisms were nitrate limited, and preferred utilization of nitrate to fulfill their N needs when ammonium source might have been exhausted (Recous and Mary 1990). Elevated nitrification activity in these two soil profiles at depth might be explained by reduced N immobilization due to lower overall microbial activities at depth, higher pH values (Tietema et al. 1992a; Persson and Wirén 1995) and availability of ammonium substrate via ammonium leaching down the soil profile and ammonification (Riaz et al. 2008). Waldrop et al. (2004) cited various studies displaying stimulation in microbial activities following N additions in several forest, grassland and wetland ecosystems. However, some other studies indicate suppression in microbial activities as a result of N additions (Smolander et al. 1994; Carreiro et al. 2000; Gallo et al. 2004), which could explain the spatial and vertical variation of our two soil profiles in N species transformation dynamics in response to similar N additions.

These acid grassland soils with low pH, low C:N ratio, and high in situ potential for nitrate and ammonium production, displayed positive net nitrification and are candidates for nitrate and

ammonium leaching into adjacent waters (Bengtsson et al. 2003; Macdonald et al. 2002; Mian et al. 2008). The in situ production of mineral-N in deeper soil layers of the soil profiles under investigation could make them susceptible to nitrate leaching from the ecosystem (Persson and Wirén 1995; Tietema et al. 1992a).

## Conclusions

- Net mineralization occurred at all depths (hypothesis 1) in both soil profiles when soils were incubated with d.w., and did not change significantly with depth even though the organic C% and N% declined significantly with depth.
- Net nitrification was positive for both soil profiles at almost all depths during incubation with d.w. Ammonium-spiking consistently, but not always significantly, enhanced nitrification rate, suggesting substrate limitation.
- The significant increase in nitrate-N in one profile following ammonium spiking demonstrated that ammonium leached down the profile might be nitrified at 80–100 cm depth.
- It might be expected that more mineral-N would be produced when soil was incubated with d.w. when organic matter content was higher; this was the case generally for profile B. The negative correlation with pH might then simply be reflecting inverse auto correlation between organic matter content, and depth and pH. However, profile A showed the reverse behavior.
- It is postulated that the difference between the two profiles probably reflects differences in mixing of organic matter by soil fauna. Further research would be needed to confirm this, however.
- Expressing ammonium-N production on a per kg of soil C basis allowed clear and consistent pH effects to be then readily seen, supporting hypothesis 4.

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