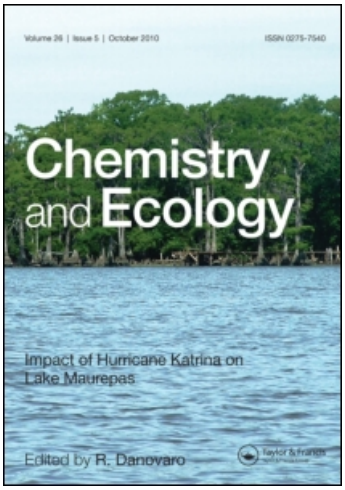

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Ishaq A. Mian[a]; Muhammad Riaz[a]; Malcolm S. Cresser[a]
[a] Environment Department, University of York, Heslington, York, UK

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What controls the nitrate flush when air dried soils are rewetted?

Ishaq A. Mian*, Muhammad Riaz and Malcolm S. Cresser

Environment Department, University of York, Heslington, York, UK

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Changes in nitrification rates of an acid grassland soil with and without air drying have been monitored over 9 days, after first flushing native nitrate from the soils with deionised water. The results confirmed that full re-establishment of nitrification after air drying takes several days, supporting the hypothesis that any immediate first flush of nitrate from air-dried soils originates from cell lysis or flushing of 'stored' nitrate. Ammonium spiking confirmed that nitrification was not ammonium substrate limited. It was also found that ammonium accumulates in the soil during the drying process, providing a substrate pool once the population of nitrifiers has re-established. Over the first week of incubation, nitrate immobilisation was less conspicuous in the soil that had been rewetted after air drying compared with the incubated field moist soil.

Keywords: nitrification; nitrate flush; ammonium; mineral nitrogen; grassland; drying; rewetting; microbial activity

1. Introduction

Reviewing the possible causes of drying-induced stress on soil microbes, Fierer et al. noted that soils are regularly subjected to drying/rewetting cycles in many parts of the world, and that mineral-N flushes have often been documented from rewetted air-dried or partly-dried soils in response to changes in microbial activities and diversity [1]. For example, reducing the water content of a coniferous forest litter layer material to 10% of dry weight for 12 days reduced microbial biomass C by 67% and reduced respiration markedly [2]. Subsequent rewetting to 340% resulted in significant flushes of respiration, soluble C and mineral N within a few hours [2]. Gordon et al. investigated the effects of drying and rewetting on the concentrations of inorganic N species in leachate from improved and unimproved grassland soils [3]. Nitrate leaching was increased by the stress from drying, especially in the improved soil. Thus soils that may be prone to extended drought periods often give a nitrate flush in the next precipitation event. A rapid increase in nitrate concentration was noted in a mineral soil under shrubland in Israel when the first winter rainfall rewetted dried soil, although in adjacent forest soil an unexpected increase in nitrite concentration accompanied by only a small increase in nitrate concentration was

*Corresponding author. Email: iam501@york.ac.uk

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observed [4]. It was suggested that this might be due to differing changes in microbial populations in response to summer stress for the two ecosystems [4].

Nitrate flushes may become a potential environmental issue when soil/vegetation mesocosms are used as biofilters for the removal of inorganic N pollution from urban runoff. When such filters were subjected to simulated drought periods of 4 to 6 weeks, nitrate concentrations always increased sharply in the subsequent first flush of outlet water [5,6]. The flush is short-lived, which could support the concept of intracellular cell solute release following cell lysis in response to osmotic shock upon rewetting [1]. However, although nitrate might make a contribution to osmo-regulation in microbial cells, its contribution is likely to be very small as the intracellular solutes released subsequent to dilution stress are predominantly organic or potassium [7]. Therefore the authors consider that the nitrate flush could be partly due to removal of nitrate accumulated in soil but not taken up by plants during drying, rather than just cell lysis. Ford et al. recently studied the effects of rewetting on mineralisation in semi-arid grassland soils from Western Australia [8]. The amount of nitrate initially extractable from their air-dried soil was small (ca. $1\,mg\,kg^{-1}$) compared with that subsequently produced when the soil was rewetted and incubated over 4 weeks. At the 40 °C incubation temperature that they used, nitrate accumulated quite rapidly over 2 to 3 days. Other researchers have concluded that microbial cells killed during soil desiccation were not major contributors to N flushes on rewetting [9]. The results of Ford et al. [8] highlight the need to distinguish between the nitrate flux available for immediate mobilisation from rewetted air-dried soils, and the nitrate subsequently produced in the soil.

It seems possible that as soils dry, water and associated solute species migrate to progressively smaller and smaller pores, so both the water and the contained solute might become unavailable to plants. Such retained nitrate would almost certainly be removed quite quickly during a subsequent rain storm event. Any nitrate flush from a sudden burst of activity of nitrifiers and possibly also ammonifiers is likely to occur later in rewetted air-dried soil.

In order to improve our understanding of how extended drought periods might influence the dynamics of nitrate leaching to an adjacent stream from soils that have been heavily N-impacted by atmospheric deposition, it was decided to:

(1) Compare how nitrate production rates in a rewetted air-dried soil and the corresponding field moist soil change over time *after* flushing out any stored residual nitrate or nitrate from cell lysis with deionised water wash.
(2) Determine if ammonium substrate availability limits the initial rate of nitrification in a heavily N-impacted, dried or field-moist soil by testing whether ammonium spiking enhances the nitrification rate.
(3) Conduct a nitrate spiking experiment to confirm that if the net nitrate production rate after rewetting appears to be slow it is not due to microbial immobilisation of nitrate and/or localised denitrification.

2. Materials and methods

2.1. *Site*

The soil used was from Hob Moor, a permanent acid grassland near the City of York in North Yorkshire, UK. Hob Moor (53°57′30″N & 1°04′48″W) is an ancient acid grassland, 36.4 ha in area, slightly south west of the York city walls. The mean annual rainfall in the area is 639 mm.

The site is a Local Nature Reserve with a management plan to maintain low nutrient status and high biodiversity. The Moor has small streams close to its edges, which have been shown in occasional analyses over a seven year time span to contain variable concentrations of nitrate up to 27 mg l^{-1}. This is a high concentration for an unfertilised site that has received no synthetic fertiliser for at least six decades. Therefore any ammonium or nitrate mobilised within the soil profiles would be from natural element cycling/recycling and/or from deposition of atmospheric pollution. The site is still used for grazing cattle over the summer months, as part of the plan to maintain a low nutrient status.

The soil chosen was a freely draining, very fine sandy loam and therefore appropriate for comparison with soils likely to be used in biofilters. In shallow layers of incubated moist soil it would not be prone to denitrification over planned incubation periods of up to 9 days.

2.2. *Soil preparation*

A bulk soil sample was collected on 14 December 2006 from the upper 20 cm of the soil profile, below approximately 1 cm of litter. The soil was carefully, but quickly, hand sorted to remove any obvious root material and the few stones present. To standardise conditions and make results from field moist soils directly comparable to those from air-dried soils, half of the thoroughly mixed bulk sample was air dried as a shallow layer (2–3 cm) on plastic trays, and the other half was stored at 4 °C in a refrigerator, for 6 days. The moisture contents of the field moist and air-dried samples were then determined in duplicate by oven drying at 105 °C overnight.

2.3. *Incubation experiment*

The masses of moist and air-dried soil equivalent to exactly 10.0 g of oven-dry soil were calculated to be 12.34 and 10.28 g, respectively. Sub-samples with these masses of moist and air-dry soil were packed into two series of 50-ml leaching tubes, each plugged at the bottom with 0.40 g of cotton wool. Duplicate blank tubes were also prepared with no soil. All tubes and contents, and the blanks, were immediately leached over 2–3 h with 100-ml portions of deionised water to remove native nitrate-N, and then left to drain for 24 h. The wash solutions were discarded.

After 24 h, one third of each set (previously air-dried or field moist) of 30 tubes was treated with 1 ml of deionised water, one third with 1 ml of solution containing 50 μg of ammonium-N (as ammonium sulphate), and the remaining third with 1 ml of solution containing 50 μg of nitrate-N as potassium nitrate. Thus 10 tubes of moist soil and 10 tubes of air-dry soil received each N treatment. The N spike size was selected to give an N species N concentration value comparable to that of the native nitrate-N and ammonium-N concentrations.

From each of these sets of 10 tubes, two tubes were leached with 100 ml of 0.5 molar potassium chloride immediately after the N or water spike additions (T0), and then further duplicates were leached after exactly 1, 2, 5 and 9 days (T1, T2, T5 and T9).

2.4. *Soil analyses*

Soil pH

Soil pH was measured using a glass/calomel electrode and a pre-calibrated Thermo Orion pH meter at a 1:5 field-moist soil:deionised water ratio. The solution was stirred thoroughly and allowed to stand for 30 min to equilibrate, then stirred again and pH was measured to the nearest 0.1 pH unit.

Soil C:N ratio

The soil C%, N% and C/N ratio were determined in duplicate using oven dry soil sub-samples with an automated Elementar Vario Macro C & N analyser. The oven dry soil samples were first ball milled into fine powder, and approximately 100-mg sub-samples were weighed to the nearest 0.1 mg into tin foil sample cups. The samples were carefully tightly wrapped in order to avoid any loss, and analysed for C (%), N (%) and C/N ratio using glutamic acid as a calibration standard.

Extractable ammonium-N and nitrate-N

Extractable ammonium- and nitrate-N in the 0.5 molar KCl extracts were measured using a standard Bran and Luebbe two channel AutoAnalyser® 3 with matrix-matched standards. Whenever necessary, sample extracts were appropriately diluted off line with the 0.5 molar KCl extractant solution to give a final concentration below 2 mg l^{-1} for measurement.

2.5. *Statistical analyses*

To investigate the significance of spike treatment effects on extractable ammonium-N and nitrate-N concentrations at individual times (T0 to T9), Tukey HSD multiple comparison was employed, taking treatment as grouping variable. Treatment effects were assumed significant at $p < 0.05$. To assess significance of differences over time, one-way ANOVA was used to compare means of extractable nitrate-N and ammonium-N for distilled water- (DW), nitrate- and ammonium-spiked soil samples. Tukey HSD multiple comparison ($\alpha = 0.05$) was used as post hoc test using time as grouping variable.

3. Results

The soil used was a very fine sandy loam which had a pH value of 4.45. The electrical conductivity of a saturated paste was 80.5 μS cm^{-1}. The mean concentrations of C and N were 4.03% and 0.361%, respectively, and the mean C:N mass ratio was 11.5.

Figure 1 shows how nitrate-N concentrations changed over the 9-day incubation period following the deionised water flush and subsequent spiking with either deionised water (the control), 5 mg of nitrate-N kg^{-1} of soil, or 5 mg of ammonium-N kg^{-1} of soil, for the air-dried soil (upper chart) and for the field moist soil (lower chart).

Comparison of the results for the air-dried and field moist soils treated only with deionised water clearly shows that there was a substantial delay in the onset of nitrification following air drying (Figure 1, upper chart, white bars). Statistical analysis (Table 1) showed that for the distilled water-spiked air dry soils, the nitrate concentration did not increase significantly compared with the mean value at T0 until T5. However, nitrification was already very rapid in the field moist soil by T0, only 24 h after the water rinse (Figure 1, lower chart, white bars), bearing in mind the fact that there was a period of 24 h between flushing with deionised water and the subsequent KCl leaching of ammonium and nitrate. By day 5 and day 9, nitrate concentration had fallen in the field-moist soil (Figure 1, lower chart, white bars) compared with the initial concentration at time zero, but the changes over time were not significant (Table 1).

Comparison of the results for the air-dried and field moist soils spiked with 5 mg kg^{-1} nitrate-N shows that over the first five days of incubation, net nitrate-N for the air-dried soil increases by approximately this amount (Figure 1, upper chart, compare grey and white bars). There is a clear sign in Figure 1 of immobilisation/loss of nitrate-N by T9 for the air-dry soil, however, and at T9 the nitrate spiking treatment had no significant effect upon the extractable nitrate concentration.

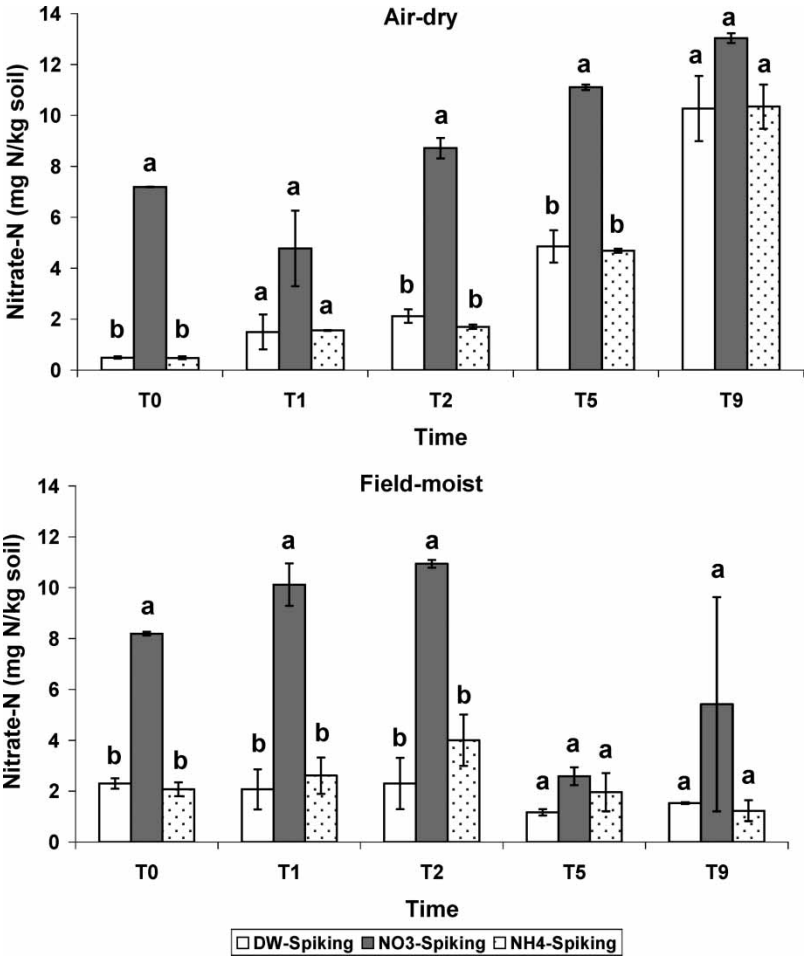

Figure 1. Changes in concentration of extractable nitrate-N in air dry (upper chart) and field moist (lower chart) soils after spiking with deionised water, nitrate-N or ammonium-N over a 9-day incubation period at room temperature. All values are means of two replicates. Error bars indicate standard errors of means. Bars at any specified time sharing different letters differ significantly at $p < 0.05$ (Tukey HSD multiple comparison using treatment as grouping variable).

In the field moist soil (Figure 1, lower chart), substantial nitrate loss occurred by T5 and nitrate loss was still marked at T9. Nitrate spiking had no significant effect upon the extractable nitrate concentration at either T5 or T9.

Ammonium spiking of the air-dried soils confirmed the slow recovery of nitrification seen for the distilled water control soils (Figure 1, upper chart, compare spotted and white bars). It also showed that nitrate production was not ammonium substrate-limited throughout the 9-day incubation period as ammonium addition did not stimulate additional nitrification. Nor did ammonium spiking stimulate substantial additional nitrate production in the ammonium-spiked field-moist soils (Figure 1, lower chart, compare spotted and white bars). At no time did ammonium spiking significantly enhance the nitrate concentration compared with distilled water spiking.

To confirm that ammonium substrate is not limiting nitrate production also requires evidence that the ammonium added during spiking has not been used by, or immobilised substantially in, microbial biomass. Figure 2 shows how ammonium-N concentration changed over the 9-day incubation period following the deionised water flush and subsequent spiking with either deionised water (the control), 5 mg of nitrate-N kg^{-1} of soil, or 5 mg of ammonium-N kg^{-1} of soil,

Table 1. One-way ANOVA for time to compare means for extractable nitrate-N and ammonium-N concentrations in soil for DW-, nitrate and ammonium spiked soil samples. Tukey HSD multiple comparison ($\alpha = 0.05$) was used as a post hoc test using time as grouping variable.

	Nitrate-N (mg N/kg soil)			Ammonium-N (mg N/kg soil)		
Time (Days)	DW-Spiked	NO3-Spiked	NH4-Spiked	DW-Spiked	NO3-Spiked	NH4-Spiked
Air-dry						
T0	0.485 c	7.190 bc	0.475 c	7.720 a	11.200 a	13.535 a
	(0.045)	(0.010)	(0.055)	(0.800)	(0.380)	(0.325)
T1	1.495 bc	4.775 c	1.560 c	7.805 a	20.830 a	16.545 a
	(0.685)	(1.485)	(0.020)	(5.785)	(5.410)	(0.215)
T2	2.115 bc	8.720 b	1.700 c	10.790 a	11.895 a	14.205 a
	(0.265)	(0.400)	(0.080)	(1.210)	(1.365)	(0.495)
T5	4.860 b	11.105 ab	4.690 b	10.335 a	11.440 a	15.505 a
	(0.630)	(0.105)	(0.080)	(0.585)	(0.610)	(1.525)
T9	10.620 a	13.040 a	10.345 a	11.500 a	12.685 a	15.080 a
	(1.280)	(0.190)	(1.223)	(1.020)	(0.742)	(1.530)
Field-moist						
T0	2.300 a	8.190 a	2.070 a	3.615 a	3.935 a	8.985 a
	(0.210)	(0.070)	(0.270)	(0.035)	(0.255)	(0.075)
T1	2.070 a	10.120 a	2.605 a	2.140 a	2.215 a	7.175 a
	(0.790)	(0.840)	(0.715)	(0.450)	(0.405)	(0.025)
T2	2.030 a	10.770 a	3.995 a	1.810 a	1.210 a	5.915 a
	(1.010)	(0.150)	(1.005)	(0.110)	(0.120)	(0.375)
T5	1.165 a	2.590 a	1.960 a	3.095 a	2.995 a	6.585 a
	(0.125)	(0.350)	(0.750)	(0.035)	(0.515)	(1.205)
T9	1.525 a	5.420 a	1.230 a	3.165 a	2.155 a	7.465 a
	(0.035)	(4.210)	(0.410)	(1.265)	(1.055)	(2.295)

All values are means of duplicate samples. Standard errors of means are enclosed in parentheses. Means in any column sharing only different letters at two times differ significantly at $p < 0.05$.

for both air-dried (upper chart) and field moist (lower chart) samples. Figure 2 suggests that, for the field moist soil, most of the added ammonium-N spike is still KCl-extractable throughout the incubation period. However the ammonium spiking only significantly enhanced the extractable ammonium concentration at T0, T1 and T2. In the air-dry soil, ammonium spiking did not significantly enhance the extractable ammonium concentration at any time except T0 (Figure 1).

It may be assumed that the nitrate production build up over the 9 days seen in Figure 1 (upper chart) for the rewetted air-dry soils (white bars) has arisen as a consequence of ammonium oxidation. It appears that this oxidation has restricted significant ammonium accumulation within the rewetted air-dried soil (Figure 2, upper chart, white bars). Similar nitrate build up may be seen for the ammonium-N and nitrate-N spiked soils (Figure 2, upper chart, grey and spotted bars and Table 1). In the field-moist soil, however, there appeared to be a consistent decline in ammonium concentration between T0 and T2, regardless of whether or not an ammonium-N or nitrate-N spike had been added (Figure 2, lower chart), but Table 1 shows that this apparent effect was not significant.

4. Discussion

The nitrate-N concentration results for the air-dried and field moist soils very clearly showed a marked delay in the onset of nitrification following air drying (Figure 1, upper chart), but that nitrification was very rapid in the field moist soil over the 24 h after the deionised water flushing (Figure 1, lower chart). In the latter, nitrate concentration was $>2\,\mathrm{mg\,kg^{-1}}$ at T0, only 24 h after

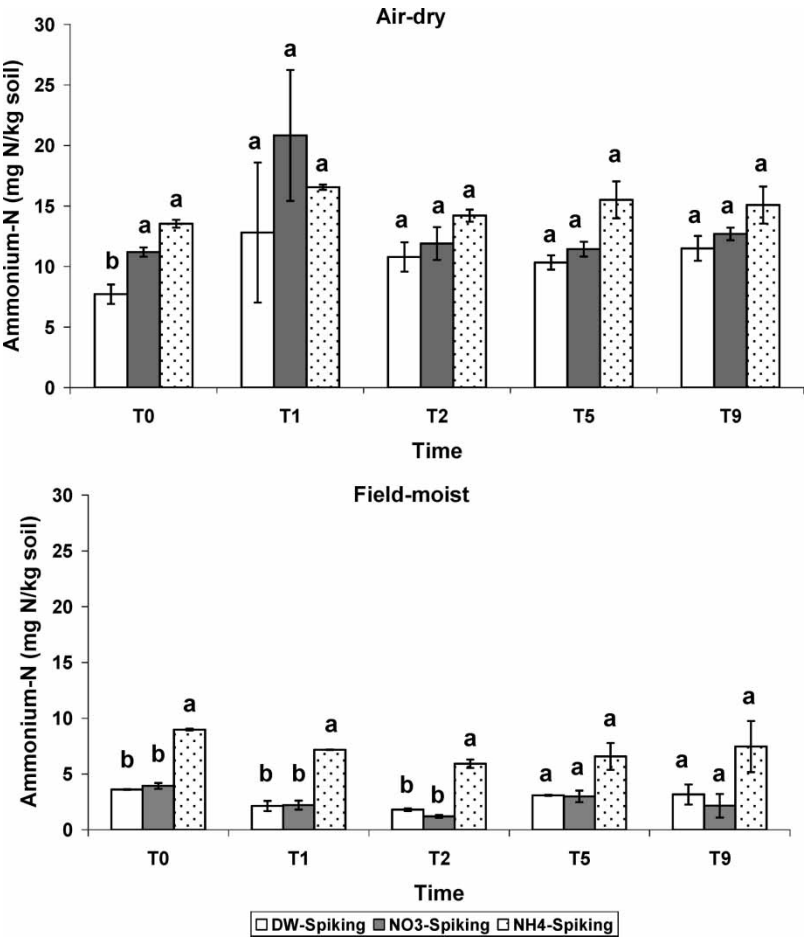

Figure 2. Changes in concentrations of extractable ammonium-N in air dry (upper chart) and field moist (lower chart) soils after spiking with deionised water, nitrate-N or ammonium-N over a 9 day incubation period at room temperature. All values are means of two replicates. Error bars indicate standard errors of means. Bars at any specified time sharing different letters differ significantly at $p < 0.05$ (Tukey HSD multiple comparison using treatment as grouping variable).

the deionised water flushing. As noted in the results section, even by day 9, nitrate-N concentration had not dropped significantly in the field-moist soil compared with the nitrate-N concentration at T0 (Table 1). Any nitrate loss could have been indicative of denitrification, but the soil layers in the bottles were shallow (<1 cm), the bottles were loosely capped, and in the field the soil was very freely drained so it would not have had a population of anaerobes. It is therefore extremely unlikely that the soils in the experiment were anaerobic which ties in with the lack of a significant effect. There was no such trend either in the air-dried soils, which were incubated under similar conditions. These samples had a lower moisture content, and would in any case be even less likely to become anaerobic.

An important conclusion from this study is that *rapid* nitrate flushes following rewetting of dry soils that have often been attributed to surges in soil microbial activity are, in practice, more likely due to flushing out of residual nitrate from the soil that has been produced and stored during the drying phase or nitrate from cell lysis, as discussed in the introduction.

The results for air-dried and field moist soils spiked with 5 mg kg^{-1} nitrate-N over the first five days (Figure 1, comparing grey and white bars) provided little evidence of immobilisation/loss

of nitrate-N for air-dried soil before T9 (Figure 1, upper chart), and although for the field-moist soil nitrate loss appeared to occur by T5, and was still marked at T9 in that the treatment effect was no longer significant (Figure 1, lower chart), the change in nitrate-N in nitrate-spiked soils over time was not significant (Table 1). It should be pointed out that the soil used had a quite low C:N ratio, well below the critical threshold value of 25, which would lower the risk of microbial immobilisation [10,11]. It is well known that nitrate additions to soils with high C:N ratios may result in immobilisation of nitrate in the short term [10,11].

Nitrate production in the previously air-dried soil was not ammonium-substrate limited throughout the 9-day incubation because ammonium spiking of the soil did not stimulate additional nitrification (Figure 1, upper chart, compare spotted and white bars). Ammonium spiking also failed to stimulate nitrate production significantly in the field-moist soils (Figure 1, lower chart). For the field-moist soil, ammonium added during spiking was not quickly immobilised into microbial biomass (Figure 2). However the ammonium spike was more rapidly immobilised in the air-dry soil. As outlined in the results, Figure 2, lower chart shows that the added ammonium-N spike was still largely KCl-extractable at T0, T1 and T2 from the field-moist soil. Atmospheric pollution of the Hob Moor soils by N deposition is high, and the soils there consequently have a low C:N ratio. Nevertheless, ammonium seems to be getting immobilised, and this may reflect the changes in biomass after removal of soil from contact with plant roots.

For the air-dry soils, the increasing (over time) oxidation of ammonium to nitrate (Figure 1, upper chart) prevented ammonium accumulation within the rewetted soil (Figure 2, upper chart and Table 1). In the field-moist soil, however, regardless of whether or not an ammonium-N or a nitrate-N spike had been added, ammonium concentration did not vary significantly over time (Table 1).

One of the most conspicuous effects of the drying/rewetting cycle is the sharp rise in ammonium-N concentration following drying/rewetting (Figure 2, compare white bars in upper and lower charts). It is almost certain that ammonification continues for a while during the drying stage, and not all residual ammonium would have been leached out by the soil washing stage with deionised water. Subsequently it appears that ammonium production and nitrification rates approximately match in the rewetted soils. For Californian oak wood and grassland soils it has been found that ammonium concentrations remained low and unaffected by wetting/drying stress cycles, but it was concluded that effects of repeated wetting/drying cycles on nitrate concentrations in soil could last for several weeks [12]. Appel reported a mineral N flush following drying/rewetting cycles from an arable soil in Germany [13], which he related to mobilisation of non-biomass organic N. Van Gestel et al. suggested that non-biomass organic residues contributed to flushes of mineral N after drying/rewetting, but thought that at least part of the flush was due to microbial biomass killed by drying [14]. Because of the sharp rise in ammonium N noted above following drying in our experiment, the net result here too was a mineral N flush, but a delay of a few days will occur before this manifests itself as an enhanced nitrate-N concentration. It should be remembered that the field moist soils had been stored at $<4\,^{\circ}C$ for six days while half of the soil was drying, and this would undoubtedly have slowed the production of ammonium-N in the stored soil during this period compared to the rate in the drying soil.

5. Conclusion

The results of this experiment support the hypothesis that for the acid soil used here, any rapid initial flush of nitrate from rewetted soil would have to have originated either from lysis of microbial cells or from residual nitrate stored in soil as it dried out. There will then be a second nitrate flush as the nitrifier population re-establishes. At this stage, ammonium accumulated in

the soil during the drying stage may be nitrified, further contributing to the delayed nitrate flush. Further work is needed however to assess the relative importance of these mechanisms. In the soil from this site, ammonium substrate was not limiting nitrification. This was as expected, bearing in mind the high level of N deposition at the site, which would also include redistributed inputs from manure from the cattle grazing the site over the summer and early autumn months.

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