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# Impact of microbial activity on the leaching of soluble N forms in soil

A. M. Carswell<sup>1</sup> · P. W. Hill<sup>2</sup> · D. L. Jones<sup>2</sup> · M. S. A. Blackwell<sup>1</sup> · P. J. Johnes<sup>3</sup> ·  
E. R. Dixon<sup>1</sup> · D. R. Chadwick<sup>2</sup>

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**Abstract** The hydrological transport of low-molecular weight organic nitrogen (LMWON) compounds has received little attention in the literature, particularly relative to inorganic nitrogen (N), with less attention given to the decoupling of the carbon (C) and N cycles following rainfall events. We determined the impacts of the soil biota on the transport of N compounds in a loam soil, using <sup>15</sup>N and <sup>13</sup>C to trace the vertical transport of <sup>15</sup>N<sup>13</sup>C-urea, <sup>15</sup>N<sup>13</sup>C-amino acids, <sup>15</sup>NO<sub>3</sub><sup>-</sup>, and <sup>15</sup>NH<sub>4</sub><sup>+</sup> through the soil profile, following simulated rainfall events. This research has demonstrated that biotic assimilation leads to rapid decoupling of the C and N cycles during leaching, with C transport limited to the soil surface (< 2 cm), whereas N which was stored within the soil profile during a single rainfall event could be remobilised and leached (a further 2–6 cm) following an additional rainfall event.

**Keywords** Amino acids · DON · Immobilisation · Leaching · Mobilisation · Urea

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✉ A. M. Carswell  
alison.carswell@rothamsted.ac.uk

<sup>1</sup> Rothamsted Research, Sustainable Agricultural Systems, Okehampton, Devon EX20 2SB, UK

<sup>2</sup> School of Environment, Natural Resources & Geography, Bangor University, Gwynedd LL57 2UW, UK

<sup>3</sup> School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS, UK

## Introduction

Hydrological transfer of nitrogen (N) in agricultural systems has typically focussed on the inorganic N forms of ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>), due to their relevance as N fertilisers (Lipiec et al. 2011; Peukert et al. 2014), and implications for eutrophication of surface waters and aquifers (Durand et al. 2011). Globally, however, urea (CH<sub>4</sub>N<sub>2</sub>O) remains the commonest form of fertiliser N applied to soil (Glibert et al. 2006), and due to its low cost and high N content its use is predicted to increase. As urea is known to rapidly hydrolyse in soil (forming (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>) and become susceptible to N losses by volatilisation (as NH<sub>3</sub>; Chambers and Dampney 2009), studies that examine urea cycling and N transport often focus on NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. Research focusing on intact urea transport is limited, although it is known to be transferred to receiving waterbodies, where it provides an N source to aquatic biota (Glibert et al. 2006). Similarly, other forms of dissolved organic N (DON) can also be lost to waterbodies, providing a bioavailable N source (Heathwaite and Johnes 1996; Durand et al. 2011). This focus on DON as a pathway of N losses (van Kessel et al. 2009) has meant that studies examining DON leaching have tended to focus on N and exclude C dynamics (Zhou et al. 2006; Abaas et al. 2012). However, uptake of low molecular weight organic N (LMWON) and its subsequent assimilation or immobilisation can primarily be driven by the C demands of soil microbes (Farrell et al. 2014). Thus, there is a need to examine both C and N dynamics to better understand the mechanisms behind mobilisation, immobilisation and leaching of DON.

Here we examine the role of single and double simulated rainfall events on leaching of <sup>15</sup>N<sup>13</sup>C-urea, <sup>15</sup>N<sup>13</sup>C-amino acids (AA), and <sup>15</sup>NO<sub>3</sub><sup>-</sup> and <sup>15</sup>NH<sub>4</sub><sup>+</sup> through soil profiles. The importance of microbial activity for decoupling the C

and N cycles (Knowles et al. 2010) through immobilisation/mobilisation processes was also assessed using sterile and non-sterile soil. A double rainfall event was simulated to examine the potential for C and N re-mobilisation following initial biotic immobilisation.

## Materials and methods

### Soil collection and processing

Soil was collected from 0 to 10 cm depth at a grassland site in the UK (Dystric Cambisol; online resource; Table S1), and stored at 4 °C in gas permeable bags before use, with three separate sites (2 m apart) forming the three replicates used throughout the study. Each soil replicate was sieved to < 2 mm and allowed to equilibrate at 20 °C overnight, prior to use in the leaching experiment, or for background characterisation of the soil. The centrifugation-drainage technique (Giesler and Lundström 1993) was used to obtain soil solution for background characterisation. All other soil properties were determined on the < 2 mm soil (see online Resource 1 for soil background characterisation methods; Table S1). A subsample was autoclaved twice at 121 °C for 20 min, for use as a sterilised control to distinguish the importance of biotic processes.

### Nitrogen and carbon transport experiment

Soil columns (9 × 235 mm; i.d. × h) were packed to a density of 0.69 g cm<sup>-3</sup> with either sterile or non-sterile < 2 mm soil to a height of 20 cm. Nitrogen treatments were applied to the surface of the soil column and consisted of a 50 µl addition of 10 mM N as <sup>15</sup>N<sup>13</sup>C-urea, <sup>15</sup>N<sup>13</sup>C-AA (equimolar mixture of asparagine acid, threonine, serine, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine; Sigma-Aldrich, 487910), K<sup>15</sup>NO<sub>3</sub>, or <sup>15</sup>NH<sub>4</sub>Cl (all labelled compounds were >98 atom%). Two leaching scenarios were examined, the first (LS1) was a single 30 min simulated rainfall event, started immediately after treatment application on both the sterile and non-sterile soils. The second (LS2) was the same as for LS1, however it was only conducted on non-sterile soils and, after the soil columns had been incubated at 10 °C for 7 days in the dark. All simulated rainfall events were conducted at an equivalent rainfall rate of 1.9 mm h<sup>-1</sup> to simulate a low-intensity rainfall event (not inclusive of the treatment addition), using locally collected rainwater delivered via a peristaltic pump. Following LS1, or LS2, soil columns were immediately cut into 1 cm sections between 0 and 10 cm depth (the maximum wetting front depth observed). Individual sections were placed in paper bags and dried at 80 °C for a minimum of

48 h to minimise microbial activity and remove soil moisture. Following drying, the 1 cm subsections were ball-milled to a fine powder, and subsamples weighed and sealed into 8 × 5 mm tin capsules prior to analysis.

### Laboratory and data analyses

Soil <sup>15</sup>N and <sup>13</sup>C, and total N and total C were measured in soil column subsections using a Carlo Erba NA 2000 linked to a Sercon 20/22 isotope ratio mass spectrometer (Sercon, Crewe, UK; Carlo Erba, CE Instruments, Wigan, UK). The % recovery of applied <sup>15</sup>N and <sup>13</sup>C was determined as:

$$\% \text{recovery} = 100 \left[ \frac{({}^{15}\text{N}^{13}\text{C}_{\text{Spl}} - {}^{15}\text{N}^{13}\text{C}_{\text{Bgd}})}{{}^{15}\text{N}^{13}\text{C}_{\text{Ap}}} \right] \quad (1)$$

where <sup>15</sup>N<sup>13</sup>C<sub>Spl</sub> is µg g<sup>-1</sup> <sup>15</sup>N<sup>13</sup>C in the enriched soil subsection, <sup>15</sup>N<sup>13</sup>C<sub>Bgd</sub> is the background µg g<sup>-1</sup> <sup>15</sup>N<sup>13</sup>C in the soil (prior to enrichment) and <sup>15</sup>N<sup>13</sup>C<sub>Ap</sub> is the µg g<sup>-1</sup> <sup>15</sup>N<sup>13</sup>C of the applied compounds. Analysis of variance test was used to examine differences between leaching scenarios, sterile and non-sterile soils, and N treatments were examined with each column treated as a main plot. Where depth was examined it was included as a split-plot. Multiple comparisons were made using either Tukey's test or Fisher's LSD test, and all analyses were performed in Genstat (v. 16; VSN International) and differences reported as significant where *p* < 0.05.

## Results and discussion

### Compound recovery

Across all treatments and transport scenarios, over the 0–10 cm depth sampled, mean <sup>15</sup>N recoveries were 92 ± 3%. In contrast, mean <sup>13</sup>C recovery was 69 ± 5%, with <sup>13</sup>C recovery in the non-sterile soils much reduced at 62 and 48% for <sup>15</sup>N<sup>13</sup>C-AA, and 6.2 and 5.9% for <sup>15</sup>N<sup>13</sup>C-urea under LS1 and LS2, respectively. The lower recovery of <sup>13</sup>C in the non-sterile soils can be related to C losses via microbial assimilation and respiration processes. We were unable to detect any culturable organisms following the culturing of sterile soil (data presented in Carswell et al. (2016)), nonetheless this method does not account for the presence of viable but non-culturable organisms (Kell et al. 1998). However, reduced <sup>13</sup>C recoveries of 73 and 65% were also observed in the sterilised soils for <sup>15</sup>N<sup>13</sup>C-AA and <sup>15</sup>N<sup>13</sup>C-urea, respectively. Wessel and Tietema (1992) suggest reduced recoveries can be caused by low <sup>13</sup>C application amounts and subsequent dilution into the natural abundance <sup>13</sup>C pool. Here, the compounds used were >98 atom% labelled, so any further addition would have required increasing the concentration of applied compounds beyond that which could reasonably be justified. It is also possible that during sterilisation microbial cells were lysed,

increasing extracellular organic C and consequently diluting the labelled  $^{13}\text{C}$  pool within the sterile soils.

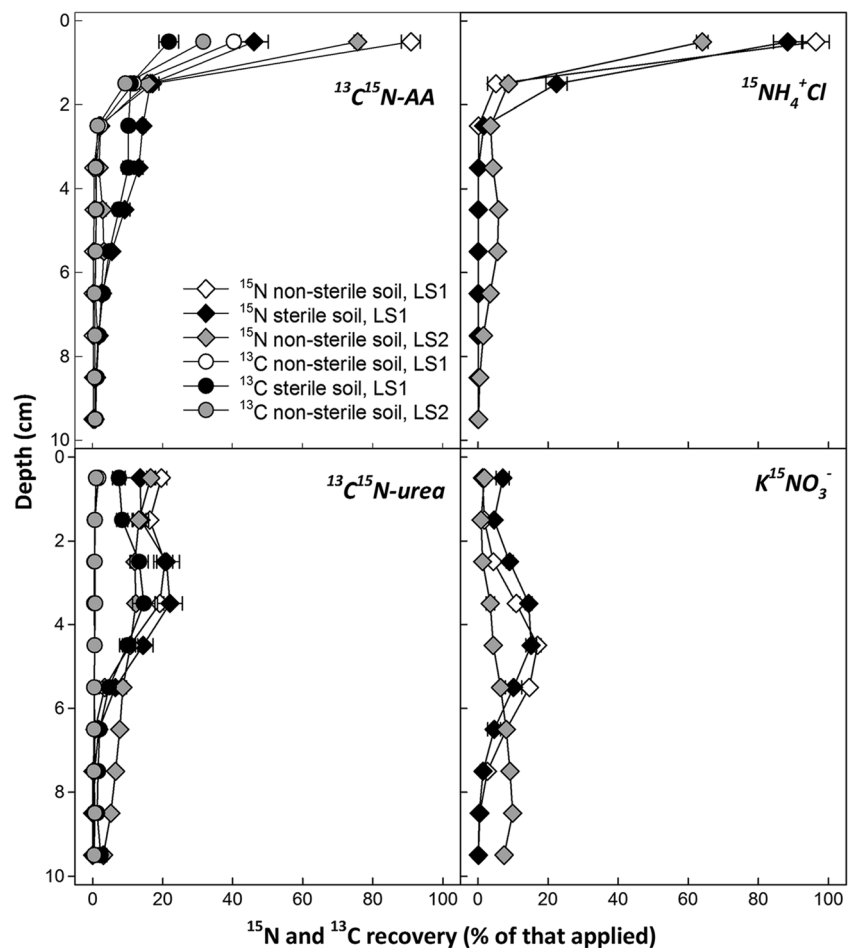
### Biotic processes influence N transport

Following LS1 significant differences were observed in  $^{15}\text{N}$  leaching between the sterile and non-sterile soils, and between N compounds ( $p < 0.01$ ; Fig. 1), indicating that biotic processes play an important role in N leaching, and this is dependent on the N compounds being examined. Under the  $^{15}\text{N}^{13}\text{C}$ -AA treatment, leaching was limited to the top 2 cm of the non-sterile soil columns, whereas leaching continued to 8 cm depth within the sterile soil columns. This indicates that microbial uptake, and subsequent assimilation and immobilisation is a key control on AA transport. Similarly,  $^{15}\text{NH}_4^+$  leaching was mostly limited to the top 2 cm of the soil columns (Fig. 1), for both the non-sterile and sterile soil columns, indicating that  $^{15}\text{NH}_4^+$  transport was limited by abiotic processes, such as adsorption to clay surfaces (Wang and Alva 2000). However, recovery was greatest at 0–1 cm in the non-sterile soil which is likely due to the additional impact of microbial  $^{15}\text{NH}_4^+$  uptake (Jackson et al. 1989). Both  $^{15}\text{N}$ -urea and  $^{15}\text{NO}_3^-$  were more mobile and leached deeper than  $^{15}\text{N}$ -AA

and  $^{15}\text{NH}_4^+$ , following LS1, with  $^{15}\text{N}$  reaching 6 and 8 cm for the  $^{15}\text{N}$ -urea and  $^{15}\text{NO}_3^-$  treatments, respectively. This greater mobility of  $\text{NO}_3^-$  was also observed by Zhou et al. (2006) who found  $\text{NO}_3^-$  leached twice as deep as  $\text{NH}_4^+$  in their sandy-loam soil. No significant differences were observed between the non-sterile and sterile soils for  $^{15}\text{N}$  recovery under the  $^{15}\text{N}^{13}\text{C}$ -urea treatment. However, significantly greater  $^{13}\text{C}$  recoveries (of 8–15%) were observed in the sterile soil columns at individual depths between 0 and 4 cm relative to the non-sterile soils (in which maximum  $^{13}\text{C}$  recovery of 1.6% occurred at 0–1 cm).

These results are indicative of rapid decoupling of the C and N cycle within the non-sterile soil columns under the  $^{15}\text{N}^{13}\text{C}$ -urea treatment. The loss of  $^{13}\text{C}$  from the non-sterile soils under the  $^{13}\text{C}^{15}\text{N}$ -urea treatment and to a lesser extent from the  $^{13}\text{C}^{15}\text{N}$ -AA treatment (see Fig. 1, total  $^{13}\text{C}$  recovery of 6.2, and 62%, respectively) is likely due to rapid N mineralisation (Knowles et al. 2010). This can occur via biotic uptake, which was observed in the same soil for  $^{14}\text{C}$ -urea and  $^{14}\text{C}$ -L-arginine (Carswell et al. 2016), or via extracellular enzymes, like urease which can rapidly mineralise urea (Tabatabai 1994), with both processes leading to  $\text{CO}_2$  losses. Following mineralisation of  $^{13}\text{C}^{15}\text{N}$ -urea or  $^{13}\text{C}^{15}\text{N}$ -AA, the

**Fig. 1** Percentage recoveries of  $^{15}\text{N}$  and  $^{13}\text{C}$  from the soil column 1 cm sections, for the treatments  $^{13}\text{C}^{15}\text{N}$ -amino acids,  $^{13}\text{C}^{15}\text{N}$ -urea  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  under leaching scenario 1 (LS1; a single simulated rainfall event performed on non-sterile and sterile soils), or leaching scenario 2 (LS2; single simulated rainfall event, followed by a 7-day 10 °C incubation and an additional repeat rainfall event performed on non-sterile soil only). Data points are mean  $\pm$  SEM ( $n = 3$ )



C and N cycle are decoupled (Knowles et al. 2010; see Fig. 1), the C is removed from the soil in a gaseous phase and the N remains in the soil matrix where it may be recycled and transformed into other N compounds.

### Repeated rainfall event remobilises and transfers N further down the soil column

After a 7-day incubation and a repeat simulated rainfall event (LS2), which was conducted on the non-sterile soil only,  $^{13}\text{C}$  recoveries of 5.9 and 48%, for  $^{13}\text{C}^{15}\text{N}$ -urea and  $^{13}\text{C}^{15}\text{N}$ -AA, respectively were observed. Mineralisation of AA, both individual and mixtures, have been observed to have a bi-phasic profile, where initially AA mineralisation is rapid, and is then followed by a slower secondary phase (Jones et al. 2009). In this study, the reduction in  $^{13}\text{C}$  recoveries after a 7-day incubation may be due to the extended duration of a slower secondary mineralisation phase. In the secondary mineralisation phase  $^{13}\text{C}$  losses are caused by turnover of the soil microbes, which had previously assimilated the  $^{15}\text{N}^{13}\text{C}$ -AA (Boddy et al. 2007).

In contrast, under LS2, re-mobilisation of  $^{15}\text{N}$  and subsequent leaching occurred under all treatments, with  $^{15}\text{N}$  reaching 8, 8, 10, and 10 cm depth after LS2, increases of 6, 5, 4, and 2 cm from LS1, for  $^{15}\text{NH}_4^+$ ,  $^{13}\text{C}^{15}\text{N}$ -AA,  $^{13}\text{C}^{15}\text{N}$ -urea, and  $^{15}\text{NO}_3^-$ , respectively. This suggests that  $^{15}\text{N}$  was released back into the soil solution by soil microbes, although not for  $^{15}\text{NO}_3^-$  which was not immobilised under LS1 and was transported with the wetting front. Once in the soil solution the  $^{15}\text{N}$  compounds would have been available for N cycling processes, including re-uptake by soil microbes. Consequently,  $^{15}\text{N}^{13}\text{C}$ -AA uptake and earlier immobilisation by microbial activity following LS1 was almost certainly driven by C rather than N demand, as the  $^{13}\text{C}$  was depleted and the remaining  $^{15}\text{N}$  was re-mobilised in LS2, suggesting an imbalance of microbial resource stoichiometry (Zhou et al. 2017). The addition of exogenous N and C to the soil in this study would have a priming effect on soil organic matter (SOM) decomposition (Chen et al. 2014). We suggest that, due to the high soil N supply in this agricultural soil, SOM decomposition would follow the stoichiometric decomposition theory of Craine et al. (2007) rather than the microbial N mining theory. Our findings are also in agreement with that of Farrell et al. (2014) who concluded that amino acid uptake by soil microbes was due to a requirement for C rather than N.

### Conclusions

To the knowledge of the authors, this dataset is the first to report the impact of two different leaching scenarios (simulated rainfall events) on the transfer of dual isotopically-labelled LMWON compounds in soil. The results support the theory

that biotic uptake of LMWON is largely driven by microbial demand for C rather than N, particularly in soils with high N supply, with large  $^{13}\text{C}$  losses via mineralisation and other metabolic processes, and the release and subsequent re-mobilisation of  $^{15}\text{N}$  into the soil solution. Transport of inorganic N compounds was also shown to be affected by biotic processes, although to a lesser extent than that of LMWON compounds. This work has shown that LMWON compounds are immobilised and transported in compound specific ways, which should be considered when modelling N pools and dynamics.

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