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

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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 15N and 13C to trace the vertical transport of 15N13C-urea, 15N13C-amino acids, 15NO3−, and 15NH4+ 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

Introduction

Hydrological transfer of nitrogen (N) in agricultural systems has typically focussed on the inorganic N forms of ammonium (NH4+) and nitrate (NO3−), 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 (CH4N2O) 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 (NH4)2CO3) and become susceptible to N losses by volatilisation (as NH3; Chambers and Dampney 2009), studies that examine urea cycling and N transport often focus on NH4+ and NO3−. 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 15N13C-urea, 15N13C-amino acids (AA), and 15NO3− and 15NH4+ 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⁻³ 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 ¹⁵N¹³C-urea, ¹⁵N¹³C-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), K¹⁵NO₃, or ¹⁵NH₄Cl (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⁻¹ 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 ¹⁵N and ¹³C, 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 ¹⁵N and ¹³C was determined as:

\[
\%\text{recovery} = 100 \left( \frac{(¹⁵N¹³C_{\text{Spl}} - ¹⁵N¹³C_{\text{Bgd}})}{¹⁵N¹³C_{\text{Ap}}} \right)
\]

where ¹⁵N¹³C_{\text{Spl}} is μg g⁻¹ ¹⁵N¹³C in the enriched soil subsection, ¹⁵N¹³C_{\text{Bgd}} is the background μg g⁻¹ ¹¹⁵N¹³C in the soil (prior to enrichment) and ¹⁵N¹³C_{\text{Ap}} is the μg g⁻¹ ¹¹⁵N¹³C 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 ¹⁵N recoveries were 92 ± 3%. In contrast, mean ¹³C recovery was 69 ± 5%, with ¹³C recovery in the non-sterile soils much reduced at 62 and 48% for ¹⁵N¹³C-AA, and 6.2 and 5.9% for ¹⁵N¹³C-urea under LS1 and LS2, respectively. The lower recovery of ¹³C 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 ¹³C recoveries of 73 and 65% were also observed in the sterilised soils for ¹⁵N¹³C-AA and ¹⁵N¹³C-urea, respectively. Wessel and Tietema (1992) suggest reduced recoveries can be caused by low ¹³C application amounts and subsequent dilution into the natural abundance ¹³C 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 $^{13}$C pool within the sterile soils.

**Biotic processes influence N transport**

Following LS1 significant differences were observed in $^{15}$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 $^{15}$N$^{13}$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, $^{15}$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 $^{15}$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 $^{15}$NH$_4^+$ uptake (Jackson et al. 1989). Both $^{15}$N-urea and $^{15}$NO$_3^-$ were more mobile and leached deeper than $^{15}$N-AA and $^{15}$NH$_4^+$, following LS1, with $^{15}$N reaching 6 and 8 cm for the $^{15}$N-urea and $^{15}$NO$_3^-$ treatments, respectively. This greater mobility of NO$_3^-$ was also observed by Zhou et al. (2006) who found NO$_3^-$ leached twice as deep as NH$_4^+$ in their sandy-loam soil. No significant differences were observed between the non-sterile and sterile soils for $^{15}$N recovery under the $^{15}$N$^{13}$C-urea treatment. However, significantly greater $^{13}$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 $^{13}$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 $^{15}$N$^{13}$C-urea treatment. The loss of $^{13}$C from the non-sterile soils under the $^{13}$C$^{15}$N–urea treatment and to a lesser extent from the $^{13}$C$^{15}$N–AA treatment (see Fig. 1, total $^{13}$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 $^{14}$C–urea and $^{14}$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 CO$_2$ losses. Following mineralisation of $^{13}$C$^{15}$N–urea or $^{13}$C$^{15}$N–AA, the

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**Fig. 1** Percentage recoveries of $^{15}$N and $^{13}$C from the soil columns 1 cm sections, for the treatments $^{13}$C$^{15}$N-amino acids, $^{13}$C$^{15}$N–urea, $^{15}$NO$_3^-$ and $^{15}$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 ± 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, 13C recoveries of 5.9 and 48%, for 13C15N–urea and 13C15N–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 15C recoveries after a 7-day incubation may be due to the extended duration of a slower secondary mineralisation phase. In the secondary mineralisation phase 13C losses are caused by turnover of the soil microbes, which had previously assimilated the 15N13C–AA (Boddy et al. 2007).

In contrast, under LS2, re-mobilisation of 15N and subsequent leaching occurred under all treatments, with 15N reaching 8, 8, 10, and 10 cm depth after LS2, increases of 6, 5, 4, and 2 cm from LS1, for 15NH4+, 13C15N–AA, 13C15N–urea, and 15NO3-, respectively. This suggests that 15N was released back into the soil solution by soil microbes, although for 15NO3– which was not immobilised under LS1 and transported with the wetting front. Once in the soil solution the 15N compounds would have been available for N cycling processes, including re-uptake by soil microbes. Consequently, 15N13C–AA uptake and earlier immobilisation by microbial activity following LS1 was almost certainly driven by C rather than N demand, as the 13C was depleted and the remaining 15N 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 13C losses via mineralisation and other metabolic processes, and the release and subsequent remobilisation of 15N 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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References

Abaas E, Hill PW, Roberts P, Murphy DV, Jones DL (2012) Microbial activity differentially regulates the vertical mobility of nitrogen compounds in soil. Soil Biol Biochem 53:120–123

Boddy E, Hill PW, Farrar J, Jones DL (2007) Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biol Biochem 39:827–835

Carswell AM, Hill PW, Jones DL, Blackwell MSA, Johnes P, Chadwick DR (2016) Short-term biotic removal of dissolved organic nitrogen (DON) compounds from soil solution and subsequent mineralisation in contrasting grassland soils. Soil Biol Biochem 96:82–85

Chambers B, Dampney P (2009) Nitrogen efficiency and ammonia emissions from urea-based and ammonium nitrate fertilisers. In Proceedings No. 657 International Fertiliser Society, York, pp 1–20

Chen R, Senbayram M, Blagodatsky S, Myachina O, Dittert K, Lin X, Blagodatskaya E, Kuzyakov Y (2014) Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob Chang Biol 20:2356–2367

Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113

Durand P, Breuer L, Johnes PJ (2011) Nitrogen processes in aquatic ecosystems. In: Sutton MA, Howard CM, Willem Erisman J, Billen G, Bleecker A, Grennfelt P, van Grinsven H, Grizzetti B (eds) The European nitrogen assessment. Cambridge University Press, Cambridge, pp 126–146

Farrell M, Prendergast-Miller M, Jones DL, Hill PW, Condron LM (2014) Soil microbial organic nitrogen uptake is regulated by carbon availability. Soil Biol Biochem 77:261–267

Giesler R, Lundström U (1993) Soil solution chemistry-effects of bulking soil samples. Soil Sci Soc Am J 57:1283–1288

Gilbert P, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea–a global change contributing to coastal eutrophication. Biogeochemistry 77:441–463
Heathwaite AL, Johnes PJ (1996) Contribution of nitrogen species and phosphorus fractions to stream water quality in agricultural catchments. Hydrol Process 10:971–983
Jackson LE, Schimel JP, Firestone MK (1989) Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biol Biochem 21:409–415
Jones DL, Kielland K, Sinclair FL, Dahlgren RA, Newsham KK, Farrar JF, Murphy DV (2009) Soil organic nitrogen mineralization across a global latitudinal gradient. Glob Biogeochem Cycles 23:1–5
Kell DB, Kaprelyants AS, Weichart DH, Harwood CR, Barer MR (1998) Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. Antonie Van Leeuwenhoek 73:169–187
van Kessel C, Clough T, van Groenigen JW (2009) Dissolved organic nitrogen: an overlooked pathway of nitrogen loss from agricultural systems? J Environ Qual 38:393–401
Knowles TDJ, Chadwick DR, Bol R, Evershed RP (2010) Tracing the rate and extent of N and C flow from $^{15}$C, $^{15}$N-glycine and glutamate into individual de novo synthesised soil amino acids. Org Geochem 41:1259–1268
Lipiec J, Nosalewicz A, Siczek A, Kotowska U (2011) Effects of land use on leaching of nitrate-N, ammonium-N and phosphate in relation to stained surface area. Int Agrophys 25:149–154
Peukert S, Griffith BA, Hawkins JMB, Orr RJ, Blackwell MSA, Murray PJ, Macleod CJA, Brazier RE (2014) Intensive management in grasslands causes diffuse water pollution at the farm scale. J Environ Qual 43:2009–2023
Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angel GS, Bottomley PS (eds) Methods of soil analysis, part 2: microbiological and biochemical properties. Soil Sci Soc Am Inc, Madison, pp 775–833
Wang FL, Alva AK (2000) Ammonium adsorption and desorption in sandy soils. Soil Sci Soc Am J 64:1669–1674
Wessel WW, Tietema A (1992) Calculating gross N transformation rates of N-15 pool dilution experiments with acid forest litter-analytical and numerical approaches. Soil Biol Biochem 24:931–942
Zhou JB, Xi JG, Chen ZJ, SX LI (2006) Leaching and transformation of nitrogen fertilizers in soil after application of N with irrigation: a soil column method. Pedosphere 16:245–252
Zhou Z, Wang C, Jin Y (2017) Stoichiometric responses of soil microflora to nutrient additions for two temperate forest soils. Biol Fertil Soils 53:397–406