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Short communication

Short-term biotic removal of dissolved organic nitrogen (DON) compounds from soil solution and subsequent mineralisation in contrasting grassland soils

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ABSTRACT

Cycling of low molecular weight dissolved organic nitrogen compounds constitutes an important component of soil organic matter turnover in soils. We determined how rapidly grassland soils can cycle urea, compared to the amino acid L-alanine, and the peptide L-trialanine. Using naturally occurring concentrations of ^{14}C-labelled compounds the rates of removal from soil solution and subsequent mineralisation were measured. Biotic removal of all three compounds and subsequent mineralisation to CO_2 occurred within minutes. This research has demonstrated, for the first time, the potential for rapid removal of urea at low concentrations by the soil microbial biomass.

Adverse ecosystem effects of excess nitrogen (N) have been observed globally (Vitousek et al., 2009). Excess N in grasslands, prone to leaching and N emissions, is typically derived from N amendments including organic manures, urine patches and excessive use of synthetic fertilisers. Of these, urea has frequently been examined due to its importance as a fertiliser (IFA, 2014), and its presence in manures and urine (Ball and Ryden, 1984).

Urea is a low molecular weight dissolved organic N (LMW-DON) compound with a C:N molar ratio of 1:2.33, and similar to nitrate and ammonium, is capable of being taken up directly by both plants and microorganisms (Berman and Bronk, 2003; Wang et al., 2008). The extent to which plants can acquire LMW-DON and the degree to which it leaches down the soil profile, is critically dependent on the activity of the soil microbial biomass (SMB; Jones et al., 2013). Recent studies indicate that uptake of LMW-DON by the SMB is frequently driven by carbon (C) rather than by N demand (Farrell et al., 2014). Therefore, the presence of C within urea may drive its rate of removal from grassland soil solutions. Although urease activity in soil (Nielsen et al., 1998; Bolado-Rodriguez et al., 2005), and to a lesser extent urea assimilation by the SMB (Smith et al., 2007) have been investigated, urea removal from the soil solution by the SMB over short time-scales has not.

Here SMB removal of ^{14}C-urea from the soil solution and its subsequent catabolic and anabolic partitioning was examined in three grassland soils. Microbial cycling of urea was directly compared to that of other typical LMW-DON compounds found in soils: the amino acid ^{14}C-L-alanine and the oligopeptide ^{14}C-L-trialanine, whose turnover have been extensively characterised and have also been implicated in direct plant LMW-DON acquisition (Hill et al., 2011; Wilkinson et al., 2014).

Soil was collected from three separate grazed grassland sites in the UK (Table 1). All soils were collected towards the end of the growing season (October), with three independent replicates collected for each type. Soil cores (10 × 8.5 cm; h × i.d.) were kept intact, at field-moisture, in gas-permeable bags, in the dark at 4 °C prior to use.

To characterise LWM-DON in each soil, porewater was obtained from intact soil cores, with the root mat removed, by centrifugation-drainage (Giesler and Lundström, 1993). Soluble N was determined as described by Farrell et al. (2013) and Sullivan and Havlin (1991). All experimentation with ^{14}C-labelled compounds was performed on < 2 mm sieved soil from separately taken soil samples, which had equilibrated to 20 °C overnight. The rate of LMW-DON depletion from soil solution was measured

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Table 1
Characteristics of the three grassland soils (upper 10 cm) used in the study. Values represent mean ± SEM (n = 3).

	Dystric Cambisol*	Stagni-vertic Cambisol*	Eutric Cambisol**
Sample location	50°46′N, 3°55′W	50°47′N, 3°57′W	53°14′N, 4°01′W
Soil texture	Loam*	Heavy clay*	Sandy clay loam**
Soil pH	5.2 ± 0.07	5.3 ± 0.07	4.8 ± 0.03
Total soil C (g kg^{-1} DW)	21.1 ± 0.04	29.1 ± 0.29	27.1 ± 0.19
Total soil N (g kg^{-1} DW)	3.6 ± 0.02	4.0 ± 0.02	4.1 ± 0.04
Soil water (kg kg^{-1} DW)	0.19 ± 0.01	0.35 ± 0.02	0.29 ± 0.00
Soil solution free amino acids (μM)	11.3 ± 1.08	6.50 ± 1.18	8.00 ± 1.48
Soil solution short peptides (<1 kDa; μM)	153 ± 47.3	145 ± 24.7	164 ± 52.0
Soil solution NO_3–N (mg N l^{-1})	10.6 ± 0.92	0.74 ± 0.23	2.22 ± 1.21
Soil solution urea (μM)	6.22 ± 0.20	7.24 ± 0.62	15.33 ± 8.12
Soil solution NH_4–N (mg N l^{-1})	0.14 ± 0.02	0.30 ± 0.07	0.23 ± 0.05
Soil solution DOC (mg C l^{-1})	29.38 ± 3.25	31.80 ± 5.51	19.98 ± 2.77
Soil solution DON (mg N L^{-1})	1.94 ± 0.38	2.17 ± 0.38	1.54 ± 1.26
Soil respiration (mg C kg^{-1} dry soil h^{-1})	0.37 ± 0.05	0.86 ± 0.32	0.60 ± 0.14

Data gained from the literature are marked with either * (described by Harrod and Hogan (2008)) or ** (described by Hill et al. (2012)).

according to Hill et al. (2008). Briefly, 1 g soil (dry weight equivalent; DW) was placed in a microcentrifuge tube with a hole pierced in the bottom. This tube was placed inside another microcentrifuge tube. 300 μl of either ^{14}C-labelled urea, L-alanine or L-trialanine (10 μM, 0.9 kBq mL^{-1}) was then applied to the soil surface and allowed to infiltrate the soil (<2.5 s, 20 °C; associated soil water content increase to 45–52%). This concentration was chosen to reflect the urea and free amino acid concentrations naturally occurring within soil solution (Table 1). At 1, 5, 10, 30 and 60 min after substrate addition, the soil was centrifuged (4000 *g*, 1 min, 4 °C; data presented for 0 min in Figs. 1 and 2 are assumed) allowing the soil solution to pass to the lower microcentrifuge tube. The ^{14}C content of the recovered soil solution was determined after addition of Scintisafe3 scintillation cocktail (Fisher Scientific, Loughborough, UK) using a Wallac 1404 liquid scintillation counter (Perkin Elmer Life Sciences, Boston, MA). To assess the mineralisation rate of LMW-DON compounds, 1 g soil was placed in a glass tube through which air was passed before being transferred through 2 successive 0.1 M NaOH traps to capture evolved $^{14}CO_2$. At 1, 5, 10, 30 and 60 min after ^{14}C-labelled substrate addition (as above), NaOH was replaced and its ^{14}C content determined as above. To separate biotic (e.g. microbial, enzymatic) and abiotic (e.g. sorption) LMW-DON removal processes, the soil solution recovery experiment was also performed on sterilised soil (autoclaved at 121 °C, 20 min). Recovery of ^{14}C-labelled compounds from the sterilised soil solutions was used to calculate the theoretical maximum ^{14}C-activity (Hill et al., 2008) that could be recovered following complete mixing of amended ^{14}C-labelled treatments with native soil solution. A two-way ANOVA was used to test for differences and interactions between soils and LMW-DON treatments.

Complete mixing of the applied treatments with native soil solution was not achieved, and after 60 min deviated between 101 and 153%. Greater than 100% recovery of ^{14}C-compounds was achieved at all incubation periods, thus demonstrating that no retention of ^{14}C-compounds occurred in the sterile soils (Wilkinson et al., 2014; see Supplementary Information for equations),

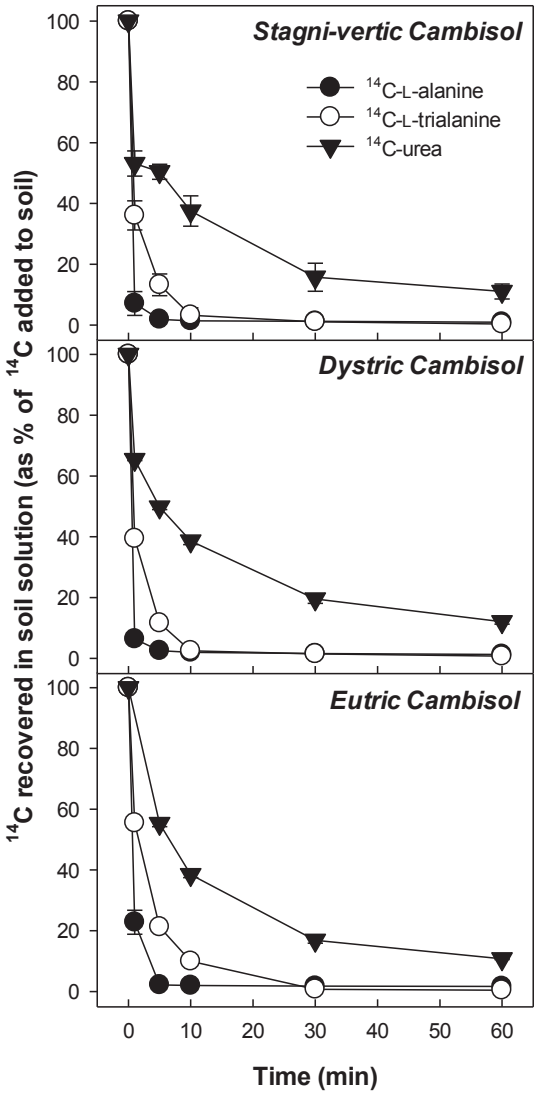

Fig. 1. Microbially-mediated depletion of ^{14}C-labelled alanine, trialanine or urea from soil solution in three grassland soils. Data points represent means ± SEM (n = 3).

consequently no evidence of abiotic loss pathways was observed. However autoclaving soils can increase the solubilisation of soil organic matter (SOM; Powlson and Jenkinson, 1976), which may block adsorption sites that would be available in the living soils. Nonetheless autoclaving has been found to be more effective that $CHCl_3$ fumigation or gamma irradiation at reducing viable cell numbers (Blankinship et al., 2014), and consequently was chosen as the optimal sterilisation method for this study.

All ^{14}C-labelled LMW-DON compounds were rapidly removed from the soil solution (Fig. 1). After 60 min, removal of ^{14}C-L-alanine and ^{14}C-L-trialanine was almost complete, at 98.7 and 99.5% respectively. However, removal of ^{14}C-urea was consistently lower at all incubation periods, and after 60 min was 88.7%. Removal from soil solution followed the series: alanine > trialanine > urea ($p < 0.001$). Across all soils, the half-life of urea, alanine and trialanine in solution was 4.15 ± 0.69, 0.30 ± 0.04, 0.94 ± 0.13 min (mean ± SEM; based on fitting first order single exponential decay to the data), respectively. In contrast, no effect of soils was observed on substrate depletion. This is perhaps unsurprising as cycling of key LMW-DON compounds can be remarkably similar across diverse soils and systems (Jones et al., 2009). Slower uptake of urea