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

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13 **ABSTRACT**

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

22

23 Keywords: Dissolved organic matter, Nutrient cycling, Urine patch, Urea

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) demand rather than N (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-Rodríguez 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

48 replicates collected for each type. Soil cores (10 × 8.5 cm; h × i.d.) were kept intact, at field-
49 moisture, in gas-permeable bags, in the dark at 4°C prior to use.

50 To characterise LWM-DON in each soil, porewater was obtained from intact soil cores,
51 with the root mat removed, by centrifugation-drainage (Giesler and Lundstöm, 1993). Soluble
52 N was determined as described by Farrell et al. (2013) and Sullivan and Havlin (1991). All
53 experimentation with ^{14}C-labelled compounds was performed on < 2 mm sieved soil from
54 separately taken soil samples, which had equilibrated to 20ºC overnight. The rate of LMW-
55 DON depletion from soil solution was measured according to Hill et al. (2008). Briefly, 1 g
56 soil (dry weight equivalent; DW) was placed in a microcentrifuge tube with a hole pierced in
57 the bottom. This was placed inside another microcentrifuge tube. 300 µl of either ^{14}C-labelled
58 urea, L-alanine or L-trialanine (10 µM, 0.9 kBq mL^{-1}) was then applied to the soil surface and
59 allowed to infiltrate the soil (< 2.5 sec, 20°C; associated soil water content increase to 45-52%).
60 This concentration was chosen to reflect the urea and free amino acid concentrations naturally
61 occurring within soil solution (Table 1). At 1, 5, 10, 30 and 60 min after substrate addition, the
62 soil was centrifuged (4000 *g*, 1 min, 4°C; data presented for 0 min in Figures 1 and 2 are
63 assumed) allowing the soil solution to pass to the lower microcentrifuge tube. The ^{14}C content
64 of the recovered soil solution was determined after addition of Scintisafe3 scintillation cocktail
65 (Fisher Scientific, Loughborough, UK) using a Wallac 1404 liquid scintillation counter (Perkin
66 Elmer Life Sciences, Boston, MA). To assess the mineralisation rate of LMW-DON
67 compounds, 1 g soil was placed in a glass tube through which air was passed before being
68 transferred through 2 successive 0.1 M NaOH traps to capture evolved $^{14}CO_2$. At 1, 5, 10, 30
69 and 60 min after ^{14}C-labelled substrate addition (as above), NaOH was replaced and its ^{14}C
70 content determined as above. To separate biotic (e.g. microbial, enzymatic) and abiotic (e.g.
71 sorption) LMW-DON removal processes, the soil solution recovery experiment was also
72 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 with native soil solution was not achieved, and after 60 min deviated between 101-153%. Greater than 100% recovery 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), 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. Although soil sterilisation via autoclaving has been found to be more effective that $CHCl_3$ fumigation or gamma irradiation at reducing viable cell numbers (Blankinship et al., 2014).

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 relative to the other compounds could be linked to lower transporter expression and affinity within the SMB. It has also been proposed that soil microorganisms exist in a C-starved state (Hobbie and Hobbie, 2013). Accordingly, in the soils

98 examined here the SMB may have exhibited a preference for compounds with a greater C
99 content and which are commonly present in soil solution (amino acids and peptides are present
100 in rhizodeposits and via protease action on SOM).

101 In contrast to removal from soil solution, soil affected ($p < 0.001$) mineralisation of
102 different compounds at 1, 5, 10 and 30 min, but not after 60 min (Fig. 2). The differences
103 between soils may, in part, be due to differences in initial soil water content, which can impact
104 net mineralisation rates (Paul et al., 2003). However, the experiment was performed on field
105 moist soils to represent the same preceding climatic conditions rather than target a specific soil
106 water content (Table 1). ^{14}C-urea had the highest mineralisation rates of the three LMW-DON
107 compounds. This may be attributed to the alternative mineralisation pathway urea can take via
108 the enzyme urease, which is encountered both intra- and extracellularly. Extracellular urease
109 has been shown to account for an average of 46% of total urease activity in a range of soils
110 (Klose and Tabatabai, 1999). This may account for the more rapid mineralisation of urea
111 relative to alanine and trialanine. Although ^{14}C-urea was most rapidly mineralised of the three
112 compounds, only 40-45% of ^{14}C-urea removed from the soil solution was subsequently respired
113 as $^{14}CO_2$ over 60 min, suggesting that the remaining ^{14}C-urea was assimilated by the SMB
114 (Nielsen et al., 2008). Another intracellular urea pathway is via ATP:urea amidolyase (Cheng
115 et al., 2005; Strope et al., 2011), which produces NH_3 and HCO_3^- via two enzyme (urea
116 carboxylase and allophanate hydrolase) reactions, making it a likely path for urea assimilation
117 in soils.

118 Although it is widely acknowledged that urea is rapidly mineralised in soils, this is the
119 first time that such rapid removal of urea from the soil solution by the SMB has been reported.
120 This suggests that there will be strong microbial competition for urea in soil which may limit
121 its capture by plant roots when present in low concentrations. The fate of urea-derived NH_4^+

requires further investigation. Assimilation of urea by the SMB at higher concentrations of urea and following application of solid urea as fertiliser requires further work.

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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 a * (described by Harrod and Hogan, 2008) or a ** (described by Hill et al., 2012).

199
200

Figure legends

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).

Fig. 2. Time-dependent cumulative mineralisation of ^{14}C-labelled alanine, trialanine or urea in three grassland soils. Data points represent means ± SEM (n = 3).