



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

Carswell, A. M.; Hill, P. W.; Jones, David; Blackwell, M. S. A.; Johnes, P.; Chadwick, D. R.

### Soil Biology and Biochemistry

DOI:

[10.1016/j.soilbio.2016.01.017](https://doi.org/10.1016/j.soilbio.2016.01.017)

Published: 01/05/2016

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*

Carswell, A. M., Hill, P. W., Jones, D., Blackwell, M. S. A., Johnes, P., & Chadwick, D. R. (2016). Short-term biotic removal of dissolved organic nitrogen (DON) compounds from soil solution and subsequent mineralisation in contrasting grassland soils. *Soil Biology and Biochemistry*, 96, 82-85. <https://doi.org/10.1016/j.soilbio.2016.01.017>

#### Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

3

4 A.M. Carswell<sup>a\*</sup>, P.W. Hill<sup>b</sup>, D.L. Jones<sup>b</sup>, M.S.A. Blackwell<sup>c</sup>, P. Johnes<sup>a</sup>, D.R. Chadwick<sup>b</sup>

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

6 <sup>b</sup>*School of the Environment, Natural Resources & Geography, Bangor University, Gwynedd,*

7 *LL57 2UW, UK*

8 <sup>c</sup>*Rothamsted Research, Okehampton EX20 2SB, Devon, UK*

9

10

11 *Corresponding author: Tel +44 117 928 8808; fax: +44 117 928 7878*

12 *e-mail address: alison.carswell@bristol.ac.uk (A.M. Carswell)*

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 <sup>14</sup>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<sub>2</sub> 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

24 Adverse ecosystem effects of excess nitrogen (N) have been observed globally (Vitousek et al.,  
25 2009). Excess N in grasslands, prone to leaching and N emissions, is typically derived from N  
26 amendments including organic manures, urine patches and excessive use of synthetic  
27 fertilisers. Of these, urea has frequently been examined due to its importance as a fertiliser  
28 (IFA, 2014), and its presence in manures and urine (Ball and Ryden, 1984).

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

40 Here SMB removal of  $^{14}\text{C}$ -urea from the soil solution and its subsequent catabolic and  
41 anabolic partitioning was examined in three grassland soils. Microbial cycling of urea was  
42 directly compared to that of other typical LMW-DON compounds found in soils: the amino  
43 acid  $^{14}\text{C}$ -L-alanine and the oligopeptide  $^{14}\text{C}$ -L-trialanine, whose turnover have been extensively  
44 characterised and have also been implicated in direct plant LMW-DON acquisition (Hill et al.,  
45 2011; Wilkinson et al., 2014).

46 Soil was collected from three separate grazed grassland sites in the UK (Table 1). All  
47 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 <sup>14</sup>C-labelled compounds was performed on < 2 mm sieved soil from  
54 separately taken soil samples, which had equilibrated to 20°C 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 <sup>14</sup>C-labelled  
58 urea, L-alanine or L-trialanine (10 µM, 0.9 kBq mL<sup>-1</sup>) 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 <sup>14</sup>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 <sup>14</sup>CO<sub>2</sub>. At 1, 5, 10, 30  
69 and 60 min after <sup>14</sup>C-labelled substrate addition (as above), NaOH was replaced and its <sup>14</sup>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 <sup>14</sup>C-labelled

73 compounds from the sterilised soil solutions was used to calculate the theoretical maximum  
74  $^{14}\text{C}$ -activity (Hill et al., 2008) that could be recovered following complete mixing of amended  
75  $^{14}\text{C}$ -labelled treatments with native soil solution. A two-way ANOVA was used to test for  
76 differences and interactions between soils and LMW-DON treatments.

77 Complete mixing with native soil solution was not achieved, and after 60 min deviated  
78 between 101-153%. Greater than 100% recovery was achieved at all incubation periods, thus  
79 demonstrating that no retention of  $^{14}\text{C}$ -compounds occurred in the sterile soils (Wilkinson et  
80 al., 2014; see supplementary information for equations), consequently no evidence of abiotic  
81 loss pathways was observed. However autoclaving soils can increase the solubilisation of soil  
82 organic matter (SOM; Powlson and Jenkinson, 1976), which may block adsorption sites that  
83 would be available in the living soils. Although soil sterilisation via autoclaving has been found  
84 to be more effective than  $\text{CHCl}_3$  fumigation or gamma irradiation at reducing viable cell  
85 numbers (Blankinship et al., 2014).

86 All  $^{14}\text{C}$ -labelled LMW-DON compounds were rapidly removed from the soil solution  
87 (Fig. 1). After 60 min, removal of  $^{14}\text{C}$ -L-alanine and  $^{14}\text{C}$ -L-trialanine was almost complete, at  
88 98.7 and 99.5% respectively. However, removal of  $^{14}\text{C}$ -urea was consistently lower at all  
89 incubation periods, and after 60 min was 88.7%. Removal from soil solution followed the  
90 series: alanine > trialanine > urea ( $p < 0.001$ ). Across all soils, the half-life of urea, alanine and  
91 trialanine in solution was  $4.15 \pm 0.69$ ,  $0.30 \pm 0.04$ ,  $0.94 \pm 0.13$  min (mean  $\pm$  SEM; based on  
92 fitting first order single exponential decay to the data), respectively. In contrast, no effect of  
93 soils was observed on substrate depletion. This is perhaps unsurprising as cycling of key LMW-  
94 DON compounds can be remarkably similar across diverse soils and systems (Jones et al.,  
95 2009). Slower uptake of urea relative to the other compounds could be linked to lower  
96 transporter expression and affinity within the SMB. It has also been proposed that soil  
97 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).  $^{14}\text{C}$ -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  $^{14}\text{C}$ -urea was most rapidly mineralised of the three  
112 compounds, only 40-45% of  $^{14}\text{C}$ -urea removed from the soil solution was subsequently respired  
113 as  $^{14}\text{CO}_2$  over 60 min, suggesting that the remaining  $^{14}\text{C}$ -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; Strobe et al., 2011), which produces  $\text{NH}_3$  and  $\text{HCO}_3^-$  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  $\text{NH}_4^+$

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

124

## 125 **Acknowledgements**

126 This work was funded by the UK Natural Environment Research Council  
127 (NE/K01093X/1), and the Lawes Trust/University of Reading Studentship scheme.

128

## 129 **References**

130 Ball, P.R., Ryden, J.C., 1984. Nitrogen relationships in intensively managed temperate  
131 grasslands. *Plant and Soil* 76, 23-33.

132 Berman, T., Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic  
133 ecosystems. *Aquatic Microbial Ecology* 31, 279-305.

134 Blankinship, J.C., Becerra, C.A., Schaeffer, S.M., Schimel, J.P., 2014. Separating cellular  
135 metabolism from exoenzyme activity in soil organic matter decomposition. *Soil*  
136 *Biology and Biochemistry* 71, 68-75.

137 Bolado-Rodriguez, S., Alonso-Gaite, A., Alvarez-Benedi, J., 2005. Characterization of  
138 nitrogen transformations, sorption and volatilization processes in urea fertilized soils.  
139 *Vadose Zone Journal* 4, 329-336.

140 Cheng, G., Shapir, N., Sadowsky, M.J., Wackett, L.P., 2005. Allophanate hydrolase, not  
141 urease, functions in bacterial cyanuric acid metabolism. *Applied and Environmental*  
142 *Microbiology* 71, 4437-4445.

143 Farrell, M., Hill, P.W., Farrar, J., DeLuca, T.H., Roberts, P., Kielland, K., Dahlgren, R.,  
144 Murphy, D.V., Hobbs, P.J., Bardgett, R.D., Jones, D.L., 2013. Oligopeptides represent  
145 a preferred source of organic n uptake: a global phenomenon? *Ecosystems* 16, 133-145.



146 Farrell, M., Prendergast-Miller, M., Jones, D.L., Hill, P.W., Condon, L.M., 2014. Soil  
147 microbial organic nitrogen uptake is regulated by carbon availability. *Soil Biology &*  
148 *Biochemistry* 77, 261-267.

149 Giesler, R., Lundstrom, U., 1993. Soil solution chemistry - Effects of bulking soil samples.  
150 *Soil Science Society of America Journal* 57, 1283-1288.

151 Harrod, T.R., Hogan, D.V., 2008. The soils of North Wyke and Rowden Revised edition of  
152 Harrod, T.R. (1981) *Soils in Devon V: Sheet SS61 (Chumleigh) Soil Survey Record*  
153 no. 70.

154 Hill, P.W., Farrar, J.F., Jones, D.L., 2008. Decoupling of microbial glucose uptake and  
155 mineralization in soil. *Soil Biology & Biochemistry* 40, 616-624.

156 Hill, P.W., Farrell, M., Jones, D.L., 2012. Bigger may be better in soil N cycling: Does rapid  
157 acquisition of small L-peptides by soil microbes dominate fluxes of protein-derived N  
158 in soil? *Soil Biology & Biochemistry* 48, 106-112.

159 Hill, P.W., Quilliam, R.S., DeLuca, T.H., Farrar, J., Farrell, M., Roberts, P., Newsham, K.K.,  
160 Hopkins, D.W., Bardgett, R.D., Jones, D.L., 2011. Acquisition and assimilation of  
161 nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS ONE* 6,  
162 1-4.

163 Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: the  
164 starving-survival lifestyle in soil and consequences for estimating microbial rates.  
165 *Frontiers in Microbiology* 4, 324.

166 IFA., 2014. Production and international trade, IFA Statistics, 2014, online.

167 Jones, D.L., Clode, P.L., Kilburn, M.R., Stockdale, E.A., Murphy, D.V., 2013. Competition  
168 between plant and bacterial cells at the microscale regulates the dynamics of nitrogen  
169 acquisition in wheat (*Triticum aestivum*). *New Phytologist* 200, 796-807.

170 Jones, D.L., Kielland, K., Sinclair, F.L., Dahlgren, R.A., Newsham, K.K., Farrar, J.F.,  
171 Murphy, D.V., 2009. Soil organic nitrogen mineralization across a global latitudinal  
172 gradient. *Global Biogeochemical Cycles* 23, GB1016.

173 Nielsen, T.H., Bonde, T.A., Sorensen, J., 1998. Significance of microbial urea turnover in N  
174 cycling of three Danish agricultural soils. *FEMS Microbiology Ecology* 25, 147-157.

175 Paul, K.I., Polglase, P.J., O'Connell, A.M., Carlyle, J.C., Smethurst, P.J., Khanna, P.K.,  
176 2003. Defining the relation between soil water content and net nitrogen mineralization.  
177 *European Journal of Soil Science* 54, 39-47.

178 Powelson, D.S., Jenkinson, D.S., 1976. Effects of biocidal treatments on metabolism in soil. 2.  
179 Gamma-irradiation, autoclaving, air-drying and fumigation. *Soil Biology and*  
180 *Biochemistry* 8, 179-188.

181 Smith, J.L., Bell, J.M., Bolton, H., Bailey, V.L., 2007. The initial rate of C substrate  
182 utilization and longer-term soil C storage. *Biology and Fertility of Soils* 44, 315-320.

183 Strope, P., Nickerson, K., Harris, S., Moriyama, E., 2011. Molecular evolution of urea  
184 amidolyase and urea carboxylase in fungi. *BMC Evolutionary Biology* 11, 1-15.

185 Sullivan, D.M., Havlin, J.L., 1991. Flow injection analysis of urea nitrogen in soil extracts.  
186 *Soil Science Society of America Journal* 55, 109-113.

187 Tabatabai, M.A., 1994. Soil enzymes, In: Weaver, R.W., Angel, G.S., Bottomley, P.S. (eds),  
188 *Methods of Soil Analysis, part 2: Microbiological and Biochemical Properties*,  
189 Madison, USA, Soil Science Society of America.

190 Vitousek, P.M., Naylor, R., Crews, T., David, M.B., Drinkwater, L.E., Holland, E., Johnes,  
191 P.J., Katzenberger, J., Martinelli, L.A., Matson, P.A., Nziguheba, G., Ojima, D., Palm,  
192 C.A., Robertson, G.P., Sanchez, P.A., Townsend, A.R., Zhang, F.S., 2009. Nutrient  
193 imbalances: Pollution remains response. *Science* 326, 665-666.

- 194 Wang, W.H., Kohler, B., Cao, F.Q., Liu, L.H., 2008. Molecular and physiological aspects of  
195 urea transport in higher plants. *Plant Science* 175, 467-477.
- 196 Wilkinson, A., Hill, P.W., Farrar, J.F., Jones, D.L., Bardgett, R.D., 2014. Rapid microbial  
197 uptake and mineralization of amino acids and peptides along a grassland productivity  
198 gradient. *Soil Biology and Biochemistry* 72, 75-83.

**Table 1** Characteristics of the three grassland soils (upper 10 cm) used in the study. Values represent mean  $\pm$  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 $\pm$ 0.07	5.3 $\pm$ 0.07	4.8 $\pm$ 0.03
Total soil C (g kg <sup>-1</sup> DW)	21.1 $\pm$ 0.04	29.1 $\pm$ 0.29	27.1 $\pm$ 0.19
Total soil N (g kg <sup>-1</sup> DW)	3.6 $\pm$ 0.02	4.0 $\pm$ 0.02	4.1 $\pm$ 0.04
Soil water (kg kg <sup>-1</sup> DW)	0.19 $\pm$ 0.01	0.35 $\pm$ 0.02	0.29 $\pm$ 0.00
Soil solution free amino acids ( $\mu$ M)	11.3 $\pm$ 1.08	6.50 $\pm$ 1.18	8.00 $\pm$ 1.48
Soil solution short peptides (<1 kDa; $\mu$ M)	153 $\pm$ 47.3	145 $\pm$ 24.7	164 $\pm$ 52.0
Soil solution NO <sub>3</sub> -N (mg N l <sup>-1</sup> )	10.6 $\pm$ 0.92	0.74 $\pm$ 0.23	2.22 $\pm$ 1.21
Soil solution urea ( $\mu$ M)	6.22 $\pm$ 0.20	7.24 $\pm$ 0.62	15.33 $\pm$ 8.12
Soil solution NH <sub>4</sub> -N (mg N l <sup>-1</sup> )	0.14 $\pm$ 0.02	0.30 $\pm$ 0.07	0.23 $\pm$ 0.05
Soil solution DOC (mg C l <sup>-1</sup> )	29.38 $\pm$ 3.25	31.80 $\pm$ 5.51	19.98 $\pm$ 2.77
Soil solution DON (mg N L <sup>-1</sup> )	1.94 $\pm$ 0.38	2.17 $\pm$ 0.38	1.54 $\pm$ 1.26
Soil respiration (mg C kg <sup>-1</sup> dry soil h <sup>-1</sup> )	0.37 $\pm$ 0.05	0.86 $\pm$ 0.32	0.60 $\pm$ 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

201 **Figure legends**

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

204

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

207