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Short Communication

Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts

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ABSTRACT

Soil extracts are routinely used to quantify dissolved organic nutrient concentrations in soil. Here we studied the loss and transformation of low molecular weight (LMW) components of DOC (^{14}C-glucose, 1 and 100 μM) and DON (^{14}C-amino acid mixture, 1 and 100 μM) during extraction of soil (0–6 h) with either distilled water or 0.5 M K_2SO_4. The extractions were performed at 20 °C, at 4 °C, or in the presence of an inhibitor of microbial activity ($HgCl_2$ and Na-azide). We showed that both glucose and amino acids became progressively lost from solution with increasing shaking time. The greatest loss was observed in H_2O extracts at 1 μM for both substances (>90% loss after 15 min). Lower temperature (4 °C) and presence of K_2SO_4 both resulted in reduced loss rates. The presence of microbial inhibitors effectively eliminated the loss of glucose and amino acids. We conclude that microbial transformation of LMW-DOC and DON during H_2O or K_2SO_4 extraction of soil may affect the estimation of their concentrations in soil. This finding has significant implications for methods that rely on chemical extractions to estimate LMW-C components of DOC and DON.

Low molecular weight (LMW) components of the soil organic matter have recently been gaining attention due to the potentially important contributions by LMW hydrocarbons such as monosaccharides, amino acids and organic acids to soil respiration (Van Hees et al., 2005, 2008; Bengtson and Bengtsson, 2007; Boddy et al., 2007; Oburger and Jones, 2009) and to the putative role of LMW organic N, including amino acids, as a N resource for plant growth (Birt and Hird, 1958; Chapin et al., 1993; Näsholm et al., 1998; Owen and Jones, 2001). The detection, quantification and turnover assessment of these compounds in soil all rely on extraction steps to facilitate chemical analysis. Common to all these extractions is, importantly, a time-lag between the start of extraction of DOC and DON from soil and its subsequent detection due both to time of extraction and interval between end of extraction and analysis. Here, we studied the loss and transformation of LMW components of DOC (glucose) and DON (amino acid mix) at typical soil concentrations in soil extracts (1 and 100 μM) as a function of extraction time (0–6 h). This was achieved by monitoring the recovery of added ^{14}C-labelled substances from the extracts. We extracted soil solutions with distilled H_2O or 0.5 M K_2SO_4 at 20 °C, at 4 °C, or in the presence of an inhibitor of microbial activity ($HgCl_2$ and Na-azide).

Soil was obtained from the surface horizons of two unfertilized, freely draining, profiles of grassland soils which had very different organic matter contents, Soil A and Soil B (Table 1). From both sites, soil (0–10 cm depth) was collected using a 5 cm diameter stainless steel corer and 10 individual samples taken within a 4 m^2 area and were pooled to form each of four representative samples ($n = 4$; sieved <2 mm; stored at 4 °C until analysis). Soil pH and electrical conductivity were determined in 1:1 (v/v) soil:H_2O extracts and total C and total N were determined with a CHN-2000 analyzer (Leco Corp., St Joseph, MI). Exchangeable cations were determined in 1:5 (w/v) soil:1 M $BaCl_2$ extracts by Inductively Coupled Plasma-Argon Emission Spectroscopy. All values provided are averages of four (organic C, total N and pH) or two (the other values) determinations (Table 1).

To extract dissolved organic (DON) and inorganic nitrogen (DIN), 1.5 g of field-moist soil was mixed with 7.5 ml of either distilled H_2O or 0.5 M K_2SO_4 in 20 ml polypropylene tubes and placed on an orbital shaker (250 rev min^{-1}, 1 h). After shaking, samples were centrifuged for 10 min at 15,000 *g* to remove suspended solids. Supernatant solutions were retained for N analysis and stored frozen (−20 °C) until analysis.

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Table 1
Selected properties of the two test soils used.

	Soil A	Soil B
Location	Snowdonia national park, UK (53° 04′ N, 4° 03′ W; 450 m elevation)	Abergwyngregyn, UK (53° 14′ N, 4° 01′ W; 150 m elevation)
Mean annual rainfall	2500 mm	1250 mm
Predominant vegetation cover	*Festuca ovina* L. var. Ovina, *Agrostis capillaries* L.	*Lolium perenne* L., *Cynosurus cristatus* L.
Horizon	O	Ah
Classification	Typic Fragiochrept	Typic dystrochrept
Grazing	Sheep	Sheep
Texture	–	Sandy loam
$CaCO_3$, g kg^{-1}	<0.1	<0.1
Organic C, g kg^{-1}	154	39.9
Total N, g kg^{-1}	9.3	3.3
C to N ratio	16.6	12.1
$EC_{1:1}$, dS m^{-1}	0.35	0.52
$pH_{(H_2O)}$	3.6	4.3
K, $mmol_c$ kg^{-1}	5.7	13.8
Na, $mmol_c$ kg^{-1}	5.7	10.8
Ca, $mmol_c$ kg^{-1}	8.1	15
Mg, $mmol_c$ kg^{-1}	2.2	2.6
Al, $mmol_c$ kg^{-1}	25.5	9.2

Spiking of the samples with a known quantity of LMW-DOC or DON was achieved by the addition of a known quantity of uniformly ^{14}C-labelled glucose (0.1 kBq ml^{-1}) or a mixture of fifteen uniformly ^{14}C-labelled L-amino acids to the extraction solution (H_2O or 0.5 M K_2SO_4) immediately prior to shaking. The standard amino acid mixture (pH 5.60) was obtained by mixing together the following ^{14}C-labelled amino acids (ICN Pharmaceuticals Inc., Irvine, CA) to give a final total amino acid concentration in the extraction solution of either 1 or 100 μM and specific activity of 0.1 kBq ml^{-1}: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine. The added concentrations of glucose and amino acids were based on previous estimates in soil solution including those for the soils used here (Monreal and McGill, 1985; Chapin et al., 1993; Boddy et al., 2007; Van Hees et al., 2005, 2008; Fujii et al., 2010). At known times after the start of shaking, at either 4 or 20 °C, aliquots (500 μl) from the extractions were collected centrifuged (at 4 or 20 °C; 15,000 *g*; 10 min) and the amount of ^{14}C remaining in the supernatant solution was determined using a Wallac 1404 liquid scintillation counter (PerkinElmer Corp., Beverly, MA). To suppress microbial activity during soil extraction, $HgCl_2$ and Na-azide were added to the extractant solution immediately prior to shaking to a final concentration of 10 mM.

DON was determined by the modified persulfate-oxidation procedure in which DON is converted to NO_3^-, as described by Williams et al. (1994). All DON standards yielded typically >95% N recovery as NO_3^- after the persulfate-autoclaving step. NH_4^+ was determined spectrophotometrically by the salicylate–nitroprusside method of Mulvaney (1996) while NO_3^- was determined colorimetrically using Cd–Cu reduction/N-1-napthylethylenediamine with a Perstorp flow-injection analyzer. DON was calculated as the difference between the persulfate-oxidation reading (Total N) and the combined NH_4^+ and NO_3^- reading (inorganic N). Total amino acid concentrations in the soil extracts (of the 100 μM glucose treatment only) were determined fluorometrically according to Jones et al. (2002). ANOVA with Tukey pair-wise comparison was used to compare treatment factors (JMP 7.0 for Mac, SAS Institute Inc., Cary, NC, USA).

More than 90% of the 1 μM glucose (Fig. 1A) and amino acid (Fig. 2A) additions were lost within 1 h of extraction in H_2O at 20 °C. The rates of loss of DOC (Fig. 1) and DON (Fig. 2) were lower in the K_2SO_4 extractions compared with the H_2O extractions for both soils ($P < 0.001$ after 15 min in all treatments except 100 μM glucose in soil A, where the same difference emerged after 45 min), and also lower in 4 °C extractions compared with 20 °C ($P < 0.001$ for all treatments after 45 min for glucose, Fig. 1, and after 1 h for amino acids, Fig. 2). These differences increased with longer times of extraction. The proportional loss of 100 μM additions of glucose and amino acids were slower than the 1 μM additions, as indicated by longer half-times, and also slower in soil B compared with soil A. The difference in loss rates in 0.5 M K_2SO_4 compared with H_2O extracts were higher in soil B compared with soil A, and after 6 h there were no consistent differences between recovery from K_2SO_4 extractions performed at 4 °C and those performed in the presence of microbial inhibitors. This is evidenced by very low losses of 100 μM glucose (Fig. 1D) and both 1 and 100 μM amino acids (Fig. 2B, D) in these treatments of soil B. The loss of added ^{14}C-labelled glucose and amino acids from solution was severely reduced by the presence of microbial inhibitors in both soils irrespective of extractant and substrate concentration. This quickly (<15 min) led to large differences in ^{14}C-glucose and amino acid concentration between the 20 °C H_2O extraction and the extractions with inhibitors ($P < 0.001$ in all eight cases).

Intrinsic total free amino acid concentrations (i.e. non-^{14}C-labelled) remained largely unchanged around 1–2 μM in both soils in the 20 °C H_2O treatment (Fig. 3). K_2SO_4 extracts yielded higher concentrations of amino acids ($P < 0.001$ for both soils at all timepoints). The addition of microbial inhibitors resulted in higher concentrations of amino acids being extracted compared with the 20 °C water treatment ($P < 0.001$ for both soils at all timepoints). In addition, the inhibitors led to a progressive increase in total amino acid concentrations with longer extractions times (Fig. 3). However, in soil B there were no additional effects by the addition of inhibitor compared with only K_2SO_4.

Extractions with distilled H_2O indicated that approximately 20% of the extractable N in the two soils was in the form of DON (Table 2). In comparison to H_2O extractions, K_2SO_4 yielded a similar DON recovery. The amount of NH_4^+ extracted from both soils was significantly greater with K_2SO_4 than with H_2O, while the amount of NO_3^- extracted appeared to be independent of the extractant used. Only small additional amounts of DON were recovered with K_2SO_4 in comparison to H_2O (increase at 4 °C in both soils = 33.2 ± 6.6%, Table 2).

Water extraction of soil is a very common method used to evaluate soil DOC concentrations (*e.g.* Embacher et al., 2007; Macdonald et al., 2007; Marinari et al., 2010). Here we show that LMW-DOC and DON, added at realistic rates in the form of glucose or an amino acid mixture, became progressively lost from solution with increasing shaking time. The greatest loss was observed in H_2O extracts performed at room temperature and when glucose and amino acid concentrations were low (1 μM). The concentration dependence of microbial uptake is consistent with the higher affinity system acting on lower LMW-C concentrations (at or below in situ concentrations) compared with a slower low-affinity system active for higher concentrations (Hill et al., 2008). As expected, the application of factors to reduce the rate of microbial activity decreased loss rates of LMW-DOC and DON and reducing the extract temperature (Lloyd and Taylor, 1994) or using K_2SO_4 (Wichern et al., 2006) both resulted in reduced rates of LMW-DOC and DON loss. Finally, the addition of microbial inhibitors ($HgCl_2$ and Na-azide) eliminated this loss suggesting that the observed losses of the ^{14}C-labelled compounds in the other treatments were related to microbial activity rather than due to solid phase sorption processes.