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Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils

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Abstract

Large amounts of low molecular weight (LMW; <250 Da) carbon (C) are lost from roots into the rhizosphere as a consequence of root turnover and exudation. Their rates of turnover after release into the soil remain poorly understood. We extracted soil solution from a temperate grassland Eutric Cambisol, isotopically labeled the glucose and amino acid components, and then re-injected the solution back into the soil. We followed the subsequent evolution of $^{14}CO_2$ and incorporation of the LMW C into the soil microbial biomass or grasses for 48 h. The experiments were performed both on grazed and un-grazed swards in the field, and in the laboratory. In the field, we showed that glucose and amino acids had short half-lives ($t_{1/2}$) in soil solution ($t_{1/2}$ = 20–40 min), but that they persisted in soil microbes for much longer. A first-order double exponential model fitted the experimental data well and gave rate constant (k) values of 1.21–2.14 h^{-1} for k_1 and 0.0025–0.0048 h^{-1} for k_2. Only small amounts of the added ^{14}C were recovered in plant biomass (<5% of total added to soil) indicating that plant roots are poor competitors for LMW dissolved organic C (DOC) in comparison to soil microorganisms. The first phase of glucose and amino acid mineralization in the laboratory was slower ($t_{1/2}$ = 40–60 min) than measured in the field reinforcing the importance of making flux measurements in situ. Whilst grazing stimulated below-ground respiration, it exerted only a small influence on the turnover of LMW DOC suggesting that the increase in respiration was due to increased root respiration and not turnover of soil organic matter (SOM). Our results suggest that some components of the LMW DOC pool are turned over extremely rapidly (ca. 4000 times annually).

Keywords: Biodegradation; Carbon cycling; Carbon dioxide; Dissolved organic carbon; Dissolved organic nitrogen; DOC; DON; Grazing; Mineralization; Rhizodeposition; Soluble carbon

1. Introduction

Soils contain a large pool of potentially mineralizable organic carbon and any net increase in soil CO_2 emissions in response to environmental change may have the potential to exacerbate global warming. Consequently, identifying the factors that regulate soil respiration is critical in predicting ecosystem responses to global change. Soil respiration is derived partly from soil organisms and partly from plant roots. Soil microbial community respiration is usually limited by the availability of C substrates (Raich and Tufekcioglu, 2000). Carbon substrates are supplied to the soil microorganisms mainly by root exudation and turnover (rhizodeposition). Typically, 1–10% of a plant's net fixed C is lost into the soil by rhizodeposition while a further 20–40% is lost as root respiration (Nguyen, 2003; Warembourg et al., 2003).

The dissolved organic carbon (DOC) pool provides the main source of C for soil microorganisms and may be the most dynamic component of soil C (Pelz et al., 2005). Although root exudates are largely composed of low molecular weight (LMW) compounds (e.g. sugars, organic acids, amino acids), the size of this C pool is very low in comparison to the C in a solid form (van Hees et al., 2005). Further, much of the C in this solid pool is probably not of recent root origin and remains relatively recalcitrant to microbial attack (Kalbitz et al., 2000). This suggests that

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the root exudate components of DOC are either rapidly removed by sorption to the solid phase, abiotically mineralized, volatilized, leached or captured by soil microorganisms and plant roots (Jones et al., 2004; Rasse et al., 2005).

Soil organic C (SOC) displays a large degree of heterogeneity in the turnover of its different pools, with turnover times estimated to range from 1 to 6000 y (Knorr et al., 2005). In grassland topsoil, the turnover of total SOM has been estimated to range from 8 to 34 y (Potvin et al., 2004). In the laboratory, however, the turnover of LMW C compounds of forest and grassland soils ranges from 1 to 12 h (Jones et al., 2005b; van Hees et al., 2005). It remains unclear whether these fast turnover times are an artifact of laboratory conditions caused by starvation of the microbial community following the removal of plant roots. Further, some previous studies have added unnaturally large concentrations of substrate to the soil, which may not reflect steady state concentrations in the soil solution (Coody et al., 1986).

Dilkes et al. (2004) showed that after a plant fixes C, its appearance in root exudates occurred within 0.5–3 h. Further, the exudation flux was dependent upon the physiological state of the plant at the time of C fixation. This implies that C flow into the rhizosphere will be a highly dynamic process both spatially and temporally. The primary aim of this study was to determine in situ whether the rate of LMW DOC mineralization to CO_2 in two grassland soils is similarly rapid to the flow of C from plant shoot to soil. This was determined with two groups of compounds which represent dominant components of root exudates: sugars and amino acids. The secondary aim was to compare the turnover time of the results obtained in the field with those obtained in the laboratory.

2. Materials and methods

2.1. Field sites and sampling regime

Soil was obtained from a temperate oceanic agricultural grassland located in Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°01′W; Table 1). The mean annual soil temperature at 10 cm depth is 11 °C and the annual rainfall is 1250 mm. The soil is derived from glacial till of Ordovician origin and is classified as a Eutric Cambisol with crumb structure and texture of 48% sand, 29% silt and 23% clay and supports a sward of *Lolium perenne* L. and *Trifolium repens* L. The field site contained four delineated areas of improved pasture used for high-density sheep grazing and four delineated areas that have not received grazing for 2 y. Field studies were undertaken during August, 2004. At this time, the soil temperature (5 cm depth) was 19.2±0.4 °C in the grazed plots and 15.0±0.1 °C in the un-grazed plots. For the laboratory studies, four intact swards, from within the grazed plots, of 10 × 8 × 5 cm were collected from the field. For soil

Table 1
Selected properties of the two grassland soils used in the mineralization studies

Grassland	Above-ground biomass ($g\,m^{-2}$)	Below-ground biomass ($g\,m^{-2}$)	Moisture content (%)	pH	Wet bulk density ($g\,cm^{-3}$)	Soil microbial C ($mg\,kg^{-1}$)	Total C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	C-to-N ratio	Gross photosynthesis ($g\,CO_2\,m^{-2}\,h^{-1}$)	Below-ground respiration ($g\,CO_2\,m^{-2}\,h^{-1}$)	Below-ground respiration (% C fixed)
Grazed	252±48	921±7	32.6±0.8	5.98±0.06	1.54±0.04	750±38	31.51±1.98	3.01±0.20	10.5±0.2	3.18±0.03	1.91±0.12	60±2
Un-grazed	779±96	959±4	31.6±0.5	5.07±0.14	1.58±0.05	693±83	29.92±1.70	3.69±2.17	8.1±1.0	2.76±0.14	1.17±0.06	43±2
	**	NS	NS	NS	NS	NS	NS	NS	NS	*	***	***

Values represent means±SEM; $n = 18$ for gas-exchange measurements and $n = 4$ for all other measurements. The symbols *, **, *** indicate significant differences at the $P<0.05$, $P<0.01$ and $P<0.001$ levels respectively between grazed and un-grazed treatments, while NS indicates no significant difference ($P>0.05$). Below-ground biomass is for the 0-5 cm soil layer.

characterization intact soil cores were taken to a depth of 5 cm using a 4.2 cm diameter soil corer.

2.2. Background plant and soil characteristics

Soil bulk density and moisture content were assessed by oven drying soil at 80 °C. Dry, root-free soil was analyzed for C and N content in a Leco CHN 2000 analyzer (Leco Corp., St Joseph, MI, USA). Soil pH was measured with a BDH Gelplas electrode (VWR International, Lutterworth, UK) in a 1:1 mixture with deionized water. Roots were separated from soil by hand washing in a 0.5 mm mesh sieve. Root and shoot biomass were assessed after drying at 80 °C. Photosynthesis was measured with a CIRAS 2 infrared gas analyzer (IRGA) (PP Systems Ltd., Hitchin, UK) with an acrylic canopy cuvette and below-ground respiration was measured with an EGM-4 IRGA with soil respiration chamber (PP Systems Ltd.).

2.3. Soil solution extraction

Soil solution was extracted according to Giesler and Lundström (1993). Briefly, intact soil cores were centrifuged (4000*g*, 15 min, 20 °C) to obtain soil solution and the collected solutions passed through a Whatman 42 filter paper before freezing at −20 °C to await chemical analysis. For the mineralization experiments, a spike of ^{14}C–U-labeled glucose or amino acids or $NaH^{14}CO_3$ (50 μl; <10 nM; 1 μCi ml^{-1}; Amersham Biosciences UK Ltd., Chalfont St. Giles, UK) was added to 450 μl of soil solution. The amino acids consisted of an equimolar mixture of fifteen uniformly ^{14}C-labeled L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine).

2.4. Carbon substrate mineralization in the field

To trace the fate of C added to the soil in situ, the soil solution containing the ^{14}C-labeled substrates was added directly to the soil in the grazed and un-grazed field plots. Briefly, 500 μl of the ^{14}C-labelled solution was injected back into the soil between the grass plants at a maximum depth of 2 cm below the root surface. This injection depth represents the zone of maximal root biomass. An open topped plastic cylinder (area 6.2 cm^2) was then placed over the labeled area and pushed into the soil to a depth of 2 cm. A 1 M NaOH trap (1 ml) was then placed inside the cylinder to catch evolved $^{14}CO_2$ and the cylinder sealed at the top. The NaOH trap was suspended above the soil to allow free diffusion of CO_2 from the soil surface. The cylinder was then covered with silver foil to prevent secondary fixation of evolved $^{14}CO_2$ by the plants within the chamber. The NaOH trap was replaced after 0.25, 0.5, 1, 2, 4, 8, 12, 20, 28, 36, and 48 h and the $^{14}CO_2$ in the 1 M NaOH traps determined by liquid scintillation counting using a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) and Optiphase 3® alkali compatible scintillation fluid (Wallac EG&G).

Following the final change of NaOH trap at 48 h, the aboveground vegetation was removed to a distance of 4.2 cm from the point of labeling and oven dried (80 °C, 24 h). Soil and roots were also removed from the same area to a depth of 5 cm and oven dried (80 °C). After oven drying, the soil was sieved (2 mm) and roots and bulk soil separated. Inevitably, a very small amount of rhizoplane soil remained attached to the root surface. The ^{14}C content of roots, shoots and soil were determined with an OX-400 Biological Sample Oxidizer (RJ Harvey Instrument Corp., Hillsdale, NJ). The liberated $^{14}CO_2$ was collected in Oxosol scintillation fluid (National Diagnostics, Hessle, UK) and then counted on the scintillation counter as described previously.

To quantify the recovery of $^{14}CO_2$ using the method outlined above (i.e. NaOH trapping efficiency) we added $NaH^{14}CO_3$ to the soil solution in place of the ^{14}C-labelled substrate and injected this into the soil. The $NaH^{14}CO_3$ was rapidly converted to $^{14}CO_2$ and thus provided a control to account for any non-biological effects.

2.5. Carbon substrate mineralization in the laboratory

Intact grass swards were collected from the grazed plots and placed in plastic trays in a climate-controlled growth chamber (Fi-totron PG660/C/RO/HQI; Sanyo-Gallenkamp Ltd., Loughborough, UK) with constant temperature of 20 °C, relative humidity of 70%, photoperiod of 16 h and light intensity of 500 μmol photons m^{-2} s^{-1} photosynthetic active radiation at canopy height. CO_2 concentrations within the growth cabinets were maintained at 350 μmol mol^{-1} by 1.5 changes per hour of external air. The swards were kept in the growth chamber for 24 h prior to experimentation, and hydration was maintained by watering daily from below. ^{14}C-labelled substrates were added to the grass swards and $^{14}CO_2$ evolution measured as described above for the field.

2.6. Chemical analysis

Soil solution samples were analyzed for DOC and total dissolved N (TDN) using a Shimadzu TOC–V–TN analyzer (Shimadzu Corp., Kyoto, Japan). NO_3^- was determined colorimetrically by the Cu–Zn–hydrazine reduction method of Downes (1978) and NH_4^+ by the salicylate-hypochlorite procedure of Mulvaney (1996). Phenolic concentrations were assessed using the Folin Ciocalteau reagent, calibrated with a phenol standard, according to Swain and Hillis (1959). Free amino acids were measured fluorometrically according to Jones et al. (2003). Glucose was determined spectrophotometrically with a Glucose (GO) Assay® kit (Sigma-Aldrich, Missouri, USA).

2.7. Statistical and data analysis

To calculate the recovery efficiency of $^{14}CO_2$ from the $NaH^{14}CO_3$ added (i.e. the amount of $^{14}CO_2$ lost and not captured by the NaOH trap; S_U), a single first order kinetic equation with asymptote was fitted to the experimental data where

$$S = S_U + [T_{CO_2} \times \exp(-k_{CO_2} t)] \quad (1)$$

and where S is the ^{14}C-label remaining in the soil, S_U is the amount of $^{14}CO_2$ unrecovered, T_{CO_2} describes the total recovery of $^{14}CO_2$ in the NaOH traps, k_{CO_2} is the rate constant describing the recovery of $^{14}CO_2$ and t is time. The half-life of $NaH^{14}CO_3$ in soil ($t_{1/2}$) was then calculated as follows:

$$t_{1/2} = \ln(2)/k_{CO_2}. \quad (2)$$

Previous studies have shown that amino acid and glucose mineralization in soils is biphasic (Chotte et al., 1998; Saggar et al., 1999; Jones et al., 2005b; van Hees et al., 2005). The two processes can be described by a double first-order decay equation

$$S = S_U + [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)], \quad (3)$$

where S is the ^{14}C-label remaining in the soil, S_U is a defined asymptote from the $NaH^{14}CO_3$ experiment (i.e. the capture efficiency of the system: $32.98 \pm 0.38\%$ is unrecovered in grazed systems and $30.71 \pm 0.95\%$ in un-grazed systems), k_1 is the rate constant describing the primary mineralization phase, k_2 is the rate constant describing the second, slower mineralization phase, a_1 and a_2 describe the size of pools with rate constants k_1 and k_2 and t is time.

We suggest that the first rapid phase of $^{14}CO_2$ production is attributable to the immediate use of the substrate in catabolic processes (i.e. respiration) and approximates to the depletion rate from the soil solution. Since glucose adsorption to soil particles is minimal (Kuzyakov and Jones, 2006) and adsorption of amino acids is low (Jones, 1999), we further suggest that the remaining substrate taken up is immobilized in the microbial biomass (i.e. formation of new biomass and metabolites; Paul and Clark, 1996). Thus, the slower second phase of $^{14}CO_2$ production is then attributable to the subsequent turnover of the soil microbial community leading to the production of CO_2. However, there may be more than one functional compartment in the decomposition system including, specific death rates of different microbes, subsequent turnover of the necromass and the decomposition of metabolites and storage compounds all contribute to measured $^{14}CO_2$ in the second phase making it difficult to differentiate between the decay rates of various compartments (Saggar et al., 1999; Toal et al., 2000).

The half-life of the first rapid phase of $^{14}CO_2$ production can be calculated using a first-order kinetic model (Paul and Clark, 1996)

$$t_{1/2} = \ln(2)/k_1. \quad (4)$$

Various models have been suggested for the fate of C substrates after entering the soil, proposing different connectivity of pool a_2 to a_1 (Moorby and Jarman, 1975; Saggar et al., 1996; Kuzyakov and Demin, 1998; Saggar et al., 1999; Toal et al., 2000). Consequently, we do not calculate the half-time for pool a_2 as we do not know enough about its connectivity to a_1.

All experiments were conducted in quadruplicate. Data were subjected to factorial ANOVA (Univariate GLM) whereby respiration (mineralization of $^{14}CO_2$) could be compared across treatments (amino acid, glucose or $NaH^{14}CO_3$) and location (laboratory versus field or grazed versus un-grazed). Component analysis of the field plots was also analyzed using factorial ANOVAs. Statistical procedures were carried out with the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) with $P < 0.05$ used as the upper limit for statistical significance.

3. Results

3.1. Soil solution chemistry

In both grassland soils DON represented approximately 70% of the total soluble N in soil (Table 2). The total free amino acids represented on average $12 \pm 3\%$ of the DON in soil (assuming an average amino acid N content of $19.6\,g\,mol^{-1}$). If it is assumed that plant roots can only directly take up inorganic N (NO_3^- and NH_4^+) and amino acids from soil (Jones et al., 2005a) then the free amino acids represented $23 \pm 2\%$ of the plant available N. The DOC-to-DON ratio of the soil solution (14 ± 2) was significantly greater than the C to N ratio of the total soil (9 ± 1; Tables 1 and 2).

On average, free glucose represented $1.4 \pm 0.4\%$ of the total DOC in soil solution. Similarly, free amino acids also

Table 2
Measured C and N components of the soil solution of grazed and ungrazed grassland soils

Grassland	Dissolved organic carbon ($mg\,C\,l^{-1}$)	Dissolved organic nitrogen ($mg\,N\,l^{-1}$)	Glucose ($mg\,C\,l^{-1}$)	Total free amino acids ($mg\,C\,l^{-1}$)	Total phenolics ($mg\,C\,l^{-1}$)	NO_3^- ($mg\,N\,l^{-1}$)	NH_4^+ ($mg\,N\,l^{-1}$)
Grazed	179 ± 20	12 ± 3	3.5 ± 0.6	6.7 ± 4.2	3.7 ± 0.1	5.7 ± 1.0	1.0 ± 0.3
Un-grazed	211 ± 67	17 ± 4	3.9 ± 0.7	6.1 ± 1.2	4.6 ± 1.1	4.3 ± 0.6	0.5 ± 0.1
	NS	NS	NS	NS	NS	NS	NS

Values represent means $\pm$ SEM ($n = 4$). The symbols *, **, *** indicate significant differences at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels respectively between grazed and un-grazed treatments, while NS indicates no significant difference ($P > 0.05$).

represented a small proportion of the total DOC ($3.3 \pm 0.4\%$ of total assuming an average amino acid C content of $62.4\,g\,mol^{-1}$). Consequently, over 95% of the DOC in soil remained unidentified. There were no significant differences in the concentration of solutes between the grazed and un-grazed treatments ($P > 0.05$; Table 2).

3.2. Inorganic C dynamics in the field

Our results showed that on average only $67 \pm 2\%$ of the added $NaH^{14}CO_3$ could be recovered as $^{14}CO_2$ by the NaOH traps with the remainder presumably lost by lateral diffusion of $^{14}CO_2$ through the soil (Fig. 1). The experimental data conformed well to a single exponential decay model with asymptote ($r^2 > 0.997$ for both grazing treatments). From this model we calculated the half-life ($t_{1/2}$) of $NaH^{14}CO_3$ in soil to be 0.47 ± 0.16 h. There was no significant difference in $t_{1/2}$ for the two grazing treatments ($P > 0.05$).

3.3. DOC mineralization dynamics in the field

Following addition of the soil solution containing radiolabeled substrates to the soil, there was an initial rapid phase of $^{14}CO_2$ evolution followed by a secondary slower phase (Fig. 2). The pattern was similar in all treatments. A double exponential decay gave a good fit to the biphasic experimental data (r^2 values > 0.985 in all cases; Eq. (3), Fig. 2). The recovery of $^{14}CO_2$ after the addition of ^{14}C-glucose to the soil was significantly greater than that recovered after the addition of ^{14}C-amino acid ($P < 0.05$). Overall, there was a close similarity between the dynamics of $^{14}CO_2$ evolution in the grazed and un-grazed treatments throughout the 48 h period (Fig. 2). ANOVA

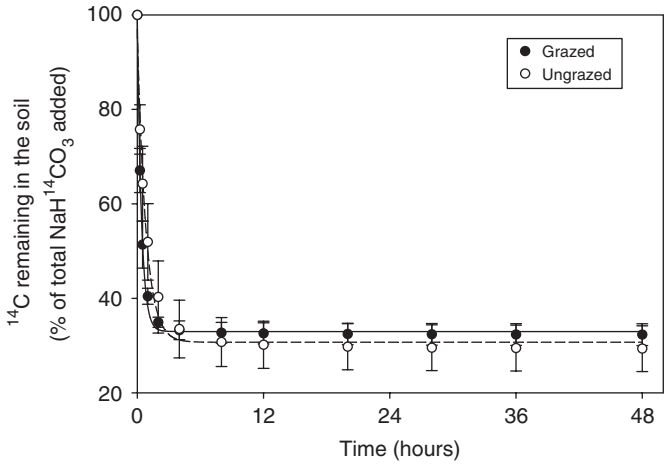

Fig. 1. Amount of ^{14}C remaining in a grassland soil after the injection of $NaH^{14}CO_3$ into the soil and its subsequent capture as $^{14}CO_2$ in a 1 M NaOH trap located at the soil surface. Values represent means SEM ($n = 4$). Lines represent fits of a single first order with asymptote kinetic equation to the experimental data (see Section 2 for further details).

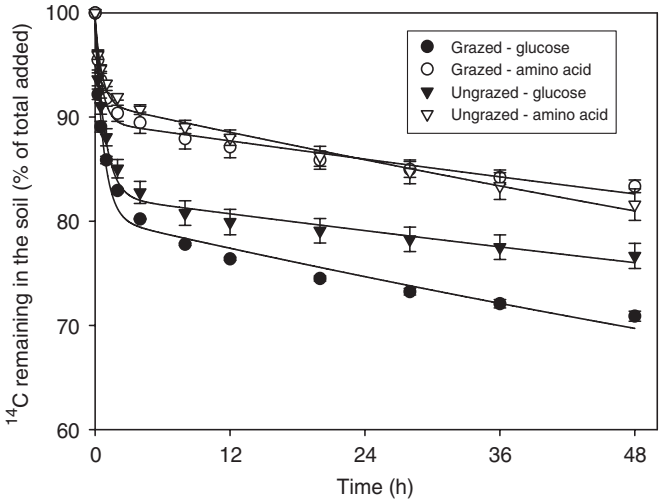

Fig. 2. Amount of ^{14}C remaining in a grazed and un-grazed grassland soil after the injection of ^{14}C glucose or amino acids into the soil. Experiments were performed in situ in the field. Values represent means SEM ($n = 4$). Lines represent fits of a double first-order with asymptote kinetic equation to the experimental data (see Section 2 for further details).

indicated that the type of C substrate was the only factor to significantly affect the rate of $^{14}CO_2$ evolution ($P < 0.001$).

The half-lives ($t_{1/2}$) of the C substrates in the soil solution calculated using the double exponential kinetic model are presented in Table 3. The results show that the turnover of both glucose and amino acids in soil solution were extremely rapid, with an average first phase (k_1) half-life of 0.51 ± 0.05 h. ANOVA indicated that neither substrate type or grazing regime had a significant effect on the rate of loss of these compounds from the soil solution ($P > 0.05$).

In contrast to substrate present in the soil solution phase, the rate constant for the second phase was significantly smaller, with k_2 having an average value of $0.0038 \pm 0.0005\,h^{-1}$ for glucose-C and $0.0032 \pm 0.0004\,h^{-1}$ for the amino acid-C (compared to a k_1 value of $1.26 \pm 0.11\,h^{-1}$ for glucose-C and $2.04 \pm 0.32\,h^{-1}$ for amino acid-C). ANOVA indicated that for the decomposition of ^{14}C-substrate in the second phase there was a significant interaction between grazing regime and the added substrate ($P < 0.001$), such that k_2 for glucose mineralization was greatest in grazed swards, whereas for amino acid mineralization it was greatest in un-grazed swards.

3.4. Inorganic C and DOC mineralization dynamics in the laboratory

The patterns of $^{14}CO_2$ evolution in the grassland swards in the laboratory were similar to those obtained in the field. Again, the data for $NaH^{14}CO_3$ fitted well to an asymptotic first order kinetic model ($r^2 = 0.985$) indicating that the half-life of $^{14}CO_2$ in the laboratory soils was 0.33 ± 0.06 h. For the DOC substrates, a greater amount of $^{14}CO_2$ was recovered after the addition of ^{14}C-labelled glucose in comparison to amino acids ($P < 0.05$; Fig. 3). Again, a double exponential decay equation gave a good fit to the

Table 3
Parameters of the double exponential equation fitted to mineralization data following the addition of LMW C substrates (glucose and amino acids) to the soil solution

Sample	Soil solution (a_1)		Soil solution half-life (hours)		Microbial pool (a_2)		Microbial pool (k_2)	
	Glucose	Amino acids	Glucose	Amino acids	Glucose	Amino acids	Glucose	Amino acids
Field								
Grazed	18.45±0.15	9.28±1.10	0.54±0.05	0.37±0.08	47.51±0.22	57.48±1.18	0.0048±0.0006	0.0025±0.0003
Un-grazed	16.69±1.05	8.40±0.44	0.63±0.10	0.49±0.14	51.65±1.16	60.48±0.57	0.0028±0.0002	0.0038±0.0006
Laboratory	15.49±1.10	6.98±0.70	1.04±0.21	0.61±0.10	38.52±1.10	47.90±0.82	0.0092±0.0005	0.0045±0.0016

Soil solution half-life is defined as $0.693/k_1$. The microbial pool includes C contained in storage and structural pools. Pool size estimates are given in a_1 and a_2. Values represent means±SEM ($n=4$).

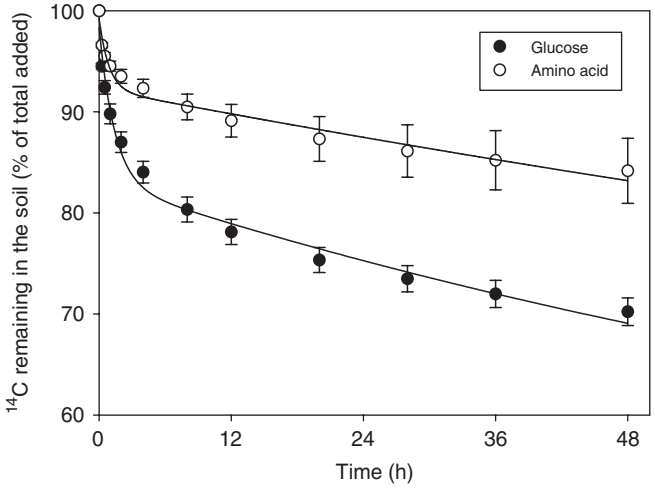

Fig. 3. Amount of ^{14}C remaining in a grazed grassland soil after the injection of ^{14}C-labelled glucose or amino acids into the soil. Experiments were performed in the laboratory. Values represent means SEM ($n=4$). Lines represent fits of a double first order with asymptote kinetic equation to the experimental data (see Section 2 for further details).

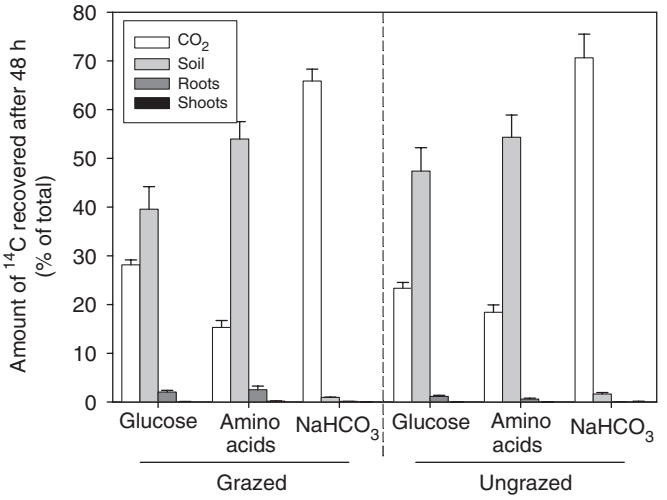

Fig. 4. Amount of ^{14}C recovered after 48 h as $^{14}CO_2$ (respiration) and in the roots, shoots and soil of a grazed and un-grazed grassland after the addition of ^{14}C glucose and amino acids to the soil. Values represent means SEM ($n=4$).

experimental data ($r^2>0.970$ in all cases; Fig. 3). An ANOVA comparison of the laboratory total recovery of $^{14}CO_2$ showed no statistical difference when compared to field data. The calculated first phase (k_1) half-life of the amino acids and glucose in the laboratory soils indicated a rapid removal from soil solution with a mean half-life of 0.83±0.14 h. There was no significant difference between the rate of turnover of the amino acids and glucose in soil solution ($P>0.05$). Comparison of the laboratory and field results indicated that the type of environment (laboratory or field plots) had a significant effect on the rate of substrate turnover in soil solution ($P<0.05$). Generally, the first phase of mineralization was significantly slower in the laboratory than in the field ($P<0.05$; Table 3).

Again the first-order kinetic model indicated that the decomposition of ^{14}C once in the second phase (k_2) was significantly slower than that of the soil solution substrate pool (Table 3). An ANOVA comparison of the laboratory and field results indicated that the type of environment had a significant effect on the secondary mineralization phase ($P<0.01$), such that it was generally faster in the laboratory than in the field. The turnover of glucose-C in the microbial biomass (second phase) was significantly faster than amino acid-C ($P<0.05$).

3.5. ^{14}C partitioning in the plant–soil system

At the end of the field experiments the ^{14}C-label in the different plant–soil pools was determined and the results are presented in Fig. 4. Only small amounts of ^{14}C could be recovered in the plants with 0.07±0.02% of the total ^{14}C recovered in the shoots and 1.6±0.3% recovered in the roots. Whilst there was no difference in shoot ^{14}C content between treatments ($P>0.05$) there was a significant difference in the amount of ^{14}C recovered in roots both between grazed and un-grazed plots ($P<0.01$) and substrate treatments ($P<0.001$). Very small amounts of $NaH^{14}CO_3$-derived ^{14}C were also recovered in the plants (0.14±0.01% of the total ^{14}C). A large proportion of the amino acid- and glucose-derived ^{14}C was recovered in the soil from all treatments at the end of the experiment

(49 ± 4% of the total ^{14}C). In contrast, only 1.3 ± 0.3% of the $NaH^{14}CO_3$-derived ^{14}C was recovered from the soil.

4. Discussion

4.1. Soil DOC concentrations

As grazing is thought to increase soil inputs of sugars and amino acids through enhanced root exudation and turnover (Paterson and Sim, 1999; Hamilton and Frank, 2001; Frank et al., 2002), we hypothesized that these compounds would be present in higher amounts in the grazed soil treatment. Overall, however, our study revealed few significant differences in the soil solution concentrations of DOC, DON or individual groups of compounds in the grazed and un-grazed plots. Further, glucose and free amino acids were present at extremely low concentrations and represented only a small proportion of the total DOC (<5% of total). Here we show in situ and under laboratory conditions that these low concentrations arise due to their rapid removal by the soil microbial community. This supports previous studies suggesting that the bottleneck in soil C and N cycling is the extracellular breakdown of macromolecules rather than the utilization of their LMW breakdown products (Jones et al., 2004).

4.2. Plant uptake of DOC

Plants roots have the capacity to take up LMW DOC from soil via a range of constitutively expressed active proton cotransport systems (Jones et al., 2005a). While it has been hypothesized that this is important for recapturing C and N lost into the soil as root exudates, these transport systems may also be important for taking up organic N directly from the soil (Jones et al., 2005a). This has been hypothesized to provide plants with a mechanism to short circuit the N cycle as it negates the need for organic N to be further microbially processed to NH_4^+ and NO_3^- before root uptake. At the end of our in situ experiments, however, only small amounts of ^{14}C were recovered in the plant (<2% of the total ^{14}C applied). Although we cannot directly account for the ^{14}C-substrate taken into the root and subsequently respired as $^{14}CO_2$, based upon previous estimates of catabolic and anabolic use of exogenously taken up C, the amount of ^{14}C substrate captured by the root would still represent <5% of that added to the soil (Owen and Jones, 2001; Kuzyakov and Jones, 2006). This supports previous studies indicating that roots are poor competitors for LMW DON when the concentrations in soil solution are low (Bardgett et al., 2003; Jones et al., 2005a).

4.3. LMW DOC turnover and soil respiration

To our knowledge this is the first report performed in situ that demonstrates that intrinsic concentrations of LMW DOC are rapidly turned over in soil. The field turnover rates derived from values of k_1 reported here for amino acids ($t_{1/2} = 0.43 \pm 0.06$ h) were more rapid than the amino acid half-life reported for a range of agricultural soils tested in the laboratory in the absence of roots ($t_{1/2} = 2.3 \pm 0.5$ h; Jones et al., 2005b). This is supported by our measured rates of substrate turnover (k_1) in intact swards in the laboratory which were consistently slower than in the field. This was unexpected; as we hypothesized that disturbance caused by sampling would increase microbial activity and stimulate mineralization. However, Vance and Chapin (2001) have previously reported that microbial activity is much higher in the field than in the laboratory. We speculate that this is partially attributable to the removal of rhizosphere component of the microbial biomass in root-free soil experiments (Jones et al., 2005b), but cannot account for the difference found here where the plants and rhizosphere were not removed. We are similarly unable to provide an obvious explanation for the effect the environment has on the k_2 values. We have assumed that k_2 describes the turnover of the applied substrate in the microbial biomass. If this assumption is correct, it implies that, low MW C cycled faster through the microbial biomass in the laboratory than in the field.

Assuming a $t_{1/2}$ value of 0.5 h for the rate of glucose and amino acid turnover in soil solution and a Q_{10} value of 2 (to account for the lower mean annual temperature of 11 °C), we estimate that these LMW C pools turn over approximately 4000 times annually. We estimate that this equates to an annual C flux of approximately $1\,g\,C\,kg^{-1}\,soil\,y^{-1}$ for both glucose and amino acids. This can be compared to a standing SOM of $33\,g\,C\,kg^{-1}$, root biomass of $1.7\,g\,C\,kg^{-1}$ and microbial biomass of $0.8\,g\,C\,kg^{-1}$.

Our double first-order kinetic model assumed that the second mineralization phase was attributable to the turnover of all substrates that had become incorporated into the microbial biomass—whether this is in storage, exudates or structural components (Saggar et al., 1999; Toal et al., 2000). Whilst our data fitted well to this kinetic model ($r^2 = 0.986 \pm 0.002$) the addition of a third exponential decay term would have given a better fit ($r^2 = 0.999 \pm 0.001$; data not shown). This three pool model could be justified in terms of internal partitioning into short term (immediate use in respiration), medium term (e.g. storage or exudates) and long term (e.g. cell wall) C pools within the microbial biomass or different turnover rates of different classes of microbes, but we currently have no experimental way of differentiating between these pools in situ.

The exponential coefficients for this second phase (k_2) of the model are significantly smaller than those of microbial metabolism of LMW C. If we extrapolate the model presented by Paul and Clark (1996) and Kuzyakov and Demin (1998) for the second phase ($t_{1/2} = \ln(2)/k_2$) then mean residence times (MRT) of 6–24 d for ^{14}C label in the soil can be calculated. Despite the drawback that this method may grossly oversimplicate C flow within this

pool(s), the MRT were similar to those reported for 16 New Zealand soils (9–49 d; Saggar et al., 1999) and 4 Moscow soils (0.6–4.4 d; Kuzyakov and Demin, 1998). However, this was much faster than reported microbial biomass turnover in some other soils, e.g. 0.25–2.5 y in forest soils (van Hees et al., 2005) and 0.17–0.42 y for soils under ryegrass (Saggar et al., 1996; Kouno et al., 2002). Kuzyakov and Demin (1998) suggest that longer half-lives can be an effect of ^{14}C being adsorbed to humus and clay minerals.

Previous work has shown that, although some C may persist in the plant for several days before exudation (Thornton et al., 2004), C fixed in photosynthesis can be exported to the root and subsequently exuded into the soil within 1 h and can be maximal within 4 h (Dilkes et al., 2004). Combined with the work presented here it suggests that a significant proportion of the C entering the soil system from root exudation will be fixed by the plant and subsequently leave the soil as CO_2 within 24 h. While the processes of photosynthesis and root exudation, and therefore to some extent rhizomicrobial respiration, are tightly coupled, this signal is damped in soil by the relatively slow rate of C passage through the microbial biomass. From the double exponential decay model we estimate that, ca. 20–30% of any given portion of exuded C is rapidly respired and 70–80% is cycled through the microbial biomass.

4.4. Influence of grazing on DOC turnover

Above-ground grazing can stimulate grassland productivity, and enhanced C inputs may increase the microbial biomass and N mineralization (Tracy and Frank, 1998; Stewart and Metherell, 1999; Hamilton and Frank, 2001; Frank et al., 2002). However, this may not be universal as some studies suggest that grazing suppresses soil respiration (Bremer et al., 1998). Our study indicated that although grazing had no effect on root or microbial biomass, the ratio of C lost as below-ground respiration to that fixed in photosynthesis was markedly higher in the grazed plots in comparison to the un-grazed plots (Table 1), implying that C is flowing faster through the grazed soil than its un-grazed counterpart. However, our findings do not necessarily imply a faster turnover of SOM. The increase in soil respiration could be attributable to a more active root system rather than a more active microbial community, and/or to higher soil temperatures in grazed plots. Our results showed similar soil solution concentrations and turnover rates of LMW DOC in the different grazing treatments. Thus, assuming steady state conditions, then the rate of soil C and N processing by the soil microbial biomass must also be similar under the two grazing regimes. We therefore hypothesize that at the time of measurement, a greater proportion of the roots in the grazed grassland were active due to the need to supply nutrients to the above-ground shoot components due to the continual removal of nutrients by defoliation. To clarify this issue, further work is required to ascertain the relative rates of root turnover and active root fraction in the two grazing regimes, and to evaluate any potential seasonal variation.

4.5. Conclusions

This study has shown both in situ and in the laboratory that two of the dominant forms of SOM input, sugars and amino acids, are cycled extremely rapidly by the soil microbial community. We estimate that the soil solution LMW pools of these compounds are turned over approximately 4000 times annually. The rates of turnover were faster in the field than in the laboratory highlighting the need for field experiments to reliably estimate soil C fluxes. Overall, grazing regime had little effect on the rate of C turnover. Our results also support previous studies showing that in comparison to soil microorganisms, plant roots are poor competitors for LMW DOC present in the soil solution.

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