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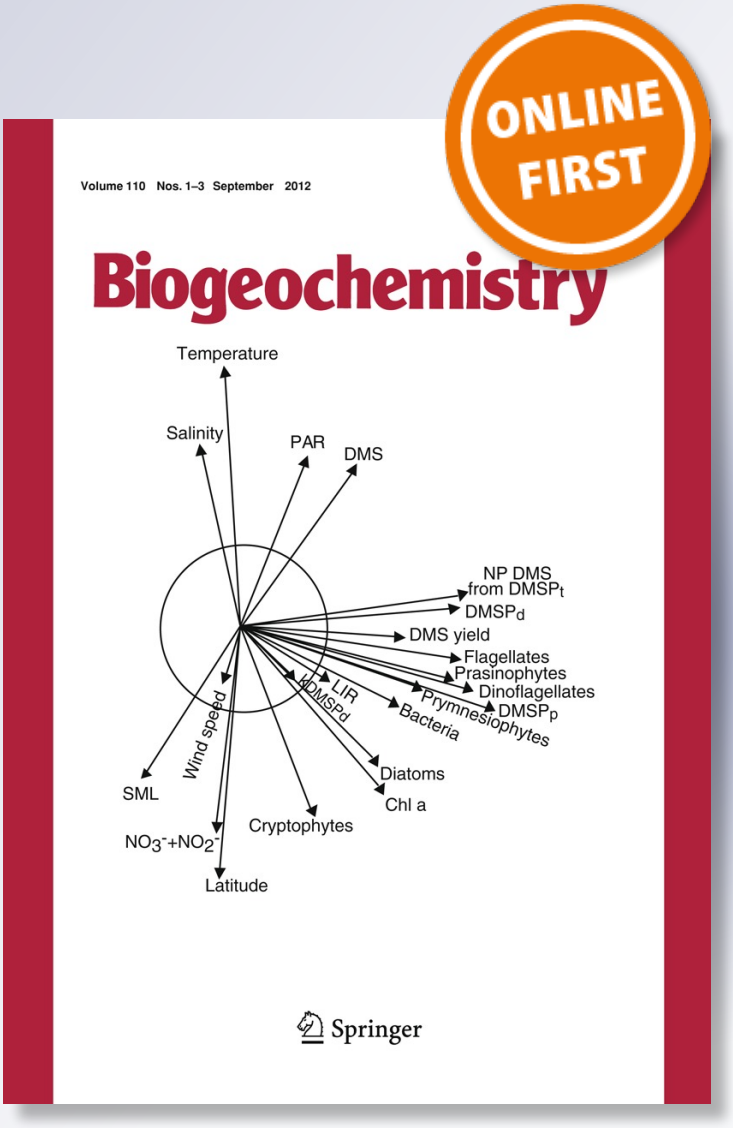

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Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth

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Abstract Plant-carbon inputs to soils in the form of dissolved sugars, organic acids and amino acids fuel much of heterotrophic microbial activity belowground. Initial residence times of these compounds in the soil solution are on the order of hours, with microbial uptake a primary removal mechanism. Through microbial biosynthesis, the dissolved compounds become dominant precursors for formation of stable soil organic carbon. How the chemical class (e.g. sugar) of a dissolved compound influences stabilization in field soils is unknown and predictions from our understanding of microbial metabolism, turnover and identity are contradictory. We show that soil carbon formation, from chronic amendments of dissolved compounds to fertilized and unfertilized grasslands, is 2.4-times greater from a sugar than an amino acid. Formation rates are negatively correlated with respiration rates of the compounds, and positively correlated with their recovery in microbial biomass. These relationships suggest that the efficiency of microbial growth on a compound is positively related to formation rates of soil organic carbon. Fertilization does not alter these findings, but together nitrogen and phosphorus additions reduce soil carbon formation. Our results highlight the need to consider both nutrient enrichment and global-change induced shifts in the form of dissolved root inputs to soils to predict future soil carbon stocks and hence phenomena such as climate warming and food security to which these stock sizes are intimately tied.

Keywords Soil organic carbon · Soil carbon formation · Microbial biomass · Root exudation · Low molecular weight carbon compounds · Dissolved organic carbon

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Introduction

Soil organic matter (SOM) comprises the largest terrestrial sink of carbon (C) and its concentration is directly related to soil fertility and hence ecosystem productivity (Lal 2004). The sink size is determined by the balance between SOM formation and loss, requiring understanding of both processes to predict global change impacts on SOM (Allison et al. 2010; Bradford et al. 2008; Trumbore 2009). Given concerns about warming and its stimulation of soil respiration, most recent attention has focused on stabilization mechanisms that protect SOM from microbial-mediated decomposition (Conant et al. 2011). An emerging thesis, however, is that soil microbes also act as agents of SOM formation (Miltner et al. 2011; Schmidt et al. 2011; Cotrufo et al. 2012) because microbial-derived compounds are the primary constituents of stable, long-term SOM stores (Grandy and Neff 2008; Lundberg et al. 2001). It seems then that, at least in systems other than those such as the arctic where microbial-mediated decomposition is severely limited by climate (Schmidt et al. 2011), plant-C inputs generally are degraded, assimilated and then biosynthesised by microbes prior to long-term SOM stabilization (Cotrufo et al. 2012). This pathway explains the apparent paradox that substantial proportions of C from plant inputs with the shortest initial residence times in soils, low molecular weight carbon (LMWC) compounds such as sugars, are recovered many years later in SOM (Voroney et al. 1989).

Low molecular weight C compounds enter the soil solution through numerous pathways, a dominant one being root exudation (Högberg and Read 2006; van Hees et al. 2005). Because they do not require microbes to produce extracellular enzymes to degrade them into simpler, soluble forms prior to uptake, LMWC compounds should have a lower energy cost of acquisition when compared to other plant-C inputs that microbes must degrade prior to assimilation. The lower energy cost should make more substrate-C available for biosynthesis (vs. respiration for energy). Given that LMWC inputs from roots fuel as much as 30–50 % of heterotrophic soil respiration (Högberg and Read 2006; van Hees et al. 2005), it then seems reasonable to suggest that they are a dominant substrate for microbial growth and turnover, and hence precursors for formation of stable SOM (Miltner et al. 2011; Schmidt et al. 2011).

All plant species exude LMWC compounds from their roots, and the amount of sugars, organic acids and amino acids varies by species and environment (Bais et al. 2006). As the environment changes, and along with it the forms of LMWC-inputs (Bais et al. 2006), the consequences for SOM formation are unknown. To redress this knowledge gap, we amended grassland plots weekly across 6 months with common and abundant LMWC compounds: the sugar 'glucose' and the amino acid 'glycine' (Fischer et al. 2007). The two compounds were uniformly labeled with ^{13}C, so we could quantify C partitioning to respiration, microbial biomass, SOM, soil solution and plants. Theory is contradictory as to whether more SOM should be formed from glucose or glycine. The reactivity-stabilization hypothesis (Yang and Janssen 2002) proposes that compounds that have initially higher respiration rates will have the largest proportion of their C stabilized in soil (Fig. 1). This is because higher initial respiration is assumed to be indicative of the speed and hence ease of uptake and metabolism of a substrate, and so low energy investment in substrate acquisition (Yang and Janssen 2002). Glycine is often initially respired more rapidly than glucose (Jones and Murphy 2007; Kuzyakov and Demin 1998; Webster et al. 1997), suggesting it should form more SOM than glucose. Similar predictions are yielded from overflow metabolism theory, where lack of nutrients such as nitrogen (N) should result in sugars shunted to waste respiration and not growth (Schimel and Weintraub 2003). As an amino acid, glycine contains N and therefore N limitation should be alleviated, favoring microbial biosynthesis of SOM precursors (Fig. 1).

Three alternate mechanisms make opposite predictions to the reactivity-stabilization and overflow metabolism hypotheses about how LMWC compound identity affects SOM formation rates. First, glycine but not glucose is preferentially assimilated by bacteria over fungi (Paterson et al. 2007; Rinnan and Bååth 2009), meaning glucose should form more SOM because the faster turnover of active bacterial biomass (days) compared to fungi (months) is hypothesized to lead to greater C losses (Strickland and Rousk 2010) (Fig. 1). Second, plants exude amino acids such as glycine to stimulate turnover of microbial biomass by promoting growth and consequent microbivory, liberating N but also stimulating C loss because of the inefficiencies of trophic transfers (Strickland and Rousk 2010) (Fig. 1). Third, where nutrients are

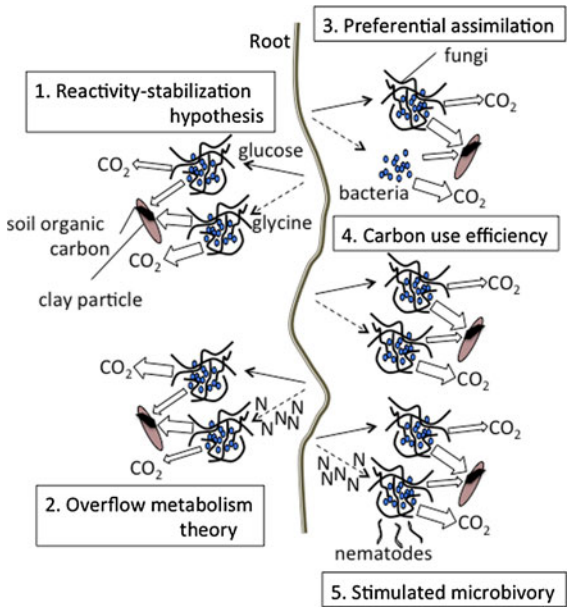

Fig. 1 Five conceptual frameworks relating the fate of low molecular weight carbon (LMWC) compounds exuded from roots to their stabilization in soil organic matter (SOM). Glucose (*solid arrows*) and glycine (*hatched arrows*) are common respresentatives of sugars and amino acids, respectively, exuded from roots. Each of the five frameworks (1–5) posit that microorganisms (bacteria and fungi) assimilate the LMWC compounds and then use them for respiration or growth. The proportion retained for growth, theoretically, is positively associated with SOM formation rates because microbial biomass and other products are an important precursor of stable SOM (e.g. Schmidt et al. 2011). Under frameworks 1 and 2 more glycine than glucose is retained in SOM (depicted by *thicker block arrows* coming from the microbes); and frameworks 3–5 make the opposite prediction. See the main text for the mechanisms that explain these predictions. The empirical results of our study (Fig. 3b) support the predictions of frameworks 3–5 in that more glucose than glycine forms stable SOM

available then growth efficiencies are higher for glucose than glycine (Kuzyakov and Demin 1998; van Hees et al. 2005), so a greater proportion of glucose should be available for microbial biosynthesis and SOM formation (Webster et al. 1997) (Fig. 1). Our study was not designed to discern between these three hypotheses suggesting that more SOM should be formed from glucose than glycine, but rather to test between these hypotheses and the competing hypotheses of reactivity-stabilization and overflow metabolism, that propose more glycine than glucose should form SOM.

Methods

Study site and experimental design

Twelve 1-m^2 plots were established in a gently sloping, annually-mown old field at the Coweeta Long Term Ecological Research site in North Carolina, USA (35°03′N, 83°25′W; 750–1,025 m elevation, 183 cm MAP, 13 °C MAT). Soils are mapped in the Saunook series and fall within the sub group of Humic Hapludults. The plots were arrayed in three spatial blocks, each consisting of four 1-m^2 plots, with 0.5-m corridors running between the plots within a block, and at least 2 m between blocks. Within blocks, plots were randomly assigned to water, N, phosphorus (P), or N × P regimes. Fertilizer was added as a 10-mm rainfall event, using a backpack sprayer, 4-times across the growing season (April through September) at applications of 100 kg N ha^{-1} y^{-1} and 50 kg P ha^{-1} y^{-1}. Nitrogen was added as $(NH_4)_2SO_4$ and P as KH_2PO_4; SO_4^{2-} was used as the counter-ion to NH_4^+ because it has minimal effects on microbes and soil pH (Bradford et al. 2008; Gulledge et al. 1997).

Within each 1-m^2 plot, we randomly placed three 15.4-cm dia. PVC collars to 5-cm depth, giving 36 collars total. Weekly for 28 weeks, collars received 100 mL of water, 99 atom% ^{13}C-glucose or ^{13}C-^{15}N-glycine, equivalent to a rate of 26.022 g C m^{-2} y^{-1} and comprising an additional 8.2 % water over mean annual precipitation. The soil surface applications of the compounds followed approaches described previously (Strickland et al. 2010; Strickland et al. 2012), with LMWC compounds added at concentrations (2.22 mM glucose, 6.65 mM glycine) dilute enough to avoid stimulating soil respiration. Note that the primary focus of our study was to investigate controls on the formation of SOM from LMWC compounds, and not on how addition of these compounds affected bulk SOM stocks. Higher replication and longer time scales are usually required to detect changes in bulk SOM given pronounced fine-scale variation in SOM concentrations and the slow response time of this pool. For example, bulk SOC concentrations were three orders of magnitude greater than the ^{13}C-label concentrations we recovered in the SOM, with for example concentrations in the glucose addition, no N, no P plots of 22.1 ± 3.73 mg C g $soil^{-1}$ (mean ± 95 %CI).

Respiration measures

At week 28, $^{13}CO_2$ respiration was measured before, 2, 5, 20 and 168 h following substrate amendment. Efflux rates were determined using a closed-chamber approach (e.g. Bradford et al. 2001), where CO_2 concentrations were determined at the start and end of a 45 min capping period (as in Strickland et al. 2010). Headspace samples were taken with 20-mL gas syringes (SGE, Ringwood, AU), transported to the laboratory in 12-mL Exetainers (Labco, High Wycombe, UK), and then CO_2 concentrations were determined using an infra-red gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA, Model LI-7000). A second sample was analyzed using continuous flow, isotope-ratio mass spectrometry (IRMS; Thermo, San Jose, CA, USA) to determine the Atom% ^{13}C value of the CO_2 in the sample. The initial headspace sampling provided the pre-addition values for the isotope mixing equations, where the mass of ^{13}C-label respired was calculated as follows (sensu Ineson et al. 1996): $C_{label\text{-}derived} = C_{total} \times (Atom\%^{13}C_{after} - Atom\%^{13}C_{before})/(Atom\%^{13}C_{compound} - Atom\%^{13}C_{before})$, where C_{total} is the total amount of C respired, $Atom\%^{13}C_{after}$ is the atom% value of the respired C after the label was added, $Atom\%^{13}C_{before}$ is the atom% value of respired C before label was added, and $Atom\%^{13}C_{compound}$ is the atom% value of the added substrate. Note that for the respiration assays, and the plant and soil measures (below), atom% and not delta values were used to calculate the mass of ^{13}C label in the gas, plant and soil carbon pools because at high enrichments delta values are meaningless (see Fry 2006). The mass of label was determined by subtracting atom% values of unlabeled materials from labeled materials, before multiplying the total carbon or nitrogen mass values for these materials by the calculated atom% excess values. Calculation of atom% excess values corrects for the natural abundance content of ^{13}C or ^{15}N in organic samples that would otherwise be treated as a component of the isotopic tracer (Fry 2006).

Plant and soil measures

Following the respiration measures, plants and soils (to 10 cm depth) in the collars were harvested and assessed for: mass, %C, %N and ^{13}C- and ^{15}N-contents. We separated plant shoots and roots, microbial biomass, dissolved organic matter (DOC and DON), and particulate OM (POM) and mineral-associated SOM pools.

Plant roots were shaken free of soil, washed and then, along with shoots, placed at 65 °C until constant mass. After weighing, samples were ball-milled to a fine powder and total carbon, nitrogen and stable isotope ratios determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy) coupled to a continuous-flow IRMS. For the IRMS working standards were calibrated to PDB (Pee Dee Belemnite) for carbon using NIST-SRM 1577b Bovine Liver as a reference, and to atmospheric N_2 for nitrogen.

Soils were passed through a 4-mm sieve, screened for fine roots that passed the sieve, and then analyzed for DOC, DON and microbial biomass carbon and nitrogen. To extract dissolved materials, soils were shaken with 0.5 M K_2SO_4 for 4 h and then filtered using Whatman #42 papers. Microbial biomass carbon and nitrogen was estimated using a modified, chloroform-fumigation extraction method as described in Fierer and Schimel (2002, 2003). The method controls for potential soil moisture differences by using soil slurries, and compares the flush of dissolved materials in fumigated samples against non-fumigated controls. To determine the total carbon and ^{13}C contents of the DOC and microbial biomass pools, the liquid extracts were introduced to the IRMS via a total organic carbon (TOC) analyzer (Shimadzu, Columbia, MD, USA). Just as with the plant material, DOC and microbial biomass values were derived for unlabeled samples (water-only collars) to provide natural abundance atom% values for calculation of ^{13}C label amounts. Total dissolved N and ^{15}N in the soil solution and microbial biomass was determined using a modified version of the alkaline persulfate oxidation procedure described by Cabrera and Beare (1993) to convert the organic and inorganic N forms in the 0.5 M K_2SO_4 extracts to NO_3^-. Next, the NO_3^- was fixed to acid-washed PTFE discs by shaking at 30 °C for 2 day with Devarda's alloy in solutions adjusted to pH 13 with 10 M NaOH. PTFE discs were then rinsed with DI water, dried and their total %N and $atom\%^{15}N$ determined as for the plant materials.

A subsample of the sieved soil was air-dried and used for SOM determinations. Although there is no ideal method for determining meaningful SOM fractions (Olk and Gregorich 2006; von Lützow et al. 2007), mineral-associated C pools are expected to have slower turnover times and greater long-term sink

capacity than POM C pools (Schlesinger and Lichter 2001). We fractionated our SOM into these two pools using a chemical dispersant (sodium hexametaphosphate: NaHMP) with shaking to break apart aggregates, followed by physical fractionation to separate pools. We classified material that passed through a 53-μm sieve as mineral-associated and material retained on this sieve as POM (Paul et al. 2001). Specifically, 30 g of air-dried soil was dispersed with 100 mL of NaHMP for 18 h in rectangular 175 mL Nalgene bottles placed on their side in an end-to-end shaker at high velocity, before being passed through the 53 μm sieve (modified from Cambardella and Elliott 1992, following Bradford et al. 2008). We ensured that all mineral-associated material was rinsed through using DI water and then both fractions were dried to constant weight at 65 °C; air-dried soil was also dried at this temperature and 105 °C to permit air-dry to oven-dry weight conversions. Fractions were ball-milled to a fine powder prior to element and isotope determination as described above. However, because it is not possible to separately quantify DOC and microbial biomass C from these fractions, we subtract the mass of label in the dissolved organic and microbial biomass pools from the sum of the POM and mineral-associated pools, when we report the mass of label in the total SOM.

To verify if the label recovered in the SOM was stabilized, we incubated the sieved soils from the glucose-amended and water-only collars for 12 weeks at 12 and 28 °C at 65 % water-holding capacity (WHC). This method is thought to provide an estimate of microbially-available carbon (e.g. Bradford et al. 2008). We reasoned that if the ^{13}C-label we recovered was stabilized, then the amount lost across 12 weeks at 28 °C (i.e. favorable conditions for microbial activity) should be equivalent to or less than the label mass in the microbial biomass and DOM pools at the start of the incubations; meaning the label in the SOM was protected from microbial decay. At the end of 12 weeks, SOC fractions were re-measured as described above.

Duplicate subsamples of sieved field soil were used to determine pH, measured after mixing soil with H_2O 1:1 by volume (Allen 1989), gravimetric soil moisture (24 h at 105 °C) and microbial biomass using modified substrate-induced respiration (SIR) (West and Sparling 1986). This latter method provides an estimate of active as opposed to total microbial biomass (Wardle and Ghani 1995).

Statistical analysis

Our study design necessarily involves spatial and temporal clustering, where for example collars in the same 1-m^2 plot, ecological materials from the same collar, and respiration measures across time, are non-independent. Linear mixed-effects models (Pinheiro and Bates 2000) contain random error structures that account for these time- and space-dependent associations, and all of our models had an error term where plot was nested within block. Fixed effects in each model were substrate identity (glucose or glycine), N addition (no or yes) and P addition (no or yes). Before P values were reported, fixed effects structures were reduced by removing interactions and main effects (when they were not part of a significant interaction) where $P > 0.10$. This must be done with mixed-effects models to generate meaningful P values (Pinheiro and Bates 2000). Because it is not clear whether it is necessary to remove terms where P falls between 0.05 and 0.1, we tested between models with and without such terms using maximum likelihood tests to identify the minimal adequate model for each response variable. For retained fixed effects, we considered $P < 0.05$ significant and $P < 0.10$ marginally significant (Hurlbert and Lomabardi 2009). Models were checked to ensure the residuals were homoscedastic, that the response variables were a reasonably linear function of the fitted values, and that errors were reasonably closely distributed in each block. All analyses were conducted using the freeware statistical package R (http://cran.r-project.org/).

Results and discussion

Inputs of LMWC compounds should be rapidly assimilated and available for metabolism by soil microorganisms (Jones and Murphy 2007). This was confirmed by compound respiration 2 h following addition and the exponential decrease in rates across the 7 days between amendments (Fig. 2a). Consistent with previous observations (Jones and Murphy 2007; Unteregelsbacher et al. 2011; Webster et al. 1997), glycine respiration was higher (~ 2-times across 24 h) than for glucose (Fig. 2b). This effect of compound identity was observed whether soils had received LMWC (Fig. 2b) or water over 6 months. In water only collars ($n = 6$), which were only measured

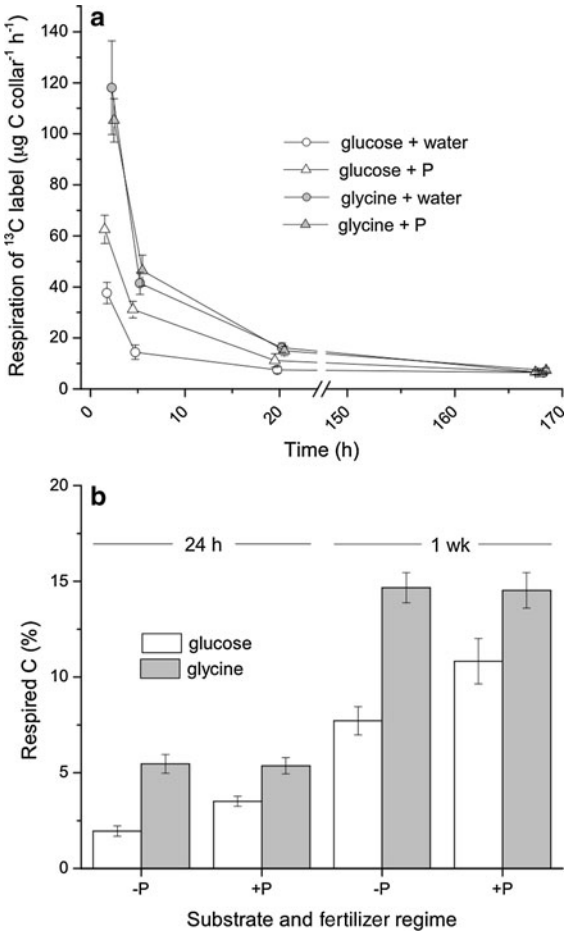

Fig. 2 Respiration of glucose and glycine amended to field soils. Respiration rates across the week following amendment (**a**) and cumulative proportions of the amounts added that are respired in 24 and 168 h (**b**). Symbols in (**a**) are slightly offset on the *x-axis* for clarity. Phosphorus fertilization increased rates and the cumulative proportion of glucose respiration but did not influence glycine respiration, which was greater than for glucose (significant substrate × P fertilizer interaction at 24 h ($P < 0.01$) and 1 week ($P < 0.05$)). Values are means ± 1SE, $n = 6$

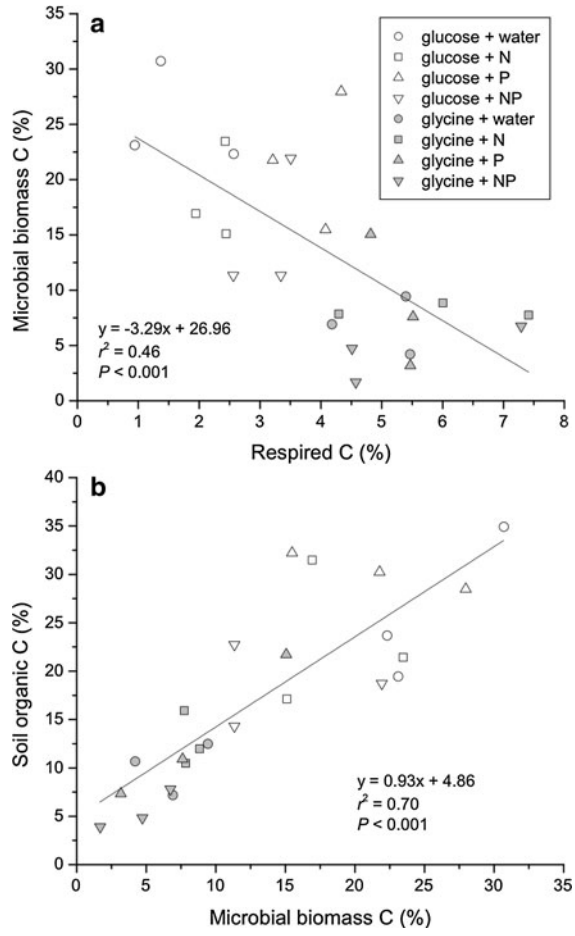

Fig. 3 Relationships between glucose- and glycine-respiration, and recovery in microbial biomass and SOM. The cumulative proportion of glucose and glycine amended to soils that is respired in 24 h is negatively related to the proportion of glucose- and glycine-carbon recovered in soil microbial biomass (**a**). In contrast, the proportion of the carbon recovered in microbial biomass is positively related to the proportion recovered in soil organic matter (SOM) (**b**). Values are for individual field plots and are shown by substrate and fertilizer regime ($n = 24$)

to 24 h, the total proportion of ^{13}C-label respired following glucose and glycine amendment was 3.9 ± 0.13 % and 4.6 ± 0.31 %, respectively (compound identity effect: $P < 0.01$).

The negative relationship between compound respiration rates across the 24 h following addition, and recovery of the ^{13}C label in the microbial biomass (Fig. 3a), supported the mechanism of lower growth efficiencies on glycine than glucose (Kuzyakov and Demin 1998; van Hees et al. 2005). Additional support came from the observation that 2.9-times less glycine- than glucose-^{13}C was recovered in the microbial biomass (Table 1). Evidence for the theory that microbial biomass is a dominant precursor for SOM (Miltner et al. 2011; Schmidt et al. 2011) came from the strong positive relationship between ^{13}C recovery in the microbial biomass and SOM (Fig. 3b), and that 2.4-times more glucose was recovered in the SOM (Table 1). Together these data on ^{13}C recovery in respiration, microbial biomass and SOM provide the first empirical field support, which we're aware of, for the idea that LMWC compound identity influences SOM

Table 1 Proportions of glucose- and glycine-C recovered in different ecosystem pools, and associated plant and soil variables

Variable	Water	Glucose	Glycine	P^a
Microbial biomass (%C added)[b]	na[c]	20.1 ± 1.77	7.00 ± 1.00	***
Dissolved organic C (%C added)	na	0.26 ± 0.020	0.14 ± 0.02	***
Soil organic matter (%C added) [f]	na	24.6 ± 1.94	10.4 ± 1.42	***
Plant biomass (%C added)	na	1.70 ± 0.12	2.45 ± 0.22	**
Soil pH (unitless)[d]	4.99 + 0.06	4.87 + 0.05	4.85 + 0.03	*
Shoot biomass (g C m^{-2})	603 ± 84	622 ± 66	903 ± 152	ns
Root biomass (g C m^{-2})	1,498 ± 313	1,201 ± 195	1,497 ± 222	ns
Soil moisture (% by mass)	0.28 ± 0.007	0.29 ± 0.006	0.29 ± 0.006	ns
Microbial biomass (μg C–CO_2 g $soil^{-1}$)[e]	6.02 ± 0.16	6.54 ± 0.13	7.46 ± 0.34	***

[a] Significance of glucose vs. glycine addition on the measured variables, where ns, *, ** and *** represents P >0.05, <0.05, <0.01 and <0.001. Given no significant interactions with the fertilization regime, data are pooled across the nitrogen and phosphorus treatments

[b] Percent C recovered of the total amount added as glucose or glycine across 6 months

[c] na is not applicable because no C was added to water-only plots

[d] pH values are back calculated from $[H^+]$ and so are presented with positive errors only

[e] Active microbial biomass was measured using substrate-induced respiration, and data are presented as uncorrected rates[10]

[f] Total recoveries across the POM and mineral-associated pools were, respectively: 24.0 ± 1.64 and 72.1 ± 1.54 % (glucose); 25.4 ± 1.48 and 60.0 ± 2.10 % (glycine). These total recoveries in the POM and mineral pools do not correct for microbial biomass and DOC contained within these pools. Values are means ± 1 SE (n = 12)

formation. Notably, microbial growth efficiencies on the third class of dominant LMWC inputs (i.e. organic acids; not investigated here) are much lower than for amino acids and sugars (van Hees et al. 2005), suggesting that compound identity may have even greater influence on SOM formation than we observed.

Soil organic matter represents a continuum of C qualities ranging from compounds whose chemistries make them easy to decompose, to those highly resistant to microbial attack (Ågren and Bosatta 2002). Further, even when their chemistry is amenable to decay, physical and chemical processes stabilize SOM compounds (Conant et al. 2011). We assayed the glucose-amended soils, using a method that estimates microbially-available C (Bradford et al. 2008), to determine what proportion of the ^{13}C recovered in the soil was stabilized against microbial decay. After 12 weeks of lab incubation at 28 °C, only 26.7 ± 1.13 % (mean ± SE) of the total ^{13}C recovered in the soil was lost. This C was most likely lost from the unprotected microbial biomass and DOC, which together accounted for almost half the initially recovered ^{13}C-label (Table 2). Microbial biomass and DOC can form stable SOM through occlusion within aggregates, and the physical disturbance itself of preparing the soil for the assays had little influence on losses, with only 3.0 ± 0.69 % of the total soil ^{13}C lost following 12-week incubations at 12 °C. Further support for the idea that the ^{13}C-SOM was stabilized came from fractionating the soils into POM and mineral-associated C, with 72.1 ± 1.54 % of the ^{13}C being recovered in the more stable mineral-associated SOM fraction, which is thought to be primarily microbially-derived (Grandy and Neff 2008; Lundberg et al. 2001). The stabilization of LMWC inputs in SOM of field soils has been shown but only following large, pulsed additions of glucose (Voroney et al. 1989). The dynamics of glucose respiration and retention in soils differ from large pulsed additions when glucose is added chronically and in trace amounts (Jans-Hammermeister et al. 1997). Chronic, trace amendments of LMWC compounds are more representative of processes such as root exudation, and our study demonstrates their potential to form stabilized SOM in field soils.

We nested compound amendments within a factorial N by P fertilization experiment to investigate which subset of microbial hypotheses might explain compound-identity effects on SOM formation. For example, we expected to find support for overflow metabolism theory if N increased SOM formation by promoting microbial biosynthesis (Schimel and Weintraub 2003). However, the effects of N and P

Table 2 Relative partitioning of carbon amendments across different ecosystem pools as influenced by substrate identity and fertilization regime

Treatment[a]	Microbes[b]	DOC	SOM	Plants
Glucose	42.9 ± 2.17	0.66 ± 0.110	52.5 ± 2.11	3.9 ± 0.45
Glycine	33.7 ± 2.02	0.67 ± 0.067	51.1 ± 2.35	14.5 ± 2.54
P[c]	*	ns	ns	***
Water	41.3 ± 3.64	0.49 ± 0.090	50.4 ± 0.22	7.9 ± 2.48
Nitrogen	39.4 ± 2.96	0.67 ± 0.083	54.3 ± 0.03	5.6 ± 0.80
Phosphorus	36.2 ± 3.33	0.79 ± 0.199	55.2 ± 152	7.8 ± 2.82
Nitrogen × phosphorus	36.3 ± 4.31	0.71 ± 0.094	47.4 ± 0.34	15.6 ± 5.04
P	ns	ns	ns	ns

[a] Given no significant interactions between substrate and fertilization regimes, data are pooled in the first two rows across fertilization regimes for the two substrates (values are means ± 1 SE, $n = 12$); and then pooled across the substrate regimes for the four fertilizer treatments ($n = 6$)

[b] Glucose- and/or glycine-carbon recovered after 6 months in microbial biomass (Microbes), dissolved organic carbon (DOC), soil organic matter (SOM) and plant biomass (Plants), expressed as the relative proportion in each pool of the carbon recovered across all four ecosystem pools

[c] There was a significant two-way interaction between pool and substrate ($P < 0.001$), and a three-way interaction between pool, nitrogen and phosphorus ($P < 0.01$). We investigated each of these interactions by pool and the results are shown in the row immediately below those data for substrate and then fertiliser regime. Significant fertilizer effects were not found for each pool, probably given reduced statistical power when pools were investigated individually as opposed to together; but note the greater relative carbon partitioning to plants compared to microbes and SOM under N × P fertilization. Significance is given as ns, *, ** and *** representing $P > 0.05$, < 0.05, < 0.01 and < 0.001, respectively

Table 3 Proportions of carbon amendments recovered in different ecosystem pools under nitrogen and phosphorus fertilization, and associated plant and soil variables

Variable[a]	Water	N	P	N × P	P[b]
Microbial biomass[c]	16.1 ± 4.37	13.3 ± 2.58	15.2 ± 3.69	9.6 ± 2.90	ns
Dissolved organic C[c]	0.16 ± 0.030	0.22 ± 0.040	0.23 ± 0.047	0.17 ± 0.031	NP*
Soil organic matter[c]	18.1 ± 4.17	18.1 ± 3.12	21.8 ± 4.29	12.1 ± 3.16	NP*
Plant biomass[c]	2.02 ± 0.33	1.71 ± 0.17	2.05 ± 0.29	2.51 ± 0.32	ns
Soil pH	4.99 + 0.04	4.83 + 0.02	5.04 + 0.03	4.79 ± 0.04	N***
Shoot biomass	661 ± 177	789 ± 161	522 ± 36	865 ± 72	N*
Root biomass	868 ± 134	1,193 ± 235	1,712 ± 335	1,822 ± 270	P*
Soil moisture	0.30 ± 0.006	0.29 ± 0.008	0.29 ± 0.007	0.27 ± 0.006	ns
Active microbial biomass	6.60 ± 0.32	6.80 ± 0.23	6.93 ± 0.50	6.36 ± 0.17	ns

[a] Units are as described in Table 1

[b] Significance of fertilization regime on the measured variables, where ns, *, ** and *** represents $P > 0.05$, <0.05, <0.01 and <0.001. N or P indicates a significant main effect of the nitrogen or phosphorus treatment, and NP a significant interaction between the treatments. Given no significant interactions with the substrate regime, data are pooled across the glucose and glycine amendments

[c] Percent C recovered of the total amount added as glucose and glycine across 6 months. Values are means ±1 SE ($n = 6$)

fertilization on ^{13}C dynamics were largely independent of compound identity. This might have been because the microbes were not nutrient-limited, but this seems unlikely given that the experiment was established on a highly-weathered, low-base status soil, and that plant production was 1.3-times greater under N fertilization (Table 3). Furthermore, fertilization did influence the ^{13}C-label dynamics, with N and P together reducing the amount of SOM formed by at least 1.5-times compared to other treatments

(Table 3). Substantial losses of SOM in other systems exposed to N and P fertilization (Mack et al. 2004), despite increased plant biomass, might then be explained by reduced SOM formation as well as enhanced decomposition.

The mechanisms underlying reduced SOM formation under N and P fertilization are not obvious because N and P tended to have significant main but not interactive effects on other aspects of the ^{13}C-dynamics, and these were sometimes dependent on compound identity. For example, although glycine respiration was not influenced by fertilization, P addition increased respiratory losses of glucose by as much as ~1.8-times (Fig. 2). Given that P availability explained variation in glucose respiration rates across forests, pastures and arable fields (Strickland et al. 2010), and P fertilization of tropical forest stimulated heterotrophic respiration of soluble C (Cleveland and Townsend 2006), these and our observations suggest an important role for P in determining the initial fate of LMWC inputs to soils.

Nitrogen fertilization decreased the total ^{13}C recovery across all pools (microbial biomass, SOM, DOC and plants). Specifically, 28.8 ± 3.89 % of the label was recovered with N and 37.9 ± 5.33 % recovered without N, perhaps reflecting more rapid cycling of recent C inputs observed in N fertilization studies (Neff et al. 2002). Similarly, more rapid cycling of the glycine inputs seemed to also be linked with lower total ^{13}C recovery across all pools (20 vs. 47 % for glucose; Table 1). For both compound identity and the N × P interaction, faster cycling seemed associated with reduced SOM formation and greater relative partitioning of the recovered C from microbial to plant biomass (Table 2). The relative shift to plant biomass was likely the result of greater mineralization of inputs followed by photosynthetic fixation of the evolved $^{13}CO_2$. Direct uptake of the amino acid seems unlikely given the strong positive relationship between total but not plant recovered glycine C and N (Fig. 4), and the low total recovery of both glucose and glycine-derived ^{13}C in plants (<2.5 % of that added, Table 1). Eutrophication of terrestrial ecosystems, and associated changes in the identity of LMWC inputs from plants, are likely to alter SOM formation rates but further work is required to quantify the impact on SOM stocks.

Fertilization and higher rates of glycine respiration were both related to lower SOM formation, suggesting that hypotheses of overflow metabolism and reactivity-stabilization (Schimel and Weintraub 2003; Yang and Janssen 2002) did not adequately explain fates of LMWC compound additions to soils (Fig. 1). In contrast, lower microbial growth efficiencies on glycine than glucose (Kuzyakov and Demin 1998; van Hees et al. 2005), in addition to glycine's preferential assimilation by bacteria (Paterson et al. 2007; Rinnan and Bååth 2009) and role in stimulating microbial biomass turnover (Strickland and Rousk

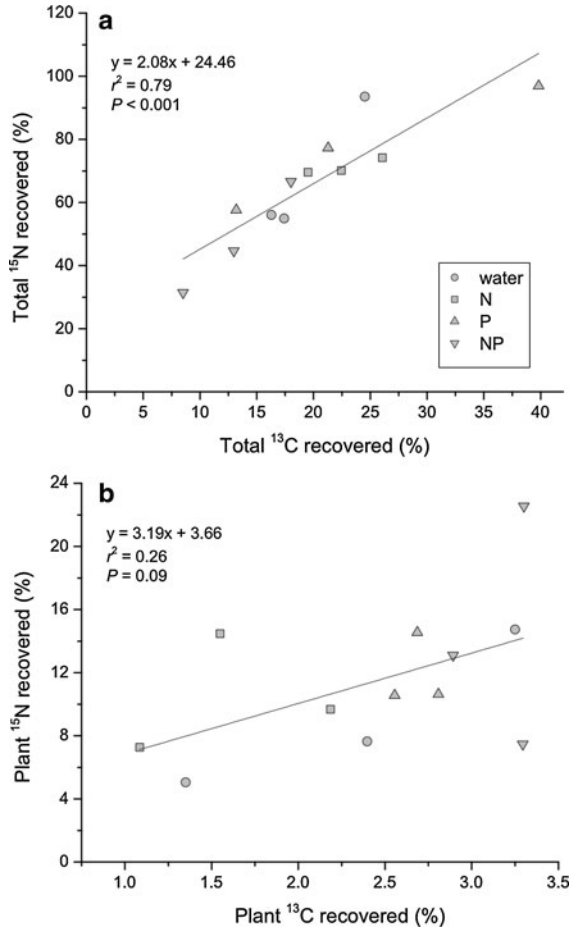

Fig. 4 Relationships between ^{13}C and ^{15}N recovery from amended glycine in total ecosystem and plant biomass pools. The cumulative proportion of glycine ^{13}C and ^{15}N amended to soils that is recovered in total across the soil microbial biomass, SOM, soil solution and plants is strongly positively related, with on average 3.4-times (range of 2.4–4.4) as much ^{15}N recovered as ^{13}C (**a**). In contrast the relationship was much weaker when only the plant biomass pool was considered (**b**), and obvious in these data is the greater range in relative recoveries (range of 2.3–9.3; mean of 4.9-times greater ^{15}N), suggesting that C and N of LMW organic inputs are more tightly cycled in the whole system vs. just the plants. Values are for individual field plots and are shown by fertilizer regime ($n = 12$)

2010), suggest that a greater proportion of glucose than glycine inputs should be stabilized as SOM (Figs. 1, 3b). These mechanisms were supported by greater active microbial biomass under glycine than glucose amendment (Table 1), and higher respiration of glycine (Fig. 2) despite lower recoveries in the microbial biomass (Fig. 3a). However, our three hypotheses relating to growth efficiencies, preferential assimilation and biomass turnover are not mutually exclusive. That is, they all predict greater formation of SOM from glucose than glycine (Fig. 1). Future work is needed to discern the relative contribution of each mechanism to the formation of stable SOM.

Microbial-derived compounds are the primary constituents of stable, long-term SOM (Grandy and Neff 2008; Lundberg et al. 2001) and explicit incorporation of microbial processes in SOM models can alter the responses of soil C stores to environmental change (Allison et al. 2010). Yet formal theory relating microbial identity, physiology and food web dynamics to SOM formation rates is in its infancy (Cotrufo et al. 2012; Schmidt et al. 2011). Our work provides empirical data to test and develop formal theory relating microbial dynamics to SOM formation. It shows the potential for LMWC inputs to form stable SOM, and that formation rates appear positively related to the mass of label first incorporated into microbial biomass. As LMWC inputs fuel a substantial proportion of belowground heterotrophic activity, our data highlight the need to understand the microbial-based mechanisms regulating the fate of these inputs to predict the dynamics of some of the most persistent soil C compounds, and hence future changes in SOM stocks.

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