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Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates

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Summary

- Root carbon (C) inputs may regulate decomposition rates in soil, and in this study we ask: how do labile C inputs regulate decomposition of plant residues, and soil microbial communities?
- In a 14 d laboratory incubation, we added C compounds often found in root exudates in seven different concentrations (0, 0.7, 1.4, 3.6, 7.2, 14.4 and 21.7 mg C g^{-1} soil) to soils amended with and without ^{13}C-labeled plant residue. We measured CO_2 respiration and shifts in relative fungal and bacterial rRNA gene copy numbers using quantitative polymerase chain reaction (qPCR).
- Increased labile C input enhanced total C respiration, but only addition of C at low concentrations (0.7 mg C g^{-1}) stimulated plant residue decomposition (+2%). Intermediate concentrations (1.4, 3.6 mg C g^{-1}) had no impact on plant residue decomposition, while greater concentrations of C (> 7.2 mg C g^{-1}) reduced decomposition (–50%). Concurrently, high exudate concentrations (> 3.6 mg C g^{-1}) increased fungal and bacterial gene copy numbers, whereas low exudate concentrations (< 3.6 mg C g^{-1}) increased metabolic activity rather than gene copy numbers.
- These results underscore that labile soil C inputs can regulate decomposition of more recalcitrant soil C by controlling the activity and relative abundance of fungi and bacteria.

Introduction

The soil is an important terrestrial carbon (C) sink, storing *c.* 4.5 times the amount of C contained in vegetation (Lal, 2004). Soil C accrual is controlled by the balance between C input via plants and C output via microbial and root respiration (Jastrow *et al.*, 2007). Root inputs contribute significantly to soil C pools because plants partition up to 40% of assimilated C below ground (Merckx *et al.*, 1986). Roots also regulate soil C output by stimulating microbial activity through the release of root exudates (Cheng & Kuzyakov, 2005). Root exudates are low-molecular-weight compounds that are passively released by living roots (Lynch & Whipps, 1990; Darrah, 1996). They consist of a complex mixture of organic acids, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, iorganic ions, gaseous molecules, enzymes and root border cells (Dakora & Phillips, 2002; Dennis *et al.*, 2010). Carbon-rich substrates, such as sugars (50–70% of total exudate), carboxylic acids (20–30% of total exudate) and amino acids (10–20% of total exudate) make up the majority of exudate compounds (Kraffczyk *et al.*, 1984; Jones, 1998; Hutsch *et al.*, 2002), and they are an important C source for C-limited soil microbial communities (Bowen & Rovira, 1991; Darrah, 1996). Nonetheless they are an often overlooked regulator of decomposition processes (Darrah, 1996; Kuzyakov, 2002; Phillips, 2007).

Both the quality and quantity of root exudate inputs to soil, and their importance in the C cycle remain controversial (Kuzyakov *et al.*, 2000) and we have a poor understanding of how root exudate inputs impact more recalcitrant C turnover. For example, in the presence of living roots, soil organic

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matter (SOM) C and N mineralization can decrease by up to 30%, or increase by as much as 100%, relative to nonrhizosphere soil (Dormaar, 1990; Cheng & Kuzyakov, 2005). An increase in plant- or soil-derived C respiration in response to labile C additions is termed a 'real positive priming effect' (Kuzyakov *et al.*, 2000), but if an increase in respired CO_2 is derived from root exudate C only, and is not accompanied by increases in the decomposition rate of recalcitrant substrates, the process is referred to as 'an apparent priming effect' (Kuzyakov, 2002). Previous research showed that the supply of easily assimilable compounds to soil may result either in large (Hamer & Marschner, 2002, 2005b) or small (Dalenberg & Jager, 1989; Kuzyakov *et al.*, 2007) real positive priming effects, or in no (Wu *et al.*, 1993) or negative (Kuzyakov & Bol, 2006; Blagodatskaya *et al.*, 2007) changes in plant residue decomposition. Understanding the causes of this variation will enable a better understanding of root impacts on the soil C cycle.

Soil microbial communities play a large role in decomposition processes, and root exudate inputs have an impact on their community structure and C-use efficiency (De Deyn *et al.*, 2008). At a coarse scale, microbial communities can be broken down into fungal and bacterial groups, and research indicates that these groups function differently in the decomposition process. In general, bacterial decomposition pathways support high turnover rates of easily available substrates, while slower fungal-dominated decomposition pathways more complex organic materials (Wardle *et al.*, 2002). Fontaine & Barot (2005) propose that observed priming effects of soil C decomposition result from the succession of opportunist microorganisms (such as bacteria) that specialize in the decomposition of easily available resources to slow-growing, efficient microorganisms (such as fungi) that feed on polymerized SOM. However, this hypothesis is the subject of some debate; a rapid increase in soil organic carbon (SOC) mineralization (e.g. priming) after substrate additions might be the result of an increase in microbial opportunist activity, rather than a shift to slow-growing microorganisms (De Nobili *et al.*, 2001; Hamer & Marschner, 2002; 2005a,b; Hamer *et al.*, 2004).

To shed light on the impact of root exudation on SOM decomposition, we examined how labile C compounds, commonly found in root exudates, alter plant residue decomposition and the relative abundances of fungi and bacteria responsible for those changes. We focus on C because it makes up over 90% of root exudates and often limits the growth of microbial communities. We predict that soil bacterial and fungal communities will preferentially use labile C compounds and that shifts in the relative proportion of bacterial and fungal communities will determine both the size and direction of the real priming effect.

Using experimental soil incubations, we added a synthetic root exudate cocktail at seven concentrations (0, 0.7, 1.4, 3.6, 7.2, 14.4 and 21.7 mg C g^{-1}) of C-input to soils with and without ^{13}C-labeled plant residue and measured the respiration rates over 14 d. These treatments enabled us to differentiate between the C respired from added plant residue and that from pre-existing soil C. The artificial 'exudate cocktail' contained the most ubiquitous C compounds of root exudates and combined them in realistic ratios (Kraffczyk *et al.*, 1984; Jones, 1998; Hutsch *et al.*, 2002). Estimates of the quantity of C released in soil via root exudates vary depending on the method of collection, ranging from 50 to 1500 μg g^{-1} soil d^{-1} (Trofymow *et al.*, 1987; Jones & Darrah, 1993; Meharg & Kilham, 1995; Cheng, 1996). The exudate cocktail in our study was applied as a single pulse of C substrate that covered the full spectrum of root-derived labile soil C concentrations measured in the soil and beyond, to assess the effect of different thresholds of added exudate on priming. To monitor decomposition, we measured ^{13}C-CO_2 efflux over 14 d, and we determined shifts in the relative abundance of fungi and bacteria at days 3 and 14 using real-time PCR. Together these data will enable us to better understand how changes in root-derived labile C inputs may impact overall SOM decomposition.

Materials and Methods

Production of labeled material

Panicum virgatum L. Alamo (hereafter referred to as switchgrass) seeds were surface-sterilized using 70% ethanol and 30% commercial bleach, vernalized at 6°C for 3 d, germinated on agar for 2 d, and then established in a sealed mesocosm filled with coarse silica sand (maintained at 60% water-holding capacity, WHC). The mesocosm consisted of a stainless steel soil bin (50 × 50 × 30 cm) and a transparent Plexiglas cuvette (50 × 50 × 45 cm) inserted into a climate-controlled growth chamber (Conviron® CMP3224, Pembina, ND, USA). The photosynthetically active (400–700 nm) photon fluence rate of 400 μmol m^{-2} s^{-1}, a 16 : 8 h day : night cycle, and a 20 : 15°C day : night temperature corresponded to a 25 : 18°C day : night temperature within the mesocosm and was maintained for the duration of the experiment.

Plants were continuously labeled with 99 atom% ^{13}C-enriched CO_2 gas (Cambridge Isotope Laboratories, Andover, MA, USA). Regulated with an IRGA (model 820-LC; Li-Cor, Lincoln, NE, USA), the CO_2 concentration in the mesocosm was maintained at *c.* 500 μmol mol^{-1}. To decrease fungal growth that was inhibiting plant germination and growth, the mesocosm was opened periodically and treated with a diluted (1 : 100) algaecide : fungicide solution (Zero Tol; BioSafe Systems LLC., East Hartford, CT, USA). The fungicide was applied at a 10-fold lower concentration than the standard agricultural application of this fungicide.

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After 2 months of growth, the labeled above- and below-ground plant biomass was harvested, dried at 70°C for *c.* 48 h, and ground to 2.5 mm using a Wiley mill. Subsamples were further ground using a ball mill and analyzed for total C concentrations, total N concentrations, and stable C isotope ratios ($^{13}C/^{12}C$) using an Integra-CN, continuous flow, isotope ratio mass spectrometer (serCon Ltd, Crewe, UK) coupled with a LECO CN-2000 elemental analyzer (LECO Corporation, St Joseph, MI, USA).

Root exudates

We made six concentrations of synthetic root exudates that mimicked observed root exudate C release by mixing the most common C compounds found in root exudates. The relative concentrations of sugars, organic acids, and amino acids were kept constant and reflect ratios observed in the field (*c.* 60% sugars, *c.* 35% organic acids and *c.* 2% amino acids) (Kraffczyk *et al.*, 1984; Jones, 1998; Hutsch *et al.*, 2002). The stock solution contained: D-glucose (28 mg ml^{-1}), D-fructose (9.4 mg ml^{-1}), D-xylose (8 mg ml^{-1}), oxalate (7.8 mg ml^{-1}), fumarate (14.6 mg ml^{-1}), succinate (1.2 mg ml^{-1}), acetate (4.0 mg ml^{-1}), L-proline (0.1 mg ml^{-1}), L-arginine (0.1 mg ml^{-1}), L-glycine (0.1 mg ml^{-1}), L-serine (0.1 mg ml^{-1}), and glutamate (0.3 mg ml^{-1}) (Kraffczyk *et al.*, 1984).

Incubation design

Soil was collected (0–10 cm) using a hammer core from a cultivated switchgrass field (24 yr of cultivation) and homogenized. Soils were sieved (2 mm), visible roots (> 2 mm) were removed, and soil was kept at 6°C until used. A subsample of soil was dried, ground to a fine powder, and analyzed for total C, N and stable C isotope ratios ($^{13}C/^{12}C$).

After the soil was removed from the refrigerator, the soil (10 g DW) was incubated (20°C) in one of two ways: with artificial root exudates, or with artificial exudates and plant residues. The samples containing just soil and exudates, but no plant residue, were included to account for CO_2 evolution from the pre-existing soil C. These data were used to calculate plant residue-derived C respiration. The amount of plant residue added to each soil was kept constant (0.1 g), but the exudates were applied to the soil in a single pulse in one of seven C concentrations: 0, 0.7, 1.4, 3.6, 7.2, 14.4, and 21.7 mg C g^{-1} dry soil (Table 1).

Plant material was mixed with soil in 120 ml specimen cups. The WHC of the soils was determined by calculating the difference in soil weight at the water saturation point and the oven-dry weight (100°C). The exudate solutions were added homogeneously to obtain 60% of WHC. Specimen cups were placed in 1 l mason jars and 5 ml of water was added to the bottom of the jar to maintain humidity.

Microbial respiration

A septum in the lid of each mason jar allowed air samples (10 ml) to be removed from the headspace with a syringe. Soil CO_2 evolution was measured at days 1, 2, 3, 5, 7, and 14. Blank jars (three) that contained no soil were included to determine the background concentrations of CO_2 and its ^{13}C-isotopic composition. Carbon dioxide samples were collected in 12 ml exetainers (Labco Ltd, High Wycombe, UK). Following gas sampling, the mason jars were opened, the soils were removed, and the jars were flushed with air for 30 min. Samples were analyzed for CO_2 and its PDB-^{13}C signature with a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20–20 isotope ratio mass spectrometer; Sercon, Crewe, UK). For the CO_2 analyses, the highest available 'certified' ^{13}C standard IAEA 309B (+535.3 per mille Vienna PDB standard (VPDB)) was used. Then, calibration mixtures for samples enriched beyond this were made. Nominal 99 atom% enriched ^{13}C carbonate was mixed with natural abundance carbonate to produce CO_2 at a range of enrichments from 14 to 48 atom% ^{13}C. Carbon mineralization data are expressed on the basis of oven-dry soil.

Results of the C-isotope analyses are expressed in atom% ^{13}C. To calculate the amount of CO_2-C derived from ^{13}C-labeled plant residues, incubated in soil with exudates, the following mass balance was used (Denef *et al.*, 2002; de Graaff *et al.*, 2004):

Table 1 Overview of treatments used in the incubation experiment. The soil was incubated (20°C) in four different combinations: soil plus exudates; soil plus exudates and plant residue; soil plus water; and soil plus water and plant residue

Treatment	Exudate addition (mg C g^{-1} dry soil)	Plant litter addition (g g^{-1} dry soil)
Soil + exudates	0.7	0.0
	1.4	0.0
	3.6	0.0
	7.2	0.0
	14.4	0.0
	21.7	0.0
Soil + exudates + plant litter	0.7	0.01
	1.4	0.01
	3.6	0.01
	7.2	0.01
	14.4	0.01
	21.7	0.01
Soil + water	0.0	0.00
Soil + water + plant litter	0.0	0.01

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$$Q_t{}^*\text{atom}\%^{13}C_t = Q_p{}^*\text{atom}\%^{13}C_p + Q_{s+e}{}^*\text{atom}\%^{13}C_{s+e} + Q_b{}^*\text{atom}\%^{13}C_b$$

where Q_t is the total amount of CO_2-C, atom% $^{13}C_t$ is its isotopic composition; Q_p is the amount of CO_2-C derived C from plant residue, atom% $^{13}C_p$ is the isotopic composition of plant residue (70 atom% ^{13}C); Q_{s+e} is the amount of CO_2-C derived from the unlabeled soil + exudate, atom% $^{13}C_{s+e}$ is the isotopic composition of the unlabeled soil + exudate (1.1 atom% ^{13}C, on average), Q_b is the CO_2-C amount in the control blank jar and atom% $^{13}C_b$ is the isotopic composition of CO_2 in the control blank jar (1.1 atom% ^{13}C on average). The CO_2-C derived from the plant residue (Q_p) during the incubation was quantified by subtracting soil respiration (Q_{s+e} + Q_b) from the respiration of soil with incorporated plant materials (Q_t).

Quantification of bacterial and fungal gene copy numbers

After mixing the soil in each container, a subsample of 5 g was collected and frozen (−80°C) for DNA extraction and to assess the relative abundance of fungi and bacteria. Microbial DNA was extracted from 0.25 g soil using the PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA) in concert with a 96-well plate shaker, using a plate adapter set (MOBIO Laboratories). After extraction, the DNA concentration and its purity were determined with a nano-drop using the ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

Quantitative PCR runs were set up in clear Low-Profile 96-Well PCR Plates (Bio-Rad) using 10 µl iQ SYBR Green Supermix (Bio-Rad), 5 µl H_2O, 2 µl of forward primer, 2 µl of reverse primer (10 pM) and 1 µl of sample. Primers used for 16S rRNA bacterial genes were EUB338 (5′-ACTCCTACGGGAGGCAGCAG-3′; Lane, 1991) and Eub518 (5′-ATTACCGCGGCTGCTGG-3′; Muyzer *et al.*, 1993); and for fungal rRNA genes were nu-SSU-1196F (5′-GGAAACTCACCAGGTCCAGA-3′) and nu-SSU-1536R (5′-ATTGCAATGCYCTATCCCCA-3′) (Borneman & Hartin, 2000). Samples were analyzed in duplicate with triplicate standard curves. We tested 1 : 10 DNA dilutions in selected samples, did not observe inhibition, and obtained similar results.

Standard curves were prepared using known amounts of DNA extracted from pure cultures carried out concomitantly with experimental samples and exhibited a linear relationship between the log of the rRNA-gene copy number and the calculated threshold (C_t) value ($R^2 > 0.99$). *Escherichia coli* was used for bacterial amplifications (*c.* 180 bp amplicon) and *Saccharomyces cerevisiae* for fungal amplifications (*c.* 340 bp amplicon). These organisms were used to calculate the number of equivalent copies in the sample according to a standard curve. Amplification efficiencies ranged from 1.6 to 1.8, which are consistent with other reported values (Fierer *et al.*, 2005; Castro *et al.*, 2010).

Statistical analyses

Statistical tests were performed on CO_2 respiration rates and fungal and bacterial gene copy numbers at each sampling date during the incubation. Statistics were performed using the Univariate GLM in SPSS STATISTICS 17.0. We conducted a one-way ANOVA for each sampling date, with treatments (i.e. labile C input treatments, and plant residue amendments) as fixed effects. We tested for homogeneity of variance using Levene's test ($P \leq 0.01$). If equal variance was assumed, we compared means using the least significant differences *post hoc* test. If equal variance was not assumed, we used the Tamhane's T2 *post hoc* test to compare means. The levels of significance were $P \leq 0.05$ and $P \leq 0.01$.

Results

Total C respiration

The addition of water and exudates to soil resulted in an immediate increase in CO_2 respiration. Total cumulative C respiration (i.e. C derived from soil + exudates + plant residue) increased with each increase in root exudate ($P < 0.001$), although cumulative total C respiration was similar when concentrations of 0.7–1.4 mg C g^{-1} were added to soils (Table 2). Increasing exudate additions also enhanced the maximum respiration rate (i.e. the greatest measured CO_2 efflux in a 24 h period during the 14 d incubation) for almost all of the treatments ($P \leq 0.001$). However, exudate additions of 0.7 and 1.4 mg C g^{-1} resulted in similar maximum respiration rates, and exudate additions of 14.4 and 21.7 mg C g^{-1} reached equal maximum respiration rates (Fig. 1a).

With increasing exudate concentrations, the time it took to reach maximum C respiration rates was delayed (Fig. 1a). Soils receiving the lowest amount of exudates reached maximum respiration rates on day 1 ($P \leq 0.001$), whereas increasing exudate additions delayed the time it took to reach the maximum CO_2 efflux rate from day 2 to day 4 (Fig. 1a).

Plant- and soil-derived C respiration

The difference in isotopic signature between soils containing exudates and plant residues and those containing exudates only allowed us to calculate plant residue-derived C respiration. Addition of the lowest amount of exudates (0.7 mg C g^{-1}) to soil resulted in an increase in total

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Table 2 Cumulative soil CO_2 respiration at 14 d

Exudate addition	Experimentally determined CO_2 respiration: Soil + exudates (mg C g^{-1} soil)	Experimentally determined CO_2 respiration: Soil + exudate + plant residue (mg C g^{-1} soil)	Calculated CO_2 respiration: Plant residue (mg C g^{-1} soil)	Calculated CO_2 respiration: Soil (mg C g^{-1} soil)
Water only (control)	0.09 ± 0.004a	1.69 ± 0.04a	1.11 ± 0.03a	0.096 ± 0.04
0.7 μg C g^{-1}	0.86 ± 0.01a	2.29 ± 0.02a	1.21 ± 0.02b	0.145 ± 0.01
1.4 μg C g^{-1}	1.54 ± 0.003b	2.31 ± 0.12a	0.93 ± 0.05a	0.02a
3.6 μg C g^{-1}	2.09 ± 0.09a,b	3.86 ± 0.30b	1.02 ± 0.02a	0.00
7.2 μg C g^{-1}	4.26 ± 0.03b,c	5.17 ± 0.06c	0.73 ± 0.004b	0.00
14.4 mg C^{-1}	6.40 ± 0.27c	8.62 ± 0.07d	0.56 ± 0.01c	0.00
21.7 mg C g^{-1}	6.26 ± 0.74c	11.5 ± 0.07e	0.50 ± 0.004d	0.00

The second column represents the amount of CO_2 respired from soils receiving exudates only. The third column represents the amount of CO_2 respired from soils receiving both exudates and plant residues. The fourth column represents the contribution of plant residue to the total soil CO_2 respiration. The fifth column represents the contribution of the native soil carbon to the total soil CO_2 respiration.
Different letters indicate differences among treatments within a column ($P \leq 0.05$) within each table column. Values are mean ± SE (n = 3).

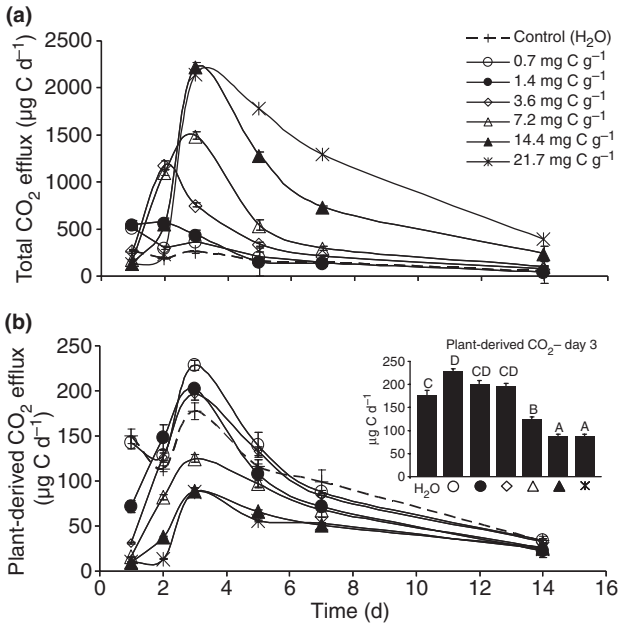

Fig. 1 Soil carbon (C) respiration rates across a range of labile C additions. (a) Carbon derived from the decomposition of native soil C, plus added labile C, plus added plant residue. (b) Carbon derived from the decomposition of added plant residue; the bar graph insert highlights plant residue decomposition differences among treatments on day 3 of the incubation. Different letters indicate significant differences ($P \leq 0.05$) among C-addition treatments. Values are means ± SE (n = 3).

cumulative plant-derived C respiration relative to water plus residue control treatments ($P \leq 0.05$; Table 2). By contrast, addition of exudates in excess of 7.2 mg C g^{-1} significantly reduced cumulative plant-derived C respiration relative to water-only treatments ($P \leq 0.001$; Table 2).

The impact of exudate additions on plant residue turnover rates was largest on day 3 of the incubation (Fig. 1b). Addition of the lowest amount of exudates (0.7 mg C g^{-1}) significantly stimulated decomposition of plant residue by 29% relative to the water-only treatment (P = 0.03; Fig. 1b). Addition of 1.4 and 3.6 mg C g^{-1} resulted in a trend towards increased decomposition of plant-derived C relative to plant-derived C respiration from soils receiving water only (+14 and +11%, respectively; Fig. 1b). Additions of 7.2, 14.4 and 21.7 mg C g^{-1} all resulted in significantly reduced plant residue-derived CO_2 respiration relative to the soils amended with plant residue and water only, by 30% (P = 0.003), 50% ($P \leq 0.001$) and 50% ($P \leq 0.001$), respectively (Fig. 1b).

Soil-derived CO_2 respiration was determined by subtracting the total cumulative amount of respiration in soils receiving exudates only from the amount of C added to the soils (Table 2). Addition of 0.7 and 1.4 mg C g^{-1} resulted in a trend toward increased cumulative soil C efflux relative to soils receiving water only (+20 and +16%, respectively; Table 2).

Quantification of bacterial and fungal gene copy numbers

Methods using qPCR-generated gene copy numbers have several limitations when used to calculate absolute abundances of microorganisms (e.g. microbial biomass or cell numbers) and are far from unambiguous (Fierer *et al.*, 2005; Rousk *et al.*, 2010; Strickland & Rousk, 2010). Interpreted from relative copy number alone, our data show a very consistent response and our study is highly controlled by design (e.g. using the same soil source, DNA extraction, PCR conditions, and DNA standards) especially when compared with other studies where qPCR is used across diverse soil types and ecosystems. We keep the limitations of the qPCR method in mind, and interpret any treatment-induced shifts in gene copy number as a relative change within a group (i.e. fungi or bacteria).

Across all treatments, exudate additions increased relative bacterial and fungal gene copy numbers. Relative increases

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in bacterial and fungal gene copy numbers resulting from C addition were generally greater in soils amended with both exudates and plant residue than in soils amended with exudates only (Table 3). The relative increase in fungal gene copy numbers in response to labile C additions was generally higher than the relative increase in bacterial gene copy numbers, specifically in soils amended with plant residues (Fig. 2). After 3 d of incubation and relative to water-only treatments, fungal : bacterial gene copy ratios significantly increased following a labile C input of 3.6 mg C g^{-1} (Fig. 2a). On day 14 of the incubation, and relative to water-only treatments, fungal : bacterial gene copy ratios significantly increased following a labile C input of 0.7 mg C g^{-1} (Fig. 2b).

After 3 d of incubation and relative to treatments that received water only, fungal gene copy numbers were significantly elevated at an input of 3.6 mg C g^{-1} in soils receiving both exudates and plant residues ($P \leq 0.05$; Table 3). After 14 d of incubation and relative to water-only treatments, fungal gene copy numbers were significantly elevated at exudate additions of 0.7 mg C g^{-1} in soils receiving exudates only and in soils receiving exudates and plant residues ($P \leq 0.05$; Table 3). After 3 and 14 d of incubation and relative to water-only treatments, bacterial gene copy numbers increased at exudate additions of 14.4 mg C g^{-1} when no plant residue was added to the soils ($P \leq 0.05$; Table 3). After 3 d of incubation and relative to water-only treatments, bacterial gene copy numbers were enhanced when exudate additions exceeded 1.4 mg C g^{-1} in soils amended with plant residue and consistently increased thereafter ($P \leq 0.05$; Table 3). After 14 d of incubation and relative to water-only treatments, bacterial gene copy numbers were enhanced at exudate additions of 0.7 mg C g^{-1} in soils amended with plant residue, but they did not consistently increase with higher concentrations of C ($P \leq 0.05$; Table 3).

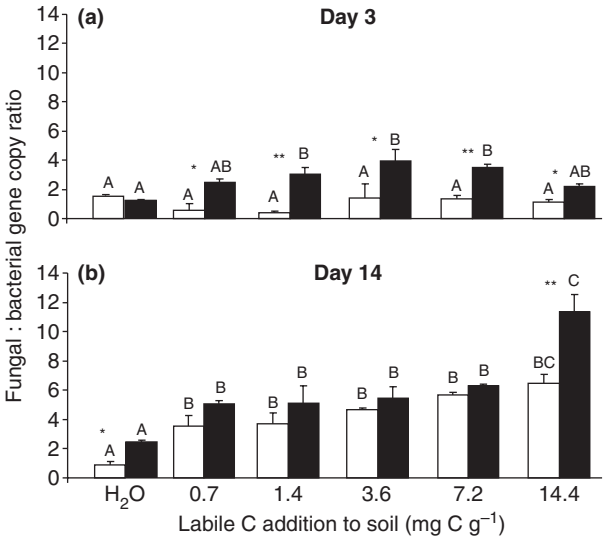

Fig. 2 Fungal : bacterial gene copy ratios in soils exposed to the different labile carbon (C) addition treatments. Open bars, samples that received labile C additions only; closed bars, samples that received labile C and plant residue at days 3 (a) and 14 (b) of the incubation. Different letters above the bars indicate significant differences among treatments ($P \leq 0.05$) as induced by different labile C inputs within a group (i.e. soil with added exudates, or soil with added exudates and plant residue). Asterisks indicate significant differences between groups (i.e. soil with added exudates, or soil with added exudates and plant residue) within each labile C addition treatment, Significance: *, $P \leq 0.05$; **, $P \leq 0.01$. Values are means ± SE (n = 3).

Table 3 Soil fungal and bacterial gene copy number for the 16S : 18S subunit (qPCR) under five different labile carbon (C) additions

	Bacterial gene copy numbers		Fungal gene copy numbers	
Treatments	Day 3	Day 14	Day 3	Day 14
Water only	(8.4 ± 0.2) × 10^5a	(7.9 ± 0.3) × 10^5a	(1.3 ± 0.3) × 10^6b	(7.1 ± 1.7) × 10^5a
0.7 mg C g^{-1}	(8.3 ± 0.4) × 10^5a	(9.4 ± 0.9) × 10^5a	(4.7 ± 0.7) × 10^5a	(3.3 ± 0.5) × 10^6b
1.4 mg C g^{-1}	(9.5 ± 0.5) × 10^5a	(1.2 ± 0.01) × 10^6a	(3.7 ± 0.9) × 10^5a	(4.6 ± 1.7) × 10^6b
3.6 mg C g^{-1}	(9.4 ± 1.7) × 10^5a	(1.2 ± 0.1) × 10^6a	(1.3 ± 0.5) × 10^6b	(5.4 ± 0.3) × 10^6b
7.2 mg C g^{-1}	(9.9 ± 1.0) × 10^5a	(1.3 ± 0.04) × 10^6a	(1.4 ± 0.1) × 10^6b	(7.4 ± 0.7) × 10^6b
14.4 mg C g^{-}1	(1.6 ± 0.1) × 10^6b	(1.6 ± 0.3) × 10^6b	(1.7 ± 0.3) × 10^6b	(1.0 ± 0.1) × 10^7c
Soil + plant residue	(1.8 ± 0.1) × 10^6b	(1.2 ± 0.1) × 10^6a	(2.1 ± 0.1) × 10^6a	(2.9 ± 0.5) × 10^6a
0.7 mg C g^{-1} + plant residue	(1.5 ± 0.1) × 10^6a	(1.5 ± 0.1) × 10^6b	(3.6 ± 0.1) × 10^6a	(7.7 ± 0.6) × 10^6b
1.4 mg C g^{-1} + plant residue	(1.4 ± 0.2) × 10^6a	(1.5 ± 0.1) × 10^6b	(4.1 ± 0.4) × 10^6a	(7.6 ± 2.6) × 10^6b
3.6 mg C g^{-1} + plant residue	(2.4 ± 0.2) × 10^6c	(1.6 ± 0.3) × 10^6a,b	(9.3 ± 1.2) × 10^6b	(8.7 ± 1.2) × 10^6b
7.2 mg C g^{-1} + plant residue	(2.5 ± 0.2) × 10^6c	(2.0 ± 0.2) × 10^6b,c	(8.9 ± 1.1) × 10^6b	(1.3 ± 0.2) × 10^7b,c
14.4 mg C^{-1} + plant residue	(3.2 ± 0.1) × 10^6d	(1.0 ± 0.2) × 10^6a	(6.9 ± 0.5) × 10^6b	(1.2 ± 0.3) × 10^7b,c

qPCR, quantitative polymerase chain reaction.
From left to right, columns represent: bacterial gene copy numbers on day 3 of the incubation, bacterial gene copy numbers on day 14 of the incubation, fungal gene copy numbers on day 3 of the incubation, and fungal gene copy numbers on day 14 of the incubation.
Different letters indicate differences among treatments ($P \leq 0.05$) among C-addition treatments within each table column. Values are mean ± SE (n = 3).

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Discussion

The addition of exudates to soil enhanced both overall CO_2 respiration and bacterial and fungal gene copy numbers. However, the composition of respired CO_2 (i.e. the abundance of soil plus exudate-derived CO_2 relative to plant residue-derived CO_2) was determined by the amount of labile C added to the soil. Additions of low amounts of substrate (i.e. 0.7 mg C g^{-1}) increased plant residue decomposition, without detectable increases in relative fungal or bacterial gene copy numbers. At intermediate additions (i.e. 1.4 and 3.6 mg C g^{-1}), decomposition of the plant residues remained similar to decomposition of residues when only water was added, although total microbial respiration and relative microbial gene copy numbers were enhanced. By contrast, the highest amount of labile C inputs to the soil (i.e. > 3.6 mg C g^{-1}) had the greatest stimulating effect on relative microbial gene copy numbers and total respiration rates, but it also significantly reduced plant residue decomposition.

Our results, that labile C inputs can increase, have no effect on, or decrease more recalcitrant C decomposition in soil, support previous findings (Kuzyakov, 2002; Blagodatskaya & Kuzyakov, 2008). For example, several studies found only apparent priming effects when labile C was added to soil (Dalenberg & Jager, 1981, 1989; Chapman & Gray, 1986; De Nobili *et al.*, 2001). By contrast, others have found either positive (Kuzyakov *et al.*, 2007; Hamer & Marschner 2005a,b), or negative priming effects when labile C was added to soil (Kuzyakov & Bol, 2006; Blagodatskaya *et al.*, 2007). In many cases this variability cannot be explained by the amount of labile C added to the soil system. For example, Hamer & Marschner (2005b) observed large (+63%) increases in native SOM decomposition following additions of labile C in similar concentrations to our 'low' additions, whereas others found no impact of labile C inputs on decomposition of more stable C following labile C additions at a 10-fold smaller rate (Blagodatskaya *et al.*, 2007; Kuzyakov *et al.*, 2007). However, none of the previous studies examined decomposition and concomitant microbial responses to a range of labile C inputs to soil. Our results suggest that the amount of labile soil C input can either increase or decrease decomposition of more recalcitrant soil C, by changing the relative abundance of bacteria and fungi originally present in the soil. Therefore, our results shed light on some of the variation found in previous studies and thus enable better predictions of when priming may occur in soils.

Clearly, soil microbial communities are important in SOM decomposition and they respond quickly to changes in labile soil C additions because they are often C-limited, (Bowen & Rovira, 1991; Darrah, 1996). Thus, their response to C additions should regulate priming effects. In a literature synthesis, Blagodatskaya & Kuzyakov (2008) proposed that the size of the soil microbial biomass C strongly regulates the impact of added substrate C on the direction of the priming effect. While we cannot explicitly test for this in our study because our qPCR-generated gene copy numbers do not reflect changes in the absolute abundance of microorganisms (Fierer *et al.*, 2005; Rousk *et al.*, 2010), the relative increase in microbial gene copy numbers within both bacterial and fungal groups with increasing C additions in our study lends support for this hypothesis. We observed a real positive priming effect when small amounts of exudate input stimulated microbial metabolic activity, leading to an increased need for C by the microbial community irrespective of its source (i.e. plant residues or native SOM). Intermediate inputs stimulated relative microbial gene copy numbers to the extent that the active microbial community could be supported by both labile C and residue C. By contrast, when high inputs of labile C were sufficient to support the relative increase in microbial abundance, the microbial community preferentially consumed the most labile substrate leading to a real negative priming effect, a mechanism proposed previously (Cheng, 1996; Cheng & Kuzyakov, 2005). Our results indicate that the threshold for a priming effect is determined by the interaction between substrate addition and the active microbial community.

Changes in microbial community structure, in addition to or instead of changes in relative abundances of bacteria and fungi, may also regulate priming effects. Fontaine *et al.* (2003) proposed that positive priming results from the succession of fast-growing, competitive microorganisms to more slow-growing microbial communities that specialize in the decomposition of more recalcitrant C. However, Hamer & Marschner (2005b) point out that rapid increases in SOC mineralization after substrate additions might indicate that fast-growing microorganisms are driving positive priming effects. Similar to previous work, we measured a greater increase in fast-growing fungi relative to bacteria upon labile C additions, resulting in enhanced fungal : bacterial gene copy ratios (Griffiths *et al.*, 1998; Broeckling *et al.*, 2008; Chiginevaa *et al.*, 2009). Chiginevaa *et al.* (2009) suggested that these fungi belong to a fast-growing fungal group that specializes in metabolizing relatively large amounts of labile C-rich substrates. The significant shift toward fungal decomposition on day 3 in our study appeared to result in an apparent or negative priming effect, whereas no detectable change in fungal : bacterial gene copy ratios on day 3 of our study yielded a positive priming effect. Given this, our data suggest that upon substrate addition, an increase in the activation of the innate soil microbial community, without large shifts in microbial functional groups, can lead to a real positive priming effect.

The response of microbial C respiration to exudate input in our study was rapid, and was followed by a steep decline.

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Such swift increases (1–3 d) and subsequent decreases in microbial CO_2 respiration following a one-time addition of labile C to soil are common (Wu *et al.*, 1993; Bremer & Kuikman, 1997; Mondini *et al.*, 2006; Jones & Murphy, 2007; Hoyle *et al.*, 2008). However, under field conditions, plants exude labile C into soil continuously, potentially reducing the need for a priming effect of more recalcitrant soil C. Nonetheless, continuous addition of C at a rate of 10 μg C g^{-1} soil d^{-1} showed a similar increase in plant residue mineralization (Kuzyakov *et al.*, 2007). This suggests that under more natural conditions, with a continuous input of labile C to soil, an overall greater C requirement by the microbial community promotes degradation (priming) of more stable soil C. Further, root exudation is not necessarily a continuous steady-state process, but can occur locally at the root tip in sudden large bursts (Jaeger *et al.*, 1999; Farrar *et al.*, 2003). These pulsed inputs are similar to our experimental design and can lead to intermittent periods of microbial growth and subsequent exhaustion of C substrates (Kuzyakov *et al.*, 2007). Such events may be important in controlling priming effects in soils, as the enhanced microbial biomass may have to be supported in the longer term by decomposition of more recalcitrant soil C, or of plant residue additions (Kuzyakov *et al.*, 2007).

Despite a general fast response in metabolic activity of the microbial community, we found a greater time lag in maximum CO_2 respiration with increasing substrate additions. In fact, the maximum respiration rate occurred at day 1 for the lowest labile C addition and at days 2, 3, and 4 with increasing C additions. Such time lags have been observed previously (Schneckenberger *et al.*, 2008) and are most likely associated with the inability of soil microbes to take up and utilize large amounts of labile C, microbial growth, and shifts in the microbial community (Blagodatskaya & Kuzyakov, 2008). While our experiment is unable to tease apart which of these three mechanisms is responsible for the observed time lag in C degradation, it supports the results of previous studies (Anderson & Domsch, 1978).

Plants can allocate C above and below ground and the amount of allocation can change in response to a variety of perturbations, such as changing atmospheric CO_2 concentration (de Graaff *et al.*, 2009; Iversen, 2010) and herbivory (Dyer & Bokhari, 1976; Guitian & Bardgett, 2000; Hamilton & Frank, 2001). If these perturbations alter the amount of C being shunted below ground, the rate of C degradation in soils may change. Of course, other factors that we did not investigate might alter the response we observed. For example, root exudates collected in natural ecosystems are complex and contain a wide array of secondary chemicals that we did not include in our cocktail (Dakora & Phillips, 2002; Dennis *et al.*, 2010). Shifts in secondary metabolites, for example, might alter the response of the microbial community (Wu *et al.*, 1993; Fontaine *et al.*, 2003; Hamer & Marschner 2005a; Dennis *et al.*, 2010). Similarly, different soil types and their associated microbial communities may respond to labile C substrate additions in different ways (Kuzyakov *et al.*, 2000). Nonetheless, our data clearly show how small changes in below-ground C allocations may change associated decomposition processes and the communities responsible for those changes.

Conclusions

The quantity of exudate input to soil appears to strongly mediate decomposition rates of plant residue by controlling the direction of the priming effect. Each step-increase in labile C addition significantly enhanced microbial CO_2 respiration. Exudate concentrations in excess of 3.6 mg C g^{-1} appeared to promote competitive fungal and bacterial groups, leading to preferential substrate utilization and a negative priming effect. By contrast, exudate additions at concentrations of 0.7 mg C g^{-1} soil d^{-1} did not significantly enhance bacterial or fungal gene copy numbers, but triggered metabolic activity of the microbial community resulting in a positive priming effect. Future work should investigate how different types of root-specific compounds alter SOM decomposition, what the role of microbial abundance vs community structure is in these processes, and how different types on perturbations may influence these patterns across ecosystems.

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