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Distinct fungal and bacterial $\delta^{13}C$ signatures as potential drivers of increasing $\delta^{13}C$ of soil organic matter with depth

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Abstract Soil microbial biomass is a key source of soil organic carbon (SOC), and the increasing proportion of microbially derived SOC is thought to drive the enrichment of ^{13}C during SOC decomposition. Yet, little is known about how the $\delta^{13}C$ of soil microbial biomass differs across space or time, or with the composition of the microbial community. Variation in soil microbial $\delta^{13}C$ may occur due to variation in substrates used by soil microorganisms, and variation in how different microorganisms synthesize biomass. Understanding these variations in soil microbial $\delta^{13}C$ would enable more accurate interpretation of patterns in the $\delta^{13}C$ of SOC. Here, we report the variation in $\delta^{13}C$ values of individual phospholipid fatty acids (PLFA) within podzolic soils from mesic boreal forests characterized by steep decreases in fungal to bacterial (F:B) ratios. By comparing trends in $\delta^{13}C$ of PLFA indicative of either fungi or bacteria to those PLFA common across both microbial groups, we tested the hypothesis that the enrichment of ^{13}C in bacterial relative to fungal biomass represents a mechanism for the increase of bulk SOC $\delta^{13}C$ with depth. We demonstrate that PLFA derived from fungi were consistently depleted in ^{13}C (−40.1 to −30.6 ‰) relative to those derived from bacteria (−31.1 to −24.6 ‰), but unlike bulk SOC the $\delta^{13}C$ of PLFA from either group did not vary significantly with depth. In contrast, the $\delta^{13}C$ of PLFA produced by both fungi and bacteria, which represent the $\delta^{13}C$ of soil microbial biomass as a whole, strongly increased with depth (increase of 7.6–8.4 ‰) and was negatively correlated with the fungi/(fungi + bacteria) ratio ($R^2 > 0.88$). The steep increase of the $\delta^{13}C$ of general PLFA with depth cannot be explained by an increase in the $\delta^{13}C$ of either fungal or bacterial biomass alone since the PLFA indicative of those groups did not vary with

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depth. Instead, these data demonstrate that the increase in soil biomass $\delta^{13}C$ with depth is driven by a change in the proportion of bacterial relative to fungal biomass. We suggest that the increased proportions of soil bacterial relative to fungal biomass with depth may represent an important mechanism contributing to increasing $\delta^{13}C_{SOC}$ with depth via contributions from 'necromass' to SOC.

Keywords ^{13}C · Necromass · Podzols · Soil organic carbon · PLFA · Soil microbial biomass

Introduction

Since the establishment of isotope-ratio mass spectroscopy (IRMS), the analysis of natural abundance stable isotope composition (isotopic signatures) has led to key insights into the ecophysiology and food webs of macroscopic organisms (e.g. Fry et al. 1978; Ehleringer et al. 1985; Peterson et al. 1985). Yet for the study of microbial communities and their processes, stable isotope analysis remains largely limited to labeling approaches (stable isotope probing), even though well established methods for the compound-specific isotopic analysis of microbial biomarkers in environmental samples exist (e.g. Hayes et al. 1990; Silfer et al. 1991; Abrajano et al. 1994).

Soil microorganisms mediate both the formation and mineralization of soil organic carbon (SOC). They not only act as gatekeepers of the respiratory release of CO_2 from SOC (Schlesinger and Andrews 2000), but are also considered an important source or precursor of SOC (Grandy and Neff 2008; Bol et al. 2009; Miltner et al. 2011). Soil microorganisms therefore exert crucial control on key feedbacks in the global carbon cycle. Linking microbial formation and degradation of SOC to the isotopic signatures of SOC and microbial biomarkers in soils could provide a tool for the investigation of these microbial processes in relatively undisturbed soils. This is sorely needed, given that most studies on microbial substrate use and SOC formation are based on laboratory incubations with labeled substrates (e.g. Waldrop and Firestone 2004; Rinnan and Bååth 2009) or labeled microbial biomass (Miltner et al. 2009). Such stable isotope probing experiments typically require severe manipulations of the soils, including the removal of the soils from their in situ location, homogenization, addition of substrates often above ambient concentrations, and the exclusion of many C sources available to soil microorganisms in situ, like litter leachates or root exudates. The patterns of C cycling found in such experiments therefore can differ drastically from in situ measurements of the same soil (Phillips et al. 2013).

Natural variations in the $\delta^{13}C$ of soil microorganisms may reveal patterns of microbial substrate use, given expectations that the $\delta^{13}C$ of soil microorganisms would co-vary with the $\delta^{13}C$ of their substrates, as noted in aquatic ecosystems (Coffin et al. 1990). Substrates available to soil microorganisms can have distinct isotopic signatures resulting from isotopic fractionation during plant biosynthesis, like the enrichment of ^{13}C in carbohydrates relative to lignin and lipids or of root-derived relative to leaf-derived carbon (Hobbie and Werner 2004 and references therein), and perhaps from the discrimination against ^{13}C-containing compounds during decay (Ehleringer et al. 2000). In turn this systematic variation in substrate $\delta^{13}C$ may influence the $\delta^{13}C$ of microbial biomass. Detection of ^{13}C-enriched D-amino acids in soil suggests the preferential use of ^{13}C enriched substrates by bacteria (Glaser and Amelung 2002), as do observations of ^{13}C enriched microbial biomass relative to available substrates in some soils (Dijkstra et al. 2006). Fungal use of more ^{13}C-deplete lignin-derived compounds in soil, for example, is also possible given their role in lignin degradation (Osono 2007) potentially making fungal $\delta^{13}C$ distinctly lower than bacterial biomass. In circumstances when fungi are not in competition with bacteria, such as fungi colonizing dead wood, however, their biomass appears enriched in ^{13}C by about 1–2 ‰ relative to cellulose extracted from that wood (Gleixner et al. 1993). The various substrates available in soil are also subjected to distinct temporal and spatial variations in $\delta^{13}C$. The $\delta^{13}C$ of plant roots and photosynthates transferred to mycorrhizal fungi may vary temporally but likely do not change with depth (Wallander et al. 2004). Conversely, bulk SOC is more enriched in ^{13}C in deeper soil horizons, a trend generally associated with SOC at depth having undergone a greater degree of microbial processing (Nadelhoffer and Fry 1988; Nakamura et al. 1990; Fig. 1a) and enrichment of ^{13}C of microbial biomass relative to the substrates used (Macko and Estep 1984), though the underlying mechanisms at play in soil remains poorly understood (Boström et al. 2007).

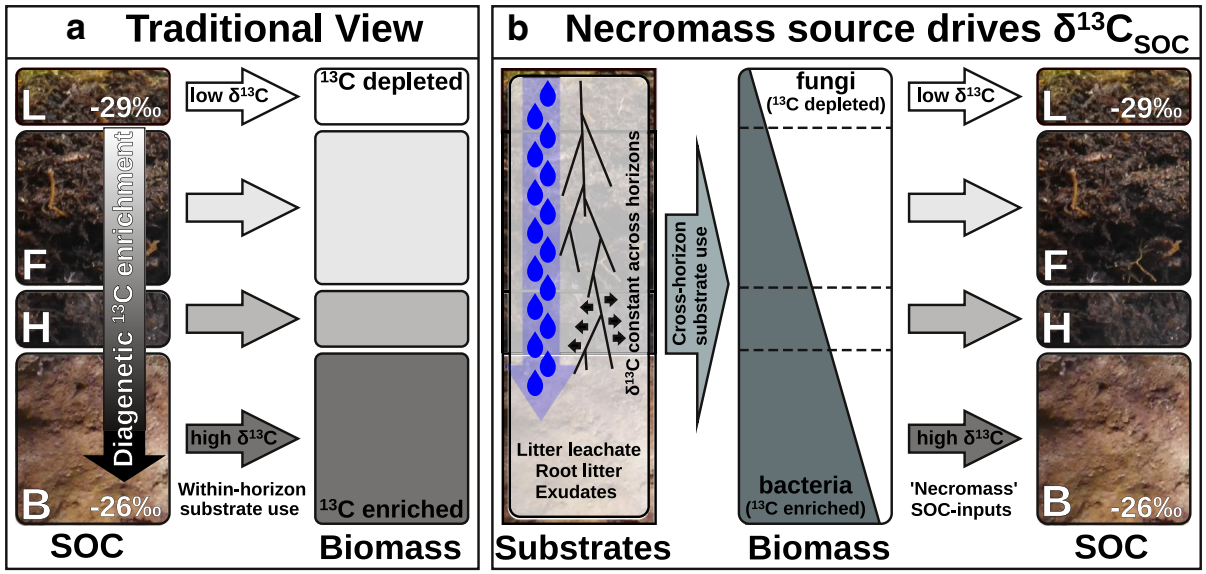

Fig. 1 Conceptual figure depicting how diagenesis and microbial substrate use have often been assumed to drive the $\delta^{13}C$ of SOC and microbial biomass within the horizons of soil profiles (**a**) versus the more complex exchange, and availability of common substrates (e.g. root exudates, litter and soil leachates) across horizons, relevant to the results of this study (**b**). While the traditional view is that soil microorganisms primarily consume the SOC from 'their' own soil horizon and that $\delta^{13}C_{biomass}$ therefore increases with depth along with $\delta^{13}C_{SOC}$, we demonstrate that fungal and bacterial biomass have distinct $\delta^{13}C$ values and that the fungi:bacteria ratio dictate the overall $\delta^{13}C_{biomass}$ through the profile. We further suggest that the depletion of ^{13}C fungal dominated biomass in shallow horizons and the enrichment of ^{13}C in bacterially dominated biomass in deeper soil horizons can contribute to the increases in $\delta^{13}C_{SOC}$ signatures with depth in these soils

The relation between the $\delta^{13}C$ signature of soil microbial biomass and $\delta^{13}C_{SOC}$, however, is bi-directional (Fig. 1b). As precursors to SOC, microbial biomass $\delta^{13}C$ signatures have the potential to contribute to changes in $\delta^{13}C_{SOC}$. Given the fungi:bacteria ratio (F:B) decreases with depth in most soils (e.g. Schnecker et al. 2015), an increase in the $\delta^{13}C$ of microbial necromass inputs to SOC from shallow horizons dominated by potentially ^{13}C depleted fungi to deep horizons dominated by potentially ^{13}C enriched bacterial necromass could, for example, represent a further mechanism for the increase in $\delta^{13}C_{SOC}$ with depth. Fungi and bacteria often fulfill different roles in soil biogeochemistry (e.g. Strickland and Rousk 2010) and may generate chemically and perhaps isotopically distinct biomass and therefore necromass (Schimel and Schaeffer 2012). Such distinct signatures may occur due to the utilization of different substrates (e.g. Baum et al. 2009) or distinct metabolic fractionations (Hayes 1993). Although potentially useful in tracing contributions of necromass to soil, it remains unclear whether distinct biomass isotopic signatures exist between broadly defined soil microbial groups (e.g. fungi and bacteria). Our knowledge about the variations in $\delta^{13}C$ of soil microorganisms so far remains largely limited to the isotopic signatures of the microbial biomass as a whole, as analyzed after chloroform fumigation extraction, without differentiation between microbial groups (e.g. Dijkstra et al. 2006; Werth and Kuzyakov 2010).

Phospholipid fatty acids (PLFA) have long been analyzed as a proxy for the abundance, composition and $\delta^{13}C$ of microbial biomass in soils (Frostegård et al. 2010). Some PLFA predominantly occur in specific functional or operationally defined groups of microorganisms (e.g. fungi, Gram positive-, Gram negative- or Actinobacteria) and allow for the measurement of the $\delta^{13}C$ of these groups (Ruess and Chamberlain 2010 and references therein). Other PLFA are produced by a broad range of microorganisms and therefore report an integrated $\delta^{13}C$ of the microbial community as a whole (e.g. Cifuentes and Salata 2001). The application of $\delta^{13}C_{PLFA}$ in natural abundance studies so far remains largely limited to case studies where major contrasts in the isotopic

composition of potential substrates occur naturally (e.g. methanotrophy; Cifuentes and Salata 2001) or were experimentally introduced (e.g. C3/C4 crop change experiments; Kramer and Gleixner 2006; or free-air in situ CO_2 enrichment (FACE) experiments; Billings and Ziegler 2008; Streit et al. 2014). Consequently, few studies have investigated the natural variations of $\delta^{13}C_{PLFA}$ to assess potential variation in microbial $\delta^{13}C$ (Baum et al. 2009; Cusack et al. 2011; Churchland et al. 2013).

Here, we report the variation in $\delta^{13}C$ values of individual PLFA within soil profiles characterized by steep decreases in F:B ratios and increases bulk $\delta^{13}C_{SOC}$ with depth. We invoked variation in $\delta^{13}C_{PLFA}$ values within profiles to test the hypothesis that the enrichment of ^{13}C in bacterial biomass relative to fungal biomass drives an increase of the overall $\delta^{13}C$ of soil microbial biomass with depth, representing a further mechanism contributing to the increasing bulk $\delta^{13}C_{SOC}$ with depth. If in fact the biomass of these two groups were consistently distinct in their $\delta^{13}C$ we would expect the $\delta^{13}C$ of 'general' PLFA—derived from both bacteria and fungi—to be negatively correlated to the F:B ratio, and increase with depth. To attribute such a trend in general PLFA $\delta^{13}C$ to an enrichment of ^{13}C in bacterial relative to fungal biomass, the $\delta^{13}C$ of 'specific' PLFA—derived from either fungi only or bacteria only—would not only need to be distinct from each other but remain invariant with depth relative to the $\delta^{13}C$ of general PLFA.

Methods

All field sampling was conducted in June 2011. Field sites were located in the end member regions of a latitudinal transect of mesic boreal forests in Atlantic Canada (Newfoundland and Labrador Boreal Ecosystem Latitudinal Transect; NL-BELT) in south-western Newfoundland and southern Labrador. In each region, three locations ('sites') were selected for similarity in vegetation, stand type, class and age, and elevation (Online Resource 1). Within each site, three soil profiles were collected at random locations within a 10 m diameter circular as described in Laganière et al. (2015). For each profile, two adjacent samples with an area of 10 × 10 cm were cut out of the organic layer. After removal of all live vegetation, organic layer samples were immediately separated into L, F, and H horizons (corresponding to the O_i, O_e, and O_a subhorizons in the U.S. soil nomenclature). Where present, the eluviated A horizon was removed and two 10 cm long cylindrical soil cores from the B horizon were taken using a 5.1 cm diameter corer. L, F, H, and B horizon samples were pooled to the plot level in the field resulting in two paired sets of 24 composite samples (2 regions, 3 sites, 4 horizons), which were designated for chemical analysis and PLFA extraction.

Bulk $\delta^{13}C_{SOC}$ values were analyzed by elemental analysis/isotope ratio mass spectroscopy (EA/IRMS). PLFA were extracted by an optimized protocol based on Ziegler et al. (2013). The recovery of PLFA was determined in each extraction batch through duplicate extractions of samples from the F and B horizons with one replicate spiked with 130 µg di-17:0-phosphatidylcholine as described in Ziegler et al. (2013). We recovered ≥73 % of this phospholipid with no significant differences between soil horizons or extraction batches. PLFA were quantified by gas chromatography/flame ionization detection (GC/FID), compound specific stable isotope ratios were measured by gas chromatography–combustion–isotope ratio mass spectroscopy (GC/IRMS), and peak identities were confirmed by gas chromatography/mass spectroscopy (GC/MS) analysis of selected samples. A detailed description of our analytical methods is provided in Online Resource 2.

We detected a total of 45 individual PLFA which were assigned to microbial groups based on Ruess and Chamberlain (2010). Briefly, we assigned 18:2ω6,9 and 18:3ω3,6,9 to fungi and branched PLFA, monounsaturated PLFA (except $18{:}1\omega9_{cis}$), cyclopropylated PLFA, and straight chained PLFA with an uneven number of C atoms to bacteria. A complete list of PLFA and their assignment to microbial groups is provided in Online Resource 3. Based on this assignment we calculated the ratio of mol% fungal to mol% bacterial PLFA, which we also transformed to fungal/(fungal + bacterial) PLFA in representation of a two-pool mixing model. For a multivariate analysis of the composition of PLFA we conducted a non-metric multi-dimensional scaling (NMDS) based on Bray–Curtis distances of the mol% of each PLFA in each sample to visualize how community structure varies with soil horizon and region. Based on this ordination, we then calculated linear fittings for the mol% of the sum of fungal or bacterial PLFA using the function 'envfit' in vegan (see below).

Carbon isotopic signatures were analyzed for nine individual PLFA that were properly separated and sufficiently abundant in all samples. Seven of the nine PLFA were specific to broad microbial groups including fungi (18:2ω6,9) or bacteria. Among bacteria, i15:0 and a15:0 are specific to Gram positive bacteria, while 16:1ω7, 18:1ω7, cy17:0, and cy19:0 occur predominantly but not exclusively in Gram negative bacteria (Grogan and Cronan 1997). The other two PLFA were general biomarkers produced by both fungi and bacteria (16:0 and 18:1ω9$_{cis}$; Ruess and Chamberlain 2010; Frostegård et al. 2010). In addition, we determined $\delta^{13}C$ values of the fungal PLFA 18:3ω3,6,9 in the fungi-rich L horizons but not in deeper soil horizons, where low concentration of this PLFA inhibited reliable measurements. Together, these 9 or 10 PLFA made up 61–74 mol% of the total PLFA content of the samples.

Preliminary analysis showed that the differences in $\delta^{13}C_{PLFA}$ between the two study regions (typically < 1 ‰) were minor compared to differences among soils horizons or among microbial groups. We therefore tested for significant differences among soil horizons in the $\delta^{13}C_{PLFA}$ of each individual PLFA using a mixed effect model with 'soil horizon' as a fixed effect and 'site' as a random effect followed by pair-wise comparison of soil horizons with Tukey HSD post hoc tests as described by Hothorn et al. (2008). The same analysis was conducted for SOC normalized $\delta^{13}C_{PLFA}$ values (i.e. $\Delta^{13}C_{PLFA-SOC}$) to test whether PLFA were more or less enriched relative to the surrounding bulk SOC in the different soil horizons. Furthermore, we tested whether $\delta^{13}C_{PLFA}$ co-varied with $\delta^{13}C_{SOC}$ by calculating the correlation coefficients (R) between these two measures for each individual PLFA. Because of the relatively low $\delta^{13}C_{PLFA}$ in L horizon samples, we repeated the analysis excluding the L horizon samples to further test whether $\delta^{13}C_{PLFA}$ was correlated with $\delta^{13}C_{SOC}$ in the deeper soil horizons. All statistical analyses were performed with the software and statistical computing environment R (Version 3.0.2) using the packages 'nlme' (Version 3.1–113), 'multcomp' (Version 1.3–7), and 'vegan' (Version 2.0–7). All results are stated as means of six field sites ± one standard deviation unless indicated otherwise.

Results

The soil horizons contained a highly stratified microbial community as characterized by proportions of fungal and other eukaryotic PLFA decreasing with depth and proportions of bacterial PLFA increasing with depth. The overall PLFA concentration varied little relative to bulk SOC among soil horizons (3.5 ± 0.4 to 3.8 ± 0.5 μmol g^{-1} SOC; Online Resource 4). The concentration of fungal PLFA decreased relative to bulk SOC from 0.97 ± 0.11 μmol g^{-1} SOC in L horizons to 0.075 ± 0.013 μmol g^{-1} SOC in B horizons. In contrast, concentrations of bacterial PLFA increased from 1.18 ± 0.14 μmol g^{-1} SOC in L

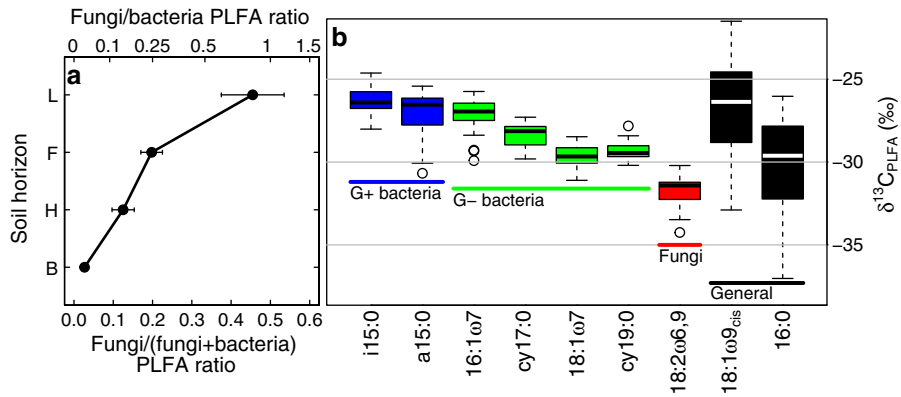

Fig. 2 Depth profile of the molar ratio of fungal to bacterial phospholipid fatty acids (PLFA). The plotted values represent the mean of 6 field sites with *error bars* indicating one standard deviation of the mean (**a**). Overall range of the $\delta^{13}C$ of individual PLFA in the organic (LFH) and mineral (B) horizons of boreal forest soils studied (n = 24) (**b**). Note the higher variance in the $\delta^{13}C$ of general PLFA (*black*) compared to PLFA specific to an individual microbial group (*in color*) and the consistent enrichment in ^{13}C in bacterial PLFA relative to fungal PLFA

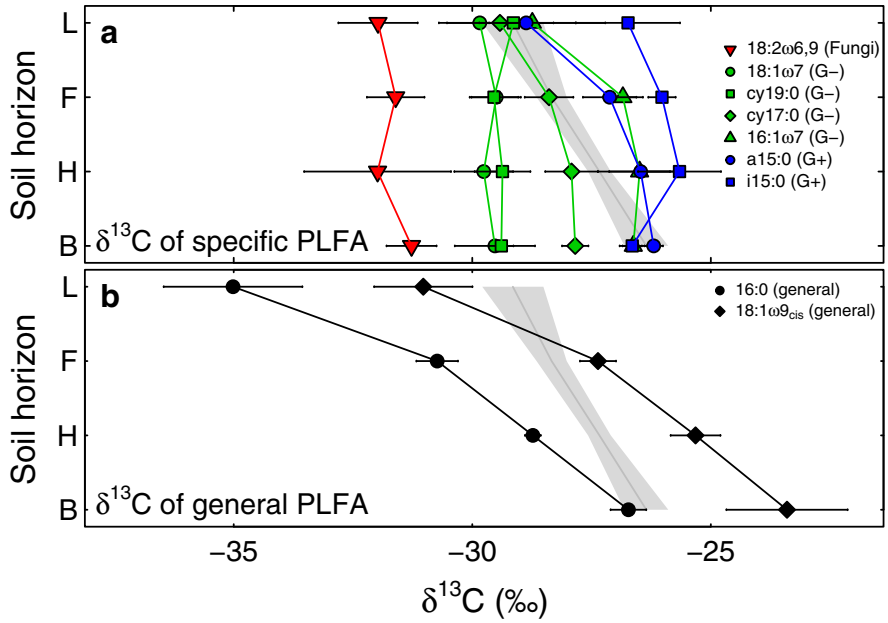

Fig. 3 Within-profile variation of the $\delta^{13}C$ of phospholipid fatty acids (PLFA) in boreal forest soils. $\delta^{13}C$ profiles are presented for the 7 PLFA specific to Gram positive bacteria (G+), Gram negative bacteria (G−), or fungi (**a**); and for 2 general PLFA produced by both, fungi and bacteria (**b**). The $\delta^{13}C$ of bulk soil organic carbon (SOC) printed in the background (*grey line*) for comparison. All *values* are given as the mean of six field sites with *error bars* and *shaded areas* indicating one standard deviation of the mean

horizons to 2.84 ± 0.36 μmol g^{-1} SOC in B horizons. The ratio of fungal to bacterial PLFA therefore decreased from 0.82 ± 0.10 in the L horizon to 0.027 ± 0.002 in the B horizon, corresponding to fungal/(fungal + bacterial) ratios of 0.45 ± 0.03 and 0.027 ± 0.002, respectively (Fig. 2a). Neither PLFA concentration nor PLFA composition (mol% fungi or bacteria) exhibited a significant difference between the two regions. Multivariate analysis (NMDS) resulted in samples clustering by soil horizon, suggesting that microbial community composition varied strongly between soil horizon but little between the studied regions (Online Resource 5).

Carbon isotopic signatures of bulk SOC increased with depth from −29.2 ± 0.3 ‰ in L horizons to −26.4 ± 0.2 ‰ in B horizons. $\delta^{13}C_{PLFA}$ was more variable relative to the SOC and ranged from −40.1 to −22.4 ‰ (Fig. 2b), varying by 8–10 ‰ among individual PLFA within a sample. The $\delta^{13}C$ of general and group-specific PLFA exhibited contrasting patterns (Fig. 3). Total variation in $\delta^{13}C$ among *specific* PLFA, that is individual PLFA produced by only one microbial group (i15:0, a15:0, 16:1ω7, cy17:0, 18:1ω7, cy19:0, and 18:2ω6,9), was captured by variation in the $\delta^{13}C$ of PLFA produced by Gram positive bacteria (G+) and fungi (Figs. 2b, 3a). In all samples, PLFA produced by fungi (18:2ω6,9 and 18:3ω3,6,9) exhibited the lowest $\delta^{13}C$ values while those PLFA produced by G + bacteria (i15:0 and a15:0) were highest. In contrast to this range in $\delta^{13}C$ among PLFA by source organism, the $\delta^{13}C$ of each specific PLFA showed remarkably little variation with depth (Fig. 3a) or within the entire dataset (Fig. 2b).

If the bulk soil substrate $\delta^{13}C$ had any influence on microbial biomarker $\delta^{13}C$ the $\delta^{13}C_{PLFA}$ would have increased with depth in parallel with $\delta^{13}C_{SOC}$. However, 4 of the 7 specific PLFA (i15:0, 18:1ω7, cy19:0, 18:2ω6) exhibited no significant difference in $\delta^{13}C$ among soil horizons. The other 3 specific PLFA (a15:0, 16:1ω7, cy17:0) varied in $\delta^{13}C$ by horizon and exhibited a significant correlation between $\delta^{13}C_{PLFA}$ and $\delta^{13}C_{SOC}$ ($R = 0.71–0.77$; $p < 0.001$), but these correlations were primarily driven by low $\delta^{13}C_{PLFA}$ values in the L horizons (Table 1) and were lost or largely diminished when L horizon samples were excluded (Online Resource 6). While the $\delta^{13}C$ of specific PLFA exhibited little difference between soil horizons in absolute terms, these PLFA were more ^{13}C-depleted relative to the surrounding bulk SOC at greater depth, as indicated by significantly lower $\Delta^{13}C_{PLFA–SOC}$ values in deeper soil horizons compared to more shallow horizons in 6 of 7 specific PLFA (Table 1), providing further evidence that $\delta^{13}C_{PLFA}$ signatures of group-specific PLFA were not directly affected by the increase of $\delta^{13}C_{SOC}$ with depth.

Unlike specific PLFA, *general* PLFA, that is PLFA produced by both bacteria and fungi (18:1ω9 and 16:0), varied by over 10 ‰ among samples (Fig. 2b) and

Table 1 Carbon isotopic signatures of 9 individual PLFA by soil horizon, presented both in absolute terms ($\delta^{13}C_{PLFA}$) and relative to the surrounding SOC ($\Delta^{13}C_{PLFA-SOC}$)

PLFA		Soil horizon				Soil horizon effect ($F_{3,15}$)[a]
		L	F	H	B	
i15:0	$\delta^{13}C_{PLFA}$	−26.7 (1.1)	−26.0 (0.3)	−25.7 (0.9)	−26.6 (0.2)	3.1
(G+ bacteria)	$\Delta^{13}C_{PLFA-SOC}$	2.4 (1.0)[a]	2.3 (0.3)[a]	1.7 (0.9)[a]	−0.3 (0.5)[b]	**19.6*****
a15:0	$\delta^{13}C_{PLFA}$	−28.9 (1.7)[a]	−27.1 (0.6)[b]	−26.5 (0.9)[b]	−26.2 (0.2)[b]	**11.9*****
(G+ bacteria)	$\Delta^{13}C_{PLFA-SOC}$	0.3 (1.4)	1.2 (0.5)	0.9 (0.9)	0.2 (0.6)	2.3
16:1ω7	$\delta^{13}C_{PLFA}$	−28.7 (0.9)[a]	−26.8 (0.4)[b]	−26.5 (0.8)[b]	−26.6 (0.3)[b]	**30.9*****
(G− bacteria)	$\Delta^{13}C_{PLFA-SOC}$	0.4 (0.7)[a]	1.5 (0.4)[b]	0.8 (0.6)[a]	−0.3 (0.6)[c]	**16.7*****
cy17:0	$\delta^{13}C_{PLFA}$	−29.4 (0.4)[a]	−28.4 (0.5)[b]	−27.9 (0.6)[b]	−27.8 (0.3)[b]	**16.2*****
(G− bacteria)	$\Delta^{13}C_{PLFA-SOC}$	−0.3 (0.7)[a]	−0.1 (0.3)[a]	−0.6 (0.6)[a]	−1.5 (0.4)[b]	**8.9*****
18:1ω7	$\delta^{13}C_{PLFA}$	−29.8 (0.9)	−29.5 (0.5)	−29.8 (0.6)	−29.5 (0.8)	0.4
(G− bacteria)	$\Delta^{13}C_{PLFA-SOC}$	−0.7 (0.3)[a]	−1.2 (0.5)[a]	−2.4 (0.6)[b]	−3.2 (0.6)[c]	**29.0*****
cy19:0	$\delta^{13}C_{PLFA}$	−29.1 (0.8)	−29.5 (0.5)	−29.4 (0.6)	−29.4 (0.3)	0.9
(G− bacteria)	$\Delta^{13}C_{PLFA-SOC}$	0.0 (0.5)[a]	−1.2 (0.3)[b]	−2.0 (0.5)[c]	−3.0 (0.4)[d]	**83.7*****
18:2ω6,9	$\delta^{13}C_{PLFA}$	−32.0 (0.8)	−31.6 (0.6)	−31.0 (1.5)	−31.3 (0.5)	1.3
(Fungi)	$\Delta^{13}C_{PLFA-SOC}$	−2.8 (0.6)[a]	−3.3 (0.6)[a]	−4.7 (1.6)[b]	−4.9 (0.5)[b]	**11.5****
18:3ω3,6,9[b]	$\delta^{13}C_{PLFA}$	−37.2 (1.8)	–	–	–	–
(Fungi)	$\Delta^{13}C_{PLFA-SOC}$	−8.0 (1.5)	–	–	–	–
16:0	$\delta^{13}C_{PLFA}$	−35.0 (1.5)[a]	−30.7 (0.5)[b]	−28.7 (0.2)[c]	−26.7 (0.4)[d]	**176.1*****
(General)	$\Delta^{13}C_{PLFA-SOC}$	−5.9 (1.2)[a]	−2.4 (0.4)[b]	−1.4 (0.4)[bc]	−0.4 (0.6)[d]	**148.8*****
18:1ω9	$\delta^{13}C_{PLFA}$	−31.0 (1.0)[a]	−27.4 (0.4)[b]	−25.3 (0.5)[c]	−23.4 (1.2)[d]	**129.0*****
(General)	$\Delta^{13}C_{PLFA-SOC}$	−1.9 (1.2)[a]	1.0 (0.5)[b]	2.0 (0.6)[bc]	2.9 (1.5)[c]	**33.8*****

The presented values are given as the average among six field sites with the standard deviations of these averages provided in brackets. Different superscript letters indicate significant differences between soil horizons across of region (Tukey HSD)

Furthermore, test metric (F) and significance level (*** $p < 0.001$; ** $p < 0.01$, * $p < 0.05$; n.s., $p > 0.05$) for soil horizon effect on PLFA isotopic signatures

Bold letters indicate a significat difference among soil horizons ($p < 0.05$)

[a] Soil horizon effects on $\delta^{13}C_{PLFA}$ and $\Delta^{13}C_{PLFA-SOC}$ were tested in mixed effects model with soil horizon as a fixed effect and site as a random effect

[b] $\delta^{13}C$ values of 18:3ω3,6,9 could not be analyzed in F, H, and B horizons due to the low concentrations of this PLFA in these horizons

became more enriched in ^{13}C with depth, both in absolute terms and relative to bulk SOC (Table 1; Fig. 3b). The increase in $\delta^{13}C$ of general PLFA with depth and lack of such change in any group-specific PLFA (fungal or bacterial) was exhibited in all soil profiles analysed (Online Resource 7). The $\delta^{13}C$ of general PLFA co-varied with the proportion of fungal versus bacterial PLFA given as the ratio fungal/(fungal + bacterial) PLFA ($R = -0.96$ and -0.94 for 16:0 and 18:1ω9$_{cis}$; $p < 0.001$; Fig. 4a) consistent with the idea that $\delta^{13}C$ of these general PLFA was largely attributed to the proportion of fungi to bacteria PLFA present in the soil and these groups' distinct $\delta^{13}C$ signatures (Figs. 3b). No such relationship with fungal/(fungal + bacterial) PLFA was observed for any specific PLFA (Fig. 4b). The $\delta^{13}C$ of general PLFA co-varied with $\delta^{13}C_{SOC}$ ($R = 0.87$–0.92, $p < 0.001$; Online Resource 6), but slopes of $\delta^{13}C_{PLFA}/\delta^{13}C_{SOC}$ were significantly greater than one (2.57 ± 0.23 (standard error) and 2.27 ± 0.27 for 16:0 and 18:1ω9$_{cis}$; $t = 6.56$ and 4.67; both $p < 0.001$; $n = 24$) indicating that the $\delta^{13}C$ of general PLFA exhibited a much steeper increase with depth than $\delta^{13}C_{SOC}$ (Fig. 3b).

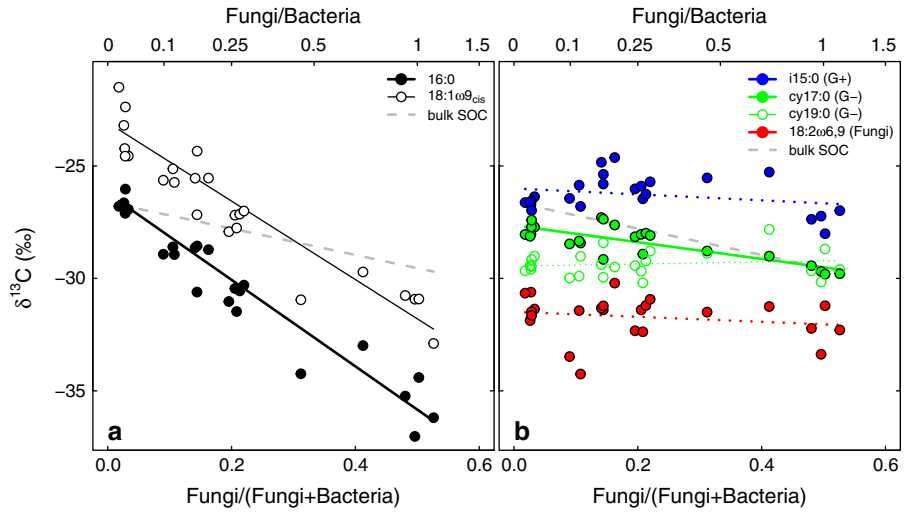

Fig. 4 Correlation between $\delta^{13}C$ of the two general PLFA (**a**) and four group specific PLFA (**b**) with the ratio of fungal/(fungal + bacterial) PLFA. *Solid lines* indicate linear regressions where significant correlations were detected; *dotted lines* indicate linear fits where no significant correlation was found. The *dashed grey lines* indicate the linear regression between $\delta^{13}C_{SOC}$ and fungal/(fungal + bacterial) PLFA

Discussion

Fungal biomass is depleted in ^{13}C relative to bacterial biomass

Our data suggest that fungal PLFA in these soils are depleted in ^{13}C relative to bacterial PLFA. This is consistent with most previous studies from which we could retrieve such data demonstrating key fungal PLFA (typically 18:2ω6,9 as used here) are typically deplete in ^{13}C relative to bacterial PLFA (Table 2a). One noteworthy exception to this generally consistent pattern was one study that found fungal PLFA enriched in ^{13}C relative to bacterial PLFA in soils from a glacial forefield chronosequence (Esperschütz et al. 2011). The lower $\delta^{13}C$ values observed in fungal PLFA in the current study are not an artefact of fungal PLFA selection; indeed, 18:2ω6,9 is generally enriched in ^{13}C relative to other fungal PLFA and fungal biomass in culture (−0.3 to +2.5 ‰; Abraham and Hesse 2003). Furthermore we observed highly ^{13}C depleted values for the second fungal PLFA 18:3ω3,6,9 analyzed in the L horizon soils across sites where it was abundant enough to analyze (Table 1).

The increasing trend with depth in $\delta^{13}C_{PLFA}$ produced by both fungi and bacteria (16:0 and 18:1ω9$_{cis}$) further supports our observation of the ^{13}C depletion of fungal relative to bacterial PLFA in these soils. The depth trend in these two PLFA was best explained by the proportion of fungal to bacterial PLFA abundance in these soils (Fig. 4a). The negative correlation between soil F:B ratio and the $\delta^{13}C$ of 16:0 and 18:1ω9$_{cis}$ implies that in a given sample the same individual PLFA was more enriched in ^{13}C when produced by bacteria than when produced by fungi. Because we found that the $\delta^{13}C$ of PLFA indicative of fungi or bacteria exhibit little to no variation with depth, the increase in $\delta^{13}C$ of 16:0 and 18:1ω9$_{cis}$ could not be explained by changes in the $\delta^{13}C$ of one or the other of these groups if, for example, shifts in community structure or substrate use with depth had an important influence on the $\delta^{13}C$ of microbial biomass.

The enrichment of ^{13}C of bacterial relative to fungal PLFA is a result of differences in biomass $\delta^{13}C$ between these two groups, and not because of a stronger discrimination against ^{13}C during fatty acid biosynthesis ($\Delta^{13}C_{PLFA\text{-}biomass} = \delta^{13}C_{PLFA}\text{-}\delta^{13}C_{biomass}$) in fungi relative to bacteria. Fatty acids were consistently depleted in ^{13}C by 2–4 ‰ relative to bulk biomass in both fungal and bacterial cultures, which is generally attributed to the fractionation associated with decarboxylation of pyruvate to acetyl-CoA, the main building block for fatty acids (DeNiro and Epstein 1977; Hayes 2001). Congruent with this, available literature values (presented for 16:0 in Table 2b) do not

Table 2 Compiled literature values of $\delta^{13}C_{PLFA}$ expressed as the difference in $\delta^{13}C$ ($\Delta^{13}C$) among (a) fungal and bacterial PLFA within soil samples and (b) among PLFA and bulk biomass in cultures of fungi and heterotrophic bacteria. The differences in the $\delta^{13}C$ derived from the weighted mean of all PLFA attributed to the groups (e.g. bacteria, Gram negative bacteria, Gram positive bacteria, fungi) is also provided in each case from the data provided in this study and Churchland et al. (2013). References for this table are provided in Online Resource 8

PLFA	Reference	Range ($\Delta^{13}C$)	Ecosystem/strain	Comments
(a) Fungal (18:2ω6,9) versus bacterial PLFA in soil samples				
All bacteria		Bacteria-fungi (‰)		
Weighted mean	This study	2.4 to 4.3	Boreal forest soil	
	Churchland et al. (2013)	1.2 to 2.2	Temperate forest soil	Clearcut > forested
Gram positive bacteria		Bacteria-fungi (‰)		
Weighted mean	This study	4.3 to 6.4	Boreal forest soil	
i15:0-18:2ω6,9	Kramer and Gleixner (2006)	7.6	Agricultural soil	
	This study	3.2 to 7.5	Boreal forest soil	
	Steinbeiss et al. (2009)	3.3 to 6.6	Agricultural & forest soil	
	Streit et al. (2014)	2.6 to 5.1	Alpine forest soil	Inter-annual difference
	Billings and Ziegler (2008)	3.7 to 3.8	Temperate forest soil	
	Baum et al. (2009)	−1.5 to 5.3	Temperate forest soil	Before litterfall > after litterfall
	Esperschütz et al. (2011)	−3.5 to 1.6	Glacier forefield	
Gram negative bacteria		Bacteria-fungi (‰)		
Weighted mean	This study	2.1 to 3.8	Boreal forest soil	
16:1ω7-18:2ω6,9	Baum et al. (2009)	2.1 to 10.1	Temperate forest soil	Before litterfall > after litterfall
	Kramer and Gleixner (2006)	6.4	Agricultural soil	
	Streit et al. (2014)	2.8 to 6.8	Alpine forest soil	Inter-annual difference
	Billings and Ziegler (2008)	4.0 to 5.1	Temperate forest soil	Fertilized > control
	This study	2.8 to 7.1	Boreal forest soil	
	Steinbeiss et al. (2009)	2.1 to 5.5	Agricultural & forest soil	
	Esperschütz et al. (2011)	−3.4 to −0.3	Glacier forefield	

Table 2 continued

PLFA	Reference	Range ($\Delta^{13}C$)	Ecosystem/strain	Comments
(b) PLFA versus bulk biomass in cultures				
Fungi		PLFA-biomass (‰)		
16:0	Abraham et al. (1998)	−3.7 to +2.4	Fusarium solani	Four substrates
	Abraham and Hesse (2003)	−1.0 to +2.4	4 fungal species	Four substrates
	Cowie et al. (2009)	−2.9 to +3.1	Acremonium sp.	Heterotrophic enriched culture from acid mine drainage
18:2ω6,9	Abraham and Hesse (2003)	−0.2 to +2.5	Fusarium solani	Four substrates
	Cowie et al. (2009)	−3.1 to +0.8	Acremonium sp.	Heterotrophic enriched culture from acid mine drainage
Heterotrophic bacteria		PLFA-biomass (‰)		
16:0	Londry and Marais (2003)[ab]	−16 to +3	4 sulfate reducing bacteria	Substrate: acetate or lactate
	Zhang et al. (2003)[ab]	−14.2 to −5.6	2 Fe(III) reducing species	Substrate: acetate or lactate
	Teece et al. (1999)[ac]	−10.3	Shewanella putrefaciencs	Substrate: lactate (anaerobic)
	L. Kohl, unpublished	−3.0 to −0.8	Pseudomonas fluorescence	Substrate: cellobiose
	Teece et al. (1999)[b]	−2.0	Shewanella putrefaciencs	Substrate: lactate (aerobic)
	Wick et al. (2003)	−0.7	Mycobacterium sp.	Substrate: glucose
	Zhang et al. (2002)[a]	+0.1 to +0.8	Thermatoga maritima	Substrate: glucose
	Wick et al. (2003)	+5.3	Mycobacterium sp.	Substrate: anthracene
All fatty acids	Blair et al. (1985)[c]	−2.5	Escherichia coli	Substrate: glucose
	Monson and Hayes (1982)[c]	−3.2	Escherichia coli	Substrate: glucose

[a] The microbial cultures in this study were grown under anaerobic conditions, which can cause a much larger depletion of ^{13}C in fatty acids compared bulk biomass than aerobic cultures (Teece et al. 1999)

[b] In these studies the highest values for $\Delta^{13}C_{\text{PLFA-biomass}}$ were found in cultures grown on acetate, which is not a common substrate in soil, and where a smaller depletion of ^{13}C in PLFA were ^{13}C enriched as expected due to the direct generation of fatty acids from acetyl-CoA as opposed to the production from pyruvate (Hayes 2001)

[c] These studies reported the $\delta^{13}C$ of fatty acids in the total lipid fraction. We report these studies because little data is available on the apparent fractionation between $\delta^{13}C$ PLFA and $\delta^{13}C$ biomass in bacteria, and the difference in $\delta^{13}C_{16:0}$ between PL and other lipid fractions is variable, but small on average (0.7 ‰; Abraham et al. 1998)

indicate any consistent difference in the fractionation of PLFA or total fatty acids relative to bulk microbial biomass between these two groups. In fungal cultures the $\delta^{13}C$ values of 16:0, 18:1ω9$_{cis}$, and 18:2ω6,9 typically fell within 2.5 ‰ of the bulk cell (Abraham and Hesse 2003). A similar but perhaps slightly greater fractionation between fatty acid and biomass has been observed in aerobic cultures of heterotrophic bacteria ($\Delta^{13}C_{PLFA\text{-}biomass} = -3$ to $+0.8$ ‰). A much larger fractionation ($\Delta^{13}C_{PLFA\text{-}biomass} = -14.7$ to -10.4 ‰), however, was found in bacteria that utilize the serine pathway which is active under anaerobic conditions (Teece et al. 1999). Together, the available data from culture studies suggests that if $\Delta^{13}C_{PLFA\text{-}biomass}$ differs between fungi and bacteria, the discrimination against ^{13}C in PLFA relative to biomass would be stronger in bacteria than in fungi. As such the $\delta^{13}C$ of bacterial biomass based upon their PLFA would lead to an underestimation of biomass $\delta^{13}C$ in bacteria relative to fungi, suggesting we could have underestimated the difference in the $\delta^{13}C_{biomass}$ of these two groups using these biomarkers in our study.

The consistent enrichment of ^{13}C in PLFA indicative of bacteria relative to those indicative of fungi in all horizons (Fig. 3a), across which substrate identity and microbial community structure varied, suggests that differences in $\delta^{13}C$ between fungal and bacterial biomass are driven by fundamental differences in metabolisms of these microbial groups. For example, fungi and bacteria likely differ in how C is allocated into different classes of microbial compounds (e.g. microbial cell walls, extracellular enzymes and polysaccharides, osmolytic compounds; Schimel and Schaeffer, 2012). This is consistent with results from Abraham et al. (1998), who reported that when grown on the same substrate, fungal PLFA 16:0 was depleted by 1.1–6.0 ‰ compared to bacterial 16:0. Results from the current study suggest that investigation into the mechanisms that may lead to differences in soil microbial $\delta^{13}C$ from natural environments are warranted given the potential significance of exploiting these signatures to further link bacterial and fungal activity to soil C cycling.

Soil $\delta^{13}C_{PLFA}$ values are driven by their fungal or bacterial origin, not by $\delta^{13}C_{SOC}$

The constant $\delta^{13}C$ values of PLFA indicative of fungi or bacteria across all soil horizons show that though $\delta^{13}C_{PLFA}$ of individual strains grown in culture on a single C source can exhibit large variability (Abraham et al. 1998), $\delta^{13}C_{PLFA}$ may vary little in natural environments where diverse organic substrates are available to complex microbial communities. Similar observation have been made in marine estuarine sediments (Bouillon et al. 2006). Constancy of fungal and bacterial PLFA across depth further suggests that the $\delta^{13}C$ of fungal and bacterial biomass were in part decoupled from the increase of bulk $\delta^{13}C_{SOC}$ with depth. This is consistent with the constant $\delta^{13}C$ values of fungal mycelia observed between 0 and 30 cm depth in spruce and mixed forest soils (Wallander et al. 2004). The $\delta^{13}C$ of general PLFA produced by both fungi and bacteria, indicative of $\delta^{13}C_{biomass}$ of the soil microbial community as a whole, increased with depth but to a much larger extent (7.6–8.3 ‰ from L to B horizon) than bulk SOC (2.8 ‰; Fig. 3b). In spite of the correlation between the $\delta^{13}C$ of general PLFA and $\delta^{13}C_{SOC}$, the variation in the $\delta^{13}C$ of general PLFA could not have been driven by $\delta^{13}C_{SOC}$. If that were the case, we would have observed increased $\delta^{13}C$ values in deeper soil horizons not only in general PLFA, but also in PLFA specific to one or more microbial groups. More likely, the increased $\delta^{13}C$ of general PLFA with depth was driven by a greater bacterial relative to fungal source of these PLFA in deeper horizons, as fungi:bacteria ratios decreased by over an order of magnitude from L to B horizons and were highly correlated with the $\delta^{13}C$ of general PLFA as discussed above.

While fungi and bacteria might consume distinct substrates with different isotopic signatures, the lack of a soil horizon effect on the $\delta^{13}C$ of fungal and bacterial biomass observed here suggests that the substrates consumed by each group of soil microorganisms had similar isotopic signatures across all soil horizons. Bulk SOM therefore is a poor representative of the substrates actually taken up by soil microorganisms in situ. Our data thus suggest that isotopic signatures of soil microorganisms are driven by substrates of common $\delta^{13}C$, found throughout soil horizons, rather than by bulk $\delta^{13}C_{SOC}$ signature of the soil horizon within which the microorganisms reside. This is feasible if the same substrate, with a constant $\delta^{13}C$ signature, were available for microbial uptake in all soil horizons. Indeed, plant transfer of photosynthate to mycorrhizal fungi likely represents such a substrate source, as do root exudates and fine root litter

(Wallander et al. 2004). A mobile SOC fraction percolating through horizons also could provide such a substrate source, and our field sites are characterized by relatively high precipitation and exhibit significant vertical DOC fluxes from the organic to the mineral horizons (10–30 g C m^{-2} $year^{-1}$; Edwards unpublished data). This is congruent with reports that in situ heterotrophic soil respiration can be derived from modern C even in deep mineral soil horizons containing centuries old SOC and even when root inputs were excluded (Phillips et al. 2013). Substrate transport across soil horizons can also occur via fungal mycelia (Strickland and Rousk 2010) and perhaps via actinobacterial mycelia, but this mechanism is unlikely in the B horizon where we detected only marginal amounts of fungal or actinobacterial PLFA.

Increased necromass $\delta^{13}C$ driven by fungal:bacterial ratios may contribute to increased $\delta^{13}C_{SOC}$ with depth

The difference in $\delta^{13}C$ between fungal and bacterial PLFA is a strong indicator that $\delta^{13}C$ of C pools influenced by microbial activity such as CO_2, DOC, and SOC are influenced by the identity of the microbial group contributing to the pool. The main trends observed in our study—the depletion of ^{13}C in fungal relative to bacterial biomass and the independence of specific $\delta^{13}C_{PLFA}$ from the increase of $\delta^{13}C_{SOC}$ with depth—have important implications for our conceptual understanding of soil C fluxes as well as the origin of SOC and its variation in $\delta^{13}C$ (Fig. 1). Ex situ laboratory incubation experiments, which dominate the study of microbial substrate use patterns, rely on the assumption that soil microorganisms primarily consume SOC that is located within 'their own' soil horizon (Fig. 1a). The reliance on this assumption occurs because other substrates available in situ are excluded in such experiments through the separation of soil horizons, pre-incubating soils to remove labile C sources liberated by the disturbance required for experimental setup, and exclusion of litter leachates or root exudates. Such experiments inevitably generate data supporting the idea that microbial biomass in deeper soil horizons is enriched in ^{13}C due to microbial access to and use of more ^{13}C enriched SOC with depth. In contrast, our in situ study suggests the $\delta^{13}C$ value of microbial biomass as a whole can be more enriched in ^{13}C in deeper soil horizons as a result of a shift in fungal to bacterial dominance—a feature common to many of the world's soils (e.g. Schnecker et al. 2015).

Deeper soil horizons are traditionally viewed as exhibiting a greater bulk $\delta^{13}C_{SOC}$ value due to the enrichment of ^{13}C during diagenesis, associated losses of ^{13}C-depleted compounds from the soil system (Fig. 1a, Nadelhoffer and Fry 1988; Nakamura et al. 1990) or increasing proportions of ^{13}C enriched compounds of microbial origin with depth (Boström et al. 2007). Our results imply that the gradual shift from fungal to bacterial dominance with increasing depth, and changes in the $\delta^{13}C$ of bulk necromass as a result of that shift, can contribute to the more ^{13}C enriched SOC in deeper soil horizons (Fig. 1b). The $\delta^{13}C$ of bulk SOC therefore would not only depend on the quantity of material of microbial origin in SOC, but the fungal or bacterial identity of its microbial precursors as well. Thus, the $\delta^{13}C$ of microbial biomass likely exerts some influence on the $\delta^{13}C$ of SOC with depth, rather than conversely.

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Conflict of interest The authors declare no conflict of interest.

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