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Increased belowground carbon inputs and warming promote loss of soil organic carbon through complementary microbial responses

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ABSTRACT

Current carbon cycle-climate models predict that future soil carbon storage will be determined by the balance between CO_2 fertilization and warming. However, it is uncertain whether greater carbon inputs to soils with elevated CO_2 will be sequestered, particularly since warming hastens soil carbon decomposition rates, and may alter the response of soils to new plant inputs. We studied the effects of elevated CO_2 and warming on microbial soil carbon decomposition processes using laboratory manipulations of carbon inputs and soil temperature. We incubated soils from the Aspen Free Air CO_2 Enrichment experiment, where no accumulation of soil carbon has been observed despite a decade of increased carbon inputs to soils under elevated CO_2. We added isotopically-labeled sucrose to these soils in the laboratory to mimic and trace the effects of increased carbon inputs on soil organic carbon decomposition and its temperature sensitivity. Sucrose additions caused a positive priming of soil organic carbon decomposition, demonstrated by increased respiration derived from soil carbon, increased microbial abundance, and a shift in the microbial community towards faster growing microorganisms. Similar patterns were observed for elevated CO_2 soils, suggesting that the priming effect was responsible for reductions in soil carbon accumulation at the site. Laboratory warming accelerated the rate of the priming effect, but the magnitude of the priming effect was not different amongst temperatures, suggesting that the priming effect was limited by substrate availability, not soil temperature. No changes in substrate use efficiency were observed with elevated CO_2 or warming. The stimulatory effects of warming on the priming effect suggest that increased belowground carbon inputs from CO_2 fertilization are not likely to be stored in mineral soils.

1. Introduction

The terrestrial carbon (C) cycle regulates atmospheric CO_2 concentrations through a balance between photosynthetic uptake and respiratory release from plants and plant residues sequestered in soils. Rising atmospheric CO_2 and concomitant global warming are likely to alter this balance by modifying the rates of these uptake and release processes, with unknown implications for long-term soil C storage. Higher atmospheric CO_2 concentrations fertilize plant C uptake, resulting in greater plant productivity that is transferred to soils in the form of increased litterfall, root biomass, and root exudation (Liu et al., 2005; Norby et al., 2005; Pregitzer et al., 2008; Phillips et al., 2011). However, it is unclear whether these enhanced inputs increase soil C storage (Norby and Zak, 2011), because elevated CO_2 also stimulates respiratory losses of C from plant tissues and soils (Drake et al., 2011).

In addition, climate warming is likely to erode stores of soil organic carbon (SOC) by increasing decomposition rates (Hopkins et al., 2012); however, the warming effect may be limited by the amount of substrate available for decomposition (Melillo et al., 2002). Through its interaction with substrate availability, warming has the potential to alter the response of soils to additional plant inputs in a high CO_2 world. Ecosystem-scale manipulations of CO_2

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and temperature have shown that the combination of these two drivers has a different effect on SOC turnover rates and soil microbial community composition than either treatment alone (Gray et al., 2011; Gutknecht et al., 2012; Nie et al., 2012).

Prior to ecosystem-scale experimental tests, it was predicted that additional C taken up by forests under elevated CO_2 would be stored in soils (e.g., Harrison et al., 1993); however, data from the Free Air CO_2 Enrichment (FACE) experiments have shown a mixed response (de Graaff et al., 2006). Despite consistent stimulation of plant productivity across forested FACE sites (Norby et al., 2005), increased C allocation belowground under elevated CO_2 has actually resulted in less accrual of C to soil stores in some experiments (e.g., Talhelm et al., 2009).

Given higher litter inputs under elevated CO_2 (eCO_2), observed reductions in SOC formation can only be explained by decreased retention of new C inputs to soil, or an acceleration of SOC decomposition rates. Specifically, eCO_2 may increase the fraction of C inputs lost from soils through respiration by changing the efficiency of microbial processes, such as the proportion of C allocated to respiration vs. growth (Ziegler and Billings, 2011). In contrast, eCO_2 may increase decomposition outputs from SOC via the rhizosphere priming effect, whereby additions of easily degradable C exuded by roots stimulates microbial activity and results in greater SOC turnover (Kuzyakov et al., 2000; Carney et al., 2007; Cheng et al., 2013). Higher root exudation rates have been observed under eCO_2 (Phillips et al., 2011), as have increased soil respiration rates (Pregitzer et al., 2006); however, the many sources of soil respiration make the detection of the priming effect in an intact ecosystem extremely challenging (Hopkins et al., 2013; Phillips et al., 2013). Nevertheless, both explanations— reduced microbial efficiency and rhizosphere priming— invoke changes in microbial metabolism as drivers for reductions in SOC under eCO_2; thus, better understanding of the microbial drivers of SOC decomposition is needed to assess the effect of CO_2 fertilization on soil C storage (Billings et al., 2010).

The effect of climate warming on the C balance of soils is also mediated by microbial decomposition— warming rapidly stimulates microbial metabolism, and results in nearly instantaneous increases in microbial respiration (Dijkstra et al., 2011). In the long term, however, warming may hasten substrate limitation for microorganisms (e.g., Melillo et al., 2002—field; Fissore et al., 2008—laboratory), and alter the temperature response of microbial respiration rates (Thiessen et al., 2013). It remains unclear whether observed decreases in the temperature sensitivity of microbial respiration over the course of long-term soil warming experiments is due to the direct effect of temperature on microbial physiology, such as through reduced substrate use efficiency (Bradford et al., 2008), or whether the decrease is owed to indirect effects of warming on microbial substrate supply (Dungait et al., 2012).

In this study, we combine short-term manipulations of temperature and substrate supply in a laboratory incubation experiment of soils from a decade-long CO_2 fertilization experiment. We used a combination of C isotope labels, respiration measurements, and microbial biomarker analysis to study the response of microbial processes to eCO_2 and warming. Soils were taken from the Aspen FACE experiment, where eCO_2 exposure had altered the amount and ^{13}C and ^{14}C isotope signature of plant-derived C inputs to soils for more than 10 years. In the laboratory, we warmed soils and added isotopically-labeled sucrose to mimic root exudation, further enabling us to track incorporation of new C inputs, and to monitor the effects of changing substrate availability on the temperature response of respiration. Our goal was to determine how global change effects on microbial community composition and activity might affect the decomposition process, and in turn, the fate of soil C stores in the future.

We evaluated the plausibility of a rhizosphere priming effect in eCO_2 soils by adding isotopically-labeled sucrose to soils in the laboratory. Sucrose and its monomers are a common component of root exudate (Grayston et al., 1996) that can induce priming effects (de Graaff et al., 2010), and are readily available to most heterotrophic soil organisms (Killham and Prosser, 2007). We hypothesized that sucrose addition would induce a positive priming effect, exemplified by increases in respiration of soil-derived C and microbial abundance relative to soils receiving water alone. We also examined the effect of eCO_2 and sucrose addition on microbial community composition to determine whether they were consistent with a priming effect. We tracked incorporation and respiration of added sucrose as a measure of microbial function, which allowed us to determine the effect of eCO_2 on microbial substrate use. We hypothesized the eCO_2 soils would retain a lower proportion of new C inputs, demonstrated by less incorporation of the sucrose $\delta^{13}C$ label into microbial biomass per unit of CO_2 respired. We also investigated the relationship between substrate availability and the warming response by using respiration of added sucrose as a proxy for substrate availability. We monitored respiration of added sucrose, using the ^{13}C and ^{14}C label, and compared temperature treatments on the basis of amount of sucrose respired rather than length of time of the experiment. We hypothesized that any apparent interactions between warming and substrate supply, e.g., higher temperature sensitivity in the sucrose addition treatment, would result from differences in amount of C available to microbes, not to changes in microbial substrate use.

2. Methods

2.1. Free air CO_2 enrichment

We studied soils from the Aspen FACE experiment near Rhinelander, WI, USA (45°40.5′N, 89°37.5′W), which was designed to study the effects of eCO_2 on a newly planted stand of deciduous trees (Dickson et al., 2000). In eCO_2 plots, CO_2 concentrations were raised during the growing season by 200 μmol mol^{-1} above background levels for 11 years (1998–2009). The CO_2 used in the experiment was derived from fossil sources, and thus had a distinct C isotope signature from background air (Pregitzer et al., 2006). Hence the SOC isotopic signature records incorporation of C into soils in eCO_2 plots over the 11-year duration of the experiment. C fixed by photosynthesis and delivered belowground in eCO_2 plots in 2009, the year of sampling, was depleted in its C isotope signature by −12‰ in $\delta^{13}C$ and −340‰ in $\Delta^{14}C$ relative to C fixed in ambient CO_2 plots (Table 1a).

2.2. Aspen FACE site

We sampled soils where the vegetation type was an aspen clonal monoculture plantation (*Populus tremuloides* Michx.). The soils are classified as mixed, frigid Alfic Haplorthods with sandy loam A horizons. After a decade of eCO_2, net primary productivity (NPP) was enhanced by an average of 26% over the aCO_2 control plots, with a 34% stimulation of litterfall and a 15% stimulation of fine root production (Zak et al., 2011). After trees were planted in 1997, SOC contents increased linearly in both eCO_2 and ambient CO_2 (aCO_2) control plots (Talhelm et al., 2009), but after a decade of eCO_2, SOC contents did not differ significantly between the CO_2 treatments (Hofmockel et al., 2011).

2.3. Soil sampling and processing

In July 2009, we sampled soils from 3 replicate eCO_2 plots, and 3 replicate aCO_2 plots. After removal of surface litter, soils were

Table 1
Isotopic signature of microbial carbon sources and products. Reported values are the mean of three replicates, with the standard error of the mean given in parentheses, in units of ‰.

a) **Microbial carbon sources**

Source	$\delta^{13}C$		$\Delta^{14}C$	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Soil organic carbon	−26.4 (0.2)	−31.1 (1.2)	51(4)	−98 (20)
Roots <2 mm	−27.9 (0.2)	−39.9 (0.5)	53 (2)	−287 (22)
IAEA-CH-6 (sucrose)	−10.2 (0.2)		490.8 (2.0)	

b) **Microbial carbon products**

Product	Treatment	$\delta^{13}C$		$\Delta^{14}C$	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Microbial respired CO_2	+*Water, 5°*	−26.4 (0.8)	−34.0 (1.6)	40 (33)	−187 (34)
	+*Water, 25°*	−27.5 (0.3)	−35.3 (0.7)	63 (29)	−169 (34)
	+*Sucrose, 5°*	−16.3 (0.2)	−18.9 (0.3)	42 (57)	−220 (66)
	+*Sucrose, 25°*	−18.2 (0.4)	−22.5 (0.4)	84 (63)	−162 (74)
Microbial PLFA-C	+*Water, 5°*	−26.7 (0.4)	−33.5 (1.1)		
	+*Water, 25°*	−26.1 (0.03)	−33.8 (1.2)		
	+*Sucrose, 5°*	−25.1 (0.4)	−31.5 (0.6)		
	+*Sucrose, 25°*	−24.7 (0.3)	−30.9 (0.3)		

collected in increments of 0–5 cm and 5–15 cm with a 5 cm diameter impact corer. 5 cores of mineral soil were sampled from each of the 6 plots, then composited and subdivided into 6 subsamples for laboratory incubation. Soils were sieved to 4 mm, and roots and rocks were removed. Soils were transported on ice and refrigerated between processing steps.

We took a 5 g subsample from each composite to determine soil moisture, C content, and C isotope ratio of bulk soil. Subsamples were dried at 60 °C, ground, and analyzed on an NA 1500 NC elemental analyzer (Fisions Instruments) coupled to an isotope ratio mass spectrometer (IRMS; Thermo Finnigan continuous flow Delta Plus) for mass percent C and $\delta^{13}C$ value.

2.4. Laboratory incubation experiment

We incubated soils in the laboratory with a factorial manipulation of temperature (3 levels: 5 °C, 15 °C, 25 °C) and substrate (2 levels: water and sucrose addition). Soils were incubated at 5 °C, the site mean annual temperature, and with two levels of warming, 15 °C and 25 °C, applied in +10 °C increments (e.g., Steinweg et al., 2008; Nie et al., 2012; Thiessen et al., 2013). Substrate levels in half of the soils were raised by addition of 20 mg of sucrose dissolved in 1 mL of deionized water, equivalent to about 70 μg sucrose C per gram soil, or <0.5% of SOC. The other half of soils served as controls, receiving 1 mL of deionized water to compensate for water lost during soil sieving. The amount of sucrose-C added was roughly equivalent to 20% of annual inputs to SOC pools (Talhelm et al., 2009), and was of the same magnitude as root exudation rates measured in other ecosystems (Phillips et al., 2011), and expected exudation rate based on root biomass observed at the site (Grayston et al., 1996). The added sucrose (IAEA-CH-6) had a known C isotope composition that was distinct from SOC (Table 1a; Coplen et al., 2006; Xu et al., 2010), so we could track its contribution to respired CO_2. We considered each of the 6 experimental plots sampled (3 eCO_2 plots, 3 aCO_2 plots) to be the level of replication for the experiment ($n = 3$).

Roughly 120 g of soil from each plot was weighed into 250 mL glass containers. Water or sucrose solution was slowly added to soil surface by drops from a needleless syringe, taking care to evenly distribute additions, and avoid pooling of water. Soils were placed in 0.5 L Mason jars with lids equipped with sampling ports (Hopkins et al., 2012). CO_2-free air was used to purge the each jar's headspace, so subsequent CO_2 accumulation was derived only from soil respiration. We measured CO_2 concentrations periodically (up to 8 times) by removing 2 mL syringe samples from the jar headspace, and injecting them into a LI-6252 (Licor) infrared gas analyzer (Davidson and Trumbore, 1995). We also periodically took subsamples of headspace air for measurements of $\delta^{13}C$ (3 times) and $\Delta^{14}C$ (once). $\delta^{13}C$ of CO_2 was measured directly on subsamples of headspace air injected into He-filled exetainers by IRMS (Thermo Finnigan Gas Bench coupled to continuous flow Delta Plus). For $\Delta^{14}C$ analysis, we collected headspace air by connecting a 0.5 L, evacuated stainless steel canister to the jar lid sampling port. CO_2 was cryogenically purified, and converted to graphite for ^{14}C measurement at the WM Keck Carbon Cycle Accelerator Mass Spectrometer Facility at UC Irvine (Xu et al., 2007).

The overall length and timing of the incubation experiment was determined by the amount of sucrose-C lost through respiration, as a means to control for differential substrate depletion amongst temperature treatments. Substrate depletion can affect observed respiration and substrate use efficiency in soils (Fissore et al., 2008; Shen and Bartha, 1996). Because of the large difference in flux rates between temperature treatments, we measured CO_2 fluxes and their isotopic composition at different times for each temperature level, but selected those times when approximately the same amount (~60%) of sucrose-C had been respired. At this point (1/e of sucrose remaining in soil), we collected the last headspace CO_2 for isotope analysis, and then immediately froze soils for phospholipid fatty acid (PLFA) analysis.

2.5. PLFA analysis

We extracted lipids from 50 g dry weight equivalent of soil by shaking with chloroform, methanol and 0.05 M phosphate buffer, following the Bligh-Dyer method as applied in Kramer and Gleixner (2006). Phospholipids were separated from other lipid fractions by sequential elution with chloroform, acetone, and methanol on silica-filled solid phase extraction columns. Phospholipids were hydrolyzed and methylated to form fatty acid methyl esters (FAMEs), which were subsequently separated into saturated (SATFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids with silver impregnated SCX columns, so they could be analyzed separately for amount and $\delta^{13}C$.

We determined PLFA amounts using the relative size of chromatographic peaks detected on an HP-6890 gas chromatograph (GC) with a flame ionization detector (Agilent) using the program described by Gude et al. (2012). Before analysis, we added a known amount of PLFA nonadecanoic acid-methyl ester (19:0) standard to allow quantification of PLFAs. We only included peaks greater than 10 ng μL^{-1} in our analysis based on the lower limit of our GC calibration.

We measured the $\delta^{13}C$ of individual FAMEs by GC-IRMS (Finnigan Delta Plus XL). We analyzed each of the three FAME groups per sample in triplicate, so $\delta^{13}C$ values reported here are the average of the three analytical replicates. We corrected $\delta^{13}C$ values for the contributions of methyl-C using mass balance for each FAME. We used the 19:0 standard ($\delta^{13}C = -30.05$) to correct for machine drift and sample fractionation. We used a standard mixture of SATFAs (Supelco) as a secondary standard. The standard deviation of 40 measurements of secondary standards was 0.53‰ in $\delta^{13}C$.

We identified 49 different FAME peaks, 37 of which were large enough for quantification in all samples, including 22 SATFA, 12 MUFA, and 3 PUFA. FAMEs were identified by comparison of retention times and peak shape primarily using an in-house database that was developed from GC–MS and mass spectral libraries (Thoms et al., 2010). Chromatograms were also checked against the

SATFA standard that was analyzed alongside samples. We used the sum of all FAMEs in the sample to estimate relative amounts of microbial biomass C between treatments.

2.6. Calculations

2.6.1. Sources of C for respiration and PLFA using C isotopes

2.6.1.1. Sucrose-derived CO_2. We used a ^{13}C mass balance equation to determine the fractional contribution of added sucrose to respired CO_2:

$$f_{\text{sucrose}} = \frac{\delta^{13}\text{CO}_{2,\text{soil+sucrose}} - \delta^{13}\text{C}_{\text{sucrose}}}{\delta^{13}\text{CO}_{2,\text{soil+water}} - \delta^{13}\text{C}_{\text{sucrose}}} \quad (1)$$

where values for $\delta^{13}C_{sucrose}$ and $\delta^{13}CO_{2,\ soil}$ for microbial respiration from sucrose addition and water addition soils are reported in Tables 1a and b, respectively.

The contribution of SOC to respired CO_2 from sucrose treatment soils was determined:

$$R_{\text{SOC, soil+sucrose treatment}} = 1 - f_{\text{sucrose}} * R_{\text{total, soil+sucrose treatment}} \quad (2)$$

The priming effect (PE) was calculated as:

$$PE = R_{\text{SOC, soil+sucrose treatment}} - R_{\text{SOC, soil+water treatment}} \quad (3)$$

We used the known $\Delta^{14}C$ value of added sucrose to determine the $\Delta^{14}C$ value of SOC-derived CO_2 using a ^{14}C mass balance:

$$\Delta^{14}\text{CO}_{2,\text{SOC}} = \frac{\Delta^{14}\text{CO}_{2,\text{soil+sucrose}} - f_{\text{sucrose}} * \Delta^{14}\text{C}_{\text{sucrose}}}{1 - f_{\text{sucrose}}} \quad (4)$$

2.6.1.2. FACE-label derived CO_2. After using $\delta^{13}C$ (equation (1)) to remove the contribution of sucrose-C from the amount (equation (2)) and isotopic signature (equation (4)) of respired CO_2, we used $\Delta^{14}C$ to calculate the fraction of respired CO_2 consisting of C fixed since the FACE experiment began (f_{FACE}) using the ^{14}C mass balance equations described in Hopkins et al. (2012). The ^{14}C end-member for FACE C was the average $\Delta^{14}C$ signature of roots in each experimental plot, and the ^{14}C end-member for pre-FACE C was based on the $\Delta^{14}C$ signature of SOC from the paired aCO_2 control plots (Table 1a).

We confirmed the results of the $\Delta^{14}C$ mixing model using a $\delta^{13}C$ mixing model for soils that did not receive sucrose addition, and found no differences in overall pattern.

2.6.1.3. Sucrose-derived PLFA-C. We calculated the fraction of sucrose-C incorporated in each individual PLFA biomarker, $f_{PLFA-sucrose}$, accordingly:

$$f_{\text{PLFA-sucrose}} = \frac{\delta^{13}\text{C} - \text{PLFA}_{\text{soil+sucrose}} - \delta^{13}\text{C} - \text{PLFA}_{\text{soil+water}}}{\delta^{13}\text{C}_{\text{sucrose}} - \delta^{13}\text{CO}_{2,\text{soil+water}}} \quad (5)$$

Values of $f_{PLFA-sucrose}$ were only retained for further analysis if they were greater than the value of their propagated standard errors (Phillips and Gregg, 2001), excluding 22% of PLFA by mass.

2.6.1.4. FACE-derived PLFA-C. Similarly, we calculated the fraction of FACE-derived C in individual PLFA biomarkers, $f_{PLFA-FACE}$, by assuming that the maximum possible difference between $\delta^{13}C$-PLFA from eCO_2 and aCO_2 soils is similar to the difference in the $\delta^{13}C$ values of fine roots between eCO_2 and aCO_2 (Table 1; see also Balesdent and Mariotti, 1996; Kramer and Gleixner, 2006):

$$f_{\text{PLFA-FACE}} = \frac{\delta^{13}\text{C} - \text{PLFA}_{\text{eCO}_2} - \delta^{13}\text{C} - \text{PLFA}_{\text{aCO}_2}}{\delta^{13}\text{C} - \text{roots}_{\text{eCO}_2} - \delta^{13}\text{C} - \text{roots}_{\text{aCO}_2}} \quad (6)$$

2.6.2. Microbial community structure and function

2.6.2.1. Whole community. We estimated relative amounts of microbial biomass C as the sum of all detectable PLFA-C amounts for each sample. Similarly, we estimated the total amount of sucrose-derived PLFA-C by summing the product of $f_{PLFA-sucrose}$ and PLFA-C amount for each biomarker for each sample. Bulk $\delta^{13}C$ of all PLFAs in a sample was the sum of the product of the amount of C for each PLFA by its $\delta^{13}C$ value, divided by total PLFA-C (Morrison et al., 2010).

2.6.2.2. Microbial functional groups. We separated the 37 microbial PLFAs into functional groups (Table S1) primarily using the standard community structure method (Hedrick et al., 2005), where monounsaturated and cyclopropyl fatty acids were classified as Gram-negative bacteria, terminally branched SATFAs were Gram-positive, mid-chain branched SATFAs were Actinobacteria, and PUFAs were Eukaryotes. The straight chain fatty acids, which are produced by all organisms, were grouped as a "general" microbial biomarker. Among PUFAs, we distinguished two types of organisms; 18:2ω6c, a biomarker for saprotrophic and ectomycorrhizal fungi (Frostegård and Bååth, 1996; Olsson, 1999); and 20:4ω6c, a biomarker for protozoa (Vestal and White, 1989). Among MUFAs, two fatty acids were not grouped as Gram-negative bacteria, and were analyzed separately or as part of the fungal PLFA group; 16:1ω5t was considered to be a biomarker for arbuscular mycorrhizal (AM) fungi (Olsson, 1999; Drigo et al., 2010), and 18:1ω9c, which is produced by both fungi and Gram-negative bacteria (Frostegård and Bååth, 1996), were analyzed separately.

2.6.2.3. Microbial community composition. We evaluated changes to the microbial community by testing treatment effects on the relative abundance of individual PLFAs using redundancy analysis (RDA), a statistical procedure which derives a set of synthetic variables from imposed treatments and explanatory environmental variables which are not of primary interest to determine how much of the variance in the data can be attributed to treatment effects. Partial RDAs allow us to remove the effect of environmental variables by including them as co-variables in ordination models, and thus determine how much variance in the data can be attributed to treatment effects (Leps and Smilauer, 2003). RDAs were performed in CANOCO software (ver. 4.5, Microcomputer Power, Inc., Ithaca, NY).

2.6.2.4. Microbial activity. Differences in the $\delta^{13}C$ signature of PLFA between eCO_2 and temperature treatments with sucrose addition were interpreted as changes in the amount of sucrose-C remaining in microbial biomass. Similarly, difference in $\delta^{13}C$ of PLFA among temperature treatments for eCO_2 soils was interpreted as changes in the proportion of FACE-derived C taken up by microbes.

We then calculated PLFA-based substrate-use efficiency (SUE; Ziegler and Billings, 2011):

$$\text{SUE} = \frac{\sum_i PLFA - C * f_{PLFA-sucrose}}{\sum_i PLFA - C * f_{PLFA-sucrose} + R_{total} * f_{sucrose}} \quad (7)$$

where i was either all PLFA measured in each sample (integrated community SUE), or individual PLFAs, for soils receiving added sucrose. Similarly, we also compared treatments on the basis of ratios of respired CO_2 to total PLFA.

2.7. Statistics

We analyzed the data using ANOVAs with a randomized block design (Phillips et al., 2002) in Proc GLM in SAS version 9.2. We tested the effect of the field CO_2 treatment, and laboratory temperature and sucrose addition effects on cumulative respiration of sucrose-derived and SOC-derived CO_2 and $\delta^{13}C$–CO_2, and amounts and $\delta^{13}C$ content of PLFAs (bulk, groups, and individual biomarkers) with Tukey's Honestly Significant Differences (HSD) test. Treatment effects were considered significant at the $\alpha = 0.05$ level, and marginally significant at the $\alpha = 0.1$ level. We adjusted the p-value threshold for significance at the $\alpha = 0.05$ level to account for multiple comparisons when testing effects of treatments on multiple PLFA (Holm, 1979).

3. Results

3.1. Fate of sucrose

Respiration of sucrose added to soils at the beginning of the experiment was highly temperature sensitive (Fig. 1a, $p = 0.0002$), but we controlled for this effect by sampling all temperature treatments after 62% (±8%, 1 standard deviation) of added sucrose-C had been respired (so there was no significant difference in cumulative sucrose respired across temperature treatments, Fig. 1b, $p > 0.9$). This represented a cumulative loss of 44 μg of sucrose-C per g soil over 31 days for soils incubated at 5 °C, 13 days for soils at 15 °C, and 6 days for soils at 25 °C (Fig. 1a).

While 62% of added sucrose had been respired as CO_2, 5% (±3.5%, 1 standard deviation) was recovered in microbial PLFA. For soils that were amended with sucrose, about 10% of PLFA-C was derived from added sucrose (Table 2). CO_2 treatment and laboratory warming had no effect on the amount of sucrose-C recovered in PLFA. The remaining 23% of added sucrose that was not accounted for by our methods was probably incorporated into other microbial products, as PLFA only accounts for a small portion of microbial biomass C (5–20%, based on observed species composition; Bååth, 1994; Frostegård and Bååth, 1996).

3.2. Higher C inputs increase respiration of SOC-derived CO_2

Soils that had experienced increased C inputs, over the long term in eCO_2 soils, and immediately following a pulse addition of sucrose in the laboratory, had higher rates of respiration from SOC sources (Fig. 1c, g). Cumulative respiration losses of SOC were about 35% higher from eCO_2 soils than aCO_2 soils on a soil mass basis (Table 3, $p = 0.0003$), reflecting a decade of increased plant inputs in the eCO_2 treatment soils. Respiration from eCO_2 soils remained consistently higher than from aCO_2 soils over the length of the experiment (Fig. 1c, $p < 0.0001$). Short-term laboratory additions of sucrose also increased respiration of SOC, inducing a positive priming effect. Soils receiving added sucrose respired 20% more from SOC sources, averaged over all treatments, than soils receiving water alone (Table 3, $p = 0.0044$). In contrast to the eCO_2 treatment, the sucrose addition effect declined over the course of the experiment (Fig. 1g, $p < 0.0001$). The priming effect induced by sucrose additions declined at a similar rate as sucrose respiration over time (Fig. 1a, e).

3.3. Warming effects on respiration and interactions with increased C inputs

Warming strongly increased respiration rates of SOC-derived CO_2 ($p < 0.0001$, Fig. 1 c, e), and cumulative SOC lost over the experiment ($p = 0.0007$). Even when controlling for the same cumulative loss of sucrose across temperature treatments, there was still greater loss of SOC to respiration in warmed soils, by about 50% between 5° and 25 °C treatments (Table 3). The combination of warming and high C inputs increased respiration rates much more strongly than either effect alone. This interactive effect was observed over the whole experimental period for the eCO_2 soils (Fig. 1d, $p = 0.0004$), but only initially for soils with added sucrose (Fig. 1e, f). The positive interaction of sucrose addition and warming declined as added sucrose was consumed. By the end of the experiment, the cumulative amount of SOC primed by sucrose addition was the same across temperature treatments (Fig. 1f).

3.4. Sources of soil-respired CO_2 with sucrose-induced priming

The C isotope signature of SOC in eCO_2 treatment soils showed significant incorporation of depleted fumigation C into SOC pools over the ten years of FACE—about 30% of the SOC stock carried the FACE isotope label (Table 1). Actively decomposing C, as measured in CO_2 respired from soils, was closer in $\delta^{13}C$ and $\Delta^{14}C$ to new roots, showing preferential degradation of more recently added C. Nonetheless, about 30% of respired C was more than 10 years old, pre-dating the FACE experiment (Table 4). Since the C isotope signature of eCO_2 soils was altered by the CO_2 treatment itself, we could not directly test whether the priming effect in eCO_2 soils changed the age of decomposing C relative to the aCO_2 treatment. Instead, we used priming caused by sucrose addition to test the effect of priming on the age of decomposed C. We used the $\delta^{13}C$ of respired CO_2 to determine the contribution of added sucrose to respiration (Eqn. (1)), and subtracted it from total CO_2 (and $^{14}CO_2$) flux (Eqns. (2) and (4)). The $\Delta^{14}C$ signature of CO_2 derived from SOC (Eqn. (4)) was used to determine the proportion of C that pre-dated the FACE experiment. Addition of sucrose stimulated respiration of both FACE and pre-FACE derived C (Fig. 1h), but did not alter the relative proportion of the two sources (Table 3). There was no statistically significant difference between the fractions of FACE-derived C (f_{FACE}) respired by soils receiving added sucrose and those receiving water alone. We also calculated the $\Delta^{14}C$ signature (and f_{FACE}) of CO_2 from the priming effect (Eqn. (3)), but due to the small size of the priming effect and spatial heterogeneity of fluxes and isotopic endmembers among plots, we were unable to resolve the source of priming effect-derived CO_2 (Fig. 2). We performed a sensitivity test, assuming all primed CO_2 originated from either entirely FACE-C, or entirely pre-FACE C. Small differences and large errors in the expected $\Delta^{14}C$ signatures of these two scenarios suggest that we cannot definitively determine the origin of primed CO_2. In aCO_2 soils, which do not have the FACE isotope label, $\Delta^{14}C$ of respired CO_2 also gives information about age of C sources in surface soils on timescales of years to decades (Trumbore, 2000). In aCO_2 soils, there was no change in $\Delta^{14}C$ of CO_2 respired from soils with sucrose-induced priming, contrary to results observed for deeper soils with natural abundance levels of ^{14}C (Fontaine et al., 2007). This provides additional support that the priming effect did not cause preferential degradation of older or younger SOC pools.

3.5. Effect of higher C inputs and warming on PLFA

Total PLFA-C was higher in eCO_2 soils compared to aCO_2 soils (Table 3, $p = 0.0112$), due to significant increases in PLFA-C of Gram-negative bacteria (+25%, $p = 0.0159$ for group), fungal biomarkers (+25%, $p = 0.0036$ for group), and protozoa (+60%, $p = 0.0281$), (Fig. 3, Table S1). Sucrose addition soils also had higher total PLFA-C (Table 2, $p = 0.0157$), attributed to growth of Gram-negative bacteria ($p = 0.0116$ for group) and all fungal biomarkers ($p = 0.0016$ for group) by 30% and 34%, respectively (Fig. 3, Table S1).

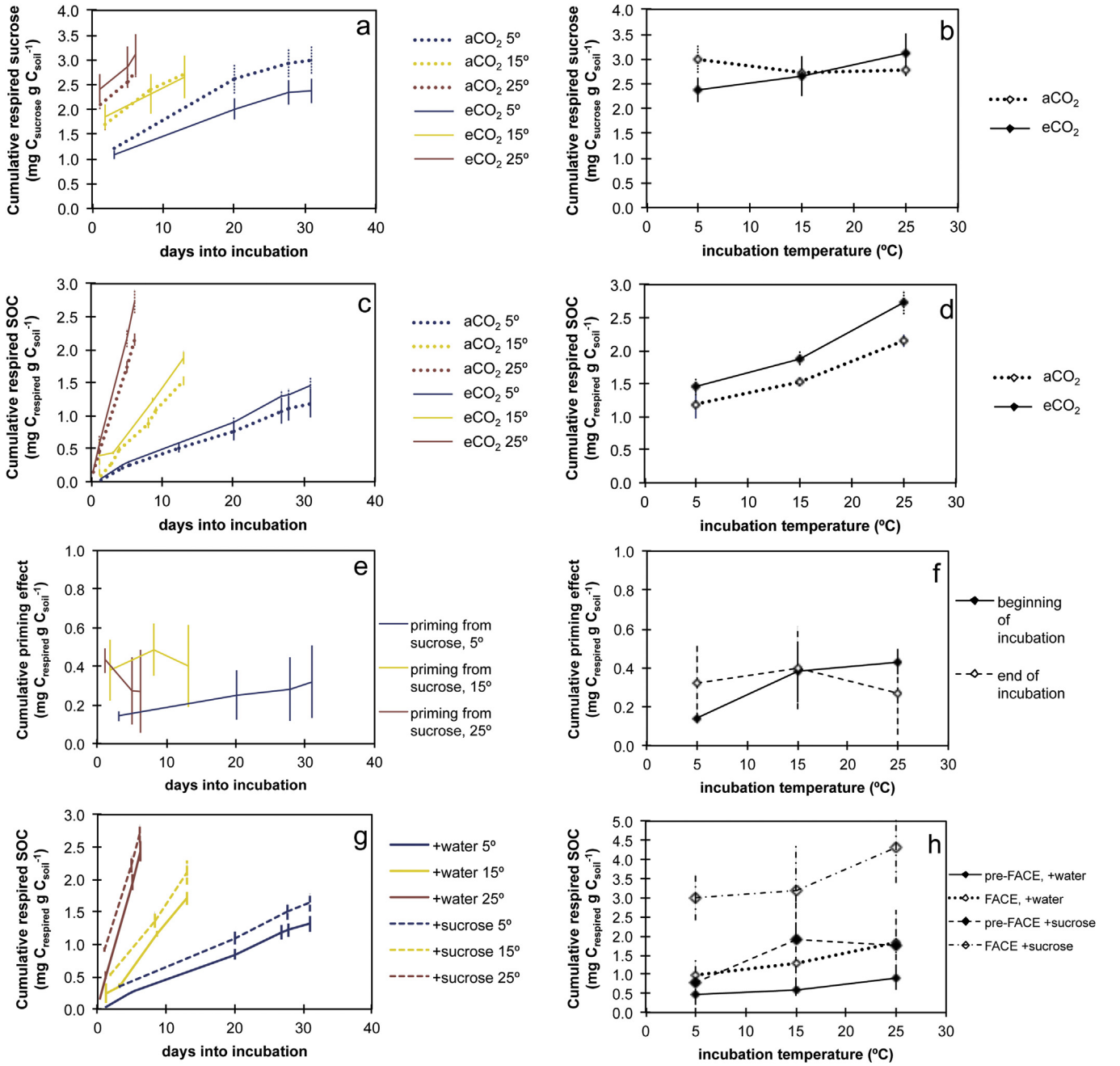

Fig. 1. Cumulative respiration and temperature sensitivity of respired CO_2 sources, in mg CO_2–C g soil C^{-1}. Left side plots show cumulative respiration for the three temperature treatments— 5°: blue, 15°: yellow, 25°: red, over the course of the experiment. Right side plots show temperature sensitivity of different respired CO_2 sources. a) Cumulative respiration of sucrose-derived CO_2 from aCO_2 soils (dotted lines) and eCO_2 soils (solid lines), b) Temperature sensitivity of cumulative sucrose-C lost by the end of the experiment, c) Cumulative respiration of SOC-derived CO_2 from aCO_2 and eCO_2 soils, d) Temperature sensitivity of cumulative SOC-derived CO_2 from aCO_2 and eCO_2 soils, e) Cumulative respiration of soil-derived CO_2 from the priming effect, calculated as $R_{SOC\,+\,sucrose} - R_{SOC\,+\,water}$ for each temperature treatment, averaging both CO_2 treatments, f) Temperature sensitivity of the priming effect, at the first respiration measurement (solid lines and symbols) and the last respiration measurement (dashed lines and open symbols), g) Cumulative respiration of SOC-derived CO_2 comparing soils receiving water addition (solid lines) and sucrose addition (dashed lines), h) Temperature sensitivity of FACE-labeled C (recent) and pre-FACE (>10 y) C respired from +water and +sucrose treatments from eCO_2 soils.

Warming had no significant effect on total PLFA-C ($p = 0.947$); however, the increase in PLFA-C caused by added sucrose was lower at 25° than at 5 °C, shown by a marginally significant interaction ($p = 0.0935$) between warming and sucrose addition on total PLFA and for many individual biomarkers. In the warming treatment, all fungal and many Gram-negative biomarkers showed less of a growth response to added sucrose than they had at the control temperature (Fig. 4). None of the treatments significantly changed the amount of PLFA-C for actinobacteria, Gram-positive bacteria, or the general microbial biomarkers.

3.6. Sources of C in PLFA

Similar to respiration, we calculated the fraction of PLFA-C coming from different C sources using the $\delta^{13}C$ signature of PLFA biomarkers. The eCO_2 ($p < 0.0001$) and sucrose addition

Table 2
PLFA-C, in μg C_{PLFA} g $soil^{-1}$, across CO_2, temperature, and substrate treatments. For eCO_2 soils, PLFA-C was partitioned using its $\delta^{13}C$ signature into C added during FACE, and C previous to FACE (pre-FACE). For soils receiving added sucrose, PLFA-C was similarly partitioned into PLFA derived from added sucrose, and PLFA derived from SOC sources.

		5 °C		25 °C	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
+Water	Total PLFA-C	22.0 (8.9)	21.1 (1.2)	22.5 (3.9)	27.0 (3.3)
	FACE C		11.8 (2.2)		15.9 (2.7)
	Pre-FACE C		9.3 (2.1)		11.1 (3.9)
+Sucrose	Total PLFA-C	25.6 (4.1)	33.3 (11.3)	23.0 (6.1)	31.2 (7.3)
	Sucrose-C	3.4 (1.1)	4.0 (2.0)	2.12 (0.7)	4.3 (2.0)
	SOC	22.3 (3.2)	29.3 (9.4)	20.8 (5.4)	26.9 (5.5)

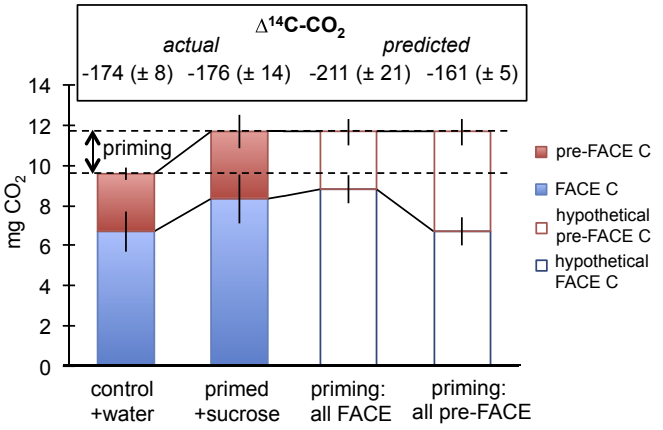

Fig. 2. Contributions of pre-FACE and FACE C to priming effect. Solid bars show SOC-derived CO_2 respired from FACE soils with additions of water and sucrose partitioned into FACE-derived (blue) and pre-FACE (red) sources using a ^{14}C mixing model. Open bars show expected contributions of FACE-derived and pre-FACE sources if priming effect was driven by increased decomposition of only new FACE-labeled C inputs, and or of only pre-FACE C sources. Error bars are the standard error of measurement means propagated through the mixing model. Box above figure shows actual and predicted $\Delta^{14}C$–CO_2 values (±standard error of the mean) for each scenario shown below.

($p = 0.0062$) treatments had large effects on the $\delta^{13}C$ composition of total PLFA, by −6.0 per mil and +1.8 per mil, respectively, associated with microbial uptake of recently added C inputs—i.e., over the past 10 y in eCO_2 soils, and from additions of sucrose in the laboratory incubation experiment. Incubation temperature had no consistent effect on $\delta^{13}C$ of PLFA for any groups or biomarkers (Figure S1), hence warming caused no detectable change in the proportion of new and decades-old C in biomass.

To explore the role and relative contribution of different microbial groups to the whole community response, we tested the effects of treatments on $\delta^{13}C$ of each PLFA biomarker and on microbial functional groups. The eCO_2 treatment caused a shift toward more depleted $\delta^{13}C$ values for all microbial functional groups, and all PLFAs except 16:1ω5t (AM fungi), 15:1ω11 (Gram-negative bacteria), and 17:1ω8t (Gram-negative bacteria), a putative methane oxidizer, hence, very low $\delta^{13}C$-PLFA value for 17:1 ω8t; Ringelberg et al., 1989; Figure S1). This shift indicates that the majority of soil microorganisms were using C fixed during the last decade, and is consistent with the finding of depleted $\delta^{13}C$ values for all physically separable soil pools under eCO_2 at this site (Hofmockel et al., 2011).

In contrast, sucrose addition only affected the $\delta^{13}C$-PLFA of certain groups. The general microbial biomarkers, Gram-negative bacteria, and PLFA 18:1ω9c (biomarker for Gram-negative bacteria and fungi) became more enriched with addition of sucrose, by +1.2‰ ($p = 0.063$), +2.3‰ ($p = 0.0011$), and 3.3‰ ($p = 0.0021$), respectively (Figure S1). The protozoa biomarker, 20:4ω6c, became more depleted, by 1.8‰ ($p = 0.0085$). Sucrose addition had no effect on the $\delta^{13}C$-PLFA of Gram-positive bacteria or actinobacteria. Tests on individual PLFA from microbial functional groups revealed similar results—the majority of PLFA classified as Gram-negative bacteria were significantly enriched in $\delta^{13}C$ with sucrose addition (Figure S1), while only 1 PLFA from the Gram-positive bacteria group was significantly enriched in $\delta^{13}C$ ($p = 0.0028$, PLFA 15:0a). Neither incubation temperature, nor the interaction of temperature

Table 3
Cumulative CO_2 respired over experiment from soil organic carbon (SOC) and added sucrose, in μg $C_{respired}$ g $soil^{-1}$. For eCO_2 soils, SOC-derived CO_2 efflux was partitioned between recent FACE C (<10 y old) and pre-FACE C (>10 y old) using its $\Delta^{14}C$ signature. For sucrose addition soils, SOC-derived respired CO_2 was partitioned from total respired CO_2 using its $\delta^{13}C$ signature. Values are reported as the mean of three replicates with the standard error of the mean given in parentheses.

		5 °C		15 °C		25 °C	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
+Water	Total respiration	17.0 (5.0)	24.3 (1.8)	22.5 (2.4)	30.6 (5.9)	32.4 (3.6)	47.0 (9.3)
	FACE		16.3 (1.6)		20.9 (4.8)		31.9 (7.8)
	pre-FACE		8.0 (3.0)		9.6 (2.4)		15.1 (4.7)
+Sucrose	Total respiration	71.5 (7.9)	63.0 (3.6)	65.3 (8.6)	87.2 (25.7)	81.8 (6.3)	103.7 (10.8)
	SOC	27.7 (5.6)	23.6 (4.1)	25.7 (4.1)	42.3 (15.3)	38.3 (5.9)	51.2 (8.9)
	FACE		18.1 (1.5)		25.9 (3.4)		37.1 (12.4)
	pre-FACE		5.5 (1.5)		16.4 (10.3)		14.1 (5.7)

Table 4
Fractional contribution of recent, FACE-label C (f_{FACE}) to microbial products in eCO_2 soils. f_{FACE} of respired CO_2 is calculated using $\Delta^{14}C$, and f_{FACE} of PLFA is calculated using $\delta^{13}C$. PLFA is given for all microbial groups, and for microbial functional groups. Values are averages of three replicates, with standard errors shown in parentheses.

	f_{FACE}-CO_2	f_{FACE}-PLFA						
		All	Actino-bacteria	General	Gram-positive	Gram-negative	Fungi	Protozoa
+Water 5°C	0.67 (0.06)	0.60 (0.11)	0.51 (0.13)	0.58 (0.10)	0.58 (0.09)	0.61 (0.09)	0.64 (0.09)	0.35 (0.11)
+Water 25°C	0.68 (0.05)	0.68 (0.11)	0.75 (0.11)	0.63 (0.11)	0.66 (0.10)	0.66 (0.12)	0.70 (0.12)	0.42 (0.07)
+Sucrose 5°C	0.87 (0.11)							
+Sucrose 25°C	0.71 (0.09)							

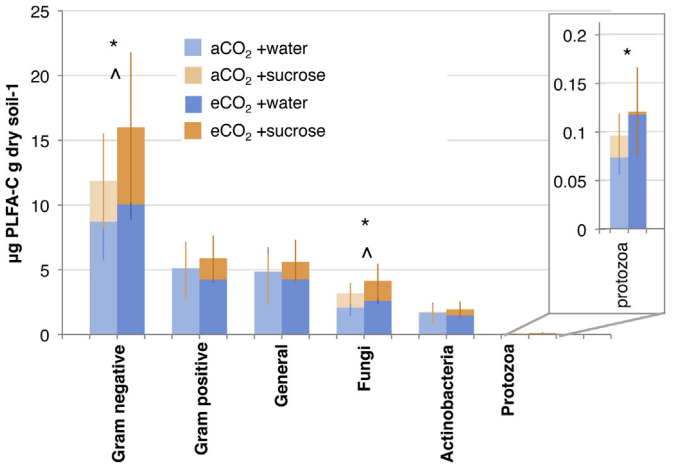

Fig. 3. Effect of eCO_2 and laboratory sucrose addition on PLFA amounts for microbial functional groups, in μg PLFA-C g dry soil^{-1}. Light-colored, left side bars for each group represent aCO_2 soils, and dark-colored, right side bars represent eCO_2 soils. The increase in PLFA abundance from sucrose addition is overlaid (orange) over the PLFA abundance in the water only addition treatment (blue). Groups that had a statistically significant difference between CO_2 treatments are denoted by *, and for sucrose addition, by ^. Error bars are the standard error of the mean of three replicate samples per treatment.

with other treatments, had a statistically significant effect on δ^{13}C-PLFA on any group.

Greater incorporation of the FACE label by certain microbial groups was demonstrated by significant differences in f_{FACE} of PLFA between microbial functional groups (Table 4; $p < 0.0001$, Tukey's HSD: fungi and Gram-negative bacteria [A] ≥ general [AB] ≥ Actinobacteria [BC] ≥ Gram-positive bacteria [C] > protozoa [D]). Warming increased f_{FACE} for Actinobacteria ($p = 0.0109$), the general microbial group ($p = 0.0006$), and fungi ($p = 0.0325$). Incorporation of the sucrose label, $f_{sucrose}$ of PLFA, also differed significantly between microbial functional groups (Table 3; $p = 0.0011$, Tukey's HSD: fungi [A] ≥ Gram-negative bacteria [AB] ≥ Gram-positive bacteria [ABC] ≥ general [BCD] ≥ Actinobacteria [DC] ≥ protozoa [D]).

3.7. Treatment effects on microbial activity

We used the total amount of sucrose-derived PLFA-C and respired CO_2 to calculate PLFA-based SUE for each individual PLFAs, and by group (Table 5). We found no significant effects of eCO_2 or warming on SUE. Combining respiration amounts and microbial biomass estimates from PLFA abundance, we found that sucrose addition ($p = 0.0001$) and warming ($p = 0.0340$) both significantly increased total respiration per unit biomass ($R_{total}/PLFA_{total}$). For respiration originating from SOC (calculated from Eqn. (2)), only warming ($p = 0.0016$) significantly increased respiration per unit biomass ($R_{SOC}/PLFA_{total}$).

3.8. Microbial community composition

PLFAs of Gram-negative bacteria dominated the microbial community in all treatments, with about 40% (by mol) of the total PLFA biomass, followed by Gram-positive (~20%), the general

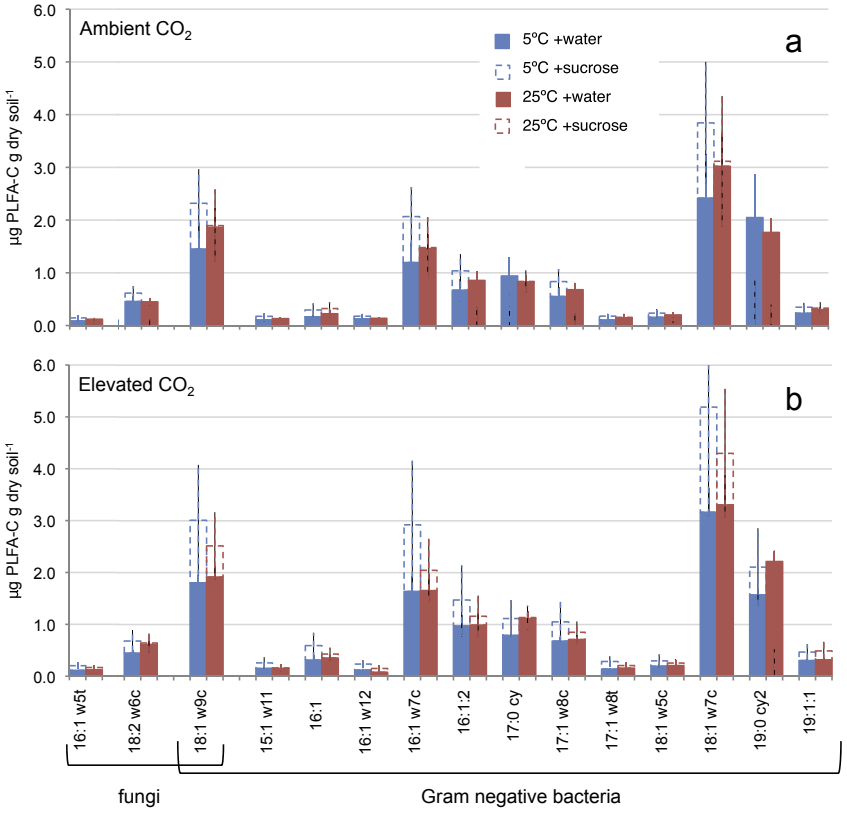

Fig. 4. Effect of warming and sucrose addition on PLFA abundance of individual fungal and Gram-negative bacterial biomarkers. Lefthand (blue) bars show PLFA abundances in the 5 °C incubation treatment, and righthand (red) bars show PLFA abundance in the 25 °C incubation treatment. The increase in PLFA abundance with sucrose addition is shown by the dashed area above each bar. Top plot (a) shows aCO_2 soils, bottom plot (b) shows eCO_2 soils. Error bars are the standard error of the mean of three replicate samples per treatment.

Table 5
Sucrose-C as a component of respired CO_2 and PLFA-C ($f_{sucrose}$). Also shown are sucrose content of PLFA (μg C g soil^{-1}), and PLFA-based substrate use efficiency (SUE).

		CO_2 level	$f_{sucrose}$		Sucrose content		SUE	
			5 °C	25 °C	5 °C	25 °C	5 °C	25 °C
Respired CO_2		Ambient	0.67 (0.06)	0.56 (0.07)				
		Elevated	0.68 (0.05)	0.53 (0.05)				
PLFA	*All*	Ambient	0.10 (0.03)	0.09 (0.02)	22 (3)	21 (5)	1.5 (0.6)	0.4 (0.3)
		Elevated	0.08 (0.05)	0.12 (0.05)	31 (10)	27 (5)	1.2 (0.6)	1.0 (0.4)
	Actinobacteria	Ambient	0.08 (0.08)	0.01 (0.01)	1.4 (0.1)	1.5 (0.3)	0.09 (0.09)	0 (0)
		Elevated	0.03 (0.02)	0.11 (0.06)	1.9 (0.5)	1.7 (0.2)	0.03 (0.02)	0.06 (0.03)
	General	Ambient	0.07 (0.07)	0.06 (0.03)	3.9 (0.2)	4.0 (0.7)	0.18 (0.18)	0.07 (0.04)
		Elevated	0.05 (0.03)	0.10 (0.05)	5.2 (1.5)	5.5 (1.2)	0.17 (0.09)	0.18 (0.10)
	Gram-positive	Ambient	0.09 (0.07)	0.07 (0.03)	4.3 (0.03)	4.2 (0.7)	0.27 (0.24)	0.08 (0.04)
		Elevated	0.07 (0.02)	0.11 (0.05)	5.4 (1.5)	5.2 (0.9)	0.20 (0.08)	0.17 (0.08)
	Gram-negative	Ambient	0.16 (0.02)	0.12 (0.02)	10 (2)	9 (3)	0.77 (0.11)	0.23 (0.04)
		Elevated	0.07 (0.03)	0.12 (0.04)	15 (5)	11 (2)	0.57 (0.29)	0.43 (0.18)
	Fungi	Ambient	0.15 (0.02)	0.14 (0.03)	2.7 (0.5)	2.2 (0.7)	0.19 (0.02)	0.06 (0.00)
		Elevated	0.10 (0.05)	0.15 (0.03)	3.7 (1.2)	2.9 (0.6)	0.21 (0.13)	0.12 (0.04)
	Protozoa	Ambient	0 (0)	0.03 (0.03)	0.10 (0.02)	0.07 (0.02)	NA	NA
		Elevated	0 (0)	0.02 (0.02)	0.12 (0.03)	0.15 (0.07)	NA	NA

microbial group (~20%), fungi (~10%), Actinobacteria (~10%), and protozoa (<0.5%) (Table S1).

Increased abundance of total PLFA-C in both eCO_2 and sucrose addition soils was mostly due to growth of Gram-negative bacteria and fungi, which suggests a change in community composition with increasing C input amounts. Despite the significant change in PLFA abundance for the Gram-negative bacteria and fungi, eCO_2, sucrose addition, and warming treatments were less important to microbial community composition than environmental variables from the field site. Specifically, there is an NPP gradient across the field site (Dickson et al., 2000) that strongly determined community composition among replicate field plots. Fine root biomass, which was closely related to NPP, was the best predictor of microbial community composition by RDA. The explained variation in community composition was 67.1% including fine roots and NPP as variables in RDA (Figure S2a). When these factors were fit as covariables, the explanatory power of RDA decreased to 20.1% (Figure S2b).

Table 6
Summary of treatment effects. Arrows indicate effect, colors indicate treatment: green, sucrose addition; blue, eCO_2; red, warming. Up arrows indicate positive treatment effects, sideways arrows indicate no effect, and down arrows indicate negative treatment effects. Statistically significant interactions between treatments are shown by two-colored arrows. n/a indicates that treatment effects cannot be determined. For example, in the first row, incubation-derived rates of CO_2 evolution increased in elevated CO_2 plots compared to controls (blue upward arrow), and warming increased the rate of CO_2 evolution in ambient CO_2 soils (red upward arrow). Elevated CO_2 soils responded more to warming (indicated by both upward blue and red arrows, and the two-color arrow indicates there was a significant positive interaction).

	eCO_2	warming	eCO_2 x warming
Specific rate of CO_2 efflux	↑	↑	↑↑
+ Sucrose	↑↑	↑↑	↑↑↑
Cumulative loss of SOC	↑	↑	↑↑
+ Sucrose	↑↑	↑↑	↑↑
Age of respired CO_2	n/a	→	n/a
+ Sucrose	→→	→→	→→
Microbial biomass	↑	→	↑→
+ Sucrose	↑↑	→→	↑↑→
Microbial community shift toward G- bacteria, fungi	↑	↓	↑↓
+ Sucrose	↑↑	↑↓	↑↑↓

4. Discussion

4.1. Reduced SOC accumulation under eCO_2 likely driven by microbial priming effect

A mechanistic explanation for observed reductions in SOC accumulation despite substantial increases in above- and belowground C inputs is an important unresolved issue for the Aspen FACE site, and eCO_2 in general (Talhem et al., 2009; Norby and Zak, 2011). In this study, we tested two potential explanations for this pattern: (a) a change in microbial efficiency under eCO_2 leads to faster, more complete decomposition of newly added C inputs, and (b) increased C inputs with eCO_2 caused positive priming of SOC decomposition, and accelerated the overall rate of SOC cycling. We found that the positive priming of SOC decomposition is a more likely explanation than altered microbial efficiency.

SOC decomposition is difficult to observe directly on the timescales of a laboratory experiment, so we inferred eCO_2 effects on SOC decomposition using isotopic and microbial measurements (Conant et al., 2011). In addition, the lack of a parallel isotope label to compare eCO_2 and aCO_2 soils required addition of a new isotope label, in the form of sucrose, which doubled as a proxy for soluble C inputs added to soils by roots. Laboratory additions of sucrose induced a positive priming of SOC decomposition, exemplified by increased respiration of SOC-derived CO_2 and greater PLFA abundance (Table 6). Moreover, PLFA measurements showed that increased microbial abundance was not just derived from added sucrose, but also from SOC sources. We also observed a shift in community composition toward microorganisms that are closely associated with the rhizosphere, particularly Gram-negative bacteria and fungi. Indeed, the δ^{13}C-PLFA of these microbial groups suggested that they took up a higher proportion of added sucrose, and likely used a greater proportion of recently fixed C sources.

The difference in SOC decomposition metrics—SOC-derived respiration, PLFA abundance, and microbial community activity and composition—between eCO_2 and aCO2 soils was qualitatively similar to the differences induced by laboratory sucrose addition, providing mechanistic support for the inference of a priming effect in eCO_2 soils. It is difficult to quantitatively compare the short-term influence of sucrose addition to the decade-long influence of increased belowground C inputs to soils under eCO_2; however, the priming effect in both soils was of similar size (Fig. 1). Indeed, if the 30% increase in respiration for eCO_2 soils measured here are representative of a priming effect, it could explain Talhelm and

colleagues' (2009) finding of 17.4 Mg C ha^{-1} less C in eCO_2 plots over 7 years given estimated input rates ($\sim$1.4 Mg C ha^{-1}).

It is possible that sucrose addition is not a good proxy for increased belowground C inputs under eCO_2. Specifically, eCO_2 experimental plots at Aspen FACE experienced increased inputs of many types of organic C substrates to soils, such as increased leaf and root litter as well as increased root exudation. However, it is very likely that higher leaf litter inputs do not contribute to the priming effect in soils at the Aspen FACE site, as demonstrated by the negative priming effect observed by Liu et al. (2009) after adding varying amounts of leaf litter in a soil incubation experiment. Changes in litter quality have been observed with eCO_2 at Aspen FACE (e.g., Parsons et al., 2008), but this effect was shown to be relatively unimportant for SOC cycling at the site (Liu et al., 2009). While we do not have direct observational evidence of increased rhizodeposition under eCO_2 at Aspen FACE, Johnson and Pregitzer (2007) report a marginally significant increase in total soluble sugars in soils of eCO_2 plots at the Aspen site. In addition, eCO_2 strongly increased fine root biomass (King et al., 2005; Zak et al., 2011), so the proportion of soil in contact with the rhizosphere likely increased along with root abundance (Phillips et al., 2011).

One of the original goals of this experiment was to determine whether the sucrose-driven priming of SOC decomposition in eCO_2 soils was due to faster cycling of new, FACE-derived C inputs, or rather from acceleration of the turnover of decades-old and new SOC in these soils. We found no difference in the fraction of FACE-derived C respired from soils with a priming effect and their controls (Table 6). In fact, the $\Delta^{14}C$ signature of soils receiving added sucrose was identical to those receiving only water after the contribution of sucrose respiration to the isotopic value of CO_2 was separated out. This suggests that the same sources of C contributed to the priming effect as to basal respiration in the water addition control soils. However, we cannot rule out the possibility of primed C consisting solely of new, FACE-labeled, root-derived C due to the small size of the priming effect and spatial heterogeneity of CO_2 fluxes and their $\Delta^{14}C$ signatures.

Other studies done at the Aspen FACE site provide additional evidence for rhizosphere priming of SOC. Despite the increase in fine root biomass with eCO_2, decomposition rates of fine root biomass did not change in eCO_2 plots relative to aCO_2 (Chapman et al., 2005), eliminating different root decomposition rates under eCO_2 as a cause of these differences. Moreover, all SOC pools separated by physical methods were labeled; new, FACE-derived C comprised at least 40% of even the slowest cycling SOC fraction (Hofmockel et al., 2011). Loss of SOC from the mineral-associated fraction of these soils with eCO_2 provides further support for the involvement of SOC decomposition in the observed increases in respiration from eCO_2 soils (Hofmockel et al., 2011). In addition, increased activity of cellulolytic enzymes with eCO_2 has been observed at Aspen FACE (Larson et al., 2002), and is considered a key indicator of microbially-driven priming effects (Kuzyakov, 2010).

4.2. eCO_2 does not affect microbial processing of newly added C substrates

The alternative hypothesis, that decomposition of newly added C inputs was fundamentally different in eCO_2 soils, was not supported by the results of this experiment. We found that SUE for added sucrose was not different between eCO_2 and aCO_2 soils, suggesting eCO_2 did not alter the proportion of new C inputs to soils that were lost to respiration vs. incorporated into microbial cell membranes, with the chance to be stabilized in soil over a longer time period (Grandy and Neff, 2008). Our findings are limited by the fact that we only could measure the SUE of added sucrose, which may not be representative of the overall suite of microbial C sources, and that we only measured sucrose-C incorporated into PLFA. However, Larson et al. (2002) found no effect of eCO_2 on microbial metabolism of a number of root-derived substrates at Aspen FACE.

4.3. Mechanisms of microbial respiration response to increased C inputs

Despite lack of change in SUE, other changes to the microbial community were observed that explain the observed increase in respiration with higher levels of C inputs. Greater microbial abundance without a change in respiration on a biomass-specific basis suggests that a larger microbial community may be more efficient at decomposing any SOC that becomes available to microorganisms, either by the action of increased extracellular enzyme activity, or through abiotic processes, such as described in the "Regulatory Gate" hypothesis (Kemmitt et al., 2008). Essentially, more microorganisms mean that the microbial decomposition process is better able to compete for soluble forms of C against abiotic stabilization factors like sorption to mineral surfaces (Conant et al., 2011), increasing the probability that any C molecule in soil is taken up into biomass and respired (Gleixner, 2013).

Microbial community composition changes may also be related to the observed priming effect. In general, greater C inputs lead to an increase in the abundance of Gram-negative bacteria and fungi that are often associated with the rhizosphere (Lu et al., 2004). Indeed, $\delta^{13}C$ of PLFA supports this interpretation— more enriched $\delta^{13}C$-PLFA values of these groups relative to the whole community with sucrose addition demonstrates the ability of this group to compete for easily decomposable C, and more depleted $\delta^{13}C$-PLFA of these groups relative to the community in eCO_2 soils suggests the importance of recently added rhizodeposits as a C source. Organisms with these characteristics are often termed as copiotrophs—organisms that thrive under high substrate availability, as opposed to oligotrophs—organisms that are better equipped to tolerate low substrate availability (Fierer et al., 2007). Copiotrophs necessarily have high turnover rates (Blagodatskaya et al., 2007), suggesting that increased rates of microbial turnover may be responsible for some portion of increased respiration with sucrose addition. This interpretation is supported by the observed increase in protozoa abundance with eCO_2, as protozoa are the primary predators in a bacteria-dominated soil food chain (Kuzyakov et al., 2000). Counterintuitively, protozoa PLFAs became more $\delta^{13}C$-depleted with addition of $\delta^{13}C$-enriched sucrose, suggesting that sucrose addition induced protozoa to consume soil microorganisms with the most depleted $\delta^{13}C$-PLFA values.

4.4. Warming enhances the rate, not amount, of priming for a given C input

Increased rates of SOC cycling caused by increased C inputs were exacerbated by warming—twice as much priming from sucrose was observed in soils at 25 °C compared to 5 °C at the time of the first respiration measurement. Enhancement of the priming effect with warming has also been observed in a soil warming experiment with live plants (Zhu and Cheng, 2011), suggesting that warming increases the rate of priming as long as sufficient levels of substrate are available. This was the case for eCO_2 soils, in which warming had a greater effect on respiration relative to aCO_2 soils for the duration of the experiment, indicating continued higher substrate availability.

In contrast, the sucrose addition experiment showed that warming affected the rate, not the amount, of priming for a given amount of added substrate. The priming effect from added sucrose declined over time in proportion to the amount of sucrose remaining in soil, so the priming effect in 5 °C soils caught up to the priming effect in 25 °C soils by the time the same amount of sucrose had been lost. This suggests that amount of priming is primarily determined by the amount of microbially-available C substrate added to soils (de Graaff et al., 2010; Dilly and Zyakun, 2008). Ultimately, there was no difference in the amount of priming effect across temperature for the same amount of sucrose respired, suggesting that SOC loss through the priming effect will be limited by the rate of substrate supply from plants relative to consumption by microorganisms.

4.5. Interactive effects of warming and increased C inputs on soil microbial community form and function

In this experiment, temperature sensitivity of microbial respiration appeared greater in soils with higher substrate availability, as previously observed in soils from the microcosm (Gershenson et al., 2009) to ecosystem scale (Curiel Yuste et al., 2007). The interaction between warming and substrate availability suggests that there are distinct mechanisms by which these two factors affect microbial respiration—warming allowed microorganisms to take up and metabolize substrates more quickly, and sucrose addition made greater amounts of C available to soil microorganisms in general. Sucrose addition increased microbial PLFA and respiration together, indicating that short-term substrate availability is the primary control over microbial community size and activity, and likely the mechanism for the priming effect. Neither elevated CO_2 nor warming changed SUE, in contrast to previous studies which found that warming reduced SUE (e.g., Steinweg et al., 2008). Lack of observed change in SUE in our experiment is likely due to the fact that we controlled for faster substrate processing rates with warming by sampling soils from higher incubation temperatures sooner, and thus eliminating the potential bias of substrate depletion (Dijkstra et al., 2011).

In contrast to eCO_2 and sucrose addition, warming had few independent effects on the microbial community, subtly increasing the abundance of Gram-positive bacteria, and decreasing the fungal to bacterial ratio. Positive responses of Gram-positive bacteria have been observed in many experiments (e.g., Frey et al., 2008; Feng and Simpson, 2009), including field warming experiments that received a continual supply of new C inputs from overlying plant communities (e.g., Bardgett et al., 1999; Gutknecht et al., 2012). In this study, the effects of warming on the microbial community were greatest in eCO_2 and sucrose addition treatments, where it appeared that warming helped Gram-positive bacteria better compete for increased C inputs. Field warming has also been observed to lessen the stimulatory effect of eCO_2 on Gram-negative bacteria (Gutknecht et al., 2012).

Indeed, the differing physiology of the two bacteria types may provide the key to the differences in warming response, particularly in the sucrose addition treatment. The strong interlinked cell walls of Gram-positive bacteria are thought to make them inherently more resistant to stress than the single layer cell wall of Gram-negative bacteria (Schimel et al., 2007). Gram-negative bacteria likely responded rapidly to sucrose additions by growing; however, active growth makes microorganisms more vulnerable to rapid substrate exhaustion with warming (Schimel et al., 2007 and references therein). Thus, warming gave Gram-positive bacteria a competitive advantage over Gram-negative bacteria that became particularly apparent with the difference in exploitation of a new resource, sucrose.

4.6. Implications of global change on future SOC balance: elevated CO_2 and warming are likely to cause net loss of SOC through increased heterotrophic respiration

The C balance of soils in the future depends on how higher atmospheric CO_2 levels and global warming alter inputs, outputs, and residence time of C in soils. We found that the fate of new C inputs, quantified as a laboratory addition of traceable sucrose into respired CO_2 and PLFA, did not change in eCO_2 or warmed soils. In contrast, we found strong effects of increased C inputs and warming on decomposition outputs. These effects were particularly pronounced when warming and substrate treatments were applied together. In addition, the isotopic composition of additional C lost by both substrate-induced priming and warming suggests that this effect will most likely not be limited to young, fast-cycling SOC, but applies to the majority of the SOC stock (53–94%, Hopkins et al., 2012). Together, these findings suggest that the residence time of C in soils will decrease, and that soils will become a net source of C to the atmosphere in the future.

Large, ecosystem-scale manipulations such as FACE are powerful because they allow us to test the accuracy of our predictions about global change, and to closely investigate the processes that determine an ecosystem-scale observation. We found that both eCO_2 and warming increased SOC decomposition rates in these soils, and had an even greater effect together, suggesting that these factors in concert will cause a net loss of SOC to the atmosphere. In contrast, global climate-C cycle models currently predict that eCO_2 will add SOC to soils, and at least partially counterbalance increased SOC decomposition with warming (Todd-Brown et al., 2013). While the amount of experimental warming (10 and 20 °C) was quite large compared to predictions for the 21st century, sucrose addition and elevated CO_2 treatments are very reasonable, even modest, estimates of changes to SOC inputs (Friedlingstein et al., 2006). Other eCO_2 experiments have observed a similar lack of accumulation of SOC in soils (e.g., Carney et al., 2007), and our work suggests that the large microbial community that develops with higher C input rates to soils will more efficiently return any available C in soils back to the atmosphere. These results highlight the urgent need to better understand soil microbial processes, and to incorporate these findings into predictive models.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.04.028.

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