

5-23-2019

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Recommended Citation

Dove, N. C., Stark, J. M., Newman, G. S., and Hart, S. C.. 2019. Carbon control on terrestrial ecosystem function across contrasting site productivities: the carbon connection revisited. *Ecology* 00(00):e02695. 10.1002/ecy.2695

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Carbon control on terrestrial ecosystem function across contrasting site productivities: the carbon connection revisited

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Citation: Dove, N. C., J. M. Stark, G. S. Newman, and S. C. Hart. 2019. Carbon control on terrestrial ecosystem function across contrasting site productivities: the carbon connection revisited. *Ecology* 00(00):e02695. 10.1002/ecy.2695

Abstract. Understanding how altered soil organic carbon (SOC) availability affects microbial communities and their function is imperative in predicting impacts of global change on soil carbon (C) storage and ecosystem function. However, the response of soil microbial communities and their function to depleted C availability in situ is unclear. We evaluated the role of soil C inputs in controlling microbial biomass, community composition, physiology, and function by (1) experimentally excluding plant C inputs in situ for 9 yr in four temperate forest ecosystems along a productivity gradient in Oregon, USA; and (2) integrating these findings with published data from similar C-exclusion studies into a global meta-analysis. Excluding plant C inputs for 9 yr resulted in a 13% decrease in SOC across the four Oregon sites and an overall shift in the microbial community composition, with a 45% decrease in the fungal : bacterial ratio and a 13% increase in Gram-positive : Gram-negative bacterial ratio. Although gross N mineralization decreased under C exclusion, decreases in gross N immobilization were greater, resulting in increased net N mineralization rates in all but the lowest-productivity site. Microbial biomass showed a variable response to C exclusion that was method dependent; however, we detected a 29% decrease in C-use efficiency across the sites, with greater declines occurring in less-productive sites. Although extracellular enzyme activity increased with C exclusion, C exclusion resulted in a 31% decrease in microbial respiration across all sites. Our meta-analyses of published data with similar C-exclusion treatments were largely consistent with our experimental results, showing decreased SOC, fungal : bacterial ratios, and microbial respiration, and increased Gram-positive : Gram-negative bacterial ratio following exclusion of C inputs to soil. Effect sizes of SOC and respiration correlated negatively with the duration of C exclusion; however, there were immediate effects of C exclusion on microbial community composition and biomass that were unaltered by duration of treatment. Our field-based experimental results and analyses demonstrate unequivocally the dominant control of C availability on soil microbial biomass, community composition, and function, and provide additional insight into the mechanisms for these effects in forest ecosystems.

Key words: ¹⁵N pool dilution; carbon-use efficiency; extracellular enzyme activity; gross N immobilization; gross N mineralization; meta-analysis; microbial ecology; microbial respiration; nitrogen cycle; phospholipid fatty acid analysis; soil carbon availability.

INTRODUCTION

Microbial growth and activity in soils are controlled by the availability of soil organic carbon (SOC; Paul 2006). Hence, reduced plant productivity and below-ground plant C allocation radically alter microbial community composition and function (Bååth et al. 1995, Siira-Pietikäinen et al. 2001b, Smith et al. 2008). This can lead to ecosystem-level effects such as increased

nitrogen (N) leaching (Vitousek and Melillo 1979), loss of obligate microbial symbionts necessary for plant growth (Perry et al. 1989), and reduced C sequestration (Baldocchi 2008). However, changes to the physical (e.g., altered temperature and moisture; Binkley and Fisher 2012) and chemical (e.g., soil acidification and N loss; Dahlgren and Driscoll 1994) environment associated with loss of plant biomass hinders a clear, mechanistic understanding of the impact of reductions in below-ground C allocation on soil microorganisms.

Distinct heterotrophic soil microbial communities emerge under different levels of SOC availability (Myers et al. 2001). Zymogenous (fast-growing, inefficient C

Manuscript received 25 October 2018; revised 8 February 2019; accepted 25 February 2019. Corresponding Editor: Serita D. Frey.

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use) and autochthonous (slow-growing, efficient C use) microorganisms reflect a spectrum of heterotrophic metabolism under gradients of C quality and quantity in soils, and promote different rates of SOC or nutrient mineralization and immobilization (Koch 2001, Fierer et al. 2007). Furthermore, fungal : bacterial ratios have been shown to correlate negatively with SOC quality (Högberg et al. 2006, Rousk and Bååth 2007, Strickland and Rousk 2010). Because of the higher microbial carbon to nitrogen mass ratio (C : N, Cleveland and Liptzin 2007) and ability to release oxidative lignin-degrading enzymes (Hanson et al. 2008, Floudas et al. 2012, Treseder and Lennon 2015), fungi are generally able to degrade higher C : N substrates than bacteria. Changes in the relative abundance of bacteria and fungi could affect the overall metabolism and nutritional needs of the microbial community (Strickland and Rousk 2010). Hence, shifts in the microbial community composition induced by changes to SOC availability could *indirectly* lead to altered microbial function (e.g., decomposition and nutrient transformations).

Soil microbial functions are also *directly* controlled by SOC availability. For instance, rates of microbial respiration are often related to SOC availability (Birge et al. 2015). As such, microbial respiration rates are strongly related to net primary productivity (NPP; Raich and Schlesinger 1992), and experimentally reduced C inputs have been shown to decrease microbial respiration (Bowden et al. 1993, Rey et al. 2002, Li et al. 2004, Wang et al. 2013). Furthermore, because N mineralization primarily occurs through the microbial cleavage of C—N bonds (McGill and Cole 1981), gross N mineralization is positively correlated with soil CO₂ evolution (SOC; Hart et al. 1994a, Booth et al. 2005) and NPP (Stark and Hart 1997). Inorganic N immobilization is also correlated with SOC availability and microbial growth because of the stoichiometric relationships of microbial biomass. This has led to numerous microbial and enzymatic models that incorporate the interaction between SOC decomposition and N mineralization (Schimel and Weintraub 2003, Saetre and Stark 2005, Moorhead et al. 2012, Abramoff et al. 2017). However, as noted above, SOC quantity and quality often covary with other physiochemical environmental conditions. Thus, separating the effect of SOC on microbial processes from pH, soil moisture, soil temperature, and nutrient availability is challenging.

Long-term laboratory incubations are able to detect changes to the soil microbial community composition and function in response to decreased SOC quality and quantity over time, while controlling for multiple physiochemical conditions. After a 707-d incubation, respiration and microbial biomass, but not extracellular enzyme activity, declined in concert with a 20% decrease in organic C (Birge et al. 2015). During an 18-month incubation, Frostegård et al. (1996) detected a decrease in the fungal : bacterial ratio, which they attributed to a loss of ectomycorrhizal fungi. However, in a 450-d

incubation, Hart et al. (1994a) showed that microbial C : N increased with time, and they attributed this to an increase in the fungal : bacterial ratio due to a reduction in the labile SOC pool (and a relative increase in recalcitrant SOC). Hart et al. (1994a) also measured overall decreases in respiration, C-use efficiency (CUE), gross N mineralization, and gross nitrification with time. Taken together, these results suggest that SOC is a major controlling factor in microbial community structure and soil N cycling. However, although laboratory experiments can be useful for controlling confounding effects, extrapolating these results to the field can be difficult because of disturbances to soil structure and changes to soil moisture and temperature regimes.

Multiple field-based SOC manipulation studies have investigated impacts of altered SOC availability on microbial properties and processes through complete C exclusion (i.e., prevention of above- and belowground C inputs) as part of the Detritus Input and Removal Treatment (DIRT) network (Appendix S1: Table S1; Nadelhoffer et al. 2004). Although some of these studies have shown altered microbial community composition, reduced microbial respiration, and reduced extracellular enzyme activity with C exclusion (Brant et al. 2006a, b, Wang et al. 2013, Lajtha et al. 2014a), others (Holub et al. 2005, Yarwood et al. 2013, Veres et al. 2015) have failed to detect a significant effect of SOC depletion on soil microbial communities. It is unlikely that all ecosystems respond similarly to C exclusion and depletion. For instance, it is currently unclear whether SOC pools are more or less sensitive to perturbations in sites with higher standing SOC stocks (Hart and Sollins 1998, Crowther et al. 2016, van Gestel et al. 2018), because differences in sampling and laboratory techniques could mask important site-specific properties or interactions with ecological gradients even if the experimental treatment is consistent (Dove and Hart 2017). Therefore, both consistent measurements of the microbial response to C exclusion across ecological gradients and robust, quantitative, and synthetic analysis of C-exclusion studies may help determine the overall response and identify significant moderators of the effect of experimentally reduced SOC availability on soil microbial communities.

We evaluated the role of SOC in controlling soil microbial biomass, community composition, and function by (1) experimentally excluding plant C inputs for 9 yr at four forest sites along a productivity gradient in Oregon, USA, and (2) integrating these findings with published data from the DIRT network into a global meta-analysis. We hypothesized that 9 yr of C exclusion in the Oregon sites would reduce C availability leading to altered microbial community composition and reduced biomass. Specifically, we expected an increase in the fungal : bacterial ratio due to greater SOC recalcitrance and an increase in the ratio of Gram-positive : Gram-negative bacteria because exclusion of plant roots should decrease the relative abundance of rhizosphere-associated bacteria, which are

predominately Gram-negative (Kennedy and de Luna 2005). We also hypothesized that decreased C availability would reduce N immobilization, increase N mineralization, and decrease microbial respiration and extracellular enzyme activity. We expected the greatest relative and absolute response to C exclusion to occur in the high-productivity sites (with the greatest standing SOC stock) because the relatively larger annual C flux in these sites would be disrupted to a greater extent. For the meta-analysis, we hypothesized that the effect size of C exclusion on SOC would increase with length of C exclusion and initial SOC concentrations. Additionally, we hypothesized that the effect of C exclusion on microbial respiration, biomass, and community composition would increase with the effect size of SOC and length of C exclusion. Our overall goal was to characterize the response of the microbial community to C exclusion and to elucidate the role of SOC availability on microbial community composition and function, especially the linkages between C- and N-cycling processes.

METHODS

Study sites, treatment, and soil sampling

This study consists of four mature, relatively undisturbed sites along an east–west moisture-productivity gradient in northwestern Oregon (Table 1; Gholz 1982, Runyon et al. 1994, Stark and Hart 1997). From east to west, the sites are named according to the dominant overstory vegetation as follows: western juniper (*Juniperus occidentalis* Hook.; WJ), ponderosa pine (*Pinus ponderosa* Douglas ex. C. Lawson; PP), mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.; MH), and Sitka spruce–western hemlock (*Picea sitchensis* (Bong.) Carr. and *Tsuga heterophylla* (Raf.) Sarg., respectively; SH). Both PP and WJ experience extreme drought in the summer months (Peterson and Waring 1994).

In August 1994, paired C-excluded (–C) and unconfined control (UC) plots were established at each site. Belowground C exclusion was achieved by trenching plots by pounding 25 cm diameter thin-walled steel pipe 30 cm deep into the ground. We decided upon a 30-cm depth because bedrock prevented us from trenching deeper at the MH site, and we wanted to keep trenching

depth consistent among sites. Trenching to 30-cm depth was sufficient in preventing root intrusion because over 50% of roots in the upper 2 m occur above this depth in coniferous forests (Jackson et al. 1996). Furthermore, ion-exchange resin (IER) bags were placed beneath the cores to evaluate nutrient retention (*unpublished data*), so at regular intervals (annually to biennially), we pulled out the cylinders to exchange the IER bags and checked for any roots that were penetrating upwards into the core, which never occurred. Aboveground litter inputs were excluded from the trenched plots by covering the pipes with a 1-mm aluminum mesh screen. The UC plots consisted of undisturbed areas within 3 m of the –C plots. Five blocks containing the two treatments were randomly located within a 0.25-ha area at each site.

In August 2003, 4.8 cm diameter by 15 cm deep soil cores, which included the A and upper part of the B horizon (amount of B horizon varied by site), were collected from the mineral soil layer (after carefully removing any O horizon material) within each plot for field ¹⁵N isotope dilution assays (Hart et al. 1994b). Additional bulk soil was collected from the 0–15-cm mineral soil layer in each plot for supplemental analyses (see below). Soils were kept cool on blue ice in a cooler during transport to the laboratory. Immediately after return from the field, these bulk soil samples were homogenized, sieved to <2 mm, and stored at 4°C until PLFA analysis, chloroform fumigation, or extracellular enzyme assays (these analyses occurred within a week after sampling).

Soil pH, total C, and total N

Freeze-dried, archived soils were analyzed for pH, total C, and total N in 2015. It is unlikely that the storage impacted these soils, given that pH, total C, and total N values are stable for long periods when freeze-dried (Sheppard and Addison 2008). We measured pH in a 1:2 ratio of soil to both water and 0.01 mol/L CaCl₂ solutions with an Orion DUAL STAR pH meter (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Prior to total C and N analysis, freeze-dried soils were ground to a fine powder using a roller mill. Approximately 40 mg of oven-dry, ground soil was weighed into tin capsules and analyzed for total C and N by continuous-flow, direct combustion, and mass

TABLE 1. Environmental characteristics of the study sites (adapted from Stark and Hart 1997).

Ecosystem	Symbol	Elevation (m)	MAT† (°C)	MAP‡ (mm)	ANPP§ (Mg·ha ⁻¹ ·yr ⁻¹)	Soil classification (great group)
Western juniper (<i>Juniperus occidentalis</i>)	WJ	930	9.0	220	1.2	Torriorthent
Ponderosa pine (<i>Pinus ponderosa</i>)	PP	1,030	7.5	540	2.2	Vitrandept
Mountain hemlock (<i>Tsuga mertensiana</i>)	MH	1,460	5.1	1,800	5.1	Cryandept
Sitka spruce/western hemlock (<i>Picea sitchensis</i> / <i>Tsuga heterophylla</i>)	SH	240	13.0	2,400	13.0	Haplohumult

† Mean annual temperature.

‡ Mean annual precipitation.

§ Aboveground net primary production.

spectrometry using a Europa Scientific 20-20 mass spectrometer (PDZ, Crewe, United Kingdom). These soils did not contain free carbonates (determined by lack of effervescence with addition of 1 mol/L HCl), so total C is equal to SOC.

Microbial community composition and biomass

We used relative differences in the amount of phospholipid fatty acids (PLFAs) to determine changes to the microbial community composition (mol%) and biomass. Back in the laboratory, subsamples of sieved soil were immediately frozen for 24 h, then freeze-dried (50°C, 70×10^{-3} mbar for 24 h, Edwards Modulyo, Crawley, UK) prior to extraction for PLFA. The extraction and analysis method we utilized is described in detail in White and Ringelberg (1998).

Individual PLFA markers were used to quantify the relative abundances of specific microbial guilds. We assigned i15:0, a15:0, i16:0, and 10me16:0 as PLFA indicators for Gram-positive bacteria, and cy17:0, 16:1 ω 9c, and cy19:0 as indicators for Gram-negative bacteria (Frostegård and Bååth 1996, White et al. 1996, O'Leary and Wilkinson 1988). The PLFA 10me16:0 was used as a specific indicator for actinobacteria (Zelles 1999). The PLFA 18:2 ω ,6,9 was used as an indicator for fungi (Frostegård and Bååth 1996). Fungal to bacterial (fungal : bacterial) ratios were determined by the PLFA 18:2 ω ,6,9 divided by the sum of Gram-positive and -negative bacteria PLFAs (Strickland and Rousk 2010). Microbial biomass was measured as the sum of all detected 14–19 C-length PLFAs. Longer PLFAs are indicators of mosses, eukaryotes, and higher plants (Zelles 1999).

We also measured microbial biomass by chloroform fumigation extraction (CFE; Vance et al. 1987, Haubensak et al. 2002). Fumigated and nonfumigated extracts were analyzed for total organic C using a Dohrmann DC-80 carbon analyzer with an infrared detector (Tekmar-Dohrmann, Cincinnati, Ohio). We used an extraction efficiency factor (k_{cC}) of 0.45 to convert chloroform-labile C to microbial C (Beck et al. 1997).

Gross and net rates of N transformations and respiration

We used ^{15}N pool dilution techniques to determine net and gross rates of N mineralization and inorganic N immobilization (Hart et al. 1994b). Briefly, we collected a 4.8 cm diameter by 15 cm long soil core from each of the two treatments in each of the five plots at each site. Immediately after collection, each core was injected with 16 mL of solution containing 1.8 mmol/L of ^{15}N as $(^{15}\text{NH}_4)_2\text{SO}_4$ (at 99 atom % ^{15}N). The solution was injected with a syringe and a 15 cm long, 18-gauge, double side-port needle. The needle was inserted into the core and the syringe plunger was depressed as the needle was withdrawn to distribute the solution throughout the length of the soil core. Four 2-mL injections were made from the core bottom and four 2-mL injections were

made from the top to further ensure uniform distribution of the solution throughout the soil. Immediately after injection, the soil in the core was homogenized by hand, and a 20-g (oven-dry equivalent) subsample was extracted in 200 mL of 2 mol/L KCl to measure the initial inorganic N pool sizes and ^{15}N enrichments (see below). A second 20-g subsample was sealed in a 1-L canning jar with a lid containing a butyl rubber septum to allow collection of headspace gas samples for CO_2 analyses. The two canning jars containing soil from control and C-exclusion treatments were then reburied at the same location in a plot for a 24-h in situ incubation. The remaining soil from the core was sealed in a polyethylene bag and kept cool during transport back to the laboratory on blue ice contained in an ice chest. After the 24-h in situ incubation, a 25-mL gas sample was collected from the headspace of each canning jar and stored in an evacuated tube, and the soil subsample was extracted in 2 mol/L KCl to measure the final inorganic N pool sizes and ^{15}N enrichments.

Initial and final inorganic N pools were determined by extracting soil with 2 mol/L KCl (1:10 soil mass : solution volume). An aliquot of each extract was analyzed for NH_4^+ and NO_3^- concentration with a Lachat Quick-Chem 8500 Series 2 Colorimetric Analyzer (Lachat Instruments, Milwaukee, Wisconsin, USA) by the indophenol and cadmium reduction methods, respectively. The remaining extract was prepared for ^{15}N analyses with a diffusion procedure described in Stark and Hart (1996), and the ^{15}N enrichment was measured by continuous-flow direct dry combustion and mass spectrometry with the use of a Europa 20-20 system (PDZ, Crewe, UK). Headspace gas samples were analyzed for CO_2 concentration using a Varian 3300 gas chromatograph (Agilent, Santa Clara, California, USA) equipped with a thermal conductivity detector. Initial and final inorganic N pool sizes and ^{15}N enrichments in soil samples injected with $^{15}\text{NH}_4^+$ were used to calculate gross N mineralization rates based on the equations from Kirkham and Bartholomew (1954). Gross inorganic N immobilization was calculated from the difference between gross N mineralization and net N mineralization rates.

With the use of microbial respiration and gross N immobilization, we calculated CUE with the use of the equations derived in Schimel (1988). We assumed the carbon-to-nitrogen mass ratio of microbial biomass ($\text{C} : \text{N}_{\text{biomass}}$) to be 6. This approach defines the minimum value of CUE because maintenance respiration of the nongrowing portion of the microbial community and organic N assimilation are assumed to be negligible, and the $\text{C} : \text{N}_{\text{biomass}}$ value used is also at the low extent of its range (Wardle 1992, Stark and Hart 2003).

Extracellular enzyme activity

We measured potential extracellular enzyme activity fluorometrically of α -glucosidase (AG), β -glucosidase

(BG), β -xylosidase (BX), cellobiohydrolase (CB), β -galactosidase (GA), N-acetylglucosaminidase (NAG), and alkaline phosphatase (AP) with the use of a microtiter method adapted from Sinsabaugh et al. (2000). The enzymes AG, BG, BX, CB, and GA are involved in the degradation of organic C, and total C-degrading enzyme activity (C_{sum}) was operationally defined as the sum of these five enzymes activities. The enzyme NAG is involved in releasing N-acetylglucosamine from aminopolysaccharides such as chitin and peptidoglycan, and thus is considered an N-acquiring enzyme. Alkaline phosphatase is involved in releasing phosphate from ester bonds, representing a P-mineralizing enzyme (Burns et al. 2013).

Extracellular enzyme activities were expressed on both soil mass and microbial biomass (measured by PLFA and CFE) bases, reflecting ecosystem- and microbial community-level properties, respectively. Expressing activity on a biomass basis represents the nutritional status of the microbial community, which allows comparisons across vastly different ecosystems (Boerner et al. 2005).

We also measured the ratio of C-, N-, and P-acquiring enzymes. Because extracellular enzymes mediate nutrient acquisition for soil microorganisms, they can be used to determine relative nutrient demand (Olander and Vitousek 2000, Sinsabaugh et al. 2009). Hence, we used $C_{\text{sum}} : \text{NAG}$, $C_{\text{sum}} : \text{AP}$, and $\text{NAG}:\text{AP}$ as C : N, C : P, and N : P relative demand ratios, respectively.

Statistical analyses

All statistical tests and visualizations were conducted in R (R Development Core Team 2008) with the vegan (Oksanen et al. 2013), car (Fox and Weisberg 2011), nlme (Pinheiro et al. 2017), and metafor (Viechtbauer 2010) packages. Changes in community composition were analyzed with nonparametric multivariate analysis of variance (PERMANOVA, Anderson 2001) using Bray-Curtis distances. PERMANOVA is a nonparametric approach to determine significant differences ($\alpha = 0.05$) of mean within-group distances among groups. PLFA (mol %) signatures were analyzed as community members, groups were based on treatment and site location, and permutations were constrained by the paired plot (i.e., block). Differences in community composition were visualized using nonparametric multidimensional scaling (NMDS) plots along two dimensions using Bray-Curtis distance.

For all univariate data, we used mixed-design analysis of variance (ANOVA) to determine significant differences among sites, treatments, and site \times treatment interaction. Treatment was nested within each paired plot. We used QQ plots and scale-location plots to inspect normality and homoscedasticity, respectively. If these assumptions were not met, data were then log-transformed and reanalyzed, which occurred for SOC, soil total N, CFE-microbial biomass, and the extracellular enzyme activities. Where significant differences were

detected, we used Tukey's Test of Honest Significant Differences (HSD) to determine which sites or interactions differed. Where data were incomplete, causing the ANOVA to become unbalanced, we used type III or type II sum of squares to calculate our ANOVA depending if the interaction was significant or not, respectively (Langsrud 2003). When ANOVAs are balanced, types I, II, and III sum of squares are equivalent (Fox 2015).

We used a meta-analysis to integrate quantitatively the effect size of C exclusion on SOC pools, microbial biomass, microbial respiration, the fungal : bacterial ratio, and the Gram-positive : Gram-negative bacterial ratio in the Oregon sites to previous field-based C-exclusion studies. We incorporated all known published measurements (as of August 19, 2018) of the effect of *complete* exclusion of plant C inputs on the 0–10-cm or 0–15-cm soil C pool from 19 studies in addition to our experiment. The effect size was calculated as the natural log of the response ratio ($\ln[R]$). The response ratio (R) is the mean of the treatment response divided by the mean of the control ($R = X_{\text{treatment}}/X_{\text{control}}$, Hedges et al. 1999). For example, if $\ln[R] = 0$, then there is no treatment effect. Post-analysis, effect sizes were converted to percent difference using the equation:

$$\text{Difference (\%)} = 100 \times (e^{\ln(R)} - 1)$$

Continuous and categorical random effects models were conducted to determine if effect sizes varied with duration of C exclusion, initial SOC pools (for effect on SOC), method of measurement (for effect on microbial biomass, the fungal : bacterial ratio, and microbial respiration), and mycorrhizal association of the dominant vegetation (for effect on the fungal : bacterial ratio). Location was added to the models as a random effect to account for the lack of independence among multiple measurements from the same site but at different times. Effect sizes were weighted by the inverse variance for each treatment. In cases where standard deviation was not reported (precluding variance calculation; Nadelhoffer et al. 2004, Yarwood et al. 2013), we used the average variance from other studies (Furukawa et al. 2006). Following Aloe et al. (2010), we report R_{Meta}^2 values rather than traditional R^2 based on ordinary least squares (OLS) for the continuous random effects models (meta-regressions), because the assumption of equal variances needed for OLS does not hold in meta-regression (i.e., effect sizes are weighted). The statistic R_{Meta}^2 describes the proportional reduction in the amount of heterogeneity in the model after including moderators, and it is useful for interpreting the practical significance and comparing the fit of competing meta-regression models (López-López et al. 2014). For all the above meta-analyses, the residuals by study variance were symmetric, indicating that published findings were not influenced by statistical power (i.e., publication bias; Duval and Tweedie 2004; Appendix S1: Fig. S1).

RESULTS

Soil pH, total C, and total N

The 9-yr soil C-exclusion treatment at the Oregon forest sites did not significantly nor consistently affect soil pH ($P = 0.505$). Soil pH was negatively correlated with site productivity ($P < 0.001$, Table 2). Nine years of soil C exclusion reduced SOC and N concentrations by an average of 13% (SE = 3%, $P < 0.001$) and 9% (SE = 3%, $P < 0.001$), respectively (Table 2). The greatest relative declines in SOC occurred in the two sites with the lowest productivity (WJ = 19%, PP = 21%). Soil organic C and N concentrations both increased with site productivity ($P < 0.001$). Soil C : N was 4.5% (SE = 1%, $P = 0.003$) lower in the C-excluded than the control plots. Mean soil C : N also differed across sites. Soil C : N in the control plots was 28% lower at the highest-productivity site (SH, C : N = 18.6) compared to PP and MH (mean C : N = 25.8 for both, $P < 0.001$).

Microbial community composition and biomass

Nonmetric multidimensional scaling (NMDS) showed that microbial community composition differed across sites ($P < 0.001$, Fig. 1a). The NMDS axis 1 was positively correlated with site productivity, suggesting a dominant role of SOC in shaping microbial communities. Nine years of C exclusion explained a very small ($r^2 = 0.044$) but significant amount of the variation in the microbial community composition across all sites ($P = 0.001$), with the C-exclusion treatment shifting microbial community composition towards lower values across NMDS axis 1 in all sites except the most productive site (SH). The relative biomass of fungi compared to bacteria was reduced in the C-exclusion treatments by an average of 45% (SE = 7%) across all sites ($P = 0.002$, Fig. 1b). The C-exclusion treatment increased the Gram-positive : Gram-negative bacterial ratio by 11% (SE = 5%) across all sites ($P = 0.002$, Fig. 1c), which was partially explained by a 10% (SE = 4%) average increase in actinobacteria because of the treatment (treatment: $P = 0.040$, location: $P < 0.001$; Fig. 1d).

Microbial biomass, as assessed by CFE, decreased by 32% (SE = 10%) following 9 yr of C exclusion across all

sites ($P = 0.031$; Fig. 2a). However, total PLFA was only marginally decreased (statistically) by the C-exclusion treatment ($P = 0.095$; Fig. 2b). Both measures of microbial biomass increased with site productivity ($P < 0.001$).

Gross and net rates of N transformations

Nine years of C exclusion resulted in a 42% (SE = 6%) reduction in gross N mineralization ($P < 0.001$) across all sites (Fig. 3a). Carbon exclusion also lowered gross rates of N immobilization by an average of 68%. Because the decline in N immobilization at the WJ site was not significant, there was a significant interaction between C exclusion and site ($P = 0.044$, Fig. 3b). Generally, both gross N mineralization and immobilization increased with site productivity (Fig. 3a, b).

The effect of C exclusion on net N mineralization also interacted with site ($P = 0.006$, Fig. 3c). Because decreases in gross N immobilization were greater than the decreases in gross N mineralization in the three highest-productivity sites, the C-exclusion treatment increased net N mineralization from -2.74 (SE = 0.76) to -0.17 mg N·kg⁻¹·d⁻¹ (SE = 0.39; $P = 0.064$) in the PP site, from -1.76 (SE = 0.57) to 2.06 mg N·kg⁻¹·d⁻¹ (SE = 1.21; $P = 0.007$) in the MH site, and from -1.16 (SE = 1.14) to 4.14 mg N·kg⁻¹·d⁻¹ (SE = 0.99; $P < 0.001$) in the SH site. The treatment did not significantly affect net N mineralization in the lowest-productivity WJ site ($P = 0.503$). Overall, the magnitude of the increase in net N mineralization with C exclusion increased with site productivity. This resulted in the microbial communities at the two highest-productivity sites shifting, on average, from functionally net N immobilizers to net N mineralizers.

Respiration, carbon-use efficiency, and extracellular enzyme activity

The C-exclusion treatment reduced in situ microbial respiration by 31% (SE = 12%, $P < 0.001$) and in situ microbial respiration normalized to the mass of soil organic C by 19% (SE = 14%, $P < 0.001$) across all sites (Fig. 4a, b). Microbial CUE was also reduced by 29% (SE = 7%; $P < 0.001$) across all sites because of C

TABLE 2. Mean (and standard error [$n = 5$]) 0–15-cm soil organic carbon (C) and nitrogen (N) concentrations and pH in unconfined control (UC) and carbon-exclusion (–C) plots across the four sites: western juniper (WJ), ponderosa pine (PP), mountain hemlock (MH), and Sitka spruce–western hemlock (SH).

Site	C (g/kg)		N (g/kg)		C:N		pH (0.01 mol/L CaCl ₂)	
	UC	–C	UC	–C	UC	–C	UC	–C
WJ	14.5 (1.3)	11.8 (0.4)	0.62 (0.04)	0.54 (0.04)	23.2 (0.9)	21.8 (0.6)	5.2 (0.4)	5.3 (0.2)
PP	22.6 (2.7)	17.9 (1.8)	0.88 (0.04)	0.73 (0.04)	25.5 (1.3)	24.4 (1.4)	4.7 (0.4)	4.7 (0.2)
MH	57.7 (0.9)	49.2 (7.1)	2.32 (0.54)	2.02 (0.40)	27.4 (3.1)	25.8 (2.0)	4.0 (0.1)	4.3 (0.3)
SH	141.5 (8.5)	135.2 (4.5)	7.46 (0.31)	7.43 (0.09)	18.9 (0.6)	18.2 (0.4)	3.9 (0.5)	3.8 (0.2)

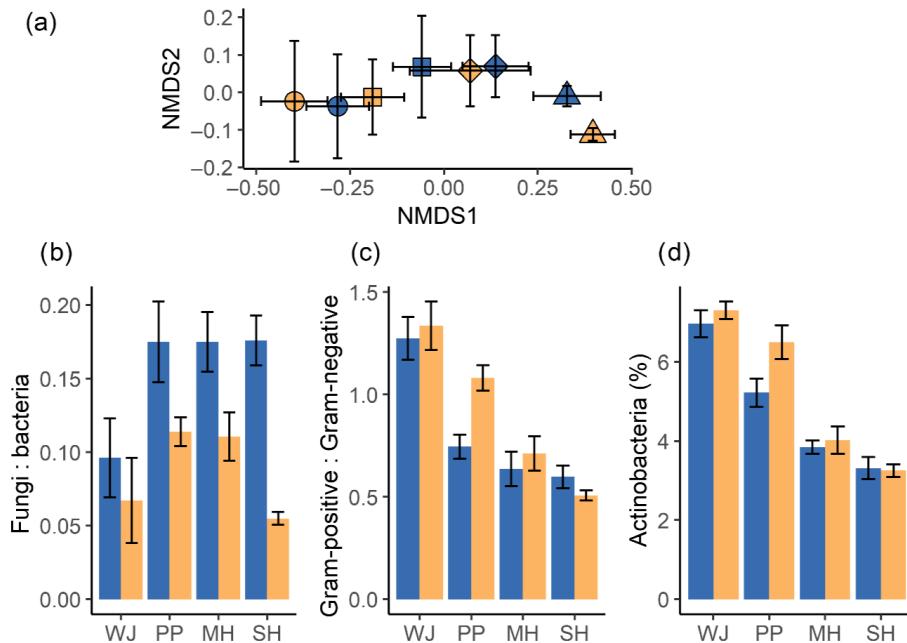


FIG. 1. Response of microbial community composition to carbon (C) exclusion. (a) Nonmetric multidimensional scaling (NMDS) plot of microbial community composition, (b) the fungal : bacterial ratio, (c) Gram-positive : Gram-negative bacterial ratio, and (d) actinobacteria (mol %) across all sites: western juniper (WJ, circles), ponderosa pine (PP, squares), mountain hemlock (MH, diamonds), and Sitka spruce–western hemlock (SH, triangles). Blue bars and shapes represent the unconfined control (UC) plots and gold bars and shapes represent the C-exclusion treatment (–C) plots. Error bars in NMDS plot show 95% confidence intervals and error bars in bar graphs show the standard error of the mean ($n = 5$). NMDS stress = 0.061.

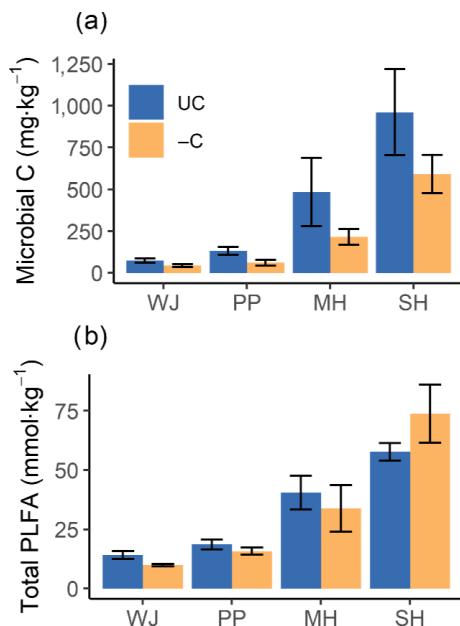


FIG. 2. Microbial biomass assessed by (a) chloroform fumigation extraction and (b) total phospholipid fatty acid (PLFA) between treatments and across sites: western juniper (WJ), ponderosa pine (PP), mountain hemlock (MH), and Sitka spruce–western hemlock (SH). Blue bars represent the mean of the unconfined control (UC) plots and gold bars represent the mean of the carbon-exclusion treatment (–C) plots. Error bars show the standard error of the mean ($n = 5$).

exclusion (Fig. 4c). Although the greatest relative reductions in microbial respiration due to C exclusion occurred at the two intermediate-productivity sites, reductions in CUE were inversely related to site productivity (Fig. 4c).

The C-exclusion treatment increased microbial biomass-normalized C-degrading extracellular enzyme activity (C_{sum}) by an average of 210% (SE = 59%) across all sites ($P < 0.001$, Fig. 5a), with the greatest increase at the most productive site (i.e., SH). Similarly, microbial biomass-normalized NAG and AP activities were increased by an average of 153% (SE = 53%, $P < 0.001$) and 127% (SE = 38%, $P < 0.001$), respectively, by the C-exclusion treatment across all sites (Fig. 5b, c), again with the greatest increase in the most productive site. For all enzymes, the highest activity normalized to microbial biomass occurred in the most productive site ($P < 0.001$). Total PLFA-normalized and absolute extracellular enzyme activities also differed by treatment ($P < 0.05$) and followed similar patterns (Appendix S1: Fig. S2). The C-exclusion treatment did not affect C_{sum} : NAG ($P = 0.543$), C_{sum} : AP ($P = 0.292$), or NAG : AP ($P = 0.107$, Appendix S1: Table S2). However, there was a significant site effect for C_{sum} : NAG and NAG : AP ($P < 0.001$ and $P = 0.016$, respectively). The highest- (SH) and lowest- (WJ) productivity sites had 61% higher C_{sum} : NAG than the intermediate-productivity sites (PP and MH), and the intermediate-productivity sites had 123% higher NAG : AP than the

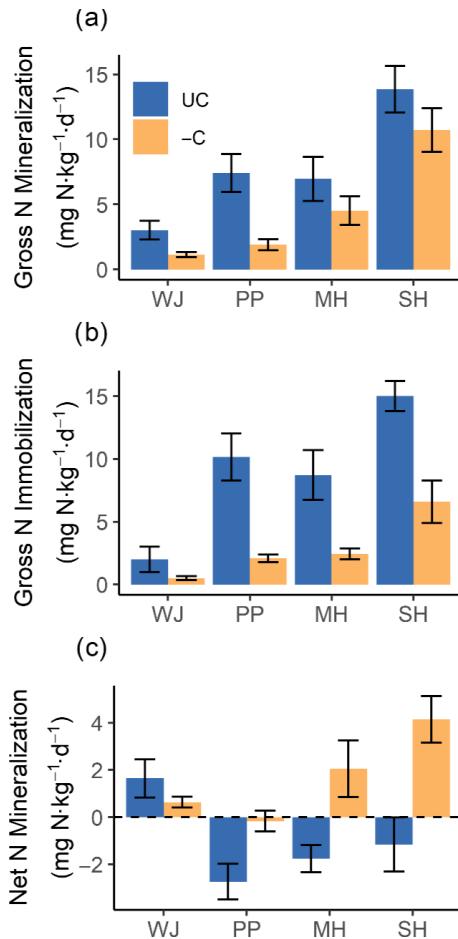


FIG. 3. (a) Gross nitrogen (N) mineralization, (b) gross inorganic N immobilization, and (c) net N mineralization across all sites: western juniper (WJ), ponderosa pine (PP), mountain hemlock (MH), and Sitka spruce-western hemlock (SH). Blue bars represent the mean of the unconfined control (UC) plots and gold bars represent the mean of the carbon-exclusion treatment (-C) plots. Error bars show the standard error of the mean ($n = 5$).

highest- and lowest-productivity sites (Appendix S1: Table S2).

Meta-analysis

Twenty-one records (four from our study) across 12 published studies (and one unpublished Ph.D. dissertation, Brewer 2010) were used to examine the effect of C exclusion on SOC (Appendix S1: Table S3). One record showed a 52% increase in SOC with 5 yr of C exclusion (Holub et al. 2005). This record had a studentized residual of 3.60 and was removed from the final model as an outlier following Lund (1975). Location and initial SOC were not significant moderators of the effect of C exclusion on SOC and were therefore removed from our final model. We found a strong negative correlation between the response ratio of mineral soil C and

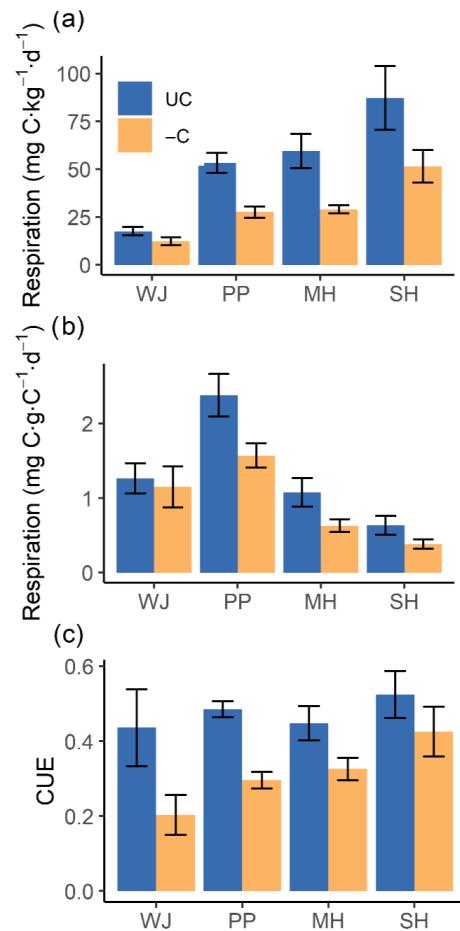


FIG. 4. (a) Microbial respiration, (b) carbon-normalized microbial respiration, and (c) carbon-use efficiency (CUE) across all sites: western juniper (WJ), ponderosa pine (PP), mountain hemlock (MH), and Sitka spruce-western hemlock (SH). Blue bars represent the mean of the unconfined control (UC) plots and gold bars represent the mean of the carbon-exclusion treatment (-C) plots. Error bars show the standard error of the mean ($n = 5$).

duration of C exclusion ($\ln[R] = -0.0106 \times \text{years of C exclusion} - 0.0824$, $R^2_{\text{Meta}} = 0.91$, $P < 0.001$; Fig. 6). Excluding our sites, at time = 9 yr (the duration of our study), the fitted value for reduction in soil C was calculated as -21% (SE = 3%), compared to the average for our sites of -13% (SE = 3%).

Ten records (four from our study) across four published studies (and one unpublished Ph.D. dissertation, Brewer 2010) were used to examine the effect of C exclusion on microbial respiration (Appendix S1: Table S4). We analyzed absolute and SOC-normalized respiration. Location and length of incubation were not significant moderators of the effect of C exclusion on either response variable. We found a negative logarithmic correlation between the response ratio of absolute microbial respiration and duration of C exclusion ($\ln[R] = -0.3489 \times \ln(\text{years of C exclusion}) + 0.1462$,

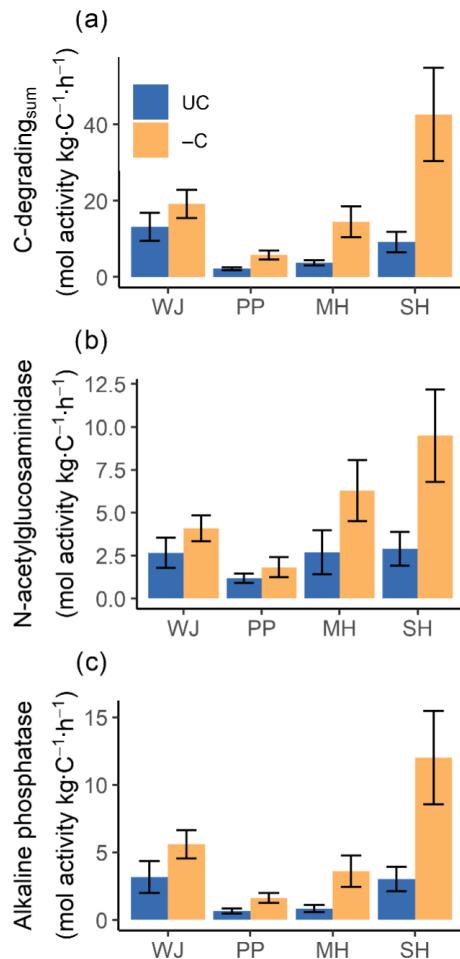


FIG. 5. Microbial biomass-normalized (assessed by chloroform fumigation extraction) extracellular enzyme activity for the sum of α -glucosidase, β -glucosidase, β -xylosidase, cellobiohydrolase, and β -galactosidase. (a) C-degrading_{sum}, (b) N-acetylglucosaminidase, and (c) alkaline phosphatase across all sites: western juniper (WJ), ponderosa pine (PP), mountain hemlock (MH), and Sitka spruce-western hemlock (SH). Blue bars represent the mean of the unconfined control (UC) plots and gold bars represent the mean of the carbon-exclusion treatment (-C) plots. Error bars show the standard error of the mean ($n = 5$).

$R_{\text{Meta}}^2 = 0.47$, $P = 0.013$; Fig. 7a). We also found a positive correlation between the response ratio of SOC-normalized microbial respiration and duration of C exclusion ($\ln[R] = 0.0085 \times \text{years of C exclusion} - 0.6511$, $R_{\text{Meta}}^2 = 0.64$, $P = 0.011$; Fig. 7a).

Twenty-one records (eight from our study) across nine published studies were used to examine the effect of C exclusion on microbial biomass (Appendix S1: Table S5). The average response to C exclusion across studies was a 39% reduction (SE = 8%, $P < 0.001$; Fig. 7b). Unlike the SOC and microbial respiration meta-analyses, the effect of C exclusion on microbial biomass was not influenced by duration of exclusion ($P = 0.951$). There was a significant difference between microbial biomass assessed by chloroform fumigation

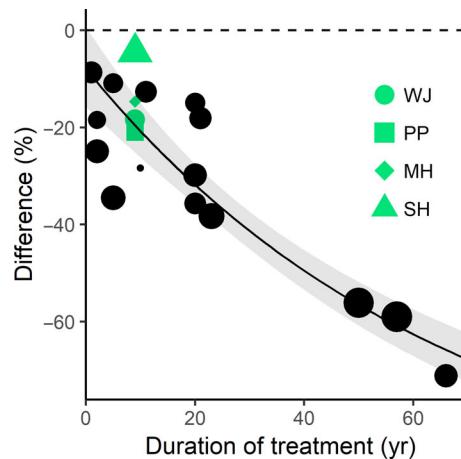


FIG. 6. Meta-analysis of mean responses of soil organic carbon (C) as a function of time since C exclusion. Solid line represents best-fit meta-regression, shaded gray area shows 95% confidence intervals, and dashed line shows no response. Green shapes show the responses of the Oregon sites in this study: circles, western juniper; squares, ponderosa pine; diamonds, mountain hemlock; triangles, Sitka spruce-western hemlock. Size of the shape is proportional to the inverse variance of the response and weight of the record in the meta-analyses.

incubation (CFI) and all other methods (i.e., CFE, substrate-induced respiration [SIR], total PLFA, direct microbial counts; $P < 0.001$).

Sixteen records (four from our study) across nine published studies were used to examine the effect of C exclusion on the fungal : bacterial ratio (Appendix S1: Table S6). The effect of C exclusion on the fungal : bacterial ratio was not influenced by duration of exclusion ($P = 0.936$). Instead, the effect was best explained by location ($P < 0.001$), specifically whether the site was dominated by ectomycorrhizal (EM) or arbuscular mycorrhizal (AM) -associated vegetation ($P = 0.022$). The average response to C exclusion in EM sites was a 45% reduction (SE = 17%) in the fungal : bacterial ratio, while there was no significant response in AM sites (Fig. 7c). We also tested the effect of pH, which did not significantly influence the response of the fungal : bacterial ratio ($P = 0.277$). The SOC concentration of the control plots did significantly influence the fungal : bacterial ratio ($P = 0.019$); however, this was caused by a single influential data point (SH from our study, Cook's distance = 0.40 [> 2.5 times the average]). With this point removed, initial C concentration was no longer significant ($P = 0.397$).

Eleven records (four from our study) across five published studies were used to examine the effect of C exclusion on the Gram-positive : Gram-negative bacterial ratio (Appendix S1: Table S7). The meta-analysis showed that there was a 18% increase (SE = 8%) in Gram-positive : Gram-negative bacterial ratio ($P = 0.015$; Fig. 7d) across all studies. Similar to the microbial biomass and fungal : bacterial ratio meta-analyses, the

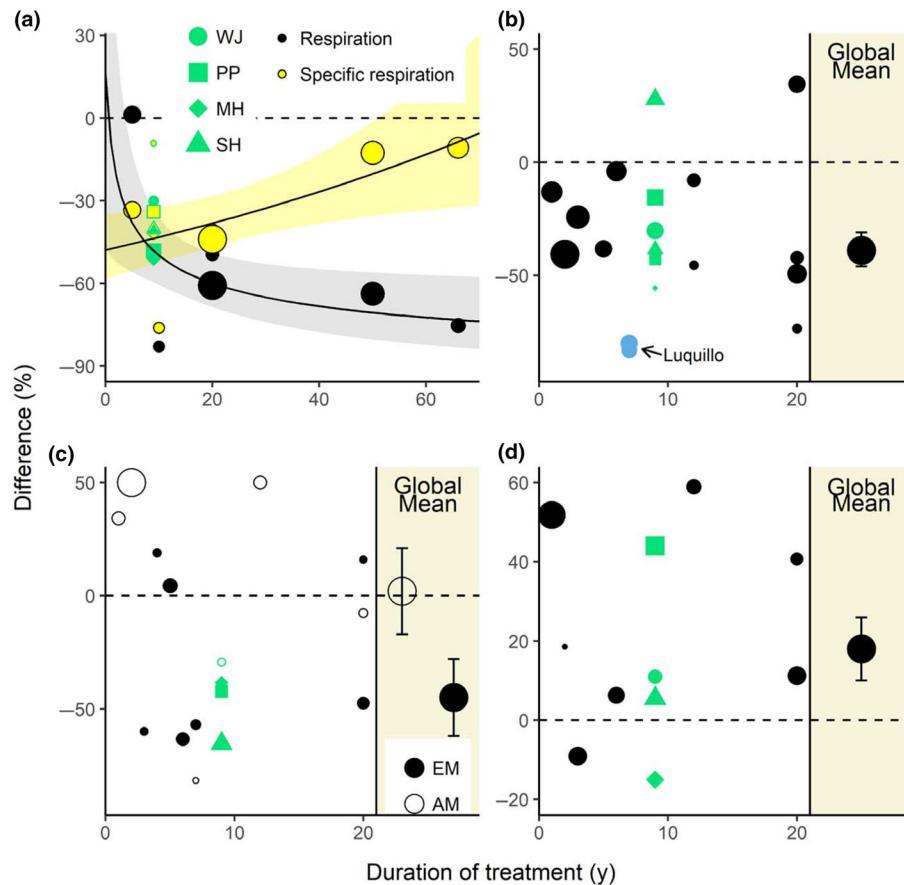


FIG. 7. Meta-analysis of mean responses of absolute and soil organic carbon [C]-normalized (a) microbial respiration (open circles, specific respiration; solid circles, respiration), (b) microbial biomass, (c) the fungal : bacterial ratio in sites dominated by arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi, and (d) Gram-positive : Gram-negative bacterial ratio as a function of time since C exclusion. Solid lines represent best-fit meta-regression where significant, shaded area shows 95% confidence intervals, and dashed line shows no response. Where meta-regressions were nonsignificant (b–d), global means are presented with error bars representing the standard error. Green shapes show the responses of the Oregon sites in this study: circles, western juniper; squares, ponderosa pine; diamonds, mountain hemlock; triangles, Sitka spruce–western hemlock. Size of the shape is proportional to the inverse variance of the response and weight of the record in the meta-analyses.

effect size was not influenced by duration of exclusion ($P = 0.851$).

DISCUSSION

Determining the influence of C availability on microbial communities is critical for understanding and predicting the response of ecosystems to altered belowground C inputs and identifying possible interactions between plants and soil microorganisms. Consistent with our hypotheses, exclusion of plant C inputs at our Oregon forests sites reduced SOC pools, the lability of the SOC, its availability to the microbial community, and, concomitantly, microbial biomass, community composition, physiology, and function. With some exceptions, these findings were supported by the meta-analysis of soil C-exclusion experiments in ecosystems of North America, Hungary, and China.

Exclusion of plant C inputs reduces C availability over time

Across our Oregon sites, the greatest absolute reduction in SOC occurred at the highest-productivity (highest C stock) sites, whereas the greatest relative reduction in SOC occurred in the lowest-productivity (lowest C stock) sites. However, the finding of decreased effect size with initial C stock was not consistent globally. Instead, duration of exclusion was the only significant predictor of SOC response in our meta-analysis.

It is likely that the C-exclusion treatment differentially reduced accessible, labile C more than recalcitrant C compounds. Although we did not measure SOC quality directly at our sites, the C-exclusion treatment reduced C-normalized microbial respiration, suggesting that the C remaining was either less labile or less accessible (Grady and Hart 2006, Rousk and Frey 2015). Other

C-exclusion studies did measure SOC quality and accessibility more directly. In both forested and grassland ecosystems, Lajtha et al. (2014b) found that 50 yr of C exclusion reduced free-light and intermediate-aggregate C fractions by over 50%, but reduced mineral-associated C fractions by less than 15% (bulk SOC was reduced by ~50%). At the Harvard Forest DIRT site, 20 yr of C exclusion decreased the relative proportion the free-light fraction (Lajtha et al. 2014a). Furthermore, 20 yr of C exclusion reduced the relative abundance of free sugar and free cyclic lipid abundance (i.e., labile C substrates) at the temperate hardwood Bousson Forest DIRT site (Wang et al. 2017). The decreased C-normalized respiration seen in our study was also observed across studies within the meta-analysis. However, the negative effect of C exclusion on C-normalized respiration decreased with duration of exclusion, which suggests that the microbial community may be able to acclimate to decreased SOC availability (DeAngelis et al. 2015).

Microbial biomass and community composition is altered by C exclusion

The meta-analysis supported our hypothesis that C exclusion would reduce soil microbial biomass (by CFE). However, results from our sites were mixed and depended on the method used (C exclusion only marginally influenced biomass assessed by total PLFA). Although microbial biomass assessed by CFE and PLFA are generally well correlated ($r^2 = 0.77-0.96$; Bailey et al. 2002, Leckie et al. 2004), they were only moderately correlated in our study ($r^2 = 0.48$, $P < 0.001$, $n = 39$). The discrepancy could be due to the drastic differences in the fungal : bacterial ratio of microbial biomass between treatments in our study. Although 18:20,6,9 is a good indicator for the relative abundance of fungi compared to bacteria, microbial biomass C per unit PLFA differs between these groups (Frostegård and Bååth 1996), so using total PLFA as an indicator for microbial C may not be appropriate when the fungal : bacterial ratio of microbial biomass changes substantially. Indeed, the two methods were better correlated within treatments (UC: $r^2 = 0.61$, $P < 0.001$, $n = 19$; -C: $r^2 = 0.76$, $P < 0.001$, $n = 19$). There was a significant effect of method for the microbial biomass meta-analysis. However, this effect was only observed for CFI; this result may be confounded by the effect of location because CFI was used only in the sole tropical site in the meta-analysis (Luquillo) that also exhibited the greatest reduction in microbial biomass ($P < 0.001$).

Our PLFA analyses supported our hypothesis that 9 yr of C exclusion would alter microbial community composition (although not in the direction that we expected). Altered microbial community composition has been corroborated by other C-exclusion DIRT studies using PLFA-based methods (Brant et al. 2006a, Rousk and Frey 2015). However, at one of the same sites (H. J. Andrews Experimental Forest) in Brant et al.

(2006a), microbial community composition assessed using 16S and 28S amplicon-based pyrosequencing was not altered by 12 yr of C exclusion (Yarwood et al. 2013). This discrepancy could be methodological where PLFA analyses broadly incorporate the entire microbial community, and amplicon-based analyses analyze bacterial/archaeal (16S) and eukaryotic communities (28S, 18S, or ITS) independently. Also, comparisons of the relative abundance of bacteria/archaea and eukaryotic (fungal) communities using qPCR may be inaccurate given that DNA/biomass relationships differ among microbial taxa (Leckie et al. 2004). Thus, if changes in the microbial community are mainly due to changes in the fungal : bacterial ratio, amplicon-based methods may fail to detect such changes.

In contrast to our hypothesis, the change in microbial community composition due to C exclusion was driven by a dramatic *decrease* in the fungal : bacterial ratio (-45% across all sites), with the largest reductions occurring in the most productive sites. This is surprising considering that fungi are assumed to be better decomposers of recalcitrant C containing compounds such as lignin (Floudas et al. 2012), which proportionately increases with C exclusion (Wang et al. 2017). However, the meta-analysis showed that this trend was consistent in sites dominated by EM-associated vegetation but not in sites dominated by AM-associated vegetation. The decrease in the fungal : bacterial ratio with C exclusion in EM sites, but not AM sites, may be explained by preferential loss of mycorrhizal fungi due to root trenching (Siira-Pietikäinen et al. 2001a) and the ability to detect changes among mycorrhizal guilds using PLFAs. Arbuscular mycorrhizal fungi do not contain high amounts of 18:10,9 and 18:20,6,9 (used as indicators for fungi, Frostegård and Bååth 1996), so loss of these species was probably undetected by PLFA methods. Although our study did not have trench-only treatments, the trench-only treatment from the EM-dominated DIRT sites had a similar effect on the fungal : bacterial ratio, suggesting that the root exclusion was responsible for changes in the fungal : bacterial ratio, whereas litter exclusion had a limited effect (Brant et al. 2006a, Rousk and Frey 2015). Furthermore, laboratory growth rates of fungi in the Harvard Forest DIRT site, which would exclude plant-associated mycorrhizal growth, were unaffected by C exclusion, suggesting that changes in the fungal : bacterial ratio were largely influenced by the loss of mycorrhizal fungi (Rousk and Frey 2015). Similarly, consistent with our hypothesis, in our sites and in the meta-analysis, we found a relative decrease in Gram-negative bacteria, which are predominately rhizosphere-associated (Kennedy and de Luna 2005). Both measurements of microbial community composition were not influenced by duration of C exclusion (i.e., SOC availability). Therefore, changes in microbial community composition from root exclusion studies are likely not due to changes in overall SOC concentrations; rather, these changes in microbial community composition are probably caused

by the loss of rhizosphere-associated microorganisms when plant root-supplied C is excluded.

Carbon exclusion increases rates of net N mineralization in high-productivity forests

Consistent with our hypothesis, increased SOC limitation induced by 9 yr of C exclusion increased N availability and dramatically altered the N cycle in these soils. Even though gross N mineralization decreased with C exclusion, net N mineralization increased in the three most productive sites because gross N immobilization declined even more than gross mineralization. This implies that in response to reduced labile plant C inputs, ecosystems are at greater susceptibility to N saturation (Aber et al. 1989) through decreased N demand by soil microorganisms. In the longer term, this could lead to losses in soil N capital. In fact, across our sites, 9 yr of C exclusion reduced total N by 9%. Considering that most temperate ecosystems are N limited (Chapin et al. 2011), recovery of ecosystems after long-term reduced C inputs could be slowed by this decrease in N stock and availability. Also, reduced plant C inputs across larger spatial scales are likely to have detrimental effects on coupled aquatic ecosystems through increased NO_3^- inputs from uplands and possible eutrophication of aquatic habitats (Dahlgren and Driscoll 1994).

Our study corroborates other laboratory studies showing the connection between the C and N cycles (Hart et al. 1994a). However, we know of only one other study (Brewer 2010) that has shown altered rates of N cycling processes with experimentally reduced plant C inputs in situ (10 yr of C exclusion at H. J. Andrews Experimental Forest). Carbon exclusion at other DIRT sites (2 yr at the Síkfökút Experimental Forest and 20 yr at Harvard Forest) did not result in significant changes in gross or net rates of N cycling processes in the mineral soil (Holub et al. 2005, Rousk and Frey 2015). This could be the result of long-term N deposition ($7\text{--}19 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) and potential N saturation at these sites (Ollinger et al. 1993, Horváth 2004, Magill et al. 2004) that could mask C-driven changes in the N cycle. However, we also did not detect a change in N immobilization or net N mineralization at our lowest-productivity site, even though N deposition at this site is low. Lack of studies investigating the impact of C exclusion on N cycling processes prevented a meta-analysis; however, differential effects by site productivity imply that the impact of C on the N cycle is positively influenced by the rate of C cycling within the ecosystem (Booth et al. 2005).

Carbon exclusion decreases carbon-use efficiency

The C-exclusion treatment significantly reduced CUE, which we attribute to both increased SOC complexity (i.e., number of enzymatic steps necessary to degrade the soil organic matter) and decreased SOC accessibility.

Although we did not chemically characterize the SOC in our study, 20 yr of C exclusion in the temperate hardwood Bousson Experimental Forest resulted in increased concentrations of suberin and decreased concentrations of free sugars (Wang et al. 2017), implying that the chemical quality of the residual SOC becomes proportionally more complex with C exclusion. Because increased C allocation towards enzyme production decreases CUE (Manzoni et al. 2012), added SOC complexity with C exclusion should reduce CUE. Numerous laboratory incubations corroborate this finding by showing that the C-use efficiency of phenol and other organic acids is significantly less than that of glucose (Brant et al. 2006b, Frey et al. 2013, Öquist et al. 2017, Jones et al. 2018). The reduced quantity of available SOC in our experiment likely also reduced CUE because under low substrate levels, a greater fraction of the available SOC is needed for cellular maintenance (Öquist et al. 2017). Although CUE may be unresponsive to the concentration of an *added* substrate (Roberts et al. 2007), in both our experiment and a long-term laboratory incubation using the same CUE estimate technique as we employed (Hart et al. 1994a), decreases in CUE were at least correlated with decreases in substrate availability. Future research separating the effects of SOC quantity and quality on CUE with experimentally reduced C inputs would further elucidate the mechanisms of SOC persistence in terrestrial ecosystems.

Although C supply and substrate quality are likely to have important regulatory effects on CUE, microbial community acclimation may serve to moderate the effects of these factors on CUE. For instance, at the H. J. Andrews Experimental Forest, neither respiration (Lajtha et al. 2005) nor gross N immobilization (i.e., growth, Holub et al. 2005) responded to 5 yr of C exclusion, suggesting that CUE (and perhaps C availability as indicated by our meta-analysis, Fig. 6) was unaltered by the relatively short duration of exclusion. However, mineral soil CUE (estimated from reported average growth and respiration) after 20 yr of C exclusion in the Harvard Forest DIRT site was also not significantly different from control plots (Rousk and Frey 2015). Therefore, we hypothesize that the Harvard Forest microbial community had acclimated to the new state of C availability (i.e., increased oligotrophy/efficient growth sensu Fierer et al. 2007) reducing the C availability effect on CUE. This acclimation of the microbial community with time is corroborated by our meta-analysis, which showed the response of C-normalized respiration to C exclusion decreased with the duration of the treatment (Fig. 7a). Furthermore, sites of contrasting C availability still have similar CUEs (Spohn et al. 2016), suggesting that under longer time scales, microbial communities will acclimate to C substrate availability, adjusting their metabolisms and growth rates to the optimal levels for that ecosystem. Nine years of C exclusion in our study resulted in decreased C availability, but relatively smaller changes to the microbial community composition. Hence, the effect

of reduced C availability on CUE may not have been fully abated by the small change in the microbial community composition.

Because the response of the microbial community composition, physiology, and metabolism to altered C inputs in situ is unknown, or at least unclear (studies to date have used PLFA analysis to characterize the microbial community composition), our hypothesis of the moderating effect of the microbial community composition is speculative. Understanding the interplay between C substrate availability and microbial community composition and metabolism (and the time scales which they respond to altered C inputs) in determining community CUE could be improved by incorporating genomic signatures (e.g., codon usage bias, Vieira-Silva and Rocha 2010) in long-term C input manipulations. This information would be useful in constraining SOC models that incorporate CUE as a variable (Wieder et al. 2013, 2015).

Carbon exclusion leads to higher enzyme activity, but lower respiration

Contrary to our hypothesis, C exclusion *increased* biomass-normalized and absolute potential extracellular enzyme activity. This is surprising, because C exclusion (2–20 yr) in DIRT sites has been shown to decrease C-degrading and S- and P- mineralizing enzyme activities across multiple ecosystems when expressed on a soil mass basis (Fekete et al. 2011, Kotrocó et al. 2014, Lajtha et al. 2014a, Veres et al. 2015). Increased enzyme activity in our sites could indicate increased demand for resources in an attempt to depolymerize more SOC. However, C_{sum} : NAG and NAG : AP, which should have increased and decreased, respectively, with greater net N mineralization and availability (Olander and Vitousek 2000), did not change with C exclusion. Although NAG is used as a model extracellular enzyme for N acquisition, aminopolypeptides are generally not a large proportion of the soil organic N pool, and they can also be an important source of C. Consequently, numerous studies show inconsistent responses of NAG to N availability (see review by Sinsabaugh and Shah 2012), which leads these authors to question its validity as a model N-acquiring enzyme. Furthermore, recent work suggests that the ratios of model extracellular enzymes may not accurately measure nutritional limitations of microbial growth or respiration (Rosinger et al. 2019).

Inconsistencies in the response of extracellular enzyme activity to C exclusion among studies may be due to the impact of C exclusion on N availability. Extracellular enzymes are nutritionally expensive (Duff et al. 1994), and fertilization studies show that increases in N availability increase production of extracellular enzymes (Ajwa et al. 1999, Saiya-Cork et al. 2002, Marklein and Houlton 2012; but see Kivlin and Treseder 2014 for a more nuanced discussion). We found significant, positive correlations between net N mineralization (a widely used

measure of N availability; Binkley and Hart 1989) and microbial biomass-normalized C_{sum} ($r = 0.71$, $P < 0.001$, $n = 32$), NAG ($r = 0.51$, $P = 0.003$, $n = 32$), and AP activities ($r = 0.71$, $P < 0.001$, $n = 32$; Appendix S1: Fig. S3). Sites from the DIRT network that detected lower extracellular enzyme activities with C exclusion (Síkfókút Experimental Forest and Harvard Forest) did not show increased N availability (Holub et al. 2005, Rousk and Frey 2015), which may explain the discrepancy. Therefore, we suggest that the response of extracellular enzyme activity to C exclusion is dependent on concomitant changes in soil N availability.

Nine years of C exclusion decreased microbial respiration even though extracellular enzyme activities increased. This finding is corroborated by the meta-analysis that showed a logarithmic decrease in respiration with duration of exclusion. It is unsurprising that respiration decreased while extracellular enzyme activity increased because the positive correlation between potential extracellular enzyme activity and microbial respiration is not always significant (Frankenberger and Dick 1983) and, again as previously noted, increased production of extracellular enzymes may occur in direct response to C limitations. Thus, our study underscores the caution that must be used when relating potential extracellular enzyme activity to in situ rates of microbial respiration. Instead, we interpret this finding as increased potential degradation of the remaining SOC, which was less likely to be degraded in the unconfined control plots.

CONCLUSION

Microbial processes are increasingly incorporated into soil C models (Wieder et al. 2013, 2015, Georgiou et al. 2017). However, the assumptions underlying these models need to be constrained by experiments across ecosystems of contrasting composition and function. Results from this study show the dramatic and extensive control of SOC over soil microbial properties and processes across large ecological gradients. The cross-site design of our study (consistent measurements over a productivity gradient) allowed us to identify effects of C exclusion that were robust across ecosystems (e.g., decreased respiration) and those that interacted with ecosystem productivity (e.g., N cycling processes). Using other field manipulations of C inputs (namely, the DIRT studies), we show that the impact of SOC availability on soil microbial communities is dependent on time and ecosystem properties (e.g., mycorrhizal association). We emphasize that C exclusion results in trends that, in many cases, go against long-held theories of microbial ecology and biogeochemistry (e.g., we found decreases in the fungal : bacterial ratios with putative increases in SOC recalcitrance). Hence, systematic and comprehensive evaluation of microbial processes in response to experimentally altered C inputs are necessary to elucidate changes in SOC stocks in response to any environmental change that results in long-term

decreases in plant C inputs (Schlesinger 1986, Crowther et al. 2016).

ACKNOWLEDGMENTS

The authors thank Sarah Benanti and Toby Hooker for providing field and laboratory assistance, Steve Overby and Jen Schweitzer for their assistance with PLFA measurements, and Dana Erickson for assistance with GC-MS analysis. Additionally, we thank the authors whose work was included in our meta-analyses. The comments made by two anonymous reviewers and Subject-Matter Editor Serita Frey significantly improved this manuscript. This work was supported by grants from the National Science Foundation Division of Ecosystem Studies (9208828 and 9807097), U.S. Department of Agriculture Co-operative State Research Service (USDA-CSRS; 92-37101-7976), the U.S. Department of Agriculture National Institute of Food and Agriculture, the Utah Agricultural Experiment Station, Logan, Utah, USA, a University of California Merced Graduate Fellowship Award (to NCD), and a Southern California Edison Graduate Fellowship (to NCD).

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