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Authors
Throckmorton, Heather M
Bird, Jeffrey A
Monte, Nick
et al.

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The soil matrix increases microbial C stabilization in temperate and tropical forest soils

Heather M. Throckmorton • Jeffrey A. Bird • Nick Monte • Tad Doane • Mary K. Firestone • William R. Horwath

H. M. Throckmorton: Los Alamos National Lab, MS 495, Los Alamos, NM 87545, USA e-mail: hthrockmorton@lanl.gov; J. A. Bird: Queens College CUNY, 65-30 Kissena Blvd., Flushing, NY 11367, USA e-mail: jbird@qc.cuny.edu; N. Monte T. Doane W. R. Horwath: University of California, One Shields Ave., Davis, CA 95616, USA e-mail: wrhorwath@ucdavis.edu; M. K. Firestone: University of California, 137 Mulford Hall, Berkeley, Berkeley, CA 94720, USA e-mail: mkfstone@berkeley.edu

Abstract

Microbial biomass represents a substantial source of labile C contributing to soil organic matter (SOM) maintenance. Microbial residues may associate with the soil matrix through a variety of mechanisms, reducing its bioavailability and increasing its persistence in soil. Our objective was to examine soil matrix effects on the stability of non-living microbial C inputs in two contrasting forest ecosystems by following microbial residues (Fungi, Actinobacteria, Gram-positive bacteria (Gm +), Gram-negative bacteria (Gm −)) into SOM fractions in a temperate forest in California (CA) and a tropical forest in Puerto Rico (PR) for 3 and 2 years, respectively. We isolated 3 SOM fractions: (i) free light fraction (FLF), (ii) occluded light fraction (OLF), and (iii) dense fraction (DF). Additionally, we characterized SOM fraction chemistry to infer quality and source of native fraction SOM. Our results showed greater stabilization as mineral-associated microbial C (i.e., as DF and OLF), compared with loose detrital C (i.e., FLF). There was no microbial group effect (i.e., differences in fraction C recovery among different microbial cell types). Our findings suggest that mineral association is more important for stabilizing non-living microbial C in soil than the cellular structure of the initial source of microbial inputs, with site specific edaphic factors as the major controllers of the amount of microbial residues stabilized.

Keywords: Microorganisms, Fungi, Bacteria, Carbon, Soil organic matter, Mineral protection, Stabilization

Introduction

Soil particles and structure play a critical role in controlling C cycling by stabilizing nonliving microbial biomass (necromass) on mineral and organic surfaces or within aggregates. The majority of detrital plant C and N added to soil is transformed into microbial biomass (Kindler et al. 2006; Simpson et al. 2007; Engelking et al. 2008, Mambelli et al. 2011), and the subsequent accumulation of microbial necromass may represent up to 80 % of total soil organic matter (SOM) (Liang and Balser 2010). There is increasing evidence

that, due to the proximity and concentration of microbial cells and extracellular products on soil particles (Or et al. 2007), microbial cells and products may preferentially stabilize on charged mineral and organic surfaces through H-bonding, van der Waal's forces, cation or anion exchange, ligand exchange, and bridging by polyvalent cations (Sutton and Sposito 2005; Kleber et al. 2007). In addition to direct complexation with minerals and other organics, microbial components may be protected via occlusion within aggregates, creating a physical barrier from enzymatic attack and mineralization (Balesdent et al. 2000; Christensen 2001; Rasmussen et al. 2005). Moreover, microbial biomass and products (including extracellular polysaccharides (EPS) and proteins) have been shown to enhance the development of soil structure and aggregate stability (Liu et al. 2005; Tang et al. 2011). For example, fungal hyphae can act as a 'sticky string bag' to entangle particles and release glue-like EPS (Oades and Waters 1991; Six et al. 2004; Jastrow et al. 2007). Understanding the relative importance of these stabilization mechanisms and whether cellular components from various microbial groups stabilizes differently is an important knowledge gap that would improve our ability to predict soil C cycling and optimize ecosystem scale C models.

The potential for microbial necromass and/or EPS to accumulate on soil particles and in aggregates has implications for understanding how microbial communities affect soil C storage and maintenance. Contrary to traditional views and assumptions, we recently reported that non-living microbial cell type had little impact on its stabilization in situ in a temperate forest or tropical forest soils (Throckmorton et al. 2012). However, this conclusion was based on the recovery of isotopically- labeled microbial biomass on a total soil basis. The stabilization of microbial cell type may be a function of its interaction with different soil SOM fractions including: (i) weakly associated with the mineral matrix (free light fraction (FLF)); (ii) occluded within aggregates (occluded light fraction (OLF)); and (iii) associated with mineral and organic surfaces (dense mineral fraction (DF)) (Rasmussen et al. 2005; Sollins et al. 2009; Wagai et al. 2009). This fractionation procedure has been shown to produce characteristically distinct fractions differing in chemistry (based on C:N ratios; nuclear magnetic resonance (NMR); and pyrolysis) and stability (mean residence times with ^{14}C, consistent with the likely accumulation and stabilization of microbial cells and products in mineral-associated fractions (Rasmussen et al. 2005; Swanston et al. 2005; Kleber et al. 2007; Sollins et al. 2009; Wagai et al. 2009).

In the present study, we assessed soil matrix effects on the stabilization of diverse non-living microbial cells to understand mechanisms of microbial C stabilization. We also characterized the chemical composition of the isolated SOM fractions using pyrolysis gas chromatography mass spectrometry (Py-GC–MS) to infer native SOM source and processes. Four microbial groups (i.e., temperate and tropical: Fungi, Actinobacteria, Gram-positive bacteria (Gm (+)), Gram-negative bacteria (Gm (−)) were isolated from CA and PR

soils. Isolated groups were recultured with labeled ^{13}C, and non-living cells with EPS (herein defined as '*residues'*) were added back to soils at both sites in a reciprocal design. The retention and partitioning of microbial inputs into and among distinct SOM fractions was monitored in situ for 3 years in CA and 2 years in PR. We hypothesized that the mineral matrix would exhibit greater influence on C retention than the source of microbial C inputs through (i) intra-aggregate occlusion (i.e. the occluded light fraction (OLF)); and (ii) complexation with particulate surfaces (i.e. dense fraction (DF)). In addition, we hypothesized that the same SOM fractions (DF, OLF, and FLF) would be chemically similar across sites, but within each site the SOM fractions would be distinct, as each has fraction has distinct processes controlling SOM stabilization and destabilization.

Materials and methods

Field sites

The study was conducted at two climatically contrasting sites, which differ ecologically in plant species and microbial community composition, as well as in management and soil properties (Throckmorton et al. 2012). Blodgett forest is a temperate conifer forest located in the Sierra Nevada near Georgetown, CA (38° 53′ N, 120° 39′ W) at an elevation of 1,315 m. Mean annual precipitation is 1,770 mm occurring mostly during winter (November–March). Mean annual temperature is 24–27 °C in the summer and 0–9 °C in the winter (Blodgett Forest Research Station; 1961-present). The overstory vegetation is dominated by Ponderosa pine (*Pinus ponderosa*), with sugar pine (*Pinus lambertiana*), white fir (*Abies concolor*), douglas fir (*Pseudotsuga menziesii)*, and incense cedar (*Calocedrus decurrens*). The soil is a fine-loamy mixed mesic, ultic Haploxeralf with a mixed andesitic lahar/granitic parent material, with less than 20 % clay content. The Luquillo forest site is located in Puerto Rico (18° 41′ N, 65° 47′ W) at an elevation of 780 m. Mean annual precipitation is 4,500 mm and mean annual temperature is 18.5° C. About forty species occur in this forest, with the dominant species being *Cyrilla racemiflora*. The soils are derived from volcanoclastic sediments with quartz diorite intrusions (Ultisols), with high clay content (up to 70 %; Templer et al. 2008). The microbial community in CA soils contains a greater fungal: bacterial ratio than Luquillo forest (Throckmorton et al. 2012).

Microbial cell growth and addition to soils in situ

Isolation, selection, production and growth characteristics of microbial isolates were described in detail in Fan et al. (2009). Briefly, soils were collected (0–15 cm depth) at 15 locations along 3 transects at each site, and homogenized separately. Microbial cells were isolated using selective media specific to each of four microbial groups (fungi, actinobacteria, Gm (+), Gm (−)) (Fan et al. 2009). Final selections produced 2–4 isolates for each microbial group from each sites for liquid medium culturing, producing 8 total microbial treatments (tropical and temperate counterparts for each of the four groups). The final 23 isolates were grown in 100 atom % ^{13}C labeled

liquid media and harvested in late stationary phase. Cell exudates (extracellular polysaccharides (EPS)) were isolated and combined with harvested cultures. Cells were killed and sterilized by autoclaving and lyophilizing, and cells within each of the 8 treatments were homogenized. Resulting microbial cells and EPS contained 101.1 ± 2.5 atom % ^{13}C (Throckmorton et al. 2012). Microbial residues with EPS were added to soils in mesocosms (PVC: 10 cm diameter and 20 cm in depth) as previously described (Throckmorton et al. 2012). A total of 80 mg of ^{13}C- labeled microbial residues and EPS of each treatment was suspended in 30 mL of deionized (DI) water and injected into the top 1–3 cm of the soil. Input microbial C amounted to approximately 0.2 % of background C and 15 % microbial biomass C. Control mesocosms (unamended) were treated with injections of equal volumes of dionized water. While the organisms cultured clearly do not represent the full diversity of a soil microbial community, they provide a substantial range in cell composition, allowing us to compare and contrast cellular and SOM biochemical characteristics among genetically and phenologically distinct soil microorganisms.

The experiment was a split-plot design, with three replicate blocks (~10–20 m diameter) at each site. Each of the 8 microbial treatments and unamended controls were randomly assigned to mesocosms in each block. Five replicate mesocosms were installed per treatment, and were excavated on five sampling dates. In CA isolates were introduced June 21, 2006 and cores were excavated at 33, 163, 370, 763, and 1,133 days after application. In Puerto Rico, isolates were added Oct. 6, 2006 and soils were excavated 17, 114, 249, 480, and 886 days after application.

Soil organic matter fractionation

Since previous analyses showed that a majority of the ^{13}C label was recovered in surface depths (0–7.5 cm) (data not shown), surface soils were fractioned for each sampling event, and examined for ^{13}C fraction recovery. Bulk soils were separated into free light fraction (FLF); an aggregate-occluded light fraction (OLF); and a dense mineral-rich fraction (DF) using density fractionation. Briefly, 20 g of freeze-dried soil (<2 mm) samples were fractioned using 150 mL of sodium polytungstate (SPT) at a density of 1.7 g ml^{-1} based on methods described by Rasmussen et al. (2005). Briefly, soils with polytungstate were gently inverted by hand several times to disperse soils and centrifuged at 4,070×*g* for 1 h to separate FLF from DF and the OLF. The FLF was isolated on 1.6 µm pore GF/A filter (Whatman, NJ, USA) and rinsed with DI water. The remaining heavy pellet was sonicated for 3 min at 70 % pulse (10 Watts g^{-1} dry soil; 168 J mL^{-1}; 1,260 J g^{-1} soil; Branson 450 digital sonifier (CT, USA), and centrifuged at 4,070×*g* for 1 h. The supernatant containing OLF was aspirated, filtered, rinsed with distilled water, and lyophilized before analysis. Once the FLF and OLF were isolated, 150 ml of DI water was added to the remaining DF pellet and shaken vigorously on a shaker table for 1 h. The suspension was centrifuged at

4,070×*g* for 20 min. The supernatant was decanted, and the DF rinsing and decanting with DI was repeated 3× prior to lyophilization of the DF.

Soil and microbial ^{13}C isotope analyses and Py-GC–MS

Soil and microbial ^{13}C enrichment was measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK) and reported relative to standards calibrated against National Institute of Standards and Technology Standard Reference Materials (NIST; Gaithersburg, MD, USA). Recovery of ^{13}C within SOM fractions was determined according to Throckmorton et al. (2012).

For Py-GC–MS analyses, replicate blocks for fractions isolated were composited on a per C basis for soils unamended with ^{13}C labeled microbial cells. Subsamples of each SOM fraction isolated (~450 µg C) were pyrolyzed at 590° in pyrofoils (Pyrofoil F590, Dychrom, Sunnyvale, CA) with a Curie-Point pyrolyzer (Japan Analytical Industry Co. Japan). Pyrolyzed compounds were transferred online to a gas chromatograph (Varian, Walnut Creek, CA). The inlet temperature was set to 280 °C, with a split injection (split ratio 10:1 for 2 min, then 100:1 for 30 s, then 10:1 for the remainder of the run, He flow rate 2.2 ml min^{-1}). Separation of pyrolysis products was done on Varian FactorFour VF5-MS (30 × 0.25 mm, film thickness 0.25 µm) using a temperature program of 55 °C for 5 min, 5 C min^{-1} to 210 C, followed by a jump (20 °C min^{-1}) to a final temperature of 280 °C. The column outlet was coupled to a Saturn 2,200 ion-trap mass spectrometer (Varian, Walnut Creek, CA) operated at 70 eV in the EI mode. For the first 2 min flow was directed to the FID, then flow was directed to the MS. The transfer line was heated to 150 °C and the trap temperature was held at 180 °C. Pyrolysis products after 2.55 min (to avoid initial flush of volatile compounds) were identified using the retention index and by comparison to reference spectra after deconvolution and extraction using AMDIS v 2.64 and NIST spectra (Pouwels et al. 1989; Schulten and Schnitzer 1998; Grandy et al. 2007; Nierop and Jansen 2009). Principal component analysis (PCA) was used to characterize compound class relative abundance, and reduce the dimensionality of the data in order to highlight similarities and differences among samples (SAS for Windows Version 9.1, SAS institute, Cary, NC, USA).

Statistical analyses for microbial ^{13}C recovery in fractions

The following terms will be used to describe variables for this study: 2 ‘sites’ (CA and PR); 8 microbial ‘treatments’, representing a factorial of the 4 microbial ‘groups’ (fungi, actinobacteria, Gm (+), Gm (−)) and 2 ‘origins’ (isolated from temperate or tropical sites). Soils were sampled at 5 ‘time’ points at both sites. Sites were separately assessed using linear mixed effects models (repeated measures ANOVA) for ^{13}C recovery in fractions (% of applied) with fixed effects for time, origin, group, fraction, and all interactions, In order to include time as a variable for the analyses, separate models for sites were necessary, as time increments between sampling

points differed, resulting in ^{13}C recoveries that were not directly comparable across sites. Pairwise comparisons were performed using the Fisher least significant difference (LSD) test. Model fit was assessed using the Shapiro–Wilk test for normality and graphical analysis of studentized residuals. Statistical significance was declared at $p < 0.05$. Statistical analyses were performed using SAS for Windows Version 9.1 (SAS institute, Cary, NC). Prior to analyses, all datasets were transformed with a natural log transformation.

Results

SOM fraction yields and composition

Total soil C (<2 mm) recovered in SOM fractions was 98.0 ± 0.7 % (CA) and 101.6 ± 0.6 % (PR), averaged across all time points ($n = 121$) relative to bulk soils. Total soil N (<2 mm) recovered in SOM fractions was111.9 ± 2.7 % (CA) and 101.5 ± 3.1 % (PR), averaged across all time points ($n = 121$). The dry matter distribution among SOM fractions differed between sites, with CA yielding a greater mass of FLF (502 g kg^{-1} dry soil) than PR (206 g kg^{-1} dry soil), while PR produced a 14.2 % greater DF yield than the CA soil (Table 2). Both sites produced similar OLF yields (0.92 ± 0.03 g kg^{-1} dry in CA; and 1.08 ± 0.06 g kg^{-1} dry soil in PR; Table 2). For both sites, the OLF contained the highest concentration of C; followed by the FLF; with the DF having the lowest C concentration (Table 2).

Table 1 Microbial cell and extra cellular polysaccharide (EPS) elemental C/N ratios and compound class relative abundances are expressed as a percent (%) (Throckmorton et al. 2012)

Treatment*		C/N	Compound class[a]				
Origin[b]	Group[c]		Lp	Ph	B	Ps	N
Temperate	Actinobacteria	7.1	15	14	13	11	45
	Fungi	13	21	6.9	7.7	35	27
	Gram + bacteria	6.0	4.6	12	13	20	49
	Gram − bacteria	6.4	15	18	18	15	33
Tropical	Actinobacteria	5.3	0.8	13	16	21	47
	Fungi	11	34	6.7	7.7	24	24
	Gram + bacteria	8.1	21	17	17	9.6	34
	Gram − bacteria	10	57	10	11	8.9	13

Values are expressed as percent relative abundance for microbial isolate group composites

Lp Lipids, *Ph* Phenol, *B* Benzene, *Ps* Polysaccharide, *N* Nitrogen-compounds

* Treatment (origin*group; $n = 8$)

[a] Py-GC–MS characterization for compound classes

[b] Origin ($n = 2$)

[c] Group ($n = 4$)

Table 2 Yields, C and N contents for SOM fractions for soils (0-7.5 cm depth; <2 mm) from CA and PR

In CA, fractions were separated for soils collected 33, 163, 370, 763, and 1,133 days after microbial additions. In PR, fractions were separated for soils collected 17, 114, 249, 480, and 886 days after microbial additions. Means and standard errors are shown in parentheses ($n = 121$)

* Produced from fractioning 20 g whole soil sample

California						
	LF		OLF		MF	
Total weight (g^{-1})*	4.12	(0.19)	0.92	(0.03)	14.90	(0.23)
C (g Kg^{-1})	311.52	(3.3)	410.93	(5.2)	52.55	(1.0)
N (g Kg^{-1})	10.44	(0.2)	13.16	(0.2)	3.40	(0.1)
C/N ratio (elemental)	35.55	(0.61)	36.71	(0.46)	17.98	(0.11)
Portion of whole soils C (g Kg^{-1})	201.90	(9.0)	44.88	(1.4)	730.22	(11.0)
Portion of whole soils mass (g Kg^{-1})	502.07	(12.5)	160.97	(5.3)	336.95	(9.6)
Puerto Rico						
	LF		OLF		MF	
Total weight (g^{-1})*	1.67	(0.18)	1.08	(0.06)	17.83	(0.29)
C (g Kg^{-1})	315.86	(4.2)	399.64	(6.4)	78.09	(2.3)
N (g Kg^{-1})	9.07	(0.2)	10.97	(0.2)	3.53	(0.1)
C/N ratio (elemental)	41.14	(0.43)	42.58	(0.42)	25.91	(0.31)
Portion of whole soils C (g Kg^{-1})	89.36	(11.4)	54.03	(3.3)	872.54	(13.0)
Portion of whole soils mass (g Kg^{-1})	206.37	(14.0)	179.42	(5.8)	614.21	(13.4)

Py-GC–MS characterization showed some variation both between sites and among SOM fractions. The DFs were enriched in benzene and N-compounds compared with other fractions; the FLFs were enriched in lignin and phenol compounds; and the OLFs were characterized by high lipid yield (Fig. 1; Supplemental Table 1). PCA analyses and biplots for PC1 and PC2 suggest that site differences are mostly explained by chemical variation within the OLFs and DFs, while the FLFs for the two sites plotted similarly on the PCA biplot (Fig. 2). PC1 explained 62.5 % of the sample variance observed among SOM fractions from both sites. Nitrogen-rich compounds, Ps, and benzene compounds were represented by negative eigenvector coefficients in PC1, while the remaining compounds (Lp, Ph, Lg) were represented by positive eigenvector coefficients in PC1. PC2 explained 24.5 % of the sample variance and was represented by Lp, N-compounds, and B, and positive coefficients for Ps, Ph, and Lg compounds (Table 3).

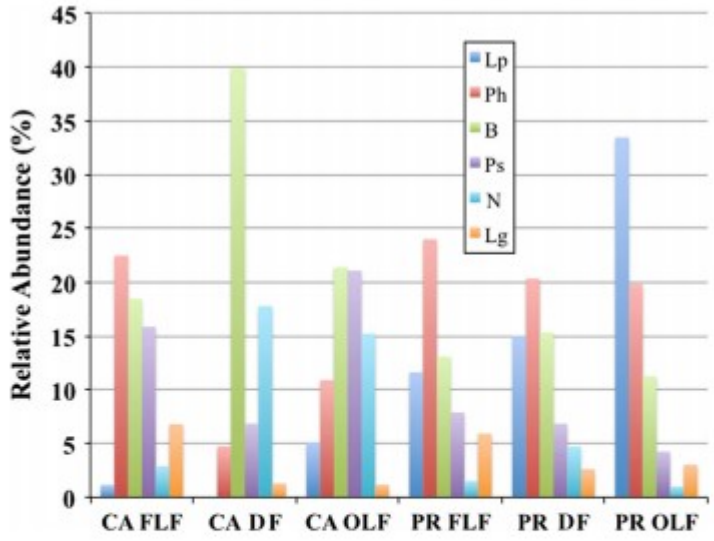

Fig. 1 Relative abundances of compound classes for all recovered compounds for SOM fractions. Results shown are from composited SOM fractions (i.e., from 3 field replicates) on a per C basis for 3 unamended soils (0-7.5 cm depth; < 2 mm) collected in June 2006 (CA) and October 2006 (PR). Abbreviations: lipid (Lp); phenol (Ph); benzene (B); polysaccharides (Ps); nitrogen-compounds (N); lignin (Lg)

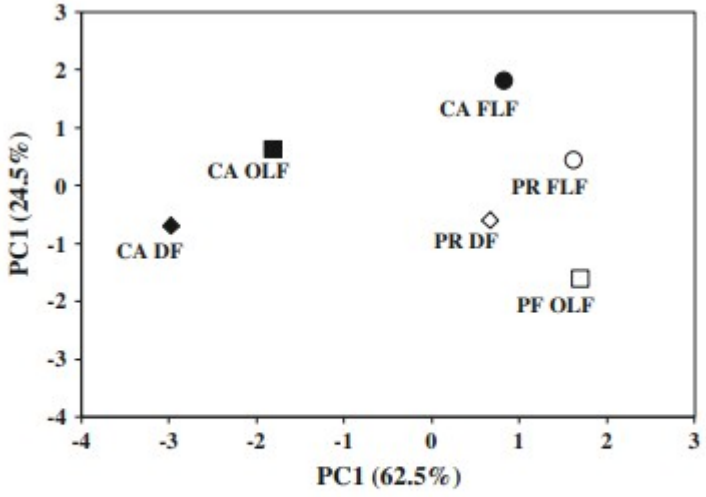

Fig. 2 PCA biplot of eigenvalues for pyrolysis compound class relative abundances shown in Table 3 for soil fractions for both sites (0-7.5 cm depth). Variance for each axis is shown in parentheses the two dimensions of the model

Table 3 Eigenvector estimates for PC1 and PC2 for compound classes

	PC1	PC2
Lipid	0.331	−0.573
N compound	−0.513	−0.021
Polysaccharide	−0.173	0.628
Benzene	−0.470	−0.026
Phenol	0.497	0.201
Lignin	0.361	0.486

Microbial residue ^{13}C recovery among SOM fractions

Tested affects (ANOVA) results for both sites are shown in Table 4 and outlined in detail in the following subsections. Some significant effects occurred for both sites, highlighting differences in total microbial C recovered among fractions. At both sites, microbial residue groups resulted in similar total ^{13}C recoveries in SOM fractions (Table 4; see following results subsections separately by site).

Table 4 Statistical parameters for analyses of variance for ^{13}C recoveries among SOM fractions (sites considered separately) for all variables and interactions

	California		Puerto Rico	
	p	F	*p*	F
Origin	0.0778	3.14	0.2265	1.47
Group	0.1720	1.68	0.4926	0.80
Origin*Group (i.e. Treatment)	0.0572	2.54	0.6195	0.59
Fraction	<.0001***	436.22	<.0001***	339.67
Origin*Fraction	0.6899	0.37	0.4292	0.85
Group*Fraction	0.1983	1.45	0.6030	0.76
Origin*Group*Fraction	0.0348*	2.31	0.1907	1.47
Time	0.0871	2.06	<.0001***	34.17
Origin*Time	0.8130	0.39	0.6731	0.59
Group*Time	0.1913	1.35	0.8546	0.58
Origin*Group*Time	0.6023	0.85	0.1672	1.40
Fraction*Time	<.0001***	6.84	0.0115*	2.54
Origin*Fraction*Time	0.3734	1.09	0.8710s	0.48
Group*Fraction*Time	0.8193	0.73	0.6281	0.88
Origin*Group*Fraction*Time	0.9494	0.57	0.8141	0.73

There were 229 and 211 degrees of freedom (df) for the CA and PR site, respectively

Significance is indicated at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

California

In CA, there were no significant differences in ^{13}C recovery among treatments during any of the five sampling dates (i.e., origin*group: $p = 0.0572$; Table 4). Total microbial ^{13}C recovery significantly differed among fractions (“Fraction” main effect, $p < 0.0001$; Fraction*Time, $p < 0.0001$; Table 4). After 33 days, significantly more microbial ^{13}C was recovered within the FLF and the DF (ca. 20 % of input) relative to the OLF (Table 5); however,

the amount of added microbial ^{13}C recovered in the FLF and DF was similar. By 1,133 days microbial ^{13}C recovered in CA FLFs had declined by 60.7 % and by 36.3 % for the DF relative to the first sampling point (compared to 51.4 % for whole soils), resulting in significantly greater microbial ^{13}C recovery in the DF than the FLF (Table 5). For all sampling points the CA OLF accounted for significantly less C microbial inputs than the FLF or the DF. However, by 1,133 days the proportion of microbial C recovered in the OLF had increased by 96 % relative to that within the OLF at 33 days, indicating a total net gain of C in the CA OLF during 1,133 days in situ (Table 5).

Table 5 Microbial ^{13}C recovery (% of applied) among SOM fractions (0–7.5 cm depth) at California site (averaged across microbial treatments)

	Time (days)										Δ (%)[a]	
	33		163		370		763		1,133			
FLF	22.3	(1.3)[b]	15.7	(0.7)[b]	14.0	(0.4)[b]	9.3	(0.6)[c]	7.4	(0.3)[c]	−60.7	(5.2)
OLF	1.9	(0.1)[c]	2.0	(0.2)[c]	2.7	(0.2)[c]	1.2	(0.1)[d]	3.2	(0.2)[d]	+96.1	(28.4)
DF	20.7	(0.8)[b]	18.6	(0.9)[b]	13.8	(0.4)[b]	15.9	(0.3)[b]	12.6	(0.4)[b]	−36.3	(6.5)
Bulk soil (< 2 mm)	54.0	(3.9)	42.7	(1.8)	34.1	(0.8)	33.2	(1.4)	24.3	(1.0)	−51.4	(3.1)

Means and standard errors are shown ($n = 24$), averaged across 8 microbial treatments. Fractions sharing a letter are not significantly different within time points ($p < 0.05$)

[a] Change in the recovery of applied microbial ^{13}C from initial (33 days) to final (1,133 days) sampling point (as a percentage of ^{13}C recovered at initial sampling point)

Puerto Rico

Similar to CA, in PR all eight microbial residue addition treatments resulted in similar total ^{13}C recoveries in SOM fractions (i.e., origin*group: $p = 0.6195$; Table 4). Also consistent with the CA site, in PR there was a significant fraction effect ("Fraction" main effect, $p < 0.0001$; Fraction*Time, $p = 0.0115$), with the DF containing the greatest amount of recovered microbial ^{13}C throughout the course of the study, followed by the FLF, and then the OLF (Table 6). By 886 days in situ, recovery of added microbial ^{13}C residues within the DF declined by 77.4 % relative to the amount of microbial C recovered in the DF at the first sampling (17 days) (Table 6). Similar to CA soils, in PR the relative losses of microbial C from the FLF were the greatest from the first to the final sampling, with an 89.1 % loss of microbial C from the FLF and a loss of 85.7 % recovery from the OLF, which was also greater than the proportion lost from the PR DF (Table 6).

Discussion

In the present study, we examined the fate and partitioning of nonliving residues from diverse microbial groups among SOM fractions in two forest soils that differed in climate, C mineralization rates and mineralogy among other edaphic properties (Rasmussen et al. 2005; Bird and Torn 2006; Throckmorton et al. 2012). The absence of significant differences in the recovery of added microbial residue ^{13}C (i.e., no microbial group effect) suggests that sorption by the mineral matrix may not strongly select for, or

differentiate amongst, microbial residues based on microbial cell type. Microbial necromass that accumulates in soils represents a spectrum of decomposing cellular material beginning as fresh cells, which likely become more and more altered over time (Mambelli et al. 2011), and potentially stabilized by increased recalcitrance (e.g., concentration of less bioavailable compounds such as aromatic/polymethylenic molecules (Derenne and Largeau 2001)) in addition to protection by the soil matrix. The accumulation of microbial necromass in soils is likely not a reflection of the living microbial cell composition, or the microbial cells and EPS added to soils in our study. Rather most of this biomass would, like plant C, undergo transformation to low molecular weight compounds as a result of enzymatic catalysis (e.g., via hydrolase), and become more likely to stabilize on mineral surfaces (e.g., Kleber et al. 2007). Nonetheless, our study attempted to simulate the turnover or death of a portion of the standing biomass by introducing non-living microbial cells and their EPS as a potential substrate for the living soil microbial community.

Table 6 Microbial ^{13}C recovery (% of applied) among SOM fractions (0–7.5 cm depth) at Puerto Rico site (averaged across microbial treatment)

	Time (days)										Δ (%)[a]	
	17		114		249		480		886			
FLF	5.1	(0.4)[b]	3.4	(0.9)[b]	2.4	(0.2)[b]	1.3	(0.4)[b]	0.5	(0.1)[b]	−89.1	(4.4)
OLF	2.1	(0.1)[c]	1.2	(0.1)[b]	1.2	(0.1)[b]	0.2	(0.0)[d]	0.3	(0.0)[c]	−85.7	(3.7)
DF	20.8	(1.1)[e]	13.2	(0.7)[e]	10.2	(0.5)[e]	5.4	(0.2)[e]	4.3	(0.3)[e]	−77.4	(3.5)
Whole Soils	33.2	(1.7)	21.8	(1.1)	16.9	(1.3)	11.8	(1.7)	8.1	(1.0)	−83.8	(2.6)

Means and standard errors are shown ($n = 24$), averaged across 8 microbial treatments. Fractions sharing a letter are not significantly different within time points ($p < 0.05$)

[a] Change in the recovery of applied microbial ^{13}C from initial (17 days) to final (886 days) sampling point (as a percentage of ^{13}C recovered at initial sampling point)

[d] Non-estimable

In this study, microbial residues were added to soils and well dispersed throughout the soil matrix. This approach did not replicate the natural spatial distribution of microbial biomass on soil particles and within pores and aggregates. A significant amount of added microbial residues were likely consumed for subsequent microbial growth, and would presumably mimic the natural temporal dynamics and mineralization of native, living soil microbial communities. Thus, the effect of the living microbial biomass cannot be differentiated from residual inputs, and could have an effect on the quality and residence time of the microbial- derived C. Nonetheless, our results support our previous findings that the initial source and chemical composition of microbial residues has little effect on its stabilization rate in soil (Throckmorton et al. 2012); and that the main stabilization mechanisms in these soils may not strongly select for microbial cell type and biochemistry. The stability of microbial C and associated protection mechanisms are likely more related to specific properties of compounds that

are released after macromolecular breakdown, rather than specific cellular biochemistry associated with the microbial groups.

Site specific effects on soil C were evidenced by different partitioning of native C and added ^{13}C microbial residues within SOM fractions at each site, with ~15 % larger FLF C in CA, and ~14 % larger DF in PR. Forest site differences in microbial C recovery in fractions related to SOM fraction's size (as a proportion of total soil C). Differences in the amount of unprotected (i.e., FLF) versus protected (OLF and DF) SOM have been widely documented across locations, and are often attributed to climate and other edaphic factors (Gregorich et al. 2006). Differences in partitioning of soil C observed in our two forest sites (i.e., larger DF in PR than at the CA site) may be explained by the higher crystalline clay content in PR soils relative to CA (Throckmorton et al. 2012), which could provide additional charged surface area for SOM sorption (Kögel-Knabner et al. 2008).

In addition, the larger FLF pool in CA and greater partitioning of microbial ^{13}C into the CA FLF relative to PR may be explained by climatic differences across sites. Greater precipitation and warmer temperatures at the PR site promote faster decomposition rates, which may preferential mineralize the unprotected FLF (Wagai et al. 2008). The proportion of FLF relative to whole soils could also be due to differences in vegetation type and litter (substrate) quality (Skjemstad et al. 2004). Slow mineralization of the coniferous litter inputs in the CA site, relative to the deciduous PR litter inputs, may influence the respective size and composition of the FLF pool (Skjemstad et al. 2004).

The net rate of microbial C loss from the FLF throughout the study was greater than other fractions at both sites, when comparing relative losses from the first to last sampling points; with disproportionately lower net loss from the DFs (Tables 5 and 6). These findings are consistent with previous studies that have shown that the DF and OLF are relatively stable SOM pools, as a result of being highly processed by the microbial community (Wagai et al. 2008; 2009; Rasmussen et al. 2005; Swanston et al. 2005; Gregorich et al. 2006; Grandy et al. 2007). In CA, the net amount of added microbial residue ^{13}C recovered in the OLF increased throughout the course of the study, suggesting movement and redistribution of microbial C into the OLF despite an overall loss from whole soils. This redistribution of input microbial C into aggregates may have been promoted by uptake or redistribution of input residue- ^{13}C by native living microbial and the faunal community. At the CA site, the microbial community has a larger proportion of fungi (Throckmorton et al. 2012), which are known to promote aggregate formation (Oades and Waters 1991; Six et al. 2004; Jastrow et al. 2007). Consequently, fungal or other faunal activity may have been a pathway for input residues to become transferred into and stabilized within the OLF.

Consistent with previous studies (Swanston et al. 2005; Wagai et al. 2009), chemical characterization of SOM fractions suggests that the mineral-rich DF from CA and PR are relatively concentrated with C from microbial cells and

products as naturally occurring OM. The DFs at both sites had low C/N ratios and high concentrations of N- rich compounds, indicative of microbial films and microbial cell biochemistry (Swanston et al. 2005; Aufdenkampe et al. 2001; Hedges and Oades 1997; Kleber et al. 2007). Some variation occurred across sites in the DF chemistry including abundant benzene and N compounds in CA DFs, while PR DFs were composed more from lipids and phenols. Such variation in DF chemistry between sites may be attributed to differential decomposition rates for native C, which could influences the quality of SOM available to interact with mineral surfaces (Grandy and Neff 2008).

We observed limited variation in OLF chemistry between the two sites, with CA OLF producing abundant polysaccharides, N-compounds, and benzene compounds, while PR OLF was relatively abundant in lipids and lignin compounds. The chemical properties of OLFs from our sites were consistent with commonly reported characteristics for OLFs from other studies, such as a high C/N ratio (Wagai et al. 2009), a high concentration of C and N, and lipid-type compounds (Golchin et al. 1994b; Kölbl and Kögel-Knabner 2004; Marin-Spiotta et al. 2008; Sohi et al. 2001; Poirier et al. 2005; Wagai et al. 2009). Despite the increased stabilization of microbial ^{13}C residues observed in the OLFs, native OLF organic matter did not strongly reflect a microbial composition, as was noted in the DF. Recent reviews summarizing numerous studies on the OLF discuss the nebulous nature of the OLF, which may be highly variable in source and composition (comprising a combination of plant and microbial- derived products), and the origin is generally not well understood (Wagai et al. 2009). Nonetheless, the dynamics of our microbial residues suggest that, once associated with this fraction, microbial C is more stabilized than free un-occluded C (FLF).

The FLFs from both sites were enriched in lignin and polysaccharide products, with low abundance of N-compounds, indicative of partially degraded plant litter and generally consistent with previous reports on FLF chemistry (Skjemstad et al. 1999; Kracht and Gleixner 2000; Gleixner et al. 2002). Chemical characterization of this fraction, along with the lesser stability of microbial C inputs throughout the course of our study, is also consistent with reports that the FLF does not strongly promote the stabilization of microbial C; but rather the FLF is likely a more plant derived pool with a fast-turnover (Bird et al. 2008; Dalal et al. 2005; Rasmussen et al. 2005; Wagai et al. 2009).

Summary

The SOM fractionation approach used in this study was developed to isolate SOM fractions that differ in their relative associations with, and protection by, the mineral matrix (Golchin et al. 1994a; 1994b). Our results show that the association and retention of C from added microbial residues is a function of differences in stabilization processes among fractions. Our findings are in agreement with previous reports that mineral-associated C, by intra-

aggregate occlusion or binding to soil particles (i.e., the OLF and DF, respectively) is more stable than uncomplexed C (as in the FLF) (Dalal et al. 2005; Rasmussen et al. 2005; Swanston et al. 2005; Rasmussen et al. 2006; Wagai et al. 2009). However at PR, the microbial C inputs were more quickly mineralized in all fractions relative to CA, which we attribute to greater precipitation and higher temperatures in PR, and possibly other edaphic factors such as greater faunal activity at PR.

For both sites, added microbial residues that associated with the FLFs were less stable than that associated with the DFs and the OLFs. Site-specific controls on SOM and added microbial ^{13}C cycling were evidenced by different partitioning of native C and added microbial C among SOM fractions, with greater importance of the DF in PR, and of the FLF in CA as a reservoir for native soil C and added microbial residues. Regardless of initial microbial source or cell type, our results suggest association of input microbial C with the mineral matrix promotes stability and increases its residence time, both within aggregates and via association with soil particles (including mineral and organic surfaces). Although the initial source of microbial C and cell type did not influence its mineralization rate or mechanism of stabilization, specific compounds may preferentially stabilize and influence mineralization rates. Studies contrasting the molecular composition of decomposed necromass with living microbial cell materials and EPS would be valuable to understand mechanisms controlling microbial C turnover. The contrasting ecosystems in this study have illustrated that protection by the mineral matrix may vary across locations, but location in the soil matrix clearly influences the stability of microbial cells and products.

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