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Author
Dane, Laura Jennifer

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Soil Microorganisms as Precursors and Mediators of Soil Carbon Stabilization

by

Laura Jennifer Dane

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requirements for the degree of

Doctor of Philosophy

in

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in the

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of the

University of California, Berkeley

Committee in Charge:

Professor Mary. K. Firestone, Chair
Professor Steven E. Lindow
Professor Céline Pallud

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Abstract

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Soil organic matter (SOM) results from a suite of microbial and geochemical processes that in combination convert carbon (C) of biological origin to stabilized, potentially long-lived materials. While it is well established that soil microorganisms are involved in the conversion of plant biomass into SOM, the conversion of microbial bodies themselves to stabilized soil organic materials is not well understood. Recent studies suggest that microbial products such as polysaccharides, amino acids, fatty acids, and a number of other biomolecules of microbial origin can remain in soils for long periods of time, and that microbial bodies play a much more important role as the precursors to SOM than previously considered. In this dissertation, I examine the flow of microbial carbon in two soil types as it is assimilated into the living microbial biomass under different climate regimes, how long it remains in the living microbial biomass, and ultimately the flow of this microbial C out of the living biomass as it is respired out as CO_2 or is incorporated into SOM. The influence of soil type and climate were examined because both are key drivers of soil biogeochemical processes and strongly affect organic matter stabilization in soils.

In Chapter 1, I report the results of a field study conducted to understand not only whether different microbial groups preferentially assimilate carbon from different microbial sources of C, but also whether climate and edaphic characteristics alter either the assimilation of necrotic microbial carbon and/or the length of residence of this carbon within the living biomass. This study followed the fate of ^{13}C labeled dead microbial bodies for three years after they were injected into field soils located in a Temperate mixed-conifer forest and a Tropical wet forest. The ^{13}C was subsequently assimilated into the standing microbial biomass and ultimately lost from the biomass over a 3 year period. In general, the saprophytic microbial groups preferentially assimilated carbon from their same groups or groups with similar molecular compositions; however, only the Gram-positive bacteria in the Tropical site and Gram-positive and Gram-negative bacteria in the Temperate site demonstrated an affinity for assimilating C from dead actinobacteria. At each harvest throughout the study, the Temperate soils retained more labeled ^{13}C from the labeled necromass groups than the Tropical soils. The faster loss of labeled carbon from the living biomass in Tropical soils may have significant implications for the relative contributions of microbial biomass to the formation of SOM. As evidenced in Chapter 3, the retention time of C in living microbial biomass may be positively correlated to the sorption of microbial C to mineral surfaces in the heavy fraction (HF) of SOM. Since the HF is

generally the longest-lived SOM pool, an increase in the proportion of microbial C sorbed to mineral surfaces in the HF due to longer residence times of C in the living biomass of Temperate soils may lead to a higher proportion of microbial C in the HF of Temperate soils which may then lead to longer residence times of microbial C in the SOM of Temperate soils.

I also conducted a 1.5-year lab incubation designed to investigate the stability and fate of microbial cell materials by following the fate of added, labeled microbial cell C as it was assimilated into the living microbial biomass, respired out as CO_2, and recovered in the SOM. Soil samples were collected from a Temperate mixed-conifer forest ecosystem and a Tropical wet forest ecosystem; a single common mixture of ^{13}C labeled bodies was added to the soils, and the soils were incubated for 520 days under 3 different climate regimes (Mediterranean mixed conifer forest, Redwood forest, and Tropical forest). Results from the analyses of the stabilization of microbial C into operationally-defined organic matter fractions in two different soil ecosystems, and the fate of microbial C as it is assimilated into the living biomass, respired out as CO_2, and stabilized in the SOM of a Tropical soil are presented in Chapters 2 and 3, respectively.

The research presented in Chapter 2 addresses the interactions of climate and edaphic characteristics on the stabilization of microbial C into soil organic matter fractions in Temperate and Tropical soils. Both climate and soil type exerted significant influences on the total amount of ^{13}C recovered in the incubated soils as well as the amount recovered in each of the three operationally-defined stabilized carbon pools: free light fraction (FLF), occluded light fraction (OLF), and heavy fraction (HF). The recovery of ^{13}C was higher in the HF fraction than the OLF and FLF fractions in all but one soil-climate combination. The high recovery of ^{13}C in the HF is consistent with the stabilization of microbial C through interactions with soil mineral surfaces. There was clear influence of climate on the ^{13}C-OLF recoveries from Tropical soils as more ^{13}C was stabilized under the Temperate climates (Mediterranean and Redwood) compared to the Tropical climate.

In Chapter 3, the analyses were designed to ask whether longer residence times of carbon in the microbial biomass increased the association of microbial carbon with mineral surfaces in the heavy fraction of SOM. For this study, I monitored the fate of labeled dead carbon as it was added to Tropical soils under three climate regimes (Mediterranean, Redwood and Tropical), assimilated into the living saprophytic biomass, respired out as CO_2, and recovered in the SOM. An increase in saprophytic fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomasses under the Mediterranean climate early in the incubation indicated that all four of these microbial communities temporarily responded favorably to the relatively cold, dry spring Mediterranean climate conditions. Interestingly, assimilation of the dead microbial carbon by actinobacteria was highest under the Tropical climate; this trend was primarily due to the atom% ^{13}C excess of the actinobacterial cells, and not increases in actinobacterial biomass, indicating that while the presence of dead microbial carbon did not cause the actinobacterial communities to increase in size, the actinobacteria in this study did assimilate dead microbial carbon under the static warm, wet climate conditions. The results of Chapter 3 demonstrate that longer residence times of carbon in the microbial biomass may indeed increase the association of microbial carbon with mineral surfaces in the HF of SOM. Here, soils with the longest retention times of ^{13}C in the living biomass and the lowest respiration rates stabilized the most labeled carbon in the HF, while soils with the lowest retention times of ^{13}C in the living biomass and the highest respiration rates stabilized the least amount of labeled carbon in the HF.

While the contribution of microbial bodies to soil organic matter have historically been overlooked, or have been considered to be negligible, the findings of this dissertation support recent research that shows that microbial bodies are central to organic matter stabilization and that soil type and climate influence the retention of microbial C in SOM. In Chapter 1, I found that Temperate mixed conifer soils retained higher amounts of microbial C in the living biomass than Tropical wet forest soils over a 3 year period. The research in Chapter 3 demonstrated that longer retention times of C in the living microbial biomass led to higher stabilization of microbial C in the the HF of SOM. Chapter 2 showed a higher retention of microbial C in the HF, as opposed to the FLF or OLF, is likely due to the stabilization of microbial C in the HF through interactions with mineral surfaces. Together, the chapters in this dissertation indicate that a higher proportion of microbial C in Temperate soils may be stabilized through association with soil mineral components than in Tropical soils; in Topical soils, C in the living biomass is lost at a faster rate, diminishing the amount of time that microbial C interacts with mineral surfaces, potentially lessening the proportion of microbial C sorbed to and stabilized on mineral surfaces.

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Chapter 1

Assimilation and Retention of Dead Microbial Carbon by Living Microbial Communities in Temperate and Tropical Soils

Abstract

While it is well understood that soil microorganisms are vital for the conversion of plant biomass into stabilized soil organic matter (SOM), the conversion of microbial bodies to soil organic materials is not well understood. Research is beginning to reveal that microbial biomass is, in fact, an important precursor of SOM. Additionally, knowledge concerning the decomposition and subsequent utilization and assimilation of dead microbial bodies by the living microbial biomass is lacking. While previous studies have utilized individual microbial organisms and/or their cellular products to monitor the movement of microbial biomass carbon (C) into SOM, this study was the first of its kind to utilize ^{13}C-labeled, complex mixtures of four different microbial groups to monitor the movement of carbon from dead microorganisms into the living soil biomass. This study utilized *in situ* incubations in both Temperate and Tropical soil ecosystems over three years to assess not only whether various microbial groups preferentially assimilate carbon from specific microbial groups, but also whether climate and edaphic characteristics alter either the assimilation of dead microbial carbon and/or the length of retention of this carbon within the living microbial biomass. The results of this study demonstrate that the assimilation of C from the dead microbial groups did not depend on whether the added necromass was cultured from isolates that were originally native or non-native to the soils. In the Temperate soil ecosystem, Gram-positive bacteria were the only saprophytic microbial consumers to demonstrate an affinity for the assimilation of actinobacterial bodies, while in the Tropical soil ecosystem, Gram-negative bacteria also assimilated carbon originally from actinobacterial bodies. The lack of assimilation of dead actinobacterial bodies into the living fungal and actinobacterial biomasses may have been attributed to the relatively small size of the actinobacterial populations that was detected by phospholipid fatty acid analysis (PLFA) in the two soil ecosystems studied, or possibly a result of antibiotic production by actinobacteria. Even though it has been reported that less than 1% of chitin digesters in soils are fungi, the results of this study indicate that living fungi preferentially assimilate dead fungi over other microbial groups. Thus, living fungi may be more important to the breakdown and assimilation of dead fungal bodies than previously thought. Temperate soils retained more labeled ^{13}C from the necromass groups than the Tropical soils over the time course of the field incubations. The faster loss of labeled C from the living biomass in Tropical soils is likely attributable to the higher microbial metabolic processing rates found in Tropical ecosystems or lower carbon use efficiencies. The retention time of carbon within the standing microbial biomass is important because the retention time in microbial communities may be correlated with the stabilization of microbial carbon in the mineral-bound heavy fraction of soil organic matter. The application of complex mixtures of dead whole cells to Temperate and Tropical soils and the tracking of the fate of the cellular C provide insight into the cycling and stability of microbial carbon in soil ecosystems.

Introduction

While it is well established that soil microorganisms are integrally involved in the conversion of plant biomass into soil organic matter (SOM), the conversion of microbial bodies to stabilized soil organic materials is not well understood. Few studies have examined the fate of microbial bodies in the formation of soil organic matter (Kindler *et al.,* 2006; Lueders, *et al.,* 2006).

The incredibly high microbial diversity in soils (Curtis *et al.,* 2002; Torsvik *et al.,* 2002; Fierer and Jackson, 2006) has been thought to render taxonomically unique microbial communities in different ecosystems functionally similar with regards to soil carbon cycling (Dalal, 1998; Liang *et al.,* 2011). However, several studies have demonstrated that soil carbon cycling is in fact influenced by the variation among microbial community structures (Saetre and Bååth, 2000; Meyers *et al.,* 2001; Waldrop and Firestone, 2003; Gutknecht *et al.,* 2012; Andresen *et al.,* 2014). Soil microbial community composition itself is influenced by many factors, such as temperature, precipitation, soil pH, soil mineralogy, soil chemistry, and plant community structure. Across ecosystems, soil microbial communities can vary significantly in their community composition, metabolic capacities and biomass chemical composition; these differences have been suggested to influence the rates, amounts, and types of organic matter stabilized in soil (Waldrop and Firestone, 2004; Bird *et al.,* 2011; Liang and Balser, 2012).

The contributions of microbial bodies to soil organic matter have historically been overlooked, or have been considered to be negligible, because the active microbial biomass in soils often constitutes less than 5% of the organic matter in soils (Wardle, 1992; Branco de Freitas Maia *et al.,* 2013), and microbial biomass carbon is frequently less than 4% of the organic carbon in soils (Sparling, 1992; Anderson and Joergensen, 1997). However, the results of recent studies suggest that microbial bodies play a substantially more important role in determining the composition of SOM than previously considered (Kögel-Knabner, 2002; Kindler *et al.,* 2006; Grandy and Neff, 2008; Malik *et al.* 2013), and the relatively small sizes of microbial biomass pools in soils do not fully reflect the potential for microbial products to exist in stabilized organic pools (Liang *et al.,* 2011). Recent studies have shown that microbial products such as polysaccharides, fatty acids, amino acids, and a number of other biomolecules of microbial origin can remain in soils for long periods of time and contribute significantly to SOM (Kiem and Kögel-Knabner, 2003; Kindler *et al.,*2006; Potthoff *et al.* 2008; Kindler *et al.,* 2009; Miltner, 2009; Yao and Shi, 2010; Liang and Balser, 2012). These studies have demonstrated that microbial communities contribute to the formation of SOM not only via their metabolic capacities to decompose plant litter, but also that their bodies themselves can become the building blocks of stabilized organic matter. Therefore, it is important to understand how microbial bodies themselves contribute to soil organic matter formation.

Elucidation of the mechanisms underlying the assimilation of microbial bodies and their transformation into soil organic matter requires an understanding of saprophytic microbial food webs. This understanding not only applies to the microbial consumption of plant litter, but also to the assimilation of dead microbial bodies into the living, active microbial biomass (Lueders, *et al.,* 2006). While it is generally understood that saprophytic microorganisms feed on dead microbes, there is little understanding of whether microbial communities exhibit preferential assimilation of different dead microbial groups.

Complex biotic interactions and trophic webs control the movement and transformation of organic carbon in soils. At the community and ecosystem levels, a multitude of trophic

interactions within and between the aboveground plant biomass and belowground saprophytic biomass components drive the retention and cycling of nutrients (Wardle *et al.,* 2004). While knowledge concerning plant-microbial interactions and the decomposition of plant matter by microbial saprophytes has increased rapidly, there is very little detail published on the specific interactions between microbes as saprophytic food sources and the living microbes that consume them (Lueders *et al.,* 2006).

To untangle the processes underlying the transformation and incorporation of dead microbial biomass into the active, living microbial biomass and, ultimately, the stabilization of microbial necromass in soil organic matter, this study followed the fate of ^{13}C labeled dead microbial bodies for approximately 3 years after they were injected into two different soils *in situ*. The labeled carbon was then followed into the living microbial biomass to quantify how much microbial carbon was incorporated and retained in indigenous microbial groups in the soil ecosystems over time.

The fate of the C from dead microbial cells was studied in two very different soil ecosystems, a tropical and a temperate ones, in order to examine the extent to which two different indigenous, active microbial communities assimilate dead microbial carbon and how climate and edaphic characteristics influence both the assimilation and retention of microbial carbon in the living biomass of soils. The microbial strains used for this study were originally cultured from both the Temperate and Tropical soil ecosystems and were applied reciprocally to each soil ecosystem to compare the effects of cell origin and microbial metabolic capacities, in conjunction with edaphic effects, on the fate of dead microbial carbon in two very different soils.

By following the ^{13}C from the dead cells into the standing, active microbial biomass, this study was able to identify which indigenous microbial groups were active in the assimilation of the four groups of dead microbial C, whether any of these microbial groups exhibited preferences for the assimilation of the dead microbial groups, and whether there were differences in the turnover of that carbon in the living biomass. The fate of the ^{13}C-label was followed for approximately 3 years by quantifying the ^{13}C in microbial biomarker components of phospholipid fatty acids (PLFA).

Following the fate of labeled microbial cell C through the use of ^{13}C-PLFA provides insight into the assimilation of dead microbial C by the living soil biomass, as well as the turnover of this C in biomass pools in two different ecosystems. While a limited number of studies have traced the flow of carbon from individual microbial organisms and/or their cellular products into the living soil microbial community, and the subsequent stabilization in the soil organic matter (Kindler *et al.,* 2006; Lueders *et al.,* 2006), this study is the first of its kind to follow a complex mixture of labeled cells *in situ*. This is important because the microbial carbon sources in soil ecosystems are not simply composed of single organisms or cellular products, but are complex mixtures of compounds whose degradation kinetics and/or physical or chemical protection mechanisms may differ substantially from those of individual components (Lueders *et al.,* 2006). This study examines which of the four dead microbial groups the living microbes assimilate, and compares the shared traits within each of the necromass groups that may have led to preferential assimilation by the living biomass.

Materials and Methods

Field sites

This study was conducted at the University of California Blodgett Forest Research Station (Temperate site) and the Colorado Forest in the Puerto Rico Luquillo Experimental Forest (Tropical site). Blodgett Forest is located in the mid-elevation Sierra Nevada Mountains, 240 km east of San Francisco (Table 1-1), and is representative of productive mixed-conifer forestlands in California. Dominant plant species include sugar pine (*Pinus lambertiana*) and ponderosa pine (*Pinus ponderosa*). The Puerto Rico Forest is located in the highlands of Puerto Rico, 35 km east of San Juan (Table 1-1), and is representative of a tropical montane wet forest in which high levels of precipitation cause soils to fluctuate between oxic and suboxic conditions. Approximately 40 plant species occur in the Puerto Rico forest, and the dominant plant species is myrtle (*Cyrilla racemiflora*). The Firestone lab at UC Berkeley has extensive experience working at both locations, and a detailed knowledge of their site characteristics (Table 1-1).

Microbial growth and additions

Microbial cell cultures were isolated from soils sampled in 2004 from the University of California Blodgett Forest Research Station and the Luquillo Experimental Forest in Puerto Rico as described below; the origin of each isolate is given in Table 1.2. At each forest site, soil samples were collected (0-15 cm depth) at 15 locations along 3 transects, homogenized separately, and stored at field moisture and ambient temperature for up to 4 days before subsampling for culturing. Each of the cultured microbial groups from both the Temperate and Tropical sites were applied to the Temperate and Tropical field sites separately to account for any differences in native versus non-native cell cultures.

The four microbial groups used in this study were fungi, actinobacteria (high-GC Gram-positive bacteria), Gram-positive bacteria, and Gram-negative bacteria; the exact composition of isolates in each group can be found in Table 1-2. The primary cell types contained in the culture group mixtures utilized in this study (of 23 total) included: *Penicillium verruculosum* and *Penicillium sacculum* (fungi), *Streptomyces sp.* and *Kitasatospora sp.* (actinobacteria), *Arthrobacter sp.* and *Bacillus sp.* (Gram-positive bacteria), *Burkholderia sp.* and *Rhizobium sp.* (Gram-negative bacteria). The four microbial groups used in this study were chosen because the groups were expected to have fairly different cellular chemistries. Nuclear Magnetic Resonance (NMR) analysis of the groups used in this study, after they had been chosen and ^{13}C labeled, revealed they had fairly distinct cellular chemistries (Fan *et al.,* 2009). For example, the Gram-negative bacteria contained a high abundance of β-hydroxybutyrate (BHB) and low levels of trehalose, whereas BHB was absent from and high amounts of trehalose was present in Gram-positive bacteria, fungi, and actinobacteria; The fungi contained more glycolipids than the actinobacteria, and demonstrated the highest synthetic capacity for glycerol production among all four of the microbial group types; The Gram-positive bacteria were more enriched in phospholipids than the Gram-negative bacteria, fungi and actinobacteria, and contained less triacylglycerides than the other three groups.

Table 1-1. Site and soil characteristics of the two soil harvest locations. Means are shown with standard errors in parentheses.

Region	Puerto Rico	Central Sierra Nevada
Forest Type	Tropical Deciduous	Temperate Mixed Coniferous
Site Name	Luquillo Experimental Forest	Blodgett Experimental Forest
Latitude	18º 41' N	38º 53' N
Elevation (m)	780	1315
Mean precipitation (mm)	4,500 [2]	1,774 [1]
Mean annual temperature (ºC)	18.5 [2]	13 [3]
Dominant plant species	*Cyrilla racemiflora*	*Pinus ponderosa*
Soil order	Ultisol [2]	Alfisol [1]
Soil texture	sandy loam	sandy loam
% sand; % silt; % clay	64.0%; 18.3%; 17.8%	54.6%; 34.8%; 10.6%
pH	4.5 [4]	5.8 [4]
Potassium (meq/100g)	0.10 (0.00)	0.36 (0.01)
Sodium (meq/100g)	0.13 (0.02)	0.02 (0.00)
Calcium (meq/100g)	1.07 (0.21)	9.00 (1.32)
Magnesium (meq/100g)	0.78 (0.10)	0.69 (0.10)
Extractable sulfate (ppm)	20.7 (3.2)	12.9 (0.4)
Total manganese (ppm)	23.3 (3.6)	1281.4 (155.5)
Phosphorus (ppm)	0.33 (0.07)	1.08 (0.03)
Extractable Iron- DTPA (ppm)	417.0 (19.7)	83.4 (5.1)
CEC (meq/100g)	16.98 (3.5)	24.46 (1.7)
Soil organic carbon (mg C/g soil)	121 (6) [4]	100 (5) [4]
Microbial biomass carbon (mg C/g soil; CFDE)	1.512 (0.116) [4]	0.432 (0.042) [4]
Microbial biomass carbon (mg fatty acid C/g soil; PLFA)	0.058 (0.003)	0.035 (0.001)
Fungal:Bacterial biomass ratio	0.20	0.32

1 Bird and Torn, 2006

2 Templer et al., 2008

3 Blodgett Experimental Forest Research Station

4 Throckmorton et al., 2012

Table 1-2. Background information for each microbial isolate that was isotopically labeled and used in this field study: soil harvest site (Temperate or Tropical), microbial group (Fungi, Actinobacteria, Gram-positive bacteria, or Gram-negative bacteria), taxonomic classification and operational taxonomic unit BLAST match, and % of each homogenized group. Since the origin (harvest site) did not lead to differences in the average overall assimilation of native and non-native necromasses, the results from the Temperate and Tropical harvest sites were combined for each group.

Harvest Site	Group	Taxonomic Classification	Operational Taxanomic Unit (BLAST match)	Taxa % of Group
Temperate	Fungi	Ascomycete	Penicillium sacculum	78.2%
		Basidiomycete (mycelial)	Holtermannia corniformis	8.8%
		Basidiomycete (yeast)	Cryptococcus terreus	6.2%
		Zygomycete	Umbelopsis nana	6.9%
Temperate	Actinobacteria	Streptomycetaceae	Streptomyces sp. strain Soll14	51.7%
		Streptomycetaceae	Streptomyces capoamus	20.0%
		Streptomycetaceae	Streptomyces sp. JL164	28.3%
Temperate	Gram-positive bacteria	Actinobacteria (non-Streptomycetaceae)	Arthrobacter sp. strain RC100	68.7%
		Firmicutes	Bacillus subtilis strain KL-077	31.3%
Temperate	Gram-negative bacteria	Alpha-proteobacteria	Rhizobium sp. strain 'USDA 1920'	37.7%
		Beta-proteobacteria	Burkholderia sp. strain A22-1	37.5%
		Beta-proteobacteria	Burkholderia sp. strain UCT 29	24.9%
Tropical	Fungi	Ascomycete	Penicillium verruculosum	87.4%
		Basidiomycete (mycelial)	Trichosporon multisporum	9.6%
		Basidiomycete (yeast)	Cryptococcus podzolicus	3.0%
Tropical	Actinobacteria	Streptomycetaceae	Streptomyces sp. strain 317	29.1%
		Streptomycetaceae	Kitasatospora sp. C2	35.8%
		Streptomycetaceae	Kitasatosporia griseola JCM 3339	35.1%
Tropical	Gram-positive bacteria	Firmicutes	Bacillus thuringiensis strain 2000031485	60.0%
		Firmicutes	Bacillus mycoides strain: S31	40.0%
Tropical	Gram-negative bacteria	Bacteroidetes	Flexibacter cf. sancti	2.1%
		Beta-proteobacteria	Burkholderia plantarii	76.8%
		Gamma-proteobacteria	Dyella japonica	21.1%

The initial culturing methods screened hundreds of isolates per site using agar amended with soil-extracts and 10% trypticase soy agar (TSA) or 10% trypticase soy broth (TSB) at pH 5.5; a pH of 5.5 was chosen to mimic the acidic conditions of both the Blodgett Forest and Luquillo Forest soils (Table 1-1). The cultures were then further isolated with additions to the media: novobiocin and potato dextrose agar (PDA) with streptomycin for fungi; chitin with cycloheximide or starch casein for actinobacteria; 10% TSA with cycloheximide for Gram-positive and Gram-negative bacteria (Alef 1995; Fan *et al.,* 2009). The identities of the final 23 isolates selected were confirmed using DNA extraction kits (PowerSoil, Mobio Inc., CA); 16S rRNA genes were amplified from the bacterial isolates using the "universal" primers 27F and R1492 (Lane *et al.*, 1985) and 18S-5.8S-28S ITS regions were amplified from fungal isolates using the ITS1F and ITS4 primers (Gardes and Bruns, 1993). Full, high quality sequences (phred score $q \geq 20$) were compared by BLAST with the public databases NCBI Genbank and greengenes (DeSantis *et al.*, 2006).

The final isolates were ^{13}C-labeled by growing cultures in liquid media to late stationary phase at $25^o C$; the media contained 20% glucose (99.9 atom% enriched with ^{13}C) and 10% yeast extract with M9 salts at pH 5.5. Whole cells were harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 7.0) to remove remaining media. Low-density cellular materials produced during culturing that were not harvested with the cells were filtered from the used

media using 0.1-μm polycarbonate filters (Sterlitech Co., Kent, WA), washed with deionized water, and combined with the whole cells. In order to focus on the assimilation and stabilization of microbially-derived carbon in soils, and not the metabolic activity of labeled microbial cells, the cultures were autoclaved and lyophilized to kill the cells while maintaining as much cellular structure as possible (Fan *et al*., 2009).

In order to widely distribute the ^{13}C-labeled, sterilized microbial necromass in the soils, all of the previously freeze-dried, dead cell material was suspended in water before syringe injection into the cores. A tissue homogenizer (Potter-Elvehjem) was then used to distribute and standardize the mixture of microbial isolates. While injection of cell-containing solutions into soil did not realistically simulate the natural circumstances of microbial growth and turnover, it did allow us to follow the assimilation of the dead cell C by the living microbial biomass.

Experimental design

This study followed the movement of carbon from dead microbial bodies into the living microbial biomass in Temperate and Tropical soil ecosystems. In 2004, soil cores (PVC, 10 cm diameter, 20 cm height) were installed at the two field sites to a soil depth of 18 cm (so that 2 cm of the core remained above the soil surface). Each soil core contained three open holes in the side (2.5 cm diameter; centered at soil depths of 3, 8 and 13 cm) that were lined with 450-μm mesh screening. These holes were created to allow fungal hyphae and fine plant roots to enter the soil cores, and to allow for limited water and gas exchange with the surrounding bulk soil. Once the cores were installed, the soils were allowed to equilibrate for 1 year before the labeled necromass was injected into the soil cores.

There were 8 different necromass groups added to soil cores in each soil ecosystem: Temperate fungi, Temperate actinobacteria, Temperate Gram-positive bacteria, Temperate Gram-negative bacteria, and Tropical fungi, Tropical actinobacteria, Tropical Gram-positive bacteria, Tropical Gram-negative bacteria. The Temperate and Tropical necromasses for each microbial group were applied separately to determine if there were any differences in microbial assimilation of native versus non-native necromass in either of the soil ecosystems.

A total of 80 mg of labeled microbial necromass (34.3 mg ^{13}C) was suspended in 30 ml of deionized water, and then injected into each soil core at five points at a depth of 3 cm. Before the necromass was injected into the soil cores, the forest litter was first removed then replaced after injection. Each site contained control soil cores that received 30 ml deionized water at multiple injection points, with no amendments of labeled microbial necromass. There were 3 field replicates for each of the 9 treatments (8 labeled cell treatments plus 1 control) and 5 harvests for each of the 2 sites for a total of 270 soil cores. While multiple injection sites were used to distribute the labeled cells as best as possible in each soil core, one limitation of this method was that there was no way to guarantee that the cells were uniformly dispersed within the soil cores; non-uniform distribution could lead to results that are not representative of true patterns of assimilation of microbial necromass by the living soil microbial biomass.

The sterilized, labeled necromass was introduced into the soil cores on June 21, 2006 in the Temperate site and on October 6, 2006 in the Tropical site. The soil cores were harvested at 5 timepoints at each location. The Tropical soil cores were harvested over a shorter time span than the Temperate soil cores because soils in tropical climates have demonstrated significantly higher metabolic processing rates than Temperate soils (Harmon *et al.,* 2009). Soil cores were harvested from the Temperate site at 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5) days post ^{13}C-labeling; from the Tropical site, soil cores were harvested at 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5) days post ^{13}C-labeling. At each timepoint, three replicates for each

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of the eight treatments were harvested, in addition to three control soil cores, for a total of 27 cores per harvest timepoint.

Soil processing

After the soil cores were harvested, each core was separated into two depth increments: 0-7.5 cm and 7.5-15 cm from the soil surface. The O_a, a highly decomposed soil horizon, was included in the 0-7.5 cm analyses. Since the labeled necromass was injected 3 cm below the soil mineral surface, the O_i (intact litter with very little decomposition) and the O_e (moderate decomposition of litter) soil horizons were removed before soil processing and not included in any analyses. Loss of label from the removal of the O_i and O_e horizons was assumed to be less than 1% (Bird and Torn, 2006). The two soil depths were analyzed separately, and because the 7.5-15 cm soils did not retain a detectable ^{13}C-signal due to extremely low retentions of the ^{13}C label by the standing biomass, analyses of this layer were not included in this study.

While the soil cores harvested from the Temperate site were transported intact directly to the lab, the two soil depths within the Tropical cores were separated in the field due to the extremely high moisture contents of the soils, which would have compacted the soils during the flight back to California. Once all the soils arrived in the lab, they were stored at 4° C and processed within 7 days after harvest. Temperate soils were homogenized and sieved to 2 mm; the Tropical soils were too wet to sieve; Thus, the soils were manually mixed within their storage bags and large particles, such as visible roots and woody debris, were removed by hand. After the soils were processed, subsamples for microbial analysis were stored at -80° C.

Microbial community composition and microbial assimilation of cellular ^{13}C

Phospholipid fatty acid analysis (PLFA) was used to characterize the total living microbial biomass and the fungal-to-bacterial ratios (PLFA; Zelles 1999). The extraction, identification and quantification of PLFAs followed the modified methods reported in Bligh and Dyer (1959) and White and Ringelberg (1998). Microbial lipids were extracted from 8 g freeze-dried soil in a monophasic mixture of chloroform, methanol and phosphate buffer (1ml:2ml:0.83ml) with di-19:0PC (1, 2-Dinonadecanoyl- sn-Glycero-3-Phosphocholine, Avanti Polar Lipids, Alabaster, AL, USA) used as a surrogate standard to quantify soil PLFA. Extracts were separated into two phases by adding chloroform and DI-water, and the lipid-containing phase was dried at 37 °C under N_2 until all the water had evaporated; the length of drying time varied considerably. Phospholipids were separated from neutral lipids and glycolipids on Inert II silica columns (Burdick & Jackson, Muskegon, MI) using sequential elution by chloroform, acetone, and methanol. Phospholipids in the methanol elution were transesterified at 37 °C, dried at room temperature under N_2, and resuspended in hexane containing 10:0 FAME (methyl decanoate, Sigma-Aldrich, St. Louis, MO, USA) added as an internal standard.

The biomarkers used to determine the fungal-to-bacterial ratios were similar to those reported in Bird *et al.* (2011) and the ratio was calculated as the sum of the fungal group yield divided by the sum of the Gram-positive bacteria, Gram-negative bacteria, and Cyclopropyl Gram-negative bacterial group yields (nmol fungal fatty acid C per g dry soil/nmol bacterial fatty acid C per g dry soil).

Thirteen indicator PLFA biomarkers were categorized into 1 fungal group and 3 bacterial groups: fungi were indicated by 18:2ω6,9c, 18:1ω9c; Gram-negative bacteria by 16:1ω7c, 18:1ω7c (Marschner 2007); Cyclopropyl Gram-negative bacteria by 17:0cyclo, 19:0cyclo (Arao 1999); Gram-positive bacteria by 15:0i, 15:0a, 16:0i, 17:0i, 17:0a (Frostegård and Bååth, 1996); and actinobacteria by 16:0-10Me, 18:0-10Me (Marschner 2007). The following 9 unassigned

biomarkers were included in calculations of total microbial biomass (nmol fatty acid C per g dry soil): 14:0, 15:0, 16:1ω5c, 16:1ω9c, 16:0, 16:0-12Me, 16:1-2OH, 18:1ω5c, 18:0 (Herman et al. 2012).

It should be noted that measurements of microbial biomass by PLFA is limited to measurements of known microbial fatty acids. Microbial biomass measurements for the 13 known fatty acids that comprise representatives of the fungal, actinobacterial, Gram-positive, and Gram-negative bacterial groups may not be representative of the actual microbial biomass for each group. Biomass measurements of the 9 unknown microbial fatty acid biomarkers, as well as additional fatty acid biomarkers not used in this study because we cannot say for certain that they are microbially derived, are often not insignificant. The redistribution of these unknown fatty acid biomarkers into known microbial groups could substantially alter the relative proportion of the groups to one another, as well as calculations of mass uptake of ^{13}C by each microbial group.

Studies that utilize PLFA often do not include results and discussions of the importance of unassigned fatty acids in microbial community profiles and assimilation of carbon into unassigned microbial biomass (Arao, 1999; Wilkinson *et al.,* 2002; Balser and Firestone, 2005; Bird *et al.,* 2011). Even though we are unable to distinguish the group identity of unassigned fatty acids, they are included in the results and discussion of the ng ^{13}C recovered in the living biomass at the end of this study because the size of their biomass and assimilation of ^{13}C accounted for a substantial portion of the ^{13}C cycling within the microbial biomass in this study.

Elemental and isotopic analysis

PLFA samples were analyzed at UC Berkeley on a Hewlett Packard (Agilent) 5890 Series II Gas Chromatograph (GC) with a 30 m × 0.32 mm × 1.0 mm ZB-5 column (Phenomenex, Inc., Torrance, California, USA) connected via a Europa ORCHID on-line combustion interface (PDZ Europa, Cheshire, UK) to an Isoprime 100 IRMS (Isoprime, Manchester, UK). The microbial biomass (mg fatty acid/g soil), atom% ^{13}C excess PLFA, and ng ^{13}C excess PLFA per microcosm were measured for all timepoints. The background atom% ^{13}C and background ng ^{13}C results were subtracted from the T1, T2, T3, T4, and T5 results to account for the natural abundance of ^{13}C in the living biomass of Temperate and Tropical soils.

To calculate the mg C for each PLFA biomarker, the nmol fatty acid C for each lipid biomarker was multiplied by the mass of the carbon chain length specific to that biomarker (Eqn 1). To calculate the ng ^{13}C excess for each biomarker, atom% ^{13}C for each biomarker was multiplied by the ng C of that biomarker (Eqn 2). The atom% ^{13}C excess and ng ^{13}C excess results for PLFA biomarkers are reported in the results and discussion as atom% ^{13}C and mg ^{13}C, respectively.

$$\text{mg C} = \text{nmol fatty acid C} * \frac{\text{mass of carbon chain length (ng C)}}{1 \text{ nmol fatty acid C}} * 10^{-6} \text{ mg ng}^{-1} \tag{1}$$

$$\text{ng } ^{13}\text{C} = \frac{\text{atom\% } ^{13}\text{C}}{100} * \text{mg C} * 10^6 \text{ ng mg}^{-1} \tag{2}$$

While PLFA analysis is designed to capture the fatty acids of living microbial groups, there is a possibility that ^{13}C from the labeled necromass was picked up early in the study. However, by 17 days (Tropical site T1) and 33 days (Temperate site T1), most of the labeled microbial bodies are assumed to have been consumed by the living microbial communities in the

soil cores. Kindler *et al*. (2006) found that living soil microbes quickly assimilated ^{13}C-labeled bacterial biomass after only 1 hour of incubation, and Lueders *et al*. (2006) found a rapid decrease in the number of introduced, genetically modified bioluminescent bacterial cells, which served as the substrate for assimilation by indigenous soil microorganisms.

Statistical analysis

Means and standard deviations were calculated for the biomass C, atom% [13]C excess and ng ^{13}C excess recovered from each soil treatment over time. Significant differences among native and non-native necromass additions for each group at each harvest at each incubation site were determined by one-way analysis of variance (ANOVA) with necromass origin as the independent variable. Significant differences among fatty acid biomasses for each group (mean of T1 through T5 harvests) were determined by two-way ANOVA with incubation site and living biomarker group as the independent variables. Significant differences among treatments in the atom% [13]C excess PLFA and in the ng ^{13}C excess PLFA recoveries throughout the entirety of the study were determined by two-way ANOVA with time and the microbial group that consumed the necromass as the independent variables. Significant differences among treatments in the atom% [13]C excess PLFA and in the ng ^{13}C excess PLFA recoveries at each timepoint were determined by one-way ANOVA with the microbial consumer group that consumed the dead labeled bodies as the independent variable. Significant differences among ng ^{13}C recovered from each necromass group at the T5 harvest were determined by two-way ANOVA with incubation site and necromass group as the independent variables. Tukey's Honestly Significant Difference (HSD) test was used to determine the significant differences among the means when the ANOVA result was significant ($p < 0.05$). Data are reported as the mean $\pm$ 1 standard error.

Results

Assimilation of native versus non-native microbial necromass

The assimilation of ^{13}C by saprotrophic microorganisms was measured by calculating the PLFA atom% [13]C excess of each consumer biomarker, derived from the uptake of each of the eight added, labeled microbial groups. A comparison of the assimilation of native and non-native necromass groups into 22 PLFA biomarkers was performed for each of the 22 fatty acid biomarkers for each harvest date in both the Temperate and Tropical soils. All the consumer groups (living fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomarkers) assimilated both native and non-native microbial necromass.

The overall average assimilation of ^{13}C did not significantly differ between native and non-native necromass in either of the two soil ecosystems. Out of 880 comparative analyses (22 fatty acid biomarkers × 8 labeled necromass treatments × 5 timepoints), only 103 cases indicated a difference in the assimilation of a biomarker from native and non-native microbial necromass (Table 1-3). However, it should be noted that fungi cultured from Tropical soils were assimilated by a number of Temperate soil microbial biomarkers slightly more than fungi cultured from Temperate soils, and Gram-positive bacteria cultured from Temperate soils were assimilated slightly more by a number of biomarkers in Temperate and Tropical soils than Gram-positive bacteria cultured from Tropical soils. Since the origin (native versus non-native) of the labeled microbial necromass did not significantly affect the average overall perceived quality of the

labeled cell material to the labeled microbial consumers, the results from the native and non-native cell treatments for each necromass group were combined for all subsequent analyses.

Standing fatty acid biomass in experimental soils

Measurements of total standing fatty acid biomass represent the sum of the fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned fatty acids in each soil core. The biomasses recovered for the Gram-positive bacteria, Gram-negative bacteria, and unassigned fatty acid biomarkers in the Tropical soil were significantly higher ($p < 0.0001$) than in the Temperate soil (Fig. 1-1). The biomasses indicated by the fungal and actinobacterial biomarkers were the same in the Tropical and Temperate soils. In the Temperate soil ecosystem, standing biomass levels followed the order: Gram-negative bacteria > unassigned fatty acids > Gram-positive bacteria = fungi > actinobacteria. In the Tropical soil ecosystem, the order was: Gram-negative bacteria = unassigned fatty acids > Gram-positive bacteria = fungi > actinobacteria. The biomass recoveries of all four standing microbial groups at each timepoint can be found in Table 1-4.

Recovery of ^{13}C in the living biomass from labeled Fungi

In both the Temperate and Tropical soils, the labeled fungal carbon contributed to relatively low ^{13}C mass recoveries in the living microbial consumer groups compared to the ^{13}C assimilated from other labeled necromasses (Figs. 1-2 and 1-3). In addition, the atom% ^{13}C values for Gram-positive (15:0a, 16:0i, 17:0a, 17:0i) and Gram-negative (18:1ω7c) fatty acid biomarkers that had consumed labeled fungi at T1 in both soil incubation sites were substantially lower than the same consumer biomarkers that consumed labeled Gram-positive and Gram-negative bacteria (Tables 1-5 and 1-6).

In both the Temperate and Tropical soils, time and microbial consumer group had significant effects ($p < 0.0001$) on the ng ^{13}C recovered in the standing fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned fatty acids from the assimilation of labeled fungal necromass. In the Temperate soil, the interaction of time and microbial consumer group also had a significant effect ($p = 0.0022$), and in the Tropical soil, the interaction of time and microbial consumer group had a significant effect at $p < 0.0001$ (Figs. 1-2 and 1-3).

At the first Temperate soil harvest, the living Gram-negative bacteria and unassigned lipid biomasses recovered more ng ^{13}C originating from labeled fungal bodies than the living fungal and actinobacterial biomasses, while the Gram-positive bacteria exhibited mid-level recoveries (Fig. 1-4). The fungal biomass as a whole recovered more ng ^{13}C than the Gram-positive bacterial biomass, although this average value was primarily due to the high atom% ^{13}C recovered in the 18:2ω6,9c biomarker. The atom% ^{13}C values and biomass pool size for the Gram-negative bacteria at the first harvest indicate that the high ng ^{13}C recovery of fungal derived ^{13}C in the Gram-negative bacteria may be primarily attributed to the large size of the Gram-negative biomass pool in the Temperate soils, and not an overall high atom% ^{13}C recovery (Tables 1-4, 1-5). The next highest atom% ^{13}C recoveries in Temperate soil at 33 days were found in the 15:0i, 15:0a, and 16:0i biomarkers (Gram-positive bacteria). The high atom% ^{13}C values for these Gram-positive biomarkers, coupled with the mid-range biomass pool of Gram-positive bacteria in Temperate soils led to mid-range assimilation of ng ^{13}C by the Gram-positive bacteria at 33 days. The living actinobacteria at the first two harvests displayed both low mass recovery and low atom% ^{13}C values.

Table 1-3. Assimilation of native versus non-native microbial necromass as measured by atom% ^{13}C for each PLFA biomarker. Values represent the number of harvests (out of 5) when the assimilation of native and non-native necromasses differed for each biomarker. Green squares indicate harvests when the isolates cultured from Tropical soils were assimilated into the living biomass more than the Temperate soil cultures. Blue squares indicate harvests when the isolates cultured from Temperate soils were assimilated into the living biomass more than the Tropical soil cultures. Out of 880 comparative harvest times, only 103 harvests differed in the uptake of native and non-native cultures.

^{13}C in necromass groups added to Temperate and Tropical soils:

Assimilation by:	Fungal ^{13}C added to		Actinobacterial ^{13}C added to		Gram-positive bacteria ^{13}C added to		Gram-negative bacteria ^{13}C added to	
	Temperate site	Tropical site	Temperate site	Tropical site	Temperate site	Tropical site	Temperate site	Tropical site
Fungi 18:2w6,9c	0	0	0	0	0	0	0	0
18:1w9c	1	0	2	1	0	0	0	0
Actinobacteria 16:0 10Me	0	0	0	0	0	1	0	0
18:0 10Me	0	1	0	0	1	0	1	1
Gram-positive bacteria 15:0i	1	0	0	0	0	2	0	0
15:0a	2	0	1	0	1	1	3	1
16:0i	0	1	2	0	2	1	2	2
17:0i	0	0	0	0	0	0	1	0
17:0a	0	1	1	0	1	2	1	0
Gram-negative bacteria 16:1w7c	1	0	0	0	0	1	0	0
17:0cyclo	0	0	1	0	1	4	0	0
18:1w7c	1	0	1	0	1	4	2	0
19:0 cyclo	0	0	1	0	1	2	1	0
Unassigned biomarkers 14:0	1	0	0	0	2	3	0	0
15:0	2	0	0	0	0	2	2	0
16:1w9c	2	0	1	0	0	1	0	0
16:1w5c	1	0	0	1	1	0	0	0
16:0	2	0	0	0	1	3	0	0
16:0 12Me	0	2	0	0	2	0	1	0
16:1 2OH	1	1	0	0	0	2	0	2
18:1w5c	2	1	0	0	1	0	0	0
18:0	2	0	0	0	0	1	0	0

Figure 1-1. Fatty acid biomass (mean ±1 standard error). Data for each group living at each incubation site are averaged over timepoints 1-5; The fungal group includes 30 replicate measurements (2 biomarkers × 3 reps × 5 timepoints), the actinobacterial group includes 30 replicate measurements (2 biomarkers × 3 reps × 5 timepoints), the Gram-positive bacterial group includes 75 replicate measurements (5 biomarkers × 3 reps × 5 timepoints), the Gram-negative bacterial group includes 60 replicate measurements (4 biomarkers × 3 reps × 5 timepoints), and the Unassigned group includes 135 replicate measurements (9 biomarkers × 3 reps × 5 timepoints). Different letters indicate significant differences among soil ecosystems and microbial biomass groups as determined by two-way ANOVA.

Table 1-4. Microbial biomass (nmol fatty acid C per g dry soil) for each standing microbial biomass group in the Temperate and Tropical soils. Means are shown with standard errors in parenthesis.

Site	Time (days)	Fungi	Actino	Gram-positive	Gram-negative	Unassigned
Temperate	33	28.1 (2.7)	8.6 (0.7)	32.0 (2.2)	68.1 (4.4)	48.9 (3.3)
Temperate	163	20.2 (1.2)	6.9 (0.3)	26.9 (1.4)	62.3 (3.3)	41.2 (2.2)
Temperate	370	17.9 (1.9)	5.4 (0.5)	21.7 (2.1)	46.1 (4.3)	31.4 (3.0)
Temperate	763	26.9 (1.6)	6.4 (0.2)	24.9 (0.8)	58.4 (2.6)	39.2 (1.7)
Temperate	1133	34.5 (2.3)	7.6 (0.4)	21.5 (1.1)	62.2 (3.6)	38.1 (1.9)
Tropical	17	19.8 (1.4)	11.8 (0.7)	40.8 (2.4)	64.6 (3.9)	63.3 (4.6)
Tropical	114	22.7 (2.6)	13.2 (1.1)	39.0 (2.9)	80.8 (6.2)	63.9 (7.0)
Tropical	249	31.6 (3.9)	17.8 (1.7)	59.1 (6.2)	122.9 (12.6)	95.7 (11.4)
Tropical	480	29.0 (4.3)	15.0 (1.4)	47.3 (5.1)	92.6 (7.4)	78.8 (10.2)
Tropical	886	30.7 (5.1)	16.1 (1.7)	57.7 (7.3)	96.5 (10.1)	95.2 (15.6)

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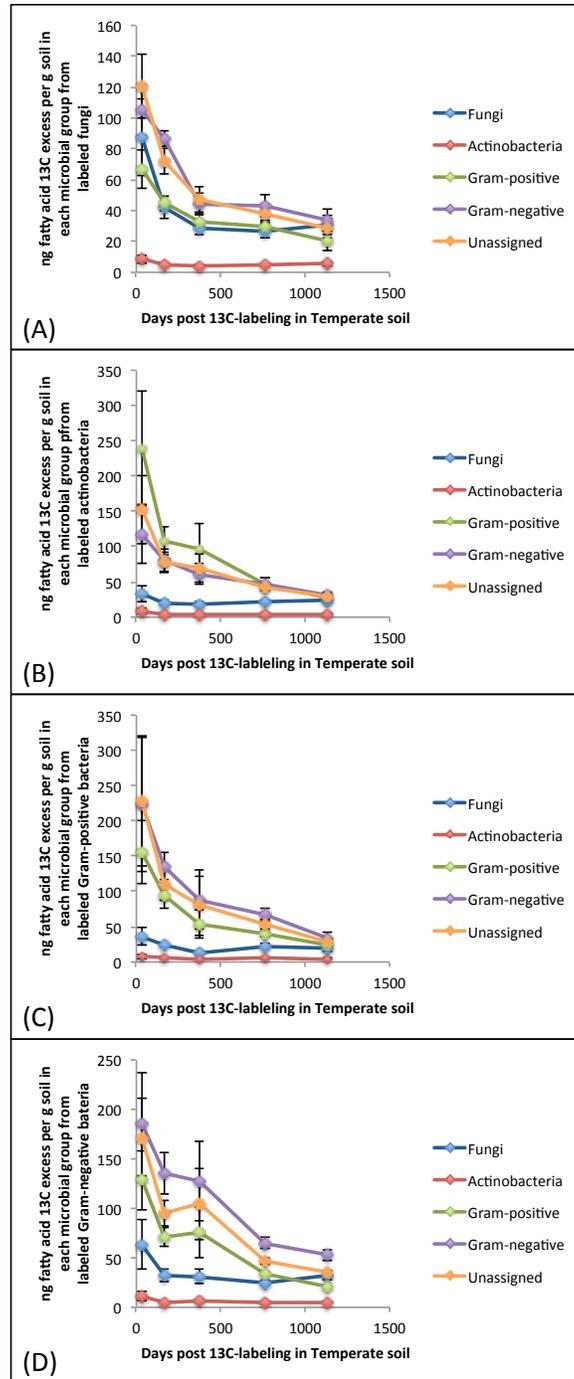
Figure 1-2. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups over time due to the assimilation of labeled microbial necromasses added to Temperate soils. Panel (A) shows the assimilation of ^{13}C from labeled fungi, (B) actinobacteria, (C) Gram-positive bacteria, and (D) Gram-negative bacteria. Measurements of the assimilation of ^{13}C by living fungi include 6 replicates, actinobacteria include 6 replicates, Gram-positive bacteria include 15 replicates, Gram-negative bacteria include 12 replicates, and the Unassigned group includes 27 replicates; the number of replicate measurements is dependent upon the number of representative fatty acid biomarkers $\times$ 3 reps.
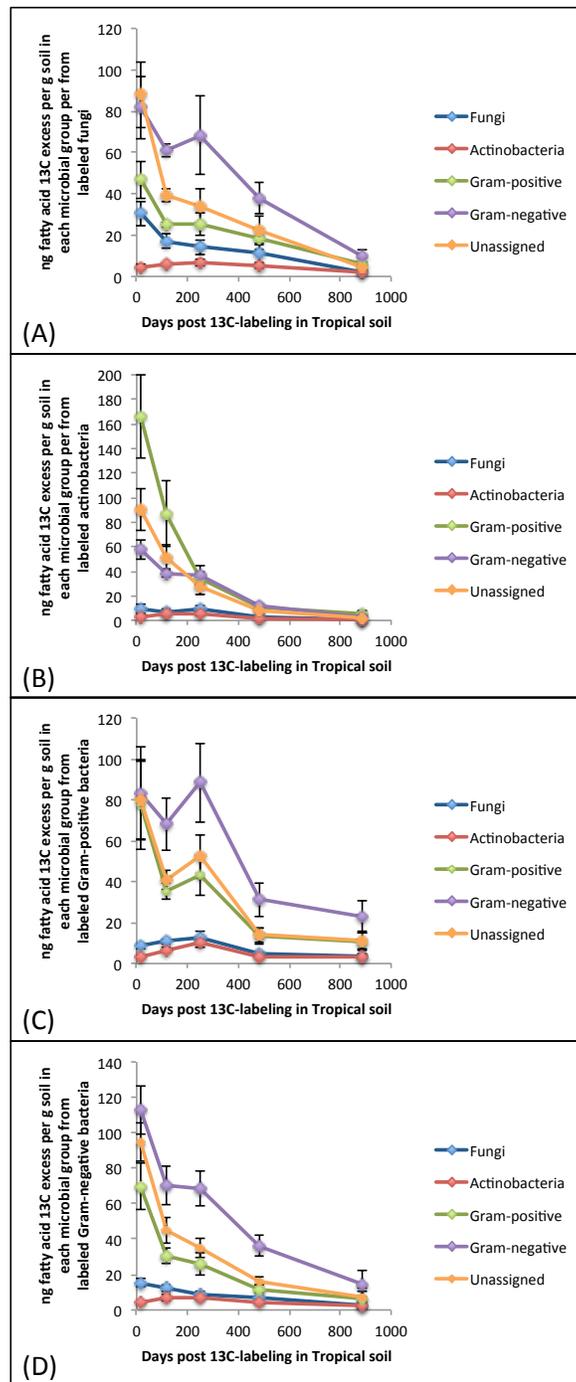
Figure 1-3. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups over time due to the assimilation of labeled microbial necromasses added to Tropical soils. Panel (A) shows the assimilation of ^{13}C from labeled fungi, (B) actinobacteria, (C) Gram-positive bacteria, and (D) Gram-negative bacteria. Measurements of the assimilation of ^{13}C by living fungi include 6 replicates, actinobacteria include 6 replicates, Gram-positive bacteria include 15 replicates, Gram-negative bacteria include 12 replicates, and the Unassigned group includes 27 replicates; the number of replicate measurements is dependent upon the number of representative fatty acid biomarkers $\times$ 3 reps.

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Table 1-5. Atom% ^{13}C excess of microbial PLFA biomarkers due to the assimilation of labeled microbial necromasses added to Temperate soils. One-way ANOVAs were performed on a by-timepoint basis with microbial consumer group as the independent variable. Biomarkers at each time not sharing the same letter are significantly different.

TEMPERATE SOILS

^{13}C from FUNGI into:

GROUP	BIOMARKER	DAY 33	DAY 163	DAY 370	DAY 763	DAY 1133
Fungi	18:2w6,9c	1.55 [a]	1.08 [a]	0.95 [a]	0.35 [bc]	0.30 [ns]
	18:1w9c	0.86 [bcd]	0.73 [ab]	0.72 [ab]	0.58 [ab]	0.43 [ns]
Actinobacteria	16:0 10Me	0.32 [d]	0.29 [cd]	0.30 [bc]	0.33 [bc]	0.23 [ns]
	18:0 10Me	0.85 [bcd]	0.63 [bcd]	0.53 [abc]	0.60 [ab]	0.52 [ns]
Gram-positive bacteria	15:0i	0.98 [abcd]	0.71 [abc]	0.74 [ab]	0.60 [ab]	0.34 [ns]
	15:0a	1.14 [ab]	1.10 [a]	0.92 [a]	0.78 [a]	0.43 [ns]
	16:0i	1.02 [abc]	0.91 [ab]	0.83 [a]	0.60 [ab]	0.33 [ns]
	17:0a	0.77 [bcd]	0.64 [bcd]	0.64 [ab]	0.58 [ab]	0.42 [ns]
	17:0i	0.96 [abcd]	0.62 [bcd]	0.67 [abc]	0.51 [abc]	0.32 [ns]
Gram-negative bacteria	16:1w7c	0.72 [bcd]	0.90 [ab]	0.65 [abc]	0.49 [bc]	0.27 [ns]
	18:1w7c	0.63 [bcd]	0.60 [bcd]	0.47 [abc]	0.37 [bc]	0.18 [ns]
	17:0cyclo	0.64 [bcd]	0.53 [bcd]	0.54 [abc]	0.45 [bc]	0.27 [ns]
	19:0 cyclo	0.37 [cd]	0.25 [d]	0.20 [c]	0.23 [c]	0.22 [ns]

^{13}C from ACTINOBACTERIA into:

GROUP	BIOMARKER	DAY 33	DAY 163	DAY 370	DAY 763	DAY 1133
Fungi	18:2w6,9c	0.30 [d]	0.17 [d]	0.21 [d]	0.11 [e]	0.07 [f]
	18:1w9c	0.49 [d]	0.53 [cd]	0.57 [bcd]	0.45 [cde]	0.40 [cdef]
Actinobacteria	16:0 10Me	0.35 [d]	0.18 [d]	0.29 [d]	0.26 [de]	0.24 [def]
	18:0 10Me	0.67 [cd]	0.46 [cd]	0.59 [bcd]	0.40 [cde]	0.42 [cdef]
Gram-positive bacteria	15:0i	1.77 [bcd]	1.23 [bcd]	1.34 [bcd]	0.60 [bcd]	0.45 [cde]
	15:0a	3.86 [abc]	2.41 [ab]	2.45 [ab]	0.95 [ab]	0.70 [abc]
	16:0i	6.78 [a]	3.70 [a]	3.85 [a]	1.30 [a]	1.03 [a]
	17:0a	4.26 [ab]	2.23 [abc]	2.31 [abc]	0.86 [abc]	0.88 [ab]
	17:0i	2.54 [bcd]	1.56 [bcd]	1.51 [bcd]	0.62 [bcd]	0.55 [bcd]
Gram-negative bacteria	16:1w7c	0.82 [cd]	0.93 [bcd]	0.80 [bcd]	0.43 [cde]	0.30 [def]
	18:1w7c	0.51 [d]	0.50 [cd]	0.49 [cd]	0.25 [de]	0.18 [ef]
	17:0cyclo	1.19 [bcd]	0.68 [bcd]	0.84 [d]	0.38 [cde]	0.29 [def]
	19:0 cyclo	0.47 [d]	0.23 [d]	0.35 [bcd]	0.23 [de]	0.19 [def]

^{13}C from GRAM-POSITIVE BACTERIA into:

GROUP	BIOMARKER	DAY 33	DAY 163	DAY 370	DAY 763	DAY 1133
Fungi	18:2w6,9c	0.71 [ab]	0.58 [cde]	0.40 [e]	0.28 [ns]	0.17 [b]
	18:1w9c	0.61 [b]	0.58 [cde]	0.48 [cde]	0.51 [ns]	0.41 [ab]
Actinobacteria	16:0 10Me	0.35 [ab]	0.31 [e]	0.32 [e]	0.31 [ns]	0.27 [ab]
	18:0 10Me	0.62 [ab]	0.50 [de]	0.40 [cde]	0.52 [ns]	0.51 [ab]
Gram-positive bacteria	15:0i	1.63 [ab]	1.33 [abcd]	1.06 [abcd]	0.70 [ns]	0.54 [ab]
	15:0a	2.73 [ab]	1.97 [a]	1.69 [a]	0.96 [ns]	0.80 [ab]
	16:0i	2.91 [ab]	1.93 [a]	1.67 [a]	0.91 [ns]	0.72 [ab]
	17:0a	2.49 [ab]	1.36 [abc]	1.32 [abc]	0.75 [ns]	0.89 [a]
	17:0i	2.70 [ab]	1.66 [ab]	1.43 [abc]	0.77 [ns]	0.70 [ab]
Gram-negative bacteria	16:1w7c	1.03 [ab]	1.42 [ab]	0.76 [bcde]	0.55 [ns]	0.33 [ab]
	18:1w7c	1.00 [ab]	0.91 [bcde]	0.69 [cde]	0.48 [ns]	0.24 [ab]
	17:0cyclo	3.49 [a]	1.30 [abcd]	1.93 [ab]	0.84 [ns]	0.54 [ab]
	19:0 cyclo	1.38 [ab]	0.47 [e]	0.71 [de]	0.39 [ns]	0.31 [ab]

^{13}C from GRAM-NEGATIVE into:

GROUP	BIOMARKER	DAY 33	DAY 163	DAY 370	DAY 763	DAY 1133
Fungi	18:2w6,9c	1.15 [b]	0.55 [b]	0.41 [b]	0.17 [d]	0.21 [c]
	18:1w9c	0.88 [b]	0.81 [b]	0.72 [b]	0.54 [bc]	0.49 [abc]
Actinobacteria	16:0 10Me	0.39 [b]	0.31 [b]	0.33 [b]	0.30 [cd]	0.29 [bc]
	18:0 10Me	1.17 [b]	0.59 [b]	0.69 [b]	0.50 [bcd]	0.62 [ab]
Gram-positive bacteria	15:0i	1.28 [b]	0.94 [b]	0.97 [b]	0.61 [bc]	0.43 [abc]
	15:0a	1.67 [b]	1.37 [b]	1.23 [ab]	0.77 [ab]	0.56 [abc]
	16:0i	4.64 [a]	2.98 [a]	2.66 [a]	1.01 [a]	0.78 [a]
	17:0a	1.36 [b]	0.95 [b]	0.86 [b]	0.57 [bc]	0.56 [abc]
	17:0i	1.40 [b]	0.89 [b]	0.98 [b]	0.59 [bc]	0.45 [abc]
Gram-negative bacteria	16:1w7c	1.19 [b]	1.41 [b]	0.97 [b]	0.56 [bc]	0.38 [bc]
	18:1w7c	1.01 [b]	0.92 [bcd]	0.79 [b]	0.47 [cd]	0.30 [bc]
	17:0cyclo	2.56 [ab]	1.57 [b]	1.58 [ab]	0.72 [ab]	0.58 [abc]
	19:0 cyclo	0.87 [b]	0.55 [b]	0.54 [b]	0.33 [cd]	0.33 [bc]

Table 1-6. Atom% ^{13}C excess of microbial PLFA biomarkers due to the assimilation of labeled microbial necromasses added to Tropical soils. One-way ANOVAs were performed on a by-timepoint basis with microbial consumer group as the independent variable. Biomarkers at each time not sharing the same letter are significantly different.

TROPICAL SOILS

^{13}C from FUNGI into:

GROUP	BIOMARKER	DAY 17	DAY 114	DAY 249	DAY 480	DAY 886
Fungi	18:2w6,9c	3.18 a	0.57 ns	0.39 ns	0.09 ns	0.01 ns
	18:1w9c	0.39 b	0.21 ns	0.19 ns	0.14 ns	0.03 ns
Actinobacteria	16:0 10Me	0.16 b	0.16 ns	0.17 ns	0.15 ns	0.05 ns
	18:0 10Me	0.13 b	0.17 ns	0.17 ns	0.15 ns	0.04 ns
Gram-positive bacteria	15:0i	0.56 b	0.25 ns	0.20 ns	0.15 ns	0.03 ns
	15:0a	0.74 b	0.35 ns	0.31 ns	0.24 ns	0.08 ns
	16:0i	0.46 b	0.23 ns	0.21 ns	0.17 ns	0.04 ns
	17:0a	0.34 b	0.22 ns	0.20 ns	0.14 ns	0.03 ns
	17:0i	0.45 b	0.20 ns	0.15 ns	0.12 ns	0.02 ns
Gram-negative bacteria	16:1w7c	1.16 b	0.32 ns	0.25 ns	0.18 ns	0.03 ns
	18:1w7c	0.65 b	0.22 ns	0.15 ns	0.11 ns	0.02 ns
	17:0cyclo	1.01 b	0.43 ns	0.39 ns	0.25 ns	0.05 ns
	19:0 cyclo	0.27 b	0.27 ns	0.24 ns	0.17 ns	0.05 ns

^{13}C from ACTINOBACTERIA into:

GROUP	BIOMARKER	DAY 17	DAY 114	DAY 249	DAY 480	DAY 886
Fungi	18:2w6,9c	0.21 c	0.05 b	0.00 d	0.00 e	0.00 ns
	18:1w9c	0.19 c	0.18 ab	0.16 cd	0.09 bcd	0.02 ns
Actinobacteria	16:0 10Me	0.08 c	0.26 ab	0.15 cd	0.08 cd	0.03 ns
	18:0 10Me	0.10 c	0.18 ab	0.18 cd	0.10 bcd	0.02 ns
Gram-positive bacteria	15:0i	1.09 c	0.88 ab	0.26 bcd	0.09 bcd	0.03 ns
	15:0a	2.38 ab	1.64 ab	0.52 ab	0.19 a	0.07 ns
	16:0i	3.14 a	2.27 a	0.58 a	0.19 a	0.08 ns
	17:0a	3.19 a	2.15 ab	0.47 ab	0.16 abc	0.07 ns
	17:0i	1.29 bc	0.97 ab	0.27 bcd	0.10 bcd	0.03 ns
Gram-negative bacteria	16:1w7c	1.07 bc	0.83 ab	0.27 bc	0.09 bcd	0.03 ns
	18:1w7c	0.32 c	0.25 ab	0.13 cd	0.05 de	0.01 ns
	17:0cyclo	2.29 ab	1.62 ab	0.46 ab	0.16 ab	0.05 ns
	19:0 cyclo	0.13 c	0.16 ab	0.13 cd	0.07 d	0.01 ns

^{13}C from GRAM-POSITIVE BACTERIA into:

GROUP	BIOMARKER	DAY 17	DAY 114	DAY 249	DAY 480	DAY 886
Fungi	18:2w6,9c	0.58 b	0.08 b	0.03 b	0.01 c	0.00 ns
	18:1w9c	0.20 b	0.23 b	0.20 b	0.09 bc	0.08 ns
Actinobacteria	16:0 10Me	0.15 b	0.27 b	0.23 b	0.11 bc	0.10 ns
	18:0 10Me	0.10 b	0.19 b	0.19 b	0.10 bc	0.07 ns
Gram-positive bacteria	15:0i	0.74 b	0.53 ab	0.28 b	0.12 bc	0.09 ns
	15:0a	1.44 ab	0.62 ab	0.47 ab	0.17 ab	0.13 ns
	16:0i	0.96 b	0.51 ab	0.40 ab	0.16 bc	0.11 ns
	17:0a	1.22 ab	0.53 ab	0.33 b	0.12 bc	0.10 ns
	17:0i	0.95 b	0.55 ab	0.30 b	0.11 bc	0.09 ns
Gram-negative bacteria	16:1w7c	1.53 ab	0.63 ab	0.36 b	0.12 bc	0.08 ns
	18:1w7c	0.63 b	0.42 ab	0.28 b	0.11 bc	0.08 ns
	17:0cyclo	2.91 a	1.00 a	0.83 a	0.32 a	0.26 ns
	19:0 cyclo	0.20 b	0.27 b	0.23 b	0.14 bc	0.12 ns

^{13}C from GRAM-NEGATIVE into:

GROUP	BIOMARKER	DAY 17	DAY 114	DAY 249	DAY 480	DAY 886
Fungi	18:2w6,9c	0.66 b	0.32 b	0.03 b	0.00 c	0.00 ns
	18:1w9c	0.30 b	0.29 b	0.29 b	0.13 b	0.05 ns
Actinobacteria	16:0 10Me	0.17 b	0.28 b	0.18 b	0.12 b	0.05 ns
	18:0 10Me	0.14 b	0.24 b	0.15 b	0.11 b	0.05 ns
Gram-positive bacteria	15:0i	0.64 b	0.41 b	0.17 b	0.10 b	0.03 ns
	15:0a	1.06 b	0.56 b	0.25 b	0.16 b	0.06 ns
	16:0i	1.30 a	0.69 b	0.27 b	0.15 b	0.08 ns
	17:0a	0.89 b	0.41 b	0.18 b	0.12 b	0.03 ns
	17:0i	0.49 b	0.34 b	0.16 b	0.09 bc	0.03 ns
Gram-negative bacteria	16:1w7c	1.91 ab	0.71 b	0.28 b	0.14 b	0.05 ns
	18:1w7c	0.81 b	0.48 b	0.23 b	0.13 b	0.04 ns
	17:0cyclo	4.53 a	2.05 a	0.67 a	0.36 a	0.14 ns
	19:0 cyclo	0.23 b	0.30 b	0.20 b	0.15 b	0.06 ns
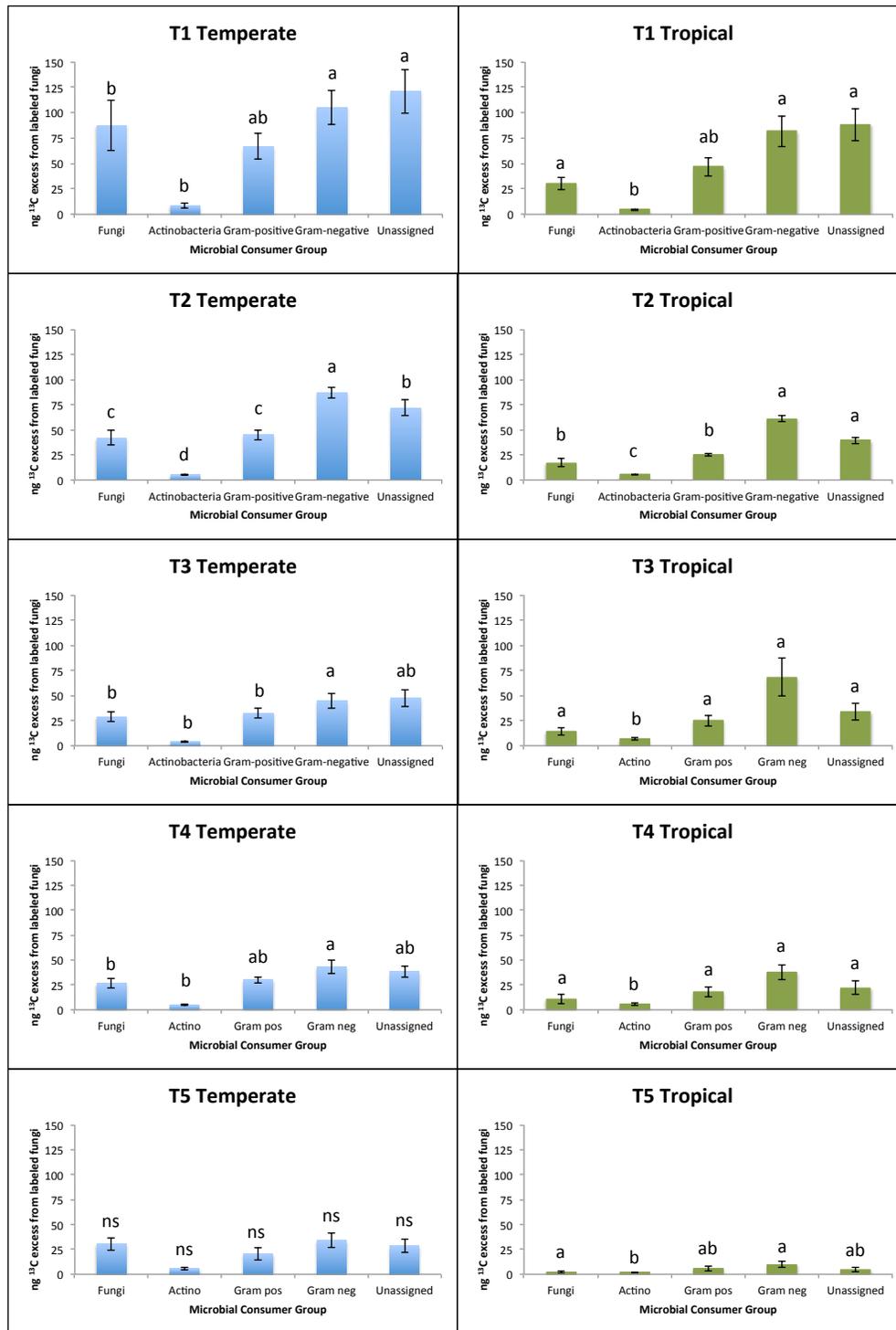
Figure 1-4. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups due to the assimilation of labeled fungal necromass. In the Temperate site, the harvest timepoints (days post ^{13}C-labeling) correspond to: 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5). In the Tropical site the harvest timepoints (days post ^{13}C-labeling) correspond to: 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5). One-way ANOVAs were performed on a by-timepoint basis for each soil; different letters indicate significant differences among microbial consumer groups.

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At the first Tropical soil harvest, the living actinobacteria recovered less ng ^{13}C from the necrotic actinobacteria than the living fungi, Gram-negative bacteria, and unassigned lipid biomasses (Fig. 1-4). Throughout the study, the lowest ng ^{13}C at each timepoint was recovered in the actinobacteria. Analysis of the atom% ^{13}C in all lipid biomarkers in Tropical soil due to addition of labeled fungal necromass reveals that there were no significant differences at any of the timepoints, except an increased atom% ^{13}C in the 18:2ω6,9c biomarker (fungi) at the first harvest (Table 1-6). Therefore, any differences in mass ^{13}C recovery in the different living microbial groups in the Tropical soil due to the assimilation of necrotic fungi must primarily be due to differences in biomass pool sizes (Table 1-4).

Recovery of ^{13}C in the living biomass from labeled Actinobacteria

In the Temperate and Tropical soils, time and microbial consumer group both had a significant effect at $p < 0.0001$ on the ng ^{13}C recovered in the standing fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomasses from the assimilation of labeled actinobacterial necromass (Figs. 1-2 and 1-3). In the Temperate soil, the interaction of time and microbial consumer group had a significant effect at $p = 0.0159$; In the Tropical soil, the interaction of time and microbial consumer group had an effect at $p < 0.0001$.

At the first harvest for both the Temperate and Tropical soils, the living Gram-positive bacteria contained high ng ^{13}C recoveries from the assimilation of labeled actinobacterial necromass (Fig. 1-5). The Gram-positive biomarkers 15:0a, 16:0i, and 17:0a in both soils demonstrated a high atom% recovery of ^{13}C from actinobacterial necromass compared to the other microbial consumer groups (Tables 1-5 and 1-6). Given the mid-range biomass pool of Gram-positive bacteria compared to other microbial groups (Table 1-4), the high ng ^{13}C recovery of the Gram-positive bacteria may be attributed, in large part, to relatively high assimilation of ^{13}C originally from the labeled actinobacterial necromass.

In both soils, the Gram-negative bacteria and unassigned lipid biomasses contained only slightly less ng ^{13}C than the Gram-positive bacteria at the first harvest. With one exception, the atom% ^{13}C of the Gram-negative bacteria and unassigned lipid biomarkers at the first harvest were not significantly high, indicating that overall, the large biomass pools of both groups led to the high mass recoveries of necrotic actinobacteria; the only Gram-negative bacterial biomarker to exhibit a high atom% ^{13}C value was that of 17:0cyclo (Table 1-6).

Recovery of ^{13}C in the living biomass from labeled Gram-positive bacteria

In the Temperate and Tropical soils, time and microbial consumer group both had a significant effect at $p < 0.0001$ on the ng ^{13}C recovered in the standing fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomasses from the assimilation of labeled Gram-positive necromass (Figs. 1-2 and 1-3). Only in the Tropical soil, did the interaction of time and microbial consumer group had an effect ($p = 0.0025$).

At the first Temperate soil harvest, the Gram-negative bacteria, Gram-positive bacteria, and unassigned lipid biomasses contained high ng ^{13}C from the addition of labeled Gram-positive bacteria, while at the first harvest in the Tropical soil there were no significant differences in the ng ^{13}C recoveries in any of the biomass groups (Fig. 1-6). The atom% ^{13}C recoveries of all the biomarkers representing Gram-positive and Gram-negative bacteria in both the Temperate and Tropical soils at the first harvest revealed substantially (although not statistically significant) higher atom% ^{13}C recoveries than the fungal and actinobacterial biomarkers (Tables 1-5 and 1-6).

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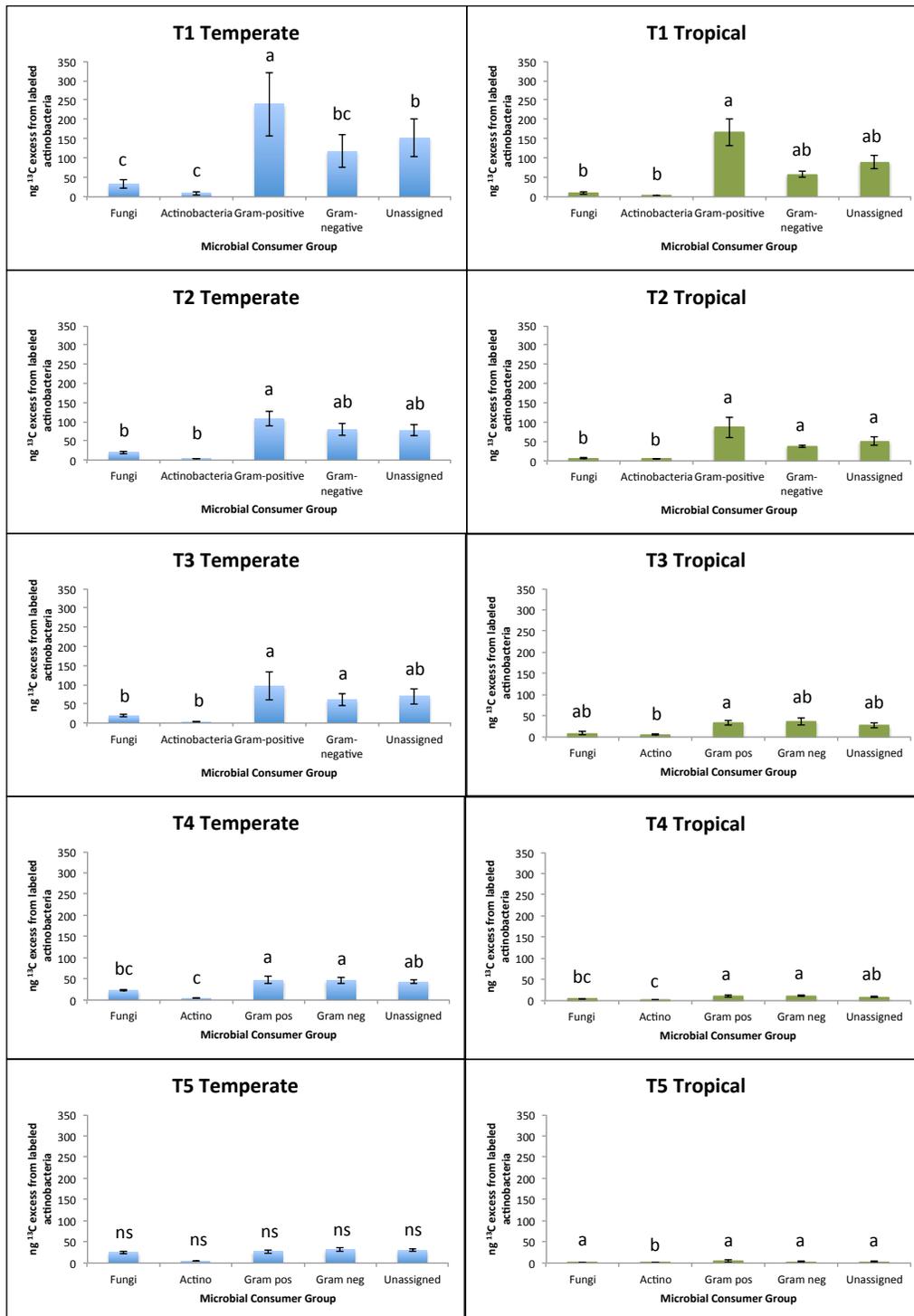
Figure 1-5. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups due to the assimilation of labeled actinobacterial necromass. In the Temperate site, the harvest timepoints (days post ^{13}C-labeling) correspond to: 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5). In the Tropical site the harvest timepoints (days post ^{13}C-labeling) correspond to: 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5). One-way ANOVAs were performed on a by-timepoint basis for each soil; different letters indicate significant differences among microbial consumer groups.

20
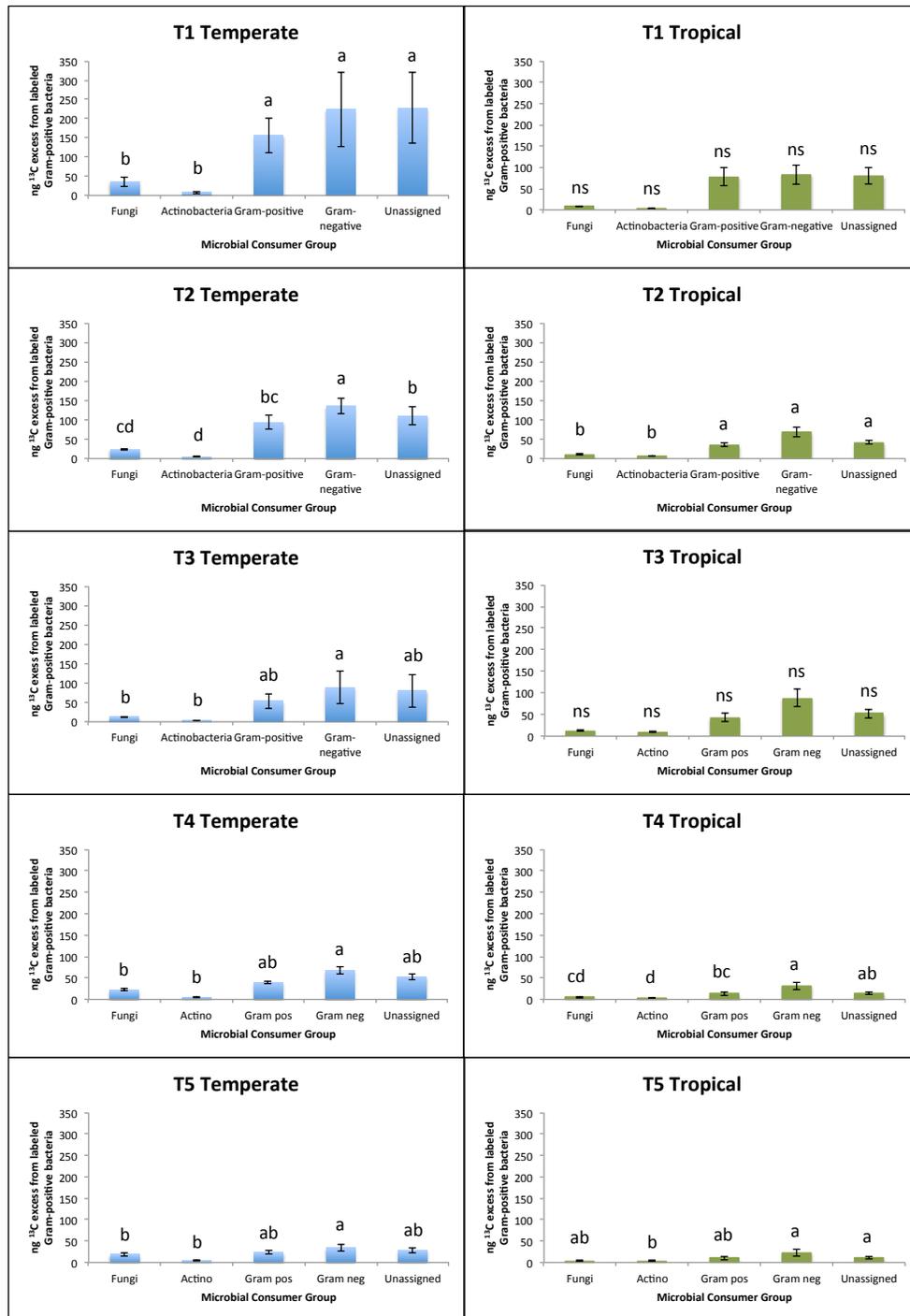
Figure 1-6. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups due to the assimilation of labeled Gram-positive bacterial necromass. In the Temperate site, the harvest timepoints (days post ^{13}C-labeling) correspond to: 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5). In the Tropical site the harvest timepoints (days post ^{13}C-labeling) correspond to: 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5). One-way ANOVAs were performed on a by-timepoint basis for each soil; different letters indicate significant differences among microbial consumer groups.

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Recovery of ^{13}C in the living biomass from labeled Gram-negative bacteria

Time and microbial consumer group both had a significant effect ($p < 0.0001$) on the ng ^{13}C recovered in the living fungi, actinobacteria, Gram-positive bacteria, and Gram-negative bacteria, and unassigned lipid biomasses in Temperate and Tropical soils after the addition of labeled Gram-negative necromass (Figs. 1-2 and 1-3). Only in the Tropical soil did the interaction of time and microbial consumer group have a significant effect ($p < 0.0001$).

At the first harvest in the Temperate soils, the living Gram-negative bacteria recovered high ng ^{13}C compared to the recoveries in living Gram-positive bacteria, fungi or actinobacteria; the unassigned lipid group also contained relatively high ng ^{13}C due to the assimilation of necrotic Gram-negative bacteria compared to the living fungi and actinobacteria (Fig. 1-7). At the first harvest in the Tropical soils, the living Gram-negative bacteria and unassigned lipid biomass groups contained higher ng ^{13}C values than the living actinobacteria (Fig. 1-7). In the Temperate soil, the 16:0i (Gram-positive bacteria) and 17:0cyclo (Gram-negative bacteria) biomarkers demonstrated high atom% ^{13}C values at the first harvest due to the addition of labeled Gram-negative bodies (Table 1-5); in the Tropical soil, the 16:1ω7c and 17:0cyclo (Gram-negative bacteria) biomarkers demonstrated high atom% ^{13}C values (Table 1-6).

Retention of ^{13}C in the living biomass at the five harvest timepoints

At each harvest, Temperate soils retained more ng ^{13}C than the Tropical soils, despite the fact that the Temperate harvest dates for each sampling were longer from the date of ^{13}C addition than those of the Tropical sites (Fig. 1-8). By the end of the study, the Temperate soils retained an approximately five fold greater amount of dead fungal derived ^{13}C than the Tropical soils, eleven fold more actinobacterial ^{13}C, two fold more Gram-positive bacterial ^{13}C, and two fold more Gram-negative bacterial ^{13}C than the Tropical soils (Table 1-7).

Within the Temperate soil, the highest ng ^{13}C recovered in the living biomass at the end of the study was due to the addition of labeled Gram-negative bacteria, and the highest recovery in the Tropical soil was due to the addition of labeled Gram-positive bacteria (Table 1-7). While incubation site had a significant effect ($p < 0.05$) on the ng ^{13}C recovered at all five harvests, the microbial consumer group did not have an effect at any harvest time.

When the ng ^{13}C remaining in the phospholipid fatty acids of the living biomass (fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and the unassigned lipid biomasses) at the end of the study from all the added necromass groups (labeled fungi, actinobacteria, Gram-positive bacteria, and Gram-negative bacteria) were summed, the Temperate soils recovered significantly more ng ^{13}C than the Tropical soils ($p = 0.0002$; Fig. 1-9 and Table 1-7).
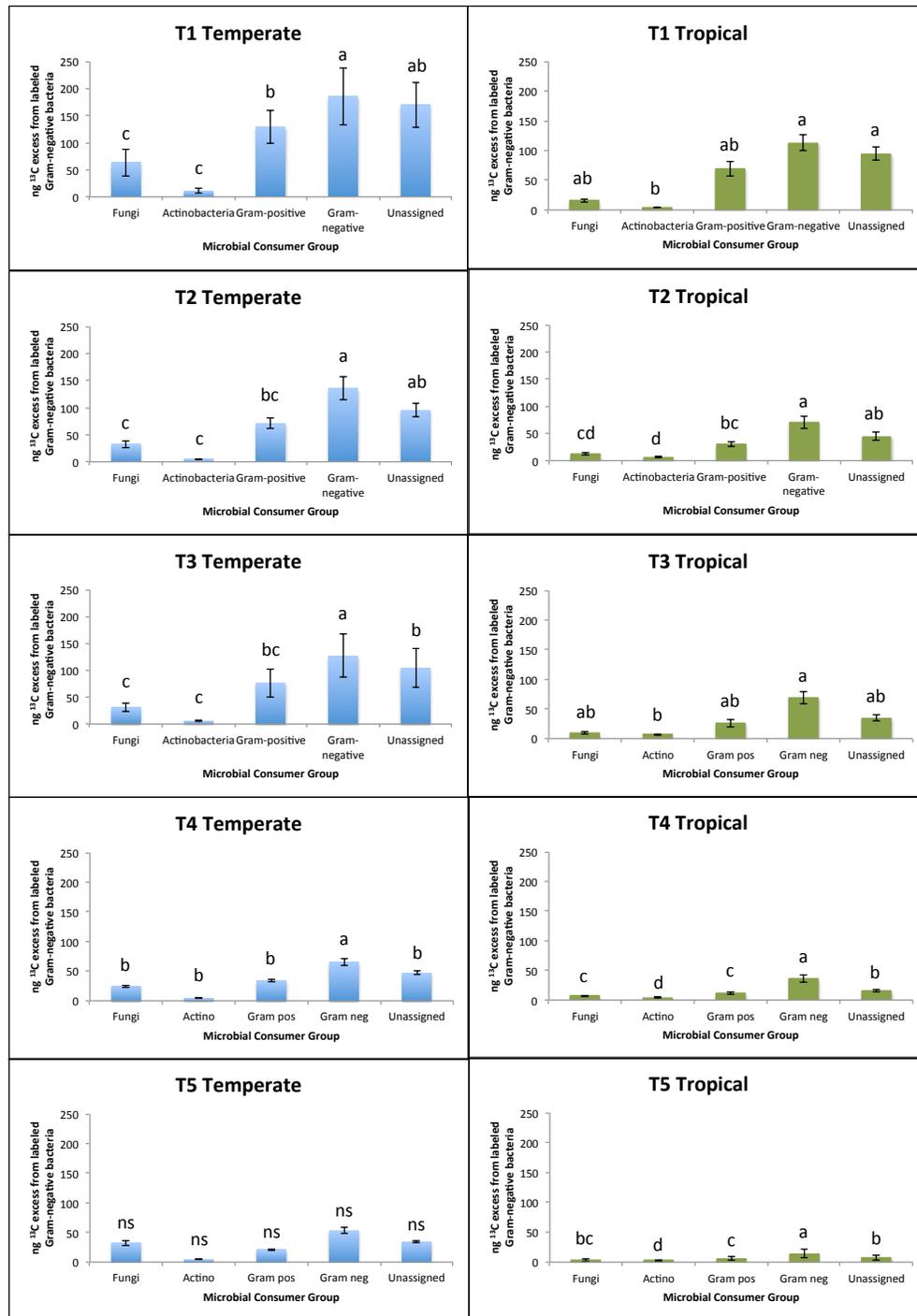
Figure 1-7. ng ^{13}C excess (means $\pm$ 1 standard error) of standing microbial groups due to the assimilation of labeled Gram-negative bacterial necromass. In the Temperate site, the harvest timepoints (days post ^{13}C-labeling) correspond to: 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5). In the Tropical site the harvest timepoints (days post ^{13}C-labeling) correspond to: 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5). One-way ANOVAs were performed on a by-timepoint basis for each soil; different letters indicate significant differences among microbial consumer groups.
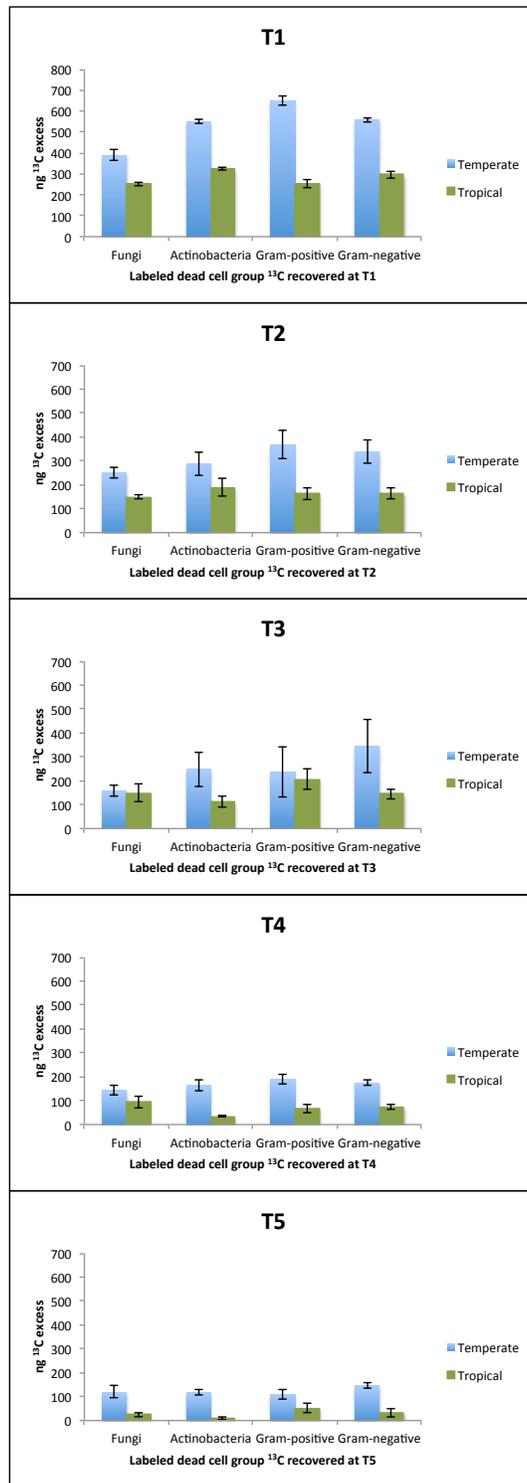
Figure 1-8. ng ^{13}C (means $\pm$ 1 standard error) recovered from each of the necromass groups in Temperate and Topical soil ecosystems at each harvest time. In the Temperate site, the harvest timepoints (days post ^{13}C-labeling) correspond to: 33 (T1), 163 (T2), 370 (T3), 763 (T4), and 1133 (T5). In the Tropical site the harvest timepoints (days post ^{13}C-labeling) correspond to: 17 (T1), 114 (T2), 249 (T3), 480 (T4), and 886 (T5). Each measurement includes 3 replicates.
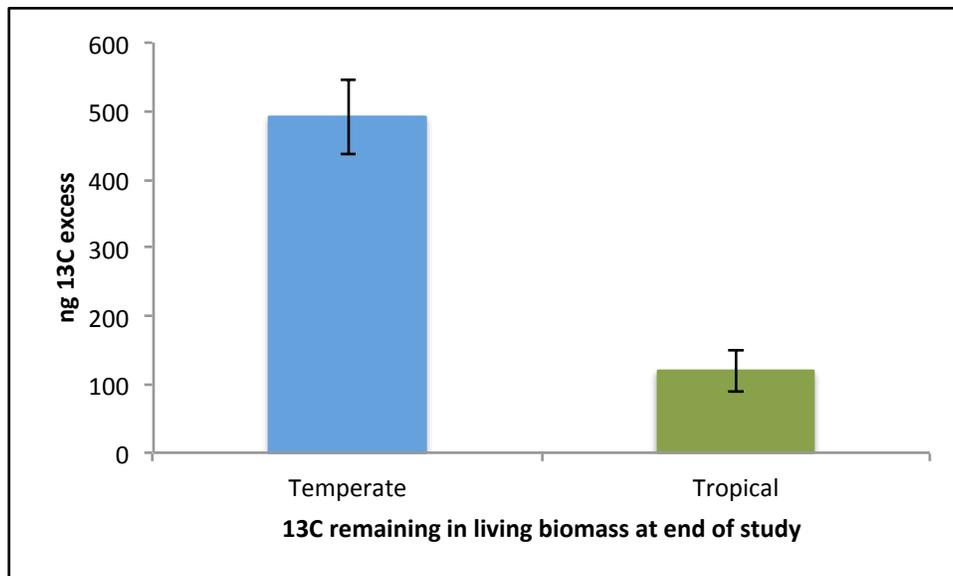
Figure 1-9. Mean sum ($\pm$ 1 standard error) of the ng ^{13}C recovered from the Temperate and Tropical soil ecosystems at the end of the study. Each measurement includes 3 replicates.

Discussion

As substrates for saprophytic microbial consumption, the composition and quality of microbial bodies is important. The addition of ^{13}C labeled dead microbial bodies to Temperate and Tropical soils *in situ* allowed for a coarse elucidation of which dead microbial groups were assimilated by other living microorganisms during the course of the study, and which living microbial carbon pools retained the added ^{13}C longest. Each of the living microbial consumer groups (fungi, actinobacteria, Gram-positive bacteria, and Gram-negative bacteria) demonstrated marked differences from one another in the assimilation of labeled microbial necromass. For example, the Gram-positive bacteria were the only microbial consumer group to preferentially assimilate ^{13}C from labeled actinobacteria in both Temperate and Tropical soils.

While there were clear differences imparted by the four added groups as to the amount of ^{13}C retained in the living microbial biomass, the cells were uniformly labeled so I cannot ascertain which specific cellular components accounted for the differences in assimilation. However, known characteristics of the four different cell groups support further discussion.

Fungal bodies as a saprophytic food source

Fungal cell walls are distinctive for the presence of chitin (Alexopoulos *et al.,* 1996), glucan (Madigan *et al.,* 2003a), and melanin (Caesar-Tonthat *et al.,* 1995; Eisenman and Casadevall, 2012; Nosanchuk and Casadevall 2003; Walker et al. 2010). The basic unit of fungal cell walls is chitin, a long-chain polymer of N-acetylglucosamine in β-(1,4)-glycosidic bonds. Similar to glucans, chitin is also highly crystalline and water-insoluble, making it difficult for some microorganisms to degrade fungal bodies (Kögel-Knabner, 2002), although many bacteria do degrade chitin for nutrition and energy (Kielak *et al.,* 2013). Chitin is depolymerized by chitinase, which is commonly produced by bacteria, actinobacteria, and fungi. Fungi produce

chitinases in order to reshape their own chitin (Atlas and Bartha, 1998), which could account for the ability of living fungi in this study to assimilate dead fungal ^{13}C. While Alexander (1991) states that less than 1% of chitin digesters in soils are fungi, the results of this study indicate that fungi may play a more dominant role in the breakdown of chitin and/or assimilation of fungal bodies than previously thought. Interestingly, actinobacteria are known to have the ability to degrade chitin in fungal cell walls (Alexander, 2005), yet the living actinobacteria in this study did not demonstrate any preferential assimilation of ^{13}C from fungal bodies. The assimilation of fungal necromass by living Gram-positive bacteria in the Temperate soil ecosystem may be explained by the prevalent ability of *Bacillus*, a genus of Gram-positive bacteria, to hydrolyze chitin. Cody (1989) found that 78% of characterized *Bacillus* isolates in chitin rich soils tested positive for the production of chitobiase.

Melanin tends to be resistant to microbial degradation (Linhares and Martin, 1978), which may account for the low overall recovery of labeled fungal biomarkers, as well as the lower levels of fungal ^{13}C necromass preferentially assimilated (as determined by atom% ^{13}C) by the living actinobacteria in the Temperate ecosystem, and by the lower recovery in the Gram-positive bacteria, Gram-negative bacteria, actinobacteria, and 18:1ω9c fungal biomarker in the Tropical ecosystem. While melanins are a minor component of fungal cell walls (Kögel-Knabner, 2002), humic-acid like melanins are common in ascomycetous fungi and fungal melanins have been linked to humic acid formation (Valmaseda *et al.*, 1989). The dead fungi mixture cultured from Temperate soils contained 78.2% Ascomycetes, and the fungi mixture cultured from Tropical soils contained 87.4% Ascomycetes. Due to the likely presence of melanins in these Ascomycete bodies, the dead fungal group mixtures used in this study may have been resistant to degradation.

In addition, fungi synthesize glucans, which are polysaccharides of glucose monomers joined by α-1,3, α-1,6, β-1,3, or β-1-6 linkages (Alexopoulos *et al.*, 1996; Madigan *et al.*, 2003a). The highly crystalline and water-insoluble nature of glucans (Kögel-Knabner, 2002), may have made degradation and assimilation of fungal bodies by the living soil microorganisms difficult.

Biochemical characterization, using CP-MAS ^{13}C NMR, of the dead microbial bodies utilized in this study revealed that the fungal groups were most similar to the actinobacterial groups (Fan *et al.*, 2009). Still, the living actinobacteria in this study did not demonstrate preferentially assimilate ^{13}C from the dead fungal bodies. If the living actinobacteria in the Temperate and Tropical study soil ecosystems depolymerized the dead fungal chitin, it appears that the actinobacteria did not then assimilate and retain the fungal ^{13}C.

Actinobacterial bodies as a saprophytic food source
Throughout the study, actinobacteria were preferentially assimilated by Gram-positive bacteria. In the Temperate soil ecosystem, no other living microbial group demonstrated a preference for actinobacteria; in the Tropical soil ecosystem, the 17:0cyclo biomarker (Gram-negative bacteria) also demonstrated high levels of actinobacterial necromass assimilation.

Not only did Gram-positive bacteria exhibit a relatively high saprophytic preference for actinobacterial necromass, but also, the atom% ^{13}C values in the living Temperate Gram-positive bacterial biomarkers (15:0a, 16:0i, 17:0a) from necrotic actinobacteria assimilation were some of the highest atom% values recorded in this study. It is well known that some actinobacteria have the ability to produce antibiotics. For example, *Streptomyces* (which was utilized in this study as representative, labeled actinobacterial necromass) produce polyenes, a group of antifungal compounds that work by binding to ergosterol, causing membrane permeability and cell death (Madigan *et al.*, 2003b). Alexander (2005) states that the significance of antibiotic production in

ecological interactions among soil microbes is still largely unknown. The low assimilation of actinobacterial necromass by fungi in this study, despite their biochemical similarities to actinobacteria (Fan *et al.,* 2009), may be attributed to the actinobacterial production of polyenes.

If the PLFA method of measuring microbial biomass was accurate for the actinobacteria, the low levels of actinobacteria necromass assimilation by many of the saprophytic microbial biomarkers could also be, in part, associated with the low amounts of standing actinobacterial biomass naturally contained within the soils of the two study sites. Relatively low amounts of actinobacterial biomass in these soils may have precluded selection for the consumption of actinobacterial cells by the general soil community due to a lack of evolutionary adaptations for the ability to degrade and assimilate actinobacterial necromass.

Gram-positive bacterial bodies as a saprophytic food source

In both soil ecosystems, Gram-positive bacterial bodies were consumed primarily by other living Gram-positive bacteria and by Gram-negative bacteria. The cell walls of Gram-positive bacteria contain high amounts of peptidoglycan, approximately 50% dry weight of the cell (Kögel-Knabner, 2002), and often have no outer membrane. Peptidoglycan is a polymer consisting of carbohydrate and amino acid elements (Rogers *et al.,* 1980; Koch, 1990) that form glycan strands that are cross-linked by short peptides (Vollmer *et al.,* 2008). The carbohydrate contains alternating β-(1-4) linked N-acetylglucosamine and N-acetylmuramic acid, and the sugars and amino acids are linked together by three types of chemical bonds (amide, glycosidic, and peptide) to form a 2-3 dimensional net-like polymer (Vollmer *et al.,* 2008) that provides both rigidity and elasticity to the cell wall (Kögel-Knabner, 2002).

The hydrolysis of peptidoglycan requires amidases, peptidases and glycosylases (Schockman *et al.,* 1996; Geisseler *et al.,* 2010). Peptidoglycan hydrolases are common in bacteria as they play vital roles in the modification of bacterial cell walls for separation, growth and sporulation (Vollmer *et al.,* 2008). The production of peptidoglycan hydrolases by bacteria may explain why the greatest assimilation of dead Gram-positive bacterial bodies was by Gram-positive bacteria (whose cell walls contain the most peptidoglycan) and Gram-negative bacteria (whose cell walls do contain peptidoglycan, albeit less than the Gram-positive bacteria). While peptidoglycan hydrolases are also common in fungi (Grant *et al.,* 1986), fungal assimilation of dead Gram-positive bacterial biomass was relatively low in both soil ecosystems.

Gram-negative bacterial bodies as a saprophytic food source

Dead Gram-negative bacterial bodies were primarily assimilated into the Gram-positive bacteria biomarker 16:0i in the Temperate soil ecosystem; in the Tropical soil ecosystem, dead Gram-negative bacterial bodies were preferentially assimilated by the living Gram-negative bacteria biomarker 17:0cyclo. The cell walls of Gram-negative bacteria contain relatively little peptidoglycan, comprising only 10% of the dry weight of the cell (Kögel-Knabner, 2002). Instead, a major portion of Gram-negative bacteria cell walls is composed of an additional wall layer made of lipopolysaccharides (LPS), which contains polysaccharides and proteins. The outer membrane of Gram-negative bacteria is often toxic to animals (Madigan *et al.,* 2003b), whether the organism is pathogenic or not. The ability for living Gram-positive and Gram-negative bacteria to consume dead Gram-negative bacteria may, in part, have been enhanced by a reduction in the toxicity of the outer membrane upon death and the subsequent deterioration of the Gram-negative bacterial cell wall because LPS loses its endotoxic properties once it is deacylated (Ulevitch and Tobias, 1995).

The Gram-negative bacteria exhibited the largest pool of biomass (living, non-labeled) in both soil ecosystems, as measured by PLFA. If the PLFA method accurately measured the biomass pool size of Gram-negative bacteria, there would naturally be high turnover of Gram-negative bacterial bodies in both soils, which may have led to adaptations by some Gram-positive bacteria (in Temperate soils) and Gram-negative bacteria (in Temperate and Tropical soils) to consume dead Gram-negative bacterial bodies. Living fungal and actinobacterial taxa may not have adapted to consume Gram-negative bacterial necromass as readily as Gram-negative and Gram-positive bacteria because the biochemical characterization of the dead Gram-positive and Gram-negative bacteria utilized in this study, using CP-MAS ^{13}C NMR, demonstrated that the Gram-positive bacteria and Gram-negative bacteria were most similar to one another (Fan *et al.,* 2009), while that of the fungi and actinobacteria were most similar to one another and substantially different than that of the Gram-positive and Gram-negative bacteria. While the Gram-positive bacteria and Gram-negative bacteria did exhibit differences in their cellular chemistries, the 1D solid-state cross-polarization magic angle spinning (CP-MAS) ^{13}C NMR spectral signature for the Gram-positive bacteria closely resembled that of the Gram-negative bacteria (Fan *et al.,* 2009). 1D CP-MAS ^{13}C NMR allows for *in situ* characterization of microbial cell components that are not readily extractable. The similarities in the biochemistry of Gram-positive bacteria and Gram-negative bacteria may provide insight into the increased ability of each saprophytic microbial group to assimilate the other upon cell death.

Importance of climate and soil ecosystem on the assimilation and retention of microbial carbon

The amount of labeled ^{13}C remaining in the standing biomass at each time in the study was highly dependent upon both the soil ecosystem to which the necromass was added, and to a lesser extent, the type of microbial necromass group that was added. The living microbial communities under the constant wet and warm climate of the Tropical site were likely characterized as having higher metabolic rates and/or low carbon use efficiencies (CUE) than the microbial communities under the colder, drier climate of the Temperate site (Chambers *et al.,* 2004; Manzoni *et al.*, 2012). The effect of these differences in metabolic rates and CUE may lead to differences in the amount of time organic C remains in the standing biomass before it is respired out as CO_2, as was observed in this study.

The differences in retention of the ^{13}C-label in the standing biomasses of the two soil ecosystems after approximately 3 years is important because the retention time of carbon within the standing microbial biomass may be positively correlated with stabilization of that carbon within the heavy fraction of soil organic matter (see dissertation Chapter 3). In other words, the longer carbon is retained within the living soil biomass, the higher the probability that the microbial carbon will ultimately become stabilized in soils and not mineralized out of the soil system as CO_2. In fact, Throckmorton *et al*. (2012) reports that dead microbial bodies added to Temperate soils demonstrated longer mean residence times (MRT) in the bulk soil than dead microbial bodies added to Tropical soils.

Conclusion

In summary, with the aid of ^{13}C-PLFA and additions of ^{13}C-labeled dead microbial cells, this study provides insight into the assimilation of dead microbial carbon by the living soil

biomass, the preferences of living microbial groups in terms of decomposing dead microbial groups, and the carbon retention in the living biomass of two very different soil ecosystems. While a limited number of studies have traced the flow of carbon from specific microbial organisms and/or their products into the living soil microbial community and soil organic matter (Kindler *et al.,* 2006; Lueders *et al.,* 2006), this study is the first of its kind to follow a complex mixture of labeled cells into natural soil microbial communities. As recent studies have shown, biomolecules of microbial origin can remain in soils for long periods of time and contribute significantly to SOM. The microbial groups examined in this study differed in terms of their preferences for utilizing dead biomass from the various groups. In general, microbial groups preferentially assimilated carbon from their same groups or groups with similar biochemical compositions, though only the Gram-positive bacteria in the Tropical site and Gram-positive bacteria and Gram-negative bacteria in the Temperate site appeared to have an affinity for assimilating actinobacterial carbon. With regards to longer-term fates, greater amounts of labeled carbon were retained in the microbial biomass in Temperate soils than in Tropical soils. This finding suggests that a higher proportion of the C in microbial bodies in temperate systems are stabilized by growth on and sorption to mineral surfaces. This finding is further confirmed by the results presented in Chapters 2 and 3.

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Chapter 2

Soil Microbes:

Precursors of Stabilized Soil Carbon

Abstract

In terrestrial ecosystems, microbial bodies and microbial processes are central to the stabilization of soil organic matter (SOM). However, many questions about the role of soil microbes as the precursors and mediators of organic matter stabilization remain unanswered. This study investigated the stability and fate of microbial cell materials by following added microbial cell carbon (C) into three operationally defined stabilized organic matter products in two different soil ecosystems. The influences of climate and edaphic ecosystem properties on SOM distribution were also examined. Soils were collected from both a Californian mixed conifer forest and a tropical Puerto Rican forest; ^{13}C-labeled microbial dead bodies were added to the soils, and the soils were incubated for 520 days under 3 different climate regimes (Mediterranean mixed conifer forest, Redwood forest, and Tropical forest). Both climate and soil type exerted significant influences on the total amount of ^{13}C recovered in the incubated soils as well as the amount recovered in each of the three operationally-defined stabilized carbon pools: free light fraction (FLF), occluded light fraction (OLF), and heavy fraction (HF). The recovery of ^{13}C was highest in the HF in all but one soil-climate combination; 3.0-11.9% of the added ^{13}C was recovered in the HF versus 0.4-2.2% in the OLF and 0.3-5.4% in the FLF. The high recovery of ^{13}C in the HF is consistent with the stabilization of microbial C through interactions with soil mineral surfaces. There was a clear influence of climate on the ^{13}C-OLF recoveries from Puerto Rico soils as more ^{13}C was stabilized under temperate climates compared to the Tropical climate. Hence, the seasonal changes of the temperate climates appear to have favored an increase in the aggregation of Puerto Rico soil particles. In contrast, the results indicate that warm and wet climate conditions promoted greater microbial activity and decomposition of FLF; the recovery of ^{13}C-FLF from the Mixed Conifer soils incubated under the Tropical climate was minimal compared to that recovered from the Mixed Conifer soils exposed to the Temperate climates. When comparing the two soils under the Tropical climate, the recovery of ^{13}C-FLF in the Puerto Rico soils was small compared to that of the Mixed Conifer soils, likely the result of a greater level of microbial metabolic activity in the Puerto Rico soils associated with their higher microbial biomass, or differences in fungal to bacterial ratios between the two soils. My research shows that climate and soil both exert significant influences on the persistence of microbial body carbon in soil organic matter pools and that microbes are important not only as metabolic pathways for the breakdown of organic material but also as nascent stabilized organic materials.

Introduction

Soil organic matter (SOM) results from a suite of microbial and geochemical processes involved in the conversion of biomolecules to stabilized, potentially long-lived materials. These processes have been studied for decades (McGill and Cole, 1981; Leinweber and Schulten, 1995; Kleber *et al*., 2007) and it is well known that soil mineral surfaces (Elliott *et al*., 1996; Baldock and Skjemstad, 2000; Kleber *et al*., 2004; Rasmussen *et al*., 2007) and climate (Krull *et al*., 2003; Lützow *et al*., 2006) are critical drivers of organic matter stabilization processes.

A long-held tenet of soil microbiology is that most carbon entering the soil passes through the microbial biomass, sometimes referred to as "the eye of the needle". While this truism is included in textbooks (Wolf and Wagner, 2005; Plante and Parton, 2007), there is little evidence of how soil microbial communities and the processes that they catalyze are actually involved in soil carbon stabilization. The primary carbon (C) input to soil is plant detritus, and both climate and edaphic characteristics impact the amount and quality of C input. These primary environmental drivers also impact the characteristics and composition of the soil microbial community, and plant inputs to soil are consumed by a complex array of soil organisms including fungi, bacteria, and mesofauna. Microbial utilization of C initializes the flow of detrital C along the pathway to stabilization or mineralization to CO_2. The movement of decomposing plant C into microbial biomass has been followed in a number of studies, primarily through the use of ^{13}C-PLFA (Waldrop and Firestone, 2004; Bird and Torn, 2006) and chloroform fumigation direct extraction (CFDE; Kelly and Burke, 1997). Over the past decade, it has become increasingly clear that microbial bodies and microbial processes are also central to organic matter stabilization (Chenu and Stotzky, 2002; Tremblay and Benner, 2006; Kleber, 2007), yet few studies have traced the flow of C from microbial bodies themselves through the process of stabilization (Lueders *et al*., 2006; Kindler *et al*., 2006; Miltner *et al*., 2012; Throckmorton *et al*., 2012).

The stabilization of SOM can be considered in terms of three broad, interactive categories: biological recalcitrance, chemical protection, and physical protection. Biological recalcitrance refers to the resistance of a molecule to biological (enzymatic) decomposition (Krull *et al*., 2003). Chemical protection encompasses interactions (such as hydrogen bonding or Van der Waals forces) between organic residues and the mineral matrix that form long-lasting bonds which make the organic matter unavailable for decomposition (Chenu, 1995). Physical protection occurs when organic matter is occluded within aggregated soil, making it inaccessible to decomposing organisms (Krull *et al*., 2003); organic matter that is physically protected may or may not be resistant to biological degradation. Soil aggregation occurs when groups of soil particles bind more strongly with each other than with adjacent soil particles, and both abiotic and biotic factors affect soil aggregation (Martin *et al.,* 1955). Abiotic factors of aggregation include climate, soil parent material, soil texture, adsorbed polyvalent cations such as Fe^{3+} and Ca^{2+}, and tillage practices (Allison, 1968; Wolf and Wagner*,* 2005; Voroney, 2007). Biotic factors that promote the binding of soil particles include the organic matter content of a soil, root exudates, polysaccharides and proteins from decomposing tissues, microbial extracellular polysaccharides, and adhesion via fungal hyphae and microbial cells (Allison, 1968; Wolf and Wagner*,* 2005; Voroney, 2007).

Soil organic matter pools are often examined by the separating organic matter via density fractionation into a free light fraction (FLF), free occluded light fraction (OLF) and dense organo-mineral (heavy) fraction (HF). The FLF is a low-density fraction that commonly resembles recent organic matter inputs both visually and chemically (Swanston *et al.,* 2004); the FLF often contains younger carbon than that found in the OLF and the HF, with turnover times from months to 10 years (Spycher *et al.*, 1983; Gaudinski *et al.,* 2000). While the OLF also contains low-density organic matter, it is often older than the FLF due to its physical protection within soil aggregates. The turnover time of occluded organic matter is dependent upon the location of the organic matter within soil aggregate sites and whether the organic matter has adsorbed to mineral surfaces (Golchin *et al.*, 1994). The HF often contains highly processed organic carbon associated with soil mineral surfaces, and frequently contains the oldest carbon of the three density gradient fractions; Trumbore (1993) found HF SOM mean residence times (MRT) of 470 and 990 years for temperate and tropical soils, respectively.

It is important to note that while previous models of SOM stabilization attributed the persistence of SOM to the chemical nature of the decomposing organic materials (Kögel-Knabner *et al.,* 1988; Cambardella and Elliott, 1992; Rovira and Vallejo, 2007), a more recent model of soil organic carbon dynamics attributes the persistence of SOM to a combination of ecosystem properties such as reactive mineral surfaces, water availability, temperature, soil acidity and other physiochemical and biological influences (Schmidt *et al.,* 2011).

Both edaphic characteristics and climate influence the stabilization of SOM. One key edaphic driver is the clay content of a soil. Soil carbon has been shown to be linearly, and positively, correlated to soil texture; as clay content increases, soil carbon storage increases (Schimel *et al.*, 1994). The clay content of a soil is also of particular importance to aggregation as clay-sized particles are frequently associated with soil aggregates (Bronick and Lal, 2005). As discussed previously, the aggregation of soil particles is also heavily influenced by the organic matter content of a soil (Chaney and Swift, 1984).

Another key edaphic influence on SOM stabilization is the presence of iron oxides. It is well known that organic matter adsorbs to mineral surfaces such as iron oxides (Gu *et al.*, 1994). Iron oxides have been demonstrated to influence SOM stabilization in multiple ways: (a) they have a high capacity to sorb simple carbon compounds (Jones and Edwards, 1998) and humics (Tipping, 1981), (b) there is a positive correlation between total organic matter concentrations and iron oxides (Wagai and Mayer, 2007), (c) there is an inverse correlation between soil organic matter turnover times and non-crystalline mineral concentrations such as that of iron oxides (Torn *et al.,* 1997; Masiello *et al.,* 2004), and (d) organic matter is stabilized onto iron oxides via ligand exchange between carboxyl and hydroxyl functional groups of organic matter (Gu *et al.*, 1994).

Climate is also a key driver of soil biogeochemical processes and strongly affects organic matter stabilization in soils. Two primary climatic drivers of SOM stabilization are temperature and precipitation. Globally, as temperature decreases, SOM concentrations increase (Schimel *et al.,* 1994; Alvarez and Lavado, 1998; Dai and Huang, 2006; Davidson and Janssens, 2006). The inverse relationship of SOM storage to temperature is in large part due to the positive relationship of decomposition rates to increasing temperature. Changes in precipitation can have variable effects on the storage of SOM (Davidson and Janssens, 2006). Increases in precipitation have been correlated with both decreases in SOM concentrations (Dai and Huang, 2006) and increases in SOM concentrations (Alvarez and Lavado, 1998); oftentimes, the influence of

precipitation on SOM stabilization is dependent upon the drainage and aeration of the soil ecosystem.

This study specifically addresses the interactive impacts of climate and edaphic characteristics on the stabilization of microbial C into operationally-defined stable organic matter fractions. In order to follow the movement and stabilization of dead microbial C among operationally defined SOM fractions, microbial C (in the form of dead microbial bodies already enriched with ^{13}C) was added to two soil types that were exposed to three different climate regimes over a period of 520 days. The two soil types were chosen because they represent typical, terrestrial forest soil ecosystems with considerable differences in edaphic and biotic characteristics (Table 2-1). The three climate regimes chosen for this study represent a redwood forest climate with consistently mid-range temperatures and relatively low moisture availability (R); a Mediterranean mid-elevation conifer forest with a cold, snowy winter and warm, dry summer (M); and a warm, wet tropical forest (T). By exposing two soil types to three climate regimes, I was able to examine the influence of both climate and soil characteristics, and their interaction, on the fate of microbial C as it is decomposed and stabilized among the three operationally-defined SOM fractions examined.

Materials and Methods

Field sites

Soil samples were collected from the University of California Blodgett Forest Research Station (Mixed Conifer soils; MC) and the Colorado Forest in the Puerto Rico Luquillo Experimental Forest (Puerto Rico soils; PR). At each forest site, soil samples were collected (0-7.5 cm depth) at 5 locations (1 m apart) along 3 transects, homogenized together, and stored at field moisture and ambient temperature for 3 days before subsampling into each microcosm. Blodgett Forest is located in the mid-elevation Sierra Nevada Mountains, 240 km east of San Francisco, and is a Mediterranean forest representative of productive forestlands in California. Dominant plant species include sugar pine (*Pinus lambertiana*) and ponderosa pine (*Pinus ponderosa*). The Puerto Rico Forest is located in the highlands of Puerto Rico, 35 km east of San Juan, and is representative of a tropical montane wet forest in which high levels of precipitation cause soils to fluctuate between oxic and suboxic conditions. Approximately 40 plant species occur in the Puerto Rico forest, and the dominant plant species is myrtle (*Cyrilla racemiflora*). The Firestone lab at UC Berkeley has extensive experience working at both locations and a detailed knowledge of their site characteristics (Table 2-1).

Table 2-1. Site and soil characteristics of the two soil harvest locations. Means are shown with standard errors in parentheses.

Region	Puerto Rico	Central Sierra Nevada
Forest Type	Tropical Deciduous	Temperate Mixed Coniferous
Site Name	Luquillo Experimental Forest	Blodgett Experimental Forest
Latitude	18º 41' N	38º 53' N
Elevation (m)	780	1315
Mean precipitation (mm)	4,500 [2]	1,774 [1]
Mean annual temperature (ºC)	18.5 [2]	13 [3]
Dominant plant species	*Cyrilla racemiflora*	*Pinus ponderosa*
Soil order	Ultisol [2]	Alfisol [1]
Soil texture	sandy loam	sandy loam
% sand; % silt; % clay	64.0%; 18.3%; 17.8%	54.6%; 34.8%; 10.6%
pH	4.5 [4]	5.8 [4]
Potassium (meq/100g)	0.10 (0.00)	0.36 (0.01)
Sodium (meq/100g)	0.13 (0.02)	0.02 (0.00)
Calcium (meq/100g)	1.07 (0.21)	9.00 (1.32)
Magnesium (meq/100g)	0.78 (0.10)	0.69 (0.10)
Extractable sulfate (ppm)	20.7 (3.2)	12.9 (0.4)
Total manganese (ppm)	23.3 (3.6)	1281.4 (155.5)
Phosphorus (ppm)	0.33 (0.07)	1.08 (0.03)
Extractable Iron- DTPA (ppm)	417.0 (19.7)	83.4 (5.1)
CEC (meq/100g)	16.98 (3.5)	24.46 (1.7)
Soil organic carbon (mg C/g soil)	121 (6) [4]	100 (5) [4]
Microbial biomass carbon (mg C/g soil; CFDE)	1.512 (0.116) [4]	0.432 (0.042) [4]
Microbial biomass carbon (mg fatty acid C/g soil; PLFA)	0.058 (0.003)	0.035 (0.001)
Fungal:Bacterial biomass ratio	0.20	0.32

1 Bird and Torn, 2006

2 Templer et al., 2008

3 Blodgett Experimental Forest Research Station

4 Throckmorton et al., 2012

Microbial growth and additions

       Microbial cell cultures were isolated from soils sampled in 2004 from the University of California Blodgett Forest Research Station and the Luquillo Experimental Forest in Puerto Rico as described below; the origin of each isolate is given in Table 2-2. The four microbial groups used in this study were fungi, actinobacteria (high-GC Gram-positive bacteria), Gram-positive bacteria, and Gram-negative bacteria. The mixture of microbial groups was arranged to include as complex a blend as possible from the unused ^{13}C-labeled cell culture materials that were produced and used in a field investigation (see dissertation Chapter 1). The primary cell types contained in the culture mixture utilized in this study (of 23 total) included: *Penicillium verruculosum* (fungus), *Burkholderia sp.* (Gram-negative bacterium), *Bacillus sp.* (Gram-positive bacterium), and *Streptomyces sp.* (actinobacterium). The four microbial groups used in this study were chosen because the groups were expected to have fairly different cellular chemistries. Nuclear Magnetic Resonance (NMR) analysis of the groups used in this study, after they had been chosen and ^{13}C labeled, revealed they had fairly distinct cellular chemistries (Fan *et al.,* 2009). For example, the Gram-negative bacteria contained a high abundance of β-hydroxybutyrate (BHB) and low levels of trehalose, whereas BHB was absent from and high amounts of trehalose were present in Gram-positive, fungi and actinobacteria; The fungi contained more glycolipids than the actinobacteria, and demonstrated the highest synthetic capacity for glycerol production among all four of the microbial group types; The Gram-positive bacteria were more enriched in phospholipids than the Gram-negative bacteria, fungi and actinobacteria, and contained less triacylglycerides than the other three groups.

       The initial culturing methods screened hundreds of isolates per site using agar amended with soil-extracts and 10% trypticase soy agar (TSA) or 10% trypticase soy broth (TSB) at pH 5.5; a pH of 5.5 was chosen to mimic the acidic conditions of both the Blodgett Forest and Luquillo Forest soils (Table 2-1). The cultures were then further isolated with additions to the media: novobiocin and potato dextrose agar (PDA) with streptomycin for fungi; chitin with cycloheximide or starch casein for actinobacteria; 10% TSA with cycloheximide for Gram-positive and Gram-negative bacteria (Alef 1995; Fan *et al.,* 2009). The identities of the final 23 isolates selected were confirmed using DNA extraction kits (PowerSoil, Mobio Inc., CA); 16S rRNA genes were amplified from the bacterial isolates using the "universal" primers 27F and R1492 (Lane *et al*., 1985) and 18S-5.8S-28S ITS regions were amplified from fungal isolates using the ITS1F and ITS4 primers (Gardes and Bruns, 1993). Full, high quality sequences (phred score $q \geq 20$) were compared by BLAST with the public databases NCBI Genbank and greengenes (DeSantis *et al*., 2006).

       The final isolates were ^{13}C-labeled by growing cultures in liquid media to late stationary phase at 25°C; the media contained 20% glucose (99.9 atom% enriched with ^{13}C) and 10% yeast extract with M9 salts at pH 5.5. Whole cells were harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 7.0) to remove remaining media. Low-density cellular materials produced during culturing that were not harvested with the cells were filtered from the used media with 0.1-μm polycarbonate filters (Sterlitech Co., Kent, WA), washed with deionized water, and combined with the whole cells. In order to focus on the assimilation and stabilization of microbially-derived carbon in soils, and not the metabolic activity of labeled microbial cells, the cultures were autoclaved and lyophilized to kill the cells while maintaining as much cellular structure as possible (Fan *et al*., 2009).

Table 2-2. Microbial isolates which were cultured, labeled with ^{13}C, mixed, and homogenized before injection into each microcosm. Percentages are based on dry cell weight.

Harvest Site	Group	Taxonomic Classification	Operational Taxonomic Unit (BLAST match)	Taxa % of Mixture
Tropical	Fungi	Ascomycete	Penicillium verruculosum	10.72%
		Basidiomycete (mycelial)	Trichosporon multisporum	1.68%
		Basidiomycete (yeast)	Cryptococcus podzolicus	0.23%
Temperate	Fungi	Ascomycete	Penicillium sacculum	3.09%
		Basidiomycete (mycelial)	Holtermannia corniformis	0.03%
		Basidiomycete (yeast)	Cryptococcus terreus	0.02%
		Zygomycete	Umbelopsis nana	0.02%
Tropical	Actinobacteria	Streptomycetaceae	Streptomyces sp. strain 317	3.71%
		Streptomycetaceae	Kitasatospora sp. C2	8.87%
		Streptomycetaceae	Kitasatosporia griseola JCM 3339	8.45%
Temperate	Actinobacteria	Streptomycetaceae	Streptomyces sp. strain Soll14	11.20%
		Streptomycetaceae	Streptomyces capoamus	2.33%
		Streptomycetaceae	Streptomyces sp. JL164	2.18%
Tropical	Gram-positive bacteria	Firmicutes	Bacillus thuringiensis strain 2000031485	5.42%
		Firmicutes	Bacillus mycoides strain: S31	3.84%
Temperate	Gram-positive bacteria	Actinobacteria (non-Streptomycetaceae)	Arthrobacter sp. strain RC100	5.60%
		Firmicutes	Bacillus subtilis strain KL-077	3.69%
Tropical	Gram-negative bacteria	Bacteroidetes	Flexibacter cf. sancti	1.23%
		Beta-proteobacteria	Burkholderia plantarii	8.72%
		Gamma-proteobacteria	Dyella japonica	1.77%
Temperate	Gram-negative bacteria	Alpha-proteobacteria	Rhizobium sp. strain 'USDA 1920'	3.64%
		Beta-proteobacteria	Burkholderia sp. strain A22-1	11.04%
		Beta-proteobacteria	Burkholderia sp. strain UCT 29	2.49%

In order to widely distribute the ^{13}C-labeled, sterilized microbial necromass in the soils, all of the previously freeze-dried, dead cell material was suspended in deionized water before injection into the microcosms. A tissue homogenizer (Potter-Elvehjem) was then used to distribute and standardize the mixture of microbial isolates. While injection of cell-containing solutions into soil did not realistically simulate the natural circumstances of microbial growth and turnover, it did allow us to follow the assimilation of the dead cell C by the living microbial biomass.

Incubation setup and soil processing

Laboratory incubations were constructed using homogenized Mixed Conifer and Puerto Rico soils. Microcosms were constructed from sealed mason jars (1 quart) that were opened and flushed with ambient air twice a week to prevent suboxic conditions in the headspace. Each microcosm contained 120 g of soil (dry-weight equivalent) with moisture contents equivalent to the average April conditions under which each microcosm was to be initially incubated (minus 2 mL water that was subsequently added when the cell mixture was injected into the soils). The microcosms were then pre-incubated in order to dissipate much of the disturbance-induced CO_2 pulse. The Mixed Conifer soils were pre-incubated for 4 weeks and the Puerto Rico soils were incubated for 2 weeks; the Mixed Conifer soils were incubated for a different period of time than the Puerto Rico soils because of logistical difficulties associated with collecting the Puerto Rico soils at the same time as the Mixed Conifer soils. After pre-incubation, 31.0 mg of the homogenized mix of ^{13}C-labeled dead microbial bodies in 2 mL deionized water were added to each microcosm (Fig. 2-1A) and the soils containing the mixture of labeled microbes were stirred with a rod for 60 seconds to evenly distribute the mixture within the microcosm. Of the total 31.0 mg of cells, 13.3 mg consisted of ^{13}C. No additional carbon was added to the microcosms during the course of the study.
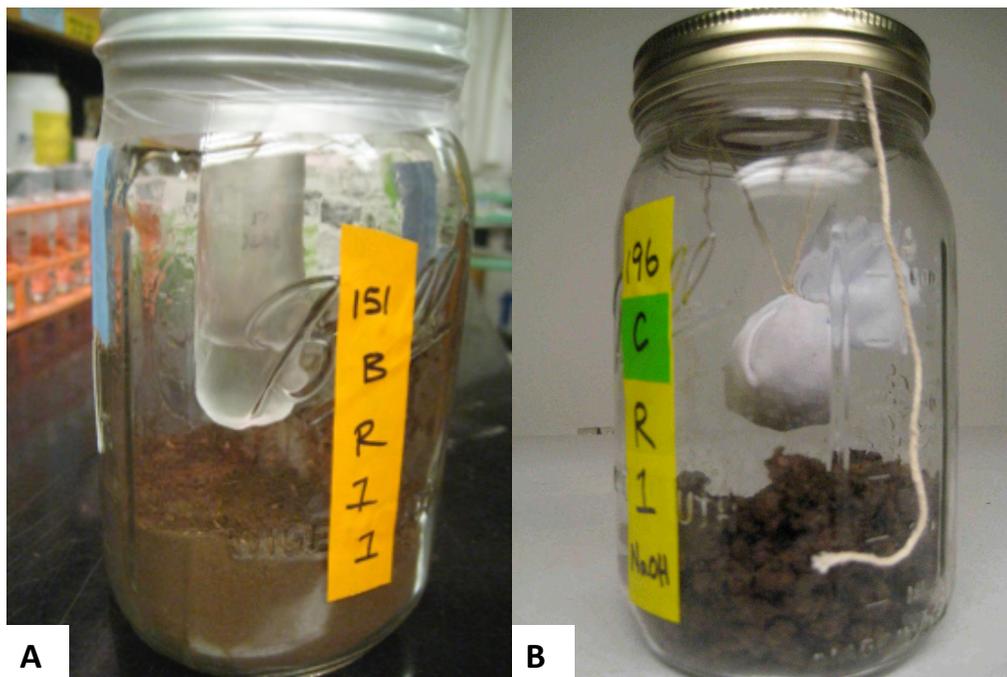
Figure 2-1. Example of soil-climate microcosm with: (A) NaOH trap to capture CO_2, and (B) desiccant ball to remove soil moisture.

There were 3 replicates for each of the 3 soil-climate combinations and 4 harvests for a sum of 36 microcosms per soil type, and 72 microcosms total. Of these 36 microcosms per soil type, 9 were harvested immediately after the application of labeled cells (T0); 9 were harvested at 7 days (T1); 9 were harvested at 70 days (T2); and 9 were harvested at 520 days (T3). For each soil type, 9 additional microcosms that did not receive labeled cells were harvested before the climate simulations began (backgrounds) and 9 additional unlabeled microcosms were harvested after 520 days (controls).

Phospholipid fatty acid analysis (PLFA) was used to characterize the living microbial biomass and the fungal-to-bacterial ratios (Table 2-1; Zelles 1999). The extraction, identification and quantification of PLFAs followed the modified methods reported in Bligh and Dyer (1959) and White and Ringelberg (1998). Microbial lipids were extracted from 8 g freeze-dried soil in a monophasic mixture of chloroform, methanol and phosphate buffer (1ml:2ml:0.83ml) with di-19:0PC (1, 2-Dinonadecanoyl- sn-Glycero-3-Phosphocholine, Avanti Polar Lipids, Alabaster, AL, USA) used as a surrogate standard to quantify soil PLFA. Extracts were separated into two phases by adding chloroform and DI-water, and the lipid-containing phase was dried at 37 °C under N_2 until all the water had evaporated; the length of drying time varied considerably. Phospholipids were separated from neutral lipids and glycolipids on Inert II silica columns (Burdick & Jackson, Muskegon, MI) using sequential elution by chloroform, acetone, and methanol. Phospholipids in the methanol elution were transesterified at 37 °C, dried at room temperature under N_2, and resuspended in hexane containing 10:0 FAME (methyl decanoate, Sigma-Aldrich, St. Louis, MO, USA) added as an internal standard.

The biomarkers used to determine the fungal-to-bacterial ratios were similar to those reported in Bird *et al.* (2011) and the ratio was calculated as the sum of the fungal group yield divided by the sum of the Gram-positive bacteria, Gram-negative bacteria, and Cyclopropyl Gram-negative bacterial group yields (nmol fungal fatty acid C per g dry soil/nmol bacterial fatty acid C per g dry soil).

Thirteen indicator PLFA biomarkers were categorized into 1 fungal group and 3 bacterial groups: fungi were indicated by 18:2ω6,9c, 18:1ω9c; Gram-negative bacteria by 16:1ω7c, 18:1ω7c (Marschner 2007); Cyclopropyl Gram-negative bacteria by 17:0cyc, 19:0cyc (Arao 1999); Gram-positive bacteria by 15:0i, 15:0a, 16:0i, 17:0i, 17:0a (Frostegård and Bååth, 1996); and actinobacteria by 16:0-10Me, 18:0-10Me (Marschner 2007). In addition, the following 9 unassigned biomarkers were included in calculations of total microbial biomass (nmol fatty acid C per g dry soil): 14:0, 15:0, 16:1ω5c, 16:1ω9c, 16:0, 16:0-12Me, 16:1-2OH, 18:1ω5c, 18:0 (Herman et al. 2012).

PLFA samples were analyzed at UC Berkeley on a Hewlett Packard (Agilent) 5890 Series II Gas Chromatograph (GC) with a 30 m × 0.32 mm × 1.0 mm ZB-5 column (Phenomenex, Inc., Torrance, California, USA) connected via an Europa ORCHID on-line combustion interface (PDZ Europa, Cheshire, UK) to an Isoprime 100 IRMS (Isoprime, Manchester, UK).

Soil moisture content and organic matter content was calculated gravimetrically by drying soil subsamples at 105 °C to a constant weight for moisture content, and ashing the same subsamples at 550°C to a constant weight for organic matter content. Soil moisture content was expressed by weight as the ratio of the mass of water in the sample to the mass dry weight of the sample, and soil organic matter content was expressed by weight as the ratio of the mass of ash in the sample to the mass oven dried soil.

Climate regimes

The characteristics of the climate regimes imposed on the Puerto Rico soils were taken from data collected with Campbell CR-10X dataloggers (Campbell Scientific Inc., Logan, UT) installed for multiple years at each site. These data were then used to determine the monthly averages of soil moisture and soil temperature for each of the climate regimes. Both soil moisture and temperature were measured at 7.5 cm soil depth at 15-minute intervals and logged hourly to the dataloggers; soil moisture was measured with CS616 water content reflectometers (Campbell Scientific Inc, Logan, UT) and soil temperature was measured with T-type thermocouples.

Each microcosm was maintained under one of three simulated climate regimes based on the monthly averages of soil temperature and moisture, and these climate averages were imposed on the microcosms each month to simulate the seasonal changes in climatic conditions for a period of 520 days (Fig. 2-2). Soil moisture was determined by gravimetric water content, and when changes in climate required drier soil conditions, desiccant bags were hung from the top of the sealed microcosms to remove soil moisture (Fig. 2-1B). The length of time required to achieve the required moisture content in each of the microcosms varied between one to five days depending on the amount of water to be removed, soil type, and temperature. The study began with the microcosms under the simulated April climate conditions and the microcosms were harvested 520 days post ^{13}C-labeling.

The three climate regimes followed those of a redwood forest ecosystem (Redwood; R), a Mediterranean-climate mixed conifer forest (Mediterranean; M), and a montane wet tropical forest (Tropical; T). The Redwood climate regime followed that of the Grove of the Old Trees, a coast redwood grove in Sonoma County that experiences cool, wet winters and warm, dry summers; soil moisture and soil temperature data was captured at this redwood grove from January 2005 through December 2006 (Ewing *et al.,* 2009). The Mediterranean climate regime represented that of a Mediterranean mid-elevation mixed conifer forest ecosystem with hot, dry summers and cold, wet, snow-covered winters (Blodgett experimental forest); soil moisture and soil temperature data was captured at this mid-elevation forest from January 2005 through July 2009. The Tropical forest climate regime was one of constant high moisture and warm temperatures typical of a montane wet tropical forest; soil moisture and soil temperature data for the tropical forest was captured at Luquillo Experimental Forest in Puerto Rico from April 2005 through April 2008.

Mean Monthly Soil Temperature

(A)

Mean Monthly Soil Moisture

(B)
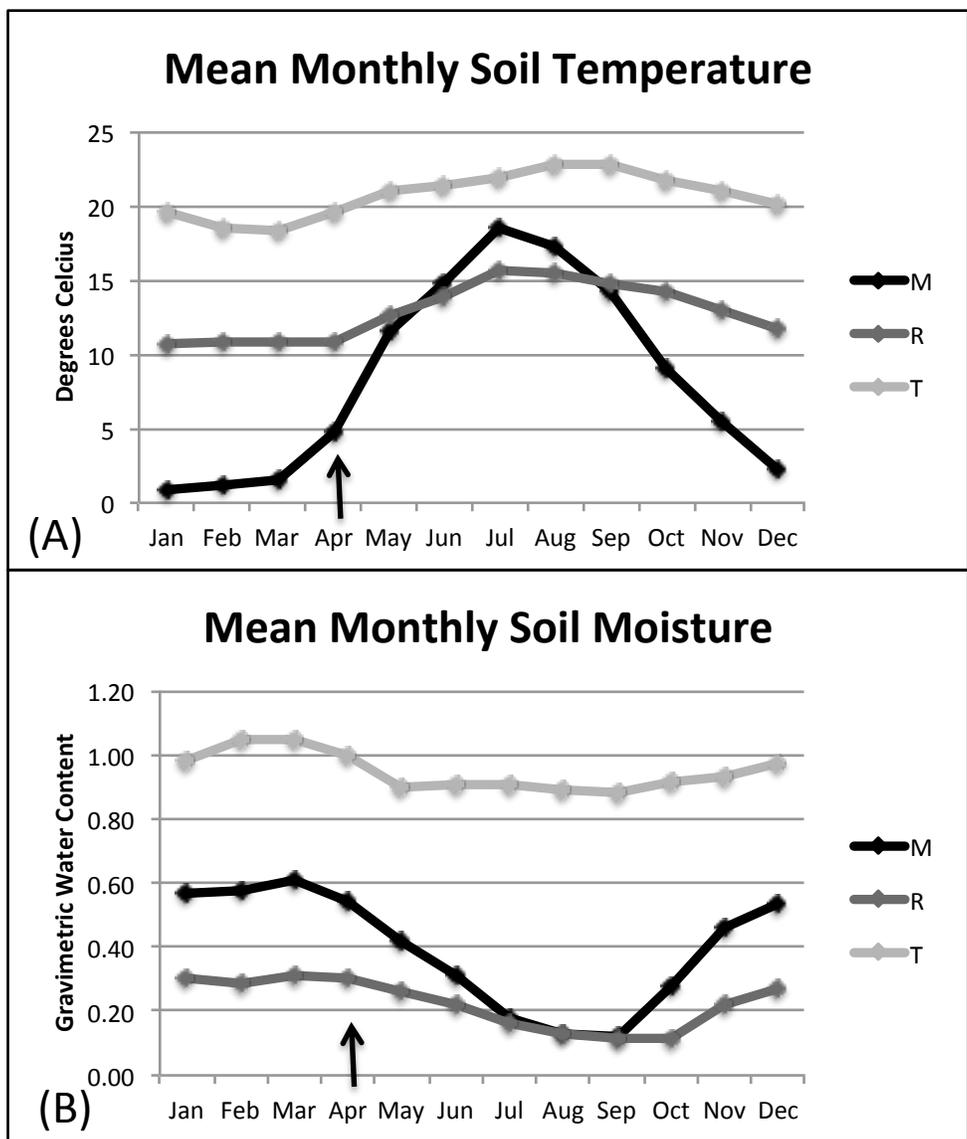
Figure 2-2. Annual soil moisture (A) and soil temperature (B) trends for the three climate regimes that were imposed in the incubations chambers during the experiment. Climate labels indicate: Mediterranean climate (M); Redwood climate (R); Tropical climate (T). Arrows denote the imposed month in which the study began.

Density fractionation

Soil organic matter fractions were separated into 3 operationally defined pools (free light fraction (FLF), occluded light fraction (OLF) and dense organo-mineral (heavy) fraction (HF) by density fractionation. The method used for soil density fractionation followed that of Swanston *et al*., (2005) with modifications as noted; this technique has been established as an efficient method by which to isolate soil organic matter and microbial cell material in three organic matter fractions in soils (Christensen, 1992; Morono *et al*., 2013).

The FLF from this density fractionation method recovers organic material in soils that is not trapped in aggregates or bound to the mineral fraction. Over time, this FLF may become

occluded in aggregates so that while the organic material may still be chemically labile for microbial consumption, it is physically protected so that the organic material is unavailable for microbial utilization. When soil aggregates are broken apart during the sonication step in the density fraction method, the organic material trapped within them becomes freed; if the organic material did not become bound to the mineral fraction during the course of the experiment, it was released into the OLF pool of organic matter, and if it did become bound to the mineral fraction while occluded it then became part of the HF recovery pool (Fig. 2-3).

Figure 2-3. The expected flow of soil carbon through 3 operationally defined soil organic matter fractions. The FLF recovers organic material in soils that is not trapped in aggregates or bound to the mineral fraction. Over time, this FLF may become occluded in aggregates. Hence, while the organic material may still be chemically labile for microbial consumption, it is physically protected and thus becomes stabilized. When soil aggregates are broken apart during the sonication step in the density fraction method, the organic material trapped within them becomes freed; if the organic material has not become bound to the mineral fraction during the course of its physical protection, it is released into the OLF pool of organic matter, if it did become bound to the mineral fraction during occlusion, it would then become part of the HF pool recovery.

To fractionate the soils via density-gradient separation, a 20 g dry-weight equivalent of soil was weighed into a centrifuge bottle and 150 ml of sodium polytungstate (SPT) prepared at a density of 1.85 g/ml (Cusack *et al*., 2011) was added, and the centrifuge bottle was inverted gently 5 times to mix the soils and SPT before centrifuging for 1 hour at 4,600 rpm (Sorvall RC-5B Superspeed Centrifuge with a 172.3 mm radius swing bucket rotor, Sorval HS-4). The FLF was then removed and rinsed with DI water through a 1.6 μm glass microfiber filter (Whatman GF/A) until all of the SPT was removed. The FLF was then lyophilized until the weight stabilized.

Once the FLF was removed, an additional 100 ml of SPT was added to the centrifuge bottle before the residual fractions were mixed for 1 min using a bench top mixer (G3U05R, Lightnin), sonicated in an ice bath for 3 min at 70% pulse for a total input of 200 J/ml (Branson 450), and centrifuged (1 hr at 4,600 rpm) to separate the OLF trapped within soil aggregates from the organo-mineral fraction (HF). The OLF was removed and rinsed in the same manner as described for the FLF and lyophilized until the weight stabilized.

After the FLF and OLF were removed, DI water was added to the pellet remaining in the centrifuge bottle, and the remaining material was shaken vigorously before the centrifugation (20 min at 4,600 rpm, 3 times) and subsequent lyophilization of the remaining HF pellet.

Elemental and isotopic analysis

The SOM density fractions (FLF, OLF, HF) were analyzed using a Vario Micro Cube elemental analyzer interfaced to an Isoprime 100 IRMS (Isoprime, Manchester, UK) to determine the elemental %C and the amount of ^{13}C label contained in each density gradient fraction. The total mg C recovered in each SOM fraction (FLF, OLF, HF), atom% ^{13}C excess within each fraction, and mg ^{13}C excess per fraction were measured for the Background and 520 day harvests. The background ^{13}C results were subtracted from 520 days results to account for the natural abundance of ^{13}C in the FLF, OLF and HF of each soil. The mg ^{13}C in each SOM fraction was calculated by multiplying the total mg C in each fraction by the atom% of that fraction (Eqn 1). Findings of ^{13}C excess in the SOM fractions are reported in the results and discussion as mg ^{13}C. C:N ratios were calculated by dividing the elemental % C by the elemental %N in each fraction, as determined by IRMS.

$$mg\ ^{13}C\ in\ fraction = mg\ C\ in\ fraction * \frac{atom\%\ ^{13}C\ of\ fraction}{100} \tag{1}$$

Statistical analyses

Significant differences among soil-climate combinations in the total mg C, in the atom% excess ^{13}C, and in the mg ^{13}C recovered in each fraction were determined by two-way analysis of variance (ANOVA) with soil type and climate as the independent variables. Tukey's Honestly Significant Difference (HSD) test was used to determine the significant differences among the means of the six soil-climate combinations when the ANOVA result was significant ($p < 0.05$). Differences among the three fractions (FLF, OLF, and HF) in the total mg C, in the atom% excess ^{13}C, and in the mg ^{13}C recovered were also determined for each soil-climate combination by performing a one-way ANOVA with SOM fraction as the independent variable, with subsequent Tukey's HSD tests performed when appropriate ($p < 0.05$). Data are reported as the mean ($\pm$ 1 standard error) for each fraction for each soil-climate combination.

Results

The results of my analyses are presented in the following order: mg C recovery (total quantity of ^{12}C+^{13}C in FLF, OLF, and HF density fractions), atom % ^{13}C (^{13}C enrichment of the density fractions), and mg ^{13}C (quantity of ^{13}C originally from the labeled microbial dead bodies that was recovered in each density fraction). The mg total C recovery is important for

understanding how much total C was contained in each density fraction. The atom% ^{13}C illustrates how enriched each fraction became with the labeled microbial-^{13}C, and the mg ^{13}C indicates the total mass of the labeled ^{13}C that was recovered from each density fraction. The results of the mg total C recovery and atom% ^{13}C analyses are presented in the results section to demonstrate whether the mg ^{13}C values can be attributed to the size of the total C pool, the enrichment of ^{13}C in each pool, or the combination of these two were factors.

All soil-climate combination treatments are labeled as follows: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

Recovery of total carbon in operationally-defined organic matter fractions

The two-way ANOVA performed for the total C recovery in the FLF indicates that the effects of soil, climate and their interaction were all significant at $p < 0.0001$ (Table 2-3). Under all three climate regimes, the amount of total C recovered in the FLF was always significantly and substantially greater in the Mixed Conifer soils compared to the Puerto Rico soils, especially under the two temperate climates (Fig. 2-4A). Furthermore, while there were no significant differences among the Puerto Rico soils incubated under the three climates, the Mixed Conifer soils incubated under the two temperate climates had significantly greater recoveries of total C in the FLF than the Mixed Conifer soil incubated under the Tropical climate.

The effect of climate ($p = 0.0129$) and the interaction of soil and climate ($p = 0.0144$) had a significant effect on the total C recovery in the OLF (Table 2-3). In Mixed Conifer soils, the recovery of total C in the OLF was higher under the Mediterranean climate than under the Redwood climate (Fig. 2-4B). The total C recovery in the OLF of Puerto Rico soils did not differ with changes in climate.

The effects of soil ($p = 0.0002$), climate ($p = 0.0007$) and their interaction ($p = 0.0012$) all had a significant effect on the total C recovery in the HF (Table 2-3). Under the temperate climates (Redwood and Mediterranean), recovery of total C in the HF was higher in the Puerto Rico soils than in the Mixed Conifer soils. Furthermore, Mixed Conifer soil under the Tropical climate regime recovered more total C in the HF than did the same soils under the temperate climate regimes (Fig. 2-4C). There was no discernable difference in total C recovery in the HF for Puerto Rico soils under all 3 climate regimes.

When the FLF, OLF and HF were summed for each soil-climate combination, the effect of soil ($p < 0.0001$), climate ($p = 0.0117$), and their interaction ($p = 0.0489$) were all significant factors in total C recovery (Table 2-3). The recovery of total C in the summed humic fractions was greatest in the HF of both soils under all climate regimes, except for the Mixed Conifer soils under the 2 temperate climate regimes; in these 2 soil-climate combinations, the recovery of total C in the FLF was highest (Fig. 2-4D).

Table 2-3. Mean recovery ($\pm$ standard error) of (A) total mg carbon ($^{12}C+^{13}C$), (B) atom% ^{13}C excess, and (C) mg ^{13}C excess for each soil-climate combination at 520 days post ^{13}C-labeling. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T). Different letters indicate significant differences among sites as determined by two way ANOVAs which were performed on a by-row-basis; different letters cannot be compared on a by-column basis. Significance of soil, climate, and the interaction of soil and climate on recovery are indicated by: ns $p > 0.05$.; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

	MC-M	MC-R	MC-T	PR-M	PR-R	PR-T	p-value (ANOVA)		
							Soil	Climate	Soil*Climate
Total mg C									
fractions summed	2093.2 (16.7) [ab]	2216.4 (43.6) [a]	1859.7 (34.0) [b]	1566.2 (6.4) [c]	1444.3 (15.9) [c]	1416.6 (136.9) [c]	****	**	*
FLF	973.9 (34.5) [b]	1366.6 (29.0) [a]	400.3 (38.3) [c]	99.3 (17.9) [d]	135.1 (9.4) [d]	117.9 (2.2) [d]	****	****	****
OLF	444.5 (12.8) [a]	243.8 (11.8) [b]	338.6 (57.4) [ab]	340.2 (38.1) [ab]	341.2 (29.0) [ab]	250.2 (15.3) [b]	ns	**	**
HF	674.8 (13.6) [b]	606.1 (42.7) [b]	1120.8 (25.6) [a]	1126.7 (25.3) [a]	968.0 (35.4) [a]	1048.6 (120.1) [a]	***	***	***
atom% ^{13}C excess									
fractions summed	0.18 (0.01) [cd]	0.21 (0.01) [c]	0.08 (0.00) [e]	0.39 (0.03) [a]	0.31 (0.02) [b]	0.14 (0.00) [d]	****	****	***
FLF	0.040 (0.002) [cd]	0.061 (0.004) [c]	0.017 (0.001) [d]	0.123 (0.011) [b]	0.156 (0.008) [a]	0.035 (0.002) [cd]	****	****	****
OLF	0.034 (0.004) [b]	0.034 (0.002) [b]	0.016 (0.002) [b]	0.104 (0.016) [a]	0.049 (0.004) [b]	0.032 (0.001) [b]	****	***	**
HF	0.105 (0.005) [bc]	0.118 (0.006) [b]	0.043 (0.002) [d]	0.166 (0.015) [a]	0.110 (0.006) [bc]	0.077 (0.002) [cd]	***	****	***
mg ^{13}C excess									
fractions summed	1.25 (0.05) [bc]	1.63 (0.12) [b]	0.60 (0.05) [d]	2.31 (0.17) [a]	1.43 (0.08) [b]	0.92 (0.11) [cd]	***	****	***
FLF	0.39 (0.02) [b]	0.83 (0.04) [a]	0.07 (0.01) [d]	0.12 (0.03) [cd]	0.21 (0.01) [c]	0.04 (0.00) [d]	****	****	****
OLF	0.16 (0.01) [b]	0.08 (0.00) [c]	0.06 (0.02) [c]	0.35 (0.02) [a]	0.17 (0.00) [b]	0.08 (0.01) [c]	****	****	****
HF	0.70 (0.05) [bc]	0.71 (0.09) [bc]	0.47 (0.04) [c]	1.85 (0.17) [a]	1.05 (0.07) [b]	0.80 (0.11) [bc]	****	****	**
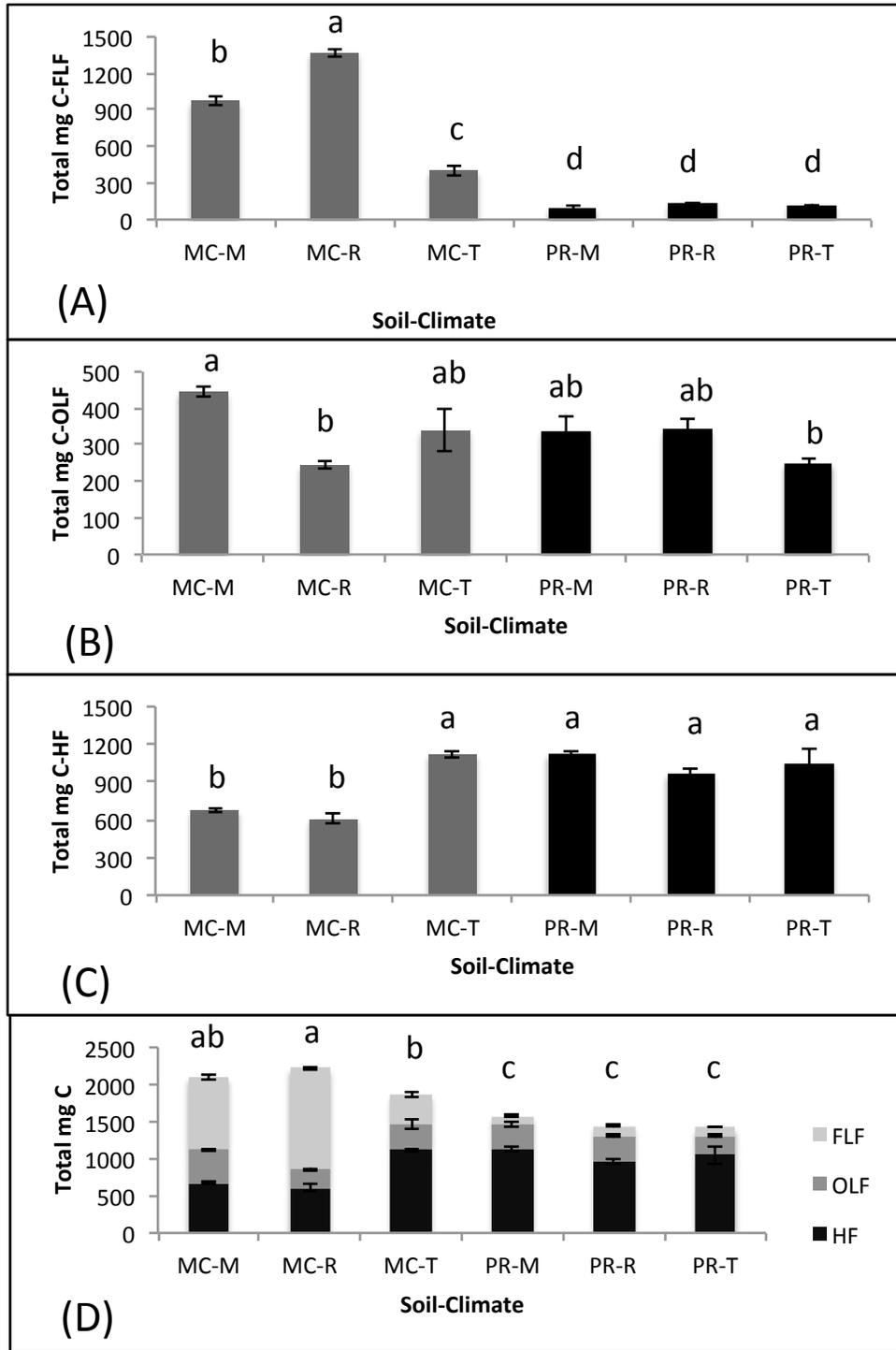
Figure 2-4. Columns represent the mean mg C ($\pm$ 1 standard error) found in each soil-climate combination at 520 days post ^{13}C-labeling in (A) the FLF, (B) the OLF, (C) the HF, and (D) all fractions. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

Atom% [13]C

The two-way ANOVA performed on the atom% [13]C in the FLF indicates that the effects of soil, climate and their interaction were all significant at $p < 0.0001$ (Table 2-3). Under the 2 temperate climate regimes (Redwood and Mediterranean), the atom% [13]C in the FLF was significantly higher in the Puerto Rico soil (Fig. 2-5A). The atom% [13]C in the FLFs of the Puerto Rico soil-temperate climate combinations were significantly higher than any other soil-climate combination, and the Puerto Rico soil-Redwood climate combination had the highest atom% [13]C. The FLF of the Mixed Conifer soil retained a lower atom% [13]C under the Tropical climate than under the Redwood climate.
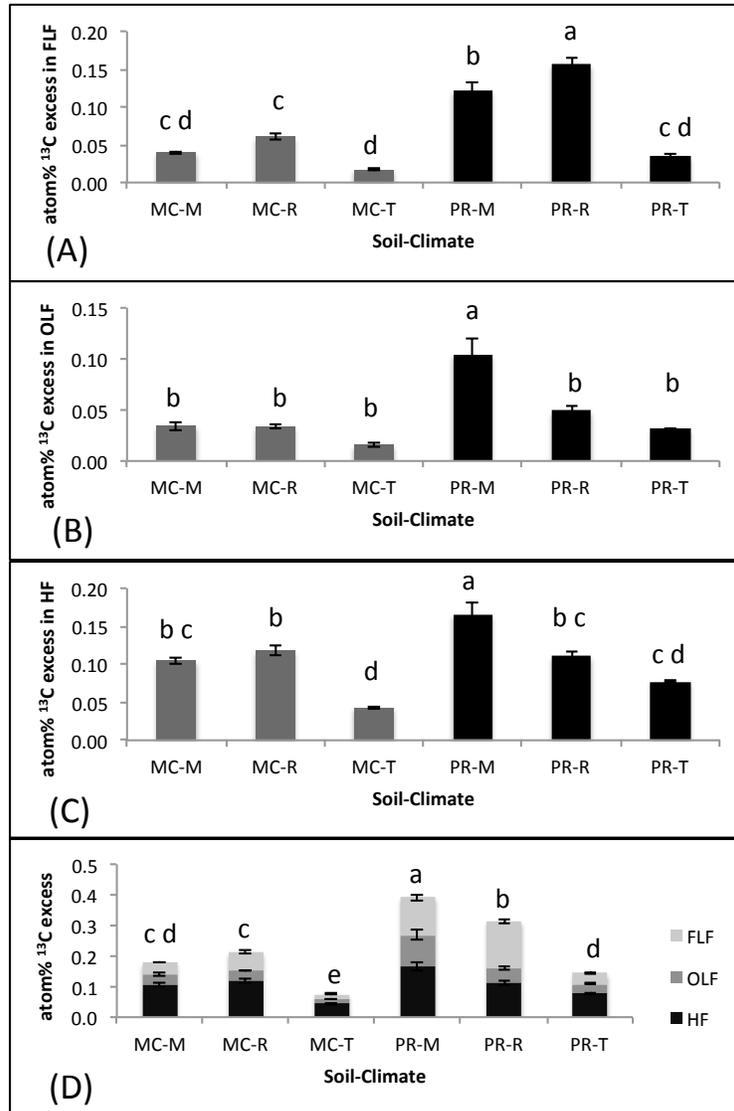
Figure 2-5. Columns represent the mean atom% [13]C ($\pm$ 1 standard error) found in each soil-climate combination at 520 days post ^{13}C-labeling in (A) the FLF, (B) the OLF, (C) the HF, and (D) all fractions. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

For the OLF atom% ^{13}C, the effects of soil ($p < 0.0001$), climate ($p = 0.0001$) and their interaction ($p < 0.0001$) were all significant (Table 2-3). Under the Mediterranean climate, Puerto Rico soils recovered the greatest OLF atom% ^{13}C of all the soil-climate combinations; the remaining soil-climate combinations were not significantly different from one another (Fig. 2-5B).

The effect of soil ($p = 0.0004$), climate ($p < 0.0001$) and their interaction ($p = 0.0018$) were all significant factors on the atom% ^{13}C recovered in the HF (Table 2-3). Under the Mediterranean climate, Puerto Rico soils had the highest atom% ^{13}C of all the soil-climate combinations (Fig. 2-5C). Under the Tropical climate, the Mixed Conifer soil had a significantly lower atom% ^{13}C than all other soil-climate combinations, except for the Puerto Rico soil under Tropical climate.

Recovery of ^{13}C from labeled necrotic microbial bodies

The two-way ANOVA performed for the ^{13}C-FLF recovery results indicates that the effects of soil, climate and their interaction were all significant at $p < 0.0001$ (Table 2-3). Under the temperate climates, the amount of ^{13}C-FLF recovered was significantly greater in the Mixed Conifer soils compared to the Puerto Rico soils, especially under the Redwood climate regime (Fig. 2-6A). Under the Tropical climate, both the Mixed Conifer and Puerto Rico soils retained the least amount of ^{13}C. In both the Puerto Rico and Mixed Conifer soils, the amount of recovered ^{13}C-FLF was minimal when the soils were incubated under the Tropical climate (0.3%-0.5% recovery; Table 2-4).

Table 2-4. Percentage of the ^{13}C label recovered in each fraction 520 days post ^{13}C-labeling. 31 mg of necrotic cells that were 42.9% carbon were added to each microcosm; therefore, 13.3 mg C was added to each microcosm. Percentages represent the mg ^{13}C divided by the 13.3 mg ^{13}C necrotic cell material added to each microcosm ($\pm$ 1 standard error). Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

Soil-Climate	HF	OLF	FLF	SUM
MC-M	5.3% (0.3%)	1.2% (0.1%)	3.0% (0.2%)	9.4% (0.6%)
MC-R	5.4% (0.6%)	0.6% (0.0%)	6.3% (0.3%)	12.2% (0.9%)
MC-T	3.5% (0.3%)	0.4% (0.1%)	0.5% (0.1%)	4.5% (0.5%)
PR-M	13.9% (1.3%)	2.6% (0.2%)	0.9% (0.2%)	17.4% (1.3%)
PR-R	7.9% (0.6%)	1.3% (0.0%)	1.6% (0.1%)	10.8% (0.6%)
PR-T	6.0% (0.8%)	0.6% (0.0%)	0.3% (0.0%)	6.9% (0.8%)

^{13}C recovery in the OLF showed that the effects of soil, climate and their interaction were all significant at $p < 0.0001$ (Table 2-3). Under the Mediterranean climate, the amount of ^{13}C-OLF was substantially greater in the Puerto Rico soil compared to the Mixed Conifer soil, and the Puerto Rico soil-Mediterranean climate combination recovered the highest amount of ^{13}C-OLF of all the soil-climate combinations (Fig. 2-6B). Puerto Rico soils retained significantly different amounts of ^{13}C in the OLF under the 3 climate regimes; Puerto Rico soil under the Mediterranean climate contained the greatest of mg ^{13}C (as described above), Puerto Rico soil under the Redwood climate contained the second most mg ^{13}C, and Puerto Rico soil under the Tropical climate contained the lowest mg ^{13}C. Mixed Conifer soil incubated under the Mediterranean climate retained a greater of ^{13}C-OLF than the Mixed Conifer soil-Redwood climate and Mixed Conifer soil-Tropical climate combinations.

The effects of soil ($p < 0.0001$), climate ($p = 0.0001$), and their interaction ($p = 0.018$) were all significant factors affecting the amount of ^{13}C-HF recovered (Table 2-3). Under the Mediterranean climate, the amount of ^{13}C-HF recovered was significantly greater in the Puerto Rico soil than in the Mixed Conifer soil; the PR-M climate combination retained the highest amount of ^{13}C-HF of any soil-climate combination (Fig. 2-6C).

When the mg ^{13}C of the FLF, OLF and HF were summed for each soil-climate combination, the effects of soil ($p = 0.0005$), climate ($p < 0.0001$), and their interaction ($p = 0.0002$) were all significant (Table 2-3). The total recovery of ^{13}C in the summed organic fractions was greatest in the Puerto Rico soil-Mediterranean climate combination (Fig. 2-6D). When comparing among fractions, for both the Puerto Rico and Mixed Conifer soils, much more ^{13}C was recovered in the HF under all the climate regimes, except for the Mixed Conifer soil-Redwood climate combination (that with the greatest total mg of FLF) for which the ^{13}C-FLF recovery was similar to that of the ^{13}C-HF.

When taking into account background data on the SOC and standing microbial biomass of each soil type, and assuming that the fate of the dead microbial bodies added represented the fate of all the microbial biomass in the soil at the time of label addition (Table 2-5A), the percentage of SOC that would have been derived from dead microbial bodies (had all of the standing microbial biomass been labeled, died, and been subject to 520 days of decomposition/stabilization processes) would have been 0.09%-0.22% in Puerto Rico soils and 0.02%-0.05% in Mixed Conifer soils (Table 2-5B).

Carbon to Nitrogen ratios
Soil ($p < 0.0001$) was a significant factor affecting the C:N ratio of the SOM fractions at 520 days. C:N ratios of Puerto Rico soils were on average, higher than that of the Mixed Conifer soils. In the HF, C:N ratios were significantly lower than the C:N ratios of the FLF and OLF. C:N ratios of the Mixed Conifer soil HFs averaged 14.4 and the C:N ratios of the Puerto Rico soil HFs averaged 18.4. Mixed Conifer soils OLF C:N ratios averaged 25.3 while the Puerto Rico soil OLF C:N ratios averaged 27.4. In the FLF, the Mixed Conifer soils C:N ratios averaged 24.8 and the Puerto Rico soils C:N ratios averaged 29.7 (Table 2-6).

Table 2-5. (A) The amount and relative proportion of ^{13}C added to the two soils compared to the soil organic carbon (SOC) content and standing microbial biomass, and the amount of ^{13}C recovered in each density fraction. **(B)** Assuming all the standing biomass had been uniformly labeled, the relative proportion of the SOC that would be accumulated from stabilized microbial cells over a period of 520 days. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

(A) RESULTS FROM THIS STUDY:

Labeled C added to microcosms: 0.111 mg ^{13}C per g dry soil

Soil-Climate	SOC (mg/g soil)	^{13}C-label as % SOC	standing microbial biomass C (mg C/g soil)	^{13}C-label as % standing microbial biomass C
MC	100	0.111%	0.432	25.67%
PR	121	0.092%	1.512	7.34%

mg ^{13}C recovered in each fraction per g soil

Soil-Climate	HF	OLF	FLF	SUM TOTAL
MC-M	0.0058	0.0013	0.0033	0.0104
MC-R	0.0059	0.0007	0.0069	0.0136
MC-T	0.0039	0.0005	0.0006	0.0050
PR-M	0.0154	0.0029	0.0010	0.0193
PR-R	0.0088	0.0014	0.0018	0.0119
PR-T	0.0067	0.0007	0.0003	0.0077

(B) ESTIMATES IF ALL STANDING MICROBIAL BIOMASS HAD BEEN UNIFORMLY LABELED:

mg ^{13}C that would be recovered in each fraction per g soil

Soil-Climate	HF	OLF	FLF	SUM TOTAL
MC-M	0.0227	0.0050	0.0128	0.0405
MC-R	0.0231	0.0027	0.0271	0.0529
MC-T	0.0152	0.0019	0.0023	0.0193
PR-M	0.2096	0.0392	0.0140	0.2627
PR-R	0.1196	0.0190	0.0239	0.1625
PR-T	0.0906	0.0092	0.0047	0.1046

% of the total SOC that would have been recovered if all standing biomass had been labeled

Soil-Climate	HF	OLF	FLF	SUM TOTAL
MC-M	0.02%	0.01%	0.01%	0.04%
MC-R	0.02%	0.00%	0.03%	0.05%
MC-T	0.02%	0.00%	0.00%	0.02%
PR-M	0.17%	0.03%	0.01%	0.22%
PR-R	0.10%	0.02%	0.02%	0.13%
PR-T	0.07%	0.01%	0.00%	0.09%
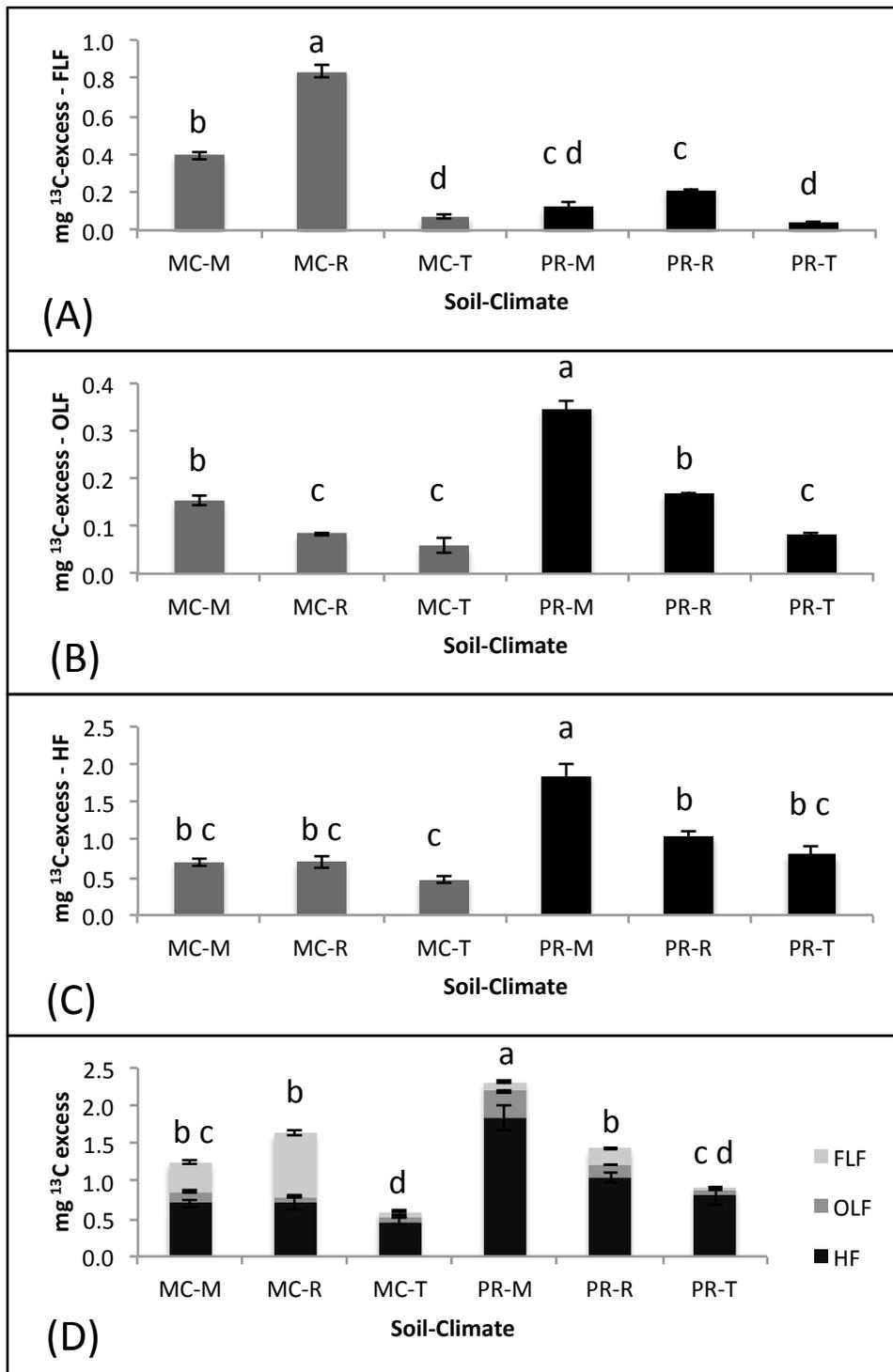
Figure 2-6. Columns represent the mean mg ^{13}C ($\pm$ 1 standard error) found in each soil-climate combination at 520 days post ^{13}C-labeling in (A) the FLF, (B) the OLF, (C) the HF, and (D) all fractions. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T).

Table 2-6. Mean C:N ratios ($\pm$ 1 standard error) recovered in each fraction at 520 days. Soil-Climate labels indicate: Mixed conifer soil-Mediterranean climate (MC-M); Mixed conifer soil-Redwood climate (MC-R); Mixed conifer soil-Tropical climate (MC-T); Puerto Rico soil-Mediterranean climate (PR-M); Puerto Rico soil-Redwood climate (PR-R); Puerto Rico soil-Tropical climate (PR-T). Different letters indicate significant differences among soil-climate combinations and SOM fractions.

Soil-Climate	HF	OLF	FLF
MC-M	13.7 (0.4)[i]	24.1 (0.4)[ef]	23.3 (0.8)[f]
MC-R	13.7 (0.6)[i]	24.6 (0.2)[def]	23.8 (0.7)[f]
MC-T	15.9 (0.5)[hi]	27.1 (0.6)[cd]	27.3 (0.3)[c]
PR-M	18.0 (0.5)[gh]	27.7 (0.5)[bc]	31.7 (0.5)[a]
PR-R	18.6 (0.5)[g]	26.5 (0.2)[cde]	27.4 (0.9)[bc]
PR-T	18.5 (0.2)[gh]	28.0 (0.3)[bc]	30.0 (0.1)[ab]

Discussion

Importance of soil and climate on carbon stabilization

A current model of soil organic carbon dynamics hypothesizes that soil organic matter persists not only due to the chemical nature of the organic materials, but because of properties such as reactive mineral surfaces, water availability, temperature, soil acidity and other physiochemical and biological influences (Schmidt *et al.,* 2011). My research supports this model in that the combined properties of soil and climate were both found to be important influences on the persistence of C from microbial bodies in soil organic matter. Climate had a significant effect on the amount of labeled ^{13}C recovered in the three soil organic matter fractions as a whole, and on each of the individual fractions: FLF, OLF, and HF. While there were only 2 soil types to compare in this experiment, soil type also demonstrated a significant effect on the amount of labeled ^{13}C recovered in the fractions as a whole, and on each of the individual fractions: FLF, OLF, and HF.

In almost all soil-climate combinations, the recovery of labeled ^{13}C was highest in the HF. High recovery of ^{13}C in the HF was likely due to the ability of mineral particles to bind microbial products. The importance of clay minerals and/or iron oxides to the stabilization of SOM is further evidenced by the effect of soil alone on the recovery of labeled ^{13}C in the HF. The Puerto Rico soil, which contained higher % clay and higher extractable Fe than the Mixed Conifer soils, stabilized a higher proportion of ^{13}C in the HF fraction (as compared to ^{13}C stabilized in the FLF and OLF fractions) than did the Mixed Conifer soil. Iron oxides are well known to influence organic matter stabilization via ligand exchange between carboxyl and

hydroxyl functional groups of organic matter (Gu *et al*., 1994), and as Schimel *et al.*, (1994) reported, soil carbon is positively correlated to clay content. Furthermore, the adhesion of microorganisms and adsorption of microbial metabolites onto clay and iron oxide surfaces increases the likelihood of the stabilization of microbial products in the HF (Chenu and Stotzky, 2002).

There was a clear influence of climate, and its interaction with soil type, on ^{13}C-OLF recoveries. Puerto Rico soils under the two temperate climates (Mediterranean and Redwood) retained higher amounts of ^{13}C-OLF than the Mixed Conifer soil counterparts under the same climate regimes. The highest mg ^{13}C-OLF was recorded in Puerto Rico soil under the Mediterranean climate. The high mg ^{13}C-OLF in Puerto Rico soils under temperate climates suggests that there may be an overall increase in aggregation in these soil-climate combinations. One hypothesis as to why Puerto Rico soils under temperate climates exhibited higher ^{13}C-OLF is that the higher clay and organic matter contents of the Puerto Rico soils produced higher levels of aggregation under the temperate climates. It is well understood that increased aggregation is often associated with an increase in clay-sized particles (Bronick and Lal, 2005). The high aggregation, and subsequent high recovery of ^{13}C-OLF in Puerto Rico soils, may also be due to organic protection. Aggregation of soil particles is heavily influenced by the organic matter content of a soil (Chaney and Swift, 1984), and the Puerto Rico soils contained 121 mg organic C/g soil while the Mixed Conifer soils contained only 100 mg organic C/g soil.

It is evident that the high clay and organic matter contents were not the only factors influencing the higher recovery of ^{13}C-OLF in the Puerto Rico soils compared to the Mixed Conifer soils because the recoveries of ^{13}C-OLF for each soil type were not consistent across climate treatments. Climate also plays an important role in soil aggregation and deaggregation, primarily through wetting and drying events, freeze-thaw events and temperature changes (Harris *et al.*, 1966; Allison, 1968). For example, the presence of water in soils is the primary cause of soil deaggregation (Lynch and Bragg, 1985), and drying events can often increase aggregate stability, especially that of small aggregates < 0.25 mm (Harris *et al.*, 1966); slow freezing events often result in the formation of large stable aggregates and the subsequent thaw results in the destabilization of aggregates (Harris *et al*, 1966); increases in temperature typically result in increased microbial activity, leading to the decomposition of the organic materials binding soil aggregates, resulting in deaggregation. The climatic factors that influence the formation, stability and breakdown of soil aggregates act in concert with one another and do not act exclusive of one another. Since the highest recovery of ^{13}C-OLF of both soils was found under the Mediterranean climate regime, and the Mediterranean climate exhibited the most extreme seasonal changes of the three climates examined, these results support the idea that climate is important to aggregation in the soil matrix.

In reality, it is likely that a combination of soil texture (the relative proportion of sand, silt, and clay in a soil), organic matter content, and climate drove soil aggregation in the microcosms. For example, the results for the ^{13}C-OLF recoveries demonstrate the importance of climate by the higher recoveries of ^{13}C-OLF for both soils under the Mediterranean climate compared to the other two climates. Soil type and/or organic matter content were also important because the Puerto Rico soil under the Mediterranean climate still recovered significantly more ^{13}C-OLF than did the Mixed Conifer soil under the Mediterranean climate. The differences in ^{13}C-OLF recovery between the two soils observed for the Mediterranean climate may have been driven by either soil texture or organic matter content since soils with high amounts of clay that experience dry climate conditions have been shown to exhibit physical aggregation and

protection of organic matter contained within the aggregates (Harris *et al.,* 1966; Causarano 1993), and higher amounts of organic residues in soils commonly lead to increased stabilization of aggregates (Martin *et al.,* 1955; Harris *et al.,* 1966).

Importance of soil and climate on decomposition of free light fraction carbon

Puerto Rico soils under the two temperate climates retained significantly less ^{13}C-FLF than their Mixed Conifer soil counterparts under the same temperate climates, likely as a result of a larger, more active microbial community in the wet tropical Puerto Rico soil (Weaver and Murphy, 1990; Wardle, 1992). The Puerto Rico soils contained over three times the amount of standing microbial biomass compared to the Mixed Conifer soils, and the microbial community in the Puerto Rico soils contained less fungi than the Mixed Conifer soils.

Puerto Rico soils under the Mediterranean climate retained significantly higher total ^{13}C (FLF+OLF+HF) than any other soil-climate combination; primarily due to the high amounts of ^{13}C retained in the OLF and HF fractions. In these soils, because there was significant loss of labeled ^{13}C from the FLF, yet high amounts of ^{13}C were stabilized in the OLF and HF, I hypothesize that a large portion of the labeled ^{13}C that was not immediately mineralized to CO_2 was quickly assimilated by the living microbial biomass. The living biomass may have then become occluded in soil aggregates or bound to mineral particles, leading to the high atom% ^{13}C recovered in the OLF and HF.

In both the Puerto Rico and Mixed Conifer soils, the amount of recovered ^{13}C-FLF was minimal when the soils were incubated under the Tropical climate. Rapid metabolism and turnover of the labeled microbial bodies under the favorable, warm and wet climate conditions likely led to increased mineralization and loss of the label as ^{13}C-CO_2, causing the disappearance of ^{13}C-FLF in both soils under the Tropical climate. Since neither soil type under the Tropical climate stabilized an increased amount of ^{13}C in the OLF or HF, the metabolized ^{13}C was likely respired out of the system as CO_2 rather than having been assimilated into the bodies of the living microbial communities. Thus, either carbon use efficiency of the microbial community was lower under the tropical climate or the rates of turnover were higher under the tropical climate. This result demonstrates the centrality of climate control over decomposition processes, and indicates that dead microbial cells can provide a quickly utilized source of C under favorable climate conditions.

Carbon to Nitrogen ratios of the SOM fractions in Puerto Rico and Mixed Conifer soils

The C:N ratio of the HF of both soils was significantly lower than the C:N ratios of the OLF and FLF. Lowered C:N ratios indicate an increased degree of microbial utilization and alteration of soil organic matter C during decomposition (Rodionov *et al.,* 2000). The low C:N ratios of the HF compared to the FLF and OLF fractions indicate that the HFs of both soils experienced increased microbial processing of C during decomposition of the added, labeled cells. The increased degree of processing of SOM in the HF is consistent with the findings of Rodionov *et al.* (2000), Six *et al.* (2001), Heckman *et al.* (2013), and Ryals *et al.* (2014).

Importance of microbial cells as stabilized organic matter in soils

Previous studies have primarily focused on the role of plant materials as the building blocks of soil organic matter. We are now starting to understand that microbial bodies themselves are building blocks of soil organic matter (Chenu and Stotzky, 2002; Kögel-Knabner 2002; Miltner 2012). These microbial bodies are susceptible to becoming stabilized in soils because they have adhesive properties, are plentiful in soils, and commonly live on soil particles

to which they can become adsorbed. My results indicate that microbial bodies do contribute a portion of the nascent materials for the stabilization of soil organic matter, albeit, likely a lower portion than that contributed by plant tissues.

Soil type was a significant factor influencing the amount of microbial cell material that was stabilized in the soil organic matter matrix. The percentage of SOC that would have been derived from dead microbial bodies (had all of the standing microbial biomass been labeled, died, and been subject to 520 days of decomposition/stabilization processes) would have been substantially higher in Puerto Rico soils than in Mixed Conifer soils. Clearly, these estimates do not reflect the actual proportion of SOC that would be derived from microbial biomass materials, which would likely be much higher, because the calculations take into account only the biomass that was standing at the start of the incubation and do not take into account growth of new microbial cells and the high and constant turnover of microbial communities in soils.

The propensity of Puerto Rico soils to stabilize a higher proportion of microbial bodies in the SOC would not solely be due to the edaphic characteristics of the soil, but rather, it would also be due to the high levels of standing microbial biomass in the soils. Soils containing higher proportions of microbial biomass would have greater opportunities for microbial products to become stabilized in the soil organic matrix. Favorable climate and edaphic characteristics, such as high temperatures and high nutrient availability, promote the growth of microbial communities, which in turn may affect the adsorption of microbial bodies to the mineral matrix. An increase in adsorption of microbial bodies may occur due to increased levels of total biomass in the soils, which would increase the proportion of SOC that is comprised of microbial bodies. A decrease in adsorption could occur if other soil organisms readily consume and metabolize the increased levels of biomass so that the carbon within the microbial biomass is respired out of the soil ecosystem before it is adsorbed to the mineral matrix. This in turn, would decrease the proportion of SOC that is comprised of microbial bodies. As climate changes globally, the size of standing microbial biomass in soils may change with transformations in temperature and precipitation, potentially negatively or positively depending on the change of climate in a given location. Thus, climate change may impact the proportion of microbial biomass carbon that becomes stabilized as organic matter in soil.

Conclusion

While the composition of microbial communities and their subsequent metabolic capacities are central to soil carbon stabilization, we have little understanding about the role of soil microbes as precursors of soil carbon. Current research shows that in addition to plant residues, microbial bodies contribute carbon to SOM pools. The research presented here addresses the role of climate and soil type on the stabilization of microbial carbon in three SOM pools over 1.5 years. Results of my study demonstrated that microbial bodies do indeed contribute to soil organic carbon pools, and that after 1.5 years, the majority of the microbial carbon remaining in the SOM was retained in the HF. The recovery of microbial C in the HF of SOM is important because it indicates stabilization of microbial C in the longest-lived organic matter pool in soils. I also found that both climate and soil type influenced the recovery of microbial carbon in SOM. In the FLF, the warm, wet Tropical climate promoted greater microbial activity and decomposition. The OLF exhibited differences in soil aggregation whereby Puerto Rico soils under the temperate climates stabilized higher amounts of ^{13}C in the

OLF than Puerto Rico soils under the Tropical climate than their Mixed Conifer soil counterparts. The influence of climate and soil characteristics on the rate and degree of the stabilization of soil organic matter, and how these environmental drivers ultimately impact the partitioning of microbial C in soil, are topics that deserve further consideration in our changing environment.

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Chapter 3

Cycling of Carbon From Microbial Bodies

in Tropical Forest Soils

Abstract

The movement of carbon from plant matter into soil microbial communities, and the turnover of carbon within these microbial communities, is an important component of the cycling and stabilization of carbon in soils. Climate, edaphic characteristics, and microbial community composition and function can influence whether dead microbial carbon is re-utilized by the microbial community, respired out as CO_2, or stabilized in the soil. This study asked whether longer residence times of carbon in the microbial biomass increased the association of microbial carbon with mineral surfaces in the heavy fraction of soil organic matter (SOM). To elucidate whether there was a relationship between residence times of microbial carbon and their association with mineral surfaces, I followed the fate of labeled dead microbial carbon in soils as it was assimilated into the living biomass, respired out as CO_2, and recovered in the SOM. Soils were collected from a tropical Puerto Rican forest; ^{13}C-labeled microbial dead bodies were introduced to the soils, and soils were then incubated for 520 days under 3 different climate regimes (Mediterranean mixed conifer forest, Redwood forest, and Tropical forest). Climate exerted significant influence on the amount of ^{13}C recovered in the standing biomass, the amount of ^{13}C-CO_2 respired, and the ^{13}C recovered in each of three operationally defined soil organic matter pools: free light fraction (FLF), occluded light fraction (OLF), and heavy fraction (HF). Climate alone however had no effect on the total biomass of soils after 70 and 520 days of incubation. An increase in saprophytic fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomasses under the Mediterranean climate early in the incubation indicated that all four of these microbial communities temporarily responded favorably to the relatively cold, dry spring Mediterranean climate conditions. Along with increases in microbial biomass at 7 days, tropical soils under the Mediterranean climate also consistently retained higher amounts of total dead ^{13}C-labeled cell material in the living biomass over 520 days than they did under their native Tropical climate. Interestingly, assimilation of the dead microbial carbon by actinobacteria was highest under the Tropical climate; this trend was primarily due to the atom% ^{13}C excess of the actinobacterial cells, and not increases in actinobacterial biomass, indicating that while the presence of dead microbial carbon did not cause the actinobacterial communities to increase in size at 520 days, the actinobacteria in this study did assimilate dead microbial carbon under the static warm, wet climate conditions. There was also clear influence of climate on the OLF of Puerto Rico soils as more ^{13}C was recovered in the OLF of these soils under temperate climates compared to the Tropical climate. Hence, the soil temperature and moisture shifts of the temperate climates appear to have favored an increase in the aggregation of Puerto Rico soil particles. In addition, Puerto Rico soils under the Tropical climate respired significantly more ^{13}C-CO_2 than soils under the two temperate climates, and

soils under the Mediterranean climate respired the lowest amounts of ^{13}C-CO$_2$, supporting the principle that microbial communities in Tropical climates have high metabolic rates and/or low carbon use efficiencies (CUE) than communities in colder, drier soils. The effect of these differences in metabolic rates and CUE may lead to differences in the amount of time organic C remains in the standing biomass before it is respired out as CO$_2$, thus impacting the likelihood of carbon becoming stabilized in soil organic matter pools. This study demonstrates that longer residence times of carbon in the microbial biomass may indeed increase the association of microbial carbon with mineral surfaces in the heavy fraction of SOM. Here, soils with the longest retention times of ^{13}C in the living biomass and the lowest respiration rates stabilized the most labeled carbon in the HF, while soils with the lowest retention times of ^{13}C in the living biomass and the highest total respiration of CO$_2$ stabilized the least amount of labeled carbon in the HF.

Introduction

   The cycling of carbon (C) in soils is critical to soil health and fertility, and is a central component of the global carbon cycle. The soil carbon pool contains approximately 1550 Gt of organic carbon and is over three times the size of the atmospheric carbon pool (Lal, 2004). Soil respiration contributes approximately 75×10^{15}g carbon per year to the atmosphere; the respiration of soil carbon at a global level can be an important driver of climate change, and significant changes in soil respiration have the potential to comprise feedback loops (Schlesinger and Andrews 2000; Davidson and Janssens, 2006). Changes in soil carbon cycling, such as alterations in decomposition and/or carbon sequestration, drive potential feedbacks to climate change (Davidson and Janssens, 2006). For example, increases in decomposition due to warming temperatures can transfer C from soil carbon pools to the atmosphere, increasing the amount of the greenhouse gas CO_2 in the atmosphere, leading to higher global temperatures, driving a positive feedback to climate change. As temperatures warm due to increased levels of greenhouse gases in the atmosphere, rates of soil microbial metabolism may increase and carbon use efficiency (CUE) may decrease (Chambers *et al.,* 2004; Manzoni *et al.*, 2012) due to the more favorable temperatures. Both an increase in microbial metabolism and an increase in CUE would increase rates of soil respiration, increasing the amount of CO_2 being respired into the atmosphere, and decreasing the residence time of carbon in the standing microbial biomass.

   It is well established that the primary C input to soil is plant tissue and that most detrital plant C entering the soil passes through the microbial biomass (Wolf and Wagner, 2005; Plante and Parton, 2007). A number of studies have followed the assimilation of detrital plant C by soil microbial communities, primarily through the use of ^{13}C-phospholipid fatty acid analysis (PLFA; Waldrop and Firestone, 2004; Bird and Torn, 2006) and chloroform fumigation direct extraction (Kelly and Burke, 1997). As shown in Fig. 3-1, both climate and edaphic characteristics impact the amount and quality of C input; but perhaps of equal importance, these primary controllers also impact the functional capabilities, community composition, and biomass sizes of soil microbes. Yet, very little is known about how soil microbial communities and the processes that they catalyze are specifically involved in the stabilization of soil organic matter (SOM). Two central roles are proposed: 1) microbial metabolic capacities are key controllers of the decomposition and transformation processes constituting soil organic matter stabilization, and 2) microbial bodies are nascent substrates for stabilized organic materials. Few studies have traced the flow of C from microbial bodies themselves through the process of decomposition and stabilization in SOM pools (Lueders et al., 2006; Kindler et al., 2006).

   Soil organic carbon exists in different SOM pools, each with different chemical characteristics and turnover times. There have been a variety of methods used to define soil organic carbon pools (Stevenson, 1994; Olk and Gregorich, 2006; Schmidt *et al.,* 2011), but one approach that is frequently used to separate and define soil organic matter fractions is density gradient fractionation. Density fractionation separates soil organic carbon pools into a free light fraction (FLF), occluded light fraction (OLF) and dense organo-mineral (heavy) fraction (HF). The FLF contains low-density organic matter that commonly resembles recent detrital inputs both visually and chemically. Carbon in the FLF is often younger than C found in the OLF and the HF (Spycher *et al*., 1983; Swanston *et al.,* 2004), with turnover times ranging from months to a decade. Like that found in the FLF, organic C in the OLF is also low-density, yet it is often older than C the FLF due to its physical protection within soil aggregates. The age of occluded organic carbon is dependent upon where it is located within the aggregate and whether or not the

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organic C has adsorbed to mineral surfaces within the aggregate (Golchin *et al*., 1994). The HF contains organic carbon associated with soil mineral surfaces. Carbon in the HF is often highly processed and older than C in the FLF and OLF with turnover times reaching upwards of a thousand years (Trumbore 1993). While previous models of SOM stabilization attributed the persistence of SOM to the chemical nature of the decomposing organic materials (Kögel-Knabner *et al.,* 1988; Cambardella and Elliott, 1992; Rovira and Vallejo, 2007), a current view of soil organic carbon dynamics attributes the persistence of SOM to "ecosystem properties" such as reactive mineral surfaces, water availability, temperature, soil acidity and other physiochemical and biological influences (Schmidt *et al.,* 2011). In current models of carbon stabilization in soils, microbial bodies are likely to become stabilized in SOM because of their abundance in soils, adhesive properties, and because they commonly live on the surface of minerals and within soil aggregates (Chenu *et al.*, 2001). The adhesion of soil microbes onto metal oxide minerals and clay surfaces increases the likelihood of microbial products becoming stabilized in the HF of SOM (Chenu and Stotzky, 2002). In fact, with increasing density, SOM fractions demonstrate progressive enrichment of microbial derived decomposition products (Liu *et al*., 2013).
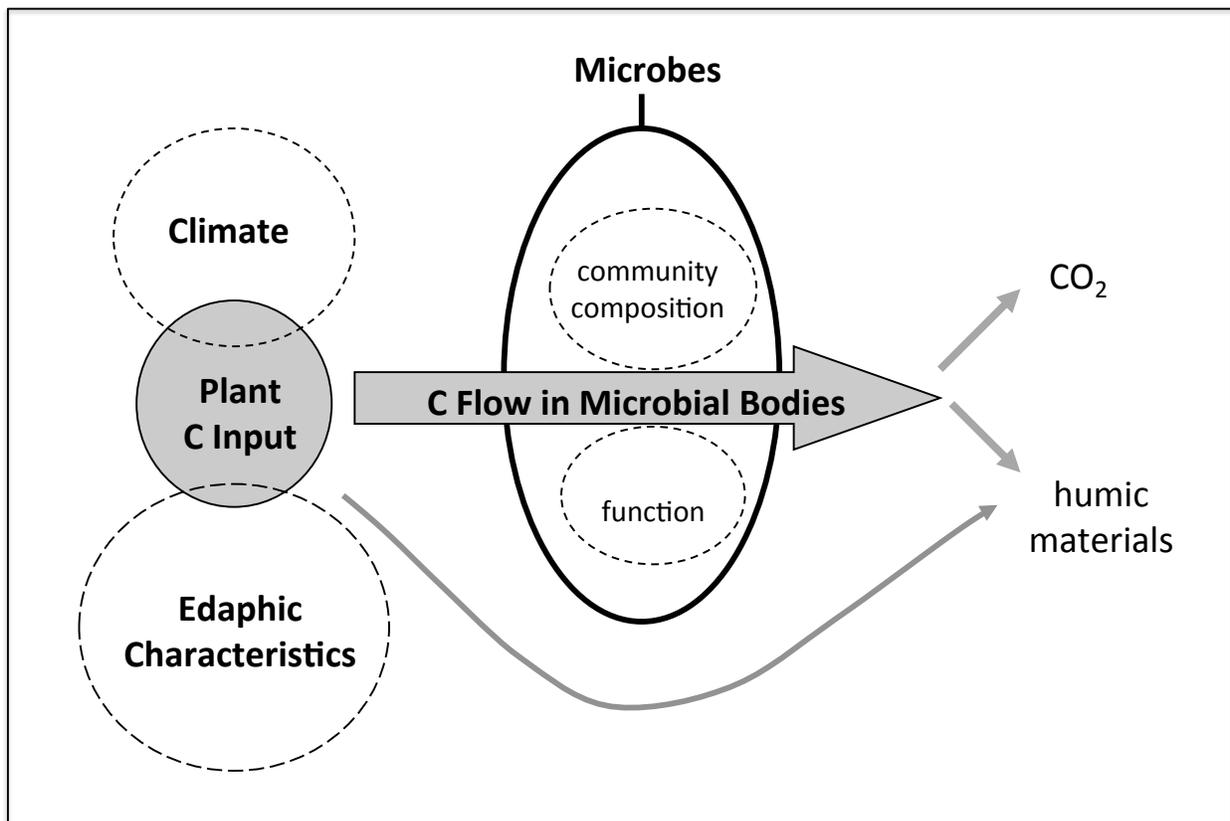
Figure 3-1. Influence of climate and edaphic ecosystem properties on carbon cycling and microbial communities.

The presence of metal oxide minerals is an important influence on SOM stabilization and it is well known that organic matter adsorbs onto metal oxide mineral surfaces such as iron oxides (Gu *et al.,* 1994). Highly weathered tropical soils typically exhibit high concentrations of iron oxides (Vitousek and Sanford, 1986) and soils in the Luquillo tropical forest of Puerto Rico are no exception (Liptzin and Silver, 2009; Dubinsky *et al.,* 2010). Iron oxide concentrations in soils are important for the retention of organic matter because they have been demonstrated to influence OM stabilization in multiple ways: 1) they have a high capacity to sorb humics (Tipping, 1981) and some simple carbon compounds (Jones and Edwards, 1998), 2) organic matter is stabilized onto iron oxides via ligand exchange between carboxyl and hydroxyl functional groups of organic matter (Gu *et al.,* 1994), 3) there is a positive correlation between total organic carbon concentrations and iron oxides in many soils (Wagai and Mayer, 2007), and 4) there is a positive correlation between soil organic carbon storage and non-crystalline mineral concentrations such as irons oxides (Torn *et al.,* 1997; Masiello *et al.,* 2004).

Another influence on the stabilization of organic matter in soil is clay content. Soil carbon storage has been shown to be positively correlated to clay content (Schimel *et al*., 1994) due to the adsorption of microbial bodies and products onto clay surfaces (Chenu and Stotzky, 2002) and increases in soil aggregation as clay-sized particles are frequently associated with soil aggregates (Bronick and Lal, 2005) that can trap and physically protect organic matter from microbial degradation. It should be noted that in addition to clay content, soil aggregation is also heavily, and positively correlated to the organic matter content of a soil (Chaney and Swift, 1984); organic matter such as extracellular polysaccharides, glomalin, and decomposing tissues bind soil particles together, creating soil aggregates that can trap and protect organic matter from further decomposition.

In addition to edaphic characteristics, climate is also an important influence on the residence time and stabilization of organic carbon in soils. Climate is an important driver of soil biogeochemical processes and thus, can strongly affect organic matter stabilization in soils. Two primary climatic drivers of SOM stabilization are temperature and precipitation. Globally, as temperature decreases, SOM concentrations increase (Schimel *et al.,* 1994; Alvarez and Lavado, 1998; Dai and Huang, 2006; Davidson and Janssens, 2006). The positive relationship between decomposition rates and temperature is a primary cause of the inverse relationship between SOM storage and temperature. Changes in precipitation lead to varying effects on SOM storage (Davidson and Janssens, 2006). Increases in precipitation have been correlated with both increases in SOM concentrations (Alvarez and Lavado, 1998) as well as decreases in SOM concentrations (Dai and Huang, 2006). Frequently, the influence of precipitation on SOM storage is dependent upon the aeration and drainage of a soil.

For the past decade much research has focused on the role of plant communities and plant carbon inputs as controllers of soil microorganisms (Zak et al., 1994; Wardle et al. 1999, Marschner et al. 2001, Eviner and Chapin, 2002). Yet, microbial community composition can be a critical factor in the cycling and sequestration of soil nutrients, including carbon and nitrogen. One proposed mechanism of how microbial community composition can alter soil nutrients is that dominant populations may have an increased ability to metabolize different substrates over minor microbial communities, and that shifts in microbial community composition in conjunction with shifts in climate may change substrate utilization patterns (Zogg *et al.,* 1997). Degens (1999) found that catabolic response profiles (CRP; profiles of the breakdown of complex molecules to simpler ones by living organisms) differed between taxa of microbes grown in the same soil, and that soil type had a significant impact on the CRP of the same taxa

grown in different soils. Schimel and Gulledge (1998) demonstrated that differences in microbial communities are responsible for differences in soil respiration and methane production in a range of soils, and that changes in climate could alter the microbial community composition in soils such that trace gas fluxes are subsequently altered as well.

The question of whether there are specific climate characteristics that are of dominant importance in controlling the composition of soil microbial communities is a topic of substantial interest (Fierer and Jackson, 2006). Recent work suggests that a limited number of climatic characteristics may be dominant controllers of the microbial communities inhabiting soils (Dubinsky *et al*., 2006; Treseder *et al.,* 2012). Two primary climate factors to which microbial communities respond include changes in temperature and water availability (Treseder *et al.,* 2012 and references therein).

This study tests whether changes in climate lead to longer residence times of carbon in the microbial biomass, and whether increases in the residence time of C in the biomass increases the association of microbial C with mineral surfaces in the HF of SOM. If organic carbon is retained in the living microbial biomass for longer periods of time, and living microbial bodies are closely associated with mineral surfaces, this could lead to an increase in the retention of microbial carbon in the stabilized mineral bound fraction of soil organic matter, leading to an increase in microbial based carbon in SOM.

In this study, microbial C (in the form of dead microbial bodies enriched with ^{13}C) was introduced to Puerto Rican tropical soils in order to follow the movement and stabilization of dead microbial C through the standing microbial biomass as it was either respired out as CO_2 or retained in operationally defined SOM fractions over a period of 520 days. The soils were exposed to three different climate regimes to study the effects of climate on the cycling of microbial derived carbon in soil ecosystems. Soil from a wet tropical forest in Puerto Rico was chosen because tropical forest soils comprise a large storage pool of organic carbon and their sensitivity to changes in temperature and precipitation may be important to the response of carbon cycling in the tropics to changing climate.

By exposing a Puerto Rico soil to three climates with different average and seasonal changes in soil temperature and moisture content I was able to examine the influence of climate on soil microbial community biomass pools, microbial respiration of microbially derived C, and the incorporation of microbial C into SOM pools in a tropical forest soil. The differences in microbial assimilation and cycling of C in tropical soils under the three climate regimes allowed me to test whether longer residence times of carbon in the microbial biomass increase the association of microbial carbon with mineral surfaces in the heavy fraction of SOM.

Materials and Methods

Field sites

Soil samples were collected (0-7.5 cm depth) from the Colorado Forest in the Puerto Rico Luquillo Experimental Forest at 5 locations (1 m apart) along 3 transects. The soil samples were then homogenized together and stored at field moisture and ambient temperature for 3 days before subsampling into each microcosm. The Luquillo Forest is located in the highlands of Puerto Rico, 35 km east of San Juan, and is representative of a tropical montane wet forest in which high levels of precipitation (Table 3-1) cause soils to fluctuate between oxic and suboxic

conditions. Approximately 40 plant species occur in the Puerto Rico forest, and the dominant plant species is myrtle (*Cyrilla racemiflora*). The Firestone lab at UC Berkeley has extensive experience working at this location and a detailed knowledge of its site characteristics (Table 3-1).

Table 3-1. Site and soil characteristics of the soil harvest location. Means are shown with standard errors in parentheses.

Region	Puerto Rico
Forest Type	Tropical Deciduous
Site Name	Luquillo Experimental Forest
Latitude	18° 41' N
Elevation (m)	780
Mean precipitation (mm)	4,500 [1]
Mean annual temperature (°C)	18.5 [1]
Dominant plant species	*Cyrilla racemiflora*
Soil order	Ultisol [1]
Soil texture	sandy loam
% sand; % silt; % clay	64.0%; 18.3%; 17.8%
pH	4.5 [2]
Potassium (meq/100g)	0.10 (0.00)
Sodium (meq/100g)	0.13 (0.02)
Calcium (meq/100g)	1.07 (0.21)
Magnesium (meq/100g)	0.78 (0.10)
Extractable sulfate (ppm)	20.7 (3.2)
Total manganese (ppm)	23.3 (3.6)
Phosphorus (ppm)	0.33 (0.07)
Extractable Iron- DTPA (ppm)	417.0 (19.7)
CEC (meq/100g)	16.98 (3.5)
Soil organic carbon (mg C/g soil)	121 (6)[2]
Microbial biomass carbon (mg C/g soil; CFDE)	1.512 (0.116) [2]
Microbial biomass carbon (mg fatty acid C/g soil; PLFA)	0.058 (0.003)
Fungal:Bacterial biomass ratio	0.19

[1] Templer et al., 2008

[2] Throckmorton et al., 2012

Microbial growth and additions

Microbial cell cultures were isolated from soils sampled in 2004 from the Luquillo Experimental Forest in Puerto Rico (described above) and the University of California Blodgett Forest Research Station. These locations were chosen because the cell cultures were originally isolated for use in a field investigation of microbial C cycling and assimilation in these two soil habitats (see dissertation Chapter 1). Blodgett Forest is located in the mid-elevation Sierra Nevada Mountains, 240 km east of San Francisco and is a Mediterranean forest representative of productive mixed-conifer forestlands in California. The origin of each microbial isolate is given in Table 3-2. The four microbial groups used in this study were fungi, actinobacteria (high-GC Gram-positive bacteria), Gram-positive bacteria, and Gram-negative bacteria. The mixture of microbial groups was arranged to include as complex a blend as possible from the unused ^{13}C-labeled cell culture materials that were produced and used in the field investigation (see dissertation Chapter 1). The primary cell types contained in the culture mixture utilized in this study (of 23 total) included: *Penicillium verruculosum* (fungus), *Burkholderia sp.* (Gram-negative bacterium), *Bacillus sp.* (Gram-positive bacterium), and *Streptomyces sp.* (actinobacterium). The four microbial groups used in this study were chosen because the groups were expected to have fairly different cellular chemistries. Nuclear Magnetic Resonance (NMR) analysis of the groups used in this study, after they had been chosen and ^{13}C labeled, revealed they had fairly distinct cellular chemistries (Fan *et al.,* 2009). For example, the Gram-negative bacteria contained a high abundance of β-hydroxybutyrate (BHB) and low levels of trehalose, whereas BHB was absent from and high amounts of trehalose was present in Gram-positive, fungi and actinobacteria; The fungi contained more glycolipids than the actinobacteria, and demonstrated the highest synthetic capacity for glycerol production among all four of the microbial group types; The Gram-positive bacteria were more enriched in phospholipids than the Gram-negative bacteria, fungi and actinobacteria, and contained less triacylglycerides than the other three groups.

The initial culturing methods screened hundreds of isolates per site using agar amended with soil-extracts and 10% trypticase soy agar (TSA) or 10% trypticase soy broth (TSB) at pH 5.5; a pH of 5.5 was chosen to mimic the acidic conditions of both the Blodgett Forest and Luquillo Forest soils (Table 1-1). The cultures were then further isolated with additions to the media: novobiocin and potato dextrose agar (PDA) with streptomycin for fungi; chitin with cycloheximide or starch casein for actinobacteria; 10% TSA with cycloheximide for Gram-positive and Gram-negative bacteria (Alef 1995; Fan *et al.,* 2009). The identities of the final 23 isolates selected were confirmed using DNA extraction kits (PowerSoil, Mobio Inc., CA); 16S rRNA genes were amplified from the bacterial isolates using the "universal" primers 27F and R1492 (Lane *et al*., 1985) and 18S-5.8S-28S ITS regions were amplified from fungal isolates using the ITS1F and ITS4 primers (Gardes and Bruns, 1993). Full, high quality sequences (phred score $q \geq 20$) were compared by BLAST with the public databases NCBI Genbank and greengenes (DeSantis *et al*., 2006).

Table 3-2. Microbial isolates which were cultured, labeled with ^{13}C, mixed, and homogenized before injection into each microcosm. Percentages are based on dry cell weight.

Harvest Site	Group	Taxonomic Classification	Operational Taxanomic Unit (BLAST match)	Taxa % of Mixture
Tropical	**Fungi**	Ascomycete	Penicillium verruculosum	10.72%
		Basidiomycete (mycelial)	Trichosporon multisporum	1.68%
		Basidiomycete (yeast)	Cryptococcus podzolicus	0.23%
Temperate	**Fungi**	Ascomycete	Penicillium sacculum	3.09%
		Basidiomycete (mycelial)	Holtermannia corniformis	0.03%
		Basidiomycete (yeast)	Cryptococcus terreus	0.02%
		Zygomycete	Umbelopsis nana	0.02%
Tropical	**Actinobacteria**	Streptomycetaceae	Streptomyces sp. strain 317	3.71%
		Streptomycetaceae	Kitasatospora sp. C2	8.87%
		Streptomycetaceae	Kitasatosporia griseola JCM 3339	8.45%
Temperate	**Actinobacteria**	Streptomycetaceae	Streptomyces sp. strain Soll14	11.20%
		Streptomycetaceae	Streptomyces capoamus	2.33%
		Streptomycetaceae	Streptomyces sp. JL164	2.18%
Tropical	**Gram-positive bacteria**	Firmicutes	Bacillus thuringiensis strain 2000031485	5.42%
		Firmicutes	Bacillus mycoides strain: S31	3.84%
Temperate	**Gram-positive bacteria**	Actinobacteria (non-Streptomycetaceae)	Arthrobacter sp. strain RC100	5.60%
		Firmicutes	Bacillus subtilis strain KL-077	3.69%
Tropical	**Gram-negative bacteria**	Bacteroidetes	Flexibacter cf. sancti	1.23%
		Beta-proteobacteria	Burkholderia plantarii	8.72%
		Gamma-proteobacteria	Dyella japonica	1.77%
Temperate	**Gram-negative bacteria**	Alpha-proteobacteria	Rhizobium sp. strain 'USDA 1920'	3.64%
		Beta-proteobacteria	Burkholderia sp. strain A22-1	11.04%
		Beta-proteobacteria	Burkholderia sp. strain UCT 29	2.49%

The final isolates were ^{13}C-labeled by growing cultures in liquid media to late stationary phase at 25°C; the media contained 20% glucose (99.9 atom% enriched with ^{13}C) and 10% yeast extract with M9 salts at pH 5.5. Whole cells were harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 7.0) to remove remaining media. Low-density cellular materials during culturing that were not harvested with the cells were filtered from the used media with 0.1-μm polycarbonate filters (Sterlitech Co., Kent, WA), washed with deionized water, and combined with the whole cells. The cultures were autoclaved and killed, while maintaining as much of the cellular structure as possible (Fan *et al.*, 2009), so that the focus of this study would be on the assimilation and stabilization of microbially-derived carbon in soils rather than the metabolic activity of labeled microbial cells.

In order to widely distribute the ^{13}C-labeled, sterilized microbial necromass in the soils, all of the previously freeze-dried, dead cell material was suspended in water before injection into the microcosms. A tissue homogenizer (Potter-Elvehjem) was then used to distribute and standardize the mixture of microbial isolates. While injection of cell-containing solutions into soil did not realistically simulate the natural circumstances of microbial growth and turnover, it did allow us to follow the assimilation of the dead cell C by the living microbial biomass.

Incubation setup and soil processing

Laboratory incubations were constructed using homogenized soils from the Puerto Rico Luquillo Experimental Forest. Microcosms were constructed from sealed mason jars (1 quart) that were opened and flushed with ambient air twice a week to prevent suboxic conditions in the headspace. Each microcosm contained 120 g of soil (dry-weight equivalent) with moisture contents equivalent to the average April conditions under which each microcosm was to be initially incubated (minus 2 mL water that was subsequently added when the cell mixture was injected into the soils). The microcosms were then pre-incubated for 2 weeks in order to dissipate much of the disturbance-induced CO_2 pulse. After pre-incubation, 31.0 mg of the homogenized mix of [13]C- labeled dead microbial bodies in 2 mL deionized water were added to each microcosm (Fig. 3-2A) and the soils containing the mixture of labeled microbes were stirred with a rod for 60 seconds to evenly distribute the mixture within the microcosm. Of the total 31.0 mg of added cells, 13.3 mg consisted of [13]C. No additional carbon was added to the microcosms during the course of the study.

There were 3 replicates for each of the 3 climate regimes and 2 harvests for a sum of 18 microcosms. Of these 18 microcosms, 9 were harvested immediately after the application of labeled cells (T0) and 9 were harvested at 520 days (T1). Nine additional microcosms that did not receive labeled cells were harvested before the climate simulations began (backgrounds) and 9 additional unlabeled microcosms were harvested after 520 days (controls).

Soil moisture content and organic matter content was calculated gravimetrically by drying soil subsamples at 105 °C to a constant weight for moisture content, and ashing the same subsamples at 550°C to a constant weight for organic matter content. Soil moisture content was expressed by weight as the ratio of the mass of water in the sample to the mass dry weight of the sample, and soil organic matter content was expressed by weight as the ratio of the mass of ash in the sample to the mass oven dried soil.
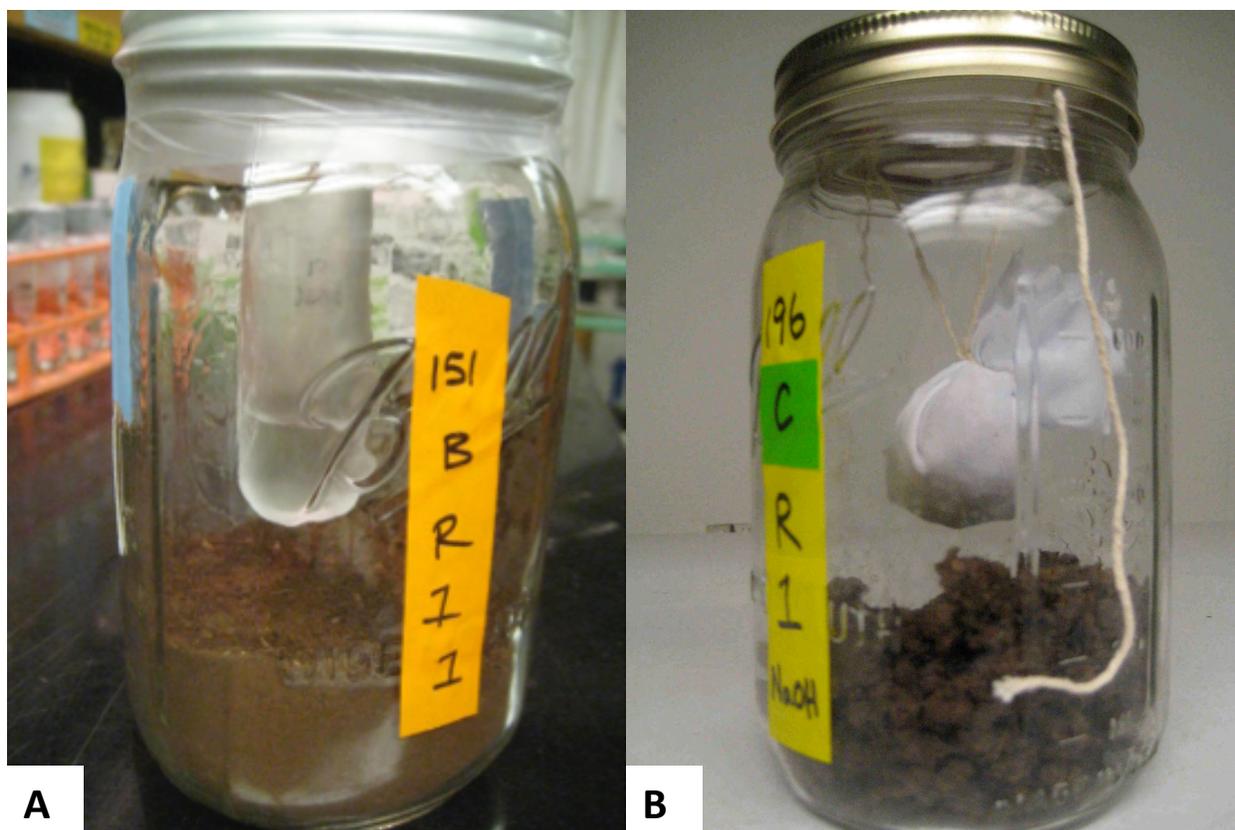
Figure 3-2. Example of soil-climate microcosm with: (A) NaOH trap to capture CO_2, and (B) desiccant ball to remove soil moisture.

Climate regimes

The characteristics of the climate regimes imposed on the Puerto Rico soils were taken from data collected with Campbell CR-10X dataloggers (Campbell Scientific Inc., Logan, UT) installed for multiple years at each site. These data were then used to determine the monthly averages of soil moisture and soil temperature for each of the climate regimes. Both soil moisture and temperature were measured at 7.5 cm soil depth at 15-minute intervals and logged hourly to the dataloggers; soil moisture was measured with CS616 water content reflectometers (Campbell Scientific Inc, Logan, UT) and soil temperature was measured with T-type thermocouples.

Each microcosm was maintained under one of three simulated climate regimes based on the monthly averages of soil temperature and moisture, and these climate averages were imposed on the microcosms each month to simulate the seasonal changes in climate conditions for a period of 520 days (Fig. 3-3). The length of time required to achieve the required moisture content in each of the microcosms varied between one to five days depending on the amount of water to be removed, soil type, and temperature. Soil moisture was determined by gravimetric water content, and when changes in climate required drier soil conditions, desiccant bags were hung from the top of the sealed microcosms to remove soil moisture (Fig. 3-2B). The study began with the microcosms under simulated April climate conditions.
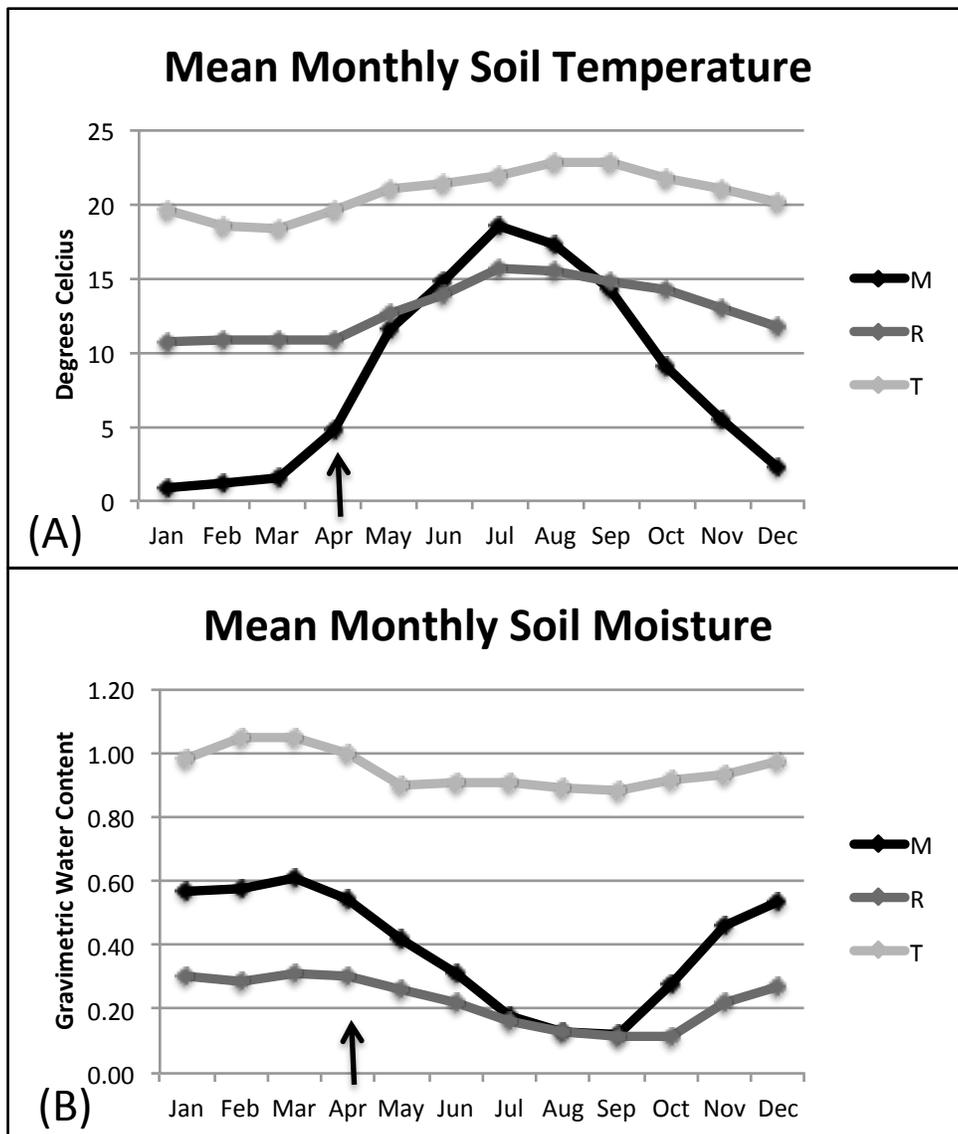
Figure 3-3. Annual soil moisture (A) and soil temperature (B) trends for the three climates that were imposed in the incubations chambers during the experiment. Climate labels indicate: Mediterranean climate (M); Redwood climate (R); Tropical climate (T). Arrows denote the imposed month in which the study began.

The three climate regimes followed those of a Mediterranean-climate mixed conifer forest (Mediterranean; M), a redwood forest ecosystem (Redwood; R), and a montane wet tropical forest (Tropical; T). These three climate regimes were chosen because soils from each forest ecosystem were included in the lab incubation discussed in Chapter 2; results of the Redwood soils were not presented due to methodological errors in the density fractionation procedures). The Mediterranean climate regime represented that of a Mediterranean mid-elevation mixed conifer forest ecosystem with hot, dry summers and cold, wet, snow-covered winters (Blodgett experimental forest); soil moisture and soil temperature data was captured at

this mid-elevation forest from January 2005 through July 2009. The Redwood climate regime followed that of the Grove of the Old Trees, a coast redwood grove in Sonoma County that experiences cool, wet winters and warm, dry summers; soil moisture and soil temperature data was captured at this redwood grove from January 2005 through December 2006 (Ewing *et al.,* 2009). The Tropical forest climate regime was one of constant high moisture and warm temperatures typical of a montane wet tropical forest; soil moisture and soil temperature data for the tropical forest was captured at Luquillo Experimental Forest in Puerto Rico from April 2005 through April 2008.

Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) was used to characterize the total living microbial biomass and the fungal-to-bacterial ratios (PLFA; Zelles 1999). The extraction, identification and quantification of PLFAs followed the modified methods reported in Bligh and Dyer (1959) and White and Ringelberg (1998). Microbial lipids were extracted from 8 g freeze-dried soil in a monophasic mixture of chloroform, methanol and phosphate buffer (1ml:2ml:0.83ml) with di-19:0PC (1, 2-Dinonadecanoyl- sn-Glycero-3-Phosphocholine, Avanti Polar Lipids, Alabaster, AL, USA) used as a surrogate standard to quantify soil PLFA. Extracts were separated into two phases by adding chloroform and DI-water, and the lipid-containing phase was dried at 37 °C under N_2 until all the water had evaporated; the length of drying time varied considerably. Phospholipids were separated from neutral lipids and glycolipids on Inert II silica columns (Burdick & Jackson, Muskegon, MI) using sequential elution by chloroform, acetone, and methanol. Phospholipids in the methanol elution were transesterified at 37 °C, dried at room temperature under N_2, and resuspended in hexane containing 10:0 FAME (methyl decanoate, Sigma-Aldrich, St. Louis, MO, USA) added as an internal standard.

The biomarkers used to the determine fungal-to-bacterial ratios were similar to those reported in Bird *et al.* (2011) and the ratio was calculated as the sum of the fungal group yield divided by the sum of the Gram-positive bacteria, Gram-negative bacteria, and Cyclopropyl Gram-negative bacterial group yields (nmol fungal fatty acid C per g dry soil/nmol bacterial fatty acid C per g dry soil).

Thirteen indicator PLFA biomarkers were categorized into 1 fungal group and 3 bacterial groups: fungi were indicated by 18:2ω6,9c, 18:1ω9c; Gram-negative bacteria by 16:1ω7c, 18:1ω7c (Marschner 2007); Cyclopropyl Gram-negative bacteria by 17:0cyc, 19:0cyc (Arao 1999); Gram-positive bacteria by 15:0i, 15:0a, 16:0i, 17:0i, 17:0a (Frostegård and Bååth, 1996); and actinobacteria by 16:0-10Me, 18:0-10Me (Marschner 2007). In addition, the following 9 unassigned biomarkers were included in calculations of total microbial biomass (nmol fatty acid C per g dry soil): 14:0, 15:0, 16:1ω5c, 16:1ω9c, 16:0, 16:0-12Me, 16:1-2OH, 18:1ω5c, 18:0 (Herman et al. 2012).

It should be noted that measurements of microbial biomass by PLFA is limited to measurements of known microbial fatty acids. Microbial biomass measurements for the 13 known fatty acids that comprise representatives of the fungal, actinobacterial, Gram-positive, and Gram-negative bacterial groups may not be representative of the actual microbial biomass for each group. Biomass measurements of the 9 unknown microbial fatty acid biomarkers, as well as additional fatty acid biomarkers not used in this study because we cannot say for certain that they are microbially derived, are often not insignificant. The redistribution of these unknown fatty acid biomarkers into known microbial groups could substantially alter the relative proportion of the groups to one another, as well as calculations of mass uptake of ^{13}C by each microbial group.

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Studies that utilize PLFA often do not include results and discussions of the importance of unassigned fatty acids in microbial community profiles and assimilation of carbon into unassigned microbial biomass (Arao, 1999; Wilkinson *et al.,* 2002; Balser and Firestone, 2005; Bird *et al.,* 2011). Even though we are unable to distinguish the group identity of unassigned fatty acids, they are included in the results and discussion of this study because the size of their biomass and assimilation of ^{13}C accounted for a substantial portion of the ^{13}C cycling in this study.

Density fractionation

Soil organic matter fractions were separated into 3 operationally defined pools (free light fraction (FLF), occluded light fraction (OLF) and dense organo-mineral (heavy) fraction (HF) by density fractionation. The method used for soil density fractionation followed that of Swanston *et al.*, (2005) with modifications as noted; this technique has been established as an efficient method by which to isolate soil organic matter and microbial cell material in three organic matter fractions in soils (Christensen, 1992; Morono *et al.*, 2013).

The FLF from this density fractionation method recovers organic material in soils that is not trapped in aggregates or bound to the mineral fraction. Over time, this FLF may become occluded in aggregates so that while the organic material may still be chemically labile for microbial consumption, it is physically protected so that the organic material is unavailable for microbial utilization. When soil aggregates are broken apart during the sonication step in the density fraction method, the organic material trapped within them becomes freed; if the organic material did not become bound to the mineral fraction during the course of the experiment, it was released into the OLF pool of organic matter, and if it did become bound to the mineral fraction while occluded it then became part of the HF recovery pool (Fig. 3-4).

Figure 3-4. The flow of soil carbon through three operationally defined soil organic matter fractions. The FLF recovers organic material in soils that is not trapped in aggregates or bound to the mineral fraction. Over time, this FLF may become occluded in aggregates so that while the organic material may still be chemically labile for microbial consumption, it is physically protected so that the organic material becomes stabilized. When soil aggregates are broken apart during the sonication step in the density fraction method, the organic material trapped within them becomes freed; if the organic material has not become bound to the mineral fraction during the course of its physical protection, it is released into the OLF pool of organic matter, if it did become bound to the mineral fraction during occlusion, it would then become part of the HF pool recovery.

To fractionate the soils via density-gradient separation, a 20 g dry-weight equivalent of soil was weighed into a centrifuge bottle and 150 ml of sodium polytungstate (SPT) prepared at a density of 1.85 g/ml (Cusack *et al.*, 2011) was added, and the centrifuge bottle was inverted gently 5 times to mix the soils and SPT before centrifuging for 1 hour at 4,600 rpm (Sorvall RC-5B Superspeed Centrifuge with a 172.3 mm radius swing bucket rotor, Sorval HS-4). The FLF was then removed and rinsed with DI water through a 1.6 μm glass microfiber filter (Whatman GF/A) until all of the SPT was removed. The FLF was then lyophilized until the weight stabilized.

Once the FLF was removed, an additional 100 ml of SPT was added to the centrifuge bottle before the residual fractions were mixed for 1 min using a bench top mixer (G3U05R, Lightnin), sonicated in an ice bath for 3 min at 70% pulse for a total input of 200 J/ml (Branson 450), and centrifuged (1 hr at 4,600 rpm) to separate the OLF trapped within soil aggregates from the organo-mineral fraction (HF). The OLF was removed and rinsed in the same manner as described for the FLF and lyophilized until the weight stabilized.

After the FLF and OLF were removed, DI water was added to the pellet remaining in the centrifuge bottle, and the remaining material was shaken vigorously before the centrifugation (20 min at 4,600 rpm, 3 times) and subsequent lyophilization of the remaining HF pellet.

Carbon dioxide

Carbon dioxide respired by the active soil microbes in each microcosm was trapped in glass centrifuge vials containing 12.2M NaOH that were hung from the top of the sealed microcosms (Fig. 3-2A). Additional NaOH traps were hung from control microcosms to quantify background ^{13}C-CO$_2$. The accumulated CO$_2$ was measured at 520 days post ^{13}C-labeling (Hagendorn et al. 2003). 2M SrCl$_2$ was used to precipitate out the trapped CO$_2$ in the NaOH solution, forming SrCO$_3$. The supernatant was vacuum aspirated so that the SrCO$_3$ pellet could be removed and dried at 80°C for analysis (Harris et al. 1997).

Elemental and isotopic analysis

PLFA samples were analyzed at UC Berkeley on a Hewlett Packard (Agilent) 5890 Series II Gas Chromatograph (GC) with a 30 m × 0.32 mm × 1.0 mm ZB-5 column (Phenomenex, Inc., Torrance, California, USA) connected via an Europa ORCHID on-line combustion interface (PDZ Europa, Cheshire, UK) to an Isoprime 100 IRMS (Isoprime, Manchester, UK). The microbial biomass (mg fatty acid C/g soil), atom% ^{13}C excess PLFA, and mg fatty acid ^{13}C excess per microcosm were measured for all timepoints. The mg ^{13}C excess results at T0 are presented to account for any labeled ^{13}C PLFA that was picked up from the dead bodies immediately upon addition of the labeled cells to the microcosms (Table 3-3). The background atom% ^{13}C and background mg ^{13}C results were subtracted from the T1, T2 and T3 results to account for the natural abundance of ^{13}C in the living biomass of Puerto Rico soils.

To calculate the mg C for each PLFA biomarker, the nmol fatty acid C for each lipid biomarker was multiplied by the mass of the carbon chain length specific to that biomarker (Eqn. 1). To calculate the mg ^{13}C excess for each biomarker, the mg C for each biomarker was multiplied by the atom% ^{13}C of that biomarker (Eqn. 2). The atom% ^{13}C excess and mg ^{13}C excess results for PLFA biomarkers are reported in the results and discussion as atom% ^{13}C and mg ^{13}C, respectively.

Table 3-3. Mean ($\pm$ 1 standard error) μg ^{13}C excess fatty acid recovered from the total biomass and the living fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomarkers in soils harvested immediately after application of ^{13}C microbial necromass mixture to the microcosms (T0).

CLIMATE	Total	Fungi	Actinobacteria	Gram-positive bacteria	Gram-negative bacteria	Unassigned lipids
Mediterranean	178 (15)	7 (1)	0.4 (0.1)	75 (6)	47 (4)	50 (5)
Redwood	233 (44)	8 (2)	0.6 (0.1)	104 (20)	56 (13)	65 (10)
Tropical	166 (18)	6 (1)	0.4 (0.1)	69 (9)	45 (3)	46 (6)

$$\text{mg C fatty acid} = \text{nmol fatty acid C} * \frac{\text{mass of carbon chain length (ng C)}}{1 \text{ nmol fatty acid C}} * 10^{-6} \text{ mg ng}^{-1} \qquad (1)$$

$$\text{mg }^{13}\text{C fatty acid} = \text{mg C} * \frac{\text{atom\% }^{13}\text{C}}{100} \qquad (2)$$

To estimate the mg ^{13}C recovered in the total biomass from measurements of mg ^{13}C phospholipid fatty acids, I multiplied the mg ^{13}C fatty acid results by 26.07 (Eqn. 3). The conversion factor of 26.07 was calculated as the difference between the total microbial biomass C as determined by chloroform fumigation direct extraction (CFDE; Table 3-1) and the mg fatty acid C PLFA results from the soils used in this study. Previous work has shown a relationship between CFDE and PLFA results (Bailey *et al.*, 2002).

$$\text{mg }^{13}\text{C total biomass} = \text{mg }^{13}\text{C fatty acid} * 26.07 \qquad (3)$$

The SOM density fractions (FLF, OLF, HF) were analyzed using a Vario Micro Cube elemental analyzer interfaced to an Isoprime 100 IRMS (Isoprime, Manchester, UK) to determine the elemental %C and the amount of ^{13}C label contained in each density gradient fraction. The total mg C recovered in each SOM fraction (FLF, OLF, HF), atom% ^{13}C excess within each fraction, and mg ^{13}C excess per fraction were measured for the Background and T3 harvests. The background atom% ^{13}C and mg ^{13}C results were subtracted from T3 results to account for the natural abundance of ^{13}C in the FLF, OLF and HF of Puerto Rico soils. The mg ^{13}C in each SOM fraction was calculated by multiplying the total mg C in each fraction by the atom% of that fraction (Eqn. 4). Findings of ^{13}C excess in the SOM fractions are reported in the results and discussion as mg ^{13}C.

$$\text{mg }^{13}\text{C in fraction} = \text{mg C in fraction} * \frac{\text{atom\% }^{13}\text{C of fraction}}{100} \qquad (4)$$

The CO_2 precipitates were analyzed using a Vario Micro Cube elemental analyzer interfaced to an Isoprime 100 IRMS (Isoprime, Manchester, UK) to determine the amount of

^{13}C-label contained in each SrCl$_2$ precipitation pellet. The mg ^{13}C-CO$_2$ excess captured in each microcosm was calculated by multiplying the atom% ^{13}C excess of each SrCl$_2$ pellet (Eqn. 5) by the mg inorganic C in each sample (Eqn. 6). Findings of ^{13}C-CO$_2$ excess are reported in the results and discussion as mg ^{13}C-CO$_2$.

$$\text{atom\% } ^{13}\text{C-CO}_2 \text{ excess} = \text{atom\% } ^{13}\text{C of labeled pellet} - \text{atom\% } ^{13}\text{C of background pellet} \quad (5)$$

$$\text{mg } ^{13}\text{C-CO}_2 \text{ excess} = \frac{\text{atom\% } ^{13}\text{C-CO}_2 \text{ excess}}{100} * \text{mg total inorganic carbon} \quad (6)$$

Statistical analyses

Significant differences among the different climate regimes in the total mg C and in the mg ^{13}C recovered in the living biomass were determined by two-way analysis of variance (ANOVA) with climate and type of microbial group that consumed the dead labeled bodies as the independent variables. Tukey's Honestly Significant Difference (HSD) test was used to determine the significant differences among the means of the three climate regimes when the ANOVA result was significant (p < 0.05). Data are reported as the mean ($\pm$ 1 standard error) for each climate regime.

Significant differences among the three fractions (FLF, OLF, and HF) in the total mg C, in the atom% ^{13}C, and in the mg ^{13}C recovered were also determined for each climate regime by performing a one-way ANOVA with SOM fraction as the independent variable, with subsequent Tukey's HSD tests performed with appropriate (p < 0.05). Data are reported as the mean ($\pm$ 1 standard error) for each fraction for each climate regime.

Significant differences in the total mg ^{13}C-CO$_2$ respired over 520 days post ^{13}C-labeling were determined for each climate regime by performing a one-way ANOVA with ^{13}C-CO$_2$ as the independent variable, with subsequent Tukey's HSD tests performed when appropriate (p < 0.05).

Results

Standing microbial biomass

Measurements of total standing microbial biomass represent the sum of the fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and unassigned biomasses in each microcosm. Two-way ANOVA performed for the recovery of total standing biomass indicated that the effects of climate and time were both significant at $p < 0.0001$, and their interaction was significant at $p = 0.0003$. Under the Mediterranean climate, the total amount of standing microbial biomass was significantly higher than in soils under the Redwood and Tropical climates at 7 days post ^{13}C-labeling (Fig. 3-5). Furthermore, there was no significant difference between the soils incubated under the Redwood and Tropical climates throughout any of the timepoints.

Two-way ANOVAs performed separately for the recovery of fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and unassigned lipid biomasses indicated that climate ($p = 0.002$), time ($p < 0.0001$), and their interaction ($p < 0.004$) all had a significant effect. The biomass recovery of all four microbial groups followed similar patterns over the

course of 520 days. At the start of the incubation, there was no significant difference in biomasses recovered from soils incubated under the three climates. Under the Mediterranean climate, the amount of standing biomass for each group was significantly greater 7 days post ^{13}C-labeling than under the Redwood and Tropical climates. Climate alone had no effect on the biomass of soils at 70 and 520 days post ^{13}C-labeling (Fig. 3-6A-E; Table 3-4A-E).
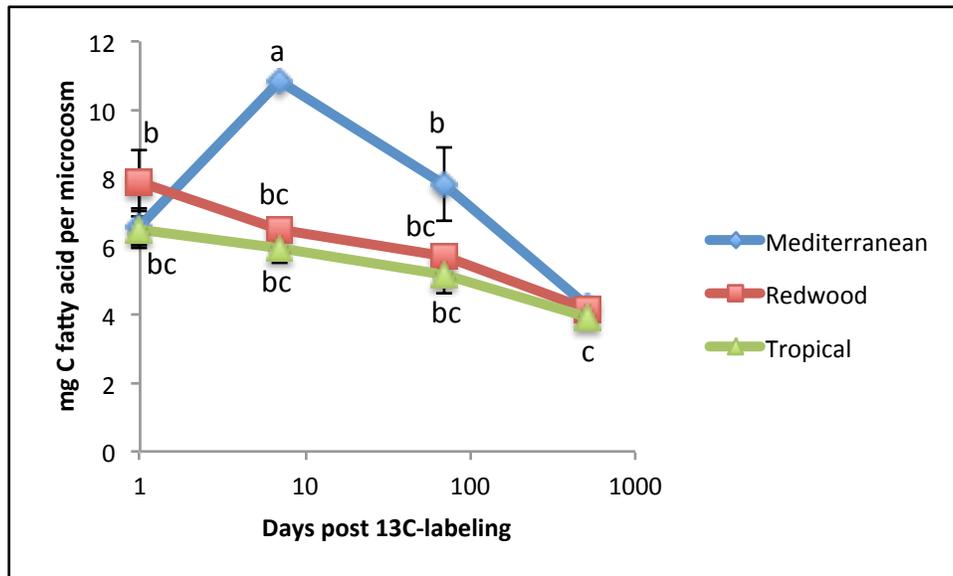
Figure 3-5. Total recovery (means $\pm$ 1 standard error) of mg C fatty acid per microcosm under three different climates over time. Data includes mg C recovered in the phospholipid fatty acids of fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and unassigned lipid biomarkers. Different letters indicate significant differences among climates and time as determined by two-way ANOVA.
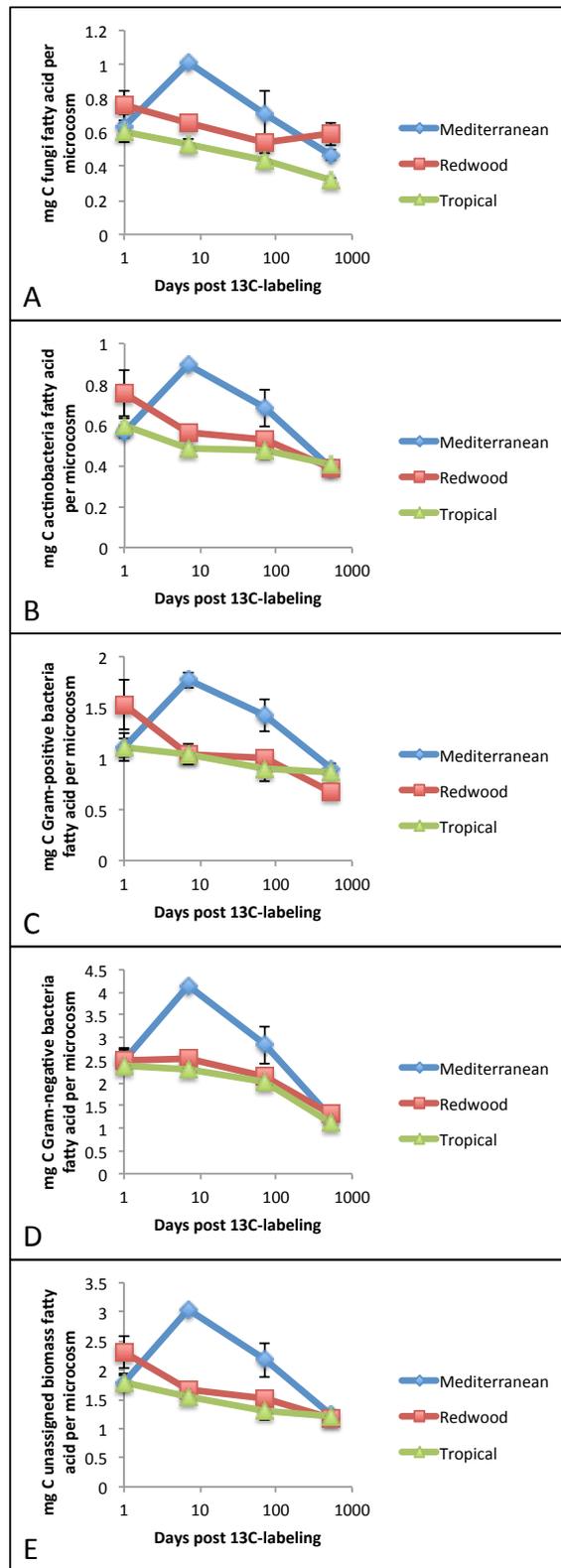
Figure 3-6. Recovery (means $\pm$ 1 standard error) of mg C fatty acid per microcosm in Puerto Rico soils under three different climates over 520 days. Levels of significance at each harvest timepoint can be found in Table 3-4.

Table 3-4. Significant differences of mean recovery ($\pm$ 1 standard error) mg fatty acid C (per g soil) among climates and time as determined by two-way ANOVA. Timepoints include: 0 days, 7 days, 70 days, and 520. Microbial biomass groups include: (A) fungi, (B) actinobacteria, (C) Gram-positive bacteria, (D) Gram-negative bacteria, and (E) unassigned lipid biomarkers. Different letters indicate significant differences among climates and time as determined by two-way ANOVA. Each ANOVA was performed on a by-column basis for each microbial consumer group. Levels not connected by the same letter are significantly different. ANOVA results presented in this table correspond to Fig. 3-6.

	(A) mg FUNGI fatty acid	(B) mg ACTINOBACERIA fatty acid	(C) mg GRAM-POSITIVE fatty acid	(D) mg GRAM-NEGATIVE fatty acid	(E) mg UNASSIGNED fatty acid
	Fig 3-6A:	**Fig 3-6B:**	**Fig 3-6C:**	**Fig 3-6D:**	**Fig 3-6E:**
0 Mediterranean	b c	b c d	b c d	b	b c d
0 Redwood	a b	a b	a b	b	a b
0 Tropical	b c d	b c d	b c d	b	b c d
7 Mediterranean	a	a	a	a	a
7 Redwood	b c	b c d	b c d	b	b c d
7 Tropical	b c d	c d	c d	b	c d
70 Mediterranean	a b c	a b c	a b c	b	b c
70 Redwood	b c d	b c d	b c d	b c	c d
70 Tropical	b c	c d	c d	b c d	d
520 Mediterranean	b c d	d	c d	c d	d
520 Redwood	b c d	d	d	c d	d
520 Tropical	d	d	c d	d	d

Assimilation of ^{13}C by standing microbial biomass groups

It is worth noting that at the beginning of the study, the mg fatty acid ^{13}C recovered from the total biomass and the fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomass groups were not significantly different among climate treatments, which was to be expected. While PLFA analysis is designed to capture the fatty acids of living microbial groups, it is likely that ^{13}C from the labeled necromass was picked up early in the study. The mg ^{13}C recovered at T0 is presented in Table 3-3. However, by 7 days after labeled biomass addition, most of the labeled microbial carbon is assumed to have been consumed by the living microbial communities in the microcosms. Kindler *et al*. (2006) found that living soil microbes quickly assimilated ^{13}C-labeled bacterial biomass after only 1 hour of incubation, and Lueders *et al*. (2006) found a rapid decrease in the number of introduced, genetically modified bioluminescent bacterial cells, which served as the substrate for assimilation by indigenous soil microorganisms.

Calculations of total mg ^{13}C within the standing microbial biomass represent the sum mg ^{13}C recovered in the fungal, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and unassigned biomasses in each microcosm. Climate and time both had a significant effect at $p <$ 0.0001 on the total mg ^{13}C recovered over time as determined by two-way ANOVA. By 7 days post labeling, the total mg ^{13}C in the biomass was significantly higher under the Mediterranean climate than under both the Redwood and Tropical climates (Fig. 3-7), and at 70 and 520 days, the mg ^{13}C in the soil biomass under both the Mediterranean and Redwood climates were significantly higher than in soils under the Tropical climate.

Climate ($p = 0.0005$) and time ($p = 0.0156$) had significant effects on the mg ^{13}C recovered in the fungal biomass. At 7, 70, and 520 days post ^{13}C-labeling, fungal biomass in soils under the Tropical climate recovered less mg ^{13}C than soils under the two temperate climates, though there was not a statistically significant difference between soils under the temperate and Tropical climates (Fig. 3-8A).
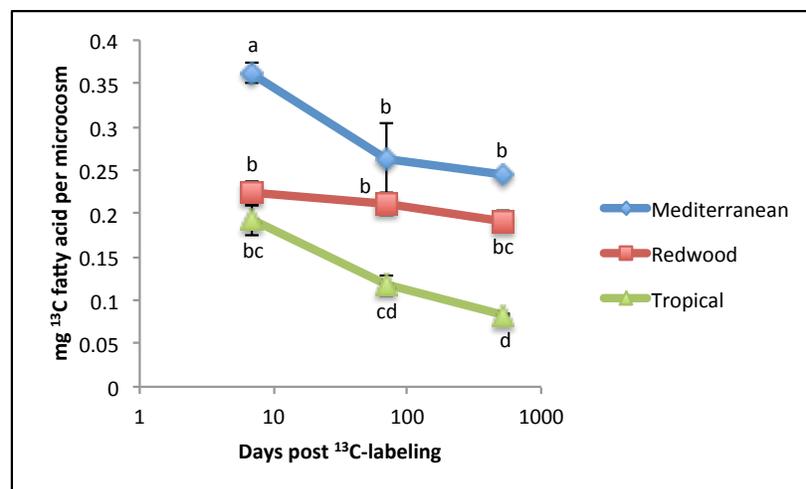
Figure 3-7. Total mg ^{13}C excess fatty acid recovery (means $\pm$ 1 standard error) under three different climates over time. Data includes mg ^{13}C recovered in the phospholipid fatty acids of fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria and unassigned lipid biomarkers. Different letters indicate significant differences among climates and time as determined by two-way ANOVA.
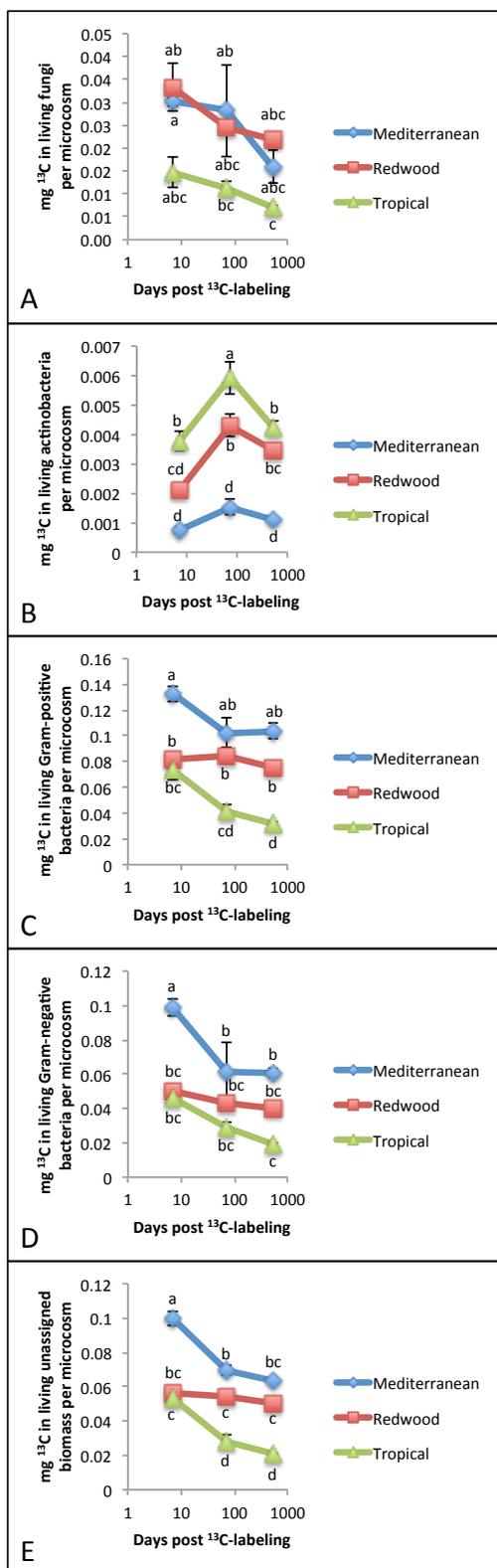
Figure 3-8. mg ^{13}C excess in fatty acids per microcosm (means $\pm$ 1 standard error) recovered in: (A) fungi, (B) actinobacteria, (C) Gram-positive bacteria, (D) Gram-negative bacteria, and (E) unassigned lipid biomarkers under 3 different climates over time.

Two-way ANOVA indicated climate and time both had a significant effect ($p < 0.0001$) on the mg ^{13}C in the actinobacterial biomass. At 7 days post ^{13}C-labeling, the mg ^{13}C in the standing actinobacterial biomass was significantly higher in soils under the Tropical climate than in soils under the Mediterranean and Redwood climates. At 70 days, ^{13}C in the living actinobacteria in soils under the Tropical climate were higher than those under the Redwood climate, and both were higher than those under the Mediterranean climate. At 520 days post labeling, soils under the Mediterranean climate retained significantly less ^{13}C in the actinobacterial biomass than soils under the Redwood and Tropical climates (Fig. 3-8B).

Two-way ANOVA performed on the mg ^{13}C in the Gram-positive bacterial biomass indicated climate ($p < 0.0001$), time ($p = 0.0004$), and their interaction ($p = 0.0450$) were all significant. At 7 days post ^{13}C-labeling, the mg ^{13}C in the Gram-positive bacteria was significantly greater in soils under the Mediterranean climate than in soils under the Redwood and Tropical climates. At 70 and 520 days, soils under the Mediterranean and Redwood climates recovered higher ^{13}C in the Gram-positive bacteria than soils under Tropical climate (Fig. 3-8C).

Climate ($p < 0.0001$) and time ($p = 0.0003$) had a significant effect on the mg ^{13}C in the Gram-negative bacterial biomass. At 7 days post ^{13}C-labeling, the mg ^{13}C in the living Gram-negative bacterial biomass was significantly greater in soils under the Mediterranean climate than in soils under the Redwood and Tropical climates. By 70 days, the amount of ^{13}C in the Gram-negative bacterial biomass was again not statistically different in any of the soils under the three climates. At 520 days, soils under the Mediterranean climate recovered higher levels of ^{13}C in the Gram-negative bacteria than soils under the Tropical climate (Fig. 3-8D).

Two-way ANOVA performed on the mg ^{13}C in the unassigned lipid biomarkers indicated climate ($p < 0.0001$), time ($p < 0.0001$), and their interaction ($p = 0.0003$) were all significant. At 7 days post ^{13}C-labeling, the mg ^{13}C in the unassigned lipid biomarkers was significantly greater in soils under the Mediterranean climate than in soils under the Redwood and Tropical climates. At 70 days, soils under the Mediterranean climate recovered greater mg ^{13}C in the unassigned lipid biomasses than soils under the Redwood climate, and both recovered higher mg ^{13}C than soils under the Tropical climate. At 520 days post labeling, soils under the Tropical climate recovered significantly less ^{13}C in the unassigned lipid biomarkers than soils under the Mediterranean and Redwood climates (Fig. 3-8E). Overall, the assimilation of microbial ^{13}C in the unassigned lipid biomarkers over time followed a similar pattern to that of the assimilation by Gram-negative bacteria (Figs. 3-8D, 3-8E).

Influence of climate on assimilation of ^{13}C by the standing microbial biomass

Time ($p < 0.0001$), what type of microbial group consumed the labeled dead microbial carbon ($p < 0.0001$), and their interaction ($p = 0.0531$) all had a significant effect on the recovery of ^{13}C in soils under the Mediterranean climate. From the start of the incubation and throughout the 520 days of the incubation, Gram-positive bacterial biomass recovered the highest mg ^{13}C of any microbial consumer group under the Mediterranean climate. At 7 and 70 days post ^{13}C-labeling, the Gram-positive bacteria, Gram-negative bacteria, and unassigned lipid biomarkers had assimilated and retained higher mg ^{13}C than the fungi and actinobacteria. By 520 days, only the Gram-positive bacteria recovered higher mg ^{13}C than the fungi and actinobacteria, and the unassigned lipid biomarkers recovered more than only the actinobacteria (Fig. 3-9A).

Under the Redwood climate, the type of microbial group that consumed the labeled dead microbial carbon ($p < 0.0001$) and time ($p = 0.0125$) both had a significant effect on the recovery of ^{13}C. From the start of the incubation and throughout the 520 days of the incubation, Gram-positive bacterial biomass recovered significantly higher mg ^{13}C under the Redwood climate than

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any other microbial consumer group. At 7 days, the unassigned lipids recovered higher mg ^{13}C than both the fungi and actinobacteria, and fungi and Gram-negative bacteria recovered more than the actinobacteria. At 70 and 520 days, the ^{13}C recovered in the Gram-negative bacteria and the unassigned lipid biomarkers were significantly higher than that in fungi, and fungi recovered more than actinobacteria (Fig. 3-9B).

Time, what type of microbial group consumed the ^{13}C, and their interaction all had a significant effect at $p < 0.0001$ on the recovery of ^{13}C in soils under the Tropical climate. From the start of the incubation and throughout the 520 days of the incubation, Gram-positive bacterial biomass recovered the highest mg ^{13}C of any microbial consumer group. At 7 days post ^{13}C-labeling, the Gram-positive bacteria recovered significantly higher mg ^{13}C than the Gram-negative bacteria and unassigned lipid biomarkers, and all three of these microbial consumer groups recovered higher ^{13}C than the fungi and actinobacteria. At 70 days, the mg ^{13}C recovered in the Gram-positive bacteria, Gram-negative bacteria, and unassigned lipids were significantly higher than that of the fungi and actinobacteria. By 520 days, only the Gram-positive bacteria recovered higher ^{13}C than the fungi and actinobacteria (Fig. 3-9C).
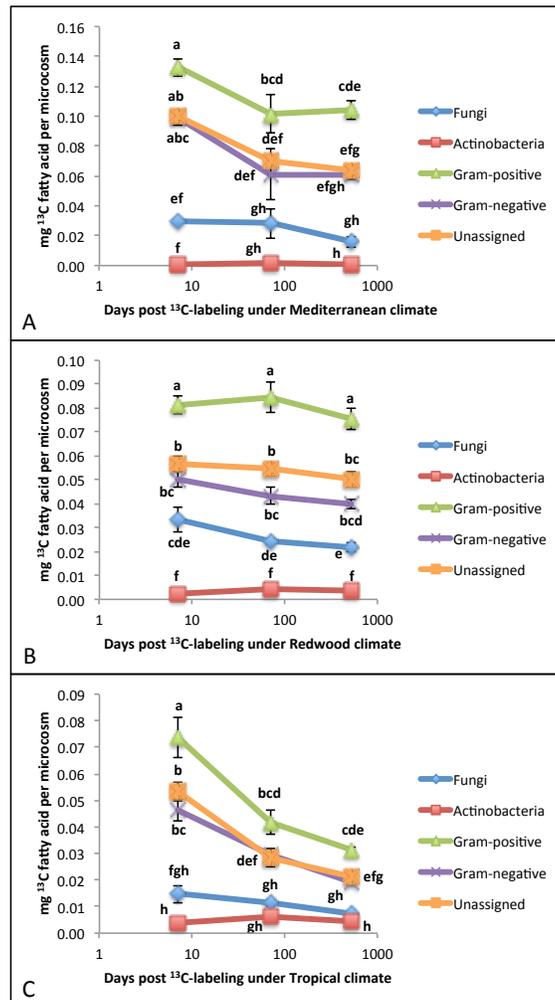
Figure 3-9. mg ^{13}C excess fatty acids recovered per microcosm (means $\pm$ 1 standard error) under the three climate regimes: (A) Mediterranean, (B) Redwood, and (C) Tropical.

Recovery of ^{13}C in soil organic matter fractions

At 520 days post ^{13}C-labeling, when the FLF, OLF and HF were summed for each climate, the effect of climate ($p = 0.0006$) was a significant factor in total SOM ^{13}C recovery. The total ^{13}C in soils under the Mediterranean climate was significantly higher than that recovered in the SOM of soils under the Redwood and Tropical climates (Fig. 3-10).

To assess the difference in all SOM recoveries under the three climate regimes at 520 days post ^{13}C-labeling, the FLF, OLF and HF of each climate treatment were analyzed together. When the FLF, OLF and HF were not summed for each climate and all 9 fractions were analyzed, the effect of climate, time and their interaction were all significant at $p < 0.0001$. The recovery of ^{13}C in the SOM fractions was greatest in the HF under the Mediterranean climate; ^{13}C-HF in soils under the Redwood and Tropical climates were the next highest recoveries. There were no significant differences in the FLF and OLF recoveries under any of the climate regimes when the HF was included in the analysis due to the large recovery of ^{13}C in the HF under all climate treatments (Fig. 3-10).
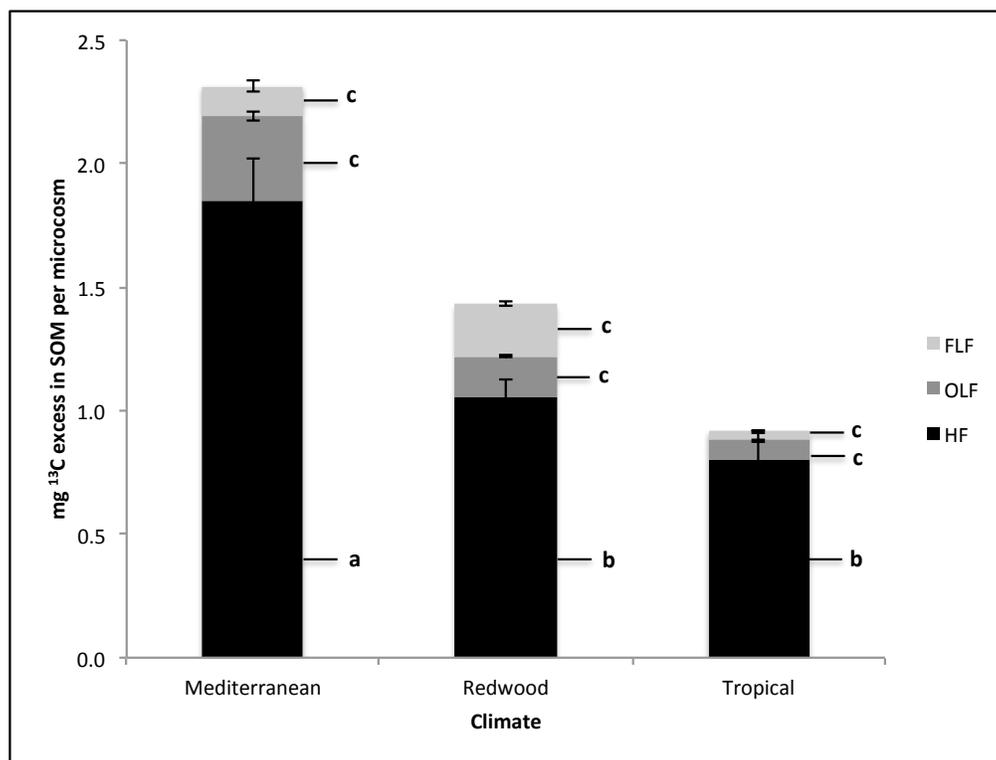
Figure 3-10. mg ^{13}C excess recovery (means ± 1 standard error) of the free light fraction (FLF), occluded light fraction (OLF), and mineral bound heavy fraction (HF) under three different climates at 520 days post ^{13}C-labeling. Different letters indicate significant differences among climates and SOM fractions as determined by two-way ANOVAs. When FLF, OLF and HF were summed, climate was significant at $p = 0.0006$.

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To assess the difference in FLF recoveries under the three climate regimes, the FLF of all climate treatments were analyzed without the OLF and HF recoveries. Two-way ANOVA performed for the ^{13}C recovery in the FLF at 520 days indicated that the effect of climate was significant at $p = 0.0008$. The amount of ^{13}C-FLF recovered was highest under the Redwood climate, second highest under the Mediterranean climate, and lowest when under the Tropical climate (Fig. 3-11A).

To assess the difference in OLF recoveries under the three climate regimes, the OLF of all climate treatments were analyzed without the FLF and HF recoveries. Climate had a significant effect ($p < 0.0001$) on the recovery of ^{13}C-OLF at 520 days post ^{13}C-labeling. The amount of ^{13}C-OLF recovered was highest under the Mediterranean climate, second highest under the Redwood climate, and lowest under the Tropical climate (Fig. 3-11B).

To assess the difference in HF recoveries under the three climate regimes, the HF of all climate treatments were analyzed together the FLF and OLF recoveries. Climate had a significant effect ($p = 0.0026$) on the recovery of ^{13}C-HF at 520 days post ^{13}C-labeling. The amount of ^{13}C-HF recovered was highest under the Mediterranean climate and there was no significant difference in the ^{13}C-HF recovered in soils incubated under the Redwood and Tropical climates (Fig. 3-11C).

When taking into account background data on the SOC and standing microbial biomass of Puerto Rican soils, and assuming that the fate of the labeled dead microbial carbon added represented the fate of all the microbial carbon in the soil at the time of label addition (Table 3-5A), the percentage of SOC that would have been derived from dead microbial carbon (had all of the standing microbial biomass been labeled and subjected to 520 days of decomposition and stabilization processes) would have been highest in soils under the Mediterranean climate (Table 3-5B).

Recovery of respired ^{13}C-CO_2

Climate had a significant effect on the amount of ^{13}C-CO_2 produced ($p < 0.0001$) at 520 days post ^{13}C-labeling. The ^{13}C-CO_2 produced in soils under the Tropical climate was significantly higher than that produced in both temperate climates, and the ^{13}C-CO_2 produced under the Redwood climate was significantly higher than that produced under the Mediterranean climate (Fig. 3-12). Under the Mediterranean climate, only 31.7% of the 13.3 mg ^{13}C from the dead microbial bodies that was added to each microcosm was respired out as ^{13}C-CO_2 whereas 76.4% was respired out of the soils under the Tropical climate (Table 3-6).

Movement of ^{13}C through standing microbial biomass and into CO_2 and SOM

At 520 days post ^{13}C-labeling, climate had a significant effect on whether ^{13}C from the dead microbial bodies was recovered in the total microbial biomass (see Eqn. 3), the SOM, or respired out as CO_2 ($p < 0.0001$). Under the Tropical climate, significantly and substantially more ^{13}C was respired out as CO_2 than remained in the standing biomass or SOM (Table 3-5). Under the Redwood and Mediterranean climates, significantly more ^{13}C was respired out as CO_2 than remained in the SOM, though differences between respired and SOM carbon under the temperate climates were not as dramatic as that found in the soils under the Tropical climate (Fig. 3-13; Table 3-5). Substantially more ^{13}C remained in the total standing biomass of soils under the temperate climate than remained in soils under the Tropical climate. Soils under the Mediterranean climate recovered the highest mg ^{13}C in the standing biomass and the lowest ^{13}C-CO_2 recoveries while soils under the Tropical climate recovered the lowest mg ^{13}C in the standing biomass and the highest ^{13}C-CO_2 recoveries (Fig. 3-13; Table 3-5).

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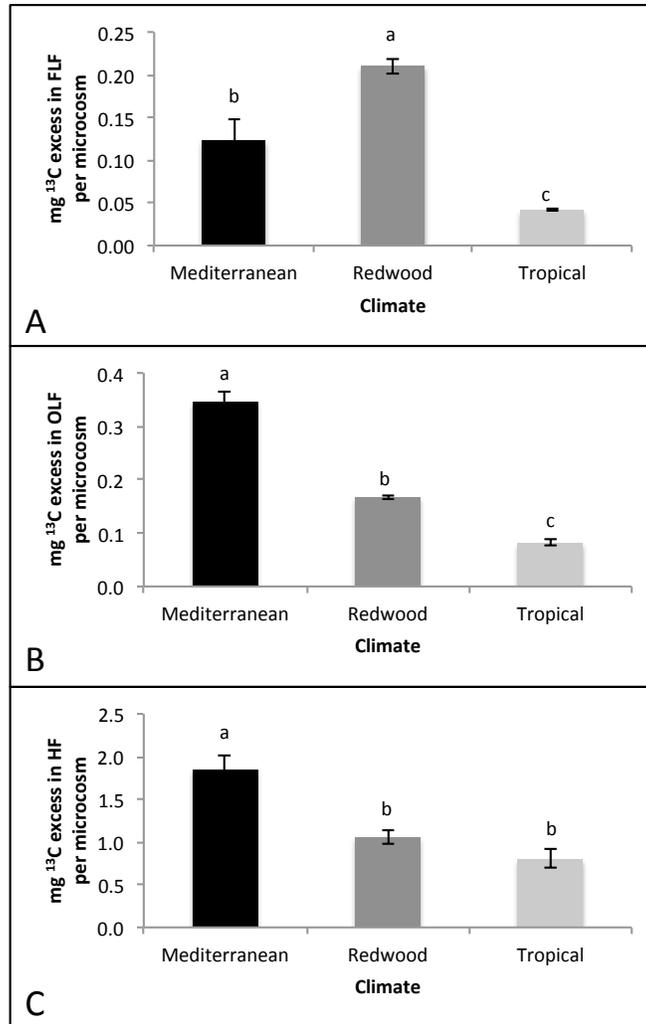
Figure 3-11. mg ^{13}C excess recovery (means $\pm$ 1 standard error) of the free light fraction (FLF), occluded light fraction (OLF), and mineral bound heavy fraction (HF) under three different climates over time. Different letters indicate significant differences among climates as determined by two-way ANOVA.

Table 3-5. (A) The relative proportion of added ^{13}C-labeled dead cells recovered in the density fractions: (A1) to standing microbial biomass and (A2) to soil organic carbon (SOC).
(B) Assuming all the standing biomass had been uniformly labeled: (B1) the mg ^{13}C that would be recovered in each fraction per gram soil and (B2) percent of the total SOC that would have been recovered.

(A) RESULTS FROM THIS STUDY:

(A1) mg ^{13}C per g soil added to microcosms: 0.111mg

Soil	standing microbial biomass C (mg C/g soil)	^{13}C label as % standing microbial biomass C	SOC (mg/g soil)	^{13}C-label as % SOC
Puerto Rico	1.512	7.34%	121	0.092%

(A2) mg ^{13}C recovered in each fraction per g soil

Climate	HF	OLF	FLF	Total SOM	CO2	Standing Biomass
Mediterranean	0.0154	0.0029	0.0010	0.0193	0.0351	0.0532
Redwood	0.0088	0.0014	0.0018	0.0119	0.0512	0.0415
Tropical	0.0067	0.0007	0.0003	0.0077	0.0847	0.0180

(B) ESTIMATES IF ALL STANDING MICROBIAL BIOMASS HAD BEEN UNIFORMLY LABELED:

(B1) mg ^{13}C that would be recovered in each fraction per g soil

Climate	HF	OLF	FLF	Total SOM	CO2	Standing Biomass
Mediterranean	0.2095	0.0392	0.0140	0.2627	0.4784	0.7247
Redwood	0.1195	0.0190	0.0239	0.1624	0.6977	0.5653
Tropical	0.0906	0.0092	0.0047	0.1045	1.1543	0.2452

(B2) % of the total SOC that would have been recovered if all standing biomass had been labeled

Climate	HF	OLF	FLF	Total SOM
Mediterranean	0.17%	0.03%	0.01%	0.22%
Redwood	0.10%	0.02%	0.02%	0.13%
Tropical	0.07%	0.01%	0.00%	0.09%
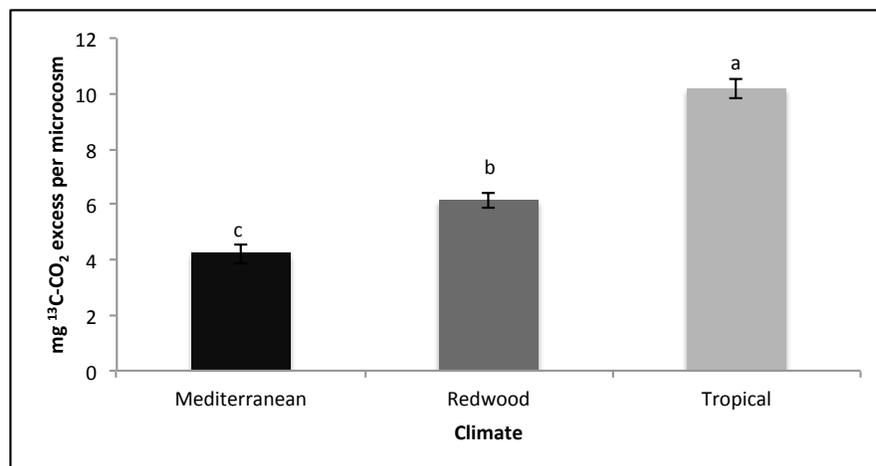
Figure 3-12. mg ^{13}C-CO$_2$ excess (means $\pm$ 1 standard error) under 3 different climates at 520 days post ^{13}C-labeling. Different letters indicate significant differences among climates as determined by two-way ANOVAs.

Table 3-6. Percentage of the ^{13}C-label recovered from the FLF, OLF, HF, total SOM, CO_2 respired, and the total standing microbial biomass 520 days post ^{13}C-labeling. 13.3 mg ^{13}C was added each microcosm. Percentages below represent the mg ^{13}C excess in each fraction divided by the 13.3 mg ^{13}C dead cell material added to each microcosm ($\pm$ 1 standard error).

CLIMATE	HF	OLF	FLF	Total SOM	CO2	Standing Biomass	Total Recovery
Mediterranean	13.9% (1.31%)	2.6% (0.15%)	0.9% (0.19%)	17.4% (1.25%)	31.7% (2.57%)	48.0% (0.44%)	97.1%
Redwood	7.9% (0.56%)	1.3% (0.03%)	1.6% (0.07%)	10.8% (0.58%)	46.2% (1.99%)	37.4% (2.22%)	94.4%
Tropical	6.0% (0.82%)	0.6% (0.04%)	0.3% (0.01%)	6.9% (0.84%)	76.4% (2.53%)	16.3% (0.38%)	99.6%
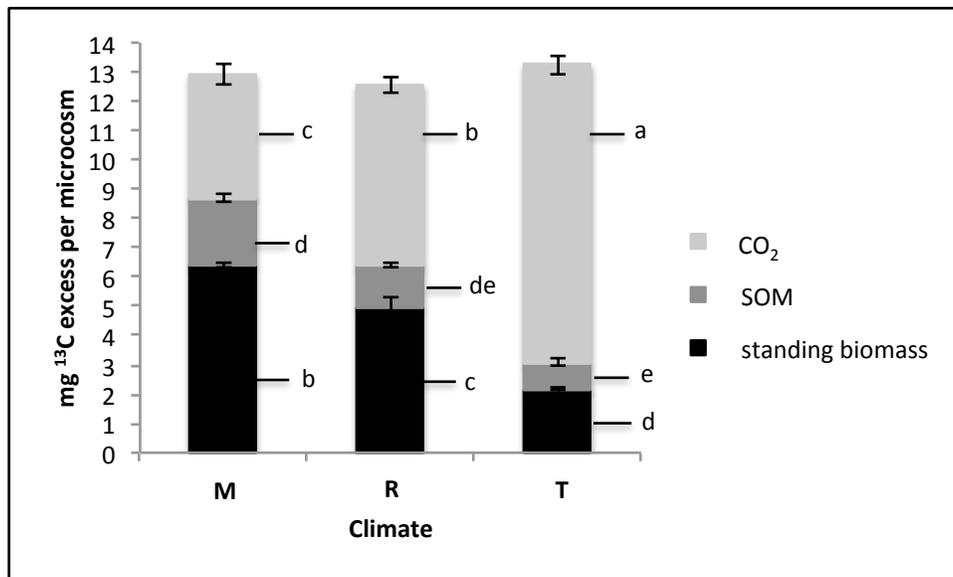
Figure 3-13. mg ^{13}C excess (means $\pm$ 1 standard error) in the total standing microbial biomass, SOM (FLF, OLF, HF) and CO_2 respired under three different climates at 520 days post ^{13}C-labeling. Different letters indicate significant differences among climates and carbon pool destination as determined by two-way ANOVA.

Discussion

Importance of climate on microbial community biomass and composition

In this study, at the start of the incubation, the biomass detected within each of the fungi, actinobacteria, Gram-positive bacteria, Gram-negative bacteria, and the unassigned lipid biomarkers were all similar among climate treatments. Yet, between 0 and 7 days, the amount of biomass in all of the microbial groups under the Mediterranean climate became significantly higher than that found under the Redwood and Tropical climates. This was surprising because the soils were all incubated for two weeks under the three climate regimes before the addition of the labeled bodies, during which time the amount of biomass in the soils under the different climates remained similar. It was only between 0 and 7 days that the soils under the Mediterranean climate exhibited an increase in microbial biomass.

A review of the atom% ^{13}C of each lipid biomarker in the microcosms at day 7 did not reveal any increased assimilation of ^{13}C by the living soil microorganisms under the Mediterranean climate, indicating that the carbon for growth must have come from indigenous carbon. In addition, the error bars for the total microbial biomass values under the Mediterranean climate were very small, indicating that all three replicates showed virtually identical growth responses to the shift in climate.

A possible explanation for the increase in biomass of tropical soils under Mediterranean climate conditions at day 7 is an increase in oxygen availability in the newly dry tropical soils. Microbial communities native to Puerto Rico soils experience, and are adapted to, frequent redox fluctuations due to precipitation events that cause changes in oxygen availability (Pett-Ridge and Firestone, 2005). An increase in oxygen availability may have led to an increase in the activity of obligate aerobes, facultative aerobes and/or microaerophiles. Yet, had an increase in oxygen availability been the primary cause of the increase in biomass, the same increase would have been expected in soils under the Redwood climate. However, soils under the Redwood climate did not experience this temporary increase in microbial biomass.

In addition, the static aerobic conditions of these soils would not have been advantageous to all portions of the soil community, especially to organisms that were obligate anaerobes; The stress of constant oxygen availability may have caused these microbes to cease to function. A decrease in biomass in Puerto Rico soils under static oxic conditions has been previously noted (Pett-Ridge *et al.,* 2006). Microbial communities in these soils are adapted to fluctuating redox conditions and differ in their community structure and biomass from microbial communities in soils under stable aerobic conditions (Pett-Ridge *et al.,* 2006). While it would have been more realistic to subject the soils under the Tropical climate regime to fluctuating redox conditions, the timeframe of this study and the time required to remove moisture from each of the microcosms made fluctuating redox conditions logistically impossible to impose on this incubation.

When the tropical soils were harvested, they were stored at ambient temperatures for 3 days before being moved into the Mediterranean climate incubators where they were then exposed to 5°C (see April climate conditions in Fig. 3-3). This reduction in temperature comprises a major climate difference between soils incubated under the Mediterranean climate and the Redwood and Tropical climates. While some studies have shown a decrease in microbial biomass and basal respiration in soils incubated under cold storage (Stenberg *et al.,* 1998), Černohlávková *et al.* (2009) demonstrated that grassland and mixed forest soils did not exhibit changes in microbial biomass C when stored at 5°C for 2, 4, 8, 16, and 32 weeks. It should be

noted that these studies were not performed on tropical forest soils that often demonstrate higher optimal temperatures for microbial growth. In fact, Balser and Wixon (2009) assessed the temperature response of microbial communities in Puerto Rico soils from the Luquillo Experimental Forest (the same location from which soils for this study were harvested), and found that the optimum temperatures for utilization of BiOLOG substrates and maximum microbial respiration rate were 36-37°C. These results are in marked contrast to the significant increase in microbial biomass of Puerto Rico soils incubated at 5°C in this study, a contrast for which we currently cannot explain.

Over the 520 days of laboratory incubation, all microbial groups under all three climate regimes exhibited a significant decrease in biomass. A contributing factor to the decrease may have been depletion of available carbon required for microbial growth. While the microcosms were monitored throughout the incubation for CO_2 production, and all microcosms were still respiring CO_2 at the end of the experiment, the rates of respiration did decline near the end of the experiment. The decrease in respiration at the end of this study under all three climate regimes is consistent with the reported decrease in microbial biomass over the 520 days of the study.

Cycling of ^{13}C within the standing microbial biomass

Overall, soils under the Mediterranean climate recovered more mg ^{13}C-labeled carbon at 7 days than the same soils under the Tropical and Redwood climates. This was largely due to the large biomass size of microbes under the Mediterranean climate at 7 days, and not due to increases in atom% ^{13}C. At days 70 and 520, soils under the Mediterranean and Redwood climates recovered more mg ^{13}C than soils under the Tropical climate. Thus, more labeled microbial carbon was retained in the standing biomass of soils under temperate climates later in the study than under Tropical climate conditions. The increased retention of microbially derived carbon in the living microbial biomass under temperate climates was likely a result of slower microbial metabolic rates or higher carbon use efficiencies (CUE) under the temperate climates.

Under the Mediterranean climate regime, the soils underwent periods of cold and wet, then warm and dry, then cold and wet conditions; under the Redwood climate, soil temperatures were constantly mild and soil moisture was consistently dry. The Mediterranean and Redwood climate conditions likely decreased the metabolic rate of soil microbial communities compared to the metabolic rates of the microbes in the same soils under the consistently warm, wet Tropical climate. Lower rates of respiration suggest that organic carbon remains in the standing biomass for longer periods of time before being respired out as CO_2. In addition to differences in metabolic rates between the Puerto Rico soils under the Tropical and temperate climates, the microbial communities in these soils may have exhibited differences in CUE. Climate variables such as temperature and soil moisture can alter microbial metabolism, shifting the balance of growth and C losses to respiration, thereby altering CUE; on broad scales, CUE decreases as temperature increases (Sinsabaugh *et al.,* 2013). While all the soils were from the same Puerto Rico forest, the decreased temperatures of soils under the Mediterranean and Redwood climates may have increased the CUE of the soil microbial communities, thereby increasing the amount of time the labeled microbial carbon remained in the living biomass before being respired out as CO_2.

Assimilation of the labeled dead microbial carbon by living fungi was lowest in soils under the Tropical climate, indicating that the saprophytic fungi in these soils were either not as competitively able to decompose the dead microbial cell material under static warm and wet Tropical conditions as they were the under cooler, drier temperate climates, or that the ^{13}C may have passed through the fungal communities quickly. If the low mg ^{13}C recovery of microbial

carbon by fungi under the Tropical climate was due to a lack of competitive ability, this could be due in part to the obligate aerobic nature of most saprophytic fungi, whereby the constant high moisture in the Tropical climate microcosms may decreased oxygen availability, slowing decomposition by the fungi (Griffin, 1963).

Actinobacteria in soils under the Mediterranean climate consistently retained the least amount of ^{13}C-labeled cell material. This is interesting because as a group actinobacteria are commonly thought to be relatively resistant to desiccation and can not only survive periods of prolonged drying, they often remain active under hot, dry conditions to a degree not usually exhibited by bacteria and some fungi (Brady and Weil, 1996; Singer and Munns, 1996; Atlas and Bartha, 1998; Alexander 2005; Madigan *et al.,* 2003). Surprisingly, assimilation of the labeled microbial carbon by actinobacteria was highest under the Tropical climate at every harvest time when compared the uptake of ^{13}C by actinobacteria under the Mediterranean and Redwood climates. This trend was primarily due to the high atom% ^{13}C in the actinobacterial lipids under the Tropical climate, and not increases in actinobacterial biomass. This indicates that while actinobacterial biomass did not expand due to the addition of the ^{13}C-labeled microbial bodies, the warm, wet Tropical climate did favor consumption and assimilation of dead microbial carbon by the PLFA-defined actinobacterial group.

Under all three climate regimes, the Gram-positive bacteria consistently retained higher mg ^{13}C in their biomass than the four other microbial groups. While the Gram-negative and unassigned lipid biomasses constituted larger soil biomasses, the Gram-positive bacteria retained more mg ^{13}C due to consistently higher atom% ^{13}C values in their biomass. After the Gram-positive bacteria, the Gram-negative bacteria and unassigned lipid biomarkers retained the next highest amounts of mg ^{13}C over the course of the study, both of which contained high amounts of biomass in the soils and mid-level atom% ^{13}C values. Gram-positive and Gram-negative bacteria have previously been shown to be important controllers of the decomposition of plant materials (Waldrop and Firestone, 2004; Bird *et al.,* 2011; Herman *et al.,* 2012). It is important to note that a sizeable portion of the labeled ^{13}C was recovered in unassigned lipid biomarkers. This indicates that there are saprophytic microbes that we cannot identify by PLFA that contribute to the assimilation of dead microbial carbon in soils. To more fully understand the assimilation of dead microbial carbon by all saprophytic communities, future studies may consider applying a much higher mg ^{13}C label per g soil to allow for the utilization of DNA-stable isotope probing techniques.

Respiration versus stabilization of dead microbial carbon under different climates

Despite the fact that Puerto Rico soils incubated under the Tropical climate started and ended with the same standing microbial biomass as Puerto Rico soils incubated under the Mediterranean and Redwood climates, soils under the Tropical climate respired significantly more ^{13}C-CO$_2$. Puerto Rico soils under the Mediterranean climate respired the lowest amounts of ^{13}C-CO$_2$. This indicates that microbial communities under the Tropical climate had the highest metabolic rates while communities in the drier Mediterranean climate had the lowest metabolic rates. A decrease in metabolic activity may lead to an increase in the amount of time that organic material remains in the living biomass of microbial communities before it is respired out of the soil ecosystem as CO$_2$. If organic carbon remains in the standing microbial biomass for longer periods of time, this could lead to an increase in the retention of microbial carbon in the stabilized mineral bound fraction of soil organic matter, leading to an increase in microbial based carbon in SOM. In fact, results of this study showed that of all the climate regimes imposed on the Puerto Rico soils, soils under the Mediterranean climate retained the highest amounts of ^{13}C

in the living biomass, respired the least ^{13}C-CO$_2$, and recovered the most ^{13}C in the HF over 520 days.

The recovery of high amounts of microbial carbon in the HF of soils where the microbial biomass retained carbon longer may be due to the life history traits of soil microorganisms. Due to their abundance, adhesive properties, and because they commonly live within aggregates and on the surfaces of soil minerals, microbial bodies are likely to become stabilized in soils (Chenu *et al.,* 2001; Chenu and Stotzky, 2002). The adhesion of these microorganisms, and the adsorption of microbial bodies and metabolites onto clay and iron oxide surfaces, increases the likelihood for these microbial products to become stabilized in the heavy fraction of SOM (Chenu and Stotzky, 2002). It stands to reason that microbial communities with lower metabolic rates, or higher CUE, would respire organic carbon out of the soil ecosystem more slowly, allowing more time for the organic carbon to become stabilized within the SOM. This study is the first of its kind to demonstrate that increases in the retention time of carbon in living microbial communities may lead to increases in microbially derived organic matter in the heavy fraction of SOM. Here, soils with the lowest retention times of ^{13}C in the living biomass and highest respiration rates stabilized the least amount of labeled carbon in the HF, and soils with the longest retention times of ^{13}C in the living biomass and lowest respiration rates stabilized the most labeled carbon in the HF.

Microbial bodies as precursors of SOM under different climates

Under all three climate regimes, the recovery of microbial derived ^{13}C in the SOM at the end of the study was highest in the heavy fraction. High recovery of microbial ^{13}C in the heavy fraction was likely due to the ability of mineral particles to bind microbial cells and products. Iron oxides, which are plentiful in Puerto Rican tropical soils, are well known to influence organic matter stabilization via ligand exchange between carboxyl and hydroxyl functional groups of organic matter (Gu *et al*., 1994), and soil carbon is often positively correlated to clay content (Schimel *et al.,* 1994). Furthermore, the adhesion of microorganisms and adsorption of microbial metabolites onto clay and iron oxide surfaces increases the likelihood of the stabilization of microbial products in the HF (Chenu and Stotzky, 2002).

In this tropical soil, climate also played an important role in the stabilization of organic matter. This was especially true when Puerto Rico soils underwent lower temperatures and lower available moisture. This research shows that the stabilization of microbial cell material in the occluded fraction of the soil SOM under temperate climates differs from that found under the constant warm, wet Tropical climate. Higher levels of occluded microbial carbon were recovered in soils that were incubated under colder, drier conditions than the warm wet Tropical climate conditions, indicating that the temperate climate conditions could have led to increased aggregation of soil particles. This increase in soil aggregation may have been due at least in part to the decrease in moisture (Harris *et al.* 1966; Wolf and Wagner, 2005); It should be noted that while Causarano (1993) found that the relationship between grassland soil aggregation and changes in moisture (field capacity to air dry) was also influenced by the organic matter content of a soil, the soils used in this study were all harvested from the same Puerto Rico field site and began the incubation with similar organic matter contents.

If the decrease in moisture was the primary driver of aggregation, soils under the Redwood climate would have been expected to recover the highest levels of ^{13}C in the OLF, yet soils maintained under the Mediterranean climate retained the highest levels of occluded microbial carbon, followed by soils under the Redwood climate. Thus, temperature must have also had a significant influence on the aggregation of soil particles during the 520 days of the

study. Lower temperatures, especially those that fall below a threshold for microbial activity, can limit decomposition, resulting in a decrease in microbial consumption of the organic residues that bind soil aggregates (Wolf and Wagner, 2005) and an accumulation of SOM over time (Franzluebbers *et al.,* 2001). This decrease in deaggregation and accumulation of SOM can then result in an increased formation of microaggregates (Niklaus *et al.*, 2001; Bronick and Lal, 2005). The colder temperatures imposed on the soils under the Mediterranean climate in this study, along with the decrease in soil moisture, likely led to increased aggregation of soil particles, trapping the highest proportion of microbial ^{13}C in the OLF. The soils under the Redwood climate, with mid-level temperatures and low soil moisture, exhibited the second highest levels of soil aggregation and recovery of microbial ^{13}C in the OLF.

While mean precipitation is predicted to increase due to climate change in many regions of the globe, decreases in precipitation are also predicted to occur in many locations (Meehl *et al.*, 2007). These decreases in precipitation will lead to decreases in soil moisture and models predict that an expansion of the tropical ITCZ belt will also likely lead to fundamental shifts in soil moisture and ecosystem function in many locations (Seidel, *et al.,* 2008; Mitchell and Ingram,1992). The Intergovernmental Panel on Climate Change reports that many tropical forests from North to South America will undergo a 1.8-5°C increase in temperature and 48% less precipitation by 2100 (IPCC, 2013). In the Caribbean, where the Puerto Rico soils were collected for this study, models predict an approximate 4°C in warming and in increase in variability of rainfall with a 40% likelihood of extremely dry seasons (Christensen *et al.*, 2007; Anderson, 2011). These changes in climate will likely lead to alterations in the way microbial carbon is stabilized in soils, as demonstrated by this research.

Importance of microbial cells as stabilized organic matter in soils

Climate was a significant factor influencing the amount of microbial cell material that remained in either the standing biomass or SOM pools, or was respired out as CO_2. The percentage of SOC that would have been derived from dead microbial carbon if all of the standing microbial biomass been labeled and subjected to 520 days of decomposition and stabilization processes would have been highest in soils under the Mediterranean climate. Clearly, these estimates do not reflect the actual proportion of SOC that would be derived from microbial biomass materials; the real proportion of SOC derived from microbial materials would likely be much higher because these calculations take into account only the biomass that was standing at the start of the incubation and do not take into account growth of new microbial cells or the high and constant turnover of microbial communities in soils.

Conclusion

Previous studies have primarily focused on the role of plant materials as the building blocks of soil organic matter. We are now starting to understand that microbial bodies themselves are building blocks of soil organic matter (Chenu and Stotzky, 2002; Kögel-Knabner 2002; Miltner 2012). As previously stated, microbial bodies are likely to become stabilized as SOM because they have adhesive properties, are plentiful in soils, and commonly live on soil particles to which they can become adsorbed. Soil microorganisms also produce extracellular polysaccharides that adsorb to clay surfaces through hydrogen bonding and Van der Waals forces. While the individual bonds of microbial extracellular polysaccharides are weak, they

occur in such high amounts that their ability to form lasting bindings of microbial products with soil clays are exceptional (Chenu, 1995; Alexander, 2005). This study demonstrated that microbial materials are in fact stabilized on mineral surfaces, and are an important part of the nascent materials for the stabilization of soil organic matter.

By following the fate of dead, labeled microbial carbon in the Puerto Rico soil as it was assimilated into the living biomass, respired out as CO_2, and incorporated into the SOM under three different climates, I was able to study whether there was a relationship between the residence time of microbial carbon and associations with mineral surfaces. The findings of this study suggest that there was indeed a positive relationship between residence times of microbial C and associations with mineral surfaces as soils with the longest retention times of C in the living microbial biomass and the lowest total respiration of labeled CO_2 recovered the most labeled C in the HF of SOM. While Chapter 1 indicates that the type of microbial source of organic C may not have an effect on the residence time of carbon in living soil microbial communities, climate has a large impact. The research presented here in Chapter 3 indicates that climate likely influences not only the residence time of C in living microbes but the stabilization of that C in the HF of SOM as well. In this study, climate demonstrated a significant effect on the recovery of labeled microbial carbon in all of the measured C pools. The interactive impacts of soil temperature and moisture upon microbial community composition and function on the stabilization of microbial C in soils is not well understood and this study demonstrates that this relationship deserves further investigation.

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