Effects of Three Climate Regimes on the Mechanisms of Carbon Stabilization in a Sierra Nevada Mixed Conifer Forest Soil

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ABSTRACT

A change in carbon residence times in soil has huge potential to alter global carbon cycling and atmospheric composition. Therefore, an understanding of how mechanisms of carbon stabilization respond to ecosystem changes is essential for accurately modeling global climate change. Soils as whole ecosystems and specific soil biotic conditions are known to respond to changes in climate. However, the response of specific mechanisms of carbon stabilization to a change in climate has not been tested. In this study, I identify how the specific stabilization mechanisms of relative recalcitrance, occlusion in aggregates, and mineral binding are affected by incubation in a seasonally fluctuating climate, consistently warm/wet climate, and consistently cool/dry climate. I incubated soil from Blodgett forest in the Sierra Nevada Mountains with ^{13}C labeled dead microbial cells under the three climates for one year and compared the amounts of ^{13}C stabilized in the three functional humic fractions: free light fraction (fLF), occluded fraction (oLF), and heavy fraction (HF). I found that the soil under the consistently warm/wet climate stabilized on average 20.7 mg ^{13}C, 9 mg less than the fluctuating climate and 18 mg less than the cool/dry climate. The warm/wet climate did, however, stabilize the most ^{13}C in the HF (12.6 mg) compared to the soils under the other two climates. I also found that the consistently cool/dry climate stabilized the most ^{13}C in the fLF (15.6 mg), 6 mg more than the warm/wet climate. There was no significant difference in the amount of ^{13}C stabilized in the oLFs under the three climates.

KEYWORDS

Functional humic fractions, density fractionation, relative recalcitrance, occlusion in aggregates, mineral binding

INTRODUCTION

Carbon stabilization in soil plays a large role in the global carbon cycle and is hypothesized to be a major factor in predicting the extent of global climate change effects (Krull et al. 2003). Carbon stabilization is the process by which biological and physical mechanisms lengthen the residence time of carbon in soil (Lützow et al. 2006). These lengthened residence times make soil one of the largest storage sites of carbon on Earth where two-thirds of all terrestrial carbon is contained as soil organic matter (Trumbore et al. 1996). Consequently, a change in residence time or size of this pool has the potential to alter atmospheric composition and global climate (Lützow et al. 2006).

Humification is a dominant process in carbon stabilization and is a useful lens for studying carbon stabilization in soil. Humification is defined as a set of biogeochemical mechanisms that convert organic carbon into heterogeneous clusters of low molecular weight organic compounds. The resulting associations of organic compounds are called humics or humic substances and have relatively long residence times in soil (Sutton and Sposito 2005). Functional humic fractions describe humics with respect to their functional characteristics like bioavailability, residence time, or mechanism of stabilization and are divided into the free light fraction (fLF), the occluded light fraction (oLF), and the heavy fraction (HF) (Christensen 2001, Swanston et al. 2005). The sum of these three fractions is the total humic fraction (THF). Measuring these functional humic fractions can allow soil carbon stabilization analysis to be applied specifically to the following mechanisms: relative recalcitrance, occlusion in aggregates, and mineral binding (Swanston et al. 2005). Analysis of humic fractions can provide information about how specific pools of carbon and the mechanisms that generate those pools are affected by different environmental conditions.

A change in climate regime may influence many functional characteristics of a soil, including those that can change how the soil cycles and stabilizes carbon. For example, microbial activity often increases under warmer, wetter conditions and increased activity requires microorganisms to use more carbon substrates for growth and maintenance (Chenu and Stotzky 2002, Lloyd and Taylor 1994). Changes in biological activity can also affect the physical structure of a soil because microbial bodies and exudates contribute to aggregation in soils (Chenu and Stotzky 2002). In addition, temperature may have an effect on the binding of organic

matter to soil minerals (Kleber et al. 2007). Ultimately, the biotic and abiotic characteristics of soil are likely to change as climate changes and this may affect the stabilization of carbon in soils. However, the directional and magnitudinal response of specific stabilization mechanisms to a change in climate has not been tested (Krull et al. 2003).

In this study, I identify how climate affects the stabilization of soil carbon by measuring the functional humic fractions of soil samples incubated in three different climate regimes: a seasonally fluctuating climate, a consistently warm and wet climate, and a consistently dry and cool climate. I hypothesized that the soil incubated in the consistently warm and wet climate would stabilize the lowest amount of carbon in the fLF because the high level of microbial activity would utilize higher amounts of available carbon substrates. I also expected the soil incubated in the warmer, wetter climate to stabilize the lowest amount of carbon in the HF because the higher temperatures and water content should cause the HF to undergo chemical changes more frequently than the soil in the climate with the lower water content and temperatures. I hypothesized that the soil in the consistently cooler and drier climate would stabilize the most carbon in these fractions and the soil in the fluctuating climate would stabilize an intermediate amount. Lastly, I hypothesized that the soil incubated in the consistently warm, wet climate would stabilize a higher proportion of carbon in the oLF because the higher microbial activity would increase aggregation and trap more carbon in the oLF than in the drier, cooler system and the seasonally fluctuating climate regime.

METHODS

To determine the effect of climate on mechanisms of soil carbon stabilization I incubated one soil type in its native climate regime and two foreign climate regimes for one year with a supply of ^{13}C labeled dead microbial cells, and compared the amounts of ^{13}C stabilized in the different humic fractions at the end of the incubation. I measured the atom percent ^{13}C and calculated the mass of ^{13}C stabilized in each fraction for comparison. To compare the relative ^{13}C enrichments in each fraction, I also measured the $\delta^{13}C$ value.

Study system

Both the soils and the data I used to create the incubation climate regimes were collected by Laura Dane (University of California, Berkeley). Dane collected soil samples from a mixed-conifer forest in the Sierra Nevada Mountains at the UC Berkeley Blodgett Study Site from the first 7.5 cm after all litter material was cleared. The soil type was a fine-loamy mixed mesic, ultic haploxeralf with andesitic lahar parent material. Dane also measured soil temperature (°C) and moisture content (g water/g soil) every 15 min from 2005-2009 at the same site. From this data, she calculated the monthly averages of both climate characteristics (Appendix A). Dane used the same method to measure the soil temperature and moisture content for two more sites: a subtropical rainforest dominated by *Cyrilla racemiflora* at the Luquillo Experimental Forest 35 km east of San Juan, Puerto Rico and from a Redwood forest in Sonoma county, California 90 km north of San Francisco (Appendix A; Dane, unpublished data).

Lab incubation

I incubated 100g dry weight equivalent of the Sierra Nevada mountain soils with a single addition of dead microbial cells labeled with ^{13}C for one year with three replicates for each climate regime. Half of the soils did not receive the labeled cells and were maintained as control samples. I reproduced the average monthly temperature and moisture conditions of the Blodgett, Puerto Rico, and Redwood forests throughout the incubation to mimic the three natural climates. During the incubation period, I aired out the jars every two weeks to keep the systems from turning anaerobic which could potentially change the microorganism communities and the mechanisms of stabilization. To make sure microbial communities were active during the entire incubation period, the microbial activity of the samples was measured throughout the incubation using soda lime to capture CO_2. Because adequate activity was measured, I did not add any additional carbon to the system during the incubation.

Labeled microbial cell addition

I added 31 mg of labeled cells to each of the 100 g dry weight soil samples. The microbial species were native to the Blodgett and Puerto Rico study sites including three types of fungi, six types of actinomycetes, four types of gram + bacteria, and six types of gram - bacteria. I added 2 mL total volume to each soil sample, which contained either the rehydrated mix of labeled microbial bodies or pure water for the control soil samples.

Separation of fractions

After one year, I harvested the soils and performed density fractionations to determine in which functional humic fractions the labeled carbon had been incorporated (Swanston et al. 2005, Strickland and Sollins 1987, Golchin et al. 1994).

Free light fraction separation

I added 50 mL of sodium polytungstate (SPT) from Fisher with a density of 1.85 g/mL to each of the 20 g dry weight equivalent soil samples and centrifuged for 1 hour at 4600 rpm. I aspirated, rinsed, and freeze-dried the supernatant to obtain the fLF of the sample (Table 1).

Table 1: Summary of Functional Humic Fractions

Name of Fraction	Mechanism of Stabilization	Relative Residence Times	Measurement
Free light fraction (fLF)	Relative recalcitrance[1]	Medium	Suspended in SPT before sonication
Occluded light fraction (oLF)	Physical protection in aggregates	Long if the aggregates are stable	Suspended in SPT after sonication[2]
Heavy fraction (HF)	Bound to minerals[3]	Long with gradient of longer to shorter as the distance from the mineral surface increases	Remains in dense pellet before and after sonication

1. Relative recalcitrance is the relative difficulty of decomposing different substrates.
2. Aggregates may trap recalcitrant or labile carbon substrates as well as free or mineral bound compounds. The oLF does not include the mineral bound organic compounds inside aggregates.
3. The exact mechanisms of binding depend on the distance to the mineral surface (Kleber et al. 2007).

Occluded light fraction separation

Next I separated the oLF from the HF. I added about 100 mL of 1.85 g/mL SPT to the dense pellets from the previous centrifugation and mixed for 1 min at 75% maximum speed using a Lightnin G3U05R variable speed bench-top mixer. Then I sonicated the samples using a Branson 450A sonifier at maximum output and 70% pulse for 3 min to break apart the aggregates and release the oLF. I centrifuged the samples again for 1 hour at 4600 rpm. Then I aspirated, rinsed, and freeze-dried the supernatant to obtain the oLF of the soil sample (Table 1).

Heavy fraction separation

Finally, I rinsed the pellet from the second centrifugation to obtain a pure sample of the HF (Table 1). I added 150 mL of water and centrifuged for 20 min at 4600 rpm for each rinse. I rinsed until the aspirated liquid was 1.00 g/mL, about 3 rinses for each sample. Finally, I freeze-dried the HF sample.

Mass spectrometry

I ran a sample of each fraction on a Europa isotope ratio mass spectrometer to determine the amount of ^{13}C contained in each fraction. I measured both the atom percent ^{13}C and the $\delta^{13}C$.

Calculations and statistics

I performed calculations to determine the mass of ^{13}C in each fraction, the total humic ^{13}C mass, and the percent of the total humic ^{13}C in each fraction. I used the following calculation to determine the mass of ^{13}C in each fraction:

(Mass of fraction recovered) x (% C of sample/100) x (atom % ^{13}C/100) = mass ^{13}C in fraction

I summed the values of the mass ^{13}C in each functional humic fraction to calculate the ^{13}C in the THF in each incubated soil. To calculate the percent of the THF labeled carbon contained in the

individual functional humic fractions I divided the mass of ^{13}C in the fraction by the mass of ^{13}C in the THF and multiplied by 100.

To determine if the mechanisms of stabilization differed between the soils incubated in the three climates, I analyzed the measured $\delta^{13}C$ values, the calculated masses of ^{13}C in the fLF, oLF, HF, and THF, and the calculated percentages of the THF ^{13}C in the fLF, oLF, and HF for a statistical difference between each of these values for the three climate regimes. I preformed Kruskal-Wallis tests using R software (R Development Core Team 2010) because most of the data was normally distributed but did not show equal variance when visualized. I also ran Kruskal-Wallis multiple comparisons using the pgirmess package for R (Giraudoux 2011).

RESULTS

Labeled carbon in the free light fraction

After separating the fLF and determining the mass of ^{13}C and the $\delta^{13}C$ value in each sample, I found a significant difference ($p=0.0273$) between the amount of carbon stabilized in the fLF of the three climates (Fig. 1). The observed significant difference was between the soil under the Redwood climate and the Puerto Rico climate with an effect size of 6.0 mg. The difference between the soil under the Blodgett climate and the Puerto Rico climate was not significant and the same was true for the difference between the soil under the Blodgett climate and the Redwood climate. The masses of ^{13}C measured in the fLF under the Redwood climate were always larger than those measured under the other two climates and the masses measured from the Puerto Rico climate were always lower than those from the two other climates (Table 2). The difference between the masses of ^{13}C in the fLF of the control samples from the three different climates was not significant ($p=0.430$).

I also found a significant difference ($p=0.0273$) between and the $\delta^{13}C$ values in the fLF of the three climates (Fig. 2). Similar to the fLF ^{13}C mass data, the significant difference between the $\delta^{13}C$ values was between the soil incubated under the Redwood climate and under the Puerto Rico climate with an effect size of 6.0 units. There was not a significant observable difference between the soil from the Blodgett climate and the Puerto Rico climate or between the soil from the Blodgett climate and the Redwood climate. The direction of difference between the $\delta^{13}C$

values in the fLF was also the same as the mass ^{13}C values with the enrichment greatest in the Redwood climate and least in the Puerto Rico climate (Table 3). The difference between the $\delta^{13}C$ values in the fLF of the control samples from the three different climates was not significant (p=0.561).

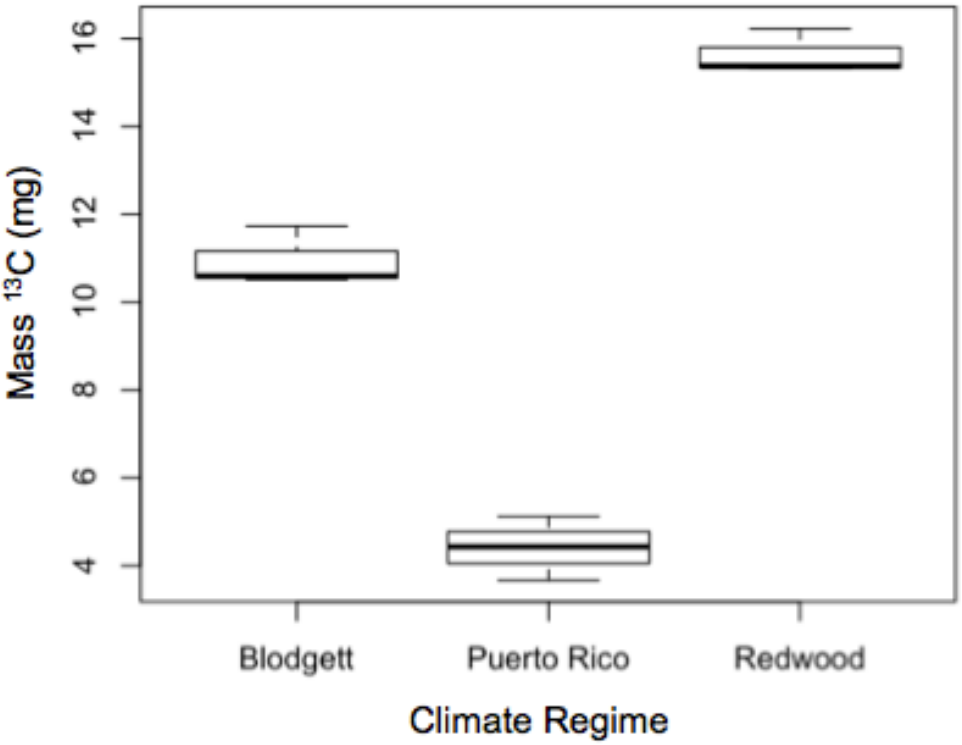

Fig. 1. Mass ^{13}C stabilized in the fLF from each incubation climate. Difference was statistically significant (p=0.0273) with an effect size of 6.0 mg between the soil from the Redwood climate and from the Puerto Rico climate.

Table 2. Mean mass ^{13}C stabilized in each functional humic fraction. Statistically significant differences (p<0.05) from the Kruskal-Wallis tests are indicated by a *.

Humic Fraction	Climate Regime	Mean Mass of ^{13}C (mg)
fLF*	Blodgett	10.945 ± 0.682
	Puerto Rico	4.406 ± 0.726
	Redwood	15.639 ± 0.506
oLF	Blodgett	4.971 ± 0.220
	Puerto Rico	3.726 ± 1.106
	Redwood	2.724 ± 0.220
HF	Blodgett	8.009 ± 0.315
	Puerto Rico	12.609 ± 0.534
	Redwood	7.279 ± 0.943

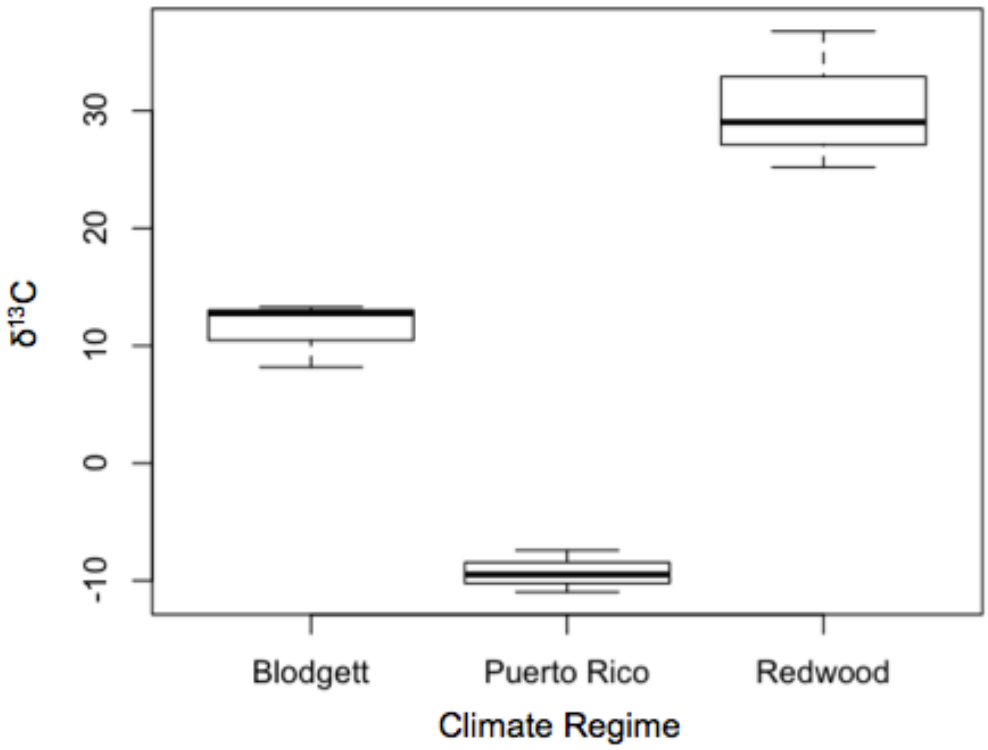

Fig. 2. $\delta^{13}C$ of the fLF from each incubation climate. Difference was statistically significant (p=0.0273) with an effect size of 6.0 mg between the soil from the Redwood climate and from the Puerto Rico climate.

Table 3. Mean $\delta^{13}C$ of each functional humic fraction. Statistically significant differences (p<0.05) from the Kruskal-Wallis tests are indicated by a *.

Humic Fraction	Climate Regime	Mean $\delta^{13}C$
fLF*	Blodgett	11.42 ± 2.82
	Puerto Rico	-9.28 ± 1.79
	Redwood	30.34 ± 5.90
oLF	Blodgett	6.67 ± 5.84
	Puerto Rico	-10.39 ± 3.06
	Redwood	6.04 ± 3.48
HF*	Blodgett	68.91 ± 7.22
	Puerto Rico	12.43 ± 2.85
	Redwood	81.06 ± 10.13

Labeled carbon in the occluded light fraction

After separating the oLF, I did not find a significant difference (p=0.0581) between the amounts of carbon stabilized in the oLF of the three climate treatments (Fig. 3). Although it was not significant, the effect size between the soils incubated under the Blodgett climate and under

the Redwood climate was 5.3 mg. The measured ^{13}C masses in the oLF were the largest in the Blodgett climate with a minimum of 4.718 mg and a maximum of 5.108 mg. The masses in the soil incubated under the Redwood climate were the lowest with a minimum of 2.592 mg and a maximum of 2.979 mg. The masses from the Puerto Rico climate ranged from a minimum of 2.693 mg to a maximum of 4.892 mg (Table 2). The difference between the masses of ^{13}C in the oLF of the control samples from the three different climates was not significant (p=0.509).

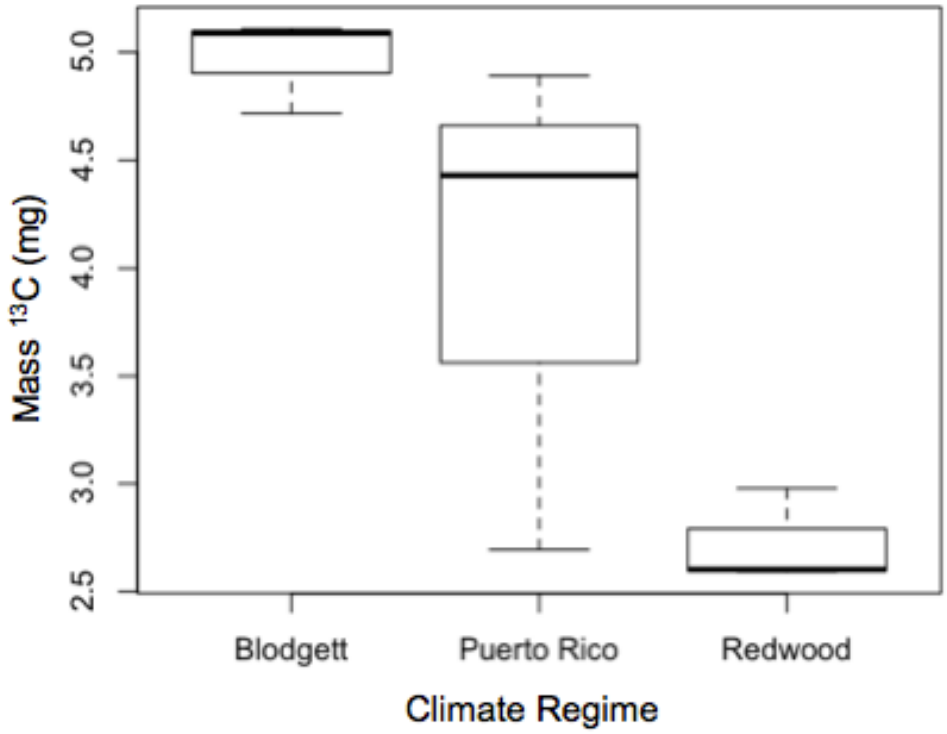

Fig. 3. Mass ^{13}C stabilized in the oLF from each incubation climate. Difference was not statistically significant (p=0.0581).

I also did not find a significant difference (p=0.0665) between the three climate treatments and the $\delta^{13}C$ values in the oLF (Fig. 4). Although they were not significant, the largest effect sizes were between the soils from the Blodgett climate and from the Puerto Rico climate (4.7 units) and between the Redwood climate and the Puerto Rico climate (4.3 units). The effect size between the Redwood climate and the Blodgett climate was only 0.3 units. While the soils from the Blodgett and Redwood climates had very similar $\delta^{13}C$ values in the oLF, the soil from the Puerto Rico climate had lower values of $\delta^{13}C$ (Table 3). The difference between the $\delta^{13}C$ values in the oLF of the control samples from the three different climates was also not significant (p=0.113).

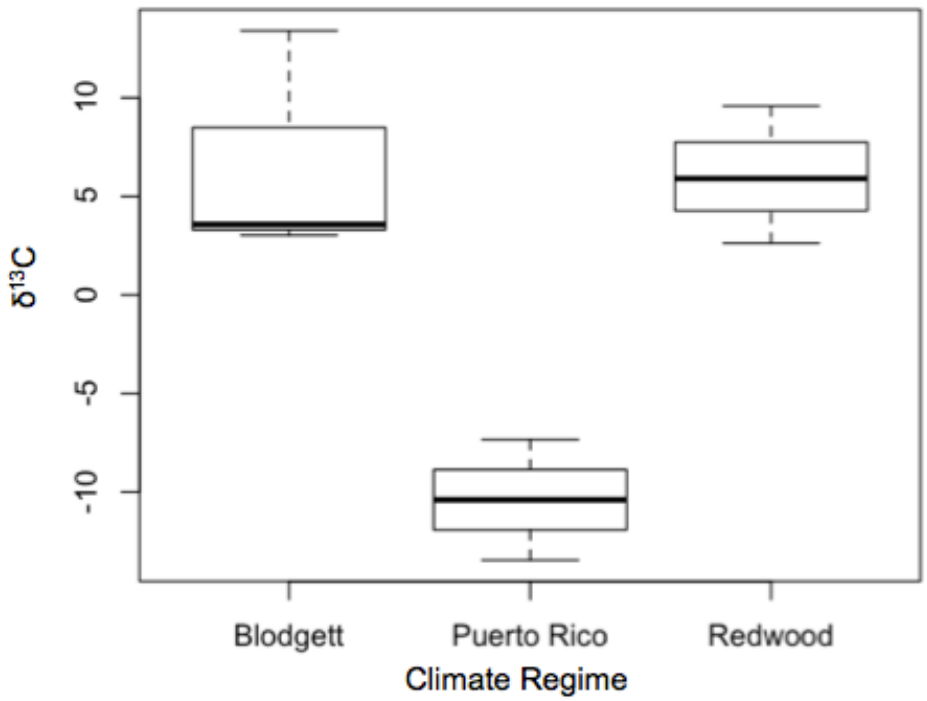

Fig. 4. $\delta^{13}C$ in the oLF from each incubation climate. Difference between treatments was not statistically significant (p=0.0665).

Labeled carbon in the heavy fraction

After separating the HF and determining the mass of ^{13}C in each sample, I did not find a significant difference (p=0.0509) between the masses of carbon stabilized in the HF under the three climate regimes (Fig. 5). Although it was not significant, the effect size between the soil incubated under the Redwood climate and the soil under the Puerto Rico climate was 5.3 mg. The ^{13}C masses in the soils from the Redwood climate and Blodgett climate were lower than the masses from the Puerto Rico climate (Table 2). The difference between the masses of ^{13}C in the HF of the control samples from the three different climates was not significant (p=0.0665).

I did find a significant difference (p=0.0390) between the three climate treatments and the $\delta^{13}C$ values in the HF (Fig. 6). The observed significant difference was between the soil under the Redwood climate and the Puerto Rico climate with an effect size of 5.7 units. The difference between the soil under the Blodgett climate and the Puerto Rico climate was not significant and the same was true for the difference between the soil under the Blodgett climate and the Redwood climate. The $\delta^{13}C$ values in the HF under the Puerto Rico climate were lower than the values from the soils incubated under the Redwood climate and the Blodgett climate (Table 3).

The difference between the $\delta^{13}C$ values in the HF of the control samples from the three different climates was not significant (p=0.0794).

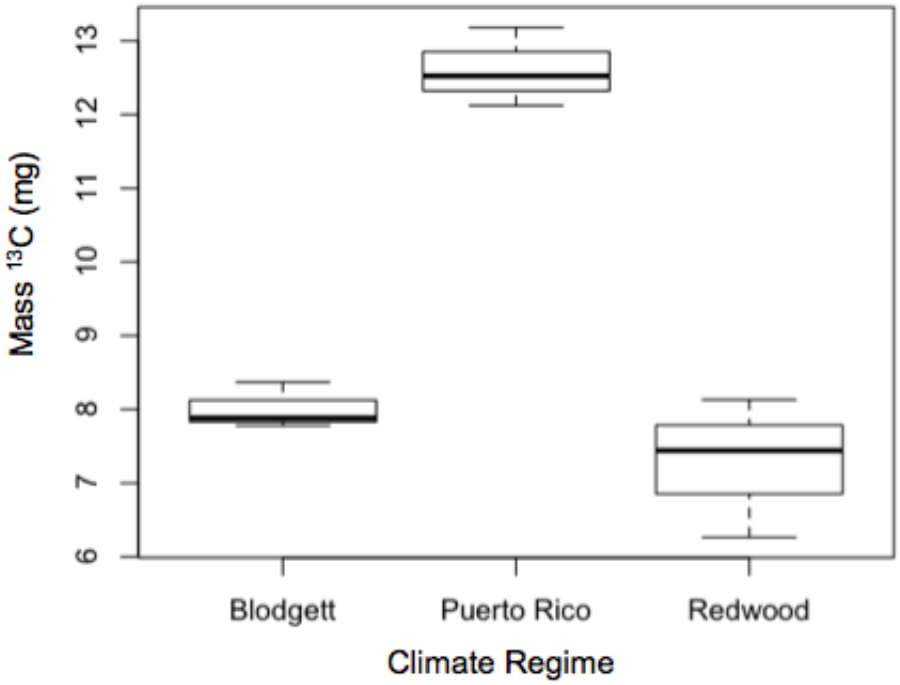

Fig. 5. Mass ^{13}C stabilized in the HF from each incubation climate. Difference was not statistically significant (p=0.0509).

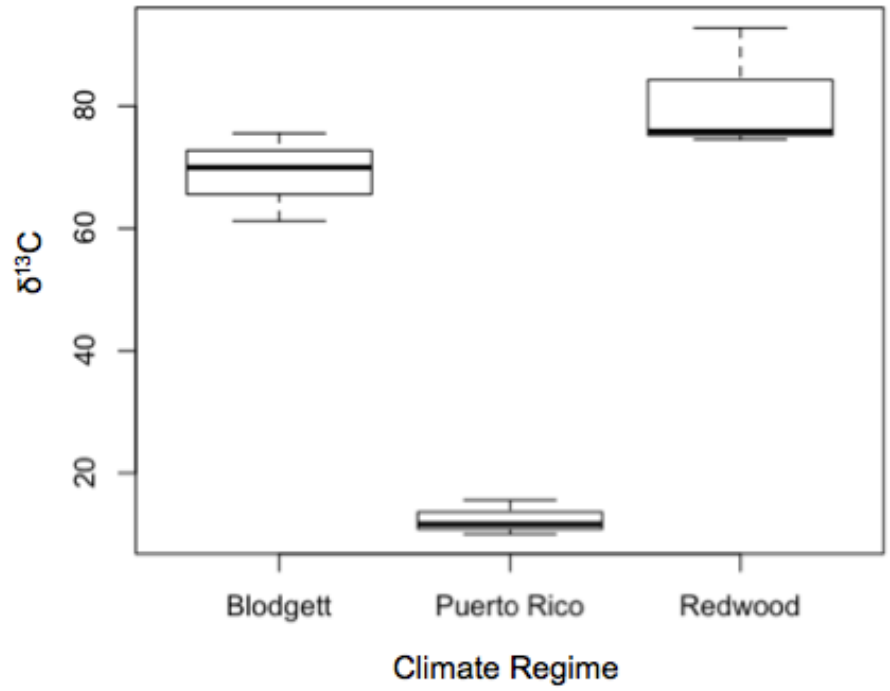

Fig. 6. $\delta^{13}C$ in the HF from each incubation climate. Difference between treatments was statistically significant (p=0.0390) with an effect size of 5.7 units between the soils from the Redwood climate and the Puerto Rico climate.

Total ^{13}C stabilized

I found the total ^{13}C stabilized in each soil sample by adding together the masses of ^{13}C in each fraction and found a significant difference (p=0.0273) in the total amount of labeled carbon stabilized under each climate regime (Fig. 7). The observable difference between the THF ^{13}C masses of the Blodgett climate and of the Puerto Rico climate was 9.0 mg. The observable difference between the Puerto Rico and Redwood climates was 18.0 mg. Finally, the observable difference between the Blodgett and Redwood climates was 9.0 mg. The Redwood climate stabilized the most ^{13}C, while the Puerto Rico climate stabilized the least (Table 4). The difference between the total masses of ^{13}C from the control samples of each incubation climate was not significant (p=0.733).

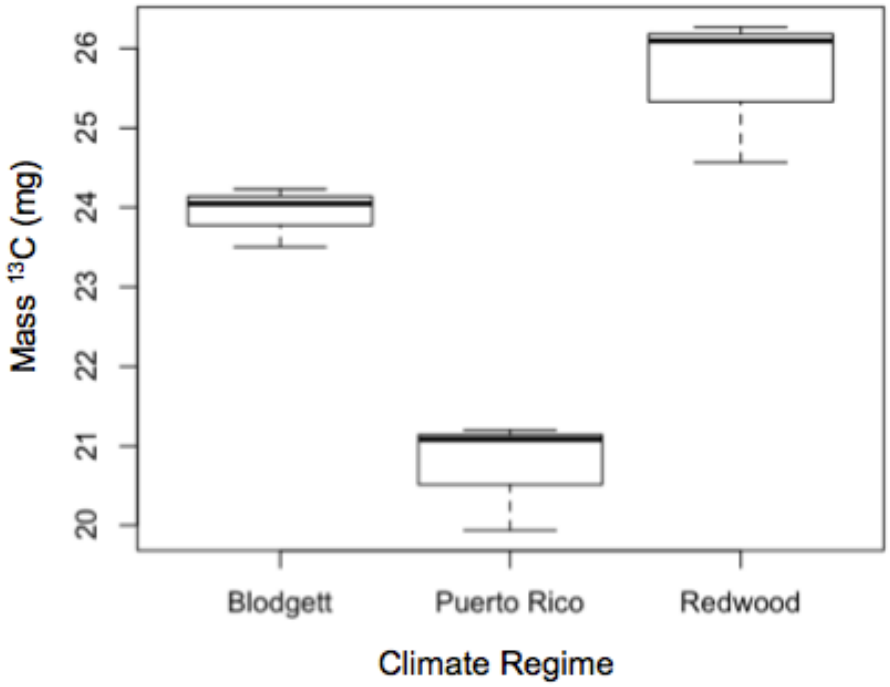

Fig. 7. Total mass ^{13}C stabilized in soil from each incubation climate. Difference was statistically significant (p=0.0273) with effect size of 9.0 mg both the Blodgett and Puerto Rico climates and the Blodgett and Redwood climates. The effect size between the Redwood and Puerto Rico climates was 18.0 mg.

Table 4. Mean THF ^{13}C stabilized in each incubation climate. Statistically significant differences (p<0.05) from the are indicated by a *.

Climate Regime	Mean Total ^{13}C
Blodgett*	23.926 ± 0.783
Puerto Rico*	20.741 ± 1.426
Redwood*	25.643 ± 1.093

Percent of the total humic ^{13}C stabilized in each fraction

After calculating the percent of the total humic ^{13}C stabilized in each fraction of the soil samples, I found a significant difference in the percentages that the fLF ($p=0.0273$) and the HF ($p=0.0273$) contributed to the THC between climate regimes. However, I did not find a significant difference ($p=0.0608$) between the percent that the oLF contributed to the THC in the three climates (Fig. 8). In both the fLF and the HF, the significant effect size was between the soils incubated under the Redwood climate and under the Puerto Rico climate and was 6.0%. The effect sizes between the Redwood climate and the Blodgett climate and between the Blodgett climate and the Puerto Rico climate were not significant. Although they were not significant, the effect sizes of the percentages oLF contributes to THF between the Blodgett and Redwood climates was 5.0% and between the Redwood and Puerto Rico climates was 4.0%. The soil incubated under the Redwood climate showed the highest percent fLF and Puerto Rico showed the lowest. The soil under the Puerto Rico climate showed high variability of percent oLF with a range of 9.69% and the soil from the Redwood climate had the lowest percent oLF. The percent HF in the soil incubated under Puerto Rico was higher than both the Blodgett and Redwood climates (Table 5).

Table 5. Percent each functional humic fraction contributes to the THF. Statistically significant differences ($p<0.05$) from the Kruskal-Wallis tests are indicated by a *.

Humic Fraction	Climate Regime	Mean %
fLF*	Blodgett	45.73 ± 2.35
	Puerto Rico	21.33 ± 4.16
	Redwood	61.01 ± 1.84
oLF	Blodgett	20.79 ± 1.17
	Puerto Rico	17.88 ± 4.91
	Redwood	10.66 ± 1.27
HF*	Blodgett	33.48 ± 1.35
	Puerto Rico	60.79 ± 1.38
	Redwood	28.33 ± 2.82

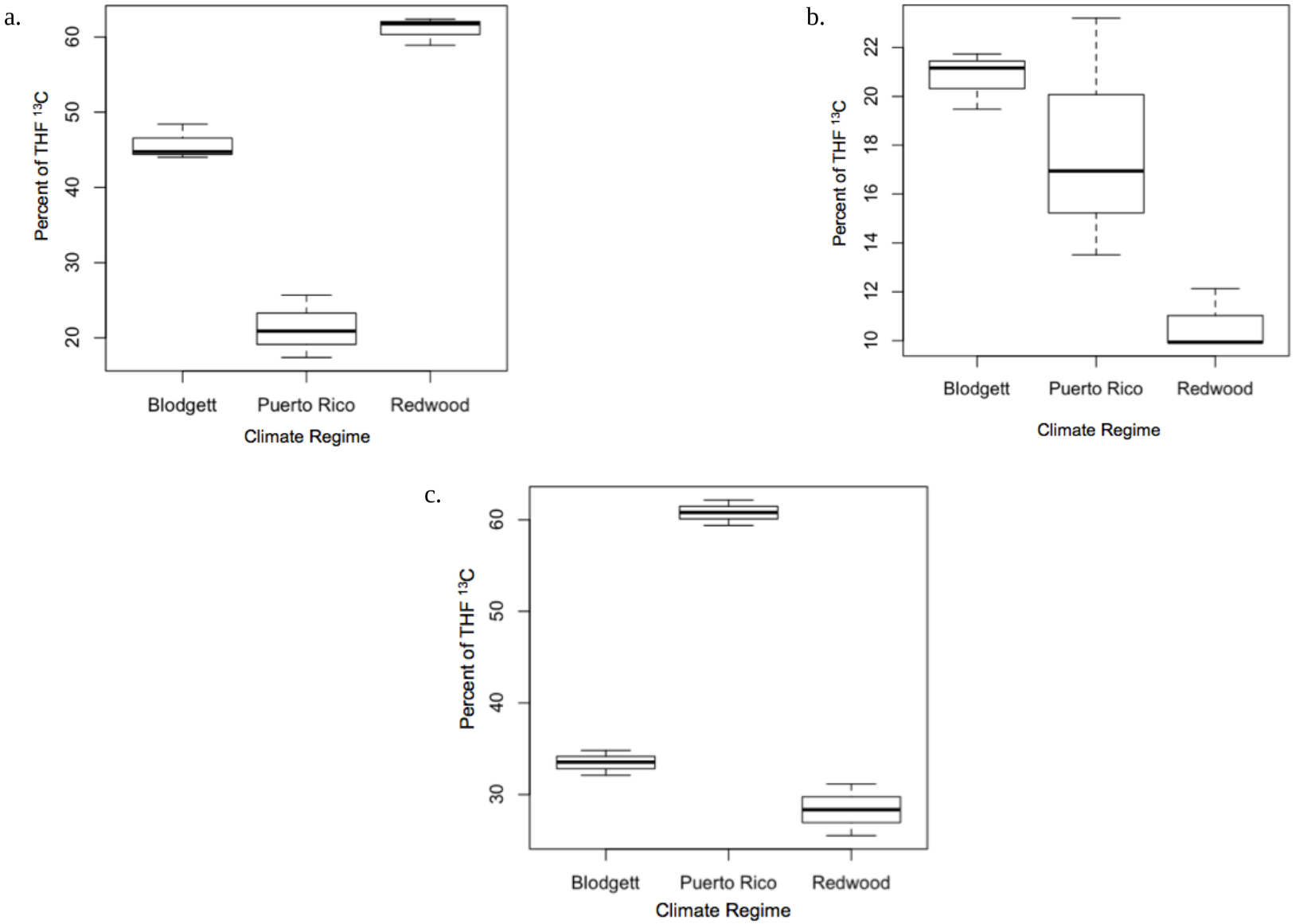

Fig. 8. Percent of total humic ^{13}C in each fraction of each incubation climate. Percent fLF (a) and HF (c) showed a significant difference (p=0.0273) and percent oLF (b) was not significantly different between climates (p=0.0608).

DISCUSSION

An understanding of how mechanisms of carbon stabilization respond to ecosystem changes is essential for accurately modeling global climate change. Soils as whole ecosystems and specific soil biotic conditions are known to respond to changes in climate. However, the response of specific mechanisms of carbon stabilization to a change in climate has not been tested. In this experiment I found that climate does affect how carbon is stabilized in soil. The data I collected suggests that differences in temperature, moisture content, and fluctuation cycles of these two climate features can affect the processes of stabilization by relative recalcitrance, occlusion in aggregates, and mineral binding. I also found that climate changes such as warming and reduction of fluctuation in a climate regime can alter the total amount of carbon stabilized in all three of these fractions. These differences in the ability to stabilize carbon are likely caused by changes in abiotic and biotic soil characteristics due to the new climate regime.

Factors affecting the ^{13}C stabilization in the free light fraction

I found a significant difference between the ability of the fLF under the three climate regimes to stabilize the added ^{13}C because of microbial activity's dependence on climate. The fLF from the Puerto Rico climate had the smallest mean mass of ^{13}C and the smallest mean $\delta^{13}C$ value because the consistently warm and wet conditions optimized microbial activity in the Sierra Nevada soils. Previous studies have found that increases in temperature and tropical climates cause greater losses of carbon as CO_2 than cooler, drier climates because microorganisms use more carbon substrate under these climate conditions (Raich et al. 2006, Wang 2002, Wagai et al. 2008, Trumbore 1993). The fLF contains the most available humic carbon and therefore it is logical that the active microorganisms would use much of the carbon in the fLF instead of allowing it to stabilize.

The fLF under the Redwood climate regime stabilized the largest mass of ^{13}C of the three climates however, the $\delta^{13}C$ value of the fLF under the Redwood conditions was not statistically different from that of the Blodgett climate. The large mass and low enrichment means that the Redwood climate stabilized a larger mass of all carbon in the fLF not just the labeled ^{13}C. With

low microbial activity, all carbon sources will be utilized less causing the unlabeled carbon in the fLF before the incubation to be minimally decomposed and therefore accumulate along with the newly added ^{13}C sources. In addition, fluctuations in the temperature and moisture content could have increased decomposition of the fLF under the Blodgett climate as compared to the soil from the Redwood climate because of a resulting increase in microbial activity. Periods of wetting and drying like that which occurs in the Blodgett climate causes increases in microbial activity for a brief time (Amato and Ladd 1980).

Factors affecting the ^{13}C stabilization in the occluded light fraction

The differences between the amounts of carbon stabilized in the oLF of the different climates were not statistically significant. Newer theories of aggregation formation suggest that macro-aggregates form by the presence of roots and other large biological structures and then degrade to form stable micro-aggregates (Six et al. 2000). The absence of plant roots in my lab incubations decreased the ability for macro-aggregates to form and therefore may have decreased the ability for any new aggregates, both macro and micro, to form. Although microbial activity promotes aggregation in soils, I found that the oLF from the Puerto Rico climate had a smaller mean $\delta^{13}C$ value than the other two climates (Chenu and Stotzky, Dormaar and Foster 1991). The increased microbial activity may have caused more decomposition of the added labeled carbon before it had a chance to be stabilized by aggregation therefore causing a lower enrichment in the oLF under the Puerto Rico climate. The ^{13}C masses in the oLF were not significantly different in part because the variance was so large in the Puerto Rico climate. The larger range in measured values could be a result of competing effects of climate like increased microbial activity and increased aggregation, where in any given sample one may dominate the other thereby decreasing or increasing the mass of stabilized ^{13}C respectively.

The mass of ^{13}C stabilized in the oLF under the Blodgett climate was not statistically different from the mass stabilized under the Redwood climate. However, the effect size was relatively large and the high enrichment coupled with the high ^{13}C mass implies a relatively large amount of all isotopes of carbon was stabilized in the oLF under the Blodgett climate. The increased stabilization of carbon in the oLF under the Blodgett climate may have occurred because of the fluctuations in temperature and moisture promoting microbial activity, which in

turn promotes aggregation (Amato and Ladd 1980, Dormaar and Foster 1991). Alternatively, the freezing and subsequent thawing of the soils under the Blodgett climate may have broken apart aggregates and decreased the amount of ^{13}C I measured in the oLF under this climate and possibly causing the difference to be not significant (Beare et al. 1994).

Factors affecting the ^{13}C stabilization in the heavy fraction

The ^{13}C enrichment of the HF of each climate was significantly different however the mass of ^{13}C stabilized was not. The soil incubated in the Puerto Rico climate had both the largest mean mass of ^{13}C stabilized and the lowest $\delta^{13}C$ value in the HF. Low enrichment and high mass means that the HF in the Puerto Rico climate stabilized a relatively large amount of all carbon isotopes. Contrary to the hypothesis presented at the beginning of this paper, the increased soil moisture and temperature under the Puerto Rico climate increased the stabilization of carbon on the mineral surface. Although warmer, wetter climates favor microbial activity which should cause an increase in carbon cycling, these or other factors in the warm, wet environment actually allow more carbon to be stabilized in the HF. Whatever is causing this increased ability to stabilize carbon is likely occurring in the outer, kinetic zone of the HF because this is the zone that is most exposed to changes in soil activity and temperature (Kleber et al. 2007). However, it is unclear how this change in climate is increasing the mineral's ability to hold carbon substances.

Total stabilized ^{13}C under each climate

The ^{13}C stabilized in the THF under each climate is consistent with findings from previous studies about stabilization and CO_2 release from soil under different climates. Previous studies have found that increases in temperature and tropical climates cause greater CO_2 release than cooler, drier climates (Raich et al. 2006, Wang 2002, Wagai et al. 2008, Trumbore 1993). Therefore, it is logical that the warm, wet Puerto Rico climate stabilized the least total ^{13}C while the drier and cooler Redwood climate stabilized the most total ^{13}C. Also, the seasonally fluctuating Blodgett climate stabilized an intermediate amount of total ^{13}C because it has periods of stabilization promotion and periods of mineralization promotion.

Relative importance of each mechanism of stabilization under each climate

Comparing the percentage of the THF that each individual fraction contributes, implies that some mechanisms are more important than others in stabilizing carbon under different climates. The soil incubated under the Puerto Rico climate stabilized most of the humic ^{13}C in the HF implying that the HF is the most important pool for stabilizing carbon under the warm, wet conditions of the Puerto Rico climate because the fLF is too readily decomposed by microorganisms and the oLF is too variable. The soil under the Redwood climate stabilized most of its humic ^{13}C in the fLF. Therefore the fLF plays the major role in carbon stabilization under the cool, dry Redwood climate because the lower microbial activity allows this pool to remain in the soil at a relatively large size. The soil under the Blodgett climate showed less variation in the relative masses of ^{13}C stabilized in each fraction, where each played a more equal role in stabilization.

Limitations

There are several limitations to my study including the possible difference in behavior between the labeled carbon and the non-labeled carbon and the small level of inference provided by the experimental design. Additionally, more replicates may have been important for providing statistical evidence for the observations made in this experiment. Amato and Lad (1980) found a difference in the rate of decomposition of labeled and non-labeled carbon. If the non-labeled and labeled carbon in my study interacted differently with the soil environment, more carbon may have been decomposed by microbes in the experiment than would have been decomposed in a natural environment where no labeled carbon was added. Also, if substances containing ^{13}C were decomposed more easily following ^{13}C may have caused underestimates of the amount of carbon stabilized in the tested soils, especially under the Puerto Rico climate where decomposition plays an even more prominent role than under the other climates. The experimental design would not work without labeled carbon, but it is important to keep this possible confounding factor in mind when interpreting the results of this study.

Also, the experimental design limits my level of inference. This experimental design only allows me to assess the differences between my three tested climates. I cannot find a correlation between climate and mechanisms of stabilization because the climate variables used were not continuous. As a result, I can only draw confident conclusions about these 3 climates in relation to one another and therefore can only hypothesize about how other climates might affect stabilization mechanisms.

The small number of replicates was also a problem in the experimental design. Only three replicates were tested because the amount of work it took to maintain the incubating samples limited the samples to a minimum. However, there were differences that had effect sizes and p-values that were very close to being significant in particular the difference in the ^{13}C mass in the oLF between the Blodgett and Redwood climates and the difference in the ^{13}C mass in the HF between the Redwood climate and the Puerto Rico climate. More replicates may have resulted in statistical significance in many of the tests that I did not find significant differences.

The lab incubation was a good experimental design for this exploratory experiment because not much is known yet about how these mechanisms of stabilization are affected by different variables including climate. However, as more is learned about these mechanisms field experiments will be important. For example, I did not find statistically significant differences in the amount of carbon stabilized in the oLF. Field experiments would allow roots to form macro-aggregates and may change results significantly because of the importance of macro-aggregates in forming micro-aggregates.

My data suggests important factors to incorporate into modeling global climate change and its affects on carbon stabilization, but more work must be done to provide information on correlations between climate factors and stabilization. Further experiments should test continuous variables of climate to make the results more applicable to model building and predicting climate change effects. Future studies should also test different soil types to see if changes in climate have the same effects on other soil types.

Broader implications

This experiment is an important first step in understanding how changes in climate due to global climate change could change the way carbon is stabilized in soil. Understanding the

directional and magnitudinal changes of specific mechanisms of stabilization can increase the accuracy of global climate change models. The more we understand the specifics of what causes a change of how much in each mechanism, the more accurate we can be in determining how any change in climate will affect any soil simply given the climate data and characteristics of the soil. Although a lot more work needs to be done to be able to understand these specifics, this project provides a basic understanding of what future studies might find.

AKNOWLDGEMENTS

First, I would like to thank the graduate students and staff in the Firestone Laboratory who provided me with valuable feedback on how to present my data and supported me throughout my data collection process. This group of minds included Mary Firestone, Don Herman, Steve Blazewicz, Rebecca Daly, Dara Goodheart, Erin Nuccio, Shengjing Shi, Katerina Estera, Tsu-Ling Julie Wang, and visiting professor, Jim Prosser. From this group at the Firestone Laboratory, I would like to especially thank Laura Dane for her dedication to teaching me everything I needed to learn about the subject matter and data collection to pull off this project. I would also like to thank her for her tireless efforts in babysitting the very needy incubations for an entire year! Without her this project really would not exist. Second, I would like to thank the instructors of ES196: Patina Mendez, Kurt Spreyer, Melissa Eitzel, and Seth Shonkoff. Without their direction to the class, this project would have been a much bigger struggle. I would especially like to thank Patina Mendez for her continuous feedback during the course through grading and office hours and Melissa Eitzel for sharing her expertise in statistical analysis. Finally, I would like to thank my peer edit group in the ES196 class for their input and support: Emily Chow, Gary Gao, Kimberly Lam, and Taichi Natake.

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APPENDIX A: Soil Temperature and Moisture Data Used in Incubations

a.

Average Temperature (°C)

Climate	***Jan***	***Feb***	***Mar***	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***
Blodgett	0.88	1.24	1.47	4.84	11.49	14.69	18.45	17.32	14.21	8.96	5.51	2.30
Puerto Rico	19.53	18.47	18.29	19.51	21.06	21.38	21.94	22.71	22.84	21.72	21.16	20.12
Redwood	10.71	10.80	10.76	10.84	12.68	13.94	15.57	15.40	14.75	14.20	13.03	11.60

b.

Average Soil Moisture (g water/g dry soil)

Climate	***Jan***	***Feb***	***Mar***	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***
Blodgett	0.57	0.57	0.61	0.54	0.42	0.31	0.18	0.13	0.12	0.27	0.46	0.53
Puerto Rico	0.78	0.84	0.84	0.79	0.71	0.73	0.72	0.71	0.70	0.73	0.74	0.77
Redwood	0.30	0.29	0.32	0.31	0.26	0.22	0.17	0.13	0.12	0.12	0.22	0.27

Figure A1. The average monthly soil temperature (a) and moisture (b) data used as the monthly targets for soil temperature and moisture during the incubation. Laura Dane (University of California, Berkeley) collected this data at field sites in the Blodgett, Puerto Rico, and Redwood forests. They are averages from measurements taken every 15 min from 2005-2009.