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Assessing biotic and abiotic controls of carbon storage in soil

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Assessing biotic and abiotic controls of carbon storage in soil

A dissertation submitted in partial satisfaction of the requirement for the degree Doctor of Philosophy in Geography

by

Avishesh Neupane

2019

ABSTRACT OF THE DISSERTATION

Assessing biotic and abiotic controls of carbon storage in soil

by

Avishesh Neupane

Doctor of Philosophy in Geography

University of California, Los Angeles, 2019

Professor Gregory Stewart Okin, Chair

Understanding the mechanisms of soil carbon (C) formation and loss is essential for predicting the C storage capacity of soils under ongoing global change scenarios. Climatic variables, vegetation structure, microbial activity, soil mineralogy, and tissue C chemistry each have the potential to affect the fate of C in soils, and the interactions among these controls vary in different environments. Our mechanistic understanding of how these factors interact with each other to determine soil C storage is still rudimentary. This dissertation used a series of field and laboratory studies to assess the interacting roles of vegetation, soil mineralogy, microbial activity, C chemistry, and temperature in regulating the fate of C in soils.

In the first experiment, we sought to understand how soil mineralogy, soil nutrient and C status, and C chemistry interact to determine warming effects on the fate of newly added soil C using a ^{13}C isotopic tracing approach. By tracking the added ^{13}C label in soil pools at 4 days and 255 days in tropical forest soils with differing weathering and mineralogical conditions, we found that initial microbial uptake of ^{13}C and average carbon use efficiency (CUE) by microbes

were strongly correlated with longer-term C retention in mineral soils. Overall, warming had a negative effect on ^{13}C retention in soil in the youngest, least-weathered soil only, with no warming effect on moderately to strongly weathered soils. Thus, soil C stocks in less weathered soils, and with lower microbial CUE, may be most vulnerable to C loss with a warming climate.

Our second study assessed the fate of newly added organic ^{13}C-labeled compounds in soils of differing fertility along weathering gradients. Comparing additions of two low molecular weight compounds, 2.9x greater retention occurred for ^{13}C-labeled glucose versus ^{13}C-labeled glycine after two years, suggesting that glucose may be a better precursor for soil organic matter formation. Soil mineralogy and nutrient availability were not significant factors in ^{13}C retention in soil. Soil spectra from ^{13}C NMR revealed an increase in the proportion of alkyl C in glucose and glycine amended soil relative to control soils, and alkyl C are commonly associated with relatively stable organic C. Thus, our results indicate that microbial incorporation of labile organic compounds like glucose into biomass may be associated with greater C retention in stable soil components.

Our third study estimated the long-term effect of grass cover loss on soil organic C (SOC) and total nitrogen (TN) storage, and the spatial heterogeneity of SOC and TN in two arid grasslands. The nine years of experimental grass removal resulted in soil deflation and 30% and 35% declines in SOC and TN respectively in 100% grass removal plots (TU100). Grass removal also led to soil deposition in downwind areas of the plot (TD100). Soil organic C and TN concentrations in the deposition plot (TD100) was variable, and likely depended on the structure of the vegetation community trapping wind-blown particulates. Geostatistical analysis showed that weaker and smaller fertile islands, compared to the control, developed in TD100 plots over nine years of aeolian transport.

The outcomes of this dissertation will add to the current body of knowledge about mechanisms of soil C stabilization across environmental conditions and with warming.

This dissertation of Avishesh Neupane is approved.

Daniela F. Cusack

Thomas Welch Gillespie

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Gregory Stewart Okin, Committee Chair

University of California, Los Angeles

2019

Dedications

To my dear family . . .

for the endless love and support

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Curriculum Vitae

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Manuscripts in preparation

Neupane, A., Vitousek, P. M., and Cusack, D.F., Effect of warming on the fate of newly added carbon to soils from a Hawaiian weathering gradient. In preparation for *Soil Biology and Biochemistry*.

Neupane, A., Vitousek, P. M., Hockaday W.C., and Cusack, D.F., Annual-scale shifts in labile carbon storage across mineralogical, vegetation and rainfall gradients. In preparation for *Ecosystems*

Neupane A, Li, J., Zhang, J. and Okin, G.S., Effect of Vegetation on Soil C Storage in an Arid Grassland. In preparation for *Plant and Soil*

Dietterich L.H., **Neupane A.,** Karpman J, Turner B.L., and Cusack D.F., Seasonal shifts in soil carbon fractions in moist tropical forests of Panama, In preparation for *Soil Biology and Biochemistry*.

Dietterich L.H., **Neupane A.**, Ciochina M, Hess N, Tfaily M., Cusack D.F. Root exudate responses to drying and mycorrhizal colonization for two tropical tree seedlings, In preparation for *New Phytologist*.

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Neupane, A., Vitousek, P. M. and Cusack, D.F., Ecological Society of America, Meeting 2018, Effects of warming on the fate of newly added carbon across a Hawaiian soil mineralogical gradient, August 5-10, New Orleans, Louisiana

Neupane, A., Cusack, D.F., A study of Carbon stabilization at different temperatures along a mineralogical soil gradient using a soil incubation experiment, Hawaii Ecosystems Meeting, July 2016, Hilo, Hawaii.

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Poster Presentations

Neupane, A., Cusack, D.F., 2017, Effects of warming on the fate of carbon across a Hawaiian soil mineralogical gradient. The Association of Pacific Coast Geographers Conference, October 25-28, 2017 CSU, Chico.

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Chapter 1 Introduction

Background

Terrestrial ecosystem stores more carbon (C) in soil as soil organic matter than is stored in atmosphere and vegetation combined (Ciais et al., 2014). Continuing storage of C in soil is essential for maintaining soil fertility and regulating the climate (Schmidt et al., 2011). Each year, soil respiration emits ~80 PG of C into the atmosphere (Raich et al., 2002), which is ~10 times the amount released through fossil fuels combustion and cement production combined (7.8 $PGyr^{-1}$) (Ciais et al., 2014). To maintain soil organic carbon (SOC) stocks, soils must receive a similar amount of new C annually from photosynthesis and plant detritus. Depending on the balance between soil respiration and inputs of new C from plants, soils can be either as net C source to the atmospheric or a terrestrial sink. This net balance may depend on how different global change factors alter ecosystem processes like plant growth and decomposition (Trumbore, 1997; Davidson and Janssens, 2006). A suite of global change factors such as warming, increased CO_2 concentration, altered precipitation, Nitrogen (N) deposition, land use change are altering ecosystem properties and the biogeochemical processes that store carbon (Vitousek et al., 1997; Cusack et al., 2016). This role of soil in regulating terrestrial C storage is one of the largest sources of uncertainty in global C models (Cox et al., 2000; Jones and Falloon, 2009). Therefore, understanding long-term controls over SOC storage is crucial for projecting atmospheric greenhouse gas concentration and global warming (Marschner et al., 2008).

New C enters soils as the byproducts of incomplete decomposition of litter, litter leachate, root exudates, root turnover, and microbial biomass turnover (Kögel-Knabner et al., 2008). Much of this SOC entering the soil is susceptible to decomposition and subsequent loss to the atmosphere

as CO_2. However, some forms of SOC (e.g. charcoal, mineral-associated SOC) have high stability and can remain protected in soil for thousands of years (Kögel-Knabner et al., 2008). Climatic variables, microbial activity, soil mineralogy, and tissue C chemistry each have the potential to affect the fate of C in soils, and the interactions among these controls vary in different environments. Our mechanistic understanding of how these factors interact with each other to determine soil C storage is still rudimentary. New knowledge about how the relative strength of these drivers change with changing environment is necessary to accurately predict the C storage capacity of soils, particularly under global change.

Given the great necessity to improve prediction of soil C stability under global change, the main objective of this dissertation is to advance understanding of how biotic and abiotic controls regulate C accumulation and stabilization in soils across a range of environmental gradients, and under warming scenarios. The research will use a series of field and laboratory studies to quantitatively assess the interacting roles of vegetation, soil mineralogy, microbial activity, C chemistry, and temperature in regulating the fate C in soils. In terms of ecosystem types, this research uses two ecosystems in extreme ends of the C density per unit area, tropical forest with very high C density and drylands with low soil carbon density (Jobbágy and Jackson, 2000), although both ecosystems store a similar amount of global carbon stocks (~30% of global SOC).

The outcomes of this dissertation will add to the current body of knowledge about mechanisms of C stabilization across environmental conditions and with warming.

Dissertation Outline

The overall aim of this research is to advance understanding of how biotic and abiotic controls regulate C accumulation and stabilization in soils across a range of environmental

gradients and under warming scenarios. All chapters excluding the introduction and conclusion are written as peer-reviewed publications for a scientific journal and will be submitted as primary research articles.

Chapter 1 provides the context and organization for the remaining work. Chapter 2 assesses how soil mineralogy, soil nutrient and C status, and C chemistry interact to determine warming effects on the fate of newly added soil C using a 13C isotopic carbon tracing approach. We used soils from three montane tropical forest sites along a soil weathering gradient, adding ^{13}C-labeled glucose and ^{13}C-labeled glycine in a lab warming incubation at ambient, +5 and +10 ^{o}C. By tracking the added ^{13}C label in soil pools at four days and 255 days, we found that Initial microbial uptake of ^{13}C and average CUE were strongly correlated with longer-term SOC retention in mineral soils. Our results indicate that microbial incorporation of organic compounds into biomass promotes longer-term C retention in soil. Overall, warming had a negative effect on ^{13}C retention in soil in the youngest soil only, with no warming effect on moderately to highly weathered soils. Thus, soil C stocks in tropical forests on less weathered soils, and with lower microbial CUE, may be most vulnerable to C loss with a warming climate.

Chapter 3 assessed the fate of added organic compounds in soils over two years, comparing C loss versus storage along a long substrate age gradient (LSAG) across wet Hawaiian forests, and along a climate and soil weathering gradient in Hawaiian grasslands. A long-term field fertilization experiment was also used at one site to assess the influence of nutrient variation on C storage. Replicate soil columns were inserted into soils at each site, and ^{13}C-labeled glucose, ^{13}C-labeled glycine, or charcoal was added to each column. Added C was tracked into microbial biomass, dissolved organic C (DOC), and the soil matrix at one- and two-year time points. After two years,

most of the added ^{13}C label from organic compounds remained within the top 0 – 5 cm of soil. After two years, an average of 8.7% and 3.0% of added ^{13}C added was recovered in mineral soil in forest and grassland soils, respectively, with ~2.8*x* greater retention for glucose versus glycine after one and two years. There was no effect of soil mineralogy or the long-term nutrient additions on ^{13}C retention in the soil after two years. ^{13}C NMR revealed an increase in the proportion of alkyl C in glucose and glycine amended soil, with alkyl C indicative of lipids such as may be produced by microbial biomass. Our results indicate that microbial incorporation of organic compounds like glucose into biomass may promote the production and storage of alkyl C compounds. Thus, the chemistry of C inputs to soils, subsequent incorporation and retention into microbial biomass, and vegetation cover type had more importance for two-year soil C storage than other ecosystem factors like soil mineralogy and nutrient availability.

Chapter 4 estimated the long-term effect of grass cover loss on soil organic carbon (SOC) and total nitrogen (TN) storage and their spatial heterogeneity in two arid grassland communities in New Mexico, United States. Enhanced wind erosion was encouraged by experimentally reducing grass cover (but not shrub) for nine years. The nine years of grass removal resulted in soil deflation in the 100% grass removal plots and deposition in the area immediately downwind to the plots. Enhanced erosion resulted in the decline of 30% of SOC and 35% of TN concentrations in the surface (0-5cm) soil in the 100 % grass removal plots. The change in SOC and TN concentration in the soil immediately downwind of the grass removal plot (downwind plot) was variable and likely depended on the structure of the vegetation community of that site. Geostatistical analysis showed a lower range of autocorrelation (A_0) and the proportion of variance that is spatially structured in the plots downwind of 100 % grass removal plots relative to control plots indicating that weaker but smaller fertile islands, compared to the control, appeared to

develop over nine years of aeolian transport.

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Chapter 2 Effects of warming on the fate of newly added carbon to soils along a Hawaiian weathering gradient

Abstract

Tropical forest soils contain some of the largest carbon (C) stocks on Earth, and these ecosystems are predicted to undergo substantial warming in the coming decades. Effects of warming on the fate of organic C entering soils, and how this will vary across landscape gradients in soil properties, is poorly understood. This research sought to understand how soil mineralogy, soil nutrient and C status, and C chemistry interact to determine warming effects on the fate of newly added soil C. We hypothesized that soils with greater microbial uptake of added C at the beginning of the experiment would promote greater soil organic C (SOC) retention on mineral surfaces after eight months, demonstrating the importance of microbial processing and incorporation into protenaceous biomass for promoting soil C retention. We predicted that soils with greater nutrient availability would promote greater initial microbial uptake of C. We used soils from three montane tropical forest sites along a soil weathering and nutrient availability gradient, adding ^{13}C-labeled glucose and ^{13}C-labeled glycine in a lab warming incubation at ambient 16 °C, + 5 °C, and +10 °C for 255 days. Added ^{13}C was tracked into microbial biomass, dissolved organic C (DOC), and the soil matrix after four days and 255 days, calculating microbial C use efficiency (CUE) over the first four days. Initial microbial uptake of ^{13}C and average CUE were strongly correlated with longer-term SOC retention in mineral soils (R^2 = 0.71, n=36). Overall, 2.9% – 17.8% of added C was retained in microbial biomass on day four, dropping to 0.3% – 6.9% by day 255. In contrast, 6.6% – 32.3% of added C was in mineral soil pools on day 4, and 6.9% – 23.3 % was retained by day 255. ^{13}C retention showed significant variation among

sites, substrates, and temperatures over time. Overall, warming had a negative effect on ^{13}C retention in soil in the youngest soil only, with no warming effect on moderately to highly weathered soils. Warming also caused a significant decline in microbial CUE, which in turn was greater for glucose versus glycine. Glucose was assimilated into microbial biomass at a greater rate than glycine, and subsequently had greater longer-term retention in soils. Overall, our results indicate that microbial incorporation of organic compounds into biomass promotes longer-term C retention in soil. Also, less reactive mineral content in younger, less weathered soils might make new SOC more vulnerable to warming relative to soil with more reactive mineral content. Thus, soil C stocks in tropical forests on less weathered soils, and with lower microbial CUE, may be most vulnerable to C loss with a warming climate.

Introduction

Tropical forests store around one-third of the world's soil C pool (Jobbágy and Jackson, 2000) and tropical regions are expected to warm significantly over the next two decades (Diffenbaugh and Scherer, 2011; Mora et al., 2013). Soils can be either a net C source or sink to the atmosphere (Sollins et al., 2007; Heimann and Reichstein, 2008; Trumbore and Czimczik, 2008), with the net balance likely sensitive to climatic warming because of changes in ecosystem processes like microbial respiration and plant growth (Trumbore, 1997; Davidson and Janssens, 2006; Cusack et al., 2016). Whether tropical forests will continue to serve as net sinks for carbon in a warmer world remains highly uncertain (Wood et al., 2012). Within tropical forests, soil responses to warming are likely to vary over gradients in nutrients and mineralogy.

A C compound newly introduced to the soil matrix could be directly sorbed to a mineral surface or occluded into microaggregates via a *mineral-regulated C storage pathway*, or could be

taken up by microbes with microbial biomass products subsequently stored in soils over longer periods via a *microbe-regulated C storage pathway*. Low molecular weight C compounds like amino acids, sugars, and carboxylic acids are an important constituent of C entering soils, and come from rhizodeposition, leaching of leaf litter, or as byproducts of decomposition. These small compounds are readily available to microbes and fuel heterotrophic respiration (van Hees et al., 2005; Fischer et al., 2010), but they can also contribute to soil C storage via direct sorption to mineral surfaces, or by longer-term recycling within microbial biomass and subsequent sorption to mineral surfaces (von Lützow et al., 2006; Miltner et al., 2012; Bradford et al., 2013; Cotrufo et al., 2015; Kallenbach et al., 2016). Longer-term stability of C in soils depends largely on spatial separation from microbial decomposers (e.g. in stable micro-aggregates) (Dungait et al., 2012; Solomon et al., 2012; O'Brien and Jastrow, 2013) and/or via formation of organo-mineral associations which can make C inaccessible to decomposers (Kleber et al., 2007; von Lützow et al., 2007; Marschner et al., 2008). Factors like the chemistry of new C moving into the soil, microbial activity and C use efficiency (CUE), soil nutrient availability, and mineralogy may influence the balance between *microbe-regulated* vs. *mineral-regulated* pathways of soil C storage. Warming is also likely to shift the balance between these two pathways differently across different sites.

First, the organic chemistry of C compounds may influence their fate. For example, microbes were able to quickly (<5 h) outcompete mineral sorption for alanine, glucose, and acetate in an Alfisol (Fischer et al., 2010), while citrate remained unprocessed by microbes much longer than glucose (over 22 h) due to rapid and strong sorption to ferric hydroxide (Jones and Edwards, 1998). Similarly, rapid sorption to the soil matrix reduced the bioavailability of glutamate, glycine, and lysine in a Eutric cambisol soil (Jones and Hodge, 1999), and of glycine, lycine and glutamate

in Calcic Palexeralf soil (Vinolas et al., 2001). Thus, some compounds like glucose can be assimilated by microbes quickly (Allison and Vitousek, 2004; Waldrop et al., 2004), yet direct sorption into mineral surface may occur more quickly for some compounds in the presence of highly reactive mineral surfaces (Keiluweit et al., 2015).

Second, soil mineralogy is likely to influence the relative strength of mineral- versus microbe-regulated soil C storage. The formation of organo-mineral associations is more rapid in soils with greater binding site densities, such as silt, clay, short-range ordered (SRO) Al-silicates (e.g. allophane and imogolite), Fe oxides, and permanently charged phyllosilicate clay minerals (Cox et al., 2000; Kleber et al., 2015).

Third, the capacity for microbes to take up C and build new biomass, rather than losing C to respiration, or CUE, will also affect the fate of C in soil. Microbial growth and turnover are increasingly recognized as principle mechanisms leading to longer-term retention of C in soils (Cotrufo et al., 2013; Kallenbach et al., 2016; Liang et al., 2017). Faster growth and microbial necromass production with greater CUE appear to promote greater C storage in soils (Sinsabaugh et al., 2013; Zheng et al., 2019). Microbial CUE, in turn, appears to depend in part on the chemical quality of available C, the soil microbial community structure (Manzoni et al., 2012; Sinsabaugh et al., 2013; Lee and Schmidt, 2014; Bölscher et al., 2016), and nutrient availability (Ågren et al., 2001).

Warming is likely to affect pathways of soil C stabilization by affecting microbial enzyme activities, CUE, and adsorption/desorption kinetics (Davidson and Janssens, 2006; Allison et al., 2010), with warming generally expected to accelerated microbial respiration (Davidson and Janssens, 2006; Crowther et al., 2016), and suppress microbial CUE across organic compounds

(Steinweg et al., 2008; Manzoni et al., 2012; Li et al., 2019; Zheng et al., 2019). Some studies have found that microbial CUE responds positively or not at all to warming (Steinweg et al., 2008; Frey et al., 2013; Sinsabaugh et al., 2013), and the factors governing microbial CUE response to warming remain poorly understood. Adsorption and desorption process rates are also temperature sensitive, but since both might increase (Conant et al., 2011), the net effect of warming on the sorption balance is expected to be low (Davidson and Janssens, 2006).

This study assessed the effects of warming on the fate of two newly added low molecular weight C compounds to soils from a weathering and soil nutrient gradient in Hawaii. We tracked two ^{13}C-labeled compounds into mineral soil, microbial biomass, and DOC over 255 days using ambient and warmed conditions. We tested three hypotheses: (H1) short-term microbial uptake of ^{13}C into biomass promotes longer-term retention in soils, with microbial uptake rates greatest in soil with low mineral sorptive capacity; (H2) low molecular weight C compounds that include nitrogen (N) (e.g. glycine) are taken up in greater proportion and therefore have greater retention in soils relative to compounds without N (e.g. glucose), with greater microbial uptake also occurring in more nutrient-rich soils; (H3) warming favors microbial uptake and respiration of new C (i.e. suppresses CUE) particularly in soils with lower sorptive capacity, where microbe-regulated pathways of C retention are dominant. We, therefore, predicted that fresh C in soils with low mineral sorptive capacity would be the most vulnerable to losses with warming.

Methods

Site Description

This study was conducted using soils from the long substrate age gradient (LSAG) in Hawaii, USA. The LSAG experimental sites included three montane forests along a soil

weathering gradient on a basaltic lava flow across the Big Island of Hawaii that have similar elevation, climate, plant species composition, topography, and parent material, but vary substantially in parent material age (Crews et al., 1995; Chorover et al., 2004; Vitousek et al., 2004). The mean annual temperature and precipitation of the sites are 16 ^{o}C and 2500 mm respectively and all the sites are located at elevation ~1200 m (Chadwick et al., 1999; Giambelluca et al., 2013). All the sites have native tree species *Metrosideros polymorpha* as the dominant vegetation (Kitayama and Mueller-Dombois, 1995). The sites included a 300-yr-old soil (Thurston N 19°25', W155°15'), a 20,000-yr-old soil (Laupahoehoe N 19 55 ^{o}.73', W 155^{o} 18.06'), and a 150,000-yr-old soil (Kohala N 20 ^{o} 03.06', W115 ^{o} 41.05'), providing a distinct soil mineralogy gradient from poorly weathered to strongly weathered (Herbert et al., 1999). The mineral soil at Thurston consists of primary minerals like olivine, glass, and plagioclase feldspar, with increasing amounts of metastable noncrystalline secondary minerals like primary ferrihydrite, allophane, and imogolite along the gradient to Laupahoehoe and then Kohala (Shoji et al., 1993; Torn et al., 1997). We categorized Thurston, Laupahoehoe, and Kohala soils respectively as "low", "intermediate", and "high" binding site density on mineral surfaces (Shoji et al., 1993; Torn et al., 1997; Torn et al., 2005).

Soil Collection and Lab Incubation

Mineral soils from 0 – 10 cm depths were collected from the three sites in 2015, removing the organic soil horizon to compare only mineral soils among sites. After collection soils were maintained at ambient temperature and shipped to UCLA within five days. Soils were passed through 2 mm sieves removing roots, visible plant and animal remains, and gravel. Initial gravimetric soil moisture content was determined by drying soil at 105 ^{o}C until mass stabilized, and initial soil pH was measured in a 4:1 water-to-soil volumetric ratio using a benchtop pH meter.

Homogenized fresh soil samples were then places in glass incubation vessels (115 ml capacity), using 15 g of dry-weight equivalent. Replicate samples received wither: (1) 35.5 mg of >99 atom% ^{13}C-labeled glucose, (2) 44.5 mg of >99 atom% ^{13}C-labeled glycine (Sigma-Aldrich, USA), or (3) 3 ml deionized (DI) water (control), such that samples with label received almost 1mg of ^{13}C/g-dry-soil. The labeled glucose and glycine were added to the soil as DOC in 3 ml DI water. Prior to the addition, a simple isotopic mixing model was used to calculate the quantity of each material required to increase the δ^{13}C in the soils by 5 – 10‰ if at least 1% of each added organic compound is incorporated into the mineral-associated soil fraction (Phillips and Koch, 2001).

Soils were then covered with perforated cellophane wraps to allow gas exchange while maintaining high soil moisture. Ambient-controlled incubations were conducted in the dark in triplicate (n = 3) at temperatures 16 °C (ambient), 21 °C, and 26 °C, with two sets of identical soils prepared for a subsequent early harvest (4 days) and a late harvest (255 days), for a total of 162 incubation vessels. Thus, the experimental design was fully factorial. Soils were maintained at field soil moisture level by watering with DI water to initial weight every 5 days.

Respiration and Chemical Analyses

The rate of CO_2 flux was measured for each soil every 15-30 days. Compressed air was used to flush the headspace for 1 minute to achieve a standard starting atmosphere at the beginning of each measurement, assessing the CO_2 concentration in the compressed air as background. Each glass vessel was fitted with a gas-tight lid with septa (Airtight M-Type Septa Type 6 mm septa, Altech Corp) and placed at the respective incubation temperature. After 1.5 to 5 hours of closure, 5 ml of headspace gas from the jars were collected using an airtight gas syringe (SGE - 10MDR-VLLMA-GT; Restek Corporation), and the CO_2 concentration was measured immediately by injection into a bench-top infra-red gas analyzer (Li-COR model LI-820, Lincoln, NE, USA).

Cumulative C lost via respiration over the incubation period was determined by integrating CO_2 efflux rate values across measurements using a linear interpolation between sampling dates (Cusack et al., 2009). Respiration measurements were extrapolated to day 1 by fitting a quadratic line for the logarithmic fit using the measured instantaneous efflux rates at 11 different time points.

Temperature sensitivity (Q_{10}) value of soil heterotrophic respiration was calculated according to Leifeld and Fuhrer (2005) as:

$$Q_{10} = (k2/k1)^{10/(t2-t1)}$$

Where (k_2) = rate of heterotrophic respiration at t_2, (k_1) = rate heterotrophic respiration at t_1. Apart from Q_{10} temperature sensitivity (i.e. 16 ºC to 26 ºC warming), temperature sensitivity was also calculated for 16 ºC to 21 ºC warming and 21 ºC to 26 ºC warming to assess whether the range of temperature increase altered temperature sensitivity.

Soils were harvested after four days and 255 days. The ^{13}C incorporated into DOC and microbial biomass was assessed by extracting soils using a chloroform fumigation slurry method (Fierer and Schimel, 2003) . Briefly, 8 – 10 g soil was extracted with 0.25 M K_2SO_4 by shaking for 4 hours and filtered through pre-rinsed Whatman #1 filter paper for DOC. A second sample was shaken with 0.5 ml ethanol-free chloroform and extracted the same way, with the difference between the two extractions used as a measure of microbial biomass. No conversion for extraction efficiency was used. Then, 1 ml of filtrate from each extraction was evaporated at 60 °C in a tin cone in a ventilated oven (Dijkstra et al., 2006). The resulting residue was rolled and analyzed at UC Davis Stable Isotope Facility to calculate total C and the $^{13}C/^{12}C$ ratio in DOC and microbial biomass. The residual soil in the filter paper after the microbial biomass extraction was also transferred to an aluminum weighing dish and air dried, and then analyzed for total C and the

$^{13}C/^{12}C$ ratio using combustion system (Costech Analytical, Valencia, CA, USA) paired to Thermo Delta V isotope ratio measuring mass spectrometer (IRMS) at UCLA. The residual soils was taken as a measure of non-microbial SOC in mineral soil. Thus, ^{13}C partitioning into DOC, microbial biomass, and mineral soil was accounted for, calculating the ^{13}C lost to respiration by difference (Hagedorn et al., 2003).

We also measured the temperature response of microbial CUE of added ^{13}C-labelled organic compounds at four days as a proxy for temperature response of microbial efficiency as:

$$\mathrm{CUE} = \frac{\mathrm{dB_C}}{(\mathrm{dB_C} + \Sigma \mathrm{CO_2} - \mathrm{C}}\,,$$

Where, dB_C is the amount of C from added organic compound incorporated into microbial biomass and ΣCO_2–C is the cumulative C lost from added organic compound (Frey et al., 2001; Frey et al., 2013). Since the estimated half-life period of glucose and glycine in microbial biomass is only few hours (Kuzyakov and Demin, 1998; Hill et al., 2008; Hobbie and Hobbie, 2013) our CUE measurement after 4 days represents whole-soil level CUE that includes microbial biomass turnover and substrate recycling (Geyer et al., 2016).

Statistical Analyses

To directly test our hypotheses, we assessed: (H1) the relationship between 4-day microbial biomass ^{13}C incorporation, and 255-day mineral soil ^{13}C retention for each site of varying mineral reactivity; (H2) temperature as a main effect determining the retention of added ^{13}C over time across sites, and the temperature effect on microbial respiration per microbial biomass at 4 days and at 255 days (an indication of microbial CUE); (H3) the retention of ^{13}C from glucose versus glycine in different soil C pools over time and sites, including site-level nutrient availability.

Across analyses, standard least square analysis of variance (ANOVA) models were used

to investigate the effects of site, warming, organic compound, and time on soil respiration, soil C, and ^{13}C retention, using backward stepwise models to identify significant factors, and comparing BIC values to identify the minimum adequate model, which was then run. We also ran a separate ANOVA for each time point individually to investigate the effects of site, warming, organic compound at that time. Since heterotrophic respiration was measured at multiple time points, repeated measures ANOVA was used to assess its predictors across time points. Models were checked to ensure there was no heteroscedasticity in the residuals and that the response variables were a reasonable linear function of the fitted values, with errors closely distributed. Tukey means separation tests were used for post-hoc comparisons. Results are presented as the mean ± one standard error unless otherwise noted, with n = 3, unless replicates were pooled because of a lack of main effect. Data for DOC and heterotrophic respiration normalized by microbial biomass was log transformed to meet assumptions of normality for ANOVA. Statistical significance was determined as $p < 0.05$ unless otherwise noted. Analyses were conducted using 14.0.0 JMP software (SAS Institute Inc. Cary, NC, USA, 2019).

Results

Soil Carbon Pools Across Sites with Warming

Bulk soil C and N concentrations varied significantly by site, with no significant effect of temperature, organic compound, or time (Appendix Table A2). Thurston had significantly lower soil C and N concentrations followed by Kohala, and then Laupahoehoe (Table A1).

For microbial biomass, temperature and time point were significant factors, with no effect of compound added, and significant interactions of site × time, and temperature × time interaction. Laupahoehoe had the greatest microbial biomass C followed by Kohala and Thurston (Figure 1a).

The site × time interaction resulted from a significant decline in microbial biomass C at 255 days versus 4 days at Kohala and Laupahoehoe sites but not at Thurston. The temperature × time interaction resulted from the significant negative effect of warming on microbial biomass C at the end of the experiment but not at 4 days (Figure 1b). Interacting effects reflected variation among treatments over time, but the overall directions of the main effects generally held (Figure 1a, 1b). Soil microbial biomass C was significantly positively correlated with soil C content (n=108, R^2 = 0.66, $p < 0.001$) and N content (n = 108, $R^2 = 0.70$, $p < 0.001$) across all samples.

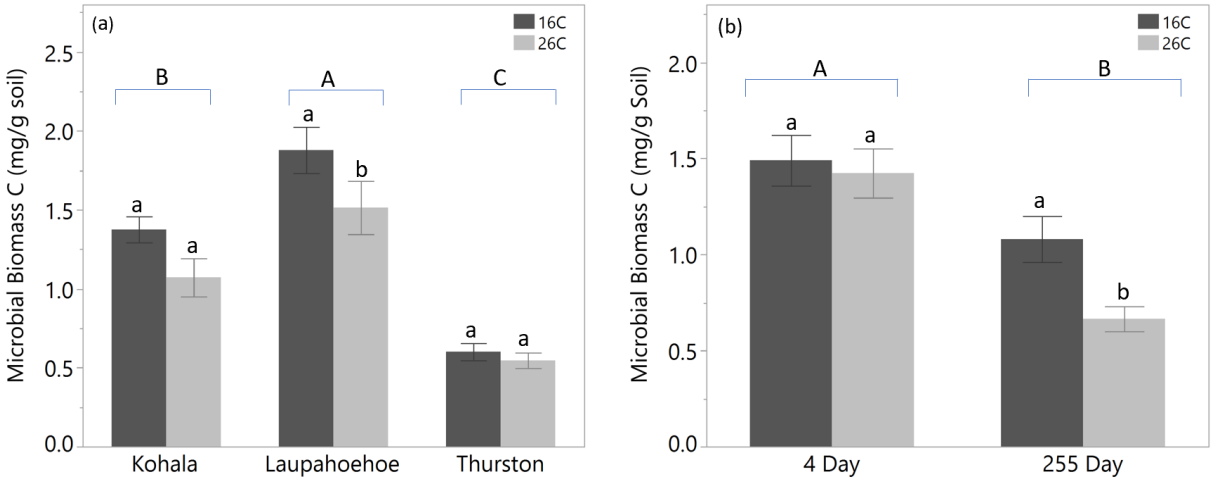

Figure 1 a.) Soil microbial biomass C is shown after 4 days and 255 days of incubation for three tropical forest sites. Data are pooled across the organic compound added and time (n = 18). Differences among sites are shown by uppercase letters, and differences between temperature treatments within sites are shown by lowercase letters. b.) Soil microbial biomass C after 4 days and 255 days of incubation. Data are pooled across organic compound added and sites (n = 27). Differences in microbial biomass C between time points are shown by uppercase letters, and differences among treatments within that time are shown by lowercase letters. Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

Total soil microbial biomass C was also standardized per g soil C, which similarly varied significantly with site, temperature, and time, with no effect of added organic compound, and a significant temperature × time interaction (Appendix Table A1; Appendix Figure A2b). Kohala had the greatest microbial biomass per unit of soil C, followed by Laupahoehoe and Thurston.

Like bulk soil C, total extractable DOC also varied significantly by site, with no experimental effects (Appendix Table A1), and was significantly positively correlated with soil C ($n = 108$, $R^2 = 0.79$, $p < 0.001$). Thurston had the lowest extractable DOC (0.4 ± 0.03 mg C g^{-1} soil), followed by Kohala (0.73 ± 0.02 mg C g^{-1} soil), and Laupahoehoe (1.14 ± 0.08 mg C g^{-1} soil).

The average pH at Kohala, Laupahoehoe, and Thurston was 4.41 ± 0.07, 3.9 ± 0.08 and 5.26 ± 0.07 respectively, while the average gravimetric field moisture content was 67.96 ± 1.96 %, 70.7 ± 2 % and 62.1 ± 5.63% respectively.

Soil Respiration Across Sites and Treatments

Repeated measures ANOVA showed that instantaneous CO_2 fluxes declined significantly over time across sites and treatments (Appendix Figure A2a). Total cumulative respiration over the incubation period varied by site and temperature, with no effect of the organic compound added. Among sites, Laupahoehoe had significantly greater cumulative respiration per g soil than Kohala and Thurston (Appendix Figure A2B). Soil cumulative heterotrophic respiration was significantly positively correlated with soil C content ($R^2 = 0.80$, $n = 81$, $p < 0.001$) and N content ($R^2 = 0.69$, $n = 81$, $p < 0.001$) across sites. Standardizing soil respiration to organic soil C, in contrast, gave the largest values for Thurston followed by Laupahoehoe and Kohala (Figure 2a, 2b, Table 1).

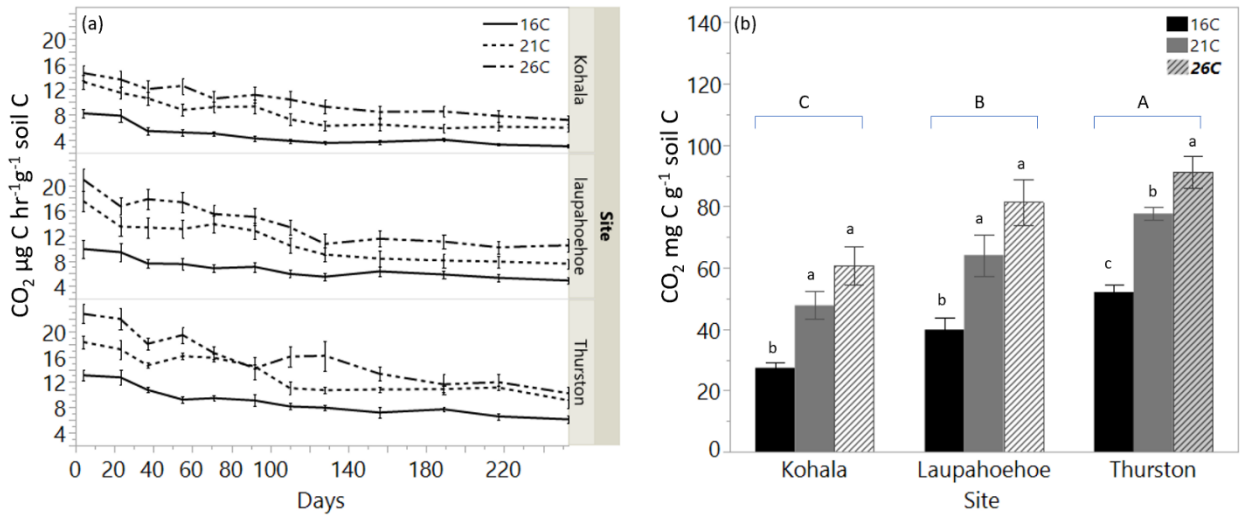

Figure 2 a.) Average hourly heterotrophic respiration rates from soil per mass of soil carbon over a 253-day incubation. b.) Total cumulative heterotrophic respiration over 253 days per mass of soil C is shown, showing differences among sites (uppercase letters), and differences among temperature treatments within sites (lowercase letters). For both graphs, data are pooled across organic compounds added (n = 9). Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

Cumulative soil respiration normalized to microbial biomass C also varied significantly by site and temperature, with no effect of time or compound added, but with a significant interaction of compound × temperature. Among the sites, Laupahoehoe and Thurston had significantly greater respiration per microbial biomass C compared to Kohala (Table 1), and respiration per microbial biomass C was significantly greater at 26 ºC versus 16 ºC (Table 1).

Table 1 Cumulative heterotrophic respiration per mass of soil C, respiration per mass of microbial biomass C, and temperature sensitivity (Q10)

Site	Cumulative Heterotrophic Respiration (CO_2 mg g^{-1}soil C)			Log (1+Respiration per microbial biomass C) (µg CO_2-C [mg microbial C] $^{-1}$ hr^{-1})		Temperature Sensitivity
	16 ºC	21 ºC	26 ºC	16 ºC	26 ºC	Q_{10} (16-26 ºC)
Kohala	27.33 ± 1.88^{Bb}	47.71 ± 4.5^{Ba}	60.82 ± 6.22^{Ba}	0.55 ± 0.05^{Bb}	1.13 ± 0.06^{Ba}	2.2 ± 0.13^{A}
Laupahoehoe	39.81 ± 3.86^{ABb}	64.12 ± 6.77^{ABa}	81.38 ± 7.47^{ABa}	0.86 ± 0.12^{Ab}	1.44 ± 0.08^{Aa}	2.08 ± 0.09^{A}
Thurston	52.06 ± 2.46^{Ac}	77.64 ± 2^{Ab}	91.33 ± 5.26^{Aa}	0.98 ± 0.05^{Ab}	1.44 ± 0.05^{Aa}	1.75 ± 0.05^{B}

Values are mean ± SE. Data were pooled across organic compound added (n = 9). Significant differences among sites for each temperature are indicated by uppercase letters down the column and differences among temperatures treatments for each site are indicated by lowercase letters across the row. For temperature sensitivity, significant differences among the sites are indicated by uppercase letters down the column. Comparisons are conducted using Tukey HSD Tests.

Temperature sensitivity (Q_{10}) of heterotrophic respiration per mass of soil varied by site, with no effect of organic compound added. Kohala had significantly greater Q_{10} values than the other sites (Appendix Table A3). Temperature sensitivity (Q_5) was greater for warming from 16 ^{o}C to 21 ^{o}C compared to warming from 21 ^{o}C to 26 ^{o}C (Appendix Table A3), such that the initial 5 ^{o}C of warming above ambient had the greatest effect on calculated Q_{10} values. Temperature sensitivity per mass of soil C also varied by site with no effect of the added organic compound, with similar patterns as above (Table 1).

The Fate of Added ^{13}C in Soil Pools

^{13}C in Microbial Biomass and CUE

Microbial CUE over the first 4 days varied significantly by organic compound and temperature, while site and interactions were not significant (Table 2). Microbial CUE was significantly greater at 16 ^{o}C versus 26 ^{o}C, and for glucose versus glycine (Table 2). Addressing our hypothesis, microbial CUE at 4 days was positively and significantly correlated to the retention of ^{13}C into mineral soil C at the end of the experiment (R^2 = 0.71, n=36, p<0.001) (Figure 3a). Microbial CUE was significantly related to long term retention of ^{13}C into mineral soil C at both 16 ^{o}C (R^2 = 0.60, n = 18, p = 0.009) and at 26 ^{o}C (R^2 = 0.81, n = 18, p < 0.001).

The percentage of added ^{13}C retained in microbial biomass represented the second largest pool for ^{13}C retention in soil (Figure 4), and varied significantly with organic compound, temperature, and time, with significant interactions of organic compound × time, and organic compound × temperature × time, which site was not significant (Figure 5a, 5b, Appendix Table A4). Overall, ^{13}C retention in microbial biomass C was significantly greater at 16 ^{o}C vs. 26 ^{o}C, was greater at four days vs. 255 days, and was greater for glucose vs. glycine (Figure 5a, 5b). The

organic compound × temperature × time interaction resulted because warming at four days caused a significant decline in ^{13}C retention in microbial biomass C only from glycine but not from glucose, whereas at 255 days it caused a significant decline in ^{13}C retention only from glucose but not from glycine (Figure 5a). Across treatments, an average of 11.5 ± 1.1% of added ^{13}C was retained in microbial biomass after four days of incubation, while only 2.4 ± 0.4% was retained after 255 days.

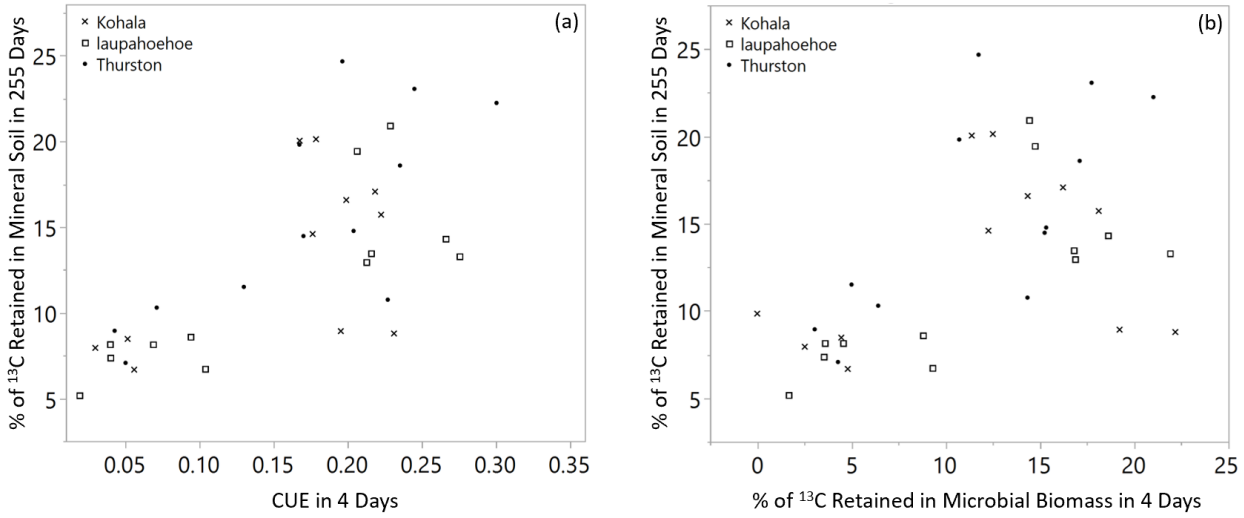

Figure 3 a.) Relationship between microbial CUE at four days versus percent of ^{13}C recovery in mineral soil at 255 days (R^2 = 0.71). b.) Relationship between the percent of ^{13}C recovery in microbial biomass at four days versus percent of ^{13}C recovery in mineral soil at 255 days (R^2 = 0.57).

Table 2 Partitioning of added ^{13}C across different soil pools after four days and 255 days of incubation is shown by organic compound added, site, and temperature. Calculated CUE after four days is also shown, with ANOVA model results shown at the bottom.

Factor	Treatment	CUE	Percent Recovery - 4 Days				Percent Recovery - 255 Days			
			DOC	microbial biomass C	Mineral	All Pools	DOC	microbial biomass C	Mineral	All Pools
Organic Compound	Glucose	0.22±0.01[b]	0.64±0.12[b]	15.65±0.76[a]	27.69±1.34[a]	43.98±1.06[a]	0.31±0.07[a]	3.72±0.56[a]	17.88±0.85[a]	21.92±1.00[a]
	Glycine	0.09±0.01[a]	7.9±3.70[a]	7.38±1.48[b]	11.87±2.07[b]	27.16±3.81[b]	0.22±0.06[a]	1.01±0.19[b]	8.78±0.50[b]	10.01±0.52[b]
Site	Kohala	0.14±0.02[a]	4.34±3.21[a]	11.5±2.05[a]	18.42±3.04[a]	34.26±3.86[a]	0.17±0.06[a]	2.81±0.76[a]	12.91±1.43[b]	15.88±1.94[ab]
	Laupahoehoe	0.15±0.03[a]	0.8±0.38[a]	11.22±1.98[a]	19.55±3.05[a]	31.58±4.23[a]	0.48±0.1[a]	2.47±0.65[a]	11.56±1.46[b]	14.51±1.93[b]
	Thurston	0.17±0.02[a]	7.67±4.80[a]	11.83±1.72[a]	21.37±3.49[a]	40.86±4.31[a]	0.14±0.03[a]	1.83±0.51[a]	15.53±1.73[a]	17.5±2.17[a]
Temperature	16 ºC	0.18±0.02[a]	7.98±3.71[a]	13.76±1.43[a]	18.82±3.07[a]	40.55±3.38[a]	0.25±0.07[a]	3.57±0.59[a]	14.19±1.32[b]	18.01±1.71[a]
	26 ºC	0.12±0.01[b]	0.57±0.13[b]	9.28±1.46[b]	20.74±1.97[a]	30.58±3.10[b]	0.28±0.06[a]	1.17±0.23[b]	12.47±1.25[a]	13.91±1.43[b]
Effect Test for the Full Model										
Site (n=12)		0.3324	0.1092	0.9473	0.6342	0.0794	0.0080	0.0701	0.0008	0.0246
Compound (n=18)		<0.0001	0.0050	<0.0001	<0.0001	<0.0001	0.2909	<0.0001	<0.0001	<0.0001
Temp (n=18)		0.0006	0.0015	0.0065	0.453	0.0057	0.6974	<0.0001	0.03	<.0001
Site × Compound		0.1231	0.0581	0.202	0.6433	0.2766	0.3070	0.8317	0.662	0.8068
Site × Temp		0.3525	0.1820	0.3689	0.1908	0.5001	0.9564	0.1408	0.0406	0.2579
Compound × Temp		0.0528	0.0005	0.0894	0.2785	0.0313	0.4780	0.0002	0.7539	0.1402
Site × Compound × Temp		0.9469	0.0868	0.981	0.5111	0.6174	0.9359	0.4232	0.3503	0.7289

Values are mean ± SE. For each factor, mean ± SE values of the treatments not connected by the same letters (in the same column) are significantly different at $p < 0.05$ using Tukey HSD tests. Effect test results are shown at the bottom, including p-values of the respective treatment effect or their interaction from the full least square ANOVA model.

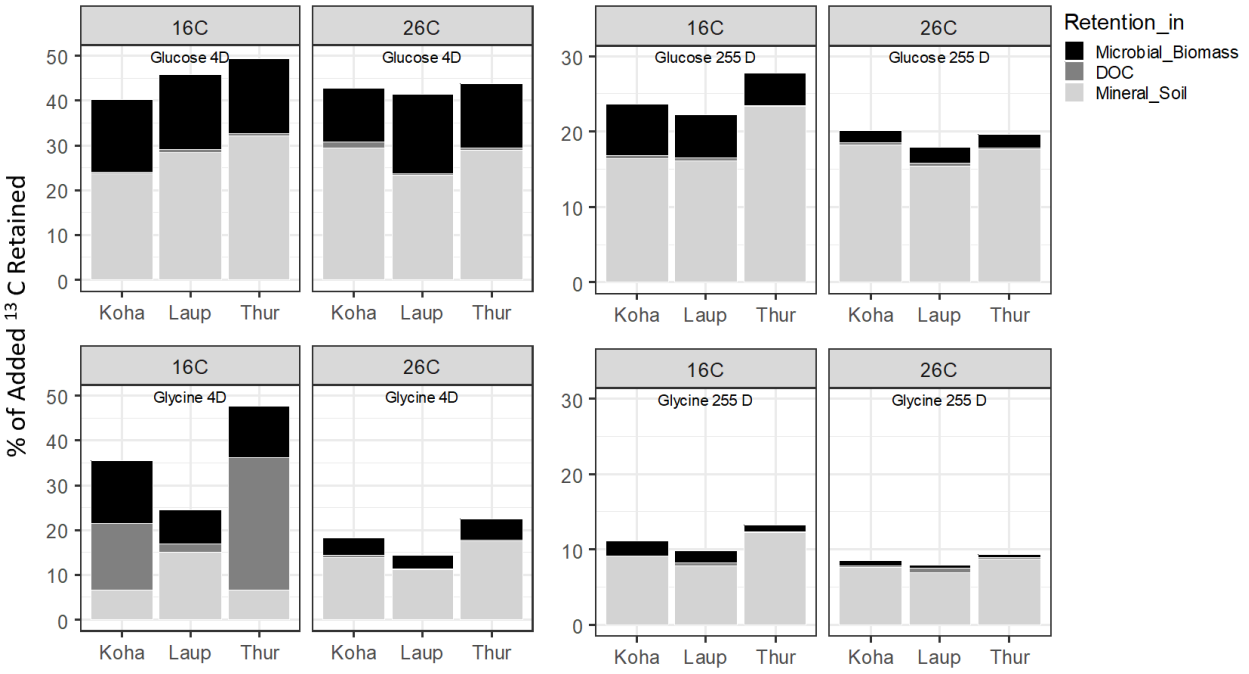

Figure 4 Overall partitioning of ^{13}C from added organic compound retained into DOC, microbial biomass and mineral soil pools is shown for sites and temperature treatments for four days and 255 days of incubation (n = 3). Percent of ^{13}C added that was recovered in each pool is shown.

The percentage of added ^{13}C retained in microbial biomass represented the second-largest pool for ^{13}C retention in soil (Figure 4), and varied significantly with organic compound, temperature, and time, with significant interactions of organic compound × time, and organic compound × temperature × time, which site was not significant (Figure 5a, 5b, Appendix Table A4). Overall, ^{13}C retention in microbial biomass C was significantly greater at 16 ºC vs. 26 ºC, was greater at 4 days vs. 255 days, and was greater for glucose vs. glycine (Figure 5a, 5b). The organic compound × temperature × time interaction resulted because warming at 4 days caused a significant decline in ^{13}C retention in microbial biomass C only from glycine but not from glucose, whereas at 255 days it caused a significant decline in ^{13}C retention only from glucose but not from glycine (Figure 5a). Across treatments, an average of 11.5 ± 1.1% of added ^{13}C was retained in microbial biomass after 4 days of incubation, while only 2.4 ± 0.4% was retained after 255 days.

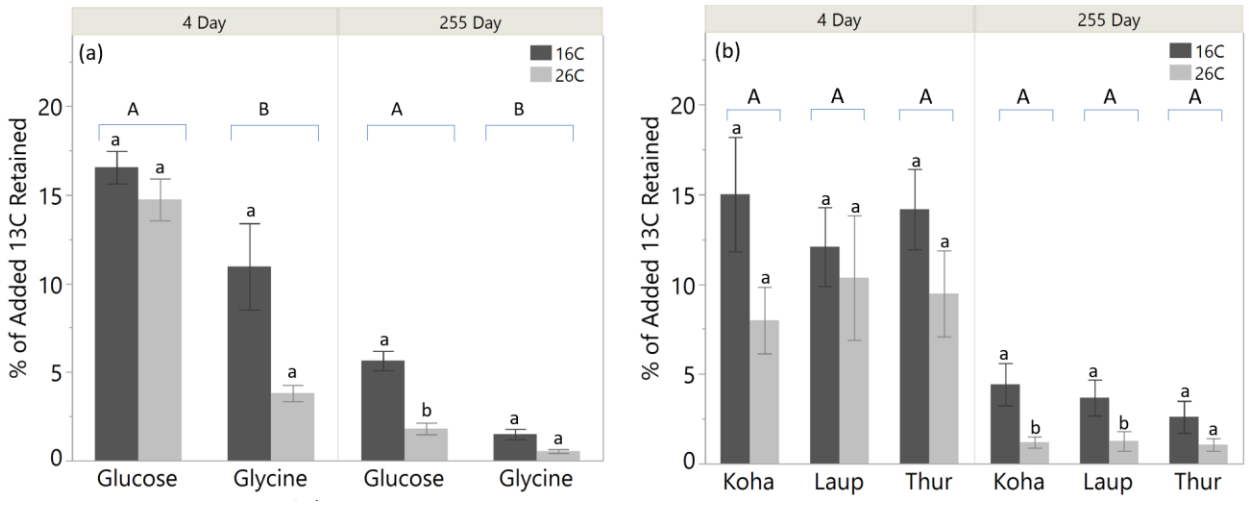

Figure 5 a.) Percent of added ^{13}C retained in soil microbial biomass C by organic compound added after four days and 255 days of incubation is shown. Differences between organic compounds in each time point are shown by uppercase letters, and differences between temperature treatments within organic compounds are shown by lowercase letters. Data are pooled across sites (n = 9). b.) Percent of ^{13}C retained in soil microbial biomass C by sites after four days and 255 days of incubation is shown. Data are pooled across compound added (n=9). Differences among sites for each time point are shown by uppercase letters, and differences among temperature treatments within sites are shown by lowercase letters. Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

Similar to patterns for CUE, the initial percentage of ^{13}C retained in microbial biomass was positively and significantly correlated to the retention of ^{13}C in mineral soil C at the end of the experiment ($R^2 = 0.57$, n=36, $p < 0.001$) (Figure 3b). When broken down by sites, this relationship was significantly positive in Laupahoehoe ($R^2 = 0.72$, n = 12, p = 0.008) and Thurston ($R^2 = 0.70$, n = 12, p = 0.011) but not in Kohala ($R^2 = 0.35$, n = 12, p = 0.270).

We also assessed the percent of ^{13}C retention in microbial biomass per unit of microbial biomass C as another indication of microbial efficiency. This value varied significantly with site, organic compound, temperature, and time, with significant interactions of site × time, and organic compound × temperature × time (Table A5). Overall, retention of ^{13}C in microbial biomass per unit of microbial biomass C was significantly greater at 16 ºC vs. 26 ºC, greater at 4 days vs. 255 days, and greater for glucose vs. glycine, similar to patterns for the total proportion of ^{13}C retained

in the microbial biomass. At 4 days, Thurston soil had 3x greater ^{13}C retention in microbial biomass per unit of microbial biomass C versus Laupahoehoe and 2x greater retention versus Kohala. At 255 days Thurston still had 1.9x greater retention than Laupahoehoe, and 1.3x greater retention in microbial biomass per unit of microbial biomass C than Kohala. These normalized retention values suggest greater microbial biomass retention efficiency for Thurston, and the lowest for Laupahoehoe, following patterns of total soil C availability in these sites.

^{13}C in Mineral Soil

The mineral soil contained the largest proportion of retained ^{13}C both at the beginning and the end of the experiment (Fig. 4), and this proportion varied significantly with the organic compound added and time, with a significant organic compound × time interaction, and no effect of site or temperature (Appendix Table A4). The ^{13}C retention in mineral soil was significantly greater for glucose vs. glycine, and greater at 4 days vs. 255 days (Figure 6a, Table 2). The organic compound × time interaction resulted from significantly a greater change over time for ^{13}C from glucose versus from glycine (Figure 6a). Overall, an average of 19.8 ± 1.8 % of added ^{13}C was retained in the mineral soil after 4 days of incubation, and 13.3 ± 0.9% was retained after 255 days (n = 36).

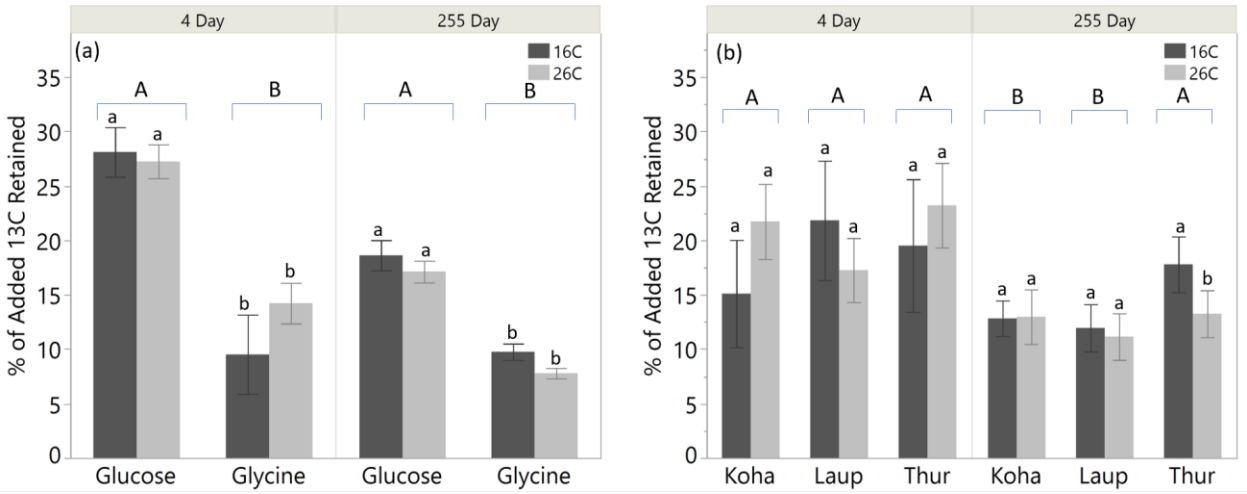

Figure 6 a.) Percent of ^{13}C retained in mineral soil by organic compound added after 4 days and 255 days of incubation is shown. Differences between organic compounds in each time point are shown by uppercase letters, and differences between temperature treatments within organic compounds are shown by lowercase letters. Data are pooled across sites (n = 9). b.) Percent of ^{13}C retained in mineral soil by sites after 4 days and 255 days of incubation. Data are pooled across compound added (n=9). Differences among sites for each time point are shown by uppercase letters, and differences among treatments within sites are shown by lowercase letters. Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

Assessing only the final time point, the proportion of ^{13}C retained in mineral soil varied significantly with site, organic compound, and temperature, and with a significant site × temperature interaction (Table 2). On average, ^{13}C retention at 255 days was significantly greater at Thurston vs Kohala and Laupahoehoe (Table 2, Figure 6b), and was significantly greater for glucose compared to glycine (Table 2). Warming had a significant negative effect on ^{13}C retention in mineral soil in Thurston at 255 days, but not in Kohala and Laupahoehoe, resulting in the significant site × temperature interaction (Table 2, Figure 6b). Thus, the greatest retention of ^{13}C in mineral soil after 255 days was for glucose added to Thurston, the least weathered soil, but ^{13}C in mineral soil at this site was also the most sensitive to warming.

^{13}C in DOC

Overall, only a tiny fraction of added ^{13}C remained in the DOC pool at 4 days, and even

less at 255 days, illustrating the rapid movement of added dissolved compounds into other soil pools (Table 2). The percentage of ^{13}C retained in DOC varied significantly with organic compound, temperature, and time, with interactions for site × organic compound, site × time, organic compound × temperature, organic compound × time, temperature × time, and organic compound × temperature × time (Appendix Table A4). Specifically, ^{13}C retention in DOC was significantly greater at 16 ºC vs. 26 ºC, greater at 4 days vs. 255 days, and greater for glycine vs. glucose, similar to trends for microbial biomass ^{13}C retention (Table 2). The temperature × time interaction resulted from greater ^{13}C retention at 16 ºC versus 26 ºC at 4 days, but not at 255 days. The organic compound × temperature interaction resulted from greater ^{13}C retention in DOC from glycine versus glucose at 16 ºC but not at 26 ºC. The organic compound × time interaction resulted from greater ^{13}C retention in DOC from glycine versus glucose at 4 days but not at 255 days. The organic compound × temperature × time interaction reflected greater retention of glycine ^{13}C in DOC at 4 days at 16 ºC vs. glucose, but not at 26 ºC or 255 days (Appendix Figure A4a). Across samples, an average of 4.3 ± 1.9 % of added ^{13}C was retained in the DOC after 4 days of incubation, and 0.3 ± 0.05% was retained after 255 days, constituting the smallest pool of ^{13}C retention (n = 36, Figure 4).

Total and Relative Retention of ^{13}C Across Soil Pools

Total ^{13}C retention across all soil pools (DOC + microbial biomass C + mineral soil pools) varied significantly with site, organic compound, temperature, and time, with a significant organic compound × temperature × time interaction (Appendix Table A4, Figure A4a). The total ^{13}C retention in all soil pools was greatest at Thurston followed by Kohala and Laupahoehoe (Table 2; Appendix Figure A4b). Total ^{13}C retention was greater at 16 ºC vs. 26 ºC, greater at four days vs.

255 days, and greater for glucose vs. glycine (Appendix Figure A4a). The interaction resulted because warming caused a significant decline in ^{13}C retention in soil at four days only for glycine but not for glucose, whereas at 255 days the trend reversed and warming caused a significant decline in ^{13}C retention for glucose but not for glycine (Appendix Figure A4a). Both at four days and at 255 days, warming had a negative effect on total ^{13}C retention in soil in Thurston, with no warming effect on Kohala or Laupahoehoe (Appendix Figure A4b). Overall, an average of 35.6 ± 2.4% of added ^{13}C was retained in soil after 4 days of incubation, and 16.0 ± 1.2% was retained in soil after 255 days (Table 4).

The relative partitioning of retained ^{13}C among soil pools helped clarify trends across sites and treatments and was less variable than the total proportion in each pool because this calculation controls for variation in respiration losses (Table 3). From 82 – 88% of recovered ^{13}C was in the soil mineral pool at the end of the experiment, and 10 - 16% of ^{13}C was retained in the microbial biomass pool at the end of the experiment. Among the sites, relative partitioning of ^{13}C to (microbial biomass): (mineral soil) at the end of the experiment was lower in Thurston versus the other two sites. Also, relative partitioning of ^{13}C to (microbial biomass): (mineral soil) was significantly greater at 16 ^{o}C vs. 26 ^{o}C.

Table 3 Relative partitioning of added ^{13}C across different soil pools after 255 days of incubation as influenced by organic compound added type, site, and temperature.

Factor	Treatment	DOC	microbial biomass C	Mineral Soil
Compound	Glucose	1.47 ± 0.33[a]	16.36 ± 2.36[a]	82.16 ± 2.37[b]
	Glycine	2.62 ± 0.85[a]	9.69 ± 1.65[b]	87.69 ± 1.51[a]
Site	Kohala	1.05 ± 0.41[b]	15.69 ± 2.73[a]	83.26 ± 2.67[b]
	Laupahoehoe	4.09 ± 1.11[a]	14.79 ± 3.03[a]	81.12 ± 2.57[b]
	Thurston	1.01 ± 0.25[b]	8.6 ± 1.71[b]	90.39 ± 1.52[a]

Temperature	16 °C	1.55 ± 0.50[a]	18.53 ± 2.24[a]	79.92 ± 2.12[b]
	26 °C	2.54 ± 0.77[a]	7.53 ± 1.02[b]	89.93 ± 1.15[a]

Values are mean ± SE. For each factor, mean ± SE values of the treatments not connected by same letters (in the same column) are significantly different at $p < 0.05$ using Tukey HSD tests.

Discussion

The Fate of Added ^{13}C in Soils

Microbial and Mineralogical Pathways of Soil C Stabilization

Overall, greater initial retention of ^{13}C in microbial biomass was related to longer-term transfer and retention of ^{13}C into mineral soil C over 255 days, supporting our initial hypothesis. Other studies have also found that microbial growth and biomass turnover rate are also important determinants of soil C formation (Hagerty et al., 2014; Zheng et al., 2019). Microbial N-rich compounds have been posited to be key for stabilization of C in mineral soil, because they can sorb strongly to mineral surfaces and have hydrophobic outward-facing protons, leading to further associations with a wide range of organic compounds (Sollins et al., 2006; Kleber et al., 2007). The relationship we observed between initial microbial uptake of ^{13}C, and longer-term retention in mineral soils supports the paradigm of microbes functioning as a "pump" moving fresh C into long-term soil C pools (Liang et al., 2017). Recovery of ^{13}C from labeled glucose in microbial biomass and soils over one year in an agricultural soil also showed that greater initial uptake by microbes in some sites corresponded to greater longer-term retention in mineral soil (Kallenbach et al., 2015). In our sites, the relationship was strongest for the less weather soils at Thurston and Laupahoehoe, with a weaker relationship at the oldest soil in Kohala. In Kohala, greater sorptive capacity on mineral surfaces might have promoted more *mineral-regulation* of C storage initially, making microbial uptake less important for longer-term storage.

Our data also indicate that microbial uptake and recycling of C likely contributed to long-term retention in soil. The loss of the majority of added ^{13}C label to respiration we observed in the first four days is consistent with studies indicating that the half-life of glucose and glycine in microbial biomass is only a few hours (Kuzyakov and Demin, 1998; Hill et al., 2008; Hobbie and Hobbie, 2013). Interestingly, ^{13}C that was not immediately respired and was still in the microbial biomass after four days appears to have had a much greater probability of remaining in the soil system. Initial uptake of low molecular weight carbon compounds for growth and maintenance can occur within seconds to hours, with some C lost to respiration, but the turnover and recycling of the initial microbial biomass by subsequent microbes can occur indefinitely (Gunina and Kuzyakov, 2015). Studies from forests, grasslands and agricultural fields suggesting that microbial biomass turns over every 18 – 140 days (Kouno et al., 2002; Perelo and Munch, 2005; Cheng, 2009; Spohn et al., 2016a; Spohn et al., 2016b). Even by four days, there may have been substantial microbial turnover and substrate recycling contributing microbial products to mineral soil pool (Geyer et al., 2016). Thus, our study likely captured at least one complete turnover of microbial biomass, such that retained the ^{13}C at the end of the experiment was most likely recycled within microbial biomass.

Also striking was the relative constant retention of soil C in the mineral soil pool from 4 days to 255 days, with this pool showing the smallest declines in ^{13}C retained from 4 days to 255 days. This observation concurs with other studies that show high initial losses followed by a slower loss, and the most stable soil C pool in mineral soils, as observed in 16 different types of pasture soils (Saggar et al., 1999), in a biofuel grass plantation (Schneckenberger et al., 2008) and in an agricultural soil (Kallenbach et al., 2015). Thus, the greatest vulnerability to the loss for new C added to soils is in the first few days, whereas fresh C that is incorporated into microbial biomass

or mineral soil is much more likely to remain in soil for the longer term.

Site Effects on ^{13}C Retention in Soil

We expected that retention of ^{13}C would be greatest in more strongly-weathered soils with greater binding site density, but in fact the youngest soil in Thurston had the greatest ^{13}C retention. While overall retention of ^{13}C was greater in Thurston, less ^{13}C was in microbial biomass and more was in mineral soil compared with Kohala and Laupahoehoe at the end of the experiment. Thurston had a smaller microbial biomass overall than the other two sites, so microbes may have had less capacity to take up and process added C initially, allowing for greater mineral sorption. Still, Thurston soil had over 2x greater ^{13}C retention in microbial biomass per unit of microbial biomass C than did Kohala and Laupahoehoe, suggesting more efficient uptake in this soil with lower mineral sorptive capacity. Differences in microbial community composition could help explain the apparent differences in microbial uptake, efficiency, and long-term recycling, with differences in microbial community composition documented for Thurston vs. Laupahoehoe (Balser, 2001). Thus, microbial biomass and community characteristics likely had a large influence over initial C retention in these soils.

Competition between microbial uptake vs. direct mineral sorption for newly added C in soil has been an active area of research, with some conflicting results. In a lab experiment with model combinations of soil minerals, microbial community composition had a greater influence over C retention than did clay mineralogy (Kallenbach et al., 2016), indicating the importance of microbial community. In contrast, a study with soils from Cowlitz River soil chronosequence (250 years – 1.2 million years) found that a relatively insoluble phenolic derivative (p-hydroxybenzoic acid) had significantly greater C retention in mid-weathered soils with the greatest reactive mineral

content, while there was no difference in initial glucose-C retention among sites (McFarland et al., 2019), indicating the importance of both C compound and soil mineralogy. Here, initial microbial uptake and CUE were the strongest drivers of longer-term retention in mineral soils, and the soil with the least reactive mineral had the greatest long-term retention of added C, arguing for a greater role of initial microbial processing for the fate of fresh C among these sites.

The Role of C Chemistry on ^{13}C Retention

We observed that respiration of ^{13}C glycine at 4 days was greater than of ^{13}C glucose, which is in agreement with other studies that have reported 1.3-2.4x greater loss of glycine versus glucose after 4 – 7 days of substrate addition in forest, pasture and farmland soil (Webster et al., 1997; Kuzyakov and Demin, 1998; Oldfield et al., 2018). Lower microbial CUE for glycine compared to glucose has been attributed to greater incorporation of C from glucose into structural components and extracellular microbial metabolites, with more catabolic use of glycine, leading to greater respiration losses (Webster et al., 1997; Kuzyakov and Demin, 1998; van Hees et al., 2005). Generally, amino acids have shorter mean residence times in microbial biomass compared to glucose (Boddy et al., 2008). Accordingly, glycine had less retention in microbial biomass early in our experiment, and less retention in microbial biomass as well as mineral soil pools at the end of the experiment, further supporting our hypothesis that incorporation of organic matter into microbial biomass promotes longer-term retention of C in soil.

Effects of Warming on ^{13}C Retention in Soil C Pools

Similar to previous studies, we found that initial glucose uptake and retention by microbes was independent of temperature (Schimel and Mikan, 2005; Bore et al., 2017). Short-term glycine retention by microbes, in contrast, was sensitive to temperature, with lower 4-day retention at

warmer temperatures. This contradicts some studies that have reported rapid microbial uptake of soluble low molecular weight sugars and amino acids irrespective of incubation temperature (Finzi and Berthrong, 2005; Hobbie and Hobbie, 2013; Bore et al., 2017). Our microbial biomass ^{13}C data at four days are an integrated measure of short-term uptake and retention in microbial biomass, so the lower values at warming for glycine could just reflect greater initial respiration losses of this compound, particularly since warming did not affect the quantity of either compound in mineral soils at four days.

We found that warming decreased microbial CUE early in the experiment, resulting in less retention of ^{13}C in mineral soil for warmed samples at the end of the experiment. Other studies have found both reductions in microbial CUE with increasing temperature (Pietikäinen et al., 2005; Steinweg et al., 2008; Tucker et al., 2013), and limited responses to increased temperature (Devêvre and Horwáth, 2000; Dijkstra et al., 2011). In agreement with our study, declines in microbial CUE with warming were observed for glycine in soils from Spanish herbaceous scrub vegetation (Vinolas et al., 2001). Similarly, a study in O horizon soil samples from a boreal coniferous forest found glucose CUE declined with warming across a temperature range of 9 to 19 ^{o}C (Öquist et al., 2017). Also, no significant change in CUE of glucose with warming was observed across temperature range of 5 to 25 ^{o}C in temperate forest soils (Frey et al., 2013; Hagerty et al., 2014).

By 255 days, warming significantly reduced the amount of ^{13}C retained in microbial biomass in all the sites, and in mineral soil pools in Thurston, the least weathered soils. Since Thurston soil has less reactive mineral content, organo-mineral association are likely less abundance, and therefore soil C may be less protected from elevated microbial decomposition

activity with warming. In a laboratory incubation experiment that used temperate forest soil, there was no main effect of 5 ºC warming on glucose or glycine retention in the mineral soil pool after 28-weeks (Oldfield et al., 2018). Thus, warming is likely to affect C retention in mineral-associated pools differently across sites.

Bulk Soil Carbon Pools and CO_2 Fluxes

Differences in bulk soil C pools and CO_2 fluxes among sites reflected differences in soil mineralogy. Kohala soils had the greatest soil C, clay, and short-range-order mineral content, followed by Laupahoehoe and then Thurston, where soils are sandier with an abundance of primary minerals (Shoji et al., 1993; Torn et al., 1997). Heterotrophic respiration rates per mass of soil C were lowest at Kohala followed by Laupahoehoe and Thurston, possibly indicating greater stability of soil C in Kohala. An incubation study with soils from 24 sites along a 4,000-km transect in Chile and the Antarctic Peninsula found lower respiration per unit C in C-rich soils, which was attributed to greater sorption of C compounds onto mineral surfaces (Doetterl et al., 2015). This could be the case for the Hawaiian sites, with greater C sorption to mineral surfaces in more strongly-weathered soils.

Warming increased the total heterotopic respiration from soil in this study, similar to results from warming incubations across other tropical and sub-tropical forests (Xu et al., 2006; Cusack et al., 2010b; Wu et al., 2016; Liu et al., 2017; Zheng et al., 2019). We expected that greater physical protection of soil C offered by more reactive soil mineralogy at Kohala would decrease temperature sensitivity compared to Thurston. On the contrary, we found the highest Q_{10} values in Kohala. Nonetheless, our finding agrees with the theory that soil C protected in organo-mineral association have higher temperature sensitivity than the unprotected C (Bosatta and Ågren, 1999;

Davidson and Janssens, 2006; Conant et al., 2011). Overall, the Q_{10} values for heterotrophic respiration observed here was similar to the global forest soil average of 2.67 (Wei et al., 2010) or 2.5 (Hamdi et al., 2013) and also close to the range of 1.43 – 2.21 reported from three Australian tropical rainforest sites (Zimmermann and Bird, 2012). Our observation of higher temperature sensitivity in warming from 16 to 21 ^{o}C versus warming from 21 to 26 ^{o}C also agrees with other studies that have shown that temperature sensitivity decreases with increasing temperature (Lloyd and Taylor, 1994; Kirschbaum, 1995; Leifeld and Fuhrer, 2005).

Bulk Soil Microbial Biomass and CUE

Overall, CUE calculated in our study was lower compared to studies that calculated CUE over shorter period (6 hours to 40 hours) (Elliott et al., 1983; Frey et al., 2001; Frey et al., 2013), but comparable to 0.17% reported for a root-free agricultural loamy sand soil after 25.5 to 36.5 hours of glucose addition (Blagodatskaya et al., 2014), 20% reported for a cultured population of indigenous soil bacteria in an agricultural clay loam soil after 33 to 39 hours (Christensen et al., 1995), and 14–51% for 8 agricultural soils after 48 or 96 hours of glucose monohydrate addition (Anderson and Martens, 2013). Because we calculated CUE after four days, it included the respiratory losses that occurred during microbial turnover and substrate recycling in that period, thus lowering the CUE (Chapman and Gray, 1986; Geyer et al., 2016).

Similar to our results after 255 days, a decline in microbial biomass was observed in tropical soils when warmed to 35 ^{o}C for 150 days (Grisi et al., 1998). Similarly, incubation of humid tropical semideciduous forest soil for 7 days at multiple temperatures ranging from 15 °C to 80 °C caused the microbial biomass to decline above 25 °C (Menichetti et al., 2015). In contrast, field warming experiments with 1 – 5 ^{o}C warming for 5 years resulted in no significant change in

the soil microbial biomass in a sub-tropical forest plot in China (Fang et al., 2016; Liu et al., 2017). Decreased bulk soil microbial biomass C with warming supports the idea posited previously that warming in sites with lower natural variability in temperature like tropical forests will result in reduced microbial growth and efficiency (Waldrop and Firestone, 2006).

Conclusion

This study indicates that the fate of fresh C added to soil is regulated by microbial CUE, with temperature sensitivity regulated largely by soil mineralogy. Greater initial microbial uptake of added C (e.g. glucose vs. glycine) was associated with greater long-term retention in soil mineral pools, supporting growing evidence for the central role of microbial biomass in soil C stabilization. Also, soils with more reactive minerals appear to provide greater protection of newly added organic C under warming scenarios. These results suggest that microbial incorporation of new C, and subsequent protection of microbial products on mineral surfaces, will both influence climate feedbacks in tropical forests.

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Appendix

Table A1: Soil variables under different sites, organic compound added and warming conditions

Site	Compo-und Added	Tempe-rature (°C)	Time (days)	Soil C (percent)	TN (percent)	DOC ($mgCg^{-1}$soil)	microbial biomass C ($mgCg^{-1}$soil)	DOC ($mgCg^{-1}$ Soil C)	microbial biomass C ($mgCg^{-1}$Soil C)
Kohala	Control	16	4	18.49 ± 1.66	1.04 ± 0.06	0.76 ± 0.06	1.57 ± 0.21	4.15 ± 0.12	8.58 ± 1.23
			255	18.39 ± 2.05	1.03 ± 0.05	0.79 ± 0.05	1.09 ± 0.18	4.34 ± 0.23	5.88 ± 0.3
		26	4	19.19 ± 1.71	1.08 ± 0.05	0.77 ± 0.07	1.67 ± 0.15	4.01 ± 0.03	8.82 ± 1.08
			255	18.11 ± 2.02	1.04 ± 0.06	0.67 ± 0.05	0.65 ± 0.14	3.71 ± 0.15	3.83 ± 1.11
	Glucose	16	4	19.25 ± 2.34	1.06 ± 0.08	0.77 ± 0.05	1.61 ± 0.19	4.04 ± 0.22	8.48 ± 1.05
			255	18.53 ± 2.04	1.02 ± 0.06	0.73 ± 0.03	1.24 ± 0.21	4.01 ± 0.25	6.83 ± 1.48
		26	4	19.24 ± 2.23	1.07 ± 0.07	0.79 ± 0.08	1.45 ± 0.18	4.13 ± 0.05	7.66 ± 1.08
			255	17.96 ± 2.12	1 ± 0.08	0.64 ± 0.06	0.53 ± 0.17	3.71 ± 0.6	3.1 ± 1.2
	Glycine	16	4	19.49 ± 2.24	1.09 ± 0.07	0.86 ± 0.11	1.38 ± 0.25	4.55 ± 0.8	7.37 ± 1.83
			255	18.35 ± 2.06	1.04 ± 0.06	0.67 ± 0.06	1.35 ± 0.18	3.67 ± 0.18	7.51 ± 1.3
		26	4	19.64 ± 2.04	1.1 ± 0.07	0.72 ± 0.07	1.41 ± 0.12	3.67 ± 0.17	7.36 ± 1.14
			255	18.05 ± 2.23	1.06 ± 0.08	0.6 ± 0.09	0.72 ± 0.15	3.34 ± 0.56	4.13 ± 1.13
Laupah-oehoe	Control	16	4	30.44 ± 6.28	1.38 ± 0.21	1.02 ± 0.05	2.4 ± 0.12	3.73 ± 0.94	8.62 ± 1.82
			255	31.27 ± 5.01	1.45 ± 0.17	0.82 ± 0.29	1.43 ± 0.66	2.54 ± 0.86	4.75 ± 2.3
		26	4	27.59 ± 5.91	1.23 ± 0.13	1.02 ± 0.06	2.2 ± 0.25	3.97 ± 0.68	8.78 ± 2.06
			255	30.51 ± 4.88	1.43 ± 0.16	1.07 ± 0.29	1.21 ± 0.24	3.43 ± 0.4	3.93 ± 0.15
	Glucose	16	4	32.88 ± 4.89	1.52 ± 0.15	1.06 ± 0.07	2.15 ± 0.26	3.36 ± 0.56	6.66 ± 0.71
			255	31.23 ± 5.14	1.46 ± 0.19	1.16 ± 0.24	1.54 ± 0.28	3.77 ± 0.59	5.01 ± 0.82
		26	4	31.95 ± 5.42	1.45 ± 0.18	1.03 ± 0.07	2.2 ± 0.25	3.38 ± 0.56	7.12 ± 0.89
			255	31.01 ± 5.09	1.44 ± 0.16	1.54 ± 0.53	0.75 ± 0.2	4.78 ± 0.96	2.38 ± 0.36
	Glycine	16	4	32.96 ± 4.71	1.53 ± 0.13	1.02 ± 0.09	2.13 ± 0.18	3.19 ± 0.38	6.63 ± 0.78
			255	31.27 ± 5.05	1.47 ± 0.17	1.11 ± 0.23	1.63 ± 0.29	3.56 ± 0.47	5.27 ± 0.79
		26	4	32.18 ± 5.12	1.49 ± 0.17	1.03 ± 0.09	1.87 ± 0.32	3.32 ± 0.45	6.23 ± 1.48
			255	31.75 ± 5.06	1.46 ± 0.17	1.81 ± 0.66	0.86 ± 0.17	5.45 ± 1.22	2.69 ± 0.37
Thurston	Control	16	4	10.12 ± 1.37	0.54 ± 0.06	0.36 ± 0.11	0.74 ± 0.14	3.42 ± 0.62	7.18 ± 0.39
			255	9.33 ± 1.09	0.51 ± 0.05	0.35 ± 0.15	0.43 ± 0.09	3.47 ± 1.12	4.79 ± 1.16
		26	4	11.19 ± 1.9	0.61 ± 0.09	0.35 ± 0.11	0.68 ± 0.11	3.01 ± 0.41	6.06 ± 0.07
			255	9.18 ± 1.89	0.5 ± 0.1	0.3 ± 0.07	0.51 ± 0.12	3.28 ± 0.34	5.48 ± 0.54
	Glucose	16	4	11.36 ± 2.41	0.59 ± 0.1	0.42 ± 0.13	0.78 ± 0.17	3.53 ± 0.38	6.8 ± 0.08
			255	9.75 ± 1.72	0.53 ± 0.08	0.35 ± 0.08	0.56 ± 0.16	3.49 ± 0.35	5.51 ± 0.64
		26	4	11.59 ± 2.91	0.6 ± 0.14	0.39 ± 0.11	0.69 ± 0.11	3.31 ± 0.16	6.26 ± 0.74
			255	9.25 ± 1.59	0.53 ± 0.07	0.33 ± 0.05	0.4 ± 0.03	3.6 ± 0.51	4.5 ± 0.53
	Glycine	16	4	11.02 ± 1.73	0.69 ± 0.21	0.72 ± 0.24	0.66 ± 0.07	6.32 ± 1.71	6.07 ± 0.31
			255	9.7 ± 1.81	0.55 ± 0.09	0.36 ± 0.07	0.44 ± 0.09	3.7 ± 0.14	4.7 ± 0.76
		26	4	10.98 ± 1.54	0.58 ± 0.08	0.41 ± 0.12	0.64 ± 0.11	3.63 ± 0.56	5.75 ± 0.23
			255	9.42 ± 1.66	0.5 ± 0.09	0.41 ± 0.04	0.36 ± 0.14	4.44 ± 0.37	3.61 ± 0.76

Values are means ± 1 SE (n = 3).

Table A2: Effect of added organic compound, site, temperature, and time on bulk soil C pools

Factor	Treatment	Soil C (percent)	TN (percent)	DOC ($mgCg^{-1}soil$)	microbial biomass C ($mgCg^{-1}soil$)	DOC ($mgCg^{-1}$Soil C)	microbial biomass C ($mgCg^{-1}$ Soil C)
Compound	Control	19.48 ± 1.63[a]	0.99 ± 0.06[a]	0.69 ± 0.06[a]	1.22 ± 0.12[a]	3.59 ± 0.16[a]	6.39 ± 0.43[a]
	Glucose	20.33 ± 1.7[a]	1.02 ± 0.07[a]	0.77 ± 0.07[a]	1.16 ± 0.11[a]	3.76 ± 0.14[a]	5.86 ± 0.36[a]
	Glycine	20.4 ± 1.71[a]	1.05 ± 0.07[a]	0.81 ± 0.08[a]	1.12 ± 0.10[a]	4.07 ± 0.23[a]	5.61 ± 0.35[a]
Site	Kohala	18.72 ± 0.5[b]	1.05 ± 0.02[b]	0.73 ± 0.02[b]	1.22 ± 0.08[b]	3.94 ± 0.1[a]	6.63 ± 0.43[a]
	Laupahoehoe	31.25 ± 1.27[a]	1.44 ± 0.04[a]	1.14 ± 0.08[a]	1.7 ± 0.11[a]	3.71 ± 0.21[a]	5.67 ± 0.45[ab]
	Thurston	10.24 ± 0.47[c]	0.56 ± 0.03[c]	0.4 ± 0.03[c]	0.57 ± 0.04[c]	3.77 ± 0.22[a]	5.56 ± 0.22[b]
Temperature	16 °C	20.21 ± 1.38[a]	1.03 ± 0.06[a]	0.74 ± 0.05[a]	1.28 ± 0.09[a]	3.82 ± 0.17[a]	6.48 ± 0.28[a]
	26 °C	19.93 ± 1.35[a]	1.01 ± 0.05[a]	0.77 ± 0.07[a]	1.04 ± 0.09[b]	3.79 ± 0.13[a]	5.43 ± 0.33[b]
Time	4 Days	20.53 ± 1.35[a]	1.04 ± 0.05[a]	0.75 ± 0.04[a]	1.46 ± 0.09[a]	3.82 ± 0.16[a]	7.25±0.26[a]
	255 Days	19.61 ± 1.38 [a]	1 ± 0.06[a]	0.76 ± 0.07[a]	0.87 ± 0.07[b]	3.79 ± 0.15[a]	4.66±0.26[b]
Effect Test for the Full Model		P value	P value	P value	P value	P value	P value
	Site (n=36)	<.0001	<.0001	<.0001	<.0001	0.6191	0.0294
	Compound (n=36)	0.7655	0.4829	0.2927	0.5504	0.159	0.1896
	Temp (n=54)	0.8059	0.6413	0.6387	0.0011	0.8571	0.0039
	Time (n=54)	0.4229	0.384	0.88	<.0001	0.9042	<.0001
	Site × Compound	0.981	0.9051	0.5506	0.9102	0.1437	0.7207
	Site × Temp	0.9402	0.8373	0.1088	0.1763	0.0467	0.4678
	Site × Time	0.8744	0.5981	0.0618	0.001	0.2948	0.1225
	Compound × Temp	0.9955	0.9787	0.982	0.5148	0.8299	0.5223
	Compound × Time	0.8611	0.6673	0.7946	0.4297	0.5755	0.3187
	Temp × Time	0.9957	0.8458	0.1819	0.0163	0.0616	0.0278
	Compound × Temp × Time	0.9976	0.9794	0.4899	0.5817	0.1552	0.5491
	Site × Temp × Time	0.9137	0.8906	0.2395	0.2146	0.3058	0.1925
	Site × Comp × Time	0.9912	0.9463	0.3978	0.8971	0.1026	0.7655
	Site × Comp × Temp	0.9973	0.962	0.8892	0.9609	0.8183	0.9885
	Site × Comp × Temp × Time	0.9999	0.9914	0.9976	0.9101	0.8394	0.9887

Values are means ± SE. For each factor, means ± SE values of the treatments not connected by the same letters across the rows are significantly different at p<0.05 when analyzed by Tukey HSD tests. The values at the bottom 15 rows are the p-values of the effect from the ANOVA model.

Table A3: Cumulative soil heterotrophic respiration per gram of soil for 253 days and temperature sensitivities (Q_5 and Q_{10}) of respiration

Site	Cumulative Heterotrophic Respiration (CO_2 mg g^{-1}soil)			Temperature Sensitivity (Q_5 and Q_{10})		
	16 °C	21 °C	26 °C	16-21 °C	21-26 °C	Q_{10} (16-26 °C)
Kohala	5.26 ± 0.53^{Bb}	9.34 ± 1.22^{Ba}	11.96 ± 1.56^{Ba}	3.07 ± 0.28^{a}	1.67 ± 0.12^{a}	2.23 ± 0.12^{a}
Laupahoehoe	13.4 ± 2.09^{Ab}	21.73 ± 3.58^{Aab}	25.97 ± 4.08^{Ba}	2.72 ± 0.29^{ab}	1.5 ± 0.1^{a}	1.97 ± 0.08^{b}
Thurston	5.62 ± 0.55^{Bb}	8.3 ± 0.72^{Ba}	10.11 ± 0.98^{Aa}	2.25 ± 0.15^{b}	1.48 ± 0.07^{a}	1.81 ± 0.05^{b}

Values are mean ± SE. Q_5 temperature sensitivities was calculated for 16-21 °C and 21-26 °C. Data were pooled across organic compound added because it had no main effect on respiration (n = 9). For cumulative heterotrophic respiration, significant differences among sites are indicated by uppercase letters down the column and differences among temperature treatments for each site are indicated by lowercase letters across the row using Tukey HSD Tests. For temperature sensitivity, significant differences are indicated by letters down columns, comparing sites, using Tukey HSD Tests.

Table A4: Percent of ^{13}C retained in different soil pools after four days and 255 days of incubation after substrate addition

Site	Compound Added	Temperature (ºC)	Time (Days)	Percent Recovery in			
				DOC	microbial biomass C	Soil Matrix	All Pools
Kohala	Glucose	16 ºC	4	0.52 ± 0.15	16.23 ± 1.09	23.58 ± 2.7	40.5 ± 1.83
			255	0.32 ± 0.11	6.91 ± 0.82	16.46 ± 0.39	23.81 ± 0.57
		26 ºC	4	1.43 ± 0.57	12.05 ± 0.34	29.45 ± 0.07	43.29 ± 0.39
			255	0.29 ± 0.19	1.64 ± 0.52	18.26 ± 1.83	20.29 ± 2.02
	Glycine	16 ºC	4	14.98 ± 12.25	13.8 ± 6.95	6.63 ± 6.5	36.31 ± 12.3
			255	0.01 ± 0.01	1.93 ± 0.2	9.19 ± 0.33	11.13 ± 0.27
		26 ºC	4	0.45 ± 0	3.93 ± 0.71	14.03 ± 0.53	18.57 ± 0.36
			255	0.08 ± 0.08	0.75 ± 0.16	7.7 ± 0.53	8.57 ± 0.58
Laupahoehoe	Glucose	16 ºC	4	0.7 ± 0.1	16.62 ± 1.21	28.52 ± 4.64	46.07 ± 4.24
			255	0.54 ± 0.32	5.68 ± 0.61	16.08 ± 2.46	22.47 ± 2.38
		26 ºC	4	0.45 ± 0.04	17.8 ± 2.13	23.31 ± 2.49	41.72 ± 1.31
			255	0.46 ± 0.15	2.01 ± 0.95	15.41 ± 2.02	18.04 ± 2.72
	Glycine	16 ºC	4	1.79 ± 1.55	7.55 ± 1.5	15.16 ± 9.19	24.82 ± 6.99
			255	0.39 ± 0.23	1.66 ± 0.75	7.84 ± 0.56	10.02 ± 0.87
		26 ºC	4	0.25 ± 0.08	2.92 ± 0.63	11.23 ± 0.82	14.5 ± 0.62
			255	0.54 ± 0.18	0.52 ± 0.14	6.91 ± 0.89	8.16 ± 0.84
Thurston	Glucose	16 ºC	4	0.36 ± 0.03	16.83 ± 2.71	32.27 ± 3.84	49.59 ± 2.08
			255	0.14 ± 0.07	4.33 ± 0.92	23.33 ± 0.71	27.86 ± 0.16
		26 ºC	4	0.41 ± 0.1	14.38 ± 1.9	28.99 ± 3.29	43.93 ± 1.93
			255	0.13 ± 0.05	1.78 ± 0.24	17.73 ± 1.52	19.69 ± 1.74
	Glycine	16 ºC	4	29.51 ± 13.77	11.53 ± 3.27	6.76 ± 2.93	49.09 ± 12.24
			255	0.14 ± 0.08	0.88 ± 0.36	12.25 ± 1.13	13.33 ± 1.14
		26 ºC	4	0.41 ± 0.07	4.58 ± 0.98	17.44 ± 5.61	22.57 ± 4.82
			255	0.16 ± 0.03	0.33 ± 0.22	8.78 ± 0.93	9.34 ± 1.11
				P value	P value	P value	P value
Site (n=24)				0.5322	0.9282	0.1369	0.0156
Compound (n=36)				0.0108	<.0001	<.0001	<.0001
Temperature (n=36)				0.0017	<.0001	0.9409	0.0001
Time (n=36)				<.0001	<.0001	<.0001	<.0001
Site × Compound				0.0465	0.2136	0.5149	0.3643
Site × Temperature				0.174	0.2676	0.1724	0.2997
Site × Time				0.0126	0.7391	0.7059	0.2867
Compound × Temp				0.0006	0.4306	0.3356	0.0712
Compound × Time				0.002	0.0007	0.0139	0.1529
Temperature × Time				0.0009	0.1823	0.1716	0.0897
Compound × Temperature × Time				0.0002	0.0105	0.2545	0.0126
Site × Temperature × Time				0.2216	0.4441	0.1416	0.7592
Site × Compound × Time				0.069	0.1959	0.8026	0.2237
Site × Compound × Temperature				0.0778	0.976	0.3371	0.6227
Site × Compound × Temperature × Time				0.1119	0.9098	0.7178	0.6186

Values are mean ± SE (n = 3). The values at the bottom 15 rows are the p-values of the effect or interaction from the full least square ANOVA model.

Table A5: Percent of ^{13}C retained in microbial biomass per unit of microbial biomass C

Factor	Treatment	Soil C
Compound	Glucose	8.84 ± 1.08[a]
	Glycine	4.04 ± 0.95[a]
Site	Kohala	5.26 ± 0.88[b]
	Laupahoehoe	3.67 ± 0.61[b]
	Thurston	10.39 ± 1.80[a]
Temperature	16 °C	7.71 ± 1.18[a]
	26 °C	5.17 ± 0.95[a]
Time	4 Days	10.00 ± 1.23[a]
	255 Days	2.87 ± 0.40 [a]
		P-value
Effect Test for the Full Model	Site (n=24)	<.0001
	Compound (n=36)	<.0001
	Temp (n=36)	0.0004
	Time (n=36)	<.0001
	Site × Compound	0.048
	Site × Temp	0.1439
	Site × Time	<.0001
	Compound × Temp	0.1914
	Compound × Time	0.0583
	Temp × Time	0.1368
	Compound × Temp × Time	0.0081
	Site × Temp × Time	0.5794
	Site × Compound × Time	0.4543
	Site × Compound × Temp	0.6909
	Site × Compound × Temp × Time	0.4454

Values are mean ± SE. For each factor, means ± 1 SE values of the treatments not connected by same letters down the columns are significantly different at p<0.05 when analyzed by Tukey HSD tests. The values at the bottom 15 rows are the p-values of the effect or their interaction from the full least square ANOVA model.

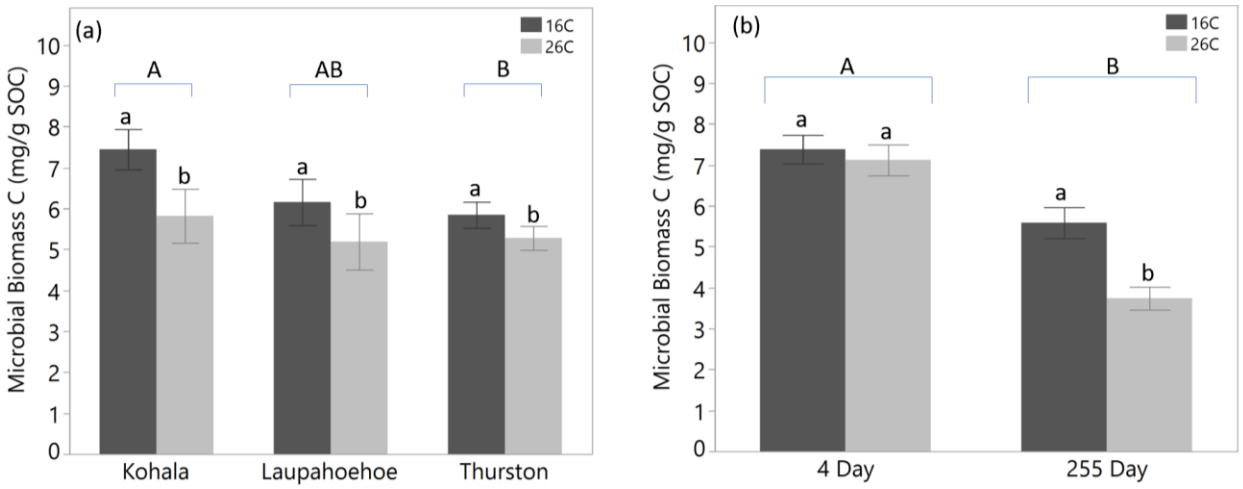

Figure A1a.) Soil microbial biomass C normalized by soil carbon concentration after four days and 255 days of incubation. Data are pooled across the substrates and time (n = 18). Differences among sites are shown by uppercase letters, and differences between temperature treatments within sites are shown by lowercase letters. b.) Soil microbial biomass C normalized by soil carbon concentration after four days and 255 days of incubation. Data are pooled across substrates and sites (n=27). Differences between two time points are shown by uppercase letters, and differences between temperature treatments within sites are shown by lowercase letters. Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

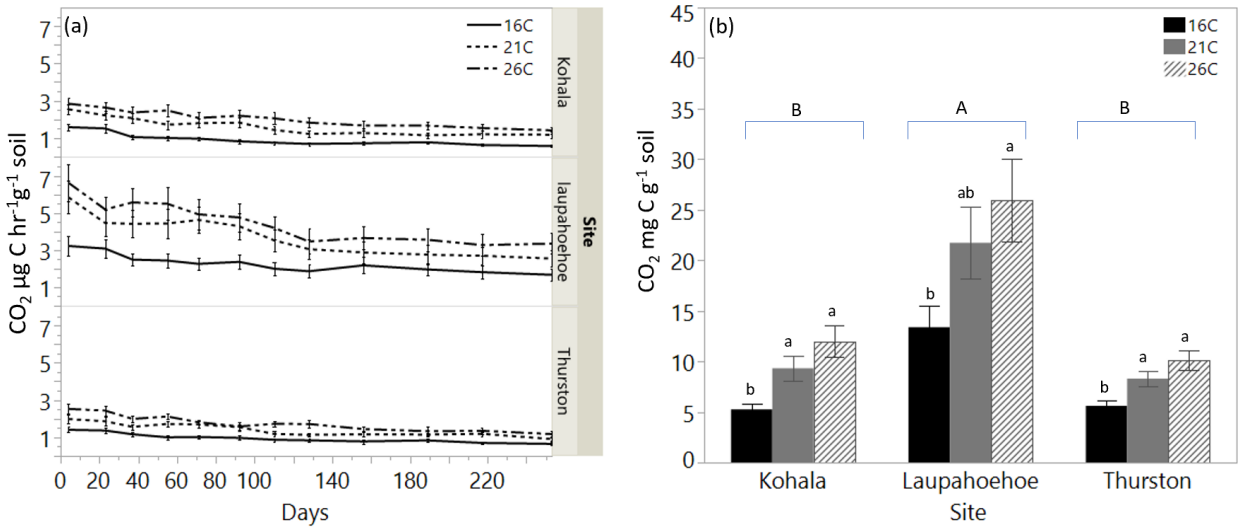

Figure A2a.) Average hourly heterotrophic respiration rate per g of soil at different time points during 253 days of incubation. Data are pooled across substrates (n = 9). b.) Average daily heterotrophic respiration from soil per g of soil during 253 days of incubation. Differences between sites are shown by uppercase letters, and differences between temperature treatments within sites are shown by lowercase letters. Data are pooled across substrates (n = 9). Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

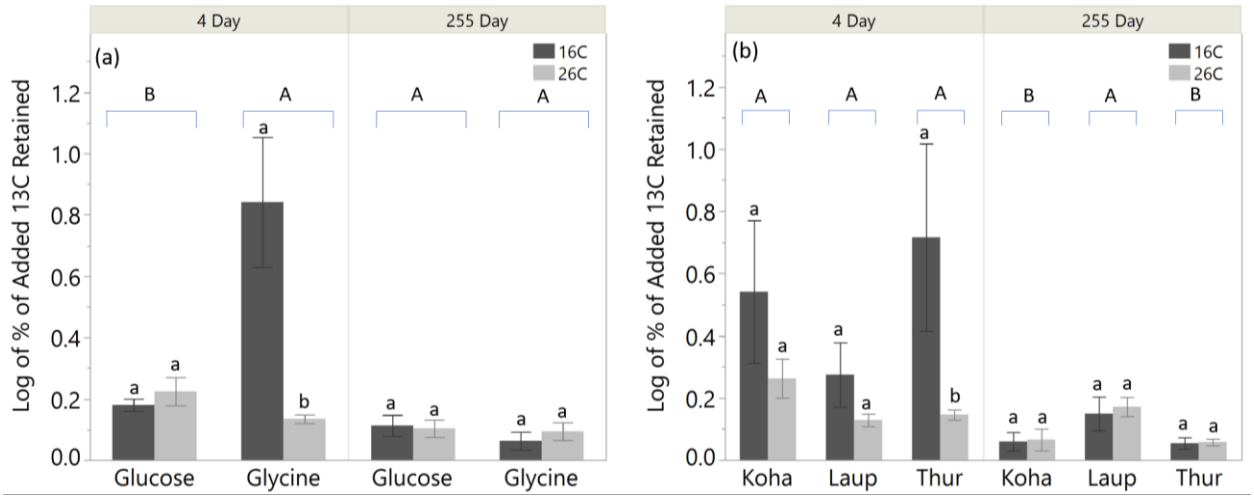

Figure A3 a.) Percent of ^{13}C retained in DOC pool after four days and 255 days of incubation (log transformed). Data are pooled across sites (n=9). Differences between organic compounds in each time point are shown by uppercase letters, and differences between temperature treatments within organic compounds are shown by lowercase letters. b.) Percent of ^{13}C retained in all soil pools after four days and 255 days of incubation. Differences between sites in each time point are shown by uppercase letters, and differences between temperature treatments within sites are shown by lowercase letters. Data are pooled across organic compounds added (n=6). Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

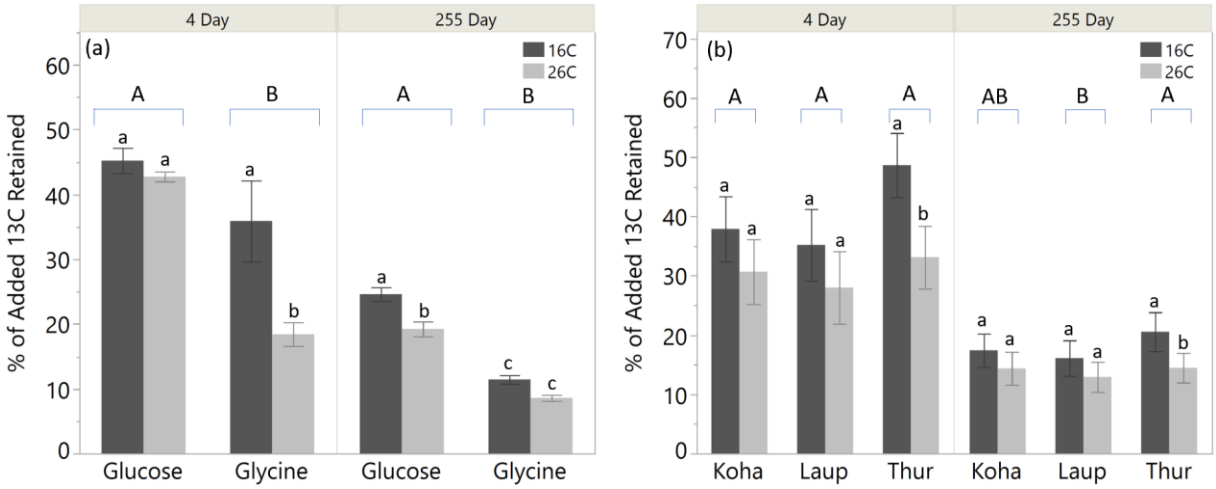

Figure A4 a.) Percent of ^{13}C retained in all soil pools (DOC + microbial biomass + mineral soil pools) by organic compound added after four days and 255 days of incubation is shown. Data are pooled across sites (n=9). Differences between organic compounds in each time point are shown by uppercase letters, and differences between temperature treatments within organic compounds are shown by lowercase letters. b.) Percent of ^{13}C retained in all soil pools by sites after four days and 255 days of incubation is shown. Differences among sites for each time point are shown by uppercase letters, and differences among temperature treatments within sites are shown by lowercase letters. Mean ± SE are shown. Letters show differences using Tukey HSD Tests.

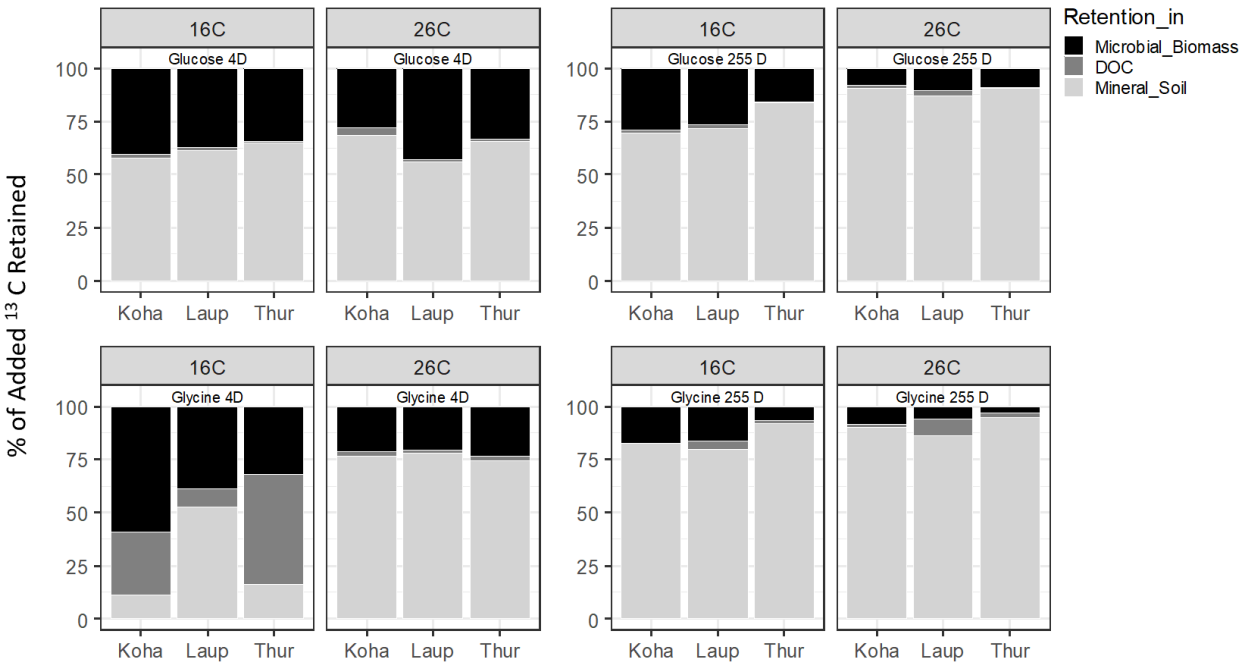

Figure A5: Relative partitioning of ^{13}C from added substrates retained into DOC, microbial biomass and mineral soil pools is shown for different sites and temperature treatments for four days and 255 days of incubation (n = 3).

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Chapter 3 The fate of added carbon in soils over two years along mineralogical gradients in Hawaiian forests and grasslands

Abstract

The balance of loss or retention of newly added carbon (C) in soils is likely regulated by environmental conditions like soil mineralogy, microbial activity and efficiency, plant cover, and nutrient availability. Understanding the relative importance of these factors would improve our ability to predict soil-climate feedbacks. We assessed the fate of added organic compounds in soils over two years, comparing C loss versus storage along a long substrate age gradient (LSAG) across wet Hawaiian forests, and along a climate and soil weathering gradient in Hawaiian grasslands. A long-term field fertilization experiment was also used at one site to assess the influence of nutrient variation on C storage. Replicate soil columns were inserted into soils at each site, and ^{13}C-labeled glucose, ^{13}C-labeled glycine, or charcoal was added to each column. Added C was tracked into microbial biomass, dissolved organic C (DOC), and the soil matrix at one and two-year time points. After two years, most of the added ^{13}C label from organic compounds remained within the top 0 – 5 cm of soil. After two years, an average of 8.7% and 3.0% of added ^{13}C added was recovered in mineral soil in forest and grassland soils, respectively, with ~2.8*x* greater retention for glucose versus glycine after one and two years. There was no effect of soil mineralogy or the long-term nutrient additions on ^{13}C retention in the soil after two years. ^{13}C NMR revealed an increase in the proportion of alkyl C in glucose and glycine amended soil, with alkyl C indicative of lipids such as may be produced by microbial biomass. Our results indicate that microbial incorporation of organic compounds like glucose into biomass may promote the production and storage of alkyl C compounds. Thus, the chemistry of C inputs to soils, subsequent incorporation and retention into

microbial biomass, and vegetation cover type had more importance for two-year soil C storage than other ecosystem factors like soil mineralogy and nutrient availability.

Introduction

Tropical ecosystems play a crucial role in regulating the global climate and biogeochemical cycles, especially C turnover and storage (Schimel, 1995). Plant productivity is expected to increase in response to elevated atmospheric carbon dioxide (CO_2) concentration thereby channeling greater C inputs to soil (Cusack et al., 2016). One crucial way the soil receives C inputs from plants is as low molecular weight C compounds like amino acids, sugars, and carboxylic acids. These compounds not only fuel heterotrophic respiration (van Hees et al., 2005; Fischer et al., 2010), but also contribute to soil C storage via direct sorption to mineral surfaces, or by longer-term recycling within microbial biomass and subsequent sorption to mineral surfaces (von Lützow et al., 2006; Miltner et al., 2012; Bradford et al., 2013; Cotrufo et al., 2015; Kallenbach et al., 2016). Longer-term stability of C in soils depends largely on spatial separation from microbial decomposers (e.g., in stable micro-aggregates) (Dungait et al., 2012; Solomon et al., 2012; O'Brien and Jastrow, 2013) and/or via formation of organo-mineral associations which can make C inaccessible to decomposers (Kleber et al., 2007; von Lützow et al., 2007; Marschner et al., 2008). Factors like soil mineralogy, soil nutrient availability and chemistry of new C moving into the soil may influence how much of the incoming compounds are incorporated and stabilized into soil versus lost via respiration.

In young soils with an abundance of primary crystalline minerals such as plagioclase, K-feldspar and quartz and highly weathered soils dominated by crystalline secondary clay minerals like halloysite, kaolinite and illite tend to accumulate C via coating, and ultimately store less C per

surface area than poorly crystalline soils (Chorover et al., 2004). Volcanic soils at the intermediate stage of weathering contain a high proportion of SRO minerals allowing them to store a large amount of SOC (Torn et al., 1997). The formation of organo-mineral associations is more rapid in soils with greater binding site densities, such as silt, clay, short-range ordered (SRO) Al-silicates (e.g., allophane and imogolite), Fe oxides, and permanently charged phyllosilicate clay minerals (Cox et al., 2000; Kleber et al., 2015).

Soil nutrient levels can also affect the fate of incoming C in soil because C and nutrient cycles are tightly coupled to the metabolic requirements of microorganisms (Finzi et al., 2011). Anthropogenic N deposition has increased substantially in tropical and subtropical regions in recent decades and is projected to continue increasing in the future (Reay et al., 2008; Bala et al., 2013). Many recent studies have shown that N fertilization in soil generally decreases soil microbial biomass and heterotrophic respiration and increase soil C pool (Cleveland et al., 2006; Cleveland and Townsend, 2006; cu et al., 2010; Janssens et al., 2010; Lu et al., 2013; Ouyang et al., 2008). Many ecosystem processes like plant growth, soil respiration and organic matter decomposition are considered P-limited in tropical forests soils (Cleveland et al., 2002; Vitousek et al., 2010). Aeolian phosphorus (P), transportation from arid regions of Africa and Eurasia, will also probably increase the future availability of P across many tropical areas (Okin et al., 2004; Yu et al., 2015; Gross et al., 2016). Similarly, the addition of P can also alter the rate of C deposition and storage. Experimental P additions in tropical forests have shown that decomposition of SOC is likely P limited (Cleveland et al., 2002). Similarly, P addition in soil was reported to increase the microbial biomass but reduce total C in the tropical forest (Liu et al., 2013). Addition of P and NP can also speed the wood decomposition in forest floor by alleviating nutrient limitation of microorganisms (Chen et al., 2016). These results indicate that soil nutrient level can also affect

C stabilization in soil.

The organic chemistry of C compounds may influence their fate. For example, microbes were able to quickly (<5 h) outcompete mineral sorption for alanine, glucose, and acetate in an Alfisol (Fischer et al., 2010), while citrate remained unprocessed by microbes much longer than glucose (over 22 h) due to rapid and strong sorption to ferric hydroxide (Jones and Edwards, 1998). Similarly, rapid sorption to the soil matrix reduced the bioavailability of glutamate, glycine, and lysine in a Eutric cambisol soil (Jones and Hodge, 1999), and of glycine, lycine and glutamate in Calcic Palexeralf soil (Vinolas et al., 2001). Thus, some compounds like glucose can be assimilated by microbes quickly (Allison and Vitousek, 2004; Waldrop et al., 2004), yet direct sorption into mineral surface may occur more quickly for some compounds in the presence of highly reactive mineral surfaces (Keiluweit et al., 2015).

Black carbon (BC) and the charcoal are a group of SOC considered to be highly recalcitrant to microbial decomposition (Cheng et al., 2008; Preston and Schmidt, 2006). Some recent studies have demonstrated that BC is not as stable in soils as initially thought. Studying BC retention in Hawaiian grasslands Cusack et al. (2012) Cusack et al. {, 2012 #677} did not find the evidence that BC is preferentially retained in soil relative to other SOC indicating that the cycling of BC might be similar to other organic C. Evidence of microbial degradation of black C from charred plant materials in 60 days and 2 years incubation (Marschner et al., 2008) shows that decomposition of black C can be significant even over short term. However, until now, the factors controlling BC degradation are not adequately understood (Marschner et al., 2008)(Hockaday et al., 2007). Studies have linked the addition of N to both increase or decrease decomposition of lignin and other recalcitrant SOC (Dijkstra et al., 2003). However, research that specifically looked

at the effect of nutrient addition on charcoal decomposition has not been found.

This study assessed the effects of vegetation cover type, mineralogy on the fate of two newly added low molecular weight C compounds and one recalcitrant material (biochar) to soils from a weathering and soil nutrient gradient in Hawaii. We tracked two ^{13}C-labeled compounds into mineral soil, microbial biomass, and DOC over 2 years period in a field incubation experiment. We tested four hypotheses: (H1) greater proportion of newly added C is stabilized (retained) in soils that have higher sorptive capacity in mineral surfaces compared to soil that have lower binding site density; (H2) in a nutrient-poor soil N and P fertilization, increases the rate of soil C retention by alleviating nutrient limitation of microorganisms (H3) low molecular weight C compounds that include nitrogen (N) (e.g., glycine) are taken up in greater proportion and therefore have greater retention in soils relative to compounds without N (e.g. glucose), with greater microbial uptake also occurring in more nutrient-rich soils; (H4) rate of charcoal decomposition varies in mineralogical and nutrient gradient even in inter-annual scale. We, therefore, expected that fresh C added to the soils will be retained in soil in a larger proportion in fertile soil and the soil that have higher sorptive capacity in its mineral surface.

Methods

Site Description

This study was conducted along a soil age gradient and a climate gradient in the Big Island of Hawaii. The soil age gradient is in native forests, and the climate gradient is in exotic grasses, providing comparisons of soil characteristics as well as plant cover types.

The soil age gradient, or long substrate age gradient (LSAG), included three montane forest sites along a soil weathering gradient on basaltic lava flows that have similar elevation, climate,

plant species composition, topography and parent material, but vary substantially in parent material age (Crews et al., 1995; Chorover et al., 2004; Vitousek et al., 2004). The mean annual temperature and precipitation of the forest sites are 16 ^{o}C and 2500 mm MAP, and all the sites are located at the elevation of ~1200 m (Chadwick et al., 1999; Giambelluca et al., 2013). These sites have a native tree species *Metrosideros polymorpha* as the dominant vegetation (Kitayama and Mueller-Dombois, 1995). The sites included a 300-yr-old soil (Thurston N 19°25', W 155°15'), a 20,000-yr-old soil (Laupahoehoe N 19° 55.73', W 155^{o} 18.06'), and a 150,000-yr-old soil (Kohala N 20 ^{o} 03.06', W115 ^{o} 41.05'), providing distinct soil mineralogies and nutrient contents gradient (Herbert et al., 1999). The mineral soil at Thurston mainly consists of primary minerals like olivine, glass, and plagioclase feldspar, with relatively low short-range-order mineral content and binding site densities in mineral soils. There are increasing amounts of metastable noncrystalline secondary minerals like primary ferrihydrite, allophane, and imogolite in Laupahoehoe, including short-range-order minerals, with the greatest content of these relatively reactive minerals in the oldest site at Kohala (Shoji et al., 1993; Torn et al., 1997). We thus refer to Thurston, Laupahoehoe, and Kohala soils respectively as "low", "intermediate", and "high" binding site density on mineral surfaces (Shoji et al., 1993; Torn et al., 1997; Torn et al., 2005).

The grassland sites, in contrast, provide a soil mineralogical gradient that results from changing rainfall levels, rather than soil parent material age. The Hawaii rainfall weathering gradient (HRWG) is on the leeward side of Kohala Volcano on the Big Island of Hawaii. Mean annual precipitation ranges from 220 to 2400 mm MAP with soil weathering and mineralogical status correlated with precipitation (Chadwick et al., 2003). Two sites were selected on this gradient for the current study at the dryer and wetter ends of the gradient. Buffel grass (*Cenchrus ciliaria*) is the dominant grass species in the arid site and *Digitaria spp.* is the dominant grass in

the mesic site.

The average annual rainfall of the drier site (N 20 º 08.81', W 155 º 50.47', altitude 476 m) and wetter grassland site (N 20º 09.17', W 155º 49.18', altitude 710 m) is estimated to be 370 and 630 mm respectively. Average annual rainfall in these sites was estimated using the elevation rainfall regression equation for this area (Chadwick et al., 2003). In general, the mesic site has more strongly weathered soil due to greater rainfall and hence higher content of reactive SRO minerals than the arid site (Chadwick et al., 2003). We refer to soils in the drier grassland and site as "low" binding site density, and mesic grassland as "high" binding site density.

Experimental Design

At each of the five sites, we inserted PVC columns into intact soils in July 2015 to create relatively undisturbed microcosms each. The columns were placed in replicates of three (n = 3), adding either: 715 mg fully labeled 99 atm% ^{13}C glucose dissolved in deionized (DI) water, 889 mg 99 atm% ^{13}C glycine in DI water, 7g of pure charcoal in DI water, or DI water alone (control). If we consider the added C to be distributed initially in 0-5 cm soil, then this addition was equivalent to 0.8% of total soil C in C rich sites Kohala and Laupahoehoe and equivalent to around 3% percent for the grassland soil. Two types of charcoal were to add a contrasting ^{13}C signature to soils. C3 pine charcoal made by controlled pyrolysis at 350 ºC (1.07512% ^{13}C [$\delta^{13}C$ of -27.93 VPDB], C content = 78.1%) was added to the microcosms in grassland soil, and C4 switch grass charcoal made by controlled pyrolysis at 480 ºC (1.09043% ^{13}C[$\delta^{13}C$ of -13.93 VPDB], C content =71.0 %) was added to the microcosms in forest soil. Both types of charcoal were prepared at high temperature in a highly controlled environment.

To assess the influence of soil nutrient availability in a controlled site, we also took

advantage of a long-term soil fertilization experiment at the Thurston site. There, microcosms were installed in 3 replicate plots that have received annual nitrogen (+N) or nitrogen and phosphorus each (+ NP) fertilization as well as in control plots which did not receive fertilization. In fertilization plots N was applied at the rate of 100 kg/ha, half as urea and half as $(NH_4)_2SO_4$) and P was applied at 100 kg/ha as triple superphosphate twice annually since 1985 (Vitousek et al., 1993; Ostertag, 2001, 2010).

At all sites, microcosms were constructed of PVC tubing 10 cm in diameter and 25cm long with small perforations (Bird and Torn, 2006), to provide general containment of labeled substrates while allowing lateral and vertical movement of water and solutes. A 2-cm rim of tubing was left aboveground to retain substrate plus native forest floor. To avoid cross-contamination, microcosms were placed at least 0.5 m away from each other and 1m away from large trees. Duplicate sets of microcosms at each site and for each treatment were then harvested at one year and at two years (July 2016 and 2017 respectively) and shipped to Dr. Cusack's biogeochemistry laboratory at UCLA, keeping soils intact in PVC columns until analysis.

Soil Analyses

Soils inside the microcosms were kept intact and shipped to the lab within five days of collection. In the lab soil inside the microcosms was divided into three depths (0-5, 5-10 and 10-23 cm). For chemical analysis, soils were passed through 2 mm sieves, removing roots, visible plant and animal remains. Initial gravimetric soil moisture content was determined by drying soil at 105 ^{o}C until the stable mass was achieved, and initial soil pH was measured in a 4:1 water-to-soil volumetric ratio using a benchtop pH meter.

Soils for bulk density was collected in July 2015 from all sites in two depths 5-10 cm and

10-25 cm, in duplicates or triplicates. Bulk density was determined by subtracting the total weight and volume of rocks, roots and any material greater than 2 mm from the soil thus only the mass of soil that passed through 2 mm sieve was accounted for bulk density. The total mass of soil in 0-5 and 5-10 and 10-25 cm soil was estimated by fitting the slope of the bulk density data of 0-10 and 10-25 cm.

The ^{13}C incorporated into DOC and microbial biomass was assessed by extracting soils using a chloroform fumigation slurry method (Fierer and Schimel, 2003). Briefly, 8-10 g soil was extracted with 0.25 M K_2SO_4 by shaking for 4 hours and filtered through pre-rinsed Whatman #1 filter paper for DOC A second sample was shaken with 0.5 ml ethanol-free chloroform and extracted the same way for microbial biomass. No conversion for extraction efficiency was used. Then, 1 ml of filtrate from each extraction was evaporated at 60 °C in a tin cone in a ventilated oven (Dijkstra et al., 2006). The resulting residue was rolled and analyzed at UC Davis Stable Isotope Facility to calculate total C and the $^{13}C/^{12}C$ ratio in DOC and microbial biomass. The residual soil in the filter paper after the microbial biomass extraction was transferred to an aluminum weighing dish and air dried, and then analyzed for total C and the $^{13}C/^{12}C$ ratio using combustion system (Costech Analytical, Valencia, CA, USA) paired to Thermo Delta V isotope ratio measuring mass spectrometer (IRMS) at UCLA. This gave the account of the ^{13}C from added C compounds that was incorporated in the soil matrix and was stable after removal of the extractable microbial biomass carbon (MBC) and DOC. Thus, ^{13}C was tracked in microbial biomass, DOC and the soil matrix (Hagedorn et al., 2003). DOC and MBC were analyzed on a Shimadzu TOC-L with TDN (total dissolved nitrogen) analyzer (Columbia, MD).

We also assessed changes in the organic chemistry of the bulk soil inside the microcosms

to understand how the addition of organic compounds altered the chemistry by using solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Soil samples for the NMR were prepared by treating the samples with hydrofluoric acid to remove mineral and glass fractions to reduce magnet interference and to concentrate SOC to reduce noise in the spectra (Schmidt et al., 1997). Here, the hydrofluoric-acid resulted in average loss of 24 ±1.7% of the organic C in mineral soils (Appendix, Table A4) which is within the range of 10–67% loss found in other studies (Skjemstad et al., 1994; Schmidt et al., 1997; Dai and Johnson, 1999; Mathers et al., 2002; Gonçalves et al., 2003).

Cross polarization ^{13}C NMR was conducted on the hydrofluoric acid treated SOC using a 300 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin, Billerica, MA) at Baylor University (Waco, TX). The 4mm sample probe was operated at a magic angle spinning (MAS) frequency of 12 kHz. Resulting spectra were analyzed and peak areas were integrated under the following seven chemical shift regions with the C functional group they represent and the common compounds in which they occur given in parenthesis: 0– 45 (alkyl group e.g., waxes, other lipids), 45–60 (N-alkyl/methoxyl group e.g. proteins, peptides), 60–95 (O-alkyl group e.g. Cellulose, other carbohydrates), 95–110 (di-O-alkyl group e.g. hemicellulose), 110–145 (aromatic group e.g., lignin, tannin), 145–165 (phenolic group e.g., acids, tannin), and 165–215 ppm (amide/carboxyl group e.g., chitin, proteins, peptides, and hemicellulose) (Baldock et al., 2004; Cusack et al., 2018). HF treatment also caused a significant shift in the proportion of all the C functional groups except for N-alkyl/methoxyl group in Kohala site. HF treatment significantly increased proportion of alkyl C (by 9.4%), amide/carboxyl C (by 18.1%), aromatic C, (by 11.8%), phenolic C (by 14.7%) groups while it decreased the proportion of di-o-alkyl (by 6.5%) and o-alkyl (by 20.0%) C groups (Figure 13).

Statistical Analyses

To directly test our hypotheses, we assessed: (H1) the relationship between 4-day microbial biomass ^{13}C incorporation, and 255-day mineral soil ^{13}C retention for each site of varying mineral reactivity; (H2) temperature as a main effect determining the retention of added ^{13}C over time across sites, and the temperature effect on microbial respiration per microbial biomass at 4 days and at 255 days (an indication of microbial CUE); (H3) the retention of ^{13}C from glucose versus glycine in different soil C pools over time and sites, including site-level nutrient availability.

Across analyses, standard least square ANOVA models were used to investigate the effects of site, organic compound, fertilization, and time on soil C, N, microbial biomass and ^{13}C retention, ^{13}C NMR chemical regions of the SOC using backward stepwise models to identify significant factors and comparing BIC values to identify the minimum adequate model, which was then run. Models were checked to ensure there was no heteroscedasticity in the residuals and that the response variables were a reasonably linear function of the fitted values, with errors closely distributed. Tukey means separation tests were used for post-hoc comparisons. Results are presented as the mean ± the standard error unless otherwise noted, with $n = 3$, unless replicates were pooled. $P < 0.05$ was determined as the level of statistical significance. We used principal components analysis (PCA) to compare general characteristics of the soil C fractions at different sites and organic C addition, using all ^{13}C NMR regions. Analyses were conducted using 14.0.0 JMP software (SAS Institute Inc. Cary, NC, USA, 2019).

Results

Bulk Soil C, MBC, and DOC

Forest Sites

Bulk soil C and N concentrations at unfertilized forest sites at both 0-5 and 5-10 cm depth varied significantly by site, with no significant effect of organic compound, or time (Figure 7, Table A1, Appendix). For 0-5 cm, Laupahoehoe and Kohala had significantly greater soil C and N concentrations compared to Thurston. Laupahoehoe had greater N concentration compared to Kohala, but C concentration was not significantly different between these two sites. For 5-10 cm Laupahoehoe had greater C and N concentration compared to Kohala and Kohala had significantly greater concentration compared to Thurston.

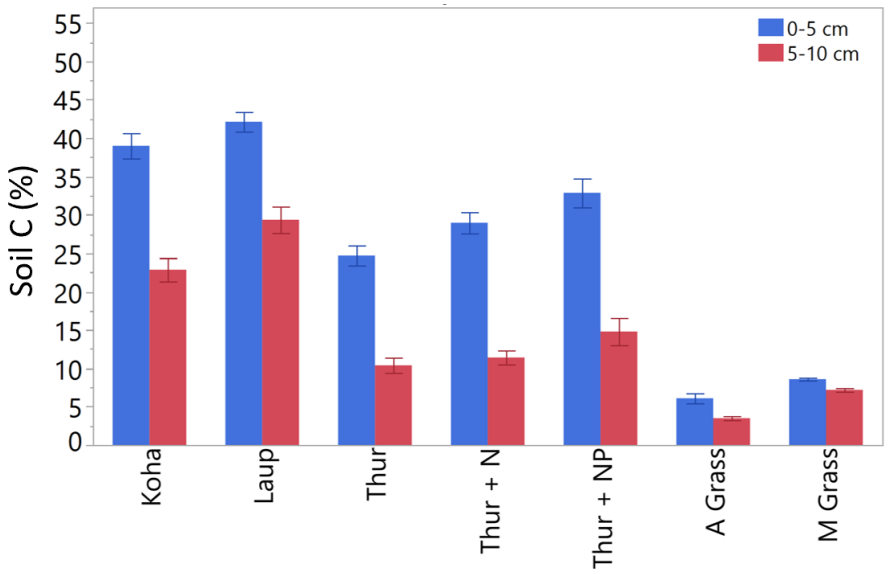

Figure 7 Soil C concentration is shown for different sites. Time and C addition have been pooled because of no significant main or the interaction effect. Mean ± SE are shown (n=24).

Soil MBC and MBN at both 0-5 cm and 5-10 cm in soil varied significantly by sites with no significant effect of organic compound added (Table A1, Appendix). Kohala and Laupahoehoe

had a significantly greater MBC and MBN compared to Thurston but there was no significant difference in MBC content between them.

Soil extractable DOC and DN at both 0-5 cm and 5-10 cm in soil varied significantly by sites with no significant effect of organic compound added (Table A1, Appendix). Kohala and Laupahoehoe had a significantly greater DOC and TN compared to Thurston for both the depths. DOC concentration between Laupahoehoe and Kohala was not significantly different in both depths. Extractable DN was greater in Laupahoehoe versus Thurston 0-5 cm soil but not significantly different in 5-10 cm.

The average gravimetric soil moisture at the top 5 cm soil varied significantly between the first year and the second year. For the first year, the average gravimetric field moisture content at Kohala, Laupahoehoe and Thurston were 79.3 ± 1.3, 80.7 ± 1.2, 77.4 ± 1.0% respectively (Table A2, Appendix). There was an average of 9.8 percent lower gravimetric soil moisture content in the soil during the second year. The average pH at top 5 cm soil at Kohala, Laupahoehoe and Thurston was 4.27 ± 0.05, 4.04 ± 0.06 and 5.16 ± 0.06 respectively and it did not vary significantly between the two sampling points (Table A2, Appendix).

Fertilization Effect

There was a significant effect of fertilization in Thurston sites on both C and N concentrations for 0-5 cm (Figure 7). C and N concentration at + NP site was significantly greater than unfertilized site but there was no significant difference in C and N concentration between + NP and + N site and the + N and unfertilized site. C and N concentration at 5-10 cm did not vary significantly with fertilization, time or the type of organic C added.

There was no significant effect of fertilization or the type of organic C added in soil MBC,

MBN and extractable DOC content in both 0-5 and 5-10 cm depth. Extractable DN content at 0-5 cm was greater at +N site compared to unfertilized and +NP sites. However, no significant fertilization effect on soil DN was seen in 5-10 cm.

Fertilization with both N and +NP significantly decreased the pH of 0-5 cm and 5-10 cm soil however only N fertilization decreased the pH at 10-23 depth (Table A2, Appendix).

Grassland Sites

Grassland sites had a lower content of bulk C, N, MBC and MBN concentration compared to forest sites (Table A1, Appendix). The moisture content was lower in the grassland sites, while pH was higher in the grassland sites (Table A2, Appendix). Bulk soil C and N concentration in the grassland sites at both 0-5 and 5-10 cm varied significantly by site, with no significant effect of the organic compound, or time. Mesic site had a significantly greater C and N concentration compared to the arid site.

In grasslands, soil extractable DN and MBN at both 0-5 and 5-10 cm soil varied significantly by site but not by the organic C added. Mesic grassland had a higher DN and MBN content compared to arid grassland in both the depths. There was no significant effect of both site and organic C in soil DOC and MBC in both 0-5 and 5-10 cm soil.

The average gravimetric field moisture content at arid and mesic grassland site was respectively 12.2 ± 0.2, and 41.4 ± 1.9 % in the first sampling point and 14.3±0.4 35±0.9 in the second sampling point. The average pH at top 5 cm soil at arid and mesic grassland was 7.15 ± 0.03 and 6.61 ± 0.03 respectively in the first sampling point and 6.96 ± 0.06 and 6.39 ± 0.03 in the second sampling point (Table A2, Appendix).

Retention of Added ^{13}C from Glucose and Glycine in Soil Pools

Total and Relative Retention of ^{13}C in Soil Pools

After one-year incubation, an average of 16.7 ± 2.1 and 6.5 ± 0.5% of added ^{13}C from glucose and glycine respectively was retained in 0-5 cm soil including mineral soil, microbial biomass and DOC in the forest sites. Grassland soils retained a significantly lower amount of added label with an average of 6.2 ± 1.0 and 2.7 ± 0.8% from glucose and glycine respectively after one year. The mineral soil after removal of microbial biomass and DOC retained the largest fraction of ^{13}C among the soil pools for both forest and grassland sites (Figure. 8, Table 4) At the end of the experiment averaged among all sites 89.4 ± 1.3%, 8.1 ±1.03% and 2.5 ± 0.6% of the ^{13}C recovered from mineral soil was recovered from 0-5, 5-10 and 10-23 cm depth respectively (Table A3, Appendix). This shows that much of the added ^{13}C remained in top 0-5 cm and almost all the ^{13}C added was contained within 0-23 cm of the soil. ^{13}C enrichment in bulk soil in these three soil depths also shows that there was a very little penetration of labeled ^{13}C in 10-23 cm depth (Figure 9). Microbial biomass represented the second largest pool of ^{13}C retention in soil after mineral soil while only a tiny fraction of added ^{13}C remained in the DOC pool after one year of organic C addition illustrating the rapid uptake, utilization and/or loss of added compounds (Table 4, Figure 8).

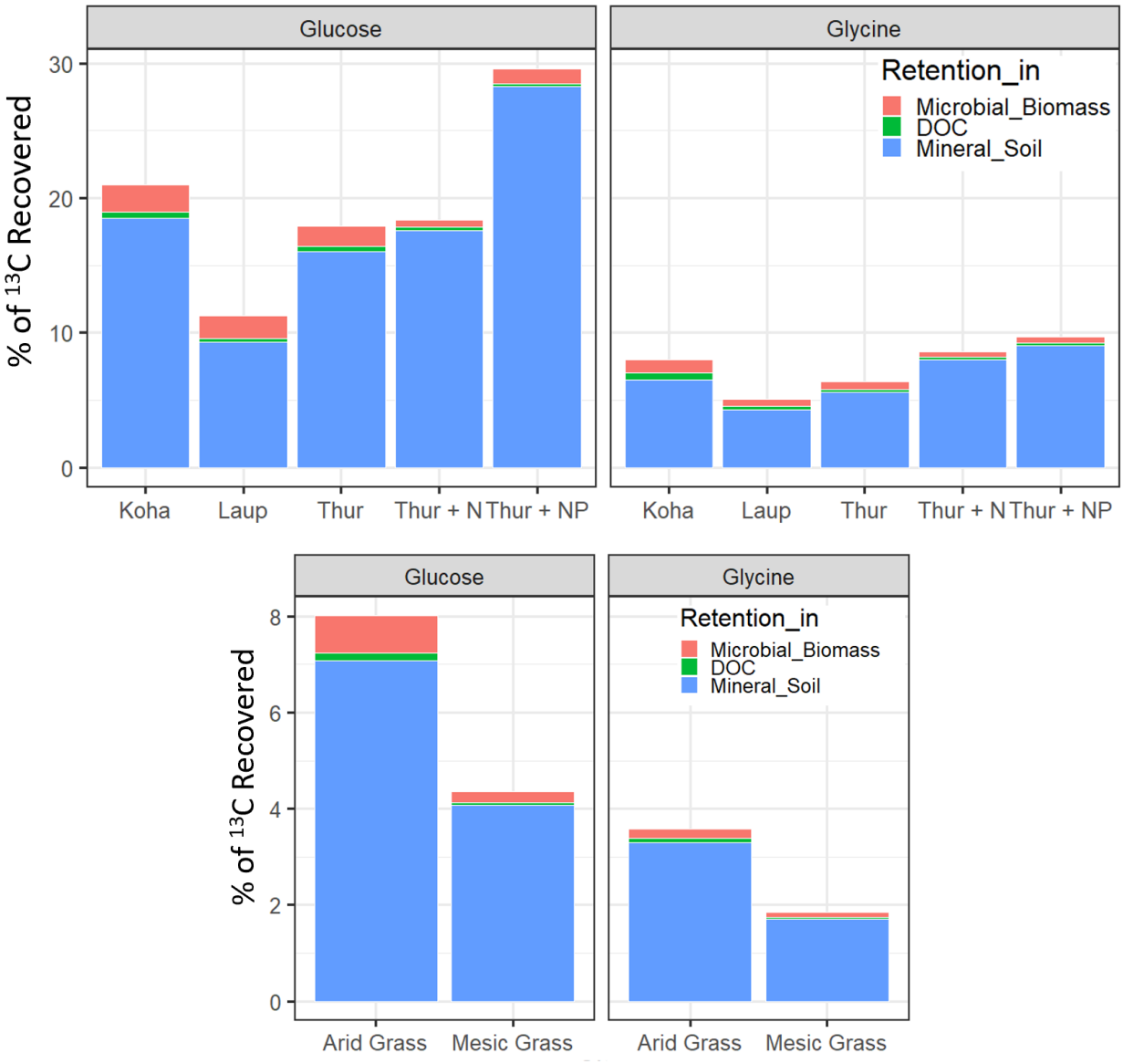

Figure 8 Overall partitioning of 13C from added glucose and glycine retained into DOC, microbial biomass and mineral soil pools is shown for different sites after one year of incubation (n = 3). Percent of 13C added recovered in each pool is shown. (Top forest sites, bottom grassland sites)

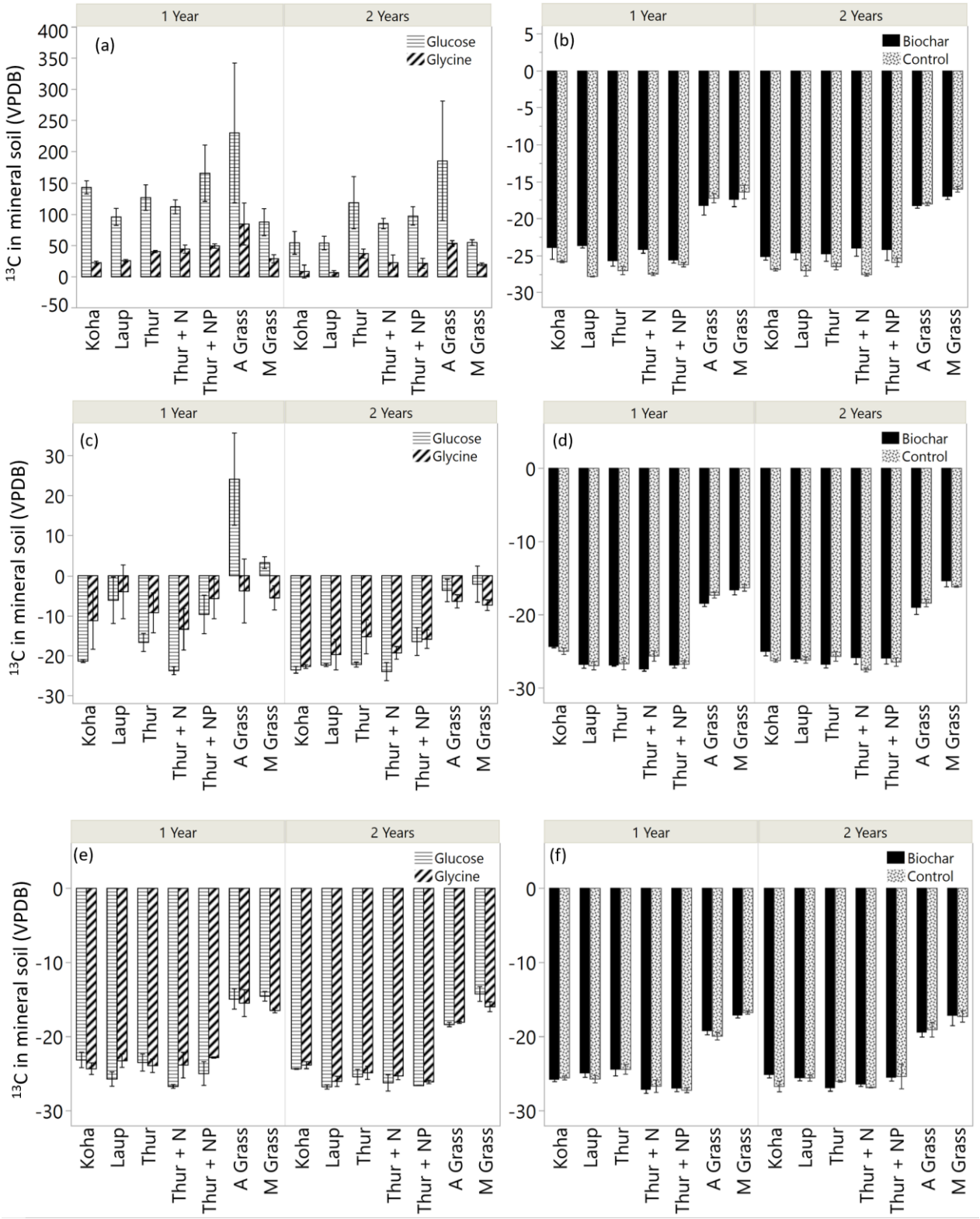

Figure 9 Effect of different organic compound addition on C isotope signature in soil after one year and two years at 0-5 cm (a-glucose and glycine, b-biochar and control), 5-10 cm (c-glucose and glycine, d-biochar and

control) 10-23 cm (e-glucose and glycine, f-biochar and control). Mean ± SE are shown (n=3).

The relative partitioning of retained ^{13}C among soil pools after one year of incubation was less variable than the total proportion retained in each pool because this calculation controls for variation in respiration losses (Table 4). Relative partitioning of ^{13}C in both microbial biomass and mineral soil varied by sites but not by the organic compound added. Among all the sites at 0-5 cm soil an average of 90.5 ± 1.3% and 89.4 ± 1.0% of ^{13}C was retained in the soil mineral pool, 7.7 ± 1.1% and 7.2 ± 0.7% of ^{13}C was retained in the microbial biomass pool and 1.7 ± 0.3% and 3.4 ± 0.4% was retained in the DOC pool from glucose and glycine respectively (Table 4).

Table 4 Partitioning of added ^{13}C across different soil pools in 0-5 cm soil after one year of field incubation is shown by site and organic compound added. Relative recovery of added ^{13}C in each pool is shown in three columns on the right side of the table. Averages are given ± 1 SE (n=3).

Site	Compound	% of added ^{13}C recovered in				Relative recovery of added ^{13}C in		
		Mineral Soil	DOC	MBC	Total	Mineral soil	DOC	MBC
Kohala	Glucose	18.51±2.49	0.47±0.08	2.02±0.58	21.0±3.07	88.4±1.5	2.2±0.2	9.4±1.7
	Glycine	6.51±0.41	0.5±0.04	0.99±0.14	8.0±0.53	81.4±0.4	6.4±0.7	12.3±1.1
Laupahoehoe	Glucose	9.3±1.42	0.25±0.06	1.7±0.43	11.26±1.9	83±1.5	2.2±0.2	14.8±1.3
	Glycine	4.27±0.25	0.29±0.06	0.49±0.03	5.05±0.33	84.7±0.7	5.7±0.9	9.7±0.2
Thurston	Glucose	16.05±3.79	0.39±0.16	1.46±0.23	17.9±4.07	89.2±1.1	2.2±0.7	8.5±0.9
	Glycine	5.56±0.54	0.22±0.01	0.56±0.06	6.33±0.58	87.7±0.7	3.5±0.5	8.8±0.4
Thurston + N	Glucose	17.6±0.72	0.25±0.04	0.55±0.07	18.4±0.76	95.7±0.3	1.3±0.2	3.0±0.4
	Glycine	8.03±0.4	0.14±0.01	0.42±0.05	8.59±0.35	93.4±0.9	1.7±0.1	5.0±0.8
Thurston + NP	Glucose	28.28±5.72	0.21±0.03	1.12±0.23	29.62±5.93	95.4±0.3	0.8±0.2	3.8±0.4
	Glycine	9.06±1.12	0.15±0.03	0.46±0.05	9.67±1.2	93.6±0.03	1.6±0.1	4.8±0.1
Arid Grass	Glucose	7.08±1	0.16±0.11	0.78±0.27	8.02±0.76	87.5±5.7	2.3±1.7	10.2±4.2
	Glycine	3.29±1.32	0.09±0.03	0.19±0.11	3.58±1.45	92.2±1.4	3.0±0.8	4.9±1.0
Mesic Grass	Glucose	4.08±0.91	0.04±0.01	0.24±0.17	4.36±1.08	94.5±1.9	1.1±0.4	4.4±2.3
	Glycine	1.71±0.25	0.04±0.01	0.1±0.04	1.85±0.27	92.6±1.1	2.3±0.5	5.1±1.6

If we assume that relative retention of ^{13}C among mineral pool, microbial biomass pools and DOC pools in 5-10 and 10-23 cm soil remains similar to that of 0-5 cm, total retention of added ^{13}C in all soil pools in the given column after 1 year of incubation amounts to an average of 20.8 ± 2.1 and loss to respiration and leaching to 79.2 ± 2.1 % for glucose and retention of 9.4

± 0.6 % and loss of 90.6 ± 0.6 % for glycine in forest sites. For the grassland sites, total retention and losses for glucose averages to 7.6 ± 1.2 % and 92.4± 1.2 % respectively for glucose and 3.3 ± 0.9 % and 96.7 ± 0.9 % for glycine. Table 5 shows the estimates of total ^{13}C retained in soil columns in all pools and the respiratory and leaching loss in 1year period.

Table 5 Retention and loss of added ^{13}C from soil columns after one year of field incubation. Averages are given ± 1 SE (n=3).

Site	Compound added	Total % of added ^{13} C retained in				Respiratory and leaching loss
		0-5 cm	5-10 cm	10-23 cm	Total column	
Kohala	Glucose	21±3.07	0.39±0.09	0.62±0.31	22±3.4	78±3.4
	Glycine	8±0.53	1.83±1.06	0.42±0.2	10.2±1.6	89.8±1.6
Laupahoehoe	Glucose	11.26±1.9	1.97±0.73	0.1±0.1	13.3±2.7	86.7±2.7
	Glycine	5.05±0.33	2.36±0.81	0.32±0.02	7.7±1.1	92.3±1.1
Thurston	Glucose	17.9±4.07	0.51±0.15	0.34±0.22	18.8±4.2	81.2±4.2
	Glycine	6.33±0.58	1.28±0.29	0.18±0.13	7.8±0.6	92.2±0.6
Thurston + N	Glucose	18.4±0.76	0.18±0.07	0±0	18.6±0.7	81.4±0.7
	Glycine	8.59±0.35	0.89±0.35	0.26±0.2	9.7±0.7	90.3±0.7
Thurston + NP	Glucose	29.62±5.93	1.45±0.48	0.19±0.09	31.3±5.4	68.7±5.4
	Glycine	9.67±1.2	1.62±0.26	0.28±0.05	11.6±1.2	88.4±1.2
Arid Grass	Glucose	8.02±0.76	1.67±0.77	0.29±0.07	10±0.1	90±0.1
	Glycine	3.58±1.45	0.33±0.13	0.33±0.21	4.2±1.7	95.8±1.7
Mesic Grass	Glucose	4.36±1.08	0.71±0.03	0.21±0.06	5.3±1.1	94.7±1.1
	Glycine	1.85±0.27	0.39±0.09	0.04±0.02	2.3±0.3	97.7±0.3

Forest Sites

The proportion of added ^{13}C that was retained in mineral soil at 0-5 cm depth in the unfertilized forest sites varied significantly with site, organic C added, and time, with no significant interaction effect (Figure 10). Retention was significantly greater for glucose versus glycine and for one year versus two years. ^{13}C retention in mineral soil was significantly greater at Thurston versus Laupahoehoe but retention between Kohala and Thurston and Laupahoehoe and Thurston was not significantly different. Across forest sites, the mineral soil at 0-5 cm retained an average

of 14.6 ± 1.9% (n = 9) and 5.4 ± 0.4% of added ^{13}C from glucose and glycine respectively after one year and 9.8 ± 2.3% and 4.0 ± 0.7% after two years.

When separate ANOVA was undertaken for two time points, ^{13}C retention in mineral soil during the first year varied by both site and substrate with no interaction effect in unfertilized 0-5 cm soil. Retention was higher for glucose versus glycine. Among the sites, Kohala had significantly greater retention compared to Laupahoehoe but there was no significantly different retention between Kohala and Thurston or Laupahoehoe and Thurston. At the end of the second year ^{13}C retention in mineral soil was still higher for glucose versus glycine but the retention did not vary significantly by site.

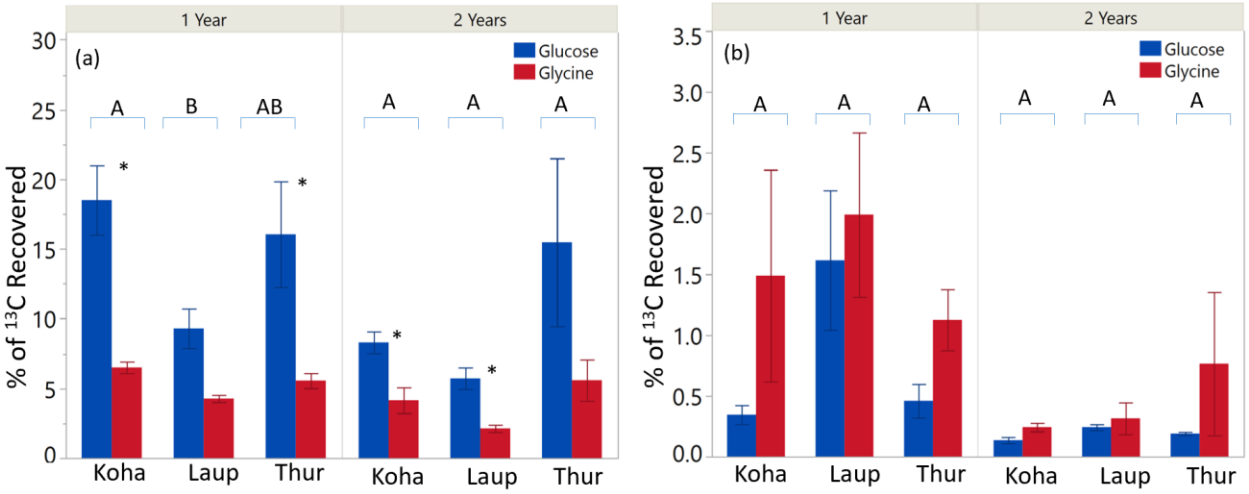

Figure 10 Percent of 13C retained in mineral soil by organic compound added after one year and two years of incubation is shown for unfertilized forest sites 0-5 cm (a) and 5-10 cm (b). Differences between sites in each time point are shown by uppercase letters, and differences between organic compound treatments within that site are shown by an asterisk. Mean ± SE are shown (n=3). Letters show differences using Tukey HSD Tests and asterisk show differences using paired Student's t-test.

At 0-5 cm depth, the percentage of ^{13}C retained in MBC varied significantly with organic C while the site was not a significant effect (Figure 13a). ^{13}C retention in MBC was significantly

greater for glucose compared to glycine (Figure 13a). Across forest sites, an average of 1.7 ± 0.2% (n = 9) and 0.7 ± 0.1% of ^{13}C from added glucose and glycine respectively was recovered in MBC after one year.

For 0-5 cm depth, the percentage of ^{13}C retained in DOC varied significantly with site but not by organic compounds. ^{13}C retention in DOC was significantly greater at Kohala compared to Laupahoehoe but retention between Kohala and Thurston and Laupahoehoe and Thurston was not significantly different. Across forest sites, an average of 0.37 ± 0.06% (n = 9) and 0.34 ± 0.05% of ^{13}C from added glucose and glycine respectively was recovered in DOC after one year.

Percentage of ^{13}C retained in mineral soil at 5-10 cm varied significantly by organic compound and time, with no significant effect of site (Figure 10 b). Unlike at 0-5 cm depth, ^{13}C recovery in mineral soil in this depth was greater from glycine versus glucose. When a separate ANOVA was undertaken at two time points ^{13}C retention in mineral soil varied significantly neither by site nor by organic C during both first year and second year.

Fertilization Effect

Among the Thurston sites, retention varied significantly with organic compound added and time, with no significant effect of fertilization (SI, Table A3, Figure 11a). Retention was significantly greater for glucose compared to glycine and greater for one year compared to two years. When separate ANOVA was undertaken for two time points, in the first year, ^{13}C retention in mineral soil in 0-5 cm varied by both fertility treatment and organic C without the interaction effect. Retention was higher for glucose versus glycine. Among the fertility treatments +NP addition had significantly greater retention compared to the unfertilized site but there was no significantly different retention between +NP and N site or between N and unfertilized site. At the

end of the second year, ^{13}C retention in mineral soil was significantly higher for glucose versus glycine but it did not vary significantly by fertility treatment.

The proportion of ^{13}C retained in MBC in Thurston sites, varied significantly by fertilization and C added and there was a significant fertilization × organic C interaction (Figure 7b). The interaction resulted because the fertilization effect was significant only for ^{13}C retention from glucose but not from glycine. Glucose retention at unfertilized Thurston site was significantly greater than glucose retention in +N site but not significantly different from +NP site. No different retention was seen for glycine retention among any of the fertilization treatment sites. The proportion of ^{13}C retained in DOC in Thurston sites showed no significant effect of either fertilization or the organic C.

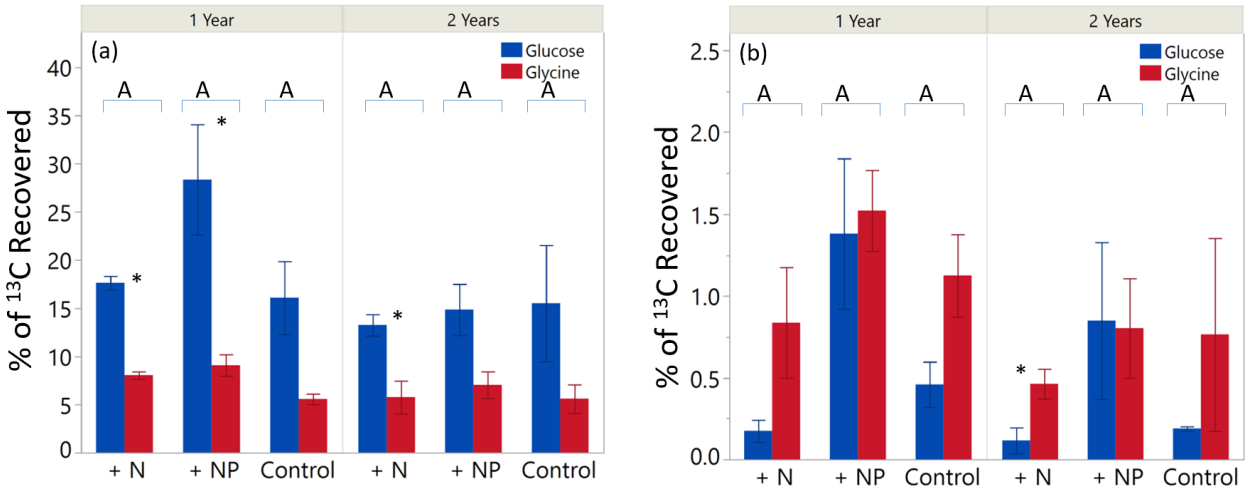

Figure 11 Percent of ^{13}C retained in mineral soil by organic compound added after one year and two years of incubation is shown for Thurston sites with +N, +NP and without fertilization. 0-5 cm (a) and 5-10 cm (b). Differences between sites in each time point are shown by uppercase letters, and differences between organic compound treatments within that site are shown by an asterisk. Mean ± SE are shown (n=3). Letters show differences using Tukey HSD Tests and asterisk show differences using paired Student's t-test.

The proportion of ^{13}C retained in Thurston sites at 5-10 cm depth varied by fertilization, organic C addition and time (5b). ^{13}C retention in mineral soil in this depth was significantly

greater at +NP site compared to +N site but retention at unfertilized Thurston site was not significantly different from both +NP and + N sites. Unlike at 0-5 cm depth, ^{13}C recovery in mineral soil in this depth was greater from glycine versus glucose. When separate ANOVA was undertaken for two time points, ^{13}C retention in mineral soil at 5-10 cm depth varied significantly by both fertility treatment and organic C during the first year. Retention was greater for glycine versus glucose. Among the fertility treatments, +NP addition had significantly greater retention compared to +N site but there was no significantly different retention between +NP and the unfertilized site or between N and the unfertilized site. ^{13}C retention in mineral soil varied significantly neither by site nor by organic C during the second year in this depth.

Grassland Sites

Grassland soils retained about three times lesser ^{13}C in all soil pools compared to forest sites. ^{13}C retention in mineral soil in the grassland sites at 0-5 cm varied significantly with site, organic compound added and time with no significant interaction effect (Figure 12a). Retention in mineral soil was significantly greater for arid vs. mesic site, for glucose vs. glycine, and at one year vs. two years. Across grassland sites, the mineral soil at 0-5 cm retained an average of 5.6 ± 0.9% (n = 9) and 2.5 ± 0.7% of added ^{13}C from glucose and glycine respectively after one year and 3.5 ± 0.9% and 1.6 ± 0.2% after two years.

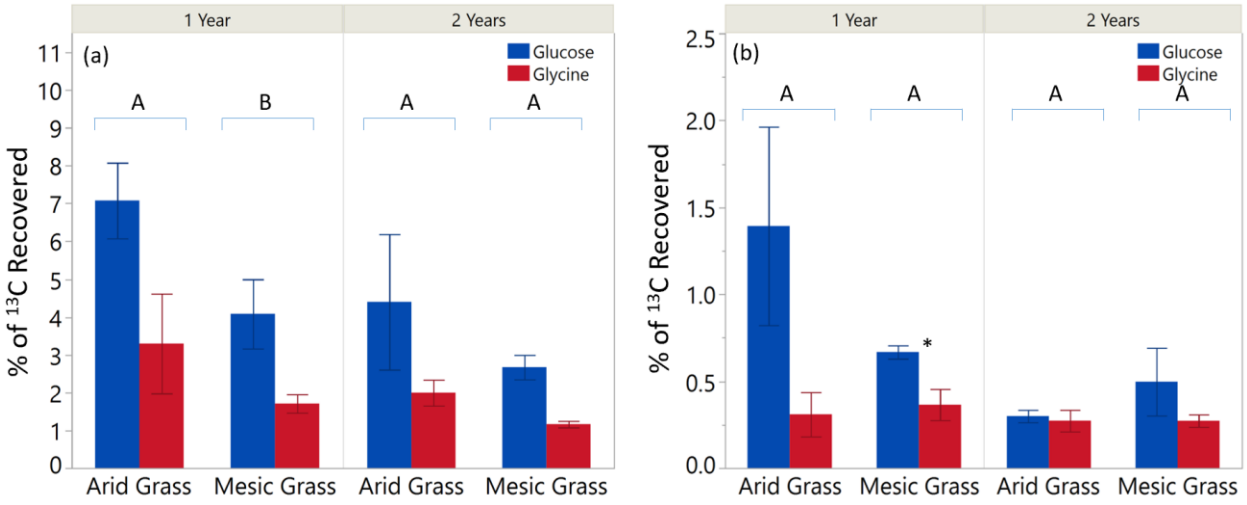

Figure 12 Percent of ^{13}C retained in mineral soil by organic compound added after one year and two years of incubation is shown for grassland sites 0-5 cm (a) and 5-10 cm (b). Differences between sites in each time point are shown by uppercase letters, and differences between organic compound treatments within that site are shown by an asterisk. Mean ± SE are shown (n=3). Letters show differences using Tukey HSD Tests and asterisk show differences using paired Student's t-test.

When ANOVA was undertaken for two time points separately, ^{13}C retention in 0-5 cm mineral soil in the first year varied both by site and organic C with no interaction effect. Retention was higher for glucose versus glycine and for arid site compared to mesic site. At the end of the second year, ^{13}C retention in mineral soil varied significantly neither by site nor by organic C.

No significant effect of site as well as organic compound was observed in the proportion of ^{13}C retention in MBC or DOC in the grassland sites after one year (Figure 13c). Across grassland sites, an average of 0.5 ± 0.2% (n = 6) of added ^{13}C was retained in the microbial biomass for glucose and 0.1 ± 0.06% was retained for glycine after one year of incubation. Similarly, an average of 0.1 ± 0.05% (n = 6) of added ^{13}C was retained in DOC for glucose and 0.04 ± 0.005% was retained for glycine after one year of incubation constituting the smallest pool of ^{13}C retention (Figure 8).

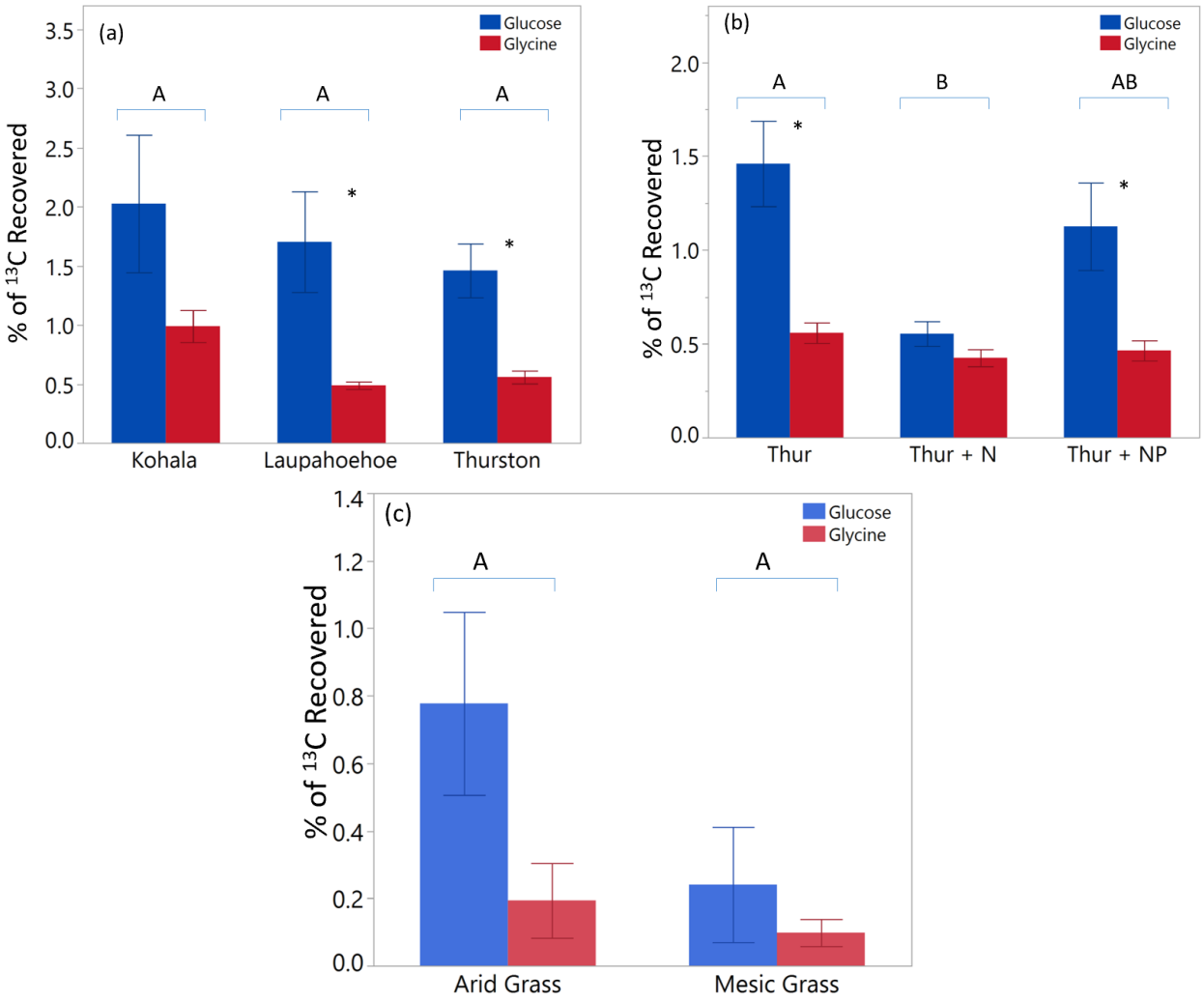

Figure 13 Percent of 13C retained in microbial biomass by organic C added after one year of incubation is shown for unfertilized forest sites 0-5 cm (a), Thurston sites 0-5 cm (b) and grassland sites (c). Differences between sites are shown by uppercase letters, and differences between organic compound treatments within that site are shown by an asterisk. Mean ± SE are shown (n=3). Letters show differences using Tukey HSD Tests and asterisk show differences using paired Student's t-test.

Percentage of added ^{13}C retained in mineral soil at 5-10 cm in grassland sites varied significantly by organic matter added and time (but it is not significant with time when the insignificant interaction term is removed from the whole model), with no significant effect of site (Figure 12 b). For the grassland sites, ^{13}C retention in mineral soil was significantly greater for glucose versus glycine and greater at one year versus two years (but it is not significant with time

when the insignificant interaction term is removed). When ANOVA was undertaken for two time points separately, ^{13}C retention in 5-10 cm in mineral soil was significantly greater for glucose versus glycine but it did not differ significantly by site during the first year. Retention was greater for glucose versus glycine. During the second year, retention in mineral soil varied significantly neither by site nor by organic C.

Organic chemistry

Difference between the sites

The solid-state ^{13}C NMR spectra reveal that soils in all analyzed samples were dominated by O-alkyl (cellulose, other carbohydrates) and alkyl C (waxes, other lipids). In general, forest, sites had a significantly greater proportion of alkyl C (waxes, other lipids), O-alkyl C (cellulose, other carbohydrates) and di-O-alkyl C (hemicellulose) groups compared to grassland sites. The grassland sites had a higher proportion of N-alkyl + methoxyl C (proteins, peptides) and amide + carboxyl C (chitin + hemicellulose) groups compared to forest sites. There was no significant difference in the proportion of Aromatic C (lignin, tannin) and Phenolic C (acids, tannin) between the forest and the grassland sites.

There also existed some differences in the composition of relative proportions of different C biomolecules among the forest sites. Among the forest sites, Thurston had lower N-alkyl + methoxyl C compared to other sites. O-alkyl C (cellulose, other carbohydrates) was greatest at Thurston followed by Kohala and Laupahoehoe. There was no significant difference between sites for other groups among forest sites. In the grassland site, mesic grass had a higher content of O-alkyl C (cellulose, other carbohydrates) and di-O-alkyl C (hemicellulose) but lower content of alkyl C (waxes, other lipids), N-alkyl + methoxyl C (proteins, peptides).

Glucose and glycine addition

Solid-state ^{13}C NMR spectroscopy revealed the effects of organic C addition on the organic chemistry of SOC after two years of field incubation by showing changes in the relative proportions of different C biomolecules (Table 6, Figure 14, 15). There was a significant effect of both site and organic C addition in the relative proportions of biomolecules for alkyl, N-alkyl + methoxyl and O-alkyl C groups. Aromatic and phenolic C proportion in soil only varied by organic C addition while Di-O-alkyl and amide + carboxyl C proportion varied only by site. There was no significant interaction effect between sites and organic C for any of the functional groups. Glucose addition caused a significant decline in the proportion of aromatic and phenolic C and glycine addition increased the proportion of N-alkyl + methoxyl and O-alkyl C functional groups relative to control. When glucose and glycine added samples are pooled, the addition of ^{13}C enriched glucose or glycine demonstrated a significant increase in the proportion of alkyl C and the decrease in the proportion of aromatic and phenolic C relative to control, while no significant change was seen in the proportion of other C groups. Alkyl C is believed to be derived from original microbial metabolites products and is considered easy to be stabilized on clay components (Deng et al., 2019).

Table 6 The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for Bulk soil for different organic C addition for the forest sites. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. Since there was no interaction effect between sites and organic C added the data from all sites are pooled. Averages are given ± 1 SE (n=9).

Organic Compound	Alkyl C (waxes, other lipids) 0–45 ppm*	N-Alkyl + Methoxyl C (proteins, peptides) 45–60 ppm*	O-Alkyl C (cellulose, other carbohydrates) 60–95 ppm*	Di-O-Alkyl C (hemicellulose) 95–110 ppm	Aromatic C (lignin, tannin) 110–145 ppm*	Phenolic C (acids, tannin) 145–165 ppm*	Amide + Carboxyl C (chitin + hemicellulose) 165–215 ppm	Ratio of (Alkyl + O-alkyl + N-alkyl):(phenolic + aromatic)*
Control	21.9±1.3^{a}	8±0.6^{b}	25.3±0.3^{ab}	7.4±0.5^{a}	17±0.3^{a}	7.2±0.4^{a}	13.2±0.2^{a}	1.3±0.4^{b}
Glucose	23.9±1.5^{a}	8.3±0.7^{ab}	25.7±0.3^{a}	7.1±0.5^{a}	15.5±0.2^{b}	6.3±0.4^{b}	13.2±0.2^{a}	1.5±0.3^{a}
Glycine	23.6±1.4^{a}	8.4±0.9^{a}	24.6±0.2^{b}	7.1±0.5^{a}	16±0.2^{ab}	6.8±0.4^{ab}	13.5±0.2^{a}	1.4±0.3^{ab}

* Significant effect of compound addition from ANOVA test

For each C functional group values down each column not connected by the same letter are significantly different from the Tukey HSD test.

Table 7 The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for Bulk soil for different organic C addition for the grassland sites. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. Sites are pooled. Averages are given ± 1 SE (n=6).

Organic Compound	Alkyl C (waxes, other lipids) 0–45 ppm $^{\pi}$	N-Alkyl + Methoxyl C (proteins, peptides) 45–60 ppm	O-Alkyl C (cellulose, other carbohydrates) 60–95 ppm*	Di-O-Alkyl C (hemicellulose) 95–110 ppm	Aromatic C (lignin, tannin) 110–145 ppm*	Phenolic C (acids, tannin) 145–165 ppm	Amide + Carboxyl C (chitin + hemicellulose) 165–215 ppm	Ratio of (Alkyl + O-alkyl + N-alkyl):(phenolic +aromatic)*$^{\pi}$
Biochar	21±1.1	7.8±0.6	23.4±0.2	7.1±0.7	20.5±0.2	7.7±0.9	12.5±0.	1.1±0.
Control	21.9±1.3	8±0.6	25.3±0.3	7.4±0.5	17±0.3	7.2±0.4	13.2±0.2	1.3±0.4

* Significant effect of compound addition from ANOVA test

$^{\Pi}$ Significant organic C × site interaction

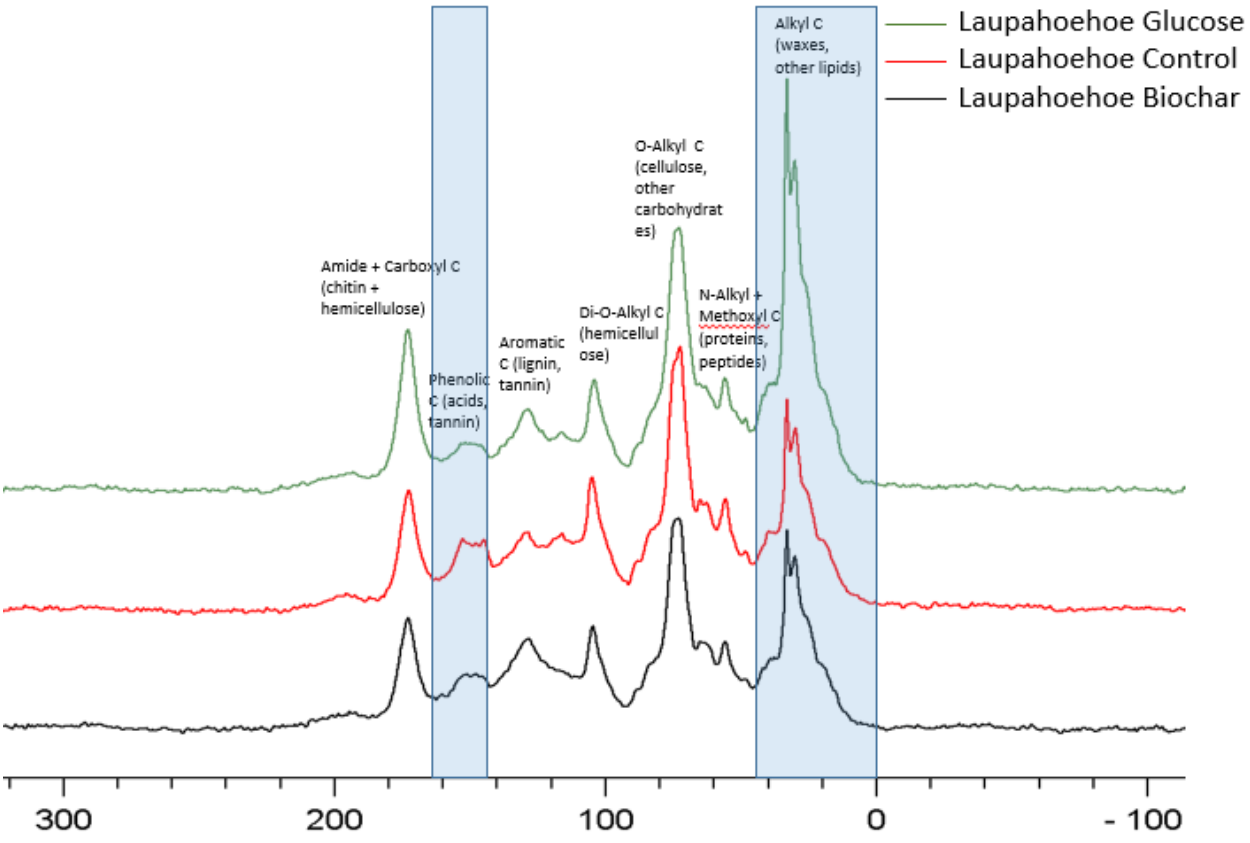

Figure 14 A representative solid state 13C NMR spectrogram of soil SOC from Laupahoehoe site showing the change in the SOC signature in soil due glucose and biochar addition vs. control after two years of addition. The regions for the C-groups that show the significant change is highlighted.

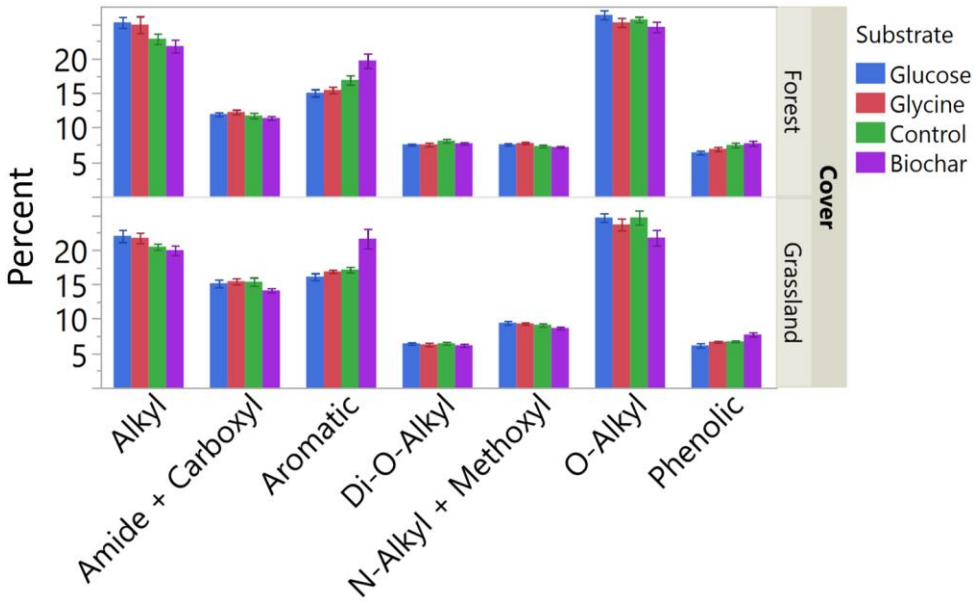

Figure 15 Average relative percentage of each C functional group from ^{13}C NMR for glucose and biochar addition vs. control. N=9 for forest, N=6 for grassland because data for sites are pooled

Biochar addition

There was a significant effect of both sites and biochar addition in the relative proportions of biomolecules only for O-alkyl C. Organic C added was the only significant effect on aromatic group, while site was the only main effect for alkyl, N-alkyl + methoxyl, Di-O-alkyl and amide + carboxyl C. Neither the site or the biochar addition had the significant effect on the proportion of phenolic C in soil. A significant interaction between site and biochar addition resulted for the proportion of alkyl group because of greater proportion of alkyl group for control at Kohala relative to biochar addition but not for other sites. Biochar addition caused a significant decline in the proportion of O-alkyl C and the increase in the aromatic C relative to control (Figure 15).

Principal component analysis also showed that the C functional groups shifted as a result of the addition of different organic C, indicating a shift in the composition of SOC as a result of the

addition of these compounds (Figure 16).

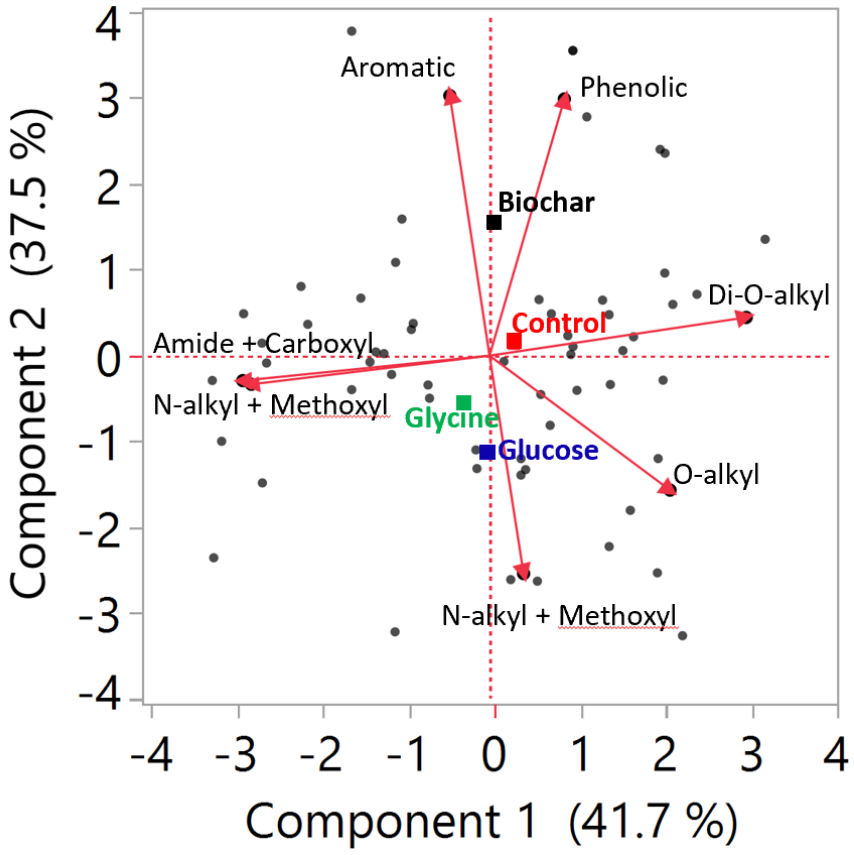

Figure 16 Principal component analysis (PCA) combined ordination plot performed on the relative abundance of 7 C functional groups across different organic compound addition treatment. Each red square represents a specific SOC chemical structure in specific organic compound addition treatment. Squares that are close together are more similar to one another than the squares that are far apart.

Decomposition of Charcoal

As expected, ^{13}C enrichment was observed in 0-5 cm soil in forest sites as a result of the addition of more enriched switchgrass biochar. Similarly, ^{13}C depletion was observed in the grassland sites as a result of the addition of more depleted pine biochar (Figure 9). Our results showed a slight gain in the ^{13}C enrichment in the second year in the grassland sites compared to the first year, an indication of loss of added biochar in the grassland during that period (Figure A3,

Appendix). On the contrary, we did not see a trend in the change of ^{13}C enrichment between the two years in any of the unfertilized forest sites (not shown). However, a slight loss in the ^{13}C enrichment in +N and +NP plots was observed in the second year, an indication of loss of added biochar (Fig. A3, Appendix). However, none of these gains of ^{13}C enrichment in grassland sites and loss in +N and +NP sites were statistically significant, indicating that there was no evidence of significant charcoal loss between the first and the second year.

Discussion

The Fate of Added ^{13}C in Soils

Site Effects on ^{13}C Retention and Stabilization in Soil

Organic C stored in soil results from the net balance between the rate of SOC inputs and the rate of mineralization in the various organic C pools (Post and Kwon, 2000). Rapid loss of ^{13}C from amended glucose and glycine from the soil results from the faster turnover of the added organic C into CO_2 by the soil microbial community. Forest soils incorporated a significantly greater amount of C from glucose and glycine into mineral soil in two years than the grassland soils suggesting that forest ecosystems are more efficient in storing available light molecular weight organic C compounds in the tropics. Grassland soils had higher pH values, lower soil moisture, lower C and N content compared to forest sites. Greater availability of moisture and C and N source probably makes microbes in forest sites more efficient users of glucose and glycine. Available studies on whether fresh C substrate is lost faster in forest or grassland sites show contradictory results. For example, a study on 150 forests and 150 grasslands showed that fine root decomposition is significantly faster in grasslands than in forests (Solly et al., 2014). Contrary to it, a greater amount of added ^{14}C from glucose was lost from an African lower montane forest than

a grassland site after 65 days incubation (Mganga and Kuzyakov, 2014).

We expected that retention of ^{13}C among the forest sites would be greatest in Kohala, the site with strongly-weathered soils with greater binding site density (Torn et al., 1997). However, at the end of two years, ^{13}C retention did not differ significantly among the forest sites. Similarly, for the grassland, the mesic site with soil having greater binding site density (Chadwick et al., 2003) did not have significantly different retention versus arid site. Although we see no difference in ^{13}C retention among forest sites after two years, Thurston and Kohala had significantly greater retention versus Laupahoehoe during the first year. Thurston soil has smaller microbial biomass overall than the other two sites, so microbes may have had a lower capacity to take up and process added C initially, allowing for greater mineral sorption in this site. On the other hand, greater sorption of added C and its metabolic products in mineral surfaces in Kohala may have slowed down the turnover of the added organic C, preventing from a rapid loss. Laupahoehoe with relatively larger microbial biomass than Thurston but lesser sorption capacity than Kohala might have lost the added organic C to respiration more quickly compared to the other sites. However, in two years, this difference in retention between the sites was likely reduced/erased as microbes continued processing the available C in all sites. In this study, we tracked the fate of a single episodic addition of organic C to the soil, thus limiting our ability to follow the continuous process of microbial turnover that happens in nature when microorganisms receive a repeated input of C (van Hees et al., 2005). Under recurrent C inputs, the initial difference between the sites that we observed during the first year may continue to manifest as microbial products continue to get added to the mineral soil. Differences in microbial community composition could also help explain the apparent differences in microbial uptake, efficiency, and long-term recycling, with differences in microbial community composition documented for Thurston vs. Laupahoehoe (Balser, 2001).

Thus, microbial biomass and community characteristics, as well as soil mineralogy, likely have a strong influence over C retention in these soils over the short term, but these differences likely become less relevant in the longer term (two years).

Unlike at 0-5 cm depth, a consistently higher retention trend was seen for glycine ^{13}C compared to glucose ^{13}C in mineral soil at 5-10 cm. This trend, however, occurred only in the forest sites and was statistically significant only when data from both years are pooled. The higher retention of glucose ^{13}C in 0-5 cm soil but of glycine ^{13}C in 5-10 cm soil probably indicates that glycine has higher mobility in soils and penetrates faster into the deeper soil compared to glucose. The higher downward mobility of glycine probably occurs only when soil moisture content is very high as seen in our forest sites. Alternatively, it also can be a result of differential microbial community composition between these two depths (Fierer et al., 2003), that metabolize these compounds differently (Rinnan and Baath, 2009).

Only a small proportion (around 7 to 8%) of ^{13}C that was retained in the bulk soil was retained in the microbial biomass pool after one year. A similar ^{13}C glucose addition in an agriculture soil showed ^{13}C recovery of 16% and 2.3% respectively in microbial biomass and 55% and 23% in bulk soil after 1 and 12 months (Kallenbach et al., 2015), which is similar to the range of ^{13}C retention we observed for glucose in our forest sites. Thus, although we did not make measurements during early period of this experiment, based on the results from our lab incubation study of soil from the same site (see chapter 1) we can deduct that microbes took up most of the added organic C and slowly converted fresh C into longer-term soil C pools (Liang et al., 2017). Although absolute retention of ^{13}C in both microbial biomass and mineral soil was much higher for glucose (2.6 times in microbial biomass pool and 2.5 times in mineral pool) compared to

glycine, there was no significant difference in the relative retention of these two organic compounds in microbial and mineral pool. This similar relative retention of glucose ^{13}C and glycine ^{13}C in these pools indicates that in annual timescale, both the compounds are lost proportionally from the microbial biomass and mineral soil pools, regardless of how much of each compound is lost in overall. This may also indicate that microbial pool and mineral soil are tightly coupled during the processing of light molecular organic compound like glucose and glycine.

Fertilization Effects on ^{13}C Retention in Soil

Our results show that both +N and +NP fertilization treatments had no significant effect on ^{13}C retention in mineral soil in both 0-5 cm and 5-10 cm at the end of two years. However, +NP site had significantly greater ^{13}C retention in mineral soil compared to +N site at the end of the first year, though there was no difference in ^{13}C retention between +NP and unfertilized site and +N and unfertilized site. Nitrogen addition in ecosystems has been generally associated with slower decomposition of soil organic matter (Zak et al., 2008; Janssens et al., 2010) (Riggs and Hobbie, 2016). Studies have shown that N additions decreased soil microbial biomass and heterotrophic respiration across a wide range of terrestrial ecosystems (Treseder, 2008; Cusack et al., 2010a; Ramirez et al., 2012; Frey et al., 2014). Other studies in contrary have demonstrated the increased decomposition of soil organic matter or added organic compounds when N and P are added, indicating nutrient limitations to decomposition (Cleveland et al., 2002; Cleveland and Townsend, 2006; Liu et al., 2013; Chen et al., 2016). Initially, microorganisms may have a different ability to obtain adequate nutrients from the soil required to process a pulse of added labile C in different nutrient conditions. N fertilization might have contributed to slightly decrease microbial efficiency of glucose and glycine use while NP fertilization might have contributed to

slightly increase microbial efficiency of glucose and glycine use thus causing a significant difference in C retention between +N and +NP sites. However, this increase and decrease were enough to have a significant difference in C retention with the unfertilized sites. The eventual lack of effect of N or P fertilization on the turnover and retention of added organic C in our study may echo the limitation of other nutrients or the limiting physiochemical environment in soil like low pH (see Table A2) which can lead to nutrient unavailability such as increased sorption of P to minerals (Olander and Vitousek, 2004). Our results demonstrate that in the long term, the quality of organic substrate added, rather than the soil fertility status more strongly regulates retention and turnover of fresh labile C entering the soil in our study sites.

Our results that nutrient treatments had no significant effect on ^{13}C retention in microbial biomass in 0-5 cm for glycine is in agreement with a study that showed that +N, +P and +NP treatments did not influence the amount of ^{13}C respired or incorporated in microbial PLFAs from added xylose and hemicellulose in soils from a 3400m tropical elevation gradient varying substantially in N and P availability. However, it is contradictory to our observation that ^{13}C retention from glucose addition was greater at the unfertilized site compared to +N site. Our result showing greater retention of glucose in microbial biomass compared to glycine also contradicts their observation, which showed no significant difference between incorporation of xylose and hemicellulose in microbial PLFA after seven days of incubation (Hicks et al., 2019).

The Role of C Chemistry on ^{13}C Retention

We observed that for all soils in 0-5 cm more glucose was incorporated in microbial biomass and mineral soil, which is consistent with other studies comparing glucose and glycine addition (Bradford et al., 2013; Oldfield et al., 2018). Lower microbial CUE for glycine compared

to glucose has been attributed to greater incorporation of C from glucose into structural components and extracellular microbial metabolites, with more catabolic use of glycine, leading to greater respiration losses (Webster et al., 1997; Kuzyakov and Demin, 1998; van Hees et al., 2005; Hartley et al., 2010). Generally, amino acids have shorter mean residence times in microbial biomass compared to glucose (Boddy et al., 2008). Higher retention for glucose in both microbial biomass and mineral soil pools at the end of the experiment also indicates that retention of C compound in microbial biomass promotes longer-term retention of C in soil.

Several studies have shown that plants compete with microorganisms to uptake glycine from soil directly (Näsholm et al., 1998; Näsholm et al., 2000; Owen and Jones, 2001; Zhu et al., 2019). However, other studies suggest direct uptake of glycine is of less ecological relevance to plants in relatively N rich temperate and tropical forests, grasslands and agricultural land (Schimel and Bennett, 2004; Kahmen et al., 2009). These studies show that some of the glycine loss from soil can also be attributed to direct plant uptake in our study. Without a full mass balance calculation of glycine's budget, these findings should be viewed with caution.

Effect on soil C chemistry

The increase in the proportion of alkyl C group in soil with glucose and glycine addition probably arises due to the formation of microbially originated products from their metabolism (Baldock et al., 1992; Lundberg et al., 2001). A study on metabolism of added ^{13}C labeled glucose in soil showed that its addition to soil resulted in the synthesis of O-alkyl C (66%), alkyl C (26%), carboxyl C (8%) and negligible if any aromatic or phenolic C during 34 days of lab incubation (Baldock et al., 1989). Our results, although showed an increased proportion of alkyl C in glucose and glycine amended soil, it did not show a significant O-alkyl C enrichment. Zhang et al. (2015)

reported that the proportion of O-alkyl C formed as a result of ^{13}C glucose addition in soil decreased at Day 31 compared with Day 21 while the proportion of alkyl C increased, suggesting that in longer-term more labile O-alkyl C compounds were further utilized. In our experiment, much of the O-alkyl C compounds, which are considered to be relatively easy to decompose compared to alkyl C (Simpson and Simpson, 2012; Solomon et al., 2012) might have already been lost during two years thus only showing the enrichment of alkyl C. Alkyl C is generally believed to derived from original microbial metabolites products and is considered to have higher stability against decomposition due to its higher adsorption affinity to get adsorbed on clay (Deng et al., 2019).

Decomposition of Charcoal

Although we observed a slight indication of loss of charcoal in grassland sites and +N and +NP Thurston sites, that there was no conclusive evidence of significant charcoal loss between the first and the second year. Since our incubation system was not fully closed, the possibility of occurrences of losses of charcoal due to wind or water erosion cannot be excluded therefore without a full mass balance calculation of charcoal budget, these findings should be viewed with caution.

Soil properties

Differences in bulk soil C pools among sites reflected differences in soil mineralogy. Kohala soils had the greatest soil C, clay, and short-range-order mineral content, followed by Laupahoehoe and then Thurston, where soils are sandier with an abundance of primary minerals (Shoji et al., 1993; Torn et al., 1997). Similarly, mesic grassland soils had higher short-range-order mineral content (Chadwick et al., 2003) also had a higher C and N contents.

N and NP addition increased the above ground productivity and litterfall of Thurston site (Vitousek et al., 1993) and at Thurston site N and P in combination significantly stimulated litter decomposition (Hobbie and Vitousek, 2000). Thus, increased litterfall combined with the increased decomposition of litter should have significantly increased the C and N stock in the soil in the +NP site. +N site also showed the increasing trend in soil CN, but the increase was not statistically significant. Increase in C stock in soil that received 22 years of chemical NP amendment was also reported for agricultural soil (He et al., 2018).

The priming effect refers to the modification of the decomposition rates of the native organic C due to the addition of labile C source (Blagodatsky et al., 2010; Kuzyakov, 2010). Priming is considered positive if the rate of decomposition of native C increases and negative if it decreases due to the addition of labile C (Blagodatskaya and Kuzyakov, 2008). We did not find any evidence of a priming effect of the added organic compound on the decomposition of the native organic C pool in our sites.

Conclusion

This study indicates that the composition of C substrates incoming to soils and the type of vegetation cover will likely be a crucial factor of soil C formation and stability. Forest soils incorporated greater amount of C from glucose and glycine into mineral soil in two years than the grassland soils suggesting that forest ecosystems are more efficient in storing available low molecular weight organic C compounds in the tropics. This study also indicates that microbial incorporation of organic compounds into biomass promotes longer-term C retention in soil. These findings have broader importance in demonstrating how vegetation cover type, soil mineralogy

and nutrient status can affect the loss and storage of different types of organic C inputs to soil.

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Appendix

Table A1: Soil variables for under different sites and depth

Site	Depth (cm)	DOC	TN	MBC	MBN	%Nitrogen	% Carbon
	0-5	0.65±0.03	0.18±0.01	1.9±0.17	0.33±0.03	1.69±0.04	38.98±1.65
	5-10	0.54±0.04	0.09±0.01	1.42±0.19	0.23±0.03	1.25±0.07	22.85±1.52
Kohala	10-23	0.45±0.04	0.05±0.01	0.48±0.08	0.08±0.01		
	0-5	0.62±0.04	0.26±0.03	1.51±0.12	0.26±0.02	1.94±0.06	42.11±1.29
	5-10	0.44±0.05	0.12±0.02	1.15±0.13	0.21±0.02	1.54±0.1	29.35±1.72
Laupahoehoe	10-23	0.51±0.06	0.04±0.01	0.56±0.06	0.12±0.01		
	0-5	0.32±0.04	0.03±0.01	0.84±0.13	0.17±0.02	1.03±0.04	24.69±1.3
	5-10	0.17±0.02	0.02±0	0.43±0.09	0.07±0.01	0.56±0.05	10.4±0.99
Thurston	10-23	0.07±0.01	0.01±0	0.05±0.01	0.01±0		
	0-5	0.36±0.05	0.09±0.01	1.05±0.11	0.16±0.02	1.19±0.04	28.96±1.39
	5-10	0.18±0.02	0.03±0.01	0.35±0.06	0.06±0.01	0.63±0.04	11.41±0.91
Thurston + N	10-23	0.07±0.01	0±0	0.06±0.01	0.01±0		
	0-5	0.3±0.04	0.04±0.01	0.99±0.1	0.15±0.02	1.26±0.06	32.86±1.88
Thurston +	5-10	0.15±0.04	0.02±0.01	0.46±0.12	0.07±0.01	0.73±0.07	14.79±1.77
NP	10-23	0.06±0.01	0±0	0.11±0.03	0.02±0		
	0-5	0.13±0.01	0.04±0	0.28±0.06	0.04±0.01	0.57±0.06	6.11±0.64
	5-10	0.09±0.01	0.02±0	0.25±0.03	0.03±0	0.39±0.02	3.51±0.26
Arid Grass	10-23	0.08±0	0.01±0	0.14±0.02	0.01±0		
	0-5	0.11±0.01	0.06±0.01	0.36±0.04	0.07±0.01	0.81±0.02	8.6±0.2
	5-10	0.1±0.01	0.03±0	0.25±0.03	0.06±0.01	0.71±0.02	7.2±0.23
Mesic Grass	10-23	0.1±0.01	0.02±0	0.19±0.03	0.04±0.01		

Table A2: Bulk density, soil moisture, and pH under different sites and depth

Site	Depth (cm)	Bulk Density	Gravimetric Moisture (%)		pH	
		g/cm	2016	2017	2016	2017
Kohala	0-5	0.24	79.3±1.3	71.3±1.2	4.29±0.09	4.26±0.05
Laupahoehoe		0.14	80.7±1.2	68.9±1.5	4.08±0.1	3.99±0.08
Thurston		0.28	77.4±1.4	74±1.2	5.15±0.12	5.17±0.05
Thurston + N			79.7±1.1	73.6±1.4	4.89±0.11	4.84±0.15
Thurston + NP			80.1±1.2	70.3±3.4	4.66±0.1	4.65±0.11
Arid Grass		0.37	12.2±0.2	14.3±0.4	6.99±0.07	6.82±0.06
Mesic Grass		0.28	41.4±1.9	35±0.9	6.3±0.03	6.11±0.04
Kohala	5-10	0.27	74.3±1.3	67.9±1.1	4.52±0.09	3.92±0.04
Laupahoehoe		0.19	76.1±1.8	68.6±0.9	3.73±0.07	3.71±0.07
Thurston		0.37	60.2±4.2	58.9±3.6	5.36±0.1	5.45±0.06
Thurston + N			65.5±2.1	55.9±3.8	5.23±0.08	5.09±0.08
Thurston + NP			64.2±5.3	51±6.1	5.06±0.09	5.1±0.13
Arid Grass		0.42	12.3±1.3	14.3±0.2	7.04±0.06	6.92±0.06
Mesic Grass		0.3	40.5±1.3	34.6±0.7	6.4±0.03	6.24±0.03
Kohala	10-23	0.33	62.9±1.4	61.5±2.1	4.48±0.06	4.26±0.07
Laupahoehoe		0.27	69.7±1.5	66.4±1.9	3.9±0.07	3.95±0.11
Thurston		0.52	40.2±3.3	39.2±3.9	5.72±0.1	5.52±0.1
Thurston + N			42.5±3	32.3±2.4	5.46±0.08	5.25±0.08
Thurston + NP			37±3.8	32.7±3	5.62±0.08	5.41±0.09
Arid Grass		0.50	15.1±0.3	15.4±0.2	7.15±0.03	6.96±0.06
Mesic Grass		0.33	37.7±0.6	31.5±1.9	6.61±0.03	6.39±0.03

Table A3: Relative percentage of ^{13}C recovered in mineral soil from different soil depths

Site	Compound	0-5 cm		5-10 cm		10-23 cm	
		Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Kohala	Glucose	95.7±1.2	95±0.9	1.7±0.3	1.5±0.2	2.5±1	3.5±1
	Glycine	80.5±7.9	84.2±4	15.6±7.6	5.4±1.4	3.9±1.9	10.4±3.7
Laupahoehoe	Glucose	85.5±3.4	95.9±0.2	14±3	4.1±0.2	0.5±0.5	0±0
	Glycine	67.2±6.9	85.3±7.5	28.6±7.3	13.1±5.9	4.2±0.5	1.6±1.6
Thurston	Glucose	95.5±1.4	97.7±0.9	2.7±0.5	1.9±1	1.8±0.9	0.3±0.3
	Glycine	81.1±2.1	88.8±5.3	16.6±3.7	9.4±5.4	2.3±1.6	1.9±1.3
Thurston + N	Glucose	99±0.4	98.9±0.9	1±0.4	0.9±0.7	0±0	0.2±0.2
	Glycine	88.6±3.1	90.6±2.6	8.8±3.1	8.4±2.7	2.5±2	1±0.8
Thurston + NP	Glucose	93.9±2.5	95.2±2.3	5.4±2.1	4.8±2.3	0.7±0.4	0±0
	Glycine	83.3±2.4	89.7±3.1	14.3±2.5	10.2±3.1	2.4±0.2	0.1±0.1
Arid Grass	Glucose	80.3±7.3	88.1±5.8	16.7±7.7	10.6±5.5	2.9±0.7	1.4±0.3
	Glycine	85.2±3.6	84.2±2.7	8.6±3.4	11.6±1.6	6.2±2.1	4.3±1.5
Mesic Grass	Glucose	81.5±3.1	80.1±4.3	14.6±2.9	13.5±3.5	3.9±1	6.4±1.7
	Glycine	80.5±5	77.6±2.4	17.5±4	18±0.8	2±1	4.5±2.4

Table A4: Carbon and nitrogen retention in the soil following hydrofluoric acid treatment

			Before HCL/HF acid demineralization				After HCL/HF acid demineralization				Mass loss during HCL/HF acid demineralization					
Site	Comp-ound added	Plot	Soil mass (gm)	N (%)	C (%)	CN Ratio	Soil mass (g)	N (%)	C (%)	CN Ratio	Soil mass loss (mg)	N mass loss (mg)	C mass loss (mg)	N loss (%)	C loss (%)	C/N of lost OM
Thur	Glucose	S1	2.5	1.15	31.3	27.2	1.52	1.26	33.2	26.3	977.3	9.6	278.3	33.4	35.6	29.1
Thur	Glycine	S1	2.8	0.74	15.8	21.4	1.49	1.07	20.9	19.6	1352.6	5.2	139.5	24.5	30.9	27.1
Thur	Biochar	S1	2.6	1.03	23.3	22.6	1.53	1.35	29.9	22.1	1038.8	5.8	140.6	22.0	23.5	24.2
Thur	Control	S1	2.5	0.92	19.8	21.5	1.39	1.34	29.7	22.2	1145.7	4.8	89.3	20.6	17.9	18.6
Thur	Glucose	S2	2.5	0.84	17.2	20.5	1.33	1.19	24.4	20.5	1193.6	5.4	110.0	25.2	25.3	20.5
Thur	Glycine	S2	2.5	0.91	22.9	25.1	1.40	1.25	31.7	25.3	1063.1	4.9	119.4	21.8	21.2	24.4
Thur	Biochar	S2	2.4	1.03	24.6	23.9	1.35	1.42	34.8	24.5	1099.9	6.1	133.8	24.1	22.2	22.0
Thur	Control	S2	2.5	0.65	14.3	21.9	0.90	1.33	30.1	22.7	1644.6	4.6	92.6	27.9	25.5	20.0
Thur	Glucose	S3	2.5	0.95	23.9	25.2	1.50	1.25	31.8	25.5	966.5	4.7	112.7	20.2	19.1	23.8
Thur	Glycine	S3	2.7	1.12	26.5	23.6	1.74	1.36	32.8	24.1	930.8	6.3	135.7	20.9	19.2	21.7
Thur	Biochar	S3	2.1	1.24	31.7	25.6	1.26	1.65	43.2	26.3	810.0	5.0	112.7	19.4	17.2	22.7
Thur	Control	S3	2.6	0.95	25.1	26.5	1.54	1.34	35.9	26.8	1107.4	4.5	112.7	17.9	16.9	25.0
Koha	Glucose	S1	2.6	1.79	45.8	25.6	2.14	1.89	50.0	26.5	415.2	5.3	100.6	11.6	8.6	19.0
Koha	Glycine	S1	2.5	1.96	46.0	23.5	2.01	2.02	50.7	25.1	483.8	8.3	127.9	16.9	11.2	15.5
Koha	Biochar	S1	2.5	1.77	46.4	26.2	1.96	1.85	50.5	27.4	575.7	8.7	185.4	19.5	15.8	21.3
Koha	Control	S1	2.6	1.54	29.0	18.8	1.28	2.48	51.0	20.6	1284.3	7.8	89.8	19.7	12.1	11.5
Koha	Glucose	S2	2.8	1.25	23.9	19.1	1.12	2.50	52.0	20.8	1702.0	7.3	94.2	20.8	14.0	12.9
Koha	Glycine	S2	2.6	1.62	33.3	20.6	1.71	2.18	45.1	20.7	851.8	4.2	83.0	10.1	9.7	19.7
Koha	Biochar	S2	2.5	1.71	33.9	19.8	1.34	2.52	53.2	21.1	1128.5	8.5	123.9	20.1	14.8	14.6
Koha	Control	S2	2.6	1.76	42.0	23.8	1.72	2.10	51.6	24.6	872.8	9.6	200.7	21.1	18.5	20.9
Koha	Glucose	S3	2.8	1.71	33.1	19.3	1.61	2.23	49.4	22.2	1181.8	11.9	127.9	25.0	13.9	10.7
Koha	Glycine	S3	2.9	1.8	42.8	23.8	2.16	1.93	49.9	25.9	706.2	10.0	149.1	19.4	12.1	14.9
Koha	Biochar	S3	2.5	1.81	44.2	24.4	1.81	1.80	53.3	29.6	651.2	12.0	122.4	26.8	11.2	10.2
Koha	Control	S3	2.6	1.68	29.3	17.4	1.26	2.81	49.5	17.6	1339.3	8.4	138.8	19.2	18.2	16.6
Laup	Glucose	S1	2.4	1.84	41.5	22.6	1.69	2.24	51.8	23.1	746.1	7.0	136.1	15.6	13.5	19.5
Laup	Glycine	S1	2.5	2.03	41.0	20.2	1.72	2.38	51.2	21.5	822.3	10.7	162.0	20.7	15.6	15.2
Laup	Biochar	S1	2.4	1.93	49.5	25.7	2.02	1.90	50.9	26.8	409.7	8.5	175.4	18.1	14.5	20.6
Laup	Control	S1	2.8	1.73	46.4	26.8	2.32	1.86	51.0	27.4	437.1	4.5	94.8	9.5	7.4	20.9
Laup	Glucose	S2	2.7	1.59	32.5	20.5	1.51	2.47	52.5	21.3	1239.2	6.5	102.1	14.9	11.4	15.7
Laup	Glycine	S2	2.8	0.9	21.0	23.3	0.92	2.01	48.3	24.0	1890.1	6.8	145.2	27.0	24.7	21.3
Laup	Biochar	S2	2.6	2.05	43.3	21.1	1.95	2.49	53.2	21.4	610.9	3.9	71.5	7.5	6.4	18.2
Laup	Control	S2	2.7	1.83	46.2	25.3	2.31	1.98	51.5	26.1	430.8	4.5	77.2	9.0	6.1	17.1
Laup	Glucose	S3	2.8	1.85	38.3	20.7	1.99	2.42	51.0	21.1	860.3	4.6	77.6	8.7	7.1	16.9
Laup	Glycine	S3	2.8	1.78	36.5	20.5	1.84	2.40	51.5	21.4	958.5	5.6	75.2	11.3	7.4	13.3
Laup	Biochar	S3	2.8	2.34	46.4	19.8	2.20	2.49	54.8	22.0	618.8	11.2	102.5	16.9	7.8	9.2
Laup	Control	S3	2.5	2.35	43.8	18.6	1.95	2.64	51.0	19.4	527.3	6.8	89.8	11.8	8.3	13.1
M Grass	Glucose	S1	2.7	0.89	9.7	10.9	0.42	3.17	32.9	10.4	2262.2	10.5	121.9	44.0	46.7	11.6
M Grass	Glycine	S1	2.9	0.82	8.8	10.8	0.44	2.79	32.4	11.6	2494.1	11.8	116.9	49.1	45.1	9.9
M Grass	Biochar	S1	2.7	0.82	9.3	11.4	0.41	3.54	36.1	10.2	2314.3	7.9	106.4	35.3	41.9	13.5
M Grass	Control	S1	2.9	0.82	8.6	10.5	0.38	3.54	37.7	10.6	2474.2	9.9	101.7	42.1	41.3	10.3
M Grass	Glucose	S2	2.9	0.82	8.6	10.5	0.46	2.89	29.2	10.1	2401.2	10.1	112.3	43.1	45.4	11.1
M Grass	Glycine	S2	3.0	0.94	9.0	9.6	0.51	3.13	30.2	9.6	2453.0	12.0	112.6	43.0	42.4	9.4
M Grass	Biochar	S2	2.9	0.88	9.5	10.8	0.45	2.99	32.0	10.7	2480.8	12.3	134.5	47.6	48.1	11.0
M Grass	Control	S2	2.8	0.82	9.0	11.0	0.45	2.94	30.0	10.2	2343.9	9.7	117.8	42.5	46.7	12.1
M Grass	Glucose	S3	2.8	0.7	7.2	10.2	0.38	2.33	32.1	13.8	2400.7	10.7	78.1	54.9	39.3	7.3
M Grass	Glycine	S3	3.0	0.67	6.8	10.2	0.37	2.19	27.3	12.5	2624.3	12.0	104.9	60.0	51.2	8.7
M Grass	Biochar	S3	2.8	0.7	9.2	13.1	0.42	2.57	30.4	11.8	2424.9	9.1	132.3	45.6	50.7	14.6

Site	Comp-ound added	Plot	Before HCL/HF acid demineralization				After HCL/HF acid demineralization				Mass loss during HCL/HF acid demineralization					
			Soil mass (gm)	N (%)	C (%)	CN Ratio	Soil mass (g)	N (%)	C (%)	CN Ratio	Soil mass loss (mg)	N mass loss (mg)	C mass loss (mg)	N loss (%)	C loss (%)	C/N of lost OM
M Grass	Control	S3	2.8	0.68	7.2	10.6	0.36	2.82	36.9	13.1	2436.4	8.9	69.0	46.7	34.1	7.8
A Grass	Glucose	S1	2.8	0.39	4.3	11.1	0.26	2.75	29.8	10.8	2559.0	3.8	43.4	34.1	35.6	11.5
A Grass	Glycine	S1	2.9	0.51	6.0	11.8	0.29	3.52	38.1	10.8	2602.9	4.6	63.6	31.1	36.6	13.9
A Grass	Biochar	S1	2.9	0.69	7.5	10.9	0.34	3.90	46.6	11.9	2578.5	6.7	60.0	33.4	27.2	8.9
A Grass	Control	S1	2.9	0.54	6.1	11.3	0.31	3.84	43.0	11.2	2567.8	3.8	43.7	24.4	24.9	11.6
A Grass	Glucose	S2	2.9	0.53	5.4	10.2	0.37	2.57	27.2	10.6	2575.9	6.0	58.4	38.6	36.5	9.7
A Grass	Glycine	S2	2.9	0.73	7.6	10.4	0.45	3.27	32.9	10.1	2443.0	6.4	71.8	30.5	32.7	11.2
A Grass	Biochar	S2	2.9	0.87	9.8	11.3	0.51	3.34	37.2	11.2	2442.5	8.8	101.4	34.3	35.0	11.5
A Grass	Control	S2	2.7	0.83	8.3	10.0	0.48	3.83	37.9	9.9	2252.0	4.3	43.4	18.8	19.2	10.2
A Grass	Glucose	S3	2.9	0.31	3.6	11.6	0.26	2.40	31.1	13.0	2677.3	2.9	24.9	31.5	23.6	8.7
A Grass	Glycine	S3	3.0	0.35	3.3	9.4	0.25	2.98	30.7	10.3	2715.0	2.8	19.6	27.1	20.0	7.0
A Grass	Biochar	S3	2.7	0.37	5.0	13.4	0.30	2.47	33.4	13.6	2414.5	2.6	33.0	25.5	24.5	12.9
A Grass	Control	S3	2.8	0.32	2.8	8.7	0.24	2.73	22.7	8.3	2554.3	2.4	23.7	27.0	30.3	9.8
Average														**26.5**	**24.1**	**15.4**
Standard Error														**1.6**	**1.7**	**0.7**

Table A5: The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for Bulk soil for different organic C addition. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. Averages are given ± one SE (n=3).

Site	Substrate	Alkyl C (waxes, other lipids) 0–45 ppm	N-Alkyl + Methoxyl C (proteins, peptides) 45–60 ppm	O-Alkyl C (cellulose, other carbohydrates) 60–95 ppm	Di-O-Alkyl C (hemicellulose) 95–110 ppm	Aromatic C (lignin, tannin) 110–145 ppm	Phenolic C (acids, tannin) 145–165 ppm	Amide + Carboxyl C (chitin + hemicellulose) 165–215 ppm	Ratio of (Alkyl + O-alkyl + N-alkyl):(phenolic+aromatic) C
Kohala	Biochar	20.1±1.4	7.3±0.2	25±0.3	7.8±0.3	20.9±1.5	8.1±0.2	10.8±0.1	1±0.1
	Control	25±1.3	7.7±0.1	25.1±0.8	7.4±0.3	15.5±0.5	6.8±0.4	12.6±0.5	1.5±0.1
	Glucose	23.7±0.6	8.1±0.3	25.8±0.1	7.7±0.2	15.8±0.5	7±0.3	11.9±0.7	1.4±0.1
	Glycine	22.9±0.7	8.3±0.2	25.4±0.5	7.8±0.2	16.5±0.2	7.4±0.2	11.6±0.8	1.3±0.02
Laupah-oehoe	Biochar	20.8±1.5	7.5±0.1	22.2±0.7	7.9±0.4	21.5±1.2	8.6±0.6	11.6±0.2	1±0.1
	Control	21.9±1.3	7.6±0.2	25.3±0.6	8.4±0.5	17.2±0.7	8.1±0.7	11.5±0.6	1.2±0.1
	Glucose	26.3±2.2	7.4±0.1	24.5±0.3	7.2±0.3	15.6±1.1	6.6±0.4	12.4±0.1	1.6±0.2
	Glycine	27.5±3	7.6±0.1	23.3±0.9	7±0.6	15.1±1	6.5±0.7	13.1±0.4	1.7±0.3
Thurston	Biochar	24.4±0.9	6.8±0.2	26.6±1.2	7.4±0.1	16.7±1.6	6.5±0.5	11.7±0.8	1.1±0.1
	Control	21.7±0.8	6.7±0.2	26.5±0.5	8.5±0.5	18±1.7	7.6±0.5	11.1±0.6	1.2±0.02
	Glucose	25.7±0.9	7.3±0.1	28.6±0.9	7.7±0.1	13.6±0.5	5.6±0.3	11.6±0.6	1.6±0.1
	Glycine	24.3±1.5	7.4±0.3	27±0.5	7.9±0.4	14.7±1	6.8±0.7	12±0.3	1.4±0.1
Arid Grassland	Biochar	21.3±0.7	9.1±0.2	20.5±1.6	5.9±0.4	21.7±2.4	7.6±0.5	13.8±0.1	1.4±0.2
	Control	21.3±0.3	9.4±0.1	22.6±0.3	6±0.1	17.9±0.2	6.8±0.2	16±0.5	1.1±0.1
	Glucose	23.8±0.6	9.9±0.3	23.5±0.3	6.1±0.2	15.6±0.9	5.7±0.4	15.4±1	1.7±0.1
	Glycine	23.1±0.7	9.7±0.2	21.8±0.1	5.8±0.1	17±0.4	6.5±0.2	16.2±0.4	1.5±0.2
Mesic Grassland	Biochar	18.6±0.1	8.3±0.1	23±1.5	6.4±0.3	21.5±2	7.9±0.4	14.4±0.6	0.9±0.1
	Control	19.6±0.4	8.9±0.4	26.7±1	6.9±0.1	16.4±0.5	6.7±0.3	14.8±1.1	1.2±0.1
	Glucose	20.3±0.7	9±0.1	25.8±0.7	6.8±0	16.7±0.2	6.7±0.1	14.9±0.6	1.3±0.03
	Glycine	20.4±0.8	9±0.1	25.5±0.4	6.8±0.1	16.8±0.3	6.9±0.1	14.7±0.4	1.2±0.05

Table A6: The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for Bulk soil for different organic C addition. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. Averages are given ± 1 SE (n=9 for forest and n=6 for grassland). For each cover type values down each column not connected by same letter are significantly different from Tukey HSD test.

Cover Type	Site	Alkyl C (waxes, other lipids) 0–45 ppm	N-Alkyl + Methoxyl C (proteins, peptides) 45–60 ppm	O-Alkyl C (cellulose, other carbohydrates) 60–95 ppm	Di-O-Alkyl C (hemicellulose) 95–110 ppm	Aromatic C (lignin, tannin) 110–145 ppm	Phenolic C (acids, tannin) 145–165 ppm	Amide + Carboxyl C (chitin + hemicellulose) 165–215 ppm	Ratio of (Alkyl + O-alkyl + N-alkyl):(phenolic+aromatic) C
Forest	Kohala	22.9±1.3^{a}	7.8±0.7^{a}	25.3±0.1^{b}	7.7±0.2^{a}	17.2±0.1^{a}	7.3±0.8^{a}	11.7±0.2^{a}	1.3±0.3^{a}
	Laupahoehoe	24.1±1.3^{a}	7.5±1.2^{a}	23.8±0.1^{c}	7.6±0.5^{a}	17.3±0.3^{a}	7.5±0.9^{a}	12.2±0.4^{a}	1.3±0.2^{a}
	Thurston	24±1.4^{a}	7±0.6^{b}	27.2±0.1^{a}	7.9±0.4^{a}	15.8±0.2^{a}	6.6±0.8^{a}	11.6±0.3^{a}	1.4±0.3^{a}
Grassland	Arid	22.4±1.3^{a}	9.5±0.4^{a}	22.1±0.1^{b}	6±0.5^{b}	18±0.1^{a}	6.6±0.9^{a}	15.4±0.3^{a}	1.3±0.4^{a}
	Mesic	19.7±1.2^{b}	8.8±0.3^{b}	25.3±0.1^{a}	6.7±0.6^{a}	17.8±0.1^{a}	7±0.8^{a}	14.7±0.2^{a}	1.2±0.3^{b}

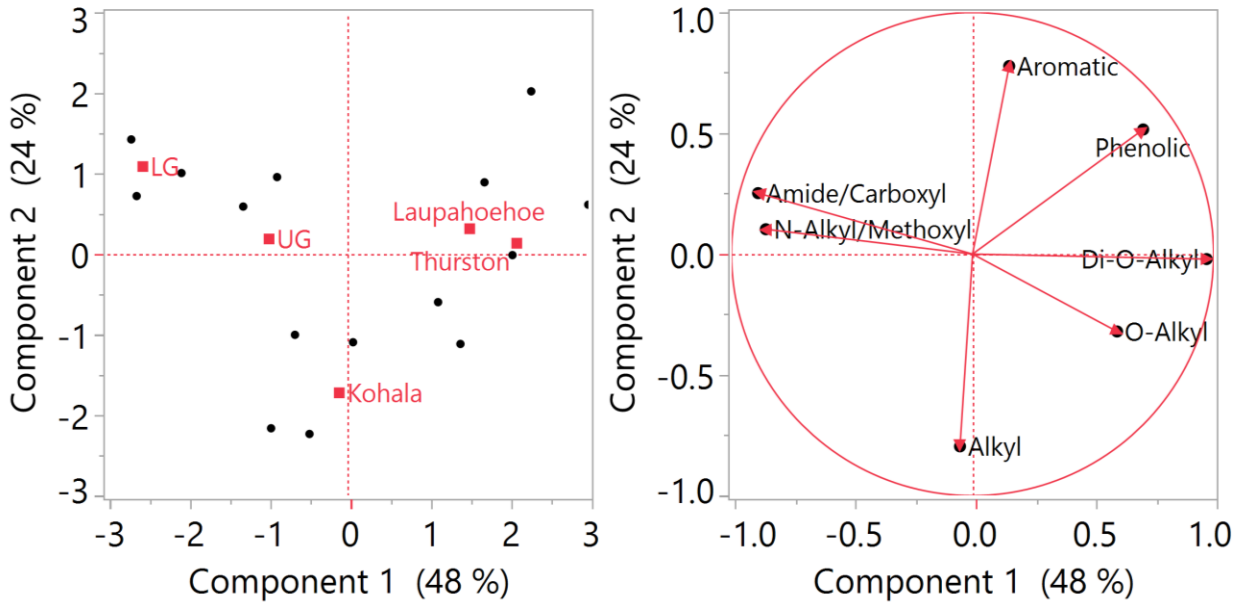

Figure A1. Principal component analysis of the composition of functional groups. Each red square represents a specific SOC chemical structure in each site. Squares that are close together are more similar to one another than the squares that are far apart.

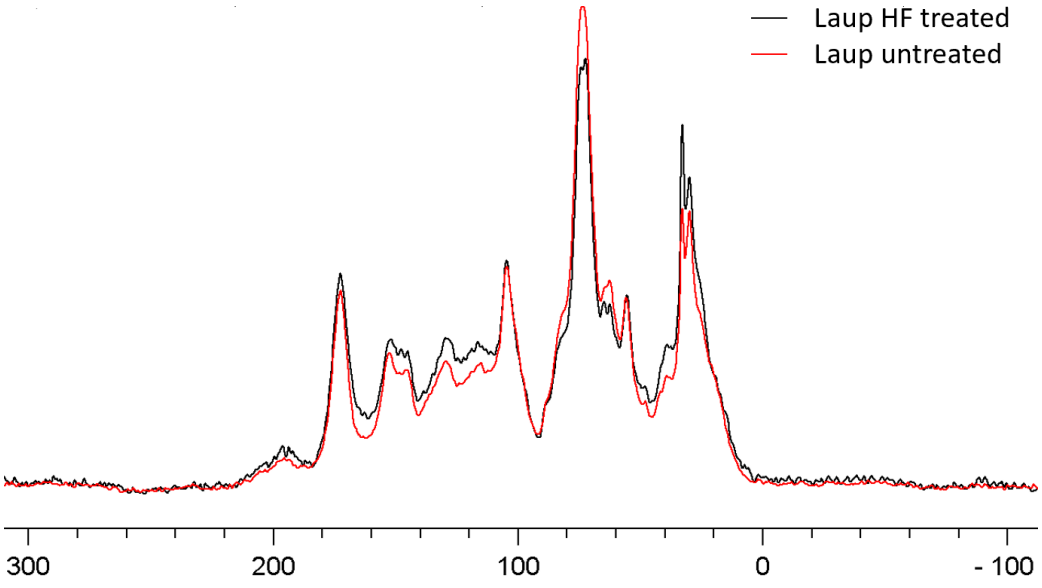

Figure A2: Representative solid state ^{13}C NMR spectrogram of soil SOC for HF treated vs. untreated soil (from water only added mesocoms at Kohala)

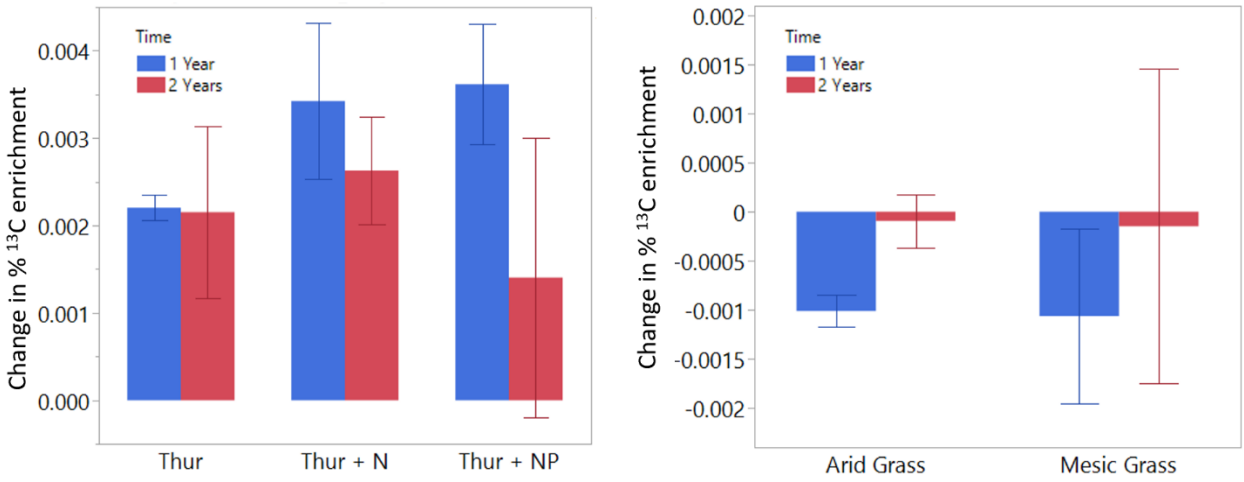

Figure A3: Change in % ^{13}C enrichment of 0-5 cm soil amended with biochar relative to control during the first and second years of incubation. More enriched switchgrass biochar was added in the forest sites, whereas less enriched pine biochar was added to the grassland soil. Note how enrichment changed over time. Mean ± SE are shown (n=24).

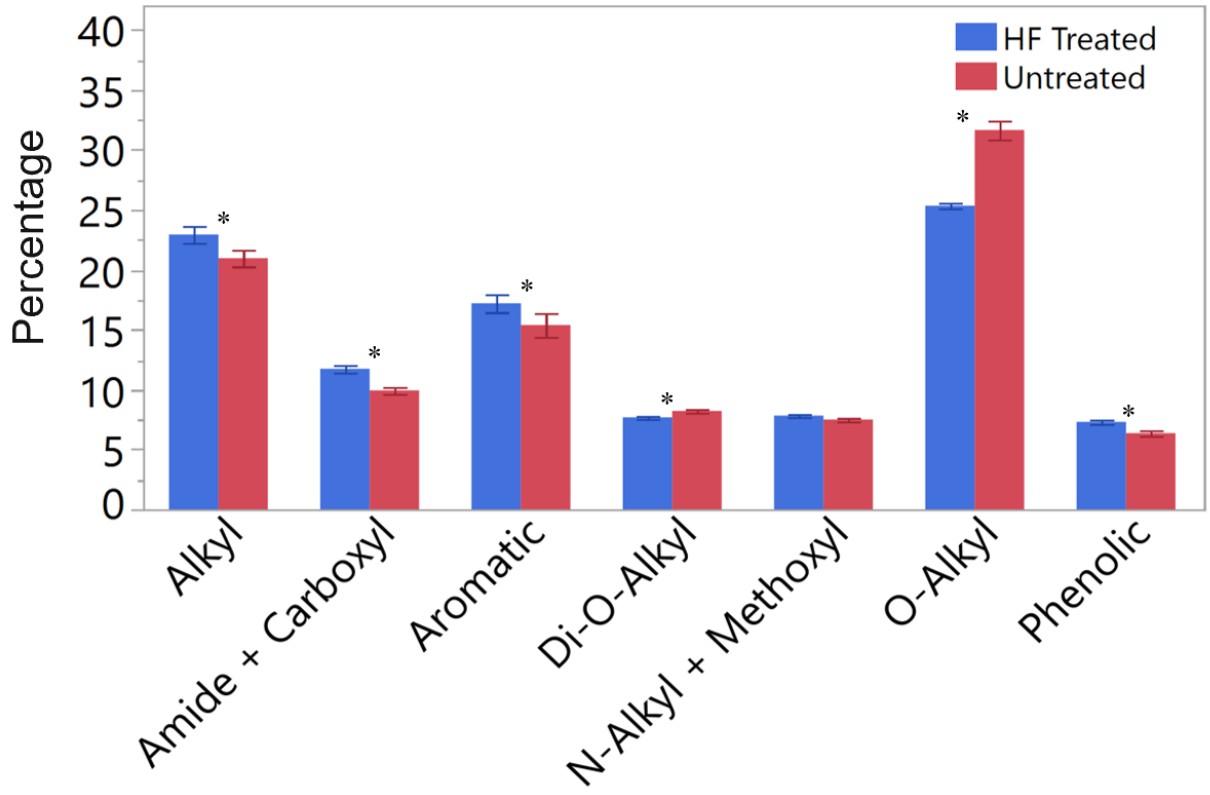

Figure A4. The average relative percentages of seven C functional groups from ^{13}C NMR for HF treated vs. untreated SOC of Kohala. Error bar represent ± 1 SE (n=3)

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Chapter 4 Effect of grass cover removal on soil carbon and nitrogen in an arid grassland

Abstract

This research estimates the long-term effect of grass cover loss on soil organic carbon (SOC) and total nitrogen (TN) storage and their spatial heterogeneity in two arid grassland communities in New Mexico, United States. Enhanced wind erosion was encouraged by experimentally reducing grass cover (but not shrub) for nine years. We compared vegetation cover, surface height and soil SOC and TN concentrations between the control versus grass removal plots at the beginning and after nine years of the experiment. We also measured the scale of spatial heterogeneity of SOC and N and how it changes through time under the influence of enhanced wind erosion. The results show that nine years of grass removal resulted in soil deflation in the grass removal plots and deposition in the area immediately downwind to the plots. Nine years of enhanced erosion caused an average decline of 30% of SOC and 35% of TN concentrations in the surface (0-5cm) soil in the 100 % grass removal plots. The change in SOC and TN concentration in the soil immediately downwind of the grass removal plot (downwind plot) showed a significant decline in the site dominated by *Sporobolus spp*. but did not show a significant change in the site dominated by *Bouteloua eriopoda*. This difference was likely due to the difference in the structural attributes of these grass species. SOC and TN concentrations were positively correlated to the total vegetation cover, grass cover, and change in soil surface height. Geostatistical analysis showed lower range of autocorrelation (A_0) and the proportion of variance that is spatially structured (C/Co+C) in the plots downwind of 100 % grass removal plots relative to control plots indicating that weaker but smaller fertile islands, compared to the control, appeared to develop over nine years of aeolian transport.

Introduction

Soil organic carbon is crucial for maintaining ecosystem health and climate regulation (Schmidt et al., 2011). Wind erosion and soil organic carbon (SOC) loss from an area is reported to increase with a decrease in vegetation cover (Li et al., 2007; Yan et al., 2013). Aeolian processes can drive SOC transport and deposition from a micro scale ranging from vegetation gaps to regional and global scales via long distance dust transport (Li et al., 2008a; Field et al., 2010). However, the impact of wind erosion on carbon (C) cycle dynamics has received little attention yet (Webb et al., 2013). Wind erosion is known to selectively remove finer soil particles including SOC and nutrients with high mobility (Su and Zhao, 2003; Tanner et al., 2016). SOC content in the dust can be much higher than that on the source soil (Webb et al., 2012). Geostatistical analyses in the arid grassland further suggest that soil organic matter related indicators such as SOC, TN (total nitrogen), available N, and $SO4^{2-}$ are most susceptible to early erosion and redistribution while Ca^{2+} and Mg^{2+} are more resilient against erosion (Li et al., 2008).

The factors that influence wind erosion and sediment transport are wind speed (He et al., 2013), vegetation cover (Mendez and Buschiazzo, 2015) and the condition of the soil (Bergametti et al., 2016) and all these factors are prone to be affected by the climate change. It is projected that the anthropogenic climate change will increase aridity in the southwestern United States (Seager et al., 2007). Increased aridity is predicted to cause a decline in perennial grass cover in the region, making the soil more susceptible to erosion by the wind (Munson et al., 2011). A large portion of American Southwest has witnessed the expansion of shrubland and decline in grassland coverage during the last century along with increased surface soil erosion and the wind is likely to play an important role in it (Buffington and Herbel, 1965; Grover and Musick, 1990; Van Auken, 2000).

Vegetation cover is considered a crucial factor for effective protection of SOC and soil nutrients will be very important for land management in the arid region. The few short-term studies that exist show that there is a threshold cover below which loss of nutrients and SOC will not be significant, but the loss increases very rapidly once the threshold is crossed. Yan et al. (2013) using a multiple short-term (9 days) vegetation cover simulation experiment in a semiarid steppe of northern China suggested that vegetation cover should be maintained at least above 35 % for the effective protection of fine soil particles and nutrients. Li et al. 2007 found that over three years only little SOC and N was lost from plots whose vegetation was removed by only 25 and 50 percent of original vegetation cover. But significant loss began at some point between 50 and 75 percent of existing grass removal.

Many recent studies have investigated the role of wind in removal and redistribution of soil resources in the natural landscape (Coppinger et al., 1991; Larney et al., 1998; Okin et al., 2001; Li et al., 2007, 2008a). Coppinger et al. (1991) demonstrated that in sagebrush steppe in Wyoming, shrub canopies can capture the wind blown materials and create shrub island. Okin and Gillette (2001) showed wind activity as a major contributing factor in shaping the observed vegetation spatial pattern in mesquite dunelands in the Chihuahuan Desert. A rapid change (within 2-3 years) of the spatial distribution of SOC and other nutrients by enhanced erosion, redistribution, and deposition of soil resources resulting from vegetation removal were demonstrated in a series of study in the arid grassland in northern Chihuahuan Desert (Li et al., 2007, 2008a; Li et al., 2008b). However, all these studies are short term and do not report the long term effect of enhanced wind erosion in removal and redistribution of soil resources.

Short-term studies indicate rapid loss of SOC and N pool after certain threshold vegetation

cover lost is reached. Earlier results from the vegetation removal experiment in northern Chihuahuan Desert showed that increased wind erosion removed up to 25% of total SOC and TN from top 5 cm of the soil in the period from 2004 to 2006 (Li et al., 2007). Li et al. (2007)estimated the lifetime of surface soil SOC and TN at this site to be ten years with 100% grass cover reduction. Although these initial results about potential SOC loss due to vegetation removal are high, there is a lack of long-term controlled experimental research that investigates how different levels of vegetation loss affect the SOC pool in long term in a wind erosion prone arid grasslands.

In this chapter, we conducted a decadal-scale vegetation-removal experiment to examine the effects of wind erosion on the soil carbon and nitrogen content and their spatial distribution in two arid grassland communities with sparse cover of mesquite (*Prosopis glandulosa*) shrub. Specifically, we studied (1) the relationship between vegetation cover change, soil erosion and deposition, (2) decadal scale changes in soil carbon and nitrogen content in soil (3) The scale of Spatial heterogeneity of SOC and N and how it changes through time under the influence of enhanced wind erosion.

Methods

Site Description

This study was conducted at the USDA-ARS Jornada Experimental Range (JER), New Mexico within the Nutrient Effects of Aeolian Transport (NEAT) experiment site (Li et al., 2007). The site lies in the northern part of the Chihuahuan Desert and represents a warm semi-desert grassland (Buffington and Herbel, 1965). The JER was established in 1912 and now is part of the National Science Foundation's Long Term Ecological Research (LTER) network. The details of the site are given at Li et al. (2007, 2008a; 2009a). Two experimental field sites were set up in on

the sand sheet geomorphic surface at the JER (Table 8). Both sites have similar topography and soils and are covered with sparse grass/shrub mixed communities. The first site is dominated by *Sporobolus spp*. grasses (mainly *S. flexuosus* and *S. contractus*) and mesquites, and the second site is dominated by *Bouteloua eriopoda* and mesquites. Based on their dominant vegetation type these sites are hereafter referred to as *Sporobolus*-mesquite (SM) site and *Bouteloua*-mesquite (BM) site respectively. Initial vegetation removal occurred in March 2004 for the SM site and in July 2004 for the BM site. Therefore, as of July 2004, the SM site had already experienced one windy season. Eighty percent of erosive wind at JER occurs from the southwesterly direction, and most of the erosion occurs from early March to May (Helm and Breed, 1999).

Aeolian activity has considerably increased in the study area due to the conversion of grasslands to mesquite shrublands (Gillette and Pitchford, 2004; Gibbens et al., 2005). A series of studies in this site by Li et al. (2007, 2008a; 2009a) have shown that enhanced wind erosion due to a varying degree of vegetation removal has led to the loss and redistribution of SOC and soil nutrients.

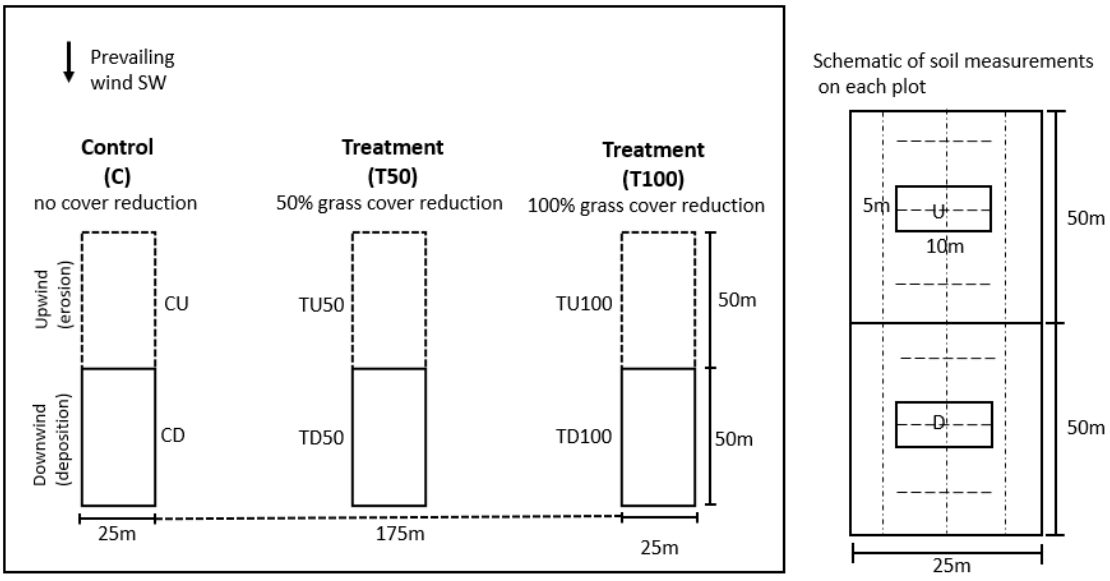

Figure 17 Sampling layout representing the control and grass reduction treatment plots. The right figure shows a more detailed layout of sampling locations in each treatment. Soil sampling was conducted in the Upwind (U) and Downwind (D) subplots where U represents upwind plot and D represents the downwind plot. Horizontal dashed lines represent the locations of erosion bridges. Location of three 50-m line point intercept transects set up for plant community monitoring in each treatment plot are represented by vertical dashed lines. The field site was fenced in 2004 and has not been grazed since.

Experimental design and sampling method

The field experiment was established in the SM site in March 2004 and in BM site in July 2004. A detailed experimental set up is described by Li et al. (2007). In each site, three 25 x 100 m plots, are aligned parallel to prevailing winds. Each plot was divided at the center to make a 25 x 50 meter upwind plot and a 25 x 50 meter downwind plot. In one of the upwind plots, the upwind plot (T100), all grasses, perennial semi shrubs and perennial forbs were removed (hereafter also referred to as ‘‘100 percent grass cover reduction’’). In the second upwind plot, the upwind plots (T50), 50 percent of grass, perennial semi-shrubs and perennial forbs were removed (hereafter also referred to as ‘‘50 percent grass cover reduction’’). No vegetation cover was removed from the third upwind control plot or any of the downwind plots. Shrub cover was low in both sites at the beginning of the experiment, and shrubs were not removed. Grass cover reduction in the upwind area caused enhanced dust and sand erosion in the upwind plots and enhanced deposition in the downwind plots Li et al (2007, 2008a; 2009a). Within each 25x 50 m upwind and downwind plots, a 5x10 m subplot named U and D respectively was set at the center of the plots to collect surface soil samples (Fig 17). Efforts to prevent grass regrowth were made by estimating grass cover during the winter, then calculating the amount of grass to be removed (if any) so that the subsequent post-removal grass cover would not exceed the original post-removal grass cover. No removals were done if grass cover was measured to be lower than the original post-removal grass cover. After nearly a decade (in 2013), this led to varying levels of grass cover at the sites that did

not necessarily follow the originally planned grass removal. Therefore, the upwind grass cover will be treated as a continuous variable somewhat independent of the naming convention.

Fifty soil samples of 2.5 cm diameter were taken from the top 5 cm of each 5x10 m sampling area (U and D) of the 25 x 50 m plots in July 2004 and July 2013. Soil sampling locations were randomly selected using a random number table and the locations of each soil sample were precisely identified for geostatistical analysis. Large debris was removed from the soil surface to facilitate sampling, but no effort was made to clear the top of the mineral soil prior to sampling. A different set of randomly selected sampling coordinates were used for each sampling period. A total of 600 soil samples were collected and analyzed for each sampling period.

Soil erosion and deflation in the experimental area was monitored by "erosion bridges," a method described by Shakesby (1993) and Gillette and Chen (2001). The metal soccer goalpost-shaped erosion bridges made from rebar were inserted 20–30 cm into the soil. Five erosion bridges were installed about 1-2 m apart along the center of each 5 m×10 m upwind and downwind subplots. Heights of the erosion bridges from the soil surface to the top of the erosion bridge were recorded in 2004 and 2014 to measure soil erosion and deposition.

Plant cover and community composition on each plot were monitored by three 50-m line point intercept transects in March 2014. Readings of plant presence and absence were taken every 25 cm along the transect. Plant cover was calculated by adding the total number of readings for each plant species along the transect and dividing it by the total number of sampling points along the length of the transect. The gap intercept method was used to measure canopy size and bare soil gap size during March 2013. The length of the canopy gap on the transect line greater than or equal to 20 cm was recorded along the three 50 m transects shown in Fig 17.

Laboratory analysis

Soil samples were air-dried in the lab and sieved through a 2-mm screen to remove gravel, roots, and debris. Any visible plant parts that passed through the sieve (e.g., roots, grass blades) were removed manually.

For the 2004 upwind plot samples, total soil organic carbon (SOC) and total nitrogen (TN) analyses were conducted on a Shimadzu TOC-VCSN total organic carbon analyzer with a SSM-5000 solid sample analyzer and a TNM-1 total nitrogen measuring unit. In this system, SOC was calculated as the difference between total carbon (TC) and total inorganic carbon (TIC). To ensure the complete decomposition of all carbonates in SOC measurements, vanadium oxide (V_2O_5) was used as a combustion catalyst with the mass ratio of catalyst to sample of 1:1.

For SOC and TN analysis of all other samples, about 8 g of the sub-sample was obtained by passing each sieved soil sample through an open pan riffle-type sample splitter (Model H-3962, Humboldt MFG. Corporation, Norridge, IL). Subsamples were ground into a powder using a ball mill (Cianflone Scientific Instruments Corporation, Pittsburgh, PA, USA). Approximately 46 mg of soil sample was weighed in the silver foil capsules and arranged on a microtiter plate. The samples were then wetted by adding 50 microliters of water and the microtiter plate was placed in a desiccator containing a beaker with concentrated (12.1 N) HCl and left for 6 hours for the inorganic carbonates to be released as CO_2 (Harris et al., 2001). The resulting inorganic carbonate free samples were dried in an oven at 60 ^oC until the stable mass was achieved and analyzed for SOC and TN content using an Elemental Combustion System (Costech Analytical, Valencia, CA, USA) calibrated using Acetanilide (C_8H_9NO) as a standard. We discarded the TN values from the downwind plot in T50 treatment because their readings from the elemental analyzer showed a high variability from the standards.

Statistical and Geostatistical Analysis

Mean and standard deviation were computed to characterize the general pattern of SOC and TN in soil samples for each experimental plot and sampling period. For both SOC and TN, paired t-tests were conducted to compare the mean values between the treatment plots and control plots of the same year. The effect of grass removal treatment on mean soil SOC and TN concentration after nine years was determined using the difference in difference (DID) estimation. DID estimation uses four data points to infer the impact of treatment on the treated plot vs. untreated plot. It assumes that the treatment group and control group would be trending in the same manner (i.e., will have a similar slope) over time if there was no treatment. Analyses were conducted using R version 3.3.1 and 14.0.0 JMP software (SAS Institute Inc. Cary, NC, USA). Statistical significance was determined as $p < 0.05$.

Geostatistical analysis was used to evaluate the spatial variation of SOC and TN in the control and grass cover reduction plots. A jacknife method (Shafer and Varljen, 1990; Huisman et al., 2003) was used to fit the experimental semivariograms using IDL 8.7.0 (Harris Geospatial Solutions Inc). SOC and TN concentrations were log-transformed to achieve an approximately normal distribution before the analysis (Webster and Oliver, 2007). We used lag intervals of 0.2 to 0.4 m and the lag distance of 2.5 to 6.0 m to calculate the experimental semivariograms for both SOC and TN concentrations. All semivariograms were fit to a spherical model using a non-linear least-squares approach employing the Levenberg-Marquardt algorithm (Press et al., 1992). The equation for the spherical model fit to semivariance [$\gamma(h)$] is given by:

$$\gamma(h) = C0 + C\,[1.5(h / A_0) - 0.5(h / A_0)^3] \quad \text{if } h \leq A_0 \quad \text{.....eq 1}$$

$$\gamma(h) = C0 + C \quad \text{if } h > A_0 \text{.....eq 2}$$

where h is the lag distance interval, C_0 is the nugget variance (≥ 0), C is the spatially structured

variance, and A_0 is the model range parameter. The effective range A, representing the distance beyond which there is no spatial autocorrelation between points, is A_0 for the spherical model.

The nugget (C_0) is the Y intercept of the semivariogram and represents the measurement error or the spatial variation on a scale smaller than the shortest distance between any two points in the sample design, variance that isn't spatially patterned, or error either in sample location or sample measurement (Diggle and Ribeiro, 2010). The measure of the proportion of sample variance (C_0+C) that is explained by spatially structured variance C was calculated using C/(C_0+C) statistic. This value approaches zero in the absence of spatially dependent variation at the range specified (pure nugget effect) while its value approaches to 1.0 for a variogram with no nugget variance. (Jackson and Caldwell, 1993). A_0 represents the model range, the distance over which spatial dependence is apparent. Samples that are located within A_0 of one another are considered to be spatially correlated, whereas samples that are separated by a distance greater than A_0 are considered to be spatially independent (Diggle and Ribeiro, 2010). The uncertainties (95% confidence limits) of variogram parameters were determined using the variance-covariance method of Pardo-Iguzquiza and Dowd (2001). Variogram parameters not overlapping 95% confidence intervals were considered significantly different.

Results

Vegetation change, soil deflation/deposition

In 2014 the effects of vegetation removal in the upwind are clearly seen in the differences in percent grass cover between the treatments (Table 8). Grass cover follows control > T50 > T100 order and this trend is clearer in SM than in BM site. Overall there is much higher shrub cover in

the SM site. Upwind at both sites, grass cover in control is 27-30%, whereas grass cover almost approaches 0 at T100.

Table 8 Vegetation cover percentage by vegetation type in 2014 for experimental plots (upland represents an average of the whole 50 m transects, downwind represents an average of only 0-25 m from upwind/downwind boundary, which corresponds to the end point of the subplot where the samples were)

Site	Vegetation type	Control	50% Removal		100% Removal	
			Upwind	Downwind	Upwind	Downwind
SM	Grass	27	12	27	1	21
	Shrub	1	9	6	5	2
	Other	2	2	4	1	1
	Total	30	23	37	7	24
BM	Grass	30	21	22	2	22
	Shrub	2	2	2	3	1
	Other	0	0	2	0	0
	Total	33	23	26	5	22

*** other include annual and perennial forbs, yucca and perennial ephedra**

Table 9 Average gap interval and gap percentage in the sites

Treatment	Wind	SM		BM	
		Avg gap (cm)	Avg gap %	Avg gap (cm)	Avg gap %
Control	U	132.3	76.7	75.0	58.9
Control	D	146.1	79.2	78.0	56.8
T50	U	250.8	81.3	104.6	78.2
T50	D	97.6	67.7	81.4	60.4
T100	U	664.9	79.1	617.7	93.3
T100	D	224.1	83.8	86.7	61.0

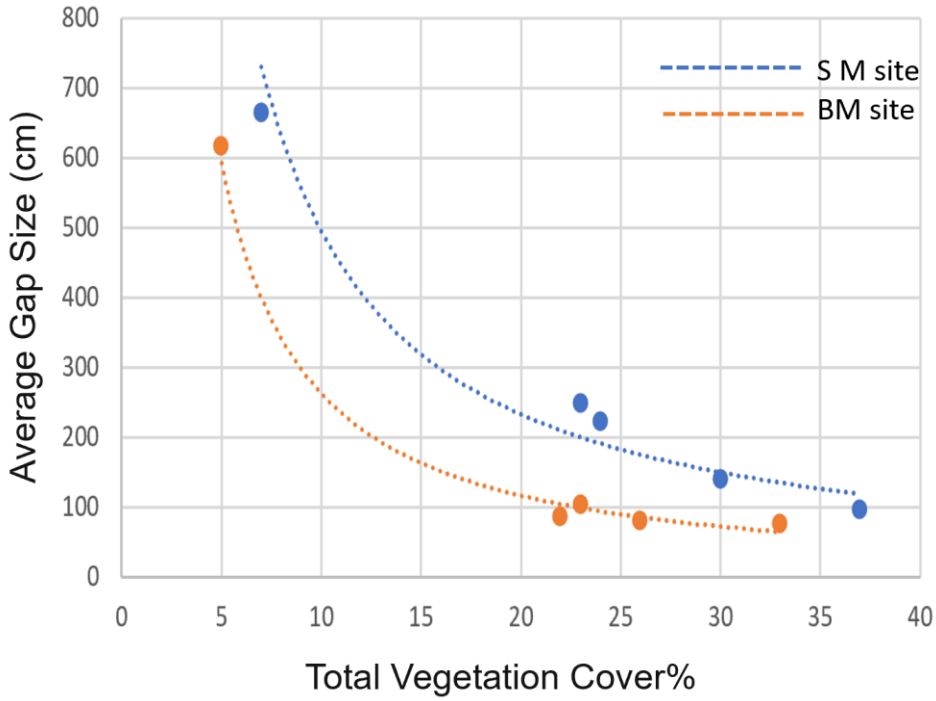

Figure 18 Relationship between average gap size and total cover percent between the two study sites. The blue line and the orange line respectively represent the site dominated by *Sporobolus* spp. and *Bouteloua eriopoda*.

Erosion bridge results show that a general deposition occurred at both sites between 2004 and 2014 (Fig. 18). The average deposition of 3.1 and 1.7 cm respectively occurred in the control plots in SM and BM sites during this period. The only place that the experiment clearly overcomes the ongoing deposition trend is in T100 plots, though there is some evidence of erosion in the upwind T50 treatment at the BM site. The clear pattern for upwind T100 plot for both SM and BM is one of upwind erosion and downwind deposition, with erosion greatest in the middle (U2) of the upwind plots and deposition greatest at the leading edge of the upwind plots (D1). For the 50% grass cover removal plots (TU50) deflation of the surface occurred only in U2 subplot at the BM site, though the dramatically lower deposition in U2 of the SM site suggests that deflation may be effectively occurring on this treatment as well.

Soil erosion/deposition follows the vegetation cover trend. Change in surface height in the

upwind subplot was most strongly correlated to the total plant cover and grass cover in the given subplot ($R^2 = 0.95$, $p < 0.01$ for total plant cover and $R^2 = 0.86$, $p = 0.02$ for grass cover). Similarly, change in surface height in the entire upwind plot was most strongly correlated to the total plant cover in the given plot ($R^2 = 0.94$, $p < 0.01$), average gap interval ($R^2 = -0.90$, $p = 0.01$) and grass cover in the given plot ($R^2 = 0.82$, $p = 0.04$). The best linear model selected to estimate the change in surface height used the total vegetation cover as a predictor variable and had a RMSE of 0.89 and R^2 value of 0.97 ($p < 0.01$). The change in the height of downwind plot was negatively correlated to change in the upwind surface height ($R^2 = -0.88$, $p = 0.01$). It was also strongly but non significantly correlated to the gap size in the upwind plot ($R^2 = 0.81$, $p = 0.05$) upwind total vegetation cover ($R^2 = -0.74$, $p = 0.09$) and downwind total vegetation cover ($R^2 = 0.70$, $p = 0.12$). The change in the height of the downwind subplot was only moderately and negatively correlated to gap size in the upwind plot and % grass cover in that subplot.

For the upwind plots (pooling both BM and SM), there is a strong negative correlation ($R^2 = -0.90$, $p = 01$) between the change in surface height and gap size. This is stronger than the correlation between grass cover and the change in surface height ($R^2 = 0.83$, $p = 0.04$).

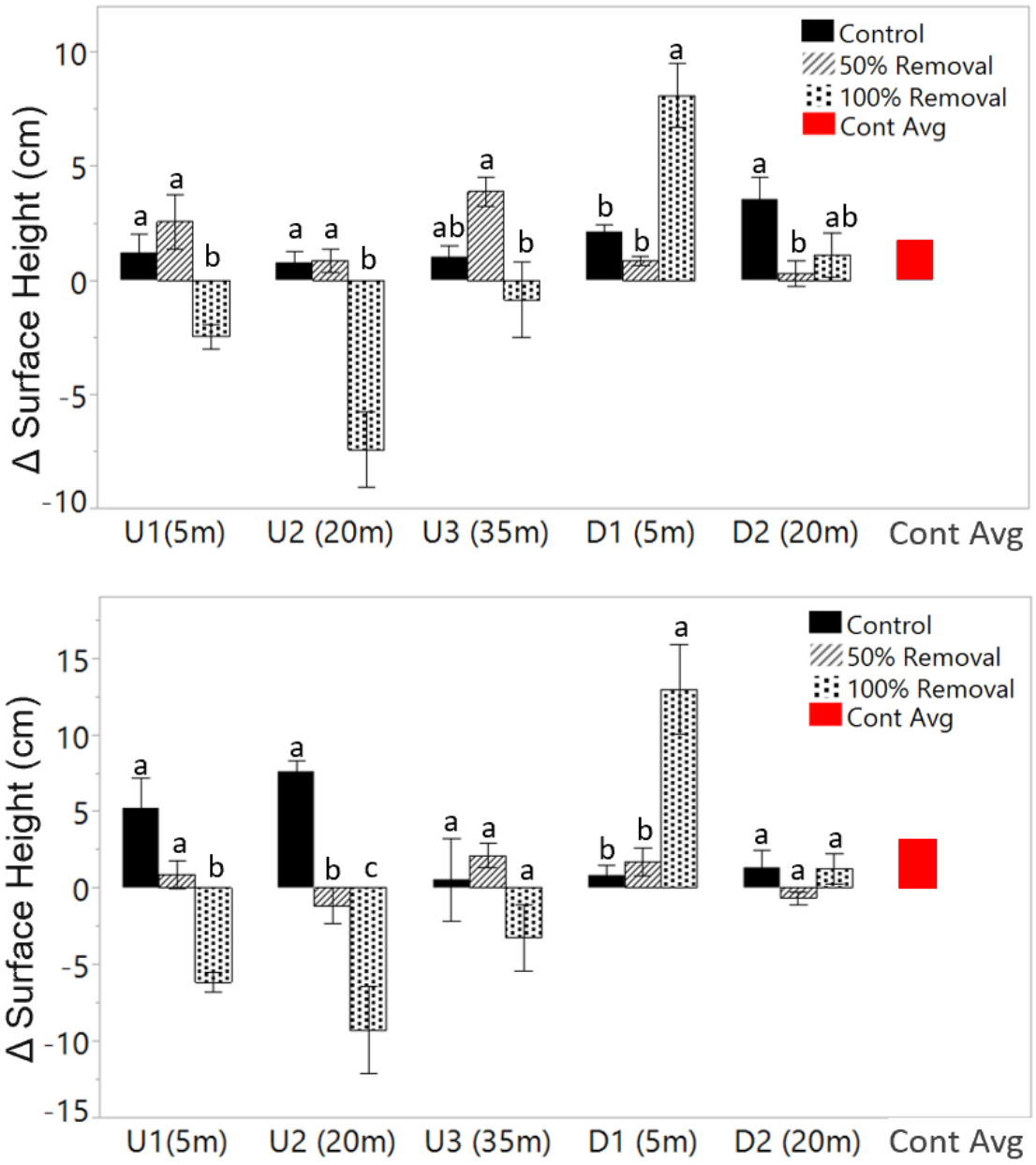

Figure 19 Total erosion (cm, negative) and deposition (positive) monitored by the erosion bridges in SM site (top) and BM site (bottom). For the upwind plots (U1, U2 and U3) numbers in the parentheses represent the distance from the beginning of the upwind subplots. For the downwind plots (D1 and D2), numbers in the parentheses are distances from the subplot to the dividing line of the upwind-downwind. The red bar at the farthest right side of the figure is the average erosion/deposition measured in the control plot. Error bars are one standard error.

Plot-scale comparisons of SOC and TN

In July 2004, the mean values of soil SOC concentration did not differ significantly

between the control plots and the T100 plots for both the upwind and the downwind plots and for both the SM and BM sites (Table 10). Mean TN concentrations at the beginning of the experiment also did not differ significantly between control and T100 plots with the exception that the upwind BM site had a higher TN concentration in the control plot compared to T100 plot (Table 10). SOC concentration in T50 plots was significantly lower relative to control plots in both upwind and downwind the SM site in both 2004 and 2013. TN concentration at T50 site was also lower relative to control in upwind SM site.

Table 10 The mean concentration of soil SOC and TN in the control plots, 50% grass removal plots (T50) and 100% grass removal plots (T100) during the experimental period. SOM values are in g kg-1 and TN in mg kg-1. The values in parenthesis are standard error. For each analyte, the means that are significantly different between 2004 and 2013 for the same treatment are denoted by asterisk. The values are considered significantly different at p<0.05 when analyzed by pairwise Student's t-test.

	Control		50% Removal				100% Removal			
			U		D		U		D	
	2004	2013	2004	2013	2004	2013	2004	2013	2004	2013
					SM Site					
SOC (g kg^{-1})	2.7 (0.1)	2.8 (0.1)	2.0 (0.1)	2.0 (0.1)	2.3 (0.1)	2.1 (0.1)	2.6 (0.1)*	1.4 (0.1)	2.4 (0.1)*	2.0 (0.1)
TN (mg kg^{-1})	282.1 (7.6)	306.1 (12.5)	229.4 (8.0)	255.1 (20.9)	194.4 (10.8)*	256.5 (11.9)	287.9 (11.3)*	186.8 (9.3)	249.6 (10.6)*	213.1 (8.0)
C:N	9.5 (0.1)	9.1) (0.1)	8.7 (0.1)*	7.7 (0.1)	12 (0.3)*	7.8 (0.1)	8.9 (0.3)*	7.5 (0.1)	9.7 (0.1)	9.5 (0.1)
					BM Site					
SOC (g kg^{-1})	2.8 (0.1)	2.7 (0.1)	3.6 (0.1)*	2.0 (0.1)	2.3 (0.1)	2.1 (0.1)	3.4 (0.1)*	2.4 (0.1)	2.6 (0.1)	2.6 (0.1)
TN (mg kg^{-1})	279.3 (7.5)*	329.9 (10.6)	378.7 (17.6)*	244.6 (8.8)	268 (14.4)	265.1 (13.4)	408.5 (12.1)	299.3 (7.1)*	296.0 (11.1)	350.8 (18.9)*
C:N	10.1 (0.1)*	8.1 (0.1)	9.1 (0.3*	8.1 (0.1)	8.6 (0.1)*	8.1 (0.1)	8.1 (0.3)	8.0 (0.1)	8.5 (0.1)*	7.4 (0.1)

The difference of difference analysis showed that for both SM and BM, nine years of 100% grass cover reduction treatment caused a significant decline in soil SOC and TN concentrations in

the T100 plots relative to the control plots in the upwind plots (Table 11, Fig 20). In the BM site, a decline in SOC and TN was seen in the T50 upwind plots, relative to the control. This was only observed for TN at the SM sites. In the downwind plots, SOC and TN decline was observed in the T100 plots and T50 plots, relative to the control, only for the SM site (Table 11). In the SM site grass cover reduction caused a significant decline in SOC in the T100 plots relative to T50 plots only in the upwind plot. No other significant differences were observed between the T50 and T100 plots.

Table 11 Difference of difference estimate of the treatment effect between control and T50 and T100 plot. The asterisk indicates significant grass removal treatment effect as measured by the difference in difference estimate between the control plots and T100 plots after nine years of grass removal treatment ($p < 0.05$). The -ve sign indicates the decline due to the treatment effect.

2004 Control Vs.	Analyte	Sporobolus-mesquite site			Bouteloua-mesquite site		
		C - T50	C - T100	T50 - T100	C - T50	C - T100	T50 - T100
Upwind 2013	SOC (g kg^{-1})	-0.10	-1.32*	-1.2*	-1.44*	-0.86*	0.58
	TN (mg kg^{-1})	1.7	-125.3*	-126.9*	-184.6*	-159.7*	24.9
Downwind 2013	SOC (g kg^{-1})	-0.30	-0.51*	-0.21	0.02	0.17	0.15
	TN (mg kg^{-1})	n/a	-60.5*	n/a	-53.4*	4.3	57.7

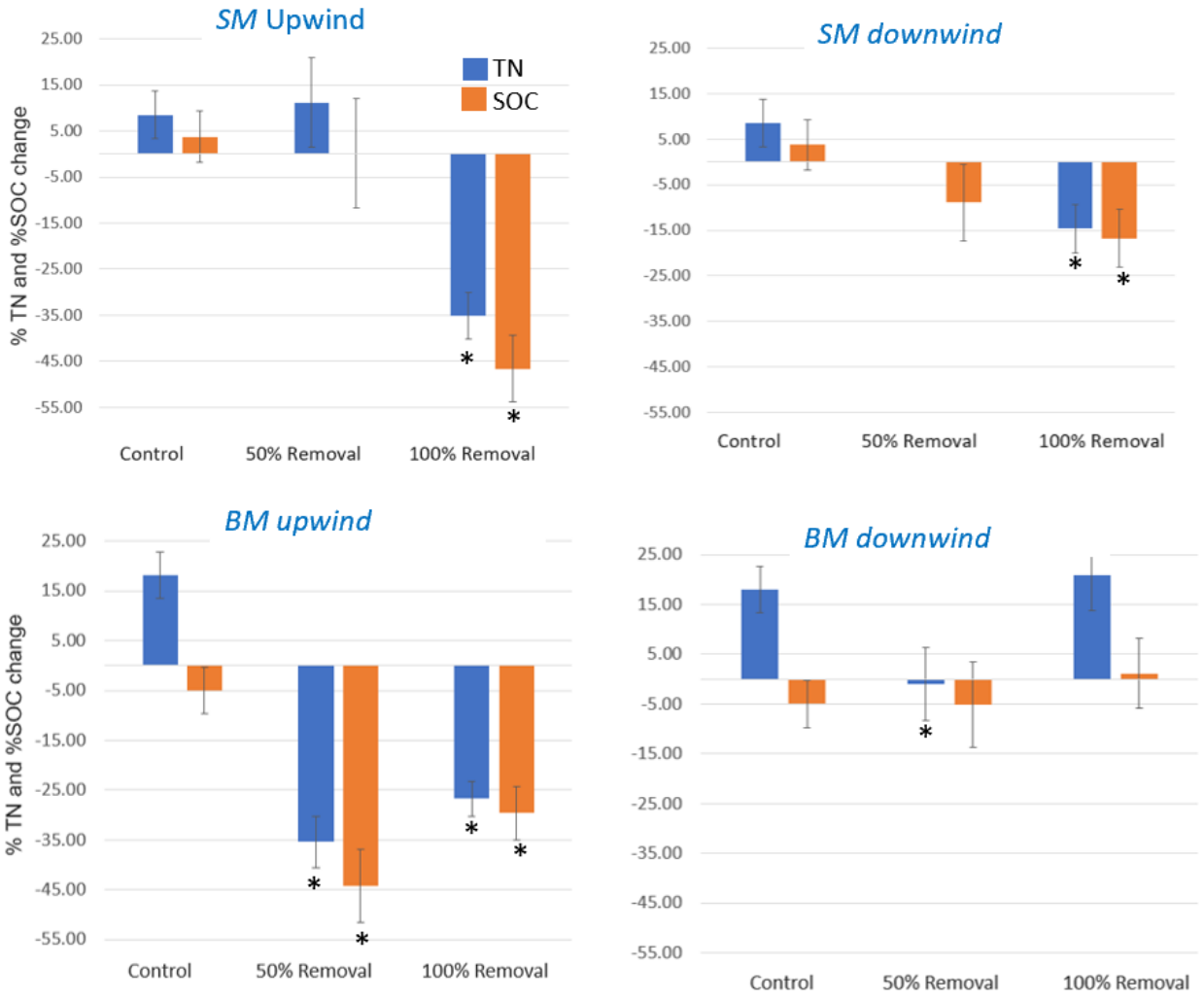

Figure 20 Net loss or gain (%) of SOC and TN on the control plot, 50% grass cover removal plot (T50) and 100% grass cover reduction plot (T100) from 2004 to 2013. Negative numbers represent net loss and positive numbers represent the net gain. The error bars represent 1standard error [generated by error propagation by using SE (x - y) =$\sqrt{SE(x)^2 + SE(y)^2}$). Where SE (x)2 is the standard error of the mean SOC or TN for 2004 and SE (x)2 is the standard error of the mean SOC or TN for 2013].

Prediction of SOC and TN concentration

Regression models were used to determine the relationship between variables. For this analysis upwind SM and BM values were pooled with downwind SM and BM values were pooled separately. No correlation was found between upwind and downwind SOC in 2004 but there was a moderate correlation between upwind and downwind TN (R^2 = 0.68, p =0.14). A strong positive

correlations between upwind and downwind TN concentrations (R^2 = 0.92 p<0.01) and SOC concentration R^2 = 0.85 = , p = 0.03) was observed during 2013.

SOC concentration in the upwind plot was most strongly correlated to average upwind grass cover of the entire upwind plot and the grass cover of U2 sublot (R^2 = 0.69, p = 0.13; R^2 = 0.62, p=0.19 respectively). SOC concentration was also positively correlated to the upwind change in the surface height in that plot. Soil TN concentration in the upwind plot generally correlated to the same variables as SOC but with slightly weaker R^2 values. Percentage change in SOC concentration in the upwind plots was moderately and positively correlated to the change in the surface height of the given subplot and change in the surface height of the entire upwind treatments. Percentage change in TN concentration in the upwind plot was positively correlated (R^2 = 0.81, p= 0.06) to the change in the surface height of the U2 subplot and change in the total vegetation cover in the entire upwind plot (R^2 = 0.76, p= 0.08).

SOC concentration in the downwind plots was moderately and positively correlated to the change in surface height of D2 subplot (R^2 = 0.71, p = 0.11) and moderately and negatively correlated to the shrub cover in the upwind treatments (R^2 = -0.71, p = 0.11) and the downwind treatments (R^2 = 0.61, p = 0.19). The change in SOC concentration in the downwind plots was similarly correlated to the same variables. TN concentration in the downwind plots was positively correlated to the change in the surface height in D2 and negatively correlated to the change in the shrub cover in the downwind and upwind treatments. Change in TN concentration in the downwind plot was moderately correlated to the average gap interval and total vegetation cover in the given D2, and grass cover at the entire downwind treatment. Downwind TOC concentration was strongly and positively correlated to the upwind TOC concentration (R^2=0.92 p<0.01).

Geostatistical analyses

For the semivariograms of both SOC and TN, we report the estimates of range A_0 and the spatial dependence index C/ (C0 + C) along with their 95% confidence limits, in July of 2004 and 2013 (Table 12).

After nine years of grass removal treatment in the SM site, the proportion of the SOC variance that is spatially structured went down relative to the control for both upwind and downwind plots (Table 12). The range of autocorrelation in this site went down relative to the control for the upwind site but did not show change for downwind plots. For BM site, the proportion of the SOC variance that is spatially structured went down only for the upwind plots but went up for the downwind plots. The range of autocorrelation for SOC in this site went down only for the upwind site but went up for the downwind site.

After nine years of grass removal treatment in the SM site, the proportion of the TN variance that is spatially structured went up relative to the control for the upwind plot but went down in the downwind plots (Table 12). The range of autocorrelation distance for TN in this site went down relative to the control for the upwind plots but went up for downwind plots. For BM site, the proportion of the TN variance that is spatially structured went down for the upwind site but went up for the downwind site. The range of autocorrelation for TN in this site went down only for the upwind site but went up for the downwind site.

Table 12 Summary of the semivariogram model parameters in both study sites before (July 2004) and after the experimental period (July 2013). Error bars represent (95% confidence interval. The asterisk indicates significant differences between control and 100 percent grass reduction plots at 95% confidence level.

Site/Parameters	Jul-04				Jul-13			
	Range (Ao)		proportion (C/Co+C)		Range (Ao)		proportion (C/Co+C)	
	control	T100	Control	T100	control	T100	Control	T100
SM site								
Upwind								
SOC	2.45 (1.74)	1.93 (0.41)	0.31 (0.24)*	0.76 (0.03)	4.03 (0.36)*	2.05 (0.47)	0.99 (0.04)	0.87 (0.14)
TN	2.46 (0.56)*	1.42 (0.26)	0.87 (0.19)	0.78 (0.09)	3.4 (0.32)*	1.56 (0.6)	0.95 (0.05)	0.95 (0.41)
Downwind								
SOC	0.9 (0.3)	0.65 (0.18)	0.92 (0.22)	1 (0.23)	2.91 (0.47)	1.96 (0.72)	0.79 (0.07)	0.6 (0.27)
TN	1.14 (0.28)*	0.68 (0.12)	0.97 (0.12)	1 (0.11)	2.94 (0.37)	2.67 (1.05)	0.96 (0.05)*	0.53 (0.17)
BM site								
Upwind								
SOC	3.58 (2.35)	4.45 (0.4)	0.39 (0.25)*	0.94 (0.03)	4.02 (0.34)	Inf (na)	1 (0.03)*	0.08 (0.29)
TN	4.12 (0.61)	2.99 (1.11)	0.74 (0.05)*	0.43 (0.16)	3.39 (0.32)	2.24 (1.16)	0.95 (0.05)*	0.4 (0.24)
Downwind								
SOC	1.72 (0.84)*	0.21 (0.0)	0.49 (0.23)	0.05 (1.62)	2.15 (1.58)	1.62 (0.5)	0.29 (0.15)	0.66 (0.23)
TN	1.86 (0.81)	>10	0.57 (0.17)*	0	1.15 (2.01)	1.42 (0.24)	0.22 (0.81)	1.0 (0.11)

Discussion

Our results show that 100% grass removal in the upwind plots (T100) resulted in an average 30% loss in SOC and 35% loss of TN from the top 0-5 cm soil between 2004 and 2013. Earlier results from the grass removal experiment on this site showed that increased wind erosion resulting from grass removal removed up to 25% of total SOC and TN from 0-5 cm of soil in the period

from 2004 to 2006 (Li et al., 2007). From these two results, we can deduce that the carbon loss rate slows down after an initial peak loss. In a nearby site in JER, eight years of continual vegetation removal resulted in an 80% and 70% loss of plant available N and P due to wind erosion (Okin et al., 2001). However, in that study, all vegetation was removed, not just grasses, so the smaller loss in the present study over a similar time frame is logical.

Although the rate of SOC loss seems to decrease over time, the rate of erosion/deflation did not slow after the initial period. From 2004 - 2006 measurements, the average deposition at T100 D1 subplots was 0.61 cm yr^{-1} in the SM site and 0.21 cm yr^{-1} in the BM site (Li et al., 2008b). Between 2004 and 2014, the average deposition in the same subplots was 0.81 cm yr^{-1} in the SM site and 1.3 cm yr^{-1} in the BM site. The SM and BM sites have similar grass and total vegetation coverage in both upwind and downwind plots. However, the type of grass coverage and the average gap size in the sites are the major factors where these sites differ significantly. At the same level of cover, the gap size for the BM site is approximately half of the SM site. This is due, in essence to the size of the plants at the two sites. The smaller *Sporobolus* plants at the SM site lead to larger gaps between plants, even at the same level of cover as at the BM site, where the *Bouteloua* plants larger (Fig. 18). Thus, the average gap size in the TD100 SM site and BM site in 2013 was ~665 cm and 618 cm, respectively (for comparison, the average gap size for the controls is 224 and 87 cm, respectively). Okin et al. (2008) hypothesized that the amount of horizontal flux that could be sustained on a vegetated surface was related to the size of unvegetated gaps between plants. According to this model, with other conditions similar, the BM site with a smaller gap should show greater deposition in the downwind D1 plot. This could also explain why SOC and TN concentration in the TD100 plots declined significantly in the SM site but not in the BM site. Greater control of erosion by evenly scattered vegetation vs. unevenly distributed pattern, given

the same percent cover was also demonstrated by Dong et al. {, 1996 #455}.

The T50 upwind treatment in the SM site, with 12% grass cover and 23% total vegetation cover, did not show significant loss of SOC. However, the T50 upwind treatment in the BM site, with 21% grass and 23% total vegetation cover, respectively, did exhibit significant SOC loss, indicating that vegetation cover as a single variable cannot successfully predict soil SOC loss.

The erosion bridge results show that there is general deposition occurring in the area. Extensive soil movement occurrences have been recorded in JER sites (Gibbens et al., 1983). The upwind SOC concentration was correlated with total grass cover and total vegetation cover at the upwind plots ($R^2 = 0.69$, $p = 0.19$, $R^2 = 0.62$, $p = 0.12$ respectively). Thus, although there is a positive correlation trend between SOC and vegetation cover, these correlations were not quite significant, suggesting exogenous factors like wind also play an important role in the SOC/TN concentrations and therefore dynamics.

Our results suggest a threshold for SOC loss can exist both below or above T50 (50% grass cover reduction) depending on the site and vegetation characteristics. Yan et al. (2013) using a multiple short-term (9 days) vegetation cover simulation experiment in a semiarid steppe of northern China suggested that vegetation cover should be maintained at least above 35% for the effective protection of fine soil particles and nutrients. In our sites, both SOC was lost significantly from TU50 plots in the BM site but not from the SM site, although both had a total vegetation cover of ~23% indicating such a high threshold as suggested by Yan et al. (2013) may not be applicable to all type of vegetation. Further, we do not see significant soil erosion in TU50 plots in both the sites because these plots still have a vegetative cover of around 23%. Thus, the threshold for sediment transport in our site was above T50, which corroborates observation from (Li et al.,

2007).

Among the upwind controls, the patterns of semivariance exhibit some dynamism. Though the ranges of autocorrelation are essentially the same for all of the controls, the proportion of variance that is spatially structured (C/Co+C) is considerably higher for in 2013 than in 2004. There is also spatial variability between upwind and downwind control sites at SM in 2004, though this disappears by 2013. Nonetheless, after nine years, in the upwind T100 sites, there are significant differences from the control. In SM the range of autocorrelation became significantly smaller than the control and the relatively high value of (C/Co+C) didn't change. In contrast, in the BM site for SOC, no good variogram could be produced, resulting in an all-nugget variogram. This can be interpreted either as making the scale of autocorrelation much, much larger than the area sampled, or making the scale of autocorrelation smaller than the average sampling distance (with 50 samples in 50 m^2, the average sampling distance is < 1 m). The TN in this case can shed some light in this case since A_0 appears to be smaller than the control in 2013. If so, then SM and BM are consistent in indicating that with vegetation removal, the scale of autocorrelation decreased in response to 9 years of increased horizontal aeolian transport. There is a significant (95%) positive correlation (R=0.96) at the upwind sites between the change in (C/Co+C) for SOC between 2013 and 2004 (expressed as the ratio) and the change in surface height for the upwind sites. In practice, this indicates that negative changes in surface height (i.e., deflation) is related to decreases in the fraction of the variance that is spatially patterned. Deflation, thus, appears to cause a weakening of fertile islands.

The downwind SM sites for both the control and T100 show the lowest values of A_0 (< 1.0) with spatially structured variance contributing to most of the sample variance ((C/Co+C)

>0.9), among all plots at all times. It is difficult to interpret this result. However, when compared to the controls, A_0 in 2013 downwind in the SM site is lower (and (C/Co+C) is lower), indicating At the SM T100, thus, weaker but smaller fertile islands, compared to the control, appeared to develop over 9 years of aeolian transport. This is consistent with the changes seen upwind at SM T100. As with the SM site, 2004 results for the downwind sites is not clear because good variograms could not be produced from BM T100. Nonetheless, smaller values of (C/Co+C), compared to the control indicates a decrease in the size of fertile islands in the downwind BMT100 treatment after nine years of enhanced aeolian transport.

Okin (2012) derived a relationship between cover, average gap size, and average canopy width: C=W/(L+W), where C is cover, W is average width, and L is average gap size. Rearranging, W=CL/(1-C). Using this equation and cover and gap data in Tables 8 and 9, the plant canopies in the BM T100 site are considerably smaller (24 cm) than in the control (55 cm). This could, in part, explain the smaller scale of autocorrelation for T100 than for the control on the BM site. However, it does not explain the SM site, because the same calculation gives average plant sizes of 71 cm in T100 and 48 cm in the control. Nonetheless, our results indicate that such a comparison may not be useful for SM given the anomalously low ranges obtained for 2004 that, perhaps, indicate some idiosyncratic feature of this downwind site.

Positive correlations between the change in SOC and the change in surface height in the Upwind plots (SM and BM) indicate that deflation is related to a loss in SOC and TN. Though these correlations were not quite significant suggesting exogenous factors like wind also play an important role in the SOC/TN concentrations and therefore dynamics. This is consistent with the previous work of Li et al. (2007). The fact that SOC concentration upwind in the BM and SM sites

is positively correlated with vegetation cover and change in surface height suggests that there may be two components to this pattern: increased deflation and decreased inputs of C and N from vegetation. Again, this is consistent with the conclusions of Li et al. (2007), who suggest that the increased aeolian transport and decreased vegetation inputs combine to reduce total nutrient concentration in the surface soil.

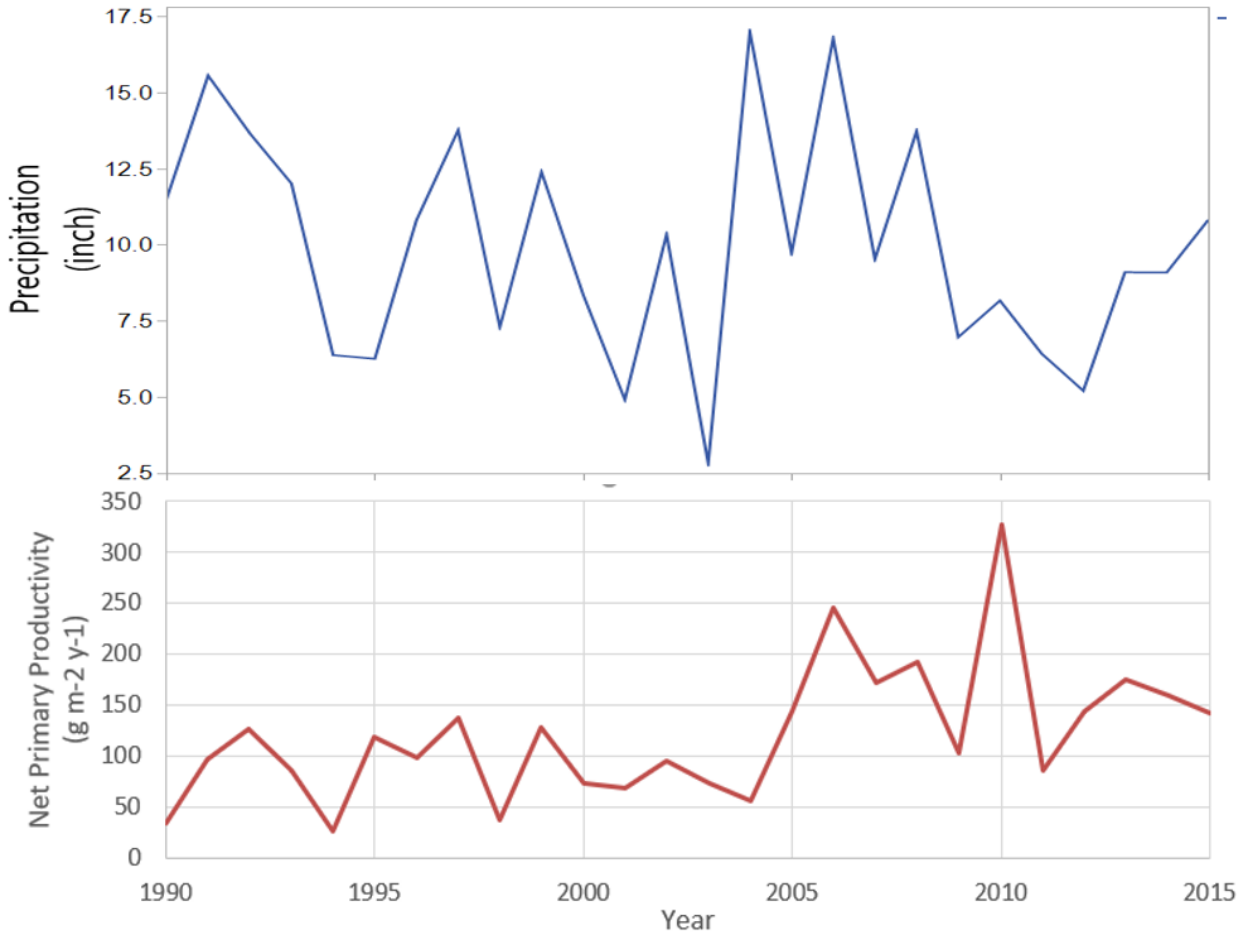

Figure 21 NPP measured at the G-IBPE site and precipitation measured at Pasture 11 A on the sand sheet at Jornada (Data courtesy of the Jornada Basin LTER Site)

Similarly, lack of significant correlations between grass cover and soil nutrients suggests that SOC and TN content is not fully controlled endogenously and, rather, that aeolian transport may be playing a significant part in controlling concentrations in the surface soil. Indeed, the

positive correlation observed between change in SOC from 2004 to 2013 and the change in surface height indicated that deposition tended to lead to increased SOC, arguing for a role of exogenous factors. This somewhat contradicts the conclusion of Li et al. (2009b), who observed a decrease in nutrients, thought to be due to winnowing, on the downwind sites. However, Li et al. (2009b)'s work investigated samples from D1, at the upwind edge of the downwind treatments. Given the longer distance, we interpret this result to suggest that deposition of more nutrient rich windborne fine nutrients in the D2 plot, led to this increase in SOC in the D2 plot.

Conclusion

The original suite of papers to emerge from the NEAT experiment Li et al., (2007) (2008a; 2009b) characterizing its first few years, indicated a relatively straightforward response of soil nutrients to increased aeolian transportation through vegetation removal: upwind, increased transport led to direct nutrient loss and a weakening and widening of fertile islands; downwind, deposited sediment was winnowed of fines leading to a decrease in nutrient availability; both the SM and BM blocks behaved similarly. After nine years of the experiment, this simple picture does not hold. For instance, relative to the controls, the size of fertile islands is smaller in both upwind and downwind treatments compared to controls. In addition, different responses between the BM and SM plots have been observed after 9 years: the SM site indicates a decrease in SOC (compared to controls) for the downwind T100 and T50 plots, but the BM site does not.

There are many possible reasons for the differences from the Li et al. papers and the present study. First, at both upwind plots, the ~ 10 cm of deflation that has been observed since 2004 means that subsurface soils layers have been exposed which likely had had different SOC and TN concentrations than the overlying soils sampled in 2004. Second, both plots have seen net

deposition, even in controls, of ~2.5 cm. This likely indicates broader landscape-scale soil redistribution. In fact, casual observations indicate considerable erosion upwind of both SM and BM, which may have led to this broader-scale deposition. In fact, the pasture that contains SM and BM has considerably changed since 2004 (Fig. 21). Again, casual observations indicate overall less grass cover and greater shrub cover in the region since the experiment originally began. Thus, what started as purposely isolated disturbances in a broader landscape are now more consistent with the wider patterns of vegetation change on the sand sheet.

In addition, there have been considerable interannual differences in precipitation since 2004. Sampling in July 2004 came at the end of a 4-year long dry spell (Peters et al., 2014). The period 2004-2008 was a wet period were at the end of a dry period, which was especially wet in 2006-8, leading to grass establishment across the Jornada in 2008 (Peters et al., 2014). 2010 experienced high net primary productivity. Between 2009 and the sampling in 2013, the Jornada experienced dry conditions with relatively high vegetation cover persisting from the 2008 recruitment episode. Differences from 2004 to 2013 necessarily reflect this variability. However, the *Sporobolus*-dominant SM site and the *Bouteloua*-dominant BM site did not necessarily respond to these changes in the same way. *Sporobolus*, for instance, is a successful sexual reproducer, whereas *Bouteloua* rarely, if ever, reproduces from seed even during the best years, but can expand via stoloniferous growth during wet years.

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