DISSERTATION

ADVANCING UNDERSTANDING OF THE FORMATION AND STABILITY OF SOIL ORGANIC MATTER IN A CHANGING ENVIRONMENT

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ABSTRACT

ADVANCING UNDERSTANDING OF THE FORMATION AND STABILITY OF SOIL ORGANIC MATTER IN A CHANGING ENVIRONMENT

Soil is one of our most precious natural resources. It plays a key role in maintaining soil fertility and water quality, and represents a major reservoir in both the global carbon (C) and nitrogen (N) cycles. Soils contain more C and reactive N than the atmosphere and all vegetation combined, the majority of which is found in soil organic matter (SOM). Despite its considerable significance, little is known about the factors that control the formation of SOM, and its stability in the environment. Key questions pertain to whether environmental changes will increase the production of CO_2 during SOM formation and decomposition, forming a large positive feedback to climate change. Answering those questions required a better understanding of how various mechanisms that confer SOM stability are affected by environmental change. My dissertation research aimed to address some of these key questions, and to advance our overall understanding of SOM formation, SOM stability, and the response of stable SOM to changes in the environment.

First, I conducted two soil incubation experiments using isotopically labeled (^{13}C and ^{15}N) plant material, which allowed me to track the incorporation of plant-derived C and N into SOM, and efflux of plant-derived C in CO_2. In one soil incubation, I tested the effects of plant litter quality and on the rate and efficiency of SOM formation (a measure of the amount of SOM formed versus the amount of CO_2 lost in the process) by comparing SOM formation from leaves versus roots. I found that plant litter chemistry (C/N ratio) was a reliable predictor of SOM

formation after the initial stage of decomposition, with low C/N ratios resulting in more SOM formation and higher formation efficiencies overall. In the second soil incubation, I tested the effect of warming on the rate and efficiency of SOM formation, as well as the rate of destabilization of stable SOM. I found that warming generally led to lower formation efficiencies, causing greater CO_2 production per unit of SOM formed. Warming also led to higher rates of destabilization of stable SOM throughout the experiment. Next, I aimed to investigate the effect of warming on SOM in the field, using soils from two multi-factor climate change experiments. Results from that study suggested that while warming increased the rate of turnover of SOM in some cases, any resulting losses of SOM were offset by increased inputs of SOM, so that total SOM stocks were unchanged. Last, I investigated the persistence of pyrogenic SOM, which is thermally transformed by fire, in the face of land use change at three agricultural sites across the US. I found that pyrogenic SOM was present in all three soils, and had persisted to a greater extent than other SOM with land use change. Many studies of SOM dynamics do not account for pyrogenic SOM, and the results of my work suggest that this lack of accounting can preclude us from fully understanding the mechanisms behind SOM stability. Overall, my work advances our understanding of stable SOM in terms of how it is formed, and whether it will persist in the face of environmental change. Changes in plant litter quality and temperature may lead to changes fluxes of CO_2 to the atmosphere during SOM formation, and while some SOM (pyrogenic SOM) is highly stable in the environment, other SOM is susceptible to loss with warming and land use change. However, in the case of warming, increased plant inputs may offset increased rates of SOM decomposition.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Soil is one of our most precious natural resources, playing a key role in maintaining soil fertility and water quality, and representing a major reservoir in both the global carbon (C) and nitrogen (N) cycles. The vast majority of soil C and N is found in soil organic matter (SOM), which contains more carbon (C) and reactive nitrogen (N) than the atmosphere and all vegetation combined (Galloway et al., 2004; Jobbágy and Jackson, 2000; Knicker, 2011). Because it is such a large reservoir, even a small change in the dynamics of SOM would have major implications for the global C and N cycles. Of major concern is whether SOM will act as a net source or a sink of atmospheric CO_2, potentially slowing or accelerating climate change (Kirschbaum, 2000). The answer hangs in the balance between inputs (SOM formation) and outputs (mainly decomposition) from the SOM reservoir. Despite its considerable significance, there is still uncertainty about how the formation and decomposition of SOM may respond to environmental changes.

Plant litter quality, a general term meant to reflect its decomposability, is thought to be a major control on the process of SOM formation. The Microbial Efficiency Matrix Stabilization hypothesis (Cotrufo et al., 2013) states that as litter quality declines, so does the efficiency of microbial processing, and ultimately less SOM is formed. There is evidence that plant tissue chemistry (Norby et al., 2001), allocation between aboveground and belowground biomass (Elser et al., 2010; Norby and Zak, 2011), and plant community composition (Bertrand et al., 2011; Kardol et al., 2010) may all change with increasing carbon dioxide concentrations and the resulting changes in our climate. We do not know how the resulting changes in litter quality will affect SOM formation and subsequent SOM storage, which motivated my first research question:

(1) How do differences in litter quality, indicated by C/N ratio, affect SOM formation and stabilization over the course of decomposition?

Our climate is warming at an unprecedented rate (IPCC, 2013), and it is yet unknown how the majority of SOM, that which cycles on a decadal to centurial timescale, is responding (Conant et al., 2011; Conen et al., 2006; Liski et al., 2000; von Lützow and Kögel-Knabner, 2009). Rates of microbial decomposition are expected to increase with warming, but only if microbes have access to SOM substrates (Davidson and Janssens, 2006). The majority of SOM is rendered inaccessible to microbial decomposition by physico-chemical stabilization mechanisms, including soil aggregation and close association with soil mineral surfaces (Kleber et al., 2007; Six et al., 2002; von Lützow et al., 2006). These stabilization processes themselves may be sensitive to temperature, in which case, warming could lead to the release of previously inaccessible SOM substrates to microbial decomposition (Conant et al., 2011; Davidson and Janssens, 2006).

In addition to rates of SOM decomposition, warming may also affect rates of SOM formation. Two of the known mechanisms of SOM formation from plant material are leaching of DOM compounds, and microbial transformation and deposition of plant-derived products, both of which can be adsorbed directly onto the surfaces of minerals or other SOM (Cotrufo et al., 2013; Kalbitz et al., 2005; 2000; Mambelli et al., 2011). Leaching has been shown to increase with temperature (Andersson and Nilsson, 2001; Kalbitz et al., 2000), but since leachable plant compounds tend to be released very quickly in the initial stages of decomposition (Marschner and Kalbitz, 2003; Soong, 2014), any effect of warming on SOM formation via that mechanism may be negligible. Microbial transformation of plant material is probably more important in terms of determining the effect of warming on SOM formation, and there are two aspects to

consider: (1) the rate of microbial transformation of plant material, and (2) the efficiency of that transformation. Aspect (2) is less well understood than aspect (1). A small number of studies suggest that the efficiency with which microbes utilize plant material, that is, the ratio of microbial products to CO_2 respired, decreases with warming (Devêvre and Horwath, 2000; Frey et al., 2013; Steinweg et al., 2008; Van Ginkel et al., 1999), but there is yet to be a consensus (Conant et al., 2011). The uncertainties associated with the effects of warming on SOM formation and decomposition motivated my next two research questions:

(2) How does warming impact the amount of litter-derived SOM formed and stabilized relative to CO_2 produced, hereafter referred to as stabilization efficiency (SE)?

(3) How does warming impact the size of SOM stocks via the balance of SOM formation and decomposition?

Pyrogenic OM (py-OM), which is thermally transformed by fire, may be one of the few cases for which the inherent chemical properties of the material hinder its decomposition in the long-term (Schmidt et al., 2011). Py-OM has been shown to persist for much longer than other SOM (Preston and Schmidt, 2006; Singh et al., 2012). It is also ubiquitous in the environment; it is constantly created by wildfire and anthropogenic activities, and subsequently spread by wind and water (Forbes et al., 2006; Schmidt and Noack, 2000). Despite a broad recognition of its presence and unique bahavior, it is often ignored in studies of SOM dynamics because it is notoriously difficult to isolate and characterize (Hammes et al., 2007). Studies on land use change provide a good context to investigate influences of py-OM. It is generally accepted that SOM is lost upon cultivation (Schlesinger and Andrews, 2000), but few studies on this topic have accounted for py-OM (Skjemstad et al., 2002). Py-OM may be contributing to pool of SOM that remains long after cultivation is initiated, which would help to explain the long residence

times observed in some studies (Follett et al., 2007; Paul et al., 2001; 1997). This idea motivated my final research question:

(4) Will measuring and accounting for py-OM improve our understanding of how soil organic matter dynamics respond to land use change?

For my dissertation work, I addressed these research questions using a combination of laboratory incubations and field experiments. My overall goal was to advance our general understanding of the processes governing SOM dynamics in order to better predict the responses of SOM stocks to environmental change.

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CHAPTER 2: RELATING LITTER QUALITY TO SOM FORMATION AND CO_2 EFFLUX USING A SLURRY INCUBATION WITH ^{13}C- AND ^{15}N-LABELED ROOTS AND LEAVES

2.1 Introduction

Soil organic matter (SOM) formation is a key, but poorly understood step in the global carbon cycle. SOM formation represents a major flux of carbon (C) and nitrogen (N) to stable pools where they may remain for decades to millennia (Knicker, 2011; Schmidt et al., 2011; von Lützow et al., 2006). The controls on SOM formation are of great interest for purposes of improving soil quality (Six et al., 2000), promoting C sequestration (Stockmann et al., 2013), and predicting effects of climate change (Kirschbaum, 2000). One of the major mechanisms of SOM formation is the direct leaching of plant-derived dissolved organic matter (DOM), which becomes intimately associated with soil minerals (Kalbitz et al., 2000). The second major mechanism of SOM formation is through microbial decomposition of plant material, which is transformed into microbial products or byproducts that may then be stabilized by the soil matrix (Cotrufo et al., 2013; Grandy and Neff, 2008). While several of the controls on these two processes have been identified, the natures of the controlling relationships are not necessarily well characterized (Cotrufo et al., 2013; Guggenberger and Kaiser, 2003; Kaiser and Guggenberger, 2000; Klotzbücher et al., 2013).

Plant litter quality is a major control on SOM formation because it determines the amount and nature of DOM that leaches into the soil (Soong et al., 2015), as well as the efficiency of the microbial "filter" through which it passes (Cotrufo et al., 2013). The term plant litter "quality" refers generally to the decomposability of the litter. It is measured in many different ways in the

literature (Prescott, 2010), but here we use the C/N ratio. Hot water extractable (HWE) material has been shown to be a reliable predictor of DOM production during the early stage of decomposition (Soong et al., 2015), while C/N ratios and other quality indicators may be good predictors of rates of microbial transformation later on in decomposition (Cotrufo et al., 2013). Key questions remain as to how plant litter quality affects (1) the amount of SOM formed from plant litter, and (2) the stabilization efficiency (SE) of SOM (defined as the partitioning of litter-C to SOM versus CO_2). We addressed these questions by comparing SOM formation from leaf- and root-derived ^{13}C and ^{15}N in isolated silt and clay soil fractions.

We hypothesized that SOM formation during the initial stage of litter decomposition would be driven mainly by direct leaching of soluble plant compounds (hypothesis 1). Based on hypothesis 1, we predicted that SOM formed during the initial stage would reflect the differences in HWEs between the litters (higher HWE-C would result in more litter-derived C in SOM (prediction 1, or P1), and higher HWE-N would result in more litter-derived N in SOM (P2)). We also predicted that stabilization efficiency would be relatively high during the initial stage, since compounds leaching directly from plant material would not have to go through microbial processing before being stabilized (P3).

We hypothesized that after the initial stage of decomposition, stabilization would be driven by microbial processing of the litter and would be controlled by non-HWE litter quality (hypothesis 2). From this, we predicted that stabilization efficiency overall would be lower than it was during the initial stage (P4), and that stabilization efficiency would be higher for leaves than for roots (P5). We based P5 on the lower C/N ratio of non-HWE of leaves in our study, along with evidence from the literature, which shows a general trend of higher quality of leaves than roots by several other indices of quality (Rasse et al., 2005; 2012).

We used isolated silt- and clay-sized fractions (2-53 μm and <2 μm, respectively) because they are realistic proxies for soil mineral surfaces, but are relatively uniform when isolated, easing interpretation of results. Silt and clay account for the majority of SOM stabilization in soils (2009; Christensen, 2001; Kögel-Knabner et al., 2008), but have different stabilization capacities (1996; Stewart et al., 2008) and tend to stabilize SOM with different chemical properties (1987; Calderón et al., 2011; Grandy and Neff, 2008; Plante et al., 2006). Comparing results between silt and clay gave us insight as to how soil characteristics might interact with litter quality to control SOM stabilization. Based on previous work showing that clay has higher surface area and more reactive surface chemistry than silt (Christensen, 2001; Joffe and Kunin, 1943; R Core Team, 2013), we predicted that clay would stabilize more C and N than silt (P6), and that it would stabilize litter-derived C from both litter types more efficiently than silt (P7), during both stages of decomposition.

We tested these hypotheses using a slurry incubation with ^{13}C and ^{15}N-labeled plant material. This approach is ideally suited to this type of study. Constant shaking in water maximizes physical contact between enzymes and substrates, greatly increasing the rates of leaching, microbial decomposition, and stabilization on mineral surfaces. SOM that was not decomposed could be interpreted as stabilized against microbial attack, since any available substrates should have been quickly decomposed. The use of ^{13}C and ^{15}N-labeled plant material allowed us to distinguish between SOM derived from the plant material we added and that which was already present in the silt or clay prior to incubation. Overall, our approach simplified the interpretation of the results and allowed for clear characterization of SOM formation dynamics.

2.2 Methods

2.2.1 Growth of isotopically labeled plant material

Big bluestem (*Andropogon gerardii*) was grown from seedling to maturity in a continuous isotopic labeling chamber at Colorado State University. A description of the chamber and the growing procedure can be found in Soong et al. (2014). This procedure produced uniformly labeled leaves that were 4.7 atom% ^{13}C and 6.5 atom% ^{15}N, and uniformly labeled roots that were 4.5 atom% C and 6.5 atom% ^{15}N. The C and N contents, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ values of the leaves and roots are shown in Table 2.1. Harvest entailed clipping aboveground biomass (which we refer to as leaves), separating belowground biomass (which we refer to as roots) from the growth media (a mixture of sand, vermiculite, and profile porous ceramic), and drying each separately in a 60 °C oven. Random subsamples of the leaves and roots were clipped to 0.5-1.5 cm in length, and sieved to remove any pieces that passed through an 800 μm opening, prior to use for this experiment. The aim was to obtain pieces of plant material that could be separated back out from the silt or clay using a 250 μm sieve after the incubation.

2.2.2 Hot water extraction

Hot water extractions were performed on four replicates each of leaves and roots according to Tappi (1999). Briefly, 0.7 g of clipped litter was combined with 40 ml of hot, deionized water in a covered test tube and kept in a digestion block at 100 °C for 3 hours. Samples were then poured over a 20 μm nylon filter to separate extracted material from the residue. Extracts were analyzed for organic C and N using a TOC analyzer (Shimadzu TOC 5000). Residues were dried overnight at 105 °C, ground, and analyzed for C and N concentrations on a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA USA) coupled to a Delta V Advantage IRMS (Thermo-Fisher, Bremen, Germany).

2.2.3 Fractionation of silt and clay

The soil used in this experiment was collected from cultivated wheat fields at Waggoner Ranch in northern Texas, south of the town Vernon in Wilbarger County (33°50' N, 99°02' W). This soil was chosen for its high silt and clay content and low SOM content, under the assumption that it would have a large capacity to stabilize additional SOM. Silt and clay was isolated from a whole soil by a physical fractionation scheme modified from Jagadamma et al. (2013). Briefly, oven dried bulk soil was dispersed by shaking for 18 hours in DI water with glass beads. After dispersion, the soil and water mixture was poured over a 53 μm mesh screen and gently sieved to remove particulate organic matter and other sand-sized material. We chose not to disperse using chemical techniques because chemical dispersants can denature enzymes and interfere with microbial activity (Allison and Jastrow, 2006; Jagadamma et al., 2013). We also chose not to employ a density separation because high density liquids such as SPT have also been shown to interfere with microbial activity and reduce microbial (Crow et al., 2007; Jagadamma et al., 2013). Chemical dispersants and high density liquids can be difficult to eliminate entirely from soils once they are introduced, and we wanted to avoid any effects they might have on microbial activity during our incubation. After sieving, the <53 μm material was sonicated to further disperse microaggregates. We chose the duration and energy of sonication based on preliminary testing which showed the maximum amount of clay that could be produced from dispersion of silt-sized aggregates by sonication for this soil. After sonication, silt and clay were separated by centrifugation at 20 °C according to Stocke's Law, and oven dried at 105°C. The C and N contents, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ values of the soil fractions are shown in Table 2.1.

2.2.4 Experimental design and initial setup

We incubated silt or clay with roots or leaves in a full factorial design with four replicates. We maintained controls, which included silt or clay that did not receive plant litter, and leaves or roots with no soil, also with four replicates. Together, the treated samples and control samples (32 total) constituted one set. We incubated two sets, allowing for one destructive harvest partway through the incubation and another at the end. The first harvest, at day 7, was timed to coincide with the sharp decrease in respiration rates early on, a common characteristic of soil incubations. The aim was to capture the dynamics within the "initial stage" or decomposition, when respiration rates are high and SOM decomposition is rapid. The second set of samples was harvested after 60 days.

Slurries consisted of 1 g of either silt or clay, 0.1 g of leaves or roots, and 20 mls of deionized water in 50 ml conical centrifuge tubes with plug seal caps fitted with rubber septa. On day zero of the incubation, the slurry components were combined, the tubes were capped (airtight), and all samples were flushed with CO_2-free air for 10 minutes. Samples were then placed on a horizontal shaker at 25 °C and shaken constantly to keep the slurries aerated.

2.2.5 CO_2 flux measurements

CO_2 concentrations were measured on one set of samples (those that were harvested at day 60) using an LI-6525 (LI-COR, Lincoln, NE) infrared gas analyzer (IRGA). CO_2 measurements were taken daily for the first seven days, and every two to three days until day 58. Immediately after CO_2 measurement, the $\delta^{13}C$ of the CO_2 was measured on the same subset of samples using a VG Optima isotope ratio mass spectrometer (IRMS) with a microgas injector and equilibration block (Isoprime Inc., Manchester, UK). These measurements were taken on days 3, 5, 8, 10, 19, 27, 34, 41, 48, and 58. For days when $\delta^{13}C$-CO_2 was not measured, the $\delta^{13}C$-CO_2 was estimated

using linear interpolation between the prior and subsequent measurements (shown with lines in Fig. 2.2b)(Murage et al., 2007; Stewart et al., 2013). After CO_2 measurements were taken, both sets of samples were flushed with CO_2-free air. The fraction of litter-derived C respired at each CO_2 sampling occasion was estimated using the mixing model:

$$f_L = \frac{\delta - \delta_C}{\delta_L - \delta_C} \quad (1)$$

where δ is the $\delta^{13}C$-CO_2 of the sample, δ_C is the average $\delta^{13}C$-CO_2 of the corresponding controls without plant litter, and δ_L is the average $\delta^{13}C$-CO_2 of the labeled plant material controls without soil, all at the same time of sampling. Fluxes of litter-derived C in CO_2 were calculated by multiplying the f_L value by the total C flux, and the remainder of the C flux was assigned to native-derived C (C present in the soil prior to incubation).

2.2.6 SOM and plant material measurements (harvests)

At each harvest, samples were poured over a 250 μm sieve to collect the remaining plant litter, and soil was collected underneath on a 1.2 μm glass microfiber filter under vacuum. The soil was rinsed of free organic matter by passing 100 ml of deionized water over the soil on the filter. Soil was dried at 60°C and analyzed for $\delta^{13}C$, $\delta^{15}N$, and C and N concentrations were measured on a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA USA) coupled to a Delta V Advantage IRMS (Thermo-Fisher, Bremen, Germany). Any C or N recovered with the soil was defined as stabilized in silt- or clay-associated SOM.

Litter-derived C in SOM was calculated according to the same mixing model used for CO_2, substituting the $\delta^{13}C$ and $\delta^{15}N$ values of solid materials (soil fractions or plant litter) from samples and controls. Stabilization efficiency (SE) was calculated using the following equation:

$$SE = \frac{\text{litter-derived C in SOM}}{\text{litter-derived C processed}} \quad (3)$$

where litter-derived C in SOM was defined as that found associated with either the soil or the clay fraction and litter-derived C processed was defined as litter-derived C in SOM + litter-derived C in CO_2.

2.2.7 Statistical analysis

We tested for differences in C and N in hot water extracts using one-way analysis of variance (ANOVA). We tested for effects of soil fraction and litter type on amounts of C and N in SOM, and cumulative CO_2 production separately for each harvest using two-way ANOVAs with Tukey's post hoc test if one or more factors were significant ($p \leq 0.05$). We tested for effects of soil fraction, litter type, and harvest on SE and C./N ratios of SOM using a three-way ANOVA with Tukey's post hoc test if one or more factors were significant ($p \leq 0.05$). All statistical tests were carried out using the statistical package in R, version 3.0.2 (R Core Team, 2013).

2.3 Results

2.3.1 Hot water extraction

Results of the hot water extraction, along with data for leaves and roots prior to extraction (bulk) are shown in Table 2.2. The two bulk litters had similar C/N ratios, but had different hot water extractable (HWE) C/N ratios (p = 6.99e-06). Leaves had significantly higher % HWE-C than roots (p = 0.0209), while roots had significantly higher % HWE-N than leaves (p = 2.75e-05). A lower proportion of total N was extracted from the leaves, and as a result the non-HWE material of the leaves had a significantly lower C/N ratio than that of the roots (p = 0.05, data not

shown).

2.3.2 Initial stage of decomposition (harvest 1)

Figure 2.1 shows the cumulative respiration of C in CO_2 (litter-derived and native-derived C-CO_2 combined), relative to the amount of total C present in each treatment or control. During the initial stage of decomposition, when respiration rates were relatively high, cumulative respiration was similar for all treatments except silt and leaves, which respired significantly more C-CO_2 per g C ($p < 0.0001$ for all contrasts, Fig. 2.1). Cumulative respiration of the litter controls was also high; respiration of the root control was similar to that of the silt and leaves treatment, but respiration of the leaf control was greater ($p = 0.015$, Fig. 2.1). Respiration of the soil fraction controls (silt and clay) was significantly lower than all of the treatments and litter controls (Fig. 2.1). Figure 2.2 shows the $\delta^{13}C$ of the CO_2, which we used to determine the relative contributions of litter-derived C and native-derived C to the total CO_2 respired in each treatment. In terms of litter-derived C-CO_2 on a per g soil basis, the silt and leaves treatment respired the most, and the clay and roots treatment respired the least, while the other two treatments were not different (Fig. 2.3a). In general, more leaf-derived C was stabilized than root-derived C (Fig. 2.3a). Clay stabilized more leaf-derived C than silt, but stabilization of root-derived C was not different between silt and clay (Fig 2.3a). SE was significantly higher for leaf-derived C in the clay, but SEs were not different between litter types in the silt (Fig. 2.3b). Samples with roots stabilized more litter-derived N in SOM than samples with leaves, but N stabilization was not different between silt and clay (Fig 1.4). The C/N ratio of litter-derived SOM was lower for samples with roots than for samples with leaves, consistent with the higher stabilization of root-derived N. While the C/N of root-derived SOM was not different between silt and clay (combined average was 5.86 ± 0.23), leaf-derived SOM had a significantly higher

C/N ratio in clay (9.38 ± 0.20) than it did in silt (7.69 ± 0.08, p=1.36e-7).

2.3.3 Later stage of decomposition (harvest 2)

Once respiration rates slowed, the samples with roots respired more total C-CO_2 per g C than samples with leaves (p = 0.0002, main effect), but there was no main effect of soil fraction (Fig. 2.1). Cumulative respiration was similar between the leaf and root litter controls, and both respired significantly more than the treatments and soil fraction controls (Fig. 2.1). Cumulative respiration of the soil fraction controls remained significantly lower than all of the treatments and litter controls, but by harvest 2, respiration of the clay control was significantly higher than that of the silt control (p = 4.45e-05, Fig. 2.1). In terms of litter-derived C-CO_2, samples with roots respired more litter-derived C-CO_2 per g soil than samples with leaves, and samples with silt respired more litter-derived C-CO_2 per g soil than samples with clay (Fig. 2.3a).

Litter-derived C stabilization followed the same pattern as in the initial stage. On average, more leaf-derived C was stabilized than root-derived C, and clay stabilized more leaf-derived C than silt, but silt and clay stabilized similar amounts of root-derived C (Fig 2.3a). In total, more root-derived C was processed than leaf-derived C, but less of it was stabilized as SOM, resulting in lower SEs for samples with roots than samples with leaves (Fig. 2.3a, b). The clay had higher a SE than the silt for leaf-derived C, but the silt and clay had similarly low SEs for root-derived C. Contrary to the initial stage of decomposition, significantly more leaf-derived N was stabilized than root-derived N (Fig 2.4). Similar to the initial stage, there was no difference in N stabilization between silt and clay (main effect, p=0.16). The increase in leaf-derived N stabilization, together with higher leaf-derived C stabilization, led to similar C/N ratios for leaf- and root-derived SOM. The one exception was the clay and leaves treatment, which had a significantly higher C/N ratio of litter-derived SOM (7.86 ± 0.28) than the other

treatments (combined average was 6.33 ± 0.14). Averaged across all treatments, C/N ratios of litter-derived SOM were not significantly different than they were for the initial stage, but the range in values was smaller (5.90-7.86 during the later stage, compared to 5.57-9.38 during the initial stage).

2.4 Discussion

In general, our results followed our predictions. In the initial phase, litter-derived C and N stabilization reflected HWE-C and -N concentrations, with more leaf-derived C stabilized (P1) and more root-derived N stabilized in SOM (P2). SE was also higher during the initial phase than it was during the later phase of decomposition (P3), which indicated that as decomposition progressed, stabilization depended more upon microbial transformation of plant material as an intermediary process. This agrees with results of Soong et al. (2015), who measured dissolved organic carbon (DOC) production throughout an incubation of different litter types. In all litters, DOC production during the initial stage of decomposition (defined in the same manner as in our study) was higher than that during the later stages of decomposition. In addition, Soong et al. (2015) observed relatively high C/N ratios of the DOM produced early on, which suggested that the DOM at that stage was not microbially transformed. Though microbial growth can take place rapidly at the start of an incubation (e.g. Blagodatskaya et al., 2009), the general agreement between stabilized C and N and HWE-C and –N, together with the very fast (within 6-9 days) stabilization of litter-derived SOM in our study, is consistent with the idea of direct stabilization of readily leachable plant compounds during the initial stage. However, the C/N ratios of litter-derived SOM were not as high as those of the HWE during the initial stage, which indicates some microbial processing of plant material and loss of litter-derived C as CO_2 prior to stabilization. So while both processes (direct stabilization of leachable plant compounds and

microbial transformation) probably contributed to stabilization early on, our results suggest that direct stabilization played a major role.

During the later stage of decomposition, stabilization efficiency overall decreased (P4), consistent with the hypothesis that microbial processing was the dominant mechanism of litter-derived C stabilization. Further support came from the narrowing of the range in C/N ratios of litter-derived SOM in the later stage of decomposition. While the average C/N ratio of litter-derived SOM was not different than it was during the initial stage, the C/N ratios during the initial stage had a wider range; the average C/N for leaf-derived SOM was higher, while the average C/N ratio of root-derived SOM was lower. After the initial stage, C/N ratios converged around an average of 6.71 ± 0.21, very near the bacterial C/N ratio and within the range of fungal C/N ratios reported by Cleveland and Liptzin (Cleveland and Liptzin, 2007), which suggests that litter-derived SOM was more microbially-processed than it was during the initial stage.

Stabilization efficiency was higher for leaves than for roots in the later stage of decomposition, consistent with our prediction (P5). We hypothesized that this was due to the differences in quality of non-HWE material between leaves and roots, and used the C/N ratio of the non-HWE as a measure of quality. While using the C/N ratio did allow us to predict stabilization dynamics, it was relatively coarse as measures of quality go. C/N does not always correlate to decomposition rates, and other, more specific indices (*i.e.* lignin:N) may serve as more reliable predictors (Prescott, 2010; Rasse et al., 2005). In our case, the C/N ratio of the non-HWE may simply have coincided with other chemical and structural differences between the leaves and roots, which were more responsible for the dynamics we observed. For example, studies that have compared leaves and roots have shown that roots tend to be higher in suberin, a highly recalcitrant biopolyester (Rasse et al., 2005). Since we did not measure any other

indicators of quality, we cannot be sure whether our observations were caused by the C/N ratio or by some other property (or combination of properties).

There were two cases where our predictions were incorrect, and both pertained to our predictions of stabilization in silt versus clay. First, we predicted that clay would stabilize more litter-derived C and N than silt at both stages of decomposition (P6). This prediction was correct for stabilization for leaf-derived C, but not for root-derived C. At both harvests, silt and clay stabilized similar amounts of root-derived C. The fact that the two soil fractions did not differ in root-derived C stabilization, and that stabilization of root-derived C was significantly lower than that of leaf-derived C, suggests that the efficiency of microbial processing may have been the limiting factor for stabilization in this case. P6 was incorrect in terms of N, as the two soil fractions did not differ in their stabilization of litter-derived N. One potential explanation is that N-rich compounds may be very prone to stabilization due to the affinity of peptides for mineral surfaces (Kleber et al., 2007; Knicker, 2011; Sollins et al., 2006). As a result, both soil fractions may have preferentially stabilized available N-rich compounds, resulting in similar N stabilization in both. Assuming the clay had a higher capacity for stabilization than the silt, any capacity that remained in the clay after stabilization of N-rich material would then have been occupied by more C-rich compounds, as was the case for leaf-derived C at both harvests.

We predicted that clay would have higher SEs than silt, regardless of litter type (P7) but this was only true for leaf-derived C. SEs for root-derived C were similarly low in silt and clay, which lends support to our previous explanation that microbial efficiency may have been the limiting factor for root-derived C stabilization. In this case, it seems that the quality of the litter can exert a stronger control on the rate of C stabilization by minerals than the characteristics of the mineral surfaces themselves. One important thing to note, however, is that stabilization of

root-derived C might be more efficient in natural systems than it was in our study. We designed our study to minimize the effects of aggregation on SOM stabilization, but in natural soils, this is a major mechanism by which plant material is protected from decomposition. Aggregation is particularly important in protecting root-derived material, as roots are already in close contact with the soil (as opposed to leaves which must fall and then move downward into the mineral soil) and the roots themselves encourage aggregate formation (Prescott, 2010; Rasse et al., 2005). This was outside the scope of our study, but should be considered in further studies of stabilization and SE of roots versus leaves.

Interestingly, our results did not agree with previous studies of rates of root versus shoot decomposition, as reviewed by Rasse et al. (2005). In that review, the authors found that roots generally decomposed more slowly than shoots. At harvest 1, our results agreed with those findings, but by harvest 2, more root-derived material was processed than leaf-derived material ($p = 0.004$, main effect). This may have to do with the fact the roots and leaves were separated in our study, so that microbes were only exposed to one material or the other, rather than having access to compounds from both leaves and roots which would allow preferential decomposition of one versus the other. Another potential explanation is that the decomposition rates of roots versus shoots depend on the plant species (Rasse et al., 2005), and the rates that we observed might simply be specific to *Andropogon geradrii*.

Based on our results, our slurry approach was an effective method to test our hypotheses. Our goal was to study C and N stabilization over time, and we saw relatively large amounts of stabilization at both harvests. The silt began at 0.84 ± 0.028 %C, and increased to 1.50 ± 0.05 %C by harvest 2, after incubation with leaves (a relative increase of 67%). The clay began at 1.31 ± 0.005 %C, and increased to 1.78 ± 0.03 %C by harvest 2, after incubation with leaves (a

relative increase of 51%). By purposely adding an excess of plant litter, we ensured that stabilization of litter-derived C and N would continue throughout the duration of the experiment. The litter also provided a large enough microbial population to justify the lack of inoculation in this experiment, based on the respiration observed from the litter controls. The fact that cumulative respiration of litter-derived C-CO_2 was lower in samples with litter and silt or clay than it was in the litter controls supported the idea that stabilization of litter-derived C took place in the presence of the soil fractions.

Overall, our results lend strong support to the idea that litter quality is a major control on the rate and efficiency of SOM formation and stabilization on mineral surfaces. When combined with the results of Soong et al. (2015), our results suggest that HWE-C and –N can be used as indicators of DOC production and subsequent SOM formation during the initial stage of decomposition. During the later stage of decomposition, C/N of non-HWE was an adequate predictor of SOM formation in our study, but other indices of quality would probably be more reliable and informative. Our results agree with the MEMS hypothesis (Cotrufo et al., 2013), and lend further support to the proposal to incorporate these controls on stabilization and SE into models of terrestrial C and N cycling.

Table 2.1. Summary data for soil fractions and plant material prior to incubation. Data are means ± one standard error (n=4).

	C (mg g^{-1})	N (mg g^{-1})	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Silt	8.39 ± 0.28	0.83 ± 0.05	10.22 ± 0.31	-18.82 ± 0.46	3.27 ± 0.48
Clay	13.13 ± 0.05	1.80 ± 0.00	7.29 ± 0.03	-18.58 ± 0.04	9.07 ± 0.09
Leaves	487.80 ± 4.72	18.20 ± 0.18	26.80 ± 0.17	3422.66 ± 3.03	17969.27 ± 54.56
Roots	456.00 ± 6.89	17.35 ± 0.35	26.33 ± 0.81	3241.43 ± 9.62	17797.47 ± 132.35

Table 2.2. Summary data for leaves and roots and their hot water extracts. Data are means ± one standard error (n=4).

	C (mg g^{-1} bulk litter)	N (mg g^{-1} bulk litter)	C/N	% HWE-C	% HWE-N
Leaves					
Bulk	487.80 ± 4.72	18.20 ± 0.18	26.80 ± 0.17		
Hot water extractable	75.65 ± 0.83	2.89 ± 0.06	26.23 ± 0.57	15.51 ± 0.17	15.87 ± 0.34
Roots					
Bulk	456.00 ± 6.89	17.35 ± 0.35	26.33 ± 0.81		
Hot water extractable	56.61 ± 1.80	4.72 ± 0.16	12.08 ± 0.80	12.41 ± 0.40	27.19 ± 0.94

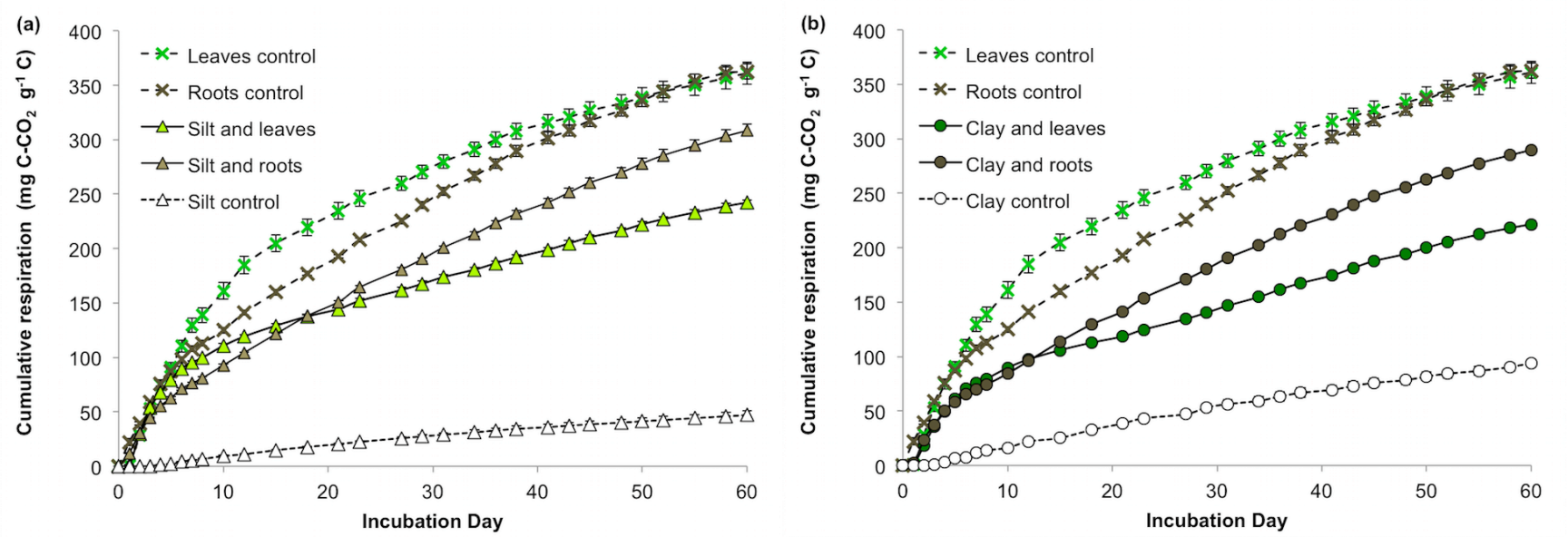

Figure 2.1. Cumulative respiration of by soil fraction and litter type, including controls, over the 60-day incubation. Data for silt fractions are shown in panel (a), and data for clay fractions are shown in panel (b). Litter control data (leaves and roots) are identical between the two panels. Points are means ± one standard error (n=4).

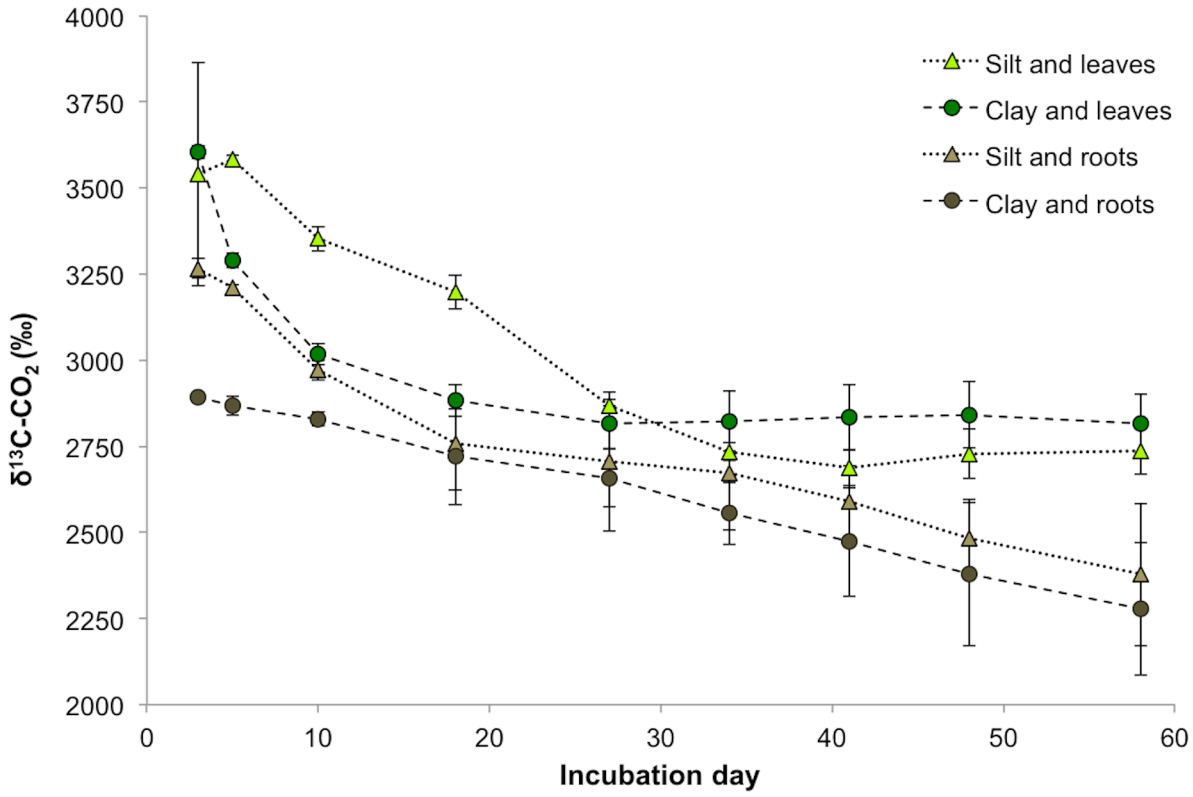

Figure 2.2. $\delta^{13}C$ of CO_2 by soil fraction and litter type over the 60-day incubation. Points are means ± one standard error (n=4).

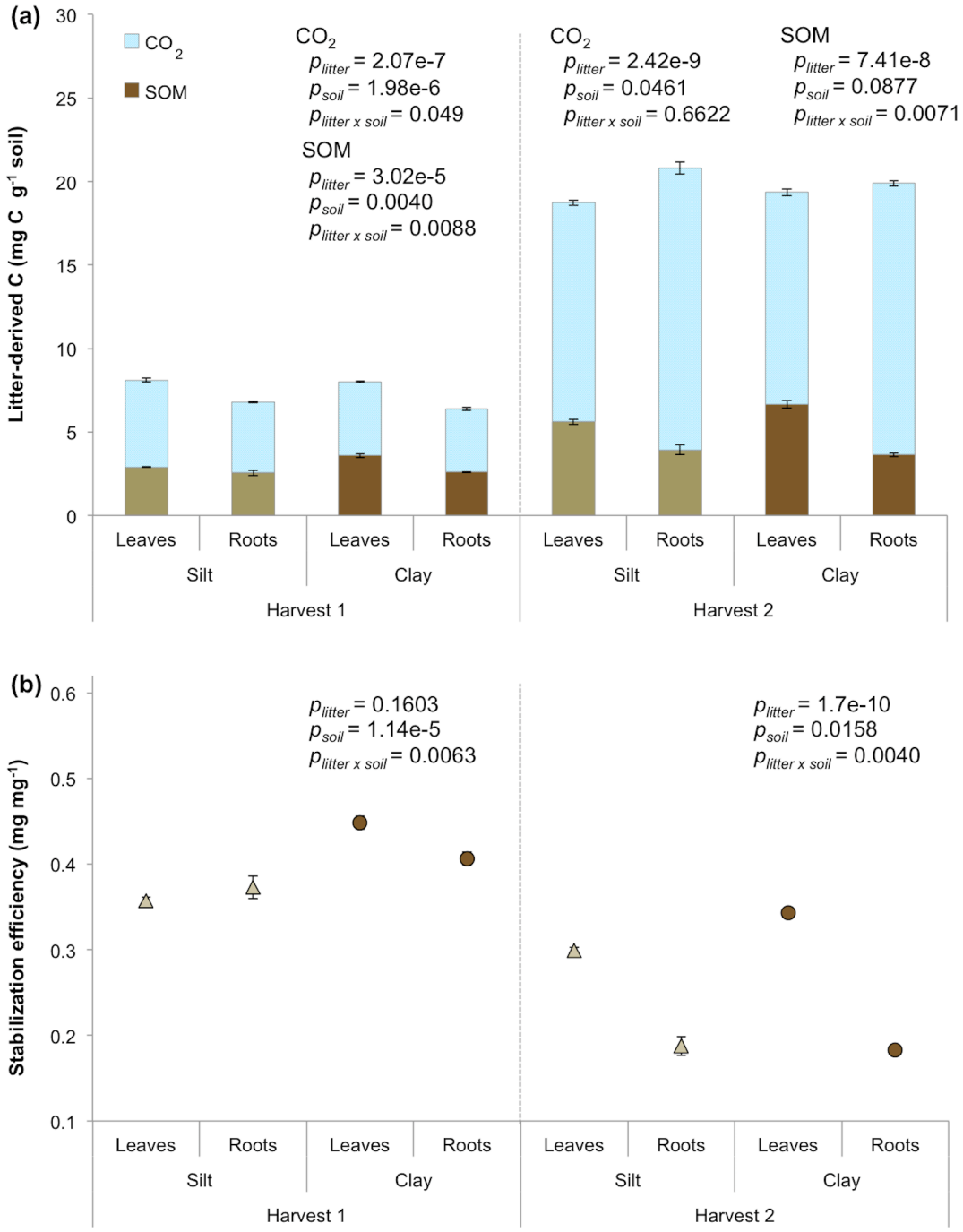

Figure 2.3. Effect of litter type on allocation of litter-derived C to CO_2 and SOM (panel a), and stabilization efficiency (SE) (panel b), in silt and clay at each harvest. In panel a, the total height of each bar represents the amount of litter processed. Data are means ± one standard error (n=4), and *p* values are results of two-way ANOVAs.

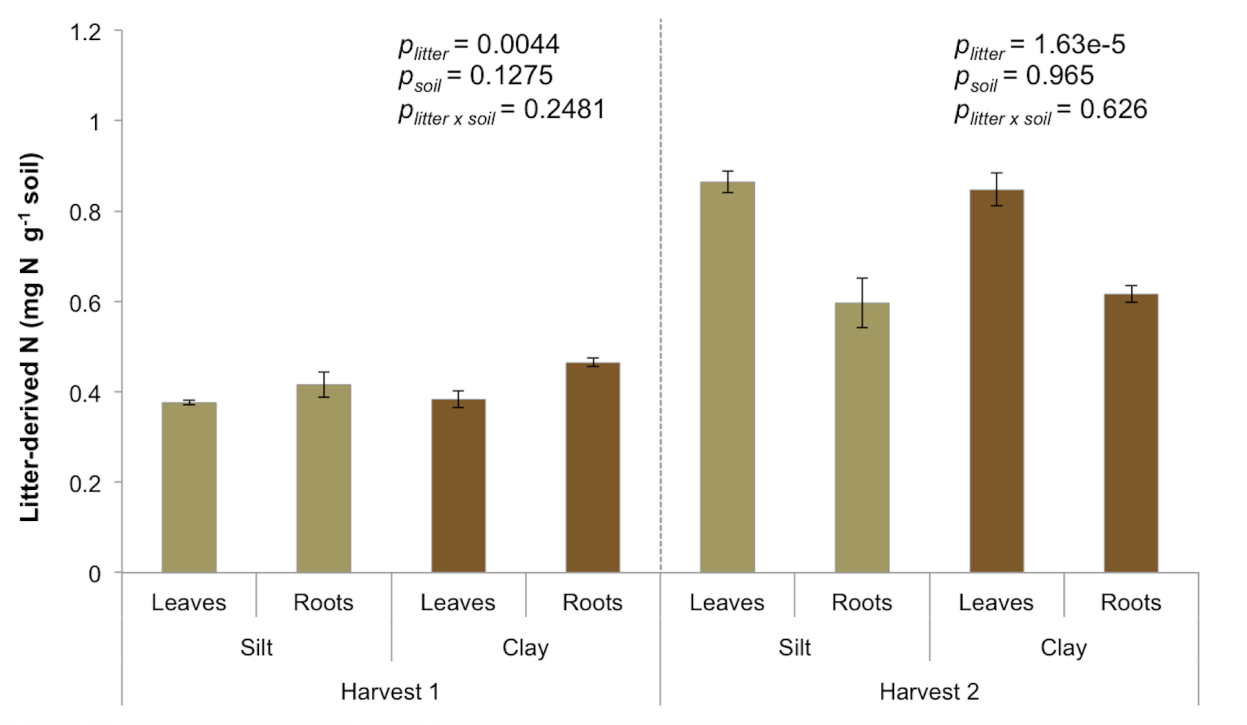

Figure 2.4. Effect of soil fraction and litter type on litter-derived N in SOM at each harvest. Data are means ± one standard error (n=4). P values are results of two-way ANOVAs.

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CHAPTER 3: DISENTANGLING TEMPERATURE CONTROLS ON MINERAL-STABILIZED SOIL ORGANIC MATTER USING A SLURRY INCUBATION WITH ^{13}C- AND ^{15}N-LABELED PLANT MATERIAL

3.1 Introduction

Mineral-associated soil organic matter (SOM) is the largest pool of soil carbon (C) and nitrogen (N), and it often contains the oldest C in a given ecosystem (Christensen, 2001; Kögel-Knabner et al., 2008; Mikutta et al., 2006). Close association between SOM and mineral surfaces limits its availability to microbial decomposition, allowing it to persist for decades to millennia (Baldock and Skjemstad, 2000; Kaiser and Guggenberger, 2003). Warming associated with climate change is expected to cause an increase in the rate of SOM decomposition, forming a large positive feedback to climate change (Davidson and Janssens, 2006). But stabilization by minerals may limit the availability of SOM to decomposition and dampen that response. Conversely, if stabilization decreases with warming, SOM decomposition could accelerate beyond current predictions (Conant et al., 2011). Because this pool of C and N is so large, an understanding of its susceptibility to warming is imperative to the accuracy of climate-carbon models, and to overall understanding of SOM dynamics. A consensus among the scientific community has not yet been reached, with some authors concluding that very old SOM is less sensitive to warming than young SOM (Hopkins et al., 2012; Liski et al., 2000), and others concluding that it is more sensitive to warming than young SOM (Knorr et al., 2005; Leifeld and Fuhrer, 2005). Thus the sensitivity of this pool to warming remains uncertain.

One of the reasons behind the disagreement in the literature is the difficulty of distinguishing between different SOM stabilization mechanisms, which may all contribute

simultaneously to the old age of SOM (von Lützow et al., 2006). Chemical recalcitrance, which refers to a molecule's inherent chemical structure being complex or energetically unfavorable for decomposition, was long thought to be a major mechanism of SOM stabilization. While substrate molecular structure is an important determinant of the initial rate of litter decomposition (Aerts, 1997; Silver and Miya, 2001), it may not be important as a long-term stabilization mechanism (Mambelli et al., 2011; Marschner et al., 2008), with the exception of some pyrogenic SOM (Schmidt et al., 2011; Singh et al., 2012). Rather, there is mounting evidence that physico-chemical stabilization, encompassing both mineral-association and encapsulation within soil aggregates, is the dominant factor contributing to the long-term persistence of SOM (Kleber, 2010; Kleber et al., 2011; Mikutta et al., 2006; Six et al., 2004). Stabilization on mineral surfaces is often confounded with encapsulation by aggregates. Both limit substrate availability to decomposers, and both occur simultaneously (even acting in tandem on the same molecule) in soils. It is virtually impossible to study them independently *in situ*, and as a result their independent temperature sensitivities are not well understood (Conant et al., 2011).

The processes contributing to stabilization and destabilization of SOM by mineral surfaces likely have differing temperature sensitivities (Conant et al., 2011; Thornley and Cannell, 2001), and the balance of these two will ultimately determine whether old SOM stocks decrease with warming. The terms "stabilization" and "destabilization" both encompass myriad processes and reactions, all with potentially differing temperature sensitivities (Conant et al., 2011). Stabilization includes the reactions that form SOM, from fragmentation, to depolymerization and solubilization, to microbial processing (the majority of mineral-associated SOM is microbially processed (Christensen, 2001; Cotrufo et al., 2013; Malik and Gleixner, 2013)), and finally, any adsorption reactions that would preclude SOM from being respired.

Destabilization includes the reversal of those adsorption reactions, making SOM available for decomposition, in addition to the microbial decomposition reactions themselves. Each of the microbially-mediated steps are governed by suites of different enzymes, each with their own individual temperature sensitivities (Hobbs et al., 2013; Schipper et al., 2014). The purpose of this work was not to speculate on the temperature sensitivity of any of those individual contributing reactions, but to better understand the collective processes of stabilization and destabilization on mineral surfaces in response to warming. We included SOM formation as part of stabilization, and so included effects of warming on stabilization efficiency (SE).

A second reason for disagreement among the literature as to the temperature response of very old SOM is that the slow turnover of old SOM makes it difficult to study. Typical incubation experiments last months to years, often not long enough to capture significant changes in rates of cycling of old SOM. Other techniques rely on environmental gradients to study very old SOM, but those come with confounding environmental variables that can mask temperature responses. Here, we investigated the effect of warming using a slurry incubation of silt and clay soil fractions with isotopically (^{13}C and ^{15}N) labeled plant material. This slurry technique has several benefits that make it ideally suited to this type of study. For one, constant shaking in water maximizes physical contact between enzymes and substrates, greatly increasing the rate of litter decomposition and SOM turnover (Wallenstein et al., 2012). It also minimizes the effect of soil aggregation, allowing for the study of organo-mineral stabilization in the relative absence of stabilization via aggregate formation. The major advantage of using ^{13}C- and ^{15}N-labeled plant litter is the ability to distinguish between old SOM that was previously stabilized on silt and clay surfaces (native-derived SOM) and new SOM that forms from the labeled plant material (litter-derived SOM). In this way, we were able to clearly distinguish

between stabilization (litter-derived SOM recovered in the silt or clay), and destabilization (respiration of native-derived SOM). We based this work on the following hypotheses:

(H1) Stabilization of C and N will increase with warming, because more litter will be microbially processed (and more microbial products and byproducts formed) at warmer temperatures.

(H2) Stabilization efficiency (SE) will decrease with warming, due to lower carbon use efficiency by microbes at warmer temperatures (Manzoni et al., 2012).

(H3) Warming will lead to higher rates of destabilization, because the rates of the chemical reactions involved in destabilization will increase with warming, consistent with kinetic theory (Conant et al., 2011).

(H4) The C/N ratio of native-derived SOM will decrease as destabilization increases, because N-rich compounds will be preferentially preserved due to their high affinity for mineral surfaces (Knicker, 2011).

We tested these hypotheses using isolated silt- and clay-sized fractions (2-53 μm and <2 μm, respectively), because they are responsible for the majority of SOM stabilization in most soils (Christensen, 2001; Kögel-Knabner et al., 2008). In previous studies of temperature sensitivity of mineral-associated SOM, silt and clay were often lumped together as one "mineral-associated" fraction (*e.g.* the <20um fraction of Hassink et al. (1997), or the <63 fraction of Leifeld et al. (2005) and Zimmerman et al. (2010)). However, when silt and clay have been separated, the two have exhibited differences in their rates of SOM turnover (Haile-Mariam et al., 2008; Virto et al., 2010), capacities for SOM stabilization (Stewart et al., 2008) and SOM chemistry (Calderón et al., 2011). We separated the two with the expectation that stabilization and destabilization in silt and clay might respond differently to warming.

3.2 Methods

Methods for this experiment followed those presented in Chapter 1, with some exceptions, which are detailed here.

3.2.1 Soil fractions and isotopically labeled plant material and soil fractions

Soil fractions were separated as in Chapter 1. Plant material used in this study was aboveground material only, prepared along with the plant material used in Chapter 1. The C and N contents, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ values of the plant material and soil fractions are shown in Table 1.

3.2.2 Experimental design and initial setup

We incubated silt or clay with leaves at three temperatures in a full factorial design with four replicates. We maintained controls, which included silt or clay that did not receive plant litter, and leaves with no soil, each also with four replicates. Together, the treated samples and control samples (60 total) constituted one set. We incubated two sets, allowing for one destructive harvest partway through the incubation and another at the end. The first harvest was timed to capture the portion of the experiment with the highest respiration rates (the "initial stage", as described in Chapter 1), which varied by temperature (Fig. 3.1). For the second harvest, at day 60, samples at all temperatures were harvested simultaneously.

Slurries consisted of 1 g of either silt or clay, 0.1 g of aboveground plant material, and 20 mls of deionized water in 50 ml conical centrifuge tubes with plug seal caps fitted with rubber septa. On day 0 of the incubation, the slurry components were combined, the tubes were capped (airtight), and all samples were flushed with CO_2-free air for 10 minutes. Samples were then placed on horizontal shakers at 15, 25, or 35 °C and shaken constantly to keep the slurries aerated.

3.2.3 Calculations

The fraction of litter-derived C respired at each CO_2 sampling occasion, and the fractions of litter-derived C and N in SOM were calculated according to the same mixing model as in Chapter 1, using values for samples and controls at the same incubation temperature. Q_{10} was calculated at representative time points throughout the incubation according to the equation:

$$Q_{10} = (k_2/k_1)^{[10/(T_2-T_1)]} \quad (1)$$

Where T_1 and T_2 are the incubation temperatures, and k_1 and k_2 are the corresponding mean rates of C-CO_2 production. SE was calculated in the same manner as in Chapter 1.

3.2.4 Statistical analysis

We tested for effects of soil fraction and incubation temperature on each factor separately for each harvest using two-way ANOVAs with Tukey's post hoc test if one or more factors were significant ($p \leq 0.05$). Three-way ANOVAs including harvest were generally avoided due to the large number of individual contrasts that would have resulted. Any comparisons made in the text regarding differences between harvests were made by comparing 95 % confidence intervals. If the intervals did not overlap, the values were assumed to be different. Q_{10} values were calculated by dividing means, and were not statistically compared. All statistical tests were carried out using the statistical package in R, version 3.0.2 (R Core Team, 2013).

3.3 Results

3.3.1 Slurry respiration

Cumulative respiration was significantly higher at warmer temperatures at both harvests (Table 3.2, Fig. 3.2a). The silt respired more than the clay early on (p = 1.1e-6 for harvest 1), except at the coolest temperature where the two were not significantly different. Later on,

however, the respiration from the clay increased relative to that from the silt (around day 10 at 35 °C, day 20 at 25 °C, and day 30 at 15 °C), due to higher respiration of litter-derived C. At the warmest temperature, the increase in the respiration rate in the clay was high enough that it overtook the silt in terms of cumulative respiration after day 20 (p = 0.001 at harvest 2) (Fig. 3.2a). The majority of C-CO_2 was litter-derived in all treatments, ranging from 86.4 % to 99.7 % of total C-CO_2 (Table 3.2).

Respiration of native-derived and litter-derived C-CO_2 both increased with warming, but respiration of the native-derived C-CO_2 was generally more sensitive to temperature. While the absolute increases in cumulative C-CO_2 were much larger for litter-derived C (Table 3.2), the relative increase in the cumulative C-CO_2 efflux from 15 °C to 35 °C was larger for native-derived C-CO_2 than for litter-derived C-CO_2 (Table 3.2). In addition, the proportion of total C-CO_2 that was native-derived was largest at 35 °C throughout the majority of the experiment (at harvest two, it was 0.14±0.01 in the clay and 0.1±0.01 in the silt at 35 °C, and 0.09±0.01 in the clay and 0.03±0.005 in the silt at 15 °C). Q_{10} was generally higher for native-derived than for litter-derived C, though there were notable exceptions after day 27 (Table 3.3), which suggest that the higher temperature sensitivity of native-derived respiration was a short-term phenomenon.

3.3.2 Stabilization of litter-derived C and N

Even though more litter-derived C was processed at warmer temperatures, less was stabilized as SOM. In the short term, stabilization of litter-derived C was lowest at the warmest temperature in both silt and clay (harvest 1, Fig. 3.3a). Short-term N stabilization was higher at 25 °C than at the other temperatures (p = 0.025), and there was no significant interaction between temperature and soil fraction. On average, the clay stabilized more N than the silt, but it

was by a very small margin (+0.03 mg per g soil, $p = 0.01$), and the C/N ratio of the stabilized litter-derived SOM was higher on average in the clay than in the silt (Table 3.2).

The SE decreased with temperature in both silt and clay, meaning that for a given amount of litter-derived C stabilized as SOM, more litter-derived C-CO_2 was respired (Fig. 3.3b). On average, the clay stabilized significantly more litter-derived C than the silt, and had significantly higher SE. However, the silt accrued more litter-derived C relative to the amount of C it began with. Over the course of just 6-9 days (harvest times varied by temperature), the clay increased from 1.31±0.005 %C to 1.48±0.04 %C (a 13 % increase), and the silt increased from 0.84±.03 %C to 1.09±0.04 %C (a 30 % increase).

Stabilization of litter-derived C was higher at harvest 2 than at harvest 1 (by comparison of 95 % CI), indicating that stabilization continued over the duration of the experiment. However, there was no clear temperature effect, as there was early on, and there was a significant interaction of temperature and soil fraction ($p = 6.72e-7$). The silt and clay stabilized comparable amounts of litter-derived C by day 60, with the exception of significantly higher stabilization by the clay at 35 °C (Table 3.2, Fig. 3.3a). This corresponded to total carbon concentrations of 1.54±0.03 %C in the silt (averaged across temperatures), and 1.76±0.041 %C in the clay (averaged over the 15 °C and 25 °C treatments). The stabilization of litter-derived N increased with warming (ANOVA main effect, $p = 3.1e-09$), and was higher in the clay than in the silt (ANOVA main effect, $p = 0.0001$) at day 60.

Though the clay stabilized more litter-derived C at the warmest temperature, it was relatively inefficient compared to the stabilization at 15 °C. In both silt and clay, SEs were highest at the coolest temperature (Fig. 3.3b). Interestingly, SE toward the end of the experiment did not decrease linearly with warming. This was due to the fact that between harvest one and

harvest two, the stabilization efficiencies at 35 °C increased significantly, while they remained the same (silt) or decreased (clay) at the other two temperatures. This also resulted in a smaller range in SE values across treatments at harvest two (0.30-0.45) than at harvest one (0.23-0.49). The clay had a higher SE than the silt on average, but the difference was much smaller than it was at harvest one, and the silt at 15 °C had the highest SE overall.

3.3.3 Destabilization of native-derived C

Respiration of native-derived C-CO_2, which can be equated to destabilization of native-derived C, was generally higher at warmer temperatures throughout the experiment. This is most clearly demonstrated when native-derived C-CO_2 is normalized to the amount of soil C originally present in each soil fraction (Fig. 3.4). As previously noted, respiration of native-derived C-CO_2 was generally more sensitive to temperature than that of litter-derived C-CO_2. In addition, the respiration of native-derived C-CO_2 from the silt was generally more sensitive to temperature than it was from the clay (Table 3.3). However, in both soil fractions, Q_{10} decreased toward the end of the incubation, indicating a decrease in temperature sensitivity of destabilization over time.

Destabilization was higher in the clay than in the silt early on (p = 6.95e-5 at harvest 1), but the two were not significantly different by day 60 (Fig. 3.4a, b). The cumulative respiration curves of clay followed the more traditional trajectory of a steep increase in the first 6-10 days, followed by a gradual tapering off, indicating decreases in the rates of destabilization after day 10. The destabilization rate at 35 °C remained higher than the rates at 25 °C and 15 °C, but decreased over time, and by day 60 the rates were similar at all three temperatures. In the silt, the pattern was a period of low respiration of native-derived CO_2 followed by an increase, then a period of near-constant rate, and finally a decline. The increase happened almost immediately at

35 °C, but did not happen until day 18 at 25 °C and day 30 at 15 °C. (The rates dropped off at 35 °C and 25 °C, but had only just begun to drop off at 15 °C by day 60. Based on the shape of the respiration curves, it appeared as though the pattern would be the same at 15 °C as it was for the other temperatures, but the incubation was not long enough to confirm this.) In summary, the rate of destabilization began high, then decreased over time in the clay, but began low, then increased and remained fairly constant in the silt before decreasing again.

3.4 Discussion

3.4.1 Warming and SOM stabilization

Early on in the incubation, more litter-derived C was processed at warmer temperatures, but less of that processed litter was stabilized in SOM. This was inconsistent with H1, as we expected to observe more stabilization at warmer temperatures. Stabilization of N was also inconsistent with H1, as there was no trend with temperature. At harvest two, there was no effect of temperature on stabilization of litter-derived C in the silt, which could indicate that it was near saturation. Though the total C content of the silt at harvest two was well below estimates of C saturation for silt fractions (Stewart et al., 2008), the artificial conditions in the slurries (most notably the decrease in physical separation of enzymes from their substrates) may have led to a lower saturation maximum. In the clay at harvest two, there was a trend of increasing stabilization with warming, though the only significant difference was at 35 °C. This higher stabilization was likely due to higher inputs of microbial byproducts, as much more litter was processed in the clay at 35 °C than in any other treatment, consistent with H1.

Regardless of the amount of litter-derived C that was stabilized, stabilization at warmer temperatures was generally less efficient, consistent with H2. Even in the one case when more litter-derived C was stabilized with warming (in the clay at harvest two) it came at a cost of

higher CO_2 production per unit of litter processed. The pattern of lower SE with warming observed in this study may be due to lower microbial carbon use efficiencies (CUE) at warmer temperatures, which has been demonstrated repeatedly in aquatic systems (see references in Manzoni et al. (2012)) and to some extent in soils (Frey et al., 2013). As temperature increases, microbes respire more CO_2 to produce the same amount of microbial biomass (Manzoni et al., 2012). Stabilization of SOM depends in large part on the production of microbial biomass and byproducts (Christensen, 2001; Cotrufo et al., 2013; Knicker, 2011), and if microbial CUE declines, so too should SE.

At harvest one, there was a clear, nearly linear decline in SE with temperature, but the relationship was no longer linear at harvest two. The significant increase in SE between harvest one and harvest two at 35 °C in both the silt and the clay may reflect a change in CUE over the course of litter decomposition. Frey et al. (2013) observed different responses of microbial efficiency to temperature for substrates of varying complexity, suggesting that microbial efficiency may respond differently to litter at different stages of decomposition. Since the litter at 35 °C was further decomposed than it was at the other temperatures, the compounds being utilized by the microbes may have been different, and that might explain the increase in SE at 35 °C by day 60. However, available theories on CUE as it relates to litter quality would predict the opposite response, that the CUE should decrease as substrate quality declines during the course of decomposition (Cotrufo et al., 2013). An alternate explanation is that the microbial communities at 35 °C began to acclimate to the temperature conditions by harvest two, increasing the efficiency with which they decomposed available substrates (Bradford et al., 2008; Frey et al., 2013), but this would not explain the decreases in SE at the other temperatures over time.

3.4.2 Warming and SOM destabilization

Consistent with H3, destabilization increased with warming in both silt and clay. Based on the comparisons of Q_{10} as well as cumulative respiration of C-CO_2 between samples incubated at the different temperatures, respiration of native-derived C-CO_2 was more sensitive to warming than respiration of litter-derived C-CO_2 for the majority of the incubation. As the litter-derived C (that which was free in solution rather than stabilized by the silt or clay) was more readily available than the native-derived C, warming had a greater impact on availability of native-derived C, in this case making it more easily accessible to decomposition.

We expected that higher rates of destabilization would correspond to lower C/N ratios (H4), under the assumption that N-containing compounds would be preferentially preserved due to strong mineral interactions (Kleber et al., 2007; Knicker, 2011), but C/N ratios and destabilization were not correlated. Our method for measuring N concentrations was somewhat coarse; many of the measured concentrations were near the detection limit of our instrument, and some of the variation in N concentrations may have been too small to capture on the elemental analyzer. As such, we do not rule out the possibility of H4, and we suggest that future work employ a more sensitive N measurement technique.

Interestingly, the effect of temperature on destabilization changed over time. Destabilization in both silt and clay followed a general pattern of a period of high temperature sensitivity of destabilization, followed by a tapering off. This suggests that some portion of mineral-stabilized SOM (between 15-30% in this case) is sensitive to warming, while the remainder may be relatively insensitive. These findings agree with those of Hopkins et al. (2012), who saw an increase in the respiration of intermediate-aged (7-13 yr) C with warming, but no effect of warming on respiration of much older C in an incubation using ^{13}C and ^{14}C

tracing techniques.

The presence of a fraction of mineral-associated C that is more accessible and therefore sensitive to temperature may be explained by applying the ideas of Conant et al. (2011) to the model of organo-mineral assemblages put forth by Kleber et al. (2007). Kleber et al. suggest a zonal structure of assemblages, with an outer layer of organic-organic associations that are relatively exchangeable compared to the inner layers where organics interact directly with mineral surfaces. The outer layer, termed the "kinetic zone", contains SOM that is retained by cation bridging, hydrogen bonding, and other reversible binding mechanisms that should be highly sensitive to temperature based on their activation energies (Conant et al., 2011). On the other hand, the inner layer, termed the "contact zone", contains SOM that is bound via very strong linkages such as ligand exchange, which may be insensitive to temperature, or may actually increase with warming (Conant et al., 2011).

3.4.3 Differences between silt and clay

One interesting difference between the silt and the clay was in the respiration of litter-derived C-CO_2 in these two treatments. The rates of respiration in the clay increased noticeably partway through the incubation, whereas they remained more constant in the silt. This may have been due to differences in microbial growth between the fractions. Due to the mixing during sonication and fractionation, the samples may have started the incubation with similar levels of microbial biomass. But clay tends to have higher microbial biomass in natural soils, suggesting it provides a better habitat for soil microbes than silt (Kögel-Knabner et al., 2008). As such, microbial biomass may have grown faster in the clay, leading to increases in respiration of litter-derived C-CO_2.

The two fractions also differed in their stabilization efficiencies. On average, SE was

higher in the clay than in the silt. As clay has a higher capacity for stabilization than silt (Christensen, 2001; Stewart et al., 2009), this confirmed our expectations that it would stabilize higher proportions of processed litter. By harvest two, temperature had less of an effect on SE in the clay than it did in the silt, which may indicate the formation of more stable organo-mineral assemblages, as the clay had higher surface area and therefore more opportunity for very strong "contact zone" interactions (Kleber et al., 2007).

Finally, the dynamics of destabilization were different between the silt and the clay. One striking contrast was the high rate of respiration of native-derived $C\text{-}CO_2$ early on in the clay, which was not observed in the silt. There are many potential explanations for this observation, including differences in microbial biomass or community structure, or differences in stabilization mechanisms of native SOM between the two fractions. However, in this case the pattern we observed in the clay during the first ten days may have been a byproduct of our fractionation scheme. Sonication of the <53 μm fraction, which dispersed aggregates and likely released unprotected SOM into solution, was followed by centrifugation and aspiration of the supernatant to obtain the clay fraction. This means that any SOM that was free in solution after sonication was aspirated along with the clay, which would explain the flush of native-derived $C\text{-}CO_2$ early on in the clay. Since respiration rates were much lower at 25 °C and 15 °C, there was more time for this free SOM to re-adsorb to the clay surfaces, which would explain the smaller initial flush of native-derived $C\text{-}CO_2$ at those temperatures. The reason that the initial flush was not observed in the controls, which would have had the same amount of free, native-derived SOM in solution, could have been that the microbial biomass differed between the controls and the samples with added plant litter. The soils were not inoculated, and the samples that received plant litter also received the microbial community that came along with it.

After the initial flush, the destabilization rates followed the same general pattern in the silt and the clay, as previously discussed, but the timing differed. Both fractions exhibited an increase in destabilization rate, followed by a decrease much later on. In the clay, the increase and tapering off occurred simultaneously at all three temperatures, but the magnitudes of the rates increased with warming. In the silt, there was a lag in respiration of native-derived C, the duration of with decreased with warming. Once destabilization began at the respective temperatures, the magnitude of the rates increased with warming as they did in the clay. Regardless of the timing, the silt and clay each lost similar proportions of their native-derived C at the respective temperatures by the end of the incubation, suggesting that there were similar stabilization mechanisms at play in the two soil fractions. The clay had a larger pool of C per g soil remaining after 60 days than did the silt, which corresponds to its higher surface area and higher capacity for stabilization via organo-mineral associations. But both soil fractions contained pools of temperature sensitive C that were respired upon warming.

3.4.4 Conclusions and implications

In general, our results showed mixed support for our hypotheses, indicating that the responses of stabilization and destabilization were complex. We expected more C and N to be stabilized at warmer temperatures, which was true for N at harvest 2, but was only true for C in the clay at 35 °C at harvest 2. Our expectation of lower SE with warming (H2) was supported at harvest 1, but was not fully supported at harvest 2. SE correlated to the amount of C stabilized in come cases, but not in others. This suggests that the amount of C stabilized was driven in part by total processing, in part by SE, and in part by other factors. There was good support for H3, that destabilization would increase with warming. The destabilization dynamics between the silt and the clay differed, which may have been attributable to our fractionation scheme. Finally, we did

not find support for H4, that the C/N ratio of native-derived SOM would decrease as destabilization progressed. This result may have resulted from our measurement method, or it may be that N-rich compounds are equally likely to be destabilized as non-N-rich compounds. Future work should address N destabilization dynamics using a method that is more appropriate for capturing very small differences in N concentrations.

Overall, our results show that mineral-associated C and N is sensitive to temperature. Destabilization may increase with warming, though there appears to be a pool of mineral-associated C and N that is strongly bound, and relatively insensitive to temperature changes. Stabilization is also sensitive to temperature, though the effect is complex. The higher temperature sensitivity of destabilization suggests that the mineral-stabilized pool may shrink with warming, but, ultimately, the response of the mineral-stabilized pool to warming will depend on the balance of stabilization and destabilization, and will depend on OM inputs. In our experiment, the pool of native-derived C was small compared to the litter-derived C pool, which undermined the impact of its relatively high temperature sensitivity. We added plant litter at a much higher rate than what it would receive naturally (48.78 mg C g^{-1} soil, about 4-5 times the amount of native C) because we wanted to ensure measurable rates of stabilization over the short-term incubation. In most soils, the mineral-associated pool of SOM is much larger than the annual input of OM, and our results suggest that a portion of mineral-associated C is highly sensitive to temperature and is readily susceptible to decomposition upon warming. In that case, warming could cause a noticeable loss of C from mineral soils unless it is counteracted by a corresponding increase in OM inputs and subsequent stabilization.

Table 3.1. Initial characteristics of soil fractions and plant material. Data are means ± one standard error (n=4).

	C (mg g^{-1})	N (mg g^{-1})	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
silt	8.39 ± 0.28	0.83 ± 0.05	10.22 ± 0.31	-18.82 ± 0.46	3.27 ± 0.48
clay	13.13 ± 0.05	1.80 ± 0.00	7.29 ± 0.03	-18.58 ± 0.04	9.07 ± 0.09
plant material	487.80 ± 4.22	18.20 ± 0.16	26.80 ± 0.15	3420.40 ± 2.71	18016.80 ± 63.60

Table 3.2. C budgets and C/N ratios by harvest, soil fraction, and temperature. Data are presented on a total basis (all CO_2 or all SOM), and split into litter-derived and native-derived values (means ± one standard error, n=4). Values in the same row with different letters were significantly different (ANOVA, $p \leq 0.05$).

		Harvest 1					
		Silt			Clay		
		15	25	35	15	25	35
C-CO_2 (mg/g soil)	total	3.874 ± 0.059 a	5.37 ± 0.06 c	7.66 ± 0.07 d	3.94 ± 0.03 a	4.59 ± 0.02 b	7.46 ± 0.08 d
	litter-derived	3.869 ± 0.060 a	5.21 ± 0.05 c	7.58 ± 0.07 e	3.67 ± 0.03 a	4.41 ± 0.02 b	6.66 ± 0.07 d
	native-derived	0.005 ± 0.002 a	0.16 ± 0.05 ab	0.08 ± 0.01 ab	0.28 ± 0.01 b	0.18 ± 0.003 ab	0.79 ± 0.01 c
C-SOM (mg/g soil)	total	11.70 ± 0.09 b	11.85 ± 0.20 b	9.17 ± 0.18 a	14.94 ± 0.37 cd	15.79 ± 0.14 d	13.65 ± 0.24 bc
	litter-derived	2.83 ± 0.06 ab	2.89 ± 0.02 ab	2.26 ± 0.07 a	3.49 ± 0.11 bc	3.59 ± 0.06 c	3.15 ± 0.09 bc
	native-derived	8.87 ± 0.07 b	8.96 ± 0.19 b	6.91 ± 0.15 a	11.46 ± 0.27 cd	12.21 ± 0.09 d	10.50 ± 0.16 c
C/N of SOM	total	9.18 ± 0.06 a	9.12 ± 0.09 a	9.42 ± 0.06 a	7.11 ± 0.06 b	6.87 ± 0.05 b	6.84 ± 0.13 b
	litter-derived	9.08 ± 0.08 b	7.69 ± 0.04 a	7.78 ± 0.09 a	10.40 ± 0.15 c	9.38 ± 0.10 bc	8.76 ± 0.23 ab
	native-derived	9.22 ± 0.06 b	9.70 ± 0.11 bc	10.11 ± 0.07 c	6.49 ± 0.06 a	6.38 ± 0.06 a	6.42 ± 0.11 a
		Harvest 2					
		Silt			Clay		
		15	25	35	15	25	35
C-CO_2 (mg/g soil)	total	8.66 ± 0.14 a	13.94 ± 0.06 bc	15.16 ± 0.12 c	9.33 ± 0.06 a	13.76 ± 0.13 b	18.44 ± 0.28 d
	litter-derived	8.36 ± 0.13 a	13.12 ± 0.07 b	13.23 ± 0.13 b	8.30 ± 0.04 a	12.71 ± 0.10 b	16.16 ± 0.24 c
	native-derived	0.30 ± 0.02 a	0.82 ± 0.07 b	1.93 ± 0.06 c	1.03 ± 0.02 b	1.05 ± 0.06 b	2.28 ± 0.06 c
C-SOM (mg/g soil)	total	16.10 ± 0.23 ab	14.97 ± 0.23 a	15.22 ± 0.22 a	17.44 ± 0.27 b	17.85 ± 0.13 b	21.20 ± 0.33 c
	litter-derived	6.78 ± 0.16 a	5.61 ± 0.07 a	5.97 ± 0.11 a	5.64 ± 0.21 a	6.64 ± 0.11 a	9.35 ± 0.11 b
	native-derived	9.32 ± 0.15 a	9.37 ± 0.19 a	9.26 ± 0.31 a	11.81 ± 0.14 b	11.18 ± 0.07 b	11.85 ± 0.22 b
C/N of SOM	total	10.39 ± 0.10 e	7.59 ± 0.05 c	8.34 ± 0.04 d	6.84 ± 0.04 b	6.27 ± 0.07 a	6.74 ± 0.05 ab
	litter-derived	12.05 ± 0.20 d	6.49 ± 0.05 a	7.50 ± 0.13 ab	9.15 ± 0.11 c	7.86 ± 0.14 b	8.16 ± 0.08 bc
	native-derived	9.45 ± 0.07 d	8.45 ± 0.06 c	8.99 ± 0.06 d	6.10 ± 0.02 b	5.59 ± 0.05 a	5.93 ± 0.03 ab

Table 3.3. Q_{10} of respiration of litter-derived and native-derived C-CO_2, calculated for silt and clay at 25 °C and 35 °C at representative time points throughout the incubation. Q_{10} values for silt at 25 °C could not be calculated until after day 27, because the silt at 15 °C did not begin respiring native-derived C-CO_2 until then. Q_{10} values were calculated by dividing means, and as such we did not provide errors on the values.

	Q_{10}			
	Silt		Clay	
	15-25°C	25-35°C	15-25°C	25-35°C
Day 10				
litter-derived	1.00	0.77	0.98	1.01
native-derived	∞	5.59	1.82	1.41
Day 18				
litter-derived	1.41	0.70	1.12	1.83
native-derived	∞	13.89	1.41	2.17
Day 27				
litter-derived	1.84	0.56	1.91	1.09
native-derived	∞	2.50	1.67	1.60
Day 58				
litter-derived	2.05	0.88	1.71	0.91
native-derived	0.85	2.28	0.57	1.38

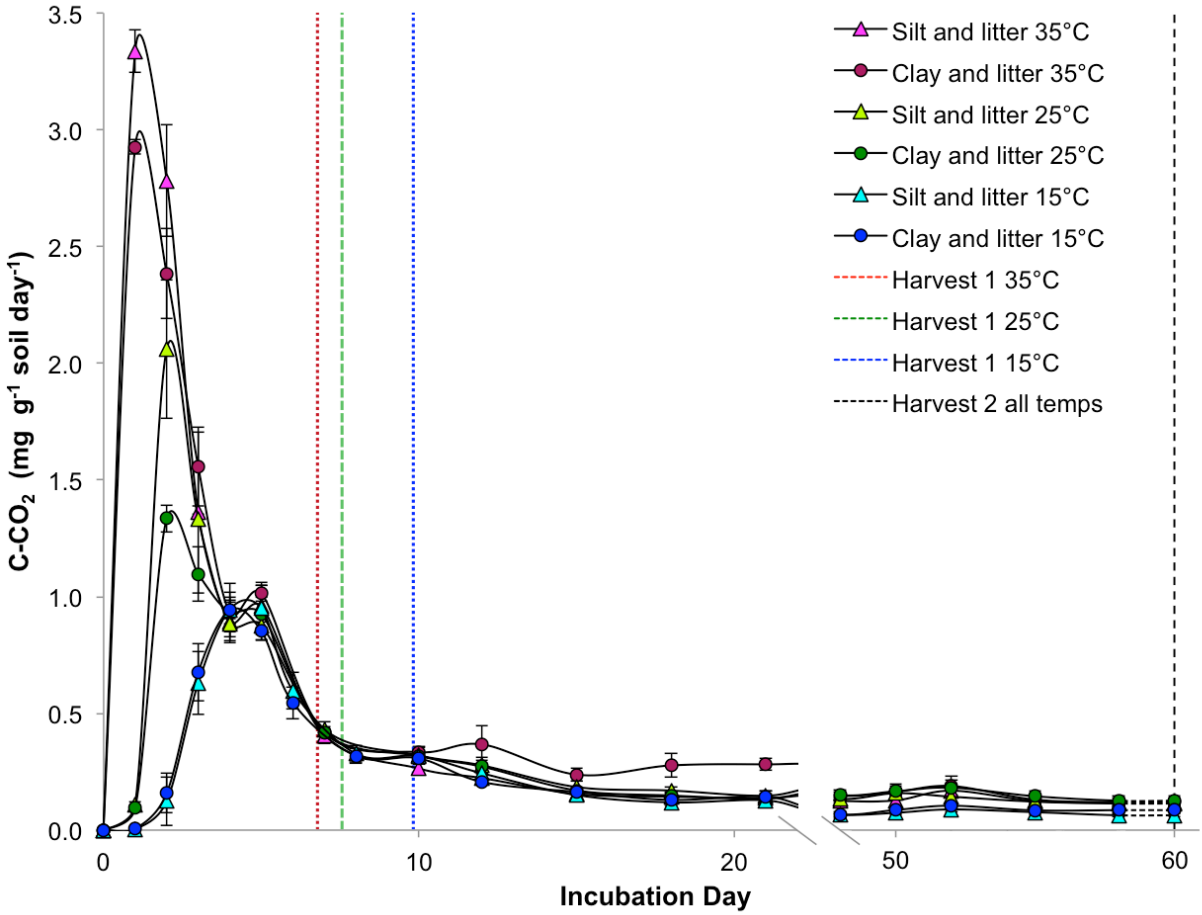

Figure 3.1. Respiration rates over the duration of the experiment. Vertical dotted lines show the times of each harvest. Points at day 60 were extrapolated from points at day 58, shown by dotted lines between points. The x-axis was broken between days 22 and 48 for clarity. Points are mean values ± one standard error (n=4).

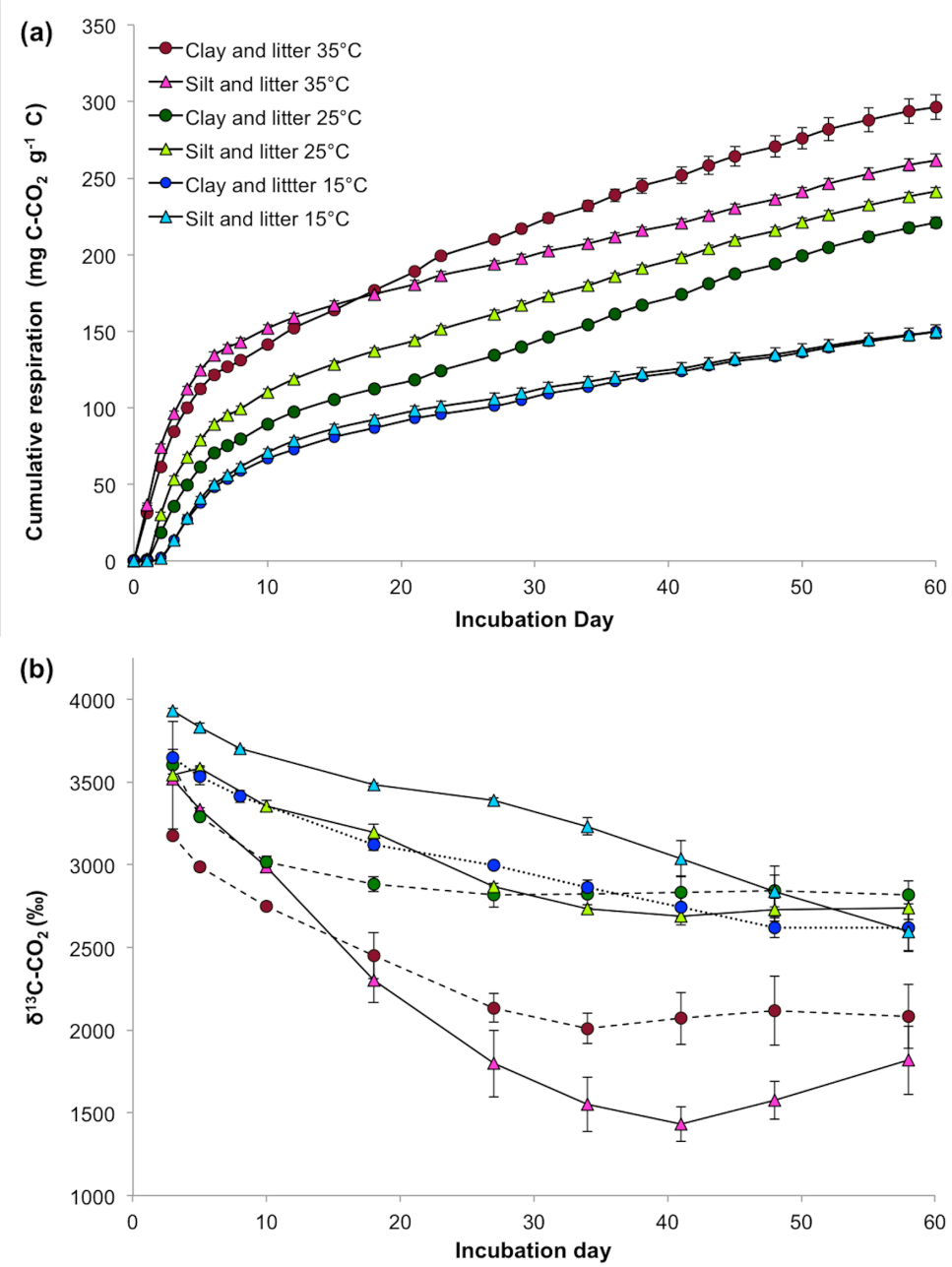

Figure 3.2. Cumulative respiration of C as CO_2 (or C-CO_2 efflux) (panel a), and $\delta^{13}C$ of C in CO_2 (panel b) throughout the experiment. Lines in panel b are the linear extrapolations used to estimate the $\delta^{13}C$ of C in CO_2 between sampling times. Averages of $\delta^{13}C$ of C in CO_2 for plant material controls over the 60 days were 3525.7, 3286.5, and 2863.2 at 15°C, 25°C, and 35°C, respectively. Points are means ± one standard error (n=4).

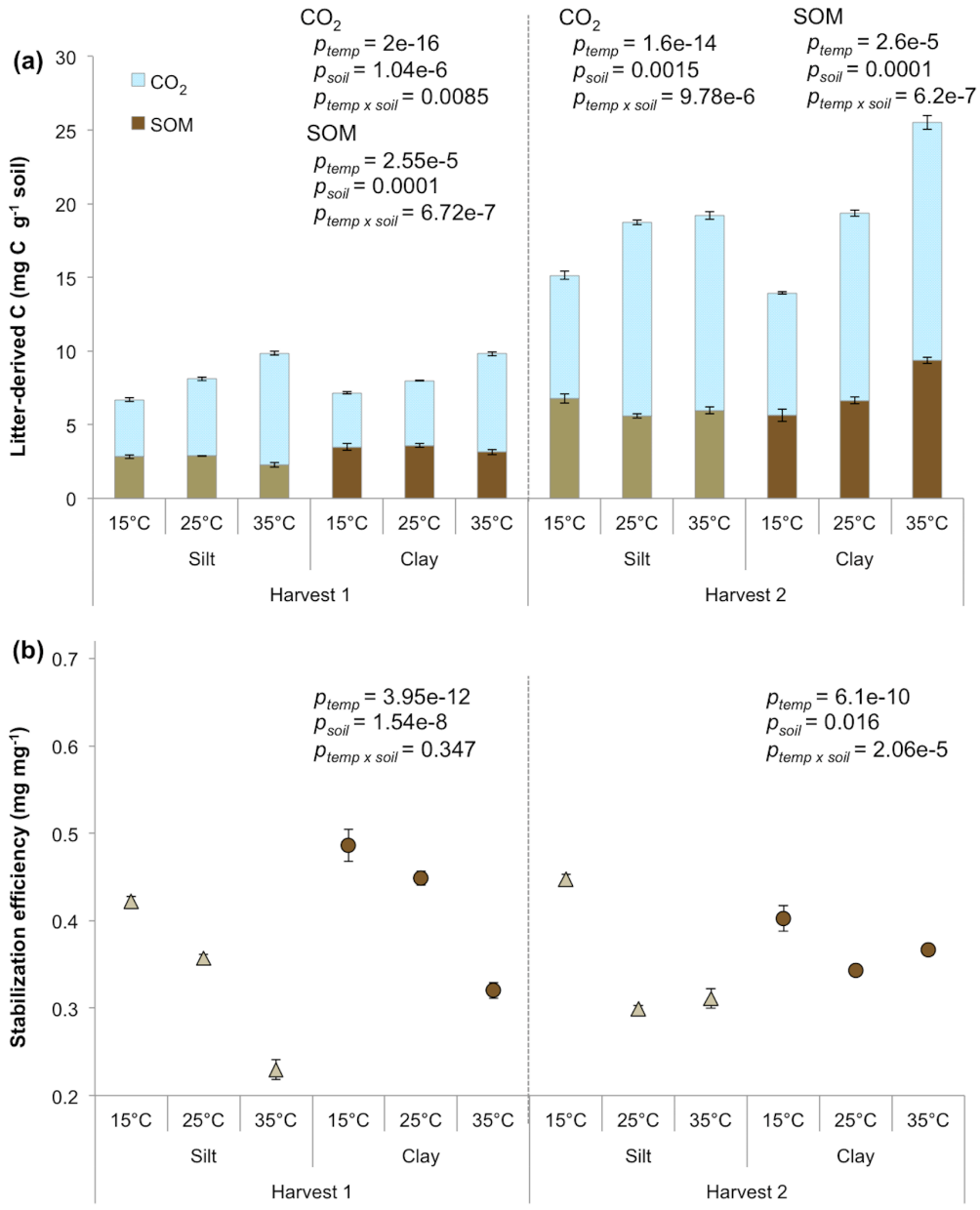

Figure 3.3. Allocation of litter-derived C to CO_2 and SOM (panel a), and stabilization efficiency of litter-derived C (panel b), by harvest, soil, and temperature. In panel a, the total height of each bar represents the amount of litter processed. *p* values for individual contrasts of values in panel a are shown in Table 2. Values are means ± one standard error (n=4), and *p* values are results of two-way ANOVAs.

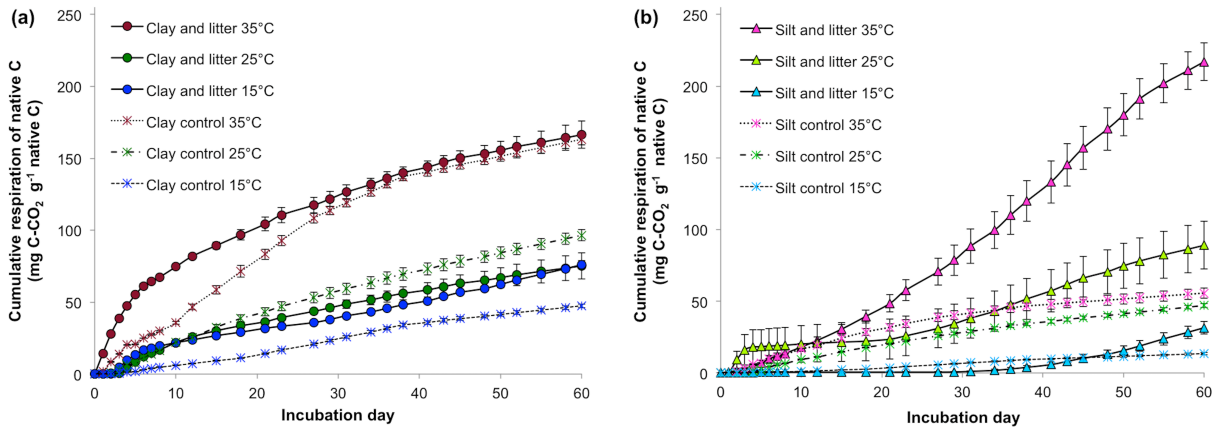

Figure 3.4. Respiration of native-derived C-CO_2 normalized to the amount of native C present in each soil fraction at the start of the experiment. Clay is shown in panel a, and silt is shown in panel b. Controls, which did not receive any plant litter, are included for comparison. Points are means ± one standard error (n=4).

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CHAPTER 4: EFFECT OF WARMING ON CARBON STOCKS IN SOIL ORGANIC MATTER FRACTIONS OF TWO MULTI-FACTOR FIELD CLIMATE CHANGE EXPERIMENTS

4.1 Introduction

As our climate warms, mineralization of soil organic matter (SOM) to carbon dioxide (CO_2) and other greenhouse gases is expected to increase, forming a large positive feedback to climate change. But this can not take place if SOM availability limits microbial decomposition (Davidson and Janssens, 2006). There is ample evidence that SOM that is readily available to microbes decomposes more quickly at warmer temperatures (see studies reviewed by Conant et al. (2011) and von Lützow & Kögel-Knabner (2009)), but the question remains as to whether decomposition of SOM that is not readily available, and therefore cycles more slowly, is sensitive to temperature. In many ecosystems, the stock of slow-cycling SOM is very large – often several times larger than the stock of fast-cycling SOM – and so determining its temperature sensitivity is key to improving the accuracy of our climate-carbon models as well as our general understanding of SOM dynamics (Davidson and Janssens, 2006). Recent thinking suggests that the processes that render SOM unavailable to decomposition (various organomineral interactions and aggregation, together referred to as "physico-chemical protection") are themselves sensitive to temperature, and as a result, warming may increase the vulnerability of large stocks of slow-cycling C to decomposition (Conant et al., 2011; Davidson and Janssens, 2006).

Researchers have been studying the effect of warming on slow-cycling C for decades, and results are mixed (Hakkenberg et al., 2008; Hopkins et al., 2012; Leifeld and Fuhrer, 2005;

Liski et al., 1999; e.g. Trumbore et al., 1996). Part of the problem is that when testing the effect of temperature in the field, confounding factors often prevent clear interpretation of the results. In studies employing elevation gradients, where location along a slope is used as a proxy for temperature, additional variables including moisture, mineralogy, and plant assemblages and production (which control organic inputs to the soil) tend to change with elevation. Soil warming experiments in the field offer a solution to this problem. These experiments manipulate temperature, moisture, and other variables in a controlled fashion within a confined area where mineralogy and – to some extent – plant assemblages are similar across plots. Soil warming experiments have weaknesses of their own, and so their results can also be difficult to interpret. Several studies using field warming experiments have found no effect of warming on total soil C stocks (Belay-Tedla et al., 2009; Garten et al., 2009; Song et al., 2012; Xu et al., 2012; Zhou et al., 2013). These results alone do not necessarily support the conclusion that SOM is insensitive to temperature. If experimental error and confounding factors are minimized, there remain several potential explanations for observing no significant change in total C stocks with warming. The most common arguments found in the literature are:

(1) Changes in inputs confound measurement of the changes in losses. If inputs to SOM also increase with warming, they will effectively compensate for any increased losses with warming, and the net change will be zero (Kirschbaum, 2000). In this case, SOM is turning over faster, but this cannot be detected if we only measure total C stocks.

(2) Responses of different SOM pools may mask one another. SOM represents a myriad of compounds, all with differing degrees of decomposability and accessibility to microbial decomposition. The degree of decomposability may be the main determinant of SOM's inherent temperature sensitivity (Davidson and Janssens, 2006). But the

accessibility of SOM to microbes has gained momentum in the literature as the main determinant of the *apparent* temperature sensitivity of SOM decomposition – that is, the temperature sensitivity that we observe in experiments (Conant et al., 2011; Davidson and Janssens, 2006; Dungait et al., 2012; von Lützow and Kögel-Knabner, 2009). Indeed, when authors who saw no effect of warming on total soil C stocks separated the SOM into different pools, they generally saw changes in some pools and not others (Belay-Tedla et al., 2009; Garten et al., 2009; Song et al., 2012; Xu et al., 2012; Zhou et al., 2013).

(3) The change in C stocks is too small to resolve. The slow turnover of SOM, especially that which is physico-chemically protected, makes it difficult to observe significant changes in C stocks on the timescales of most experiments. Estimates of SOM turnover times in the literature range from years to decades for relatively accessible SOM, to decades to millennia for physico-chemically protected SOM (Baisden et al., 2002; Bol et al., 2009; Kögel-Knabner et al., 2008; Liao et al., 2006). The vast majority of warming manipulation studies last under a decade. Even if warming did increase the rate of turnover of SOM, it's very unlikely that one would observe the resulting changes in C stocks in that amount of time.

We investigated the effect of warming on SOM in two multi-factor climate change experiments, and designed our analysis to overcome these potential pitfalls. The experiments we chose – the Old-field Community Climate and Atmospheric Manipulation in Oak Ridge, Tennessee and the Jasper Ridge Global Climate Change Experiment in Jasper Ridge, California – combined warming with elevated CO_2, which is depleted in ^{13}C relative to ambient air. We used the signature of the CO_2 added to elevated CO_2 treatments to differentiate between C that

was fixed after the treatments began ("new C") and C that was fixed before the treatments began ("old C") (Hungate et al., 1995). This allowed us to partition the effects of warming on losses of old C versus inputs of new C to different pools of SOM to overcome pitfall (1). SOM fractionation is broadly used to separate SOM into pools with different physical or chemical characteristics, which correlate to its rate of turnover. While SOM fractionations have been used previously in soil warming studies (Belay-Tedla et al., 2009; Xu et al., 2012; Zhou et al., 2013), and even on the same experimental plots (Garten et al., 2009), our particular fractionation procedure has not. Our procedure focuses on physical soil characteristics (particle size and density), with the aim of isolating SOM fractions of increasing physiochemical protection and turnover times to overcome pitfall (2) above. Pitfall (3) is difficult to avoid, as it is caused by the nature of the experiments themselves. However we attempted to address it by sampling the soils after the treatments had been in effect longer than compared to earlier studies on the same sites (five years compared to four (Garten et al., 2009) at Oak Ridge; 12 years compared to two (Shaw et al., 2002) or five (Dukes et al., 2005) at Jasper Ridge).

4.2 Methods

4.2.1 Field Sites and Experimental Designs

In 2002, 12 open-top chambers were established near Oak Ridge, Tennessee (hereinafter referred to as Oak Ridge). Vegetation was killed with glyphosate and then reestablished with plantings of seven common old-field community plant species (Wan et al., 2007). Warming and elevated CO_2 treatments, administered by fans, began in May 2003. A drought treatment was also administered, but we did not use soils from the drought treatment for this study (Garten et al., 2009). Soils were collected from Oak Ridge in July 2008, after treatments had been in effect for just over 5 years.

In the late 1990s, the Jasper Ridge Global Climate Change Experiment was established in Jasper Ridge, California (hereinafter referred to as Jasper Ridge). The experimental area is on an annual grassland on sandstone-derived soil, and experiences a typical Mediterranean climate. The study consists of 32 plots surrounded by infrared heaters and free-air CO_2 emitters (Henry et al., 2005). Treatments were initiated at the start of the 1998-1999 growing season, and included elevated CO_2 and warming, in addition to several other factors that were not used for this study. Soils from Jasper Ridge were collected in May 2010, after treatments had been in effect for 12 years. Additional information about both sites and experimental setups are given in Table 4.1, and more detailed descriptions of Oak Ridge and Jasper Ridge can be found in Wan et al. (2007) and Dukes et al. (2005), respectively.

Both experiments tested the effects of elevated CO_2 and warming alone and in combination. Each treatment had two levels (ambient or elevated) administered at the plot-level in full-factorial designs with replicate blocks. This resulted in four treatment combinations: ambient CO_2 and ambient temperature (ACAT), ambient CO_2 and elevated temperature (ACET), elevated CO_2 and ambient temperature (ECET), and elevated CO_2 and elevated temperature (ECET). Plots at both sites were then split to test additional factors such as moisture and nitrogen application, but the soils used in this study were from subplots where all other variables were maintained at ambient levels. Relevant data on soil respiration, aboveground plant production, and soil moisture at these sites, collected during previous studies, are shown in Table 4.2. At Oak Ridge, the average whole soil $\delta^{13}C$ value was -24.16±0.34 ‰ in ambient CO_2 plots, and -29.17±0.87 in elevated CO_2 plots. At Jasper ridge, the average whole soil $\delta^{13}C$ value was -27.34±0.07 ‰ in ambient CO_2 plots and -32.04±0.37 ‰ in elevated CO_2 plots.

We chose these two sites because they are both grasslands, but they have contrasting

moisture regimes and rates of plant production, and they were established on very different soils (Table 4.1). In addition, Jasper Ridge is relatively undisturbed, while the plots at Oak Ridge were cleared and replanted immediately before the experiment began. We aimed to test whether the effects of warming on SOM dynamics would be similar at the two sites, given their inherent differences.

4.2.2 Soil sampling and preparation

Oak Ridge soils were collected in July 2008. Three cores (5 cm diameter to 15 cm depth) were taken per plot and combined, and stored moist at 4°C prior to processing. Subsamples were sieved to 2 mm and air dried in 2010, and stored at room temperature until fractionation. Jasper Ridge soils were collected in May 2010. Two cores (7/8 inch diameter to 15 cm depth) were collected per plot and combined. These were sieved to 2 mm and air-dried within six days of sampling, and stored at room temperature. Due to quarantine precautions, the Jasper Ridge soils were heated to 110 °C for 18 hours prior to fractionation. There were no detectable carbonates in the soils at either site.

4.2.3 Soil fractionation and analysis

Soils were separated into fractions using a procedure modified from (Denef et al., 2013) and presented by Soong and Cotrufo (2014), shown in Fig. 4.1. The purpose of the fractionation is to isolate SOM fractions with varying turnover times, according to the principle that increasing physico-chemical protection of SOM will cause a longer turnover time. Briefly, soils were dispersed by shaking overnight with glass beads in sodium polytungstate (SPT) at a density of 1.85 g/cm^3. The light fraction (LF, <1.85 g/cm^3) was separated by centrifugation and aspirated over a 45 μm filter. The heavy fraction was then rinsed to remove any remaining SPT. Each rinse entailed filling centrifuge tubes with deionized water, shaking to disperse the pellet, centrifuging,

and removing the supernatant. The heavy fractions were further separated by rinsing over a 53 μm sieve to obtain the sand and particulate organic matter, which we refer to as the sand-sized fraction. The soil that passed through the sieve was then centrifuged according to Stocke's Law to separate the silt-sized (2 μm – 53 μm) from the clay-sized material (<2 μm). We did not deflocculate or use aggressive disruption, and so there were very likely microaggregates in both the silt-sized and clay-sized fractions (Chenu and Plante, 2006; Virto et al., 2008). Hence, we use the terms "silt-sized" and "clay-sized" rather than "silt" and "clay". All soil fractions were oven-dried at 60°C and ground, then analyzed them for % C, % N, $\delta^{13}C$ and $\delta^{15}N$ on an elemental analyzer coupled to an IRMS (EA-IRMS, Carlo Erba NA 1500 elemental analyzer and VG Isochrom continuous flow IRMS, Isoprime Inc., Manchester, UK). Percent recovery of whole soil C and whole soil mass after fractionation is shown in Table 4.3.

4.2.4 Isotope calculations and proxy for turnover

The added CO_2 has a unique isotopic signature (about -51 ‰) that can be used to track the fate of newly incorporated carbon through the soil. The fraction of new carbon (f_N) in soil fractions in elevated CO_2 plots was calculated using an equation described by Balesdent and Mariotti (1996):

$$f_N = \frac{\delta - \delta_{refA}}{\delta_{vegB} - \delta_{vegA}} \quad (1)$$

where δ is the $\delta^{13}C$ of the soil fraction , δ_{refA} is the $\delta^{13}C$ of the same type of soil fraction under ambient CO_2, δ_{vegB} is the $\delta^{13}C$ of the vegetation under elevated CO_2, and δ_{vegA} is the $\delta^{13}C$ of the vegetation under ambient CO_2. We calculated δ_{vegB} for Jasper Ridge plots from plant functional group data collected at the time of soil sampling. The plant data were collected by plant functional group, and we used the $\delta^{13}C$ and biomass for each plant functional group to calculate

weighted average $\delta^{13}C$ values for all vegetation in each plot. For the Oak Ridge calculations, we used the δ_{vegB} values from Garten et al. (2009), which were calculated in a similar manner, but then averaged for each CO_2 treatment. Specifically, they used a value of -43.6‰ for elevated CO_2 plots, and a value of -28.3‰ for ambient CO_2 plots. They found using average values for elevated and ambient CO_2 vegetation – rather than plot-level values – did not alter their results. Two paired ambient and elevated CO_2 plots were used for each f_N calculation (eg ACAT and ECAT, or ACET and ECET). The fraction of old C remaining, f_O, was then calculated by subtraction:

$$f_O = 1 - f_N \quad (2)$$

The nature of the equation allows for calculation of f_N and f_O for elevated CO_2 plots only. Hence, were only able to investigate the effect of warming in conjunction with elevated CO_2, by comparing f values between ECAT plots and ECET plots. We used of f_O as proxy for turnover under the assumption of steady state, *i.e.* that total carbon stocks (C_T) did not change (Balesdent and Mariotti, 1996). We also used f_O to calculate pre-treatment, or "old" C stocks (C_O) and post-treatment, or "new" C stocks (C_N).

4.2.5 Statistical analyses

We compared general soil characteristics – including percent C, relative masses of the soil fractions, and C stocks – between temperature treatments for the elevated CO_2 plots only. While we did collect the data for the ambient CO_2 plots, we wanted the data used here to be consistent with the data used for the f_O comparisons. Because f_O is a bounded metric (0-1), we used a logit transform function before statistical analyses.

All statistical tests were carried out in R using linear mixed models (lme4 and lmerTest packages) with the Kenward-Roger technique for determining denominator degrees of freedom

(anova function) (R Core Team, 2013). When testing for effects of warming, we performed one-way ANOVAs on each soil fraction separately, rather than performing two-way ANOVAs (warming by fraction) on all soil fractions at once. The latter would have violated the assumption of independence for ANOVA since the fractions from the same soil sample are related to one another. Since none of these effects was significant, we did not take any steps to adjust for a multiple testing problem. When testing for differences in f_O between soil fractions, we pooled ambient and warmed plots and performed a two-way ANOVA (fraction by site). Since site was not significant, we then pooled the data for the two sites and performed a one-way ANOVA with pairwise comparisons between fractions using the lsmeans function. We tested for homogeneity of variances using the Fligner-Killeen method prior to all statistical analyses.

4.3 Results

4.3.1 General characteristics of soil fractions

Overall, the Oak Ridge soils contained significantly more C than the Jasper Ridge soils, the additional C being located in the sand-sized and silt-sized fractions (Table 4). The Oak Ridge soils had considerably more silt-sized material than the Jasper Ridge soils, and while they had less sand-sized material, they had a much a higher C concentration than they did at Jasper Ridge. At both sites, the silt-sized fraction was the largest fraction by mass and contained the largest C stock (44.62 ± 3.8 % of total C at Oak Ridge, 40.89 ± 2.0 % of total C at Jasper Ridge).

Rates of C turnover differed between soil fractions at both sites. The fraction of old C remaining (f_O) in the soil fractions varied predictably: there was a trend of increasing f_O with increasing mineral interaction (LF < Sand-sized < Silt-sized < Clay-sized), though not all contrasts were significant (Fig. 4.2). Old C made up about 50% or less of the LF- and sand-sized-C, while it made up about 75% or more of the silt- and clay-sized-C. The LF and sand-sized

fraction had lower f_O than the whole soil, while the silt-sized and clay-sized fractions had higher f_O than the whole soil. This indicates that, in general, the LF and sand-sized fractions cycled more quickly, while the silt-sized and clay-sized fractions cycled more slowly.

The f_O values of each soil fraction were not different between the two sites. Since the experiment at Jasper Ridge lasted over twice as long as the experiment at Oak Ridge, this indicates that SOM turnover at Jasper Ridge was about half as fast as it was at Oak Ridge.

4.3.2 Effect of warming on turnover

At Oak Ridge, there were no significant differences in in C_T between warmed and ambient plots in any of the soil fractions (Fig. 4.3). C_T was slightly larger in the fast-cycling LF and sand-sized fractions, and slightly lower in the slow-cycling silt-sized and clay-sized fractions. There was a notable, consistent trend of higher C_N and lower C_O (Fig. 4.3) in warmed plots, though none of the differences within the soil fractions were statistically significant. When compared to ambient plots, C_O in warmed plots was smaller by 18 % in the LF, 9 % in the sand, 11 % in the silt, and 7 % in the clay. Comparing C_N in the same manner, in warmed plots it was larger by 155 % in the LF, 51 % in the sand, 49 % in the silt, and 20 % in the clay.

The trends in C_N and C_O suggest warming caused faster turnover of old C and faster incorporation of new C at Oak Ridge. Indeed, using f_O as a measure of turnover, it was lower in warmed than in ambient plots (Fig. 4.4). Looking across the soil fractions, the difference in f_O with warming decreased as the average rate of turnover increased. The largest difference in average f_O between warmed and ambient plots was in the LF (-0.24), and the smallest difference was in the clay-sized fraction (-0.02). When we compare the changes in f_O with warming in the soil fractions to those in the whole soil, we see that the responses of the fast- and slow-cycling fractions masked one another. The difference in average f_O between warmed and ambient plots in

the whole soil (-0.06) was smaller than that in the fast-cycling fractions, but larger than that in the slow-cycling fractions.

The variance of f_O was always larger in warmed plots at Oak Ridge, even though it was not significantly different from that of the ambient plots. This slightly larger variance of the warmed plots was due to one plot with consistently high f_O values (in the whole soil and all of the soil fractions) compared to the other two plots in the warmed treatment.

Similar to Oak Ridge, warming did not cause any differences in C_T at Jasper Ridge. However, unlike at Oak Ridge, there were no trends in C_N, C_O (Fig. 4.5) or f_O (data not shown) with warming at Jasper Ridge. There did appear to be larger C stocks (C_O and C_T) in the silt-sized fraction in the warmed plots. This apparent effect was due to one plot, which had an unusually high C concentration and unusually high f_O, resulting in a large C_T and C_O compared to all of the other plots.

4.4 Discussion

At Oak Ridge, where productivity was higher and the warming treatment was larger (+3°C), we observed a consistent trend of faster turnover of old C with warming across soil fractions. At Jasper Ridge, where productivity was lower and the warming treatment was smaller (+1°C), we saw no effect of warming on C turnover. The common finding for both sites was that C_T was unaffected by warming. It was not that C_T decreased slightly but insignificantly with warming – which is what we would expect if warming caused a loss of soil C at a rate too slow to adequately capture over the length of these experiments – but that any losses of C_O were counteracted by gains in C_N. This lends support to the argument that warming-induced increases in SOM decomposition will be offset by increases in plant inputs, so that total soil C stocks will remain unchanged (Giardina et al., 2014; Selmants et al., 2014). Our results are congruent with

many of the other soil warming studies that showed no change in C_T with warming (Belay-Tedla et al., 2009; Garten et al., 2009; Song et al., 2012; Xu et al., 2012; Zhou et al., 2013) and see studies reviewed by Lu et al. (2013) and Dieleman et al. (2012).

We took the trend in f_O with warming at Oak Ridge to be meaningful, despite a lack of statistical significance. We did so for two reasons. The first is that it is often difficult to find statistical significance in these types of experiments, even in a metric like f_O, for the same reasons that we often don't observe significant changes in C_T. In field experiments with so few replicates, natural variability can preclude statistical significance, even where there is a clear trend. A simple power analysis reveals that in order to find significance at the α=0.05 level with power equal to 0.8, we would need an extremely large effect size of 1.5 (a "small" effect size is 0.1 and a "large effect size is 0.4, according to Cohen (1992)). The second reason that we take the trend in f_O to be meaningful is that the pattern that we observed at Oak ridge is the pattern that we would expect if warming has a similar effect on the turnover of all of the SOM pools. That is, the magnitude of the change should be proportional to the rate of turnover. Over a given period of time, SOM that turns over most slowly (clay-sized fraction in our case) will show the smallest magnitude of change in f_O. But, relative to the rate of turnover, that change will be similar across soil fractions. Indeed, this is what we observed. In other words, because the changes in f_O follow this pattern, they do not seem due to random chance.

An important consideration in interpreting the results from both sites is that we are testing for the effect of warming in plots that also received elevated CO_2. Our method utilizing the ^{13}C-label of the added CO_2 limits us to calculating f_O, C_N, and C_O for elevated CO_2 plots only. This means that any effects of elevated CO_2 on C stocks are included by design, and may be overwhelming any warming effects. At Oak Ridge, there was no effect of elevated CO_2 on plant

production or soil C stocks, but it did increase soil respiration (Garten et al., 2009; Wan et al., 2007). The increase in soil respiration with elevated CO_2 was larger (+35.8% when averaged across all elevated CO_2 plots) than the increase due to warming (+9.9% in plots with elevated CO_2), and it may have washed out any effect of warming on soil C stocks. At Jasper Ridge, warming caused increases in plant production, except when combined with elevated CO_2 (Shaw et al., 2002). The CO_2 treatment was quite large at Jasper Ridge (+680 ppm compared to +300 ppm at Oak Ridge), adding to the likelihood that elevated CO_2 may have negated any effect of warming on soil C stocks there.

In addition to the differences in CO_2 treatments, the fact that we saw a warming trend at Oak Ridge but not Jasper Ridge is most likely because of the magnitudes of the warming treatments and the different ambient moisture regimes. At Jasper Ridge, the warming treatment was about +1°C, whereas at Oak Ridge the treatment was about +3°C. Previous studies on these plots did not show a consistent effect of warming on net primary production (Dukes et al., 2005; Shaw et al., 2002). Dukes et al. (2005) posited that the warming treatment at Jasper Ridge was "quite conservative" and suggested that plant production and soil C stocks may have been affected if the treatment had been larger. In addition, Jasper Ridge is a much drier than Oak Ridge, and is generally less productive (Table 4.1). In their 2009 study, Garten et al. concluded that soil moisture was more important than temperature in controlling SOM turnover at Oak Ridge. Considering that Jasper Ridge gets half as much precipitation as Oak Ridge, it is even more likely that soil moisture dominates over temperature as a control of SOM turnover there. This is supported by the fact that SOM at Jasper Ridge turned over half as fast as it does at Oak Ridge. Therefore, the combination of a relatively dry site and a small degree of warming could easily explain the contrasting responses of SOM turnover to warming between Jasper Ridge and

Oak Ridge.

We chose to use the fraction of old C remaining (f_O) as a proxy for SOM turnover because it is more sensitive metric than C stocks; it captures the differences in both C_O and C_N. It is also easier to compare differences in f_O between different plots, because all of the values are effectively normalized to the same scale (0-1). But f_O can only be used as a proxy for the rate of turnover of old C if total soil C stocks are not larger in warmed plots. Otherwise, a smaller f_O could be due to larger C_N alone, with no difference in turnover of old C (same or even larger C_O). Strictly speaking, C_T was not significantly different between ambient and warmed plots, and the fact that there was no consistent trend in C_T with warming further supports our use of f_O.

Our results also lend support to our choice of fractionation scheme. At both sites, f_O increased (meaning the rate of loss of old C decreased) as physico-chemical protection increased. At Oak Ridge, the magnitude of the differences in f_O with warming, while not significant, also decreased as physiochemical protection increased. This is consistent with the literature on this topic (Baisden et al., 2002; Bol et al., 2009; Christensen, 2001; Kögel-Knabner et al., 2008) and confirmed our expectations when establishing this procedure using this fractionation scheme. The slow-cycling fractions constituted a large portion of the total C in these soils, which is consistent with results from many other ecosystems (Kögel-Knabner et al., 2008). If we had analyzed whole soils rather than separate fractions, the slow-cycling fractions would have dominated the signal and masked response of the fast-cycling fractions.

Recovery of whole soil mass was generally higher than 100 %, which was due to soil retention of SPT after density flotation. While multiple rinsing steps were incorporated into the protocol to minimize SPT contamination, it is difficult to rid the soil completely of SPT. It is important to consider that too many rinsing steps can lead to low recovery of soil C, as soluble C

is lost in the supernatant that is poured off at each step, in addition to the C that is lost in the SPT during the initial density flotation (Chenu and Plante, 2006; Crow et al., 2007; Moni et al., 2012; Six et al., 1999). This is the most likely reason for our low C recovery for Oak Ridge soils. The Oak Ridge soils had a much higher proportion of their total mass present in small particle size fractions (silt-sized and clay), whereas the Jasper Ridge Soils had a higher proportion of their total mass present in the larger, sand-sized fraction. The smaller size fractions contain C that is more easily lost in supernatant, since particulate organic matter in the sand-sized fraction is too large to be dissolved and poured off. Regardless, recovery of whole soil C and whole soil mass was not different between treatments at either site, and so any issues with recovery should not have affected our results in terms of the effects of warming.

The trend of faster turnover with warming at Oak Ridge suggests that soil C pools, including those that turnover slowly, are sensitive to warming. But the results from Jasper Ridge suggest that the degree of sensitivity depends on other environmental factors, such as moisture. Taken together, the results from these two sites do not support the idea that total soil carbon stocks will decrease with warming. There were no differences in in C_T with warming at either site, even where warming led to faster turnover of SOM, which agrees with the results from several other soil warming experiments. Though numerous studies, and especially soil incubations, have concluded that soil C losses will increase in response to warming (Conant et al., 2011; von Lützow and Kögel-Knabner, 2009), studies in the field (ours included) generally find that increased plant inputs will offset most or all of that response(Dieleman et al., 2012; Giardina et al., 2014; Giardina and Ryan, 2000; Lu et al., 2013; Selmants et al., 2014). Given these results in the context of previous studies, warming-induced losses of soil C from mineral soil may not be as large as some previous estimates, and may even be negligible.

Table 4.1. General site characteristics for the field experiments used in this study. Biomass values are the means ± standard error (n=12 for Oak Ridge, n=16 for Jasper Ridge).

Experiment	Old-field Community Climate and Atmospheric Manipulation (Oak Ridge)	Jasper Ridge Global Climate Change Experiment (Jasper Ridge)
Location	Oak Ridge, TN (25° 54' N, 84° 21' W)	Woodside, CA (37° 40' N, 122° 22' W)
Soil type	Fine-silty, siliceous, active, mesic Typic Fragiudult (Captina series)	Fine, mixed, active, thermic Typic Haploxeralf (Francisquito series)
Bulk density	1.49 g m^{-2}	1.19 g m^{-2}
Whole soil %C❖	1.94 ± 0.06 %	1.23 ± 0.05 %
Mean annual precipitation	1300 mm	582 mm
Vegetation	Constructed old-field communities (annual grasses, forbs, and shrubs) planted in 2002	Annual grassland (including perennial grasses but dominated by annual grasses and forbs)
Biomass in year soils were sampled❖	756.6 ± 337.4 g m^{-2}	412.7 ± 74.0 g m^{-2}
CO_2 treatment levels	Ambient +300 ppm	Ambient +680 ppm
Warming treatment levels	Ambient +3.5°C	Ambient +1°C
Duration of treatments prior to sampling	5 years	12 years
References	Wan et al., 2007; Garten et al., 2008; Garten et al., 2009	Shaw et al., 2002; Dukes et al., 2005; Henry et al., 2005

❖combined mean for ACAT, ACET, ECAT, and ECET plots ± one standard error

Table 4.2. Background data on effects of CO_2 and warming on soil respiration, aboveground plant production, and soil moisture at Oak Ridge and Jasper Ridge, with citations of data sources. Arrows indicate direction of significant effects if numerical values were not provided. Abbreviations are: NF, not found in the literature; NS, not significant.

	Oak Ridge				Jasper Ridge			
Factor and contrast	Soil respiration	Plant production	Soil moisture	Citations	Soil respiration	Plant production	Soil moisture	Citations
CO_2 ECAT-ACAT	+35.8 %	NS	↑	Respiration: Wan et al., 2007	NF	NS	+8.9%	Production: Dukes et al., 2005
Warming ACET - ACAT	-7.6 %	NS	↓	Production: Garten et al., 2008	NF	NS	+6.1%	Soil moisture: Zavaleta et al., 2003
Warming with CO_2 ECET - ECAT	+9.9%	NS	↓	Soil moisture: Dermody et al., 2007	NF	NS	+6.1 %	

Table 4.3. Percent recovery of whole soil C and whole soil mass after fractionation. Values are means ± standard error (n=3 for Oak Ridge, n=4 for Jasper Ridge).

	Percent recovery	
Site	Whole soil C	Whole soil mass
Oak Ridge	84.84 ± 2.7†	103.34 ± 1.0†
Jasper Ridge	96.89 ± 2.2	106.05 ± 0.9†

† indicates significant difference from 100 % in a one-sample t-test.

Table 4.4. General characteristics of soil fractions in elevated CO_2 plots. Values are means ± standard error (n=3 for Oak Ridge, n=4 for Jasper Ridge).

Site	Fraction	Warming treatment	%C	% of whole soil mass	% of whole soil C	C stock (g m^2 to 15 cm)
Oak Ridge	LF	0	38.74 ± 1.11	0.69 ± 0.09	14.38 ± 1.10	589.77 ± 59.02
		+	36.77 ± 1.70	1.15 ± 0.25	20.16 ± 3.58	946.96 ± 207.62
	Sand	0	1.31 ± 0.23	10.89 ± 2.03	7.35 ± 0.46	302.22 ± 29.43
		+	1.49 ± 0.60	13.34 ± 4.24	7.49 ± 0.69	350.31 ± 45.82
	Silt	0	1.12 ± 0.13	78.47 ± 1.99	48.26 ± 7.12	1952.94 ± 203.77
		+	1.13 ± 0.16	76.82 ± 4.08	40.98 ± 2.67	1913.86 ± 210.43
	Clay	0	1.97 ± 0.18	11.89 ± 0.75	12.75 ± 1.14	518.47 ± 25.69
		+	2.00 ± 0.04	10.99 ± 0.48	10.67 ± 0.85	491.61 ± 14.35
Jasper Ridge	LF	0	28.73 ± 1.84	1.08 ± 0.18	22.78 ± 2.01	538.88 ± 72.33
		+	31.69 ± 2.40	0.97 ± 0.17	21.78 ± 1.32	531.36 ± 74.57
	Sand	0	0.37 ± 0.04	37.01 ± 1.00	10.30 ± 0.55	241.89 ± 30.54
		+	0.33 ± 0.07	41.26 ± 0.46	10.13 ± 1.15	245.52 ± 53.43
	Silt	0	0.82 ± 0.06	55.01 ± 1.10	35.23 ± 7.12	804.22 ± 57.14
		+	1.09 ± 0.19	52.26 ± 2.69	42.48 ± 2.67	1009.54 ± 151.50
	Clay	0	2.46 ± 0.12	13.05 ± 0.97	24.62 ± 8.79	567.98 ± 34.09
		+	2.31 ± 0.20	14.08 ± 0.28	24.90 ± 4.97	582.09 ± 59.42

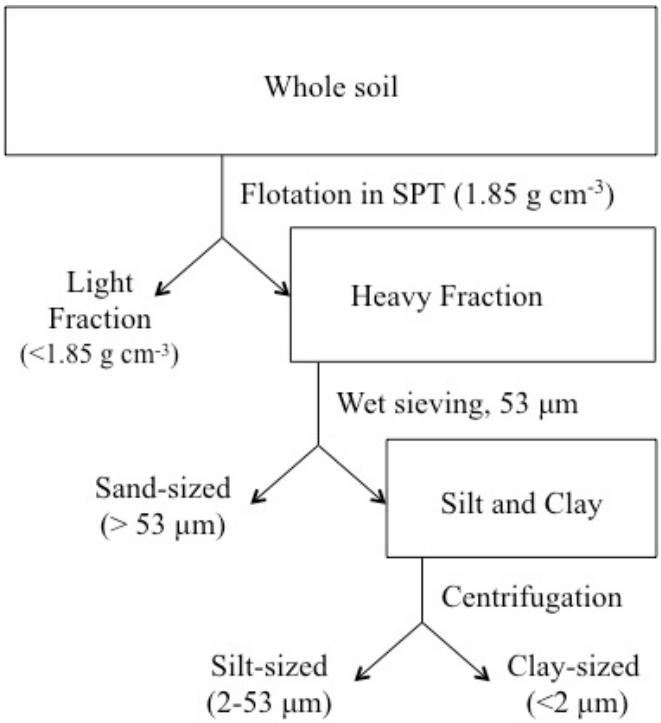

Figure 4.1. Schematic of the fractionation scheme to isolate fractions of increasing physicochemical stabilization.

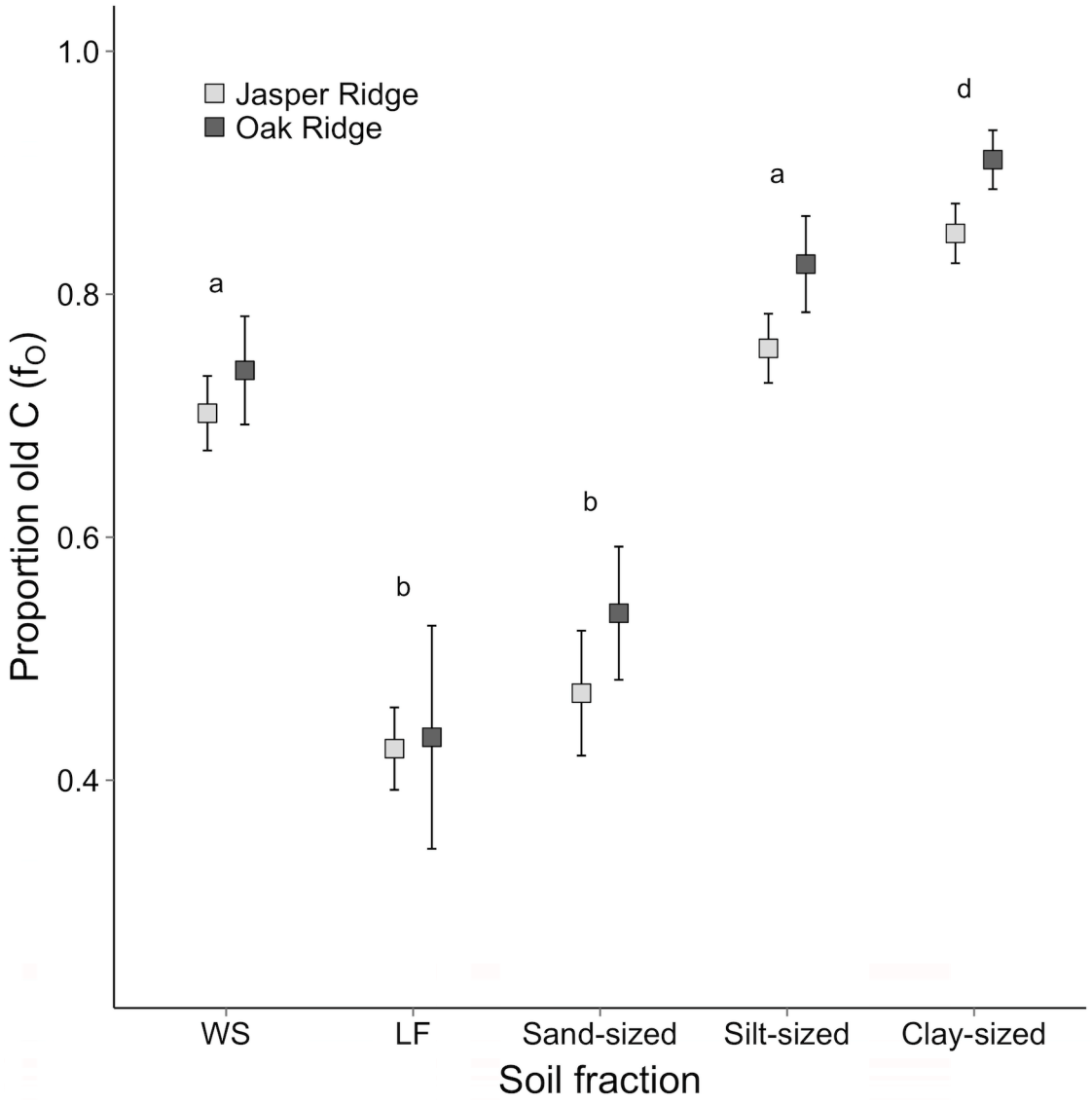

Figure 4.2. Fraction of pre-treatment C (f_O) remaining in whole soil and soil fractions at each site. Whole soil and soil fractions for the two sites are grouped together because site was not significant in a two-way ANOVA (fraction by site). Statistics were performed after logit transformation, but data shown are not transformed. Values are means ± 1 standard error (n=3 for Oak Ridge, n=4 for Jasper Ridge). Different letters show statistically significant differences between soil fractions ($p < 0.05$).

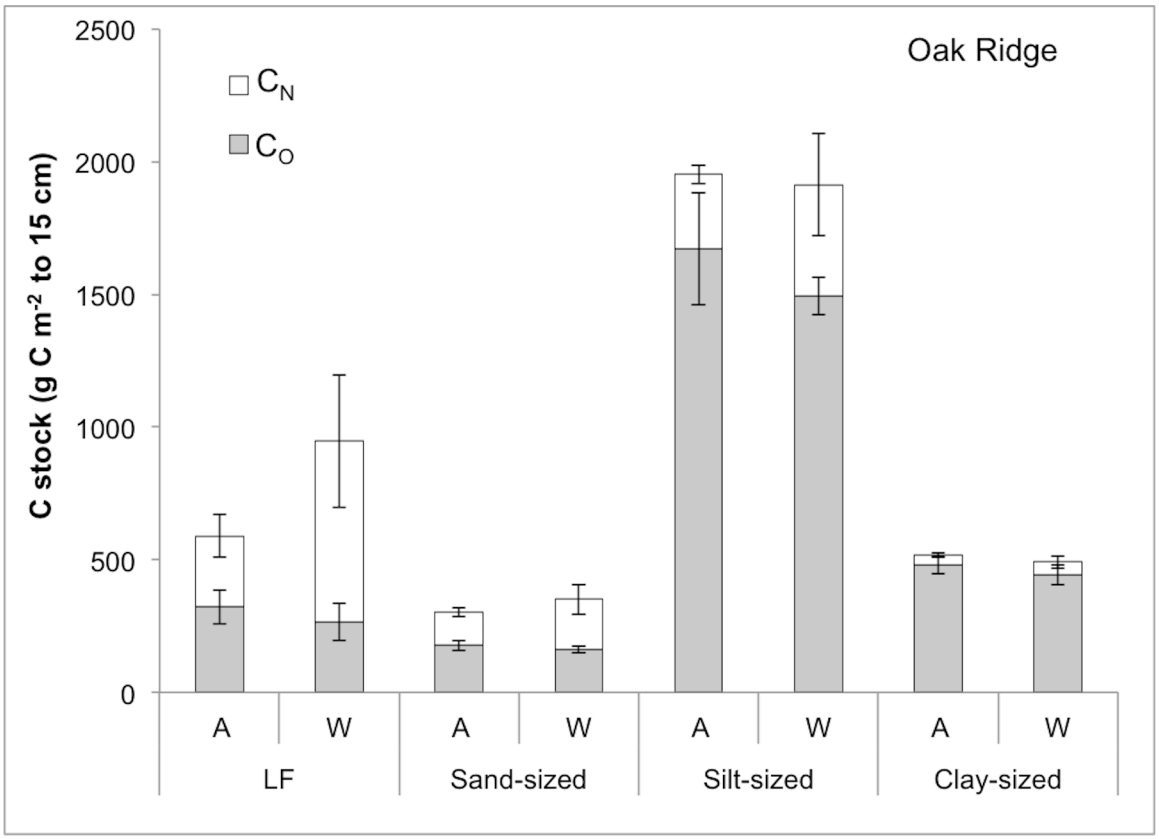

Figure 4.3. Total carbon stocks at Oak Ridge, separated into pre-treatment C (C_O) and post-treatment C (C_N), in ambient plots (A) and warmed plots (W). Values are means ± 1 standard error (n=3).

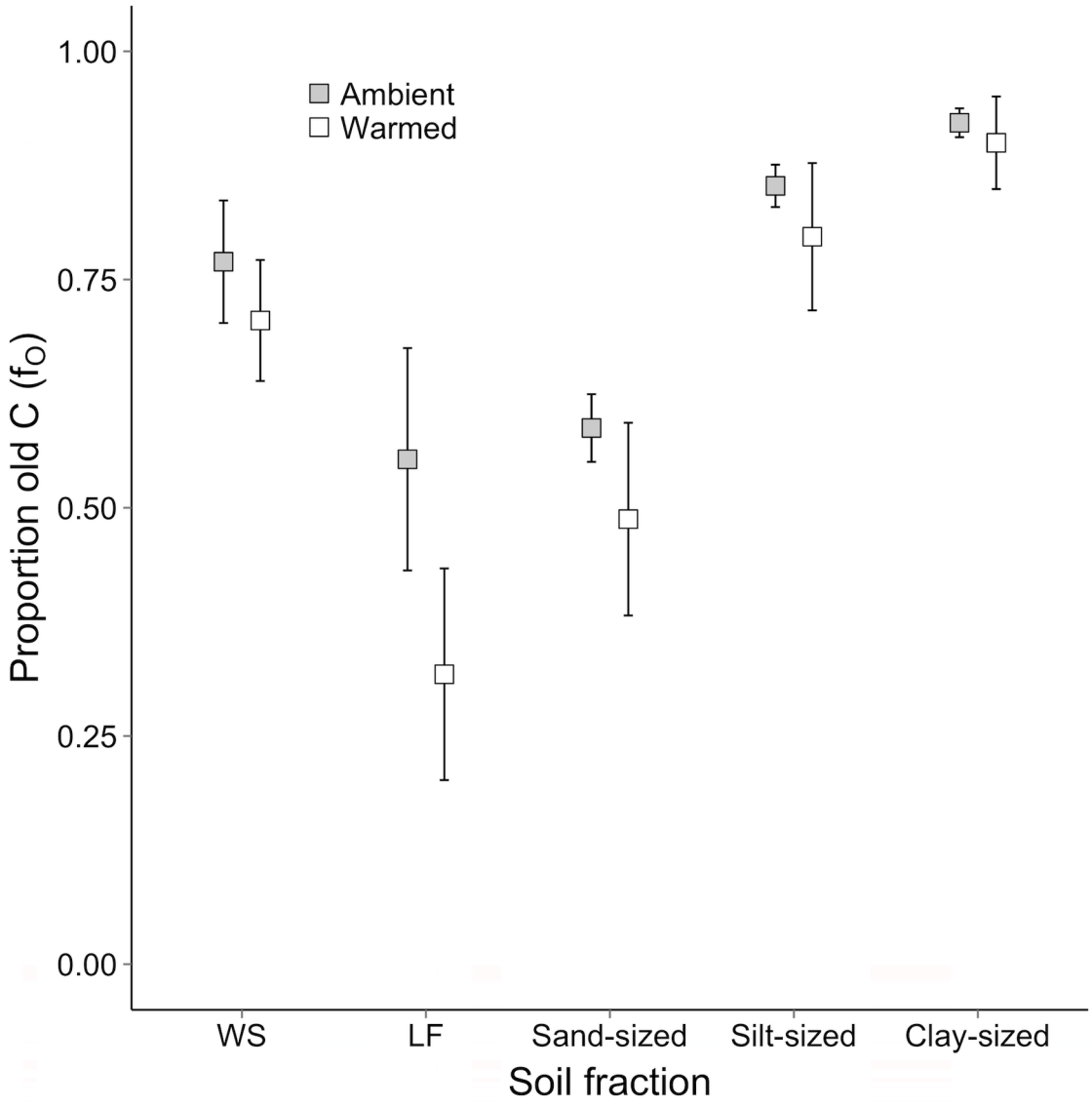

Figure 4.4. Fraction of pre-treatment C (f_O) remaining in whole soil and soil fractions at Oak Ridge. Statistics were performed after logit transformation, but data shown are not transformed. Values are means ± 1 standard error (n=3).

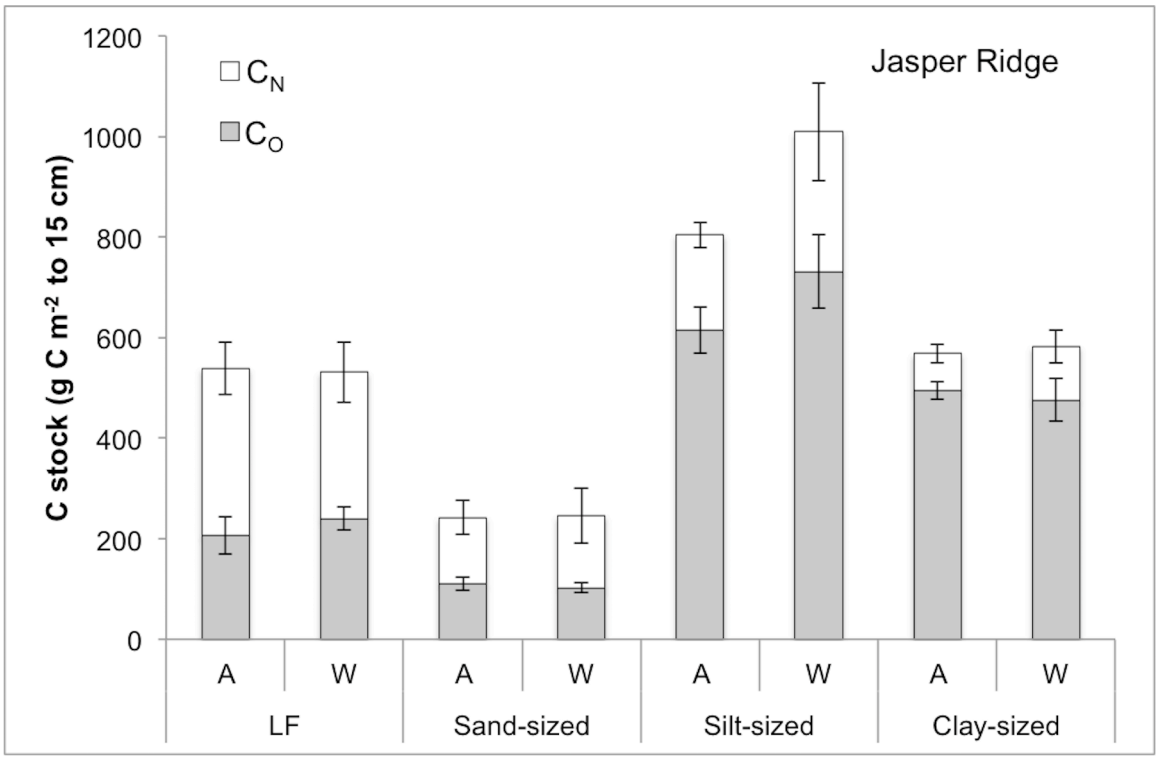

Figure 4.5. Total carbon stocks at Jasper Ridge, separated into pre-treatment C (C_O) and post-treatment C (C_N), in ambient plots (A) and warmed plots (W). Values are means ± 1 standard error (n=4).

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CHAPTER 5: CONTRIBUTION OF PYROGENIC CARBON TO SOIL ORGANIC MATTER FRACTIONS IN AGRICULTURAL SOILS AND ITS INFLUENCE ON CALCULATED MEAN RESIDENCE TIMES

5.1 Introduction

Soil is one of our most precious natural resources, providing a fertile medium for agriculture, maintaining water quality, and representing a major reservoir in the global carbon (C) cycle. Soil organic matter (SOM) is a component of soil that plays a major role in all of the ecosystem services that soil provides. The dynamics of SOM have been a major area of research over the last century, with more recent emphasis on what causes SOM to persist across ecosystems (Paustian et al., 1997). Pyrogenic organic matter (py-OM), organic material that has been thermally altered by fire, is ubiquitous in the environment and tends to persist for much longer than other SOM (Forbes et al., 2006; Preston and Schmidt, 2006; Schmidt and Noack, 2000). However, it is often ignored in studies of SOM dynamics because it is notoriously difficult to isolate and characterize (Hammes et al., 2007). As a result, the characteristics and dynamics of py-OM are lumped in with those of non-py-OM, precluding us from fully understanding either one. Hydrogen pyrolysis has recently been identified as an effective tool to isolate py-OM from non-py-OM (Meredith et al., 2013; 2012; Wurster et al., 2012), and a reliable pre-treatment for stable isotope and radiocarbon measurements (Ascough et al., 2009; Wurster et al., 2012).

In this study, we used hydrogen pyrolysis (hypy) to isolate py-OM from three agricultural sites (one grassland and two forested sites) that have undergone switches from C_3 to C_4 vegetation or vice versa, allowing tracking of native-derived C using ^{13}C. The soils at these sites

have been intensively studied and are extremely well characterized, including measurements of ^{13}C contents in the field (Follett et al., 1997; Paul et al., 2001) and upon long-term incubation (Follett et al., 2007; Haile-Mariam et al., 2008), in addition to radiocarbon dating. At all three sites, the radiocarbon ages of the cultivated plots were much older than the native plots. In the grassland the MRT of the native soil was 193 yr, while that of the cultivated soil was 1015 yr (Paul et al., 1997). Further, the MRT of the cultivated soil non-hydrolyzable fraction, which is thought to contain the SOM that is most resistant to decomposition (Paul et al., 2006), was 2685 yr. The ^{13}C data confirmed the ^{14}C dates in that the SOC of the cultivated site was primarily a residue of native-derived SOC (Follett et al., 2007). Results from the forested sites were similar. At the forested site with higher SOC content, the SOC in the cultivated soil had an MRT of 920 yr and the non-hydrolyzable fraction had an MRT of 1720 yr (Paul et al., 2001). The other forested site had lower overall MRTs as determined by both ^{13}C and ^{14}C (Collins et al., 1999; Haile-Mariam et al., 2008), nevertheless the cultivated soil contained a non-hydrolyzable fraction with an MRT of 1435 yr (Paul et al., 2001). These results suggest there was a fraction of SOM at all of these sites that could persist over millennia. There has always been the question as to how much py-C accounts for these very significant ages of the whole soils and the SOM fractions thereof, but to date it has not been quantified.

To address this question, we characterized the soils and their textural fractions before hypy, and then re-evaluated our results after hypy, accounting for py-OM. The overall aim of our study was to elucidate whether py-OM influenced our initial characterization of SOM dynamics in the soils.

5.2 Methods

5.2.1 Field sites

Soils for this experiment were from three agricultural research stations that underwent shifts from C_3 to C_4 vegetation or vice versa when they were converted from native vegetation to cultivation (Table 5.1). This allowed us to track the turnover of the native-derived SOM and the incorporation of the crop-derived SOM using stable C isotopes.

The first site is located at the Central Great Plains Research Center near Akron, Colorado, run by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS). A general site description is given in Follett et al. (2007). We used a native site and two cultivated plots, a conventional till plot and a conservation till plot (plots 307 and 205, respectively, in Follett et al. (2007)).

The second site is the Kellogg Biological Station (KBS), located in southwest Michigan. A general site description is provided by Collins et al. (1999). The native plot is a late-successional hardwood forest, and the cultivated treatment has been under continuous corn with conventional tillage since 1993. Those plots were initially cultivated in 1908, with changing rotations that included alfalfa, corn, soybean, and wheat at various times. Because of these changes in crops since the start of cultivation, the conversion from C_3 vegetation to C_4 was not abrupt, making calculations of native-derived C turnover difficult.

The third site is at the Ohio Agricultural Research and Development Center Northwest Agricultural Research Station in Hoytville, Ohio. A general site description for Hoytville is also given by Collins et al. (1999). The two cultivated soils have been under continuous corn since 1962. One cultivated treatment was under no-till and the other was under conventional tillage.

5.2.2 Soil collection and fractionation

Soils from all three sites were sampled in the fall of 2007 (Akron, KBS) or spring of 2008 (Hoytville). In each plot sampled, multiple soil cores were taken to 20 cm and combined to produce one field replicate. Four field replicates were taken per treatment at Akron, and three were taken per treatment at Hoytville. At KBS, four field replicates were taken in the native forest plot, and three were taken in the cultivated plot. Samples were stored at 4°C for up to one week before processing, at which point they were gently passed through a 2 mm and then air dried. Any coarse plant material (> 2 mm) was removed by hand during the sieving procedure.

Soil carbonates were removed by addition of 100 ml of 0.03 M H_3PO_4 to 5 g of soil and shaking for 1 h. The procedure was repeated until the pH of the soil solution remained within 0.2 pH unit of that of the original acid solution. Samples were then oven dried at 55°C.

Soils were separated into fractions using the procedure shown in Fig. 5.1. The purpose of the fractionation is to isolate SOM fractions with varying mean residence times (MRT), according to the principle that increasing physico-chemical protection of SOM will cause a longer MRT (Haile-Mariam et al., 2008). First, the light fraction (LF) was floated off using a procedure modified from Sollins et al. (2006). Two 30 g subsamples were placed in 225 ml polycarbonate centrifuge bottles with 170 ml of sodium polytungstate (SPT) at a density of 1.85 g cm^{-3} and shaken at low speed for one hour so as to mix the soil with minimal disruption of aggregates. Samples were then centrifuged for 90 min, and the floating material (LF) was aspirated and rinsed with deionized water on a 20 μm nylon filter. The heavy fraction that remained was then rinsed to remove SPT. Each rinse entailed filling centrifuge tubes with deionized water, shaking to disperse the pellet, centrifuging, and removing the supernatant.

After separating of the LF, the sand and particulate organic matter (Sand+POM) fraction

was isolated using the procedure described by Cambardella and Elliott (1992). Briefly, the heavy fractions remaining were dispersed by addition of 5 g L^{-1} sodium hexametaphosphate and shaking for 15 h with glass beads. The dispersed samples were then passed through a 53 μm sieve and rinsed thoroughly. Material that passed through the sieve (silt and clays) was then centrifuged, first to separate the silt-sized (2 μm – 53 μm) from the clay-sized material (<2 μm), and then to separate the coarse clay-sized (0.2 μm – 2 μm) from the fine clay-sized (<0.2 μm) material. All soil fractions were oven-dried at 60°C and ground prior to analysis.

5.2.3 Soil characterization

Initial soil fractions were analyzed for % C, % N, and $\delta^{13}C$ on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Carlo Erba NA 1500 elemental analyzer and VG Isochrom continuous flow IRMS, Isoprime Inc., Manchester, UK). Total recovery of whole soil C and whole soil mass are shown in Table 5.2. We refer to the C from these initial soil fractions as soil organic carbon (SOC); it includes both non-py-C and py-C. This is to differentiate the SOC from the later measurements of py-C alone.

5.2.4 Hydrogen pyrolysis and post-hypy analysis

Hydrogen pyrolysis (Hypy), along with elemental and isotope analysis of post-hypy samples were performed at the Cairns Analytical Unit of James Cook University, AU. Hypy was performed on all replicates of all soil fractions according to the procedure of Wurster et al. (2012) and Ascough et al. (2009). Briefly, 250 mg subsamples were combined with a molybdenum catalyst using an aqueous/methanol solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$]). The amount of catalyst added was approximately 1 % of SOC for each subsample. The dried, catalyst-loaded subsamples were placed in the hypy reactor (Strata Technology Ltd., Middlesex, UK) and pressurized with 15 MPa of hydrogen with a sweep gas

flow of 5 L min^{-1}. The temperature was ramped to 250 °C at a rate of 300 °C min^{-1}, then ramped more slowly at 8 °C min to a final temperature of 550 °C, which was held for 8 min. The material remaining after hypy was then weighed, and run for %C and $\delta^{13}C$ using an elemental analyzer/continuous-flow isotope ratio mass spectrometer (EA/CF-IRMS, ECS 4010 CHNSO elemental analyzer, Costech Analytical Technologies Inc., Valencia, CA coupled to a ThermoFisher Scientific DeltaV^{PLUS} isotope ratio mass spectrometer). The precision (standard deviation) on internal standards was less than ± 0.1 ‰ for $\delta^{13}C$ and less than ± 2 % of the known value for C concentration. This was the same precision as the analysis used for initial soil fractions, and a random subset of samples was run at both facilities to ensure comparability.

5.2.5 Turnover calculations

The proportion of crop-derived C in cultivated plots (f_C) was calculated according to the equation described by Balesdent and Mariotti (1996):

$$f_C = \frac{\delta - \delta_N}{\delta_{vegC} - \delta_N} \quad (1)$$

where δ is the $\delta^{13}C$ of the soil fraction in the cultivated plot, δ_N is the $\delta^{13}C$ of the same soil fraction in the native plot, and δ_{vegC} is the $\delta^{13}C$ of the crop vegetation. The proportion of native-derived C remaining in cultivated plots was calculated by subtraction ($1 - f_N$).

MRT of native-derived C was calculated in accordance with methods described by Balesdent et al. (1987):

$$MRT = t/-\ln\left(C_{f_N}/C_N\right) \quad (2)$$

where t is the duration of the experiment, C_{fN} is the native-derived carbon stock remaining, and

C_N is the average C stock in the corresponding fraction in the native plot. Due to the inconsistency of the cultivated treatments at KBS, we were unable to calculate f_C or MRT for that site.

Once py-C was quantified and analyzed for $\delta^{13}C$, we adjusted our SOC stocks and $\delta^{13}C$ values, producing estimates of non-py-C stocks and $\delta^{13}C$ values. Estimation of non-py-C stocks was done by subtracting the measured quantities of py-C from measured SOC for each sample. Estimation of non-py-C $\delta^{13}C$ was performed using the mixing model:

$$\delta_{\text{non-py-C}} = \frac{\delta - \delta_{\text{py-C}} \cdot f_{\text{py-C}}}{f_{\text{non-py-C}}} \quad (3)$$

where δ is the $\delta^{13}C$ of the SOC, $\delta_{\text{py-C}}$ is the $\delta^{13}C$ of the py-C, $f_{\text{py-C}}$ is the proportion of SOC that is py-C, and $f_{\text{non-py-C}}$ is the proportion of SOC that is non-py-C. Because we could not accurately calculate MRTs for samples from KBS, we did not adjust those SOC $\delta^{13}C$ values. MRTs for non-py-C were then calculated in the same manner as for the SOC, using the estimated non-py-C $\delta^{13}C$ values.

5.2.6 Statistical analyses

All statistical tests were carried out using the statistical package in R, version 3.0.2 (R Core Team, 2013). Unless otherwise indicated, all statistical tests were analyses of variance (ANOVAs) with Tukey's post hoc test applied if one or more factors were significant ($p \leq 0.05$). Metrics shown in Tables 1 and 3 were tested across soil fractions and treatments, within site. Metrics shown in Table 5.3 were tested across treatments only (within soil fraction and site). An analysis of covariance (ANCOVA) was used to test for significant differences between regressions of whole soil SOC content (x variable) versus py-C as a percent of SOC (y variable) by site, omitting the native plots at Hoytville because they violated the assumption of linearity.

5.3 Results

5.3.1 Soil characteristics of native plots

All of the soils under native vegetation were at least 25% silt by mass, but the Akron and Hoytville soils had significantly more silt (about 50% of their mass) than KBS (Table 5.2). The Hoytville soil was more finely textured than the other two sites, having significantly less Sand+POM and significantly more coarse clay. The KBS soil was significantly sandier than the other two sites; the Sand+POM fraction made up 66.17 ± 4.43 % of its mass.

The native plots at Akron and KBS had similar whole soil SOC contents, while the native plots at Hoytville contained almost four times as much whole soil SOC (Fig. 5.2). The Sand+POM fraction at KBS contained about the same amount of SOC (as a percent of whole soil mass) as it did at Akron (Table 5.2). This was due to the relatively low SOC concentration of the Sand+POM at KBS (Table 5.3), which suggests that it is primarily sand rather than POM. In contrast, the Sand+POM at Hoytville had a relatively high SOC concentration, suggesting that it consisted of more POM and less sand than the Sand+POM fractions at the other two sites. As a result, Hoytville had significantly more SOC in the Sand+POM fraction than the other two sites.

In general, the distribution of SOC (SOC as a % of whole soil SOC, Table 5.2) among the soil fractions was similar between sites. At all three sites, the fine mineral fractions (silt, coarse and fine clay) accounted for over half of the SOC, the majority of which was in the silt (Table 5.3 and Fig. 5.2). The only significant differences in SOC distribution between sites were in the LF and the silt. The LF accounted for a 25.04 ± 1.7 % of SOC at Akron, 18.04 ± 1.4 % of SOC at KBS, and only 7.85 ± 0.4 % of SOC at Hoytville. The percent of whole soil SOC in the silt increased in the order Akron < KBS < Hoytville, but was only significantly different between Akron and Hoytville.

In general, C/N ratios were higher in the LF and Sand+POM fractions and lower in the fine mineral fractions, especially the clays (Table 5.3).

5.3.2 Changes with cultivation

In general, the relative contributions of each soil fraction to whole soil mass changed with cultivation. The contribution of the LF and Sand+POM decreased with cultivation, and that of the fine mineral fractions increased, but the changes in the individual fractions were not always statistically significant (especially at Hoytville). The most striking change with cultivation was the decrease in the contribution of the Sand+POM fraction at KBS, which we attribute to the variation in texture between the native and cultivated plots, rather than an effect of cultivation.

Whole soil SOC decreased significantly with cultivation, regardless of tillage treatment (Figure 2). Nearly all of the soil fractions had lower SOC concentrations under cultivation, but the largest relative decreases were in the fine mineral fractions, especially at KBS and Hoytville (Table 5.3). Though their C concentrations decreased, the percent contribution of the clay fractions to SOC tended to increase under cultivation because of their large relative sizes (Table 5.2). In contrast, the percent contribution to SOC of the LF and Sand+POM generally decreased with cultivation. So while cultivation caused C to be lost from all of the soil fractions, it was lost to a lesser extent from the clays and lost to a greater extent from the LF and Sand+POM. In general, the silt fractions fell somewhere in between, except at Hoytville where the silt lost much more C upon cultivation (it decreased from 20.5 to 6.7 mg C per g soil) than at the other sites.

In general, C/N ratios of the whole soils and soil fractions decreased with cultivation (Table 5.3). The only exceptions were the LF, silt, and fine clay at Akron, and the Sand+POM at KBS.

5.3.3 Py-C content and distribution

Py-C was present at all of the sites and in all of the soil fractions (Table 5.4 and Fig. 5.3). Similar to the SOC, the majority of py-C was found in the fine mineral fractions, especially in the silt. While the amount of py-C in whole soils was small on an absolute mass basis (values ranged from 0.057 % to 0.12 % of whole soil mass), py-C accounted for a significant portion of SOC in the whole soils and soil fractions (Fig. 5.4). In the native treatments, py-C accounted for 7.74 ± 1.0 % of the whole soil SOC at Akron, 4.19 ± 1.1 % of the whole soil SOC at KBS, and 2.72 ± 0.1 % of the whole soil SOC at Hoytville. Py-C contribution to SOC was even larger in the finer soil fractions, particularly at Akron where py-C accounted for 21.6 ± 1.4 % of SOC in the silt under conventional tillage. The Hoytville soils contained more py-C than the Akron and KBS soils by mass, but py-C made up the largest percentage of SOC in the Akron soils with their lower SOC contents.

5.3.4 Preferential preservation of py-C with cultivation

When soils were cultivated, the contribution of py-C to SOC generally increased (Table 5.4, Fig. 5.4). This indicates that as soil C was lost with cultivation, py-C remained behind. This is not to say that py-C wasn't also lost – py-C content of whole soils and soil fractions decreased with cultivation in some cases – but it was lost to a lesser extent than the SOC. We show an alternative demonstration of the preferential preservation of py-C in Fig. 5.5. If py-C were lost at the same rate as SOC, then as SOC decreased, the relationship of py-C/SOC to SOC would remain constant. As the linear regressions in Fig. 5.5 show, the slopes of the lines are all negative, meaning that as SOC decreases, py-C comprises a greater percentage of SOC. The slopes of the lines in Fig. 5.5 indicate the degrees of preferential preservation at each site. At Akron and KBS, where the slopes of the lines are steeper, py-C was preserved to a greater degree

than it was at Hoytville. The native soils at Hoytville fall well outside of the slopes of the regressions due to their very high SOC contents, and so were omitted from linear regression analysis. If included, the slope of the line for Hoytville is no longer linear, but the general pattern of a faster rate of loss of py-C than at the other sites remains.

5.3.5 Py-C $\delta^{13}C$

The $\delta^{13}C$ values of py-C were generally different from the $\delta^{13}C$ values of the SOC in whole soils and within soil fractions (Fig. 5.6). At all three sites, the range in $\delta^{13}C$ values of the py-C was larger than that of the SOC. When testing for differences, there were significant interactions between treatment and soil fraction ($p \leq 0.05$), which indicates that the differences between $\delta^{13}C$ of the SOC and $\delta^{13}C$ py-C were not consistent between plots or soil fractions. We omitted the $\delta^{13}C$ data for conservation till and no till plots from Fig. 5.6 for clarity, but those data are shown in Table 5.5. In general, the $\delta^{13}C$ values for the conservation till and no till plots followed the same pattern as the conventional till plots.

The py-C at KBS and Hoytville had similar $\delta^{13}C$ values, but the py-C at Akron was more enriched, on average. In addition, the $\delta^{13}C$ values of py-C in the individual soil fractions at Akron covered a larger range (from -24.0 ± 0.1 ‰ in the LF to -12.9 ± 0.2 ‰ in the coarse clay) than they did at KBS and Hoytville (those values fell between -24 and -19).

5.3.6 Non-py-C adjustments and effects on MRT calculations

While the differences in $\delta^{13}C$ values between SOC and py-C were generally significant, the magnitudes of the differences (on absolute terms) were never more than 5 ‰, and the average difference was 1.2 ‰ (Table 5.5). As a result, our estimates for non-py-C $\delta^{13}C$ values were always within 1 ‰ of the original SOC $\delta^{13}C$ values (data not shown). However, these seemingly small adjustments to the SOC $\delta^{13}C$ values, combined with adjustments to SOC

concentrations, yielded different MRT calculations (Table 5.6). The MRTs estimated with SOC values were generally longer than those calculated using the non-py-C values; in other words, accounting for the py-C generally produced a shorter MRT estimate. In some of the fine mineral fractions at Akron, the estimated MRT of non-py-C was over 20% shorter than the previous estimate for the SOC (Fig. 5.6). The changes in MRT estimates were generally larger at Akron, where there was more py-C, than they were at Hoytville.

In general, the MRT of the native-derived SOC was not different between tillage treatments, but there were some notable exceptions, which remained after adjustment for py-C. At Akron, the Sand+POM fraction had a longer MRT under conventional tillage than under conservation tillage. At Hoytville, both the silt and coarse clay fractions had longer MRTs under conventional tillage than under no till. These longer MRTs correspond to more drastic losses of SOC under more intense cultivation, and the ^{13}C values of the SOC show that they consist of a greater proportion of native-derived C than the same fractions under the other treatments.

5.4 Discussion

5.4.1 Distribution of SOC and changes with cultivation

At all three sites, SOC decreased with cultivation which agrees with previous work at these sites (Collins et al., 1999; Paul et al., 1997; Plante et al., 2006). Though the conventionally tilled plots, which represent the more intense cultivation treatment, generally showed the largest losses of SOC, the differences between tillage treatments were often statistically insignificant. At Hoytville, there was a much larger relative decrease in SOC with cultivation because the site is located on a former wooded wetland . When the plots at Hoytville were converted to agriculture, tile drainage was installed and the soil quickly transitioned from near saturation to a well-aerated state. This change in aeration, in addition to cultivation, caused the relatively large decrease in

SOC at Hoytville. At all of the sites, C was lost from all of the soil fractions, but the clay fractions retained C more effectively than the LF and Sand+POM fractions. This, along with the longer MRTs of the clay fractions, is consistent with the literature showing that clays protect SOM from decomposition (Christensen, 2001; Kögel-Knabner et al., 2008; Mikutta et al., 2006).

One surprising result was the prevalence of SOC (as well as py-C, which will be discussed subsequently) in the silt fractions at all three sites. If all aggregates are completely dispersed (as was the aim of our fractionation scheme), the mechanism of protection of the SOC in the silt is not as clear as in the case of clay (Christensen, 2001). Silt has lower specific surface area than clay, and is generally less reactive as it contains more silicon dioxide and fewer iron and aluminum oxides by mass than clay (Joffe and Kunin, 1943). Silt particles do often have fine clay coatings and approximately one half the sesquioxide content of clays, but their lower specific surface area should still preclude them from protecting as much SOC as the clays on a per mass basis. Information on the reactivity of the silt and clay can be obtained by investigating their SOC per unit mass (SOC as % of soil fraction mass). At Akron and KBS, the silt fractions contained one-third the amount of SOC on a per mass basis as the clays, supporting the idea that clays have a higher capacity to stabilize C than silt. (The reason that C stocks were so large in the silt fractions at those sites was simply that there was a lot of silt.) In the native plots at Hoytville, the SOC per unit mass of the silt was similar to that of the clay fractions, suggesting that under very wet conditions, they stabilized as much SOC as the clays. However, the silt fractions at Hoytville lost much more of their SOC with cultivation than the clays did, so although there was a lot of silt-associated SOC to begin with, it was not as effectively protected as the clay-associated SOC.

5.4.2 Distribution of Py-C in soil fractions and possible sources

Py-C was present at all of the sites, in all of the soil fractions. Its presence in these soils confirms the results from Skjemstad et al. (2002), who found py-C in five agricultural soils across the United States, where it accounted for between 10 and 35% of SOC. Similar results have been shown in Candada (Murage et al., 2007), across Europe and Asia (Rodionov et al., 2010; Schmidt et al., 2002), and Australia (Skjemstad et al., 1996). The fact that it was present in all of the fractions also agrees with previous studies on py-C in different soil fractions (Brodowski et al., 2005; 2006; Laird et al., 2008). Py-C has a variety of physical forms, from large, low-density fragments to small, reactive particles that can interact with soil minerals (Brodowski et al., 2005). As with the SOC, the prevalence of py-C in the silt fraction at the three sites was initially surprising. The same explanations for the SOC apply to the py-C, that is, the concentration of py-C on a per mass basis was lower in the silt fractions than in the clay fractions and it was not preserved as effectively in the silt with cultivation. The prevalence of py-C in the silt fraction may also be explained by the fact that there are multiple sizes of py-C in that size fraction due to association with silt- and/or clay-sized particles. Brodowski et al. (2005) found py-C in the silt fraction of a German Chernozem in several forms, including larger py-C particles with attached clay-sized mineral particles, and smaller py-C particles attached to silt-sized mineral particles. These were primary organomineral complexes, rather than microaggregates. The many possible combinations of py-C particles and mineral particles that could produce a silt-sized complex might help to explain the large contribution of py-C to the silt fractions in our study.

These sites have not burned since cultivation (at least). The lack of recent fire at these sites suggests that py-C is left over from fires that took place before these plots were cultivated,

or that it originated outside the plots and has been deposited on them, most likely by wind. The $\delta^{13}C$ values of the py-C give us clues as to its origin. At Akron, the large range in $\delta^{13}C$ of the py-C in different soil fractions suggests that the py-C came from several different sources. Py-C with more negative values, as in the LF and the Sand+POM, likely originated from the burning of C3 vegetation, while py-C with less negative values, as in the clays, likely originated from the burning of C4 vegetation (Bird and Ascough, 2012; Krull et al., 2003). The native vegetation at Akron is mixed C3/C4 grasses and shrubs (Follett et al., 1997), and the grasslands in the great plains are known to burn fairly regularly, which would explain the presence of both types of py-C at the Akron plots. Forest fires in the mountains nearby - with smoke and ash transported for hundreds of kilometers - are also common, which would further explain the presence of more C3-derived py-C there.

At KBS and Hoytville, the py-C did not have as large a range in values as it did at Akron, which suggests that it came from less disparate sources. In the native plots, the $\delta^{13}C$ of the py-C was slightly less negative than that of the SOC. This suggests that the py-C was from the burning of the native vegetation, but was older than the SOC, and therefore reflected changes in the atmospheric $\delta^{13}C$ that occurred over the past century (Keeling, 1979). The $\delta^{13}C$ of the py-C in the cultivated plots had a larger range than it did in the native plots at Hoytville and KBS, which suggests that py-C from different vegetation sources might have been deposited in the cultivated plots. Since the native plots are densely forested, it is possible that py-C could blow onto cultivated plots, but be excluded from native plots. As for the source of that deposited py-C, the $\delta^{13}C$ was closer to that of C4 vegetation. It may have come from fires in the tall grass prairie to the west of these sites, or the burning of corn stover nearby. Additionally, the KBS experimental design includes a treatment with annual burning. The vegetation that burns is a complex mixture

that includes some C4 species (Chapman and Brewer, 2008), which would explain the range of $\delta^{13}C$ values for the py-C in the KBS cultivated plots. Another potential source of the less negative $\delta^{13}C$ values of py-C in the cultivated treatments is adsorption or direct deposition of crop-derived non-py-C by microbes (Laird et al., 2008). This latter mechanism is less likely, however, given the effectiveness of the hydrogen pyrolysis method at eliminating non-py-C (Ascough et al., 2009; Wurster et al., 2012).

The difference in $\delta^{13}C$ of the py-C among the soil fractions can be explained by the physical properties of pyrogenic material from different sources. The burning of woody (C3) plants tends to produce larger, chunkier pyrogenic material (Schmidt et al., 2002) than the burning of grasses (C3 or more often C4), which produces wispier, lighter pyrogenic material that quickly crumbles into fine particles. Since large, chunky pyrogenic material is isolated along with the LF during fractionation, the py-C in the LF tends to have $\delta^{13}C$ values similar to C3 vegetation. Finer pyrogenic material can more easily associate with soil minerals, so the py-C in the fine mineral fractions has $\delta^{13}C$ values similar to C4 or mixed C3/C4 grasses.

5.4.3 Preferential preservation vs. continual deposition of py-C

We use the increase in the percent contribution of py-C to SOC with cultivation as evidence of its preferential preservation, but it could also be due to continual deposition. Both processes probably contribute to the py-C content at all three sites. At KBS and Hoytville, the difference in $\delta^{13}C$ of py-C between native and cultivated plots suggests that the relative contribution of the two processes to py-C content may differ between the plot types. Whereas the py-C in the native plots seems to have remained from biomass burning when the $\delta^{13}C$ of atmospheric CO_2 was 1-3 ‰ less negative, the cultivated plots seems to have had some C4-derived py-C that may have been deposited more recently. At Akron, continual deposition from

prairie and forest fires is highly likely. But there is also a large body of evidence showing that once py-C enters the soil, whether by burning on-site or by deposition, it is relatively resistant to microbial decomposition and can remain there for millennia (Criscuoli et al., 2014; Liang et al., 2008; Major et al., 2010; Marschner et al., 2008; Preston and Schmidt, 2006; Schmidt and Noack, 2000; Singh et al., 2012).

The fact that continual deposition likely accounts for a significant portion of py-C at these sites precludes us from accurately comparing dynamics of py-C to those of non-py-C. However, regardless of whether the contribution of py-C to SOC increased with cultivation because of preferential preservation or continual deposition, its behavior would have been inherently different from that of the non-py-C. As such, we believe it should be accounted for and studied separately from non-py-C, especially with regards to C dynamics and turnover.

5.4.4 Importance of accounting for py-C in studies of SOM dynamics

We demonstrated that the presence of py-C influenced MRT estimates of C in different soil fractions. Py-C contributes substantially to SOC concentrations and can have different $\delta^{13}C$ values than the SOC, both of which can cause the SOC to appear older when using this stable isotope technique to calculate MRTs (Murage et al., 2007). Accounting for py-C generally decreased our MRT estimates, so that the estimated MRTs of the non-py-C were shorter than those of the SOC. This was especially true for the fine mineral fractions at Akron. In that case, neglecting to account for py-C would cause mineral associations to appear more important for preservation of C than they actually are. Krull et al. (2006) drew similar conclusions from their work with Australian soils, where protection by minerals only accounted for C preservation on the range of decades, whereas py-C was much older.

Another important finding was that the effect of py-C on SOC MRTs varied by soil

fraction and by site, suggesting that it must be accounted for in a specific manner, as opposed to performing a constant correction across soils and soil fractions. The correction cannot be inferred from py-C concentrations alone; in the cultivated plots at Hoytville, where py-C accounted for 11-12 % of SOC in the fine clay, adjusting for py-C actually had very little influence on the MRT estimates as determined with ^{13}C. This was due to the fact that the $\delta^{13}C$ of the py-C in those soil fractions was similar to that of the SOC, and shows that the $\delta^{13}C$ of the py-C in each soil fraction, in addition to its concentration, is integral to these adjustments.

We presented just one example of a method of measuring turnover that can be influenced by the presence of py-C; there are several other methods of calculating or inferring turnover that could also be influenced. For example, radiocarbon measurement is a common method for dating C in soils and soil fractions. Several studies have shown that py-C has an older average ^{14}C age than SOC (Pessenda et al., 2001; Preston and Schmidt, 2006; Schmidt et al., 2002), and so contamination of SOC by py-C, even at low concentrations, could easily lead to overestimates of ^{14}C ages of SOC presented for these soils(Paul et al., 2001; 1997). Paul et al. (2001; 1997) found the non-hydrolysable fractions in the surface horizons of cultivated plots at Akron, KBS, and Hoytville had mean radiocarbon ages of 3326, 1435 and 1770 years, respectively. Those ages correlate roughly with the contributions of py-C to SOC at each of the sites. In addition, the radiocarbon ages of non-hydrolyzable fractions in cultivated plots were all older than the respective native plots, which correlates with the increase in the contribution of py-C to SOC with cultivation. Our measurement of py-C in these soils helps with interpretation of these very old ^{14}C ages. The effects of py-C can also influence results when using laboratory incubation to estimate the rate of turnover of different pools of soil C, as py-C has been shown to decompose more slowly than SOC, in laboratory incubations (Kuzyakov et al., 2009; Liang et al., 2008;

Nguyen and Lehmann, 2009). If py-C is present but unidentified, experimenters could mistakenly attribute the persistence of that C to some other mechanism.

Knowledge of py-C content and association with different components of the soil matrix can influence our understanding of basic SOM properties in addition to turnover. One example from this study is the C/N ratio of SOM in different soil fractions. The decrease in C/N ratios with cultivation suggested that SOM that was richer in N was preferentially preserved, which agrees with the literature on the topic (Christensen, 2001; Diekow et al., 2005; Sollins et al., 2006). However, in some soil fractions there weren't significant decreases in C/N ratios with cultivation, and this may be due in part to the presence of py-C. Py-C tends to have C/N ratios resembling those of the vegetation it originated from, which are wider than the C/N ratios of most SOM fractions (Keiluweit et al., 2010; Preston and Schmidt, 2006). In the cultivated plots at Akron, py-C made up larger portions of the SOC in the silt and fine clay than it did in the same fractions at the other sites. The larger amount of py-C combined with its presumably wide C/N ratio might explain why the C/N ratios of SOM in those soil fractions did not decrease with cultivation, as they did at the other sites.

The results of this work highlight the importance of accounting for py-C in studies of SOM properties and dynamics. If it is not properly accounted for, its unique behavior can confound our understanding of non-py-C. The widespread prevalence of py-C and its variable physical properties mean that it must be accounted for in a manner specific to each site and soil fraction studied. In this study, accounting for py-C resulted in shorter MRT estimates for soil fractions (especially fine mineral fractions) at one agricultural site, but had little influence on turnover estimates at another site.

Table 5.1. Characteristics of the three agricultural sites used in this study. AP is annual precipitation, and MAT is mean annual temperature.

Site	AP (mm)	MAT (°C)	Geographic location	Soil classification	Vegetation	Year cultivated plots established
Akron, CO	420	9.2	40°8'N 103°9'W	Aridic Paleustoll	Grassland, predominantly C_4 grasses	
					Wheat-fallow (*Triticum aestivum L.*)	1928
Kellogg Biol. Station, MI	728	9	42°18'N 85°30'W	Typic Hapludalf	Deciduous hardwood forest (C_3)	
					Continuous corn (*Z. mays L.*)	1986
Hoytville, OH	835	9.5	41°00'N 84°00'W	Mollic Ochraqualf	Deciduous hardwood forest (C_3)	
					Continuous corn (*Z. mays L.*)	1963

Table 5.2. Mass and SOC distribution data for soil fractions at the three sites. Soil fraction abbreviations are: light fraction (LF), sand and particulate organic matter (Sand+POM), coarse clay (Cclay), fine clay (Fclay), and whole soil (WS). Treatments are native vegetation (Native), conservation tillage (Cons. till), conventional tillage (Conv. till), and no tillage (No till). Data are means ± one standard error. Means within the same soil fraction with different letters indicate a significant ($P \leq 0.05$) difference between the sites and treatments.

		Akron			KBS		Hoytville		
	Soil fraction	Native	Cons. till	Conv. till	Native	Conv. till	Native	No till	Conv. Till
Soil fraction mass (% of whole soil)	LF	1.75 ± 0.23 a	0.72 ± 0.10 b	0.21 ± 0.02 b	0.75 ± 0.05 b	0.32 ± 0.03 b	1.84 ± 0.50 a	0.58 ± 0.04 b	0.47 ± 0.09 b
	Sand+POM	35.89 ± 0.94 ab	25.08 ± 0.44 cd	29.84 ± 0.55 ac	66.17 ± 4.43 e	42.70 ± 3.02 b	20.47 ± 0.57 d	18.99 ± 0.26 d	18.25 ± 0.76 d
	Silt	49.88 ± 0.77 ab	55.93 ± 0.82 b	50.36 ± 1.06 ab	28.84 ± 4.36 c	46.01 ± 2.86 a	48.95 ± 1.14 ab	45.01 ± 0.24 a	46.19 ± 0.58 a
	Cclay	5.74 ± 0.09 a	9.77 ± 0.89 b	7.53 ± 0.09 a	2.23 ± 0.34 c	6.47 ± 0.25 a	20.98 ± 0.36 d	23.66 ± 0.34 e	24.95 ± 0.12 e
	Fclay	8.41 ± 0.64 abc	8.83 ± 0.92 abc	10.53 ± 0.77 abe	4.17 ± 0.13 d	5.67 ± 0.33 cd	8.43 ± 0.93 abc	12.27 ± 0.84 be	9.61 ± 0.27 abe
	Total recovery	101.7 ± 0.38 ab	100.3 ± 0.33 ac*	98.47 ± 0.41 d	102.2 ± 0.19 b	101.2 ± 0.16 abc	100.7 ± 0.59 abc*	100.5 ± 0.42 abc*	99.46 ± 0.38 cd*
SOC amount (mg/g whole soil)	LF	3.05 ± 0.355 ab	1.06 ± 0.126 c	0.45 ± 0.032 c	2.19 ± 0.146 bd	0.79 ± 0.075 c	3.71 ± 0.360 a	1.45 ± 0.100 cd	1.12 ± 0.164 cd
	Sand+POM	1.74 ± 0.271 ab	0.73 ± 0.048 a	0.59 ± 0.064 a	1.76 ± 0.185 ab	0.92 ± 0.093 a	5.85 ± 0.827 c	2.43 ± 0.125 b	1.77 ± 0.060 ab
	Silt	3.45 ± 0.203 ab	2.75 ± 0.231 a	2.27 ± 0.157 a	4.76 ± 0.272 b	2.05 ± 0.050 a	20.50 ± 0.930 c	6.70 ± 0.171 d	6.69 ± 0.467 d
	Cclay	1.40 ± 0.066 a	2.04 ± 0.269 a	1.08 ± 0.059 a	1.34 ± 0.261 a	1.60 ± 0.023 a	8.40 ± 0.526 b	5.62 ± 0.160 c	4.78 ± 0.173 c
	Fclay	1.56 ± 0.075 a	1.37 ± 0.139 a	1.28 ± 0.091 a	1.82 ± 0.094 a	1.24 ± 0.060 a	2.71 ± 0.225 b	2.50 ± 0.199 b	1.60 ± 0.028 a
SOC distribution (% of whole soil SOC)	LF	25.04 ± 1.66 a	11.44 ± 1.07 b	6.88 ± 0.39 bc	18.04 ± 1.35 d	10.37 ± 0.71 bc	7.85 ± 0.35 bc	6.60 ± 0.34 bc	6.27 ± 0.74 c
	Sand+POM	14.10 ± 0.91 ab	7.92 ± 0.40 d	8.86 ± 0.80 cd	14.41 ± 0.94 a	12.22 ± 1.42 abc	12.37 ± 1.23 abc	11.07 ± 0.47 abcd	10.01 ± 0.17 bcd
	Silt	28.87 ± 1.87 ab	30.02 ± 3.10 ab	34.59 ± 1.40 abc	39.44 ± 3.68 bc	27.08 ± 0.58 a	43.81 ± 2.62 c	30.57 ± 1.01 ab	37.74 ± 2.29 abc
	Cclay	11.84 ± 1.10 a	22.12 ± 2.92 bcd	16.54 ± 1.06 ab	10.93 ± 1.71 a	21.10 ± 0.64 bcd	17.94 ± 1.24 abc	25.67 ± 1.21 cd	27.02 ± 1.24 d
	Fclay	13.26 ± 1.60 ab	14.81 ± 1.47 abc	19.56 ± 1.79 c	14.97 ± 0.22 abc	16.43 ± 0.88 bc	5.74 ± 0.18 abd	11.44 ± 1.12 abd	9.10 ± 0.57 ad
	Total recovery	93.11 ± 4.54 a*	86.30 ± 3.11 a	86.43 ± 0.70 a	97.79 ± 3.50 a*	87.20 ± 3.39 a	87.71 ± 2.20 a	85.35 ± 1.40 a	90.14 ± 2.97 a*

* indicates that total recovery was not significantly different from 100% in a one-sample t-test

Table 5.3. SOC and C:N data for whole soils and soil fractions at the three sites. Abbreviations as in Table 1. Data are means ± one standard error. Means within the same site and soil fraction with different letters indicate a significant (P≤0.05) difference between the treatments.

		Akron			KBS		Hoytville		
	Soil fraction	Native	Cons. till	Conv. till	Native	Conv. till	Native	No till	Conv. Till
SOC (% of soil fraction mass)	LF	17.56 ± 0.45 a	15.00 ± 0.65 b	21.48 ± 0.43 c	29.23 ± 0.65 a	24.20 ± 0.50 b	22.45 ± 4.36 a	25.32 ± 0.46 a	24.15 ± 1.35 a
	Sand+POM	0.49 ± 0.08 a	0.29 ± 0.02 ab	0.20 ± 0.02 b	0.27 ± 0.02 a	0.21 ± 0.01 a	2.85 ± 0.34 a	1.28 ± 0.10 b	0.98 ± 0.07 b
	Silt	0.69 ± 0.04 a	0.49 ± 0.04 b	0.45 ± 0.03 b	1.70 ± 0.16 a	0.45 ± 0.01 b	4.19 ± 0.11 a	1.49 ± 0.05 b	1.45 ± 0.09 b
	Cclay	2.45 ± 0.14 a	2.07 ± 0.15 a	1.44 ± 0.08 b	5.95 ± 0.33 a	2.47 ± 0.07 b	4.02 ± 0.32 a	2.38 ± 0.04 b	1.92 ± 0.07 b
	Fclay	1.87 ± 0.12 a	1.55 ± 0.01 b	1.22 ± 0.06 c	4.36 ± 0.16 a	2.20 ± 0.14 b	3.24 ± 0.23 a	2.04 ± 0.03 b	1.67 ± 0.06 b
	WS	1.22 ± 0.12 a	0.92 ± 0.03 b	0.66 ± 0.02 b	1.22 ± 0.05 a	0.76 ± 0.03 b	4.70 ± 0.25 a	2.19 ± 0.06 b	1.77 ± 0.08 b
C:N	LF	12.94 ± 0.61 a	13.77 ± 0.56 a	15.86 ± 0.33 b	20.06 ± 1.13 a	16.02 ± 0.75 b	19.55 ± 0.84 a	14.68 ± 0.09 b	16.57 ± 0.82 b
	Sand+POM	8.37 ± 0.72 a	7.13 ± 0.64 a	5.67 ± 0.40 b	7.67 ± 0.42 a	7.19 ± 0.37 a	13.68 ± 0.37 a	11.22 ± 0.34 b	10.78 ± 0.36 b
	Silt	6.49 ± 0.36 a	7.16 ± 0.40 a	6.09 ± 0.22 a	10.86 ± 0.14 a	6.71 ± 0.09 b	11.13 ± 0.18 a	8.65 ± 0.20 b	8.49 ± 0.29 b
	Cclay	6.07 ± 0.10 ab	6.65 ± 0.52 a	5.41 ± 0.05 b	8.86 ± 0.09 a	6.67 ± 0.07 b	7.36 ± 0.13 a	6.03 ± 0.05 b	5.67 ± 0.11 b
	Fclay	6.72 ± 0.46 a	7.08 ± 0.38 a	6.33 ± 0.08 a	9.13 ± 0.33 a	6.83 ± 0.22 b	7.51 ± 0.16 a	5.94 ± 0.22 b	5.57 ± 0.05 b
	WS	8.65 ± 0.25 a	8.13 ± 0.17 a	7.09 ± 0.18 b	10.83 ± 0.38 a	7.37 ± 0.23 b	10.44 ± 0.30 a	7.99 ± 0.03 b	7.58 ± 0.17 b

Table 5.4. Summary data for py-C in whole soils and soil fractions at the three sites. Labels follow the scheme as Table 1. Data are means ± one standard error. Means within the same site and soil fraction with different letters indicate a significant ($P \leq 0.05$) difference between the treatments.

		Akron						KBS				Hoytville					
	Soil fraction	Native		Cons. till		Conv. till		Native		Conv. till		Native		No till		Conv. till	
py-C amount (µg/g whole soil)	LF	63.1 ± 16.5	a	62.8 ± 4.8	a	46.7 ± 1.0	a	62.8 ± 19.8	a	60.2 ± 8.7	a	124.7 ± 19.6	b	56.6 ± 5.6	a	43.9 ± 8.0	a
	Sand+POM	108.8 ± 9.1	a	101.7 ± 14.1	a	73.0 ± 9.9	a	59.5 ± 4.0	a	59.1 ± 12.2	a	92.4 ± 8.6	a	122.2 ± 66.8	a	34.8 ± 4.1	a
	Silt	472.9 ± 7.9	ab	536.6 ± 6.3	a	483.9 ± 9.7	ab	258.7 ± 42.6	c	173.5 ± 11.2	d	860.9 ± 14.4	e	466.1 ± 14.2	ab	418.5 ± 4.4	b
	Cclay	98.5 ± 2.4	a	243.2 ± 39.5	b	135.0 ± 1.9	a	89.8 ± 11.8	a	122.8 ± 11.8	a	446.2 ± 12.5	c	412.9 ± 6.7	c	443.9 ± 3.7	c
	Fclay	185.5 ± 22.1	ab	220.7 ± 39.0	ab	202.3 ± 23.7	ab	104.6 ± 11.1	a	121.0 ± 26.9	a	98.6 ± 23.8	a	284.8 ± 47.5	b	191.6 ± 26.9	ab
	WS	904.3 ± 8.9	a	920.0 ± 5.7	a	951.3 ± 1.8	a	503.1 ± 117.7	b	615.6 ± 39.9	b	1280.2 ± 95.7	c	859.7 ± 1.9	a	885.0 ± 5.7	a
py-C contribution to SOC (mg/g SOC)	LF	20.27 ± 3.70	a	59.97 ± 2.80	bc	106.55 ± 8.54	d	28.34 ± 7.77	a	75.81 ± 5.20	c	33.71 ± 4.49	a	38.82 ± 1.73	ab	38.83 ± 2.08	ab
	Sand+POM	69.39 ± 15.32	abc	139.58 ± 17.37	a	128.99 ± 21.84	ab	34.24 ± 2.84	c	65.24 ± 12.97	abc	16.38 ± 2.66	c	52.83 ± 30.11	bc	19.59 ± 2.01	c
	Silt	138.92 ± 9.60	a	199.03 ± 16.47	b	216.27 ± 13.48	b	53.83 ± 6.02	c	84.42 ± 3.48	c	42.09 ± 1.19	c	69.71 ± 3.16	c	63.21 ± 4.47	c
	Cclay	70.96 ± 4.92	ab	117.28 ± 7.29	cd	125.60 ± 6.53	d	68.43 ± 4.07	ab	77.08 ± 8.07	ab	53.66 ± 4.51	a	73.52 ± 0.97	ab	93.21 ± 3.95	bc
	Fclay	118.36 ± 9.72	ab	162.65 ± 24.52	a	157.08 ± 8.36	a	57.34 ± 4.95	bc	99.80 ± 26.09	abc	35.97 ± 6.53	c	111.34 ± 11.19	abc	120.00 ± 18.61	ab
	WS	77.43 ± 10.00	ab	99.73 ± 2.19	a	145.14 ± 4.71	c	41.93 ± 11.04	d	81.52 ± 6.60	a	27.19 ± 1.14	d	39.25 ± 1.05	d	50.14 ± 2.52	bd
py-C distribution (% of whole soil py-C)	LF	7.02 ± 1.87	ab	6.82 ± 0.51	ab	4.91 ± 0.10	a	12.21 ± 1.47	b	9.81 ± 1.41	ab	9.64 ± 0.90	ab	6.59 ± 0.67	ab	4.97 ± 0.90	a
	Sand+POM	12.03 ± 1.02	a	11.02 ± 1.46	a	7.67 ± 1.04	a	13.43 ± 3.49	a	9.89 ± 2.49	a	7.21 ± 0.27	a	14.21 ± 7.77	a	3.94 ± 0.49	a
	Silt	52.31 ± 1.10	a	58.34 ± 0.89	ac	50.87 ± 1.10	a	53.21 ± 4.55	a	28.46 ± 2.67	b	67.90 ± 4.44	c	54.22 ± 1.77	a	47.30 ± 0.71	a
	Cclay	10.88 ± 0.17	a	26.43 ± 4.28	bc	14.19 ± 0.23	a	19.51 ± 4.17	ab	19.87 ± 0.70	ab	35.33 ± 3.27	c	48.03 ± 0.77	d	50.16 ± 0.49	d
	Fclay	20.55 ± 2.55	ab	24.02 ± 4.33	ab	21.27 ± 2.49	ab	22.38 ± 3.82	ab	20.16 ± 5.13	ab	7.78 ± 1.81	a	33.13 ± 5.53	b	21.69 ± 3.19	ab
	Total recovery	102.80 ± 5.52	ab*	126.64 ± 6.74	bc	98.91 ± 2.70	ab*	120.73 ± 13.58	ab*	88.18 ± 7.16	a*	127.86 ± 7.40	bc*	156.18 ± 3.01	c	128.05 ± 5.24	bc

* indicates that total recovery was not significantly different from 100% in a one-sample t-test

Table 5.5. Comparison of $\delta^{13}C$ values between SOC and py-C for soil fractions. Abbreviations as in Table 1. Value are means ± one standard error. Means within the same site and soil fraction with different letters indicate a significant ($P \leq 0.05$) difference between the $\delta^{13}C$ values.

Site	Soil Fraction	Treatment	SOC $\delta^{13}C$ (‰)	py-C $\delta^{13}C$ (‰)
Akron	LF	Native	-18.38 ± 0.58 a	-22.97 ± 0.19 c
		Cons. till	-23.57 ± 0.05 cd	-23.15 ± 0.08 cd
		Conv. till	-21.38 ± 0.10 b	-24.02 ± 0.09 d
	Sand+POM	Native	-18.42 ± 0.55 a	-21.42 ± 0.37 b
		Cons. till	-23.50 ± 0.13 c	-21.88 ± 0.24 b
		Conv. till	-21.70 ± 0.40 b	-21.39 ± 0.15 b
	Silt	Native	-16.22 ± 0.42 a	-17.11 ± 0.29 ab
		Cons. till	-19.82 ± 0.28 c	-17.55 ± 0.20 b
		Conv. till	-18.98 ± 0.19 c	-16.88 ± 0.13 ab
	Cclay	Native	-15.12 ± 0.32 c	-13.26 ± 0.24 ab
		Cons. till	-18.13 ± 0.26 d	-14.72 ± 0.46 b
		Conv. till	-17.16 ± 0.26 d	-12.94 ± 0.18 a
	Fclay	Native	-15.56 ± 0.39 a	-14.40 ± 0.29 a
		Cons. till	-17.79 ± 0.48 b	-15.23 ± 0.10 a
		Conv. till	-17.00 ± 0.27 b	-14.65 ± 0.13 a
KBS	LF	Native	-27.17 ± 0.38 c	-24.76 ± 0.49 b
		Conv. till	-20.50 ± 0.32 a	-23.89 ± 0.24 b
	Sand+POM	Native	-26.95 ± 0.38 c	-22.17 ± 0.57 b
		Conv. till	-19.00 ± 0.39 a	-20.13 ± 0.29 a
	Silt	Native	-26.04 ± 0.16 c	-22.95 ± 0.34 b
		Conv. till	-21.51 ± 0.07 a	-20.90 ± 0.25 a
	Cclay	Native	-25.16 ± 0.13 d	-22.51 ± 0.20 c
		Conv. till	-21.28 ± 0.38 b	-19.43 ± 0.49 a
	Fclay	Native	-24.87 ± 0.15 b	-24.00 ± 0.83 b
		Conv. till	-21.86 ± 0.38 a	-21.74 ± 0.57 a
Hoytville	LF	Native	-26.75 ± 0.12 c	-24.57 ± 0.12 b
		No till	-18.73 ± 0.53 a	-24.51 ± 0.27 b
		Conv. till	-19.29 ± 0.58 a	-24.26 ± 0.20 b
	Sand+POM	Native	-26.33 ± 0.18 e	-22.64 ± 0.20 d
		No till	-16.41 ± 0.29 a	-20.48 ± 0.19 c
		Conv. till	-18.17 ± 0.07 b	-19.86 ± 0.16 c
	Silt	Native	-26.01 ± 0.05 d	-23.50 ± 0.18 c
		No till	-21.61 ± 0.12 a	-23.02 ± 0.04 bc
		Conv. till	-22.97 ± 0.11 b	-23.21 ± 0.08 bc
	Cclay	Native	-25.24 ± 0.06 d	-22.85 ± 0.02 c
		No till	-22.06 ± 0.02 a	-22.25 ± 0.12 ab
		Conv. till	-22.63 ± 0.03 bc	-22.24 ± 0.17 ab
	Fclay	Native	-25.16 ± 0.08 c	-24.19 ± 0.17 bc
		No till	-21.88 ± 0.13 ab	-21.26 ± 0.08 a
		Conv. till	-21.62 ± 0.36 a	-20.44 ± 1.21 a

Table 5.6. Estimates of MRT for native-derived SOC and non-py-C. Labels follow the scheme as Table 1. Data are means ± one standard error. Means within the same site and soil fraction with different letters indicate a significant (P≤0.05) difference between the treatments. Values within the same site and soil fraction with no letters were not significantly different.

Site	Soil Fraction	Treatment	Native-derived SOC MRT (years)		Native-derived non-py-C MRT (years)	
Akron	LF	Cons. till	29.6 ± 1.2		28.8 ± 1.1	
		Conv. till	31.1 ± 1.2		30.9 ± 1.2	
	Sand+POM	Cons. till	32.6 ± 1.3	ab	28.6 ± 1.9	a
		Conv. till	43.8 ± 3.4	c	41.1 ± 3.2	bc
	Silt	Cons. till	112.8 ± 21.8		89.1 ± 17.9	
		Conv. till	99.7 ± 9.1		79.0 ± 7.5	
	Cclay	Cons. till	198.3 ± 5.1	b	171.0 ± 3.6	ab
		Conv. till	164.2 ± 16.7	ab	130.3 ± 12.9	a
	Fclay	Cons. till	254.8 ± 101.3		231.6 ± 117.6	
		Conv. till	250.9 ± 60.6		191.2 ± 38.1	
Hoytville	LF	No till	23.8 ± 0.8		23.1 ± 0.8	
		Conv. till	21.8 ± 0.7		21.3 ± 0.6	
	Sand+POM	No till	18.8 ± 0.7		17.9 ± 1.4	
		Conv. till	20.3 ± 0.2		20.2 ± 0.2	
	Silt	No till	103.2 ± 3.5	a	98.2 ± 3.6	a
		Conv. till	161.0 ± 6.7	b	155.1 ± 6.3	b
	Cclay	No till	141.5 ± 1.1	a	135.9 ± 1.0	a
		Conv. till	178.0 ± 2.4	b	173.4 ± 3.8	b
	Fclay	No till	135.7 ± 6.4		138.3 ± 7.4	
		Conv. till	126.4 ± 14.2		132.4 ± 8.9	

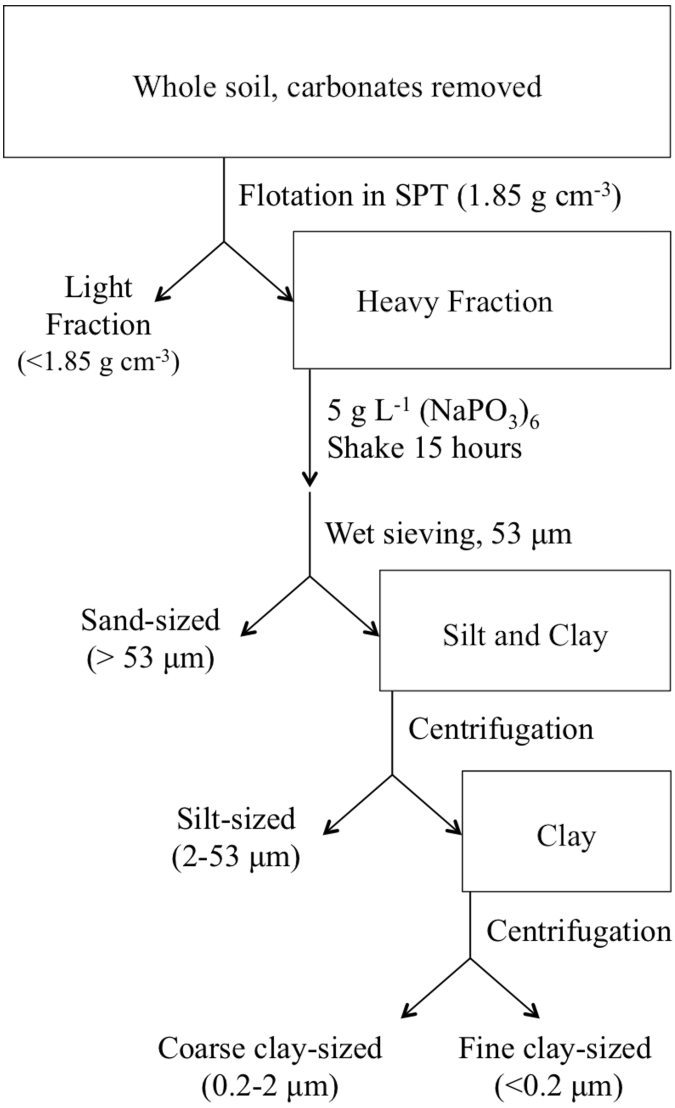

Figure 5.1. Schematic diagram of the fractionation scheme used in this study.

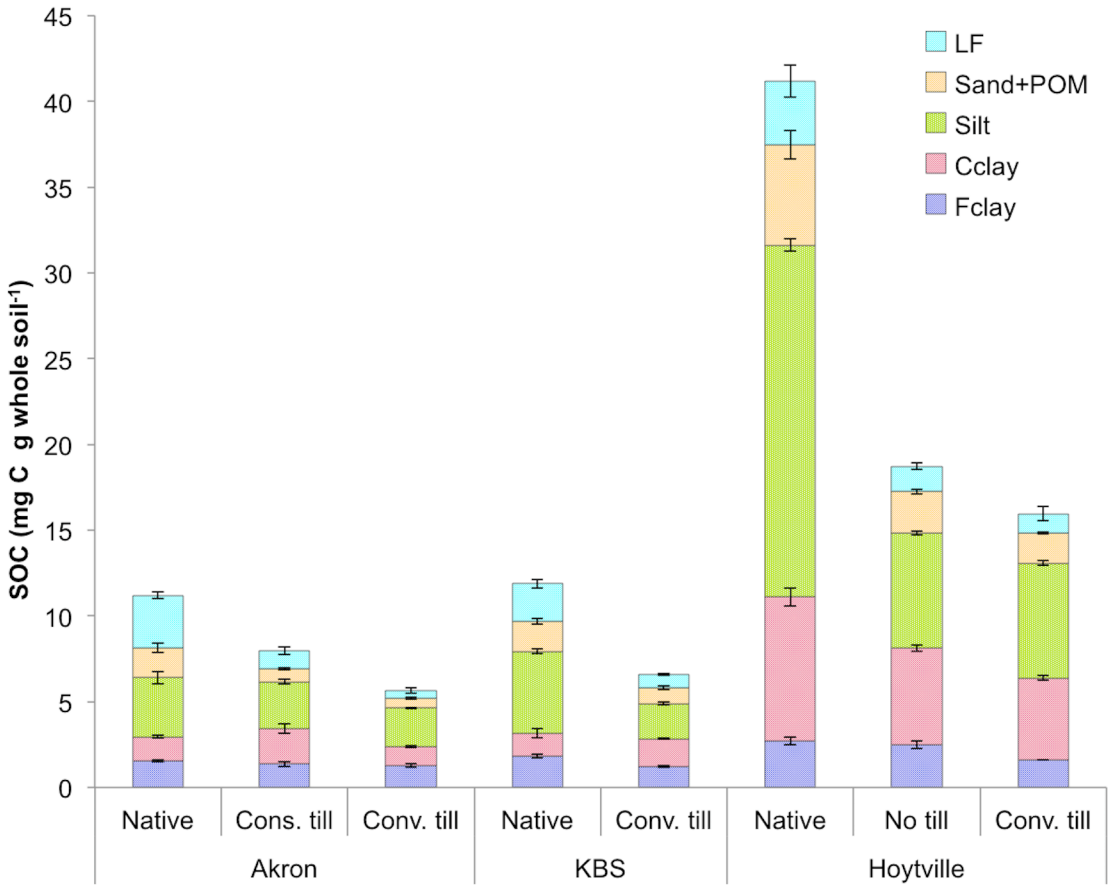

Figure 5.2. Distribution of SOC across soil fractions under each treatment for the three sites. The height of each bar represents the total OC recovered when all soil fractions are summed, and the shaded sections represent the proportion of total C recovered in each soil fraction. Error bars represent ± 1 standard error from the mean.

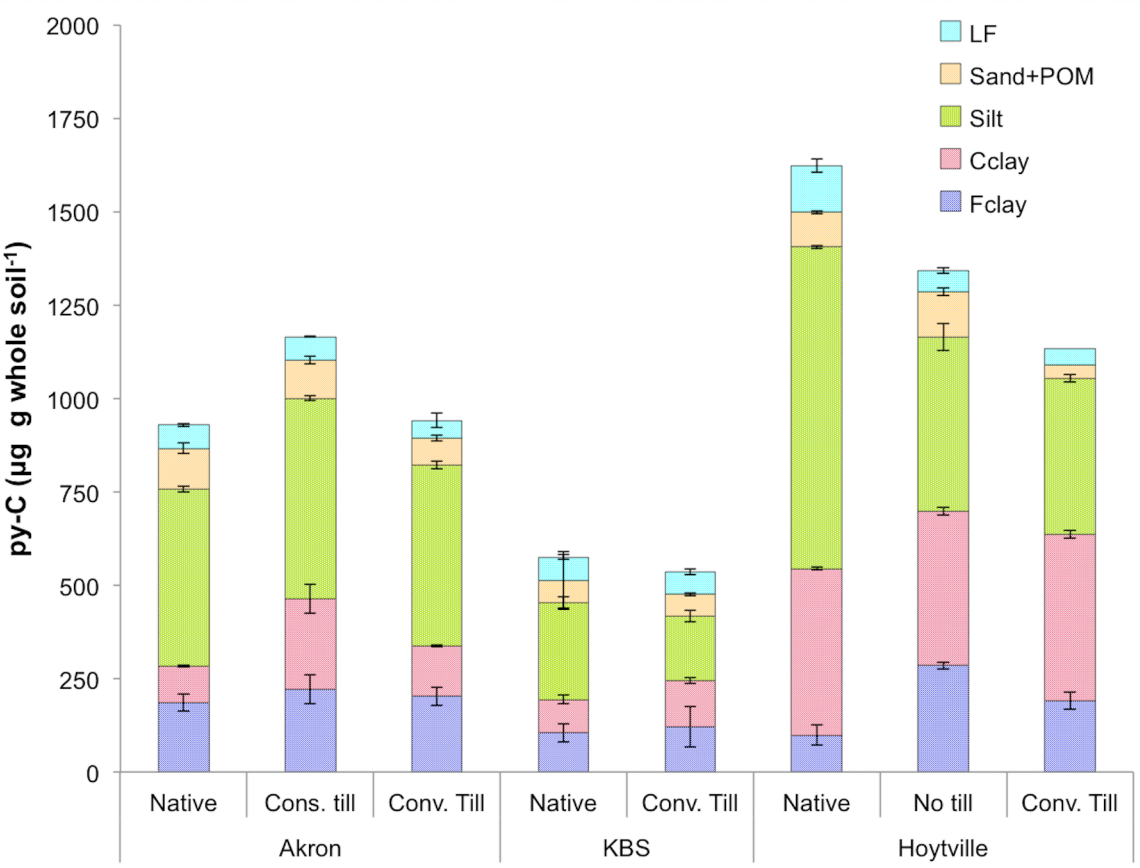

Figure 5.3. Distribution of py-C across soil fractions under each treatment for the three sites. The height of each bar represents the total py-C recovered when all soil fractions are summed, and the shaded sections represent the proportion of total C recovered in each soil fraction. Error bars represent ± 1 standard error from the mean.

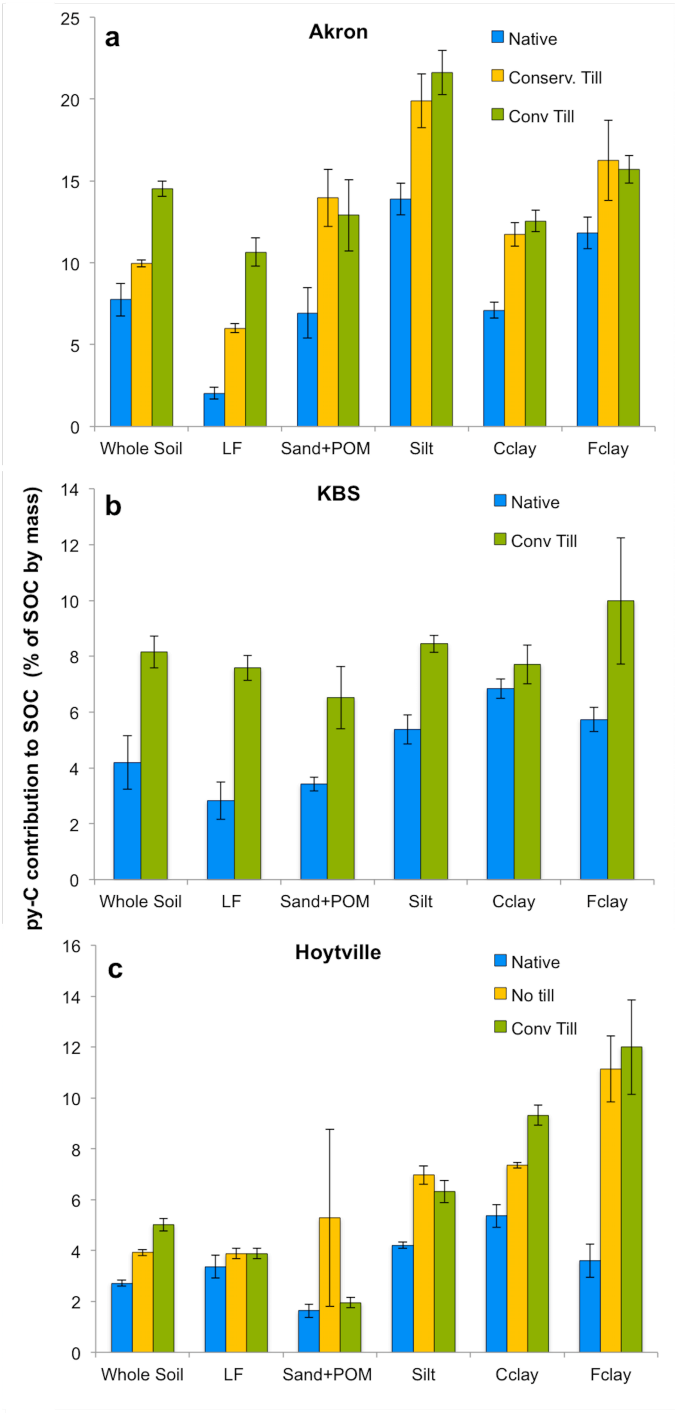

Figure 5.4. Py-C as a percent of SOC in whole soils and in each of the soil fractions at Akron (panel a), KBS (panel b), and Hoytville (panel c). Error bars represent ± 1 standard error from the mean.

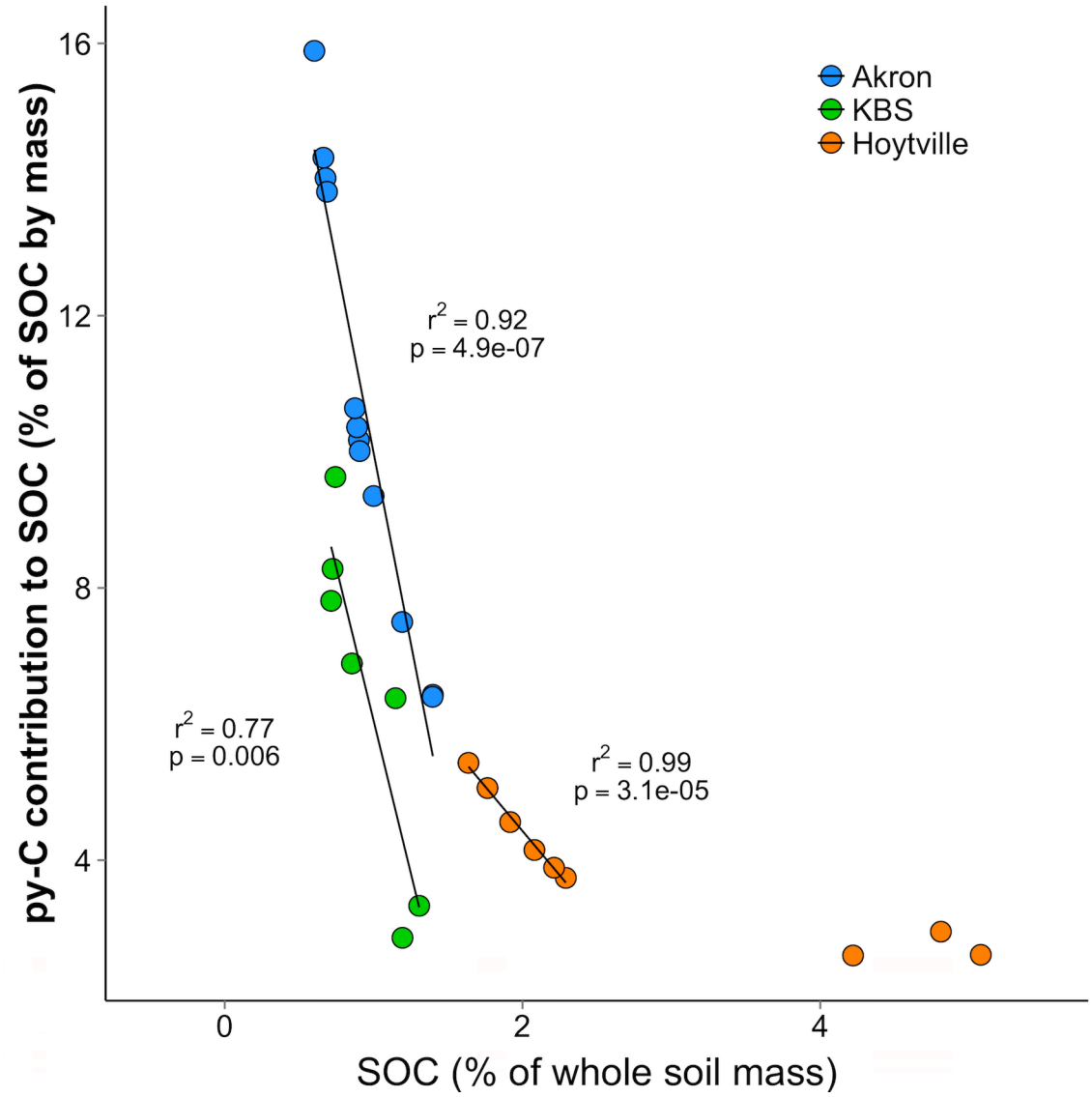

Figure 5.5. Linear regressions of py-C as a percent of SOC versus whole soil SOC at the three sites. All three linear relationships were significantly different from one another (ANCOVA, $p \leq 0.05$). The native Hoytville soils are plotted here but were omitted from the analysis due to their extremely high SOC contents.

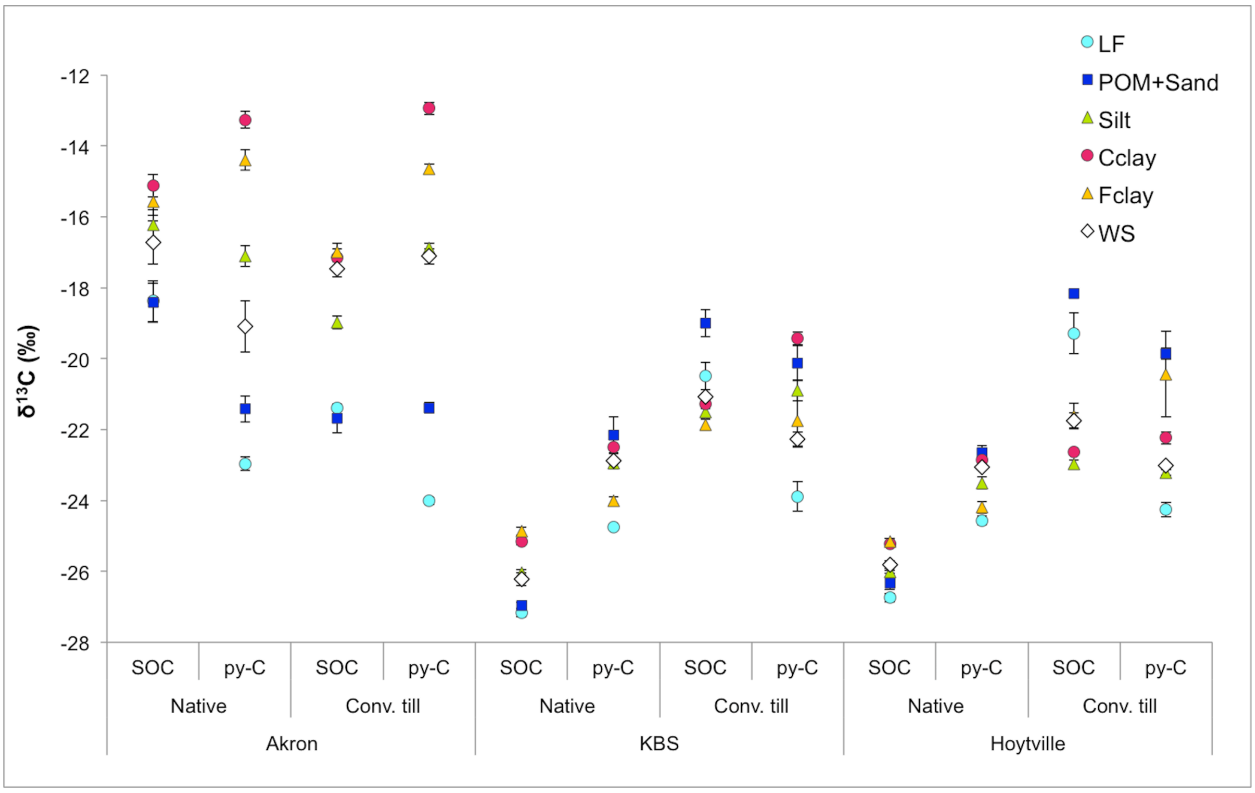

Figure 5.6. Comparison of $\delta^{13}C$ values of TOC and py-C in whole soils and soil fractions for native and conventional till plots at the three sites. At Akron, $\delta^{13}C$ of the cultivated wheat was -24.87 ‰ and that of the native vegetation was -16.1 ‰. At Hoytville and KBS, the $\delta^{13}C$ of the cultivated corn was -13.57 ‰ and -14.1 ‰, respectively, and that of the native vegetation was approximately -27.0 ‰ (Collins et al., 1999; Follett et al., 2007; Haile-Mariam et al., 2008). Error bars represent ± 1 standard error from the mean.

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CHAPTER 6: CONCLUSION

The goal of my dissertation work was to improve our overall understanding of the processes that govern SOM dynamics. I set out to address four research questions:

(1) How do differences in litter quality, indicated by C/N ratio, affect SOM formation and stabilization over the course of decomposition?

(2) How does warming impact the amount of litter-derived SOM formed and stabilized relative to CO_2 produced, hereafter referred to as stabilization efficiency (SE)?

(3) How does warming impact the size of SOM stocks via the balance of SOM formation and decomposition?

(4) Will measuring and accounting for py-OM improve our understanding of how soil organic matter dynamics respond to land use change?

I addressed question (1) using a slurry incubation with ^{13}C- and ^{15}N-labeled leaves or roots, and found that litter quality, defined by C/N ratio, was a major control on the rate and efficiency of SOM formation and stabilization on mineral surfaces, in agreement with the MEMS hypothesis (Cotrufo et al. 2013). This is one of the first studies to compare rates of stabilization and SE for litters of different quality, and it helps emphasize the need to incorporate litter quality controls on stabilization and SE into models of terrestrial C and N cycling.

I used a similar approach to address questions (2) and (3). I performed a slurry incubation with ^{13}C- and ^{15}N-labeled leaves at three temperatures, and found that the responses of stabilization and destabilization to warming were complex. However, there was strong evidence that rates of destabilization from mineral surfaces increased with warming. Our results suggest that a portion of mineral-associated C is highly sensitive to temperature and is readily susceptible

to decomposition upon warming. This study represents a step toward determining whether slowly cycling SOM is sensitive to temperature, and provides a more mechanistic context for much of the previous work on temperature sensitivity of slowly cycling SOM to warming.

I aimed to further address question (3) by investigating the temperature sensitivity of C stocks in different SOM fractions in the field. I used soils from two soil warming experiments, using the unique C isotopic signature of elevated CO_2 to trace the fate of new and old C. I found a trend of faster turnover with warming at one site, and no effect of warming on turnover at the other site. But warming had no effect on total C stocks with warming at either site, suggesting that warming may not alter the balance of inputs and outputs from SOM pools.

Finally, I addressed question (4) using hypy to quantify py-OM from three US agricultural soils. I reevaluated previous results from these soils to better understand how the presence of py-OM may have influenced our previous interpretation. Results of this study suggested that py-OM is ubiquitous, and is preferentially preserved compared to other SOM. Accounting for py-C shortened MRT estimates at one agricultural site, but had little influence on turnover estimates at another site, highlighting the importance of accounting for py-OM in a site-specific manner.

In all, I studied multiple controls on SOM formation, stabilization, and destabilization, and rates of SOM turnover, with the overall goal of advancing mechanistic understanding of SOM dynamics to better predict responses to environmental change. While there remains much to be learned about SOM dynamics and the processes that govern them, I hope to have made an important contribution to the body of knowledge, and that the results of my work will be used to better understand, predict, and manage SOM.