# SOIL-CLIMATE FEEDBACKS: UNDERSTANDING THE CONTROLS AND ECOSYSTEM RESPONSES OF THE CARBON CYCLE UNDER A CHANGING CLIMATE

by

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A DISSERTATION

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DISSERTATION ABSTRACT

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Doctor of Philosophy

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Title: Soil-Climate Feedbacks: Understanding the Controls and Ecosystem Responses of the Carbon Cycle Under a Changing Climate

Soil organic matter (SOM) decomposition and formation is an important climate feedback, with the potential to amplify or offset climate forcing. To understand the fate of soil carbon (C) stores and fluxes (i.e., soil respiration) under future climate it is necessary to investigate responses across spatial and temporal scales, from the ecosystem to the molecular level, from diurnal to decadal trends. Moreover, it is important to question the assumptions and paradigms that underlie apparently paradoxical evidence to reveal the true nature of soil-climate feedbacks. My dissertation includes research into the response of soil respiration in Pacific Northwest prairies to warming and wetting along a natural regional climate gradient (Chapter II), and then delves deeper into the mechanisms underlying SOM decomposition and formation, examining the temperature sensitivity of SOM decomposition of prairie soils that were experimentally warmed for ~2 yr, and a forest soil in which litter-inputs were manipulation for 20 yr (Chapter III), and finally testing soil C cycling dynamics, including mineral-associated C pools, decomposition dynamics, and the molecular nature of SOM itself, under litter-manipulation in order to understand the controls on SOM formation and mineralization (Chapter IV).

This dissertation includes previously published and unpublished coauthored material; see the individual chapters for a list of co-authors, and description of contributions.

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For my parents for teaching me to look,
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# CHAPTER I

## INTRODUCTION

Soil carbon (C) research has become characterized by apparent paradoxes that can only be resolved through careful examination of responses across a range of scales, from regional to local, ecosystem to microbial, diurnal to decadal. The research presented herein falls under two larger themes, (1) the ecosystem-scale response of soil organic matter (SOM) decomposition, as indicated by soil respiration, to simulated climate change across a regional climate gradient (Chapter II) and (2) understanding the mechanisms underlying SOM decomposition and accumulation, specifically (i) the temperature sensitivity of SOM decomposition (Chapter III) and (ii) how litter quantity and quality shape soil C cycling (Chapter IV).

Climate and soils are intrinsically linked. Soils play a key role in regulating climate, acting as a store for carbon in the form of soil organic matter, and the rate of SOM decomposition is in turn regulated by temperature and moisture (Schimel *et al.*, 1994). SOM represents a major C store, estimated at more than twice the C contained in the atmosphere and terrestrial vegetation together (Jobbagy & Jackson, 2000). Soil respiration, the process by which C is released back into the atmosphere as $CO_2$ due to a combination of SOM decomposition and plant root respiration, is among the largest terrestrial C fluxes (Raich & Schlesinger, 1992). Thus perturbations to the balance between soil C storage and soil respiration as SOM is decomposed may have profound repercussions for the C cycle and global climate.

Microbial decomposition of SOM, like any enzymatic process, is sensitive to temperature (Davidson & Janssens, 2006); global warming is thus predicted to increase SOM decomposition and soil respiration, potentially amplifying global warming (Cox *et al.*, 2000). However, soils also have the capacity to absorb C, acting as a C sink even as atmospheric C continues to increase (Stocker *et al.*, 2013), and there has been extensive speculation as to the mechanisms that control and the potential to manipulate this sink (see Dungait *et al.*, 2012). Thus the question is, will soils act as a source or sink of C under ongoing climate change?

Numerous Earth system models (Friedlingstein *et al.*, 2006; Anav *et al.*, 2013) and manipulative field experiments (see meta-analyses Rustad *et al*,. 2001 and Wu *et al.*,. 2011) over the last three decades have endeavored to answer this question, and have deepened both our understanding and our uncertainty of the C cycle and its role in regulating global climate. Different Earth system models range by as much as 15 Pg C in their projection of the response of the terrestrial C sink to climate change (Friedlingstein *et al.*, 2006), depending upon the underlying assumptions. Manipulative experiments have shown a wide range of responses to warming, including positive, negative, and neutral (Rustad *et al.*, 2001; Wu *et al.*, 2011), and soils have frequently been observed to 'acclimate' to warming over time, returning to ambient levels of soil respiration after only a couple of years (Melillo *et al.*, 1993; Luo *et al.*, 2001; Rustad *et al.*, 2001). Such contradictory results have revealed the complexity of soil-climate feedbacks, and provided opportunities to pry open the 'black box' of soil. Efforts in recent years have focused on key areas of uncertainty, including ecosystem-level responses such as the environmental and physical drivers of soil respiration across local to global scales, to the

molecular, mineral, and microbial controls on the formation and decomposition of SOM itself.

Soil respiration has been repeatedly demonstrated to increase exponentially with temperature across biomes and spatial scales in observational studies (Lloyd & Taylor, 1994; Davidson *et al.*, 2006) and with ongoing climate change on a global scale (Bond-Lamberty & Thomson, 2010). This can be attributed to an increase in microbial decomposer activity and thus SOM decomposition, as demonstrated by removing plant roots *in situ*, thus isolating 'heterotrophic soil respiration' (Boone *et al.*, 1998; Luo *et al.*, 2001). Many experiments have reported an increase in soil respiration due to experimental warming in the field (e.g., Melillo et al. 1993, Saleska et al. 1999, Luo et al. 2001), but in some cases the response to warming attenuated after only a couple years and had no measurable effect on SOM content (Rustad *et al.*, 2001). As warming studies have accumulated, this pattern has been attributed variously to a depletion of labile C substrates (Melillo *et al.*, 1993), moisture limitation due to the drying effect of experimental warming (Saleska *et al.*, 1999; Wan *et al.*, 2007; Liu *et al.*, 2009), or an acclimatization of the microbial community to higher temperatures (Luo *et al.*, 2001) such as a change in the microbial C use efficiency (Frey *et al.*, 2013). However, these remain hypotheses without conclusive evidence to support their overall importance or prevalence.

The range of responses to warming has led to an intensive effort to delve into the once apparently unambiguous relationship between soil respiration and temperature. Warming experiments have proliferated in recent decades, exploring the environmental and physical drivers across temporal and spatial scales (Wu *et al.*, 2011), and

emphasizing the potential dominance of context-dependent conditions over large-scale drivers such as climate (Shaver *et al*., 2000). Of particular interest is the role of soil moisture in mediating the temperature response. The importance of soil moisture has been demonstrated in arid, semi-arid (Almagro *et al.*, 2009; Liu *et al.*, 2009; Carbone *et al.*, 2011; Matías *et al.*, 2011; Correia *et al.*, 2012), and experimentally droughty systems (Schindlbacher *et al.*, 2012; Suseela *et al.*, 2012). But though the role of soil moisture has long been recognized (Schimel *et al.*, 1994; Davidson *et al.*, 1998), there has recently been increased interest in its contribution to the attenuation of the warming response and potential to offset warming (Schindlbacher *et al.*, 2012; Suseela *et al.*, 2012). Additionally, the effect of climate change on precipitation regimes is uncertain (Stocker *et al.*, 2013) but an increase in severe and prolonged drought is expected, with as-yet unknown consequences for soil C cycling and soil-climate feedbacks. To resolve this uncertainty, we must examine the influence of precipitation and soil moisture on soil respiration, both locally and across natural climate gradients as soil moisture will interact with both temperature and physical factors, including soil texture, to shape soil respiration dynamics (Schimel *et al.*, 1994). However, to understand these dynamics, ecosystem-level studies must be paired with finer-scale examinations of the biogeochemical and microbial mechanisms.

The wide range of responses to warming has forced the scientific community to examine many of the assumptions underlying our understanding of SOM formation and decomposition and how they are parameterized in Earth system models. Though there are many factors known to be highly predictive of SOM content and decomposition (Schimel *et al.*, 1994), the nature of SOM itself and the microbes that consume it have

been largely treated as a series of black boxes (Pendall *et al.*, 2004). SOM is typically conceptualized as multiple pools of C with varying decomposition rates determined by chemical complexity; thus there is a smaller, rapid turnover pool that represents most of the C respired, and two or more larger, more slow turnover pools that represent the C store (Parton *et al.*, 1987; Coleman & Jenkinson, 1996). The wide range in model projections reported by Friedlingstein *et al*., (2006) demonstrated that models routinely under- or over-estimate soil C responses and many in the literature have argued the need to explicitly model underlying microbial and molecular mechanisms (Davidson *et al.*, 2012; Frey *et al.*, 2013; Tang & Riley, 2015).

Several key factors contributing to uncertainty in modeled SOM dynamics have been identified and received critical attention in recent years. There has been an effort to incorporate enzyme kinetics as defined by the Michaelis-Menton equation to explicitly model substrate, oxygen, and moisture limitation and their effects on the typically Arrhenius-type soil respiration-temperature relationship (Davidson *et al.*, 2012), potentially helping to explain the threshold and acclimation dynamics seen in warming experiments. Additionally, there have been attempts to explicitly parameterize microbial physiological dynamics, especially C use efficiency as it may be the underlying mechanism behind soil respiration acclimation to warming (Allison *et al.*, 2010; Frey *et al.*, 2013). However, perhaps no decomposition dynamic has been more debated than the temperature sensitivity of the so-called 'recalcitrant' soil C pool (Davidson & Janssens, 2006; von Lützow & Kögel-Knabner, 2009a; Sierra, 2011).

Typically, SOM decomposition is predicted to increase exponentially with temperature as described by an Arrhenius function with a temperature sensitivity ($Q_{10}$,

quotient of rate for every 10$^{o}$C increase) of two (Todd-Brown *et al.*, 2013). Many have criticized the use of a single temperature sensitivity for all SOM pools (Davidson & Janssens, 2006; Conant *et al.*, 2008, 2011; von Lützow & Kögel-Knabner, 2009a) given that they are thought to vary in chemical complexity and thus temperature sensitivity. However, this debate has been complicated by a challenge to assumptions about the nature of SOM and soil C accumulation itself (Kleber & Johnson, 2010; Schmidt *et al.*, 2011).

The temperature sensitivity of SOM decomposition is a key parameter shaping model predictions of future SOM stores and soil respiration rates (Tang & Riley, 2015). If the older, slower, larger C pool is more chemically complex and thus resistant to decomposition, then enzyme kinetics predicts it will be more sensitive to temperature due to its higher activation energy (Davidson & Janssens, 2006). Consequently, models that use a single temperature sensitivity may be underestimating the potential loss of soil C in a warming world. The idea that the larger, slower decomposing pool is more temperature sensitive is called the carbon quality-temperature (CQT) hypothesis (Bosatta & Ågren, 1999). Though the theoretical basis for this hypothesis is not in question, there is no consensus in the literature as to the empirical nature of the temperature sensitivity of SOM. Results vary widely, from neutral, positive, to negative (Conant *et al*., 2011) depending upon the biome and method used. Conant *et al*., (2011) argued that much of the confusion has stemmed from a lack of consistency in methodology, failing to isolate the 'slow' C pool effectively.

However, a separate but related body of literature has begun to shift the view on the nature of SOM itself, challenging the foundation upon which the CQT hypothesis is

built (Kleber, 2010; Schmidt *et al.*, 2011). The chemical nature of SOM is difficult to determine; it exists as an amalgam of organic molecules, from fresh plant residues to microbial byproducts in varying states of oxidation/degradation, all decomposing simultaneously (Kleber & Johnson, 2010). It is well understood that C entering soils as plant litter varies in 'quality' or chemical complexity, dictating its decomposition rate in the litter layer (Kleber & Johnson, 2010). Historically, it was hypothesized that this relationship would continue in the soil itself, with more complex plant residues such as lignin being preferentially stabilized in the 'recalcitrant' SOM pool, and this view was supported by evidence of large molecular weight molecules termed 'humic residues' (Kleber & Johnson, 2010). However, recent studies using advanced molecular-imaging techniques to examine the nature of SOM have found little evidence for 'humics' in mineral soils (Lehmann *et al.*, 2008); rather, relatively low complexity compounds, such as polysaccharides, have been found to have turnover times on the order of decades to centuries (Kleber *et al.*, 2011; Schmidt *et al.*, 2011). Thus chemical complexity is not a necessity for stabilization in mineral soils and temperature sensitivity need not necessarily increase as decomposition rate decreases if the SOM itself is not chemically 'resistant'.

It has become apparent that interactions between SOM and the mineral matrix itself determine C accumulation and decomposition rates as SOM is physically or chemically protected from degradation (Conant *et al.*, 2011; Dungait *et al.*, 2012). The degree of mineral-association is highly correlated with SOM turnover rate and degree of degradation as it becomes less plant-like and more microbial (Sollins *et al.*, 2006). Mineral-association ranges from minimal, as in particulate organic matter, to occlusion of

SOM within soil aggregates that range in size and stability, to adsorption onto the surfaces of minerals themselves (Christensen, 2001). These soil characteristics have long been recognized as critical to soil C cycling and methods have been developed to quantify them as ecologically meaningful C pools, e,g., sequentially separating them via density fractionation in a heavy liquid (Golchin *et al.*, 1994; Christensen, 2001; Sollins *et al.*, 2006). However, the turnover dynamics and relative temperature sensitivity of these mineral-defined C pools have only begun to be tested (Conant *et al.*, 2011), much less incorporated into Earth system models.

In addition to uncertainty about the controls on soil C stabilization, it has become clear that the exact pathway C takes between litter and soil is poorly understood, including the influence of litter quantity and quality, as well as physical and climate factors (Lajtha *et al.*, 2014a). Long-term litter manipulation studies have been developed with the aim of understanding soil C cycling on decadal and longer scales (Nadelhoffer *et al.*, 2004; Bird *et al.*, 2008; Lajtha *et al.*, 2014a; Lefèvre *et al.*, 2014; Hatton *et al.*, 2015), as well as the potential to augment the natural C sink in an effort to offset climate change (Dungait *et al.*, 2012). These studies have revealed responses to litter-input manipulation to be as variable as the soil respiration response to warming.

Increases in soil C due to litter addition have been found after as little as 8 yr (Fekete *et al.*, 2014) to 28-50 yr (Lajtha *et al.*, 2014a), while other sites showed no response after 5 yr (Crow *et al.*, 2009) and 20 yr (Bowden *et al.*, 2014; Lajtha *et al.*, 2014b). Notably, although there was no overall increase in soil C in a site located in the Bousson Experimental Forest, Pennsylvania (Bowden *et al.*, 2014), there was an increase in the C content of some aggregate fractions, indicating that accumulation may be

ongoing (Mayzelle *et al.*, 2014). However, these results are complicated by the potential for priming (Kuzyakov *et al.*, 2000), whereby a lack of response or decrease in C content under litter addition may have been due to either short-term or ongoing decomposition of native C in the presence of fresh, labile inputs (Crow *et al.*, 2009; Bowden *et al.*, 2014; Lajtha *et al.*, 2014a). Density fractionation revealed that both C accumulation and losses (due to litter exclusion) were largely attributable to the free, light fraction (<1.6-1.85 g $cm^{-3}$ sodium polytungstate) (see Lajtha *et al*., 2014a, 2014b), with two sites reporting C loss from the mineral fractions (Lajtha *et al.*, 2014a; Mayzelle *et al.*, 2014). This indicates that the mineral-associated fractions were relatively stable and that C likely cannot be sequestered in the oldest fractions through direct litter addition in the short-term (Bowden *et al.*, 2014) even if the soils are not yet C saturated (Mayzelle *et al.*, 2014).

Additionally, the role of litter 'quality' in soil C accumulation has proven largely context dependent. It has been hypothesized that root C may be preferentially stabilized as it is already in the soil and roots tend to contain slowly decomposing compounds such as suberin (Rasse *et al.*, 2005). However, there is evidence that roots may contribute more to the younger, free particulate fraction than the mineral fraction in coniferous forest sites (Bird *et al.*, 2008; Crow *et al.*, 2009; Hatton *et al.*, 2015), whereas roots may contribute more to the 'stable' fractions in a deciduous forest site (Crow *et al.*, 2009). However, comparisons of root versus aboveground litter exclusion in forests have found relatively few differences in C content or stability (Crow *et al.*, 2009; Bowden *et al.*, 2014; Lajtha *et al.*, 2014a).

**Dissertation research**

Chapter II is entitled "Soil respiration response to climate change in Pacific Northwest prairies is mediated by a regional Mediterranean climate gradient" and was co-authored by Bart R. Johnson, Laurel Pfeifer-Meister, and Scott D. Bridgham. We examined the response of soil respiration to a full factorial warming and wetting experiment along a 520 km climate gradient in prairies in the Pacific Northwest (PNW), USA. We used this regional climate gradient to ask (1) how temperature and soil moisture interact seasonally to control soil respiration and (2) whether the response of soil respiration is primarily mediated by site-specific factors or regional climate. We hypothesized that (1) the stimulatory effect of warming on soil respiration would be inhibited by seasonal soil moisture limitation, and the duration of inhibition would increase with drought severity along the latitudinal climate gradient; (2) projected climate changes in the PNW would (i) deepen the seasonal moisture deficit in southern PNW prairies and (ii) shift the moisture-deficit gradient northward, resulting in inhibition of annual soil respiration in the south and no response in the north; and (3) that climate effects are more important than site-specific effects in determining soil respiration at a regional scale.

Chapter III is entitled "The carbon quality-temperature hypothesis fails to consistently predict temperature sensitivity in two manipulative ecosystem experiments" and was co-authored with Kate Lajtha, Richard D. Bowden, Bart R. Johnson, and Scott D. Bridgham. We tested the temperature sensitivity of SOM decomposition in soils from four sites representing two different biomes and two experimental manipulations of soil carbon dynamics: (1) a Northeastern deciduous forest under 20 years of chronic *in situ*

input manipulation (Detritus Input and Removal Treatment, hereafter DIRT), and (2) three Pacific Northwest prairies differing in carbon content and basal respiration, and exposed to ~20-26 months of experimental warming and wetting (Heating of Prairies Study, hereafter HOPS). If the CQT hypothesis is true, temperature sensitivity should increase (1) as labile substrates are depleted following leaf or root litter exclusion, (2) by soil depth, given that fresher SOM is found at the surface, (3) in experimentally warmed soils, where accelerated decomposition may have reduced labile SOM, (4) as soil respiration rates decrease, assuming high rates reflect greater soil carbon availability, and (5) with increasing incubation time with the progressive depletion of labile carbon. Furthermore, we examined temperature sensitivity with two different metrics: apparent $Q_{10}$ calculated from the ratio of time to decompose an initial labile and second, more 'recalcitrant' percentage of carbon and activation energy ($E_a$) throughout the duration of the incubation. Overall, we asked whether temperature sensitivity was consistent with the CQT hypothesis across carbon quality proxies, temperature sensitivity metrics, and experimental and environmental contexts.

Chapter IV is entitled "Insights into soil C cycling from long-term input-manipulation and high-resolution mass spectroscopy" and was co-authored with Kate Lajtha, Richard D. Bowden, Malak Tfaily, Bart R. Johnson, and Scott D. Bridgham. We asked how C cycles through terrestrial soils by combining a 20 year long chronic root and litter input manipulation in a northeastern deciduous forest with a long-term laboratory incubation, and comparing whole soil responses with C pools defined by mineral association. We asked if litter input manipulation changed C quantity and which C pools were most vulnerable to change. Furthermore, we directly examined the molecular

nature of C in the fine mineral fraction, demonstrated to be the oldest and thus considered the most stable pool of C (Christensen, 2001; Sollins *et al.*, 2006), to test its chemical complexity and putative stability. Finally, we asked whether changes in the total amount, density fractions, or molecular composition of mineral-associated C could explain C mineralization rates.

In Chapter V I summarize the results of the proceeding chapters (II-IV) and discuss the implications for future research.

# CHAPTER II

# SOIL RESPIRATION RESPONSE TO CLIMATE CHANGE IN PACIFIC NORTHWEST PRAIRIES IS MEDIATED BY A REGIONAL MEDITERRANEAN CLIMATE GRADIENT

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## Contributions

L.L. Reynolds collected the soil respiration data, analyzed, and wrote the manuscript. B.R. Johnson, L. Pfeifer-Meister, and S.D. Bridgham designed and established the warming experiment and edited the manuscript.

## Introduction

Soil respiration is the second largest terrestrial ecosystem carbon (C) flux (Schimel, 1995). An increase in $CO_2$ flux from soils may amplify climate forcing (Cox *et al.*, 2000), and this has led to intensive efforts to determine the drivers of soil respiration and to model respiration's potential response to climatic perturbations. Enzyme kinetic theory predicts that microbial respiration will increase with temperature (Davidson & Janssens, 2006), and this prediction has been repeatedly supported by field observations (e.g., Raich and Schlesinger 1992; Lloyd and Taylor 1994; Bond-Lamberty and Thomson

2010). Accordingly, Earth system models include a direct positive effect of temperature on soil respiration, typically in terms of an Arrhenius or $Q_{10}$ function (Todd-Brown *et al.*, 2013).

Manipulative climate change experiments have demonstrated that the response of soil respiration to warming can be complex. Studies have found an initial increase in soil respiration that attenuated after only a few years, an increase but no attenuation, a decrease, or no response at all (Saleska *et al.*, 1999; Rustad *et al.*, 2001; Wan *et al.*, 2007; Zhou *et al.*, 2007; Liu *et al.*, 2009). However, a meta-analysis by Rustad *et al.* (2001) suggested a trend of a decreasing effect size of warming in studies of three years or longer. The apparent inconsistency of the temperature response has been attributed to interactions with biotic and environmental factors, including changes in microbial C use efficiency (Frey *et al.*, 2013), substrate availability (Melillo *et al.*, 2002), and soil moisture limitation (Saleska *et al.*, 1999; Wan *et al.*, 2007; Liu *et al.*, 2009). In addition, differences in initial conditions and site-specific factors may underlie the variability in observed responses (Shaver *et al.*, 2000). To accurately project soil respiration and C cycling responses to climate change requires understanding the multiple interactive controls on the temperature response of soil respiration (Norby & Luo, 2004; Conant *et al.*, 2011).

The importance of soil moisture in controlling soil respiration has been long recognized (Schimel *et al.*, 1994; Davidson *et al.*, 1998), but its role in mediating the temperature response of soil respiration is poorly understood. It is well documented that soil moisture, rather than temperature, primarily controls soil respiration in arid and semi-arid ecosystems (Almagro *et al.*, 2009; Liu *et al.*, 2009; Carbone *et al.*, 2011; Matías *et*

*al.*, 2011; Correia *et al.*, 2012). Antecedent soil moisture conditions may be more predictive of soil respiration than instantaneous rates in semi-arid (Cable *et al.*, 2008, 2011) and dry Mediterranean (Almagro *et al.*, 2009) systems. Soil moisture limitation also has been demonstrated to inhibit the temperature response of soil respiration under experimental drought in mesic ecosystems (Schindlbacher *et al.*, 2012; Selsted *et al.*, 2012; Suseela *et al.*, 2012; Suseela & Dukes, 2013). Anomalies in both annual temperature and precipitation were found to be important controls over soil respiration in a global dataset (Bond-Lamberty & Thomson, 2010) and relatively low-frequency extreme events such as severe droughts may strongly influence the overall terrestrial C cycle (Reichstein *et al.*, 2013). However, current Earth system model projections do not agree on the response of soil respiration to soil moisture, particularly to drought and anaerobic conditions (Falloon *et al.*, 2011). The models do not fully capture the influence of soil moisture on soil respiration (Falloon *et al*., 2011), and thus shifts in precipitation regimes with changing climate, such as increased frequency and severity of drought, could further complicate the already challenging task of projecting soil respiration responses to increasing global temperatures.

Intra- and inter-annual variations in climate along natural gradients have been used to examine the interactive influences of soil temperature and soil moisture on soil respiration (e.g., Davidson *et al*., 1998; Lavigne *et al*., 2004). However, these relationships may not be readily extrapolated to the novel conditions expected under climate change (Schindlbacher *et al.*, 2012). Meanwhile, most manipulative climate change studies are restricted to a single location, limiting the ability to deconvolve context-specific versus regional-scale dynamics. This limitation may be particularly

crucial when considering how changes in precipitation regimes may interact with temperature and site-level factors, such as soil texture, nitrogen (N) availability, and plant productivity and phenology, to control soil respiration.

To address these issues, we examined soil respiration response to a full factorial warming and increased precipitation intensity experiment embedded within a 520 km natural temperature and moisture gradient in prairies in the Pacific Northwest (PNW), USA. This region experiences a Mediterranean climate with cool, moist winters and warm, dry summers (i.e., seasonal drought), creating an asynchrony between maximum temperatures and soil moisture availability. In general, average annual temperatures and the duration of summer drought increase from north to south. Furthermore, climate models for the PNW project an increase in temperature and a shift toward increased wet-season precipitation, and in some cases drier summers (Mote & Salathé, 2010), potentially deepening and lengthening summer drought. We used this regional climate gradient to ask (1) how temperature and soil moisture interact seasonally to control soil respiration and (2) whether the response of soil respiration is primarily mediated by site-specific factors or regional climate. We hypothesized that (1) the stimulatory effect of warming on soil respiration would be inhibited by seasonal soil moisture limitation, and the duration of inhibition would increase with drought severity along the latitudinal climate gradient; (2) projected climate changes in the PNW would (i) deepen the seasonal moisture deficit in southern PNW prairies and (ii) shift the moisture-deficit gradient northward, resulting in inhibition of annual soil respiration in the south and no response in the north; and (3) that climate effects are more important than site-specific effects in determining soil respiration at a regional scale.

## Materials and Methods

### *Site Description and Climate Gradient*

Our study was conducted at three upland remnant prairies spanning 520 km south to north, located in southwestern Oregon (SOR), central-western Oregon (COR), and central-western Washington (WA) (Table 2.1). Extant plant cover, largely introduced perennial grasses at all sites, was removed with the herbicide glyphosate, raking, and mowing during Summer 2009. Plots were seeded in Fall 2010 with a common mix of native graminoid and forb species (Pfeifer-Meister *et al.*, 2013), which established alongside the native and non-native prairie species that had re-emerged. Plots quickly reached typical aboveground plant biomass by the following winter and spring when soil respiration measurements were begun (as determined by Normalized Difference Vegetation Index [NDVI]; see Supplemental Figures in Appendix A, Fig. S2.1). The dominant species in SOR by cover were mostly annuals (e.g., *Bromus hordeaceus, Trifolium subterraneum*, and *Erodium cicutarium)*; in COR, a mixture of perennials and annuals (*Agrostis capillaris, Briza minor, Koeleria macrantha, Prunella vulgaris, Achillea millefolium*, and *Trifolium subterraneum);* and in WA, primarily perennials (*Leucanthemum vulgare*, *Prunella vulgaris, Eriophyllum lanatum*, and *Agrostis capillaris*).

While these sites are all remnant upland prairies, it is important to note that they are distinct in soil texture, organic C content, and N availability (Table 2.1), as is likely for any three widely dispersed sites. Any of these factors may influence soil respiration and its response to warming. In particular, WA had a sandy soil which reduced its water

**Table 2.1.** Location, climate, soil descriptions (assessed on 10 cm deep soil cores), and productivity for each site. Standard errors are in parentheses. SOR = southern Oregon, COR = central Oregon, WA = Washington. Small letters indicate significant site differences ($p < 0.05$).

| Location | | | SOR | COR | WA |
|---|---|---|---|---|---|
| Latitude; Longitude | | | 42°16'41"N; 123°38'34" W | 44°01'34"N; 123°10'56" W | 46°53'47" N; 122°44'06" W |
| Elevation (m) | | | 394 | 165 | 134 |
| Air Temperature (°C) | | | | | |
| Monthly | Mean | PRISM* | 12.3 | 11.4 | 10.5 |
| | | 2011 | 9.8 (1.9) | 10.3 (1.7) | 9.6 (1.5) |
| | | 2012 (Jan-Jun) | 8.3 (2.0) | 8.4 (1.7) | 8.2 (1.7) |
| | Maximum | PRISM* | 20.2 | 17.3 | 15.6 |
| | | 2011 | 19.3 (2.6) | 16.5 (2.2) | 15.8 (1.9) |
| | | 2012 (Jan-Jun) | 16.9 (2.6) | 14.5 (2.0) | 14.1 (2.1) |
| | Minimum | PRISM* | 4.4 | 5.4 | 5.3 |
| | | 2011 | 2.1 (1.0) | 5.6 (1.2) | 4.2 (1.1) |
| | | 2012 (Jan-Jun) | 1.07 (1.1) | 3.9 (1.3) | 3.1 (1.2) |
| Precipitation (mm) | | | | | |
| | Annual | PRISM* | 1434 | 1134 | 1196 |
| | | 2011 | 1203 | 918 | 1242 |
| | | 2012 (Jan-Jun) | 967 | 857 | 801 |
| Soil | | | | | |
| Taxonomy** | | | Loamy-skeletal, mixed, superactive, mesic Entic Ultic Haploxerolls | Very-fine, smectitic, mesic Vertic Haploxerolls | Medial-skeletal over sandy or sandy-skeletal, amorphic over isotic, mesic Typic Melanoxerands |
| | | Series | Takilma cobbly loam | Hazelair silty clay loam | Spanaway gravelly sandy loam |
| Texture (%) | | sand | 31.5 (0.5)$^{a}$ | 36.5 (0.5)$^{b}$ | 75.0$^{c}$ |
| | | clay | 31.0 (2.0)$^{a}$ | 14.5 (0.5)$^{b}$ | 3.5 (0.5)$^{c}$ |
| | | silt | 37.5 (1.5)$^{a}$ | 49.0 (1.0)$^{b}$ | 21.5 (0.5)$^{c}$ |
| | | gravel | 20 | 16 | 20 |
| Bulk density (g soil $cm^{3\ -1}$) | | | 1.04 | 0.60 | 0.82 |
| | | pH | 6.45 (0.01)$^{a}$ | 5.83 (0.04)$^{b}$ | 5.57 (0.05)$^{c}$ |
| | | %C | 3.42 (0.06)$^{ac}$ | 4.18 (0.12)$^{bc}$ | 3.78 (0.20)$^{c}$ |
| | | %N | 0.34 (0.01)$^{a}$ | 0.46 (0.01)$^{a}$ | 0.37 (0.06)$^{a}$ |
| Resin N availability (µg 10 $cm^{-2}$ $year^{-1}$) 2011 | | | 207 (32)$^{a}$ | 21 (2)$^{b}$ | 17 (2)$^{b}$ |
| Aboveground NPP ($g/m^2$) 2011 | | | 378 (16)$^{a}$ | 357 (44)$^{a}$ | 214 (17)$^{b}$ |
| Belowground NPP ($g/m^2$) 2011 | | | 190 (13)$^{ab}$ | 278 (48)$^{bc}$ | 338 (43)$^{c}$ |

* PRISM model for the period 1981– 2010 (http://www.prism.oregonstate.edu/) ** Natural Resources Conservation Service, United States Department of Agriculture (USDA), Web Soil Survey (http://websoilsurvey.nrcs.usda.gov/)

holding capacity and thus its overall moisture content, though more of that moisture may be biologically available compared to the more clay-rich COR and SOR soils (see Kramer and Boyer 1995). SOR and COR had the highest percent soil C, which may indicate increased C substrate availability, while SOR had the highest inorganic N availability. The sites also varied in above- and belowground net primary productivity (NPP) in 2011 (Table 2.1), with WA having lower aboveground NPP than the other two sites, and higher belowground NPP than SOR. Reflecting their mixture of annual and perennial plants and climate, SOR vegetation reached peak biomass and senesced earlier in the year than COR and WA vegetation (Fig. S2.1).

Long-term climate data for this region shows the highest average and maximum temperatures in SOR and lowest in WA, with the lowest minimum temperatures in SOR (Table 2.1). On average, SOR receives greater mean annual precipitation than COR and WA (Table 2.1), but it falls primarily between November and March, with less precipitation than the other two sites in spring and summer (Fig. S2.1). COR and WA have more similar precipitation patterns. Climate data collected at each site (see Soil respiration and climate measurements) for 2011 and 2012 indicate less divergence in precipitation and monthly mean and maximum daily air temperature among sites than the long-term trends would suggest, but greater divergence for monthly minimum daily air temperature (Table 2.1; Fig. S2.2, S2.3). Both 2011 and 2012 were La Niña years, which typically results in wetter and colder conditions than average in the Pacific Northwest (www.esrl.noaa.gov/psd/people/klaus.wolter/MEI/). This may have contributed to a narrowing of the temperature gradient, as SOR experienced lower mean minimum air temperatures in 2011 and 2012 than its historical average (Table 2.1, Fig. S2.3c). Despite

the La Niña influence, total annual precipitation was lower in both SOR and COR than the long-term average (Table 2.1). However, the seasonality of precipitation created a gradient of soil moisture availability: Soil began to dry down in mid to late spring (Fig. 2.1) as precipitation waned (Fig. S2.2), occurring earlier in SOR (Apr.) than COR and WA (May); soil wet-up began in September at all sites but reached saturation more rapidly in WA (Oct.) than in COR and SOR (Nov.).

*Experimental Design*

Each site consisted of twenty 7.1 $m^2$ circular plots in a fully factorial design of 2.5-3$^{o}$C warming, 20% increased precipitation intensity, and ambient conditions (n=5 for each treatment). Precipitation treatments were begun in spring 2010, and all heating treatments were in operation by fall 2010. The plots were heated so that the surface temperature was 3°C above ambient until August 2011, after which the heating was reduced to 2.5°C above ambient to reduce electricity costs. The degree of warming was intended to reflect 3$^{o}$C mean warming predicted for this region by the 2080s (Mote & Salathé, 2010). Warming was achieved with six overhead 2000 W Kalglo infrared heaters (Bethlehem, PA, USA) angled at a 45° angle to the surface of each plot (Kimball *et al.*, 2008); dummy lamps were hung in control plots to control for shading. Precipitation was collected on site with polycarbonate sheets and stored in a cistern. The quantity of water needed to achieve the precipitation treatment was calculated from the total precipitation measured onsite, and applied with a gauged hose within two weeks of

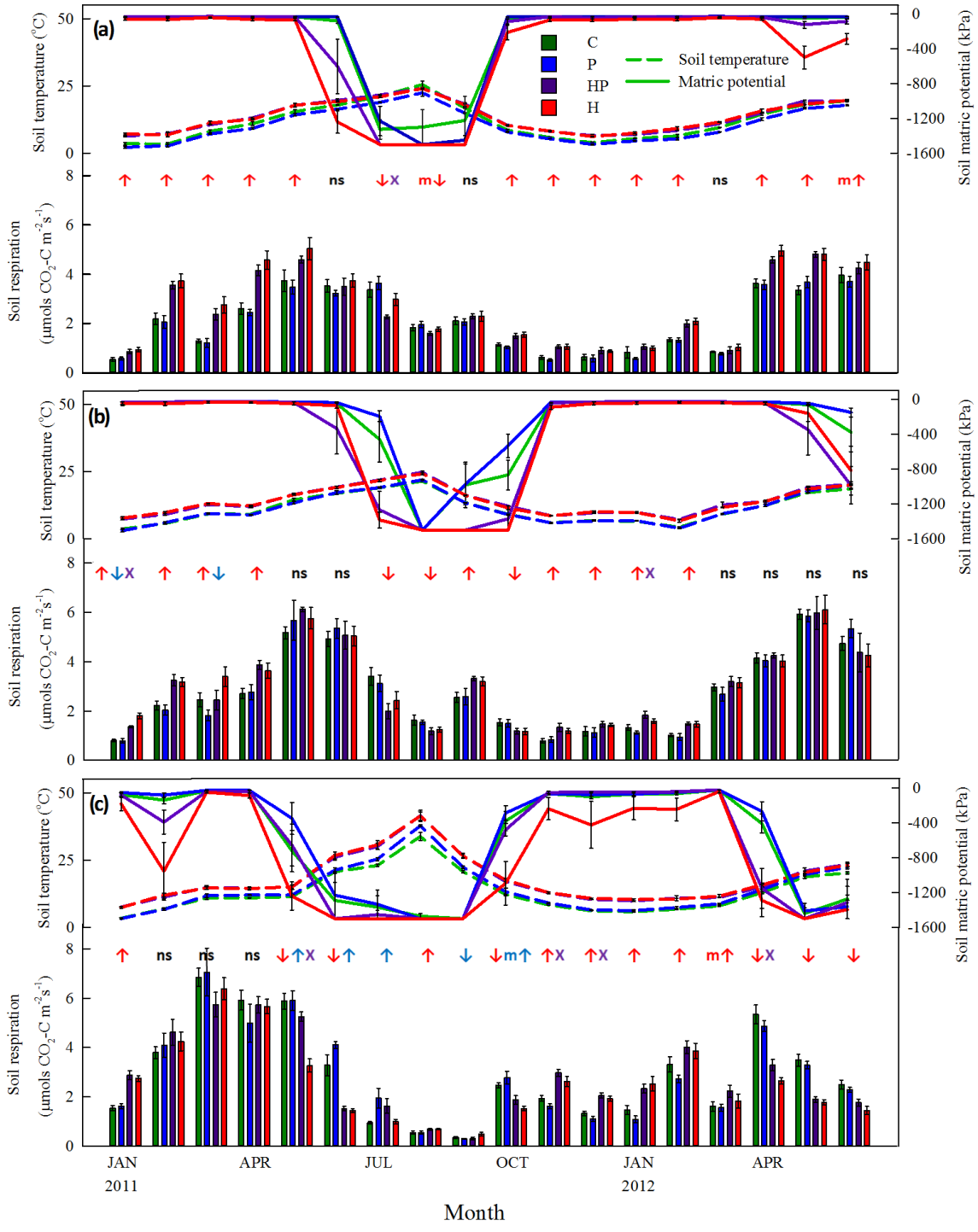


**Fig. 2.1.** Soil respiration, matric potential, and temperature for (a) WA, (b) COR, (c) SOR from Jan. 2011 to Jun. 2012. Arrows indicate significant ($p < 0.05$), marginal (m, $0.05 < p < 0.10$) and non-significant (ns, $p > 0.10$) site-level heat (red) and precipitation (blue) effects. X's indicate heat x precipitation interactions.

collection, resulting in an increase in wet season precipitation intensity but little change in dry season precipitation. Our precipitation treatment is consistent with predictions from Mote and Salathé (2010) that the PNW will experience an enhanced seasonal precipitation cycle, with more rainfall during the normal rainy season and decreased summer rainfall. An increase in precipitation intensity has been observed in the U.S. over the 20th century (Groisman *et al.*, 2004) and is predicted to occur globally with future climate change (Meehl *et al.*, 2007).

*Soil Characterization, NDVI, and Productivity*

Soil taxonomy was obtained from the Natural Resources Conservation Service, United States Department of Agriculture (USDA), Web Soil Survey (Table 2.1). Soil texture, pH, and total soil C content were determined from soil collected May 2011, dried at 60$^{o}$C for 48 hours and sieved to 2 mm. Percent clay was determined by the hydrometer method (Gee & Bauder, 1986), percent sand from the weight after sieving soil to 53 μm, with percent silt being the difference. Soil pH was determined in a 1:1 by weight fresh soil to water slurry. Total C and N was measured with dried, ground soil using a Costech Analytical Technologies 4010 elemental combustion analyzer (Valencia, CA, USA). Total inorganic N availability was monitored with PRS$^{TM}$ resin strips (Western Ag Innovations, *http://www.westernag.ca/*) incubated *in situ* for 4 month periods throughout the study period. Monthly inorganic N availability was calculated as the average rate of total available inorganic N detected with the PRS$^{TM}$ resin strips.

NDVI was measured for each plot biweekly to monthly throughout the study period using a hand-held Holland Scientific Crop Circle$^{TM}$ (Lincoln, NE, USA). NDVI

measures green biomass and thus can be used as an index of plant activity and phenology (Huemmrich *et al.*, 1999). In 2011, belowground NPP was estimated using four composited in-growth root cores in each plot ~20 cm deep and 0.05 m diameter made from ¼" mesh. The cores were deployed in January 2011 and collected in June or July 2011. Aboveground NPP was measured in June or July (depending upon phenology) by clipping 0.3 $m^2$ per plot. Roots and plants were dried for 48 hrs at 60$^oC$, sorted to exclude thatch, and weighed.

*Soil Respiration and Climate Measurements*

Soil respiration was measured monthly from January 2011 to June 2012 using a LI-6400 infrared gas analyzer with a 6400-09 chamber attachment (LI-COR Inc., Lincoln, NE) with two 10 cm diameter PVC collars per plot, which were inserted ~2 cm into the soil and weeded to remove aboveground plant biomass. No attempt was made to exclude plant roots; therefore soil respiration measurements represent both microbial and root respiration. Sites were sampled in succession within one week between 10:00 and 16:00 hours.

Air temperature (Campbell CS215-L Temperature & Relative Humidity Probe) and rainfall (Campbell TE 525 WS-L Rain Gauge) were continuously monitored at each site (Table 2.1; Fig. S2.2, S2.3). Volumetric soil moisture (0-30 cm depth; CS616-L Water Content Reflectometer, Campbell Scientific) and soil temperature (10 cm depth; 107-L thermistor, Campbell Scientific) were monitored at the center of each plot and logged continuously with 30 min averages (via AM16/32B Multiplexors connected to CR1000 datalogger, Campbell Scientific). Soil temperature at 10 cm depth was also

measured coincidentally with soil respiration measurements using the 6400-09 chamber temperature probe inserted within 4 cm of the PVC collar; these values were used in all soil respiration analyses as they are more representative of the micro-site conditions around the soil collar.

*Analyses*

To determine how soil respiration responded to the climate treatments, and how this varied with the climate gradient, monthly data was initially analyzed with repeated-measures ANOVA to account for time-dependence among sampling events. Sphericity could not be assumed ($\chi^2$=339, $p<0.0001$) and reported values are for the more conservative Greenhouse-Geisser test. Repeated-measures analysis revealed significant month x treatment and month x site interactions, and thus each month was subsequently analyzed as a 2 x 2 x 3 factorial ANOVA with heat, precipitation, and site as fixed effects. Significant site x treatment interactions necessitated repeating these analyses as a 2 x 2 factorial ANOVA for each site to determine the degree and direction of each heat and precipitation response. Consequently, we report many statistical effects, including marginally significant effects (i.e., $0.05 < p < 0.10$), and do not attempt to correct for the inflation in the family-wise error rate because they are overly conservative in terms of inflating Type II errors (Moran, 2003). However, we do not emphasize any particular individual effect but rather rely upon the overall trends in treatment response through time and along the climate gradient. We transformed data as necessary to meet assumptions of normality and equal variance. Site differences in soil organic C and N content, soil texture, above and belowground NPP, and total inorganic N availability

were tested using ANOVA followed by Tukey's post-hoc tests (Table 2.1). We were unable to accurately model soil respiration using the continuous climate data (see Modeling soil respiration below), and thus we calculated cumulative soil respiration by smoothly incrementing between monthly soil respiration rates across the number of days elapsed, and tested for site and treatment differences as described above. We also examined the relationship between cumulative soil respiration and below- and aboveground NPP for 2011 using linear regression. All ANOVAs and linear regressions were performed with SPSS version 19.0 (IBM SPSS Statistics for Windows, 2010, Armonk, NY).

ANOVA could not directly indicate whether the climate gradient, rather than site-specific factors, drove site and treatment differences as 'site' is necessarily a composite of local and climate effects. We used a variety of regression models from the literature (Table 2.2) to examine how soil respiration and its response to temperature varied with the natural climate gradient. We selected models to represent a range of mathematical temperature and moisture relationships developed in either seasonally (Eqn. 1, Davidson *et al*., 1998) or experimentally moisture-limited systems (Eqn. 2, Suseela *et al*., 2012; Eqn. 3-7, Almagro *et al*., 2009). We modified these models to include NDVI because we measured whole soil respiration, and NDVI may represent an index of root respiration. Matric potential was calculated from volumetric moisture content and soil texture (Saxton and Rawls 2006) to correct for texture-driven differences in soil moisture among sites; this conversion has been previously used to model soil respiration across a spatial and seasonal soil moisture gradient (Davidson *et al.*, 1998). Each model was run using either volumetric moisture or matric potential. To capture antecedent conditions, we ran

**Table 2.2.** Nonlinear soil respiration models from literature and modifications.

| Eqn | Reference | Model |
|---|---|---|
| 1 | Davidson 1998 | $R = ae^{bT}ce^{dM}$ |
| | | $R = ae^{bT}ce^{dN}$ |
| 2 | Suseela *et al*., 2011 | $R= ae^{bT}(d(M\text{-min. }M)(\text{max. }M\text{-}M)^{c})$ |
| | | $R= ae^{bT}(d(N\text{-min. }N)(\text{max. }N\text{-}N)^{c})$ |
| 3 | Almagro *et al*., 2009 | $R = ae^{bT}e^{(cM)+(d(M\text{^}2))}$ |
| | | $R = ae^{bT}e^{(cN)+(d(N\text{^}2))}$ |
| | | $R = ae^{bT}e^{(cM)+(d(M\text{^}2))}e^{(eN)+f(N\text{^}2)}$ |
| 4 | | $R = ae^{bT}cM$ |
| | | $R = ae^{bT}cN$ |
| | | $R = ae^{bT}cMdN$ |
| 5 | | $R = ae^{bT}e^{cM}$ |
| | | $R = ae^{bT}e^{cN}$ |
| | | $R = ae^{bT}e^{cM}e^{dN}$ |
| 6 | | $R = ae^{bT}\text{-}(M\text{-}c)^2$ |
| | | $R = ae^{bT}\text{-}(N\text{-}c)^2$ |
| 7 | | $R = ae^{bT}(M/(M+c))$ |
| | | $R = ae^{bT}(N/(N+c))$ |

a, b, c, d, e, f are fitted constants
R = soil respiration, T = temperature, M = volumetric soil moisture or matric potential (kPa), min. M = minimum M, max. M = maximum M, N = NDVI

the models against daily, 7 day, 14 day, or 30 day cumulative volumetric moisture or average matric potential (30 day values hereafter 30-CVM or 30-AMP). Models were fitted to the data using Levenberg-Marquardt and sequential quadratic programming nonlinear regression parameter estimation procedures in SPSS version 19.0. Global

stability of fitted constants was confirmed by increasing or decreasing each by 50% and confirming that the model converged on the original value.

We used classification and regression trees (CARTs) to further explore how trends in regional climate, NDVI, site, and the site-specific variables soil texture, organic C content, and monthly inorganic N availability, singly and interactively explained patterns in soil respiration. CART analysis is a nonparametric regression method that can reveal structure and parse high-order interactions in nonlinear, multi-collinear data, and thus is highly suited to exploring complex ecological relationships (De'ath & Fabricius, 2000). Both categorical and continuous explanatory variables can be included in models, enabling 'site' itself to be used as a predictor. The CART method bifurcates the response variable recursively based on which predictor variable maximizes the deviance explained, creating homogeneous subgroups, or 'leaves', which are then 'pruned' to maximize the deviance explained while minimizing the predictive error. Pruning is accomplished by an iterative cross-validation method whereby 10% of the data is randomly excluded and the resulting trees are validated against the entire dataset. Unlike non-linear regression, CARTs do not impose a predetermined function upon the data, but prioritize predictors according to which captures the highest percent deviance, revealing the underlying data structure. The 'leaves' are based upon specific values of each predictor, enabling us to parse seasonally shifting controls. Regression trees have been used successfully to investigate a range of complex ecosystem-level responses including C and nutrient dynamics (Johnson *et al.*, 2009; Cleveland *et al.*, 2011), and soil respiration (Fernandez *et al.*, 2006; Vargas *et al.*, 2010; Geng *et al.*, 2012; Leon *et al.*, 2014). CARTs were run

with the *tree* package on R version 2.11.1 (R Foundation for Statistical Computing, 2010, Vienna, Austria).

## Results

### *Climate Treatments*

Warming increased the daily average soil temperature by an average of 3.3 ± 0.05$^{o}$C from January to August 2011 and 2.83 ± 0.04$^{o}$C thereafter. Warming also generally reduced soil matric potential at times of the year when the soil was not saturated (spring dry-down through fall wet-up), while also accelerating the spring dry-down and retarding the fall wet-up period (Fig. 2.1). The precipitation treatment only modestly increased the matric potential above ambient as the additional water likely exceeded the soils' holding capacity during the wet season and relatively little was applied during the dry season.

### *Soil Respiration Along a Regional Mediterranean Climate Gradient*

Across all sites, ambient soil respiration showed a strong seasonality consistent with the increasing asynchrony of temperature and soil moisture availability from north to south (Fig. 2.1). Ambient soil respiration increased in the spring as temperatures began to warm, with the peak rate occurring 1 to 2 months earlier in SOR (Mar.-Apr.) than in COR and WA (May-Jun.) in both study years (Fig. 2.1). Soil respiration declined sharply after the spring peak in all sites as the soils began to dry down, and remained low through the summer drought (Jun. or Jul.-Sept. or Oct.). Soil respiration remained low

until the following spring in WA, but it increased again in COR (Sept. 2011) and SOR (Oct.-Nov. 2011) with the fall wet-up.

NDVI similarly tracked soil matric potential, being high under moist conditions and rapidly declining as the sites dried (Fig. S2.1). Senescence was more complete (i.e., NDVI ~ 0.2) and occurred earlier in SOR than in the more northern sites, consistent with the climate gradient. Also in 2012, SOR had relatively high NDVI throughout the rainy season, particularly in the warmed plots.

*Soil Respiration Response to Climate Treatments*

*Seasonal and Site Responses*

The response of soil respiration to the climate treatments varied complexly with season and location along the climate gradient. Soil respiration varied significantly with month, site, and warming (repeated measures ANOVA; $p < 0.0001$; Table S2.1), and these effects were interdependent ($p < 0.0001$). Within individual months, the effect of warming often varied by site (Table S2.2a), and occasionally precipitation treatment (Nov. 2011, $p = 0.06$; Jan. 2012, $p = 0.04$) (Table S2.2b).

In general, site-level trends in the response of soil respiration to warming tracked trends in soil matric potential (Fig. 2.1; Table S2.2b), with warming increasing soil respiration when soil matric potential was high, and either decreasing soil respiration or having no effect when soil matric potential was low. The response of soil respiration to warming was more variable during seasonal transitions of dry-down and wet-up. The number of months with positive responses to warming also increased from SOR (7), to

COR (9), to WA (13), consistent with decreasing drought intensity and soil moisture limitation. Soil respiration in WA increased due to warming in all but five months. The exceptions occurred when there was no response as soil matric potential was drying down (Jun. 2011) or wetting up (Sept. 2011), or when there was a negative response when soil matric potential was very low (Jul. 2011, Aug. 2011). Similar to WA, there were significant increases in COR due to warming when soil matric potential was high (Jan.-Apr. and Nov. 2011.- Feb. 2012), no response when it was initially drying down (Jun. 2011, May-Jun. 2012), and significant decreases when it was drying down or low (Jul.-Aug., Oct. 2011). The positive effects of heating were more muted in SOR, and the negative effects were more pronounced. Soil respiration in SOR increased significantly with warming when matric potential was relatively high and temperatures low (Jan. 2011 and Nov. 2011-Mar. 2012); there was no response February through April 2011 and when soil matric potential was very low (Jul., Sept. 2011), and significant decreases when soil matric potential was drying down (May-Jun. 2011, Apr.-Jun. 2012) or wetting up (Oct. 2011). There were a few exceptions to this overall pattern: Soil respiration increased slightly due to warming during the summer drought in September 2011 in COR, possibly due to a small rain event (Fig. S2.2), and in August 2011 in SOR, though there was no rainfall in this case. There was an overall low soil respiration rate and lack of response to warming in March 2012 in WA, despite high soil matric potential, which was likely due to a large rain event during sampling (personal observation).

There were fewer soil respiration responses to the precipitation treatment but they were also generally consistent with north-south gradient of increasing summer drought intensity (Fig. 2.1; Table S2.2b). Positive responses occurred only in SOR and only

when soil matric potential was drying-down (May-Jul. 2011) or wetting-up (Oct. 2011). The precipitation treatment offset the negative effect of heat in the heat x precipitation treatment plots during dry-down in May 2011 and April 2012, while warming offset the inhibitory effect of the precipitation treatment in November and December 2011 by drying out the soils when they may have been waterlogged otherwise (Fig.1; Table S2.2). The decrease in soil respiration due to the precipitation treatment in SOR in September 2011 may have been due to sampling after immediately after a rainfall event (personal observation). There were two negative precipitation responses in COR (Jan., Mar. 2011) when soil matric potential was high and thus the soils potentially waterlogged. Similar to SOR, warming offset the inhibitory effect of the precipitation treatment during January 2011 and January 2012 when the soils were very wet (Fig. 2.1; Table S2.2). There were no responses to the precipitation treatment in WA. The one exception occurred in July 2011 when the lowest soil respiration occurred in the heat plus precipitation treatment; this may have been due to soil respiration being measured immediately after the application of water to this treatment (personal observation).

*Mean Cumulative C Flux*

The response of cumulative soil respiration to warming depended upon location along the north-south gradient (Fig. 2.2; Table S2.3). In 2011, warming did not affect cumulative soil respiration in SOR, but caused a marginally significant increase in COR (13.5%) and significant increase in WA (28.6%). For January through June 2012, warming significantly decreased cumulative soil respiration in SOR (12.4%), had no effect in COR, and significantly increased it in WA (32.7%). The increased precipitation

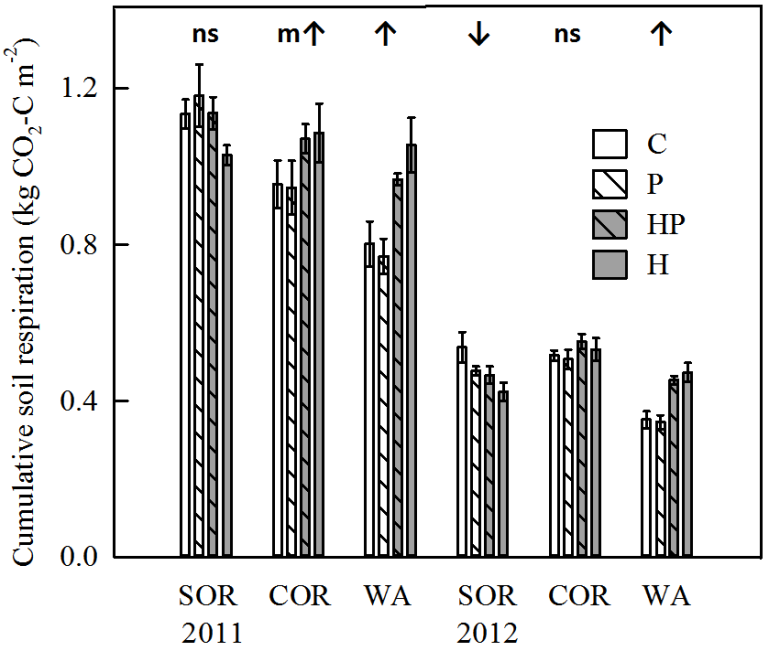


**Fig. 2.2.** Cumulative kg $CO_2$-C $m^{-2}$ respired for 2011 (Jan.-Dec.) and 2012 (Jan.-Jun.). Arrows indicate significant ($p < 0.05$), marginal (m, $0.05 < p < 0.10$) and non-significant (ns, $p > 0.10$) effects due to heat. Precipitation and its interaction with heat were never significant.

intensity treatment did not significantly affect cumulative soil respiration nor mediate the response to warming. Cumulative soil respiration across all plots in 2011 was positively related to aboveground NPP ($R^2 = 0.23$; $p < 0.0001$), though it was not related to belowground NPP ($p = 0.24$).

*Modeling Soil Respiration*

Many of the non-linear regression models tested either failed to stabilize at all sites or fit relatively poorly (Table S2.4). An exponential relationship of temperature with either NDVI, 30 day cumulative soil moisture (30-CVM), or 30 day average matric potential (30-AMP) (Eqn. 6, modified) improved the models modestly over other soil moisture periods (Table S2.4). Temperature with NDVI captured more variance than

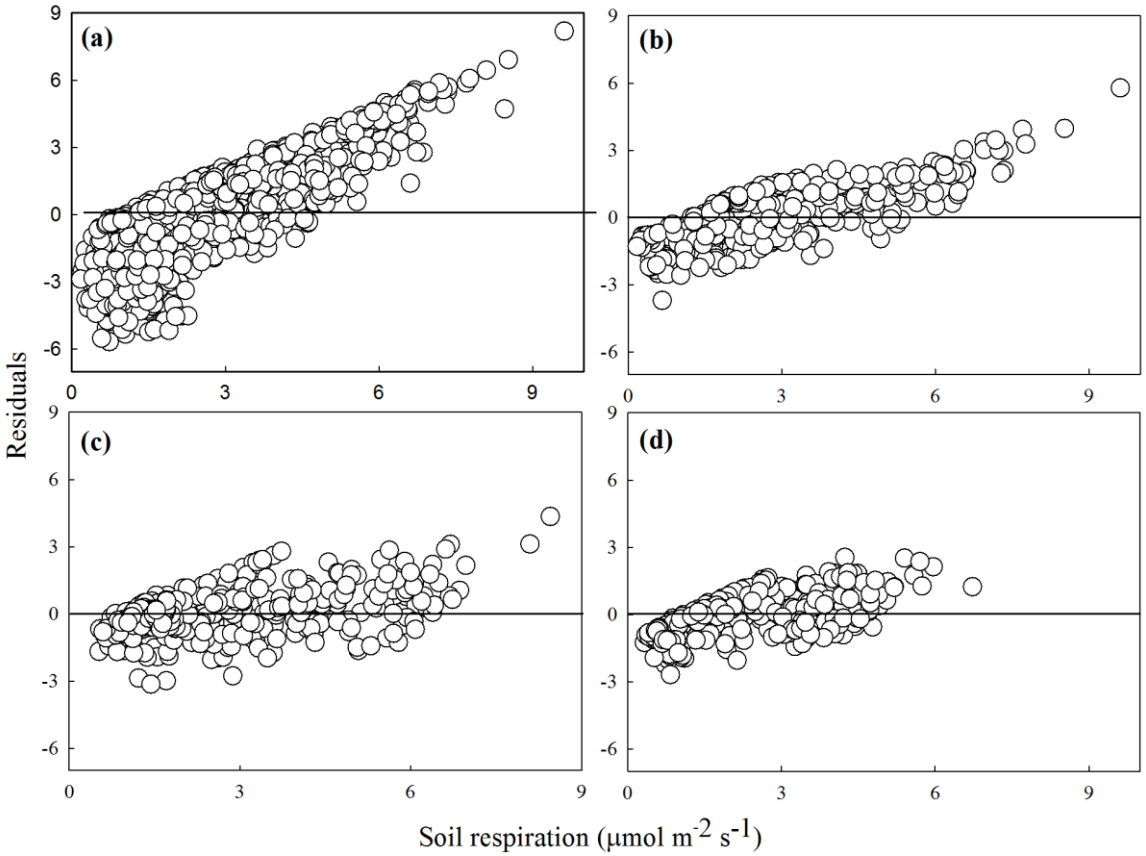


**Fig. 2.3.** Residuals versus soil respiration for $R = ae^{bT}e^{cM}e^{dN}$ for (a) all sites, (b) SOR, (c) COR, (d) WA.

temperature with soil moisture. An exponential function with temperature, NDVI, and 30-AMP achieved the best fit both across and within sites ($R^2$ = 0.55 to 0. 68, Table 2.3). The improvement over NDVI alone was relatively small, but larger in COR and SOR than in WA, consistent with greater moisture limitation in these sites. Although the model explained a reasonably high proportion of the variance in soil respiration, examination of residuals revealed that it consistently overestimated low soil respiration values, and underestimated high values (Fig. 2.3), and all other models showed the same pattern (data not shown). Due to this result, we chose not to use the model to predict cumulative soil respiration.

CART analysis (Fig. 2.4d) further revealed both the seasonally alternating and site-level controls on soil respiration across the climate gradient, and was more predictive than even the best nonlinear regression model across all sites (pseudo-$R^2$ = 0.62). Site-specific factors, such as soil texture, organic matter content, or total inorganic N availability, did not enter the final model, despite significant differences among sites (Table 2.1). Each leaf (1-7) represents how NDVI, temperature and matric potential explain the levels of soil respiration across all sites and treatments. Fig. 2.4a, b, and c show how the timing of these seasonal controls shifts from one site to another by plotting the samples belonging to each leaf over time using colored circles. The CART split on NDVI first, breaking broadly into late autumn-winter and summer drought, and when green plant biomass was low, versus seasonal transitions and spring. Soil respiration was lowest when the sites both had lower green biomass (NDVI < 0.54) and were cool (T < 8.9$^o$C); this largely coincides with the un-warmed plots during cool, wet months at all sites (leaf 1; Fig. 2.4; Fig. S2.1). When temperatures were > 8.9$^o$C, the CART split further into summer drought (NDVI < 0.41; leaf 2) and dry-down and wet-up periods (NDVI > 0.41; leaf 3), though in WA this period extended though spring 2011 as NDVI remained low at this site in the un-warmed plots (Fig. S2.1). When NDVI was > 0.54, soil respiration was either low during late winter or early spring (though higher than in the un-warmed plots) (T < 10.2$^o$C; leaf 4) or approaching its peak (T > 10.2$^o$C). Soil respiration peaked in the spring when temperatures were > 10.2$^o$C and 30-AMP was > -314.0 kPa (leaves 6, 7). Soil respiration was reduced when the 30-AMP was < -314.0 kPa (leaf 5), coinciding with the warmed and thus drier plots. Site itself divided soil respiration at its peak, with the WA peak (leaf 6; mean 3.84) lower than the SOR and

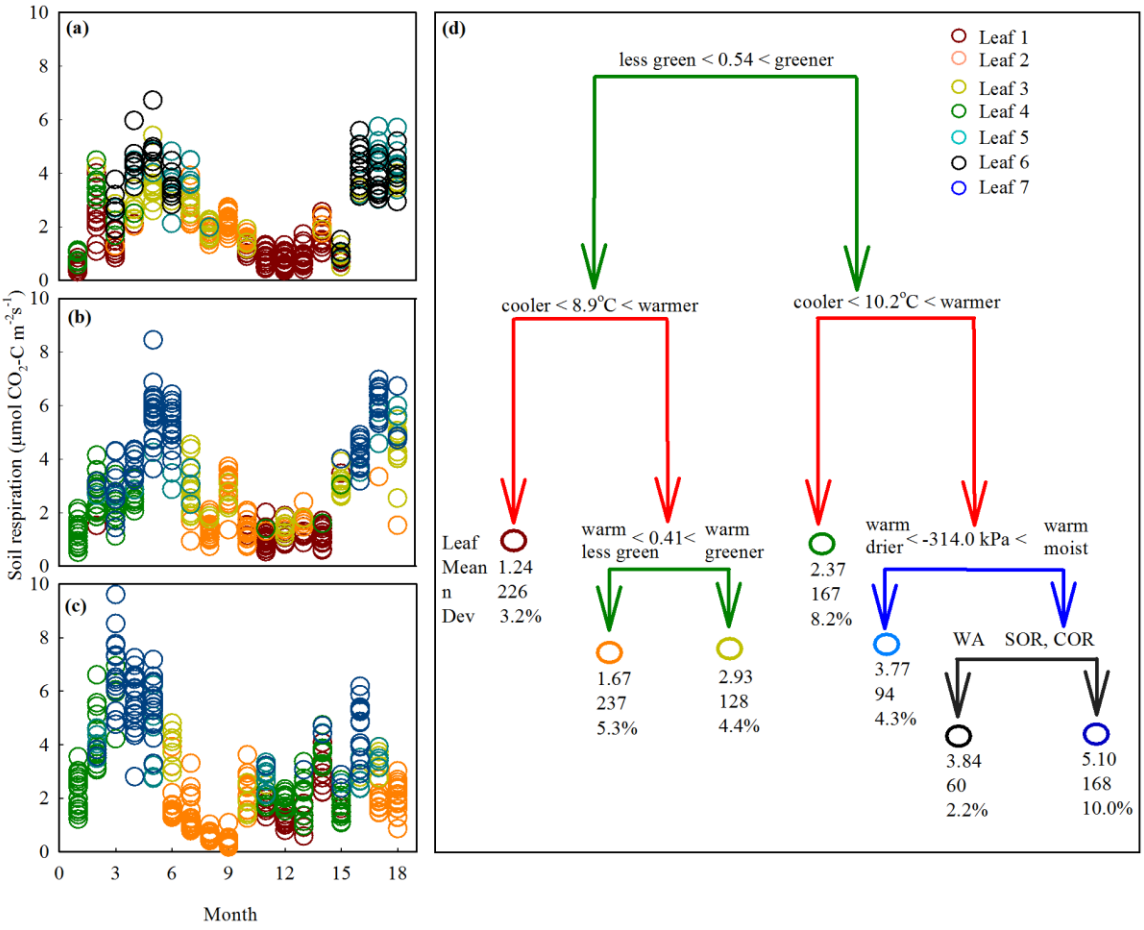


**Fig. 2.4.** CART of soil respiration versus temperature (°C), NDVI (less green, greener), 30-AMP (kPa), and site. Shown are seasonal patterns in soil respiration for January 2011 – June 2012 (months 1-18) for (a) WA, (b) COR, (c) SOR; colored circles correspond to same color leaves in (d) summary of CART results. Colored circles represent 7 terminal leaves with mean soil respiration, number of samples (n), and % deviance (Dev) represented by each leaf. Psuedo-$R^2$ = 0.62.

COR (leaf 7; mean 5.10). It is important to note that while site explained 10.0% of the deviance in this model, this represented only a 2% improvement in the pseudo-$R^2$ over a model without site (data not shown), indicating that the climate gradient and NDVI largely drive this model.

**Table 2.3.** $R^2$ and coefficient mean (se) for best fitting nonlinear regression models of soil respiration versus temperature (T), NDVI (N), and 30 day average matric potential (M) (note: all Eqn. 5 with modifications). All = all sites, SOR = southern Oregon, COR = central Oregon, WA = Washington.

| Model | Site | $R^2$ | Coefficients | | | |
|---|---|---|---|---|---|---|
| | | | a | b | c | d |
| $R = ae^{bT}e^{cN}$ | All | 0.532 | 0.366 (0.027) | 0.042 (0.002) | 2.62 (0.083) | |
| | SOR | 0.481 | 0.365 (0.068) | 0.033 (0.006) | 2.79 (0.192) | |
| | COR | 0.594 | 0.440 (0.047) | 0.056 (0.004) | 2.02 (0.119) | |
| | WA | 0.618 | 0.319 (0.035) | 0.052 (0.004) | 2.60 (0.165) | |
| $R = ae^{bT}e^{cM}$ | All | 0.325 | 1.97 (0.073) | 0.057 (0.003) | 0.001 (0.00005) | |
| | SOR | 0.432 | 2.73 (0.175) | 0.054 (0.006) | 0.001 (0.0001) | |
| | COR | 0.634 | 1.28 (0.079) | 0.089 (0.004) | 0.001 (0.00005) | |
| | WA | 0.499 | 1.03 (0.073) | 0.085 (0.005) | 0.001 (0.00005) | |
| $R = ae^{bT}e^{cM}e^{dN}$ | All | 0.564 | 0.472 (0.036) | 0.052 (0.003) | 0.0003 (0.00004) | 2.19 (0.096) |
| | SOR | 0.554 | 0.623 (0.112) | 0.053 (0.006) | 0.001 (0.0001) | 1.99 (0.208) |
| | COR | 0.684 | 0.718 (0.074) | 0.078 (0.004) | 0.001 (0.00006) | 1.06 (0.144) |
| | WA | 0.632 | 0.372 (0.043) | 0.062 (0.005) | 0.0002 (0.00005) | 2.21 (0.195) |

## Discussion

We found that soil respiration response to warming depended mainly on position along a Mediterranean climate gradient. The climate gradient effects were both direct through soil moisture and temperature and indirect through climate-mediated plant activity (i.e., NDVI). Across all three sites, alternating limitations from temperature and soil moisture consistently led to increased respiration from warming during cooler, wetter conditions and decreased respiration, or no response, during warmer, drier conditions. The net effects of these temporal dynamics differed across the climate gradient (Fig. 2.1), leading to differences in cumulative respiration response to warming (Fig. 2.2). Warming can inhibit soil respiration by decreasing soil matric potential during spring dry-down and autumn wet-up periods (Fig. 2.1) and potentially by accelerating summer

senescence of plant communities (Fig. S2.1). Furthermore, the onset of inhibition by, or lack of response to, warming occurred earlier from north to south along the climate gradient, in line with trends in summer soil moisture availability. These trends were consistent across the three sites, despite considerable differences in site characteristics, such as nutrient availability, NPP, and soil texture.

*Modeling Soil Respiration Across a Regional Climate Gradient*

Modeling soil respiration with temperature, antecedent soil moisture or matric potential, and NDVI greatly improved fit over soil moisture and temperature alone (Table S2.4; Table 2.3), indicating the interactive role of climate and plant phenology in driving soil respiration along the gradient. Interestingly, NDVI alone captured a larger portion of the variance in soil respiration than 30-AMP for all but COR (Table 2.3), likely because it integrated temperature, soil moisture, and plant effects into a single variable. NDVI was itself highly responsive to soil moisture; its correlation with 30-AMP across all sites was 0.64 ($p < 0.0001$), and this correlation increased from WA (0.37; $p < 0.0001$) to COR (0.62; $p < 0.0001$) to SOR (0.84; $p < 0.0001$). The positive correlation of cumulative soil respiration with annual aboveground NPP in 2011 suggests that aboveground plant biomass and productivity were indeed related to root respiration, or potentially release of root exudates. Surprisingly, cumulative soil respiration was not correlated to belowground NPP, which may indicate the lack of this relationship or that the in-growth root core method was inadequate to capture root dynamics in this system, despite the fact that it has been used successfully in other studies (Lauenroth, 2000; Weltzin *et al.*, 2000).

The nonlinear models that we tested did not fully capture soil respiration dynamics in this Mediterranean system (Fig. 2.3). Previous studies applied these models to wet and dry periods separately rather than attempting to capture shifting seasonal dynamics (see Almagro *et al.*, 2009; Suseela *et al.*, 2012). The exponential relationship between soil respiration and temperature appears to break down during drought, exhibiting a threshold response to moisture (Suseela *et al.* 2012), which these simple models cannot capture.

Variability in the predictions of terrestrial C flux among Earth systems models has been shown to be strongly influenced by the type of soil moisture functions used (Falloon *et al.*, 2011). A test of reduced-complexity CMIP5 Earth system models against observed C dynamics found that the inclusion of soil moisture did not improve model performance, but the authors noted that the simple exponential moisture function used may have failed to capture moisture extremes, while strong correlations with other ecosystem variables, such as net primary productivity, may mask the influence of soil moisture (Todd-Brown *et al.*, 2013). These findings, in conjunction with ours, suggest that current models do not adequately account for the complex and nonlinear nature of soil respiration and soil moisture dynamics. However, our results also show that the performance of simple models may be improved by the inclusion of antecedent conditions and plant productivity.

*Regional Versus Local Controls of Soil Respiration*

The CART analysis demonstrated that other site factors did not take priority over NDVI, temperature, and matric potential in explaining soil respiration. It also provided a

composite statistical and visual view across all plots across all sites, revealing how the predictors interacted seasonally across the three sites (Fig. 2.4). As in the nonlinear models, NDVI was the strongest predictor of soil respiration across the three sites, again acting as an integrative variable that reflected the seasonal effects of soil moisture and temperature on plant phenology and site-specific effects of nutrient availability on NPP. Most splits in the CART were based upon temperature and NDVI, with matric potential only entering the model from early spring through the transition from peak soil respiration into dry-down, or during autumn wet-up following summer drought. This is likely because of the close correlation between matric potential and NDVI (described above) that, in concert with the multiple CART splits based on temperature, subsume the effects of soil moisture limitation on soil respiration. The site factor only explained a minor amount of additional variance, indicating that plant productivity and phenology (as captured by NDVI) is adequate to largely account for site effects on soil respiration, despite significant differences in soils and plant communities (i.e., relative annual and perennial dominance) among sites. We emphasize that while maximum NDVI reflected differences in nutrient availability among sites, its seasonality largely reflected the climate gradient and the warming treatments. This leads us to conclude that climate is the primary driver of regional-scale trends in both ambient soil respiration and its response to warming.

*Soil Moisture Limitation May Mediate Response of Soil Respiration Under Future Climate*

Our study region experiences a Mediterranean climate with its accompanying asynchrony between temperature and moisture availability, whereby increasing temperatures coincide with the onset of summer drought, and decreasing temperatures coincide with autumn re-wetting (Fig. 2.1). Though this is true across all three sites, the severity and duration of the summer drought decreases from south to north, and it is this gradient, rather than total annual precipitation or local site-level factors, that appears to exert spatial and temporal controls on the response of soil respiration to warming. Our southernmost site (SOR) has the highest mean annual precipitation but is the most moisture limited during the growing season. In contrast, even though the northernmost site (WA) has a sandy and thus well-drained soil, its milder summer drought (see Fig. 2.1 and Fig. S2.3) results in less moisture limitation and higher relative soil respiration rates when dry. Thus only WA showed relatively consistent stimulation and a significant increase in cumulative C flux in 2011 (Fig. 2.2) due to warming, while COR and SOR were less responsive to warming overall, and during the summer, experienced a longer period of soil respiration inhibition due to warming. The long-term climate gradient for this region is more extreme than is represented by our study years (Table 2.1), leading us to conclude that our results provide a conservative estimate of the gradient's influence on soil respiration. However, it should be noted that our study was relatively short-term and that important ecosystem feedbacks may emerge over a longer study period as has been reported previously (Rustad *et al.*, 2001), such as shifts in plant phenology or plant

community composition, or microbial acclimation to warming, any of which could dramatically change responses.

Climate models for the PNW project an increase in temperature and a concurrent shift toward increased wet-season and decreased dry-season precipitation (Mote & Salathé, 2010). Our study suggests that this may be sufficient to shift the moisture gradient such that prairies in Washington and central-northern Oregon may experience drought conditions more like those in southern Oregon (and those in southern Oregon become even more drought prone) due to higher temperatures during seasonal transitions and drier summers. We suggest this may inhibit soil respiration response to warming temperatures. We further posit that if mesic systems continue to experience increased severity and rate of drought years as proposed by recent multi-factorial warming and rain-fall exclusion studies (Wan *et al.*, 2007; Schindlbacher *et al.*, 2012; Suseela *et al.*, 2012), they may more closely resemble the Mediterranean PNW, with cumulative soil respiration reduced considerably by growing-season moisture limitation.

Climate models have commonly predicted an increase in global soil respiration rates under a warming climate; however, variable and contradictory empirical results have challenged these projections. In particular, studies in arid and semi-arid ecosystems and those using experimental drought in mesic ecosystems have revealed inherent weaknesses in published non-linear models of soil respiration. A growing literature indicates that increasing moisture-limitation may offset soil respiration increases due to warming (Schindlbacher *et al.*, 2012) and decrease soil respiration's temperature sensitivity (Suseela *et al.*, 2012). To our knowledge, ours is the first study to document this using a manipulative warming and increased precipitation intensity study performed

at a regional scale, thus providing more robust evidence than either single-site studies or models. Our results strongly suggest that current projections may overestimate ecosystem C loss in areas where growing season soil moisture becomes more limiting.

**Bridge to Chapter III**

Though soil respiration has commonly been shown to have an exponential relationship with temperature in many cases, it is now clear that other factors, such as soil moisture, can pre-empt or even negate it, as we have demonstrated here. Many experimental warming studies have reported apparently anomalous soil respiration responses, and a number of reasons have been posited to explain them. One of these is a change in the temperature sensitivity of soil respiration *in situ* resulting in an attenuated response to temperature under warming. However, to understand the role of temperature sensitivity in determining the response of soil respiration, and thus C storage, to climate change, the nature of the temperature sensitivity of SOM decomposition itself must be understood. In the next chapter I tested the temperature sensitivity of SOM decomposition in soils that had been either warmed for ~2 years or that had litter-inputs manipulated for 20 years to understand the role of soil C content and soil type in shaping this key SOM characteristic.

# CHAPTER III

# THE CARBON QUALITY-TEMPERATURE HYPOTHESIS FAILS TO CONSISTENTLY PREDICT TEMPERATURE SENSITIVITY IN TWO MANIPULATIVE ECOSYSTEM EXPERIMENTS

## Contributions

Lorien L. Reynolds helped design the experiment, collected the data, analyzed the data, and wrote the manuscript. Richard D. Bowden manages the DIRT experimental site and collected the soils. Bart R. Johnson and Scott D. Bridgham designed and managed the HOPS experiment. Kate Lajtha, Richard D. Bowden, Bart R. Johnson, and Scott D. Bridgham edited the manuscript.

## Introduction

The temperature sensitivity of soil organic matter (SOM) decomposition has been much debated (Davidson & Janssens, 2006; von Lützow & Kögel-Knabner, 2009b; Conant *et al.*, 2011) and is a key unknown in modeling soil-climate feedbacks (Lloyd & Taylor, 1994; Zhou *et al.*, 2009; Tang & Riley, 2015). SOM is typically modeled as multiple pools with differing chemical complexity and hence different rates of decomposition (Parton *et al.*, 1987; Coleman & Jenkinson, 1996) but each pool is usually treated as having a single temperature sensitivity (Friedlingstein *et al.*, 2006). The use of a single temperature sensitivity value has been criticized because enzyme kinetics predict that if a slowly decomposing fraction of SOM is more chemically complex, i.e., of lower

carbon quality as defined by the number of enzymatic steps required for its decomposition (Bosatta & Ågren, 1999), then it should be more sensitive to temperature (Davidson & Janssens, 2006). This concept has been formalized as the carbon quality-temperature (CQT) hypothesis (Bosatta & Ågren, 1999; Davidson & Janssens, 2006). It has been supported by the detection of operationally-defined large molecular weight molecules, i.e., humic residues (see Kleber 2010, Kleber and Johnson 2010), and empirically observed patterns in soil respiration rates (see von Lützow and Kögel-Knabner 2009, Conant *et al*., 2011). If the CQT hypothesis is correct, then models may be underestimating the soil-climate response (Davidson & Janssens, 2006).

The temperature sensitivity of SOM remains elusive, however, because numerous studies have yielded mixed results. Moreover, SOM is an amalgam of substrates decomposing simultaneously so that carbon 'quality' must be operationally defined. Experiments have demonstrated an increase in temperature sensitivity with various proxies of decreasing soil carbon quality, including decreasing respiration rate (Craine *et al.*, 2010a), increasing depth (Fierer & Schimel, 2003; Karhu *et al.*, 2010), the progressive depletion of carbon over the course of an incubation (Conant *et al*., 2008; Hartley & Ineson, 2008; Haddix *et al.*, 2011), and long-term exclusion of carbon inputs (Lefèvre *et al.*, 2014). However, other studies showed no difference in temperature sensitivity with depth (Fang *et al.*, 2005; Reichstein *et al.*, 2005) or incubation time, even when other metrics respond otherwise (see Reichstein *et al*., 2005, Curiel Yuste *et al*., 2007, Karhu *et al*., 2010). Moreover, single studies have shown mixed results depending upon which carbon quality proxy is considered (e.g., across time, depth, etc.) (see Koch *et al*., 2007, Karhu *et al*., 2010). Discrepancies have been attributed to differences in

methods, including the length of incubation or influence of the 'mineral matrix' (Conant *et al.*, 2011), and calculation and interpretation of temperature sensitivity metrics (Sierra, 2011). However, some authors conclude that carbon quality is not sufficient to explain temperature sensitivity of SOM decomposition dynamics (Koch *et al.*, 2007; Haddix *et al.*, 2011).

Although the kinetic theory underlying the CQT hypothesis is inarguable, holds only if SOM chemical complexity solely determines decomposition rates. Recent investigations have found no evidence that slower decomposing SOM is more chemically complex or 'humic' than rapidly decomposing SOM (Lehmann *et al.*, 2008; Conant *et al.*, 2011; Schmidt *et al.*, 2011). Instead, interactions with the mineral matrix, such as soil aggregation and mineral absorption, may be more important drivers of soil carbon stabilization. Thus the question arises, if carbon quality is not always the primary driver of SOM decomposition rates, should we expect temperature sensitivity to consistently increase as decomposition rate decreases? Conant *et al*., (2011) established a conceptual framework to explore this new paradigm, whereby carbon quality, soil aggregation, and sorption reactions, among other factors, simultaneously drive SOM decomposition; however, little is known about the temperature sensitivity of many of these drivers. Lefèvre *et al*., (2014) speculated that an increase in the mineral-adsorbed fraction with depletion of labile, non-mineral associated pools may result in an increase in the temperature sensitivity of decomposition due to the increased energy required to mobilize this carbon. However, we might expect temperature sensitivity responses to vary widely if SOM decomposition is a function of many interacting factors, such as carbon quality,

mineral adsorption, soil aggregation, substrate diffusion, and microbial substrate-use efficiency (see Davidson and Janssens 2006, Kleber 2010, Tang and Riley 2015).

To examine the validity of the CQT hypothesis under a diversity of conditions, we tested the temperature sensitivity of SOM decomposition in soils from four sites representing two different biomes and two experimental manipulations of soil carbon dynamics: (1) a northeastern deciduous forest under 20 yr of chronic *in situ* input manipulation (Detritus Input and Removal Treatment, hereafter DIRT), and (2) three Pacific Northwest prairies differing in carbon content and basal respiration, and exposed to ~20-26 months of experimental warming and wetting (Heating of Prairies Study, hereafter HOPS). If the CQT hypothesis is true, temperature sensitivity should increase (1) as labile substrates are depleted following leaf or root litter exclusion, (2) by soil depth, given that fresher SOM is found at the surface, (3) in experimentally warmed soils, where accelerated decomposition may have reduced labile SOM, (4) as soil respiration rates decrease, assuming high rates reflect greater soil carbon availability, and (5) with increasing incubation time with the progressive depletion of labile carbon. Furthermore, we examined temperature sensitivity with two different metrics: apparent $Q_{10}$ calculated from the ratio of time to decompose an initial labile and second, more 'recalcitrant' percentage of carbon and activation energy ($E_a$) throughout the duration of the incubation. Overall, we asked whether temperature sensitivity was consistent with the CQT hypothesis across carbon quality proxies, temperature sensitivity metrics, and experimental and environmental contexts.

## Methods and Materials

### *Site Descriptions*

Soils were collected from DIRT plots in the Bousson Experimental Forest (41°36'N, 80°3'W, 381 m elevation), Pennsylvania, USA in fall 2011 after 20 yr of experimental manipulation. The experimental design and methods are described in Bowden *et al*., (2014). Briefly, the site consists of three 3 x 3 m replicate plots with either No Inputs (NI; no roots or leaf litter), No Litter (NL), No Roots (NR), ambient or COntrol conditions (CO), or Double annual leaf Litter (DL). Aboveground litterfall is excluded with screens and a portion transferred to the DL plots; roots are excluded via impermeable plastic barriers buried from the surface to the C horizon (~1.4 m).

Daily temperatures in the Bousson Experimental Forest average -4°C in January and 21°C in August with average precipitation of 105 cm $yr^{-1}$. Soils are coarse loamy mixed superactive mesic Oxyaquic Fragiudalfs (Cambridge series) derived from glacial till overlying shale and sandstone (USDA-SCS, 1979) with a fragipan present at 60 cm; pH is 4.0 (Bowden *et al.*, 2000). The site is dominated by black cherry (*Prunus serotina*) and sugar maple (*Acer saccharum*).

The organic (O) horizon from CO and DL plots was sampled by hand using 15 x 15 cm templates. For mineral soil, two cores from 0-10 cm (all plots) and 10-20 cm (CO and DL plots only) depths were collected and bulked per plot using a gas-powered 9.62 cm diameter, diamond-bit, stainless-steel soil corer (Earthquake, 9800B). The O-horizon samples were sieved to 5.6 mm and mineral soil was sieved to 2 mm; all samples were

sorted for rocks, roots, and other debris. Soils were then subsampled and stored field moist at 4$^{o}$C.

The HOPS sites are located in southwestern Oregon (SOR; 42°16'41"N, 123°38'34" W), central-western Oregon (COR; 44°01'34"N, 123°10'56" W), and central-western Washington (WA; 46°53'47" N, 122°44'06" W). A complete description of the sites and experimental design can be found in Pfeifer-Meister *et al*., (2013) and Reynolds *et al*., (2015). Briefly, the sites are remnant upland prairies dominated by introduced grasses and forbs. Mean annual temperature and precipitation, modeled with PRISM for the period 1981– 2010, are 12.3$^{o}$C and 143 cm for SOR, 11.4$^{o}$C and 113 cm for COR, and 10.5$^{o}$C and 120 cm for WA (http://www.prism.oregonstate.edu/). The SOR soil is a loamy-skeletal, mixed, superactive, mesic Entic Ultic Haploxeroll; the COR soil is a very-fine, smectitic, mesic Vertic Haploxeroll; the WA soil is a medial-skeletal over sandy or sandy-skeletal, amorphic over isotic, mesic Typic Melanoxerand (Web Soil Survey (http://websoilsurvey.nrcs.usda.gov/)).

Each site was prepared using standard restoration practices (mowing, raking, herbicide application), then seeded with a mix of 32 native annual and perennial grasses and forbs. Twenty 7.1 $m^2$ plots were established in a fully factorial design with five replicates each of 2.5-3$^{o}$C warming, 20% increased precipitation intensity, warming and wetting, and ambient conditions. Precipitation treatments began in spring 2010; warming was initiated in April 2010 in SOR and COR and October 2010 in WA. Plots were warmed with six overhead 2000 W Kalglo infrared heaters (Bethlehem, PA, USA) angled at a 45° angle to the surface of each plot (Kimball *et al.*, 2008); dummy lamps were hung

in control plots. Augmented precipitation was collected on site with polycarbonate sheets, stored in a cistern, and applied by hand within 2 wk of when it fell.
Soil was collected in July 2012. Four 10 cm deep cores from the mineral (A) horizon were collected and bulked for each plot. Soil was sieved to 2 mm, sorted for rocks, roots, and debris, and stored field moist at 4$^{o}$C.

*Incubation*

For all soils, percent carbon (C) and nitrogen (N) were determined on dried, ground soil with a Costech Analytical Technologies 4010 elemental combustion analyzer (Valencia, CA, USA). Soil pH was determined with a 1:1 by volume slurry with distilled water. Soil moisture was determined by drying soil at 60$^{o}$C for 48 hr. Moisture content at 60% of saturation was determined by saturating a subsample of each soil, draining for 4 hr, and drying at 60$^{o}$C. Approximately 20 g dry weight (dw) equivalent of mineral soil and ~7 g dw equivalent of organic soil (DIRT CO and DL plots only) was weighed into 120 mL serum bottles and moisture content adjusted to 60% of saturation with distilled water. Serum bottles were left open between measurement days; the initial moisture content was monitored by weight and distilled water added when necessary. To determine the soil respiration rate, each serum bottle was moved into an incubator at its target temperature (see below) for 24 hr, and then sealed and headspace $CO_2$ concentrations measured initially and after ~ 3 hr via direct injection with a LI-7000 (LICOR Inc., Lincoln, NE).

The DIRT incubation began in January 2012 and continued for 525 days. Soil respiration was measured every day for the first week, each week for the first month,

every month for 260 days, and every other month thereafter. The incubation included soils from 0-10 cm for all treatments, as well as the O-horizon and 10-20 cm soils of CO and DL plots. Soils were divided into 5 sets: one each incubated constantly at 25 or 35$^{o}$C, and three incubated at 25$^{o}$C, then rotated randomly on sampling days (see above) for 27 hr into 15, 35, or 25$^{o}$C. Thus soils were at the same state of decomposition relative to each other throughout the experiment. This method has been used in similar incubation experiments (Craine *et al.*, 2010a). Measurements were made using a LI-6400 (LICOR Inc., Lincoln, NE) plumbed for direct injection on days 56-57 and 84-85 while the LI-7000 was repaired.

The HOPS incubation began August 2012 and continued for 303 days. Incubations were set up as for DIRT, minus the sets kept at 25 and 35$^{o}$C constantly. Soil respiration was measured every day for a week, every week for a month, every month for 272 days, and every other month thereafter.

*Analysis*

All data were inspected for normality and soil respiration was log transformed to meet the assumptions of the statistical tests. All analyses were performed with SPSS version 19.0 (IBM SPSS Statistics for Windows, 2010, Armonk, NY, USA).

For the DIRT experiment, differences in percent C due to 20 years of input manipulation were tested across treatments (0-10 cm depth only) and depths (for CO and DL treatments only) with ANOVA followed by Tukey's post-hoc tests, and across treatments within a depth for O-horizon and 10-20 cm CO and DL soils with t-tests. For the HOPS soils, differences in percent C across sites, warming, and precipitation

treatments were tested as a 3 x 2 x 2 factorial ANOVA followed by Tukey's post-hoc tests.

For both experiments, differences in soil respiration rates among treatments over time at 25$^{o}$C were analyzed with repeated measures ANOVA. For DIRT soils, respiration in soils from 0-10 cm and across depths for CO and DL treatments showed significant treatment x day interactions (Supplemental Table 1), and thus each day was analyzed separately as described for percent C above. Respiration in soil within the O-horizon and 10-20 cm depth showed no interaction (Supplemental Table 1); results are for repeated-measures ANOVA. HOPS soils showed a significant day x site interaction (Supplemental Table 3); each day was analyzed separately, followed by 2 x 2 factorial ANOVAs within site when it interacted with treatment.

Temperature sensitivity of the DIRT soils expressed as $Q_{10}$ was calculated as in Conant *et al*., (2008), whereby soils were incubated constantly at 25 and 35$^{o}$C, and the sensitivity calculated as the ratio of the time it took to respire the initial 1% and then a final 1% of total initial soil organic carbon at each temperature. This equates to 4-5% total loss of initial carbon for 0-10 cm soils, the maximum percent carbon respired by the NI treatment, and 2-3% for CO and DL treatments across depths, the maximum respired by 10-20 cm depth. Hereafter we refer to this metric as $Q_{10}$ at constant incubation temperature, or $Q_{10C}$. Differences for $Q_{10C}$ across treatments were tested as described above for percent C. Differences within each treatment through time, including between CO and DL O-horizon and 10-20 cm depths, were tested with paired t-tests.

Temperature sensitivity was also assessed as activation energy as described with the Arrhenius equation,

$$SR = A * e^{-Ea/RT} \quad \text{eqn (1)}$$

where SR is the soil respiration rate, A is a fitted constant, $E_a$ is the activation energy, R is the gas constant (8.314 J mol $K^{-1}$), and T is the temperature in Kelvin. $E_a$ was calculated as the slope of the relationship between the natural log of SR at 15, 25, and 35$^oC$ versus -1/RT for each sampling day, treatment, and depth. As this metric of temperature sensitivity was calculated on soils rotating through a range of temperatures periodically, hereafter we will refer to this metric as $E_a$ rotating, or $E_{aR}$. $E_{aR}$ was analyzed separately for 0-10 cm, O-horizon, and 10-20 cm depths with repeated-measures ANOVA. There was a significant day x $E_{aR}$ interaction for 0-10 cm depth and across depths for the CO and DL treatments (Supplemental Table 4), and thus each day was tested separately as described above. Soil within the O-horizon and 10-20 cm depth showed no interaction (Supplemental Table 4); results are for repeated-measures ANOVA. For HOPS soils, differences in $E_{aR}$ between sites and for warming and precipitation treatments were analyzed by repeated measures ANOVA; there were significant day x $E_{aR}$ interactions (Supplemental Table 5) and thus each day was tested separately as described for percent C.

$E_{aR}$ within each DIRT input treatment and depth was tested with linear regression to determine if it increased over incubation time. Additionally, we compared our data against that of two other published studies, Craine *et al*., (2010) and Lefevre *et al*., (2014). Following methods established by these authors, we used linear regression to test whether the log of the $E_{aR}$ increased as the log of the respiration rate at 20$^oC$ ($SR_{20}$; calculated from modeled SR across incubation temperatures) decreased.

## Results

*Percent C, Soil Respiration, and Cumulative Respiration*

For the DIRT soils, %C at the 0-10 cm depth was lowest in the NI treatment ($p = 0.016$; Table 3.1). There were no significant differences in %C among any other treatments at any depth. The O-horizon in the CO and DL treatments contained greater %C than either the 0-10 or 10-20 cm depths ($p = 0.002$), while the 0-10 and 10-20 cm depths did not differ.

**Table 3.1**. Mean (se) %C for DIRT and HOPS soils. Small letters represent treatment differences within DIRT 0-10 cm soil, between CO and DL within O-horizon and 10-20 cm depths, or across HOPS sites. Numbers represent differences within DIRT CO and DL across depths. NI = no inputs, NL = no litter, NR = no roots, CO = control, DL = double litter, C = control, P = precipitation, HP = heat x precipitation, H = heat.

| Experiment | | | | | | |
|---|---|---|---|---|---|---|
| DIRT | Depth | ----------Treatment mean ---------- | | | | |
| | | NI | NL | NR | CO | DL |
| | O-horizon | | | | 22.66 (4.05)$^{a,2}$ | 28.14 (4.85)$^{a,2}$ |
| | 0-10 cm | 3.60 (0.32)$^{a}$ | 4.47 (0.36)$^{b}$ | 4.71 (0.35)$^{b}$ | 5.13 (0.13)$^{b,1}$ | 5.07 (0.03)$^{b,1}$ |
| | 10-20 cm | | | | 2.85 (0.23)$^{a,1}$ | 2.26 (0.29)$^{a,1}$ |
| HOPS | Site | Site mean | ---------- Treatment mean ---------- | | | |
| | | | C | P | HP | H |
| | WA | 4.63 (0.58)$^{b}$ | 4.86 (0.54) | 4.28 (0.22) | 4.80 (0.36) | 4.59 (0.42) |
| | COR | 6.87 (0.07)$^{c}$ | 7.10 (0.21) | 7.11 (0.46) | 6.84 (0.36) | 6.43 (0.32) |
| | SOR | 3.24 (0.35)$^{a}$ | 3.42 (0.14) | 3.06 (0.13) | 3.17 (0.13) | 3.31 (0.35) |

Carbon turnover (i.e., g C respired/initial g C) for DIRT soil at 25$^{o}$C was significantly reduced by input exclusion in the 0-10 cm depth (Fig. S3.1a; Table S3.1). Overall, soil respiration in the NI treatment was generally lower than in the CO and DL treatments. The NL and NR treatments were not different from one another, nor were the CO and DL treatments. The NL and NR treatments were intermediate and frequently not

different from either the NI, CO, and DL treatments. There was no difference in soil respiration between the CO and DL treatments in the O-horizon or 10-20 cm depths. Soil respiration was generally higher in the 0-10 than 10-20 cm depth and higher in the O-horizon than the mineral horizons in both the CO and DL treatments, though this was not always significant.

Cumulative C turnover for DIRT 0-10 cm soils at 25ºC was lowest in the NI treatment and highest in the CO and DL treatments (Table 3.2; Table S3.2). The DL

**Table 3.2**. Mean (se) soil C turnover (cumulative mg $CO_2$-C g $C^{-1}$) respired over 525 days for DIRT and 303 days for HOPS. Small letters represent significant differences within a depth for DIRT and among sites or among treatments for HOPS. Numbers represent significant differences across depths for DIRT. NI = no inputs, NL = no litter, NR = no roots, CO = control, DL = double litter, C = control, P = precipitation, HP = heat x precipitation, H = heat.

| DIRT | Depth | | |
|---|---|---|---|
| Treatment mean | O-horizon | 0-10 cm | 10-20 cm |
| NI | | 70.71 (2.83)[a] | |
| NL | | 98.95 (7.19)[b] | |
| NR | | 92.91 (4.00)[b] | |
| CO | 237.76 (8.28)[a;3] | 126.29 (2.33)[c;2] | 87.09 (5.86)[1] |
| DL | 289.46 (13.99)[b;2] | 138.97 (3.18)[c;1] | 80.94 (18.65)[1] |
| HOPS | WA | COR | SOR |
| Site mean | 53.2 (1.32)[b] | 35.23 (1.00)[a] | 87.49 (1.55)[c] |
| Treatment mean | | | |
| C | 47.05 (1.96)[a] | 38.28 (2.31)[b] | 90.02 (2.90)[b] |
| P | 53.28 (1.74)[a] | 36.83 (2.17)[b] | 91.84 (3.19)[b] |
| HP | 55.75 (3.34)[b] | 31.59 (1.21)[a] | 84.73 (1.31)[a] |
| H | 56.7 (2.68)[b] | 34.2 (1.83)[a] | 83.39 (4.11)[a] |

treatment had a higher cumulative C turnover than the CO treatment in the O-horizon, but there was no difference between the CO and DL treatments for the 10-20 cm depth. Cumulative C turnover decreased significantly by depth in the CO and NL treatments.

For the HOPS sites, %C was lowest in SOR and highest in COR ($p < 0.0001$; Table 3.1), but the treatments had no effect on %C ($p \geq 0.41$). The effect of the heat treatment on soil respiration depended upon day and site (Fig. S3.1 c-e; Table S3.3). Soil respiration was slightly reduced in warmed soil from SOR and COR and slightly elevated in warmed soil from WA, though there were many exceptions and the effects were only significant in 6 out of 17 sampling times for soils from SOR and COR, and 9 sampling times for soil from WA. Cumulative C turnover was highest in SOR soil and lowest in COR soil (Table 3.2; Table S3.2). There was no effect of precipitation treatment on cumulative soil C turnover. Warming treatments decreased cumulative C turnover from COR and SOR soils, but increased cumulative C turnover in soil from WA.

*Temperature Sensitivity*

The $Q_{10C}$ ($Q_{10}$ at constant temperature) of DIRT soil respiration decreased with incubation time in all depths and treatments, although this change was not always significant (Table 3.3). For the 0-10 cm depth, the NI treatment generally had a significantly lower $Q_{10C}$ than all other treatments for both the initial and final 1% of total C respired, whereas the $Q_{10C}$ of the NL treatment was highest for the final 1% respired. The DL treatment had a higher $Q_{10C}$ than the CO treatment in the O-horizon but the two treatments did not differ in the 10-20 cm depth.

The $E_{aR}$ ($E_a$ at rotating temperature) of soil respiration in the DIRT treatments decreased over incubation time at all depths, the treatment effect changed over time for the 0-10 cm depth, and there were no treatment effects in the other depths (Fig. 3.1a; Table S3.4). The NI treatment tended to have the lowest $E_{aR}$ in the 0-10 cm depth,

**Table 3.3.** Mean (se) $Q_{10}$ at constant temperatures ($Q_{10C}$) for an initial and final 1% C from DIRT soils. Small letters indicate significant differences within a depth among treatments. Numbers indicate significant differences between the initial and final 1% C within depth and treatment. Capital letters indicate significant differences across depth for CO and DL. NI = No Inputs, NL = No Litter, NR = No Roots, CO = Control, DL = Double Litter.

| | Treatment | Q10 | |
|---|---|---|---|
| | | Percent of total C respired | |
| Depth | | Initial | Final |
| O- horizon | CO | 1.95 (0.15)$^{a,1,A}$ | 1.33 (0.1)$^{a,1,A}$ |
| | DL | 2.52 (0.19)$^{b,2,B}$ | 1.72 (0.06)$^{b,1,B}$ |
| 0-10cm | NI | 1.54 (0.05)$^{a,2}$ | 1.08 (0.02)$^{a,1}$ |
| | NL | 2.20 (0.02)$^{b,2}$ | 1.65 (0.09)$^{c,1}$ |
| | NR | 1.85 (0.09)$^{b,2}$ | 1.55 (0.07)$^{ab,1}$ |
| | CO | 1.88 (0.11)$^{ab,2,A}$ | 1.41 (0.02)$^{b,1,A}$ |
| | DL | 1.84 (0.03)$^{b,2,A}$ | 1.54 (0.13)$^{ab,1,A}$ |
| 10-20 cm | CO | 1.74 (0.04)$^{a,1,A}$ | 1.45 (0.2)$^{a,1,A}$ |
| | DL | 1.51 (0.19)$^{a,1,A}$ | 1.35 (0.02)$^{a,1,A}$ |

although differences were not always statistically significant. Despite the significant differences among dates for $E_{aR}$ (Table S3.4), at the 0-10 cm depth these effects were inconsistent and only the CO treatment declined weakly over time (Fig.3.1a; Table 3.4, $R^2 = 0.12$). Similarly, the CO and DL treatments in the 10-20 cm depth showed a weak decrease in $E_{aR}$ over time ($R^2 = 0.07$ and $R^2 = 0.10$, respectively) (Fig. 3.1b; Table 3.4). In contrast, the $E_{aR}$ weakly increased over time in the O-horizon of the CO treatment ($R^2 = 0.19$).

The $E_{aR}$ of soil respiration in the HOPS experiment varied inconsistently among sites through time ($p < 0.0001$; Table S3.5) and there were no treatment effects. The $E_{aR}$ increased significantly during the incubation for all sites and treatments,

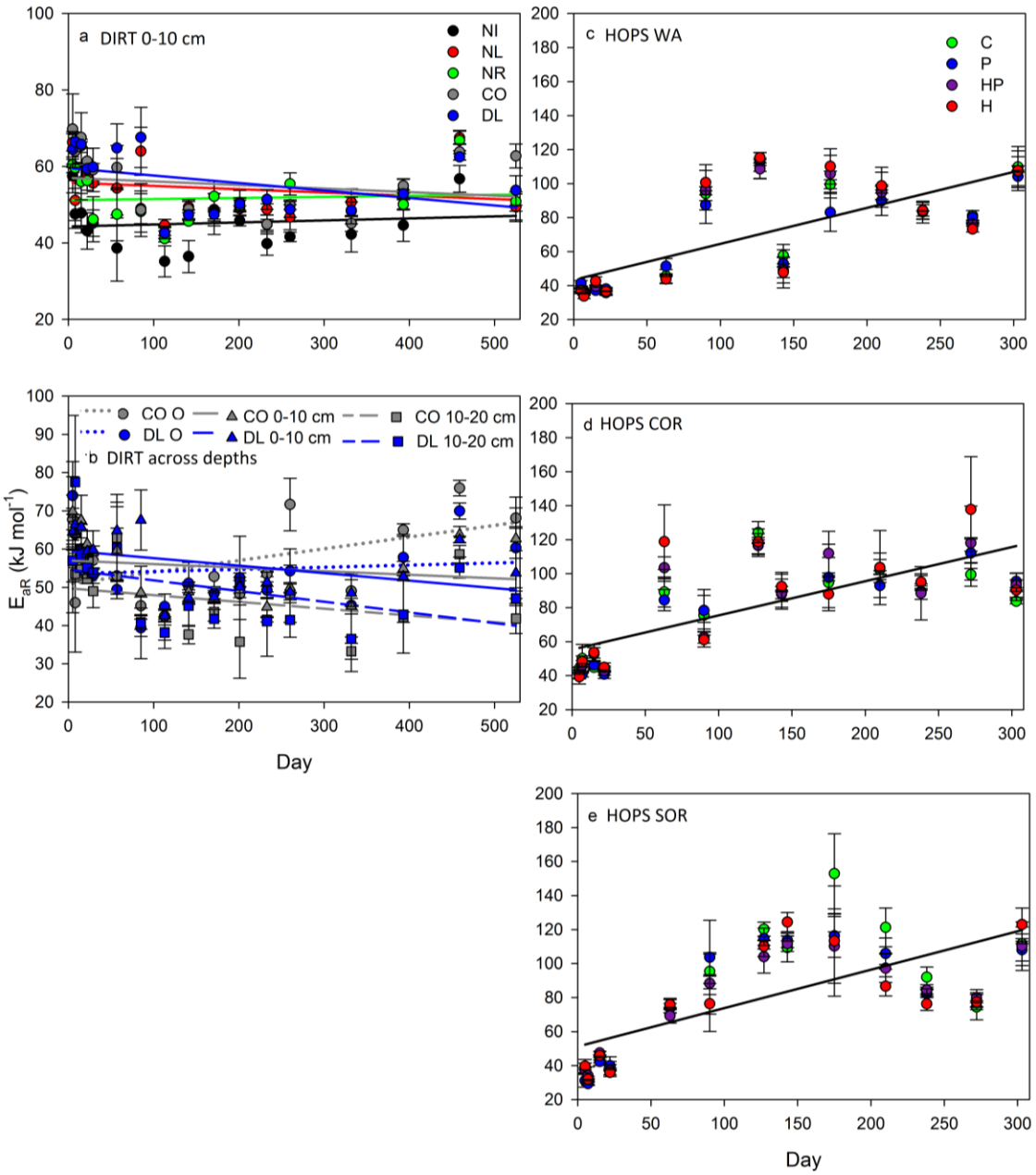


**Fig. 3.1**. Energy of activation ($E_{aR}$) through incubation time for DIRT 0-10 cm soils (a) and O-horizon, 0-10 cm, and 10-20 cm CO and DL soils (b) and HOPS WA (c), COR (d), and SOR (e). Lines represent linear regressions with statistics in Table 3.4. Note that a-b and c-e are on different y-axis scales. C = Control, P = Precipitation, HP = Heat x Precipitation, H = heat, NI = No Inputs, NL = No Litter, NR = No Roots, CO = Control, DL = Double Litter, O = Organic.

with higher correlation ($R^2$ 0.37 - 0.48) and larger slopes than in the DIRT experiment soils (Fig 3.1 c-e; Table 3.4).

**Table 3.4**. Linear regression $R^2$ and slope (b) of $E_{aR}$ over incubation time for DIRT and HOPS. Significant p-values in bold. NI = no inputs, NL = no litter, NR = no roots, CO = control, DL = double litter.

| Soil | | Treatment | $R^2$ | b | p-value |
|---|---|---|---|---|---|
| DIRT | Depth | | | | |
| | O-horizon | CO | 0.187 | 0.031 | 0.002 |
| | | DL | 0.006 | 0.006 | 0.588 |
| | 0-10 cm | NI | 0.014 | 0.005 | 0.413 |
| | | NL | 0.027 | -0.009 | 0.249 |
| | | NR | 0.003 | 0.003 | 0.707 |
| | | CO | 0.117 | -0.020 | 0.014 |
| | | DL | 0.027 | -0.009 | 0.250 |
| | 10-20 cm | CO | 0.067 | -0.018 | 0.067 |
| | | DL | 0.104 | -0.028 | 0.021 |
| HOPS | | Site | | | |
| | | WA | 0.480 | 0.24 | < 0.0001 |
| | | COR | 0.386 | 0.24 | < 0.0001 |
| | | SOR | 0.396 | 0.26 | < 0.0001 |

## Discussion

According to the carbon-quality temperature hypothesis (CQT), the temperature sensitivity of SOM decomposition should increase as labile substrates are depleted and carbon quality declines (Conant *et al*., 2011). Here we show that long-term incubations of soils from two manipulative experiments encompassing four sites showed divergent trends in temperature sensitivity that were inconsistent with proxies of carbon quality and previously published studies using similar methodology. There was no increase in the

temperature sensitivity of soil respiration in a forest soil following a 20-year depletion of soil C, although we did find a weak positive response in the $E_{aR}$ of the O-horizon in the CO (control) treatment. Similarly, we found no change in temperature sensitivity by depth. In contrast, soils from three Pacific Northwest prairies in a manipulative climate change experiment (HOPS) showed significant increases in temperature sensitivity with incubation time across all sites and treatments, consistent with the CQT hypothesis. The different temperature sensitivity responses, despite substantial losses of soil C, indicate that the CQT alone is not sufficient to explain the decay of SOM. Carbon-quality may interact with a number of other soil characteristics to create observed trends in apparent temperature sensitivity.

Overall, the response of DIRT soils was inconsistent with the CQT hypothesis across a number of operationally defined carbon quality parameters. The temperature sensitivity of soil carbon from the NI and CO treatments did not differ and did not increase with incubation time, though %C, soil respiration, and cumulative C turnover were significantly lower in the NI treatment. Although the NL and NR treatments resulted in nonsignificant changes in %C, they exhibited occasionally lower soil respiration rates than the CO treatment and this had no effect on $Q_{10C}$ or $E_{aR}$. The CO and DL treatments never differed in %C, soil respiration, cumulative C turnover, or temperature sensitivity; thus, although input exclusion led to a depletion of labile substrates, input addition did not apparently increase the pool of labile carbon. Despite often substantial decreases in %C, soil respiration rates, and cumulative C turnover with depth in the CO and DL treatments, the $E_{aR}$ of the 10-20 cm soil was never higher than that of the 0-10 cm or O-horizon and was frequently lower.

The response of the HOPS soils also was not completely consistent with the CQT hypothesis. If carbon quality is the primary driver of soil respiration and $E_a$, we would expect that relationship to hold true throughout the duration of incubation as carbon quality presumably continued to decline. The increase in $E_{aR}$ with incubation time appeared to be asymptotic, with $E_{aR}$ reaching a maximal value between day 126 and 174 (Fig. 3.1 c-e). Thus the response of $E_{aR}$ appears to be driven by an increase early in the incubation; excluding data prior to day 63 in SOR and COR results in no significant relationship with time, and similarly for data prior to day 89 in WA (data not shown). $E_a$ was also fairly stable after day 100 in Craine *et al*., (2010a) although soil respiration rates continued to fall. Furthermore, other carbon quality proxies did not indicate a strong relationship with temperature sensitivity. Though there was no measureable effect of warming on %C, there were occasional daily effects on soil respiration resulting in changes in cumulative C turnover from warmed soil, potentially indicating either an increase (for WA soil) or decrease (SOR and COR soil) in labile C, but these differences did not result in changes in $E_{aR}$. Furthermore, although the SOR soil had a substantially higher respiration rate throughout the incubation, its $E_{aR}$ was rarely lower than the COR and WA soils.

It has been proposed that variability in the temperature sensitivity of soil respiration across studies may be due to differences in methodology, especially a failure to sufficiently deplete labile substrates (Conant *et al.,* 2008) or otherwise minimize the influence of the mineral matrix (Conant *et al.,* 2011). Our results indicate otherwise. We replicated the methods of Conant *et al.*, (2008) with the DIRT soils and did not see an increase in $Q_{10}$ either with time or *in situ* carbon depletion due to the DIRT treatments

(Table 3.3). Conant *et al*., (2008) argued that Fang *et al*., (2005) failed to see an increase in $Q_{10}$ because not enough carbon was respired (<6% of the initial total). However, we did not find an increase in $Q_{10C}$ of soil respiration in DIRT soils even when considering total %C losses that were comparable to Conant *et al*., (2008a), i.e., 7-8% in all replicates (data not shown). All of our soils exhibited substantial losses of carbon and thus presumably depletion of labile substrates. DIRT input exclusion (NI) depleted soil carbon concentrations by nearly 30%, resulting in a 54% reduction in respiration rate at the beginning of the incubation. The 525-day incubation of the DIRT soils caused a further carbon depletion of 6-7%, and the 303-day incubation of HOPS soils caused a carbon depletion of 11-13%.

It also has been proposed that the organic horizon (Mikan *et al.*, 2002) or the non-mineral associated components of SOM (Wagai *et al.*, 2013) may be more likely to follow the CQT hypothesis because there is no mineral fraction to interfere with carbon losses. However, only the O-horizon of the CO treatment of the DIRT soils showed a weak positive trend of $E_{aR}$ over incubation time (Fig. 3.1b; Table 3.4), while the $Q_{10C}$ actually decreased during incubation (Table 3.3). Our results from the O-horizon do not support the supposition that organic carbon unprotected by mineral matter more closely follows the CQT hypothesis. Thus the temperature sensitivity response cannot be sufficiently explained by degree of carbon depletion or minimization of the soil matrix alone.

We directly compared our results against data from Craine *et al*., (2010) and Lefevre *et al*., (2014) to put them into context with studies using similar methods. Craine *et al*., (2010) incubated 28 soils, rotating sets of soils between 10-30$^{o}$C daily, and found a

negative correlation between the log of soil respiration at 20ºC ($logR_{20}$) and the log of temperature sensitivity calculated as $E_a$. Lefevre *et al*., (2014) incubated soils constantly at 4-35ºC from four sites left fallow for 25 to 79 years and found a similar relationship to that published by Craine *et al*., (2010). In contrast, when averaged across incubation time (as by Craine *et al*., 2010), neither HOPS nor DIRT soils showed a significant increase in the log $E_{aR}$ with a decrease in log $R_{20}$ (Fig. 3.2). DIRT soils exhibited a much lower

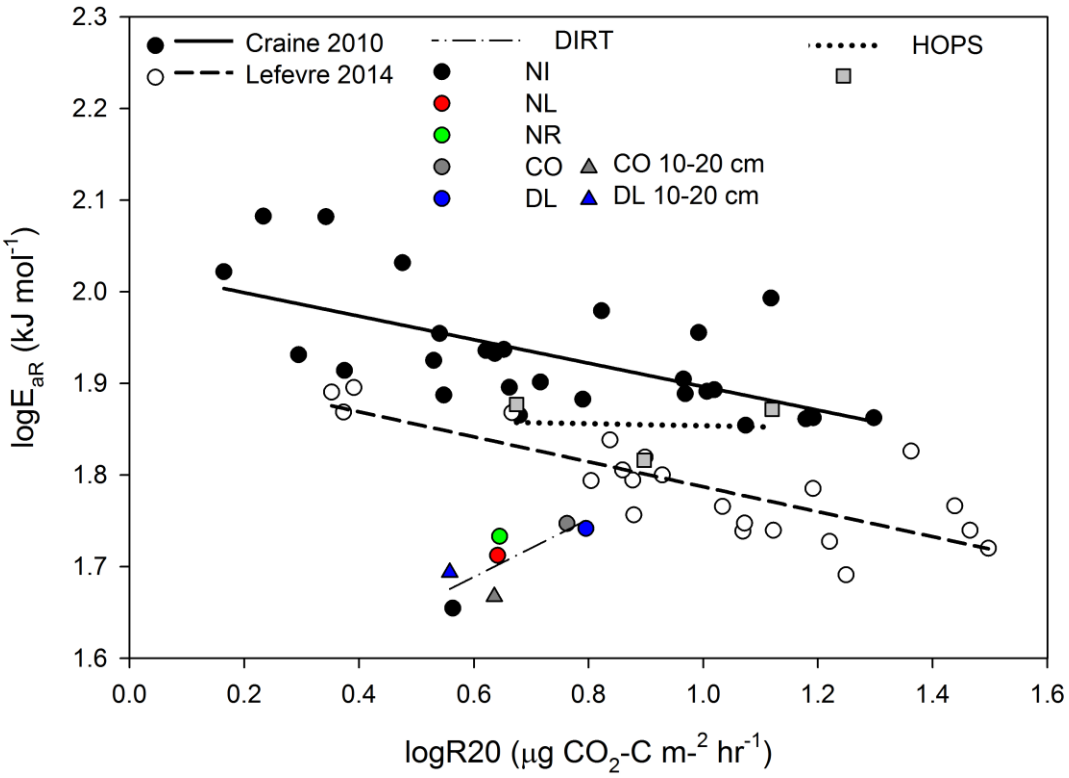


**Fig. 3.2.** Linear relationship between the log of energy of activation ($E_{aR}$) and the log of the soil respiration rate at 20ºC for DIRT ($y = 0.32x + 1.50$; $R^2 = 0.62$; $p = 0.036$) and HOPS ($y = -0.01x + 1.86$; $R^2 = 0.006$; $p = 0.95$) soils. Each point is averaged across all days and replicates, and across all treatments for HOPS. Also plotted are data from Craine *et al.*, (2010) ($y = -0.13x + 2.02$; $R^2 = 0.41$; $p < 0.0001$) and Lefevre *et al.*, (2014) ($y = -0.136x + 1.920$; $R^2 = 0.642$; $p < 0.0001$).

range of $E_{aR}$ than HOPS or the other two studies' soils, which may indicate that its responses are driven by factors unique to this soil, although Craine *et al*., (2010) also included soil from a northeastern deciduous forest.

Lefèvre *et al*., (2014) reasoned that higher $E_a$ may be due to adsorption reactions rather than chemical recalcitrance as their fallow-soils were depleted of non-mineral bound carbon. However, we did not observe such an increase in $E_a$ with carbon depletion and presumably an increase in the proportion of adsorbed carbon in the DIRT soils. It is possible that the DIRT soils showed a different response as they were not as severely carbon depleted, with at most a 30% loss between CO and NI versus 33-68% reported by Lefèvre *et al*., (2014). However, Leifeld and Fuhrer (2005) showed that the 63 µm fraction was not more temperature sensitive then bulk soil, though carbon in this fraction is thought to be chemically adsorbed. Moreover, Plante *et al*., (2010) reported particulate organic matter, considered to be labile, to have a higher temperature sensitivity than the mineral fraction. Taken together, these results suggest that trends in $E_a$ cannot be explained by an increase in the degree of mineral adsorption.

Recently it has been posited that the temperature sensitivity of SOM decomposition cannot be fully captured by a single metric but is a product of many interacting soil, microbial, and environmental factors (Kleber and Johnson 2010) and that modeling of soil-climate feedbacks should reflect these complex dynamics (Tang & Riley, 2015). However, incorporating these dynamics into models requires a set of empirical relationships that are generalizable across soils. The results of two parallel soil incubations presented here indicate that apparent contradictions in empirical findings may be due to context-specific conditions that have yet to be fully elucidated rather than

methodological differences. This is consistent with the emerging understanding that SOM stabilization is not solely a function of chemical complexity but of soil aggregation and physio-chemical interactions with the soil matrix affecting substrate availability (Kleber & Johnson, 2010; Conant *et al.*, 2011).

**Bridge to Chapter IV**

The temperature sensitivity of SOM decomposition is commonly expected to vary with decomposition rate due to a change in C quality; and this trend has been repeatedly demonstrated. However, the theoretical understanding of the nature of SOM upon which this theory is founded is undergoing a paradigm shift, leading important exceptions to the aforementioned trend to be re-examined not as errors but as important insights. If SOM decomposition rate is not controlled primarily by its chemical complexity but by its physical and chemical relationship with soil, then how do these determine soil C accumulation and mineralization? In the following chapter I delve deeper into the DIRT soil samples, determining the C content of ecologically important pools, the decomposition dynamics of SOM, and directly examine changes to the molecular nature of SOM itself under litter-input manipulation.

# CHAPTER IV

# INSIGHTS INTO SOIL C CYCLING FROM LONG-TERM INPUT-MANIPULATION AND HIGH-RESOLUTION MASS SPECTROMETRY

## Contributions

Lorien L. Reynolds helped design the experiment, collected the data, analyzed the data, and wrote the manuscript. Richard D. Bowden manages the DIRT experimental site and collected the soils. Malak Tfaily ran soil samples with FTICR-MS. Kate Lajtha, Richard D. Bowden, Bart R. Johnson, and Scott D. Bridgham edited the manuscript.

## Introduction

Soils play a key role in regulating climate, by storing vast quantities of carbon (C) as soil organic matter (SOM) (Jobbagy & Jackson, 2000; Houghton, 2005) that is subsequently released into the atmosphere via soil respiration (SR), one of the largest terrestrial C fluxes (Raich & Schlesinger, 1992). Global change is expected to perturb the balance between SOM storage and SR by altering net primary productivity, and thus plant-litter inputs, while increasing SOM decomposition (Friedlingstein *et al.*, 2006; Anav *et al.*, 2013). Meanwhile, there is speculation that soils may offer a means to offset climate forcing by using management to enhance natural C accumulation mechanisms and thus store additional carbon (Lal, 2005; Canadell *et al.*, 2007; Smith & Fang, 2010; Dungait *et al.*, 2012).

Soil C research, however, is riddled with apparent paradoxes (see Dungait *et al*.. 2012), in part because the mechanisms driving C cycling dynamics are poorly understood (von Lützow *et al.*, 2007, 2008; Schmidt *et al.*, 2011). Model projections of soil responses to climate range widely in both magnitude and direction (Friedlingstein *et al.*, 2006; Anav *et al.*, 2013). Experiments simulating climate change have reported a variety of soil responses (Rustad *et al.*, 2001; Wu *et al.*, 2011) indicative of underlying complexities. Importantly, some studies showed that the decomposition of older SOM did not have a higher temperature sensitivity (Liski *et al*., 1999, Giardina and Ryan 2000, Dalias *et al*., 2001) which contradicts enzymatic kinetic theory. Some of this apparent complexity may be due to our poor understanding of the nature of SOM. It is not clear how plant litter quality, quantity, and source (i.e., roots versus aboveground litter) are linked to C retention in soils (Dungait *et al.*, 2012; Lajtha *et al.*, 2014a, 2014b). Thus, the potential to sequester additional C within soils through management remains speculative (Dungait *et al.*, 2012), especially as C addition may potentially 'prime' decomposition of native C stocks (Kuzyakov, 2002; Bowden *et al.*, 2014; Lajtha *et al.*, 2014a, 2014b). To predict, and possibly manipulate, soil-climate feedbacks, we must understand the pathways C takes through soil, from the incorporation and transformation of litter inputs to its ultimate mineralization.

Historically, SOM was thought to become stabilized in soils as it became increasingly chemically complex during decomposition leading to organic compounds that were resistant to microbial degradation (see Kleber and Johnson 2010). However, direct investigations of the biochemical nature of SOM with high-resolution imaging techniques revealed it to be a complex amalgam of interacting, low molecular-weight

organic molecules in a matrix of minerals (Lehmann *et al.*, 2008; Schmidt *et al.*, 2011). Furthermore, SOM that has typically been considered to be chemically labile can have turnover times on the order of centuries (Kleber *et al.*, 2011). Thus the hypothesis that SOM decomposition is determined by chemical complexity alone has been challenged in favor of spatial and soil-matrix mediated controls on availability (Ekschmitt *et al.*, 2005; Kleber, 2010; Schmidt *et al.*, 2011). SOM age, and thus 'stability', tends to increase with its degree of mineral association, with free, particulate organic matter being the youngest and most plant-like, and organic matter occluded within microaggregates and adsorbed onto the surface of minerals being older and primarily microbial in origin (Sollins *et al.*, 2006). Organic matter becomes incorporated into these pools due to microbial activity in close association with mineral particles (Kleber & Johnson, 2010); chemical complexity *per se* is not the sole determinant of SOM stability.

However, the turnover rates of these mineral-associated C pools are largely unknown (Conant *et al.*, 2011). Although dissolved organic matter chemistry controls C adsorption on mineral particles and thus stabilization (Kleber *et al.*, 2007), it is not clear how this may determine turnover rates within these pools. Moreover, the pathway by which organic matter enters C pools with the longest residence time is not well known, nor is the role of litter C quantity and quality (Bird *et al.*, 2008; Prescott, 2010; Dungait *et al.*, 2012; Lajtha *et al.*, 2014b; Hatton *et al.*, 2015). Long-term litter-input experiments are perhaps best able to elucidate soil C sequestration and stabilization rates that are manifested over decades to millennia. However, the mechanisms driving the response to these long-term manipulations can be best discerned with modern methods that allow direct characterization of SOM chemistry and its relation to the mineral matrix. For

example, advances in mass spectrometry have allowed a direct examination of the biochemical constituents of terrestrial and aquatic C pools (Kellerman *et al.*, 2014; Ohno *et al.*, 2014; Tfaily *et al.*, 2015). Additionally, measures such as decomposition dynamics and catabolic profiles (Campbell *et al.*, 2003) can provide insight into shifts in microbial function, and thus SOM cycling, under differing input regimes.

The Detritus Input and Removal Treatment (DIRT) sites were designed to directly test the effects of chronic input-manipulation on SOM decomposition and accumulation on decadal time scales (Nadelhoffer *et al.*, 2004). Thus far these experiments have revealed indirect and nonlinear responses (Bowden *et al.*, 2014; Lajtha *et al.*, 2014a, 2014b) in total C and mineral-associated SOM pools, including evidence for context-specific dynamics and priming. Overall the mineral-associated fractions are reported to be less vulnerable to decomposition and less responsive to C accumulation from aboveground inputs, indicating that direct litter addition does not necessarily result in rapid or direct accumulation of additional C in the putatively 'stable' pools (Bowden *et al.*, 2014; Lajtha *et al.*, 2014a, 2014b) even when soils are not 'carbon saturated' (Mayzelle *et al.*, 2014). Moreover, the variability of responses among sites may be due to differences in litter decomposition rate as controlled by climate (Fekete *et al.*, 2014), litter quality (Bowden *et al.*, 2014), and soil mineralogy that in turn drive the litter-to-soil pathway.

Litter quality may play a primary role in controlling soil C stabilization, and it has been proposed that root C may be preferentially incorporated into soils and stabilized (Schmidt *et al.*, 2011), possibly because of its proximity to the mineral matrix or due to the layer of suberin which slows decomposition (Rasse *et al.*, 2005). However, Hatton et

al. (2015) reported that while root C contributed more particulate matter to the free, light fraction, likely due to slower decomposition of the litter, leaf C contributed more to the mineral-associated and potentially more stable fraction in a coniferous forest. Thus labile inputs such as foliar litter may actually contribute more than roots to mineral-associated SOM pools and be preferentially stabilized as they stimulate microbial activity and exudation of microbial products (Cotrufo *et al.*, 2013). This is in contrast to the DIRT findings that there is little evidence of C accumulation in the mineral-fractions due to aboveground litter addition (see above). It is possible that the foliar C that is deposited in the mineral-associated fractions may remain active as there is evidence for multiple turnover rates within this C pool (see Torn *et al*., 2013). Additionally, the relative importance of root versus aboveground litter C may vary with forest type: Though there was no overall loss of C due to root exclusion, Crow *et al*., (2009) reported preferential stabilization of foliar-derived aliphatics in a coniferous forest and root-derived aliphatics in a deciduous forest. Meanwhile, root exclusion has been found to cause greater (Fekete *et al.*, 2014), lower (Bowden *et al.*, 2014; Lajtha *et al.*, 2014b), or no difference (Lajtha et al. 2014b; grassland sites) in C losses compared to aboveground litter exclusion.

We asked how C cycles through terrestrial soils by combining a 20-year long chronic root and litter input manipulation in a northeastern deciduous forest with a long-term laboratory incubation, and comparing whole soil responses with C pools defined by mineral association. We asked if litter input manipulation changed C quantity and which C pools were most vulnerable to change. Furthermore, we directly examined the molecular nature of C in the fine mineral fraction, demonstrated to be the oldest and thus considered the most stable pool of C (Christensen, 2001; Sollins *et al.*, 2006), to directly

test its chemical complexity and putative stability. Finally, we asked whether changes in the total amount, density fractions, or molecular composition of mineral-associated C could explain C mineralization rates.

## Materials and Methods

### *Site Descriptions and Soil Collections*

Soils were collected from the Detritus Input and Removal Treatment (DIRT) plots located in the Bousson Experimental Forest (41°36'N, 80°3'W, 381 m), Pennsylvania, USA. Details about the site and experiment can be found in Bowden *et al*., (2014). Average annual precipitation is 1050 mm $yr^{-1}$ and daily temperatures average -4°C in January and 21°C in August. Soils are classified as coarse loamy mixed superactive mesic Oxyaquic Fragiudalfs (Cambridge series) derived from glacial till overlying shale and sandstone (USDA-SCS, 1979) with a fragipan present at 60 cm and with a pH of 4.0. Black cherry (*Prunus serotina*) and sugar maple (*Acer saccharum*) are the dominant tree species. The Bousson Forest DIRT plots were initiated in 1991 and consist of three 3 x 3 m replicate plots under five litter manipulation treatments: no inputs (NI; no roots or litter), no litter (NL), no roots (NR), control conditions (CO), or double litter (DL). Screens are used to exclude aboveground litterfall which is transferred to the DL treatment plots. Roots were meant to be excluded via impermeable plastic barriers buried from the surface to the C horizon (~1.4 m), but some roots have entered the plots from below the barriers and the litter treatment also affected root biomass (Bowden *et al.*,

2014). Root biomass from 0-10 cm depth was 129%, 90%, 51%, and 10% of the control in the DL, NL, NR, and NI treatments, respectively, at the time of sampling.

In fall 2011, two cores from 0-10 cm depth were collected and bulked per plot using a gas-powered 9.62-cm-diameter diamond-bit stainless steel soil corer (Earthquake, 9800B). All samples were passed through a 2 mm sieve and sorted for rocks, roots, and other debris, then subsampled and stored field moist at 4$^{o}$C. Leaf litter was collected fall 2014 and air dried for further chemical analysis (see FTICR-MS below).

*Incubation*

Incubations began January 2012 and continued for 525 days. Total C was determined on dried, ground soil with a Costech Analytical Technologies 4010 elemental combustion analyzer (Valencia, CA, USA). Soil pH was determined with a 1:1 by volume slurry with distilled water, and soil moisture was determined by drying ~10 g of soil at 60$^{o}$C for 48 hr. Approximately 20 g dry-weight equivalent of mineral soil was weighed into 120 mL serum bottles and moisture content adjusted to 60% of saturation with distilled water. Moisture content was monitored by weight and water applied when necessary.

Soils were incubated at 35$^{o}$C. Soil respiration was measured every day for the first week, each week for the first month, and every other month thereafter. To measure the soil respiration rate, each serum bottle was sealed, over-pressurized by injecting 5 mL of air, and the headspace $CO_2$ concentration measured via direct injection with a LI-7000 infrared gas analyzer (LICOR Inc., Lincoln, NE), then measured again after 3 hr; a $CO_2$ standard curve was determined on each measurement day. Measurements were made

using a LI-6400 (LICOR Inc., Lincoln, NE) plumbed for direct injection on days 56-57 and 84-85 while the LI-7000 was repaired.

*Catabolic Profile*

Substrate-induced respiration techniques such as MicroResp$^{TM}$ (Aberdeen, Scotland) can be used to assess a microbial community's ability to utilize substrates of varying quality (Campbell *et al.*, 2003). Following the MicroResp$^{TM}$ protocol, in January 2012 we measured the response of soil from each DIRT treatment to 18 carbon substrates, including simple sugars, amino acids, carboxylic acids, and complex structural carbohydrates. Briefly, soils with equivalent soil moisture contents were loaded into 96-well microtiter plates, inoculated with 25 μL of 30 mg substrate $g^{-1}$ soil water, and incubated for 6 hr at 25$^{o}$C. The detector plate, filled with a creosol red-agar solution, was analyzed with an Infinite 200 PRO® plate reader (Tecan, Switzerland) at 570 nm to determine the amount of $CO_2$ respired during the incubation. $CO_2$ standards were run in a similar manner.

*Density Fractionation*

Soils were separated by density fractionation with sodium polytungstate (SPT) using methods modified from Crow *et al*., (2006). Approximately 5 g of soil from day 0 and day 525 of the incubation were mixed with 20 ml of 1.6 g $cm^{-3}$ SPT and reciprocally shaken for 24 hr. The floating fraction (hereafter the light fraction or LF) was aspirated off, triple rinsed with DI, dried at 60$^{o}$C for 48 hr, and weighed. The heavy fraction (HF) was dried at 60$^{o}$C and sieved, with the fine HF (fHF; < 53 μm) consisting of silt and clay

and the remaining HF ($\geq$ 53 µm) consisting of sand and aggregates. The remaining HF was then mixed with 20 mL of 5% hexametaphosphate (HMP) and reciprocally shaken for 24 hr to disperse aggregates. It was then vacuum-filtered and triple rinsed with DI water to remove the HMP, dried at 60$^{o}$C, and fractionated again with SPT as described above. The floating fraction derived from dispersed aggregates was defined as the occluded LF (OLF). The HF remaining was defined as coarse HF (cHF; $\geq$ 53 µm) consisting of sand as well as the clay and silt released from the dispersed aggregates. All fractions were dried at 60$^{o}$C for 48 hr, weighed, and total C and N determined as described above for bulk soil.

*FTICR-MS*

Fine HF soil and leaf litter were processed and analyzed with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) as described in Tfaily *et al*., (2015). Briefly, 100 mg of dried SOM was extracted with 1ml of methanol, which Tfaily *et al*., (2015) determined preferentially captures organic matter with low O/C ratios ( O/C <0.6), particularly lipids and lignin-like compounds in addition to unsaturated hydrocarbons especially for soils with low % C as the ones used in this study. Thus while our results do not represent a complete inventory of SOM in the fHF, they do represent a consistent chemical footprint across the various treatments. Soils were extracted while shaking for two hours (Tfaily *et al*., 2015) and were then centrifuged. The supernatant was then injected directly intoa 12 T Bruker SolariX FTICR mass spectrometer at the Environmental Molecular Sciences Laboratory (EMSL), a Department of Energy (DOE) national user facility in Richland, WA. Negatively charged

molecular ions were generated using a standard Bruker electro-spray ionization (ESI) source and the ion accumulation time was adjusted to account for differences in the % C between samples. Extraction efficiency was estimated to be around 7% based on UV/vis spectroscopy (Tfaily *et al*., 2015). Samples were injected into the ESI source equipped with a fused-silica tube (200 μm i.d.) through a syringe pump at a flow rate of 3.0 μL/min. Experimental conditions were optimized as follows: needle voltage, +4.4 kV; Q1 set to 150 m/z; and the heated resistively coated glass capillary operated at 180°C. Each sample signal was averaged from 96 individual scans internally calibrated using OM homologous series separated by 14 Da ($-CH_2$ groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (i.e., 200< m/ z <1200). The Compound Identification Algorithm (CIA) from Kujawinski and Behn (2006) and modified by Minor *et al*., (2012) was used to assign chemical formulas based on the following criteria: S/N > 7σ, and mass measurement error <1 ppm taking into consideration the presence of C, H, O, N, S, and P and excluding other elements. Singly charged ions were confirmed by the 1.0034 Da spacing found between isotopic forms of the same molecule (between $^{12}C_n$ and $^{12}C_{n-1}$–$^{13}C_1$).

To interpret the large data set, the assigned compounds were visualized on van Krevelen diagrams. The compounds were plotted on the van Krevelen diagram on the basis of their molar H/C ratios (yaxis) and molar O/C ratios (x-axis) (Fenn *et al.*, 1990; Kim *et al.*, 2003). The van Krevelen diagrams provide a means to compare the average properties of OM and enable identification of the major biochemical classes. For this study, the chemical compounds were grouped into the 8 main families: condensed aromatic compounds, unsaturated hydrocarbon, tannins, lignin, lipids, protein, amino

sugars and carbohydrate derived. From the formula assignment, average (by number-weighted) abundance of each class was calculated and compared between samples.

## *Analysis*

### *C Concentration of Bulk Soil and Density Fractions*

Data were assessed and transformed as necessary to meet the assumptions of each analysis. Soil responses were analyzed with one-way ANOVA followed by Tukey's post-hoc tests. Differences in C concentration in density fractions between incubation days were tested with repeated-measures ANOVA to account for dependence through time, followed by ANOVAs within day and t-tests within treatment when there was a significant interaction. We then used multiple regression to test whether changes in C concentration of bulk soil and density fractions explained soil respiration rates at the beginning and end of incubation. All ANOVAs and regressions were performed using SPSS version 19.0 (IBM SPSS Statistics for Windows, 2010, Armonk, NY).

### *Cumulative Soil Respiration Modeling*

Cumulative C respired was calculated by smoothly incrementing the respiration rate g $C^{-1}$ (i.e., the C turnover rate) and g dw $soil^{-1}$ across non-measurement days. Treatment differences for total cumulative C respired were analyzed as described above. Cumulative soil respiration was fitted against a one-pool model,

$$X_t = X_0 (1-e^{-kt}) \qquad \text{equation (1)}$$

where $X_t$ is the total C respired, $X_0$ is the mineralizable C pool, *k* is the mineralization rate, and t is day. Nonlinear regressions were fitted to the cumulative respiration data using Levenberg-Marquardt and sequential quadratic programming nonlinear regression parameter estimation procedures in SPSS version 19.0. Global stability of fitted constants was confirmed by increasing or decreasing each by 50% and confirming that the model converged on the original value. A two-pool model was also attempted but never stabilized (data not shown). Treatment differences for $X_0$ and *k* were analyzed as described above.

*Catabolic Profile*

The response to each substrate was standardized by subtracting the baseline soil respiration rate and dividing by the average response for each treatment (Campbell *et al.*, 2003). Treatment differences in the response to each substrate were analyzed as described above. We assessed the overall response of each treatment to the full suite of substrates with non-metric multidimensional scaling (NMS) (McCune & Grace, 2002) on a Bray-Curtis dissimilarity matrix, and then tested for treatment differences with PERMANOVA. Both analyses were performed using the package vegan (Oksanen *et al.*, 2012) in R v. 3.2.0.

*FTICR-MS*

We tested for differences in the proportion of each biochemical class detected in the fHF due to input treatment and incubation as described above. Most biochemical compounds (all except carbohydrates, proteins, and 'other') had a significant interaction

between incubation day and litter treatment in the repeated measures ANOVA, thus the p-values for treatment differences are from ANOVAs within each incubation day and differences due to incubation are from t-tests within treatments. Changes in biochemical content between input treatments and leaf litter was also compared visually using van Krevelen diagrams (Kim *et al.*, 2003). We visualized changes in biochemical classes with non-metric multidimensional scaling (NMS) performed on a Bray-Curtis dissimilarity matrix, and tested for differences among treatments and between incubation days within this non-parametric space with PERMANOVA. Both NMS and PERMANOVA were performed using the package vegan in R v. 3.2.0. We then used multiple regression to test whether changes in biochemical classes in the fHF, as expressed by axis scores from NMS, explained soil respiration rates at the beginning and end of incubation.

## Results

### *C Concentration*

C concentration was reduced in the NI treatment versus all other treatments (Table 4.1; $p = 0.016$), with a 30% depletion versus the control. There was also a non-significant 13% and 8% reduction in C concentration in the NL (pairwise comparison: $p = 0.47$) and NR (pairwise comparison: $p = 0.80$) treatments, respectively.

### *Cumulative C Respired and Model Coefficients*

Cumulative C respired was reduced by input exclusion but largely unaffected by input addition (though there was a general decline from the DL to NI treatments), while

litter and root exclusion appeared equivalent. Similar results occurred regardless of whether results were expressed per g soil or as a turnover rate of the soil C pool (Table 4.1). The modeled size of the mineralizable pool, $X_0$, per g soil was significantly reduced

**Table 4.1.** Mean (se) C concentration, cumulative C respired, and decomposition model coefficients. Different small letters indicate significant differences.

| Parameter | Treatment | | |
|---|---|---|---|
| C concentration | | mg $CO_2$-C g dw soil$^{-1}$ | |
| | p-value | 0.016 | |
| | NI | 36.0 (3.2)$^{a}$ | |
| | NL | 44.7 (3.6)$^{b}$ | |
| | NR | 47.1 (3.5)$^{b}$ | |
| | CO | 51.3 (1.3)$^{b}$ | |
| | DL | 50.7 (0.30)$^{b}$ | |
| Cumulative C respired | | mg $CO_2$-C g dw soil$^{-1}$ | mg $CO_2$-C g$C^{-1}$ |
| | p-value | <0.0001 | <0.0001 |
| | NI | 2.36 (0.13)$^{a}$ | 70.7 (2.83)$^{a}$ |
| | NL | 3.99 (0.14)$^{b}$ | 98.9 (7.19)$^{b}$ |
| | NR | 4.01 (0.39)$^{b}$ | 92.9 (4.00)$^{b}$ |
| | CO | 5.76 (0.18)$^{c}$ | 126.3 (2.33)$^{c}$ |
| | DL | 6.19 (0.13)$^{c}$ | 139.0 (3.18)$^{c}$ |
| $X_0$ | | | |
| | p-value | <0.0001 | 0.01 |
| | NI | 4.14 (0.099)$^{a}$ | 139.7 (12.09)$^{a}$ |
| | NL | 4.59 (0.014)$^{a}$ | 121.1 (11.36)$^{a}$ |
| | NR | 5.56 (0.049)$^{ab}$ | 143.6 (6.61)$^{ab}$ |
| | CO | 6.53 (0.018) $^{bc}$ | 153.7 (4.8)$^{ab}$ |
| | DL | 7.66 (0.11)$^{c}$ | 194.8 (16.97)$^{b}$ |
| *k* | | | |
| | p-value | 0.007 | 0.001 |
| | NI | 0.0016 (0.00001)$^{a}$ | 0.0013 (0.00021)$^{a}$ |
| | NL | 0.0032 (0.00001)$^{c}$ | 0.0029 (0.00014)$^{c}$ |
| | NR | 0.0023 (0.00002)$^{ab}$ | 0.0019 (0.00006)$^{ab}$ |
| | CO | 0.0035 (0.000003)$^{c}$ | 0.0029 (0.0001)$^{c}$ |
| | DL | 0.0029 (0.00002)$^{bc}$ | 0.0023 (0.00031)$^{bc}$ |

in the NI and NL treatments versus the CO and DL treatments, while $X_0$ of the NR treatment was only different from the DL treatment. The overall trend for $X_o$ was similar

when expressed as a C turnover rate, but only the NI and NL treatments were significantly reduced versus the DL treatment. The mineralization rate, *k*, was significantly reduced in the NI treatment versus the NL, CO and DL treatments. The *k* of the NR treatment was also lower than the NL and CO treatments.

*Catabolic Profile*

In general, the NI and NR treatments respired less in response to substrate addition than the NL, CO, and DL treatments, with the exception of urocanic acid, iso-leucine, and glucosamine. However, there was a significant treatment effect for only 6 out of 18 substrates (Table S4.1). NMS explained 99.6% of the variance in soil

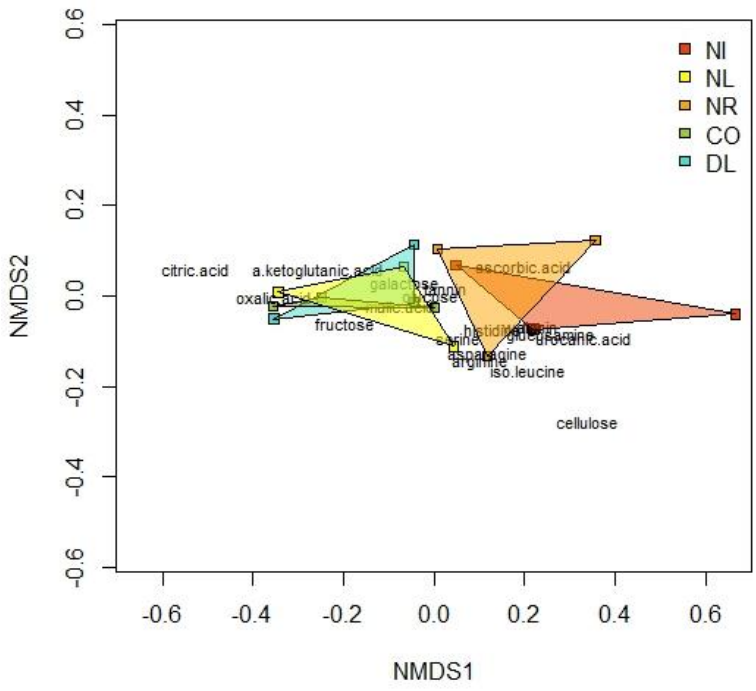


**Fig. 4.1.** Non-metric dimensional scaling of substrate induced respiration for catabolic profile of DIRT soil. NI = No Inputs, NL = No Litter, NR = No Roots, CO = Control, DL = Double Litter.

respiration and showed that the NI and NR treatments separated from the NL, CO, and DL treatments (Fig. 4.1; stress = 0.064; p = 0.047) due to differences in their response to cellulose versus sugars, and some carboxylic and amino acids.

*Density Fractionation*

The soil C concentration in the LF was reduced by 25% in the NL treatment, 35% in the NR treatment and 49% in the NI treatment versus the CO treatment, though only the NI treatment was significantly different (Fig. 4.2; p = 0.049). On day 0, exclusion of all inputs had reduced the size of the OLF versus the NL, NR, and CO treatments, though this was only significant versus the NR treatment (omnibus ANOVA: p = 0.021) and

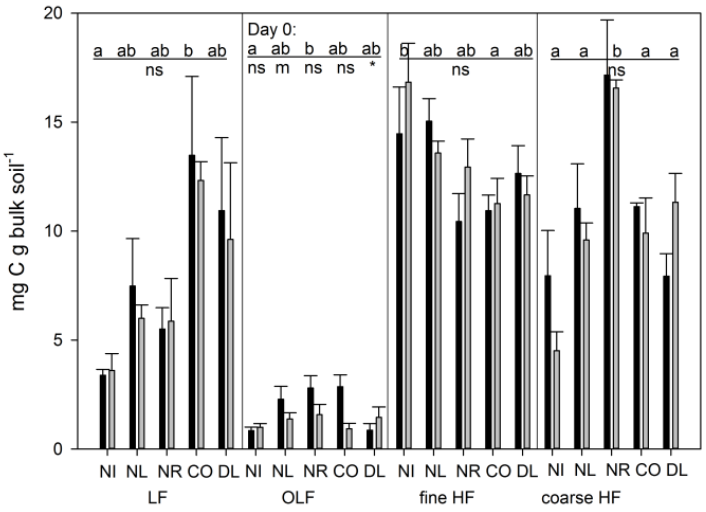


**Fig. 4.2.** Mean (se) mg C g bulk soil$^{-1}$ in density fractions for DIRT soil at the beginning (Day 0, black bars) and end (Day 525, gray bars) of incubation. Treatment differences are shown by different small letters, and incubation differences by * = significant, m = marginal, and ns = non-significant. NI = No Inputs, NL = No Litter, NR = No Roots, CO = Control, DL = Double Litter. Treatment effects are broken out by day if there was a significant treatment x incubation interaction. LF = Light Fraction, OLF = Occluded Light Fraction, fHF= fine Heavy Fraction, cHF = coarse Heavy Fraction.

marginally different from the CO treatment (pairwise comparison: p = 0.077). These differences were lost on day 525 (omnibus ANOVA: p = 0.20). In the fHF, C

concentration was significantly higher in the NI treatment versus CO ($p = 0.031$) and marginally higher than the NR treatment (pairwise comparison: $p = 0.085$). In the cHF, the NR treatment had a significantly higher C concentration than all other treatments ($p = 0.001$). The NI treatment was marginally reduced versus the NL treatment (pairwise comparison: $p = 0.067$) and the CO treatment (pairwise comparison: $p = 0.054$).

The soil C concentration of the DL treatment on day 0 was 19% lower than the control in the LF, 70% lower in the OLF, and 29% lower in the cHF, though these differences were not significant (pairwise comparisons: $p \geq 0.10$). The concentration of C in the OLF decreased during incubation in the NL, NR, and CO treatments, though only the NL treatment was even marginally significant ($p = 0.099$). The C concentration in the OLF of the DL treatment increased during incubation ($p = 0.026$).

*FTICR-MS*

There were substantial changes due to input manipulation and incubation in the classes of biochemical compounds in the fHF detected by FTICR-MS (Fig. 4.3). Considering the effect of input manipulation first (i.e., day 0), the NI treatment was very similar to the CO treatment, differing only by a marginally significant 5.1% absolute loss of proteins (Fig. 4.3 Day 0; statistics in Table S4.2; repeated measures ANOVA: $p = 0.068$; day x treatment interaction: $p = 0.23$; pairwise comparison: $p = 0.072$), though these treatments diverged during incubation (see below). In contrast, the DL treatment differed from the control treatment on day 0, with a much greater proportion of lipids (56.6% versus 18.6% respectively) (omnibus day 0 ANOVA: $p < 0.0001$), and a lower proportion of amino sugars (-0.7%, omnibus day 0 ANOVA: $p = 0.054$), condensed

hydrocarbons (-7.3%, omnibus day 0 ANOVA: p = 0.023), lignin (-11.9%, omnibus day 0 ANOVA: p = 0.035), tannins (-4.8%, omnibus day 0 ANOVA: p = 0.009), and unsaturated hydrocarbons (-8.2%, omnibus day 0 ANOVA: p = 0.001).

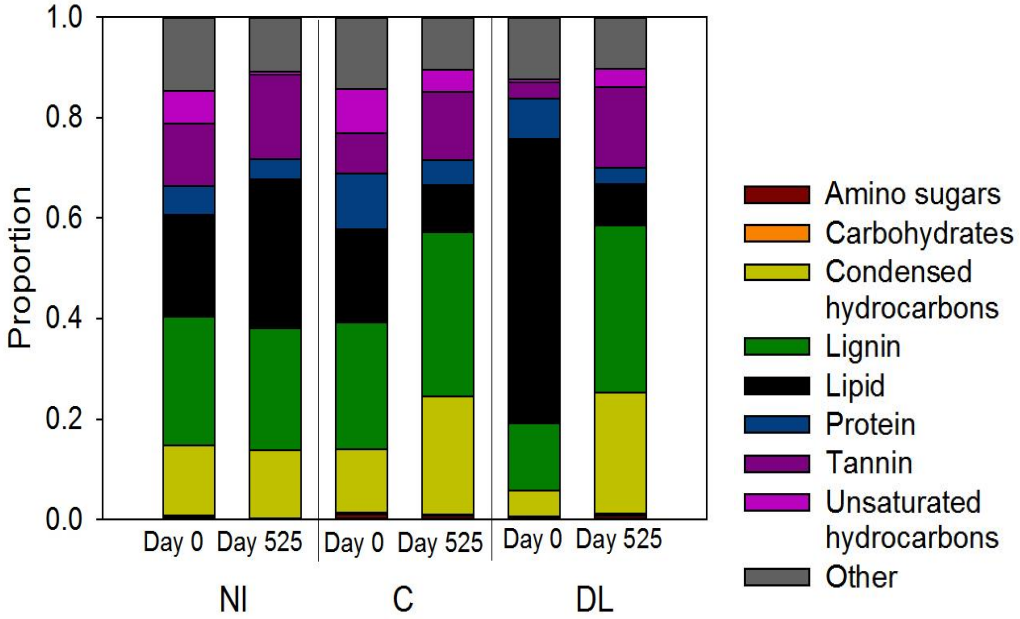


**Fig. 4.3.** Proportion of each biochemical class detected with FTICR MS in the fine heavy fraction (fHF) of no inputs (NI), control (CO), and double litter (DL) treatments at the beginning (Day 0) and end (Day 525) of incubation.

The classes of biochemical compounds changed very little over the course of the incubation in the NI treatment, with small but significant losses of amino sugars (Fig. 4.3; -0.3% absolute percentage change, p = 0.076), proteins (-2%, p = 0.001), unsaturated hydrocarbons (-6.1%, p = 0.017), and 'other' unidentified compounds (-3.9%, p = 0.039). However, though they started very similar, the biochemical composition of the CO treatment diverged from the NI treatment over the course of the incubation: The CO treatment had substantial decreases in lipids (-9.3%, p = 0.024), proteins (-5.9%, p = 0.001), unsaturated hydrocarbons (-4.4%, p = 0.019), and 'other' compounds (those that did not correspond to the main biogeochemical groups) (-3.8%, p = 0.039), and increases

in condensed hydrocarbons (+10.9%, p = 0.001), lignins (+7.6%, p = 0.035), and tannins (+5.4%, p = 0.052). The chemical signature of the DL treatment became more similar to the CO treatment over the incubation: The majority of the large lipid pool disappeared over the incubation (-48.3%, p = 0.013), 'other' compounds also declined (-2%, p = 0.039), accompanied by an enrichment in amino sugars (+0.5%, p = 0.012), condensed hydrocarbons (+19%, p = 0.006), lignin (+19.8%, p = 0.031), tannins (+12.7%, p = 0.023), and unsaturated hydrocarbons (+3%, p = 0.041). There was no change in the proportion of carbohydrates (p = 0.36), which remained small, though it should be noted that this method is not optimal for detecting carbohydrates (see Tfaily et al. 2015).

NMS explained 78.8% of the variance in biochemical composition and showed separation on both treatment (p = 0.012) and incubation day (p = 0.001), while treatments also differed between days (p = 0.001) (Fig. 4.4; stress = 0.046). On day 0, the NI and

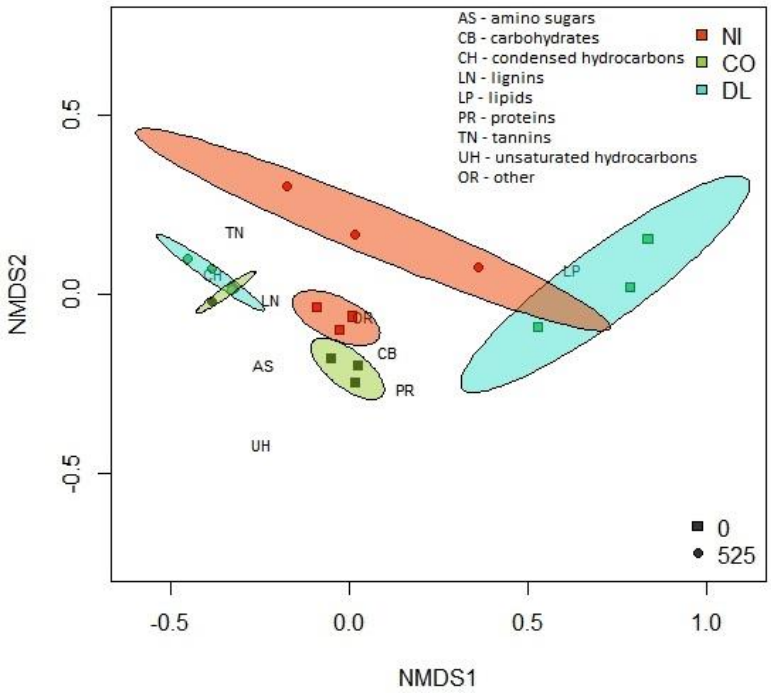


**Fig. 4.4.** Non-metric dimensional scaling of biochemical classes detected with FTICR MS in the fine heavy fraction (fHF) of no inputs (NI), control (CO), and double litter (DL) treatments at the beginning (Day 0) and end (Day 525) of incubation.

CO treatments differed from the DL treatment due to the lipid enrichment in the latter ($p = 0.001$). On day 525, the DL treatment had become more similar to the CO treatment as both lost lipids and became relatively more enriched with tannins and lignin ($p = 0.015$).

**Discussion**

The no inputs treatment resulted in a 30% *in situ* loss of soil C, and suggested more modest but proportional losses due to aboveground litter or root exclusion alone (Table 4.1). These losses were largely attributable to decreases in LF C (Fig. 4.2), representing 13, 8, and 10% reductions of the total C recovered from density fractionation versus the control treatment in the no inputs, no litter, and no roots treatments, respectively. The reduction in both cumulative respiration and mineralizable C ($X_o$) due to exclusion of leaves and roots, combined (Table 4.1), as well as a change in the catabolic profile for the no inputs and no roots treatments (Fig. 4.1), indicates a decrease in both availability and quality of the remaining carbon as light fraction C was depleted. This is consistent with the idea that the mineral matrix facilitates the stabilization of C through organo-mineral associations and aggregation (Conant *et al.*, 2011; Dungait *et al.*, 2012), whereas particulate organic matter (i.e., light fraction C) is more available and turns over more rapidly, and thus its decomposition dynamics are dictated more strongly by chemical quality (von Lützow *et al.*, 2007; Conant *et al.*, 2011). However, there was no decrease in C concentration in the LF due to incubation, despite an overall 6-12% loss of total soil C, complicating the interpretation of the LF as the most labile and available fraction on an annual timescale.

Effects of input exclusion on the mineral-fractions were not as straightforward, but there was some evidence for active turnover. There was a small, marginally significant (see Results; Fig. 4.2) decrease of occluded light fraction C in the no inputs treatment versus the control treatment, representing 3% of the total soil C recovered, indicating that there may be greater losses from this fraction in the future. This is consistent with losses from the 'intermediate density' C pools roughly equivalent to our occluded light fraction (i.e., not the heaviest clay and silt fraction) reported for other DIRT sites (see Lajtha *et al*., 2014a, 2014c). The occluded light fraction of the no litter, no roots, and control treatments also showed a decline in C concentration during incubation which may indicate that a portion of the OLF is actively turning-over on a relatively rapid timescale (i.e., 525 days) despite being trapped within soil aggregates, and this is exposed in the absence of fresh inputs.

Conversely, the soil C concentration of the no inputs treatment fine heavy fraction actually increased versus the control treatment (Fig. 4.2), possibly due to an increase in mineral content as soil aggregation decreased in the absence of fresh organic inputs, as observed by Mayzelle *et al*., (2014). The C concentration of the fine heavy fraction of the no inputs treatment was also marginally higher than the no roots treatment (see Results), however, perhaps due to the incomplete exclusion of roots (see Methods; Bowden *et al*., 2014, Mayzelle *et al*., 2014) leading to differences in the C dynamics between these two treatments.

There was actually an increase in the C concentration of the coarse heavy fraction in the no roots treatment (Fig. 4.2). In contrast, the no inputs treatment experienced a marginally significant decrease in C concentration versus the no litter and control

treatments (see Results). At first glance, this may seem like an anomalous result because the no litter and no root treatments had comparable C losses in the other soil density fractions and in whole soil C, which was also true for cumulative C respiration. However, the long-term mineralization model suggests that root exclusion left a larger mineralizable pool ($X_o$) that decayed at a slower rate ($k$) than litter exclusion (Table 4.1, only $k$ significant). Moreover, root exclusion caused a substantially different catabolic profile than the control, whereas the litter exclusion did not (Fig. 4.1). Our results provide support for the hypothesis that roots contribute disproportionately to soil C stocks (Rasse *et al.*, 2005) and C mineralization dynamics, though previous studies in this experiment reported no change in C quality under root versus aboveground litter exclusion as determined by pyrolysis (Bowden *et al.*, 2014) and mineralization rate during incubation (Crow *et al.*, 2009).

Increasing aboveground input quantity had no effect on soil C quantity, which has been previously reported for this site (Crow *et al.*, 2009; Bowden *et al.*, 2014; Mayzelle *et al.*, 2014). There was also no indication that C concentration increased in any density fraction (Fig. 4.2), similar to results reported after 12 years of litter addition (Crow *et al.*, 2009), or that the microbial community's catabolic profile was altered (Fig. 4.1). However, there were large (7 to 27%), if non-significant, increases in cumulative respiration and the size of the labile C pool ($X_o$) in the double litter treatment compared to the control (Table 4.1). In support of this, Mayzelle *et al*., (2014) showed evidence of C accumulation in some aggregate fractions of the Bousson Forest soil under double litter (a somewhat different fractionation scheme than used in our study). Results from other litter-input addition experiments have shown both increases (Leff *et al.*, 2012; Fekete *et*

*al.*, 2014; Lajtha *et al.*, 2014a) and no response (Garten, 2009; Bowden *et al.*, 2014; Lajtha *et al.*, 2014b) in soil C concentrations, indicating that C accumulation may be dictated by site-specific conditions, such as litter quality and abiotic controls, or by time. Bowden *et al*., (2014) noted that the foliar litter at Bousson Forest is nitrogen-rich and decomposes rapidly. Additionally, past studies have posed the possibility that priming early in the experiment may have reduced soil C content, with subsequent accumulation (Crow *et al.*, 2009). We also found limited evidence of priming: The soil C concentration of the light fraction, occluded light fraction and coarse heavy fraction of the double litter treatment were somewhat reduced versus the control treatment, although this was never significant (Fig. 4.2).

Though there was little evidence of change in the C concentration of the fine heavy fraction over the course of the incubation or under the double litter treatment, examination of its biochemical content with FTICR-MS revealed changes in the molecular species present (Fig. 4.3, 4.4; Table S4.2). This indicates that although this mineral-associated C fraction was 'stable' in terms of total C concentration, it was not metabolically inert. Although the scope of this investigation is limited as we only examined three treatments, one density fraction, and used only one extractant which removed an un-quantified proportion of the organic C and preferentially extracted lipids (Tfaily *et al.*, 2015), these biomarkers should not be biased to any treatment. The fine heavy fraction of the double litter treatment was enriched with lipids over 20 years (Fig. 4.3, Day 0 comparisons) and the molecular nature of these lipids differed (i.e., are not shared in common) from that of the dominant leaf litter, as shown in a van Krevelen diagram (Fig. S4.1); thus they are likely of microbial origin. This indicates that as

aboveground litter decomposed in the organic layer, a portion of the microbial byproducts were preferentially captured by this mineral fraction. The control soils also contain a large lipid pool that was reduced during incubation (Fig. 4.3) and is likely composed of similar compounds (data not shown). The conversion of lipids under incubation in both treatments, indicates this is a typical pathway that is amplified under an excess of aboveground litter. As there was no concurrent increase in C concentration, these lipids may have resulted in C turnover of other SOM compounds by competing for adsorption sites (Kleber *et al.*, 2007; Mitchell & Simpson, 2013).

These results in part support a recently proposed C stabilization pathway in which labile inputs stimulate microbial activity and their byproducts are then preferentially deposited on mineral surfaces (Cotrufo *et al.*, 2013), where SOM can reach extreme age (Kleber *et al.*, 2011). However, 'stabilization' may be relative as these lipids were rapidly (compared to 20 years of input addition) depleted over the course of a 525-day incubation (Fig. 4.3), indicating that either this pool is constantly turning-over *in situ*, or the microbial community is choosing to utilize other pools of C in the presence of fresh inputs.

Soil respiration rates (data not shown) at the beginning and end of the experiment for the no inputs, control, and double litter treatments were more strongly linked to the biochemical content of the fine heavy fraction than to the C concentration of the bulk soils or density fractions themselves. Soil C concentration (as mg C g soil$^{-1}$) of the bulk soil and the light fraction, occluded light fraction, fine heavy fraction, and coarse heavy fraction in a multiple regression model explained 71% of the variance in soil respiration ($p = 0.097$); the bulk soil C concentration ($R^2 = 0.58$, $p = 0.012$) and the bulk soil C

concentration and LF together explained the largest proportion of the variance ($R^2 = 0.59$, $p = 0.040$). Changes in proportions of biochemical classes in the fine heavy fraction as expressed by the NMS axis scores predicted 74.9% of the variance in soil respiration ($p < 0.0001$). This indicates that the fine heavy fraction is not only active, it is closely linked to overall microbial mineralization rates. However, it should be noted that an average of 24% of the soil C by mass was solubilized by density fractionation, as has been reported previously (Crow *et al.*, 2007, 2009). Thus we cannot account for the role of this large and potentially active pool, which complicates comparisons between C pool estimates from density fractions and whole soil responses. If the soluble pool represents a large proportion of the active C, but was largely removed by the fractionation method, then density fraction C concentrations would poorly explain soil respiration rates when compared to bulk soil C concentration.

**Conclusions**

Twenty years of input exclusion revealed patterns in the turnover of decades-old C that are consistent with mineral-association acting as the primary soil C stabilization mechanism. Overall, shifts in the C concentration of the mineral-associated fractions were either relatively small or inconsistent with input quantity, indicating that these fractions were relatively stable in terms of C concentration on a decadal timescale. However, FTICR-MS revealed shifts in the biochemical composition of the fine heavy density fraction in response to both input exclusion and addition, which in turn explained a large portion of the variability in soil respiration. Our results are consistent with recent reports that mineral-associated C contains an active pool (Torn *et al.*, 2013) that may play

a role in C uptake from fresh litter (Cotrufo *et al.*, 2013). We also present evidence consistent with previously published results that the pathway from litter to soil C is complex, neither linear nor direct (Bowden *et al.*, 2014) and may include continued biochemical transformations. Importantly, many of the trends we report here were not apparent at the scale of gross C content or operationally defined density fractions, and we propose that understanding the controls over C decomposition and stabilization will require pairing these metrics with observations at the ecological and molecular scale. Our findings shed new light on the path that C takes from litter to soil C pools, and the role of litter source and quantity in maintaining C stocks and lability.

# CHAPTER V

# CONCLUSIONS

Defining the linkages between soils and climate has become one of the most important and complex challenges in modern science, particularly as global climate change has the potential to re-organize the global carbon cycle. Soils, by storing and releasing C as soil organic matter (SOM) is accumulated and decomposed, have the potential to either amplify or offset warming depending upon their response to temperature and other climate drivers (Friedlingstein *et al.*, 2006; Anav *et al.*, 2013). Numerous empirical and modeling studies over recent decades have sought to deepen our understanding of soil-climate feedbacks. But even as data proliferated, so too have questions: Why does the warming response of soil respiration attenuate? Which SOM pools are susceptible to changing temperature and why? What is the nature of SOM and how does it form? In my dissertation research I sought to answer these questions using a combination of ecosystem-level manipulation and biogeochemical metrics, and revealing the nuanced and complex world of soil C cycling.

In Chapter II I presented my work examining the response of soil respiration to experimental warming and wetting along a natural regional climate gradient. Soil respiration is expected to increase with global warming (Davidson & Janssens, 2006), but the magnitude of this response is likely to be mediated by other physical and climate factors, including precipitation and soil moisture (Schindlbacher *et al.*, 2012; Suseela *et al.*, 2012). This is particularly important to deconvolving the causes underlying the

varied responses soil respiration has shown to warming (Rustad *et al.*, 2001; Wu *et al.*, 2011), as studies from single sites cannot determine whether a response is context-dependent or generalizable (Shaver *et al.*, 2000). I examined soil respiration for 18 months along a 520 km climate gradient in three Pacific Northwest, USA prairies representing increasingly severe Mediterranean conditions from north to south. At each site we implemented a fully-factorial combination of 2.5-3$^{o}$C warming and 20% added precipitation intensity. I concluded that the soil respiration response to warming was driven primarily by the latitudinal climate gradient, specifically the gradient of drought severity, and not context-dependent, despite the influence of different soils and plant communities. Warming increased respiration at all sites during months when soil moisture was not limiting but these gains were offset by reductions in respiration during seasonal transitions and summer drought (Fig. 2.1). Furthermore, the degree of this offset varied along the north-south climate gradient such that in 2011 warming increased cumulative annual soil respiration 28.6% in the northern site, 13.5% in the central site, and not at all in the southern site (Fig. 2.2). Precipitation also stimulated soil respiration more frequently in the south, consistent with an increased duration of moisture limitation (Fig 2.1). The best predictors of soil respiration in non-linear models were the Normalized Difference Vegetation Index (NDVI) as a measure of plant 'greenness', antecedent soil moisture, and temperature but these models provided biased results at high and low soil respiration (Fig. 2.3). NDVI was an effective integrator of climate and site differences in plant productivity in terms of their combined effects on soil respiration. These results suggest that soil moisture limitation can offset the effect of warming on soil respiration, and that greater growing-season moisture limitation, which is possible under

ongoing climate change (Stocker *et al.*, 2013), would constrain cumulative annual responses to warming.

Though soil moisture variability modulates the temperature response of soil respiration as an ecosystem-level response (Davidson *et al.*, 1998), it is unclear what mechanisms control the potential temperature sensitivity of SOM decomposition (von Lützow & Kögel-Knabner, 2009a), especially the sensitivity of the larger, more slowly decomposing pool that represents the majority of soil C stores. The temperature sensitivity of soil organic matter (SOM) decomposition is a key source of uncertainty in models of soil-climate feedbacks (Tang & Riley, 2015). However, empirical studies have given contradictory results concerning the temperature response of different SOM fractions, and the understanding of the chemical nature of SOM has been rapidly evolving (Conant *et al.*, 2011; Lefèvre *et al.*, 2014). The carbon-quality temperature (CQT) hypothesis states that more 'recalcitrant' organic matter should have higher temperature sensitivity (Bosatta & Ågren, 1999; Conant *et al.*, 2011). In support of this hypothesis, incubation studies have often shown a negative correlation between soil respiration rates and temperature sensitivity (Craine *et al.*, 2010b; Lefèvre *et al.*, 2014). There have been important exceptions to such results, however, and the underlying assumption that older SOM is more chemically complex and thereby recalcitrant to decomposition has been challenged (Kleber, 2010; Schmidt *et al.*, 2011). In Chapter III I presented my research asking whether a universal relationship between temperature sensitivity and soil respiration rates would be expected given that SOM decomposition is influenced by factors other than chemical complexity. I examined temperature sensitivity in long-term incubations of four soils representing two biomes and two experiments.

Soils from a manipulative climate experiment in Pacific Northwest grasslands demonstrated an increase in temperature sensitivity with incubation time, consistent with predictions of the CQT hypothesis, but soil from a 20-year input manipulation study in a Northeastern forest showed no relationship of temperature sensitivity with either carbon depletion or incubation time (Fig. 3.1). Furthermore, across all four soils we found that the temperature sensitivity of soil respiration was frequently inconsistent with indices of carbon quality and did not show a negative correlation with soil respiration rate (Fig. 3.2), as has been reported previously (Craine *et al.*, 2010b; Lefèvre *et al.*, 2014). I concluded that the CQT hypothesis failed to universally capture the temperature sensitivity of SOM decomposition across environmental contexts, consistent with an emerging understanding of the multiplicity of factors that control soil C cycling (Conant *et al.*, 2011; Dungait *et al.*, 2012).

Questions and contradictory evidence about the temperature sensitivity of SOM decomposition spurred my interest in the chemical nature of SOM and the processes by which it becomes stabilized in soils. The pathway C takes from plant litter to soil organic matter (SOM) is crucial to understanding how soil C stocks and microbial decomposition will respond to ongoing climate change (Lajtha *et al.*, 2014a), and whether the natural soil C sink can be enhanced to offset potential losses (Dungait *et al.*, 2012). In Chapter IV, I addressed these questions by applying innovations in the molecular characterization of SOM to the soils of a long-term ecosystem-scale litter manipulation. I incubated soils from a 20-year litter-input experiment for 525 days and examined how litter quantity and source (i.e., roots versus aboveground litter) affected soil C cycling, including microbial function and the size and molecular composition of C pools defined by mineral-

associated. Input exclusion led to a 30% loss of soil C, attributable largely to the non-mineral-associated C fraction (Table 4.1; Fig. 4.2). This was accompanied by declines in soil C decomposition rates (Table 4.1) and a shift in the microbial catabolic profile in the absence of roots (Fig. 41.), though there was no evidence that root litter was preferentially stabilized. Carbon did not accumulate under litter addition; however, direct examination of the chemical composition of the finest mineral fraction with Fourier transform ion cyclotron resonance mass spectroscopy (FTICR-MS) revealed dramatic changes (Fig. 4.3). Lipids increased due to input addition and were subsequently mineralized during incubation, indicating that this fraction was both preferentially stabilized (on a decadal time-scale) and metabolically available. Moreover, non-metric dimensional scaling (NMS) showed divergence of the molecular composition of soil C due to the effects of litter treatments and incubation, and explained 75% of the variance in soil respiration in a multiple regression (Fig. 4.4). I concluded that the path from litter to soil is more complex than previously thought, and that further fine-scale and long-term investigations are needed to further illuminate the nature and behavior of SOM.

Soil C research is on the verge of an explosion of discovery and transformative insights, resulting from rapid technological innovation and the need to project the impacts of anthropogenic climate forcing. My contribution to this body of literature has raised as many questions as answers: How will variability in precipitation regimes shape soil respiration and its role in climate forcing? Could soil moisture limitation underlie the acclimation of soil respiration to warming? What underlies the increase in temperature sensitivity demonstrated by many studies if the decomposition rate is not determined by chemical complexity? How does C move through soils? Do the mineral-associated

fractions represent a store only or are they integral to the flow of C from the litter to active soil C pools?

Soil organic matter has been a topic of human interest and investigation for generations (Kleber & Johnson, 2010), and yet it remains largely a black box, defined almost entirely by pools and fluxes. The mechanisms by which these pools form and fluxes change are only recently coming to light as the microbial actors become knowable, and the molecular and mineral constituents become measurable on meaningful scales. Resolving these patterns across spatial and temporal scales will require long-sighted, ecosystem-to-landscape level manipulations to fully reveal the nuances and complexity which underlies this once obscure realm.

## APPENDIX A

## SUPPLEMENTAL MATERIALS FOR CHAPTER II

### Supplemental Figures:

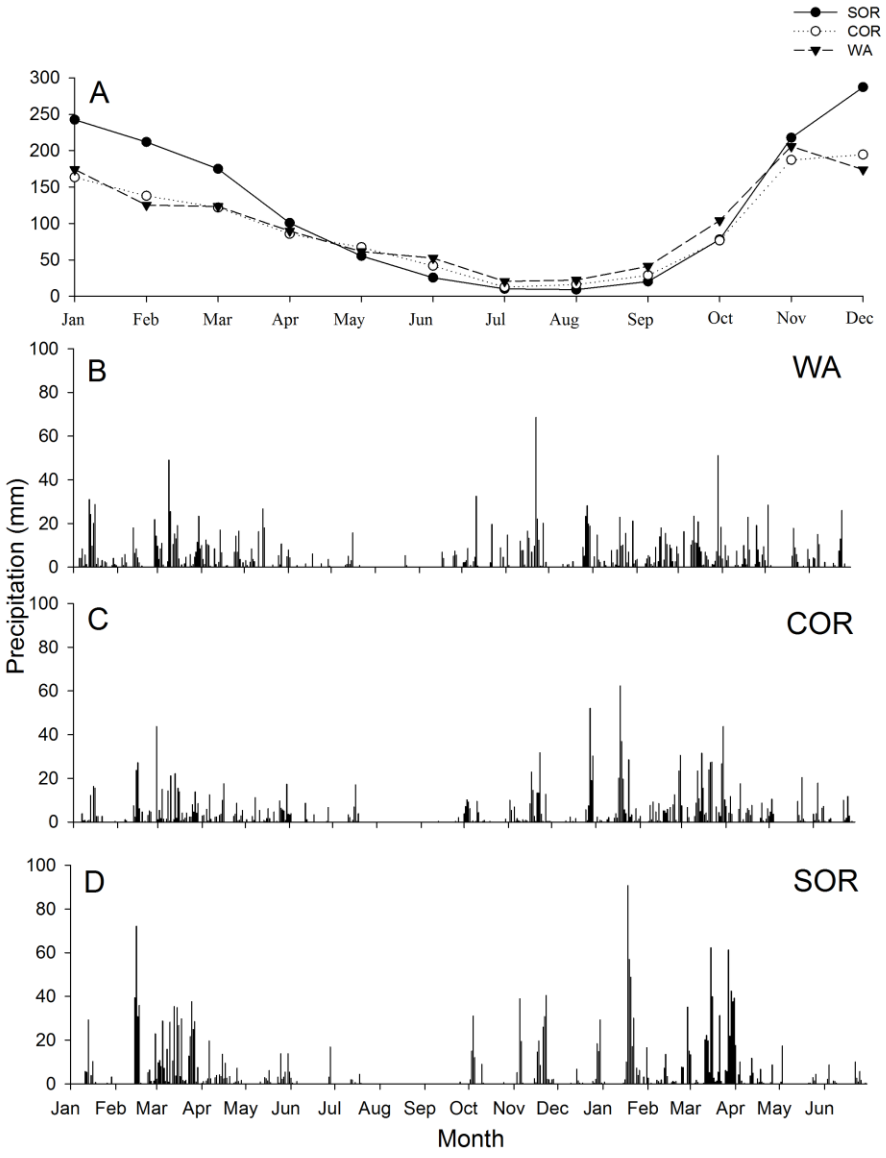


**Fig. S2.1.** (A) Modeled mean monthly precipitation at each site from 1981-2010 (http://www.prism.oregonstate.edu/), and measured daily precipitation from January 2011 to June 2012 for (B) WA, (C) COR, and (D) SOR.

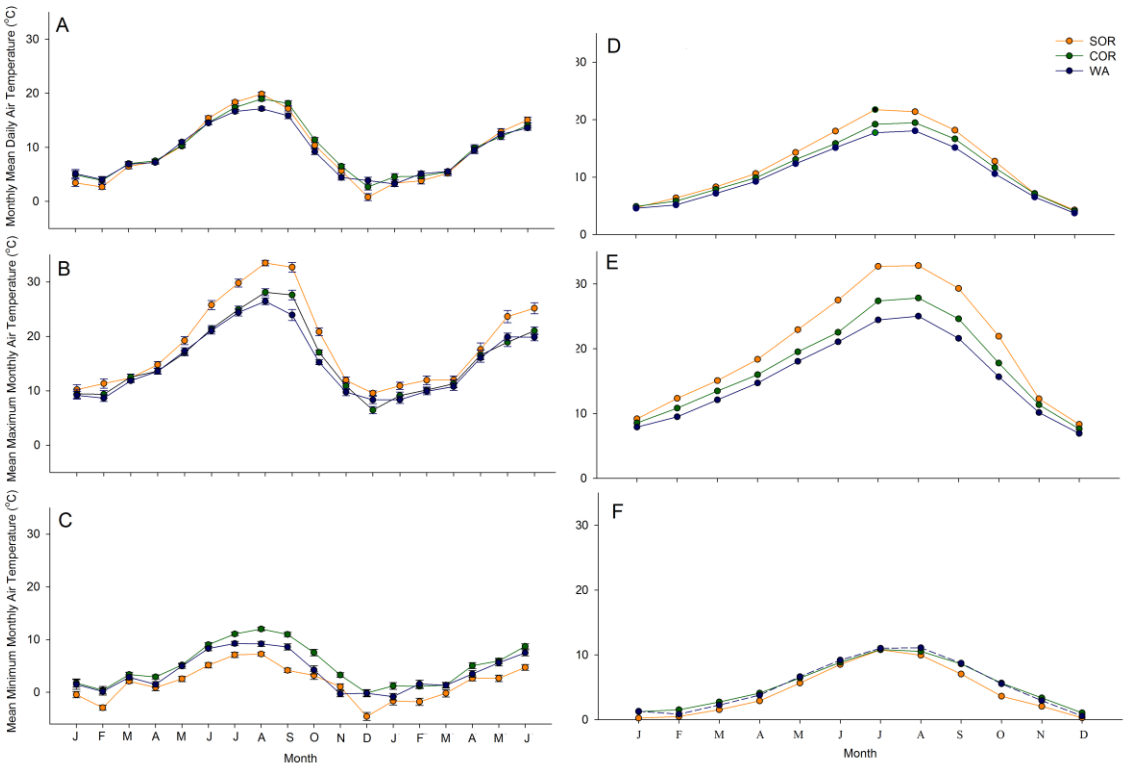


**Fig. S2.2.** Measured monthly (A) daily, (B) maximum, and (C) minimum air temperature for each site from January 2011 – June 2012, and modeled (http://www.prism.oregonstate.edu/) mean (D), maximum (E) and minimum (F) daily air temperature from 1981-2010.
Supplemental Tables:

**Table S2.1.** Repeated measures ANOVA results. M = month, S=site, H=heat, P=precipitation.

| Between subjects | p-value | Within subjects | p-value |
|---|---|---|---|
| M | <0.0001 | | |
| M x S | <0.0001 | S | <0.0001 |
| M x H | <0.0001 | H | <0.0001 |
| M x P | 0.11 | P | 0.61 |
| M x S x H | <0.0001 | S X H | <0.0001 |
| M x S x P | 0.01 | S X P | 0.35 |
| M x H x P | 0.10 | H X P | 0.52 |
| M x S x H x P | 0.81 | S X H X P | 0.55 |

**Table S2.2.** P-values for omnibus (A) and site-level (B) factorial ANOVAs. Significant ($p < 0.05$) and marginally significant ($0.05 < p > 0.1$) effects are in bold. S = site, H= heat, P = precipitation.

A Site x Heat x Precipitation Factorial ANOVA

| Year | Month | S | H | P | S x H | S x P | H x P | S x H x P |
|---|---|---|---|---|---|---|---|---|
| 2011 | Jan | <0.0001 | <0.0001 | 0.38 | 0.02 | 0.15 | 0.17 | 0.49 |
| | Feb | <0.0001 | <0.0001 | 0.98 | 0.01 | 0.56 | 0.68 | 0.93 |
| | Mar | <0.0001 | 0 | 0.02 | 0 | 0.27 | 0.47 | 0.96 |
| | Apr | <0.0001 | <0.0001 | 0.35 | 0 | 0.44 | 0.38 | 0.5 |
| | May | <0.0001 | 0.76 | 0.08 | <0.0001 | 0.03 | 0.11 | 0.05 |
| | Jun | <0.0001 | <0.0001 | 0.46 | <0.0001 | 0.19 | 0.32 | 0.69 |
| | Jul | <0.0001 | <0.0001 | 0.29 | 0.1 | <0.001 | 0.11 | 0.74 |
| | Aug | <0.0001 | 0.15 | 0.63 | <0.01 | 0.94 | 0.46 | 0.38 |
| | Sept | <0.0001 | <0.01 | 0.24 | 0.18 | 0.19 | 0.7 | 0.55 |
| | Oct | <0.0001 | <0.01 | 0.47 | <0.0001 | 0.14 | 0.57 | 0.99 |
| | Nov | <0.0001 | <0.0001 | 0.99 | 0.49 | 0.53 | 0.06 | 0.46 |
| | Dec | <0.0001 | <0.0001 | 0.56 | 0.18 | 0.96 | 0.23 | 0.78 |
| 2012 | Jan | <0.0001 | <0.0001 | 0.13 | 0.03 | 0.48 | 0.04 | 0.95 |
| | Feb | <0.0001 | <0.0001 | 0.28 | 0.82 | 0.88 | 0.23 | 0.39 |
| | Mar | <0.0001 | 0.01 | 0.75 | 0.69 | 0.37 | 0.3 | 0.64 |
| | Apr | 0.26 | 0.01 | 0.87 | <0.0001 | 0.44 | 0.1 | 0.07 |
| | May | <0.0001 | 0.11 | 0.88 | <0.0001 | 0.76 | 0.97 | 0.62 |
| | Jun | <0.0001 | 0.02 | 0.81 | 0 | 0.64 | 0.8 | 0.4 |

B ANOVA by Site

| | | SOR | | | COR | | | WA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Month | H | P | H x P | H | P | H x P | H | P | H x P |
| 2011 | Jan | <0.0001 | 0.45 | 0.95 | <0.0001 | 0.03 | 0.08 | 0 | 0.88 | 0.51 |
| | Feb | 0.25 | 0.48 | 0.93 | <0.0001 | 0.67 | 0.49 | <0.0001 | 0.58 | 0.94 |
| | Mar | 0.19 | 0.61 | 0.56 | 0.04 | 0.03 | 0.78 | <0.0001 | 0.33 | 0.72 |
| | Apr | 0.53 | 0.34 | 0.28 | <0.01 | 0.59 | 0.69 | <0.0001 | 0.28 | 0.75 |
| | May | <0.0001 | <0.01 | <0.01 | 0.24 | 0.41 | 0.99 | <0.01 | 0.38 | 0.87 |
| | Jun | <0.0001 | 0.05 | 0.16 | 0.8 | 0.68 | 0.65 | 0.43 | 0.32 | 0.98 |
| | Jul | 0.64 | <0.01 | 0.49 | 0.01 | 0.29 | 0.69 | <0.01 | 0.26 | 0.04 |
| | Aug | 0.01 | 0.92 | 0.95 | 0.02 | 0.69 | 0.93 | 0.08 | 0.8 | 0.19 |
| | Sept | 0.3 | 0.04 | 0.26 | 0.01 | 0.84 | 0.8 | 0.21 | 0.89 | 0.87 |
| | Oct | <0.0001 | 0.07 | 0.69 | 0.02 | 0.94 | 0.83 | <0.0001 | 0.33 | 0.66 |
| | Nov | <0.0001 | 0.85 | 0.03 | <0.01 | 0.48 | 0.73 | <0.0001 | 0.45 | 0.4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dec | <0.0001 | 0.45 | 0.08 | 0.04 | 0.9 | 0.78 | 0.01 | 0.76 | 0.66 |
| 2012 | Jan | <0.0001 | 0.16 | 0.42 | <0.0001 | 0.95 | 0.04 | 0.01 | 0.45 | 0.26 |
| | Feb | <0.0001 | 0.39 | 0.16 | <0.0001 | 0.69 | 0.57 | <0.0001 | 0.58 | 0.79 |
| | Mar | 0.07 | 0.45 | 0.35 | 0.13 | 0.54 | 0.42 | 0.17 | 0.27 | 0.89 |
| | Apr | <0.0001 | 0.45 | 0.04 | 0.83 | 0.79 | 0.39 | <0.0001 | 0.3 | 0.45 |
| | May | <0.0001 | 0.99 | 0.36 | 0.9 | 0.78 | 0.91 | <0.0001 | 0.36 | 0.38 |
| | Jun | <0.0001 | 0.52 | 0.11 | 0.17 | 0.68 | 0.6 | 0.06 | 0.38 | 0.95 |

**Table S2.3.** Site, heat, and precipitation effects on annual cumulative $CO_2$-C respired across and within sites. Data shown are P-values. Site = site, H = heat, P = precipitation.

| | 2011 | | | | 2012 | | | |
|---|---|---|---|---|---|---|---|---|
| Factor | ALL | SOR | COR | WA | ALL | SOR | COR | WA |
| H | 0.005 | 0.153 | 0.055 | <0.0001 | 0.042 | 0.028 | 0.187 | <0.0001 |
| P | 0.945 | 0.143 | 0.861 | 0.259 | 0.682 | 0.745 | 0.797 | 0.497 |
| S | <0.0001 | | | | <0.0001 | | | |
| H x P | 0.996 | 0.551 | 0.96 | 0.591 | 0.133 | 0.065 | 0.497 | 0.713 |
| H x S | 0.001 | | | | <0.0001 | | | |
| P x S | 0.210 | | | | 0.825 | | | |
| H x P x S | 0.751 | | | | 0.189 | | | |

**Table S2.4.** Nonlinear regressions for soil respiration across and within sites. $R^2$ and stability are shown for each model.

| Model | Eqn | Site | | | | Reference |
|---|---|---|---|---|---|---|
| | | All | SOR | COR | WA | |
| $R = ae^{bT}$ | 1 | 0.02 (Y) | 0.10 (Y) | 0.20 (Y) | 0.31 (Y) | |
| $R = ae^{bT}ce^{dM}$ | 2 | 0.11 (N) | 0.14 (N) | 0.50 (N) | 0.46 (N) | Davidson 1998 |
| $R= ae^{bT}(d(M\text{-minimum M})(\text{maximum M-}M)^{c})$ | 3 | 0.10 (N) | 0.18 (N) | 0.64 (N) | 0.52 (N) | Suseela et al. 2011 |
| $R = ae^{bT}\, e^{(cM)+(d(M\text{^}2)}$ | 4 | 0.15 (Y) | 0.18 (Y) | 0.64 (Y) | 0.56 (Y) | Almagro et al. 2009 |
| $R = ae^{bT}cM$ | 5 | 0.11 (Y) | 0.14 (Y) | 0.50 (Y) | 0.46 (Y) | |
| $R = ae^{bT}(M/(M+c))$ | 6 | 0.12 (Y) | 0.16 (Y) | 0.61 (Y) | 0.54 (Y) | |

a, b, c, d are fitted constants
R = soil respiration, T = temperature, M = volumetric soil moisture
N = model fit not stable, Y = model fit stable

# APPENDIX B

# SUPPLEMENTAL MATERIAL FOR CHAPTER III

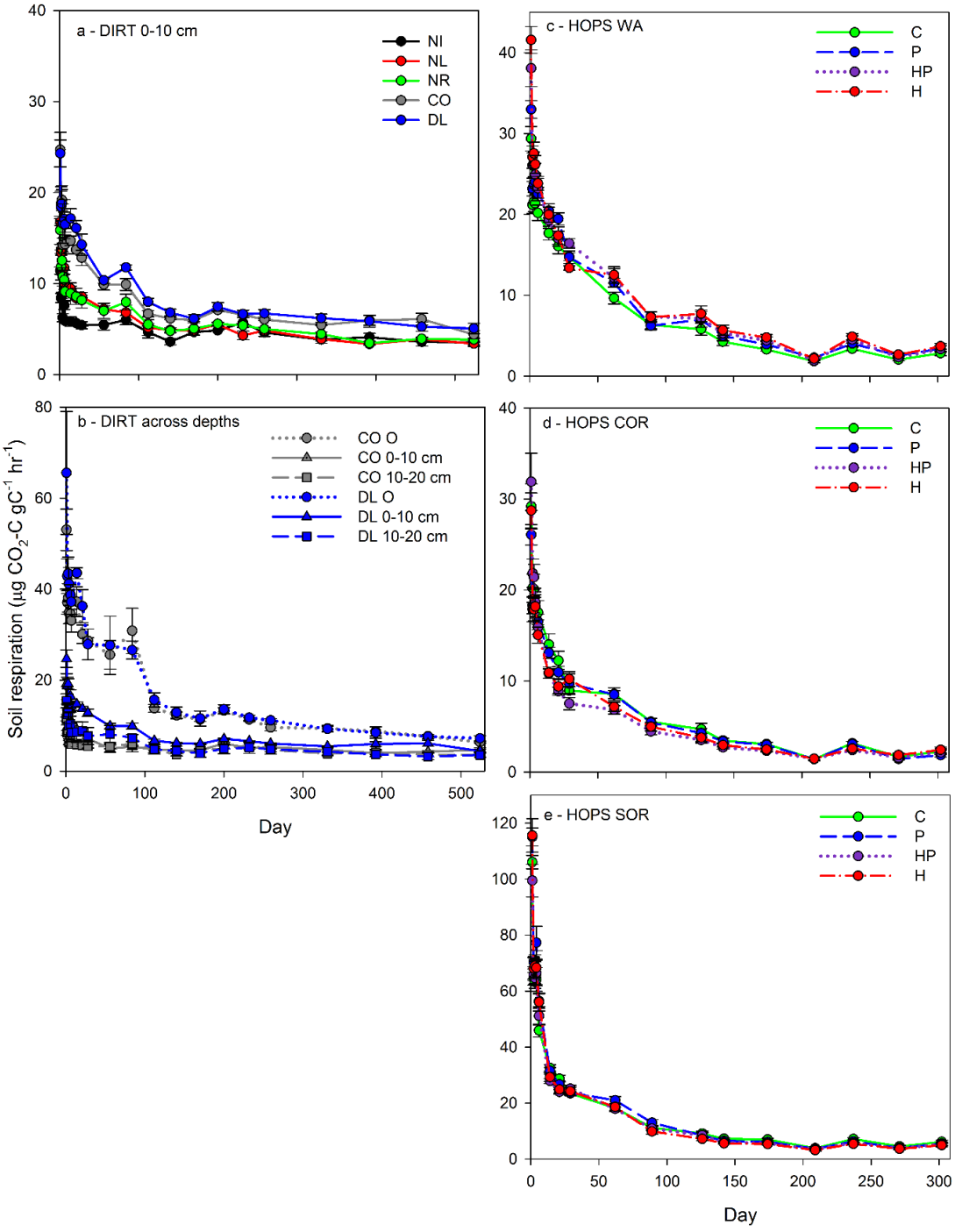


**Fig. 3.1.** Soil respiration for DIRT 0-10 cm soils (a) and O, 0-10 cm, and 10-20 cm CO and DL soils (b) and HOPS WA (c), COR (c), and SOR (e). Data were log-transformed for analysis but raw data are shown in figures. Note that sub-figures are on different y-axis scales. C = Control, P = Precipitation, HP = Heat X Precipitation, H = Heat, NI = No Inputs, NL = No Litter, NR = No Roots, CO = COntrol, DL = Double Litter, O = Organic.

**Table 3.1.** DIRT mean (se) soil respiration and p-values for repeated-measures ANOVA, ANOVA, and Tukey's results. NI = No Inputs, NL = No Litter, NR = No Roots, CO = COntrol, DL = Double Litter.

| Repeated measures ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| Within depth | | | | | | |
| | Depth | Day | Day x Trmt | Trmt | | |
| | O-horizon | <0.0001 | 0.631 | 0.506 | | |
| | 0-10 cm | <0.0001 | <0.0001 | <0.0001 | | |
| | 10-20 cm | <0.0001 | 0.151 | 0.766 | | |
| ANOVA and Tukey's results 0-10 cm depth | | | | | | |
| Day | p-value | NI | NL | NR | CO | DL |
| 1 | <0.0001 | 11.45 (0.41)a | 16.78 (0.38)b | 15.88 (1.56)b | 24.71 (1.92)c | 24.32 (1.47)c |
| 2 | <0.0001 | 8.42 (0.52)a | 13.41 (0.29)bc | 11.85 (1.45)ab | 18.79 (1.86)c | 18.42 (1.75)c |
| 3 | <0.0001 | 8.7 (0.35)a | 13.79 (0.18)bc | 12.56 (1.24)b | 19.19 (1.15)c | 18.81 (1.97)c |
| 4 | <0.0001 | 6.26 (0.44)a | 11.02 (0.1)b | 10.76 (1)b | 14.25 (0.77)bc | 16.59 (2.15)c |
| 6 | 0.001 | 7.61 (0.46)a | 11.73 (0.7)bc | 10.41 (1.06)ab | 16.47 (1.4)c | 17.09 (2.14)c |
| 7 | <0.0001 | 5.9 (0.24)a | 9.99 (0.35)b | 9.13 (0.97)b | 14.31 (1.03)c | 16.49 (0.85)c |
| 14 | <0.0001 | 5.83 (0.52)a | 9.45 (0.28)b | 8.91 (1.18)b | 14.71 (0.52)c | 17.15 (1.09)c |
| 21 | <0.0001 | 5.67 (0.24)a | 8.41 (0.19)b | 8.61 (0.89)b | 13.73 (0.45)c | 16.1 (0.83)c |
| 28 | <0.0001 | 5.43 (0.37)a | 8.59 (0.13)b | 8.18 (0.85)b | 12.84 (0.83)c | 14.28 (1.17)c |
| 56 | 0.001 | 5.47 (0.55)a | 7.21 (0.34)abc | 7.02 (0.84)ab | 9.91 (0.61)bc | 10.37 (0.32)c |
| 59 | 0.001 | 5.49 (0.56)a | 7.24 (0.34)abc | 7.06 (0.84)ab | 9.99 (0.62)bc | 10.46 (0.33)c |
| 84 | 0.002 | 6 (0.51)a | 6.82 (0.81)ab | 7.97 (0.88)abc | 9.9 (0.66)bc | 11.78 (0.29)c |
| 112 | 0.028 | 4.68 (0.65)a | 5.17 (0.86)ab | 5.51 (0.11)ab | 6.69 (0.35)ab | 8.01 (0.38)b |
| 140 | <0.0001 | 3.62 (0.34)a | 4.89 (0.25)b | 4.79 (0.2)b | 6.17 (0.1)bc | 6.82 (0.38)c |
| 170 | 0.009 | 4.68 (0.33)a | 4.88 (0.21)ab | 5.05 (0.16)ab | 5.99 (0.13)b | 6.15 (0.44)b |
| 200 | <0.0001 | 4.87 (0.01)a | 5.52 (0.22)a | 5.58 (0.21)a | 7.1 (0.16)b | 7.46 (0.47)b |
| 232 | 0.007 | 5.72 (0.53)ab | 4.31 (0.47)a | 5.41 (0.18)ab | 6.58 (0.11)b | 6.66 (0.42)b |
| 260 | 0.004 | 4.63 (0.42)a | 4.85 (0.26)ab | 5.02 (0.2)ab | 6.06 (0.11)bc | 6.73 (0.41)c |
| 331 | 0.001 | 3.85 (0.39)a | 3.91 (0.2)a | 4.46 (0.22)ab | 5.45 (0.06)bc | 6.21 (0.45)c |
| 392 | 0.001 | 4.12 (0.43)ab | 3.37 (0.08)a | 3.47 (0.22)a | 5.96 (0.53)b | 5.88 (0.66)b |
| 458 | 0.011 | 3.61 (0.48)a | 3.89 (0.24)a | 3.94 (0.11)ab | 6.1 (0.64)b | 5.27 (0.52)ab |
| 524 | 0.063 m | 3.54 (0.44)ab | 3.41 (0.15)a | 3.85 (0.15)ab | 4.41 (0.43)ab | 5.08 (0.56)b m 0.77 |
| Across Depth | | | | | | |
| | Trmt | Day | Day x Depth | Depth | | |
| | CO | <0.0001 | <0.0001 | <0.0001 | | |
| | DL | <0.0001 | 0.009 | 0.005 | | |
| ANOVA and Tukey's results within treatment | | | | | | |
| Day | p-vlaue | CO O | CO 10 | CO 20 | | |
| 1 | 0.001 | 53.05 (4.55)b | 24.71 (1.92)a | 19.19 (2.22)a | | |
| 2 | 0.001 | 37.08 (2.55)b | 18.79 (1.86)a | 14.09 (1.65)a | | |
| 3 | <0.0001 | 38.07 (3.12)b | 19.19 (1.15)a | 14.1 (1.3)a | | |

| | | | | |
|---|---|---|---|---|
| 4 | <0.0001 | 34.79 (2.41)b | 14.25 (0.77)a | 11.85 (1.22)a |
| 6 | <0.0001 | 34.54 (1.99)c | 16.47 (1.4)b | 11.27 (0.83)a |
| 7 | <0.0001 | 33.09 (2.49)c | 14.31 (1.03)b | 9.95 (0.75)a |
| 14 | <0.0001 | 37.23 (3.28)c | 14.71 (0.52)b | 9.12 (0.57)a |
| 21 | <0.0001 | 30.06 (2.02)c | 13.73 (0.45)b | 8.71 (0.74)a |
| 28 | <0.0001 | 28.64 (0.7)c | 12.84 (0.83)b | 7.13 (0.62)a |
| 56 | <0.0001 | 25.55 (3.15)c | 9.91 (0.61)b | 4.93 (0.78)a |
| 59 | <0.0001 | 26.05 (3.25)c | 9.99 (0.62)b | 4.95 (0.79)a |
| 84 | 0.001 | 30.83 (4.98)b | 9.9 (0.66)a | 5.4 (1.17)a |
| 112 | <0.0001 | 13.81 (0.86)c | 6.69 (0.35)b | 4.74 (0.3)a |
| 140 | <0.0001 | 12.24 (0.76)c | 6.17 (0.1)b | 4.56 (0.32)a |
| 170 | <0.0001 | 11.34 (0.42)c | 5.99 (0.13)b | 4.59 (0.22)a |
| 200 | <0.0001 | 13.29 (0.64)b | 7.1 (0.16)a | 6.07 (0.29)a |
| 232 | <0.0001 | 11.35 (0.38)c | 6.58 (0.11)b | 5.09 (0.22)a |
| 260 | 0.001 | 9.6 (0.75)b | 6.06 (0.11)a | 5.3 (0.4)a |
| 331 | 0.001 | 9.37 (0.66)b | 5.45 (0.06)a | 4.65 (0.45)a |
| 392 | 0.018 | 8.74 (1.03)b | 5.96 (0.53)ab | 3.97 (0.78)a |
| 458 | 0.005 | 7.65 (0.14)b | 6.1 (0.64)b | 4.24 (0.31)a |
| 524 | 0.042 | 6.33 (0.18)b | 4.41 (0.43)a | 4.68 (0.49)ab |

| Day | p-value | DL O | DL 10 | DL 20 |
|---|---|---|---|---|
| 1 | 0.001 | 65.55 (13.52)b | 24.32 (1.47)a | 15.44 (1.55)a |
| 2 | 0.001 | 42.85 (3.8)b | 18.42 (1.75)a | 12.44 (2.27)a |
| 3 | 0.001 | 43.41 (3.62)b | 18.81 (1.97)a | 12.86 (2.18)a |
| 4 | 0.004 | 41.18 (3.62)b | 16.59 (2.15)a | 9.98 (2.89)a |
| 6 | 0.004 | 38.74 (2.26)b | 17.09 (2.14)a | 10.34 (2.64)a |
| 7 | 0.001 | 37.21 (2.74)c | 16.49 (0.85)b | 8.46 (1.98)a |
| 14 | 0.001 | 43.53 (1.21)c | 17.15 (1.09)b | 8.56 (2.28)a |
| 21 | 0.001 | 36.25 (3.64)c | 16.1 (0.83)b | 8.88 (1.92)a |
| 28 | 0.003 | 27.86 (3.4)b | 14.28 (1.17)ab | 7.69 (1.87)a |
| 56 | 0.014 | 27.64 (6.41)b | 10.37 (0.32)a | 8.14 (1.87)a |
| 59 | 0.014 | 28.23 (6.64)b | 10.46 (0.33)a | 8.19 (1.89)a |
| 84 | <0.0001 | 26.6 (1.98)c | 11.78 (0.29)b | 7.27 (0.78)a |
| 112 | 0.004 | 15.64 (1.57)b | 8.01 (0.38)a | 4.77 (1.04)a |
| 140 | 0.007 | 12.86 (1.22)b | 6.82 (0.38)ab | 4.41 (1.08)a |
| 170 | 0.009 | 11.56 (1.66)b | 6.15 (0.44)ab | 3.92 (0.87)a |
| 200 | 0.006 | 13.58 (1.02)b | 7.46 (0.47)ab | 4.91 (1.07)a |
| 232 | 0.023 | 11.74 (0.52)b | 6.66 (0.42)ab | 5.13 (1.21)a |
| 260 | 0.012 | 11.14 (0.39)b | 6.73 (0.41)ab | 4.79 (1.05)a |
| 331 | 0.017 | 9.26 (0.63)b | 6.21 (0.45)ab | 4.24 (0.86)a |
| 392 | 0.012 | 8.45 (1.2)b | 5.88 (0.66)ab | 3.7 (0.54)a |
| 458 | 0.011 | 7.58 (0.49)b | 5.27 (0.52)ab | 3.2 (0.68)a |
| 524 | 0.011 | 7.18 (0.6)b | 5.08 (0.56)ab | 3.55 (0.48)a |

m – marginally significant Tukey's test ($0.05<p<0.1$)

**Table S3.2.** ANOVA results for cumulative C turnover for the DIRT and HOPS experiments. For DIRT, ANOVAs are on (i) treatment within depths and (ii) depth within treatments (CO and DL only). For HOPS, (iii) a factorial ANOVA is done on site, heat, and precipitation, and separate ANOVAs are done on (iv) site and (v) heat within site. S=site, H=heat, P=precipitation

DIRT

| (i) ANOVA within depth | | (ii) ANOVA across depths | |
|---|---|---|---|
| Depth | Treatment | Treatment | Depth |
| O-horizon | 0.045 | CO | <0.0001 |
| 0-10 cm | <0.0001 | DL | <0.0001 |
| 10-20 cm | 0.778 | | |

HOPS

| (iii) Factorial ANOVA | | (iv) ANOVA across sites | | | |
|---|---|---|---|---|---|
| Factor | p-value | Factor | | | |
| S | <0.0001 | S | <0.0001 | | |
| H | 0.215 | | | | |
| P | 0.618 | (v) ANOVA within site | | Site | |
| S x H | 0.001 | Factor | WA | COR | SOR |
| S x P | 0.396 | H | 0.020 | 0.018 | 0.026 |
| H x P | 0.317 | | | | |
| S x H x P | 0.592 | | | | |

**Table S3.3** Repeated measures, factorial ANOVA, and ANOVA p-values for HOPS incubation soil respiration. D = day, S = site, H = heat, P = precipitation.

| Repeated measure ANOVA:Day x Site x Heat x Precipitation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D | <0.0001 | | | | | | | | |
| | D x S | <0.0001 | | | | | | | | |
| | D x H | 0.063 | | | | | | | | |
| | D x P | 0.6 | | | | | | | | |
| | D x S x H | 0.071 | | | | | | | | |
| | D x S x P | 0.793 | | | | | | | | |
| | D x H x P | 0.7 | | | | | | | | |
| | D x H x P x S | 0.153 | | | | | | | | |
| ANOVA: Site x Heat x Precipitation | | | | | | | | | | |
| Day | | WA | COR | SOR | H | P | H x P | S x H | S x H x P | S x P |
| 1 | <0.0001 | b | a | c | 0.159 | 0.559 | 0.136 | 0.04 | 0.441 | 0.825 |
| 2 | <0.0001 | b | a | c | 0.063 | 0.18 | 0.917 | 0.042 | 0.014 | 0.864 |
| 3 | <0.0001 | b | a | c | 0.038 | 0.521 | 0.908 | 0.193 | 0.065 | 0.649 |
| 4 | <0.0001 | b | a | c | 0.459 | 0.178 | 0.086 | 0.226 | 0.715 | 0.844 |
| 6 | <0.0001 | b | a | c | 0.567 | 0.242 | 0.058 | 0.056 | 0.029 | 0.835 |
| 14 | <0.0001 | b | a | c | 0.001 | 0.944 | 0.377 | 0.005 | 0.259 | 0.435 |
| 21 | <0.0001 | b | a | c | <0.0001 | 0.916 | 0.651 | 0.071 | 0.178 | 0.123 |
| 29 | <0.0001 | b | a | c | 0.919 | 0.739 | 0.794 | 0.554 | 0.006 | 0.012 |
| 62 | <0.0001 | b | a | c | 0.258 | 0.238 | 0.05 | 0.004 | 0.719 | 0.602 |
| 89 | <0.0001 | b | a | c | 0.237 | 0.504 | 0.805 | 0.007 | 0.928 | 0.219 |
| 126 | <0.0001 | b | a | c | 0.45 | 0.975 | 0.998 | 0.108 | 0.287 | 0.837 |
| 142 | <0.0001 | b | a | c | 0.248 | 0.646 | 0.822 | 0.003 | 0.239 | 0.726 |
| 174 | <0.0001 | b | a | c | 0.225 | 0.588 | 0.932 | <0.0001 | 0.097 | 0.695 |
| 209 | <0.0001 | b | a | c | 0.679 | 0.608 | 0.759 | 0.573 | 0.475 | 0.883 |
| 237 | <0.0001 | b | a | c | 0.246 | 0.588 | 0.925 | <0.0001 | 0.103 | 0.671 |
| 271 | <0.0001 | b | a | c | 0.726 | 0.518 | 0.626 | 0.075 | 0.083 | 0.534 |
| 302 | <0.0001 | b | a | c | 0.56 | 0.524 | 0.942 | 0.161 | 0.246 | 0.569 |
| ANOVA: Heat x Precipitation | | | | | | | | | | |
| | SOR | | | COR | | | WA | | | |
| Day | H | P | H x P | H | P | H x P | H | P | H x P | |
| 1 | 0.297 | 0.42 | 0.015 | 0.907 | 0.647 | 0.906 | <0.0001 | 0.801 | 0.022 | |
| 2 | 0.737 | 0.357 | 0.177 | 0.775 | 0.348 | 0.057 | 0.001 | 0.628 | 0.173 | |
| 3 | 0.506 | 0.781 | 0.666 | 0.816 | 0.354 | 0.085 | 0.003 | 0.666 | 0.139 | |
| 4 | 0.444 | 0.238 | 0.222 | 0.705 | 0.621 | 0.784 | 0.019 | 0.47 | 0.049 | |
| 6 | 0.435 | 0.295 | 0.007 | 0.172 | 0.844 | 0.409 | 0.041 | 0.38 | 0.101 | |
| 14 | 0.006 | 0.435 | 0.979 | 0.002 | 0.789 | 0.787 | 0.554 | 0.251 | 0.053 | |
| 21 | 0.007 | 0.295 | 0.553 | 0.003 | 0.427 | 0.662 | 0.535 | 0.089 | 0.061 | |
| 29 | 0.503 | 0.59 | 0.597 | 0.511 | 0.072 | 0.059 | 0.746 | 0.016 | 0.005 | |
| 62 | 0.252 | 0.243 | 0.151 | 0.009 | 0.905 | 0.619 | 0.092 | 0.271 | 0.128 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 89 | 0.042 | 0.081 | 0.939 | 0.062 | 0.448 | 0.676 | 0.062 | 0.761 | 0.931 |
| 126 | 0.245 | 0.781 | 0.205 | 0.133 | 0.66 | 0.883 | 0.261 | 0.79 | 0.287 |
| 142 | 0.002 | 0.65 | 0.091 | 0.027 | 0.374 | 0.845 | 0.11 | 0.814 | 0.329 |
| 174 | 0.002 | 0.511 | 0.081 | 0.012 | 0.467 | 0.851 | 0.007 | 0.746 | 0.131 |
| 209 | 0.324 | 0.936 | 0.601 | 0.744 | 0.55 | 0.989 | 0.965 | 0.677 | 0.215 |
| 237 | 0.003 | 0.571 | 0.087 | 0.014 | 0.419 | 0.919 | 0.007 | 0.738 | 0.146 |
| 271 | 0.101 | 0.703 | 0.215 | 0.281 | 0.306 | 0.873 | 0.225 | 0.623 | 0.029 |
| 302 | 0.207 | 0.825 | 0.31 | 0.336 | 0.3 | 0.84 | 0.202 | 0.74 | 0.135 |

**Table S3.4.** DIRT $E_a$ mean (se), repeated measures ANOVA, ANOVA, and Tukey's results. NI = No Inputs, NL = No Litter, NR = No Roots, CO = COtrol, DL = Double Litter.

| Repeated-measures ANOVA | | | |
|---|---|---|---|
| Within depth | | | |
| Depth | Day | Day x Trmt | Trmt |
| O-horizon | 0.006 | 0.326 | 0.402 |
| 0-10 cm | <0.0001 | 0.029 | 0.001 |
| 10-20 cm | 0.02 | 0.462 | 0.46 |

| ANOVA and Tukey's results 0-10 cm depth | | | | | | |
|---|---|---|---|---|---|---|
| DAY | p-value | NI | NL | NR | CO | DL |
| 5 | 0.119 | 57.53 (2.86) | 66.33 (2.34) | 60.45 (2.54) | 64.43 (1.95) | 69.78 (4.96) |
| 8 | 0.043m | 47.57 (4.21)a | 51.11 (4.31)ab | 59.61 (6.5)ab | 66.45 (3.08)b | 63.99 (1.52)ab |
| 15 | 0.001 | 47.84 (1.32)a | 65.39 (1.95)b | 56.01 (5)ab | 65.78 (0.83)b | 67.56 (1.1)b |
| 22 | <0.0001 | 43.15 (1.35)a | 57.76 (0.88)b | 56.29 (2.89)b | 59.41 (0.85)b | 61.34 (0.66)b |
| 29 | 0.01 | 45.17 (2.37)a | 55.62 (0.34)abc | 46.2 (5.83)ab | 59.83 (1.16)c | 59.12 (0.46)ab |
| 57 | 0.050m | 38.65 (1.39)a | 54.32 (7.2)ab | 47.52 (5.85)ab | 64.81 (6.31)b | 59.71 (4.95)ab |
| 85 | 0.072 | 48.97 (3.69) | 63.97 (3.34) | 48.21 (6.46) | 67.6 (8.22) | 48.61 (4.06) |
| 113 | 0.084m | 35.19 (3.91)a | 44.63 (1.09)b | 41.12 (0.81)ab | 42.56 (1.48)ab | 42.76 (1.96)ab |
| 141 | 0.003 | 36.46 (3.05)a | 49.39 (1.51)b | 45.69 (0.59)b | 47.3 (1.66)b | 48.84 (1.17)b |
| 171 | 0.862 | 48.71 (4.03) | 47.6 (4.47) | 52.18 (4.89) | 47.46 (1.87) | 47.43 (1.39) |
| 201 | 0.167 | 45.79 (0.74) | 48.9 (0.84) | 49.68 (1.86) | 50.15 (1.82) | 50.54 (0.97) |
| 233 | 0.021 | 39.82 (0.46)a | 48.77 (1.01)ab | 44.79 (1.47)ab | 51.38 (3.12)b | 44.87 (2.68)ab |
| 260 | 0.003 | 41.6 (1.36)a | 46.73 (1.79)ab | 55.43 (0.98)c | 48.8 (2.54)abc | 49.99 (1.5)bc |
| 332 | 0.444 | 42.26 (3.34) | 50.68 (4.19) | 48.33 (2.55) | 48.52 (4.08) | 45.09 (1.34) |
| 393 | 0.082m | 44.63 (3.25)a | 53.99 (3.23)ab | 50.06 (1.67)ab | 52.82 (1.15)ab | 54.94 (2.25)b |
| 459 | 0.014 | 56.76 (0.54)a | 67.5 (2.12)b | 66.8 (3.06)b | 62.48 (0.69)ab | 63.8 (1.57)ab |
| 525 | 0.124 | 49.46 (4.75) | 49.4 (3.53) | 50.85 (2.62) | 53.75 (3.85) | 62.74 (3.14) |

| ANOVA and Tukey's results within CO | | | | |
|---|---|---|---|---|
| Across depth | | Day | Day x Depth | Depth |
| | CO | <0.0001 | 0.024 | 0.402 |
| | DL | <0.0001 | 0.43 | 0.195 |
| DAY | p-vlaue | CO O-horizon | CO 0-10 cm | CO 10-20 cm |
| 5 | 0.019 | 67.72 (2.1)b | 64.43 (1.95)ab | 56.88 (1.72)b |
| 8 | 0.296 | 45.97 (14.31) | 66.45 (3.08) | 53.52 (0.48) |
| 15 | 0.026 | 55.71 (3.46)a | 65.78 (0.83)b | 55.39 (1.47)a |
| 22 | 0.04 | 57.38 (2.1)ab | 59.41 (0.85)b | 52.2 (1.42)a |
| 29 | 0.197 | 57.04 (4.29) | 59.83 (1.16) | 49 (4.92) |
| 57 | 0.547 | 52.96 (0.85) | 64.81 (6.31) | 62.91 (11.89) |
| 85 | 0.028 | 45.17 (1.25)ab | 67.6 (8.22)b | 40.87 (4.49)a |

| | | | | |
|---|---|---|---|---|
| 113 | 0.251 | 42.49 (1.8) | 42.56 (1.48) | 38.22 (2.26) |
| 141 | 0.01 | 50.36 (1.21)b | 47.3 (1.66)b | 37.67 (2.78)a |
| 171 | 0.038 | 52.81 (1.79)b | 47.46 (1.87)ab | 43.57 (2.03)a |
| 201 | 0.293 | 48.25 (1.73) | 50.15 (1.82) | 35.78 (10.69) |
| 233 | 0.009 | 53.5 (0.54)b | 51.38 (3.12)b | 41.54 (0.69)a |
| 260 | 0.035 | 71.66 (4.81)b | 48.80 (2.54)ab | 48.31 (7.55)b |
| 332 | 0.039 | 49.05 (4.26)a | 48.52 (4.08)a | 33.28 (2.56)a |
| 393 | <0.0001 | 64.99 (1.72)c | 52.82 (1.15)b | 42.22 (1.65)a |
| 459 | 0.001 | 75.94 (1.56)b | 62.48 (0.69)a | 58.67 (2.2)a |
| 525 | 0.01 | 68.13 (3.25)b | 53.75 (3.85)ab | 41.85 (4.67)a |

m – marginally significant Tukey's test ($0.05<p<0.1$)
ns – non-significant Tukey's test ($p>0.1$)

**Table S3.5.** HOPS $E_a$ mean (se), repeated-measures ANOVA, ANOVA, and Tukey's results. D = day, S = site, H = heat, P = precipitation.

| Repeated measures ANOVA | | | | |
|---|---|---|---|---|
| | Factor | p-value | | |
| | D | <0.0001 | | |
| | D x S | <0.0001 | | |
| | D x H | 0.614 | | |
| | D x P | 0.966 | | |
| | D x S x H | 0.213 | | |
| | D x S x P | 0.83 | | |
| | D x H x P | 0.588 | | |
| | D x S x H x P | 0.986 | | |
| ANOVA | | Mean (se) and Tukey's results | | |
| Day | p-value | SOR | COR | WA |
| 5 | 0.047 | 36.62(0.86)a | 41.31(0.47)b | 38.37(0.46)ab |
| 7 | <0.0001 | 31.44(0.45)a | 46.2(0.9)b | 35.42(0.3)a |
| 15 | 0 | 45.14(0.47)b | 49.32(0.99)b | 39.49(0.51)a |
| 22 | 0.004 | 38.13(0.44)a | 42.94(0.37)b | 36.68(0.21)a |
| 63 | <0.0001 | 73.52(0.67)b | 99(3.46)c | 46.16(0.8)a |
| 90 | 0.013 | 90.89(2.58)b | 69.38(1.98)a | 94.55(1.23)b |
| 127 | 0.163 | 112.24(1.54) | 119.09(0.79) | 111.35(0.72) |
| 143 | <0.0001 | 114.77(1.49)c | 89.76(0.44)b | 51.81(1)a |
| 175 | 0.078 | 123.17(4.46)a | 98.14(2.25)a | 99.58(2.66)a |
| 210 | 0.571 | 102.74(3.27) | 100.63(1.15) | 95.29(0.89) |
| 238 | 0.112 | 83.72(1.45) | 92.05(0.79) | 83.92(0.1) |
| 272 | <0.0001 | 77.52(0.51)a | 116.84(3.56)b | 77.67(0.82)a |
| 303 | 0.003 | 113.2(1.49)b | 90.68(1.13)a | 107.21(0.51)b |

| SOR | C | P | HP | H |
|---|---|---|---|---|
| 5 | 38.49 (2.97) | 31.05 (3.76) | 37.30 (1.45) | 39.65 (3.99) |
| 7 | 30.47 (2.01) | 29.34 (1.08) | 34.05 (5.23) | 31.91 (1.76) |
| 15 | 44.90 (3.34) | 42.38 (2.30) | 47.40 (1.03) | 45.86 (2.57) |
| 22 | 39.63 (2.80) | 39.92 (5.31) | 37.01 (3.53) | 35.95 (1.45) |
| 63 | 75.28 (4.22) | 73.73 (2.85) | 69.24 (4.21) | 75.84 (2.92) |
| 90 | 95.32 (10.16) | 103.59 (21.86) | 88.34 (18.07) | 76.32 (16.22) |
| 127 | 120.20 (4.19) | 114.71 (6.14) | 103.98 (9.54) | 110.08 (6.01) |
| 143 | 109.39 (8.35) | 113.65 (4.29) | 111.67 (4.52) | 124.39 (5.61) |
| 175 | 152.87 (23.46) | 116.31 (12.71) | 110.24 (21.88) | 113.25 (32.25) |
| 210 | 121.26 (11.46) | 105.84 (9.19) | 97.23 (8.39) | 86.61 (5.64) |
| 238 | 91.98 (5.93) | 82.27 (2.23) | 84.35 (3.24) | 76.27 (3.85) |
| 272 | 74.41 (7.49) | 78.57 (4.30) | 79.75 (4.98) | 77.36 (4.39) |
| 303 | 111.52 (10.06 | 108.12 (9.30) | 110.23 (14.25) | 122.94 (9.78) |

| COR | C | P | HP | H |
|---|---|---|---|---|
| 5 | 44.17(0.86) | 40.36(1.96) | 41.45(3.37) | 39.24(4.17) |
| 7 | 50.02(8.43) | 40.75(1.55) | 45.8(3.13) | 48.21(3.33) |
| 15 | 44.8(1.81) | 46.24(2.42) | 52.67(3.74) | 53.56(4.9) |
| 22 | 43.24(1.36) | 40.78(2.44) | 42.94(2.75) | 44.82(2.64) |
| 63 | 89.21(8.82) | 84.51(6.21) | 103.45(6.52) | 118.83(21.66) |
| 90 | 75.57(11.65) | 78.34(12.31) | 62.42(3.24) | 61.18(4.43) |
| 127 | 124.18(6.52) | 116.55(4.76) | 116.86(6.72) | 118.76(7.54) |
| 143 | 89.96(10.85) | 88.1(4.34) | 88.51(8.25) | 92.48(7.18) |
| 175 | 94.8(14.71) | 97.83(19.55) | 111.9(13.03) | 88.02(9.77) |
| 210 | 102.42(5.97) | 92.97(6.92) | 103.6(21.76) | 103.52(8.65) |
| 238 | 95.09(3.79) | 89.52(4.55) | 88.52(15.71) | 95.08(4.85) |
| 272 | 99.45(6.75) | 112.15(9.08) | 118.04(21.82) | 137.72(31.15) |
| 303 | 83.79(1.44) | 95.45(4.97) | 93.17(6.97) | 90.31(6.03) |

| WA | C | P | HP | H |
|---|---|---|---|---|
| 5 | 37.78(1.15) | 41.44(1.58) | 37.33(1.32) | 36.93(1.68) |
| 7 | 36.99(0.42) | 35.81(0.96) | 35.08(0.79) | 33.8(1.38) |
| 15 | 38.6(1.69) | 37.19(0.8) | 39.58(0.73) | 42.57(2.6) |
| 22 | 35.77(1.01) | 37.98(0.96) | 36.37(2.21) | 36.59(1.3) |
| 63 | 45.49(4) | 51.38(4.49) | 43.99(2.77) | 43.79(2.24) |
| 90 | 94.05(4.06) | 87.41(10.84) | 96.18(11.64) | 100.57(10.53) |
| 127 | 108.9(6.07) | 112.66(5.52) | 108.56(5.53) | 115.29(3.01) |
| 143 | 57.66(6.51) | 52.84(8.23) | 49.03(7.51) | 47.73(9.11) |
| 175 | 99.46(7.86) | 83(11.08) | 105.78(10.86) | 110.1(10.39) |
| 210 | 97.76(11.74) | 89.83(8.54) | 94.9(4.43) | 98.69(7.98) |
| 238 | 84.11(5.19) | 83.24(6.44) | 84.22(3.36) | 84.08(4.88) |
| 272 | 81.19(2.89) | 80.13(3.98) | 76.11(1.16) | 73.25(1.74) |
| 303 | 109.76(12.11) | 104.18(6.29) | 107.28(8.56) | 107.62(11.71) |

## APPENDIX C

## SUPPLEMENTAL MATERIAL FOR CHAPTER IV

### Supplemental Figure

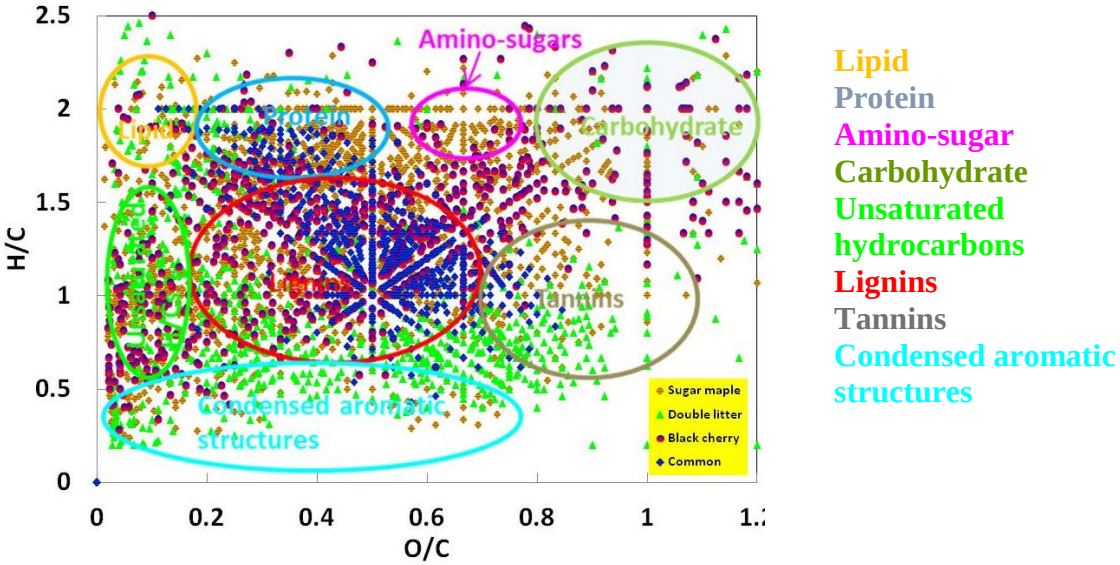


**Fig. S4.1.** Van Krevelen diagram of biochemical compounds detected with FTICR-MS in the fHF of the DL treatment and the leaf litter of the dominant tree species.

## Supplemental Tables

**Table S4.1.** Mean (se) and ANOVA results for soil respiration (μg $CO_2$-C g dw $soil^{-1}$ $hr^{-1}$) for catabolic profile. Different small letters indicate significant differences.

| Substrate | p-value | NI | NL | NR | CO | DL |
|---|---|---|---|---|---|---|
| Glucose | 0.018 | 0.62 (0.04)a | 1.27 (0.09)b | 0.78 (0.15)ab | 1.16 (0.08)ab | 1.18 (0.20)ab |
| Fructose | 0.010 | 0.50 (0.11)a | 1.41 (0.14)b | 0.64 (0.17)a | 1.23 (0.11)ab | 1.21 (0.25)ab |
| Galactose | 0.058 | 0.61 (0.05)a | 1.29 (0.18)b | 0.78 (0.18)ab | 1.15 (0.12)ab | 1.18 (0.22)ab |
| α-ketoglutanic acid | 0.17 | 0.65 (0.26) | 1.08 (0.22) | 0.72 (0.16) | 1.29 (0.21) | 1.27 (0.21) |
| ascorbic acid | 0.24 | 0.84 (0.07) | 1.06 (0.06) | 0.95 (0.13) | 1.09 (0.05) | 1.05 (0.07) |
| citric acid | 0.49 | 0.44 (0.22) | 1.21 (0.64) | 0.70 (0.11) | 1.43 (0.55) | 1.22 (0.39) |
| malic acid | 0.16 | 0.67 (0.21) | 1.17 (0.10) | 0.86 (0.16) | 1.20 (0.07) | 1.10 (0.18) |
| oxalic acid | 0.17 | 0.52 (0.22) | 1.16 (0.44) | 0.61 (0.15) | 1.32 (0.28) | 1.39 (0.26) |
| urocanic acid | 0.47 | 1.36 (0.23) | 0.95 (0.42) | 1.23 (0.30) | 0.64 (0.36) | 0.82 (0.07) |
| Arginine | 0.41 | 0.79 (0.11) | 1.09 (0.17) | 0.78 (0.21) | 1.21 (0.27) | 1.13 (0.16) |
| Asparagine | 0.047 | 0.91 (0.08)ab | 1.29 (0.13)b | 0.64 (0.10)a | 1.15 (0.16)ab | 1.01 (0.17)ab |
| iso-leucine | 0.97 | 0.84 (0.32) | 0.97 (0.20) | 1.13 (0.60) | 1.07 (0.05) | 0.99 (0.03) |
| Serine | 0.009 | 0.81 (0.13)ab | 1.06 (0.07)ab | 0.65 (0.05)a | 1.30 (0.07)b | 1.19 (0.18)b |
| Histidine | 0.023 | 0.84 (0.09)ab | 0.98 (0.11)ab | 0.74 (0.08)a | 1.12 (0.11)ab | 1.32 (0.14)b |
| Glucosamine | 0.018 | 1.15 (0.21)a | 1.20 (0.15)b | 0.68 (0.16)ab | 0.74 (0.30)ab | 1.23 (0.34)ab |
| Cellulose | 0.32 | 0.71 (0.08) | 1.00 (0.40) | 0.93 (0.10) | 1.04 (0.05) | 1.32 (0.17) |
| Lignin | 0.56 | 0.93 (0.13) | 1.09 (0.11) | 0.89 (0.07) | 1.05 (0.05) | 1.04 (0.10) |
| tannic acid | 0.61 | 0.93 (0.27) | 1.03 (0.22) | 0.77 (0.15) | 1.29 (0.25) | 0.97 (0.23) |

**Table S4.2**. P-values for analyses of proportions of FTICR-MS detected compounds.

| Repeated measures ANOVA | | Incubation day x DIRT treatment | | | |
|---|---|---|---|---|---|
| Compound | Day | Day x trmt | trmt | Tukey's | |
| Amino sugars | 0.407 | 0.005 | | | |
| Carbohydrates | 0.359 | 0.124 | 0.17 | | |
| Condensed hydrocarbons | 0.001 | 0.006 | | | |
| Lignin | 0.003 | 0.006 | | | |
| Lipid | 0.001 | 0.001 | | | |
| Protein | 0.001 | 0.227 | 0.068 | NI | a |
| | | | | CO | b |
| | | | | DL | ab |
| Tannin | 0.003 | 0.067 | | | |
| Unsaturated hydrocarbons | 0.001 | <0.0001 | | | |
| Other | 0.039 | 0.764 | 0.498 | | |

| ANOVA | | DIRT trmt w/in day | | t-tests | |
|---|---|---|---|---|---|
| | | Day | | Between days | |
| | Order of Tukey's letters | 0 | 525 | trmt | |
| Amino sugars | | 0.054 | 0.006 | NI | 0.076 |
| | NI, CO, DL | ab,a,b | a,b,b | CO | 0.286 |
| | | | | DL | 0.012 |
| Condensed hydrocarbons | | 0.023 | 0.059 | NI | 0.9 |
| | NI, CO, DL | b,b,a | a,a,a | CO | 0.001 |
| | | | | DL | 0.006 |
| Lignin | | 0.035 | 0.061 | NI | 0.716 |
| | NI, CO, DL | b,b,a | a,a,a | CO | 0.035 |
| | | | | DL | 0.031 |
| Lipid | | <0.0001 | 0.01 | NI | 0.34 |
| | NI, CO, DL | a,a,b, | b,a,a | CO | 0.024 |
| | | | | DL | 0.013 |
| Tannin | | 0.009 | 0.782 | NI | 0.455 |
| | NI, CO, DL | b,b,a | a,a,a | CO | 0.052 |
| | | | | DL | 0.023 |
| Unsaturated hydrocarbons | | 0.001 | <0.0001 | NI | 0.017 |
| | NI, CO, DL | b,b,a | a,b,b | CO | 0.019 |
| | | | | DL | 0.041 |

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