AN ABSTRACT OF THE THESIS OF

Virginia L. W. Murphey for the degree of Master of Science in Environmental Science presented on March 21, 2014

Title: Soil Organic Carbon Response to Six Years of Warming: Assessing the Impacts of Altered Diurnal Temperature Range

Abstract approved:

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There is a growing consensus that anthropogenic warming will impact soil organic matter (SOM). Globally, soil contains 2-3 times more carbon (C) than plants, and like plants, temperature induced change of SOM could have significant climate repercussions. Although, the majority of warming experiments have increased day and night temperatures equally, there is evidence of greater increase in global minimum temperatures compared to midday maxima, resulting in an altered mean diurnal temperature range (DTR). Here, intact grassland mesocosms (Terracosm facility, Corvallis OR) were used to assess soil organic C (SOC) changes resulting from altered DTR over a six year period. We assessed the response of SOC to a mean temperature increase of 3.5^{o}C with three temperature treatments: symmetric (SYM) warming of +3.5^{o}C, asymmetric (ASYM) warming of +5^{o}C/ +2^{o}C at minimum and

maximum daily temperature, respectively, and control treatments that were kept at ambient (AMB) temperatures. After six years of warming SOC was surprisingly unaltered by increased temperature and was more strongly influenced by plant dynamics than expected. The strongest temperature response was from soil respiration (reported as *In-situ* cumulative soil C mineralization) with at least 33% higher cumulative C mineralization in warming treatments. In agreement with the soil respiration response, light fraction C (<1.8g/cm^3) was depleted in warming treatments (8-10% less C than AMB) at shallow depths. However, laboratory cumulative soil C mineralization, a measure of most easily degraded SOC, had a lack of response to temperature, and actually had trends for greater C in warming treatments. This response was further validated by increased soil water dissolved organic C (DOC) concentration in warming treatments. An increase in these measures suggested that plants were more influential than temperature for highly labile SOC. Aggregates, an important part of SOC allocation and sequestration, had a differential response to SYM and ASYM treatments but this was likely more directly caused by root dynamics than temperature treatment. Due to varying responses of SOC indicators, C budgets were used to assess total ecosystem C balance. We found that under AMB conditions soil was an atmospheric C sink and under both SYM and ASYM conditions soil was an atmospheric C source, with ASYM having a higher source potential than SYM. Overall, SOC responded to both temperature increase via soil respiration and differentially to temperature treatments based on plant response. We expect that a reduced diurnal temperature range could affect soil C differently than mean temperature increase, if plant differences are sustained.

Soil Organic Carbon Response to Six Years of Warming: Assessing the Impacts of Altered Diurnal Temperature Range

by
Virginia L. W. Murphey

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Virginia L. W. Murphey, Author

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CONTRIBUTION OF AUTHORS

Dr. Kate Lajtha contributed to all sections of Chapter 2 and guided data collection. Dr. Jillian Gregg ran the warming experiment and contributed to the Abstract, Introduction and Results sections of Chapter 2 and provided overall organizational guidance. Dr. Claire Phillips assisted with soil respiration interpretation, temperature sensitivity of soil respiration analysis, statistical analyses and total carbon budgets.

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Chapter 1: Introduction to Altered Diurnal Temperature Range and Soil Organic Carbon

From 1951-2012 mean temperature of the Northern Hemisphere increased 0.72^{o}C (IPCC 2013). A continued increase in atmospheric greenhouse gas concentrations is expected to cause an additional rise in global mean temperature another 0.4^{o}C in the next twenty years (IPCC 2013). Changes in temperature affect ecosystem processes such as carbon (C) uptake and release. For example, increased temperatures decreased plant productivity in a temperate steppe (Alward *et al.* 1999) and exposed previously frozen C-rich soils at northern latitudes resulting in an increased CO_2 flux to the atmosphere (Günther *et al.* 2013). Since changes to terrestrial C stores and fluxes (*e.g.,* soil C, soil respiration, plant inputs) affect atmospheric CO_2 concentration, the main greenhouse gas of concern, accurate information on the effect of increased temperature on C cycling is of critical importance (IPCC 2013).

Soil C accumulation and turnover are important processes in the global C cycle: soils contain about 1.5 x 10^{18} g C, which is 2-3 times more C than in vegetation (Schlesinger and Andrews 2000). Additionally, the C flux between soils and the atmosphere is large, with soil respiration representing about 10 times the C flux relative to fossil fuel combustion (IPCC 2013, Schlesinger and Andrews 2000). Thus, any temperature-induced change in rates of soil C turnover will markedly affect the global C cycle, and it is critical to understand how warmer temperatures will alter soil C flux from natural systems.

Most research on soil C cycle temperature sensitivity has determined impacts of equal day and night temperature increase (reviewed by Brzostek *et al.* 2012, Dieleman *et al.* 2012, Lu *et al*. 2012, Bond-Lamberty and Thomson 2010, and Rustad *et al.* 2001). However, the 2013 IPCC reported a two- to

three-fold greater increase in minimum dawn (T_{MIN}) than maximum midday (T_{MAX}) temperatures through 1993, when averaged across the globe. This phenomenon is commonly referred to as "night-warming" (Michaels and Stooksbury 1992). Reanalysis of data from 1997 through 2004 has shown an equal complement of sites showing the greatest temperature increase at the hottest time of day (T_{MAX}), and future warming scenarios are expected to vary between sites with the greatest temperature increase at the hottest or coldest time of day, depending on location (IPCC 2013, Vose *et al.* 2005). Because photosynthesis and respiration are differentially affected by altered T_{MIN} and T_{MAX}, there is no *a priori* reason to expect that changes in T_{MIN} would result in the same response as changes in T_{MAX}. In fact, several studies that assessed ecosystem response to changes in T_{MIN} reported different net effects than equal day and night warming (Peng *et al.* 2013, Zhang *et al.* 2011, Beier *et al.* 2008 and Alward *et al.* 1999). Therefore, it is imperative that we expand our understanding of potential impacts of global warming to include the effects of asymmetrically elevated temperature profiles.

Despite a consensus on the temperature sensitivity of biochemical processes, soil organic carbon (SOC) turnover is complex and it is important to try to assess the temperature sensitivity of both biochemical reactions and environmental factors in order to find the net effect of temperature on SOC turnover (Davidson and Janssens 2006). Chemical properties of SOC substrates, supplied mostly from net primary production (NPP) but also from soil organisms, vary from low to high activation energies. Traditional multi-pool SOC models state that low molecular weight compounds (sugars, organic acids, amino acids) with low activation energies are easily degraded by soil microbes and are quickly

decomposed (labile soil C), whereas, higher molecular weight compounds (phenols, lignin, lipids) with high activation energies are less easily degraded (resistant soil C) and remain in soil for longer periods of time (Davidson *et al.* 2000). In addition to C substrates' chemical properties, sorption of compounds to clay particles, physical occlusion in soil aggregates, and water and temperature regimes are environmental factors that also control the rate of soil C degradation (Davidson and Janssens 2006).

There has been extensive research on SOC response to warming, and research to date is split over which pools will respond most strongly to elevated temperatures, and whether mineral soil C will have a long-term response to altered temperatures. A critical question is, how, if at all, temperature sensitivity differs between the labile ("young") and resistant ("old") soil C pools. Some studies suggest that soil microbial communities acclimate to altered temperature conditions and SOC decomposition rates have no sustained response to temperature increase, despite an initial response (Dieleman *et al.* 2012, Carrillo *et al.* 2011, Allison *et al.* 2010, Bradford *et al.* 2008). Other studies have suggested that the long-term positive feedback of soil decomposition in a warming world may be stronger than predicted by global models (Conant *et al.* 2008, Knorr *et al.* 2005). Models and experiments suggest that the labile pools should respond most quickly to elevated temperature (Carrillo *et al.* 2011, Cheng *et al.* 2011, Townsend *et al.* 1997). However, there is growing consensus that resistant pools should have the highest temperature sensitivity, or Q_{10} (Frey *et al.* 2013, Sierra 2012, Conant *et al.* 2011, , Conant *et al.* 2008a, Conant *et al.* 2008b, Hartley and Ineson 2008, Leifeld and Fuhrer 2005, Waldrop and Firestone 2004, Bol *et al.* 2003, Bosatta and Ågren 1999, but see Zimmerman *et al.* 2012, and Conen *et al.* 2006,

for an opposing view). Temperature response of soil C is further complicated by inconsistent definitions of the resistant soil C pool, with some defining resistant soil C by high activation energies (Sierra 2012, Conant *et al.* 2008b, Hartley and Ineson 2008, Fierer *et al.* 2005, Ågren 2000, Bosatta and Ågren, 1999), and others defining it by chemical and physical occlusion (Carrington *et al.* 2012, Gentile *et al.* 2011, Feng and Simpson 2009, Conen *et al.* 2006, Fang *et al.* 2005).

The variety of methods used to assess SOC temperature sensitivity was cited by Sierra (2012) as the cause of confusion and a lack of scientific consensus. In general, there are three different types of soil temperature sensitivity experiments, (1) laboratory incubations, (2) *in-situ* field experiments, or (3) mesocosms (reviewed by Dieleman *et al.* 2012 and Rustad *et al.* 2001). Lab studies, although useful for theory-based evaluations, are not able to replicate responses of intact systems. Davidson *et al.* (2000) and many others have pointed to this inherent problem with short-term soil laboratory incubation experiments that use disturbed and sieved soils and do not include plant response components such as soil water balance, root exudate inputs, root turnover, and detrital inputs. *In-situ* field warming treatments are often applied either to soils, and hence do not provide a realistic assessment of altered vegetative inputs (e.g. Bradford *et al.* 2008), or aboveground which generally do not penetrate the soils (e.g. Aguilos *et al.* 2011). By contrast, mesocosms provide easily manipulated intact systems with warming of both above- and belowground compartments (Tingey *et al.* 1996).

Mesocosms were used in this study and due to the unique precision climate controlled system are uniquely called terracosms. The terracosms are reconstructed native grassland mesocosms that have

been exposed to ambient (AMB), symmetric (SYM) and asymmetric (ASYM) warming treatments. The warming treatments provide the same increase in mean temperature (ambient +3.5^{o}C/day) but with different patterns. The SYM treatment has constant temperature increase of +3.5^{o}C over AMB while the ASYM treatment has greater warming at dawn (+5^{o}C over AMB) than by midday (+2^{o}C over AMB). These treatments stand in stark contrast to existing less-realistic "night-warming" experiments that increase night-time temperatures only rather than providing asymmetrical elevation of T_{MIN}, which is at dawn (Phillips *et al.* 2011). This makes the terracosms a unique climate change experiment of whole-system driven SOC response to warming in both SYM and ASYM warming treatments.

Here, we compared SOC responses to AMB, SYM and ASYM treatments after six years of treatment application. We assessed SOC dynamics through total soil C, labile and resistant SOC pools, soil C fluxes (soil respiration, plant inputs and dissolved organic C (DOC) outputs), temperature sensitivity of soil respiration (Q_{10}) and soil enzyme activities. Labile soil C was assessed looking at C in light density soil fractions (<1.8 g/cm^3) and laboratory cumulative soil C mineralization. Resistant soil C was measured as the amount of C in the heavy density fractions (>2.4 g/cm^3), microaggregates (250-53µm) and the silt and clay aggregate fraction (<53 µm). Soil respiration was reported as *in*-situ cumulative soil C mineralization and was evaluated with other fluxes (above and belowground plant biomass, and soil water leachate DOC) to create C budgets for each treatment. Temperature sensitivity of soil respiration (Q_{10}) was also measured to compare the relationship between microbial metabolism and temperature and to assess whether microbes had acclimated to the altered conditions. Lastly,

activities of common soil enzymes (β-glucosidase and phosphatase) were measured in order to assess potential temperature driven changes to microbial function or community.

We expected that warming would result in SOC losses caused by increased microbial metabolism, and that SYM treatments with the highest T_{MAX} would result in greatest soil C losses due to the exponential relationship between microbial metabolism and temperature. We expected that these losses would be evident in sensitive outputs of soil C (soil respiration and soil water leachate). More specifically, we expected that due to fast turn-over times, labile SOC indicators would have the strongest response to warming with the greatest depletion of labile C expected in SYM, because of higher T_{MAX}. In addition, if resistant soil C has a greater temperature sensitivity than labile soil C, the resistant C should be more destabilized by SYM than ASYM treatments. Also, increased plant productivity (measured as plant biomass C) in response to warming was expected to increase rhizosphere activity, leading to increased soil macroaggregate formation and soil enzyme activities.

Chapter 2: Soil Organic Carbon Response to Six Years of Warming: Assessing the Impacts of Altered Diurnal Temperature Range

Abstract

There is a growing consensus that anthropogenic warming will impact soil organic matter (SOM). Globally, soil contains 2-5 times more carbon (C) than plants, and soil respiration accounts for ten-times the flux of C to the atmosphere than C produced by anthropogenic sources. Although, the majority of warming experiments have increased day and night temperatures equally, there is evidence of a greater increase in global minimum temperatures compared to midday maxima, resulting in an altered diurnal temperature range (DTR). Here, intact grassland mesocosms (Terracosm facility, Corvallis OR) were used to assess soil organic C (SOC) changes resulting from altered DTR over a six year period. We assessed the response of SOC to a mean temperature increase of 3.5^{o}C with three temperature treatments: symmetric (SYM) warming of +3.5^{o}C, asymmetric (ASYM) warming of +5^{o}C/ +2^{o}C at minimum and maximum daily temperature, respectively, and control treatments that were kept at ambient (AMB) temperatures. The strongest temperature response was from soil respiration with at least 33% higher cumulative C mineralization in warming treatments. Light fraction C (<1.8g/cm3) was also depleted in warming treatments (8-10% less C than AMB) at shallow depths. However, laboratory cumulative soil C mineralization, under constant temperature and soil moisture conditions, showed a trend towards greater stores of easily microbially accessible or, labile C in warming treatments. This response was further validated by increased soil water dissolved organic C (DOC) concentration in warming treatments. Aggregates, an important part of SOC allocation and sequestration, had a differential

response to SYM and ASYM treatments. C budgets were used to assess total ecosystem C balance. We found that under AMB conditions soil was an atmospheric C sink and under both SYM and ASYM conditions soil was an atmospheric C source, with ASYM having a higher source potential than SYM. Overall, after six years of warming SOC was surprisingly unaltered by increased temperature and was more strongly influenced by plant dynamics than expected (*i.e.* rhizodeposition and litter).

Introduction

There has been an unprecedented 0.72^{o}C rise in mean temperature from 1951-2012 and subsequent changes to ecosystem processes, such as carbon (C) uptake and release, have the potential to impact future climate conditions (IPCC 2013). An important process in the global C cycle is soil C accumulation and turnover. Globally, soils contain about 1.5 x 10^{18} g C, which is 2-5 times greater than in vegetation (IPCC 2013). As a large terrestrial C store, any temperature-induced change in rates of soil C turnover could markedly affect the global C cycle. However, soil organic carbon (SOC) response to temperature increase is complex and often dependent on the temperature response of other ecosystem components (Davidson and Janssens 2006). For example, plant activity can both positively (via storage) or negatively (via priming) affect soil respiration, and microbial activity can affect plant nutrient uptake and thus growth (Drake *et al.* 2011). Therefore, an accurate assessment of the temperature response of SOM must come from an intact ecosystem.

Most research on soil C responses to warmer temperatures has examined impacts of equal day and night temperature increases (Brzostek *et al.* 2012, Dieleman *et al.* 2012, Lu *et al.* 2012, Bond-

Lamberty & Thomson 2010, Rustad *et al.* 2001). However, the 2013 IPCC reported a two- to three-fold greater increase in minimum dawn (T_{MIN}) temperatures than maximum midday (T_{MAX}) temperatures, when averaged across the globe, through 1993. This phenomenon is commonly referred to as "night-warming" (Michaels and Stooksbury 1992). Reanalysis of 1979-2004 data has shown an equal complement of sites showing the greatest temperature increase at the hottest time of day (T_{MAX}). Future warming scenarios are expected to vary between sites with the greatest temperature increase at the hottest or coldest time of day, depending on location (IPCC 2013, Vose *et al.* 2005). Because photosynthesis and respiration are differentially affected by altered T_{MIN} and T_{MAX}, there is a potential for SOM to be affected differently by reduced diurnal temperature range (DTR) (Peng *et al.* 2013, Zhang *et al.* 2011, Beier *et al.* 2008, Alward *et al.* 1999). Therefore, it is imperative that we expand our understanding of potential impacts of global warming on SOC to include the effects of asymmetrically elevated temperature profiles.

There has been extensive research on SOM response to warming, and research to date is split over which pools will respond most strongly to elevated temperatures, and whether mineral soil C will have a long-term response to altered temperatures. A critical question is, how, if at all, temperature sensitivity differs between the labile ("young") and resistant ("old") soil C pools. While some studies have suggested that soil microbial communities acclimate to altered temperature conditions and soil C decomposition rates have no sustained response to temperature increase, despite an initial response (Dieleman *et al.* 2012, Carrillo *et al.* 2011, Allison *et al.* 2010, Bradford *et al.* 2008), other studies have

suggested that the long-term positive feedback of soil decomposition in a warming world may be stronger than predicted by global models (Conant *et al.* 2008, Knorr *et al.* 2005). Models and experiments suggest that the labile pools should respond most quickly to elevated temperature (Carrillo *et al.* 2011, Cheng *et al.* 2011, Townsend *et al.* 1997). However, there is growing consensus that resistant pools should have the highest temperature sensitivity (Frey *et al.* 2013, Sierra 2012, Conant *et al.* 2011, Conant *et al.* 2008a, Conant *et al.* 2008b, Hartley and Ineson 2008, Leifeld and Fuhrer 2005, Waldrop and Firestone 2004, Bol *et al.* 2003, Bosatta and Ågren 1999, but see Zimmerman *et al.* 2012, and Conen *et al.* 2006, for an opposing view). Temperature response of soil C is further complicated by inconsistent definitions of the resistant soil C pool, with some defining resistant soil C by high activation energies (Sierra 2012, Conant *et al.* 2008, Hartley and Ineson 2008, Fierer *et al.* 2005, Ågren 2000, Bosatta and Ågren, 1999), and others defining it by chemical and physical occlusion (Carrington *et al.* 2012, Gentile *et al.* 2011, Feng and Simpson 2009, Conen *et al.* 2006, Fang *et al.* 2005).

Experiments on soil C temperature sensitivities can be divided into three categories: (1) laboratory incubations, (2) *in-situ* field experiments and (3) mesocosms (reviewed by Dieleman *et al.* 2012, Conant *et al.* 2008, Rustad *et al.* 2001). Influence of plant feedbacks (plants present or not), forms of temperature alteration (*e.g.*, heaters, heating cables, reflective curtains, etc.) and implemented temperature change (*e.g.*, constant temperature change, night-warming, etc.) vary between experiment types and all contribute to the lack of consensus on SOC temperature responses.

There are inherent problems with both laboratory and *in-situ* SOC temperature sensitivity experimental techniques. Lab studies, although useful for theory-based evaluations, are not able to replicate responses of intact systems that include plant inputs or rhizosphere activity. Davidson *et al.* (2000) pointed to this inherent problem with short-term soil laboratory incubations that use disturbed and sieved soils and do not include plant response components such as soil water balance, root exudate inputs, root turnover, and detrital inputs. *In-situ* field warming treatments are often applied either to soils, and hence do not provide a realistic assessment of altered vegetative inputs (*e.g.* Bradford *et al.* 2008), or only to aboveground, where warming treatments generally do not penetrate the soils (*e.g.* Cheng *et al.* 2011).

Mesocosms were used in this study and due to the unique precision climate controlled system are uniquely called terracosms. The terracosms are reconstructed native grassland mesocosms that have been exposed to ambient (AMB), symmetric (SYM) and asymmetric (ASYM) warming treatments. The warming treatments provide the same increase in mean temperature (ambient +3.5^{o}C/day) but with different patterns. The SYM treatment has constant temperature increase of +3.5^{o}C over AMB while the ASYM treatment has greater warming at dawn (+5^{o}C over AMB) than by midday (+2^{o}C over AMB). These treatments stand in stark contrast to existing less-realistic “night-warming” experiments that increase night-time temperatures only rather than providing asymmetrical elevation of T_{MIN}, which is at dawn (Phillips *et al.* 2011). This makes the terracosms a unique climate change experiment of whole-system driven SOC response to warming in both SYM and ASYM warming treatments.

Here, we compared SOC responses to AMB, SYM and ASYM treatments after six years of treatment application. We assessed SOC dynamics through total soil C, labile and resistant SOC pools, soil C fluxes (soil respiration, plant inputs and dissolved organic C (DOC) outputs), temperature sensitivity of soil respiration (Q_{10}) and soil enzyme activities. Labile soil C was assessed looking at C in light density soil fractions (<1.8 g/cm^3) and laboratory cumulative soil C mineralization. Resistant soil C was measured as the amount of C in the heavy density fractions (>2.4 g/cm^3), microaggregates (250-53µm) and the silt and clay aggregate fraction (<53 µm). Soil respiration was reported as *in*-situ cumulative soil C mineralization and was evaluated with other fluxes (above and belowground plant biomass, and soil water leachate DOC) to create C budgets for each treatment. Temperature sensitivity of soil respiration (Q_{10}) was also measured to compare the relationship between microbial metabolism and temperature and to assess whether microbes had acclimated to the altered conditions. Lastly, activities of common soil enzymes (β-glucosidase and phosphatase) were measured in order to assess potential temperature driven changes to microbial function or community.

We expected that warming would result in SOC losses caused by increased microbial metabolism, and that SYM treatments with the highest T_{MAX} would result in greatest soil C losses due to the exponential relationship between microbial metabolism and temperature. We expected that these losses would be evident in sensitive outputs of soil C (soil respiration and soil water leachate). More specifically, we expected that due to fast turn-over times, labile SOC indicators would have the strongest response to warming with the greatest depletion of labile C expected in SYM, because of higher T_{MAX}. In

addition, if resistant soil C has a greater temperature sensitivity than labile soil C, the resistant C should be more destabilized by SYM than ASYM treatments. Also, increased plant productivity (measured as plant biomass C) in response to warming was expected to increase rhizosphere activity, leading to increased soil macroaggregate formation and soil enzyme activities.

Methods

Terracosm Facility and Assembly

The terracosms are reconstructed native Oregon grassland mesocosms with precision climate controlled above- and belowground compartments (Figure 1). The Terracosm facility is located at the USEPA in Corvallis OR, US (44.565, -123.293, elevation 77 m). Mean annual temperature is 11^{o}C and mean annual precipitation is 117.1 cm (National Climatic Data Center 2012). Corvallis, OR has a Mediterranean climate with wet winters and dry summers (mean precipitation July-October is 6.2 cm).

The Terracosm facility has four replicates per temperature treatment for a total of twelve terracosms (Figure 1). Control treatments are kept at ambient (AMB) temperatures, symmetric (SYM) treatments are elevated by 3.5^{o}C, and asymmetric (ASYM) treatments are elevated to 5^{o}C above AMB at dawn (T_{MIN}) and 2^{o}C above AMB at midday (T_{MAX}).

The terracosms cover a ground area of 1m by 2m, have a 1.7m tall sloping (11.3^{o} from horizontal) top enclosure and a 1.0m deep sloping (16.7^{o} from horizontal slope) belowground soil enclosure (Figure 2). The aboveground chamber is sun-lit and enclosed with Teflon film on the top and

on three sides, October-June, with a permanent hard plastic fourth side. Terracosms are kept at ambient temperature in the summer (July-September) while plants are dormant. Rainfall was captured from terracosms roofs and added with a real-time irrigation system. Further details of terracosm function and experimental design are described in Phillips *et al.* (2011).

The belowground soil compartment, which is insulated with 15cm foam (R value 60), was filled in 2005 from a native Willamette Valley, OR grassland site (Corvallis Oregon). Soils in these grasslands had not previously been disturbed for the past 100+ years and are classified Fine, mixed, superactive, mesic Pachic Ultic Argixerolls (Dixonville Series, USDA, 2003). Soils were excavated by horizon (A: 0-20cm, 20-40cm; AB: 40-60cm; B: 60-80cm, 80-100cm) to a depth of 1m during the dormant dry period in mid-September. Soil was then sieved through 2.45 cm mesh, and tamped in-place to match site bulk-density above a layer of drainage gravel. The top 5 cm of soil was steam-sterilized to prevent germination of the seed bank. Terracosm soil was implemented with temperature and moisture monitoring (CS107-L thermisters, CS6110 TDR probes, CR10T data loggers and MD9 network, Campbell Scientific, Logan UT; Tektronics 1502b cable tester, Portland, OR) at 5, 15, 35, 55 and 75cm depths. Temperature treatment began in 2007 (April 2007-July 2007 = Year 1) with one year for settling and leaching. The conditions in this experiment are believed to be representative soil in an early successional system.

Soil Sampling

Three soil cores were collected from each terracosm at two depths (0-10cm and 10-20cm) on June 27 and 28, 2012 (Year 6). After cores were taken, soil was replaced by tamping fill soil to match existing bulk density. Two of the three soil cores, with 2-inch diameter, were composited by depth. Composited soils were root-picked for 4 hours, sieved through 2mm mesh and stored at 4^{o}C until C analyses. C concentrations were measured with a LECO CHN analyzer. The third cores, used for aggregate analysis, were immediately air-dried without any further disturbance. Seven gram subsamples (sieved and root-picked) were stored at -18^{o}C for extracellular enzyme assays.

Analyses

Sequential Density Fractionation

Sequential density fractionation, using sodium polytungstate (SPT), separated soils into three density classes (<1.8g/cm^3, 1.8-2.4g/cm^3, and >2.4g/cm^3) following Sollins *et al.* (2006). Light fraction soil had a density less than 1.8g/cm^3 and consisted of physically and chemically unprotected organic matter and represented labile soil C. The intermediate density fraction (1.8-2.4g/cm^3), was interpreted as protected intra-aggregate ("occluded") LF separated from remaining sediment (Crow *et al.* (2007), and as the organic matter associated with alumino-silicate clays usually of microbial origin in Sollins *et al.* (2009). Heavy fraction soil, with density greater than 2.4g/cm^3, was considered to represent the organo-mineral associated resistant soil C (Crow, *et al.* 2007; Sollins *et al.* 2009). Density fractions were analyzed for C content (g C/g soil). The difference between total soil C and the sum of C in all density fractions was identified as C lost during sequential density fractionation.

Laboratory Cumulative Soil C Mineralization

Laboratory cumulative soil C mineralization, like light density fraction C, is another way to assess labile soil C. Composited soil from each terracosm at two depths was homogenized and incubated for 97 days. Glass jars (100 mL) were filled with 25g of field moist (30% water content) soil and covered with polyethelyene film. Soil was maintained at constant laboratory temperatures and field moist conditions and CO_2 headspace was measured with a LI-COR 6400 on days 1, 3, 6, 9, 13, 17, 28, 42, 57, 77 and 97. CO_2 production rates were used to calculate cumulative C mineralization (mg C/g initial C).

Aggregate Classes

Air-dried cores for aggregate analysis ranged in weight from 20g to 65g. The wet-sieving procedure as described by Six *et al.* (2000) was used to obtain four aggregate classes: >2000 µm, 2000-250 µm, 250-53 µm, <53 µm. C content was found for each aggregate class. The difference between total soil C and the sum of the C in all aggregate classes was identified as C lost during wet sieving. Aggregates were reported both by weight (aggregate hierarchy) and C content (aggregate C allocation).

In-Situ Cumulative Soil C Mineralization

Soil respiration rate has been measured hourly at the terracosm facility starting in Year 4 using *in-situ* InfraRed Gas Analyzers (IRGA) with permanent soil collars. Soil collars had a 10cm diameter, were 2.5cm tall and were buried 1cm into soil to prevent atmospheric CO_2 influence and aboveground plant growth. Soil collar closed once an hour for measurement of CO_2 accumulation but otherwise remained open. Authentication of *in-situ* hourly measured soil respiration was performed with a portable LI-COR

6400 in the spring of Year 3. Hourly data were analyzed from October 2009 – July 2011 (Year 4 and 5) without summer dormant periods. Unrealistic values and outliers were removed from the data set using a filtering process. For all soil respiration data, yearly data was analyzed for % missing data before filtering and linear interpolation was used to replace missing values. Hourly respiration rates were summed based on plant growth year (*e.g.* Oct 2009- July 2010 = Year 4) and reported as *in-situ* cumulative soil C mineralization.

DOC Concentration of Terracosm Leachate

To maintain water balance, soil leachate drains from the soil chamber of each terracosm (Figure 2). Eleven samples were taken throughout Year 5 (across all seasons), frozen after collection, thawed for analysis, decanted, analyzed on a Shimadzu TOC Analyzer and assessed for treatment differences. In addition to yearly assessment, seasons were analyzed separately due to large seasonal differences in the magnitude and variance of DOC. Season lengths were defined as spring growth (Feb-July), summer dormancy (Aug-Sept), greening (Oct-Nov) and winter dormancy (Dec-Jan) (based on Phillips *et al.* 2011).

Aboveground Plant Biomass

Peak plant biomass C represents total aboveground primary production for the previous seasonal year. Aboveground biomass measurements were taken each spring as described in Phillips *et al.*, (2011). Briefly, peak cover green surface area was measured nondestructively using a $1cm^2$ grid. Cover was converted into g C using species-specific relationships between area, dry mass and carbon content, determined from subsamples of dried leaves collected in Year 1. Plant biomass C was used as a

proxy for yearly litter input. In an annual system, grassland species die each year, therefore, all aboveground C produced each year will enter the soil (Facelli and Pickett 1991). Therefore plant inputs based on aboveground biomass are overestimated.

Roots/Belowground Plant Biomass

Roots picked from soil cores were used to estimate total roots (g roots/m^2) and total root C content (g C/m^2). One-inch soil cores from Year 4 assessed roots from 0-10cm, 10-20cm, and then at 20cm increments to the bottom the terracosm. Roots in Year 4 were measured by weight removed by root-picking for 40 minutes from each depth. These roots were then analyzed for C content. Root collection in Year 6 only assessed soil from the top 20cm, therefore, data from the Year 4 sampling event was used to estimate total terracosm root content in Year 6. Additionally, root C content was estimated in Year 6 by converting total roots (g root/m^2) using the group mean of Year 4 root C concentration from all treatments (21.81%C).

Soil Respiration Temperature Sensitivity (Q_{10})

Soil respiration temperature sensitivity (Q_{10}) was assessed when plants were dormant in order to avoid plant influences over soil respiration values. Using summer and winter months using the following equation:

$$Q_{10} = \frac{R[T]}{R[T+10]}$$

Where *R[T]* is the rate of soil respiration at a specific temperature and *R[T+10]* is the rate of soil respiration 10^{o}C warmer than *R[T]*. Phillips *et al.* (2011) analyzed aboveground net primary production (NPP) and showed time periods of plant dormancy. We found sections of time while plants were dormant that had most reliable soil respiration data for all terracosms. For summer Year 4 we analyzed temperature sensitivity of July 16-30, and in winter Year 4 we analyzed December 23-31. The summer of Year 5 we analyzed July 10-26.

Enzyme Assays

According to Caldwell (2005), phosphatase (PASE) is an enzyme associate with phosphorus (P) uptake and β-glucosidase (β-GLC) is an enzyme associated with C uptake. Therefore, the ratio of these two common enzymes, β-GLC and PASE, can be used to assess C and P limitations in soil. Soil PASE and β-GLC activities were measured using modified *p*-nitrophenyl-ester based assays as described in Caldwell *et al.* (1999). Frozen soil samples (previously sieved with 2mm mesh and root-picked) were thawed and used to make soil slurries. For PASE activity, one mL of slurry was incubated at 30^{o}C for 1 hour after which 0.5 mL of 0.5M $CaCl_2$ was added and the reaction was stopped using 2 mL of 0.5 M NaOH. For β-GLC activity, 1 mL of soil slurry was incubated at 30^{o}C with 1 mL of 20 mM *p*-nitrophenyl-β-glucoside for 3 hours after which 0.5 mL of 0.5 M $CaCl_2$ was added and the reaction was stopped using 2 mL 0.1 M *tris*(hydroxyl-methyl)aminomethane. Centrifugation was used to obtain supernatant and absorbance was read at 410nm in a spectrophotometer (Hitachi U2001).

Statistical Analyses

Treatment differences were assessed using analysis of variance (ANOVA). Tukey's Honest Significant Difference test and the orthogonal contrast test were used to further investigate differences between ASYM and SYM treatments and/or differences between warming and ambient treatments. A p-value less than 0.05, represents a statistically significant difference and a p-value less than 0.1, but greater than 0.05, represents a notable statistical trend.

Results

Total Soil C and N

Counter to our expectation, six years of warming did not deplete total soil C or N stores (Table 1). Mean total soil C, N, and C:N did not differ between treatments and there was no difference between 0-10cm and 10-20cm depths.

Sequential Density Fractionation

Overall, significant warming effects on C contents of soil density fractions were not observed; only slight temperature effects were measured in light density C and intermediate density C in surface samples (Figure 3). At 0-10cm, light fraction C (<1.8g/cm^3) was depleted by 8-10% in both warming treatments, intermediate fraction C (1.8-2.4g/cm^3) was marginally greater in SYM than AMB (Figure 3:a), and heavy fraction soil C (2.4g/cm^3) was not different between any treatments at either depth (Figure 3). In all treatments, intermediate fraction contained the majority of C, with an average of 52% total soil C. The heavy fraction C was the next largest C store with approximately 21% of total soil C and light fraction C had an average of 15% of total soil C. The difference between total C accounted for in density

fractionation and total soil C, C "loss", accounted for an average of 12% of total C. C "loss" was only indirectly measured, and therefore was reported but not statistically analyzed.

Laboratory Cumulative Soil C Mineralization

In the short-term laboratory cumulative soil C mineralization, which is believed to be a measure of labile C, there was also no significant response in soils from warming treatments (Figure 4). There were no differences between treatments or depth over the 97 day soil C mineralization. However, there are slight trends for higher cumulative soil C mineralization in warming treatments in surface samples, and a divergent trend of ASYM from SYM and AMB at depth.

Aggregates Classes

Soil aggregate size distribution and C content responded differently to temperature treatments (Figure 5 and Figure 6). Large macroaggregates (>2000μm) had the most significant result with 39% fewer large macroaggregates in SYM than ASYM at both depths (Figure 5). Similarly, there were 31% fewer microaggregates (250-53μm) and 26% less silt and clay (<53μm) in ASYM than SYM at 10cm (Figure 5:a). Aggregate C content results followed those in aggregate distribution but significant differences were only found in macroaggregates of deeper samples (10-20cm). SYM large macroaggregates had significantly less C (33% less) than ASYM but significantly more small macroaggregate C (2000-250μm) than ASYM at 20cm (Figure 6:b). As with the sequential density fractionation, the difference between total C accounted for in the aggregate analysis and total soil C was

identified as C “loss” during analysis. C “loss” was only indirectly measured, and therefore was reported but not statistically analyzed.

In-Situ Cumulative Soil C Mineralization

Despite no observed difference in total C or N soil content, differences between treatments in *In-situ* cumulative soil C mineralization, a measure of soil respiration (*i.e.*, microbial metabolism and root respiration), were observed in Year 4 and 5 (Figure 7). *In-situ* cumulative soil C mineralization was significantly greater in warming treatments in Year 4, although there was a trend for ASYM to be greater than SYM cumulative soil C mineralization. In Year 5, both warming treatments had higher in-situ soil C mineralization than AMB and ASYM cumulative soil C mineralization was greater than SYM.

DOC Concentration of Terracosm Leachate

DOC concentration of soil leachate in warming treatments was 1.5 times greater during spring growth of Year 5 (

Figure 8). DOC concentration did not vary between treatments in either winter or fall of Year 5.

Plant Biomass and Roots

Aboveground plant biomass varied between treatments in Year 5 of the study (Figure 9). Plant biomass C did not differ between treatments in Years 1 through 4. In Year 5 ASYM had an average of 65g more plant biomass C than SYM and both warming treatments had significantly less plant biomass C than AMB. However, roots measured in June of Year 6 did not correlate to aboveground biomass. There was no apparent difference in root C content or root density to 20cm depth (Table 2). Using Year 4

whole terracosm root data (Figure 10), we found that approximately 56% of roots are present in the top 20cm of soil.

Temperature Sensitivity of Soil Respiration (Q_{10})

During winter dormancy, with saturated soil moisture conditions, warming treatments exhibited mean a Q_{10} of 1.75, while mean AMB Q_{10} was 1.02 (Figure 11). Warming treatments had a Q_{10} that was 73% higher than AMB treatments ($p=0.06$). For Q_{10} assessed during the summer dormancy in Year 3 and 4, no significant differences were observed between treatments.

Enzyme Assays

Soil enzyme activities did not vary between treatments but the ratio of β-GLC:PASE was slightly lower in ASYM than SYM and AMB treatments in surface samples, despite no differences between treatments in the either individual enzyme activity (Table 3).

Discussion

Total C and N

Total soil C is a large ecosystem C store that was not depleted, despite observed temperature driven increases in soil respiration (Table 1and Figure 7, respectively). Throughout six years of warming, plants continued to supply C to the soil via litter and rhizodeposition and these C inputs, in part, contributed to the lack of response of total soil C. A more important factor when considering the response of total soil C is the relative size of soil C as an ecosystem C pool. Soil C represents 95% of

ecosystem C in the terracosms and comparatively small fluxes (*i.e.,* DOC losses and soil respiration) have a limited ability to affect total C stocks over relatively short periods of time. For example, using data in Figure 7, we estimated a soil C loss of 3.0kg C to be the maximum potential terracosm soil C loss after six years of warming. This is calculated using a sustained maximum cumulative soil C mineralization value obtained from ASYM treatments in Year 5 of the study (Figure 7). In this calculation, no plant inputs were considered, but in the intact ecosystem, plants were contributing to soil C. Therefore, a maximum loss of 3kg C is a large over estimate. A 3.0kg C loss would have resulted in a 27.4% decline in total soil C and would have been measureable but with plant inputs equaling approximately the same amount over 6 years (3.0kg C) any difference would be hard to measure. In general, our data suggests that SOC temperature sensitivity experiments assessing soil alone, without considering plant inputs (*e.g.*, Bol *et al.* 2003), overestimate soil C losses.

Labile C Indicators

Indices of labile C had a weak response to temperature manipulation. Light fraction C (<1.8g/cm^3) lacked a strong response to warming and only showed slight trends for depletion in the surface horizon in the warmed treatments (Figure 3). When soil is fractionated by density, light fraction C (<1.8g/cm^3) is usually considered to be the most "labile" fraction of soil and has been identified as physically and chemically unprotected organic matter (Crow *et al.* 2007). Light fraction of soils is generally considered to respond most rapidly to land use or detrital manipulation (Davidson and Ackerman 1993), so we therefore expected a more significant depletion of this pool compared to the

more resistant heavy fraction of soil. Studies using controlled temperature experiments with soil samples without plants have often seen significant declines in light fraction C with increasing temperature (*e.g.,* Leifeld and Fuhrer 2005). The lack of a large decline in light fraction C in our grassland, after six years of warming, suggests a strong influence of plants over soil C dynamics; increased plant inputs or increased rhizodeposition likely moderated the enhanced microbial respiration that was observed in the warmed treatments.

Soil C that has the fastest turnover time is by definition the most easily accessed by soil microbes. Many soluble light molecular weight molecules present in soil, such as root exudates, are not isolated in the light density fraction (Boddy et al. 2007). C respired in the laboratory cumulative soil C mineralization is a better measure of the most "labile" or microbially accessible pool. In the laboratory cumulative soil C mineralization, soils that were under AMB, SYM and ASYM treatments for six years were kept at the same temperature and soil moisture condition for 97 days. Under these conditions laboratory cumulative soil C mineralization had a surprising lack of response, and had non-significant trends for greater cumulative C mineralization in warming treatments (Figure 4). Counter to this response, we expected that increased *in-situ* microbial activity in the warming chambers would lead to soils with less labile C. Plant associated light molecular weight C inputs from increased root exudates or root turnover under warming conditions were likely responsible for the laboratory mineralization response, which is further supported by increased leachate DOC concentration observed in both warming treatments in the spring of Year 5 (

Figure 8). Since plant inputs in warming treatments were greater in the year preceding the soil coring event, it is likely that roots were more active the year immediately preceding sampling. Even without increased peak aboveground biomass among treatments, it is quite possible that root activity and root exudation increased under warming, creating an additional source of easily mineralized – and leached – soluble C. Had we conducted a more traditional laboratory soil warming experiment, where soils are isolated from plant inputs and kept under extreme temperature conditions (as in Conant et al. 2008:b), we would have expected significant depletion of all measures of labile C. The fact that we did not see drastic depletion of labile C shows how significantly plant activity affects soil.

Resistant C Indicators

Counter to our expectation, measures of resistant soil C showed no evidence of higher temperature sensitivity in warming treatments (*i.e.,* heavy density fraction C, microaggregate C, and silt and clay aggregate fraction C). No change in resistant soil C indicators could be the result of extremely long turn-over times (>than 8 years) associated with chemically occluded C (Potvin *et al.* 2004). Future analyses of terracosm soils at greater than 6 years may reveal a depletion of resistant C in warming treatments. The lack of change in resistant C between treatments could also have resulted from a lack of relationship between chemical occlusion and molecular activation energies as suggested by Carrington *et al.* (2012). Alternatively, it is also possible that plant inputs are mediating temperature response of resistant soil C. We found no direct evidence of this, however, plant inputs were a mediating factor in labile C response, and therefore, it is important to mention the same possible plant effects on resistant

C. In general, studies that intended to assess links between chemical recalcitrance and microbial degradation, performed in laboratories with high temperatures and no inputs, have reported inconsistent results; differences in heavy fraction C (Bol *et al.* 2003; Lefevre *et al.* 2014), response of only labile pools (Zimmerman *et al*. 2012), and no difference in any soil C pools (Conen *et al.* 2006).

Soil C Inputs and Outputs

Plants proved to be important drivers of SOM dynamics not only in labile C dynamics but also in aggregate C allocation (aggregate C content, Figure 6) and distribution (aggregate weights, Figure 5). Differences in SYM and ASYM aggregates were likely a result of differential root activity. Aggregate C allocation and hierarchy are often highly influenced by root dynamics (Blanco-Canqui and Lal 2004). We did not observe any root density differences between treatments but root density does not necessarily correlate with root activity. Greater *in-situ* cumulative soil C mineralization in ASYM than SYM in Year 5 provides indirect evidence that root activity was greater in ASYM than SYM treatments via *in-situ* cumulative soil C mineralization in Year 5. Additionally, it is possible that root density during springtime growth, when plants are most active, was not represented by root density at the time of sampling (Gill and Jackson 2000). Based on indirect evidence of increased root activity through *in-situ* cumulative soil C mineralization, it is possible that root activity was also greater in ASYM than SYM in Years 5 and 6.

The expected exponential response of microbial metabolism to temperature (in SYM treatments, with highest T_{MAX}) was not evident in any analysis; in fact, if any overall trend emerged, it was for ASYM soil C to experience highest *in-situ* C mineralization (Figure 7). *In-situ* cumulative soil C

mineralization was calculated from soil respiration, a CO_2 efflux from soil to which both microbial respiration and root respiration contribute. Since plant inputs were not different in Year 4, one assumption we can make is that root respiration did not vary between treatments. If root respiration did not vary between treatments in Year 4, differences in *in-situ* cumulative soil C mineralization can be attributed to increases in microbial respiration. Also important to note, is that even though warming treatments were not significantly different from each other in Year 4, there is a consistent trend for higher C mineralization in ASYM treatments, even though plant inputs do not vary. This can be interpreted in two different ways. Either, the expected exponential response of microbial metabolism to temperature is not relevant to temperature effects on intact ecosystems and decreased DTR actually increases microbial metabolism more than T_{MAX}, or, root respiration actually was greater in ASYM than SYM treatments and root activity was not directly related to aboveground biomass.

Aggregates

Large macroaggregate C, often associated with root activity, was depleted by 33% in SYM compared to ASYM. Additionally, large macroaggregates are thought to be comprised of small macroaggregates and microaggregates (Blanco-Canqui and Lal 2004). Therefore, the increase of small macroaggregates and microaggregates in SYM is likely supplied from the breakdown of large macroaggregates. This also explains why we see a difference in the weights of SYM microaggregates (Figure 5) but not their C content (Figure 9). Large macroaggregate breakdown in SYM is further supported by response of intermediate fraction C (Figure 3). Intermediate fraction C, defined by Crow *et*

al. (2007) as protected intra-aggregate ("occluded") LF, was greater in SYM than AMB and ASYM. This result is atypical from all other analyses but it is possible that the breakdown of large macroaggregates caused increased intra-aggregate C availability. Aggregates are an important part of soil C sequestration and release (Blanco-Canqui and Lal 2004). Differences in ASYM and SYM aggregates, likely caused by differences in root dynamics, could result in long-term soil C differences.

Carbon Budgets

Temperature response of different ecosystem C stores and fluxes were variable and at times conflicting, therefore, treatment effect on net ecosystem C was best analyzed with a total C budget (Figure 12). Phillips *et al.,* (2011) assessed net changes to ecosystem C balance using Carbon exchange rates measures in the terracosms in Year 4 and found both warming treatments to be a net source of C to the atmosphere. Different from the technique used in Phillips *et al.,* (2011), Figure 12 was created using the treatment mean response of measured C stores and fluxes, from data composited from Year 5 and 6. This technique is less exhaustive than that used in Phillips *et al*., (2011) and simply assesses the balance between soil C inputs and outputs. Several important pools and fluxes were not measured, or measured by proxy. The rate of litter input was estimated from previous growth year plant biomass C, an overestimate. Root C was not directly measured in Year 6, nor were there any direct indications of root activity or microbial biomass C. Soil total C and macroaggregates C were assessed as ecosystem C stores. Soil respiration, root C, leachate DOC, and plant biomass were assessed as ecosystem C fluxes.

Plant biomass C and root C were considered as a total potential C flux into the soil since no litter or direct root input data was available, which resulted in an overestimate of inputs.

Whole C balance assessment reveals that AMB soil was an atmospheric C sink and that SYM and ASYM soil had the potential to be atmospheric C sources. Additionally, ASYM had a greater source potential than SYM, because of higher mean *in-situ* cumulative soil C mineralization. Although the C balance gives a clearer picture of total C dynamics, it is important to note that sustained increased plant inputs in ASYM as well as root aggregate C stabilization could mediate the ASYM response over the long term.

Soil Respiration Temperature Sensitivity (Q_{10})

Soil respiration temperature sensitivity (Q_{10}) was measured in order to assess potential microbial acclimation to temperature increases. Many warming studies have reported microbial acclimation to temperature increase (*e.g.,* Bradford *et al.* 2008, Carrillo *et al.* 2010) but here, after six years, we saw an increase in temperature sensitivity with increased mean temperature (Figure 11). Although temperature sensitivity can be difficult to measure *in-situ* because of plant influences, we have evidence of plant dormancy during assessed time periods via Phillips *et al.* (2011). We were also able to assess when soil moisture was a controlling factor of soil respiration and we feel confident that winter Q_{10} values are useful measures of soil temperature sensitivity. Other studies agree with our findings. Aguilos *et al.* (2011) saw no difference in soil respiration temperature sensitivity after 3 years in a warmed forested study site and, through microbial community analysis; Haugwitz *et al.* (2014) found no

evidence of acclimation after 5 years of warming in a low quality shrub land. However, the conflicting evidence from Bradford *et al.* (2008) and Carillo *et al.* (2010) suggests different response of microbial communities in different ecosystems.

Enzyme Activities

Enzyme activities did not vary between treatments and did not follow plant patterns as expected. However, the ratio of β-GLC:PASE had a trend to be lower in ASYM treatments (Table 3). Since these enzymes can be related to C and P uptake, we can surmise more energy was put into P uptake than C uptake in ASYM treatments. Since no other analysis assessed P dynamics in terracosm soils, further investigation is needed for any conclusion to be drawn.

"Night-Warming" Comparison

Our use of an asymmetric temperature profile in a grassland for ecosystem warming is in contrast to existing research on the "night-warming" phenomenon. "Night-warming" studies increase night-time temperatures rather than minimum daily temperature, which occurs at dawn. Comparison of our results and other "night-warming" studies highlights similarities in ecosystem responses. Most often, these studies reported increased soil respiration associated with night-time warming. However, plant response and interpretations vary. One "night-warming" study concluded that increased soil respiration resulted from increased plant inputs and that the increase in plant inputs was greater in night-time warming because of compensatory photosynthesis after plant respiration losses from the previous night (Zhang *et al.* 2011, Wan *et al.* 2009, Xia *et al.* 2009). We found no evidence of

compensatory photosynthesis as an underlying driver of SOC dynamics. Another "night-warming" study found an independent response of soil respiration in European shrublands (Beier *et al.* 2008). They saw both increased plant C uptake and increased soil respiration but found that soil respiration responded predictable to Q_{10} functions, independent of plant inputs. In contrast, we found close links between plant response and SOM dynamics and less predictable soil respiration responses. Overall, our soil response to asymmetric warming was more closely related to plant response than in these night-warming studies. Although not directly related to soil, Alward *et al.* (1999) assessed 23 years of recorded natural data and found a correlation between increased T_{MIN} and a decrease in native plant communities. Although this is different form the plant trend reported here, it is important to note that in a natural system, T_{MIN} affected plant productivity and, I n turn, could have affected SOC dynamics.

We compared the response of SOC after six years of ASYM and SYM warming in an intact primary succession native Oregon grassland mesocosm and we found, across most analyses, a larger plant influence over SOC dynamics than expected. The lack of significant responses of SOC pools after six years of warming, was unexpected, although, soil respiration (*in-situ* cumulative soil C mineralization) behaved more predictably and increased in warming treatments. Counter to our original hypothesis, no analysis showed greater temperature effects in SYM (high T_{MAX}) compared to ASYM. When all analyses were assessed together, we found warming treatments experienced soil C losses (through soil respiration) that outweighed inputs, resulting in a net loss of soil C to the atmosphere. ASYM treatments showed the greatest soil C loss potential, as a result of high *in-situ* soil C mineralization (Figure 12).

Conclusions

We found that plants exhibited greater controls over SOC dynamics than anticipated and therefore, SOC response temperature change is highly dependent on plant response to temperature change. There was no evidence supporting the original hypothesis expecting exponential response of microbial respiration to temperature but soil respiration did respond to temperature increases. Through total ecosystem C budgets, we found that increased soil C efflux outweighed soil C influx in warming treatments, with greatest potential losses in ASYM treatments. Since SOC dynamics are so are so closely related to plant dynamics, and terracosms only experienced plant differences in the last two years of the study, it is important to consider the future impacts of asymmetric warming on plants in order to accurately assess SOC response.

Figures and Tables

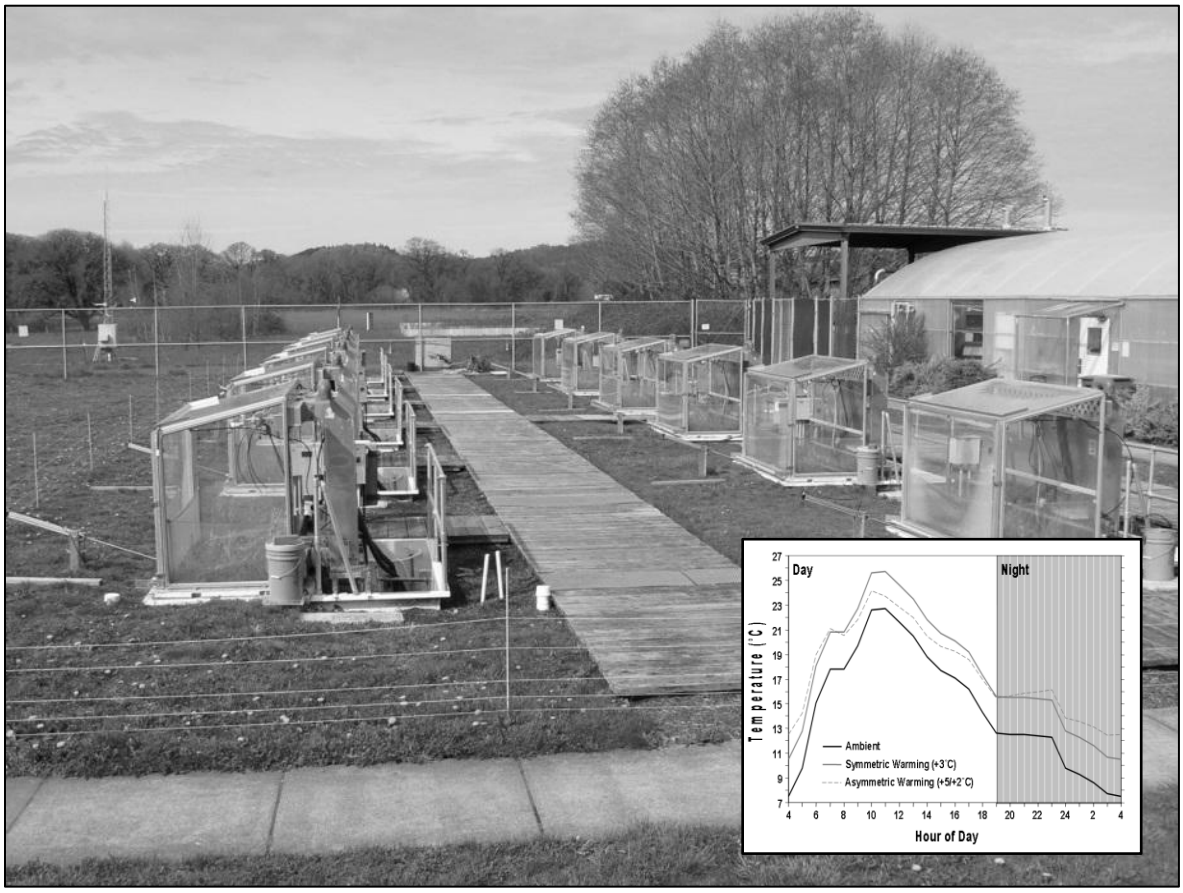

Figure 1. Terracosm facility at US EPA in Corvallis OR, USA. The terracosms (reconstructed native Oregon grassland) have precision climate controlled above- and belowground chambers for ecosystem temperature change research. The Terracosm facility is a three-way factorial experiment with four replicates per temperature treatment for a total of twelve terracosms. The three treatments are represented in the temperature profile in the bottom right of the figure and are: ambient (no warming), symmetric (+3.5^{o}C), and asymmetric (+5^{o}C/+2^{o}C at dawn/midday).

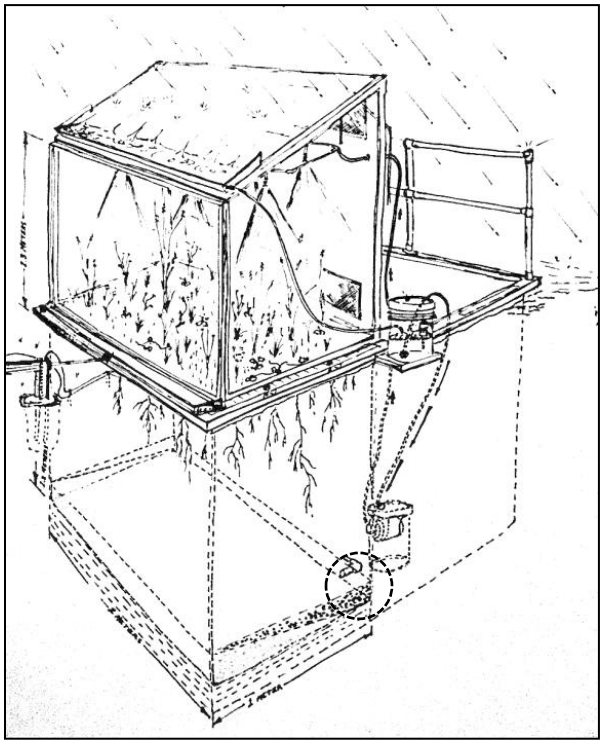

Figure 2. Schematic of one mesocosms (terracosm), with a ground print of 1m by 2m. Sun-lit aboveground compartment has a 1.7m to 1.5m tall sloping top and is enclosed with Teflon film on roof and three sides, October-June, with a permanent hard plastic fourth side. Belowground compartment is insulated with 15cm of foam and is 1.0m to 1.3m deep with a sloping bottom. Black dotted circle at the bottom of the image outlines the output of terracosm leachate.

Table 1. Total soil carbon (mg C/g soil, mean ± s.e.), nitrogen (µg N/g soil) and C:N for ambient (AMB), symmetric (SYM), and asymmetric (ASYM) treatments at two depths (N = 4 chambers per treatment). There were no significant differences between treatments ($p > 0.1$).

Depth	Treatment			
		C (mg C/g soil)	N (µg N/g soil)	C:N
0-10cm	AMB	25.59 ± 1.184	1003 ± 64.39	25.63 ± 0.677
	SYM	26.68 ± 1.501	1050 ± 61.80	25.46 ± 0.892
	ASYM	23.79 ± 0.777	950.8 ± 69.73	25.37 ± 1.797
10-20cm	AMB	27.32 ± 0.657	1109 ± 41.41	24.68 ± 0.498
	SYM	26.42 ± 0.839	1033 ± 80.66	25.90 ± 1.517
	ASYM	26.01 ± 0.608	1045 ± 98.13	25.49 ± 2.160

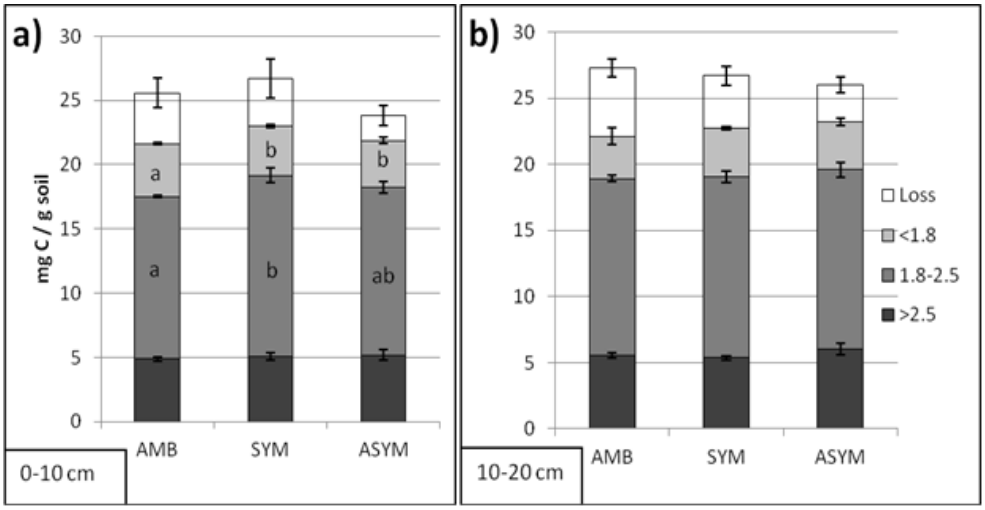

Figure 3. Carbon content (mean ± s.e.) of soil density fractions at a) 0-10cm and b) 10-20cm for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Soils were separated by density into light (<1.8g/cm^3), intermediate (1.8-2.4g/cm^3) and heavy (>2.4g/cm^3) fractions. The difference between total soil C before fractionation and the sum of C in all fractions was identified as C lost (Loss). Letters indicate p <0.1.

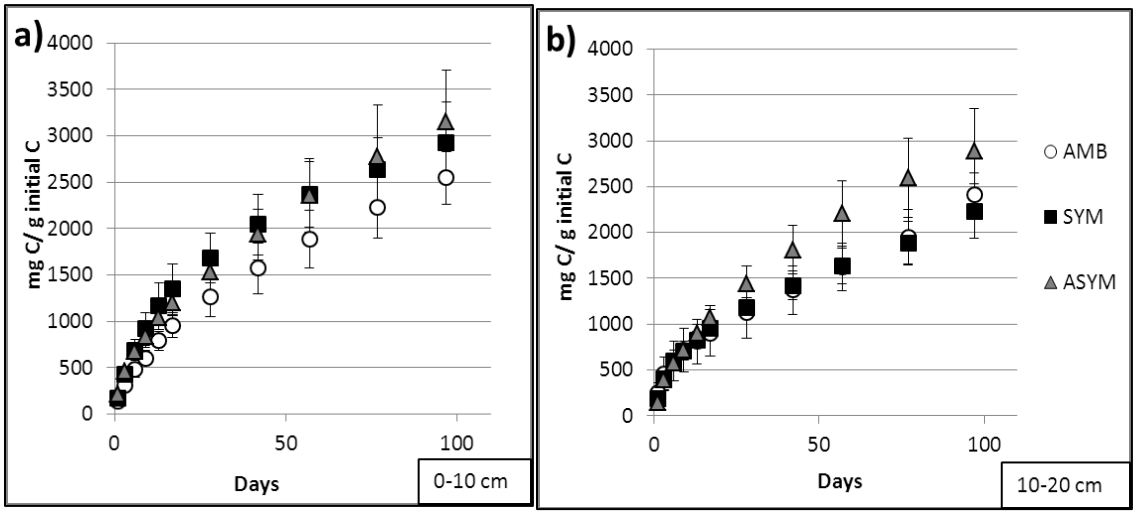

Figure 4. Ninety-seven day laboratory cumulative soil C mineralization (mean ± s.e.) with constant temperature and soil moisture conditions. Samples collected from a) 0-10cm and b) 10-20cm in chambers exposed to ambient (AMB), symmetric (SYM) and asymmetric (ASYM) warming treatments (N = 4 chambers per treatment). There were no significant differences in treatment or depth.

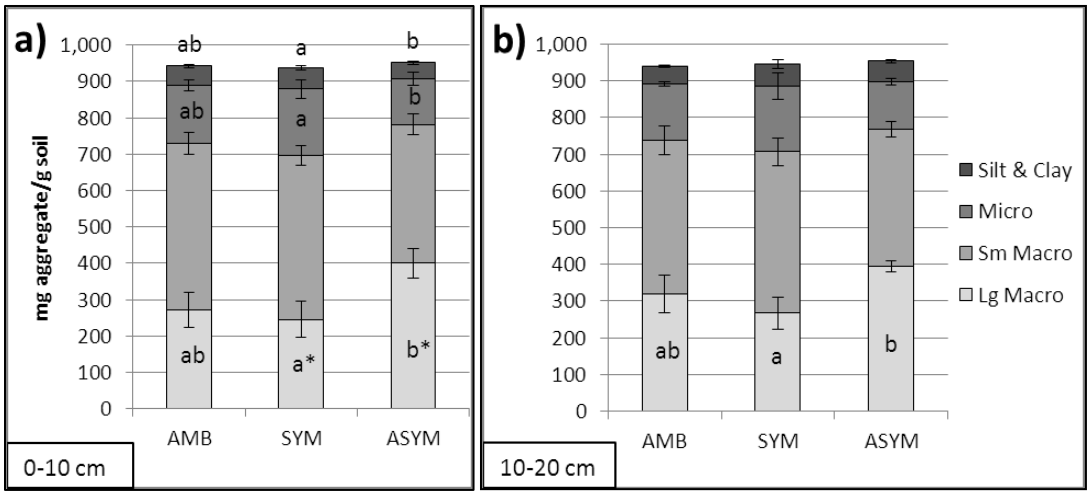

Figure 5. Weight distribution of aggregate classes (mean ± s.e.) at a) 0-10cm and b) 10-20cm depths for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Letters indicate p-value of <0.1, asterisk indicates p <0.05.

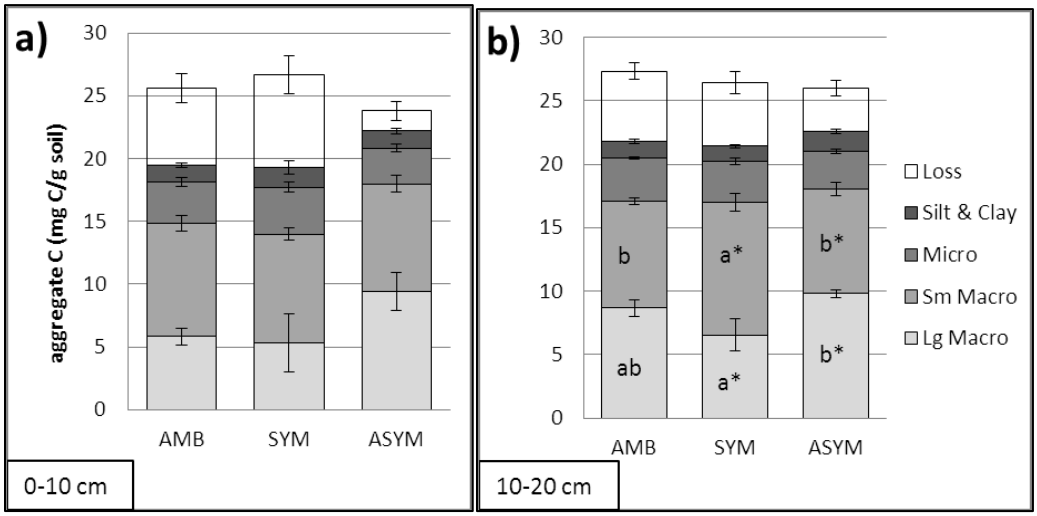

Figure 6. Carbon content (mean ± s.e.) of aggregate classes at a) 0-10cm and b) 10-20cm depths for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N=4 chambers per treatment). The difference between total soil C and the sum of C in all aggregate classes was identified as C lost (loss). Letters indicate p <0.1, asterisk indicates p <0.05.

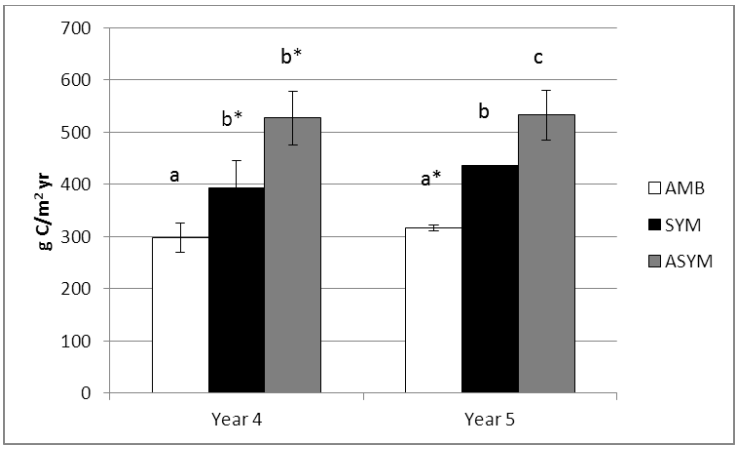

Figure 7. *In-situ* cumulative soil C mineralization (mean ± s.e.) from two growth years, Year 4 (October 2009-July 2010) and Year 5 (July 2010-June 2011) for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment in Year 4 and N=3,2 and 4 for AMB,SYM and ASYM, respectively in Year 5). Letters indicate p <0.1, asterisk indicates p <0.05.

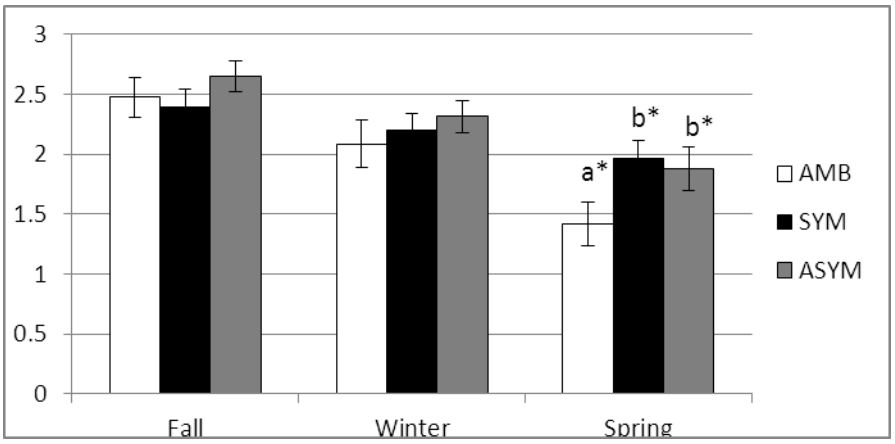

Figure 8. Dissolved organic carbon (DOC) concentrations (mean ± 95% C.I.) of terracosm leachate collected from ambient (AMB) symmetric (SYM) and asymmetric (ASYM) treatments in Year 5 (July 2010-June 2011) (N = 4 chambers per treatment). Letters indicate p-value of <0.1, asterisk indicates p <0.05.

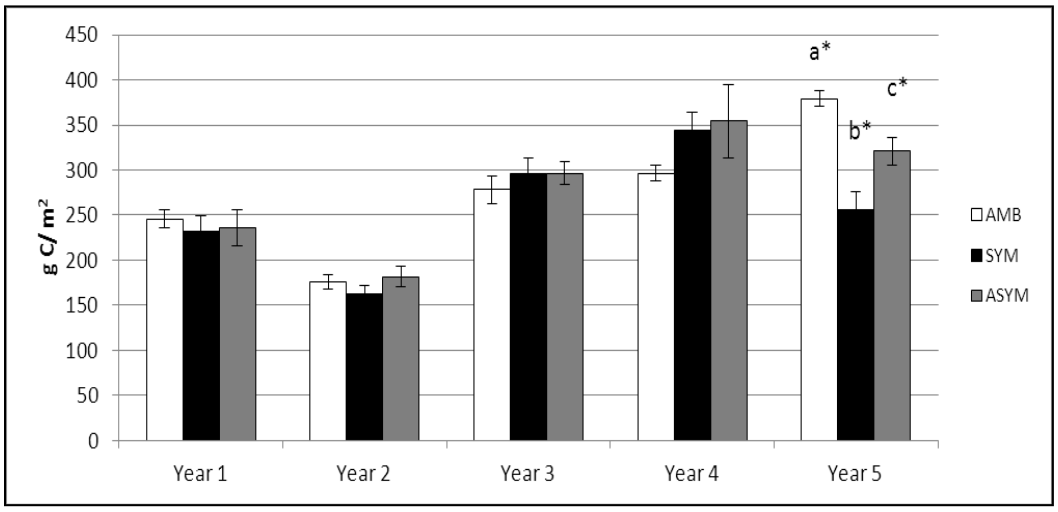

Figure 9. Carbon content of aboveground plant biomass (mean ± s.e.) collected at peak growing season for all years of study up to June 2011 for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Letters indicate p <0.1, asterisk indicates p <0.05.

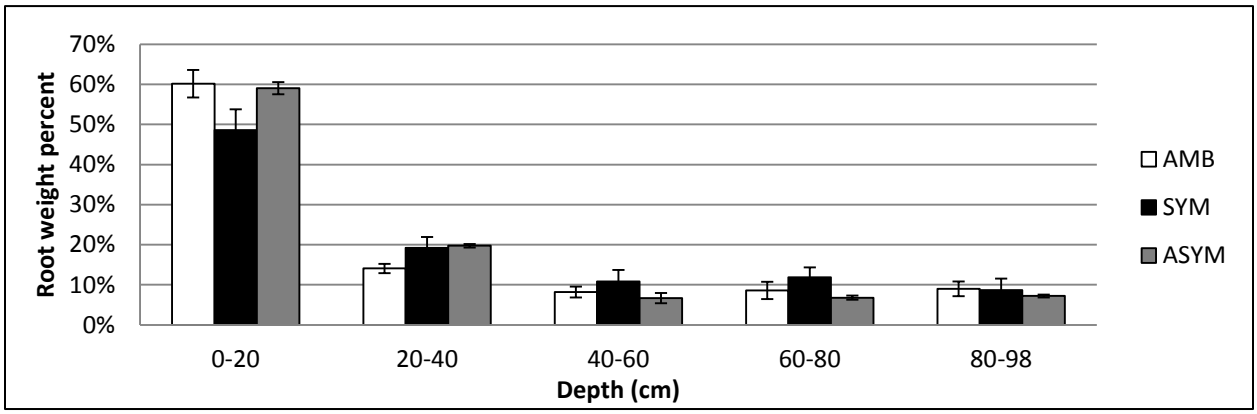

Figure 10. Distribution of root weight in Year 4 sampling event (mean ± s.e.) for ambient (AMB), symmetric (SYM) and asymmetric (AYM) warming treatments (N=4 chambers per treatment).

Table 2. Root mass (g roots/ m^2, mean ± s.e.) and root C content (g C/m^2, mean ± s.e.) from sampling event in Year 6 (June 2012) (N = 4 chambers per treatment). There were no significant differences between treatments ($p > 0.1$).

Treatment	Mass (g roots/ m^2)	Root C (g C/m^2)
AMB	595.44 ± 127.49	64.94 ± 13.90
SYM	602.08 ± 116.25	65.66 ± 5.91
ASYM	583.41 ± 54.15	63.63 ± 12.68

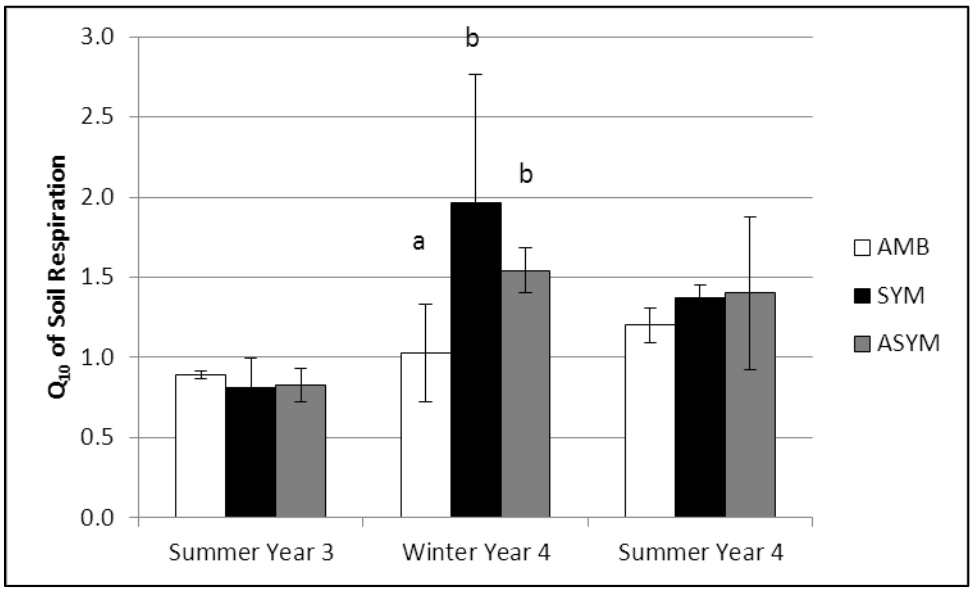

Figure 11. Temperature sensitivity (Q_{10}) of soil respiration from summer of Year 3 and 4 and winter of Year 4 for ambient (AMB) symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Letters indicate p <0.1.

Table 3. Enzyme activities (µmol *p*-NP/g/hr, mean ± s.e.) of phosphatase (PASE), β-glucosidase (β –GLC) and the ratio of the two for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Letters indicate p <0.1.

		Treatment:		
Enzyme	Depth	AMB	SYM	ASYM
PASE	0-10cm	10.59 ± 2.21	9.95 ± 3.33	12.50 ± 3.70
	10-20cm	10.81 ± 0.99	10.71 ± 1.10	12.03 ± 1.81
β –GLC	0-10cm	1.06 ± 0.21	1.03 ± 0.19	0.81 ± 0.14
	10-20cm	1.07 ± 0.08	1.30 ± 0.09	1.51 ± 0.20
β –GLC:PASE	0-10cm	0.11 ± 0.02 (ab)	0.12 ± 0.02 (a)	0.07 ± 0.01 (b)
	10-20cm	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01

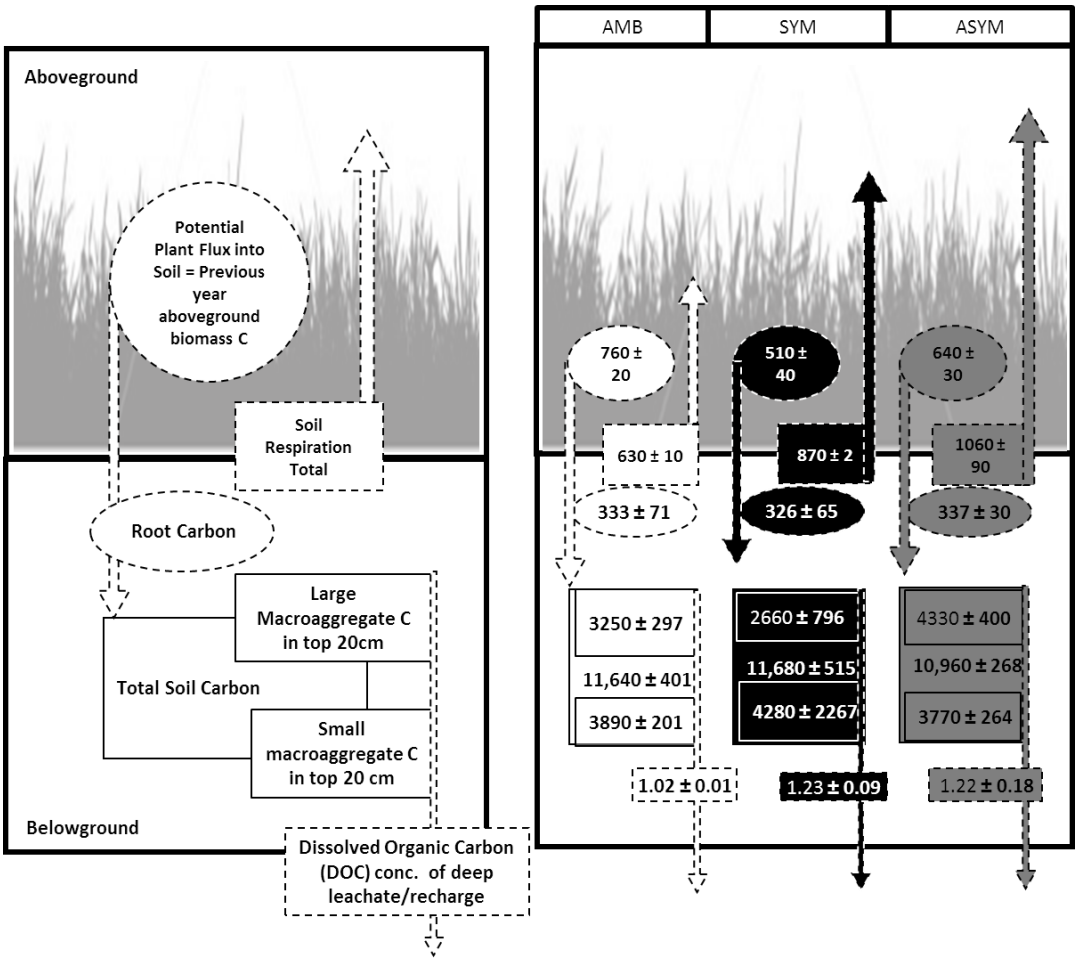

Figure 12. Carbon budget for ambient (AMB), symmetric (SYM) and asymmetric (ASYM) treatments (N = 4 chambers per treatment). Dashed ovals show plant C fluxes into the system. Boxes show soil C pools (solid lines) and fluxes (dashed lines) from soil. Units for pools are g C (mean **±** s.e.); units for fluxes are g $C/m^2/yr$ (mean ± s.e.).

Chapter 3: General Conclusion of the Assessment of Soil Organic Carbon Response to Altered Diurnal Temperature Range

In order to assess the viability of existing research on SOM temperature sensitivity, we compared SOM responses to ASYM and SYM warming in a primary succession native Oregon grassland mesocosm. Total soil C was not depleted, despite observed temperature driven increases in soil respiration. Throughout six years of warming, plants continued to contribute to soil C via litter and root dynamics and these C inputs, in part, contributed to the lack of response of total soil C. The expected exponential response of microbial metabolism to temperature (in SYM treatments, with highest T_{MAX}) was not evident in any analysis; in fact, if any overall trend emerged it was for ASYM soil C to experience greatest losses via *in-situ* C mineralization. Labile C indicators did not behave as expected. Light fraction C ($<1.8 g/cm^3$) lacked a strong response to warming and only showed slight trends for depletion in surface samples. Laboratory cumulative soil C mineralization, a measure of most easily accessible C substrate, had a very surprising lack of response, and if anything had trends for greater cumulative C mineralization in warming treatments. Root associated light molecular weight C inputs were likely responsible for the laboratory mineralization response. This was further supported by increased leachate DOC concentration observed in both warming treatments in the spring of Year 5. Counter to our expectation, measures of resistant soil C showed no evidence of higher temperature sensitivity in warming treatments. Overall, we found plants to not only control aggregate dynamics but to overall have a much greater impact on soil C dynamics than expected. When all analyses were assessed as a

whole, we found that soil C losses (through soil respiration) outweighed inputs and resulted in net losses that were greater in warming treatments and were potentially greatest in ASYM treatments. Since differential responses were found for ASYM and SYM treatments it is important to incorporate more studies utilizing asymmetric temperature profiles into climate change research. Additionally, since we found plants to be such a large influence, it is important to consider the SOC response to temperature increase in intact ecosystems with both air and soil warming.

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