THE EFFECT OF WARMING ON BELOWGROUND CARBON DYNAMICS

by

MEGAN BETH MACHMULLER

(Under the Direction of Jacqueline E. Mohan)

ABSTRACT

This dissertation explores the effects of warming on soil respiration and soil organic matter decomposition in forest soils. I utilized a field warming experiment located in the Southeastern US Piedmont, the first of its kind on highly-weathered soils. Both field and laboratory approaches were utilized to assess the microbial (heterotrophic) response to increased temperature. My results suggest that warming results in no significant change in soil respiration (carbon dioxide efflux), soil carbon stocks, or soil organic matter chemistry, unlike previous studies. Hence, rising temperatures will not universally increase carbon dioxide efflux from forest soils. To explain the lack of a response, I performed a fully-factorial laboratory incubation to determine the importance of soil moisture in regulating heterotrophic respiration response to temperature but the nature of the interaction varies with season. I observed that moisture and temperature interact to regulate heterotrophic respiration. The temperature sensitivity of heterotrophic respiration does not change with moisture treatments but increases ~ 30% from winter to summer. This finding demonstrates that key ecosystem processes vary by season and modulate the response of heterotrophic respiration. By including moisture and temperature into models of heterotrophic respiration, we improved predictions of field respiration and explained 50-70% of the variability associated with *in situ* soil respiration. Finally, I investigated how *in* *situ* warming affected six different extracellular enzyme activities involved in soil organic matter decomposition. The microbial response to soil warming was minor relative to seasonal variation in microbial activity. We observed significant seasonal variation in enzyme activities, temperature sensitivities and microbial nutrient acquisition ratios, but the response was inconsistent across enzyme type. Additionally, we found low C:P enzyme ratios suggesting P limitation in this ecosystem. Our study suggests that a major effect of climate change on soil organic matter decomposition may be the result of changes in the seasonal patterns rather than directly altering the kinetics of enzymes in the soil through warming alone.

INDEX WORDS: soil respiration, heterotrophic respiration, soil organic matter decomposition, extracellular enzymes, soil carbon

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MEGAN BETH MACHMULLER

Bachelor of Science, Creighton University 2005

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSPHY

ATHENS, GEORGIA

2014

THE EFFECT OF WARMING ON BELOWGROUND CARBON DYNAMICS IN HIGHLY WEATHERED SOILS

by

MEGAN BETH MACHMULLER

Major Professor: Jacqueline Mohan

Committee: Daniel Markewitz
Aaron Thompson
Nina Wurzburger

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
August 2014

DEDICATION

To my family (Mom, Dad, and Rachel) who have encouraged and supported me every step of the way, and to Bethie who taught me to not sweat the small stuff.

ACKNOWLEDGEMENTS

My dissertation research would not have been possible without the help and collaboration of numerous people. I would like to thank my advisor, Jacqueline Mohan, for all of her support and encouragement over the last five years at the Odum School. I am also grateful for my doctoral committee: Aaron Thompson, Nina Wurzburger, and Daniel Markewitz, who dedicated time and resources for this research. Aaron Thompson provided guidance and resources for the soil chemistry work and introduced me to the mineralogical and geochemical realm of soils. Nina Wurzburger provided critical feedback and resources for laboratory experiments. Daniel Markewitz offered feedback on data analysis and interpretation. I want to thank Tom Maddox, Yi-ting (Emmy) Deng, and Michael Marshall of the Odum School of Ecology Analytical Laboratory for their analytical expertise and timely analysis of samples. Johannes Leisen, Georgia Tech Chemistry and Biochemistry Department, provided support with the 13C-NMR analysis. I would like to thank Carly Phillips and Jeff Minucci, graduate students in the Wurzburger lab, who performed important roles in the laboratory experiments. Shialoh Wilson, Austin Martin, and Meryom Patillo of the Wurzburger lab have provided lab assistance with enzyme assays and soil incubations. Taylor Cyle and Jared Wilmoth provided stimulating conversations on soil organic matter stabilization dynamics, environmental chemistry, and mineralogy. Thank you to Kris Miller, Lynn Faust, and Lamara Martin - of the UGA Biology Department- for all their support while teaching Biology. I also want to thank the previous and current members of the Mohan lab including Paul Frankson, Peter Baas, Charles Cowden, Fern Lehman, Melanie Taylor, Shafkat Khan, Katy Bridges, Anurag Dawadi, Kaitlin McLean, Allen

Witt, Ashley Lang, Elizabeth Hughes, and Allison Hughes. Paul Frankson was responsible for the construction and maintenance of the warming experiment at Whitehall Forest and was the engine that kept things running in the field. A big thank you to Shafkat Khan for your helpful conversations and critique of this research. Charles Cowden provided extremely helpful critique on data interpretation and manuscripts. I especially want to thank Peter Baas, my partner and forever colleague, for all of your support over the last five years. And thank you to all the graduate students, staff, and faculty of the Odum School of Ecology who have made these last five years an unforgettable experience. My doctoral research was funded, in part, by the grants I received through the Sigma Xi Scientific Society and the Odum School of Ecology. Last, but certainly not least, I want to thank Eugene P. Odum for establishing a school of Ecology and providing me with a foundation of ecosystem and holistic science to build on for the rest of my career. If I have seen further it is by standing on the shoulder of giants.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

An understanding of the global carbon (C) cycle has become of central importance in the field of biogeochemistry, ecosystem ecology and climate science due to its pivotal role in the regulation of life on earth. In particular, soils play an important role in the global C cycle, storing significantly more C than is present in the atmosphere or terrestrial vegetation (Jobbágy and Jackson 2000, Schlesinger and Bernhardt 2013). Since the late 1800s, human activities such as fossil fuel combustion, forest clearing, and the conversion of land for agriculture have led to a net transfer of terrestrial C to the atmosphere (Falkowski et al. 2000). As a result, atmospheric (CO_2) dioxide concentrations have increased from 280 ppm in pre-industrial time to 400 ppm at present, potentially reaching 700 ppm by the end of the twenty-first century (Pachauri 2008). Consequently, the buildup of CO_2 and other greenhouse gases in the atmosphere have caused the global surface temperature to increase by 0.74°C since 1850 (Pachauri and Reisinger 2007). Current models of climate change suggest that the global average temperature will increase by an additional 1-6°C in the next 50-100 years (Pachauri 2007) and without strong emission reduction or technological breakthroughs will continue to increase after this time (Solomon et al. 2009). Many aspects of the terrestrial C cycle are affected by temperature, and the net effect of how climate change alters C uptake (photosynthesis) versus loss (soil organic matter decomposition) will ultimately determine the C sink strength of our ecosystems.

C enters the ecosystem through photosynthesis and is returned to the atmosphere via soil respiration. Soil respiration includes the flux of microbial (heterotrophic) and plant derived (autotrophic) CO_2 to the atmosphere and is the second largest terrestrial carbon flux (Raich and Schlesinger 1992, Raich and Potter 1995). A terrestrial feedback to climate change stems from the perspective that temperature is the dominant factor regulating respiration (Jenkinson et al. 1991, Davidson and Janssens 2006), while photosynthesis is regulated by multiple factors including light, CO_2, water, and nutrients (Farquhar et al. 1980). It has been hypothesized that microbial decomposition of soil organic matter would be more sensitive to warming than gross primary productivity (Kirschbaum 2000). Under such a scenario, in a warmer world, net C uptake from the atmosphere would be less than the release of C from soils. Yet, much uncertainty exists on the dynamics of soil respiration and the terrestrial global C flux remain poorly constrained (Trumbore 2006).

Coupled C-climate models examine potential feedbacks between global C storage and greenhouse gas emissions (Cox et al. 2000, Friedlingstein et al. 2006). These models suggest a reduced efficiency of terrestrial ecosystems to absorb atmospheric carbon dioxide (Friedlingstein et al. 2006). However, there is uncertainty in the feedback potential between the biosphere and atmosphere and estimates of additional atmospheric CO_2 at the end of the 21st century, due to a feedback mechanism, vary from 20 – 200ppm (Friedlingstein et al. 2006). Much of this uncertainty stems from the large variability associated with predicting terrestrial C sinks and sources. While the vegetation component of these models has developed rapidly over recent decades (Sitch et al. 2003), the microbial component, controlling SOM decomposition, has not (Chapin III et al.

2009, Todd-Brown et al. 2012). CO_2 released via SOM decomposition is a large component of the terrestrial C balance, releasing ~7 times more carbon than anthropogenic CO_2 emissions (Schlesinger and Bernhardt 2013). However, there still is no consensus on the temperature sensitivity of decomposition and the significance of this positive feedback is still uncertain (Davidson and Janssens 2006, Davidson et al. 2006, Conant et al. 2011).

Previous studies have found broad geographic relationships between climate and soil organic matter, which are suggestive of the regulating factors on soil C storage (Jenny 1980, Burke et al. 1991). In general, higher temperatures correlate to lower soil organic C or nitrogen (N). In his classic work on soil forming factors, Jenny described how soil organic N (and C) increased with decreasing temperature and increasing precipitation across central North American Great Plains (Jenny 1941, 1980). Kirschbaum (2000) pointed out that Jenny's findings suggest that with an increase of temperature, decomposition increases more than net primary production. On a global scale, (Post et al. 1982) showed that soil C increases with a decrease in temperature but this depends on soil moisture. The relationship between soil C and temperature was strong in very wet and dry conditions, but was inconsistent with intermediate moisture levels. This highlights the important role of soil moisture regulation on decomposition and soil C stocks across large geographic regions. The C and nutrient chemistry (stoichiometry) of leaf litter can also have strong effects on the abundance and activity of decomposers (Melillo et al. 1982). Global trends of litter decomposition rates vary as a function of litter quality, representing a connection between whole plant C and nutrient strategies and biogeochemical cycling (Cornwell et al. 2008). While the general climatic and environmental variables that control respiration materialize at large geographic scales, there are insufficient details that are critical for a quantitative prediction of how changing climate and environmental variables affect respiration fluxes at any given location (Trumbore 2006). The ability to predict soil C stocks across space does not necessarily extrapolate to accurate predictions of climate change-driven changes in soil C stocks (Agren and Bosatta 2002, Ise and Moorcroft 2006). Recent research and insight from long-term field experiments, and new isotopic, spectroscopic and molecular-marker techniques have challenged the long-standing assumptions of the factors regulating soil organic matter decomposition (Conant et al. 2011, Schmidt et al. 2011).

In order to assess how climate change will alter soil organic matter decomposition and soil C stocks it may be useful to apply thermodynamics, or kinetic theory (Arrhenius 1889), as suggested by Conant et al. (2011) and briefly outlined here. First, kinetic theory suggests that temperature will increase decomposition rates when substrate availability and enzyme activities do not constrain reactions (Davidson and Janssens 2006). Ecosystem models often utilize this aspect of kinetic theory to model rates of decomposition (Davidson and Janssens 2006). Some models are consistent with the second component of kinetic theory, stating that cold climates would experience the greatest increases in decomposition with warming (Del Grosso et al. 2005). Field (Lloyd and Taylor 1994) and laboratory incubations (Kirschbaum 1995) have supported this aspect of kinetic theory. The third component of kinetic theory implies that high activation energies of decomposition will experience greater increases with warming than low activation energy. Due to their complexity, global models depict soil C as a generic C type and operate under assumptions that thermodynamic principles are broadly applicable across ecosystems and biomes (Davidson et al. 2006, Treseder et al. 2012). In particular, these models depict that warming will stimulate soil respiration (Treseder et al. 2012). However, while long term warming experiments have shown an initial increase in soil respiration with warming (Rustad et al. 2001), within a few years soil respiration declines to ambient levels (Luo et al. 2001, Melillo et al. 2002). This suggests that the kinetics of the reactions change over the course of warming. Unraveling the response of warming on soil organic matter decomposition is particularly difficult given the wide range of intrinsic kinetic properties in any given ecosystem that can change with time and environmental stressors.

Investigations of mechanisms constraining increases of soil respiration after long term warming have been examined and include substrate limitation and decreased temperature sensitivities of soil microbial communities responsible for soil organic matter decomposition (Strickland et al. 2009, Allison et al. 2010, Sheik et al. 2011, Zhou et al. 2012, Wieder et al. 2013). Recent modeling efforts have found that warming effects on soil respiration are dependent on microbial physiology (Allison et al. 2010). Specifically, they find that reduced C-use efficiency can explain the attenuation of soil respiration to warming. In other words, after long-term warming the microbial community invests less assimilable carbon to extracellular enzyme production and growth that leads to a decrease in microbial biomass and soil respiration.

Microbial communities including bacteria, fungi, and archaea control the rate of C and nutrient cycling in nearly all terrestrial ecosystems (Schlesinger 2004). Consequently, as temperatures increase and microbial physiology is affected, the rates of biogeochemical cycling may be significantly altered. Thus, it is essential to consider the response of microbial communities to changing climates. A major uncertainty in coupled C-climate models is to what extent microbial decomposition of soil organic matter may feedback to affect atmospheric CO_2 and ecosystem functioning (Moorhead and Sinsabaugh 2006, Allison et al. 2010, Todd-Brown et al. 2012). Microbes produce extracellular enzymes to facilitate the uptake of C and nutrients stored in SOM. Measurements of extracellular enzyme activities involved in SOM decomposition and nutrient acquisition have become a common tool for examining soil microbial response to climate change (Wallenstein et al. 2011, Weedon et al. 2011, Henry 2012). At an ecosystem scale or global scale, environmental variables (e.g. temperature, plant composition, litter inputs) can affect the rate of SOM decomposition and soil C stocks. At the molecular level, microbial resource demand and extracellular enzyme production regulate soil organic matter decomposition (Billings and Ballantyne 2013). An ecosystem level understanding may require molecular-level knowledge to be able to accurately predict biogeochemical transformations. Because extracellular enzymes control the rate of soil organic matter solubilization- the rate limiting step of decomposition (Schimel and Bennett 2004, Bengtson and Bengtsson 2007)- shifts in enzyme production or allocation could have major consequences for carbon and nutrient cycling in a warmer world (Henry 2012).

Results from a global meta-analysis, from ecosystem warming experiments, show that across many ecosystem types, 1-9 years of 0.3 - 6°C warming increased soil CO_2 efflux on average by 20%, with the largest effect size in forested sites (Rustad et al. 2001). Until the installation of the experimental system used in my dissertation, all forest ecosystem warming experiments have been conducted on nutrient-rich glacial soils with relatively abundant organic matter, of over 30% C in the upper organic horizon (Rustad et al. 2001). It is unknown whether a similar response is to be expected in ecosystems on highly weathered soil with lower C contents that exist in low latitudes that cover ~20% (NRCS Soil Survey Staff 2014) of the land surface area. Therefore, I utilized an ecosystem warming experiment located in the unglaciated, southeastern U.S. Piedmont physiographic province of Georgia (Whitehall Forest; 33°53'17.87"N, 83°21'40.92"W). The forest consists of naturally-recruited un-managed forest stands dominated by mixed-deciduous trees (*Quercus alba, Q. rubra,* and *A. rubrum)* that originated in the first half of the 20th century following agricultural and land abandonment. The soils are Typic Kanhapludults with low organic matter content (low C), low fertility, medium to slow permeability, and a pH of 4.5. In **Chapter 2**, my overarching question was: Does *in situ* warming stimulate soil respiration in this unexplored ecosystem? I measured *in situ* soil respiration for the first three years of warming manipulation. I also determined how warming affected soil carbon stocks and SOM chemistry. In **Chapter 3**, I utilized a controlled laboratory experiment to examine how both temperature and moisture influence heterotrophic respiration. The field soil respiration measurements include both autotrophic and heterotrophic respiration and it is difficult to differentiate the two in the field. Therefore, excluding root contributions in the laboratory experiment allowed us to specifically identify the microbial response to fluctuations in both temperature and moisture. In **Chapter 4**, I explored how 2-3 years of warming affected extracellular enzymes involved in soil organic matter decomposition. I measured extracellular enzyme activities as well as the temperature sensitivity of enzyme activity. My dissertation research combined interdisciplinary techniques spanning chemistry and microbiology to elucidate the response of and mechanisms controlling soil organic matter decomposition in warmer conditions.

CHAPTER 2

WARMER TEMPERATURES FAIL TO INCREASE SOIL RESPIRATION IN HIGHLY-WEATHERED FOREST SOIL[1]

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[1] Megan B. Machmuller, Aaron Thompson, Ford Ballantyne, Daniel Markewitz, Nina Wurzburger, Jacqueline E. Mohan. To be submitted to *Nature Climate Change*.

Abstract

An acceleration of global warming due to a terrestrial carbon-cycle feedback is of central importance to future climate. A positive feedback, meaning an increase in carbon dioxide (CO_2) soil-atmosphere efflux with increasing temperature, can affect the storage capacity of the terrestrial biosphere and can potentially change an ecosystem from a C sink to C source. The magnitude of this microbial response is dependent on the temperature sensitivities of soil microbial communities responsible for soil C decomposition and on mechanisms protecting soil C from decomposition. Most field warming studies have observed an increase in CO_2 efflux from soils to the atmosphere, however the majority of these studies have been conducted in higher and primarily northern latitudes on relatively young soil substrates. Whether a similar response is to be expected in ecosystems on highly weathered soil in low latitudes is unknown. Here we show that warming highly-weathered, sub-tropical forest soil by 3°C or 5°C for three years results in no significant changes in CO_2 efflux, soil C stocks or C chemistry. Hence an increase in CO_2 efflux from soils due to rising temperatures is not a universal response. This warming study demonstrates that an increase in CO_2 efflux from soils due to rising temperatures is not a universal response.

Introduction

An important consideration for policy on greenhouse gas emissions concerns how climate change will affect carbon (C) storage in forests. Since the Industrial Revolution, human activities have significantly altered the C cycle and, given current projections of human population growth and increasing demand for energy (Hoffert et al. 1998, Canadell et al. 2007), the magnitude of anthropogenic climate forcing is likely to increase. Current models of climate change suggest that the global average temperature will increase by an additional 1-6°C in the next 50-100 years (Pachauri 2007) and without strong emission reduction or technological breakthroughs shall continue to increase after this time (Solomon et al. 2009). Warming trends associated with global climate change have the potential to increase soil CO_2 efflux to the atmosphere (Melillo et al. 2002, Fang et al. 2005, Knorr et al. 2005) and most ecosystem models predict that warming will stimulate soil organic matter (SOM) decomposition resulting in a positive feedback to climate (Lloyd and Taylor 1994, Cox et al. 2000, Friedlingstein et al. 2006, Heimann and Reichstein 2008). The net influence of climate change on C uptake (photosynthesis) versus loss (plant and soil respiration) determines the C sink strength of ecosystems.

Current global C models, due to their complexity, depict soil C as a generic C type and apply similar thermodynamic principles broadly across ecosystems and biomes (Davidson et al. 2006, Treseder et al. 2012). For instance, most models depict soil respiration to increase with temperature (Treseder et al. 2012). Results from a meta-analysis show that across many ecosystems types, 1-9 years of 0.3 - 6°C warming increased soil CO_2 efflux on average by 20%, with the largest effect size in forested sites (Rustad et al. 2001). Critically, however all the forest studies in this meta-analysis occurred in northern latitudes. Warming-induced increases in CO_2 efflux can lead to a loss of soil C because microbial decomposition of SOM is responsible for approximately 50- 80% of the CO_2 released from soils (Schlesinger and Andrews 2000, Melillo et al. 2002, Ryan and Law 2005). Soils release 60-75 Pg C to the atmosphere annually

(Schimel 1995, Schlesinger and Andrews 2000), thus a 20% increase in soil respiration corresponds with an additional 12-15 Pg C released each year—higher than the ~9Pg C released from anthropogenic emissions (Le Quéré et al. 2009). Although long-term warming experiments support an increase in soil respiration with warming (Rustad et al. 2001), these studies often show that after a few years of elevated temperature soil respiration declines back to ambient rates (Luo et al. 2001, Melillo et al. 2002). The magnitude and direction of this particular response can be largely driven by substrate limitation and/or the temperature sensitivities of soil microbial communities responsible for SOM decomposition (Strickland et al. 2009, Allison et al. 2010, Sheik et al. 2011, Zhou et al. 2012, Wieder et al. 2013). Yet, how these theories apply to subtropical and tropical forested ecosystems remains unknown.

Soils of subtropical and tropical ecosystems account for approximately 20% of the global land base (NRCS Soil Survey Staff 2014), but are biogeochemically distinct from higher-latitude soil types (Schlesinger and Bernhardt 2013). Kinetic theory predicts reaction rates are directly proportional to temperature up to a thermal optimum. At temperatures beyond thermal optimum, either microbial physiological constraints or substrate availability limit reaction rates (Davidson et al. 2006). Therefore, in colder climates decomposition is likely limited by temperature, whereas in warmer climates, it may be limited by microbial physiology and/or substrate availability (Davidson et al. 2006). Since warm-adapted soil microbes often have quite high thermal optima (Balser and Wixon 2009) and retain activity well beyond their native temperature range (Holland et al. 2000, Balser and Wixon 2009), microbial physiology may not pose a strong limitation on decomposition. However, many tropical and subtropical soils—especially in the southeastern US—are nutrient poor due to tillage-driven SOM loss (Trimble 1974, Hendrix et al. 1998) or the from extensive weathering-driven loss of rock-derived nutrients (e.g. phosphorus) (Crews et al. 1995). Together, these data suggest that in warmer climates, substrate availability will more likely constrain decomposition than microbial physiology or temperature.

Until the installation of the experimental system used in this study, all forest ecosystem warming experiments have been conducted on nutrient-rich glacial soils with relatively abundant organic matter, containing over 30% C in the upper organic horizon (Rustad et al. 2001). Therefore, the objective of this study was to determine how sustained warming affects the magnitude of CO_2 efflux, apparent temperature sensitivity of soil respiration, soil C stocks, and SOM chemistry in the highly-weathered, low C soils of a southeastern U.S. Piedmont forest. We hypothesized that warming would stimulate soil respiration and lead to a decrease in soil C stocks. As decomposition proceeds, microbes preferentially utilize o-alkyl constituents, leaving behind alkyl C (Baldock et al. 1997). Therefore, we hypothesized the SOM chemistry would shift towards a lower ratio of o-alkyl C compounds to alkyl C compounds.

Methods

Site Description

The warming experiment used in this study is located in the unglaciated, southeastern U.S. Piedmont physiographic province in Georgia (Whitehall Forest; 33°53'17.87"N, 83°21'40.92"W). The forest stand consists of naturally-recruited, unmanaged deciduous tree species (*Quercus alba, Q. rubra,* and *A. rubrum)* that originated in the first half of the 20th century following agricultural land abandonment. The soils are Typic Kanhapludults (Pacolet and Cecil series) which in the surface have low organic matter content, low fertility, medium to slow permeability, and a salt pH of ~4.5 (Appendix A). Mean annual temperatures throughout the experiment were 17.6°C (2010-2012), with January and July means of 6.6°C and 27.5°C (2010-2013), respectively. Mean annual precipitation from 2010-2012 was 98.5 cm, while in 2013 the annual precipitation was 132.7cm (NOAA 2013).

Experimental Design

The 18 experimental plots follow a randomized complete block design consisting of 9 plots each in cleared gap (trees removed) and forest understory habitats. Plots were blocked according to landscape position (upper, mid, and lower slope) and were warmed continuously to +3°C or +5°C above ambient temperature (Appendix A), which, along with habitat type, affects soil moisture (Appendix A). Resistance heating cables buried 10 cm deep into the soil and 20 cm apart controlled warming homogenously throughout the soil profile to 20 cm deep. The +3°C (n=6) warming treatment was initiated in October 2010 and a ramp to +5°C was implemented in February 2012 to three of the +3°C plots. The temperature-increase gradient was imposed to avoid effects of sudden high-temperature increases in this warm ecosystem. Soil temperature (n=3 per plot) and moisture (n=1 per plot) were continuously monitored using field sensors placed 10 cm deep and temperature was continuously regulated at +3°C and +5°C above ambient.

Soil Respiration and Soil Carbon Analysis

Soil respiration measurements were taken in the field between 10 am and 2 pm on an approximately monthly basis using a LiCOR 6400XT on top of permanently installed PVC collars buried 3 cm deep. Two PVC collars per treatment plot were installed 3 months prior to experimental warming treatments to minimize disturbance effects. After three years of warming (September 2013), three soil cores (down to 20 cm) per treatment plot were composited for soil C chemistry. Bulk density was also taken using a separate core (2cm diameter, 0-10 and 10-20 cm depth). Soils were sieved (<2mm) and analyzed for %C using a NA 1500 C/H/N Analyzer (Carlo Erba, Strumentazione, Milan). After one year of warming (December 2011), one soil core (top 5 cm) per treatment plot was taken and analyzed for Solid State ^{13}C-^{1}H Cross-Polarization Magic-Angle Spinning Nuclear Magnetic Resonance spectroscopy (CP/MAS) NMR to analyze carbon functional group chemistry. ^{13}C NMR data was recorded using a Bruker AVANCE500 spectrometer equipped with an 11.74T magnet at Georgia Technical University. Solid powdered soil samples were loaded in a 4 mm zirconia rotor with Kel-F end cap. NMR larmor frequencies for ^{13}C and ^{1}H were 125 and 500 MHz. The rotor was spun at 15 kHz to eliminate the spinning sidebands. A CP pulse sequence with ramped-amplitude mixing power and a two-pulsephase-modulated (TPPM) decoupling technique was used, with a contact time of 1 ms, a recycle delay of 1 s, and a spectrum width of 500 KHz. 100-300 Hz line broadening was used. Spin counting on the samples was used to compare amount of C observed against pure litter reference standards. Spectral intensities for each sample were determined by integrating signal intensities in seven chemical shift region regions: 0-45, 45-60, 60-95, 95-110, 110-145, 145-165, 165-215.

Statistics

The soil respiration measured at the two collars were averaged per treatment and used for statistical analyses. For both gap and forest habitats, there were 3 ambient plots, 3 plots regulated at +3°C above ambient, and 3 plots that were ramped from +3°C to +5°C ~1.5 years after the beginning of the experiment. Repeated-measures ANOVAs were performed on soil respiration data and split according to the time at which the +5°C ramp was initiated (Appendix A, Statistics and Data Analysis). The differences in soil respiration, within month, were assessed using an ANOVA ($\alpha=0.05$).

For repeated measures analyses and cumulative flux calculations (Appendix A) we analyzed the data in two time periods: Period 1 (before +5°C ramp was initiated) and Period 2 (after +5°C ramp). Three ambient plots (no heating treatment but included buried cables) served as the control. Ambient plots without buried cables (n=3) performed similarly to the cable controls suggesting the disturbance of cable installation did not affect our results (data not shown).

Results and Discussion

In the highly-weathered soils of this site, 3 years of warming did not cause a significant change in soil CO_2 efflux (Figures 2.1 and 2.2) or soil C stocks (Figure 2.3). While warming increased soil respiration during some months there was no overall significant effect of warming at either +3°C or +5°C on soil respiration in forest or gap habitat (Figures 2.1). Additionally, warming did not increase the overall cumulative flux in either forest or gap habitats (Figure 2.2).

When warming does matter

In late winter/early spring- when soil moisture is typically higher (Supplemental Information Figure 6) warming did increase soil respiration in some months (February and March 2012, March and May 2013; $p<0.05$) and the cumulative flux (Period 2 of the experiment) was also higher in the +5°C treatment in the forest but not the gap plots in the spring seasons (+5°C > +3°C and Ambient; $p=0.02$; Appendix A Table 6). Overall, temperature and moisture interact to affect soil respiration in both forest and gap habitats ($p < 0.01$). However, the response of soil respiration to both temperature and moisture vary depending upon the season. Generally, temperature and moisture alone positively affect soil respiration in the forest habitat ($p<0.05$), although the spring temperature is the only important variable ($p<0.0001$). In the gap, temperature is the most important variable controlling flux in the fall ($p<0.0001$), while temperature and moisture interact in spring and summer ($p<0.05$).

Why there is no observed impact of warming

In previous field studies (Oechel et al. 2000, Luo et al. 2001, Melillo et al. 2002), long-term exposure to warming resulted in decreased temperature sensitivity of soil respiration. The activation energy (Ea) or the respiratory quotient (Q_{10}) of soil respiration are mathematical interpretations of the temperature sensitivity of soil respiration (Figure 2.5, Appendix A Table 2.1, 2.2). A decrease in apparent temperature sensitivity (Q_{10} or Ea), of soil respiration could result from an environmental stress (e.g. drought) reducing root (Saleska et al. 1999) or microbial activity (Suseela et al. 2012), from substrate limitation (Bradford et al. 2008), and/or microbial thermal acclimation (Bradford et al.

2008, Bradford et al. 2010). In our study, we found both forest and gap habitats to have an overall trend of lower Ea and Q_{10} in warmed treatments despite non-significant statistical differences (Figure 2.5, Table 2.1 and 2.2). However, the temperature sensitivity of soil respiration increased during Period 2 of the experiment ($p=0.025$), which had over 30% more precipitation (and consequently a higher soil moisture) than Period 1. This observation suggests moisture may alter the magnitude of the temperature sensitivity.

Consistent with our observation of no CO_2 efflux effects from warming, we also detected no change in SOM alkyl:o-alkyl C ratios with warming (Figure 2.4). The individual C components in warmed and ambient plots, yielded no significant differences in the forest habitat. Alkyl C was higher in the gap heated plots ($p<0.05$), but did not significantly change the alkyl:o-alkyl decomposition ratio (Figure 2.4). The lack of change in SOM chemistry in the warmed plots (Figure 2.4), may also be explained by the absence of a warming effect on heterotrophic respiration.

Since warming did not stimulate CO_2 efflux (except for some spring months when moisture was not limiting) and did not cause changes in soil C stocks or C chemistry, it is possible that several mechanisms may be constraining decomposition in this ecosystem. Results here demonstrate that temperature and moisture influence soil respiration and that these effects can change per season. Drought conditions throughout the Southeastern U.S. Piedmont during the first 1.5 years of our experiment (NOAA 2013) may have constrained any increases in soil respiration. Others have shown that low soil moisture conditions can constrain increases in soil respiration in a New England old field ecosystem where warming only increased soil respiration during the spring and winter season; periods when moisture was not limiting (Suseela and Dukes 2013). In addition, increases in temperature are likely to be greater at higher latitudes where conditions are generally freezing for most of the year (Del Grosso et al. 2005) which has been observed in field experiments (Lloyd and Taylor 1994). Increases in temperature in lower latitudes may result in a much different response as many organisms already occur near thermal optimum temperatures.

Soil of the southeastern U.S. are characteristic of low nutrient availability due to decades of poor farming practices that led to SOM loss (Trimble 1974, Hendrix et al. 1998). Additionally highly-weathered soils have experienced extensive weathering which has led to the loss of rock-derived nutrients (e.g. phosphorus) (Crews et al. 1995). Therefore, the lack of warming response may also be a consequence of microbial limitation for both nutrients and substrate. These results demonstrate the complexity of soil respiration responses to warming and how this response may vary between two different habitats within one ecosystem.

Conclusion

In order to fully understand the response of biogeochemical cycles to warming we must close gaps in our basic understanding of how increases in temperature relate to C cycle feedbacks. This is the first ecosystem warming experiment conducted in a highly-weathered soil and to our knowledge the first to test the influence of soil warming in different habitats within a forested ecosystem. Most warming studies have observed a significant increase in CO_2 efflux with warming. However, our research suggests that warming-enhanced increases in soil respiration is not a universal response, and additional studies addressing highly-weathered soil biogeochemical responses are required.

Table 2.1: Modeled temperature sensitivities, Ea (kJ mol^{-1}) and Q_{10}, in the first ~1.5 years of warming before +5°C was initiated (November 2010-February 2012) in both Forest and Gap habitats.

		Period 1 (November 2010- February 2012)				
Habitat	**Treatment**	***Ea (kJ mol^{-1})**	**r^2**	***Q_{10}**	**r^2**	****N**
Forest	Ambient	55.7 ± 2.7	0.62	2.3 ± 0.1	0.62	33
	+3°C	54.8 ± 2.6	0.55	2.0 ±0.1	0.67	65
Gap	Ambient	**66.3 ± 4.5**	0.75	2.4 ± 0.1	0.71	33
	+3°C	**54.6 ± 11.1**	0.66	1.8 ± 0.2	0.59	65

*Ea and Q_{10} averaged between treatment plots [n=3 for Forest and Gap ambient plots; n=6 for +3°C plots] with associated standard error. Significant differences ($p \leq 0.05$) are in bold.

**N is the total number of observations used in the model; fit of all models was significant ($p<0.0001$)

Table 2.2: Modeled Ea (kJ mol^{-1}) and Q_{10} after the +5°C was initiated (March 2012-October 2013) in both Forest and Gap habitats.

		Period 2 (March 2012-October 2013)				
Habitat	**Treatment**	***Ea (kJ mol^{-1})**	**r^2**	***Q_{10}**	**r^2**	****N**
Forest	Ambient	78.3 ± 12.9	0.71	2.2 ± 0.2	0.72	60
	+3°C	71.0 ± 8.6	0.65	2.2 ± 0.2	0.69	59
	+5°C	55.6 ± 2.5	0.48	2.0 ± 0.1	0.46	60
Gap	Ambient	80.0 ± 12.4	0.63	2.7 ± 0.4	0.63	60
	+3°C	84.3 ± 6.8	0.66	3.0 ± 0.3	0.63	60
	+5°C	59.5 ± 6.2	0.52	2.2 ± 0.2	0.51	60

*Ea and Q_{10} averaged between treatment plots [n=3 for Forest and Gap ambient plots; n=6 for +3°C plots] with associated standard error.

**N is the total number of observations used in the model; fit of all models was significant ($p<0.0001$)

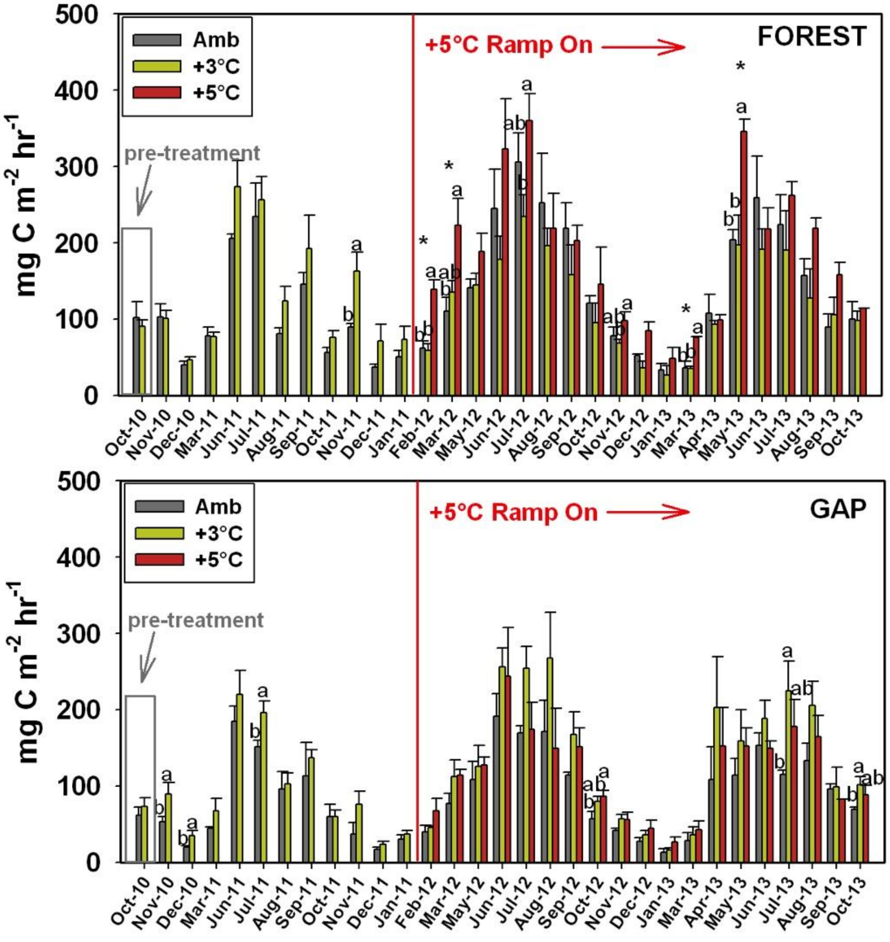

Figure 2.1: Forest (top panel) and gap (bottom panel) soil respiration (both heterotrophic and autotrophic) for the first three years of warming in warmed and ambient plots. *Denotes different letters are significant at p<0.05, all others correspond to different letters being significant of p< ~0.10.

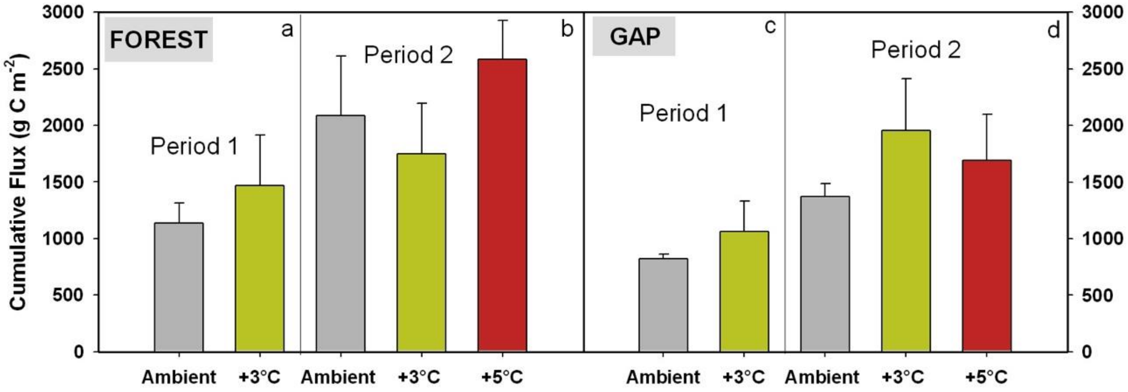

Figure 2.2: Cumulative flux values calculated for Period 1 over 16 months (November 2010- February 2012) and Period 2 over 18 months (March 2012- October 2013). Error bars represent standard deviation of the mean (Period 1 n=3 ambient, n=6 +3°C and Period 2 n=3 for each treatment).

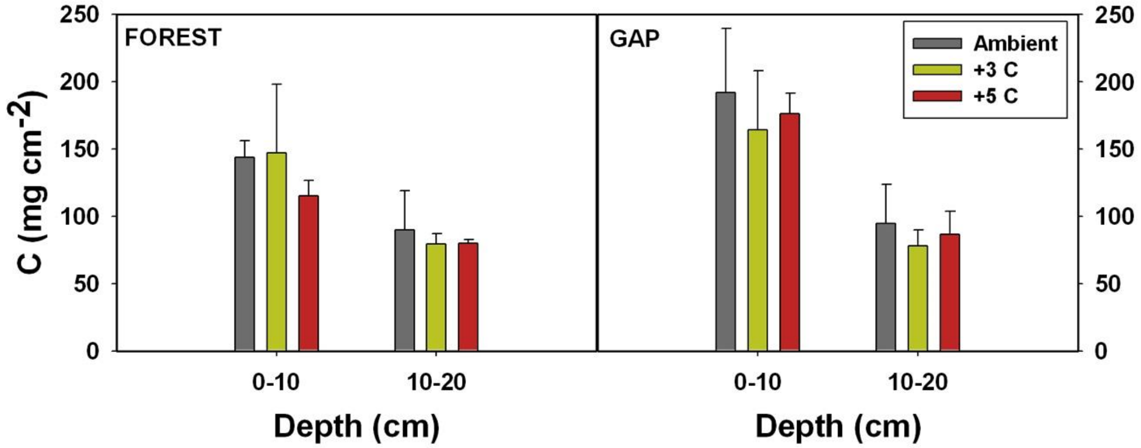

Figure 2.3: Soil C stocks in September 2013 (after ~3 years of warming treatment) in heated and ambient plots in both forest and gap habitat at depths of 0-10 and 10-20 cm. Three soil cores taken from each treatment plot and composited. C (mg cm^{-2}) calculated using bulk density measured in each treatment plot.

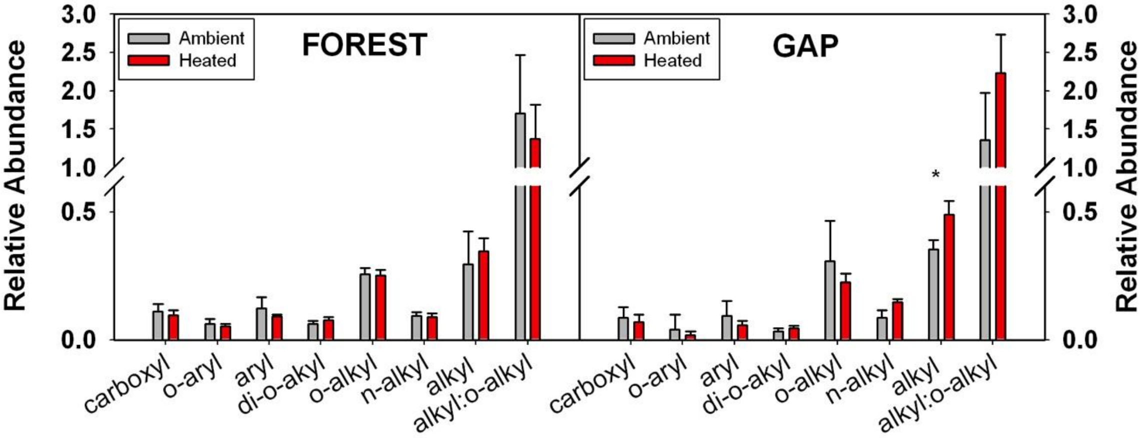

Figure 2.4: Soil C chemistry measured using solid state ^{13}C NMR in heated and ambient treatments from forest (left) and gap (right). * denotes statistical differences between treatments ($p<0.05$).

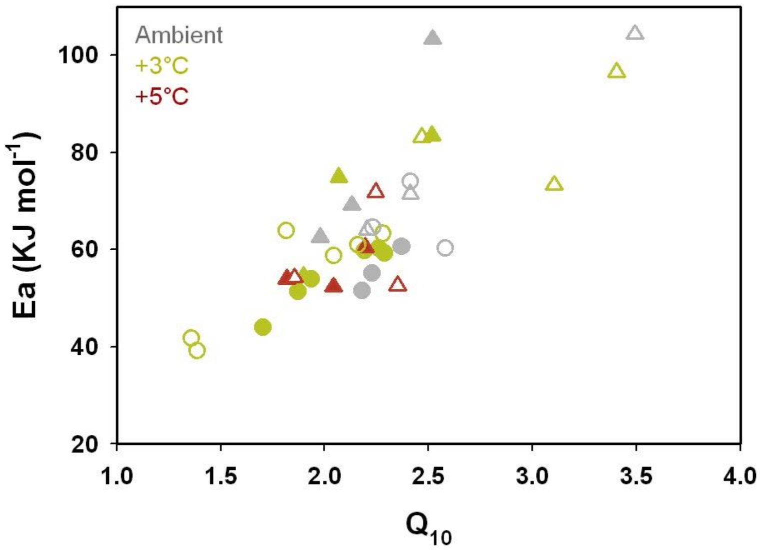

Figure 2.5: Two methods (Ea and Q_{10}) of modeled apparent temperature sensitivity of soil respiration. Different treatments are represented in different colors in both forest (filled symbols) and gap (closed symbols) habitat. Period 1 (November 2010- February 2012) represented by circles and Period 2 (March 2012- October 2013) in triangles.

CHAPTER 3

SOIL MOISTURE, TEMPERATURE, AND SEASON AFFECT HETEROTROPHIC SOIL RESPIRATION IN A SOUTHEASTERN U.S. FOREST FIELD WARMING EXPERIMENT[1]

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[1] Megan B. Machmuller, Nina Wurzburger, Carly A. Phillips, Jacqueline E. Mohan. To be submitted to *Biogeochemistry*.

Abstract

Microbial decomposition of organic matter contributes a large flux of carbon dioxide to the atmosphere that can act as a positive feedback to climate change. However, it is uncertain how diverse terrestrial ecosystems will respond to warming. We previously observed no consistent response of experimental warming on soil respiration in a temperate deciduous forest on highly weathered soils of the southeastern US. The objective of the current study was to elucidate whether and how low soil moisture conditions constrain warming-induced increases of soil respiration in these clay-rich Ultisols under field conditions. We performed fully factorial laboratory incubations to examine how soil moisture and temperature affect heterotrophic respiration and, in particular, how this response differs by season. Our results indicate the importance of season for heterotrophic respiration. We also show that the temperature sensitivity of heterotrophic respiration does not change with moisture treatments but changes by approximately 30% from winter to summer, demonstrating the importance of including season-specific temperature sensitivity parameters in ecosystem carbon flux models. We utilized laboratory moisture and temperature sensitivities of heterotrophic respiration to extrapolate to the field using *in situ* soil temperature and moisture data from a warming experiment in a Southeast U.S. forest. This modeling exercise demonstrated that by including moisture and temperature into models of heterotrophic respiration, we explain 50-70% of the variability associated with *in situ* soil respiration.

Introduction

Global climate change is expected to include both increases in land surface temperature (Mearns et al. 2003, Pachauri and Reisinger 2007) and altered precipitation regimes (Pachauri and Reisinger 2007). In response to these changes, uncertainty remains about the fate of soil carbon (C), which is the largest reservoir of terrestrial C (Jobbagy and Jackson 2000). Climatic warming is expected to accelerate the decomposition of soil organic matter (SOM) and the transfer of C to the atmosphere; however, the magnitude of this response may be constrained by reductions in soil moisture as a result of drought.

Critical questions remain about the modulating effect of moisture on the temperature response of SOM decomposition. Kinetic theory informs that warming should increase respiration at an exponential rate (Arrhenius 1889), however, the temperature effect can be dampened due to low soil moisture. Low soil moisture can suppress microbial activity and decrease soil respiration (Carlyle and Than 1988, Allison and Treseder 2008, Schindlbacher et al. 2012, Suseela et al. 2012), likely due to limitations on enzyme, substrate and nutrient diffusion (Koch 1990, Steinweg et al. 2012, Steinweg et al. 2013). Thus the temperature sensitivity of heterotrophic respiration generally declines with reductions in soil moisture (Craine and Gelderman 2011). However, current biogeochemical models often use a fixed temperature sensitivity parameter, Q_{10}, (Davidson et al. 2006) with a reducing function for soil moisture (Bauer et al. 2008). But the interactive effects of temperature and moisture on soil respiration are inconsistent across ecosystem types, hampering our ability to broadcast predictions of heterotrophic respiration and ecosystem C fluxes in response to climate change.

Uncertainty also exists about how global change factors overlay the strong seasonality of SOM decomposition. The interaction of temperature and moisture changes temporally in a given ecosystem (Reichstein et al. 2005) and seasonal changes in heterotrophic respiration and microbial activity are often of greater magnitude than are responses to warming in manipulative experiments (Henry 2012, Suseela et al. 2012, Weedon et al. 2013). The temperature sensitivity of extracellular enzyme activity can vary seasonally (Wallenstein et al. 2009) and during warmer seasons, low soil moisture more greatly limits *in situ* enzyme activity (Steinweg et al. 2012). These findings indicate that in order to improve predictions of climate-C feedbacks we require a mechanistic framework of the temperature sensitivity of SOM decomposition and how it is regulated by soil moisture across seasons.

From an *in-situ* warming experiment in a southeastern U.S. forest (Athens, GA), we observed no significant increase of soil respiration after three years of warming, but we observed modest responses to warming during wet months (Machmuller, in prep). Because the southeastern region experienced drought conditions for the first two years of our field experiment (NOAA, 2013), we speculate that low soil moisture constrained warming-induced responses of soil respiration.

The objective of this study was to determine how soil moisture and temperature interact to affect heterotrophic respiration, and how these responses varied seasonally. Specifically, our objectives were to quantify moisture and temperature sensitivities of heterotrophic respiration in the laboratory, and then use these relations to model respiration in the field using continuous soil temperature and moisture data from our *in situ* warming experiment. We hypothesized that: (1) temperature and moisture would interact to determine soil respiration such that temperature sensitivity would decline with decreasing soil moisture and that (2) low moisture would more greatly reduce the temperature sensitivity of soils sampled in May and September, when soil respiration is at its greatest, relative to February when respiration is lower. Lastly, we hypothesized that (3) a model including laboratory-derived temperature and moisture functions would demonstrate a constraint of soil moisture on the response of soil respiration to warming in our field experiment and that (4) moisture would have the greatest diminishing effect on a warming response in September relative to other months.

Methods

Study Site:

The soils were collected from Whitehall Forest in Athens, GA, located in the Southeastern U.S. Piedmont region (33°53'17.87"N, 83°21'40.92"W). The forest consists of naturally-recruited unmanaged stands dominated by mixed-deciduous trees (*Quercus alba, Q. rubra,* and *A. rubrum)* that originated in the first half of the 20th century following agricultural land abandonment. The soils are Typic Kanhapludults (Pacolet Series) with low organic matter content, low fertility, medium to slow permeability, and a pH of ~4.5. Monthly mean air temperatures of the sampling dates in February, May, and September 2013 were 7.3, 19.7, and 22.9°C and monthly mean precipitation was 16.2, 9.2, and 6.2 cm (NOAA 2013).

Soil Sampling and Experimental Design:

Mineral soils (0-5 cm) were sampled from 9 random locations in the forest during February, May, and September 2013 using a 10 cm diameter soil corer. Soils were immediately taken back to the lab and prepared for the experiment. Roots were removed by passing the soil through a 2 mm sieve. Soil moisture was determined gravimetrically by drying ~ 5 g of field moist soil at 60°C for 48 hrs.

We implemented a two-way factorial laboratory experiment (temperature by moisture), where temperature had 5 levels (5, 10, 20, 30, and 35°C) and soil moisture had 4 levels (5, 10, 20, and 30% gravimetric moisture content), for a total of 20 treatments, and 5 mesocosms per treatment. Each mesocosm (237 cm^3) contained 30 g of field moist soil, which was further adjusted with DI water or by drying. Temperature treatments were maintained in laboratory incubators. Soils acclimated to moisture and temperature conditions for ~14 days, during which the moisture level of each mesocosm was maintained by adding DI water. The mesocosms were placed in incubators and covered with perforated parafilm to allow adequate ventilation but to prevent excessive drying.

CO_2 Efflux

After the acclimation period, mesocosms were flushed with N_2 and capped with a gas tight lid that had been fitted with a septum for headspace sampling. Over a period of 24 hours, 4 headspace samples were taken by removing 3 ml of gas and transferring it into a gas tight vial that had been previously flushed with nitrogen. Gas samples were analyzed for CO_2 on a LiCor 6252 (LiCor Biosciences, Lincoln, NE) within a week. CO_2

standard gases were utilized as reference to calculate specific CO_2 concentrations in headspace and rates of heterotrophic respiration were calculated as μg C g dry soil^{-1} day.

Microbial Biomass

Sieved field moist soils that were composited were used to measure microbial biomass using a modified chloroform fumigation method (Fierer and Schimel 2003). At each sampling, 5 replicate soil samples (~ 5 g) were fumigated with and without ethanol-free cholorform ($CHCl_3$). Unfumigated and fumigated samples were transferred to a 75 ml glass tube and 40 ml of 0.5 M potassium sulfate (K_2SO_4) was added to each sample. For fumigated samples, 0.5 ml $CHCl_3$ was added to the soil sample and K_2SO_4 slurry. All samples were shaken at 150 rpm for ~3 hours and then gravity filtered using Whatman No. 1 paper (Whatman, Maidstone, UK). The samples were then vigorously bubbled with air for 30 minutes to remove $CHCl_3$. Extracts were stored at -20°C until analysis for DOC analysis on a Shimadzu TOC 5000A (Tokyo, Japan). Microbial biomass samples were calculated by taking the difference between the fumigated and unfumigated samples and expressed as μg DOC g dry soil^{-1}.

Modeling and Statistical Procedures:

Moisture and Treatment Effects

To assess the effects of temperature and moisture on heterotrophic respiration, we used ANOVA with CO_2 efflux (μg C g dry soil^{-1} day^{-1}) as the dependent response variable and temperature and moisture treatments (and moisture by temperature interaction) as the independent variables. If there was a significant ($p < 0.05$) or marginally significant ($p <$

0.10) interaction, moisture effects were assessed per temperature treatment and vice versa. To compare among months an ANOVA was performed with CO_2 efflux (per month) as the dependent variable and treatment (both moisture and temperature) as independent variables. For ANOVA statistics, data were assessed for normality and if normal distribution assumption was met, an ANOVA with a Tukey's post hoc test was performed. If normal distribution was not met and log transformation of data still resulted in non-normal distribution, then a non-parametric Wilcoxon test with multiple comparisons (Wilcoxon each pair) was utilized (JMP 10.0, SAS Institute, Cary, North Carolina).

Temperature Sensitivity

The temperature sensitivity of heterotrophic respiration was assessed using the data from the fully factorial laboratory experiment. We calculated the temperature sensitivity (or activation energy, *Ea*) per moisture treatment using the Arrhenius model:

(1) $R_h = ae^{(-Ea/RT)}$

Where R_h is the measured heterotrophic CO_2 efflux (µg C g dry soil^{-1} day^{-1}), T is the fixed temperature treatment in degrees Kelvin, a is the basal respiration (y-intercept when temperature is 0°C), R is the ideal gas constant. ANCOVA (JMP 10.0, SAS Institute, Cary, North Carolina, USA) was used to assess the effects of the moisture treatment (fixed categorical variable) and temperature (continuous independent variable) on the dependent variable heterotrophic respiration (µg C g dry soil^{-1} day^{-1}). Significant differences were determined using $p < 0.05$ and marginally significant results refer to p values < 0.10, unless otherwise specified.

To assess moisture sensitivity, we used separate linear regressions for each temperature treatment per sampling time to test differences in the slopes (moisture sensitivity), based on the following equation from (Steinweg et al. 2012):

(2) $R_h = m_{sm} x_{sm} + b_{sm}$

Where R_h is the estimated *in situ* heterotrophic respiration based on moisture effects found in the laboratory experiment, m_{sm} is the slope of the linear regression and b_{sm} is the intercept, and x_{sm} is the hourly *in situ* soil moisture measurements. Flux (µg C g soil^{-1} day^{-1}) was the dependent variable and the soil moisture content was used as a continuous independent variable while temperature treatment was used as a fixed independent variable. A significant interaction between temperature and soil moisture indicated that the slopes (e.g. moisture sensitivity) of the linear models were significantly different and multiple comparisons were then performed in order to assess the ordered differences.

Modeling In Situ Heterotrophic Respiration

Environmental Data

For model extrapolation procedures, we utilized hourly *in situ* soil temperature and moisture measurements (2 weeks prior and 2 weeks post soil sampling) from an ecosystem warming experiment at Whitehall Forest, GA, located in the same area where soils were collected for lab incubations. The warming experiment consisted of plots that were continuously warmed at +3°C and +5°C above ambient soil temperatures using resistance heating cables. Ambient plots were not warmed but contained buried cables. We compared temperature and moisture effects on *in situ* heterotrophic respiration based on a temperature only model, a moisture only model, and an integrated temperature and moisture model. Model validation was performed using *in situ* soil respiration measurements (Li-Cor 6400XT, Lincoln, NE) from warmed and ambient plots during the same time frame soil was collected for laboratory incubations.

Model Specifics

We parameterized the models using the laboratory incubation data and the temperature sensitivity and moisture sensitivities were assessed using Equation (1) and (2), respectively. Temperature sensitivity did not vary among moisture treatments, but did vary by sampling date; therefore different parameters were used for each model. Within all sampling months, the moisture sensitivity of heterotrophic respiration followed a significant linear relationship, and all laboratory data were used to parameterize the moisture model. To estimate the effects of both soil temperature and moisture on heterotrophic respiration, we combined equation (1) and (2) as follows:

(3) $R_h = ae^{(-Ea/RT)} * (m_{sm}\, x_{sm} + b_{sm})$

Since the majority of C turnover occurs in near-surface soils (Trumbore et al. 1996), field respiration rates were converted to $\mu g\ g^{-1}\ h^{-1}$ assuming the majority of respiration to occur in the top 5 cm. We also assumed mid-day respiration measurements to be representative of the entire day as shown to be reasonable by Bremer et al. (1998) who demonstrated that after a diel correction was made on daily CO_2 efflux measurements, the adjusted flux was ~96% of the daily maximum.

In addition, for model validation we performed linear regressions (JMP 10.0, SAS Institute, Cary, North Carolina) to compare *in situ* respiration measurements to laboratory incubations. We used an ANOVA to determine if average estimated heterotrophic respiration from each sampling period differed depending on the model used (e.g. temperature, moisture, temperature by moisture) and then examined treatment differences within each model.

Results

Microbial Biomass

Microbial biomass C was greatest in February soils (164.87 ± 3.55 µg DOC g dry soil^{-1}) compared to September (50.25 ± 24.23 µg DOC g dry soil^{-1}) (ANOVA, p=0.032), while May (127.81 ± 40.99 µg DOC g dry soil^{-1}) was not significantly different from either February or September.

In Situ Warming Treatments

Average *in situ* soil temperature was significantly different between treatment plots and soil temperatures in the +3°C and +5°C treatments, and were on average 2.2 and 4.1°C higher than ambient plots, respectively (Table 3.1). Although not statistically different, soil moisture was on average 17-20% lower in the +3°C and 1-13% lower in the +5°C treatments (Table 3.1).

Temperature and moisture effects on laboratory heterotrophic respiration

Temperature and moisture interacted to influence heterotrophic respiration at each sampling date (2-way ANOVA temperature by moisture interaction: February $F_{1,76}$ = 2.74, $p = 0.10$, May $F_{1,96} = 23.27$, $p < 0.0001$, September $F_{1,78} = 5.22$, $p = 0.03$) (Figure 3.1). An Arrhenius fit was used to calculate the temperature sensitivities and all model fits were statistically significant (February $F_{1,78} = 116.12$, $p < 0.001$, May $F_{1,98} = 424.36$, September $F_{1,80} = 404.24$, $p < 0.0001$). Temperature sensitivity did not differ with soil moisture (February $F_{3,72} = 0.12$, $p = 0.95$, May $F_{3,92} = 0.08$, $p = 0.97$, September $F_{3,74} = 0.47$ $p = 0.71$) (Figure 3.2). There was, however, a seasonal pattern in temperature sensitivity ($F_{2,9} = 42.18$, $p < 0.0001$), where respiration in September and May had greater temperature sensitivities than did February ($p < 0.001$) (Figure 3.2).

Extrapolation of Temperature and Moisture Models to Field Plot Measurements

The laboratory incubations resulted in a 3 – 18 fold higher rate of respiration compared to that measured in the field. Models generated from laboratory incubations explained 2 – 67% of the variation in field respiration, where the least predictive model only had a moisture function and the most predictive model included both a temperature and moisture function (Table 3.2).

Our modeling approach demonstrated the constraint of soil moisture on soil respiration in our field warming experiment. The model using only temperature sensitivity predicted that both warming treatments resulted in higher respiration than ambient plots (February $F_{2,6} = 8.10$, $p = 0.02$, May $F_{2,6} = 135.41$, $p < 0.0001$, September $F_{2,6} = 176.03$, $p < 0.0001$), where +5°C > +3°C > Ambient (Tukey post hoc: February $p < 0.05$, May $p < 0.001$, September $p < 0.001$). In contrast, the model with both temperature and moisture sensitivity functions predicted higher estimated heterotrophic respiration in only the +5°C treatment compared to ambient in May and September, (May $F_{2,6} = 4.94$, $p = 0.05$, September $F_{2,6} = 5.59$, $p = 0.04$) and higher than +3°C in February ($F_{2,6} = 4.21$, $p = 0.07$ (Figure 3.3, Table 3.1).

Models of soil respiration also demonstrated seasonal patterns in soil respiration with moisture limiting efflux more in September compared to other months (Figure 3.3, Table 3.1). In February, May, and September the model using a temperature function alone and the model using a combined temperature and moisture function resulted in the same average heterotrophic respiration in all treatments ($p < 0.05$). On average, September had 54% lower *in situ* soil moisture than February and 29% lower than May. Despite this, models including both temperature and moisture parameters estimated the greatest soil respiration in September ($F_{2,24} = 32.42$, $p < 0.0001$).

Discussion

Predicting how climate change will alter heterotrophic soil respiration, and ultimately soil C storage, is of global importance. Based on kinetic theory (Arrhenius 1889), microbial decomposition of organic matter should increase with temperature. Under these circumstances, increases in global temperature should lead to greater heterotrophic respiration and result in a positive feedback to climate change. However, respiration responses are often confounded by several environmental variables (e.g. soil moisture) that can co-vary with mean temperature and soil type (Davidson et al. 2006). Uncertainty also exists about how global change factors overlay the strong seasonality of SOM decomposition. We used a controlled laboratory experiment to determine how temperature and moisture interact to control heterotrophic respiration and how this response varied seasonally. We also demonstrated the utility of using temperature and moisture sensitivity to model *in situ* heterotrophic respiration.

Supporting our first hypothesis, we observed that temperature and moisture interact to affect soil respiration in all sampling dates. However, the nature of this interaction was contrary to our expectation, in which the temperature sensitivity was not significantly different between moisture treatments. Previous studies in different ecosystems have demonstrated that soil moisture can constrain increases in microbial activity or soil respiration (Liu et al. 2009, Suseela et al. 2012). However, this is not always a consistent result. For example, others have shown temperature sensitivity of SOC decomposition to be insensitive to decreased moisture (Reichstein et al. 2005) and the nature of the temperature and moisture interaction to vary spatially among soils within a single ecosystem (Craine and Gelderman 2011).

Seasonal changes in heterotrophic respiration and microbial activity are often of greater magnitude than are responses to warming in manipulative experiments (Henry 2012, Suseela et al. 2012, Weedon et al. 2013). In our laboratory experiment, we found no evidence for differential moisture effects between seasons, contrary to our second hypothesis. Instead, we found the magnitude of temperature sensitivity differences were greater between seasons than moisture treatments within season. In February, respiration had a ~ 30% lower temperature sensitivity relative to September and May. In February, we observed greater microbial biomass in soils relative to those collected in September. This finding suggests that in colder months, more C is allocated towards microbial growth than respiration within the microbial community, which may consequently decrease the temperature sensitivity of respiration. Seasonal changes may also manifest as altered extracellular enzyme production (Wallenstein et al. 2011) and reduced substrate and nutrient availabilities (Wallenstein et al. 2009), which may contribute to the temperature sensitivities observed in this study. Microbial-enzyme explicit models have demonstrated that a decline in microbial biomass and/or available substrate may lead to an attenuation of soil respiration under warmer temperatures (Allison et al. 2010). In our study, microbial biomass and temperature sensitivities were inversely related; suggesting substrate and nutrient availabilities which change seasonally may be more important predictors of respiration in this ecosystem.

Supporting our third hypothesis, we found that using combined temperature and moisture functions in our model, treatment differences disappear. Additionally, we improve our ability to predict *in situ* heterotrophic respiration (except in September when *in situ* soil moisture was lowest). The models with a temperature function alone estimated that warming should increase heterotrophic respiration (+5°C > +3°C >Ambient) in all months. However, when a moisture function was included in the model, only the +5°C treatment in May and September exhibited greater rates of heterotrophic respiration relative to ambient plots. This finding supports the idea that the lack of a consistent warming effect on *in situ* soil respiration (Machmuller In preparation) can be driven by low soil moisture in May and September.

Our fourth hypothesis stated that moisture would have the greatest diminishing effect on a warming response in September relative to other months. When a moisture parameter is included in the model in addition to the temperature parameter, there are no differences in the mean heterotrophic respiration rates between models. This suggests no decline in heterotrophic respiration when moisture is added to the models in the driest month, September, contrary to our hypothesis. These results infer different seasonal dynamics, suggesting that by including season-specific parameters into models we may provide more accurate predictions than including constant yearly parameters (Yuste et al. 2003, Suseela et al. 2012, Suseela and Dukes 2013).

In addition to higher annual and growing season temperatures, the southeastern U.S. may also experience reduction in growing season precipitation in the future (Mearns et al. 2003). In a previous study in the same forest system, we found three years of experimental warming did not result in a significant increase of soil respiration (Machmuller, in prep). Due to this region experiencing low precipitation and drought conditions for the first few years of the field experiment (NOAA, 2013), we hypothesized that low soil moisture may have constrained warming-induced increases in soil respiration. Here, we demonstrate the effects of temperature and moisture controls on heterotrophic respiration in a Southeastern U.S. forest in the context of season. We have demonstrated that shifts in the temperature sensitivity of respiration among seasons can only partly be explained by soil moisture. By including moisture parameters into our model, treatment differences diminished offering evidence that moisture exerts some control on heterotrophic respiration during part of the year. Although we explained 50-67% of the variation observed in the field, there are more mechanisms left to identify. This study refines our understanding of microbial responses to temperature and moisture, and leads to new questions for why elevated temperature may not result in elevated CO_2 efflux in the field.

Table 3.1: *In situ* soil temperature and moisture measurements from warming experiment plots averaged over 2 weeks prior and 2 weeks post soil sampling using hourly logged data. Standard error represents variation among treatment plot averages (n=3). Different letters represent significant differences between treatments per month ($p<0.05$).

Month	**Treatment**	**Soil Temperature (°C)**	**Soil Moisture (%)**
February	Ambient	^{c} 9.74 ± 0.22	^{a} 22.27 ± 2.93
	+3°C	^{b} 11.69 ± 0.06	^{a} 17.76 ± 3.59
	+5°C	^{a} 13.68 ± 0.03	^{a} 22.45 ± 2.93
May	Ambient	^{c} 18.01 ± 0.32	^{a} 17.28 ± 2.11
	+3°C	^{b} 20.32 ± 0.04	^{a} 14.26 ± 1.56
	+5°C	^{a} 22.11 ± 0.02	^{a} 16.90 ± 1.71
September	Ambient	^{c} 23.03 ± 0.30	^{a} 15.15 ± 1.75
	+3°C	^{b} 25.39 ± 0.01	^{a} 12.29 ± 1.75
	+5°C	^{a} 27. 24 ± 0.02	^{a} 13.25 ± 0.96

Table 3.2: Average estimated *in situ* R_h (µg C g dry soil^{-1} day^{-1}) using temperature, moisture, and combined temperature and moisture models. Different non-capitalized letters identify significant differences between models per treatment. The capitalized letters indicate significant differences between treatments within the same model. Error indicates standard deviation among plots (N=3). Models were validated using field measurements and bolded R^2 signifies a significant relationship between measured flux and modeled flux using models from lab incubation.

		---------------------------Model and Model Validation---------------------------					
Month	Treatment	Temperature	R^2	Moisture	R^2	T x M	R^2
Feb	Ambient	^{b,B}11.10 ± 0.39	**0.50**	^{a,A}17.87 ± 0.95	0.25	^{b,AB}12.21 ± 0.74	**0.56**
	+3°C	^{b,A}11.81 ± 0.001		^{a,A}16.83 ± 0.84		^{b,B,}12.17 ± 0.64	
	+5°C	^{b,A}12.52 ± 0.50		^{a,A}17.91 ± 1.17		^{b,A}13.73 ± 1.10	
May	Ambient	^{b,C}13.53 ± 1.25	**0.64**	^{a,A}18.95 ± 2.31	0.05	^{b,B}14.02 ± 1.40	**0.67**
	+3°C	^{b,B}15.14 ± 1.29		^{a,A}17.55 ± 1.80		^{b,AB}14.61 ± 1.07	
	+5°C	^{b,A}16.51 ± 1.43		^{a,A}18.77 ± 2.25		^{b,A}16.83 ± 1.29	
Sept	Ambient	^{a,C}17.29 ± 0.74	**0.64**	^{a,A}18.03 ± 2.28	0.02	^{a,B}17.04 ± 1.91	**0.54**
	+3°C	^{a,B}19.33 ± 0.78		^{a,A}16.62 ± 1.93		^{a,AB}17.90 ± 1.71	
	+5°C	^{a,A}21.09 ± 0.98		^{b,A}17.64 ± 1.99		^{a,A}20.34 ± 1.95	

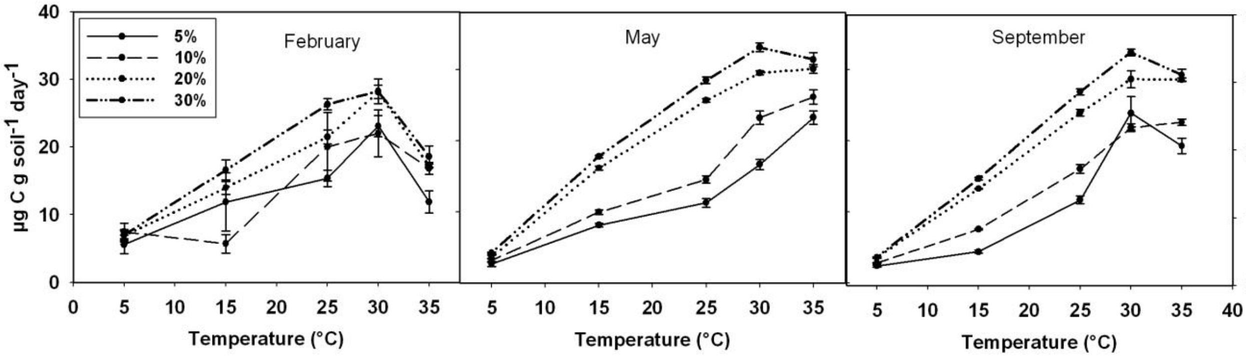

Figure 3.1: Heterotrophic soil respiration (µg C g soil^{-1} day^{-1}) in response to varying soil moisture and temperature treatments with soils taken during February, May and September.

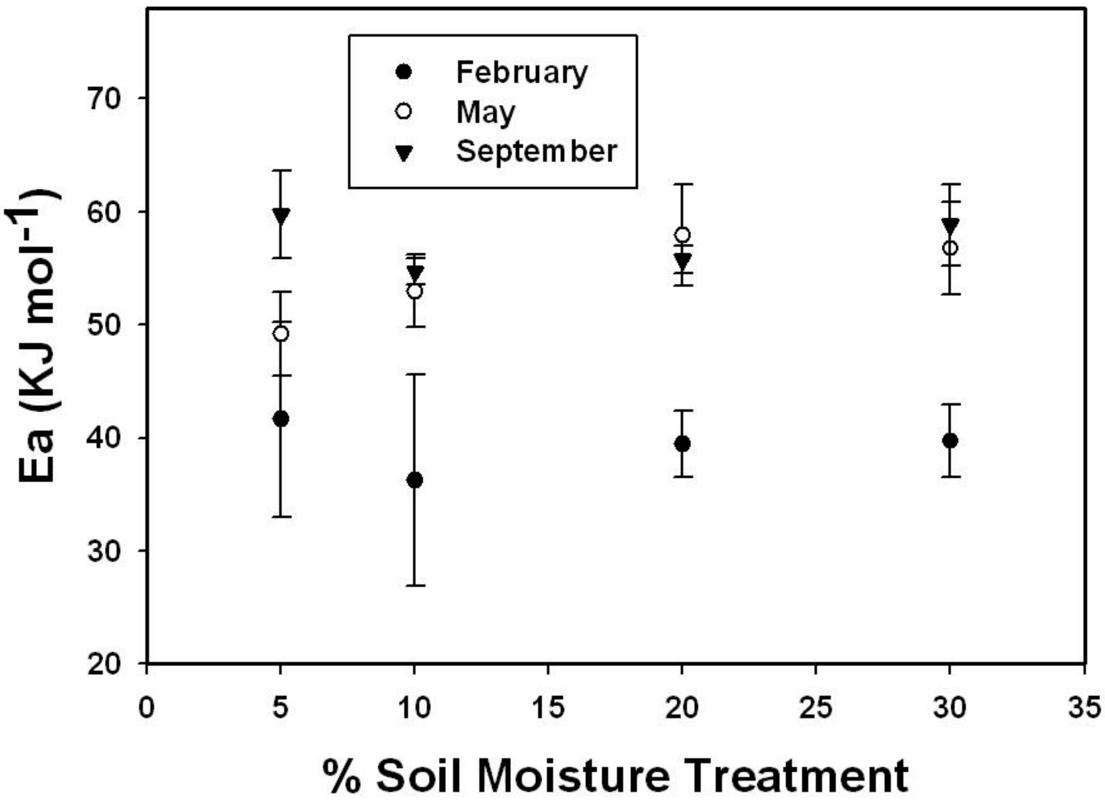

Figure 3.2: Temperature sensitivity (Ea) by moisture treatments of 0-5 cm soils collected during February, May and September.

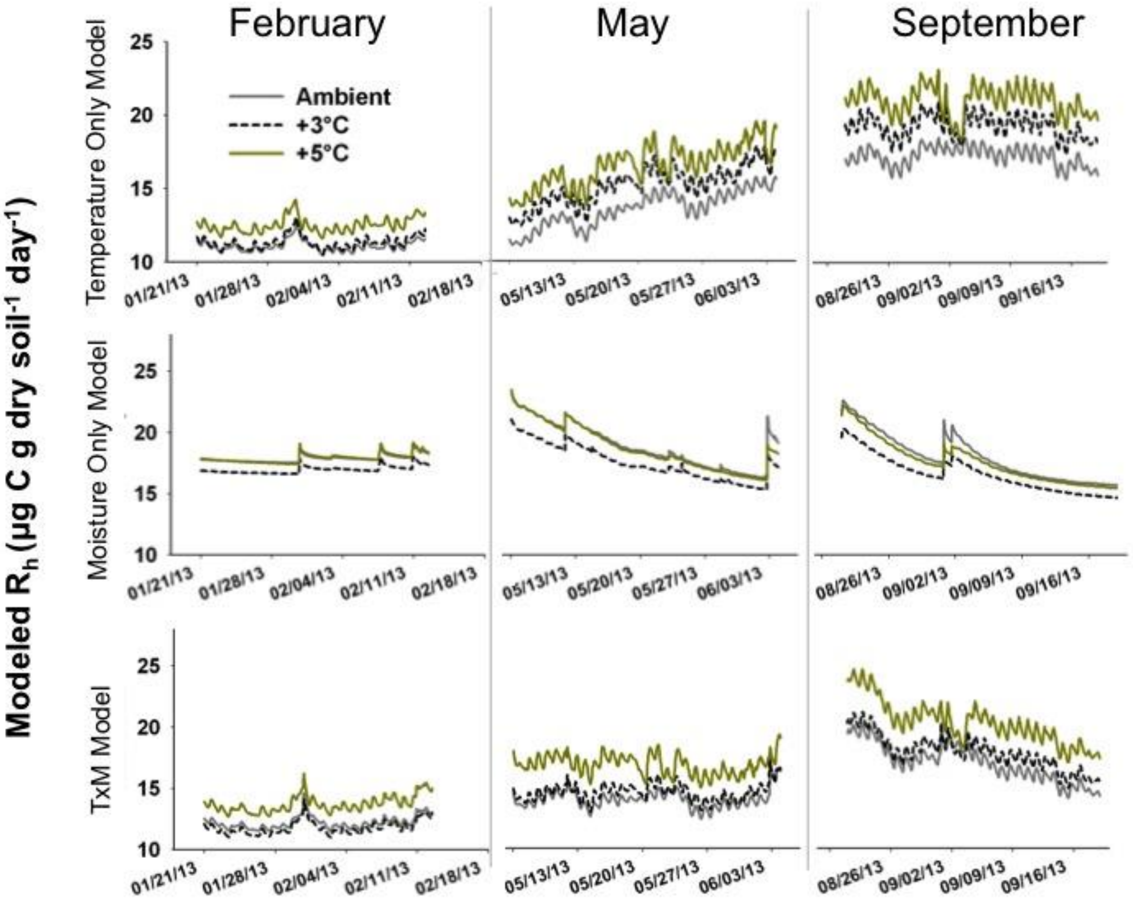

Figure 3.3: Modeled field plot R_h using laboratory temperature sensitivity (top panel) moisture sensitivity (middle panel), and temperature and moisture sensitivity combined (bottom panel).

CHAPTER 4

SEASON INFLUENCES THE ACTIVITY AND TEMPERATURE SENSITIVITY OF EXTRACELLULAR ENZYMES MORE SO THAN WARMING [1]

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[1] Megan B. Machmuller, Jacqueline E. Mohan, Jeffrey Minucci, Carly A. Phillips, Nina Wurzburger. To be submitted to *Global Change Biology*.

Abstract

Climate change may have direct and indirect effects on soil microbial communities and their ability to decompose organic matter. Microbes produce extracellular enzymes to facilitate the release of carbon and nutrients stored in soil organic matter (SOM). Therefore, measurements of extracellular enzyme activity and temperature sensitivities may provide insight into climate-driven changes of SOM decomposition. We measured the response of six hydrolytic enzymes involved in carbon (C), nitrogen (N), and phosphorus (P) degradation to experimental warming in a mixed-deciduous forest in two habitats (forest and a cleared gap) consisting of highly-weathered Ultisol soils (Whitehall Forest, Georgia, US). We found that warming at +3°C above ambient decreased the enzyme activities of only one carbon-degrading enzyme. The microbial response to soil warming was minor relative to seasonal variation in microbial activity. We observed significant seasonal variation in enzyme activities, temperature sensitivities and microbial nutrient acquisition ratios, but the response was inconsistent across enzyme type. Additionally, we found low C:P enzyme ratios suggesting P limitation in this ecosystem. Seasonal dynamics that alter both organic matter substrate supply and microbial nutrient and energy demand may have a larger control on enzyme dynamics than warming alone. Our study suggests that a major effect of climate change on soil organic matter decomposition may be the result of changes in the seasonal patterns rather than directly altering the kinetics of enzymes in the soil through warming alone.

Introduction

Soil microbial communities control the rate of carbon (C) and nutrient cycling in terrestrial ecosystems (Schlesinger 2004). Because warmer temperatures affect microbial physiology, elucidating the microbial response to changing climates is essential for understanding the subsequent effects on soil nutrient and C cycling. Yet, a major uncertainty in coupled C-climate models is to what extent microbial decomposition of soil organic matter (SOM) will respond to elevated temperatures and feed back to global climate (Moorhead and Sinsabaugh 2006, Allison et al. 2010, Todd-Brown et al. 2012).

Extracellular enzymes control the rate of SOM solubilization, the rate limiting step of decomposition (Schimel and Bennett 2004, Bengtson and Bengtsson 2007). Microbes produce these enzymes to hydrolyze C for energy and nutrient acquisition (Nannipieri et al. 2002, Sinsabaugh et al. 2008). A warmer climate could cause shifts in enzyme production and have major consequences for biogeochemical cycling and ecosystem function (Henry 2012). Therefore, the incorporation of enzyme activity into soil C decomposition models may provide a more accurate prediction of how warming affects C-feedback to the atmosphere (Allison and Martiny 2008, Allison et al. 2010, Todd-Brown et al. 2012).

We have limited empirical evidence for how soil microbes respond to warming and how these changes will manifest at the ecosystem scale (Henry 2012). Temperature can directly influence the kinetics of metabolic processes (Davidson et al. 2006). Over longer time scales, alterations in temperature can cause shifts in the microbial community structure, physiology and enzyme production (Zogg et al. 1997). Experimental warming in a northeastern US temperate forest decreased the temperature sensitivity of microbial soil respiration (Bradford et al. 2008), leading to a diminished response of respiration at the ecosystem scale (Melillo et al. 2002). However, other studies have found no evidence of microbial thermal adaptation in response to experimental warming (Hartley et al. 2008, Rinnan et al. 2009), leading to questions of how to predict microbial responses across diverse terrestrial ecosystems.

The temperature sensitivity of extracellular enzymes provides insight into how temperature controls the rate of SOM decomposition (Wallenstein et al. 2011), and may provide useful information in experimental manipulations of climate (Steinweg et al. 2012, Weedon et al. 2013). At higher temperatures, microbial communities can thermally adapt by synthesizing enzymes with a reduced maximum reaction rate (V_{max}) and an increased half-saturation constant (K_m) (Allison et al. 2010). In this case, heterotrophic respiration would decrease and potentially lead to no overall change in SOM decomposition with warming.

Recent decomposition modeling efforts have attempted to incorporate microbial physiology and the extracellular enzyme production (Moorhead and Sinsabaugh 2006, Allison et al. 2010, Todd-Brown et al. 2012). Often these microbial models represent all enzymes as following similar dynamics in response to environmental conditions. However, it is possible that C, N, and P enzyme activities respond differently to climate change (Weedon et al. 2013). For example, it has been shown that nitrogen-cycle enzymes are less sensitive to temperature than carbon-cycle enzymes (Wallenstein et al. 2009, Stone et al. 2012). This could lead to N-limitation under warmer climate scenarios, increasing the microbial synthesis of N-degrading enzymes and decreasing the synthesis of C-degrading enzymes. The response of microbial enzyme production is a product of ecological trade-offs between resource availability and demand, and the physiological constraints of the microbial community (Sinsabaugh et al. 2008, Allison et al. 2011, Billings and Ballantyne 2013). To maintain microbial stoichiometry (Cleveland and Liptzin 2007), microbes produce enzymes targeting specific C, N, or P compounds (Sinsabaugh et al. 2008, Sinsabaugh et al. 2009). Microbes should produce enzymes only when it leads to greater resource acquisition (Koch 1985) and when the appropriate substrate is present (Schimel and Weintraub 2003, Allison and Vitousek 2005, German et al. 2011). Substrate availability changes on a seasonal basis, and thus can exert influence on enzyme production and synthesis (Wallenstein et al. 2009).Thus, by examining the enzyme ratios, we better understand the coupling between cycles in regards to microbial nutrient acquisition under warmer climates across seasons.

The goal of this study was to determine how three years of *in situ* warming influenced enzyme activities and kinetics (temperature sensitivity of V_{max}) and how these responses varied by season and forest location (forest understory vs. cleared gap). We measured potential enzyme activities of six different hydrolytic enzymes involved in C, N, and P degradation (Table 1) in warmed and control treatment plots located in two habitats: forest and gap (cleared of trees). In addition, we measured the temperature sensitivity of four (of these six) enzymes. Our overarching expectation was that warming would reduce potential enzyme activities and the temperature sensitivities (of potential enzyme activities). However, we expected this response to be affected by season and forest location. First, we hypothesized that the warming would reduce potential enzyme activities and temperature sensitivities more strongly in the forest vs. gap habitat. Second, we hypothesized that potential enzyme activities and their temperature sensitivities would decline more strongly in response to experimental warming in the winter and fall relative to spring and summer. Third, we hypothesized that both C:N and C:P enzyme ratios would be greatest in May relative to other months, due to the increase in P and N enzyme activities relative to C enzyme activities.

Methods

Study Site

The soils were collected from Whitehall Forest warming experiment, located in the piedmont region of Georgia (33°53'17.87" N, 83°21'40.92" W). The forest consists of naturally-recruited forest stands dominated by mixed-deciduous trees (*Quercus alba, Q. rubra,* and *A. rubrum)* that originated in the first half of the 20th century following agricultural and land abandonment. The soils are Typic Kanhapludults with low organic matter content, low fertility, medium to slow permeability, and a pH of ~4.5. Monthly mean temperatures of the sampling times were 16.7, 7.3, 19.7, 29.6 °C and average monthly mean precipitation was 4.9, 16.2, 9.2, 14.4 cm in October 2012, February 2013, May 2013, and August 2013 respectively (NOAA 2013).

The soil warming experiment was initiated in October of 2010 and consisted of 9 plots in three blocks (i.e., 3 blocks of 3 treatments) in forested habitat and in a cleared gap. Treatment plots were warmed at +3 °C, +5 °C above ambient temperatures with resistance heating cables and control plots were not warmed but contained buried cables (n = 3 per treatment). The +5 °C experienced a ramp from +3 °C to +5 °C after 1.5 years in order to prevent a sudden increase in high temperatures. In each treatment plot, soil moisture and temperature were measured hourly at 10 cm deep with a TDR moisture sensor (CS616 Water Content Reflectometer, Campbell Scientific) and temperature probe (108 soil thermistor, Campbell Scientific) (Table 4.2).

Soil Sampling

Soils were sampled in October 2012, and February, May, and August 2013 (year three of the warming treatment) for extracellular enzyme extraction. During each sampling event, 3 soil cores (0-5 cm deep) were sampled with a 2 cm diameter corer and composited by treatment plot. Soils were immediately taken back to the lab and sieved (<2 mm), picked free of larger roots and stored at 4 °C until analysis.

Extracellular Enzyme Assays

Hydrolytic enzyme assays were performed for three enzymes involved in C acquisition (breakdown of carbohydrates and polysaccharides): β-glucosidase (BG), β-xylosidase (XYL), β-D-cellobiosidase (CB); two enzymes involved in the mineralization of N from chitin and proteins: β-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP); and one enzyme involved in the release of inorganic P: acid phosphatase (AP) (Table 4.1). In February, May, and August 2013 the temperature sensitivity of potential enzyme activity was determined for BG, XYL, CB, and NAG.

Extracellular enzyme activity (EEA) was measured using a modified method involving flourometrically-labeled substrate (Saiya-Cork et al. 2002) and the standard substrates, 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUB), were used to calculate standard curves. Soil pH was determined from field soils (1:5 ratio of soil:DI water) and buffer solutions were subsequently adjusted to pH 5.0 (with glacial acetic acid). Enzymes were extracted from ~2 g of field moist soil with 125 mL of 50 mM sodium acetate buffer mixed, blended for 1 minute, then continuously mixed on a stir plate while 200 µL aliquots were transferred to two 96 well plates (one for assay and one for standard curve). We utilized four wells as method replicates on the assay plate and 8 wells for standard curve calculation on the standard curve plate. Following the addition of soil samples, 100 µM of MUB- or AMC- (LAP only) labeled substrate was added to each well. For the 6 hydrolytic potential enzyme assays, the plates were incubated for 3 hours at 25 °C.

To measure temperature sensitivity, potential assays were conducted at 5, 15, 25, 30, and 35°C (only 15, 25, and 25°C in February) and were incubated for 24, 6, 3, and 1.5 hours respectively. Following incubation, fluorescence was measured immediately on a Synergy H1 spectrofluorometer with wavelengths set at 365 nm for excitation and 450 nm for emission. The standard curve plates were used to calculate a linear regression to determine potential enzyme activity for each sample as nmol g^{-1} dry $soil^{-1}$ hr^{-1}. We calculated temperature sensitivity of enzyme activities using the Arrhenius model:

$$R_s = ae^{(-Ea/RT)}$$

Where R_s is the measured enzyme activity (nmol activity g^{-1} dry soil hr^{-1}), T is the incubation temperature (Kelvin), a is the pre-exponential coefficient, R is the ideal gas constant, and Ea is the activation energy. In cases of poor Arrhenius fit, values were removed from analysis.

Enzyme Ratios

Enzyme ratios (nutrient acquisition ratios) were calculated from potential enzyme assays conducted at 25 °C. Enzyme C:N ratios were calculated by dividing β-glucosidase (BG) activity by the sum of β-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) activity. Enzyme C:P ratios were calculated by dividing β-glucosidase (BG) activity by acid phosphatase (AP) activity.

Statistical Procedures

In order to test our first hypothesis on the effect of warming and forest location on enzyme activity and temperature sensitivity, we performed a 2-way analysis of variance (ANOVA, JMP 10.0, SAS Institute, Cary, North Carolina, USA) for each enzyme. The ANOVA included the following factors: warming (3 levels, fixed effect), habitat (2 levels, fixed effect), followed by a Student's t-test for the effect of habitat (forest vs. gap).

To test our second hypothesis on the seasonality of enzyme activities and temperature sensitivities, we performed a 2-way ANOVA with warming (3 levels, fixed effect) and season (4 levels enzyme activity, 3 levels temperature sensitivity; fixed effect) for each enzyme (split by habitat), followed by a Tukey's posthoc test for comparison of warming treatment and season effects.

To test our third hypothesis on the ratios of EEA we performed a one-way ANOVA with season (4 levels) as the main effect, followed by a Tukey's posthoc test for seasonal differences. For all statistical analyses, treatment plots ($n = 3$ each) were used as replicates. Data were tested for normality and were log transformed if necessary to acquire normal distribution. Log transformed data was then back-transformed for graphical representation.

Results

Potential Enzyme Activities

We did not find broad support for our first hypothesis. Among the six hydrolytic enzymes, there were no differences of warming treatment between habitats (no significant interaction between location and warming treatment). The only enzyme that responded to warming was XYL (Figure 4.1), where in the gap habitat ($F_{2,24} = 6.99$, $p = 0.004$), activity was greater in ambient plots compared to the +3°C treatment. Overall NAG enzyme activity was higher in the forest than gap ($F_{1,66} = 2.65$, $p = 0.03$).

We did not observe any differences in warming effects between seasons (no significant interaction of warming treatment and season), but overall seasonal effects were observed in every enzyme (Figure 4.1). For AP enzyme activity, season was significant in both the forest ($F_{3,22} = 12.29$, $p < 0.0001$) and gap ($F_{3,23} = 11.95$, $p < 0.0001$), where activity was lowest in October relative to other months. For BG enzyme activity, season was significant in the forest habitat ($F_{3,24} = 6.50$, $p = 0.002$), where it was higher in May and February relative to August and October. In the gap ($F_{3,24} = 8.56$, $p = 0.0005$) BG activity was higher in May relative to August and October. XYL enzyme activity in the forest ($F_{3,24} = 5.23$, $p = 0.010$) had higher activity in May relative to August and in the gap ($F_{3,24} = 3.86$, $p = 0.005$), higher activity than October and August. CB enzyme activity in the forest ($F_{3,24} = 5.53$, $p = 0.005$) was highest in May relative to February and August, while in the gap ($F_{3,24} = 8.59$, $p = 0.0005$), May had greater activity overall. In the forest ($F_{3, 23} = 6.29$, $p = 0.0028$), LAP enzyme activity was greater in February than August and May. NAG enzyme activity in the forest ($F_{3, 24} = 6.71$, $p = 0.002$) was lowest in October. There were no significant seasonal patterns observed in the gap for LAP and NAG.

Enzyme Temperature Sensitivity

For the 4 hydrolytic enzymes where we estimated temperature sensitivity there were no interactions of warming treatment and location, refuting our first hypothesis (Figure 4.2). However, there were overall location effects observed for NAG and CB temperature sensitivities (CB $F_{2,41} = 7.76$, $p = 0.008$; NAG $F_{2,48} = 13.21$, $p = 0.0007$), where forest had the highest temperature sensitivities.

In contrast to our second hypothesis, we did not observe any differences in temperature sensitivities between warming treatment and season (no significant interaction of warming treatment and season). There was a significant seasonal effect of temperature sensitivities for all enzymes (BG gap $F_{2,18} = 9.51$, $p = 0.0015$; XYL forest $F_{2,18} = 11.05$, $p = 0.0007$; CB forest $F_{2,16} = 9.54$, $p = 0.002$; NAG forest $F_{2,18} = 3.67$, $p = 0.04$; NAG forest $F_{2,18} = 3.09$, $p = 0.02$). In 5 of 8 enzyme by location combinations, May had greater temperature sensitivities than August and February BG in the forested plots had lowest temperature sensitivity in February ($F_{2,18} = 18.67$, $p < 0.0001$). The lowest XYL temperature sensitivity in the gap plots was measured in August ($F_{2,18} = 14.35$, $p = 0.0002$). The NAG temperature sensitivities in the forested plots were higher in May than February ($F_{2,18} = 3.66$, $p = 0.04$).

Enzyme Ratios

Nutrient acquisition ratios varied seasonally for C:N ($F_{3,67}$=11.82, $p < 0.0001$) and C:P (F3,67 = 5.01, p = 0.0034) (Figure 4.3), where May had higher C:N (BG: NAG+LAP) enzyme ratios overall. C:P (BG:AP) enzyme ratios were highest in October and May.

Discussion

In this warm temperate forest ecosystem, seasonal dynamics in enzyme activity are stronger than are the responses to 3 years of experimental warming up to 5°C above ambient. We observed seasonal patterns in enzyme activity, temperature sensitivity, and enzyme ratios that were more pronounced and more consistent than those resulting from soil warming.

Potential enzyme activities

Contrary to our overarching hypothesis, we observed no strong and consistent response after three years of experimental warming in extracellular enzyme activities. At higher temperatures, enzymes have greater reaction efficiencies (Koch et al. 2007), and therefore, microorganisms may reduce the synthesis of enzymes while achieving the same function. In our study, this response would have been detected as a reduction in potential enzyme activity. While we observed a decline in XYL potential activities in the +3°C treatment, this was not a consistent response among enzymes and habitat. Although our results are counter to metabolic theory, previous studies (reviewed in Henry 2012) have also demonstrated a lack of, inconsistent or weak warming effect on enzyme activities (Kardol et al. 2010, Henry 2012, Steinweg et al. 2013, Weedon et al. 2013).

We hypothesized that during the winter and fall months enzyme activities would decline more strongly in response to warming relative to spring and summer months. While we did not observe any responses to warming, we observed strong seasonal effects. Season-driven changes in soil moisture and temperature can influence enzyme activity. In our study, we observed both the lowest soil moisture and enzyme activities in October. This suggests, that low soil moisture suppresses enzyme production at our study site. Reduced soil moisture can limit the diffusion of substrates (Allison and Vitousek 2005) and negate the positive effects of temperature on enzyme activity (Steinweg et al. 2012). Yet, in our study, seasonal patterns in enzyme activity are not solely driven by soil moisture. The decrease in enzyme activity that was measured from May to August only corresponded to a 3% reduction in soil moisture, suggesting that other factors, such as the demand and availability of substrates contribute to these patterns.

Enzyme Temperature Sensitivity

Warmer temperatures can induce thermal adaptation of respiratory enzymes through ecological and evolutionary changes in the microbial community (Allison et al. 2010), decreasing the temperature sensitivity of extracellular enzymes. Additionally, a decrease in temperature sensitivity may arise due to an increase in substrate supply (Davidson and Janssens 2006). We hypothesized that warming would decrease enzyme temperature sensitivity, however, we observed no effect of manipulative warming. A more striking pattern arises in the seasonality of enzyme temperature sensitivity.

Temperature sensitivity decreased from May to August in most cases, suggesting thermal adaptation of the enzymes (Allison et al. 2010) either due to physiological changes in the microbial community or the amount of substrate available.

Enzyme ratios, microbial resource demand, and seasonality

We observed no response of enzyme ratios to warming, but observed strong seasonal patterns. Enzyme ratios are indicators of nutrient acquisition patterns in the microbial community. In our study, enzyme C:N and C:P followed the same general pattern, with a peak in enzyme C:N (forest and gap) and C:P (gap) in May relative to other months. This pattern is mostly driven by the reduction in BG activity from May to August, suggesting microbial demand for labile C declines in summer while N and P demands remain relatively constant. In other studies, both C- and N-degrading enzymes change with season (Weintraub and Schimel 2005, Weedon et al. 2011), suggesting differences in both nutrient limitation and substrate availability can drive unique seasonal patterns across ecosystems. The lack of a significant seasonal pattern in enzyme C:P in the forest is likely a reflection of greater variance in both BG and AP activities.

Our study generated lower enzyme C:P ratios compared to the global average observed across several ecosystems (i.e., C:P enzyme acquisition ratio of 1:1; Sinsabaugh et al. 2008). This finding suggests that microbes allocate more energy for the synthesis of P-liberating enzymes relative to those for C, which may reflect the tendency for P limitation in these highly-weathered soils (Walker and Syers 1976, Crews et al. 1995). This is consistent with the findings of Sinsabaugh et al. (2008) who found C:P acquisition ratios declined as mean annual temperature and precipitation increased.

Conclusion

In our study, experimental warming did not consistently influence enzyme activity, temperature sensitivity or nutrient acquisition ratios; however, we observed strong seasonal patterns in all these response variables. Our results suggest that seasonal dynamics have a greater control on enzyme dynamics than does a uniform increase in temperature throughout the year. In order to predict the effect of climate warming on soil C processes, it is critical to understand how soil temperature, moisture and organic matter substrates regulate soil extracellular enzyme activity in a season context. Furthermore, as changes in temperature and precipitation are predicted to manifest unevenly across seasons under future climates, understanding seasonal consequences of climate change on soil C dynamics may become even more important.

Table 4.1: List of enzymes and their general function.

Enzyme Abbreviation	Enzyme	Substrate	General Function
BG	β–Glucosidase	4-Methylumbelliferyl β-D-glucopyranoside	Hydrolysis of cellobiose to glucose
XYL	β-Xylosidase	4-Methylumbelliferyl β-D-xylopyranoside	Hemicellulose degradation
CB	β-D-cellobiosidase	4-Methylumbelliferyl β-D-cellobioside	Cellulose degradation
AP	Acid phosphatase	4-Methylumbelliferyl phosphate	Phosphorus mineralization
NAG	β-N-acetylglucosaminidase	4-Methylumbelliferyl N-acetyl-β-D-glucosaminide	Chitin degradation
LAP	Leucine aminopeptidase	L-Leucine-7-amido-4-methylcoumarin hydrochloride	Protein degradation

Table 4.2: Average monthly soil temperatures and soil moisture for each treatment plot.

		-----------------------------Sampling Month---------------------------			
Habitat	Treatment	October 2012	February 2013	May 2013	August 2013
		°C -- % SM	°C -- % SM	°C -- % SM	°C -- % SM
Forest	Ambient	18.0 – 12.5	8.6 – 23.0	17.4 – 18.5	23.3 – 18.1
	+3°C	20.6 – 9.4	10.3 – 19.1	19.7 – 15.3	25.7 – 14.6
	+5°C	22.6 – 11.2	13.7 – 23.4	21.6 – 18.2	27.5 – 17.0
Gap	Ambient	18.7 – 14.6	9.1 – 23.0	18.9 – 18.5	24.5 – 18.1
	+3°C	22.7 – 12.0	11.7 – 19.1	20.9 – 15.3	28.2 – 14.6
	+5°C	25.4 – 11.6	13.7 – 23.4	24.5 – 18.2	29.3 – 17.0

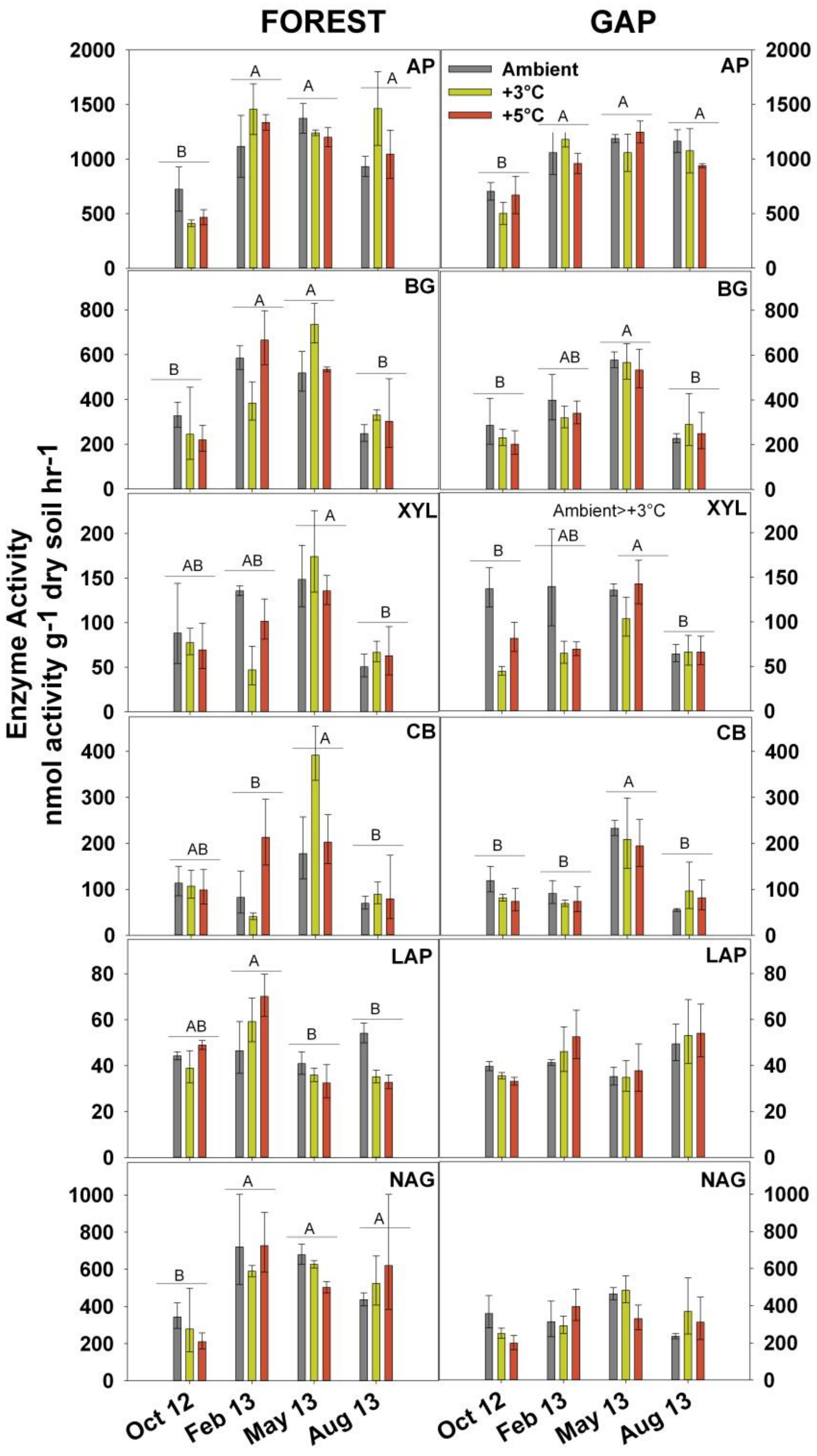

Figure 4.1: Extracellular enzyme activity for BG, XYL, CB, LAP, AP, and NAG in warmed plots (colored) and non-warmed plots (gray) in both forest and gap habitat. Error bars indicate standard error of the treatment mean and back transformed from log scale if necessary (n=3). Different letters indicate significant differences in the average monthly enzyme activities. Overall treatment differences noted on graph.

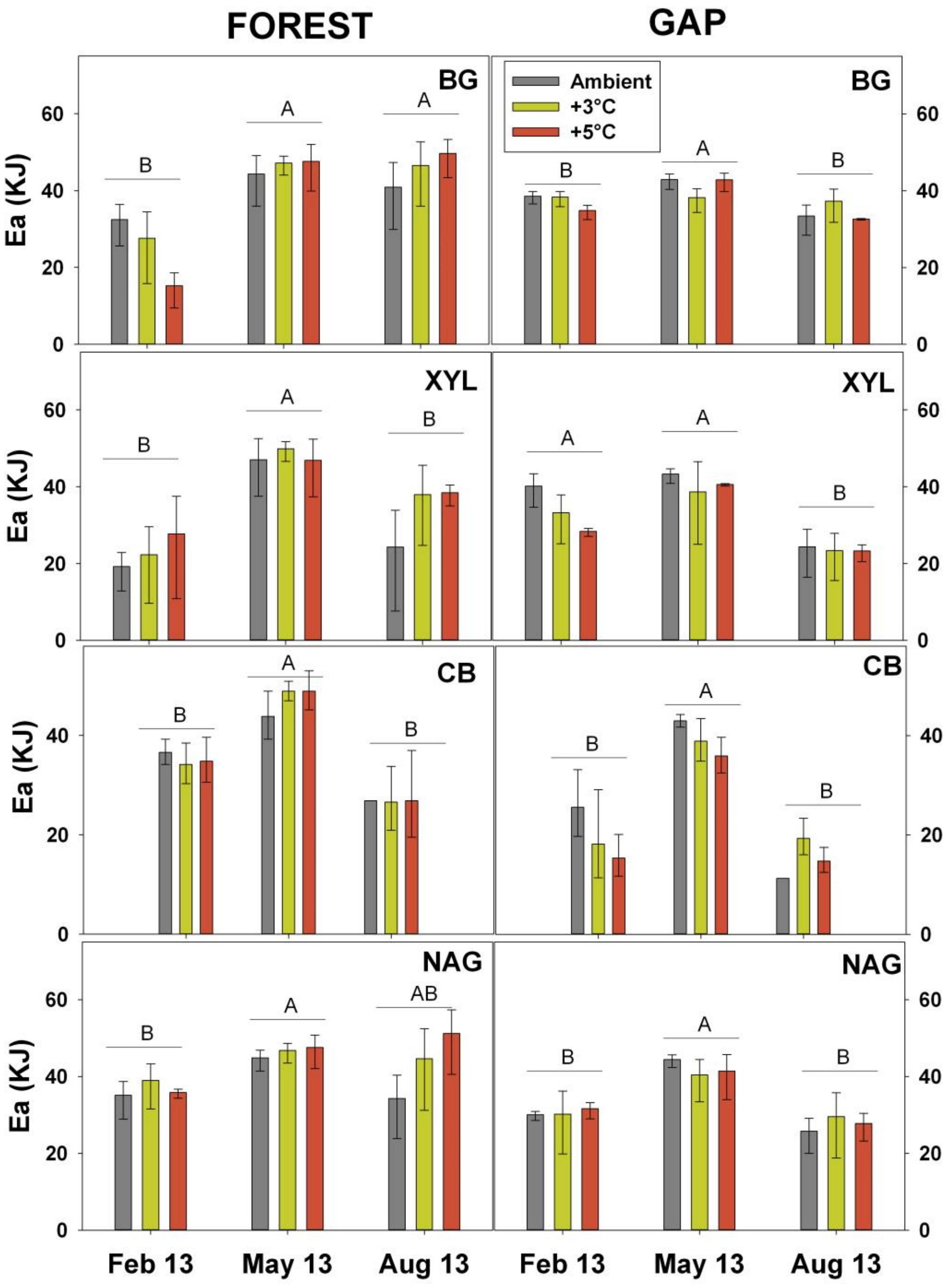

Figure 4.2: Temperature sensitivity, or activation energy (kJ), of enzyme activity BG, XYL, CB, and NAG in warmed plots (colored) and non-warmed plots (gray) in both forest (left panel) and gap (right panel) habitat. Error bars indicate standard error of the treatment mean and back transformed from log scale if necessary (n=3, except CB August ambient plots where n=1). Different letters indicate significant differences in the average monthly enzyme activities.

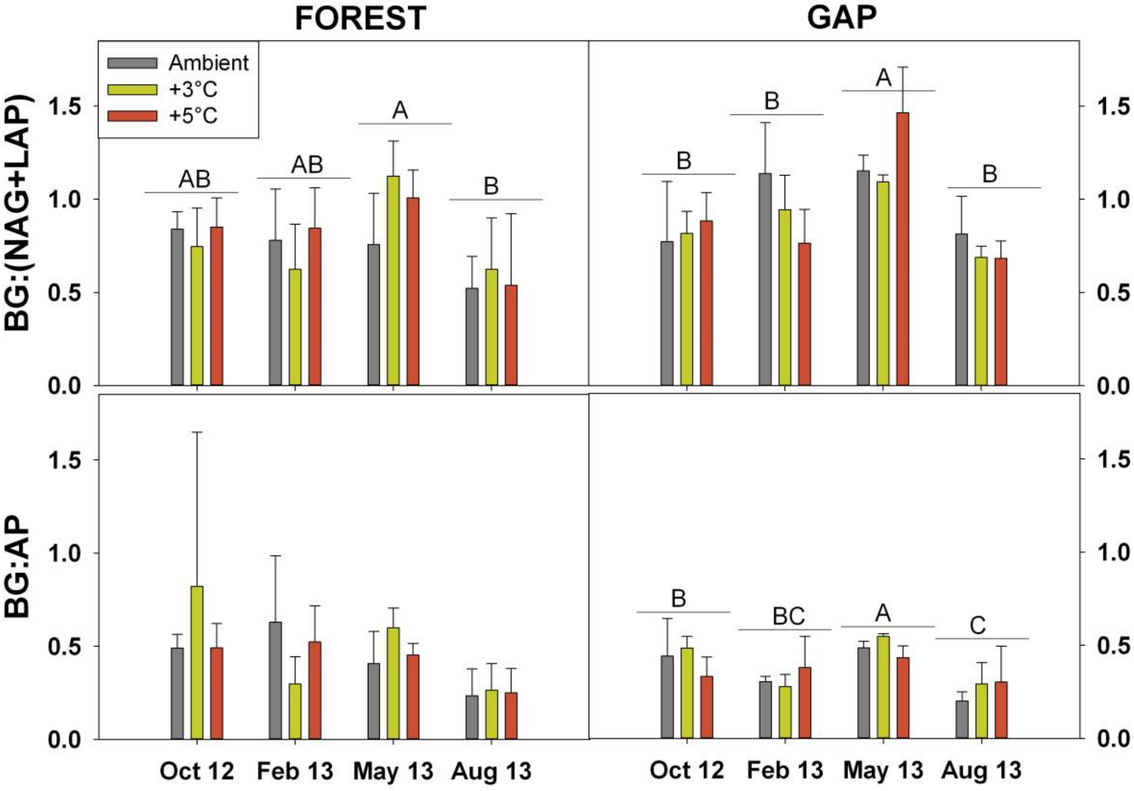

Figure 4.3: Stoichiometric ratios of potential extracellular enzyme activities displayed at different sampling dates. Different letters indicate significant differences ($p \leq 0.05$) between sampling dates. No significant differences were found between treatments.

CHAPTER 5

CONCLUSION

James Lovelock, and his Gaia hypothesis, postulates that the earth is a self-regulating entity that is able to control its environment and sustain a healthy and suitable place for life to inhabit (Lovelock 1979). The past and current trajectory of the Earth's climate, however, is strongly contingent upon human influence extracting ancient carbon, of which otherwise would remain stable for millennia. Anthropogenic fossil fuel burning has resulted in unprecedented increases in atmospheric CO_2 concentration. As a result, a novel climate is emerging at a rate many of the organisms or ecosystems on earth have not experienced. Thus, we must ask: What is the ability of our earth to keep up and is there a threshold of which anthropogenic perturbations become irreversible? These overarching questions have driven scientists from all over the world to quantify and predict the carbon sink strength under the current and projected trends in earth's climate.

Since the Devonian period of earth's history, soils and forests have been a major part of the biosphere (Richter 2001). While many plants and animals have gone extinct, soils and forest ecosystems have persisted playing a pivotal role in the regulation of earth's biogeochemical cycles and climate. In particular, soils and the microbes inhabiting this environment play an important role in the global carbon cycle, currently storing significantly more carbon than is present in the atmosphere or terrestrial vegetation (Jobbágy and Jackson 2000, Schlesinger and Bernhardt 2013). Carbon dioxide efflux, or soil respiration, to the atmosphere is the second largest terrestrial carbon flux (Raich and Schlesinger 1992, Raich and Potter 1995). An increase in soil carbon dioxide efflux with increasing temperature can change the terrestrial biosphere from a carbon sink to a carbon source exacerbating climate change. Our current understanding of how warming affects soil organic matter decomposition (carbon dioxide efflux to the atmosphere) is biased. Current empirical evidence is based on warming experiments that have been conducted in primarily northern latitudes consisting of glaciated soils high in organic matter and nutrients (Rustad et al. 2001). Herein lies the motivation behind my dissertation research: To quantify how warming affects soil respiration and soil organic matter decomposition dynamics in a previously unexplored physiographic region, a forest ecosystem that contains highly-weathered soil (Whitehall Forest, Georgia Piedmont physiographic province). While previous studies have suggested that warming will stimulate soil organic matter decomposition (Peterjohn et al. 1994, Harte and Shaw 1995, Rustad et al. 2001, Melillo et al. 2002, Melillo et al. 2011, Zhou et al. 2012), my dissertation research suggests otherwise.

For my dissertation, I examined belowground carbon dynamics in the first ecosystem warming experiment located in a forest containing highly-weathered soils. In Chapter 2, I present results that suggest manipulative warming, in this ecosystem, does not stimulate *in situ* soil respiration or change soil carbon stocks and chemistry. While warming increased soil respiration during some months there was no overall significant effect of either +3°C or +5°C on soil respiration. Although, long term warming experiments support an increase in soil respiration with warming (Rustad et al. 2001), these studies show that after a few years of elevated temperature soil respiration declines back to ambient rates – exhibiting a decrease in temperature sensitivity (Luo et al. 2001, Melillo et al. 2002). The results of Chapter 2 show that the first few years of warming resulted in a minimal affect on the apparent temperature sensitivity of soil respiration. However, temperature sensitivity increased during the later part of the experiment that had over 30% more precipitation. This suggests an important role of moisture in regulating the response of soil respiration to temperature increases.

Heterotrophic respiration of soil organic matter contributes ~50% of total respiration (Ryan and Law 2005). It is predicted that the future climate of the Southeastern U.S. experience substantial decreases (20-30%) in summer precipitation and an increase in annual temperatures (Mearns et al. 2003). In Chapter 3, I explored how moisture and temperature interact to affect heterotrophic respiration. Results indicate that temperature and moisture interact to influence heterotrophic respiration, but the strength of this interaction varied seasonally. Often, models of the temperature responses of respiration use a fixed temperature sensitivity parameter (Tjoelker et al. 2001, Davidson et al. 2006), however findings of this dissertation showed that the temperature sensitivity of heterotrophic respiration changes by ~30% from winter to summer. Including both season-specific temperature and moisture sensitivities of heterotrophic respiration improved model predictions. These models explained 50-70% of the variability measured in the field, suggesting that temperature and moisture interactions do not entirely explain the observations in the field (Chapter 2).

Temperature and moisture exert both direct and indirect effects on the microbial community responsible for soil organic matter decomposition. Microbial communities control the rate of C and nutrients in nearly all terrestrial ecosystems (Schlesinger 2004).

Consequently, as temperatures increase and microbial physiology is affected, the rates of biogeochemical cycling may be significantly altered. The results of Chapter 4 demonstrate how warming influenced microbial extracellular enzyme activities involved in soil organic matter decomposition. Warming effects on soil enzyme activities involve short-term changes driven by thermodynamics (Trasar-Cepeda et al. 2007) and longer-term effects on the enzyme pool driven by microbial production and turnover (Sowerby et al. 2005). In our study, experimental warming did not consistently influence enzyme activity, temperature sensitivity or microbial nutrient acquisition ratios; however, we observed strong seasonal patterns in all these response variables. Our results suggest that seasonal dynamics have a greater control on enzyme dynamics than does a uniform increase in temperature throughout the year. In order to predict the effect of climate warming on soil C processes, it is critical to understand how soil temperature, moisture and organic matter substrates regulate soil extracellular enzyme activity in a season context. Furthermore, as changes in temperature and precipitation are predicted to manifest unevenly across seasons under future climates, understanding seasonal consequences of climate change on soil C dynamics may become even more important.

My dissertation research established that an increase in carbon dioxide efflux from soils due to rising temperatures is not a universal response. I established how temperature and moisture manipulations affected the temperature sensitivity of respiration and how this changed seasonally. Additionally, I showed how warming and season influence extracellular enzyme activity and temperature sensitivity. Previous studies have demonstrated that microbial physiological shifts or substrate limitation drive changes after several years of warming (Allison et al. 2010). Here I show that a

Southeastern U.S. (subtropical) forest consisting of highly-weathered soils, behaves more like previous experiments after several years of warming. These results demonstrate the major uncertainties that still remain regarding the mechanisms that regulate carbon dioxide efflux in soils. Ultimately, in order to understand the carbon sink strength and ‘regulation’ potential of the biosphere that Lovelock once proposed, it is essential to understand soil microbial and ecosystem responses, including those on highly-weathered soils in subtropical latitudes that make up a large portion of this globe.

REFERENCES

Agren, G. I. and E. Bosatta. 2002. Reconciling differences in predictions of temperature response of soil organic matter. Soil biology & biochemistry **34**:129.

Allison, S. D. and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences **105**:11512-11519.

Allison, S. D. and K. K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology **14**:2898-2909.

Allison, S. D. and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry **37**:937-944.

Allison, S. D., M. D. Wallenstein, and M. A. Bradford. 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience **3**:336-340.

Allison, S. D., M. N. Weintraub, T. B. Gartner, and M. P. Waldrop. 2011. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. Pages 229-243 Soil enzymology. Springer.

Arrhenius, S. Z. 1889. Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. Zeitschrift für Physikalische Chemie **4**:226-248.

Baldock, J. A., J. M. Oades, P. Nelson, T. M. Skene, A. Golchin, and P. Clarke. 1997. Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. Australian Journal of Soil Research **35**:1061-1083.

Balser, T. C. and D. L. Wixon. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology **15**:2935-2949.

Bauer, J., M. Herbst, J. Huisman, L. Weihermueller, and H. Vereecken. 2008. Sensitivity of simulated soil heterotrophic respiration to temperature and moisture reduction functions. Geoderma **145**:17-27.

Bengtson, P. and G. Bengtsson. 2007. Rapid turnover of DOC in temperate forests accounts for increased CO2 production at elevated temperatures. Ecology Letters **10**:783-790.

Billings, S. A. and F. Ballantyne. 2013. How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. Global Change Biology **19**:90-102.

Bradford, M. A., C. A. Davies, S. D. Frey, T. R. Maddox, J. M. Melillo, J. E. Mohan, J. F. Reynolds, K. K. Treseder, and M. D. Wallenstein. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters **11**:1316-1327.

Bradford, M. A., B. W. Watts, and C. A. Davies. 2010. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. Global Change Biology **16**:1576-1588.

Bremer, D. J., J. M. Ham, C. E. Owensby, and A. K. Knapp. 1998. Responses of Soil Respiration to Clipping and Grazing in a Tallgrass Prairie. J. Environ. Qual. **27**:1539-1548.

Burke, I., T. Kittel, W. Lauenroth, P. Snook, C. Yonker, and W. Parton. 1991. Regional analysis of the central Great Plains. BioScience **41**:685-692.

Canadell, J., C. Le QuÈrÈ, M. Raupach, C. Field, E. Buitenhuis, P. Ciais, T. Conway, N. Gillett, R. Houghton, and G. Marland. 2007. Contributions to accelerating atmospheric CO2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proceedings of the National Academy of Sciences **104**:18866.

Carlyle, J. C. and U. B. Than. 1988. Abiotic Controls of Soil Respiration Beneath an Eighteen-Year-Old Pinus Radiata Stand in South-Eastern Australia. Journal of Ecology **76**:654-662.

Chapin III, S., J. McFarland, A. David McGuire, E. S. Euskirchen, R. W. Ruess, and K. Kielland. 2009. The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. Journal of Ecology **97**:840-850.

Cleveland, C. C. and D. Liptzin. 2007. C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry **85**:235-252.

Conant, R. T., M. G. Ryan, G. I. Ågren, H. E. Birge, E. A. Davidson, P. E. Eliasson, S. E. Evans, S. D. Frey, C. P. Giardina, and F. M. Hopkins. 2011. Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward. Global Change Biology.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, and N. Pérez- Harguindeguy. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters **11**:1065-1071.

Cox, P., R. Betts, C. Jones, S. Spall, and I. Totterdell. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature **408**:184-187.

Craine, J. M. and T. M. Gelderman. 2011. Soil moisture controls on temperature sensitivity of soil organic carbon decomposition for a mesic grassland. Soil Biology and Biochemistry **43**:455-457.

Crews, T. E., K. Kitayama, J. H. Fownes, R. H. Riley, D. A. Herbert, D. Mueller-Dombois, and P. M. Vitousek. 1995. Changes in Soil Phosphorus Fractions and Ecosystem Dynamics across a Long Chronosequence in Hawaii. Ecology **76**:1407-1424.

Davidson, E. and I. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature **440**:165-173.

Davidson, E. A., I. A. Janssens, and Y. Luo. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q10. Global Change Biology **12**:154-164.

Del Grosso, S., W. Parton, A. Mosier, E. Holland, E. Pendall, D. Schimel, and D. Ojima. 2005. Modeling soil CO 2 emissions from ecosystems. Biogeochemistry **73**:71-91.

Falkowski, P., R. Scholes, E. e. a. Boyle, J. Canadell, D. Canfield, J. Elser, N. Gruber, K. Hibbard, P. Högberg, and S. Linder. 2000. The global carbon cycle: a test of our knowledge of earth as a system. science **290**:291-296.

Fang, C., P. Smith, J. Moncrieff, and J. Smith. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature **433**:57-59.

Farquhar, G., S. v. von Caemmerer, and J. Berry. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta **149**:78-90.

Fierer, N. and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal **67**:798-805.

Friedlingstein, P., P. Cox, R. Betts, L. Bopp, W. Von Bloh, V. Brovkin, P. Cadule, S. Doney, M. Eby, and I. Fung. 2006. Climate-carbon cycle feedback analysis: Results from the C4MIP model intercomparison. Journal of Climate **19**:3337-3353.

German, D. P., S. S. Chacon, and S. D. Allison. 2011. Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology **92**:1471-1480.

Harte, J. and R. Shaw. 1995. Shifting dominance within a montane vegetation community: results of a climate-warming experiment. Science (New York, NY) **267**:876.

Hartley, I. P., D. W. Hopkins, M. H. Garnett, M. Sommerkorn, and P. A. Wookey. 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. Ecology Letters **11**:1092-1100.

Heimann, M. and M. Reichstein. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature **451**:289-292.

Hendrix, P. F., A. J. Franzluebbers, and D. V. McCracken. 1998. Management effects on C accumulation and loss in soils of the southern Appalachian Piedmont of Georgia. Soil and Tillage Research **47**:245-251.

Henry, H. A. 2012. Soil extracellular enzyme dynamics in a changing climate. Soil Biology and Biochemistry **47**:53-59.

Hoffert, M., K. Caldeira, A. Jain, E. Haites, L. Harvey, S. Potter, M. Schlesinger, S. Schneider, R. Watts, and T. Wigley. 1998. Energy implications of future stabilization of atmospheric CO2 content. Nature **395**:881-884.

Holland, E. A., J. C. Neff, A. R. Townsend, and B. McKeown. 2000. Uncertainties in the temperature sensitivity of decomposition in tropical and subtropical ecosystems: implications for models. Global Biogeochemical Cycles **14**:1137-1151.

Ise, T. and P. R. Moorcroft. 2006. The global-scale temperature and moisture dependencies of soil organic carbon decomposition: an analysis using a mechanistic decomposition model. Biogeochemistry **80**:217-231.

Jenkinson, D., D. Adams, and A. Wild. 1991. Model estimates of CO2 emissions from soil in response to global warming. Nature **351**:304-306.

Jenny, H. 1941. Factors of soil formation.

Jenny, H. 1980. The soil resource, Origin and behaviour. The soil resource, Origin and behaviour.

Jobbagy, E. G. and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological applications **10**:423-436.

Jobbágy, E. G. and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications **10**:423-436.

Kardol, P., M. A. Cregger, C. E. Campany, and A. T. Classen. 2010. Soil ecosystem functioning under climate change: plant species and community effects. Ecology **91**:767-781.

Kettler, T., J. W. Doran, and T. Gilbert. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses.

Kirschbaum, M. U. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry **27**:753-760.

Kirschbaum, M. U. 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? Biogeochemistry **48**:21-51.

Knorr, W., I. Prentice, J. House, and E. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature **433**:298-301.

Koch, A. L. 1985. The macroeconomics of bacterial growth. Special Publications of the Society for General Microbiology. 1985.

Koch, A. L. 1990. Diffusion the crucial process in many aspects of the biology of bacteria. Pages 37-70 Advances in microbial ecology. Springer.

Koch, O., D. Tscherko, and E. Kandeler. 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochemical Cycles **21**.

Le Quéré, C., M. R. Raupach, J. G. Canadell, and G. Marland. 2009. Trends in the sources and sinks of carbon dioxide. Nature Geoscience **2**:831-836.

Liu, W., Z. Zhang, and S. Wan. 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Global Change Biology **15**:184-195.

Lloyd, J. and J. Taylor. 1994. On the temperature dependence of soil respiration. Functional ecology:315-323.

Lovelock, J. 1979. Gaia: A new look at life on earth. Oxford University Press, Oxford, UK.

Luo, Y., S. Wan, D. Hui, and L. L. Wallace. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature **413**:622-625.

Machmuller, M. B., Thompson, A., Ballantyne, F., Markewitz, D., Wurzburger, N., Mohan, Jacqueline E. In preparation. Soil warming fails to increase soil respiration in a forest with highly-weathered soil.

Mearns, L., F. Giorgi, L. McDaniel, and C. Shields. 2003. Climate scenarios for the southeastern US based on GCM and regional model simulations. Climatic Change **60**:7-35.

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH_4. North Carolina Soil Test División, Publ, 1-53.

Melillo, J., P. Steudler, J. Aber, K. Newkirk, H. Lux, F. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. science **298**:2173.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology **63**:621-626.

Melillo, J. M., S. Butler, J. Johnson, J. Mohan, P. Steudler, H. Lux, E. Burrows, F. Bowles, R. Smith, and L. Scott. 2011. Soil warming, carbon-nitrogen interactions, and forest carbon budgets. Proceedings of the National Academy of Sciences **108**:9508.

Moorhead, D. L. and R. L. Sinsabaugh. 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs **76**:151-174.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. Enzymes in the environment. Marcel Dekker, New York:1-33.

Nelson, D. W. 1983. Determination of ammonium in KCl extracts of soils by the salicylate method 1. Communications in Soil Science & Plant Analysis **14**:1051-1062.

NOAA. Palmer Severity Drought Index data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their Web site at http://www.esrl.noaa.gov/psd/.

NOAA. 2013. National Oceanic and Atmospheric Administration, National Climate Data Center, Athens, Georgia, Ben Epps Airport Climate Station.

NRCS Soil Survey Staff. 2014. Natural Resources Conservation Service, United States Department of Agriculture online at http://websoilsurvey.nrcs.usda.gov/ Accessed 5/2014.

Oechel, W. C., G. L. Vourlitis, S. J. Hastings, R. C. Zulueta, L. Hinzman, and D. Kane. 2000. Acclimation of ecosystem CO2 exchange in the Alaskan Arctic in response to decadal climate warming. Nature **406**:978-981.

Pachauri, R. and A. Reisinger. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: Authors.

Pachauri, R. K. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. oChange **446**.

Pachauri, R. K. 2008. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. **446**.

Perkins, H. F. 1970. A rapid method of evaluating the zinc status of coastal plain soils 1. Communications in Soil Science & Plant Analysis **1**:35-42.

Peterjohn, W., J. Melillo, P. Steudler, K. Newkirk, F. Bowles, and J. Aber. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. Ecological Applications:617-625.

Post, W. M., W. R. Emanuel, P. J. Zinke, and A. G. Stangenberger. 1982. Soil carbon pools and world life zones. Nature **298**:156-159.

Raich, J. W. and C. S. Potter. 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochemical Cycles **9**:23-36.

Raich, J. W. and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B **44**:81-99.

Reichstein, M., J. A. Subke, A. C. Angeli, and J. D. Tenhunen. 2005. Does the temperature sensitivity of decomposition of soil organic matter depend upon water content, soil horizon, or incubation time? Global Change Biology **11**:1754-1767.

Richter, D., Markewitz, D. 2001. Understanding Soil Change: Soil sustainability over millenia, centuries, and decades. Cambridge University Press, New York, NY.

Rinnan, R., J. Rousk, E. Yergeau, G. A. Kowalchuk, and E. Bååth. 2009. Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. Global Change Biology **15**:2615-2625.

Rustad, L., J. Campbell, G. Marion, R. Norby, M. Mitchell, A. Hartley, J. Cornelissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia **126**:543-562.

Ryan, M. G. and B. E. Law. 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry **73**:3-27.

Saiya-Cork, K., R. Sinsabaugh, and D. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry **34**:1309-1315.

Saleska, S. R., J. Harte, and M. S. Torn. 1999. The effect of experimental ecosystem warming on CO2 fluxes in a montane meadow. Global Change Biology **5**:125-141.

Schimel, D. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology **1**:77-91.

Schimel, J. P. and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591-602.

Schimel, J. P. and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry **35**:549-563.

Schindlbacher, A., S. Wunderlich, W. Borken, B. Kitzler, S. Zechmeister-Boltenstern, and R. Jandl. 2012. Soil respiration under climate change: prolonged summer drought offsets soil warming effects. Global Change Biology **18**:2270-2279.

Schlesinger, W. and J. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry **48**:7-20.

Schlesinger, W. H. 2004. Better living through biogeochemistry. Ecology **85**:2402-2407.

Schlesinger, W. H. and E. S. Bernhardt. 2013. Biogeochemistry: an analysis of global change. Academic press.

Schmidt, M. W. I., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. K√∂gel-Knabner, J. Lehmann, and D. A. C. Manning. 2011. Persistence of soil organic matter as an ecosystem property. Nature **478**:49-56.

Sheik, C. S., W. H. Beasley, M. S. Elshahed, X. Zhou, Y. Luo, and L. R. Krumholz. 2011. Effect of warming and drought on grassland microbial communities. The ISME journal **5**:1692-1700.

Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature **462**:795-798.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, and M. E. Gallo. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters **11**:1252-1264.

Sitch, S., B. Smith, I. C. Prentice, A. Arneth, A. Bondeau, W. Cramer, J. Kaplan, S. Levis, W. Lucht, and M. T. Sykes. 2003. Evaluation of ecosystem dynamics, plant geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. Global Change Biology **9**:161-185.

Solomon, S., G. Plattner, R. Knutti, and P. Friedlingstein. 2009. Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences **106**:1704.

Sowerby, A., B. Emmett, C. Beier, A. Tietema, J. Penuelas, M. Estiarte, M. J. Van Meeteren, S. Hughes, and C. Freeman. 2005. Microbial community changes in heathland soil communities along a geographical gradient: interaction with climate change manipulations. Soil Biology and Biochemistry **37**:1805-1813.

Steinweg, J. M., J. S. Dukes, E. A. Paul, and M. D. Wallenstein. 2013. Microbial responses to multi-factor climate change: effects on soil enzymes. Frontiers in microbiology **4**.

Steinweg, J. M., J. S. Dukes, and M. D. Wallenstein. 2012. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biology and Biochemistry.

Stone, M. M., M. S. Weiss, C. L. Goodale, M. B. Adams, I. J. Fernandez, D. P. German, and S. D. Allison. 2012. Temperature sensitivity of soil enzyme kinetics under N- fertilization in two temperate forests. Global Change Biology **18**:1173-1184.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the Functional Significance of Microbial Community Composition. Ecology **90**:441-451.

Suseela, V., R. T. Conant, M. D. Wallenstein, and J. S. Dukes. 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old- field climate change experiment. Global Change Biology.

Suseela, V. and J. S. Dukes. 2013. The responses of soil and rhizosphere respiration to simulated climatic changes vary by season. Ecology **94**:403-413.

Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 2001. Modelling respiration of vegetation: evidence for a general temperature- dependent Q10. Global Change Biology **7**:223-230.

Todd-Brown, K. E., F. M. Hopkins, S. N. Kivlin, J. M. Talbot, and S. D. Allison. 2012. A framework for representing microbial decomposition in coupled climate models. Biogeochemistry **109**:19-33.

Trasar-Cepeda, C., F. Gil-Sotres, and M. Leiros. 2007. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biology and Biochemistry **39**:311-319.

Treseder, K. K., T. C. Balser, M. A. Bradford, E. L. Brodie, E. A. Dubinsky, V. T. Eviner, K. S. Hofmockel, J. T. Lennon, U. Y. Levine, and B. J. MacGregor. 2012. Integrating microbial ecology into ecosystem models: challenges and priorities. Biogeochemistry **109**:7-18.

Trimble, S. W. 1974. Man-induced soil erosion on the southern Piedmont. Soil Conservation Society of America, University of Wisconsin Department of Geography, Milwaukee, WI.

Trumbore, S. 2006. Carbon respired by terrestrial ecosystems-recent progress and challenges. Global Change Biology **12**:141-153.

Trumbore, S., O. Chadwick, and R. Amundson. 1996. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. science **272**:393.

Walker, T. and J. Syers. 1976. The fate of phosphorus during pedogenesis. Geoderma **15**:1-19.

Wallenstein, M., S. D. Allison, J. Ernakovich, J. M. Steinweg, and R. Sinsabaugh. 2011. Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates. Soil Enzymology:245-258.

Wallenstein, M. D., S. K. Mcmahon, and J. P. Schimel. 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology **15**:1631-1639.

Weedon, J., R. Aerts, G. Kowalchuk, and P. Bodegom. 2013. No effects of experimental warming but contrasting seasonal patterns for soil peptidase and glycosidase enzymes in a sub-arctic peat bog. Biogeochemistry:1-12.

Weedon, J., R. Aerts, G. Kowalchuk, and P. vanBodegom. 2011. Enzymology under global change: organic nitrogen turnover in alpine and sub-Arctic soils. Biochemical Society Transactions **39**:309.

Weintraub, M. N. and J. P. Schimel. 2005. Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biology and Biochemistry **37**:1469-1475.

Wieder, W. R., G. B. Bonan, and S. D. Allison. 2013. Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change.

Yuste, J. C., I. Janssens, A. Carrara, L. Meiresonne, and R. Ceulemans. 2003. Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. Tree physiology **23**:1263-1270.

Zhou, J., K. Xue, J. Xie, Y. Deng, L. Wu, X. Cheng, S. Fei, S. Deng, Z. He, J. D. Van Nostrand, and Y. Luo. 2012. Microbial mediation of carbon-cycle feedbacks to climate warming. Nature Clim. Change **2**:106-110.

Zogg, G. P., D. R. Zak, D. B. Ringelberg, D. C. White, N. W. MacDonald, and K. S. Pregitzer. 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal **61**:475-481.

APPENDIX A

SUPPORTING INFORMATION CHAPTER 2

General Soil Characteristics

Soil samples (sieved <2 mm), analyzed for P, K, Ca, Na, Mn, Fe, and Zn following a Mehlich I extraction (Mehlich 1953, Perkins 1970, Nelson 1983) on an ICP-OES at the University of Georgia Soil, Plant, and Water Laboratory. Soil pH was measured in a 1:1 soil:0.01 $CaCl_2$ suspension. The cation exchange capacity (CEC) was estimated from the charge equivalents of base cations in the Mehlich I-extracts (Na, Ca, Mg, K) and the exchangeable hydrogen activity determined by titration with 0.023 M $Ca(OH)_2$. Percent base saturation (BS) is calculated as the ratio of Na, Ca, Mg, K to that of CEC.

Particle size analysis was performed using the rapid hexametaphosphate method(Kettler et al. 2001). Briefly, 3% hexametaphosphate was added to air-dried soils (2mm sieved) and used as a dispersal agent, which is followed by fractionation and quantification of each particle size by sieving.

Statistics and Data Analysis

We used a mixed-model, MANOVA with repeated measures analysis to identify main (temperature, time) and interactive effects (temperature x time) of warming on soil respiration (JMP 10.0). Treatment means were calculated from the 2 subplots (collars) and utilized for further statistical analysis. Before ANOVA analyses, data were tested for a normal distribution. Further analysis was performed on all data without transformation after meeting the normality assumption. Statistical analyses were performed separately for the different habitats (forest and gap). A separate repeated measures analysis was conducted for before (Period 1) and after (Period 2) the +5°C ramp was initiated. Another repeated measures analysis was conducted on plots (Ambient, +3°C) that did not experience a ramp throughout the entire time period (3 years). For the first 1.5 years of data collection, 6 plots were warmed at 3°C above ambient, and 3 plots were held at ambient. Therefore, an unbalanced (different number of replicates) repeated measures ANOVA was performed (univariate approach; $\alpha=0.05$). Once the warming treatment at 5°C above ambient was applied, there were $n=3$ of each treatment. A separate repeated measures ANOVA was performed on this data set ($\alpha=0.05$). For both analyses, the block was not used in the ANOVA repeated measures model because it was found not to be significant. A Greenhouse-Geisser sphericity correction was applied to the p-values. To identify warming effects per month or season, separate ANOVA analyses were conducted. Seasons were separated according to Spring (March-May), Summer (June-August), Fall (September-November) and Winter (December-February).

To test the disturbance effect of the buried warming cables, non-cable controls were measured at least on a quarterly basis and an ANOVA was used for statistical comparison to ambient (no cable) controls ($n=3$ per treatment).

Cumulative Soil Respiration Calculations

We calculated annual and seasonal cumulative flux. We assumed the measured soil respiration measurements (taken between 1000 – 1400 hours) to be representative of the daily maximum flux. We then corrected (multiplied) the daily flux with the number of days between each measurement. The calculated flux was then summed over annual and seasonal time frames. Diel fluctuations in CO_2 efflux were not measured. Others have shown that even after a diel correction was made on daily CO_2 efflux measurements, the adjusted flux was ~96% of the daily maximum measured flux(Bremer et al. 1998). Therefore, extrapolating our monthly measured flux measurements to cumulative flux may be a slight overestimate.

Statistical Power of Experiment

It is possible if warming does increase soil respiration in this study ecosystem, the magnitude of change is small enough that detection within our experimental framework was not possible. Using a sample size estimation test and a 95% alpha, this experimental set-up could detect a difference ranging from 12-20% based on the coefficient of variation and mean cumulative fluxes observed (Figure 2.2). Previous warming studies have utilized similar sample sizes and statistical power (Rustad et al. 2001).

Temperature sensitivity Calculations

We calculated the temperature sensitivity (Ea) of soil respiration per treatment plot using the Arrhenius model:

$$R_s = ae^{(-Ea/RT)}$$

Where R_s is the measured CO_2 efflux (µmol m-2 s-1), T is the soil temperature in K at 10cm depth, a is the basal respiration (intercept when temperature is 0), R is the ideal gas constant, and Ea is the activation energy.

We also used an exponential function to calculate the respiratory quotient (Q_{10}) :

$$R_s = ae^{bT}$$

Where R_s is the measured CO_2 efflux (µmol m-2 s-1), T is the soil temperature in K at 10cm depth, a is the basal respiration (intercept when temperature is 0), b is the temperature sensitivity of CO_2 efflux. The Q_{10} was then calculated using:

$$Q_{10} = e^{10b}$$

Table A1**:** General Soil Characteristics at Duke and Whitehall Forests.

Location	**Habitat**	**Depth**	**pH**[1]	**Base Saturation**	**CEC**[2]	**Ca**	**Cd**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**Zn**
		(cm)	**$CaCl_2$**	**%**	**meq/100g**							**(mg/kg)**							
Georgia (Whitehall Forest)	Forest	0-10	4.47	30.98	6.81	274.64	0.07	0.15	0.48	49.83	71.48	68.91	53.02	0.22	16.90	0.47	6.69	1.44	2.88
		10-20	4.52	31.63	4.59	135.34	0.04	0.09	0.47	24.21	65.63	64.55	23.68	0.18	20.74	0.22	3.99	0.90	1.95
	Gap	0-10	4.39	23.12	6.46	152.10	0.07	0.16	0.41	48.13	57.96	60.56	26.04	0.29	20.98	0.57	5.10	1.48	2.38
		10-20	4.52	27.62	4.58	71.78	0.04	0.10	0.34	22.90	75.33	76.51	13.62	0.21	18.40	0.24	3.06	0.85	1.43
North Carolina (Duke Forest)	Forest	0-10	3.52	10.43	16.54	202.69	0.07	0.11	0.73	54.04	110.08	32.06	28.76	0.29	36.47	0.44	6.62	2.44	2.11
		10-20	3.52	7.54	12.71	106.45	0.04	0.10	0.69	31.84	39.41	27.81	5.14	0.24	21.63	0.46	3.12	1.88	1.38
	Gap	0-10	3.65	9.92	11.47	115.70	0.08	0.10	0.44	136.45	96.14	27.49	6.28	0.30	19.42	0.56	5.02	1.95	1.77
		10-20	3.55	8.20	12.99	98.09	0.05	0.05	0.48	45.61	68.65	33.80	7.80	0.26	26.79	0.59	4.15	2.17	1.80

[1] pH was measured in a 1:1 soil:water mixture of 0.01 M $CaCl_2$

[2] CEC and cations listed were determined by Mehlich 1 extraction

Table A2**:** Soil Texture Analysis from both Duke and Whitehall Forests.

Forest	**Habitat**	**Depth**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**Soil Type**
Whitehall	Forest	0-10	63.92	18.04	18.04	Sandy Clay Loam
		10-20	52.92	21.04	26.04	Sandy Clay Loam
Whitehall	Gap	0-10	63.92	19.04	17.04	Sandy Clay Loam
		10-20	55.92	20.04	24.04	Sandy Clay Loam
Duke	Forest	0-10	13.92	64.04	22.04	Silt Loam
		10-20	14.5	52.13	33.38	Silty Clay Loam
Duke	Gap	0-10	19.92	60.04	20.04	Silt Loam
		10-20	9.92	62.04	28.04	Silty Clay Loam

Table A3: Statistical p values from a repeated measures ANOVA using Period 1 soil respiration data from Whitehall Forest (Georgia).

Repeated Measures Results **Period 1** (November 2010- February 2012)			
Habitat	**Treatment Effect**	**Time Effect**	**Treatment x Time**
Forest	0.3874	<0.0001	0.2708
Gap	0.2684	<0.0001	0.7363

Table A4: Statistical p values from a repeated measures ANOVA using Period 2 soil respiration data from Whitehall Forest (Georgia).

Repeated Measures Results **Period 2** (March 2012- October 2013)			
Habitat	**Treatment Effect**	**Time Effect**	**Treatment x Time**
Forest	0.3004	<0.0001	0.2448
Gap	0.2050	<0.0001	0.4636

Table A5: Whitehall Forest cumulative flux (g C m^{-2}) estimated for warmed and ambient plots. Period 1 is over 16 months from November 2010-February 2012 (before +5°C ramp was initiated). Period 2 is over 19 months from March 2012- October 2013.

Habitat	**Treatment**	**Cumulative Flux Period 1**	**Cumulative Flux Period 2**
Forest	Ambient	1137.3 ± 102.1	2087.1 ± 303.4
	+3°C	1470.6 ± 181.3	1749.0 ± 447.7
	+5°C		2583.5 ± 199.2
Gap	Ambient	821.6 ± 24.5	1373.8 ± 63.7
	+3°C	1061.7 ± 111.0	1957.5 ± 264.0
	+5°C		1689.2 ± 236.4

Tables A6: Whitehall Forest seasonal cumulative flux (g C m^{-2}) calculations for Period 1 (November 2010 - February 2012) and Period 2 (March 2012- October 2013). Statistical differences ($p<0.05$) are bolded and listed below:

Period 2: Fall: Gap +3, +5°C > Ambient ($p < 0.10$); Spring: Forest +5°C > +3°C, Ambient ($p < 0.05$); Summer: Gap +3 > Ambient ($p < 0.10$)

Cumulative Flux (g m^{-2})
Period 1 and Period 2

Period	Season	Treatment	Forest	Gap
1	Fall	Ambient	175.95 ± 13.50	122.46 ± 34.79
		+3°C	107.25 ± 43.79	157.38 ± 21.65
		+5°C	---	---
	Winter	Ambient	60.22 ± 9.86	34.50 ± 6.52
		+3°C	93.11 ± 23.20	43.62 ± 4.93
		+5°C	---	---
	Spring	Ambient		
		+3°C		
		+5°C	---	---
	Summer	Ambient	303.57 ± 39.81	237.22 ± 3.92
		+3°C	367.44 ± 44.56	289.10 ± 23.13
2	Fall	Ambient	217.87 ± 36.04	**111.41 ± 5.08**
		+3°C	164.33 ± 25.24	**156.34 ± 10.83**
		+5°C	214.00 ± 21.04	**181.53 ±17.59**
	Winter	Ambient	105.99 ± 25.98	70.20 ± 23.23
		+3°C	108.24 ± 32.61	90.25 ± 29.14
		+5°C	181.38 ± 49.56	100.87 ± 26.71
	Spring	Ambient	**181.53 ± 17.59**	136.96 ± 20.44
		+3°C	**168.65 ± 22.55**	223.49 ± 65.52
		+5°C	**259.04 ± 8.21**	189.69 ± 24.95
	Summer	Ambient	419.21 ± 65.84	**269.24 ± 36.41**
		+3°C	327.74 ± 45.40	**413.92 ± 50.12**
		+5°C	461.93 ± 47.99	**300.96 ± 47.86**

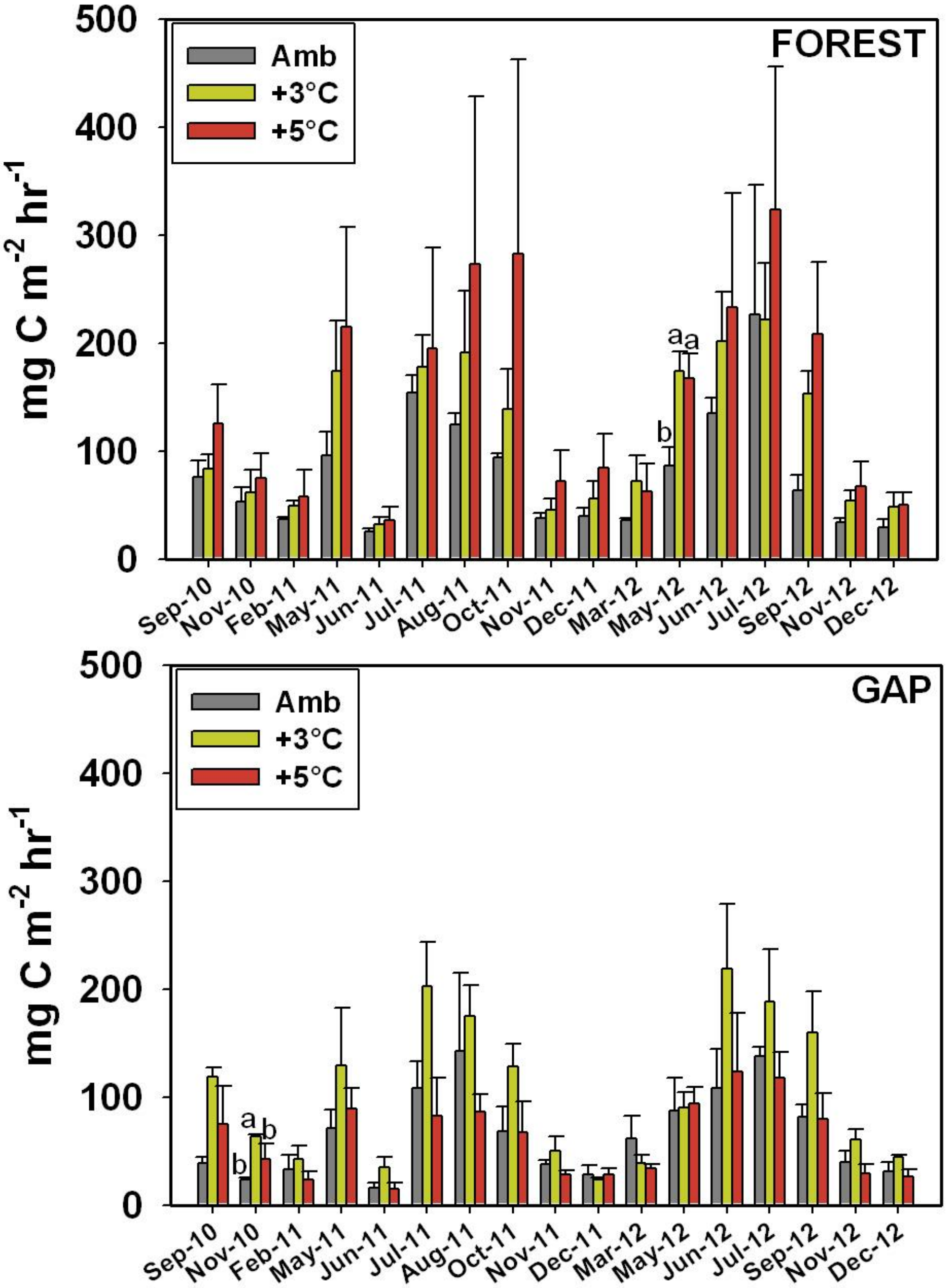

Figure A1: Duke Forest Respiration, North Carolina taken in both Forest (top panel) and Gap (bottom panel) during year three of warming manipulation.

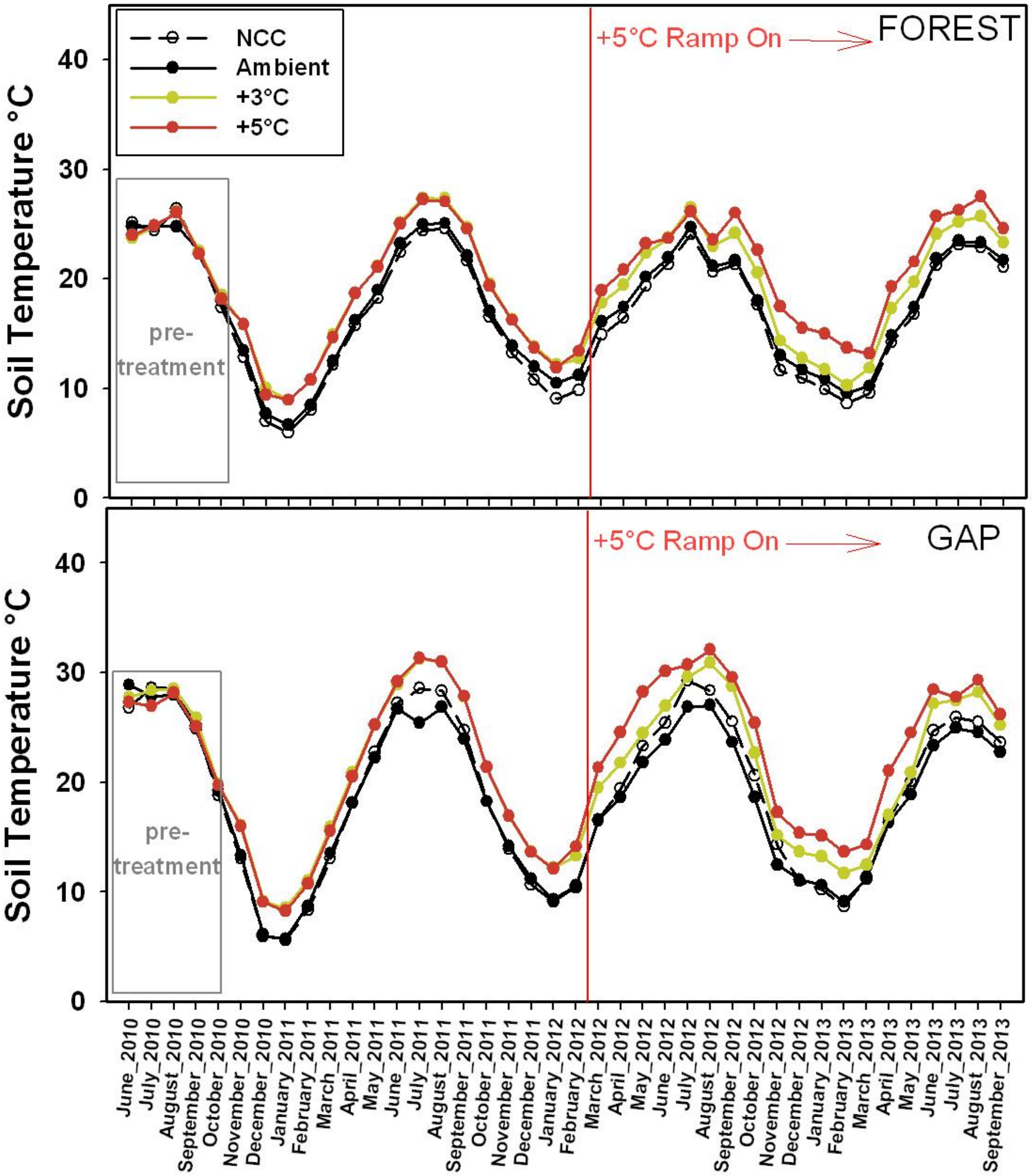

Figure A2: Soil temperatures in warmed and non-warmed treatment plots in both forest (top panel) and gap (bottom panel) habitats at Whitehall Forest. NCC is the disturbance control plots.

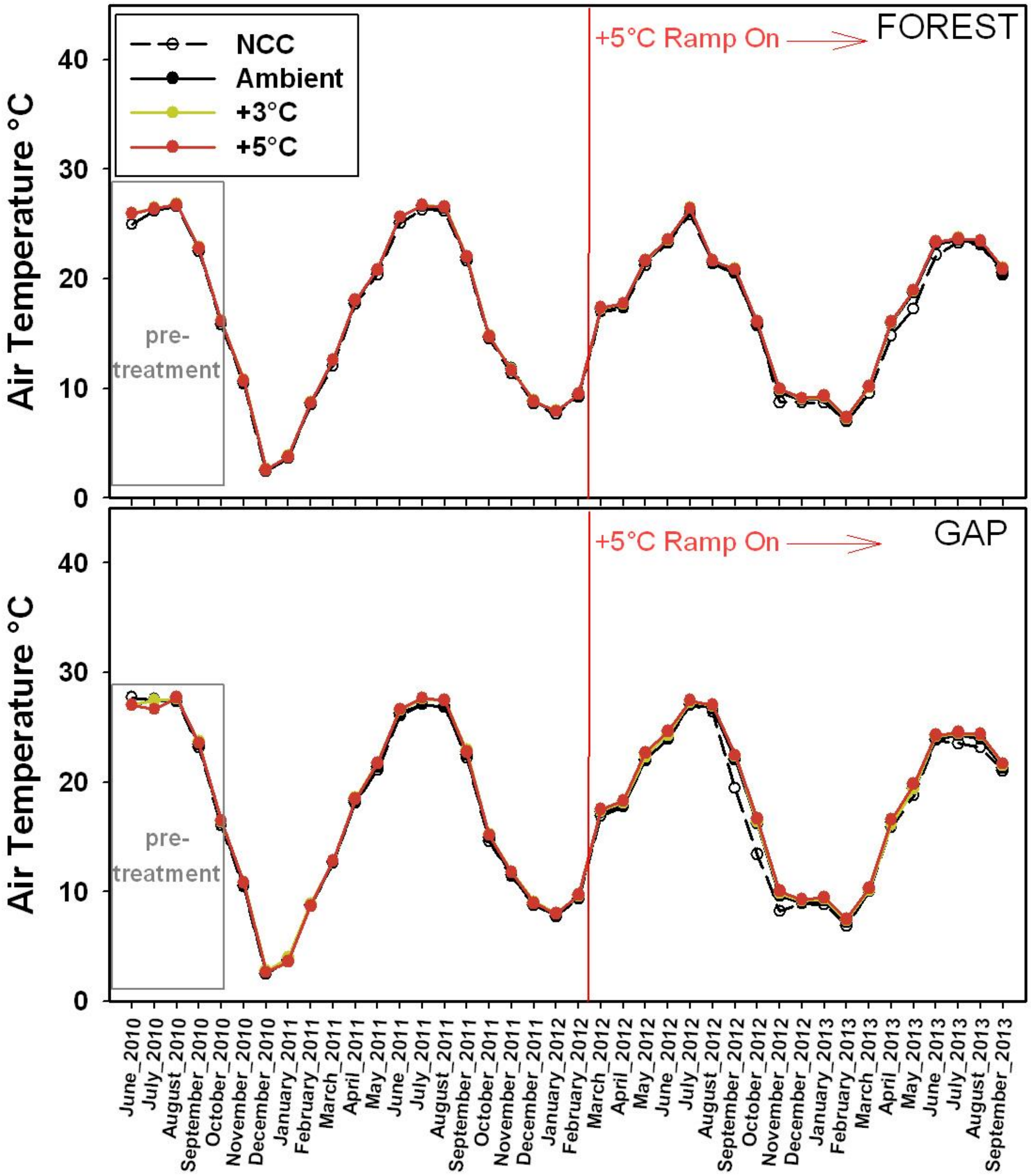

Figure A3: Air temperatures in warmed and non-warmed treatment plots in both forest (top panel) and gap (bottom panel) habitats at Whitehall Forest. NCC is the disturbance control plots.

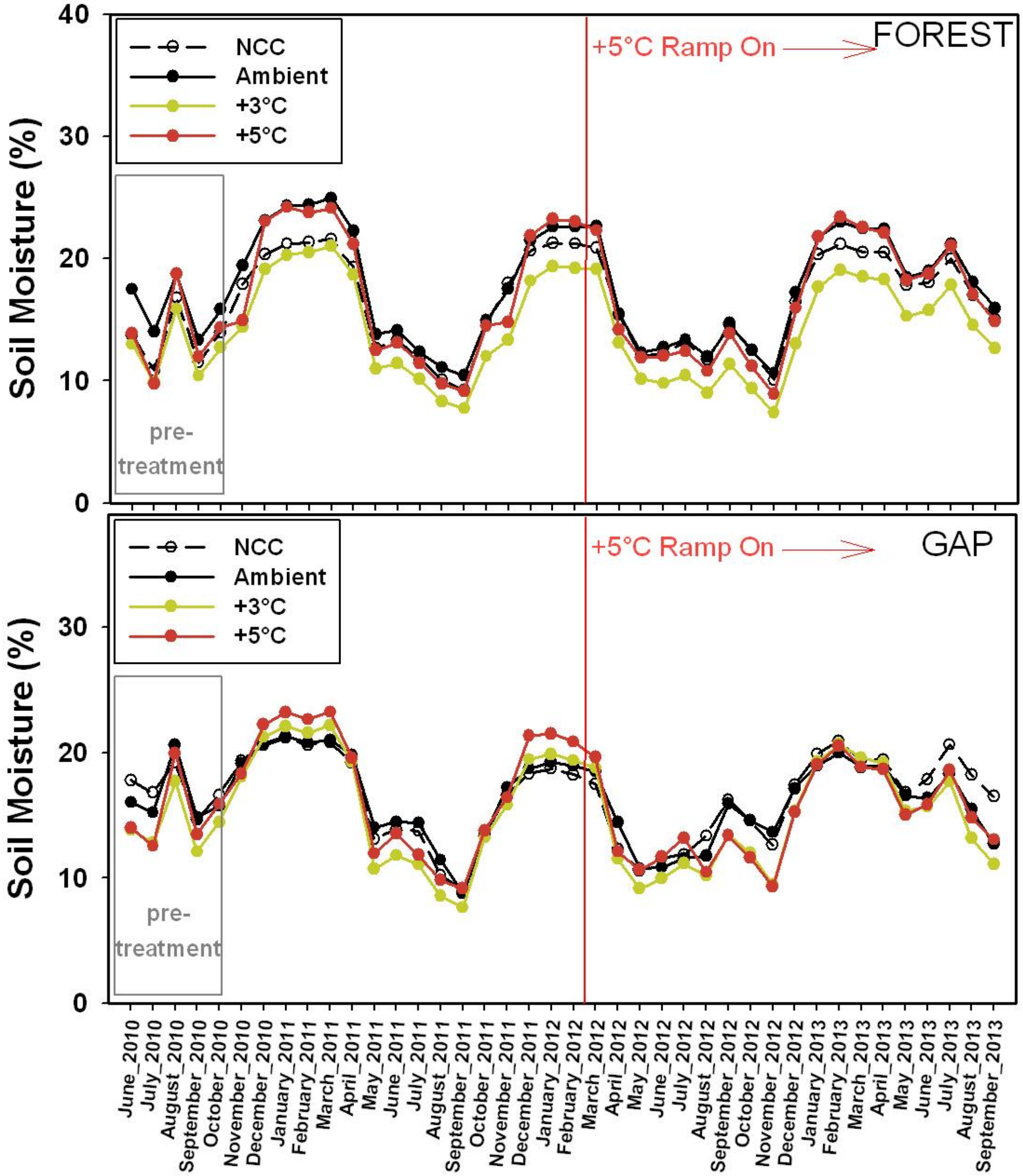

Figure A4: Soil moisture in warmed and non-warmed treatment plots in both forest (top panel) and gap (bottom panel) habitats at Whitehall Forest. NCC is the disturbance control plots.