View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by The University of North Carolina at Greensboro

INCREASED RATES OF SOIL RESPIRATION AND MICROBIAL ACTIVITY UNDER TWO COMPLIMENTARY EXPERIMENTAL WARMING METHODS: IMPLICATIONS FOR CARBON-CLIMATE FEEDBACKS

A Thesis
by
TIMOTHY GORDON WHITBY

Submitted to the Graduate School
Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

December 2011
Department of Biology

INCREASED RATES OF SOIL RESPIRATION AND MICROBIAL ACTIVITY UNDER TWO COMPLIMENTARY EXPERIMENTAL WARMING METHODS: IMPLICATIONS FOR CARBON-CLIMATE FEEDBACKS

A Thesis
by
TIMOTHY GORDON WHITBY
December 2011

APPROVED BY:

____
Michael D. Madritch
Chairperson, Thesis Committee

____
Howard S. Neufeld
Member, Thesis Committee

____
Ray S. Williams
Member, Thesis Committee

____
Steven W. Seagle
Chairperson, Department of Biology

____
Edelma D. Huntley
Dean, Research and Graduate Studies

Copyright by Timothy Gordon Whitby 2011
All Rights Reserved

FOREWORD

The two experiments detailed Chapter 2 and 3 of this thesis are intended to be submitted to the Journal of Soil Biology and Biochemistry, a peer-reviewed journal. The thesis has been prepared in accordance with the submission guidelines for this journal. Chapter 1 gives an introduction to the field of soil warming research and places the two experiments into context.

ABSTRACT

Anthropogenic climate change is expected to increase global temperatures and potentially increase soil carbon (C) mineralization, leading to a positive feedback between global warming and soil respiration. Forests contain large stores of soil C that may be respired, but forest soils also have great potential to store C. The interaction between above- and belowground C fluxes is not well understood, but important controlling factors for both soils and plants are moisture content, community composition, nutrient quality and quantity, and current climate conditions. Soil responses to warming have been studied for some time in an effort to elucidate mechanistic relationships between the structural complexity, or recalcitrance, of C and the temperature sensitivity of its decomposition. Some studies have indicated that there is no difference between the temperature sensitivity of labile and recalcitrant C, while others have indicated that recalcitrant C decomposition will increase more than will decomposition of labile C under climate change. As a result of anthropogenic climate change, soils have been shown to lose C and trees have been shown to incorporate more C into their woody tissues; therefore the future increase or decrease in either C flux is important to accurately predicting climate change. Some warming studies attempt to determine ecosystem responses by preserving natural interactions of abiotic and biotic components, and often the response to warming can be system dependent. Studies must typically make a trade-off between the scope of the experimental treatment and statistical power. High replication often means over-simplifying variables found naturally, while

large scale warming observations may only be a case-study. One approach that can balance these two shortcomings is using a natural temperature gradient provided by elevation or latitude, where soils can be warmed under natural field conditions with relatively high replication and low effort.

I employed two complimentary studies to investigate how soils will respond to future climatic warming. Both studies focused on soils along elevation gradients in the southern Appalachian Mountains; one was a laboratory microcosm experiment, while the second was a field mesocosm experiment. Both studies maintained the integrity of the soil horizons and leaf litter layer and subjected soils to realistic temperature increases. The microcosm study demonstrated that soils from lower native temperatures respired more in response to warming than did soils from high native temperatures. The mesocosm study also employed intact soil cores, but were much larger (29000 cm^3) than what are typically used. These larger cores were able to accommodate a tree sapling to estimate the C and N sequestration of new growth under simulated warming. A natural temperature gradient along an elevation gradient was used to simulate a 3 °C temperature increase in concordance with climate change scenarios, and this was enough to induce increased respiration from soils that were transplanted from cooler, high elevations to lower, warmer elevations. Plant growth was more correlated to soil nutrient concentrations, and was not increased by either temperature or higher soil respiration rates. Although these were both relatively short-term studies, and atmospheric gases were constant, the increased soil respiration rates shown here suggest that C dense forest soils from cooler climates may contribute to positive climate-carbon feedbacks as the climate warms.

ACKNOWLEDGEMENTS

Funding for this research was provided by Appalachian State University. My adviser Mike Madritch provided guidance with the planning and execution of the experiments detailed in this thesis, as well as substantial comments and revisions on the manuscript. My committee members, Ray Williams and Howie Neufeld, also provided comments and revisions on this thesis and assisted with the experimental and analytical design of the project. Jacqui Bryant and Audrey Brown assisted in data collection and processing samples in the laboratory. Laura Boggess assisted in the field collection at Coweeta as well.

TABLE OF CONTENTS

Abstract v

Acknowledgements vii

Chapter 1: Introduction to experimental warming 1

Chapter 2: Native temperature regime determines soil response to simulated warming 4

Abstract 5

Introduction 6

Methods 10

Results 14

Discussion 17

References 22

Tables 29

Figures 30

Chapter 3: Soil and tree sapling responses to simulated climate change along an elevation gradient: implications for climate-carbon feedbacks 42

Abstract 43

Introduction 44

Methods 49

Results 52

Discussion 57

References 61

Tables 71

Figures 74

References 82

Vita 95

CHAPTER 1

Introduction to experimental warming

Global climate change is predicted to warm the Earth by 3 to 6 °C within the next century, with greater relative warming occurring at high latitudes (IPCC 2007). These currently cold environments contain a higher proportion of carbon in the soils than do soils in warm climates, because low temperatures reduce litter turnover and soil organic matter decomposition rates (Berg 2000, Hobbie et al. 2000, Scowcroft et al. 2000, Garten and Hanson 2006). Soil and litter decomposition has been shown to be either more sensitive (Kirschbaum 1995, Scowcroft et al. 2000, Schindlbacher et al. 2010), equally sensitive, or insensitive (Giardina and Ryan 2000, Davidson and Janssens 2006) to temperature increases when initially incubated at lower temperatures. Studies investigating temperature sensitivity of lower quality substrates, which account for a greater proportion of C stored in cold-climate soils, also found contradicting results of positive, negative, or neutral responses of soil processes to artificial warming (Fang et al. 2005, Davidson et al. 2006, Kirschbaum 2006, Conant et al. 2008, Hartley and Ineson 2008). However, many of the studies on temperature sensitivity do not take into account the effects of the natural soil micro-environment and microbial community, which are often severely disturbed in artificial field and laboratory experimental warming experiments (Bradford et al. 2010, Thomson et al. 2010).

Various experimental approaches have been employed to simulate the effects of global climate change, each with their own strengths and weaknesses. Some studies are

reductionist and examine one process under simulated climate change in order to better understand the mechanistic relationships between ecosystems and climate change, while others try to maximize the realism by incorporating intact ecosystem components (Norby and Luo 2004, Garten et al. 2009). Passive systems attain higher temperatures by preventing energy loss and are powered by solar radiation, while active methods of warming increase the input of thermal energy and require an additional power source. Passive enclosures perform well for relatively little cost in grassland and tundra ecosystems, but would not be effective in forests with closed canopies (Beier et al. 2004, Aronson and McNulty 2009). Active heating with underground cables have been used in forests, but can cause reduced soil moisture that is inconsistent with warming from the atmosphere (Verburg et al. 1999, Aronson and McNulty 2009). Infrared lamps are very similar to actual predicted warming and can accurately maintain a temperature differential for long-term studies, but issues of operational costs associated with any active warming system can prohibit sufficient replication (Aronson and McNulty 2009, Hanson et al. 2011). The methods used in this study address some of the limitations of past incubation and field warming experiments.

To produce an accurate approximation of field responses to warming, intact cores were used in two complimentary experiments. The first study was a microcosm study that incorporated the historical effects that elevation and latitude have on soils, which is reflected in the amount and quality of belowground C stores. By sampling cores along elevation gradients within a larger latitude gradient, results may be more applicable on a regional scale. In a second field mesocosm study, intact soil cores large enough to accommodate a tree seedling were reciprocally transplanted along an elevation gradient

that provided a ~3 °C climate change. To my knowledge, employing mesocosms that are large enough to contain even a single tree is a novel experimental method. In addition, I employed realistic temperature increases that are predicted within the next century (IPCC 2007). In the reciprocal transplant study, warming of soils already at higher temperatures was not possible, but warming microcosms from the same sites in the laboratory can account for this methodological constraint. Combining the two approaches of a controlled laboratory experiment with a reciprocal transplant in the field provides insights towards how intact soils will respond to future climate change.

CHAPTER 2

Native temperature regime determines soil response to simulated warming

For submission to Soil Biology and Biochemistry

Timothy G. Whitby[a]*, and M. D. Madritch[a]

[a]Department of Biology, Appalachian State University, Boone, NC,28608, USA

*Corresponding author. Current Address: Department of Biology 161 Rankin West, 572 Rivers St. Appalachian State University, Boone, NC 28608. Tel.: +1 828 262 3025.

E-mail address: whitbytg@appstate.edu

Abstract

Anthropogenic climate change is expected to increase global temperatures and potentially increase soil carbon (C) mineralization, leading to a positive feedback between global warming and soil respiration. However the magnitude and spatial variability of belowground C cycling is not yet fully understood. Previous warming studies on the temperature sensitivity of C cycling in soils have been inconclusive. Soils that are currently at low temperatures may have a greater potential to release C than those currently at higher temperatures, because of more stored carbon and greater warming projections for high latitudes. I investigated whether more soil C would be respired from low temperature sites with intact soil cores collected from three elevation transects along a latitude gradient in the forests of southern Appalachian Mountains. Microcosms were incubated at ambient, ambient +3 °C, and ambient +6 °C to with diurnal temperature and light regimes that simulated realistic temperature changes likely to occur within the next century. After six months, temperatures for all treatments were increased to simulate annual seasonal variation. High temperature treatments increased the average soil respiration as well as the proportion of available soil C that was being respired (respiration/soil C). Average respiration was highest for microcosms containing soils that originated from low native temperature sites for simulated fall and summer incubations. However, this trend was strongest during the simulated summer incubation, after much of the labile C in the soil had already been mineralized. Respiration/soil C was highest for soils from high native temperatures. Increased nitrogen (N) mineralization and microbial enzyme activity in soils from high native temperatures shown here have the potential to mitigate soil C losses by increasing allocation of C into plant and microbial biomass.

However, this effect may not be sufficient to offset the larger potential losses of C in soils from low native temperature sites. This suggests that soils from low native temperatures have a greater potential to release C over time, since C stocks in these soils are not depleted as quickly as are carbon stocks in soils with high native temperature sites. Increased temperature-induced respiration combined with large soil carbon stocks and low N mineralization rates may make soils from low native temperatures regimes more likely to further increase atmospheric carbon dioxide levels.

1. Introduction

Forests ecosystems account for approximately half of the Earth's terrestrial surface and their responses to increased temperature are of great concern (Dixon et al. 1994). The amount of carbon dioxide (CO_2) respired from all soils is over 11 times larger than the CO_2 pumped into the atmosphere via anthropogenic processes (Bader and Korner 2010) and forests account for approximately 40 % of global soil C (Dixon et al. 1994). Increasing global temperatures can induce greater soil respiration (Bond-Lamberty and Thomson 2010), and the potential for a positive feedback between soil carbon (C) release and temperature remains unclear (Campbell et al. 2009, Bader and Korner 2010). The fate of soil C is therefore of paramount importance for projected climate change scenarios. The distribution of this C stored in soils will also affect potential mineralization of soil C since climate change is variable at regional scales (CCSP 2007, Christensen et al. 2007). The objective of this study is to determine whether the temperature dependent responses of soil respiration and belowground nitrogen cycling are influenced by regional and local variation in edaphic factors.

Within the United States, forest ecosystems cover the most area and contain the most C dense soils in the biologically active layers relative to other land use types (Guo et al. 2006). Multiple C pools within soils makes the task of predicting responses to atmospheric warming difficult. Carbon inputs to soils from plant litter are comprised of simple, easily-decomposed substrates (labile C) as well as complex, structural molecules that are not easily degraded (recalcitrant C). Many functional and simulation models simplify soil C into two or three pools, with varying turnover times ranging from years to millennia (Paustian et al. 1997, Falloon et al. 1998, Tague and Band 2004, Zhang et al. 2007). In reality there is a continuum of soil C recalcitrance, and the proportion of recalcitrant C increases over the course of decomposition during the process of humus formation (Berg 2000). Humus accumulation is often associated with climatic regimes that have short growing seasons and colder temperatures, such that these ecosystems accumulate C in recalcitrant pools. Since increases in latitude or elevation generally decrease mean annual temperature, forests at high elevations/latitudes contain more stored C than forests lower in the gradients do (Dixon et al. 1994, Garten and Hanson 2006, Griffiths et al. 2009).

With varying amounts and qualities of soil C across large ranges in mean annual temperature, it is important to know how different pools of C in different climates will respond to warming. An early meta-analysis suggested that the Q_{10} for decomposition, a change in reaction rate standardized to a 10 ^{o}C increase, was greatest at low temperatures and decreased exponentially with increasing temperature (Kirschbaum 1995). However, Katterer et al. (1998), found an average Q_{10} value of ~2 was suitable for the temperature sensitivity of decomposition when incubated at 5 -35 °C, but conceded that the Q_{10}

function may not be suitable outside of this range in temperatures. Furthermore, Giardina and Ryan (2000) found that mineral soils from 82 different sites from five continents had the same turnover times when incubated together in the lab at 5, 20, and 35 °C. Fang et al. (2005) also showed that the Q_{10} of soil respiration did not change over time in soil incubation, suggesting that labile C, which is depleted early, has a similar response to warming as does recalcitrant C. These data suggest that there should be little reason to expect any positive feedbacks from soils that are found in cold climates. However, there remains some uncertainty regarding the issue of soil C sensitivity to warming, and more recent studies have supported the theory that recalcitrant C pools will be more sensitive to warming than will labile C pools. When soils were incubated at constant temperatures, Conant et al. (2008) found that the Q_{10} increased with incubation duration. As incubation time increased, more labile C was mineralized; therefore an increasing Q_{10} value can be attributed to the recalcitrant C remaining in the soils. Similarly, Hartley and Ineson (2008) manipulated the labile fraction of C in soils by freezing soils and thawing sub-samples at 5-week intervals until all the soils were thawed. Soils that had been thawed for longer periods of time had less labile C, and these soils displayed an increase in the temperature sensitivity of soil respiration with time since thaw (Hartley and Ineson 2008). However, the magnitude of their experimental warming may be far beyond what soils experience under climate change predictions, and may also be unrealistic because substrate availability to microbes was inherently altered when subjected to sieving.

Few of the aforementioned studies have incorporated measures of microbial community dynamics, and recent focus has shifted away from intrinsic temperature

sensitivity to apparent temperature sensitivity that is more dependent on physical soil characteristics and field conditions that may better reflect actual ecosystem responses to temperature change (Davidson et al. 2006, Kirschbaum 2006). In addition, mean annual temperature is only one factor that influences the quality of soil C. Other fine-scale variables such as plant species identity or gross primary production can influence C turnover time (Knorr et al. 2005). Some of the discrepancies between studies could also be an artifact of experimental designs that disturb soils greatly through homogenization, especially when soils are sieved and/or divided into fractions. Using sieved soils is common practice because it homogenizes the soils and removes rocks and roots. However, sieving simplifies the soil matrix, making physically protected substrates in the soil more accessible to microbial decomposition, and altering the microbial community (Thomson et al. 2010). Although the results of past experiments are extremely useful, changes in substrate availability that are altered by homogenization of soils should be addressed to better approximate actual ecosystem responses with intact soils.

A potential limitation of some previous studies of temperature sensitivity of C decomposition is that they all used homogenized soils and employed time to alter the amount of recalcitrant C present (Conant et al. 2008, Hartley and Ineson 2008). Natural variations in soil C quality along environmental gradients have been used to better approximate *in situ* responses of soil C, but have not maintained the soils completely intact (Niklinska and Klimek 2007, Zimmermann et al. 2009). My study was designed to better approximate field conditions within soils by using relatively large intact cores that also included the leaf litter layer. Although the potential variation between samples was increased with this microcosm approach, it may have provided more realistic outcomes

than would well-mixed soils, since sieving disturbs the natural factors that influence substrate availability, such as water filled pore space, soil aggregates, and bulk density to name but a few (Hartley et al. 2007) . By collecting soil cores from a large latitude and elevation range, I was able to address regional scale variation in native soil temperatures. My goal was to determine whether rates of C and N cycling in soils from different native temperature regimes, and with inherently different substrate qualities, have different sensitivities to experimental warming.

2. Methods

2.1Site description

Soils were sampled along three elevation transects in the southern Appalachian Mountains of North Carolina spanning most of the north-south range within the state (~ 135 km). Three different elevation sites (high, medium, and low) were chosen along each transect, with differences in aspect minimized within each transect. The southernmost site was within the Coweeta Hydrologic Laboratory in Otto, NC (USDA – USFS), and contained the greatest elevation range (~ 700 m, Table 1). The next largest range (~ 450 m Table 1) was in the Pisgah National Forest in Avery County, and the final and northernmost site was on Appalachian State University's Gilley Field Station in Watauga County, containing the smallest elevation range (~ 200 m, Table 1).

2.2 Field collection

PVC tubes 10.3 cm in diameter were inserted into the ground to a depth of 15 cm. Due to the brittle nature of the PVC cores I necessarily avoided large roots or rocks, which would have encroached upon the amount of soil within each core and added large amounts of C for decomposition in the form of severed roots. I allowed severed fine

roots to remain within cores. Soil cores were then carefully excavated from below in order to retain material upon extraction, and capped at both ends for transport to the laboratory. Six cores were randomly excavated from each elevation at each site, for a total of 54 cores. Hobo data loggers (Onset Computer Corp., Bourne, MA, USA) were placed in the native soils to 10 cm depth before or at the time of collection at each site and logged hourly. Soil temperature data were compiled to characterize the native temperature regimes of each collection site.

In order to standardize starting conditions, cores were stored at 4 °C for up to 14 days until all cores were collected. Leaf litter mass was standardized among all cores to a mean value of approximately 0.7 g to normalize C inputs into soils during the incubation period. Litter type varied among sites, but all were mixed deciduous stands. The initial mass of litter within each microcosm was compared to the final mass at the end of the incubation period to determine the rate of litter decomposition.

2.3 Laboratory incubation

Three incubators (I-36LL, Percival Scientific Inc., Perry, IA, USA) were set on a diurnal light and temperature regime that was consistent with field conditions reported by NOAA for day length and by soil data loggers at the Pisgah site for temperature (mid-latitude, average of elevations). From this starting value, the other two incubators were increased by 3 °C increments to reflect an average and extreme warming scenario for the next century (IPCC 2007). Two replicates of each site-elevation combination were randomly assigned to each temperature; 10, 13, or 16 °C. Soils were kept moist with DI-H_2O evenly among all cores, and the specific amount was adjusted as needed to approximately maintain field moist conditions. Soil moisture was measured using a

Hydrosense probe (Campbell Scientific, Inc. Logan, Utah, USA) with 10 cm long probes. After six months (182 days) at the initial temperatures (fall), incubators were warmed by 14 °C (summer) so that the cores were at 24, 27, and 30 °C for an additional 4 months for a total incubation time of 10 months. The diurnal temperature and light cycle was adjusted to summer conditions as stated above. Incubators remained at this temperature for another three months. At three times, after 0, 124, and 304 days of incubation, subsamples of the upper 10 cm were collected with a 1 cm diameter soil corer. The corer was passed through the litter layer and the resulting leaf disks were separated from the soils for separate analyses. Soil and litter samples were freeze-dried and ground to a fine powder for C and nitrogen (N) determination by flash combustion in a Flash EA 1112 NC analyzer (Thermo Fischer Scientific, Delft, The Netherlands) .

2.4 Microbial enzyme assays

I measured microbial extracellular enzyme activity (EEA) by colorimetric reaction based on Madritch et al. (2007). I assayed three enzymes, cellobiohydrolase (CB), β-glucosidase (BG), and leucine aminopeptidase (LA). These enzymes degrade cellulose (CB and BG) and amino acids (LA) and their activity can reflect microbial allocation to C and N acquisition, respectively (Allison et al. 2008). I extracted ~1 g soil samples in 15 mL of 5 mM acetate buffer, and duplicate aliquots of 400 µL of extract were given 100 µL of substrate. I determined enzyme activity after two hours by fitting results to a p-nitrophenol standard curve. Relative change in activity over time ((Final – Initial) / Initial) was used to measure the microbial response to warming during the overall incubation. I measured N mineralization by comparing ammonium concentration

of the soils in the initial and final soil samples using the sodium salicylate/sodium dichloroisocyanurate method also described in Madritch et al. (2007).

2.5 Respiration measurements and analysis

Soil CO_2 flux (respiration) was measured with a Li-8100 automated soil CO_2 flux system with a 10 cm chamber (LI-COR Biosciences, Lincoln, NE, USA). Measurements were initially taken three times per week, and then incrementally reduced to once per month after prolonged duration of incubation to capture the most variation as microbial respiration declines exponentially with time (Bradford et al. 2008). On days when respiration measurements were taken, cores were not watered until after the respiration was measured since wetting events have been shown to cause a peak in heterotrophic respiration of soils (Chatterjee and Jenerette 2011). After each measurement, cores were rotated by one row and column within the incubator, and rotated to another incubator every month to avoid incubator effects.

2.6 Statistical analysis

Statistical analyses were performed in JMP (v9.0.0 SAS Institute Inc., Cary, NC, USA) using simple linear regressions. Using the average temperature over a one-month period at the end of the data logger record, I calculated the native temperature for all 9 sites. Experimental responses were regressed with either elevation or native temperature as the main effects, and relationships were considered significant at $p < 0.05$ or marginally significant at $p < 0.1$. I include the marginally significant category since low replication may limit the significance of a trend, but it is likely that the observed trend is still biologically significant. When effects were significant, I performed a Tukey test to determine which means were different within each effect. I transformed data as

necessary to meet the assumptions of normality. Untransformed data are presented in figures; however the interpretations and conclusions are based on statistical results, not necessarily graphical representations.

3. Results

Variation in the initial state of the soils from each site was best explained by native temperature and not site elevation since latitude also affects native temperature. C and N concentration of the upper 10 cm of soil within the 15 cm microcosms decreased with the native temperature of the collection site (Fig. 1A, B). The C:N ratio of the soils was also influenced by the native temperature; the ratio dropped by approximately 33 % over the 4 °C span in native temperatures (Fig. 1C). Bulk density of the soils increased linearly with native temperature as well (Fig. 1D). Therefore, as native temperatures rose, organic matter content was depleted, which increased the concentration of the heavier mineral content of the soils as well as reducing air space. The leaf litter C reflected the same trend as soil C (r^2=0.09, p = 0.0275) but litter N and C:N were not correlated with native soil temperature (data not shown).

Respiration from the microcosms increased with incubation temperature over the entire duration of the incubations (r^2=0.12, p=0.0112 data not shown). Respiration declined with increasing native temperature over the entire course of the incubation experiment, as well as for the fall temperature regime, and most strongly during the summer temperature regimes (Fig. 2A, E, I, respectively). In low temperature incubation treatments, the respiration decreased more strongly with increasing native temperature during the summer incubation (Fig. 2J) than during the fall incubation (Fig. 2F), primarily because there was a greater relative increase in respiration of microcosms that

were taken from low native temperatures during the summer incubation (Fig. 2J). Among incubation temperature treatments, increased variability during fall temperature incubation precluded any apparent decrease in respiration of soils from high native temperature (Fig. 2F-H). However, soil respiration decreased with native temperature of soils for all three temperature treatments during the summer incubation period (Fig. 2J-L).

Overall, respiration/soil C was greater for soil cores from warmer native temperatures, and this relationship had a greater r^2 during the fall temperature regime (Fig. 3A, E). Under summer incubation conditions, this positive trend is only present at the lowest temperature (24 °C) incubation treatment (Fig. 3J). The lack of a relationship at the two greater incubation temperature regimes could suggest a shift in the effect of native temperature as soils are warmed (Fig. 3F-H,J-L). Incubation temperature increased respiration/soil C for the average of all cores regardless of native temperature, and this effect was consistent among total, fall, and summer incubation temperatures (Fig. 4A- C). A greater respiration/soil C indicates that more of the available substrate was being used for microbial metabolism when cores were incubated at higher temperatures in the laboratory. Average soil respiration showed no relationship with elevation (data not shown); however, respiration/soil C was less at higher elevations on average across both time periods (Fig. 4D). The strength of this relationship was low, and may be driven primarily by the Coweeta high elevation site at the final incubation period at summer temp (Fig. 4F). The contrasting trends with respect to elevation and native temperature is likely due to the fact that elevation is confounded with latitude between transects with similar elevations.

Litter decomposition increased with incubation temperature (Fig. 5). Litter decomposition was also greatest in microcosms from high native temperatures across the entire incubation period (Fig. 6A). At low incubation temperatures decomposition seemed to operate on a basal level regardless of native temperature (Fig. 6B), but as the incubation temperature increased litter from higher native temperatures decomposed much more than litter from lower native temperature (Fig. 6D). This proportional decrease in mass remaining corresponds with lower C content of the litter from high native temperature sites. Nitrogen mineralization rates over the course of the entire incubation period were also positively correlated with the native temperatures of the soil cores (Fig. 7A-D). However the rate of mineralization did not seem to respond to incubation temperature, because the slope and intercept of the regression were fairly uniform in all incubators.

Initial enzyme activity for all three enzymes was greater for soils from cold native temperatures when data from all three incubation temperatures were pooled together (Fig. 8A-C). However, the final EEA values for all cores were the same across all native temperatures (data not shown). This was attributed to a loss of EEA at low native temperatures, but an increase in EEA for high native temperatures (Fig. 9A,E). However, at the intermediate temperature treatment (13, 27 °C), it was unclear why the change in EEA did not follow the same trend as when all incubation temperatures were pooled (Fig. 9C,G). The x-intercept of the trend line shifted left when incubation temperatures went from low to high, indicating that warming can help maintain greater EEA even in low native temperature cores (Fig. 9B,D,F,I). Unlike these two enzymes, leucine aminopeptidase showed no consistent trend with native temperature (data not shown),

indicating that microbial responses were driven by C and not N budgets (Allison and Vitousek 2005).

4. Discussion

I have shown that carbon dense soils originating from sites with lower native temperatures may release more carbon to the atmosphere under projected warming due to global climate change. Intact soil cores from a varied spatial scale in the southern Appalachians were sensitive to increased temperatures that are predicted for the end of the century (Meehl et al. 2007). Respiration in microcosms with soil cores from sites with low native temperatures was more sensitive to warming, as shown in the increased respiration rates after at the onset of summer incubation temperatures. In addition to the increased respiration response, larger stores of C within soils from low native temperatures may prolong increased respiration in comparison to soils from higher native temperatures. Potential for greater C loss is also reflected by low respiration/soil C in soils from sites with low native temperature, which indicates that less of the available substrate was being mineralized over the course of the entire incubation. The combination of increased average respiration and greater amount of available C will likely increase the amount of C released to the atmosphere under warmer climates. Positive feedbacks from this increased sensitivity of soils to warming are also possible if more recalcitrant C stocks in soils from low native temperature sites are also more sensitive to an increase in surface air temperature (Knorr et al. 2005, Davidson and Janssens 2006, Bonan 2008, Conant et al. 2008, Hartley and Ineson 2008).

Soil respiration/soil C for summer temperatures only showed a weak positive trend in the coldest of the three temperature treatments, but high variation and low

replication precluded finding any significant trends in the other two higher temperature treatments. However, there appeared to be a shift in the relationship of respiration/soil C to native temperature from positive to neutral or negative as incubation temperature increases. If soils from low native temperatures were beginning to respire as much or more of the available C as the soils from higher native temperatures were, the loss of C from low temperature environments could be accelerated (Knorr et al. 2005). The cause of the loss of a positive trend with increased incubation temperature was unclear; however, changes in substrate availability over time may be an important driver of the observed increase in soil respiration as the native temperature of the soils declined.

Other research on the temperature sensitivity of soil respiration along environmental gradients is not in concordance with my results, but the lack of concordance may be caused by different methodological approaches and not the soils themselves. Schindlbacher et al. (2010) found that high elevation soils from Austria and Spain were not more sensitive to warming than low elevation soils at each respective site, but respiration measurements were carried out over the course of only two or three days of incubation. Also a short, 15-day incubation time prior to experimental warming may not have been adequate to allow labile C to be respired (Schindlbacher et al. 2010). In contrast, the initial incubation temperatures I employed persisted for 182 days of incubation, giving sufficient time for labile C to be respired. Furthermore, Niklinska and Klimek (2007) also did not observe a change in Q_{10} values for soil respiration rates collected from elevation transects on four different mountains. However, the C:N ratio of the soils was not different among various elevation sites, and the total incubation time was only 6 weeks (Niklinska and Klimek 2007). Therefore, the observed Q_{10} values of

the two previous studies may not have reflected the mineralization of recalcitrant soil C, because the higher respiration rate of labile C may overwhelm the recalcitrant C signal. In addition, elevation may not always be directly correlated with native temperature regime if sites are also spread over a latitude gradient. Therefore, when interpreting studies of soil carbon sensitivity to warming it is important to distinguish between the native temperature of soils and the substrate quality, since both of these factors may interact to produce the observed effects of apparent temperature sensitivity.

In addition to apparent temperature sensitivity, the possibility of soil C feedbacks with temperature increases may also be influenced by concomitant changes in the amount of C sequestered by the forest's plant community (Bonan 2008). Similar to respiration/ soil C, N mineralization rates also increased with native temperature. Increased available NH_4^+ in soils from high native temperatures could account for the observed increase in respiration/soil C and plant litter decomposition, if reduced N limitation allowed microbes to allocate more resources to C acquisition (Allison et al. 2007, Sinsabaugh et al. 2008). In addition to this potential microbial fertilization effect, plant species at high native temperature sites may also be fertilized by increased NH_4^+ availability and increased C uptake into biomass (Ineson et al. 1998). Tree growth and physiology at high elevations is limited by lower temperatures and the length of growing season (Bresson et al. 2011), and the lower rates of N mineralization observed at low native temperatures would also limit the fertilization effect. Others have demonstrated that N mineralization rates decrease with elevation if corrected by soil N content, but N mineralization was controlled by soil moisture and substrate quality more so than by elevation (Powers 1990, Knoepp and Swank 1998). Generally, rainfall increases with

elevation in the Southern Appalachian Mountains, and soil moisture positively influences N mineralization rates (Garten and Hanson 2006, Knoepp et al. 2008). In the microcosms, the moisture regime was held constant among replicates, and substrate quality increased with native temperatures. Therefore, when lower quality substrates at low native temperature sites are warmed without increased moisture, N mineralization may be reduced, which could potentially limit CO_2 fertilization of plant growth.

The quality of substrates and the availability of limiting nutrients along the native temperature gradient may have also influenced microbial extracellular enzyme activity (EEA). The initial enzyme activity in soils from low native temperatures sites was lower than that of soils from high native temperature sites, and this coincides with an increase in the C:N ratios of the soils. Previous research has shown that enzyme production can be induced when the target substrate is limiting microbial metabolism (Allison et al. 2007). The change in EEA over time is also consistent with the resource allocation theory, where an up-regulation of enzyme production to degrade complex substrates when additional nutrients are readily available (Allison and Vitousek 2005). Even in the presence of larger concentrations of complex substrates in low native temperature soils, enzyme activity decreased over time. However, enzyme activity in soils from in high native temperature sites increased despite lower soil C concentrations. The greater N availability from increased mineralization rates in soils from high native temperature sites can induce carbon limitation of microbial growth since substrate acquisition of C and N in microbes is approximately 1:1 globally (Sinsabaugh et al. 2008). Allison and Vitousek (2005) found that although EEA increased with the addition of nitrogen and complex carbon source to soil microbes cumulative respiration over the course of the incubation

decreased. Similar substrate and readily-assimilated nutrient conditions in high native temperature soils may account for the high ΔEEA but lower respiration. However, the cause of the microbial response is not certain because microbial biomass or community composition was not measured here.

Microbial enzyme activity at the final sampling period was similar amongst all cores, and this convergence to a common value may have been caused by changes in the microbial community. In alpine soils, EEA was more temperature sensitive when collected in the winter relative to spring and summer collections (Koch et al. 2007). Greater temperature sensitivity of the enzyme activity in low native temperature soils is somewhat evident in the shift of negative ΔEEA at low incubation temperature to a positive ΔEEA for the high incubation temperature. Greater temperature sensitivity of the EEA of low native temperature soils may be explained by microbial acclimation to the incubation temperatures, and not necessarily a compositional shift. Microbial communities native to colder temperatures have been shown to increase their respiration rate per biomass more than those that were native to warmer temperatures when exposed to hotter conditions (Bradford et al. 2010) which is in concordance to the greater rate of respiration of low native temperature soils observed here. The increase in respiration despite EEA decreasing would suggest that the microbial biomass was not substantially reduced if at all, since decreases in microbial biomass are correlated with lower respiration rates (Bradford et al. 2008). Also, the loss of labile C in the soils could have reduced the observed EEA of low native temperature soils, since microbes would be more limited by lower substrate quality at later time periods in the incubation (Bradford et al. 2010). Although the effects of shifting microbial communities cannot be ruled out,

all of the observed changes in respiration and EEA may be explained by other factors of microbial acclimation, resource quality, and substrate availability.

At regional scales, historical temperature regimes may determine the magnitude of temperature-induced soil microbial respiration in response to climate change. Although elevation generally correlates with temperature, if latitude changes in temperature are present between multiple sites, then the more direct measurement of native temperature should be used to remove confounding effects. Temperature induced changes in N mineralization and microbial activity may mitigate some losses of C through increased allocation to biomass of plants and microbes through N fertilization, but mitigation may be predominantly in soils from high native temperatures. Nonetheless, respiration from soils was more sensitive to warming in cores from low native temperatures. Higher respiration rates combined with larger C stores, and decreased N mineralization rates, make historically colder soils along mountainsides a greater potential source of C release to the atmosphere and will likely contribute to positive feedbacks with atmospheric warming.

References

Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14, 1156-1168.

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

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along

carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biology & Biochemistry 39, 1770-1781.

Bader, M.K.F., Korner, C., 2010. No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Global Change Biology 16, 2830-2843.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133, 13-22.

Bonan, G.B., 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 320, 1444-1449.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 464, 579-U132.

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

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

Bresson, C.C., Vitasse, Y., Kremer, A., Delzon, S., 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiology 31, 1164-1174.

Campbell, J.L., Rustad, L.E., Boyer, E.W., Christopher, S.F., Driscoll, C.T., Fernandez, I.J., Groffman, P.M., Houle, D., Kiekbusch, J., Magill, A.H., Mitchell, M.J., Ollinger, S.V., 2009. Consequences of climate change for biogeochemical cycling

in forests of northeastern North America. Canadian Journal of Forest Research 39, 264-284.

CCSP, 2007. The First State of the Carbon Cycle Report (SOCCR): The North American Carbon Budget and Implications for the Global Carbon Cycle. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, NC, USA, 242 pp.

Chatterjee, A., Jenerette, G.D., 2011. Changes in soil respiration Q_{10} during drying-rewetting along a semi-arid elevation gradient. Geoderma 163, 171-177.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Rueda, V.M., Mearns, L., Menéndez, C.G., Rinke, J.R.A., Sarr, A., Whetton, P., 2007. Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89, 2384-2391.

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

Davidson, E.A., Janssens, I.A., Luo, Y.Q., 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. Global Change Biology 12, 154-164.

Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263, 185-190.

Falloon, P.D., Smith, P., Smith, J.U., Szabó, J., Coleman, K., Marshall, S., 1998. Regional estimates of carbon sequestration potential: linking the Rothamsted Carbon Model to GIS databases. Biology and Fertility of Soils 27, 236-241.

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

Garten, C.T., Hanson, P.J., 2006. Measured forest soil C stocks and estimated turnover times along an elevation gradient. Geoderma 136, 342-352.

Giardina, C.P., Ryan, M.G., 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature (London) 404, 858-861.

Griffiths, R.P., Madritch, M.D., Swanson, A.K., 2009. The effects of topography on forest soil characteristics in the Oregon Cascade Mountains (USA): Implications for the effects of climate change on soil properties. Forest Ecology and Management 257, 1-7.

Guo, Y.Y., Gong, P., Amundson, R., Yu, Q., 2006. Analysis of factors controlling soil carbon in the conterminous United States. Soil Science Society of America Journal 70, 601-612.

Hartley, I.P., Heinemeyer, A., Ineson, P., 2007. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. Global Change Biology 13, 1761-1770.

Hartley, I.P., Ineson, P., 2008. Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biology & Biochemistry 40, 1567-1574.

Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E., Woof, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. Global Change Biology 4, 143-152.

IPCC, 2007. Climate change 2007 : the physical science basis : contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Katterer, T., Reichstein, M., Andren, O., Lomander, A., 1998. Temperature dependence of organic matter decomposition: A critical review using literature data analyzed with different models. Biology and Fertility of Soils 27, 258-262.

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

Kirschbaum, M.U.F., 2006. The temperature dependence of organic-matter decomposition - still a topic of debate. Soil Biology & Biochemistry 38, 2510-2518.

Knoepp, J.D., Swank, W.T., 1998. Rates of nitrogen mineralization across an elevation and vegetation gradient in the southern Appalachians. Plant and Soil 204, 235-241.

Knoepp, J.D., Vose, J.M., Swank, W.T., 2008. Nitrogen deposition and cycling across an elevation and vegetation gradient in southern Appalachian forests. International Journal of Environmental Studies 65, 389-408.

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

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

Madritch, M.D., Donaldson, J.R., Lindroth, R.L., 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry 39, 1192-1201.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., 2007. Global Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Niklinska, M., Klimek, B., 2007. Effect of temperature on the respiration rate of forest soil organic layer along an elevation gradient in the Polish Carpathians. Biology and Fertility of Soils 43, 511-518.

Paustian, K., Levine, E., Post, W.M., Ryzhova, I.M., 1997. The use of models to integrate information and understanding of soil C at the regional scale. Geoderma 79, 227-260.

Powers, R.F., 1990. Nitrogen mineralization along an altitudinal gradient - interactions of soil - temperature, moisture, and substrate quality. Forest Ecology and Management 30, 19-29.

Schindlbacher, A., de Gonzalo, C., Diaz-Pines, E., Gorria, P., Matthews, B., Inclan, R., Zechmeister-Boltenstern, S., Rubio, A., Jandl, R., 2010. Temperature sensitivity of forest soil organic matter decomposition along two elevation gradients. Journal of Geophysical Research-Biogeosciences 115.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Tague, C.L., Band, L.E., 2004. RHESSys: Regional Hydro-Ecologic Simulation System-An Object-Oriented Approach to Spatially Distributed Modeling of Carbon, Water, and Nutrient Cycling. Earth Interactions 8.

Thomson, B.C., Ostle, N.J., McNamara, N.P., Whiteley, A.S., Griffiths, R.I., 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83, 69-73.

Zhang, C., Tian, H., Chappelka, A.H., Ren, W., Chen, H., Pan, S., Liu, M., Styers, D.M., Chen, G., Wang, Y., 2007. Impacts of climatic and atmospheric changes on carbon dynamics in the Great Smoky Mountains National Park. Environmental Pollution 149, 336-347.

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., 2009. Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. European Journal of Soil Science 60, 895-906.

Table 1. Soil collection site parameters.

Site	Elevation (masl)	Coordinates
Coweeta	1381	35.032039°N, 83.465392°W
	1189	35.040219°N, 83.460303°W
	702	35.056291°N, 83.432384°W
Pisgah	1146	35.919023°N, 81.888838°W
	917	35.917964°N, 81.895639°W
	701	35.914050°N, 81.901584°W
Gilley	1025	36.290717°N, 81.586530°W
	973	36.290864°N, 81.584395°W
	897	36.291378°N, 81.582769°W

Figure Legend

Fig. 1. Average initial soil characteristics with respect to native temperature of collection site for (A) Nitrogen (%) of upper 10 cm of soil, (B) Carbon (%) of upper 10 cm of soil, (C) C:N ratio of soil, and (D) bulk density. Regression lines are shown if $p < 0.1$ n = 54.

Fig. 2. Average respiration rates of soils with respect to native temperature. Columns are arranged by incubation time period, and rows are arranged by incubation temperature. (A) Soil respiration at average temperature over the total incubation time period, (B) average low temperature, (C) average intermediate temperature, (D)average high temperature, (E) average fall temperature, (F) low fall temperature, (G) intermediate fall temperature, (H) high fall temperature, (I) average summer temperature, (J) low summer temperature, (K) intermediate summer temperature, (L) high summer temperature. Regression lines are shown if $p < 0.1$ n = 54 for average and 18 for each incubation temperature.

Fig. 3. Average respiration rates of soils per initial soil carbon with respect to native temperature. Columns are arranged by incubation time period, and rows are arranged by incubation temperature(A) Soil respiration at average temperature over the total incubation time period, (B) average low temperature, (C) average intermediate temperature, (D)average high temperature, (E) average fall temperature, (F) low fall temperature, (G) intermediate fall temperature, (H) high fall temperature, (I) average summer temperature, (J) low summer temperature, (K) intermediate summer temperature, (L) high summer temperature. Regression lines are shown if $p < 0.1$ n = 54 for average and 18 for each incubation temperature.

Fig. 4. Average soil respiration rate per soil C (%) versus incubation temperature or native elevation. Rows are organized by incubation time period. (A) Average respiration/soil C at all temperatures, (B) fall temperatures, (C) summer temperatures, (D) average respiration/soil C at all temperatures versus site elevation, (E) average respiration/soil C at fall temperatures versus site elevation, (F) average respiration per C at summer temperatures versus site elevation. Solid regression lines are shown if $p < 0.05$ and dotted lines are shown for $p<0.1$ n = 54 for average and 18 for each incubation temperature.

Fig. 5. Percent litter mass remaining after total incubation with respect to fall/summer temperature. Regression lines are shown if $p < 0.1$ n = 54.

Fig. 6. Percent litter mass remaining after total incubation with respect to native temperature at (A) average of all incubation treatments, (B) average of low incubation temperature, (C) average of intermediate incubation temperature, (D) average of high incubation temperature. Solid regression lines are shown if $p < 0.05$ and dotted lines are shown for $p<0.1$ n = 54 for average and 18 for each incubation temperature.

Fig. 7. Net mineralization after total incubation with respect to native temperature at (A) average of all incubation treatments, (B) average of low incubation temperature, (C) average of intermediate incubation temperature, (D) average of high incubation temperature Regression lines are shown if $p < 0.1$ n = 54 for average and 18 for each incubation temperature.

Fig. 8. Initial microbial extracellular enzyme activity relative to native temperature for (A) cellobiohydrolase activity, (B), β-glucosidase activity, and (C) leucine aminopeptidase (C). Regression lines are shown if $p <0.1$ n = 54.

Fig. 9. Change in microbial extracellular enzyme activity relative to native temperature ((final-initial)/initial) for (A) average cellobiohydrolase (CB) activity for all incubation treatments, (B) at low temperature, (C) at intermediate temperatures, (D) at high temperatures, and (E) average β-glucosidase (BG) activity for all incubation treatments (F), at low temperature, (G) at intermediate temperatures, (H), at high temperatures. Solid regression lines are shown if $p < 0.05$ and dotted lines are shown for $p<0.1$ n = 54 for average and 18 for each incubation temperature.

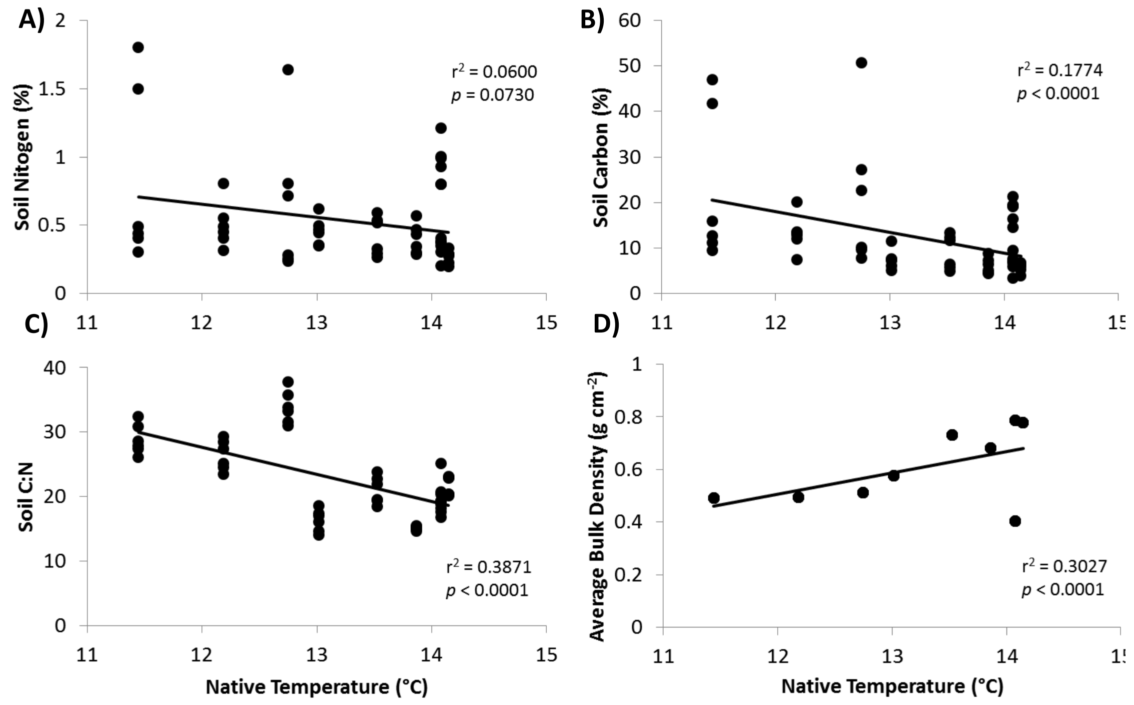


Fig. 1

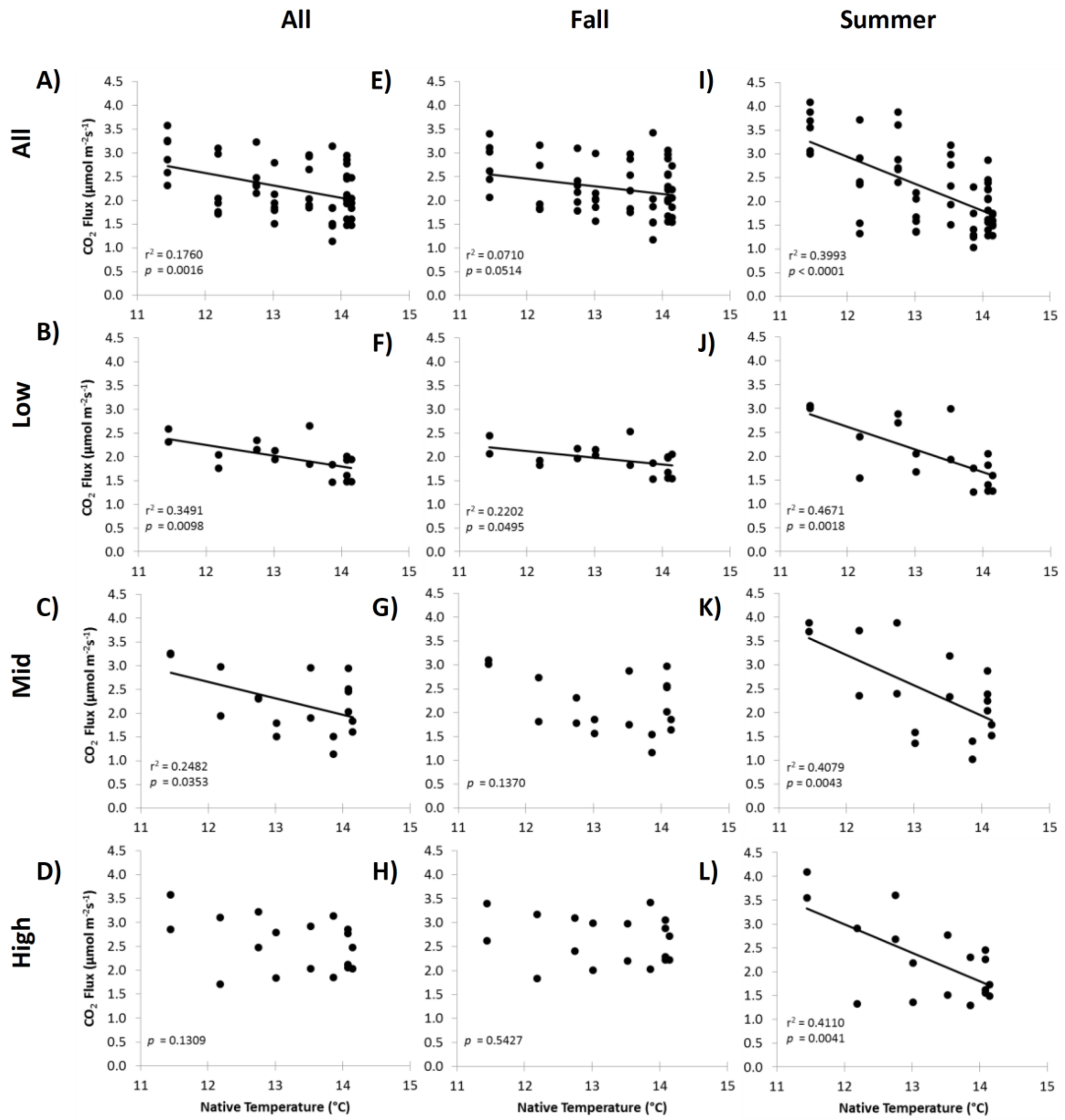


Fig. 2

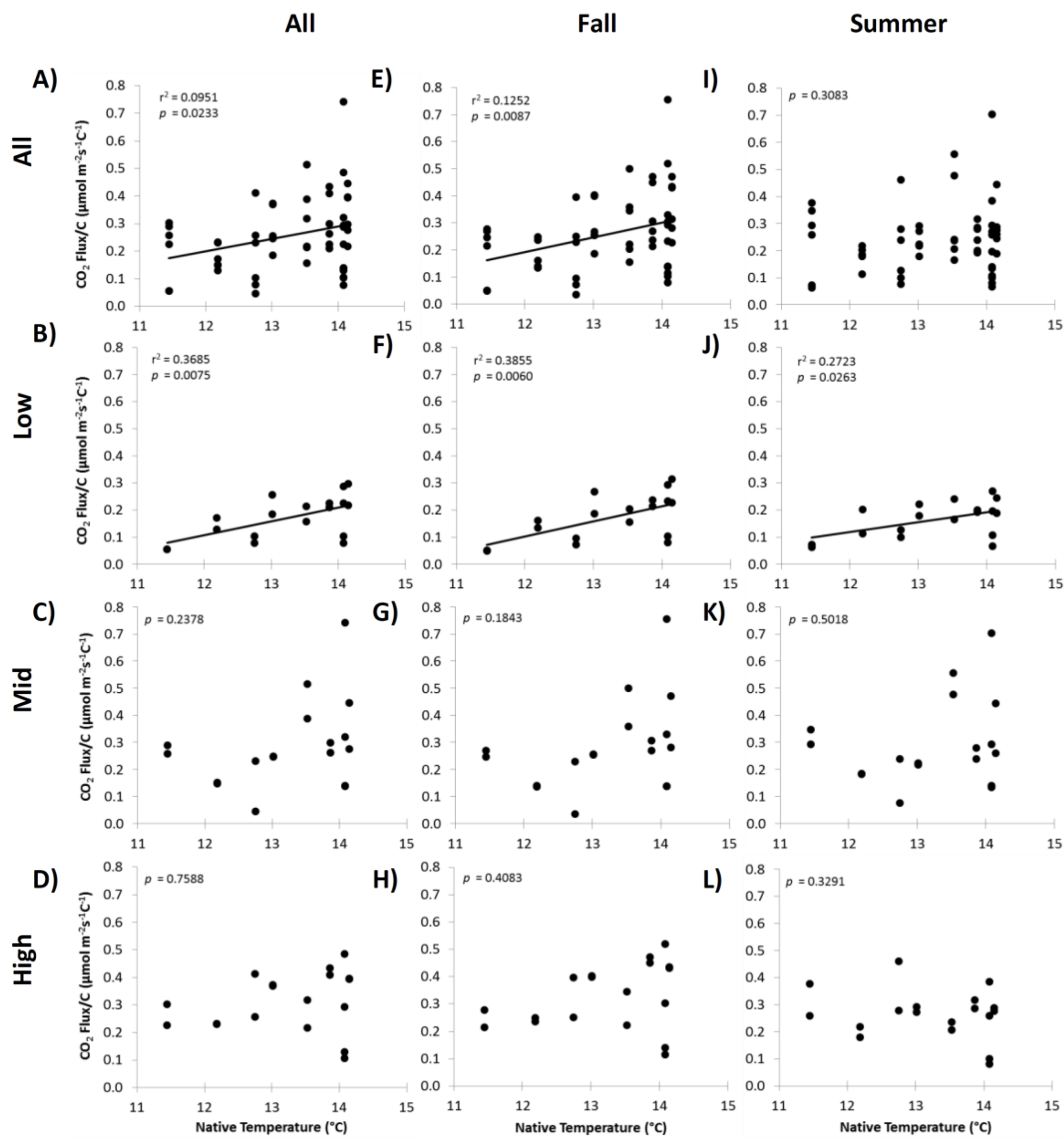


Fig. 3

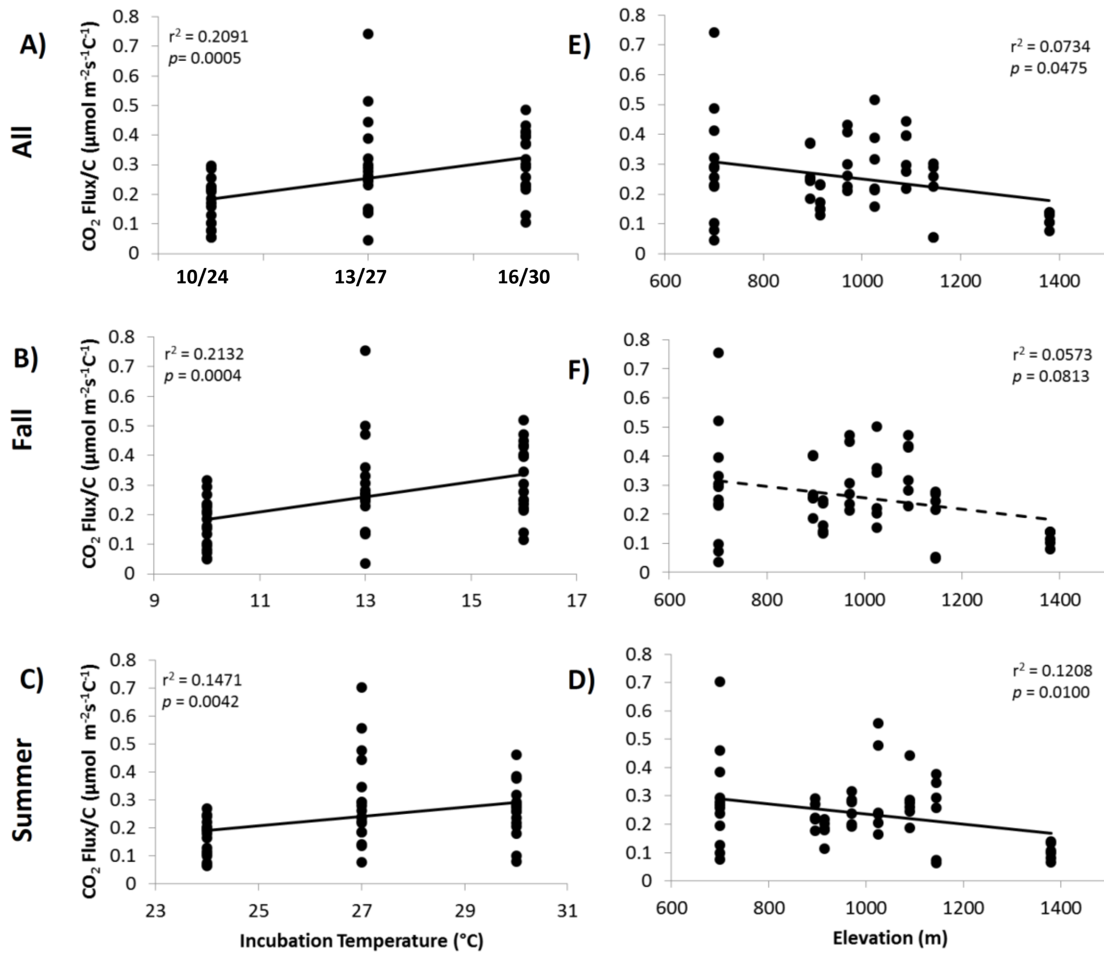


Fig. 4

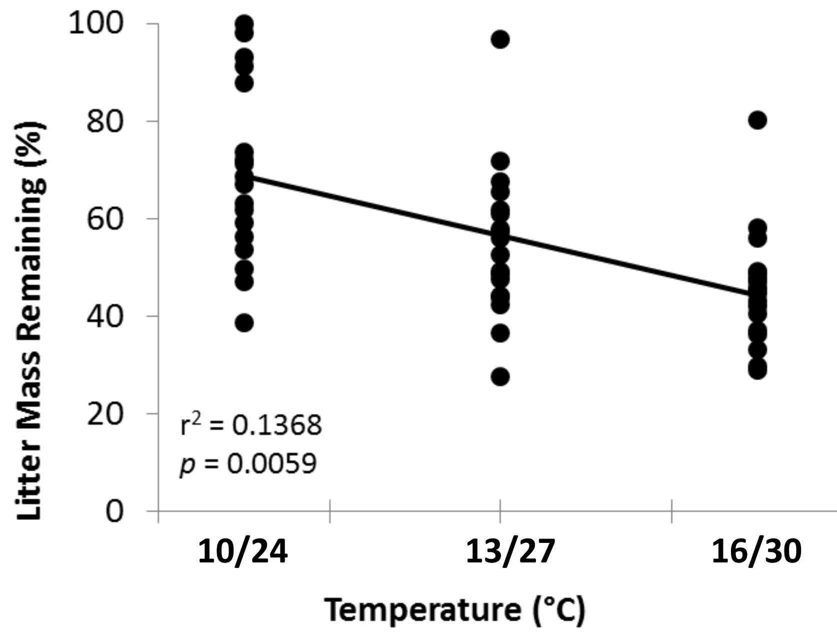


Fig. 5

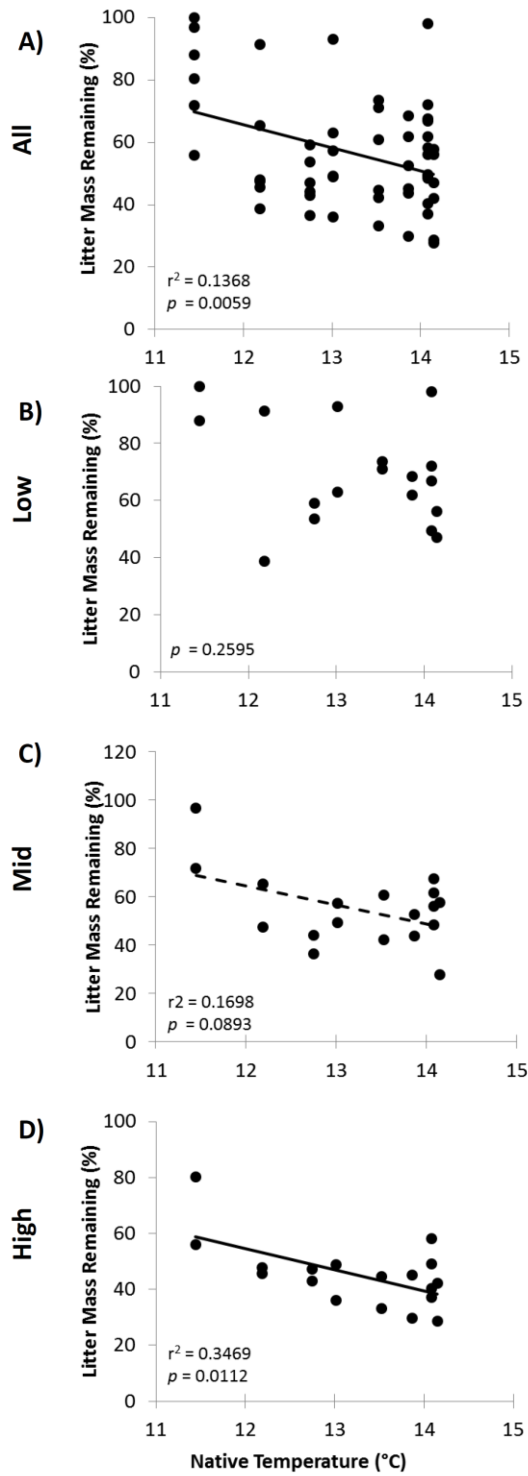


Fig. 6

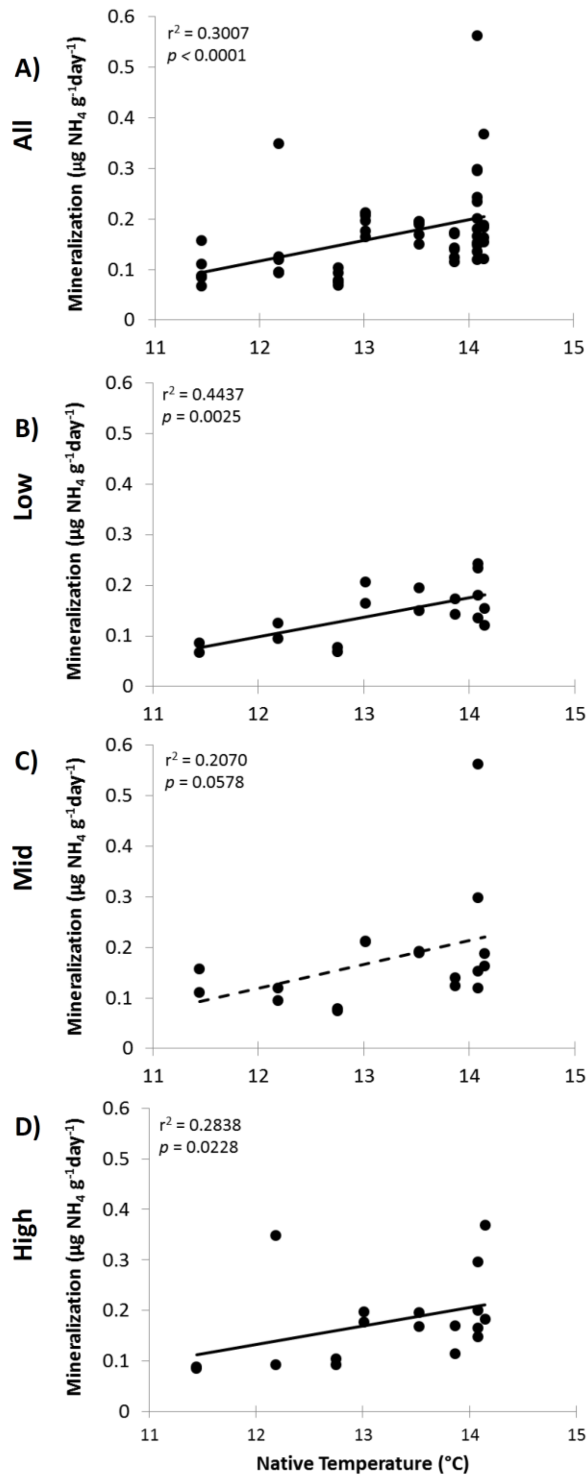


Fig. 7

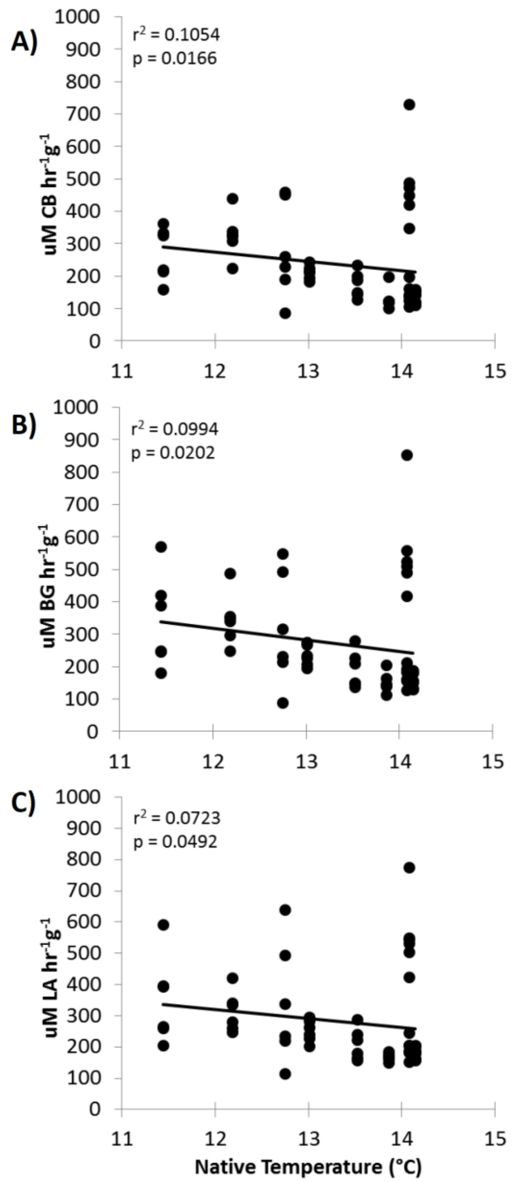


Fig. 8

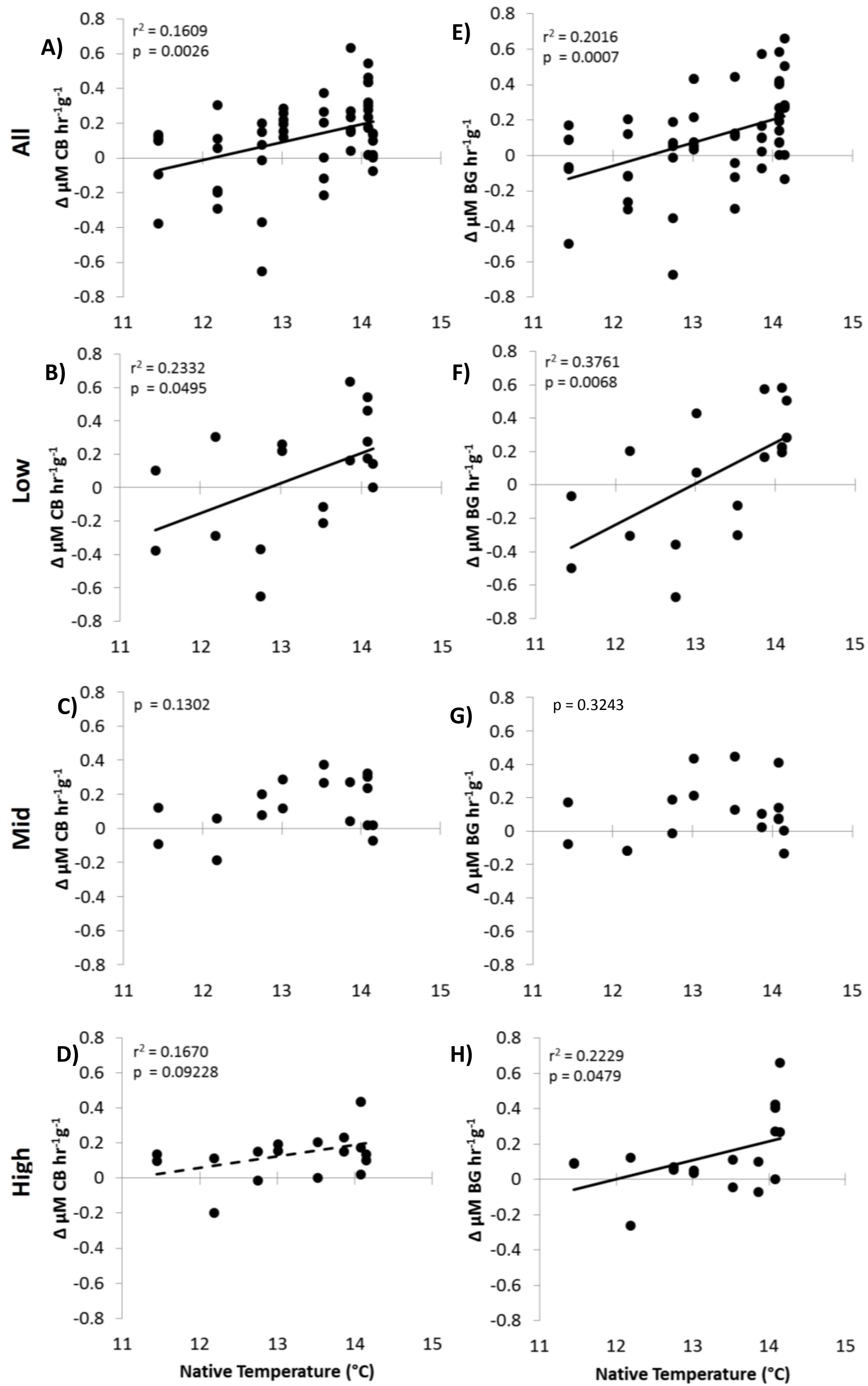


Fig. 9

CHAPTER 3

Soil and tree sapling responses to simulated climate change along an elevation gradient: implications for climate-carbon feedbacks

For submission to *Soil Biology and Biochemistry*

Timothy G. Whitby[a]*, and M. D. Madritch[a]

[a]Department of Biology, Appalachian State University, Boone, NC, 28608, USA

*Corresponding author. Current Address: Department of Biology 161 Rankin West, 572 Rivers St. Appalachian State University, Boone, NC 28608. Tel.: +1 828 262 3023.

E-mail address: whitbytg@appstate.edu

Abstract

Forest soils contain ~40 % of global soil carbon (C) and ~80 % of plant biomass C, but whether forested ecosystems will be a source or sink of C under projected climate change is uncertain. In addition to temperature, many other factors, such as precipitation, community composition, atmospheric CO_2 concentration, and nutrient availability, will affect forest C cycling on global to local scales. Many methodological approaches have been developed to actively or passively warm soils from forested ecosystems, but the difficulty in observing plant and soil interactions limits the applicability of many warming experiments. Elevation gradients provide natural temperature tenet differentials that can be used with a transplant approach and have the benefit of observing responses *in situ* where microclimatic conditions in the soils are preserved. I incorporated tulip poplar (*Liriodendron tulipifera*) saplings in large (29000 cm^3) soil cores which were reciprocally transplanted along a 700 m elevation gradient at three southern Appalachian hardwood sites in the Coweeta Hydrologic Laboratory, NC. A range of ~3.25 °C from the high to low site was enough to increase respiration of soils, as measured by a Licor portable soil CO_2 flux meter, that were transferred from the high elevation site to lower elevations. The activity rates of microbial enzymes involved in the degradation of cellulose, protein, and phenolic C-compounds also increased when soils were transferred from high elevations to low elevations. Carbon and nitrogen stores and rates of nitrogen mineralization increased with the elevation of soil origin, which may influence plant productivity by making resources more available. Decreasing levels of plant available nitrates with decreasing elevation indicated that nitrate availability may limit plant growth more so in warmer and drier soils. However, plant growth was highest for plants

in soils that originated from the low elevation. With only one growing season of data it appears that a small increase in temperature will not positively affect plant growth, but increased available nitrates will. The increased rates of microbial respiration and activity combined with low rates of plant growth indicate that C dense soils from lower temperature regimes (higher elevations) are more susceptible to C loss than are soils from high temperature regimes under realistic temperature change under field conditions.

1. Introduction

Soils are estimated to contain more than twice the amount of carbon (C) currently held in the Earth's atmosphere (Batjes 1996). In the recent historical record, anthropogenic global warming has increased soil respiration rates and losses of C in soil stocks (Bellamy et al. 2005, Bond-Lamberty and Thomson 2010). Within the next century, global surface temperatures are expected to increase by an additional 2 to 6 °C depending on the predicted rate of anthropogenic increases in greenhouse gases, including carbon dioxide (CO_2). Predicted increases in release of CO_2 from terrestrial ecosystems as a result of climate change are uncertain due to the complexity of the interactions with terrestrial biomass, relative temperature sensitivity of the decomposition of soil C from different climates and pools, and the alterations to precipitation and disturbance regimes on regional scales (Dixon et al. 1994, Pacala et al. 2001, Christensen et al. 2007, Kurz et al. 2008). Soil surveys linked to GIS data allow for global estimates of soil C stocks and can be correlated with climatic and geographic patterns to better understand the factors controlling soil C storage. Within the United States, soils from forested ecosystems cover the largest area and have the most C dense upper soil layers compared to any other land cover type (Guo et al. 2006). Forests also contain the most C

stored in belowground biomass, which makes them important study sites for ecosystem interactions that will determine the potential change in C cycling with climate change (Bonan 2008).

Belowground soil C cycling is inherently complex because of the variation in biotic and abiotic factors on both large and small spatial scales. Within the soils there is an extremely diverse community of microbes and fungi that are responsible for processing detritus (Carney and Matson 2005, Bryant et al. 2008). In addition to heterotrophic respiration by saprophytes, the network of plant roots is also constantly respiring, and often times these two CO_2 sources are lumped together because of the difficulty in distinguishing the two (Schlesinger and Andrews 2000, Giardina and Ryan 2002, Litton and Giardina 2008). Leaf litter input quality, often measured by C:N ratios, differs by plant type and species, and microbial decomposition has shown to be locally adapted to the species of leaf litter present (Ayres et al. 2009). Many biotic controls that influence soil C cycling are subject to stronger controls by abiotic factors, such as temperature and moisture that also vary over different spatial scales (Garten et al. 2009, Griffiths et al. 2009).

Trends in temperature and precipitation directly influence the rates of both plant primary production and soil C decomposition (Conant et al. 2000, Hamrick 2004, Chmura et al. 2011, Prentice et al. 2011). Soil moisture content can exert more control over soil respiration than does temperature (Garten et al. 2009), with drought and flooding both inhibiting aerobic respiration. However, prolonged flooding and resulting anaerobic decomposition will increase the relative contribution of methane, an even more potent greenhouse gases than CO_2 (Dunn et al. 2007). Topographic factors such as slope

and aspect have a more local control over temperature and moisture regimes within large latitude gradients of climate. More moisture is retained in soils whose aspects point towards the poles (Becker et al. 2007, Griffiths et al. 2009). The adiabatic lapse rate has a predictable effect of decreased temperature with elevation (Komatsu et al. 2010), while precipitation generally increases with elevation (Garten and Hanson 2006, Di Luzio et al. 2008, Knoepp et al. 2008). In higher latitudes and higher elevations, decomposition can become temperature limited and soil carbon stocks accumulate over long periods of time, resulting in soils that are very C dense (Swift et al. 1998, Hobbie et al. 2000, Leifeld et al. 2005, Garten and Hanson 2006). These soils could be a large source of CO_2 to the atmosphere in the future because of their large soil C stores and greater projected warming in polar regions (Christensen et al. 2007, Dunn et al. 2007, Niklinska and Klimek 2007, Natali et al. 2011).

Predicted increases in plant growth have the potential to offset the loss of carbon from soils, but the plant response is mediated by multiple factors, making estimates of C sequestration uncertain. Increased temperatures have already been shown to induce species distribution shifts up elevation and latitude gradients (Pounds et al. 1999, Beckage et al. 2008, Lenoir et al. 2008), and the positive effects of temperature increases on plant biomass are greatest at the upper limits of population distributions in cooler climates (De Frenne et al. 2011). The potential rate at which a species can migrate with increasing climate is dependent on its natural history traits, and also the amount of genetic variation and phenotypic plasticity that will allow it to adapt or acclimate to changing climate (Bresson et al. 2011); however migration may be limited by the new soil types encountered with range expansion (Beckage et al. 2008). Phenotypic plasticity

was shown to have more of an impact on seedling survival and growth rates along elevation gradients than genotype (Bresson et al. 2011). Therefore, acclimation of plants to climate change may potentially mitigate some of the effects of climate change without necessarily requiring shifts in plant distributions.

Various experimental approaches have been employed to simulate the effects of global climate change, each with their own strengths and weaknesses (Verburg et al. 1999, Aronson and McNulty 2009). One of the common weaknesses is that the presence of a warming apparatus may influence other, non-target, ecosystem parameters such as soil disturbance or water balance (Norby and Luo 2004, Aronson and McNulty 2009). Other than the initial disturbance of soils after coring and transplant, elevation or latitude reciprocal transplant studies offer a continuous temperature gradient and may provide more realistic soil responses to global warming (Becker et al. 2007). However, there are some concerns with transplanting along a gradient; typically, the farther sites are away from each other, the more dissimilar they are likely to be, which makes finding similar sites along large gradients difficult (Knoepp and Swank 1998, Scowcroft et al. 2000). Rainfall typically increases with elevation (Di Luzio et al. 2008, Knoepp et al. 2008), depth of soils decreases with elevation (Guo et al. 2006), and canopy species dominance will change with elevation as well (Wang et al. 2005, Knoepp et al. 2008).The spatial separation of treatments is much different than a manipulative warming experiment, where treatment and control plots are immediately adjacent to one another in most cases. Nonetheless, this difference among sites can be compensated for with higher replication of sites, careful site selection, and reciprocal transplant techniques. By using a reciprocal transplant approach and subjecting a single soil type to field "incubations" at different

elevations, responses of different soil types to temperature change can be compared at all the treatment plots to determine site specific effects (Hart 2006). One technical caveat to this approach is that the coring process causes considerable disturbance, especially if the soils are mixed and sieved, which will alter the microenvironment and soil microbial communities (Thomson et al. 2010). Semi-intact soil cores have more recently been used, as well as better techniques to access deeper soil horizons which are of great interest to long-term carbon cycling (Zimmermann et al. 2009). Very few soil transplant studies have included a live plant within transplanted soil cores (Link et al. 2003), and to my knowledge no studies have observed the concomitant changes in C and N dynamics in soil cores with a tree present.

The interactions among soils and woody species will ultimately determine whether forest ecosystems become a net source or sink for C under climate change. Here, I warmed soils by using a reciprocal transplant approach that included a tree sapling to assess whether plant growth and nutrient uptake will interact with the net release of C from soil respiration in warmer climates. Large, 29000 cm^3, soil cores were used to limit edge effects and maintain the majority of the soil micro-environment intact so that microbial activity could be observed in the absence of excess disturbance. Realistic simulated climate change and preservation of soil microenvironment in the presence of a tree seedling within these mesocosms may help determine how above- and belowground ecosystems interact to affect carbon cycling and climate feedbacks.

2. Methods

2.1 Site Description

I chose three sites along an elevation gradient in the Coweeta National Hydrologic Laboratory in Otto, North Carolina. Sites were selected to minimize differences in slope, aspect, and plant community composition. Elevation ranged approximately 700 m between sites (702, 1189, and 1381 m) along a relatively short horizontal distance of 5 km. Rainfall at high elevations is typically higher than low elevations year-round (Table 1).

2.2 Reciprocal Transplant

Soils were excavated using a custom-built soil corer modeled after a tree spade that was 50 cm x 50 cm square at the soil surface, tapered to a point at approximately 35 cm depth (29000 cm^3). Twelve plant-free cores at each site were excavated and four undisturbed areas of equal size were designated as ambient controls. Soil cores were transferred to a plywood container for transport to other sites. Four cores were randomly redistributed within their site of origin (cored controls), and four cores were randomly transplanted to each of the other two elevations.

After all cores were transplanted and acclimated to new site conditions for two weeks, a tulip poplar (*Liriodendron tulipifera* L.) seedling was planted within each core. Seedlings were obtained from the Tennessee Department of Agriculture Division of Forestry and were all from the same seed source that was selectively bred for restoration projects throughout the state. Tulip poplars were one year-old at the time of purchase and were grown in potting soil for the spring until they were transplanted to the field in mid-summer. Initial diameter and height were measured on the day trees were planted in the

field. All other plants that colonized the cores after transplantation were removed whenever field sites were visited at least once every three months, except over winter.

2.3 Field Collections

The bulk density of the soils at each site was determined prior to core transplants. Data loggers (Onset Computer Corp., Bourne, MA, USA) were installed 10 cm deep in the soils at the center of each plot. Light levels (μmol photons) were recorded (Licor Biosciences, Lincoln, NE, USA) above each sapling in leaf out conditions in late summer both years to compare among replicates. All other field data were collected every three months beginning in June, 2010, except for December when weather conditions prevented access to the sites. Soil sub-samples within each core were taken with a 2 cm diameter corer to a depth of 15 cm. Mixed resin beads in nylon mesh bags were inserted into the upper 10 cm of the soil to estimate plant available nitrogen (N) in the form of NO_3^- and NH_4^+ (Binkley 1984). Resin bags were replaced after each sampling interval. Soil respiration was also measured *in situ* with a Li-8100 automated soil CO_2 flux system with a 10 cm chamber (LI-COR Biosciences, Lincoln, NE, USA). Seedling growth was measured as an approximate volume of woody material represented by a cone with the diameter 5 cm above the soil surface and the height to the tallest living bud or leaf petiole. Green leaves of the seedlings were also collected when they were present in the two spring and fall sampling periods.

2.4 Laboratory analyses

Soil samples were passed through a 2 mm sieve to remove rocks and coarse roots, and stored at -20 °C until analysis. Approximately 2 g of field moist soils were dried at 70 °C to determine volumetric water content. Soil subsamples and leaf samples from

each collection date were also freeze-dried and ground for %C and %N content by flash combustion on a ThermoFisher Flash EA112 analyzer (Thermo Fischer Scientific, Delft, The Netherlands).

The activity of six different enzymes were assayed: cellobiohydrolase (CB), β-glucosidase (BG), leucine aminopeptidase (LA), urease (U), phenol oxidase (PO), and peroxidase (Per). The first three enzymes degrade cellulose (CB and BG) and amino acids (LA) and their activity can reflect microbial allocation to C and N acquisition, respectively (Allison et al. 2008). All assays were performed on ~1 g soil samples extracted in 15 mL of 5 mM acetate buffer according to Madritch et al. (2007). Briefly, duplicate aliquots of 400 µL of extract were used for each individual enzyme assay. Extracts were mixed with 100 µL of substrate and enzyme activity determined after two hours by fitting results to a p-nitrophenol standard curve. Urease activity was determined as the difference in ammonium levels between control tubes that were given 40 µL of buffer and substrate tubes that were given 40 µL of urea. Ammonium concentration was determined by the sodium salicylate/sodium dichloroisocyanurate method (Mulvaney 1996). For PO and Per assays, duplicate tubes were given 100 µL of L-dopa and 40 µL DI H_2O or L-dopa and 40 µL 0.3 % H_2O_2. PO and Per assays ran for 4 to 6 hours since these enzymes break down more stable phenolic C compounds and have much lower reaction rates.

For ammonium and nitrate determination, resin beads were extracted in 15 mL of 2M KCl for 24 hours. KCl extracts were decanted and resin beads were dried at 70 °C to determine the amount of extracted NO_3^- and NH_4^+ per g of resin. Ammonium concentrations were determined by the sodium salicylate/sodium dichloroisocyanurate

method (Mulvaney 1996). Extractable NO_3^- was determined via manual vanadium (III) reduction as described in Miranda et al. (2001).

2.5 Data analysis

In order to account for the effects that coring had on all mesocosms, each response variable was corrected by the ambient and cored control responses. This isolated the effects of transplantation to other sites. I subtracted cored control values (C_n), where n is the origin, from ambient control values (A_n) for each data collection period, and the resulting value was added to all cores (X_n) to approximate ecosystem responses without the effects of disturbing the soils ($X_n + (A_n - C_n)$). The direction of the coring effect could be positive or negative depending on the parameter measured, and this correction makes it possible to have negative responses for some variables. All treatment data were analyzed in SAS JMP (v9.0.0 SAS Institute Inc., Cary, NC, USA) using a two-way ANOVA with site of origin, site of destination, and the interaction term as main effects. Initial carbon and nitrogen content (n=36), and bulk density (n=12) were compared by a one-way ANOVA with site. Sample sizes for all other ANOVA's were 36. Trends were considered significant at $\alpha < 0.05$ and marginally significant at $\alpha < 0.1$. When effects were significant, I performed a Tukey test to compare the difference between the means. Data were Ln transformed to conform assumptions of normality where necessary. Light data were non-normal and tested with a Kruskal-Wallis test with site.

3. Results

Initial carbon and nitrogen stocks in the soils increased with origin ($F_{2,36} = 101.67$, $p < 0.0001$) such that highest site soils were over twice as carbon dense as were soils from the lowest site (data not shown). Maximum (max) and minimum (min) soil

temperatures of the high site were lower by approximately 3°C than they were at the low site for the entire study period (Table 2). However, the average annual difference in daily max and min temperature between the mid and low sites was less than 0.5°C. Average max temperatures for the mid site tended to be higher than those for the low site during the winter and spring time periods. It is possible that seasonal temperature inversions in the valley during winter and spring months were responsible for the lack of a temperature difference between the mid and low sites. Soils were frozen at the high site intermittently for nearly two months in the winter, while mid and low soils never went below 0 °C.

Rain fall at the high site was generally greater in 2010 (Table 1), but rainfall data were not available for 2011. Gravimetric water content (GWC) of the soils was dependent on both the type of soil (origin), and the transplant treatment (destination) (Fig. 1). Soil moisture increased with the elevation of origin which may reflect the decreased bulk density of soils, which was lowest at the high site (data not shown, $F_{2,12} = 45.08$, $p < 0.0001$). The significant interaction between origin and destination may be due to the decrease in GWC of high elevation soils that were transplanted to lower elevations, whereas soils from mid and low elevations remained very similar to their cored controls when transplanted (Fig. 1). Soil cores from low and mid elevations had very similar values for native parameters of temperature and water content, and this has important consequences when comparing destination effects among these sites. There was also no difference among light availability to plants among sites in either summer (data not shown, $\chi^2 = 1.88$ $p=0.3915$ and $\chi^2 = 0.01$ $p=0.9538$) to effect photosynthetic rate of the seedlings.

Respiration rates of the soils responded consistently among origin and destinations. Respiration data (as well as all of the following parameters) were corrected for the coring effect, as stated in section *2.5*. Results of two-way ANOVA of origin and destination are found in Table 3. Soils originating at the mid site respired less on average than did soils from either the low or high sites (Fig. 2A). However among destination treatments, soils transferred to the mid site always had the highest respiration rates, followed by low and then by high (Fig. 2A). When respiration was corrected for initial soil C content, respiration rates decreased from low to high site origins because of the increasing C density of soils along the gradient (Fig. 2B). When cores originating from the high elevation site were transplanted to the low site, respiration/soil C increased, and the converse was true when soils originating from low elevation sites were transplanted to high sites. This observed increase in respiration/soil C indicates that more of the available C in high elevation soils may be respired in response to a small temperature increase.

Extracellular enzyme activity (EEA) rates are often the limiting step in decomposition, and any increase in these rates will likely affect total C mineralization and nutrient cycling rates. Cellulose and protein decomposition, as measured by cellobioside (CB), β-glucosidase (BG), and leucine aminopeptidase (LA), were differentially affected by destination depending on the origin of the soils (Fig. 3A-C). Low elevation cores decreased in microbial activity when moved up slope, while mid elevation cores increased in activity when transferred either up or down in elevation. The destination and interaction effects were likely driven by the relatively large linear increase in activity when high elevation cores were transplanted to either site, whereas

the changes in EEA of low and mid sites appeared to be more weakly affected by the transplant. High elevation origin cores increased with transplant to the extent that EEA of the high origin cores at the low destination was over 2-fold greater than high elevation cored controls. The high to low transplant cores had the greatest observed activity among all other origin and transplant combinations.

Urease activity was not sensitive to destination treatments, but was highest for soils that originated from the mid site (Fig. 3D). Although peroxidase (Per) and phenol oxidase (PO) both degrade phenolic compounds, activities of these enzymes were dependent on different site parameters from one another (Fig. 3E,F). PO was lowest in cores originating from the low sites but was not significantly affected by destination (Fig. 3E). However, Per had no origin effect, but its activity decreased for cores transplanted up and increased for cores transplanted down (Fig. 3F). This may indicate that Per activity is sensitive to small increases in temperature, whereas PO activity is not.

Plant available nitrogen in the form of ammonium (NH_4^+) was greatest in soils that originated at the high site (Fig. 4A). Although the effect of destination was not significant, factors controlling the effect of transplantation may vary depending on the origin. Low cores that were transplanted up tended to increase in available NH_4^+, but high cores that were transplanted down also increased in available NH_4^+. A decrease in the rate of N mineralization and nitrification was seen when soils were transplanted from low to high elevation in an evergreen forest, which is contrary to the findings of this study (Hart and Perry 1999). Perhaps, the initial concentration of NH_4^+ could be a determining factor for the temperature response of N mineralization (Bonito et al. 2003), or increased soil moisture at the high site could also be responsible for increased

inorganic N availability when low elevation cores were transplanted to high elevation. Additionally, soil nitrate (NO_3^-) availability was highest in the high site regardless of the origin of the soils, which may indicate an abiotic variable associated with high elevation's increased NO_3^- levels (Fig. 4B) such as increased N deposition at high elevation (Hart and Perry 1999, Knoepp et al. 2008). The increase in available nitrogen at high elevations may interact with the lower temperature affecting plant growth in opposite ways.

The combined effects of temperature, moisture, and nutrient availability made trends in plant growth and uptake more variable than were soil processes. Soils originating at the low elevation were the most favorable to plant growth, regardless of destination site (Fig. 5). Trees grown in soils originating from low elevation were positively affected by transplant up the elevation gradient. This is contrary to what I expected from decreased temperatures and shortened growing season, but does follow the availability of soil NO_3^- as measured by resin bags. Additionally increased average precipitation at the high site could have fostered growth of seedlings in low elevation soils that were transferred up the elevation gradient. Seedlings in soils that originated from high and mid elevation soils had no net change in tree volume over time, but they both appeared to grow at mid, die back at low, and remain the same at high elevations. Negative values are present because of die-back and limited re-growth of the plants.

When considering carbon sequestration potential, the rate of nutrient uptake from the soils will also influence the %C of plant biomass. Leaves increased in %C over time relative to the end of the first growing season when transplanted to the high site, but leaves at low and mid elevations were less carbon dense (Fig. 6A). This decrease in %C

of leaves is likely responsible for the observed increase in C:N ratio of leaves, which may indicate that plants that have higher C assimilation during the growing season are more efficient at resorbing nutrients from leaves before leaf fall. Trees in mid or low origin soils that had low %C of leaves, actually had lower C:N ratios than the previous fall when transplanted to the high site. The C:N ratio also increased with the elevation of origin for soils despite increasing available ammonium with site of origin elevation. Plant response trends may not show a simple relationship with the experimental manipulations, but current soil quality clearly will influence the potential growth of saplings. Slight increases in temperature may not directly benefit plant growth, but concomitant changes in nutrient supply and water availability may influence the rate that plants take up C and N.

4. Discussion

I show that carbon cycling in a soil-plant mesocosm increases under realistic temperature and moisture changes under natural field conditions. Soil carbon and nitrogen increased along an elevation gradient, as reported by others (Garten and Hanson 2006, Guo et al. 2006, Leifeld et al. 2009). Studies which have observed decreased soil carbon and humus with increasing mean annual temperature often make the assumption that differences in current temperature regimes are similar to what ecosystem states under future climate change may be; however factors other than warming should also be considered. High bulk density of the soils from low and mid sites did not allow them to retain the rainfall that has historically been higher at the high elevations in Coweeta (Knoepp and Swank 1998, Bonito et al. 2003, Knoepp et al. 2008), and high soils were drier when transferred down elevation. Despite a decrease in water content of soils, the

respiration rate of the carbon dense soils from the high elevation site was shown to increase over a relatively small, ~ 3°C, temperature range. Past microcosm experiments typically simulate climate change using 10 °C increments to investigate the temperature sensitivity of forest soils (Kirschbaum 2006, Niklinska and Klimek 2007, Conant et al. 2008, Hartley and Ineson 2008), which is approximately 3 times greater than conservative estimates of climate change for the next century (IPCC 2007). These results demonstrate increased nutrient cycling rates under more realistic temperature changes and over a relatively short (~ 1.25 year) time period.

Carbon loss from soils may have a considerable effect on the global climate, but the net effect is also dependent on the rate of C sequestration in plant biomass. Although many studies have employed cores with homogenized soil along elevation gradients (Hart and Perry 1999, Hart 2006, Zimmermann et al. 2009), those that have retained intact soils with a plant have been limited to grassland systems which may not play as important a role in sequestering carbon under climate change as would tree seedlings (Ineson et al. 1998, Link et al. 2003). The 0.5 m x 0.5m cores (29000 cm^3) used in this study limited the edge effects apparent in smaller cores. Furthermore, I accounted for the physical disturbance of transplanting cores by using ambient and transplanted controls. Microbial communities have been shown to change with sieving and drying of soils (Thomson et al. 2010), and the coring method likely retained enough undisturbed soils in the center of each mesocosm to avoid large microbial community changes. The large size, intact microenvironment, and presence of a tree seedling may better simulate the actual forest ecosystem responses to climate change when compared to other reciprocal transplant studies.

One important aspect preserved by limiting soil disturbance is the activity of microbial enzymes, which usually control the rate limiting steps in organic matter decomposition. Four of six microbial enzymes, CB, BG, LA, and Per, were shown to increase their activities when transferred from high to low sites. Conversely, cores that were transplanted from low to high elevation demonstrated decreased activity, which suggested that the soil enzymes measured here were temperature limited. The cellulose and protein degrading enzymes act on relatively labile substrates which are rapidly decomposed and may not make a contribution to long-term positive climate feedbacks (Allison et al. 2007, Koch et al. 2007, Bradford et al. 2010), but the peroxidase breaks down phenolic compounds which are more recalcitrant C sources that accumulate in soils over time. The increase in peroxidase activity may indicate that some of the increased respiration was caused by using a previously stabilized carbon source. This increased enzyme activity may increase the C loss from high elevation soils, which have greater stocks of recalcitrant humic C substrates. Also, nitrogen in soils has been found to stabilize phenolic C-compounds in soils (Berg 2000, Allison et al. 2008, Keeler et al. 2009) and a reduction in nitrates when high soils were transplanted down the elevation gradient may have made these phenolic molecules more accessible to enzymes. The lack of a destination effect for urease or PO may indicate that the substrates these enzymes target were not limiting, indicating that constitutive expression of enzymes may be enough to satisfy microbial nutritional requirements (Sinsabaugh et al. 2008). Increases in some enzyme activity with temperature indicate that microbes may increase decomposition rates, but a lack of change with destination may also indicate that

constitutive expression levels will not be altered because increased uptake of that target substrate is not needed for increased growth.

There was some indication that seedling growth and nutrient uptake would respond to small amounts of future warming, although data were limited to one growing season. Increased C content of leaves transplanted to the high elevation site is in concordance with a recent study that demonstrates that phenotypic plasticity allows for more C and N dense leaves at high elevations (Bresson et al. 2011). Leaves at high elevations can also become more photosynthetically active by increasing the concentration of photosynthetic machinery, which could offset the shorter growing season (Bresson et al. 2011), and may partially explain why trees in cores transplanted from low sites to higher elevation sites had higher growth rates. Variation in tree growth may have also been in response to nitrate availability, as nitrates increase in soils that were transplanted from low to high elevations. Although CO_2 fertilization did not exist in this study, CO_2 induced biomass increases are predicted to reach a threshold once N in soils becomes limiting (Chmura et al. 2011). The decrease in plant-available N when high cores were transplanted from high sites to low sites may indicate that available N will limit the CO_2 fertilization effects on plants under warmer climates (Finzi et al. 2006). In addition to lower N availability, reduced rainfall at low elevations could also increase drought stress which may also limit plant growth. Since saplings were grown in a nutrient-rich potting soil for the first year of growth, these data may be biased towards showing an overall decrease in litter quality. Nonetheless, with the current data, changes in nitrogen availability and lower moisture as a result of warming may decrease growth rates of saplings and limit their C sequestration potential.

High concentrations of carbon in soils from high elevation may be susceptible to increased microbial decomposition in future warmer climates. Both soil respiration and microbial enzyme activity increased when soils were transplanted from a cooler, high elevation site to a warmer, low elevation site. Preservation of soil microenvironments by using large scale intact cores with tree seedlings may more accurately predict *in situ* responses of C and N cycling. Warming increased respiration and microbial enzyme activity, and plant growth or uptake was not increased in cores that were losing soil C, which indicate that warming may cause a net C loss from forest ecosystems.

References

Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14, 1156-1168.

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biology & Biochemistry 39, 1770-1781.

Aronson, E.L., McNulty, S.G., 2009. Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. Agricultural and Forest Meteorology 149, 1791-1799.

Ayres, E., Steltzer, H., Berg, S., Wall, D.H., 2009. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. Journal of Ecology 97, 901-912.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47, 151-163.

Beckage, B., Osborne, B., Gavin, D.G., Pucko, C., Siccama, T., Perkins, T., 2008. A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. Proceedings of the National Academy of Sciences of the United States of America 105, 4197-4202.

Becker, A., Korner, C., Brun, J.J., Guisan, A., Tappeiner, U., 2007. Ecological and land use studies along elevational gradients. Mountain Research and Development 27, 58-65.

Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M., Kirk, G.J.D., 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437, 245-248.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133, 13-22.

Binkley, D., 1984. Does forest removal increase rates of decomposition and nitrogen release. Forest Ecology and Management 8, 229-234.

Bonan, G.B., 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 320, 1444-1449.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 464, 579-U132.

Bonito, G.M., Coleman, D.C., Haines, B.L., Cabrera, M.L., 2003. Can nitrogen budgets explain differences in soil nitrogen mineralization rates of forest stands along an elevation gradient? Forest Ecology and Management 176, 563-574.

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

Bresson, C.C., Vitasse, Y., Kremer, A., Delzon, S., 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiology 31, 1164-1174.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., Green, J.L., 2008. Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. Proceedings of the National Academy of Sciences of the United States of America 105, 11505-11511.

Carney, K.M., Matson, P.A., 2005. Plant communities, soil microorganisms, and soil carbon cycling: Does altering the world belowground matter to ecosystem functioning? Ecosystems 8, 928-940.

Chmura, D.J., Anderson, P.D., Howe, G.T., Harrington, C.A., Halofsky, J.E., Peterson, D.L., Shaw, D.C., St Clair, J.B., 2011. Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. Forest Ecology and Management 261, 1121-1142.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Rueda, V.M., Mearns, L., Menéndez, C.G., Rinke, J.R.A., Sarr, A., Whetton, P., 2007. Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Conant, R.T., Klopatek, J.M., Klopatek, C.C., 2000. Environmental factors controlling soil respiration in three semiarid ecosystems. Soil Science Society of America Journal 64, 383-390.

Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89, 2384-2391.

De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B.J., Chabrerie, O., Cousins, S.A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J., Verheyen, K., 2011. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. Global Change Biology 17, 3240-3253.

Di Luzio, M., Johnson, G.L., Daly, C., Eischeid, J.K., Arnold, J.G., 2008. Constructing retrospective gridded daily precipitation and temperature datasets for the conterminous United States. Journal of Applied Meteorology and Climatology 47, 475-497.

Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263, 185-190.

Dunn, A.L., Barford, C.C., Wofsy, S.C., Goulden, M.L., Daube, B.C., 2007. A long-term record of carbon exchange in a boreal black spruce forest: means, responses to interannual variability, and decadal trends. Global Change Biology 13, 577-590.

Finzi, A.C., Moore, D.J.P., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., Kim, H.S., Matamala, R., McCarthy, H.R., Oren, R., Pippen, J.S., Schlesinger,

W.H., 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. Ecology 87, 15-25.

Garten, C.T., Classen, A.T., Norby, R.J., 2009. Soil moisture surpasses elevated CO_2 and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. Plant and Soil 319, 85-94.

Garten, C.T., Hanson, P.J., 2006. Measured forest soil C stocks and estimated turnover times along an elevation gradient. Geoderma 136, 342-352.

Giardina, C.P., Ryan, M.G., 2002. Total belowground carbon allocation in a fast-growing Eucalyptus plantation estimated using a carbon balance approach. Ecosystems 5, 487-499.

Griffiths, R.P., Madritch, M.D., Swanson, A.K., 2009. The effects of topography on forest soil characteristics in the Oregon Cascade Mountains (USA): Implications for the effects of climate change on soil properties. Forest Ecology and Management 257, 1-7.

Guo, Y.Y., Gong, P., Amundson, R., Yu, Q., 2006. Analysis of factors controlling soil carbon in the conterminous United States. Soil Science Society of America Journal 70, 601-612.

Hamrick, J.L., 2004. Response of forest trees to global environmental changes. Forest Ecology and Management 197, 323-335.

Hart, S.C., 2006. Potential impacts of climate change on nitrogen transformations and greenhouse gas fluxes in forests: a soil transfer study. Global Change Biology 12, 1032-1046.

Hart, S.C., Perry, D.A., 1999. Transferring soils from high- to low-elevation forests increases nitrogen cycling rates: climate change implications. Global Change Biology 5, 23-32.

Hartley, I.P., Ineson, P., 2008. Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biology & Biochemistry 40, 1567-1574.

Hobbie, S.E., Schimel, J.P., Trumbore, S.E., Randerson, J.R., 2000. Controls over carbon storage and turnover in high-latitude soils. Global Change Biology 6, 196-210.

Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E., Woof, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. Global Change Biology 4, 143-152.

IPCC, 2007. Climate change 2007 : the physical science basis : contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Keeler, B.L., Hobbie, S.E., Kellogg, L.E., 2009. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. Ecosystems 12, 1-15.

Kirschbaum, M.U.F., 2006. The temperature dependence of organic-matter decomposition - still a topic of debate. Soil Biology & Biochemistry 38, 2510-2518.

Knoepp, J.D., Swank, W.T., 1998. Rates of nitrogen mineralization across an elevation and vegetation gradient in the southern Appalachians. Plant and Soil 204, 235-241.

Knoepp, J.D., Vose, J.M., Swank, W.T., 2008. Nitrogen deposition and cycling across an elevation and vegetation gradient in southern Appalachian forests. International Journal of Environmental Studies 65, 389-408.

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

Komatsu, H., Hashimoto, H., Kume, T., Tanaka, N., Yoshifuji, N., Otsuki, K., Suzuki, M., Kumagai, T., 2010. Modeling Seasonal Changes in the Temperature Lapse Rate in a Northern Thailand Mountainous Area. Journal of Applied Meteorology and Climatology 49, 1233-1246.

Kurz, W.A., Dymond, C.C., Stinson, G., Rampley, G.J., Neilson, E.T., Carroll, A.L., Ebata, T., Safranyik, L., 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452, 987-990.

Leifeld, J., Bassin, S., Fuhrer, J., 2005. Carbon stocks in Swiss agricultural soils predicted by land-use, soil characteristics, and altitude. Agriculture Ecosystems & Environment 105, 255-266.

Leifeld, J., Zimmermann, M., Fuhrer, J., Conen, F., 2009. Storage and turnover of carbon in grassland soils along an elevation gradient in the Swiss Alps. Global Change Biology 15, 668-679.

Lenoir, J., Gegout, J.C., Marquet, P.A., de Ruffray, P., Brisse, H., 2008. A significant upward shift in plant species optimum elevation during the 20th century. Science 320, 1768-1771.

Link, S.O., Smith, J.L., Halvorson, J.J., Bolton, H., 2003. A reciprocal transplant experiment within a climatic gradient in a semiarid shrub-steppe ecosystem: effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. Global Change Biology 9, 1097-1105.

Litton, C.M., Giardina, C.P., 2008. Below-ground carbon flux and partitioning: global patterns and response to temperature. Functional Ecology 22, 941-954.

Madritch, M.D., Donaldson, J.R., Lindroth, R.L., 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry 39, 1192-1201.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62-71.

Mulvaney, R.L., 1996. Nitrogen—inorganic forms, In: Bartels, J.M. (Ed.), Methods of Soil Analysis: Part 3 Chemical Methods. Soil Science Society of America, Madison, WI, USA, pp. 1123–1184.

Natali, S.M., Schuur, E.A.G., Trucco, C., Pries, C.E.H., Crummer, K.G., Lopez, A.F.B., 2011. Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. Global Change Biology 17, 1394-1407.

Niklinska, M., Klimek, B., 2007. Effect of temperature on the respiration rate of forest soil organic layer along an elevation gradient in the Polish Carpathians. Biology and Fertility of Soils 43, 511-518.

Norby, R.J., Luo, Y.Q., 2004. Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytologist 162, 281-293.

Pacala, S.W., Hurtt, G.C., Baker, D., Peylin, P., Houghton, R.A., Birdsey, R.A., Heath, L., Sundquist, E.T., Stallard, R.F., Ciais, P., Moorcroft, P., Caspersen, J.P., Shevliakova, E., Moore, B., Kohlmaier, G., Holland, E., Gloor, M., Harmon, M.E., Fan, S.M., Sarmiento, J.L., Goodale, C.L., Schimel, D., Field, C.B., 2001. Consistent land- and atmosphere-based US carbon sink estimates. Science 292, 2316-2320.

Pounds, J.A., Fogden, M.P.L., Campbell, J.H., 1999. Biological response to climate change on a tropical mountain. Nature 398, 611-615.

Prentice, I.C., Harrison, S.P., Bartlein, P.J., 2011. Global vegetation and terrestrial carbon cycle changes after the last ice age. New Phytologist 189, 988-998.

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

Scowcroft, P.G., Turner, D.R., Vitousek, P.M., 2000. Decomposition of *Metrosideros polymorpha* leaf litter along elevational gradients in Hawaii. Global Change Biology 6, 73-85.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and

nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729-743.

Thomson, B.C., Ostle, N.J., McNamara, N.P., Whiteley, A.S., Griffiths, R.I., 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83, 69-73.

Verburg, P.S.J., Van Loon, W.K.P., Lukewille, A., 1999. The CLIMEX soil-heating experiment: soil response after 2 years of treatment. Biology and Fertility of Soils 28, 271-276.

Wang, L., Ouyang, H., Zhou, C.P., Zhang, F., Song, M.H., Tian, Y.Q., 2005. Soil organic matter dynamics along a vertical vegetation gradient in the Gongga Mountain on the Tibetan Plateau. Journal of Integrative Plant Biology 47, 411-420.

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., 2009. Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. European Journal of Soil Science 60, 895-906.

Table 1. Average daily precipitation by month at two sites (685 and 1362 masl) in the Coweeta basin from long term climate stations

Month	Precipitation Low (mm)	Precipitation High (mm)	Difference (mm)
January	189.5	220.2	30.7
February	147.8	173.2	25.4
March	115.3	151.9	36.6
April	113.8	138.7	24.9
May	133.4	179.8	46.4
June	87.6	119.4	31.8
July	66.3	134.6	68.3
August	95.0	155.4	60.4
September	136.9	175.8	38.9
October	119.6	117.1	-2.5
November	190.5	241.3	50.8
December	80.0	83.6	3.6
Average	123	157.6	34.6

Table 2. Average daily minimum and maximum temperature in different time periods. Numbers are shown ± standard error.

Time	Average Daily Maximum Temperature (°C)					
	High	Mid	Low	High-Mid	High-Low	Mid-Low
Average	11.8 ±0.35	14.9 ±0.29	15.1 ±0.30	-3.1±0.10	-3.4 ±0.09	-0.3 ±0.04
Summer 2010	18.3 ±0.14	20.2 ±0.12	21.2 ±0.15	-2.0 ±0.03	-2.9 ±0.03	-0.9 ±0.05
Fall 2010	7.4 ±0.53	11.2 ±0.51	11.3 ±0.48	-3.9 ±0.02	-4.0 ±0.18	-0.0 ±0.09
Winter 2010	1.2 ±0.25	6.6 ±0.38	6.3 ±0.34	-5.4 ±0.27	-5.1 ±0.24	0.2 ±0.09
Spring 2011	13.8 ±0.40	16.3 ±0.26	16.1 ±0.26	-2.5 ±0.20	-2.4 ±0.18	0.1±0.10
Summer 2011	19.0 ±0.12	20.7 ±0.14	21.5 ±0.13	-1.7 ±0.07	-2.5 ±0.06	-0.8 ±0.03

	Average Daily Minimum Temperature (°C)					
	High	Mid	Low	High-Mid	High-Low	Mid-Low
Average	10.5 ±0.34	13.2 ±0.30	13.5 ±0.30	-2.8 ±0.07	-3.0 ±0.07	-0.3 ±0.03
Summer 2010	17.6 ±0.14	19.4 ±0.13	19.7 ±0.18	-1.8 ±0.02	-2.1 ±0.05	-0.3 ±0.06
Fall 2010	6.1 ±0.51	9.6 ±0.47	9.7 ±0.43	-3.4 ±0.09	-3.5 ±0.15	-0.1 ±0.01
Winter 2010	0.6 ±0.16	4.7 ±0.27	4.9 ±0.26	-4.1 ±0.19	-4.3 ±0.18	-0.2 ±0.07
Spring 2011	11.0 ±0.45	13.8 ±0.33	14.2 ±0.29	-2.8 ±0.14	-3.2 ±0.18	-0.4 ±0.08
Summer 2011	17.9 ±0.14	19.4 ±0.14	19.9 ±0.13	-1.5 ±0.03	-2.0 ±0.05	-0.4 ±0.04

Table 3. Results of two-way ANOVA for ambient-corrected data and effects are considered significant at $p<0.05$ (bold) or marginally significant at $p<0.1$ (*bold and italic*) n=36.

Source	Origin†		Destination*		Origin*Destination‡	
	F	p	F	p	F	p
GWC (%)	*24.429*	*0.000*	*8.674*	*0.001*	*4.039*	*0.011*
CO_2 flux (μmol $m^{-2}s^{-1}$)	*5.421*	*0.011*	*37.467*	*0.000*	0.316	0.865
CO_2 flux (μmol $m^{-2}s^{-1}C^{-1}$)	*49.628*	*0.000*	*11.628*	*0.000*	0.335	0.852
CB Activity (uM $hr^{-1}g^{-1}$)	*3.366*	*0.050*	*4.155*	*0.027*	*3.289*	*0.026*
BG Activity (uM $hr^{-1}g^{-1}$)	0.217	0.134	*5.177*	*0.013*	*2.875*	*0.042*
LA Activity (uM $hr^{-1}g^{-1}$)	*6.324*	*0.006*	*5.014*	*0.014*	*4.252*	*0.009*
Urease Activity (μg$NH_4hr^{-1}g^{-1}$)	0.108	0.898	*10.089*	*0.001*	0.998	0.426
PO Activity (μM $hr^{-1}g^{-1}$)	*9.194*	*0.001*	1.2054	0.316	0.543	0.706
PO-Per Activity (μM $hr^{-1}g^{-1}$)	*4.704*	*0.018*	4.269	0.025	0.078	0.999
Average NH_4 (μg/g resin)	*12.842*	*0.000*	2.217	0.128	1.700	0.179
Average NO_3 (μg/g resin)	0.225	0.800	*8.444*	*0.001*	0.612	0.657
Δ Tree Volume	*6.737*	*0.042*	*3.299*	*0.052*	0.169	0.952
Δ(Leaf C) Fall	0.580	0.567	5.9	0.095	0.493	0.741
Δ(Leaf C:N) Fall	*5.082*	*0.014*	*5.9*	*0.007*	0.180	0.947

†df=2 ‡df=4

Figure Legend

Fig. 1. Gravimetric water content (GWC) of soils averaged over all sampling intervals ± standard error (n=36). Different uppercase letters indicate significant difference among origin, and lower case letters above individual bars indicate significant differences among origin and destination combinations.

Fig. 2. Respiration rate of soils averaged over all sampling intervals ± standard error (n=36) for (A) raw respiration data and (B) respiration per soil C. Different uppercase letters indicate significant difference among origin, and lower case letters left of the legend indicate significant differences among destinations.

Fig. 3. Microbial extra cellular enzyme activity averaged over all sampling intervals ± standard error, n=36 for (A) cellobiohydrolase (CB) activity, (B) β-glucosidase (BG) activity, (C) leucine aminopeptidase (LA) activity, (D) urease activity, (E) phenol oxidase (PO) activity, (F) and peroxidase (PO-Per) activity. Different uppercase letters indicate significant difference among origin, lower case letters left of the legend indicate significant differences among destinations, and lower case letters above individual bars indicate significant differences among origin and destination combinations.

Fig. 4. Plant available nitrogen from mixed resin bags averaged over all sampling intervals ± standard error (n=36) for (A) ammonium (NH_4^+) and (B) nitrates (NO_3^-). Different uppercase letters indicate significant difference among origin and lower case letters left of the legend indicate significant differences among destinations.

Fig. 5. Change in tree volume ((final cm^3 - initial cm^3)/ initial cm^3) from June 2010 to September 2011 ± standard error (n=36). Different uppercase letters indicate significant difference among origin.

Fig. 6. Change in green leaf nutrient content ((final %C or C:N - initial %C or C:N)/ initial %C or C:N) from June 2010 to September 2011 ± standard error (n=36) for (A) %C and (B) C:N ratio. Different lower case letters left of the legend indicate significant differences among destinations.

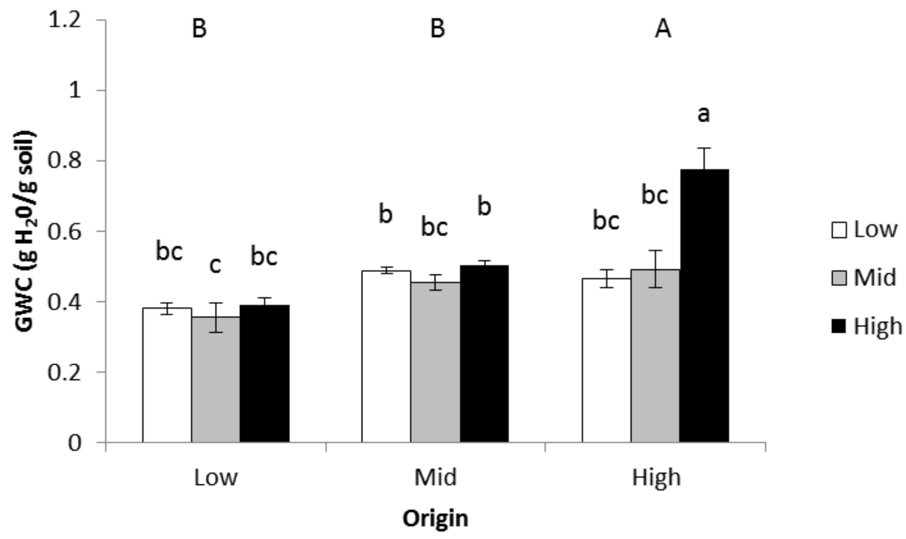


Fig. 1

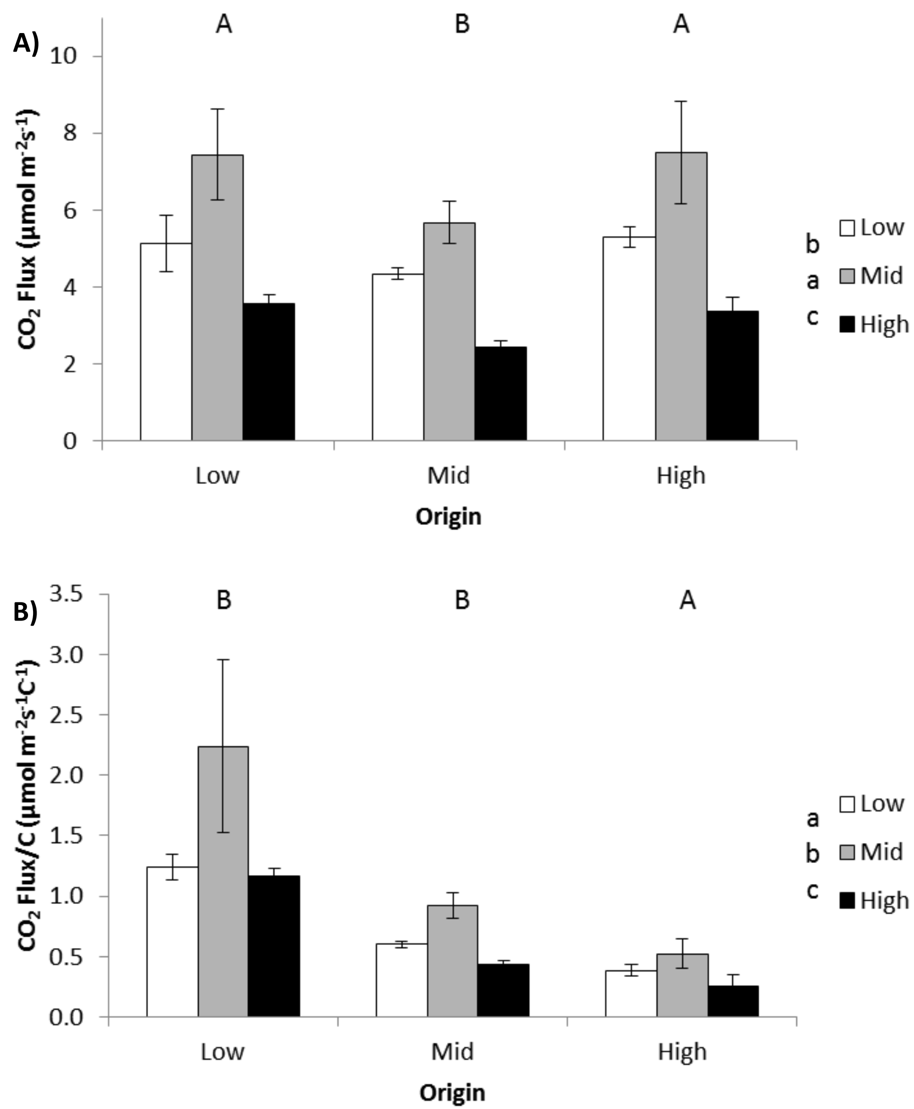


Fig. 2

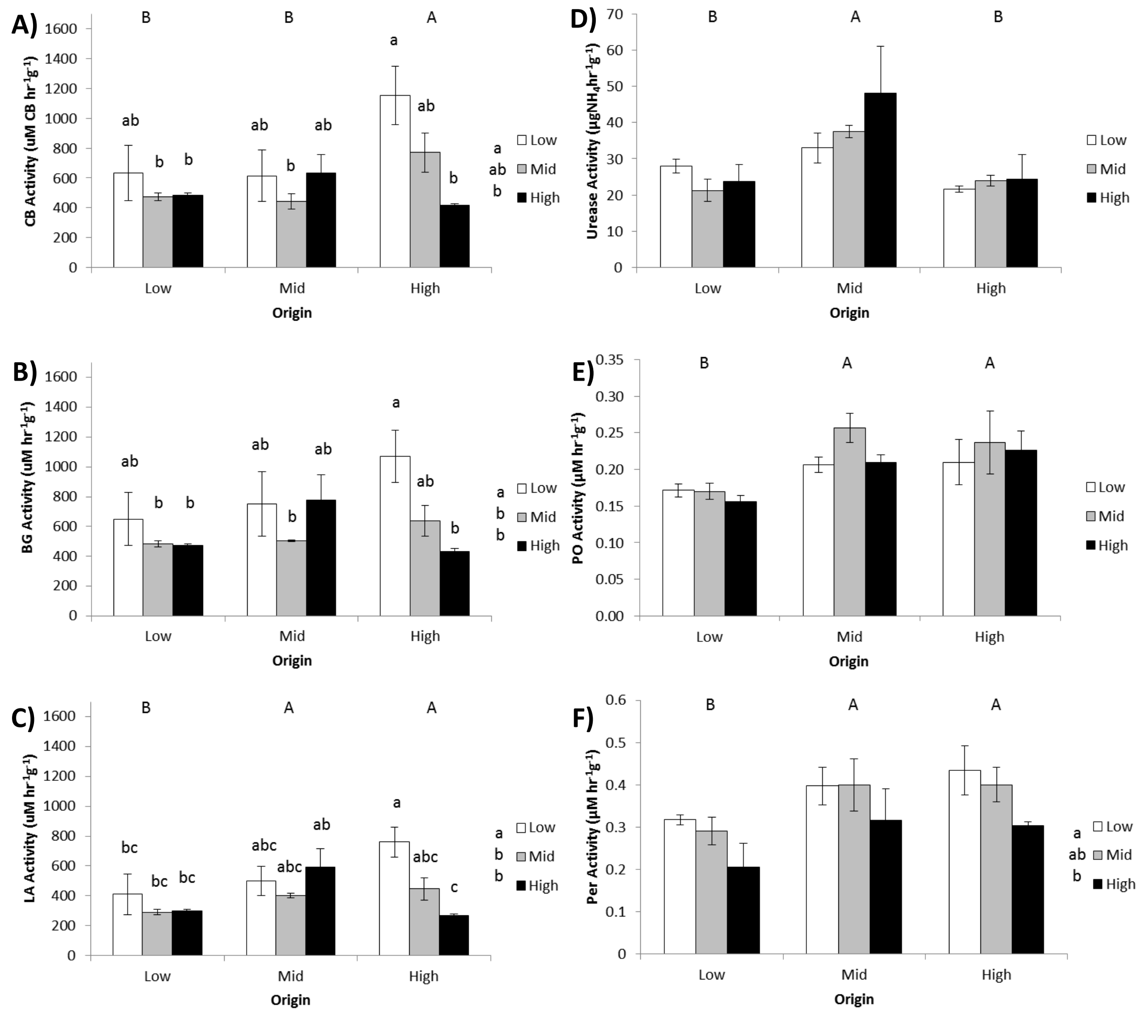
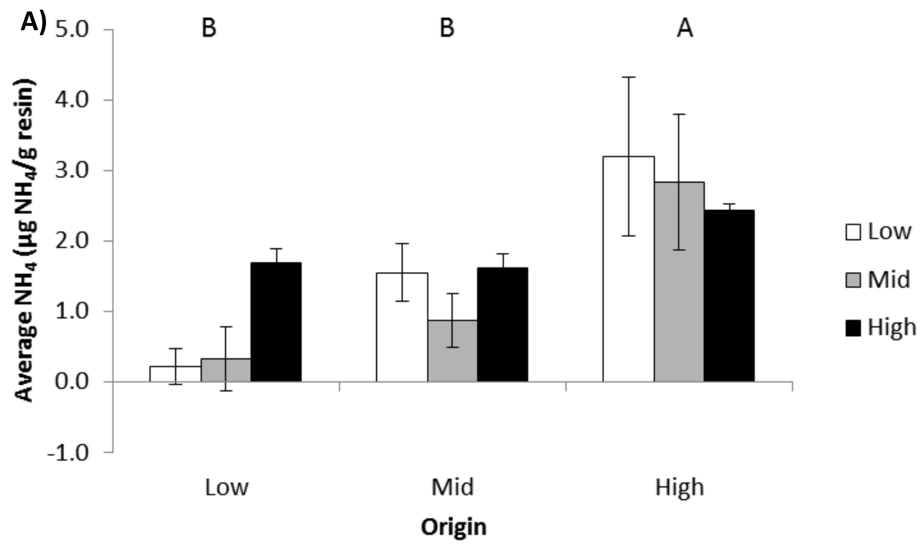


Fig. 3



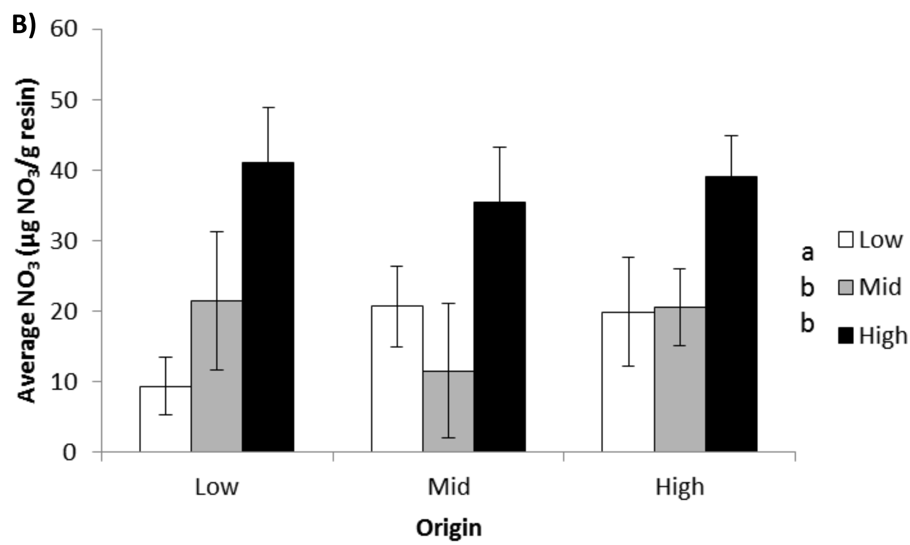


Fig. 4

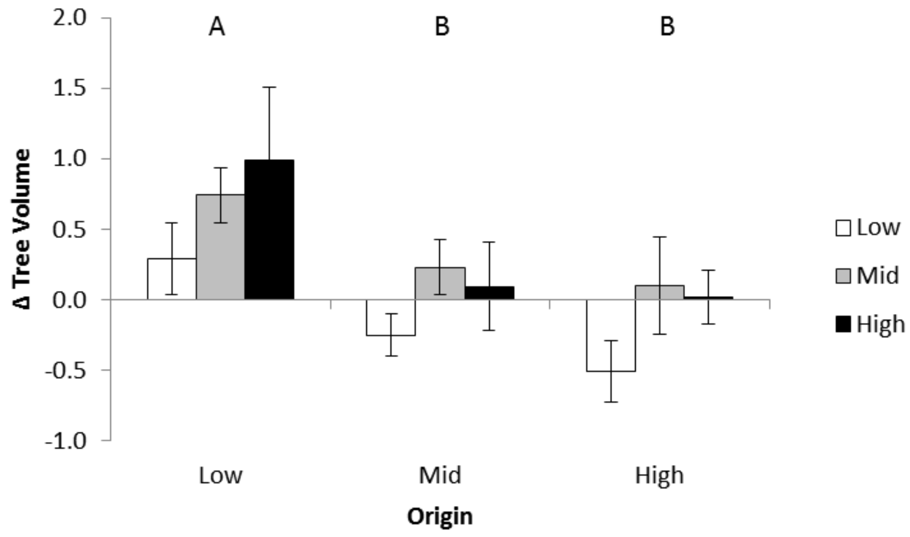


Fig. 5

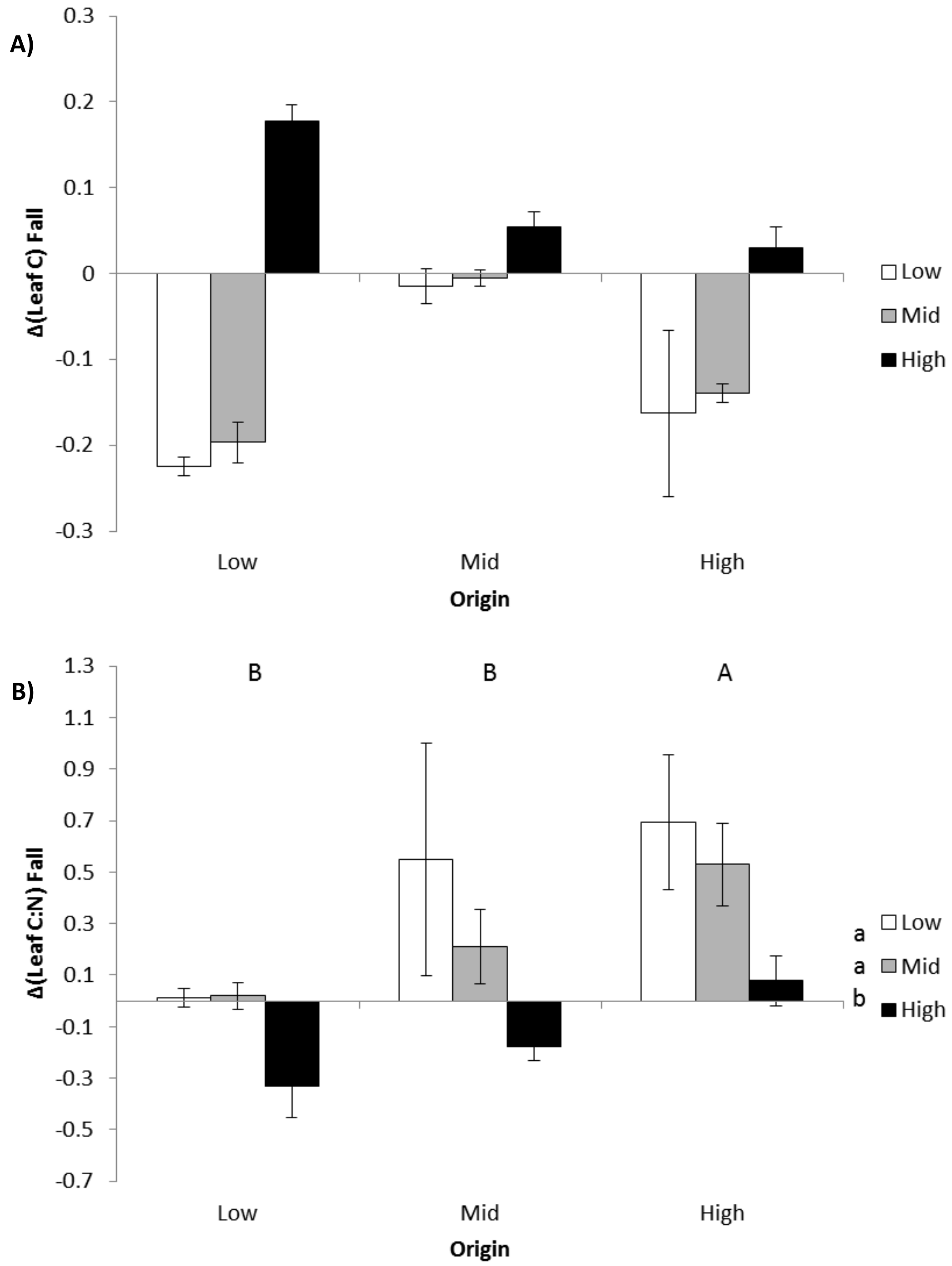


Fig. 6

REFERENCES

Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14, 1156-1168.

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

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biology & Biochemistry 39, 1770-1781.

Aronson, E.L., McNulty, S.G., 2009. Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. Agricultural and Forest Meteorology 149, 1791-1799.

Ayres, E., Steltzer, H., Berg, S., Wall, D.H., 2009. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. Journal of Ecology 97, 901-912.

Bader, M.K.F., Korner, C., 2010. No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Global Change Biology 16, 2830-2843.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47, 151-163.

Beckage, B., Osborne, B., Gavin, D.G., Pucko, C., Siccama, T., Perkins, T., 2008. A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. Proceedings of the National Academy of Sciences of the United States of America 105, 4197-4202.

Becker, A., Korner, C., Brun, J.J., Guisan, A., Tappeiner, U., 2007. Ecological and land use studies along elevational gradients. Mountain Research and Development 27, 58-65.

Beier, C., Emmett, B., Gundersen, P., Tietema, A., Penuelas, J., Estiarte, M., Gordon, C., Gorissen, A., Llorens, L., Roda, F., Williams, D., 2004. Novel Approaches to Study Climate Change Effects on Terrestrial Ecosystems in the Field: Drought and Passive Nighttime Warming. Ecosystems 7, 583-597.

Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M., Kirk, G.J.D., 2005. Carbon losses from all soils across England and Wales 1978-2003. Nature 437, 245-248.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133, 13-22.

Binkley, D., 1984. Does forest removal increase rates of decomposition and nitrogen release. Forest Ecology and Management 8, 229-234.

Bonan, G.B., 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 320, 1444-1449.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 464, 579-U132.

Bonito, G.M., Coleman, D.C., Haines, B.L., Cabrera, M.L., 2003. Can nitrogen budgets explain differences in soil nitrogen mineralization rates of forest stands along an elevation gradient? Forest Ecology and Management 176, 563-574.

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

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

Bresson, C.C., Vitasse, Y., Kremer, A., Delzon, S., 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiology 31, 1164-1174.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., Green, J.L., 2008. Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. Proceedings of the National Academy of Sciences of the United States of America 105, 11505-11511.

Campbell, J.L., Rustad, L.E., Boyer, E.W., Christopher, S.F., Driscoll, C.T., Fernandez, I.J., Groffman, P.M., Houle, D., Kiekbusch, J., Magill, A.H., Mitchell, M.J., Ollinger, S.V., 2009. Consequences of climate change for biogeochemical cycling in forests of northeastern North America. Canadian Journal of Forest Research 39, 264-284.

Carney, K.M., Matson, P.A., 2005. Plant communities, soil microorganisms, and soil carbon cycling: Does altering the world belowground matter to ecosystem functioning? Ecosystems 8, 928-940.

CCSP, 2007. The First State of the Carbon Cycle Report (SOCCR): The North American Carbon Budget and Implications for the Global Carbon Cycle. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, NC, USA, 242 pp.

Chatterjee, A., Jenerette, G.D., 2011. Changes in soil respiration Q_{10} during drying-rewetting along a semi-arid elevation gradient. Geoderma 163, 171-177.

Chmura, D.J., Anderson, P.D., Howe, G.T., Harrington, C.A., Halofsky, J.E., Peterson, D.L., Shaw, D.C., St Clair, J.B., 2011. Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. Forest Ecology and Management 261, 1121-1142.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Rueda, V.M., Mearns, L., Menéndez, C.G., Rinke, J.R.A., Sarr, A., Whetton, P., 2007. Regional Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Conant, R.T., Klopatek, J.M., Klopatek, C.C., 2000. Environmental factors controlling soil respiration in three semiarid ecosystems. Soil Science Society of America Journal 64, 383-390.

Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89, 2384-2391.

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

Davidson, E.A., Janssens, I.A., Luo, Y.Q., 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. Global Change Biology 12, 154-164.

De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B.J., Chabrerie, O., Cousins, S.A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J., Verheyen, K., 2011. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. Global Change Biology 17, 3240-3253.

Di Luzio, M., Johnson, G.L., Daly, C., Eischeid, J.K., Arnold, J.G., 2008. Constructing retrospective gridded daily precipitation and temperature datasets for the conterminous United States. Journal of Applied Meteorology and Climatology 47, 475-497.

Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263, 185-190.

Dunn, A.L., Barford, C.C., Wofsy, S.C., Goulden, M.L., Daube, B.C., 2007. A long-term record of carbon exchange in a boreal black spruce forest: means, responses to interannual variability, and decadal trends. Global Change Biology 13, 577-590.

Falloon, P.D., Smith, P., Smith, J.U., Szabó, J., Coleman, K., Marshall, S., 1998. Regional estimates of carbon sequestration potential: linking the Rothamsted Carbon Model to GIS databases. Biology and Fertility of Soils 27, 236-241.

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

Finzi, A.C., Moore, D.J.P., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., Kim, H.S., Matamala, R., McCarthy, H.R., Oren, R., Pippen, J.S., Schlesinger, W.H., 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. Ecology 87, 15-25.

Garten, C.T., Classen, A.T., Norby, R.J., 2009. Soil moisture surpasses elevated CO_2 and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. Plant and Soil 319, 85-94.

Garten, C.T., Hanson, P.J., 2006. Measured forest soil C stocks and estimated turnover times along an elevation gradient. Geoderma 136, 342-352.

Giardina, C.P., Ryan, M.G., 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature (London) 404, 858-861.

Giardina, C.P., Ryan, M.G., 2002. Total belowground carbon allocation in a fast-growing Eucalyptus plantation estimated using a carbon balance approach. Ecosystems 5, 487-499.

Griffiths, R.P., Madritch, M.D., Swanson, A.K., 2009. The effects of topography on forest soil characteristics in the Oregon Cascade Mountains (USA): Implications for the effects of climate change on soil properties. Forest Ecology and Management 257, 1-7.

Guo, Y.Y., Gong, P., Amundson, R., Yu, Q., 2006. Analysis of factors controlling soil carbon in the conterminous United States. Soil Science Society of America Journal 70, 601-612.

Hamrick, J.L., 2004. Response of forest trees to global environmental changes. Forest Ecology and Management 197, 323-335.

Hanson, P.J., Childs, K.W., Wullschleger, S.D., Riggs, J.S., Thomas, W.K., Todd, D.E., Warren, J.M., 2011. A method for experimental heating of intact soil profiles for application to climate change experiments. Global Change Biology 17, 1083-1096.

Hart, S.C., 2006. Potential impacts of climate change on nitrogen transformations and greenhouse gas fluxes in forests: a soil transfer study. Global Change Biology 12, 1032-1046.

Hart, S.C., Perry, D.A., 1999. Transferring soils from high- to low-elevation forests increases nitrogen cycling rates: climate change implications. Global Change Biology 5, 23-32.

Hartley, I.P., Heinemeyer, A., Ineson, P., 2007. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. Global Change Biology 13, 1761-1770.

Hartley, I.P., Ineson, P., 2008. Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biology & Biochemistry 40, 1567-1574.

Hobbie, S.E., Schimel, J.P., Trumbore, S.E., Randerson, J.R., 2000. Controls over carbon storage and turnover in high-latitude soils. Global Change Biology 6, 196-210.

Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E., Woof, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. Global Change Biology 4, 143-152.

IPCC, 2007. Climate change 2007 : the physical science basis : contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Johnson, D., Leake, J.R., Read, D.J., 2001. Novel in-Growth Core System Enables Functional Studies of Grassland Mycorrhizal Mycelial Networks. New Phytologist 152, 555-562.

Katterer, T., Reichstein, M., Andren, O., Lomander, A., 1998. Temperature dependence of organic matter decomposition: A critical review using literature data analyzed with different models. Biology and Fertility of Soils 27, 258-262.

Keeler, B.L., Hobbie, S.E., Kellogg, L.E., 2009. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. Ecosystems 12, 1-15.

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

Kirschbaum, M.U.F., 2006. The temperature dependence of organic-matter decomposition - still a topic of debate. Soil Biology & Biochemistry 38, 2510-2518.

Knoepp, J.D., Swank, W.T., 1998. Rates of nitrogen mineralization across an elevation and vegetation gradient in the southern Appalachians. Plant and Soil 204, 235-241.

Knoepp, J.D., Vose, J.M., Swank, W.T., 2008. Nitrogen deposition and cycling across an elevation and vegetation gradient in southern Appalachian forests. International Journal of Environmental Studies 65, 389-408.

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

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

Komatsu, H., Hashimoto, H., Kume, T., Tanaka, N., Yoshifuji, N., Otsuki, K., Suzuki, M., Kumagai, T., 2010. Modeling Seasonal Changes in the Temperature Lapse Rate in a Northern Thailand Mountainous Area. Journal of Applied Meteorology and Climatology 49, 1233-1246.

Kurz, W.A., Dymond, C.C., Stinson, G., Rampley, G.J., Neilson, E.T., Carroll, A.L., Ebata, T., Safranyik, L., 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452, 987-990.

Leifeld, J., Bassin, S., Fuhrer, J., 2005. Carbon stocks in Swiss agricultural soils predicted by land-use, soil characteristics, and altitude. Agriculture Ecosystems & Environment 105, 255-266.

Leifeld, J., Zimmermann, M., Fuhrer, J., Conen, F., 2009. Storage and turnover of carbon in grassland soils along an elevation gradient in the Swiss Alps. Global Change Biology 15, 668-679.

Lenoir, J., Gegout, J.C., Marquet, P.A., de Ruffray, P., Brisse, H., 2008. A significant upward shift in plant species optimum elevation during the 20th century. Science 320, 1768-1771.

Link, S.O., Smith, J.L., Halvorson, J.J., Bolton, H., 2003. A reciprocal transplant experiment within a climatic gradient in a semiarid shrub-steppe ecosystem: effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. Global Change Biology 9, 1097-1105.

Litton, C.M., Giardina, C.P., 2008. Below-ground carbon flux and partitioning: global patterns and response to temperature. Functional Ecology 22, 941-954.

Madritch, M.D., Donaldson, J.R., Lindroth, R.L., 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry 39, 1192-1201.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., 2007. Global Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62-71.

Mulvaney, R.L., 1996. Nitrogen—inorganic forms, In: Bartels, J.M. (Ed.), Methods of Soil Analysis: Part 3 Chemical Methods. Soil Science Society of America, Madison, WI, USA, pp. 1123–1184.

Natali, S.M., Schuur, E.A.G., Trucco, C., Pries, C.E.H., Crummer, K.G., Lopez, A.F.B., 2011. Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. Global Change Biology 17, 1394-1407.

Niklinska, M., Klimek, B., 2007. Effect of temperature on the respiration rate of forest soil organic layer along an elevation gradient in the Polish Carpathians. Biology and Fertility of Soils 43, 511-518.

Norby, R.J., Luo, Y.Q., 2004. Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytologist 162, 281-293.

Pacala, S.W., Hurtt, G.C., Baker, D., Peylin, P., Houghton, R.A., Birdsey, R.A., Heath, L., Sundquist, E.T., Stallard, R.F., Ciais, P., Moorcroft, P., Caspersen, J.P., Shevliakova, E., Moore, B., Kohlmaier, G., Holland, E., Gloor, M., Harmon, M.E., Fan, S.M., Sarmiento, J.L., Goodale, C.L., Schimel, D., Field, C.B., 2001. Consistent land- and atmosphere-based US carbon sink estimates. Science 292, 2316-2320.

Paustian, K., Levine, E., Post, W.M., Ryzhova, I.M., 1997. The use of models to integrate information and understanding of soil C at the regional scale. Geoderma 79, 227-260.

Pounds, J.A., Fogden, M.P.L., Campbell, J.H., 1999. Biological response to climate change on a tropical mountain. Nature 398, 611-615.

Powers, R.F., 1990. Nitrogen mineralization along an altitudinal gradient - interactions of soil - temperature, moisture, and substrate quality. Forest Ecology and Management 30, 19-29.

Prentice, I.C., Harrison, S.P., Bartlein, P.J., 2011. Global vegetation and terrestrial carbon cycle changes after the last ice age. New Phytologist 189, 988-998.

Schindlbacher, A., de Gonzalo, C., Diaz-Pines, E., Gorria, P., Matthews, B., Inclan, R., Zechmeister-Boltenstern, S., Rubio, A., Jandl, R., 2010. Temperature sensitivity of forest soil organic matter decomposition along two elevation gradients. Journal of Geophysical Research-Biogeosciences 115.

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

Scowcroft, P.G., Turner, D.R., Vitousek, P.M., 2000. Decomposition of Metrosideros polymorpha leaf litter along elevational gradients in Hawaii. Global Change Biology 6, 73-85.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and

nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729-743.

Tague, C.L., Band, L.E., 2004. RHESSys: Regional Hydro-Ecologic Simulation System-An Object-Oriented Approach to Spatially Distributed Modeling of Carbon, Water, and Nutrient Cycling. Earth Interactions 8.

Thomson, B.C., Ostle, N.J., McNamara, N.P., Whiteley, A.S., Griffiths, R.I., 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83, 69-73.

Verburg, P.S.J., Van Loon, W.K.P., Lukewille, A., 1999. The CLIMEX soil-heating experiment: soil response after 2 years of treatment. Biology and Fertility of Soils 28, 271-276.

Wang, L., Ouyang, H., Zhou, C.P., Zhang, F., Song, M.H., Tian, Y.Q., 2005. Soil organic matter dynamics along a vertical vegetation gradient in the Gongga Mountain on the Tibetan Plateau. Journal of Integrative Plant Biology 47, 411-420.

Zhang, C., Tian, H., Chappelka, A.H., Ren, W., Chen, H., Pan, S., Liu, M., Styers, D.M., Chen, G., Wang, Y., 2007. Impacts of climatic and atmospheric changes on carbon dynamics in the Great Smoky Mountains National Park. Environmental Pollution 149, 336-347.

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., 2009. Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. European Journal of Soil Science 60, 895-906.

VITA

Timothy Gordon Whitby received his Bachelor of Science in biology with a concentration in environmental and biological conservation from the University of Virginia in 2008. Pursuing his interest in ecological responses to climate change, he started his Master of Science at Appalachian State University in the fall of 2009 and finished in the fall of 2011.