EXPERIMENTAL WARMING EFFECTS ON SOIL ORGANIC MATTER DYNAMICS AT THE TEMPERATE-BOREAL FOREST ECOTONE

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Dedication

This dissertation is dedicated to the three most important women in my life.

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Abstract

Laboratory and field experiments have both shown that warming will increase soil CO_2 flux to the atmosphere, but do not agree on the importance of this feedback as a future climate driver. As such, we lack the process-based understanding necessary to predict how warming will change soil carbon stocks in the future. Here we measured warming effects on soil organic matter (SOM) decomposition in a southern boreal forest warming experiment (B4WarmED) at two sites in northern Minnesota, USA. We used two laboratory incubations and measurements of soil extracellular enzymes to examine mechanisms that are predicted to alter the warming response of soil respiration, including soil microbial community thermal adjustment; differential temperature response of fast and slow cycling carbon pools to warming; and the potential for upland soil drying to reduce the response of respiration to temperature.

We measured soil respiration in the first 5-yr of *in situ* warming at B4WarmED. Total soil respiration increased in the first three years of experimental warming (by 8 and 21%, for the +1.7 and +3.4°C treatments, respectively, relative to ambient temperature treatments), but warming responses decreased substantially in the fourth and fifth years of warming. In contrast, warming effects on root-excluded bulk soil respiration were relatively constant during the five years of treatment: the +1.7°C treatment showed little response to warming, whereas warming of +3.4°C increased bulk soil respiration by 13%. Warming treatments both decreased the long-term temperature response (i.e. Q_{10} parameter) and increased the soil moisture response for total soil respiration, but not for bulk soil respiration. Yet, *in situ* soils, even with warming decreases in soil moisture,

were rarely dry enough to substantially alter the response of soil respiration to warming treatment.

We used two laboratory incubations to test whether soil drying and soil microbial thermal adjustment reduced the temperature response of decomposition in B4WarmED soils. SOM decomposition was less responsive to temperature in dry soils, and in soils incubated at higher temperatures, suggesting that drying and thermal adjustment could reduce the warming effects on decomposition, although the effects of thermal adjustment were small. Finally, in both incubations, we found that the decomposition of slow cycling soil carbon responded more to warming than rapidly cycling carbon.

Soil extracellular enzymes are important catalysts of SOM decomposition, as they break down organic matter polymers and increase the carbon substrates available to the soil microbial community. We examined warming effects on four soil extracellular enzymes at the B4WarmEd sites. In contrast to our predictions, we found little evidence of microbial thermal adjustment of enzyme kinetics to warming that would reduce the warming response of SOM decomposition.

In summary, in both field and laboratory measurements SOM decomposition increased with experimental warming. Soil drying was found to decrease SOM decomposition and the response of SOM decomposition to increasing temperature, although warming-enhanced soil drying was not found to substantially decrease *in situ* soil respiration. Finally, in laboratory incubations, slowly cycling SOM was more temperature sensitive than fast cycling SOM. Together, these results suggest that warming will increase SOM decomposition, and that loss of large pools of slowly cycling

SOM, in particular, could contribute significant carbon the atmosphere over the next century. Soil drying could, however, moderate the response of soil respiration to warming, especially if increases in evapotranspiration or changes in precipitation results in drier soils than were observed in the field component of this study.

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Introduction

Climate models project that global mean annual temperature will increase by 1.2 – 4.8°C by the end of the 21st century (Collins et al. 2013). Whether terrestrial ecosystem feedbacks will mitigate or enhance warming represents a significant uncertainty in such projections of future climate. One of the largest potential positive feedbacks to climate warming is that arising from enhanced release of CO_2 from warmer soils (Davidson et al. 2006). Soils contain approximately 3-4x as much carbon as the atmosphere (Collins et al. 2013) and microbial decomposition of soil carbon is expected to increase with climate warming, as temperature is often the most important factor determining rates of soil organic matter decomposition (Lloyd and Taylor 1994). Thus, greater microbial respiration of soil organic matter could contribute substantially to enhancing the greenhouse effect in a warmer world.

Field warming experiments, which are able to integrate the multiple effects of temperature increase on processes controlling soil respiration (e.g. net primary production, substrate supply, soil moisture, microbial community composition, and enzymatic function), overwhelmingly have found rapid loss of C from soils in the first few years after warming begins. Within a few years, however, soil respiration decreases to ca. pre-warming rates (Oechel et al. 2000, Rustad et al. 2001, Luo et al. 2001, Melillo et al. 2002). Several mechanisms could explain these results. First, roots and soil microorganisms could adjust to increased temperature by down-regulating their activity, through physiological, genetic, or community change, reducing the soil respiration rate (*thermal adjustment*)(Oechel et al. 2000, Luo et al. 2001, Bradford et al. 2008). Second,

the *substrate depletion mechanism* explains these results as depletion of a fast cycling carbon pool after several years of warming. Microbial respiration rates, in turn, depend on the remaining slow cycling SOM, which because of its low turnover rate, exhibits limited absolute increases in respiration with warming, even as it may respond more to warming in *relative* terms than fast cycling C, and over time may prove to be a important flux of CO_2 from soils (Kirschbaum 2004, Eliasson et al. 2005, Knorr et al. 2005). Third, the inhibitory effects of *soil drying* on microbial activity induced by warming could offset the direct stimulatory effects of soil warming thereby decreasing the effect of warming on respiration through time (Harte et al. 1995, Davidson et al. 2006, Luo 2007). All of these mechanisms likely contribute to influencing the apparent respiration response to warming (Reichstein et al. 2005a, Bradford et al. 2008). Yet, results to date have not clearly distinguished the relative importance of these mechanisms in influencing the dynamics of the response of soil respiration to warming

The overarching goal of my dissertation work was to combine measurements of experimental warming effects on soil respiration at a highly replicated *in situ* warming experiment located in two southern boreal forests in northern Minnesota, USA, with laboratory experiments and measurements directed at understanding how thermal acclimation, carbon substrate depletion, and soil drying influence the response of soil respiration to warming.

Chapter 1 examines the soil respiration response to *in situ* warming at the B4WarmED (*Boreal Forest Warming at an Ecotone in Danger*) project, located in two boreal forest sites in northern Minnesota. In five years of study (2009-2013), we made

approximately 8000 measurements of total or microbial soil respiration, in order to quantify the effects of warming and canopy treatments, season, and year on soil CO_2 flux. Empirical models were used to examine soil temperature and moisture controls on soil respiration, and to further examine how warming treatments altered these relationships. Finally, we measured radiocarbon in soil microbial respiration in order to determine whether warming alters the relative contributions of fast vs. slow carbon pool decomposition to soil microbial respiration.

Chapter 2 uses two laboratory incubations to examine whether soil drying and soil microbial thermal adjustment reduced the temperature response of decomposition. Chapter 2 also examines whether the decomposition of fast vs. slow cycling carbon pools differ in their temperature response.

Soil extracellular enzymes are important catalysts of carbon decomposition, as they break down organic matter polymers and increase the carbon substrates available to the soil microbial community. Chapter 3 examines warming effects on four soil extracellular enzymes at the B4WarmEd project. As would be expected with microbial thermal adjustment, the activities of extracellular enzymes were predicted to decrease with warming, which could reduce the warming enhancement of the soil carbon decomposition.

Chapter 1 – Lasting stimulation of bulk soil respiration but not of rhizosphere respiration by warming at the boreal-temperate ecotone

Abstract

One of the largest potential positive feedbacks to climate warming is CO_2 arising from the enhanced decomposition of soil organic matter (SOM) in warmer soils. This project examined *in situ* warming effects on soil respiration across five years (2009-2013) of warming at three levels (ambient, +1.7°C, and +3.4°C) in a highly replicated free air warming experiment in two southern boreal forest sites in Minnesota, USA. The first three years of experimental warming increased annual snow-free season total soil respiration by 8% and 21%, for the +1.7 and +3.4°C treatments, respectively, after which the effects of warming treatments decreased. In the fourth and fifth years of warming, the +1.7°C treatment respired less 4% less, and the +3.4°C treatment 5% more relative to the ambient treatments. In contrast, warming effects on bulk soil respiration were relatively constant during the five years of treatment: the +1.7°C treatment showed little response to warming, whereas warming of +3.4°C increased bulk soil respiration by 13%. Soil respiration was positively related to both treatment and temporal variation in soil temperature and moisture. Warming treatment decreased the long-term temperature response of total soil respiration, but not of bulk soil respiration. Warming also increased the effects of moisture on total soil respiration, yet, exclusion of soil respiration measured on dry soils (5% of measurements) increased annual respiration rates by less than 2%, suggesting that these soils were rarely dry enough to substantially alter the response of soil respiration to warming treatment. These results suggest that warming will increase SOM decomposition, with the potential to release large stores of soil carbon to the

atmosphere as CO_2, but only above some threshold level of warming. Soil drying could, however, moderate the response of soil respiration to warming, especially if increases in evapotranspiration or changes in precipitation result in drier soils than were observed in this study.

Introduction

Greater microbial respiration of soil organic matter (SOM) could contribute substantially to enhancing the greenhouse effect in a warmer world (Bond-Lamberty and Thomson 2010). The global mean annual temperature over land is expected to increase by 1.2–4.8°C by the end of the 21st century (Collins et al. 2013), and SOM decomposition generally increases with temperature (Lloyd and Taylor 1994, Kirschbaum 1995, 2000, 2006). Furthermore, soils contain 2-3x more carbon (1500 – 2400 PgC) (Batjes 1996, Jobbágy and Jackson 2000, Ciais et al. 2013) than the atmosphere (840 PgC), such that even a relatively small loss of soil carbon could result in a substantial increase in atmospheric CO_2 (e.g. a 4-7% loss of soil carbon would result in a 50 ppm atmospheric CO_2 increase). Because ecosystem responses over long time scales and involving multiple warming effects on plants and soils are not well understood, the degree to which terrestrial ecosystem feedbacks will mitigate or enhance warming remains an important uncertainty in projections of future climate (Friedlingstein et al. 2006, Heimann and Reichstein 2008).

Field warming studies overwhelmingly have found that warming increases soil respiration (Oechel et al. 2000, Luo et al. 2001, Melillo et al. 2002, Bronson et al. 2008,

Natali et al. 2011, Melillo et al. 2011, Schindlbacher et al. 2012), but in most multi-year field studies, soil respiration decreases to *ca*. pre-warming rates after several years (Oechel et al. 2000, Rustad et al. 2001, Luo et al. 2001, Melillo et al. 2002). Few field-warming experiments (only Bronson et al. (2008) in the above studies) have independently and realistically warmed both aboveground and belowground ecosystem components together. Warming one ecosystem component but not the other, at least partially decouples warming effects on soil respiration from those on plant productivity, and could alter the observed warming response of soil respiration.

Several mechanisms could explain the transient response of *in situ* soil respiration to warming. First, soil respiration could decrease as soil microbial and plant communities thermally adjust to warmer temperatures, though physiological, genetic, and community changes (Oechel et al. 2000, Tjoelker et al. 2001, Luo et al. 2001, Bradford et al. 2008, Tjoelker et al. 2009, Allison et al. 2010, Conant et al. 2011, Bradford 2013). Second, depletion of faster cycling SOM after several years of warming could result in apparent declines in soil respiration, even as the decomposition of slower cycling SOM is enhanced by warming (Kirschbaum 2004, Eliasson et al. 2005, Knorr et al. 2005). Third, drying of non-saturated soil (resulting from warming) could reduce the response of soil microbial respiration to soil warming over shorter time scales, due to reduced connectivity of microbes, enzymes, and substrates and microbial desiccation (Grant and Rochette 1994, Davidson et al. 2000, Davidson and Janssens 2006, Gershenson et al. 2009).

Kinetic theory, modeling studies, and experiments together predict that the decomposition of recalcitrant SOM will be more temperature sensitive than that of labile substrates (Kirschbaum 2004, Fierer et al. 2005, Knorr et al. 2005, Davidson and Janssens 2006). Yet, a changing paradigm of the factors that influence SOM decomposition suggests that carbon turnover relates more to processes with uncertain temperature sensitivity, such as the physical accessibility of SOM to microbial decomposers, rather than its molecular recalcitrance (Schmidt et al. 2011, Marín-Spiotta et al. 2014). Still, physical and chemical stabilization of carbon in soil may at least in part be conferred by temperature sensitive processes, as many studies to date have shown that slowly cycling carbon pools are more temperature sensitive than fast pools (Fierer et al. 2005, von Lützow and Kögel-Knabner 2009, Conant et al. 2011).

Climate warming is predicted to decrease near-surface soil moisture for much of the Earth's land (except where increased evapotranspiration is offset by increased precipitation) (Dai 2012, Collins et al. 2013). Drying of non-saturated soil could reduce the response of soil respiration to warming, due to plant and soil microbial desiccation stress, and reduced connectivity of microbes, enzymes, and substrates via diffusion (Grant and Rochette 1994, Stark and Firestone 1995, Davidson et al. 2000, Davidson and Janssens 2006, Schimel et al. 2007, Gershenson et al. 2009). While links between soil moisture and respiration are better established for xeric ecosystems (Xu and Qi 2001, Rey et al. 2002), soil respiration in mesic ecosystems has been shown to decrease and exhibit reduced responses to temperature during seasonally dry periods. For example, soil respiration declined at low soil moisture availability in mixed hardwood forests stands

(Davidson et al. 1998, Savage and Davidson 2001), a beech forest (Epron et al. 1999), a pine forest (Yuste et al. 2003), and an old field (Suseela et al. 2011). Additionally, studies have found that soil respiration responded less to increasing temperature when soils were dry (Rey et al. 2002, Janssens and Pilegaard 2003, Curiel Yuste et al. 2004, Craine and Gelderman 2011, Suseela et al. 2011).

The temperature sensitivity of total soil respiration also depends on the temperature sensitivities of decomposition of different SOM components. For example, total soil respiration is composed primarily of the saprotrophic decomposition of bulk SOM (hereafter referred to as bulk soil respiration), and respiration from roots, mycorrhizae, and microbes located in the rhizosphere (hereafter referred to as rhizosphere respiration). Bulk soil and rhizosphere respiration both have been shown to increase with warming (Boone et al. 1998, Rey et al. 2002, Suseela et al. 2011), but with different temperature sensitivities and dynamics of response (Boone et al. 1998, Melillo et al. 2011). For instance, Melillo et al. (2011) found that experimental soil warming increased rhizosphere respiration only in the first three years of experimental warming, while warming enhanced bulk soil respiration for the duration of the experiment (i.e. seven years).

Here we report the warming effects on soil respiration in a highly replicated free air combined surface and soil warming experiment in two southern boreal forest sites in Minnesota, USA. Southern boreal forests cover parts of the northern U.S., from Minnesota to Maine and a much larger area of southern Canada. Upland southern boreal forest soils have low to moderate amounts of soil carbon (100-150 Mg C ha^{-1} to a depth

of 1-m) (Grigal and Ohmann 1992, Dixon et al. 1994), relative to poorly drained boreal forests (Rapalee et al. 1998). Still, warming-enhanced respiration in southern boreal upland forests could be an important source of CO_2 to the atmosphere given that higher latitudes are expected to warm more than lower latitudes (Collins et al. 2013), and respiration in colder soils is expected to be more temperature sensitive than that in warmer soils (Davidson and Janssens 2006).

We examined *in situ* warming effects on soil respiration across five years of warming at three levels (ambient, +1.7°C, and +3.4°C). These warming treatments align with predictions of warming in the next 75-100 years (Collins et al. 2013). Our *a priori* expectation was that *in situ* experimental warming would increase soil respiration in the short-term (for several years), after which respiration would return to near pre-warming levels. We expected that this return to pre-warming levels would occur sooner for rhizosphere than for bulk soil respiration, as prior studies have found (Melillo et al. 2011) Furthermore, we expected that warming effects on soil respiration would be greatest early and late in the growing season, reflecting earlier and later plant activity, respectively, as well as soil drying in the middle of the growing season in warmed treatments. We expected that soil respiration in the warmed treatments would be less temperature sensitive due to microbial thermal adjustment, and that the well-drained upland soils of these southern boreal forests sites would likely be sensitive to decreases in soil moisture caused by warming. Finally by measuring the radiocarbon in bulk soil respiration, we investigated whether warming increased the decomposition of older (presumably slow cycling) SOM more than younger, fast cycling SOM.

Methods

B4Warmed experiment

We established an *in situ*, free-air warming experiment on coarse-textured upland soils in both understory and open habitats at two southern boreal forest sites in northern Minnesota, USA, approximately 150 km apart (Fig 1-1) (Reich et al. 2015, Rich et al. 2015). The experiment was established in summer 2008 in (1) a warmer lower latitude site, the Cloquet Forestry Center (CFC), Cloquet, MN and (2) a colder, higher latitude site, the Hubachek Wilderness Research Center (HWRC), Winton, MN (See Table 1-1 for site properties and Fig 1-2 for photographs of sites). At both sites, the warming experiments were located in (≈40-60 year old) mixed aspen-birch-fir stands (with scattered pine, spruce, and other species). Because forest clearing is an important disturbance in this region (Frelich and Reich 1995), we included both a closed (≈5-10% of full light) and open (≈40-60% of full light) canopy treatment by cutting the overstory trees 1 to 3 years prior to the start of the warming treatments.

Experiment Design

Experimental plots were established in the summer 2008, at both sites (CFC and HWRC) in two canopy conditions (open and closed canopies) and at three warming treatment levels (ambient, +1.7°C, and +3.4°C). Within each site-canopy combination, we established three blocks. Within each block there were six plots, two of each warming treatment, for a total of 72 plots. Individual circular plots were 7.1-m^2. Warming was accomplished using simultaneous, independent surface and soil warming with feedback

control to maintain warming treatments at a constant differential from ambient. Surface warming was accomplished with six (+1.7°C) or eight (+3.4°C) ceramic heating elements positioned in a hexagonal or octagonal pattern on the edge of the plots (Reich et al. 2015, Rich et al. 2015). Lamps were initially positioned 1.6-m above the plots, and were raised as planted saplings grew (plantings discussed in next paragraph). Soil warming was achieved by using buried resistance-type heating cables (146-m), at a depth of 10-cm and in an east-west orientation, spaced 20-cm apart. In the ambient warming treatment, PVC tubing was buried in place of heating cables, in order to control for soil disturbances during cable installation.

Every plot across both sites and canopy treatments was divided into 162 400-cm^2 subplots, of which 121 were planted with roughly equal proportions of 5 boreal forest tree species (*Abies balsamifera, Betula papyrifera, Picea glauca, Pinus banksiana, and Populus tremuloides*), 5 temperate tree species (*Acer rubrum, A. saccharum, Quercus macrocarpa, Q. rubra, and Pinus strobus*), and one invasive tree species (*Rhamnus cathartica*). All trees were planted as seedlings. Twenty-two of the 162 subplots were used for seed germination experiments, while 11 were used for soil respiration collars and for soil sampling. The remaining subplots contained only the extant ground vegetation. Though distinct from the work here, the overall aim of the plantings was to understand the potential for climate warming to alter tree species at this temperate-boreal ecotone, through differential germination and seedling establishment (See Sendall et al. 2015, Reich et al. 2015).

Treatments bracketed the domain of anticipated warming during the next 75-100 years (Ciais et al. 2013) and enabled assessment of non-linear responses to warming. The warming treatments were operational only during the snow-free season (early spring to late fall) in order to minimize artifacts of experimental warming in the winter (e.g. increases in snow melt and freeze-thaw cycles), and to reduce treatment costs during times of low biological activity. In 2012, rain exclusion treatments were added to one-half of the open canopy plots. Here we report data from the ambient precipitation treatments only; thus the number of experimental plots used in this study decreased from 72 to 54 at the beginning of the 2012 field season.

We used infrared radiometers to measure the surface temperature, and at least two thermocouples (at 10-cm) to monitor soil temperature in each plot. Both measurements were taken continuously, and data logged every 15 minutes. Volumetric water content (VWC, 0-22.5 cm) was also measured continuously in every plot, and was logged hourly. Experimental design, setup, monitoring, and performance (2009-2011 only) are extensively detailed in Rich et al. (2015). Temperature treatment effects on soil temperature and VWC for years 2009-2013 are shown for CFC (Fig 1-3) and HWRC (Fig 1-4), and detailed (with surface temperature) in Table 1-2.

Soil Respiration

In fall 2008, we installed three PVC collars (10.2 cm diameter) in every plot to measure the *in situ* soil respiration response to warming treatments. Two of the respiration collars per plot extended approximately 2 cm into the soil, and enabled measurement of total soil respiration (i.e. bulk soil and rhizosphere respiration). The third collar in every

plot extended approximately 40 cm into the soil, and represented only bulk soil microbial respiration (i.e. no rhizosphere respiration). All collars were kept free of plant cover. Soil CO_2 flux (i.e. either total or bulk soil respiration) was measured using a LICOR 6400 infrared gas analyzer (IRGA) with soil chamber attached (LI-COR Biosciences, Inc., Lincoln, NE, USA). Soil respiration was measured approximately every two to three weeks during the snow-free season (when warming treatments were active; mean of 13.2 measurements per year for CFC, and 12.6 measurements per year for HWRC over the 2009-2013 period). For each measurement, every collar was sampled twice (3 times before September 2009) to improve sample accuracy.

Temperature and moisture controls of soil respiration

We examined soil temperature and moisture controls of both total and bulk soil respiration, using two models. The first model examined soil temperature control of soil respiration using a simple exponential relationship (*Equation 1)*, while the second model also contained parameterization for soil volumetric water content, in addition to temperature, to predict soil respiration (*Equation 2).* The two models are shown below:

$$F = R_T \times Q_T^{\frac{T-10}{10}} \quad \textit{(Equation 1)}$$

$$F = R_{TM} \times D_{TM}^{(M_{opt} - M)^2} \times Q_{TM}^{\frac{T-10}{10}} \quad \textit{(Equation 2)}$$

where F is the soil respiration flux (μmol CO_2 m^{-2} s^{-1}), R is the basal respiration rate, scaled to 10°C, Q is the factor by which soil respiration increases for every 10°C increase in temperature (i.e. equivalent to Q_{10}), D is a fitted parameter describing the soil moisture effects on soil respiration, T is hourly soil temperature in degrees Celsius from

the plot-level thermocouples, M_{opt} is the maximum volumetric water content, in percent water content, measured concurrently with respiration measurements in each plot over the 5 years of the experiment, and M is the hourly soil VWC. Subscripts refer to whether a parameter is fitted to temperature only (subscript T) or temperature and moisture models (subscript TM). Models are taken from Savage et al. (2009) and Davidson et al. (2012). We investigated multiple time scales of temperature and soil moisture controls of respiration. We fit *equations 1* and *2* using mean soil temperature and VWC from 12 different time intervals: the hour of soil respiration measurement, the hour of respiration measurement + the previous hour (i.e. 2 hours), and 4h, 6h, 8h, 12h, 24h, 36h, 48h, 96h, 168h, and 336h. The one hour models fit best most often; thus, we restricted further analyses to this time interval.

Radiocarbon

Once during 2012, we measured radiocarbon of bulk soil respiration to understand whether warming altered the relative contribution of decomposition of newer vs. older SOM to soil CO_2 flux. We modified a LICOR 6400 with a soil chamber attached to a zeolite molecular sieve (Alltech 13X, Alltech Associates, Deerfield, IL, USA) in order to trap CO_2. Prior to trapping CO_2 for radiocarbon measurement, we scrubbed atmospheric CO_2 from the measurement chamber. Twenty or more cycles (depending on the measured volume of each collar) of scrubbing (of at least 60 ppm CO_2 per cycle) was done for each respiration collar. Without removing the soil flux chamber, the IRGA was switched to

use the molecular sieve to scrub CO_2 from the chamber (for 30-40 cycles of 60 ppm per cycle).

After CO_2 capture, molecular traps were heated at 625°C to desorb CO_2 (Bauer et al. 1992), which was then purified using liquid N_2 on a vacuum line, and reduced to graphite by Fe reduction in H_2 (Vogel et al. 1987). The graphite was analyzed on the University of California Irvine W.M. Keck accelerator mass spectrometry (AMS). $\delta^{13}C$ was measured on a ThermoFinnigan continuous flow isotope ratio mass spectrometer at the University of Florida. The $\delta^{13}C$ was used to correct the $\Delta^{14}C$ values for contamination of atmospheric CO_2 using a two pool mixing model (atmosphere vs. bulk soil respiration) (Schuur and Trumbore 2006). The average fraction air contamination was 12%, (range 3-18%). Due to the expense of these measurements, radiocarbon was only measured on ambient and +3.4°C warming treatments, in the closed canopy, one time during June 2012. The soils in two collars located (in one block) at HWRC were water-saturated, and could not be measured (reducing *n* from 24 to 22).

Statistics

We used a repeated-measures linear mixed model ANOVA to test for effects of warming treatment, season, canopy, and year on total and bulk soil respiration. Warming treatment was included as a continuous variable. In order to test for season by warming effects, we divided the snow-free season into three seasons, *early* (March-May), *mid* (June-August), and *late* (September-November). We used an autoregressive AR(1)

covariance structure for the fit of the repeated measurements. Respiration rates were ln-transformed to meet the assumptions of normality.

Linear mixed models were used to test for the effects of warming treatment and canopy on the temperature response parameters (i.e. *R, D,* and *Q*) and to analyze the effects of warming treatment on $\Delta^{14}CO_2$. Where applicable, models were factorial to 3-way interactions, and block and site were treated as random effects. Statistical analyses were performed using JMP 11.0 (SAS Institute, Cary, NC, USA). Significance was accepted for all statistical tests at $\alpha = 0.05$.

Results

Treatment effects on soil respiration

As predicted, *in situ* experimental warming increased total soil and bulk soil respiration (Table 1-3, Figure 1-5). Across the two sites and two canopies (i.e. closed or open), warming effects on soil total respiration were greater in the first three years of the experiment relative to the subsequent two years (warming x year interaction, Table 1-3, Figure 1-5): total soil respiration in the +3.4°C treatment increased by 20.9% relative to the ambient treatment in 2009-2011, and by 5.0% in 2012-2013. Warming increased total soil respiration less at +1.7°C than at +3.4°C, by 8.1% for 2009-2011, while for 2012-2013, warming decreased total soil respiration relative to the ambient treatment.

In contrast to total soil respiration, warming effects on bulk soil respiration were of a consistent magnitude for all 5 years of treatment, an average increase of 12.8% with 3.4°C warming, with no warming x year interaction (Table 1-3, Figure 1-5). The effects

of the +1.7°C treatment on bulk soil respiration were small and inconsistent, indicating that there were no effects of this treatment.

Bulk soil respiration in all treatments decreased as the experiment progressed (year effect, Table 1-3), from a high of 4.3 μmol CO_2 m^{-2} s^{-1} in the first year of experimental warming to 3.2 μmol CO_2 m^{-2} s^{-1} in the last year of measurement. Bulk soil respiration was a relatively constant proportion of total soil respiration, 87%, across sites, canopies, and warming treatments in the first year (2009) after root-exclusion collar installation, and 77 – 82% in all years thereafter. Bulk soil respiration declined in the second year after root exclusion collar installation in both the early- and late-season, but not until the fifth year for mid-season respiration (season x year effect; Table 1-3). The higher proportion of respiration derived from the rhizosphere during 2009 likely resulted from decay of roots severed during installation of root-exclusion respiration collars; subsequently, soil carbon flux declined because of lack of new rhizosphere carbon inputs.

Seasonal effects on soil respiration were pronounced, and as expected, total and bulk soil CO_2 fluxes were greatest in the mid snow-free season (June to August), when soil temperatures peaked (season effect; seasonal patterns for soil temperature in Figures 1-3 & 1-4 and soil respiration in Figure 1-6 & Appendix A). In contrast to our expectations, the warming effect on soil CO_2 fluxes did not vary significantly with season (Table 1-3). Despite this, other controls on carbon fluxes did vary seasonally. Early in the snow-free season (March to May), total and bulk soil respiration were much greater in the open canopy treatment relative to the closed canopy treatment, likely because soil temperature in open canopy warmed more rapidly in the early snow-free season due to

greater penetration of solar radiation to the soil surface (canopy x season effect; Figure 1-6). In the mid-season (June to August), total and bulk soil respiration were similar in both canopies; while at the end of the snow-free season (September to November), bulk soil respiration was greater in the closed canopy treatments, but total soil respiration did not differ by canopy treatment (Table 1-3; Figure 1-6). There was a significant canopy by year interaction for bulk soil respiration: respiration decreased more with year in open canopy than in the closed canopy (Table 1-3). Overall canopy effects were of greater bulk soil respiration in the open than in the closed canopy (canopy effect; mean 3.72 vs. 3.6 μmol CO_2 m^{-2} s^{-1} for the open and closed canopies, respectively; Table 1-3).

Temperature and moisture controls of soil respiration

Models that included both soil temperature and moisture content (*Equation 2*) were better predictors of both total and bulk soil respiration than models that only included soil temperature (*Equation 1*). Across all plots, the mean R^2 for the models including both soil temperature and moisture was 0.63 (range 0.07-0.91) vs. 0.56 (range 0.02-0.88) for models without soil moisture as a predictor (Table 1-4). Thus, we focus on the results from the soil temperature and moisture model.

In situ experimental warming did not significantly alter the basal rate (i.e. R_{TM}) of total or bulk soil respiration (i.e. the rate at 10°C). Warming decreased the temperature sensitivity of total soil respiration (i.e. decreased Q_{TM}), but not bulk soil respiration (Parameters in Table 1-4 & Statistics in Table 1-5). Respiration was more temperature

sensitive, and warming effects were more pronounced in the closed relative to the open canopy (statistics in Table 1-5*;* data not shown)

Soil respiration was highest at soil volumetric water contents between 0.10 and 0.20 cm^3 H_2O cm^{-3} soil (top panel; Figure 1-7). Soil moisture tended to limit soil respiration below an approximate threshold of 0.10 cm^3 H_2O cm^{-3} soil, evident in that residuals from the fitted soil temperature model (*Equation 1*) tended to over-predict soil respiration rates in these dry soils (middle panel; Figure 1-7). Including soil moisture in the model largely reduced, but did not eliminate over-prediction of respiration for these dry soils (bottom panel; Figure 1-7). Soil respiration also declined at water contents greater than 0.20 cm^3 cm^{-3} (top panel; Figure 1-7). Soil moisture tended to be higher in the colder fringe seasons (Figures 1-3 & 1-4), resulting in a pattern of declining soil temperature with increasing soil water content (Figure 1-8). Thus, decreasing temperature, and not the direct effects of increasing soil moisture, likely explain soil respiration declines at high soil water content. This is evident in that the temperature only model of soil respiration adequately predicted soil respiration when soil water content was greater than 0.10 cm^3 cm^{-3} (middle panel; Figure 1-7).

The parameter D_{TM} describes the relationship between soil moisture and respiration, with a D_{TM} value less than one indicating that soil respiration increases with soil water content and a value greater than one indicating that soil respiration decreases with soil water content. Greater deviations from one indicate greater soil moisture effects on soil respiration. For the majority of models, D_{TM} values were less than one (60/72 for

bulk soil and 69/72 for total soil respiration), indicating that soil respiration increased with soil water content (Table 1-4).

For both total and bulk soil respiration, D_{TM} significantly decreased with warming (Table 1-4). Since the mean D_{TM} values for all warming treatments were less than one, lower values for D_{TM} in the warmed treatment indicate that respiration in these treatments increased more with soil moisture content, likely because warming increased the frequency of soils dry enough (VWC < 0.10) to limit soil respiration. The effects of soil moisture on both total and bulk soil respiration were greater in the open canopy than in the closed canopy (statistical analyses in Table 1-5; data not shown). Moreover, warming effects on D_{TM} were greater in the open than in the closed canopy. This pattern held for both total and bulk soil respiration, but a significant warming x canopy interaction was only detected for total soil respiration (statistical analyses in Table 1-5*;* data not shown)

The relative age of bulk soil efflux

We expected that *in situ* experimental warming would increase bulk soil respiration of older slower cycling soil carbon more than of younger fast cycling soil carbon, causing the isotopic age of soil CO_2 flux to increase in response to warming. Contrary to our expectations, $\Delta^{14}CO_2$ decreased marginally (by ≈6.99‰) in the +3.4°C relative to the ambient treatment (ANOVA main effect, p=0.052, Figure 1-9). We assume that the bulk of soil microbial respiration resulted from the mineralization of SOM that was fixed during the past 50 years since nuclear testing caused a spike in atmospheric $\Delta^{14}CO_2$ (Vogel et al. 1987, Trumbore 2000). As such, we interpret the decrease in $\Delta^{14}CO_2$

in the warming treatment to indicate that more of the bulk soil CO_2 flux at this measurement time derives from more recently assimilated carbon than in the ambient temperature treatments.

Discussion

Warming effects on soil respiration

As hypothesized, *in situ* experimental warming increased soil respiration during five years of above and belowground warming in two southern boreal forest sites. Both total and bulk soil respiration responded to warming at the highest treatment level (+3.4°C). Warming at +1.7°C, however, was found to only enhance total soil respiration. Warming responses of total soil respiration, but not bulk soil respiration were found to be transient: total soil respiration responses to warming decreased from the first three years of warming to the next two years, while bulk soil respiration responses to warming were nearly constant throughout the five years of study.

Q_{10} is the factor by which soil respiration increases for every 10°C increase in temperature, and provides a commonly used parameter to compare warming effects across different studies. We estimated Q_{10} (using the modified van 't Hoff equation from Davidson et al. (2006) on the basis of differences in average yearly (in the snow-free season only) respiration rates between temperature treatments. Estimated Q_{10} values of total respiration decreased from 1.7 (+3.4°C vs. ambient) and 1.4 (+1.7°C vs. ambient) for the first three years, to 1.2 (+3.4°C) and 0.8 (+1.7°C) for the last two years of this study. For bulk soil respiration, Q_{10} values were 1.4 (+3.4°C) and 1.1 (+1.7°C) across all

years of this experiment. The calculated Q_{10} values align closely with those reported in Bond-Lamberty and Thomson (2010) for changes in the global soil respiration record for 1987 – 2008 (i.e. $Q_{10} = 1.5$).

Surprisingly, Q_{10} estimates based on warming treatment differences in respiration were markedly lower than the temperature sensitivities predicted using flux rate responses to hourly, daily, and seasonal changes in temperature *within* a warming treatment (i.e. Q_{TM} values). It is possible that the constant warming treatment differentials (+1.7 and +3.4°C) promoted greater soil microbial physiological, genetic, and/or community changes than seasonal temperature shifts, even as warming treatments are nested within seasonal temperature. Alternatively, differential respiration responses to warming treatment and seasonal temperature fluctuations might reflect how these two parameters were determined. Q_{10} values are based on *mean* respiration rates by treatment, whereas Q_{TM} is calculated using *instantaneous* respiration rates across seasons and years. The determination of Q_{TM} is more likely to be influenced by the extreme values, i.e. temperature maxima and high rates of respiration that are rare, but that could influence the non-linear estimation of $Q_{TM.}$ Lastly, because soil moisture is largely accounted for in *Equation 2*, Q_{TM} values represent temperature responses at optimal soil moisture. This should increase Q_{TM} values relative to those for Q_{10}, which is calculated from soil respiration measurements averaged over the full range of soil moisture values that occurred in the field. This doesn't fully explain the Q_{TM} - Q_{10} differential, however, as Q_T values (temperature only fits; *Equation 1)* are less than those for Q_{TM}, but still greater than to those of Q_{10}.

With two warming treatment levels, we were able to identify nonlinear responses of soil respiration to warming. For total soil respiration, across all years, warming effects were smaller for the +1.7°C treatment (mean treatment Q_{10} = 1.2) than for the +3.4°C treatment (Q_{10} = 1.5). Warming effects on bulk soil respiration followed a threshold response with only small, inconsistent effects of +1.7°C warming (Q_{10} = 1.1), while the +3.4°C warming treatment consistently stimulated bulk soil respiration across all years of measurement, with a Q_{10} value of 1.4.

Nonlinear responses to climate change have been shown in other studies (Gill et al. 2002, Porporato et al. 2004, Burkett et al. 2005), yet few studies to date used multiple warming levels to examine nonlinear responses. Causes of nonlinear responses of SOM decomposition are not obvious, but could include thermal adjustment of microbial communities that offset warming effects nearly entirely with experimental warming of +1.7°C but not at +3.4°C.

In a mixed hardwood forest in Massachusetts, USA, (Melillo et al. 2002) found that soil respiration increased 28% during the first 6 years of +5°C soil-only warming (corresponding to a Q_{10} of 1.6 using the aforementioned calculation), but decreased after 6 years to approximately 5% (Q_{10} = 1.10). In our study, stimulation of total respiration by the highest level of warming was also short-lived, lasting 3 years before declining. Bulk soil respiration, however, was increased in the 3.4°C warming treatment, for the duration of the 5 years of measurement.

Rhizosphere respiration, as operationally defined by our root exclusion collars, not only included specific root respiration, but respiration from root growth and turnover,

mycorrhizae, and microbial decomposition within the rhizosphere (including rhizosphere priming effects). Previous studies have shown that rhizosphere respiration is more temperature sensitive than that of bulk SOM (Boone et al. 1998, Davidson et al. 2006), although studies have found conflicting results (Zhu and Cheng 2011). Warming at +3.4°C increased total more than bulk soil respiration in the first three years of the experiment, indicating that rhizosphere respiration responded more to warming than bulk soil respiration during this time. In the last two years of the experiment, however, bulk soil respiration in the +3.4°C warming treatment responded more than total respiration to warming, demonstrating apparent decreases in the rhizosphere respiration warming responses.

The decrease in warming response of rhizosphere respiration through time could result from decreasing root biomass, turnover rate, or carbon allocation to mycorrhizae or exudates, as well as thermal adjustment of root and soil microorganisms in the rhizosphere (Pregitzer et al. 2000, Loveys et al. 2003, Majdi and Öhrvik 2004, Bai et al. 2010, Melillo et al. 2011). For instance, (Melillo et al. 2011) found that warming increased fine root respiration (per hectare) during the first two years of *in situ* warming, after which fine root biomass decreased, resulting in warming reductions in fine root respiration. These authors suggest that declining root biomass resulted from decreased belowground allocation as warming increased plant available resources such as nitrogen in soil (Rastetter et al. 1997, Rastetter 2011, Melillo et al. 2011). Due to the high density of seedlings planted in every plot (25 seedlings m^{-2}), root biomass is unlikely to have declined, although warming could have decreased plant allocation to roots relative to

ambient treatments (even though root biomass continued to increase in both). We did not measure root biomass, turnover, or specific root respiration in our warming treatments. Without a more detailed study of warming effects on the component fluxes of rhizosphere respiration and because root phenology co-varies with soil temperature and soil moisture (Steinaker and Wilson 2008) we cannot determine the mechanisms underlying the transient rhizosphere responses to warming.

In contrast to rhizosphere respiration, there was no evidence that warming effects on bulk SOM decomposition were short-lived. It is conceivable that five years of *in situ* experimental warming in these two southern boreal forests was too short to see diminished responses. In a meta-analysis of warming studies, (Rustad et al. 2001) identified declines in respiration by the fourth year of warming. Likewise, while (Melillo et al. 2002) found that warming increased soil respiration for seven years, warming effects decreases noticeably by the fifth year. That the warming responses of SOM decomposition were not transient to date suggests that SOM could be a significant source of CO_2 to the atmosphere in a warmer world. Furthermore, it is possible that transient responses found elsewhere are incorrectly attributed to decreases in bulk soil respiration, instead of reductions in rhizosphere respiration. This could occur where only total soil respiration was measured, or where soil respiration was poorly partitioned between rhizosphere and bulk soil sources.

We found no evidence that warming effects on either total or bulk soil respiration differed by season. We expected that warming would increase belowground allocation (due to enhanced plant productivity) in the early- and late-season (relevant for total soil

respiration only), and that warming would increase mid-season soil drying, decreasing warming effects. Soil drying effects on soil respiration were less than expected (see discussion of moisture controls of soil respiration in next section). Early- and late-season dynamics were highly variable between years of measurement, perhaps due to highly variable late winter and spring climate, resulting in considerable variation in plant phenology at these sites.

Temperature and moisture controls of soil respiration

We fit total and bulk soil respiration to multiple time increments for soil temperature and VWC. The shortest interval, that for hourly soil temperature and VWC, was a better predictor of soil respiration that longer intervals ranging from 2 hours to 2 weeks. This could indicate that roots and soil microorganisms responded without a time lag to soil temperature and moisture, or that lags differed between individual measurements such that the hourly soil temperature and moisture were the best predictors of soil respiration across all measurements.

The temperature sensitivity of total soil respiration decreased with warming treatment. We found similar patterns for bulk soil respiration, as well, but due to higher variability, these differences were not significant. Decreases in Q_{TM} are expected as reaction rates in higher energy (i.e. warmer) systems are less responsive to additional inputs of energy (Davidson et al. 2006, von Lützow and Kögel-Knabner 2009). Additional decreases in Q_{TM} with warming could occur as roots and soil microorganisms thermally adjust to higher temperatures, or as the result of decreased belowground

allocation and soil carbon depletion. That the temperature sensitivity of total, but not bulk soil respiration, decreased with warming treatment, points to declining temperature response of rhizosphere respiration as a key driver of declining Q_{TM} with warming treatment (Tjoelker et al. 2001, Davidson and Janssens 2006, von Lützow and Kögel-Knabner 2009)

It is important to note that while the model included a soil moisture parameter (i.e. D_{TM}), it is possible that within this model structure, parameters describing basal respiration (R_{TM}) and temperature sensitivity of respiration (Q_{TM}) could also be influenced by soil water availability. Warmer treatments were drier on average than the control treatment (Table 1-2), which could decrease parameter estimates of R_{TM} and Q_{TM}, as desiccation stress and limited diffusion of carbon substrates (or their enzymes) limited respiration and its response to temperature.

Total and bulk soil respiration increased as a function of increasing volumetric water content (i.e. $D_{TM} < 1$). Studies in upland ecosystems have found similar patterns of reduced soil respiration at low soil moisture availability (Davidson et al. 1998, Epron et al. 1999, Savage and Davidson 2001, Yuste et al. 2003, Suseela et al. 2011). Several of these studies also found declining respiration at higher soil moisture availability (e.g. (Davidson et al. 1998, Suseela et al. 2011)). Soil respiration in this study declined at high soil water content (VWC > 0.30; Figure 1-7). Because soil temperature declined as VWC increased (Figure 1-8), declines in respiration may have been caused by higher soil moisture and/or lower soil temperature. Supporting the latter interpretation, we found no evidence that the fitted models (which accounted for temperature effects) overpredicted

respiration when VWC was greater than 0.30 cm^3 cm^{-3}. One caveat is that we avoided measuring soil respiration during or immediately after rain events, when soils were likely to be saturated. Because of this, we most likely failed to adequately capture declines in soil respiration due to oxygen limitation in saturated soils (Davidson et al. 2012).

As predicted, warming treatment increased the modeled effects of soil moisture on respiration (i.e. warming treatments had lower predicted D_{TM}), likely reflecting the greater frequency of drier soils in the warming treatments. This demonstrates that soil drying in these forests could limit the warming response of respiration. Yet, only 2.7% of respiration measurements in ambient treatments were made when soils were very dry (VWC < 0.10 cm^3 cm^{-3}). This value increased to 5.5% for the +1.7°C treatment, and 8.0% for the +3.4°C treatment. Thus, very dry soils during this experimental period were rare, even in the warming treatments. Removing the effects of very dry (VWC < 0.l0 cm^3 cm^{-3}) soil respiration increased warming treatment average soil respiration by less than 2%, indicating that the overall effects of dry soils on soil respiration were negligible. For total respiration, the effect of dry soils increased with warming treatment, but for bulk soil respiration this was not the case. Thus, experimental warming as high as +3.4°C enhanced evapotranspiration, decreasing soil moisture, yet soil drying had only small effects on average snow-free season soil respiration response to warming treatment. Still, future changes in the amount or seasonality of precipitation, combined with enhanced evapotranspiration by warming, could reduce soil moisture enough to limit the warming response of soil respiration (Suseela et al. 2011, Schindlbacher et al. 2012). Currently underway at both sites are rain exclusion shelters, which, crossed with warming treatment

in the open canopy, should help to further reveal patterns of soil drying effects on respiration.

The relative age of bulk soil efflux

Recent reviews and kinetic theory suggest that decomposition of slower cycling carbon is generally more temperature sensitive than that of fast cycling carbon (von Lützow and Kögel-Knabner 2009, Conant et al. 2011). For these reasons, we expected that the isotopic age of soil CO_2 flux would increase in response to warming, as the decomposition of slower cycling (i.e. older on average) carbon responds more to warming than that of fast cycling carbon (i.e. younger on average). Contrary to our expectations, we found that bulk soil CO_2 flux contained more recently assimilated carbon in the +3.4°C warming treatment than in the ambient temperature treatments. This provides some evidence that warming might increase the loss of young soil carbon, more than of older soil carbon. On a cautionary note, we extrapolated from only one measurement day, assumed that changes in $\Delta^{14}CO_2$ resulted primarily from changes in the decomposition of post 'bomb' carbon, and did not inventory soil radiocarbon (as in Gaudinski et al. 2000 and Sierra et al. 2012), which would have allowed us to better link changes in respired $\Delta^{14}CO_2$ to its many source pools, especially as SOM stabilization mechanisms might be expected to respond differentially to warming. Thus, we consider these measurements a preliminary exploration of warming effects on radiocarbon, particularly because two laboratory incubations using sieved and intact soil cores from

these sites found that the decomposition of slow cycling carbon pools was more temperature sensitive than that of fast cycling carbon pools (See Chapter 2).

Conclusions

The primary aim of this study was to examine the response of total and bulk soil respiration to *in situ* experimental warming in a highly replicated, open air warming experiment at two southern boreal forests in Minnesota. In agreement with our predictions, experimental warming initially increased total more than root-excluded respiration, however the response of total respiration to warming was transient; respiration responded in the first three years of warming, but not afterward. This transient response of total soil respiration can likely be linked to root and rhizosphere down-regulation, although the specific underlying mechanism is unknown. In contrast, bulk soil respiration showed constant enhancement across five years to experimental warming of +3.4°C, but showed little responses to warming of +1.7°C. Thus, these soils could release substantial carbon in a warmer world above some threshold warming level.

Soil respiration decreased in dry soils, indicating that soil moisture loss could moderate the response of soil respiration to warming. Yet, soils rarely were dry enough to limit soil respiration, even with warming. Consequently, soil drying did not substantially decrease realized respiration or the warming response of respiration. One limitation of this study is that we did not warm canopy trees in the closed canopy plots. We expect that evapotranspiration from canopy trees would increase with warming, depleting soil moisture to a greater extent than we observed without canopy tree warming. Finally, soil

drying could decrease warming effects on soil respiration, if real-world warming exceeds warming treatments in this experiment, or if warming is accompanied by changes in precipitation.

Table 1-1. Geographic, climatic, and edaphic characteristics of the two B4WarmED sites, located in two southern boreal forests in northern Minnesota, USA. Standard error values are shown in parentheses. Lettered superscripts correspond to notes at the bottom of the table.

Characteristics	Cloquet Forestry Center (CFC)						Hubacheck Wilderness Research Center (HWRC)					
Latitude	46°40'46"N						47°56'49"N					
Longitude	92°31'11"W						91°45'29"W					
Mean annual precipitation, 1981-2010 (mm) [A]	808						740					
Mean annual temperature, 1981-2010 (°C) [B]	4.8						4.0					
Soil Taxonomy [B]												
Order	Inceptisols						Entisols					
Suborder	Udepts						Orthents					
Subgroup	Typic Drstrudepts						Lithic Udorthents					
Series	Cloquet Series						Conic Series					
Parent material	Outwash plain						Glacial Till					
Canopy	Closed			Open			Closed			Open		
Overstory Age, 2009 (yr)	40 - 60			2-3			40 - 60			1		
O horizon (g m^{-2}) [C]	699(126)			688(126)			696(121)			1154(185)		
Mineral Soil Depth (cm)	*0-5*	*5-10*	*10-20*	*0-5*	*5-10*	*10-20*	*0-5*	*5-10*	*10-20*	*0-5*	*5-10*	*10-20*
pH [D]	5.23 (0.04)	5.21 (0.03)	5.23 (0.04)	5.38 (0.04)	5.43 (0.03)	5.24 (0.07)	5.45 (0.04)	5.38 (0.05)	5.02 (0.05)	5.51 (0.05)	5.38 (0.04)	5.25 (0.03)
mg C g^{-1} [E]	35 (0.96)	18.27 (0.98)	8.92 (0.51)	32.44 (1.51)	18.95 (0.93)	9.6 (0.4)	35.13 (1.39)	15.89 (0.95)	6.99 (0.32)	29.85 (1.85)	17.7 (1.51)	9.84 (1.27)
mg N g^{-1} [E]	2.17 (0.06)	1.17 (0.06)	0.58 (0.03)	1.83 (0.06)	1.16 (0.05)	0.62 (0.02)	1.85 (0.08)	0.97 (0.06)	0.49 (0.03)	1.49 (0.07)	0.97 (0.06)	0.51 (0.05)
Soil C:N [E]	16.2 (0.16)	15.6 (0.16)	15.3 (0.26)	17.6 (0.29)	16.4 (0.39)	15.6 (0.41)	19.2 (0.43)	16.3 (0.35)	14.5 (0.55)	19.6 (0.56)	17.9 (0.45)	19.3 (0.88)
% Clay [D]	7.1 (0.44)	12.4 (1.90)	12.0 (0.94)	7.5 (0.46)	10.1 (0.83)	12.4 (0.94)	12.2 (0.66)	15.8 (1.23)	22.7 (2.48)	8.2 (0.67)	7.7 (0.54)	12.9 (1.1)

% Silt [D]	29.9 (1.9)	35.1 (2.74)	33.8 (2.02)	26.5 (2.39)	30.8 (1.59)	29.4 (1.59)	28.8 (1.60)	29.0 (0.83)	30.3 (1.85)	15.6 (1.16)	17.9 (0.63)	14.6 (0.82)
% Sand [D]	63.0 (2.13)	52.5 (1.84)	54.2 (2.14)	66.1 (2.56)	59.1 (1.98)	58.2 (2.31)	58.9 (2.01)	55.2 (1.65)	47.1 (2.45)	76.3 (1.6)	74.5 (0.96)	72.5 (0.76)
Bulk density (g cm^{-3}) [F]	0.72 (0.06)	1.17 (0.13)	0.92 (0.07)	0.78 (0.1)	1.52 (0.17)	1.13 (0.1)	0.81 (0.08)	1.85 (0.15)	1.26 (0.1)	0.92 (0.07)	1.63 (0.18)	1.00 (0.09)
Root density (g kg^{-1}) [F]	5.6 (3.0)	2.8 (0.6)	5.3 (4.1)	2.2 (0.7)	6.6 (4.1)	1.1 (0.3)	11.9 5.5)	6.6 (1.5)	2.1 (0.6)	6.8 (0.8)	21.8 (11.8)	0.6 (0.1)

A. 30-year mean climate data from NOAA National Climatic Data Center from the Winton Power Plant (less than 2 km from HWRC) and Cloquet (CFC) stations.
B. For CFC, soil taxonomy from USDA Web Soil Survey, and for HWRC, soil taxonomy inferred from Minnesota Geospatial Commons. For HWRC, soil series was inferred based on characteristics from three potential series; Quetico, Insula, or Conic.
C. O horizon (mostly O_i / O_e) was sampled in 2008,using a 20 x 20cm at three locations in each B4WarmED block.
D. pH was measured in a 2:1 ratio of water to soil. Texture was measured using the hydrometer method. Soil for both analyses was sampled in 2008 and consisted of a composited sample of six cores (2.5-cm) taken from within each B4WarmED plot.
E. Soil carbon and nitrogen were determined on soil from each plot on an Elementar VarioMax elemental analyzer (Elementar Analysensysteme GmbH) from soils collected in 2008.
F. Bulk density was measured at three locations in each B4WarmED block in 2008 (outside of treatment plots). Three cores (5-cm diameter) per B4WarmED plot were sampled and the dry mass of each determined. The volume of the soil removed was determined by filling a flexible plastic bag placed in the soil hole with a known volume of water. Root density from roots sieved from these samples.

Table 1-2. Mean warming treatment effects on aboveground temperature, soil temperature (at 10cm), and soil volumetric water content by year, canopy, and site. Ambient temperature and soil moisture values are shown, with warming effects (difference from ambient) shown for +1.7 and +3.4°C treatments. Annual means and means across years, sites, and canopies are also shown.

		Aboveground temperature (°C)				Soil temperature (°C, 10cm)				Soil volumetric water content (cm^3 cm^{-3}, 0-22.5cm)			
		CFC		HWRC		CFC		HWRC		CFC		HWRC	
Year	Warming	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open
2009	Ambient	10.4	10.5	11.4	11.8	10.5	11.3	11.1	12.3	0.23	0.24	0.30	0.16
	+1.7C	*+2.1*	*+1.9*	*+2.1*	*+1.8*	*+1.9*	*+1.9*	*+2.0*	*+2.1*	*-0.02*	*-0.04*	*-0.03*	*-0.01*
	+3.4C	*+4.0*	*+3.4*	*+3.9*	*+3.7*	*+3.7*	*+3.5*	*+3.7*	*+3.9*	*-0.03*	*-0.05*	*-0.06*	*-0.04*
2010	Ambient	11.4	11.3	11.9	12.3	11.7	12.1	11.8	12.7	0.23	0.24	0.28	0.16
	+1.7C	*+2.0*	*+1.8*	*+2.2*	*+1.6*	*+1.9*	*+1.9*	*+2.1*	*+2.0*	*-0.02*	*-0.04*	*-0.03*	*-0.01*
	+3.4C	*+3.7*	*+3.2*	*+4.1*	*+3.4*	*+3.7*	*+3.7*	*+3.8*	*+3.8*	*-0.04*	*-0.06*	*-0.05*	*-0.03*
2011	Ambient	11.1	11.0	12.9	13.0	11.6	12.0	12.7	13.1	0.22	0.23	0.25	0.13
	+1.7C	*+1.7*	*+1.6*	*+1.8*	*+1.4*	*+1.8*	*+1.8*	*+1.8*	*+2.3*	*-0.03*	*-0.04*	*-0.03*	*+0.01*
	+3.4C	*+2.9*	*+2.7*	*+3.3*	*+3.0*	*+3.5*	*+3.4*	*+3.5*	*+3.9*	*-0.04*	*-0.05*	*-0.04*	*-0.01*
2012	Ambient	9.8	10.3	10.5	10.9	10.8	11.3	10.9	11.7	0.17	0.19	0.24	0.14
	+1.7C	*+1.7*	*+1.2*	*+1.6*	*+1.2*	*+1.6*	*+1.9*	*+1.9*	*+2.1*	*-0.01*	*-0.02*	*-0.02*	*-0.01*
	+3.4C	*+3.3*	*+2.5*	*+3.2*	*+3.0*	*+2.9*	*+2.9*	*+3.3*	*+3.5*	*-0.01*	*-0.04*	*-0.04*	*-0.02*
2013	Ambient	11.5	11.5	12.7	13.0	11.1	11.9	12.1	13.5	0.21	0.21	0.27	0.18
	+1.7C	*+1.7*	*+1.7*	*+1.8*	*+1.5*	*+2.1*	*+2.0*	*+1.9*	*+2.0*	*-0.03*	*-0.03*	*-0.01*	*-0.03*
	+3.4C	*+3.4*	*+3.2*	*+3.5*	*+3.1*	*+3.7*	*+3.6*	*+3.5*	*+3.4*	*-0.03*	*-0.05*	*-0.04*	*-0.04*
Overall	**Ambient**	**10.8**	**10.9**	**11.9**	**12.2**	**11.1**	**11.7**	**11.7**	**12.6**	**0.21**	**0.22**	**0.27**	**0.16**
	+1.7C	***+1.8***	***+1.6***	***+1.9***	***+1.5***	***+1.8***	***+1.9***	***+1.9***	***+2.1***	***-0.02***	***-0.03***	***-0.02***	***-0.01***
	+3.4C	***+3.5***	***+3.0***	***+3.6***	***+3.2***	***+3.5***	***+3.4***	***+3.6***	***+3.7***	***-0.03***	***-0.05***	***-0.04***	***-0.03***
	Ambient	**11.4**				**11.8**				**0.21**			
	+1.7C	**+1.7**				**+1.9**				**-0.02**			
	+3.4C	**+3.3**				**+3.5**				**-0.04**			

Table 1-3. Results from repeated measures mixed models identifying the effects of warming treatment, canopy, season, and measurement year on soil total (left) and bulk soil respiration (right).

Effects	*Total respiration*	*Bulk soil respiration*
Warming	****	*
Canopy		*
Season	****	***
Year		****
Warming x Canopy		
Warming x Year	*	
Warming x Season		
Canopy x Year		*
Canopy x Season	*	***
Season x Year	†	**
Warming x Canopy x Year		
Warming x Canopy x Season		
Warming x Season x Year		
Canopy x Season x Year		†

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ***P≤0.0001

Table 1-4. Mean parameters for temperature and temperature + soil water content models of soil respiration. Standard error is shown in parentheses.

Flux	*Soil temperature and moisture model*				*Soil temperature model*		
Bulk soil respiration	R_{TM}	D_{TM}	Q_{TM}	R^2_{TM}	R_T	Q_T	R^2_T
Ambient	2.59 (0.12)	0.99913 (0.00025)	2.34 (0.08)	0.61 (0.03)	2.47 (0.09)	2.27 (0.08)	0.57 (0.03)
+1.7°C	2.36 (0.15)	0.99876 (0.00033)	2.38 (0.13)	0.48 (0.04)	2.29 (0.17)	2.25 (0.14)	0.42 (0.04)
+3.4°C	2.45 (0.14)	0.99788 (0.00038)	2.29 (0.06)	0.53 (0.03)	2.28 (0.13)	2.06 (0.07)	0.45 (0.03)
Total respiration							
Ambient	3.13 (0.09)	0.99924 (0.00030)	2.55 (0.09)	0.75 (0.02)	3.02 (0.11)	2.45 (0.09)	0.71 (0.03)
+1.7°C	3.05 (0.10)	0.99832 (0.00027)	2.42 (0.05)	0.71 (0.02)	2.86 (0.10)	2.19 (0.06)	0.63 (0.03)
+3.4°C	3.34 (0.14)	0.99732 (0.00043)	2.30 (0.04)	0.68 (0.02)	3.11 (0.15)	1.96 (0.04)	0.55 (0.03)

Table 1-5. Results from mixed model ANOVAs identifying the effects of warming treatment and canopy effects on parameters from soil temperature or soil temperature + moisture models of bulk soil respiration (top) and total soil respiration (bottom). *R* is the basal respiration rate, scaled to 10°C, *Q* is the temperature sensitivity of respiration, i.e. the factor by which respiration increases for every 10°C increase in temperature, and *D* is a factor relating soil respiration to soil water content. The subscript *TM* indicates the combined soil temperature and moisture model, while the subscript *T* denotes the soil temperature model.

Effects	*Soil temperature and moisture model*			*Soil temperature model*	
Bulk soil respiration	R_{TM}	D_{TM}	Q_{TM}	R_T	Q_T
Warming		***			†
Canopy		*			†
Warming x Canopy					
Total respiration					
Warming		****	**		****
Canopy		*	***		****
Warming x Canopy		*	***		***

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ***P≤0.0001

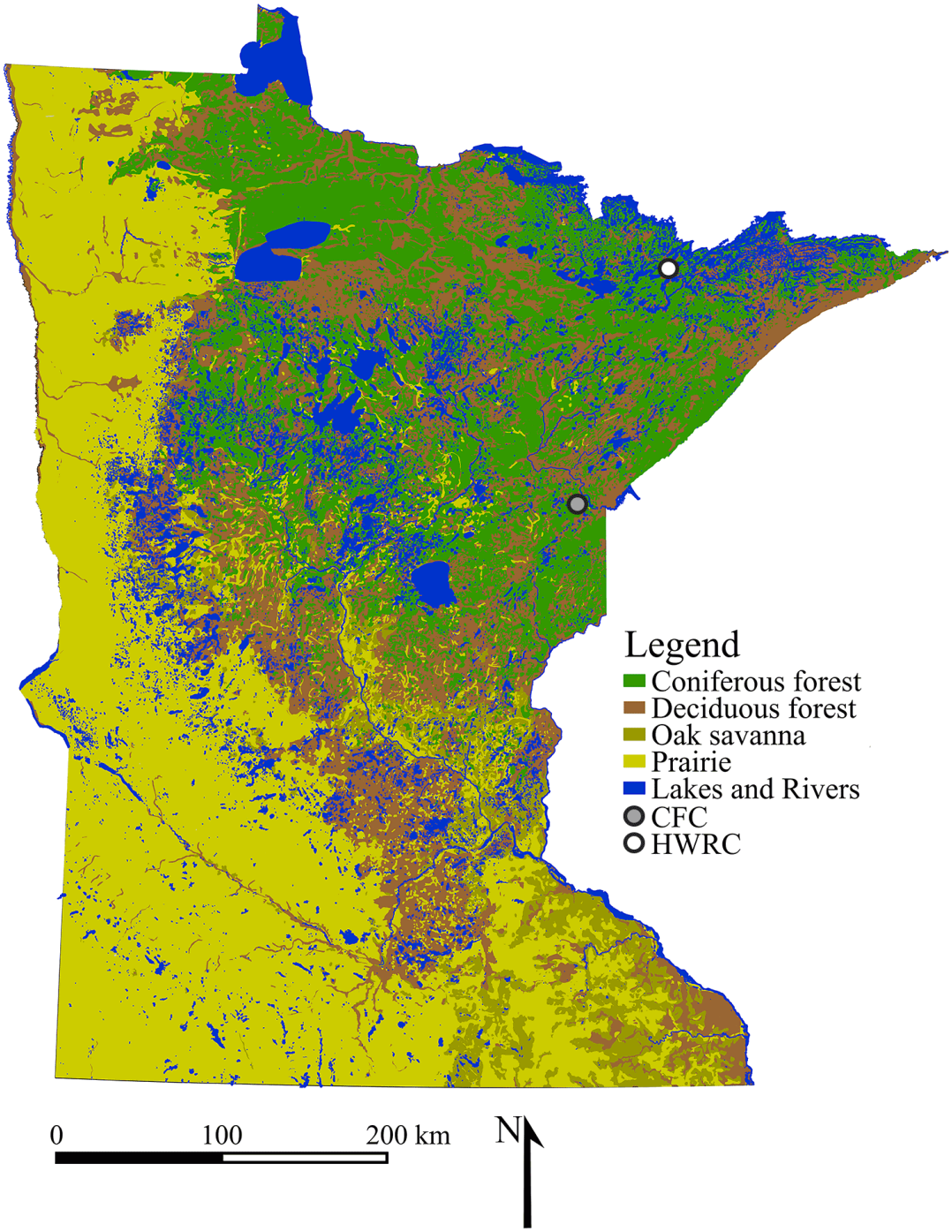

Figure 1-1. Map of Minnesota, USA, with simplified pre-settlement vegetation and B4WarmEd site locations identified. Geospatial vegetation data are from Minnesota Geospatial Commons.

Figure 1-2. Photographs of B4WarmeD sites showing warming plots in open (left) and closed (right) canopies. Both photographs were taken in 2008, during experimental setup, and show planted tree seedlings. The eight infrared heat lamps shown provide aboveground heating. Photo credit W. Eddy.

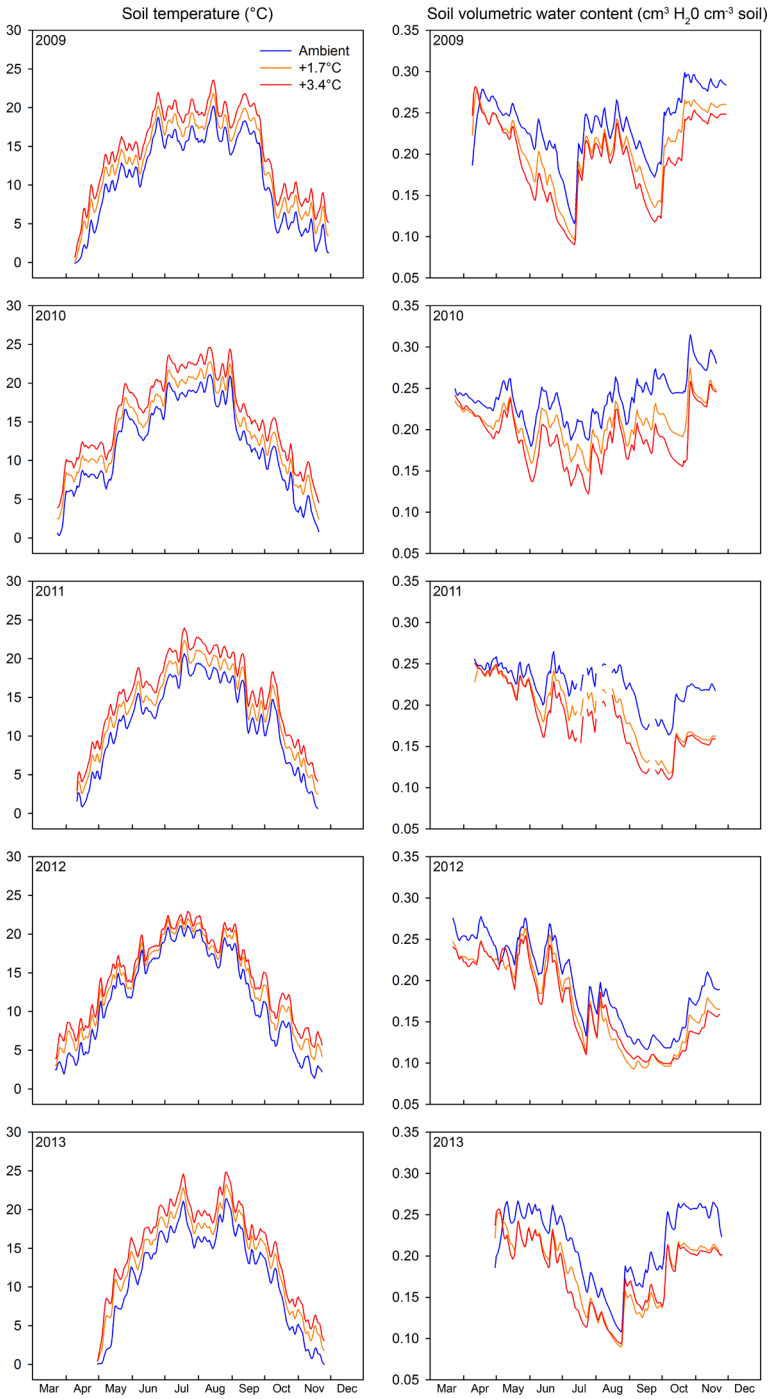

Figure 1-3. Warming treatment effects on soil temperature and soil moisture during five years (2009-2013) of *in situ* experimental warming at Cloquet Forestry Center. Soil temperature (°C, left-column) was measured at 10-cm, and VWC (cm^3 H_20 cm^{-3} soil) was measured at the soil depth of increment 0 – 22.5cm. Plotted data for soil moisture and temperature values are 3-day running means.

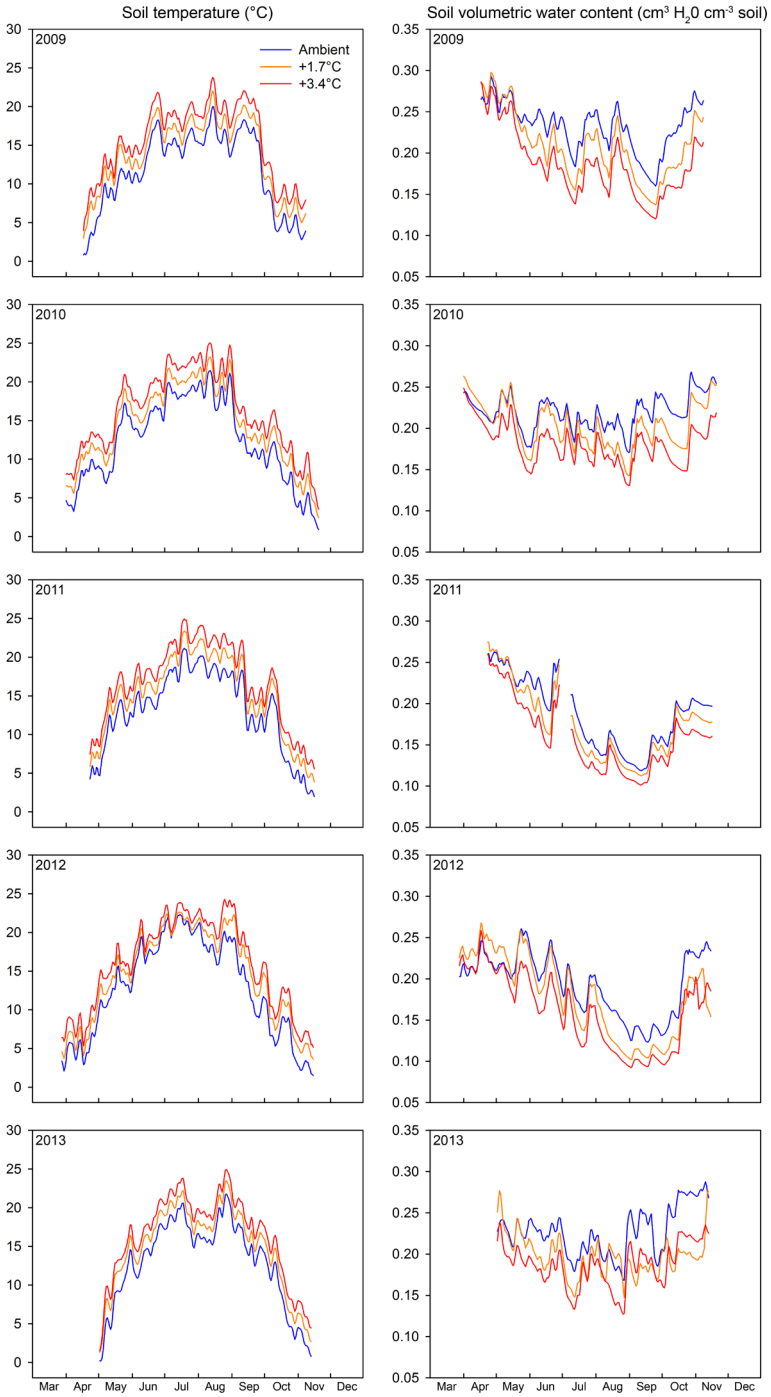

Figure 1-4. Warming treatment effects on soil temperature and soil moisture during five years (2009-2013) of *in situ* experimental warming at Hubachek Wilderness Research Center. Soil temperature (°C, left column) was measured at 10-cm, and VWC (cm^3 H_20 cm^{-3} soil, right column) was measured at the soil depth of increment 0 – 22.5cm. Plotted data for soil moisture and temperature values are 3-day running means.

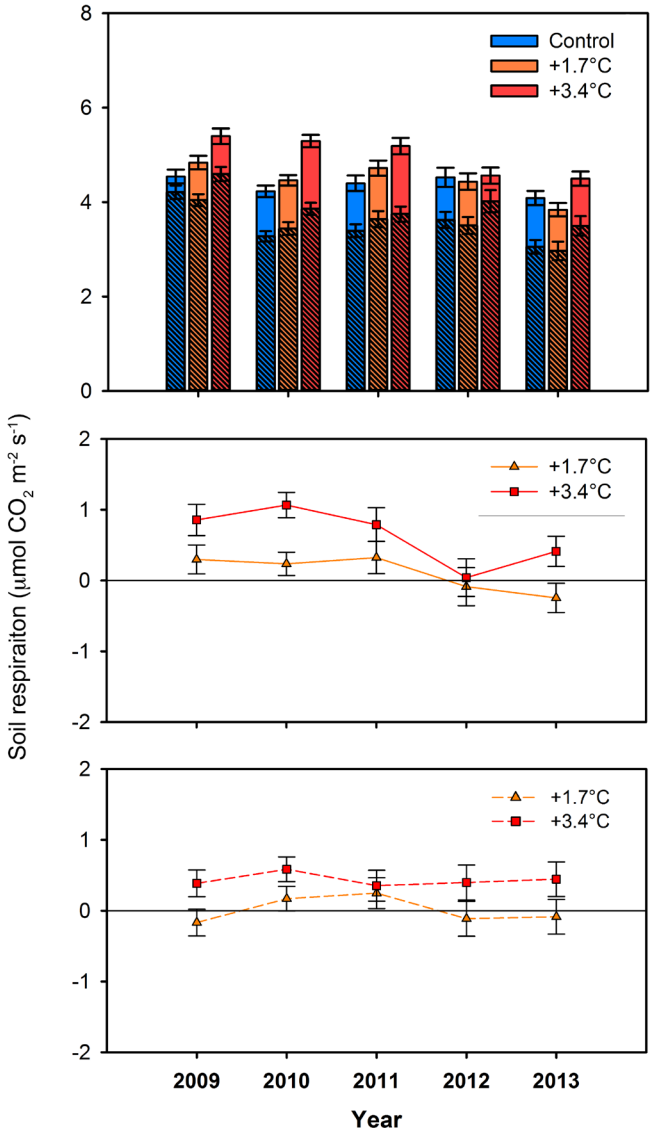

Figure 1-5. Main effects of experimental *in situ* warming on soil respiration by year. Top: Warming increased soil total (solid bars) respiration (Mixed model $p<0.0001$) and bulk soil (hatched bars) respiration (Mixed model $p<0.05$). Warming effects on soil total respiration decreased with year (Mixed model warming x year interaction $p<0.05$). Error bars are ±1SE. Middle and bottom: Warming treatment effects (warming treatment respiration – ambient respiration) for total soil (middle) and bulk soil (bottom) respiration. Values greater than zero indicate positive effects of warming on respiration, while negative values indicate negative effects warming on respiration. Error bars are propagated standard error calculated as $\sqrt{(SE_{AMB}^2 + SE_{WARM}^2)}$ (Lehrter and Cebrian 2010).

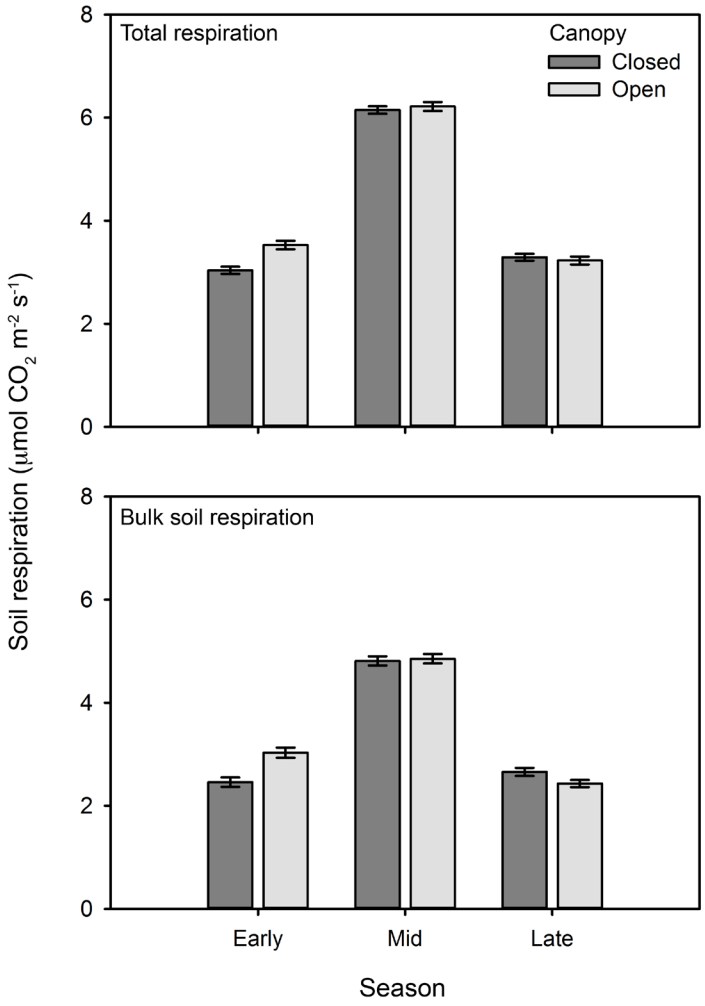

Figure 1-6. Effects of canopy x season interactions on total soil respiration (i.e. bulk + rhizosphere respiration; top), and bulk soil respiration (bottom). Error bars are ±1SE.

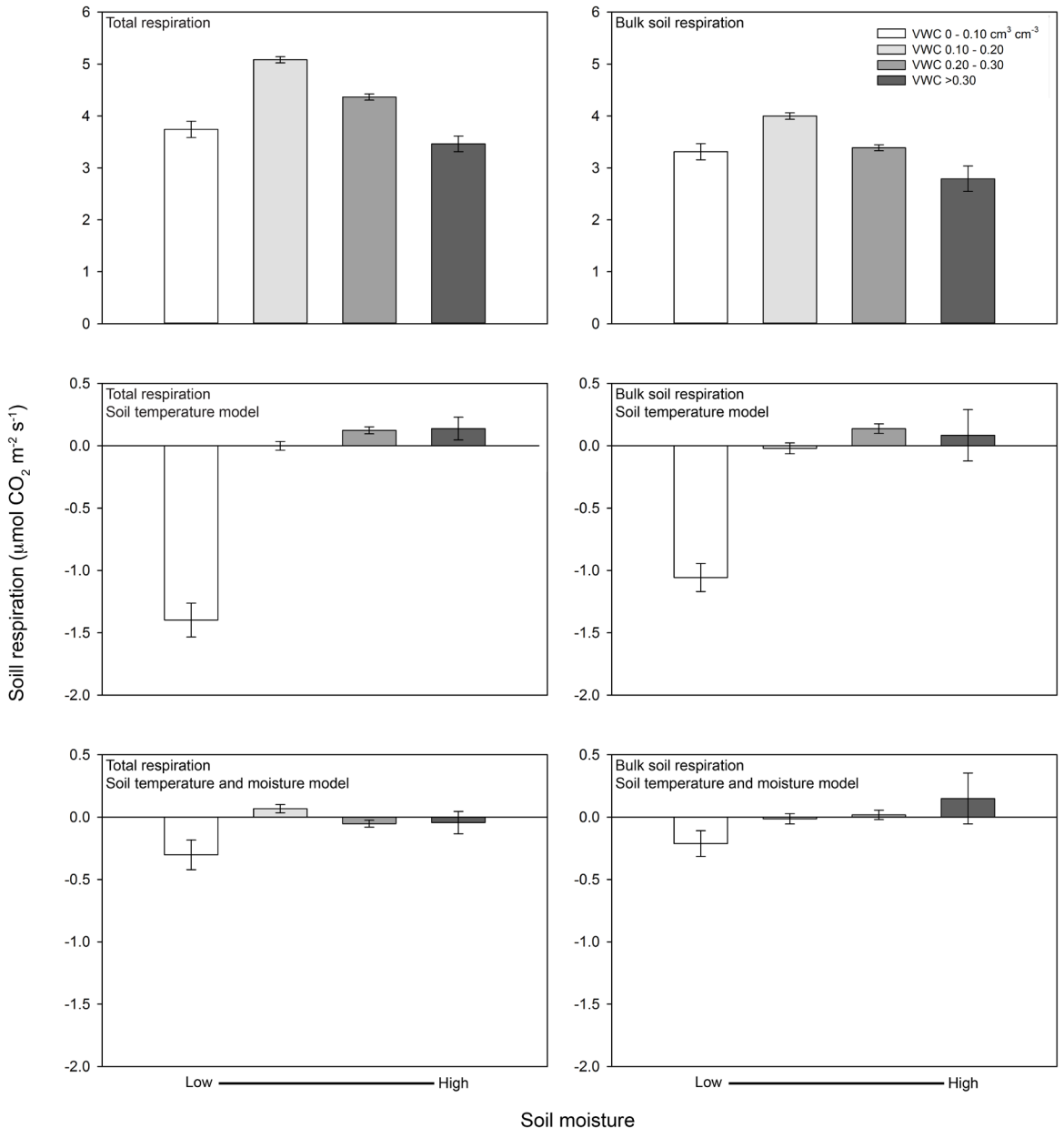

Figure 1-7. Soil respiration and model residuals (actual - predicted) across four soil moisture categories. Soil CO_2 flux rate (top row), and residuals from the soil temperature model (middle row) and soil temperature and moisture model (bottom row) for total soil (left) and bulk soil (right) respiration. Means less than zero indicate that the models tended to overpredict respiration relative to actual values, while means greater than zero indicate the models tended to underpredict respiration relative to actual values. Error bars are ±1SE across all soil respiration measurements.

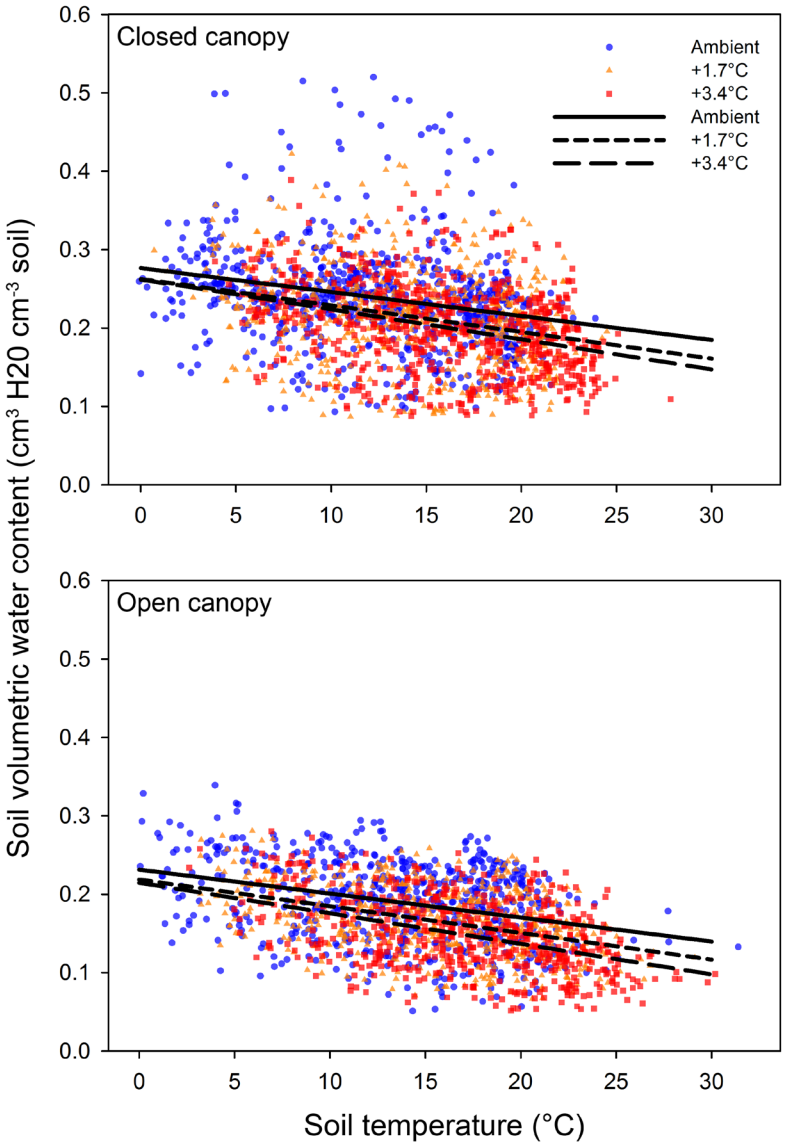

Figure 1-8. Soil volumetric water content tended to decrease with soil temperature, shown separately for the closed (top) and open canopy (bottom) treatments. Linear regression was used to fit relationships separately for the three temperature treatments.

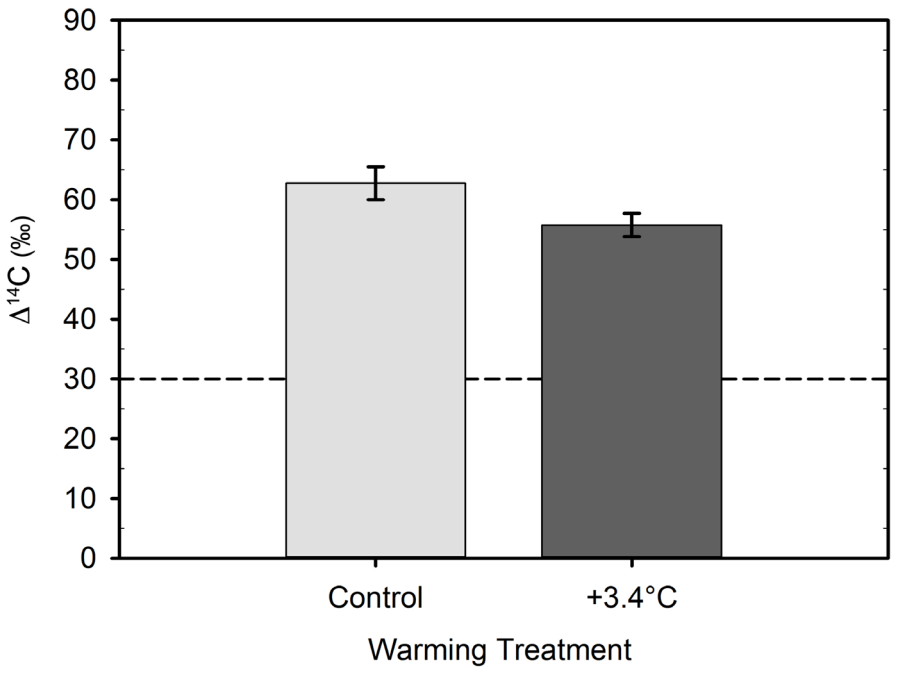

Figure 1-9. $\Delta^{14}CO_2$ by warming treatment. Dashed line at $\Delta^{14}C$ = 30‰ shows atmospheric radiocarbon in 2012, when samples were taken. (ANOVA $p<0.10$)

Chapter 2 - The temperature sensitivity of soil organic matter decomposition in southern boreal forests is altered by soil moisture and carbon pool, but not by incubation temperature

Abstract

One of the largest potential positive feedbacks to climate warming is CO_2 flux from soils to atmosphere arising from the enhanced decomposition of soil organic matter (SOM) in warmer soils. Soil drying, microbial thermal adjustment, and differences in temperate sensitivity of decomposition among soil carbon pools, however, all could influence the soil respiration response to warming, yet their effects are not well understood. The goal of this study was to examine the effects of these three mechanisms on the temperature response of SOM decomposition. Two laboratory incubations, using intact soil cores or sieved soil, tested the hypotheses that soil drying and microbial thermal adjustment would decrease the temperature response of decomposition, and that the decomposition of slow-cycling soil carbon pools would be more temperature sensitive than rapid-cycling carbon pools. Soils collected from two southern boreal forests in northern Minnesota, USA were incubated for 388 days at factorial combinations of three soil moisture levels and two temperatures (16 or 20°C). Measurements of soil respiration at incubation temperature were fit to a two-pool decomposition model, and the short-term temperature response (i.e. Q_{10}) of respiration (at 12, 16, 20, and 24°C) was measured at six time points during the long-term incubation. In both incubations, cumulative carbon respired was lower in dry treatments and at 16 vs. 20°C. Q_{10} (of soil respiration) deceased in the dry treatment, suggesting that warming-induced reductions in soil moisture could - offset warming effects on respiration in upland soils. Accounting for differences in

carbon loss between basal temperature treatments, we identified decreases in Q_{10} in soils incubated at 20 vs. 16°C. Microbial thermal adjustment effects were small and suggest that this mechanism is unlikely to counteract warming effects on respiration, at least at the time scales addressed in this study. Q_{10} increased over the course of the long-term incubation, as labile carbon was depleted and a greater proportion of respiration was derived from decomposition of slower cycling carbon. Together, these results suggest slow cycling carbon decomposition will increase with warming, which due to the large size of this pool, could represent an important positive feedback to warming.

Introduction

Climate models project that global mean annual temperature over land will increase by 1.2–4.8°C by the end of the 21st century (Collins et al. 2013). Whether terrestrial ecosystem feedbacks will mitigate or enhance warming represents a significant uncertainty in such projections of future climate (Friedlingstein et al. 2006, Heimann and Reichstein 2008). One of the largest potential positive feedbacks to climate warming is that arising from enhanced release of CO_2 from warmer soils (Davidson and Janssens 2006, Collins et al. 2013). Two to three times as much carbon (C) is contained in soils (1500 – 2400 PgC) (Batjes 1996, Jobbágy and Jackson 2000, Ciais et al. 2013) as in the atmosphere (840 PgC), and microbial decomposition of soil carbon is expected to increase with climate warming, as temperature is often the most important factor in determining rates of soil organic matter (SOM) decomposition (Lloyd and Taylor 1994, Kirschbaum 1995, 2000, 2006). Thus, greater microbial respiration of SOM could contribute substantially to enhancing the greenhouse effect in a warmer world

(Bond-Lamberty and Thomson 2010).

Laboratory (Fierer et al. 2006) and *in situ* warming experiments (Luo et al. 2001, Melillo et al. 2002, 2011, Schindlbacher et al. 2012), as well as decadal warming trends (Oechel et al. 2000, Bond-Lamberty and Thomson 2010) have detected warming responses of soil respiration. *In situ* warming studies have found that within a few years of warming manipulation, however, soil respiration has been found to decrease to *ca*. pre-warming rates (Oechel et al. 2000, Rustad et al. 2001, Luo et al. 2001, Melillo et al. 2002).

Mechanisms that could cause variation in the warming response of soil respiration include warming-induced soil drying (Harte et al. 1995, Davidson et al. 2006, Luo 2007), thermal adjustment by the microbial community (Luo et al. 2001, Bradford et al. 2008, Bradford 2013), and changes in the pool structure of SOM, particularly the depletion of labile carbon relative to more stable carbon pools (Kirschbaum 2004, Eliasson et al. 2005, Knorr et al. 2005). All of these mechanisms likely influence the respiration response to warming (Reichstein et al. 2005b, Bradford et al. 2008, Conant et al. 2011, Bradford 2013).Yet, it is difficult to test the relative importance of these mechanisms in influencing the dynamics of the response of soil respiration to warming using field measurements alone.

Warming-induced soil drying, caused by enhanced evapotranspiration(Rich et al. 2015), could offset the direct stimulatory effects of soil warming, thereby decreasing the effect of warming on respiration through time. Microbial respiration is highest at intermediate soil water content, declining at low moisture because of desiccation stress and limitations to diffusion, and at high moisture because of oxygen limitation (Grant and Rochette 1994, Davidson et al. 2000). The apparent temperature sensitivity of soil respiration is inhibited

in dry soils in field (Xu and Qi 2001, Rey et al. 2002, Janssens and Pilegaard 2003, Curiel Yuste et al. 2004, Lellei-Kovács et al. 2008, 2011) and laboratory (Craine and Gelderman 2011) studies. The decrease in temperature sensitivity in dry soils is expected due to the limited diffusion of substrates or extracellular enzymes, which could result in substrate limitation, but could also result if soils dry sufficiently to decrease plant inputs to soils.

Soil microbial communities are predicted to minimize their increase in respiration in response to a given temperature increase through physiological shifts (i.e. acclimation) and selection for genes, individuals, or species with lower rates of respiration at higher temperatures (Oechel et al. 2000, Luo et al. 2001, Bradford et al. 2008, Allison et al. 2010, Conant et al. 2011, Bradford 2013). Additionally, decreasing carbon use efficiency by the soil microbial community could lead to declines in microbial biomass, which is likely to decrease the warming responses of soil decomposition (Frey et al. 2008, Allison et al. 2010). There is considerable debate over whether this suite of warming-induced changes in soil microbial communities (thermal adjustments, hereafter) is important in driving the soil respiration responses to warming. Studies of single species (*Escherichia coli*; (Leroi et al. 1994), studies of mycorrhizae (Heinemeyer et al. 2006, Malcolm et al. 2008), laboratory incubations (Bradford et al. 2008) and field studies (Luo et al. 2001) have reported that microbial species and communities adjust to warming. Yet apparent thermal adjustments by the microbial community might be attributable to the other mechanisms outlined above, namely soil drying or depletion of a labile carbon pool (Eliasson et al. 2005, Knorr et al. 2005), as these mechanisms can be difficult to differentiate.

Bradford et al. (2008) found that carbon depletion, reductions in soil microbial biomass, and microbial adjustment decreased soil respiration responses to warming. Conversely, Hartley et al. (2008) found little evidence of thermal adjustment in a laboratory experiment where arctic organic soils were treated with pulses of chilling.

Kinetic theory predicts that the decomposition of biochemically recalcitrant organic matter is more temperature sensitive than that of biochemically labile substrates (Arrhenius 1889, Fierer et al. 2005, Davidson et al. 2006). Reactions that break down more complex molecules require greater inputs of energy (i.e. activation energy, E_a) to occur. Reactions with high E_a, in turn, will increase more with temperature gains than reactions with lower E_a as the added energy at higher temperatures increases the fraction of substrates at E_a (Davidson and Janssens 2006)

Complicating this interpretation, however, is the emerging understanding that soil carbon stability, rather than being primarily a function of molecular structure and complexity, is strongly controlled by the accessibility of organic matter to the soil microbial community in space and time (Schmidt et al. 2011, Marín-Spiotta et al. 2014). The accessibility of SOM, in turn, is related to its solubility and its physical and chemical protection, and the composition and function of the microbial community (Schmidt et al. 2011, Marín-Spiotta et al. 2014). Many physical, environmental, and biological constraints on decomposition are expected to be temperature sensitive, such as aggregate formation and turnover, adsorption and desorption, and the diffusion of substrates and enzymes (Davidson et al. 2006, Davidson and Janssens 2006). Still, many studies have found that the decomposition of more slowly cycling carbon pools was more temperature

sensitive than that of labile carbon and that controls of SOM stability are temperature sensitive (Knorr et al. 2005, Fierer et al. 2006, Ågren and Wetterstedt 2007, Conant et al. 2008a, 2008b, von Lützow and Kögel-Knabner 2009, Conant et al. 2011)

We used two laboratory incubations of sieved and intact soil cores, incubated at three different soil moisture treatments and two different temperatures to simultaneously examine potential for soil drying, microbial thermal adjustment, and differences in soil carbon pool temperature sensitivity to influence the response of soil respiration to warming. These incubations were done in conjunction with a replicated open-air warming experiment that uses soil heating cables and infrared lamps to raise temperature by 1.7 and 3.4°C with the aim of providing greater mechanistic understanding of the processes influencing warming effects on soil respiration in this experiment (Chapter 1).

We hypothesized that all three mechanisms will influence the apparent temperature sensitivity of soil respiration such that:

(1) Drier soils will respire less, and will be less temperature sensitive.

(2) Soils incubated at higher temperature will respire more, but will be less temperature sensitive due to microbial thermal adjustment. We term the predicted decrease in the temperature sensitivity at warmer temperature "thermal adjustment" rather than acclimation, because we measured soil respiration, but did not scale respiration to microbial biomass, or characterize the microbial community (Bradford et al. 2008).

(3) The respiration of the slow-cycling SOM pool will be more temperature sensitive than that of the fast-cycling pool.

Materials and methods

Two long-term laboratory incubations were established to examine the effects of soil moisture, basal temperature, and incubation stage on the temperature sensitivity of soil organic matter decomposition. Both experiments used soil collected from two upland southern boreal forest sites in Minnesota, USA (Table 2-1). These sites are part of the B4WarmED (*Boreal Forest Warming at an Ecotone in Danger)* forest warming experiment. See Chapter 1, Reich et al. (2015), Rich et al. (2015), and Sendall et al. (2015) for more information on B4WarmED experiment.

The two B4WarmED sites are located ~150 km apart in forests that span the ecotone transition from temperate to boreal biomes in northern Minnesota, USA. One site was located at the Cloquet Forestry Center (CFC), Cloquet, MN, USA and the other higher latitude site was at the Hubachek Wilderness Research Center (HWRC) near Ely, MN (See Table 2-1 for further site descriptions). Both sites (>6 ha) have poorly developed sandy loam soils derived from glacial outwash (CFC) or till (HWRC) (Table 2-1), and a modest O horizon. Warming treatments of ambient, +1.7°C, and +3.4°C using infrared lamps and soil cables (see Rich et al. 2015) were located in an intact, closed canopy (≈5-10% of full sunlight) and in a recent clearcut (≈80% of full sunlight). The laboratory incubations presented here, however, did not directly use the warming treatments, but were instead designed as direct tests of the importance of soil microbial thermal adjustment, differing temperature response of carbon pools to decomposition, and soil moisture declines in controlling the warming response of decomposition in these coarse textured upland soils.

Soil was collected from both sites in August 2011. Fifteen soil cores (2.1cm diameter sharpened PVC cores, 0-10cm) were collected from a central location from each of the six forested (closed canopy) and six clearcut (open canopy) blocks at each site, but outside of any treatment plots, and plugged with rubber stoppers. Three of the fifteen cores per block were sieved (2mm) and visually sorted into mineral soil, plant matter and detritus, and rock fractions in the laboratory within several days of sampling. These three fractions were weighed, and used for block-level estimates of core composition. At the same time, forty mineral soil cores (2.1cm diameter, 0-10cm depth) were collected from each of the three closed canopy blocks at each site along three transects per block using a sharpened PVC corer, again only collecting soil from outside of the B4WarmED treatment plots. This soil was composited by site, and sieved (2mm) to isolate the mineral soil fraction. After collection and processing, all cores and sieved soils were stored at 4°C until experimental setup.

Two different laboratory incubations were established. The intact core incubation consisted of the 144 intact cores (12 cores per block x 6 blocks per site x 2 sites) and was designed to assess interactions between moisture and temperature in soils that were undisturbed, and as close to field conditions as possible. The sieved soil incubation consisted of 96 incubations of 100g of well-mixed soils (48 samples per site x 2 sites), designed to minimize variation other than that caused by the experimental treatments. Both incubation experiments were a balanced factorial design with respect to soil moisture treatment and incubation temperature.

Soil moisture was maintained at one of three different gravimetric moisture contents (0.075, 0.150, or 0.225g H_20 g^{-1} soil), termed *dry*, *mesic*, and *wet* treatments, using repeated distilled water additions. Across five years (2009-2013), mean hourly time-domain reflectometry (TDR) measurements indicated that across all warming treatments (ambient, +1.7°C, and +3.4°C), closed canopy soils had gravimetric moisture contents of <0.075, 0.075-0.150, and 0.15-0.225 g H_20 g^{-1} soil for <0.001, 30, and 39% of the snow-free season, respectively. Open canopy soils were considerably drier with gravimetric moisture contents <0.075, 0.075-0.150, and 0.15-0.225 g g^{-1} for 2, 44, and 40% of the snow-free season, respectively (unpublished data). In the both canopies, soils were drier during the warmest months (June to August), although for the closed canopies, the frequency of soils where gravimetric moisture contents was less <0.075 g g^{-1} was still extremely rare (≈0.001% of measurements). In the open canopy treatment, from June to August, 3% of hourly measurements of gravimetric moisture contents (across all warming treatments) were less than 0.075 g H_20 g^{-1} soil (for +3.4°C warming, this was 5%). Thus, the dry treatment approximated soil moisture decreases in excess of those typically observed for experimental *in situ* warming of +3.4°C. Yet, the dry treatment is relevant given future changes in precipitation could add to warming enhanced evapotranspiration, resulting in substantially drier soils than observed at the field warming experiment (Dai 2012). Furthermore, the dry treatment is important for understanding drought period effects on SOM decomposition. For example, a drought at the northern site (HWRC) from July to September 2011 reduced soil moisture such that during this time, such that 27% of all TDR measurements in the open canopy were at or

below the dry treatment level. Warming enhanced this drying further, so that in the +3.4°C treatments, gravimetric soil moisture content was at or below the dry treatment level 42% of the time. Dry soils during droughts, particularly if they occur during the warm-season, could disproportionately mitigate warming effects on SOM decomposition.

We assume that water treatments were at levels below saturation, as there was no evidence for water pooling in the bottom of the translucent cups used for the sieved soil incubations. Furthermore, there is no evidence from *in situ* measurements that water saturation decreased soil respiration, even at soil moisture contents greater than the wet treatment in this study (Chapter 1). This is not surprising given the coarse nature of the soils.

Water was added immediately after gas flux measurements, and as needed between measurements, to maintain moisture treatments, but never within 3 days of a measurement in order to avoid measuring a pulse of microbial respiration from rewetting (Fierer and Schimel 2002). At the time of water additions, mean moisture treatments were 0.012, 0.014, and 0.017 g H_20 g^{-1} soil below target moisture content, for dry, mesic, and wet treatments. Because these measurements were done after respiration measurements, these deficits represent the minimal mean moisture values during the experiment, and it is likely that soil moisture during respiration measurements was higher. Soil incubation temperature was maintained at either at 16 or 20°C using near-ambient temperature dark incubators.

Because the initial soil moisture was greater than the lowest soil moisture treatment, we exposed all soils to an initial drying period. Soils were incubated

uncovered at 30°C for 13 days, until all soils were drier than the "dry" soil treatment. Moisture and temperature treatments were initiated on 3 October 2011.

Soil carbon respiration

We measured carbon flux of soils incubated at their respective temperature treatments (basal respiration, hereafter) at 0.5, 2, 3, 5, 6, 8, 11, 15, 19, 30, 47, and 55 weeks after incubation start. In between carbon flux measurements, intact and sieved soils were covered with low-density polyethylene (LDPE) film that was permeable to O_2 and CO_2, but not to water. For soil respiration measurements, cores or cups were placed into sealed 1L canning jars with lids fitted with Hungate septa for six hours. Initial CO_2 concentrations were determined on 2.5mL headspace samples analyzed with an infrared gas analyzer (IRGA; Li-7000, Licor Bioscience, Lincoln, Nebraska, USA) calibrated using 645, 1025, and 10,000 ppm CO_2 standards. Six hours after initial CO_2 measurements, CO_2 concentrations were determined on a second 2.5mL sample. At 30 weeks into the incubation, the headspace volume sampled was doubled to 5mL in order to improve the CO_2 detection limit as respiration rates declined. The difference between CO_2 concentrations, accounting for jar headspace volume, incubation time, and volume headspace removed during initial CO_2 measurement, was used to calculate a respiration rate.

Decomposition models

We fit one and two-pool decomposition models in order to understand the decomposition of soil carbon at the two basal temperatures (Hobbie et al. 2012, Reid et al. 2012). The one-pool decomposition model fit was:

$$C_{resp}(t)=k_f[C_f e^{-k_f t}]$$

The two-pool decomposition model fit was:

$$C_{resp}(t)=k_f[C_f e^{-k_f t}] + k_s[(C_t - C_f)e^{-k_s t}]$$

where C_{resp} is the daily respiration rate (mg C g soil^{-1}day^{-1}), C_f is the fast-cycling C pool (mg C g soil^{-1}), C_t is total C (mg C g soil^{-1}), k_f is the decomposition rate of the fast-cycling (day^{-1}) and k_s is the decomposition rate of the slow-cycling pool (day^{-1}), and t is time in days. The slow pool size was calculated as the difference between C_t and C_f. A difference of Corrected Akaike Information Criteria (AICc) greater than 3 was used to indicate a significant difference in model fits (Burnham and Anderson 2002). Decomposition models were fit in R (version 3.0.2, R Core Team 2013) using maximum likelihood estimation (mle2, Package bbmle, Boker and R Development Core Team 2014).

Two-pool decomposition models fit better than the one-pool models for both the intact core and sieved soil experiments (see appendix B for actual by predicted plots for the decomposition models). The two-pool decomposition model fit 129 out of the 144 experimental cores better than the one-pool model, the one-pool model fit 6 of the cores better, and the two models were indistinguishable (difference in AICc values less than 3) for 9 cores. For the sieved soil experiment, the two-pool decomposition model fit better

than the one-pool model in 79/96 cases, the single pool model fit better in 2/96 cases, and the two models were indistinguishable in 15/96 cases. Because the two-pool model fit best for the large majority of cases in both incubations, we chose to only present parameters based on this model fit. Additionally, we excluded model fits with $R^2 < 0.40$. This resulted in the exclusion of decomposition data from 4 out of 144 intact cores, and one out of 96 sieved soil incubations. The mean R^2 for the remaining model fits was 0.93 and 0.90 for the intact core and sieved soil incubations, respectively.

Parameter equifinality results from models where different parameter combinations produce equally good fits of the data (Schulz et al. 1999, Hyvönen et al. 2005). Equifinality might be problematic in the fitting of these decomposition models, as respiration can be modeled as either the fast decomposition of a smaller carbon pool or the slow decomposition of a larger carbon pool. In order to investigate the extent that equifinality might impact our predictions of decomposition parameters, we fit randomly generated parameter sets no less than 50,000 times for each microcosm, and compared the R^2 for the random fits. Random parameter sets were generated from a large parameter space, chosen to include all likely parameter estimates. No randomly chosen parameters resulted in a fit that had as high or higher R^2 as the MLE fit, suggesting there were no equally good model fits of the decomposition data, and thus no problems with equifinality (Appendix C provides key evidence and several examples to support these conclusions).

Temperature response models

In addition to measurements of basal respiration, temperature response curves were used to assess the short-term response of soil microbial respiration to changes in temperature. Temperature response was assessed by measuring respiration at 12, 16, 20, and 24°C, in ascending order over four days at six time points over the course of the incubation. Soils were allowed to adjust to a new temperature for 12 hours, after which soil CO_2 flux was measured over 6 hours as described previously. Temperature response curves were measured at 0.5, 6, 19, 30, 47, and 55 weeks in the incubations.

We compared several models before choosing the van 't Hoff equation, a simple exponential relationship (Lloyd and Taylor 1994, Fierer et al. 2005, Davidson et al. 2006, Fierer et al. 2006), to analyze the temperature response of soil respiration. Appendix D provides information on the models, their fits, and the rational for our choosing the van 't Hoff equation. The van 't Hoff equation is:

$$C_{resp}=Be^{kT}$$

where C_{resp} is the daily respiration rate (mg C g soil^{-1}day^{-1}), T is temperature in degrees Celsius, B is a fitted parameter describing the basal respiration, and k is a fitted parameter describing the temperature sensitivity of the reaction. Basal respiration likely scales to carbon substrate quality and bioavailability (Fierer et al. 2005), but could also be affected by soil microbial biomass and carbon use efficiency. Q_{10} was determined from k as

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

We excluded Q_{10} fits where $R^2 < 0.40$, where 2 or more temperature response data points were missing, or where low or high temperature (12 or 24°C) response was missing. Of the 864 temperature response curves done on the intact cores, 839 curves were included in the final analyses of these data. The mean R^2 for the temperature response curves from the intact cores was 0.96. Of the 576 sieved soil temperature response curves, 516 were included in the data analyses, with a mean $R^2 = 0.94$.

Statistical Analyses

We used a three-way mixed model ANOVA to test for effects of soil moisture, basal temperature, site, and canopy on cumulative respiration and decomposition model parameters (C_f, k_f, k_s) for each incubation experiment separately. We used a repeated-measures linear mixed model to test for effects of soil moisture, basal temperature, site, canopy, and time on the temperature response parameters (B and Q_{10}).

Thermal adjustment by the decomposer community in the warmer (20°C) incubations is predicted to lower Q_{10}. However this could be in part offset because the warmer incubations should also have had more cumulative respiration at any given measurement date, and therefore be respiring more of the slow-cycling carbon pool, which is predicted to increase Q_{10} relative to the 16°C incubations. Therefore, we examined the effects of basal temperature on Q_{10} using a simplified linear mixed ANCOVA model with cumulative C respired at each temperature response curve as the model covariate.

For all model structures, dependent variables were ln-transformed to meet the assumptions of normality. Tukey's HSD multiple comparison procedure was conducted when there was a significant main effect. Significance was accepted for all statistical tests at $\alpha = 0.05$. Analyses of the linear mixed model were performed using JMP 11.0 (SAS Institute, Cary, NC, USA).

Results

As predicted, drier soils respired less, and were less temperature sensitive, but we only found minor evidence that soil thermal adjustment altered respiration responses to warming. The temperature response of respiration increased as faster cycling pools were depleted, and a greater proportion of respiration was derived from slow cycling carbon. This indicates, as predicted, that the temperature sensitivity of the decomposition of slow cycling carbon responds more to temperature than the decomposition of fast cycling carbon.

Moisture effects on pool sizes and decomposition rates

The decomposition rate constant relates the size of a soil carbon pool to the CO_2 flux from that pool. Where differences were found, increasing soil moisture and temperature shifted carbon from the slow to the fast pool, and decomposition rate constants decreased for the fast pool, while increasing for the slow pool. Increases in fast pool size and slow pool decomposition rate constant dominated overall effects, so that both the fast and slow pool respiration rate increased with soil moisture (Figure 2-1).

Dry soils respired less CO_2 than wetter soils over the course of the 388-day experiment in both intact core and sieved soil incubations (Table 2-2, Figure 2-2a,b). In the intact core experiment, the mesic and wet soil moisture treatments respired 1.5x and 1.7x more, respectively, than the dry treatment (Figure 2-2a). Respiration increased in the wet relative to the mesic treatment, but we did not identify significant differences between these treatments (Figure 2-2a). Similarly, in the sieved soils, the mesic and wet soil moisture treatments respired 3.0x and 3.8x more on average, respectively, than the dry moisture treatment, and the wet sieved soils also respired more (1.3x) than mesic sieved soils (Figure 2-2b). Moisture effects was larger for sieved soils from the northern (HWRC) than the southern (CFC) site and at a basal temperature of 20 vs. 16°C (Table 2-2).

The size of the fast pool (C_F) increased significantly with increasing soil moisture in both incubation experiments (Table 2-2, Figure 2-3c-d). In the intact core incubation, the fast soil pool was 1.5x and 1.9x larger in the mesic and wet treatments relative to the dry treatment, but the mesic and wet treatments did not differ (Figure 2-3c). Moreover, the effect of soil moisture treatment on C_F was greater in the closed vs. open canopy (Table 2-2, Moisture x Canopy, data not shown). In the sieved soil incubation, the size of the fast-cycling pool increased significantly as soil moisture increased, by 3.6x and 5.8x in the mesic and wet treatments, respectively, relative to the dry treatment (Figure 2-3d).

The fast pool decomposition rate (k_F) was significantly higher in the dry treatment (1.6x on average) than in the mesic and wet treatments for the sieved soil incubation, and these effects were larger at HWRC than at CFC (Table 2-2; Moisture x Site). There was

no main effect of moisture treatment on k_F in the intact core incubation, however several interactions with moisture treatment were important predictors of k_F. Soil moisture increased the fast pool decomposition rate, at 16°C, while at 20°C, the opposite pattern emerged, and soil moisture decreased the fast pool decomposition rate. Soil moisture had contradictory effects on k_F in the two canopies, increasing k_F in the open, and decreasing it in the closed canopy. (Table 2-2; moisture x canopy; data not shown). This interaction was further influenced by site (Table 2-2, moisture x site x canopy, data not shown). At HWRC, k_F increased in the open canopy and decreased in the closed canopy with increasing soil moisture. At CFC, the mesic treatment replaced the wet treatment in having the fastest k_F in the open canopy, and slowest in the closed canopy,

For both the intact core and sieved soil incubations, the slow pool decomposition rate (k_S) increased and the slow pool size (C_S) decreased with soil moisture (Table 2-2, Figure 2-4). As with previous decomposition model parameters, for the intact core incubation, the slow pool decomposition rate (k_S) in the mesic (1.4x) and wet (1.6x) treatments differed significantly from the dry treatments, but not each other, while for the sieved soil incubation, all moisture treatments differed from each other (mesic and wet treatments were 2.8x and 3.4x, respectively, greater than the dry treatments, Figure 2-4a-b). Moisture treatment effects on k_S were greater at HWRC than at CFC, and at 20 than at 16°C for intact cores and sieved soils, respectively. Slow pool size (C_S) decreased with increasing soil moisture, also following the pattern of greater treatment effects in the sieved soil incubation relative to the intact core experiment. In the sieved soil incubation,

soil moisture effects on slow pool size were larger at a basal temperature of 20 vs. 16°C (moisture x basal temperature) and at HWRC vs. CFC (Table 2-2; moisture x site).

Basal temperature effects on pool sizes and decomposition rates

Higher basal temperature accelerated organic matter decomposition in both incubations. Soils incubated at 20°C respired 1.2x more carbon than soils incubated at 16°C over the course of this 388 day experiment in both incubations (Table 2-2, Figure 2-2c-d). Fast pool size (C_F) was greater in 20°C relative to the 16°C incubations by 1.2x in the intact core and by 1.3x in the sieved soil incubation (Figure 2-5c-d). In the intact core incubation, fast pool decomposition rate (k_F) was significantly higher (1.3x) in the 20°C relative to the 16°C incubations (Figure 2-5a), but basal temperature did not affect k_F in the sieved soil incubation (Figure 2-5b). Slow pool decomposition rate (k_S) was 1.2x higher in both incubations in the 20°C relative to the 16°C incubations (Figure 2-6a-b). Slow pool size (C_S) did not differ between basal temperatures in the intact core incubation, but in the sieved soil incubation, C_S was greater at 16 vs. 20°C (Figure 2-6c-d), and this effect was larger (though still minor) at the northern (HWRC) site.

Carbon decomposition differed significantly between the two sites, but only in the sieved soil incubations (Table 2-2, data not shown). For sieved soil, cumulative respiration was 1.4x greater in soils from northern site (HWRC) than in soils from the southern site (CFC). The fast (C_f) and slow (C_S) pools were 2.4x and 1.1x larger in HWRC than CFC soils, respectively. The fast pool decomposition rate (k_f) was 1.2x

greater in soil from CFC than HWRC, while the slow pool decomposition rate (k_S) was faster (1.1x) for HWRC than for CFC soil.

Canopy condition (closed vs. open) where the soils were collected did not affect cumulative respiration, k_F, or k_S, but the fast pool was larger in the soils from open canopy relative to the closed canopy (Table 2-2, data not shown).

Moisture and basal temperature effects on temperature response

Mean temperature response curves by soil moisture and basal temperature treatments are shown in Figure 2-7. Soil moisture enhanced the basal respiration rate (B), a metric of soil organic carbon available for decomposers. In the intact core incubation, B was 1.5x and 1.8x higher in the mesic and wet treatments, respectively, than in the dry treatment (Table 2-3, Figure 2-8a). The mesic and wet treatments did not differ significantly. In the sieved soil incubation, B was 1.9x and 2.5x higher in the mesic and wet treatments, respectively, than in the dry treatment, with significant differences between the mesic and wet treatments (Table 2-4, Figure 2-8b). In both incubations the effect of moisture on B decreased through the course of the incubations (Significant moisture x day effect, Tables 2-3 & 2-4, Figure 2-9a-b).

As hypothesized, soil carbon decomposition was more temperature sensitive in soil with higher moisture (Tables 2-3 & 2-4, Figure 2-8c-d) in both incubation experiments. For the intact core incubation, moisture effects were relatively small, with mean Q_{10} values of 2.2, 2.3, and 2.3 for the dry, mesic, and wet, treatments respectively (Figure 2-8c). For the sieved soil incubation, moisture effects were larger, with mean Q_{10}

values of 2.2, 2.5, and 2.5, for dry, mesic, and wet, treatments respectively (Figure 2-8d). The effect of soil moisture on Q_{10} varied by day (significant moisture x day interaction) in the sieved soil incubation, likely due to decreasing moisture effects on Q_{10} as the incubations progressed, as well as variability in temperature response measurements on days 329 and 385 of the incubations (Figure 2-9d).

In contrast to what we hypothesized, we found little evidence for microbial adjustment to temperature. There was no main effect of basal incubation temperature (16 vs. 20°C) on *B* in either the intact core or sieved soil incubation. (Tables 2-3 & 2-4, data not shown). The sieved soil incubation alone had a basal temperature by day interaction, although there was no consistent pattern to the interaction. Similarly, basal temperature did not affect Q_{10} in the intact soil incubation (Table 2-3). In the sieved soil incubation, higher basal temperature (20°C) reduced Q_{10} by a small amount over the entire experiment relative to the 16°C treatment, from 2.45 to 2.42. Reductions in Q_{10} were observed for three temperature response curves beginning on days 43, 130, and 210, but disappeared by the last two measurements (on days 329 and 385) (Basal temperature x day effect; Table 2-4, Figure 2-9d)

We found a significant three-way interaction of moisture, basal temperature, and site on *B* in the intact core experiment (Table 2-3, data not shown). Moisture effects on *B* were greatest at 20°C in soil from the southern site (CFC), and at 16°C in soil from the northern site (HWRC). In addition, we found significant moisture x basal temperature x day interactions on *B* and Q_{10} in the sieved soil incubation (Table 2-4, data not shown). These interactions underlie relatively minor effects, driven primarily by differences in

moisture x basal temperature effects at one or two of the six temperature response curves. For *B,* moisture treatment effects were greater at 16°C for the temperature response curve at 210 days, and greater at 20°C at 385 days. For Q_{10}, moisture treatment effects were greater at 20°C for the temperature response curve at 385 days.

As hypothesized, there was a significant increase in Q_{10} over the course of both the intact and sieved soil incubations, consistent with a shift from more labile C to more slowly cycling C over time (Tables 2-3 & 2-4; Figure 2-9). Q_{10} increased nearly linearly from 1.9 to 2.6 in the intact core incubation and from 1.9 to 3.0 in the sieved soil incubations across the 388 day incubation period. At the same time, the soil organic carbon available for decomposers (*B*) decreased with incubation time, with the major losses occurring in the first 150 days of the incubation (corresponding to fast pool depletion, Figure 2-1).

Because Q_{10} increased as carbon was respired, thermal adjustment effects could be masked because more cumulative carbon had been respired in the 20°C incubations than the 16°C incubations at any given measurement date. As a result, we would expect these soils to have a higher Q_{10} because the decomposers would be respiring more slow cycling carbon. To investigate whether there was evidence for thermal adjustment, accounting statistically for the total amount of carbon respired, we compared the relationship between Q_{10} and cumulative carbon respired for soils incubated at 16 vs. 20°C. For both incubations, we found evidence of soil microbial thermal adjustment to temperature (ANCOVA, cumulative respiration $p < 0.0001$, basal temperature $p < 0.01$). The effects, however, were small – the Q_{10} of soils incubated at 16°C was higher by an

average of 0.08 (intact core) and 0.12 (sieved soil) relative to soils incubated at 20°C (Figure 2-10).

Basal respiration (*B)* was higher at the northern site (HWRC) than the southern site (CFC) in both experiments, although this effect decreased over the time of the incubation (Tables 2-3 & 2-4, data not shown). In the intact core incubation, we found significant effects of canopy and canopy x day on *B*, with soil from the open canopy having higher *B*, especially early in the incubations. Soil carbon decomposition was more temperature sensitive at the southern site (CFC) than at the northern site (HWRC) in the sieved soil incubation. Site by day interactions were significant for sieved *(B* and Q_{10}) and intact core (Q_{10}) incubations. In the sieved soil, *B* was greater at the northern site (HWRC) early in the incubations, but differences diminished as the incubations progressed. For both incubations, site x day effects on Q_{10} were inconsistent, and thus did not appear to be ecologically important.

Discussion

This study identified important effects of soil moisture, microbial thermal adjustment, and carbon pool on the temperature sensitivity of SOM decomposition using two laboratory incubations of upland soils from two southern boreal forests in Minnesota. As expected, low soil moisture was found to limit decomposition and its temperature sensitivity, warmer incubation temperature accelerated decomposition, and less easily decomposable carbon was found to be more temperature sensitive. Contrary to

expectations, microbial adjustment to temperature had only relatively small impacts on the temperature sensitivity of decomposition.

Effects of soil moisture on decomposition and its temperature sensitivity

As hypothesized, dry soils respired less, and their respiration was less temperature sensitive. These results are consistent with other studies that have reported soil respiration decreases at low soil moisture content (Xu and Qi 2001, Rey et al. 2002, Janssens and Pilegaard 2003, Curiel Yuste et al. 2004, Craine and Gelderman 2011, Lellei-Kovács et al. 2011, Suseela et al. 2011, Zhou et al. 2014). Based on the decomposition parameters of the two-pool model, the inhibition of respiration in the dry soils can likely be attributed to smaller rapidly turning over carbon pools (i.e. C_f), or lower accessibility to fast cycling carbon pools caused by slow diffusion of enzymes and substrates, resulting in effectively smaller C_f, as well as slower decomposition of the slow pools (k_s).

Similarly, in both incubations, basal respiration (B) increased with soil moisture, as diffusion of soluble carbon substrates and extracellular enzymes was enhanced, and desiccation of soil microbes reduced (Davidson et al. 2006, Zhou et al. 2014). Likewise, Q_{10} increased with soil moisture, consistent with previous studies (Reichstein et al. 2005a, Craine and Gelderman 2011, Moyano et al. 2012, Zhou et al. 2014), and perhaps due to increased carbon available for decomposition with increased moisture.

These results suggest that moisture could be an important control of the feedback of soil carbon decomposition to warming in upland soils prone to drying. Dry soils could

have disproportional effects on soil respiration as drying at these sites often occurs during the warmest months of the year, when soil respiration is at it annual peak (Chapter 1) and has the potential to show the greatest absolute increases in response to warming, increase that could be dampened by dry conditions. Moreover, future climate change is expected to alter patterns of evapotranspiration, leading to lower soil moisture, especially in the spring and summer in temperate zones (Collins et al. 2013, Dirmeyer et al. 2013), which would increase the occurrence of dry soils, as seen in this experiment (Rich et al. 2015). Globally, climate change is expected to increase evapotranspiration and alter precipitation in ways that would drastically alter soil moisture (Dai 2012).

Effects of basal temperature on decomposition and its temperature sensitivity

As expected, temperature increased cumulative soil respiration, and the decomposition of both the fast and slow carbon pools. Soil microbial adjustment, however, has the potential to reduce the response of soil respiration to temperature. Yet, we found no effect of incubation temperature on basal respiration (*B*), and only a small effect of incubation temperature on Q_{10}, even accounting for differences in carbon depletion (and thus changes in carbon quality) resulting from different incubation temperatures. This relatively small effect will not likely be a major control over the temperature response of decomposition

These results diverge from other studies that have reported strong compensatory thermal adjustment (Luo et al. 2001, Bradford et al. 2008). Still, the lack of microbial adjustment is not without precedent. In cold-exposed incubations, (Hartley et al. 2008)

found no evidence that the soil microbial community acclimated through metabolic up-regulation. Furthermore, as pointed out by (Bradford et al. 2008), acclimation is typically measured in terms of respiration *per unit microbial biomass*. Since we did not measure microbial biomass, we are unsure if the small changes observed in Q_{10} with incubation temperature resulted from physiological changes, shifts in soil microbial community composition, or changes in microbial biomass (Bradford et al. 2008, Balser and Wixon 2009).

Effects of incubation time on decomposition and its temperature sensitivity

As hypothesized, the temperature sensitivity of respiration increased with incubation time and cumulative carbon respired. Because there were no new inputs of carbon into the incubations, it follows that decomposition was increasingly dominated by that of slow cycling carbon as the incubation progressed, an interpretation that is supported by both the decline in the respiration rate per gram of soil over the course of the incubation and by the superior fit of a two-pool decomposition model over a one-pool model (see appendix B for actual by predicted plots for the decomposition models). Thus, these results are consistent with other studies that have found that the temperature sensitivity of decomposition increased with apparent soil carbon recalcitrance (Conant et al. 2008b, Craine et al. 2010, Conant et al. 2011, Zhou et al. 2014). The mean residence time (MRT) of the fast pool across all treatments was 24.3 days (SE = 1.5) for the intact cores and 28.4 days (SE = 1.4) for sieved soils. For the slow pool, MRT was 13.4 years (SE = 0.7) and 40.8 years (SE = 2.6) for intact core and sieved soil incubations,

respectively. Thus, this 388 day experiment examines soil moisture and basal temperature effects on carbon which turns over on scales of days to years. Based on (von Lützow et al. 2006), C stabilization mechanisms that operate on these time scales include biochemical recalcitrance (0-10 yr), occlusion in macroaggregates (>250μm; 0-10 yr), and hydrophobicity (1-10 yr). Yet, whether the decline in the decomposability over the course of the experiment resulted because more of the carbon was biochemically recalcitrant or protected via physio-chemical mechanisms (e.g., within aggregates or via interactions with mineral surfaces) is unknown.

Intact versus sieved: a comparison of the two incubations

Due to differences in collection protocols and experimental setup, the soil core and sieved soil incubations were not statistically comparable. Yet, qualitative comparisons are warranted in order to understand the potential effect of soil structure on temperature sensitivity of decomposition. We expected that an intact soil matrix would affect carbon substrate availability, soil water films, and the soil microbial community. We found similar main effects of soil moisture, basal temperature, and incubation time on decomposition and its temperature response. Soil moisture increased cumulative respiration in both incubations, although differences between mesic and wet treatments were generally more apparent in the sieved soil incubation. Both incubations showed little thermal adjustment, and increasing temperature sensitivity of decomposition as the incubation progressed. We expected to see faster respiration in the sieved soil treatments, as the disturbance of the soil structure would increase soil carbon available to soil

microbes. Instead, carbon pools were smaller in the sieved soil incubations, resulting in lower cumulative respiration, and longer mean residence times. It is possible that, in the sieved soil incubation, large amounts of fast cycling carbon turned over so rapidly that it was lost in the dry-down period prior to the first measurements of soil flux. Alternatively, sieving could have represented a major disturbance of the soil microbial community, which could have resulted in lower respiration rates in the sieved soils.

It was unclear whether sieving would impact the temperature response of respiration, as the many physical, environmental, and biological constraints on decomposition such as aggregate formation and turnover, adsorption and desorption, and the diffusion of substrates and enzymes, are expected to be temperature sensitive. Still, we expected the main effect of sieving would be to increase substrate availability, and that the sieved soil incubation would be more temperature sensitive. Averaged across treatments and temperature response curves, we observed higher Q_{10} in the sieved soil ($Q_{10} = 2.42 \pm 0.03$ SE) vs. intact core incubations ($Q_{10} = 2.28 \pm 0.02$ SE). It is possible that these differences are an artifact of the two different incubations; however, if the temperature response of respiration is greater in processed soils, laboratory studies using sieved soil to determine the temperature sensitivity of soil microbial respiration might overestimate this parameter.

Implications

We examined three mechanisms that could influence the response of soil organic carbon decomposition to warming. Two of the mechanisms, drying in upland soils, and

soil microbial adjustment, are expected to reduce warming effects on soil respiration, The third mechanism, greater temperature response of slow-cycling carbon pools, could promote losses of large amounts of carbon stored in soils to the atmosphere, as slow cycling carbon pools were two orders of magnitude larger than fast cycling pools in these study sites. These potentially offsetting effects of climate change on soil carbon release to the atmosphere contribute to uncertainty about carbon cycle-mediated feedbacks to future climate change.

Treatment effects on soil microbial community thermal adjustment were small, and do not support thermal adjustment as an important control on the response of soil respiration to warming. This study, though long for a soil laboratory incubation (von Lützow and Kögel-Knabner 2009), might not be sufficiently long to see the full effects of soil microbial thermal adjustment. Nevertheless, we expect that physiological shifts in the standing microbial biomass to occur rapidly, followed in time by selection for genes, individuals, or species, and possible shifts in microbial biomass. It is possible that additional incubation time would have revealed greater effects. Furthermore, microbial communities were responding to both temperature and rapidly changing soil carbon pools (i.e. depletion of fast carbon pools) concurrently. Thus, it is also possible that the effects of shifting carbon pool were more important drivers of respiration than the temperature effects on the soil microbial community.

Fast cycling carbon pools represented a small fraction of total soil carbon (2-3%) in this study. Thus, even as the warming response of fast cycling carbon dominated, at least initially, responses were short-lived, as fast cycling pools were rapidly depleted. The

slow cycling carbon pools were found to have greater relative responses to temperature, even though absolute responses to temperature were small. Extrapolating two pool decomposition model parameters to 10 years, sieved soils and intact cores incubated at 20°C will lose on average 4% and 9% more, respectively, soil carbon than soils at incubated at 16°C. Including soil moisture treatment in decomposition model predictions, we find that soil respiration decreased by 19 and 4% for sieved soils and intact cores, respectively, in the dry treatment at 20°C, relative to the mesic and wet treatments at 16°C.

While overly simplistic, these extrapolations demonstrate that small increases in slow pool decomposition over long time periods can represent large positive feedbacks to warming (Davidson and Janssens 2006). Yet, small increases in the decomposition of slow cycling carbon might be difficult to detect, especially in highly variable *in situ* warming experiments where the majority of the soil CO_2 efflux is derived from the decomposition of rapidly cycling soil pools, and from root respiration. Furthermore, we found strong evidence that decreasing soil moisture could limit warming effects on soil carbon decomposition in well-drained upland ecosystems, as widespread reductions in soil moisture are predicted due to increased evapotranspiration and reductions in precipitation (Dai 2012).

Table 2-1. Climatic and edaphic characteristics of two southern boreal forest stand northern hardwood stands located a latitudinal gradient in Minnesota, USA.

Characteristics	Cloquet Forestry Center (CFC)		Hubacheck Wilderness Research Center (HWRC)	
Latitude	46°40'46"N		47°56'49"N	
Longitude	92°31'11"W		91°45'29"W	
Mean annual precipitation (mm)[†]	807		722	
Mean annual temperature (°C) [†]	4.5		3.0	
Soil Taxonomy				
Order	Inceptisols		Entisols	
Subgroup	Typic Dystrudepts		Lithic Udorthents	
Series	Cloquet Series		Conic Series	
Parent material	Outwash plain		Glacial Till	
Canopy status	Closed	Open	Closed	Open
Overstory Age, 2011 (yr)	40 - 60	5 - 6	40 - 60	4
pH [√]	5.22 (0.029)	5.41 (0.032)	5.41 (0.041)	5.44 (0.038)
% Clay (0-10cm) [√]	10.5 (1.4)	9.2 (0.64)	14.7 (0.96)	8.0 (0.51)
% Silt (0-10cm) [√]	33.0 (2.0)	29.4 (1.6)	29.1 (0.88)	16.9 (0.42)
% Sand (0-10cm) [√]	56.5 (1.7)	61.4 (2.0)	56.3 (1.5)	75.1 (0.85)
Bulk density (0-10cm; g cm^{-3}) [◊]	0.95 (0.045)	1.13 (0.078)	1.33 (0.068)	1.28 (0.12)
mg C g^{-1} (Sieved Soil) [±]	33.28	n/a	35.87	n/a
mg N g^{-1} (Sieved Soil) [±]	2.00	n/a	2.11	n/a
Soil C:N (Sieved Soil) [±]	16.67	n/a	17.00	n/a
mg C g^{-1} (Intact Cores) [±]	31.86 (1.46)	37.51 (2.96)	43.22 (7.05)	29.79 (7.63)
mg N g^{-1} (Intact Cores) [±]	2.00 (0.15)	2.20 (0.12)	2.35 (0.34)	1.61 (0.31)
Soil C:N (Intact Cores)[±]	16.03 (0.50)	17.08 (0.88)	18.23 (0.61)	18.15 (1.40)

[†] 30-year mean climate data (1981-2010) from NOAA National Climatic Data Center from the Winton Power Plant and Cloquet stations.

[√] Soil pH and soil texture are mean values from two depth increments (0-5 and 5-10cm). pH was measured in a 2:1 ratio of water to soil. Texture was measured using the hydrometer method. Soil for both analyses was sampled in 2008 and consisted of a composited sample of six cores (2.5-cm) taken from within each B4WarmED plot.

[◊] Bulk density was measured at three locations in each B4WarmED block in 2008. Three cores (5-cm diameter) per B4WarmED plot were sampled and the dry mass of each determined. The volume of the soil removed was determined by filling a flexible plastic bag placed in the soil hole with a known volume of water.

[±] Soil carbon and nitrogen were determined on soil from both incubations on an Elementar VarioMax elemental analyzer (Elementar Analysensysteme GmbH).

Table 2-2. Significance of statistical analyses (mixed-model ANOVA) comparing moisture treatment, basal temperature, sites, and canopies (intact core only) effects on cumulative carbon respiration, and two-pool decomposition model parameters. Analyses were restricted to 3-way interactions only, and were separate for the two incubation experiments. R^2 is the proportion of variation explained by each statistical model. Response variables were ln-transformed for analyses.

Effects	Cumulative Respiration	k_f	C_f	k_s	C_s
Intact core					
Moisture	****		****	****	***
Basal temp	**	***	*	***	
Site					
Canopy			*		
Moisture x Basal temp		*			
Moisture x Site	†			*	
Moisture x Canopy		*	*		
Basal temp x Site	†				
Basal temp x Canopy					
Site x Canopy					
Moisture x Basal temp x Site	†				
Moisture x Basal temp x Canopy					
Moisture x Site x Canopy		*	†		†
Basal temp x Site x Canopy					
R^2	0.58	0.45	0.55	0.68	0.99
Sieved soil					
Moisture	****	****	****	****	****
Basal temp	****		****	****	****
Site	****	***	****	**	****
Moisture x Basal temp	***			**	****
Moisture x Site	****	*			****
Basal temp x Site					*
Moisture x Basal temp x Site					
R^2	0.98	0.39	0.95	0.94	0.99

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ***P≤0.0001

Table 2-3. Significance of statistical analyses (repeated measures mixed-model ANOVA) comparing moisture treatment, basal temperature, sites, canopies, and time effects on the available carbon pool (*B*) and the temperature sensitivity of decomposition for the intact core incubation. Analyses were restricted to 3-way interactions only, and were separate for the two incubation experiments. R^2 is the proportion of variation explained by each statistical model. Response variables were ln-transformed for analyses.

Effects	*B*	Q_{10}
Intact core		
Moisture	****	*
Basal temp		
Site	*	
Canopy	*	
Day	****	****
Moisture x Basal temp		
Moisture x Site		
Moisture x Canopy		
Moisture x Day	**	
Basal temp x Site		
Basal temp x Canopy		
Basal temp x Day		†
Site x Canopy		
Site x Day		**
Canopy x Day	**	
Moisture x Basal temp x Site	*	
Moisture x Basal temp x Canopy		
Moisture x Basal temp x Day		
Moisture x Site x Day		
Moisture x Canopy x Day		
Moisture x Site x Canopy		†
Basal temp x Site x Day		
Basal temp x Canopy x Day		
Basal temp x Site x Canopy		
Site x Canopy x Day		
R^2	0.62	0.43

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ***P≤0.0001

Table 2-4. Significance of statistical analyses (repeated measures mixed-model ANOVA) comparing moisture treatment, basal temperature, sites, and time effects on the available carbon pool (*B*) and the temperature sensitivity of decomposition for the sieved soil incubation. Analyses were restricted to 3-way interactions only, and were separate for the two incubation experiments. R^2 is the proportion of variation explained by each statistical model. Response variables were ln-transformed for analyses.

Effects	*B*	Q_{10}
Sieved soil		
Moisture	****	****
Basal temp		*
Site	****	**
Day	****	****
Moisture x Basal temp		
Moisture x Site		
Moisture x Day	***	****
Basal temp x Site		
Basal temp x Day	****	**
Site x Day	****	**
Moisture x Basal temp x Site		
Moisture x Basal temp x Day	**	**
Moisture x Site x Day		
Basal temp x Site x Day		
R^2	0.69	0.41

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ***P≤0.0001

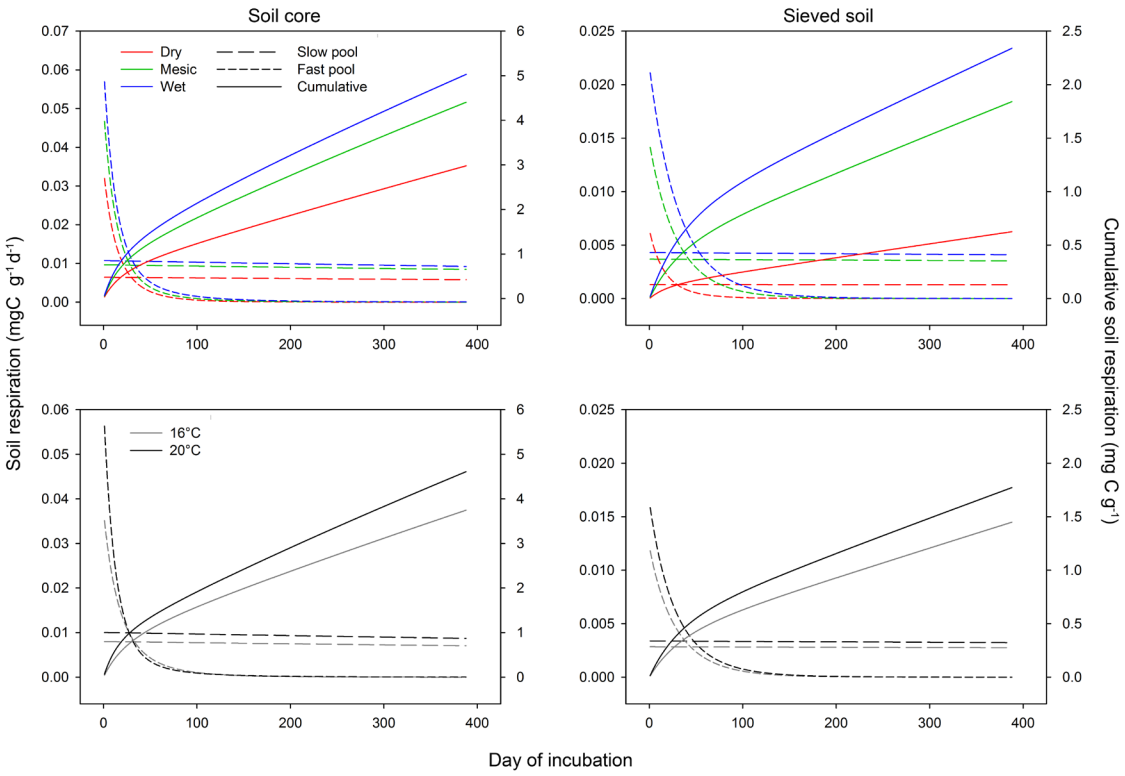

Figure 2-1. Modeled carbon respired by soil moisture (top) and basal temperature (bottom) treatments for intact core (left) and sieved soil (right) incubations. Fast (short dash) and slow (longer dash) pool respiration scale to the left axes, while cumulative carbon respired (solid lines) scales to the right axes.

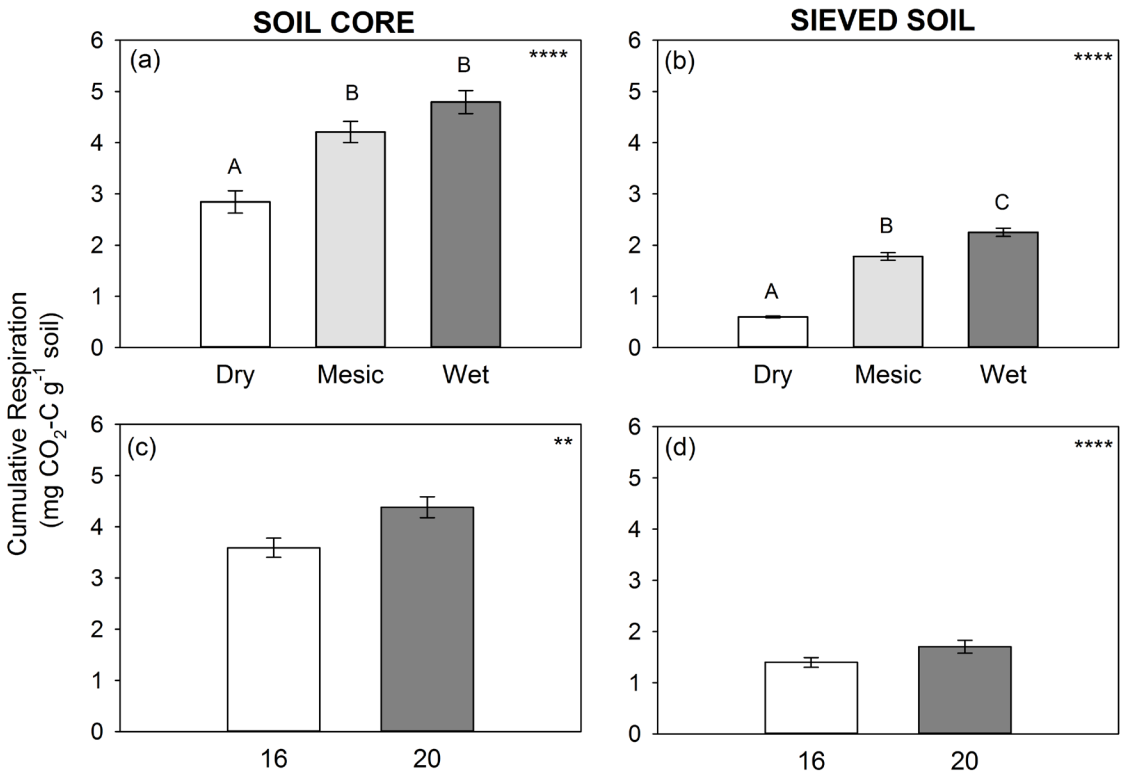

Figure 2-2. Cumulative carbon respired by soil moisture (a and b) and basal temperature (c and d) treatments for intact core (a and c) and sieved soil (b and d) incubations. An asterisk indicates significant treatment effects, and means not sharing the same letter are significantly different (Tukey HSD, $p < 0.05$). Error bars are ± 1 SE.

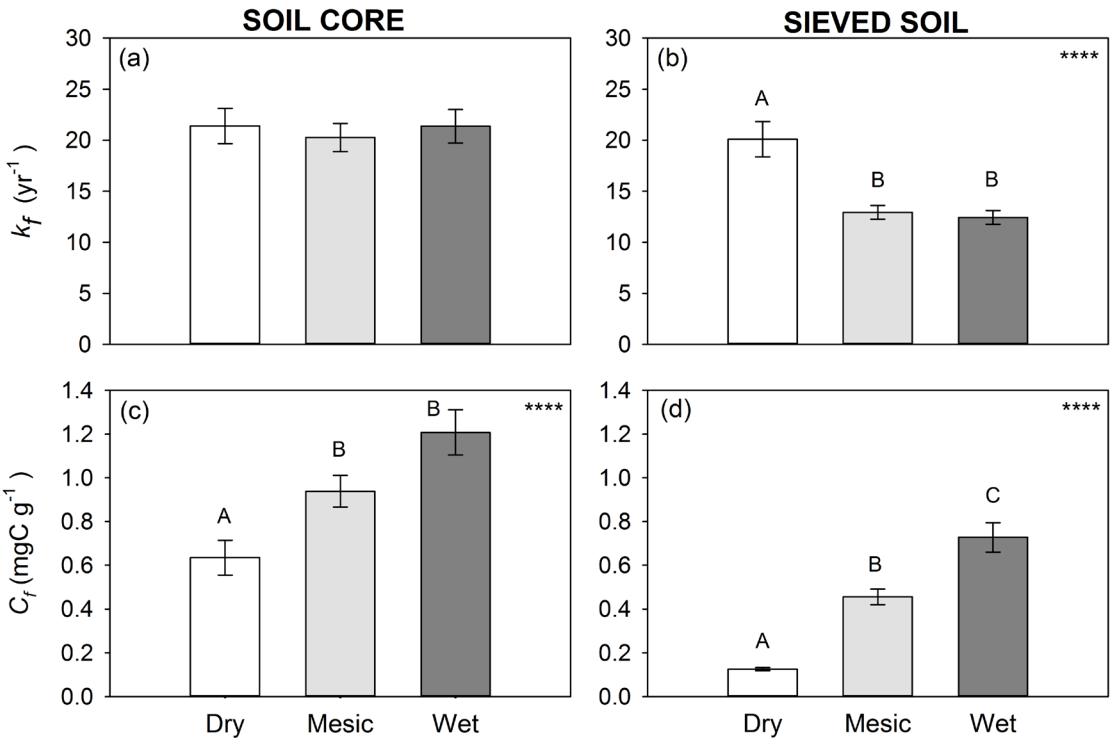

Figure 2-3. Fast pool decomposition parameters from the fit of two-pool model by soil moisture treatment for intact core (a and c) and sieved soil (b and d) incubations. Fast pool decomposition rate (k_F) is shown in (a) and (b), and fast pool size (C_F) in (c) and (d). An asterisk indicates significant moisture effects, and means not sharing the same letter are significantly different (Tukey HSD, $p < 0.05$). Error bars are ± 1 SE.

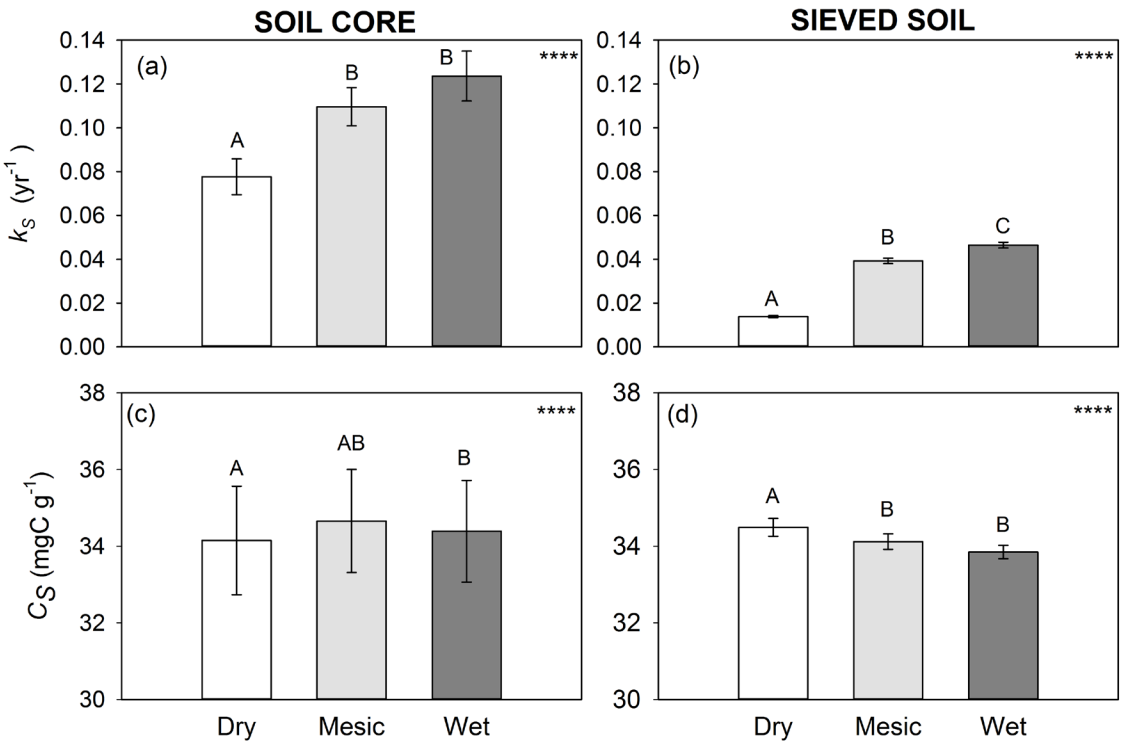

Figure 2-4. Slow pool decomposition parameters from the fit of two-pool model by soil moisture treatment for intact core (a and c) and sieved soil (b and d) incubations. Slow pool decomposition rate (k_S) is shown in (a) and (b), and slow pool size (C_S) in (c) and (d). An asterisk indicates significant moisture effects, and means not sharing the same letter are significantly different (Tukey HSD, $p < 0.05$). Error bars are ± 1 SE

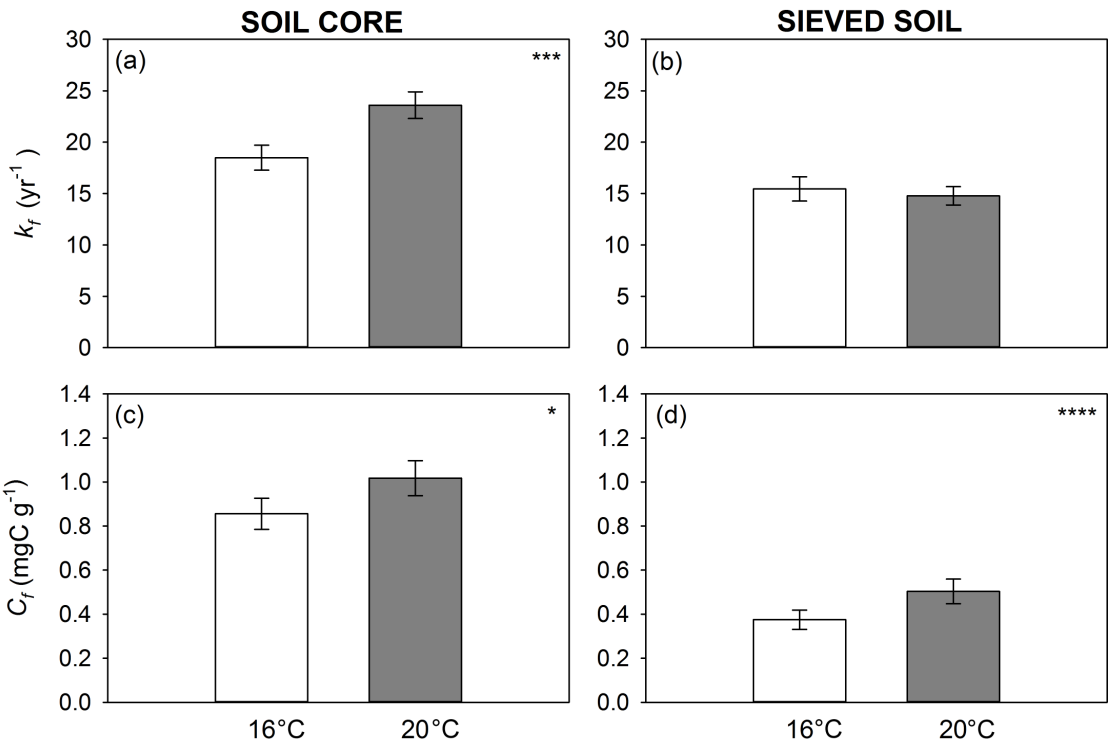

Figure 2-5. Fast pool decomposition parameters from the fit of two-pool model by basal temperature treatment for intact core (a and c) and sieved soil (b and d) incubations. Fast pool decomposition rate (k_F) is shown in (a) and (b), and fast pool size (C_F in (c) and (d). An asterisk indicates significant basal temperature effects. Error bars are ± 1 SE.

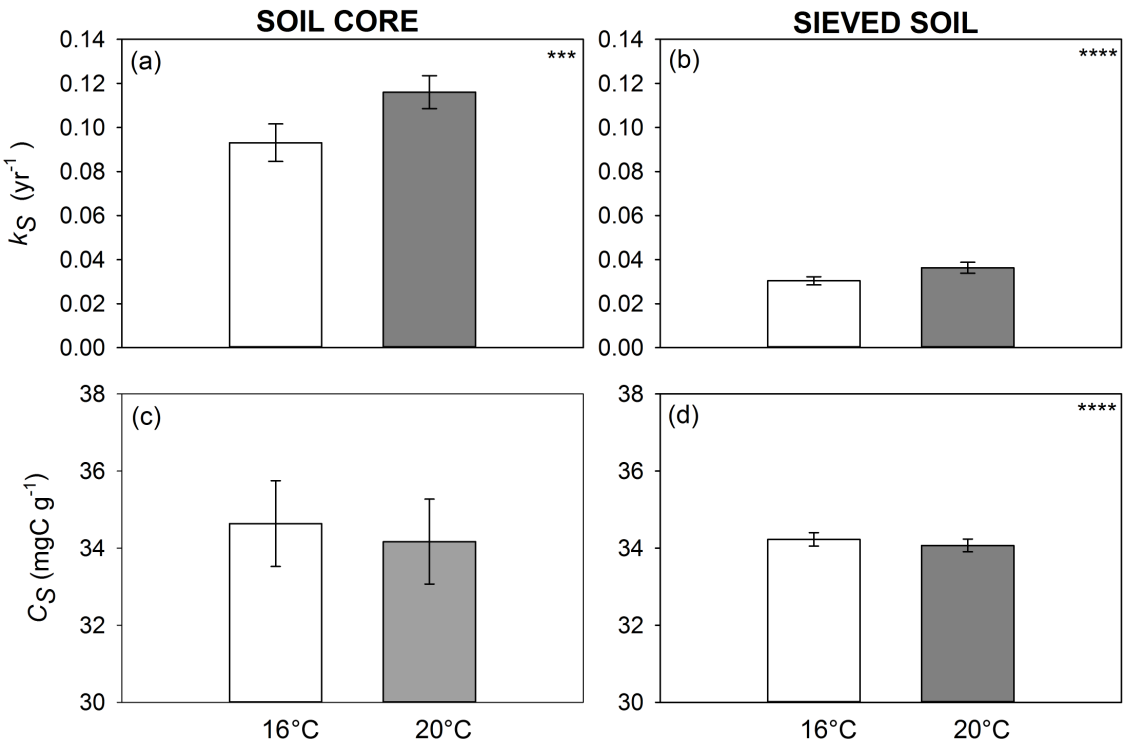

Figure 2-6. Slow pool decomposition parameters from the fit of two-pool model by basal temperature treatment for intact core (a and c) and sieved soil (b and d) incubations. Slow pool decomposition rate (k_S) is shown in (a) and (b), and slow pool size (C_S) in (c) and (d). An asterisk indicates significant basal temperature effects. Error bars are ± 1 SE.

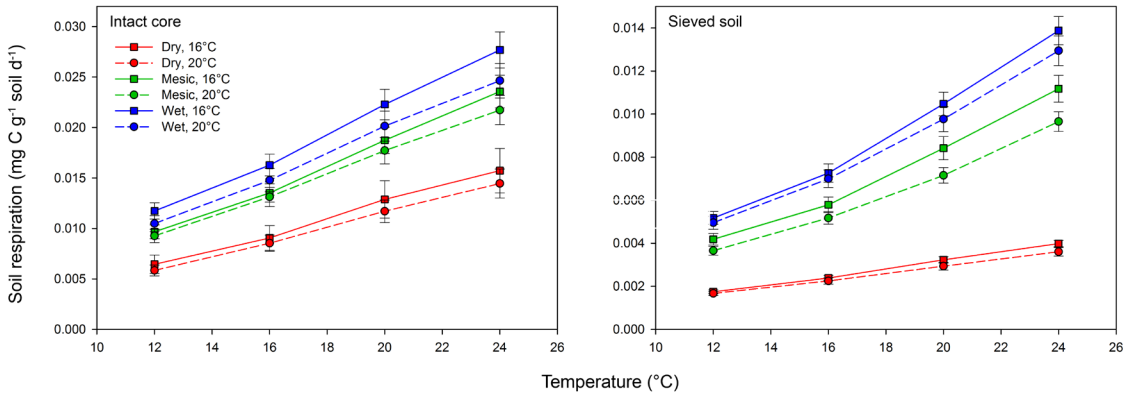

Figure 2-7 Mean respiration across six temperature response curves showing moisture and basal temperature treatments effects for intact core (left) and sieved soil (right) incubations. Error bars are ± 1 SE.

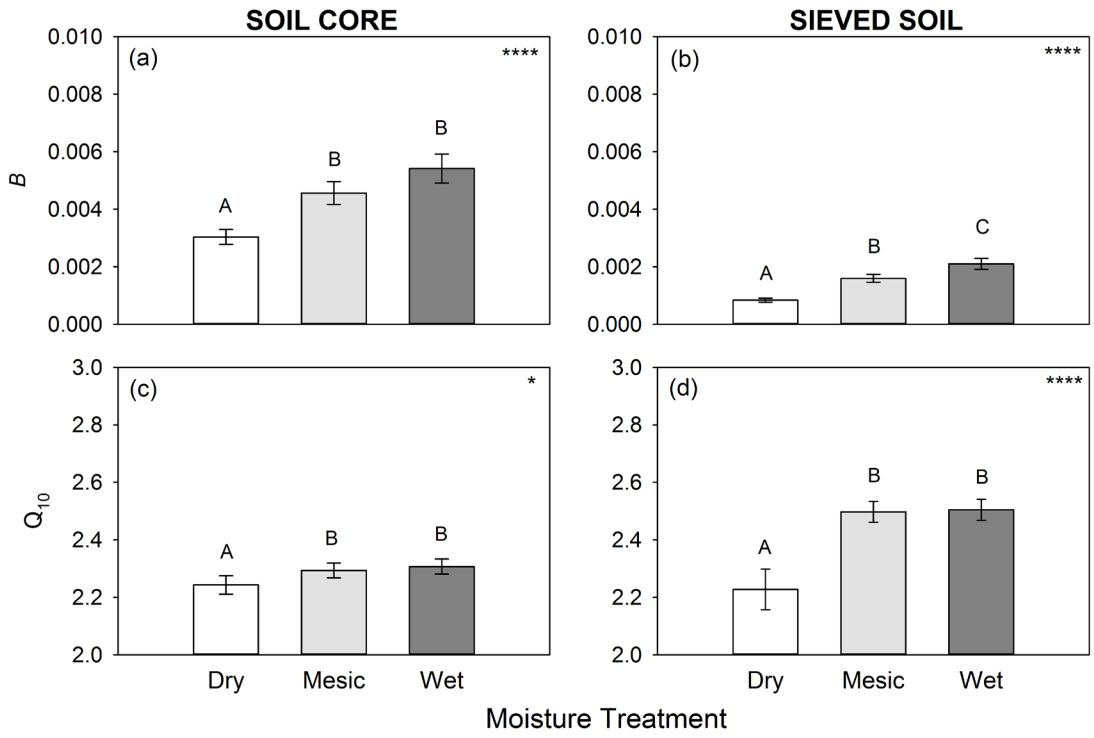

Figure 2-8. Soil organic carbon availability (*B*; top) and temperature sensitivity of soil carbon decomposition (Q_{10}; bottom) by soil moisture treatment for intact core (a and c) and sieved soil (b and d) incubations. An asterisk indicates significant moisture effects, and means not sharing the same letter are significantly different (Tukey HSD, $p < 0.05$). Error bars are ± 1 SE.

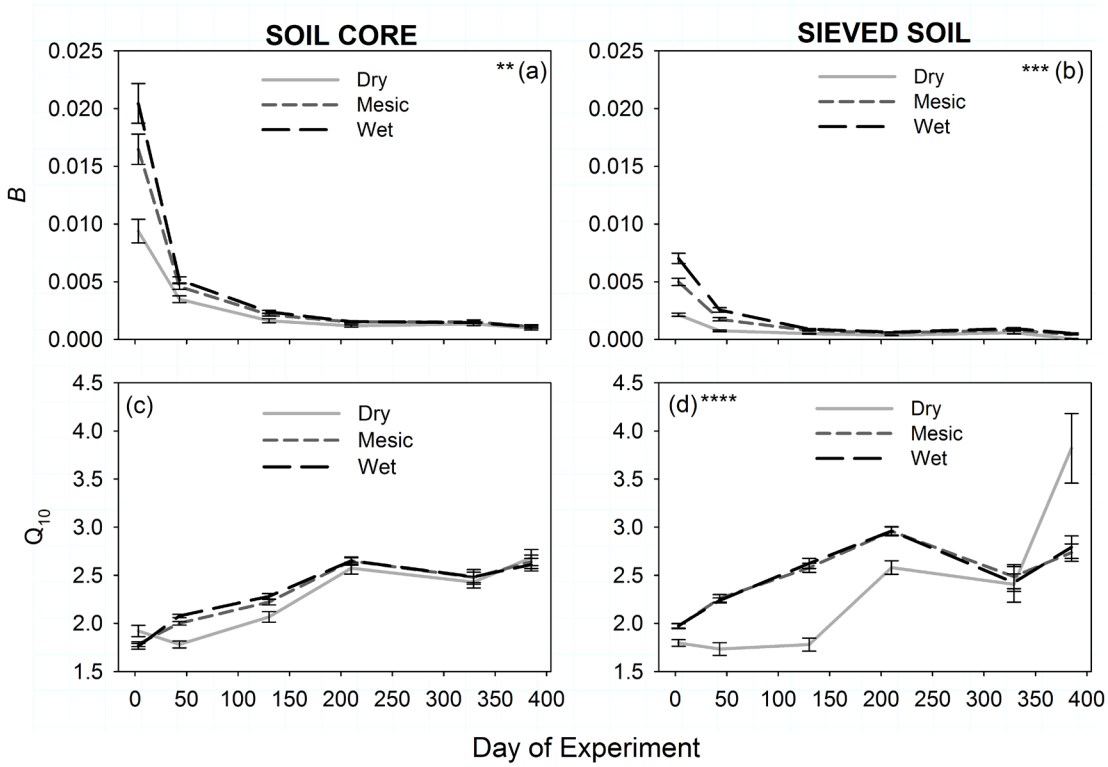

Figure 2-9. Soil organic carbon availability (*B*; top) and temperature sensitivity of soil carbon decomposition (Q_{10}; bottom) by soil moisture treatment and time for intact core (a and c) and sieved soil (b and d) incubations. An asterisk indicates significant moisture effects. Error bars are ± 1 SE.

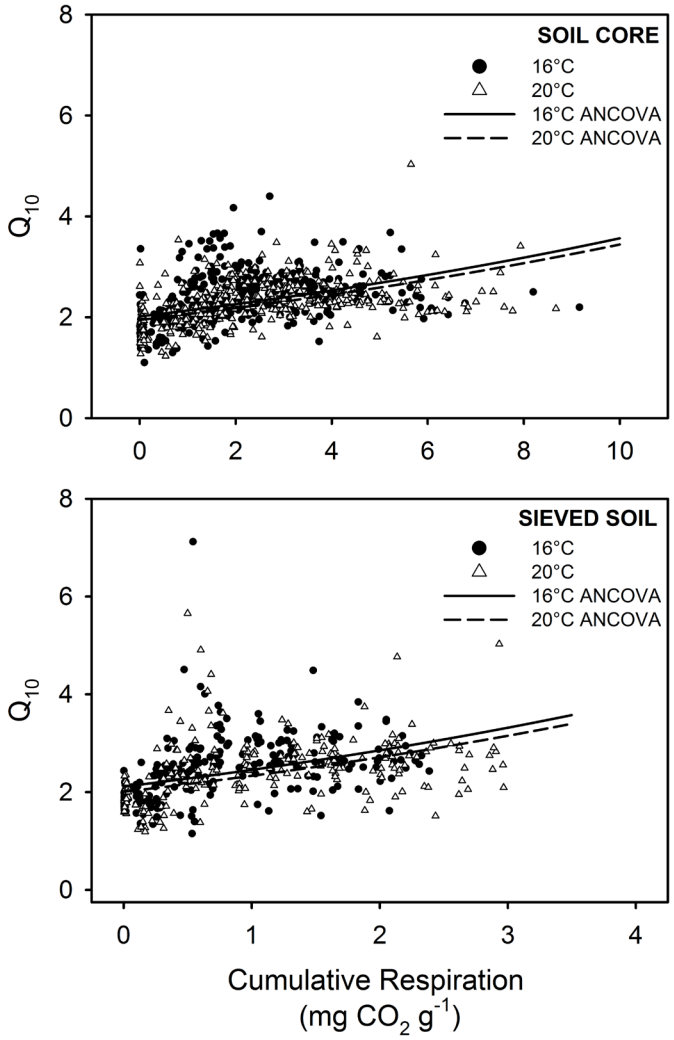

Figure 2-10. The temperature sensitivity of soil carbon decomposition (Q_{10}) as a function of cumulative carbon respired for intact core (top) and sieved soil (bottom) incubations. Regression lines are from mixed model ANCOVA fits of the effects of cumulative carbon respired and basal temperature on (ln) Q_{10}. For both incubations, cumulative carbon respired ($p < 0.0001$) and basal temperature treatment ($p<0.01$) were significant predictors of Q_{10}. X-axes were scaled according carbon respired in each incubation.

Chapter 3: *In situ* experimental warming has few effects on the temperature sensitivity of hydrolytic soil enzyme Michaelis-Menten kinetics in two southern boreal forest ecosystems.

Abstract

The decomposition of soil organic matter is expected to increase with climate warming, which could release large amounts carbon stored in soils to the atmosphere as CO_2. Soil extracellular enzymes are important catalysts of organic matter decomposition, as they break down organic polymers and increase the carbon substrates available to the soil microbial community. Soil microbial communities are expected to thermally adjust to higher temperatures by down-regulating enzyme production, and producing isoenzymes, which together could reduce the warming enhancement of the soil carbon decomposition.

We examined the effects of warming on four hydrolytic extracellular enzymes important for soil carbon and nitrogen decomposition. Soils were sampled three times across the snow-free season in the fourth year of *in situ* warming (+3.4°C) at the B4WarmED project, a highly replicated open-air warming experiment located at two southern boreal forests (~150 km apart) in Minnesota, USA. We measured the Michaelis-Menten kinetics (and their temperature response) for these soil extracellular enzymes. We expected that soil microbial community responses to warming would decrease the maximum enzymatic rate (V_{max}) and Michaelis-Menten constant (K_m), the binding affinity between substrate and enzyme, as well as the temperature sensitivity of these kinetic parameters, when measured at a common laboratory temperature.

Contrary to predictions, *in situ* experimental warming increased V_{max} and the temperature sensitivity of V_{max} of *N*-acteyl glucosaminidase. For the kinetics of α-

glucosidase, β-glucosidase, and cellobiohydrolase, warming effects differed by site and season, and included both positive and negative effects of warming on kinetic parameters and their temperature sensitivity.

In addition, we predicted that enzyme kinetic parameters (V_{max} and K_m) from colder soils, either from the northern site (Δ Temperature = 0.3°C), or from measurements in October (vs. June, ΔT = 6.7°C, and August, ΔT = 8.6°C) would be greater and more temperature sensitive. As predicted, enzyme kinetic parameters and their temperature sensitivity tended to be higher in the colder soils from the northern site, but we did not find any evidence for changes in seasonally colder soils.

Taken together, these results indicate that the activity and response to temperature for four soil extracellular enzymes important in decomposition are not reduced by warming, suggesting that changes in soil enzymes are unlikely to moderate warming response of soil carbon decomposition.

Introduction

Climate models project that global mean annual temperature over land will increase by 1.2–4.8°C by the end of the 21st century (Collins et al. 2013). One of the largest potential positive feedbacks to climate warming is that arising from the enhanced decomposition of soil organic matter (SOM) (Davidson and Janssens 2006). Soils contain approximately three to four times as much carbon as the atmosphere (Ciais et al. 2013) and microbial decomposition of soil carbon is expected to increase with climate warming, as temperature is often the most important factor determining rates of SOM decomposition (Lloyd and Taylor 1994, Kirschbaum 1995, 2000, 2006). Thus, greater microbial respiration of SOM could enhance

the greenhouse effect in a warmer world.

Soil extracellular enzymes catalyze the break down of soil organic carbon polymers into oligomers and monomers. Soluble breakdown products are free to diffuse and are able to be metabolized by soil microorganisms (Nannipieri and Kandeler 2002, Allison 2005, German et al. 2012). Extracellular enzyme activity may be the rate-limiting step in SOM decomposition (Sinsabaugh and Moorhead 1994, Allison 2005, Stone et al. 2012) and changes in extracellular enzyme activity have been shown to result from climate change (Allison and Treseder 2008, Cusask et al. 2010, Kardol et al. 2010, McDaniel et al. 2013, Zhou et al. 2013), nitrogen deposition (DeForest et al. 2004, Waldrop et al. 2004, Sinsabaugh et al. 2005, Keeler et al. 2008, Hobbie et al. 2012, Stone et al. 2012), and other global change factors (Larson et al. 2002, Finzi et al. 2006, Chung et al. 2007).

The response of extracellular enzymes to warming has been studied in comparisons across different times of year and latitudes, laboratory warming experiments, and field warming studies. Extracellular enzyme activity has been shown to increase with decreasing seasonal temperature (Fenner et al. 2005, Koch et al. 2007, Wallenstein et al. 2009, Bell and Henry 2011) and at higher latitudes (German et al. 2012). Hydrolytic soil extracellular enzyme activity has been shown to decrease with experimental laboratory warming (Cusask et al. 2010). *In situ* warming experiments have yielded mixed results. For instance, Bell et al. (2010) found no effects of experimental warming on six hydrolytic and two oxidative enzymes in an old-field site in Ontario. Studies in a boreal forest (Allison and Treseder 2008), an old field (Kardol et al. 2010), a

temperate grassland (Zhou et al. 2013), and a post-harvest temperate deciduous forest (McDaniel et al. 2013) all found declines in extracellular enzyme activity with warming, although the effects were often limited to a small proportion of the enzymes assayed. Moreover, the warming effects on soil enzymes often vary with depth (Zhou et al. 2013), plant community (Kardol et al. 2010), and soil moisture (Geisseler and Horwath 2009, Baldrian et al. 2010, Zhou et al. 2013).

Such inconsistent and sometimes counterintuitive effects of warming on extracellular enzyme activity could be informed by studies of the effects of warming on enzyme kinetics (Davidson et al. 2006). The kinetics of hydrolytic extracellular enzymes can be determined using the Michaelis-Menten equation:

$$V = \frac{V_{max} \times S}{K_m + S}$$

where V is the velocity (reaction rate), S is the substrate concentration, V_{max} is the maximum reaction rate at a given temperature, and K_m is the Michaelis-Menten constant, the substrate concentration at which the reaction velocity equals $V_{max}/2$, a measure of the binding affinity between substrate and enzyme. Most studies of soil extracellular enzymes only measure V_{max}. Recent work has used measurements of soil extracellular enzyme activity across substrate concentrations to fit the Michaelis-Menten kinetics, allowing for estimates of K_m (in addition to V_{max}) (German et al. 2011a, Stone et al. 2012, German et al. 2012), although making such measurements in soil has methodological challenges, including competitive inhibition by naturally occurring substrates (Stone et al. 2012).

Given that carbon substrates in soil are often found at low concentrations (Davidson and Janssens 2006), understanding soil decomposition responses to warming would be informed by better understanding of temperature effects on both V_{max} and K_m, particularly as the warming effects on V_{max} and K_m can potentially cancel one another out (Davidson et al. 2006). V_{max} and K_m are expected to increase with temperature. Increasing V_{max} should increase V, the reaction rate, but increasing K_m, should reduce the affinity of the enzyme for substrate. This could lead to a counteracting reduction in the reaction rate, particularly at low substrate concentrations. Indeed, both V_{max} and K_m has been found to be temperature sensitive (Davidson et al. 2012, Stone et al. 2012, German et al. 2012).

The response of extracellular enzymes to warming at high substrate concentration is expected to follow Arrhenius kinetics and the rate of the reaction should follow the temperature sensitivity of V_{max} (Davidson and Janssens 2006). As substrate concentrations decrease, however, K_m becomes more important in determining the apparent temperature sensitivity of the reaction rate, as low substrate concentrations limit the reaction rate more than the response of activation energy to low temperature. This could result in little apparent temperature sensitivity of extracellular enzymes.

In addition to the influence of substrate concentration on the apparent temperature sensitivity of soil enzyme activity, as described above, warming may influence soil enzyme activity because of physiological changes in the microbial community. With prolonged warming, the microbial community is expected to thermally adjust through physiological acclimation, genetic adaptation, and microbial community shifts (Davidson et al. 2006, Ågren and Wetterstedt 2007, Bradford et al. 2008). Hypothesized physiological

changes include the down-regulation of enzyme production and the production of isoenzymes, which together are expected to reduce the temperature sensitivity of enzyme V_{max} and K_m (Davidson et al. 2006, Allison et al. 2010). Warming may also decrease carbon use efficiency (CUE) (Devêvre and Horwath 2000, Steinweg et al. 2008, Allison et al. 2010, Frey et al. 2013). Both down-regulation and declines in CUE with warming may result in reductions in soil microbial biomass (Frey et al. 2008, Allison et al. 2010). Overall, thermal adjustments could reduce potential extracellular enzyme activity both directly through reduced enzyme production and indirectly through reduced microbial biomass arising from decreased CUE.

The goal of this study was to examine the effect of *in situ* warming on soil extracellular enzyme activity and the temperature sensitivity of enzyme kinetics in two southern boreal forests in Minnesota. Soil extracellular enzymes were measured across the snow-free season in the fourth year of a highly replicated open-air warming experiment that used infrared lamps and soil warming cables to maintain warming treatments of +3.4°C during the snow-free season. We measured the Michaelis-Menten kinetics of four hydrolytic enzymes important for organic carbon and nitrogen decomposition in soils in order to understand the effects of warming on V_{max} and K_m and to examine whether warming-induced thermal adjustments by the microbial community reduced the temperature sensitivity of extracellular enzyme activity.

We hypothesized that experimental warming would reduce soil extracellular enzyme activity and the temperature sensitivity of enzyme kinetics (i.e. V_{max} and K_m) as microbes down-regulate enzyme production at higher temperatures. Furthermore, we

predicted that such warming effects on enzyme activity would occur regardless of whether warming was caused by the experimental warming treatment, more southerly latitude, or variation across the season in ambient climate conditions.

Methods

Sites and Sampling

We examined extracellular enzyme kinetics in soils collected from the B4WarmED (*Boreal Forest Warming at an Ecotone in Danger)* warming experiment, located in two upland southern boreal forest sites in Minnesota, USA. The two sites are located ~150 km apart, in forests that span the transition from temperate to boreal biomes. One site was located at the Cloquet Forestry Center (CFC), in Cloquet, MN, USA (1981-2010 mean annual temperature (MAT) = 4.8°C, July mean temperature = 19.7°C) and the other higher latitude site was at the Hubachek Wilderness Research Center (HWRC) near Ely, MN (1981-2010 MAT = 4.0°C, July mean temperature = 19.4°C). Both sites have poorly developed sandy loam soils derived from glacial outwash (CFC) or till (HWRC). The O horizon is modest. At both sites, field warming experiments were established in mixed aspen-birch-fir forest stands (Reich et al. 2015, Rich et al. 2015). Plots (3-m diameter) were located in both closed (≈5-10% of full sunlight) and relatively open (≈80% of full sunlight) overstory conditions. Each site contained 36 plots (72 plots in total) organized into 6 blocks per site (3 closed canopy and 3 open canopy). Within each block, 6 plots were divided equally among three

temperature levels, the ambient temperature control, and two warming treatment levels (+1.7°C and +3.4°C).

Experimental warming treatments consisted of both soil heating cables and infrared heat lamps operating independently with feedback control to maintain both surface and soil warming treatments at a constant differential from ambient temperature. Warming treatments were initiated in May 2009, and were operational only during the snow free season (early spring to late fall) in order to minimize artifacts of experimental warming in the winter, (e.g. increases in snow melt and freeze-thaw cycles), and to reduce treatment costs during a period of low biological activity. Sites and warming treatments are described more completely in (Sendall et al. 2015, Reich et al. 2015, Rich et al. 2015).

For the purposes of this study, soil was collected from the ambient and +3.4°C warming treatments in the closed canopy treatment only. We sampled one replicate of each treatment in each block three times across the growing season (June, August, and October 2012) in the fourth season of warming (Figure 3-1) (2 sites x 2 heat treatments x 3 blocks per site x 1 replicate per block x 3 sampling dates = 36 total samples). Four soil cores (2.54-cm diameter, 0-9cm depth) were collected from random locations in each plot and composited. After collection, soils were stored at 4°C for up to 5 days until soils could be sieved (2mm). Soil was subsampled for dry weight determination by drying at 60°C for at least 48 hours. Soils were stored at -20°C until analyses were completed in March – April 2013.

Soil extracellular enzymes analyses

Hydrolytic soil extracellular enzymes were measured using 4-Methylumbelliferone (MUB) linked substrates (Darrah and Harris 1986, Freeman et al. 1995, Marx et al. 2001, Saiya-Cork et al. 2002, German et al. 2011b, 2011a, Stone et al. 2012, German et al. 2012). We chose four enzymes important for organic carbon decomposition (i.e. α-glucosidase, β-glucosidase, cellobiohydrolase, and *N*-acetyl glucosaminidase; see Table 3-1). For each enzyme, activity was measured at eight different substrate concentrations, representing a 100x range in concentrations, in order to determine Michaelis-Menten kinetics (Table 3-1). Kinetics were determined at 4, 12, 20, and 30°C in order to determine the effects of *in situ* warming, site, and season on the instantaneous temperature sensitivity of each enzyme. One gram of dry-weight equivalent soil was combined with 125 mL 50mM sodium acetate buffer (pH =5.0) and homogenized for one minute using a blender. On each black 96 well microplate, we measured two substrates on one soil, at one temperature. Assay fluorescence for each enzyme on a plate was determined in two rows (of 8 wells each) by combining 200 μL of soil homogenate and 50 μL of MUB-linked enzyme substrate (two rows per enzyme per plate). The MUB standard curve consisted of 200 μL soil homogenate and 50 μL of 8 MUB standards in water (final concentrations 0.05 – 5 μM MUB; two rows per plate). We accounted for background fluorescence by enzyme substrate blanks (200 μL buffer + 50 μL substrate; 2 rows per substrate per plate), homogenate blank (200 μL soil homogenate + 50 μL buffer; one row per plate), and buffer alone (250 μL buffer; one row per plate). All soils were incubated for one hour, after which 10 μL 1*M* NaOH was added

to each well in order to terminate the reaction and enhance fluorescence. Plates were allowed to rest for 10 minutes (DeForest 2009, German et al. 2011a). Fluorescence was measured on a Biotek Synergy fluorometer (Winooski, VT, USA) with 365 nm excitation and 450 nm emission.

Calculations

Enzyme activities were calculated using assay fluorescence after subtracting substrate and homogenate blank fluorescence. The MUB standard curve was used to calculate activity in units of nmol g^{-1} h^{-1}. The Michaelis-Menten equation (Equation 1) was fit using nonlinear maximum likelihood estimation (R package BBLME) to determine the parameters V_{max} (nmol g^{-1} h^{-1}) and K_m (μmol L^{-1}).

The temperature dependence of V_{max} was examined using an Arrhenius function (Davidson et al. 2012)

$$V_{max} = A \times e^{\frac{-E_a}{RT}} \text{ (Equation 2)}$$

where V_{max} is the maximum reaction velocity (nmol g^{-1} h^{-1}); A is pre-exponential factor (nmol g^{-1} h^{-1}), E_a is the required activation energy (KJ mol^{-1}); R is the gas constant (0.008314 KJ $K^{-1}mol^{-1}$); and T is the temperature in Kelvin. Arrhenius fits were done in JMP 11.0 (SAS Institute, Cary, NC, USA).

The temperature dependence of K_m was examined using a linear fit of K_m versus temperature according to the following equation (Davidson et al. 2012):

$$K_m = C_{Km} + M_{Km}T \text{ (Equation 3)},$$

where C_{Km} is the y-intercept, T is the temperature in Celsius, and M_{Km} is the slope of the linear relationship between K_m and temperature.

We also examined a simple exponential model of K_m by temperature using the equation

$K_m = A_{Km} + e^{B_{Km}T}$ (*Equation 4*),

where A_{Km} is a scaling factor, and B_{Km} is a fitted parameter describing the temperature sensitivity of K_m. Differences in Akaike's Information Criteria (AIC) greater than 3 were used to identify significant differences in the fits of these two models (Burnham and Anderson 2002).

Statistical analyses

We used a repeated-measures linear mixed model ANCOVA to test for effects of *in situ* warming treatment, site, and season (repeated measure) on Michaelis-Menten kinetics (V_{max} and K_m), with the measurement temperature (4, 12, 20, or 30°C) as a co-variate. We also examined measurement temperature sensitivity by analyzing the temperature response parameters for V_{max} (A and E_a) and K_m (C_{Km} and M_{Km}) using the same model. For all model structures, dependent variables were ln-transformed where necessary to meet the assumptions of normality, except in the case of M_{Km} which was log-modulus transformed in order to maintain positive and negative signs (John and Draper 1980). To determine if K_m responded significantly to temperature, we used linear regression to test the hypothesis $M_{Km}=0$. Significance was accepted for all statistical tests at $\alpha = 0.05$, although due to the small sample size we consider trends at $\alpha = 0.10$.

Analyses of the linear mixed models were performed using JMP 11.0 (SAS Institute, Cary, NC, USA).

Results

Warming treatment effects on Michaelis-Menten kinetics

Main effects of warming treatment on soil extracellular enzyme activity (i.e. V_{max}) were only detected for one of the four enzymes (*N*-acetyl glucosaminidase; statistical models shown in Table 3-2, data in Appendix E). Contrary to the prediction that warming would reduce soil enzyme activity (when measured at common temperatures), warming increased *N*-acetyl glucosaminidase activity. All other warming effects observed were nested within two and three-way interactions within site and season (Figure 3-2).

For β-glucosidase, cellobiohydrolase, and *N*-acetyl glucosaminidase, we identified significant two-way interactions between warming and season. Warming increased enzyme activity in June, decreased activity in October, and had neutral to negative effects on enzyme activity in August. For *N*-acetyl glucosaminidase, warming effects also differed by site, with warming increasing enzyme activity at the northern site (HWRC) and decreasing activity at the southern site (CFC). For all four enzymes, we found significant warming treatment by site by season interactions (Figure 3-2). Warming treatment increased V_{max} for all enzymes at CFC in June, but had no effect at HWRC. In August, for the four enzymes measured, warming increased V_{max} at HWRC, and decreased V_{max} at CFC. In October, warming effects were mixed, but generally small. In short, enzyme V_{max} is complicated by the interactions among of warming, site, and

season. Generally, where effects were significant, the warming treatment increased V_{max} in June at the warmer site (CFC), in August at the colder site (HWRC), and but did not alter V_{max} in October.

V_{max} was significantly greater at the northern site (HWRC) for β-glucosidase and *N*-acetyl glucosaminidase (Table 3-2). For all four enzymes, V_{max} differed by season, decreasing from June and August to October (Table 3-2). V_{max} also differed significant by site and season for α-glucosidase and cellobiohydrolase, but effects were small and did not appear to be meaningful. Finally, for all enzymes, as expected, V_{max} increased with measurement temperature (Table 3-2).

As predicted, warming tended to decrease the Michaelis-Menten constant (K_m), i.e. the binding affinity between substrate and enzyme, although we detected no main effects of warming on K_m. There were significant warming treatment by season interactions on K_m for three of the four enzymes (Table 3-2, Appendix E, Table E-1). For β-glucosidase and cellobiohydrolase, the warming treatment increased K_m in June, had neutral effects in August, and reduced K_m in October (Table 3-2). For *N*-acetyl glucosaminidase, the warming treatment increased K_m in June, but not in August or October.

K_m was significantly greater at the northern site (HWRC) for α-glucosidase, β-glucosidase, and cellobiohydrolase (Table 3-2). Season was a significant main effect for K_m for β-glucosidase only, with K_m decreasing from a high in June to October to August. Lastly, for all enzymes measured except *N*-acetyl glucosaminidase K_m increased with measurement temperature (Table 3-2).

The temperature sensitivity of V_{max}

The activation energies (E_a) of the four extracellular enzymes measured were positive, indicating that V_{max} was sensitive to instantaneous increases in temperature (i.e. measurement across four temperatures in the laboratory). For *N*-acetyl glucosaminidase, there was a significant main effect in that warming increased the E_a and the pre-exponential factor (A) (Table 3-3, Figure 3-3), indicating that warming increased the basal rate and temperature sensitivity of V_{max} for chitin decomposition. For cellobiohydrolase, we found significant warming by site by season effects on E_a and A (Table 3-3, Figure 3-4, data in Appendix E, Table E-2), where A and E_a were greater in the warming treatment at CFC in June, but had little effect on either parameter later in the season. At HWRC, there were no warming effects in June, but warming increased A and E_a in August, and reduced A in October. There was no effect of warming on the Arrhenius parameters for α-glucosidase or β-glucosidase. The E_a and A of *N*-acetyl glucosaminidase were higher at HWRC than at CFC, although main effects of site were only statistically significant for A, and marginally significant for E_a (Table 3-3).

The temperature sensitivity of K_m

We chose to only consider the linear fit of K_m by temperature for several reasons. First, the fit of linear and exponential models of K_m as a function of temperature did not significantly differ for 120 out of 144 soils (across four enzymes), indicating that neither model was more appropriate. Next, the linear model more simply represents both positive

and negative relationships between K_m and temperature. Finally, other authors have found linear fits appropriate (Davidson et al. 2012).

There is some evidence that K_m increased with temperature. Most fits of K_m as a function of temperature had positive slopes (Figure 3-5), but M_{Km} was only significantly greater than zero for 12, 1, 3, and 2 of the 36 assayed soils, for α-glucosidase, β-glucosidase, cellobiohydrolase, and *N*-acetyl glucosaminidase, respectively. We found no soil-enzyme measurement where M_{Km} was significantly less than zero. Moreover, we found that measurement temperature was a significant covariate in analyses of K_m for α-glucosidase, β-glucosidase, and cellobiohydrolase, but not for *N*-acetyl glucosaminidase.

We found little evidence that M_{Km} differed by warming treatment (Figure 3-6), site, or season. The sole significant warming effect on K_m was warming by site by season effects on M_{Km} for cellobiohydrolase (Table 3-3). (Table 3-3, Figure 3-4)

Discussion

The temperature sensitivity of Michaelis-Menten kinetics

Soil extracellular enzyme V_{max} was highly sensitive to near-instantaneous changes in temperature, across the measured temperature range of 4 - 30°C. Relatively few studies to date, however, have measured temperature effects on K_m (Davidson et al. 2012, Stone et al. 2012, German et al. 2012). At low substrate concentrations, an increase in K_m with temperature could counteract increasing V_{max}, thus resulting in little apparent increase of extracellular enzyme activity with temperature (Davidson et al. 2006). Slopes of K_m vs. measurement temperature (i.e. M_{Km}) were most often positive, but rarely significantly

different then zero. Thus, at most we provide weak evidence for the temperature sensitivity of K_m, indicating that reaction rates should increase with near-instantaneous warming.

Warming treatment effects on extracellular enzyme kinetics

In response to warming treatment, the soil microbial community might be expected to down-regulate enzyme production, and shift to warm-adapted isoenzymes. Isoenzymes are thought to result from primary protein structural differences that alter enzyme flexibility vs. stability, with a tradeoff between more flexible active sites that allow greater enzyme activity, and stable active sites that confer greater substrate affinity. (Fields 2001, Johns and Somero 2004, Wallenstein et al. 2010, German et al. 2012). Cold-adapted isoenzymes are thought to have more flexible active sites, increasing the enzyme-substrate turnover rate (K_{cat}), at the cost of enzyme-substrate binding affinity (K_m) (D'Amico et al. 2002). Warm-adapted isoenzymes have more rigid active sites, which increases enzyme affinity, but at the cost of K_{cat} (Fields 2001). Isoenzyme shifts would be expected to maintain relatively steady enzymatic rates across temperatures shifts caused by, e.g., long-term warming treatment or latitudinal variation, although when measured at the same temperature, isoenzymes from warmer conditions would be expected to have lower activity than those from colder conditions. Thus, for near-instantaneous measurements in the laboratory at common temperatures, we expected that more than three years of *in situ* experimental warming (+3.4°C) would decrease V_{max}, K_m, and their temperature responses (Somero 2004, Allison et al. 2010, German et al. 2012).

In contrast to our expectations, the single main effect of warming was an increase in *N*-acetyl glucosaminidase V_{max} and E_a (i.e. the temperature sensitivity of V_{max}) in response to warming. However, for all enzymes, a lack of significant main effects of warming treatment masked complex, highly idiosyncratic significant warming interactions with site and season. Such interactions are challenging to interpret, but emphasize the likelihood that warming effects on enzyme kinetics vary across spatial and temporal scales in ways that require further study. For example, it is possible that the soil microbial community was adjusting to warmer temperatures through reductions in enzyme production or enzyme-substrate turnover rate (i.e. isoenzyme shift to warm-adapted enzymes), but that these effects were masked by changes in microbial biomass. As such, future studies may benefit from concurrent measurements of enzyme kinetics, soil carbon and nitrogen dynamics, and soil microbial biomass and composition with better sampling across temporal and spatial scales (Burns et al. 2013). Nevertheless, taken as a whole, soil enzymes in warmed treatments were no less active or temperature sensitive than in ambient temperature treatments when measured at equivalent temperatures, indicating that ecosystem-scale soil enzymatic capacity did not appear to substantially decrease with warming.

Positive effects of warming on *N*-acetyl glucosaminidase could have resulted from the temperature dependence of enzyme synthesis or from changes in carbon and nitrogen pools or microbial community in ways that fed back to extracellular enzyme production (Sinsabaugh and Moorhead 1994, Burns et al. 2013). Warming might be expected to increase soil carbon availability through increases in plant productivity and

the destabilization of SOM. If the carbon available to soil microorganisms in the warmed treatments increased more than nitrogen availability, it could follow that a greater demand for nitrogen would result in greater microbial investment in extracellular enzymes (i.e. *N*-acetyl glucosaminidase) that decompose nitrogen containing compounds (i.e. chitin) (Sinsabaugh and Moorhead 1994, Burns et al. 2013). Supporting this interpretation, (Dorodnikov et al. 2009) showed that elevated CO_2 increased *N*-acetyl glucosaminidase activity, likely due to greater nitrogen limitation. Moreover, other studies have found that carbon mineralization was more temperature sensitive than nitrogen mineralization (Koch et al. 2007).

Several mechanisms could explain the lack of apparent down-regulation of starch-degrading (i.e. α-glucosidase) and cellulose-degrading (i.e. β-glucosidase and cellobiohydrolase) with warming. First, down-regulation might be not expected if warming increases carbon available to soil microorganisms. Next, three years of warming might not be sufficient to elicit a sustained response in the microbial community. This seems unlikely as other studies have shown that extracellular enzymes respond rapidly to warming treatments (Allison and Treseder 2008, Cusask et al. 2010) and to seasonal shifts in temperature (Fenner et al. 2005, Koch et al. 2007, Bell et al. 2010). Another possibility is that drier soils in the warmer treatments (Figure 3-1) could counteract warming effects on extracellular enzymes, although the likely effect of lower moisture on extracellular enzymes in these coarse-textured upland soils would be to reduce soil microbial biomass and extracellular enzyme production (Kardol et al. 2010, Baldrian et al. 2010, Zhou et al. 2013). Reduced enzyme production would be expected to reduce

V_{max} and K_m, but we found no evidence that enzyme kinetics followed seasonal or warming treatment patterns of soil moisture (Figure 3-1). Finally, it is possible that the two sites experience different seasonal patterns of enzyme production, and that warming alters the seasonality of enzyme production on scales finer than our three measurements.

Site and seasonal effects on extracellular enzyme kinetics

Soil microbial communities are expected to adapt to the local temperature regimes (Belotte et al. 2003, German et al. 2012) in ways that alter soil enzyme production. The annual air temperature difference between the CFC (MAT = 4.8°C) and HWRC (MAT = 4.0°C) sites was 0.8°C (1981-2010 MAT measured at National Climatic Data Center stations less than 3 km from each site). On-site measurements of soil temperature (at 10-cm) in 2012 (04/09/12 – 11/15/12) revealed differences of 0.3°C (CFC > HWRC). In spite of the small differences in temperature between the two sites, we expected that extracellular enzymes in colder soils (i.e. HWRC) would have higher activities (V_{max}) and a lower affinity for substrates (i.e. a higher K_m), and that both V_{max} and K_m would be more temperature sensitive. Consistent with this, we found that *N*-acetyl glucosaminidase activity (i.e. V_{max}) and A were higher and more temperature sensitive and that K_m for α-glucosidase, β-glucosidase, and cellobiohydrolase was greater at the higher latitude (i.e. colder) site. It is possible that a small temperature difference of 0.3°C in 2012 could explain the observed increases in enzyme kinetic parameters in the northern site, although with only two sites (one at each latitude), it is impossible to attribute the increase to

temperature unequivocally, as differences in carbon and nitrogen pools, soil texture, soil moisture, and pH might also contribute to site differences in enzyme kinetics.

We expect ecosystems to respond seasonally through community and physiological shifts that will increase values of Michaelis-Menten parameters in colder seasons. Yet, across warming treatments and sites, season did not appear to alter Michaelis-Menten kinetic parameters substantially for the four extracellular enzymes measured. Where differences were observed, kinetic parameters were not, as predicted, highest in the coldest soil (i.e. October), in spite of soil temperatures in October that considerably colder than in June ($\Delta T=6.7°C$) or August ($\Delta T=8.6°C$) across both sites.

The lack of well-defined responses of Michaelis-Menten kinetics to seasonal temperature shifts (Figure 3-1) might be attributable to seasonal patterns of soil moisture, inputs of carbon and nitrogen to the soil, and changes in the soil microbial community. For instance, soil moisture was lower in August and October than in June, which could have limited enzyme production, and obscured seasonal effects. Enzyme activity would be expected to increase with seasonal peaks in belowground carbon allocation, nitrogen availability, or microbial biomass (Wallenstein et al. 2009, Bell et al. 2010, Kaiser et al. 2010). That the lowest enzyme activity in October was surprising, given this sampling occurred after litterfall, when belowground carbon inputs are likely to be high. However, microbial enzyme production during this late season at these high latitude sites was likely limited by low temperature (Figure 3-1). Lastly, past studies showing strong seasonal effects on soil enzymes have often compared enzyme activity in winter or near-winter

with that in summer, and have found smaller differences in extracellular enzyme activity within the growing season (Koch et al. 2007, Wallenstein et al. 2009).

Conclusions

For four extracellular soil enzymes, we predicted that more than three years of *in situ* experiment warming (+3.4°C) would decrease V_{max}, K_m, and the temperature sensitivities of V_{max} (i.e. E_a), and K_m (i.e. M_{Km}). *N*-acetyl glucosaminidase activity (V_{max}) was higher and more temperature sensitive in the warmed treatment across sites and season. For α-glucosidase, β-glucosidase, and cellobiohydrolase, the warming effects differed by site and season. Generally, for these three enzymes, we found more positive effects of *in situ* warming on V_{max} and K_m , approximately the same number of positive and negative effects of warming on E_a, and only one negative effect of warming on M_{Km}. The lack of warming-induced decreases in enzyme kinetics (V_{max} and K_m) and their temperature sensitivity (E_a and M_{Km}), and the significant interactions between warming, site, and season suggest that the responses of the microbial community to *in situ* warming are complex and that warming might alter other soil properties (e.g. microbial community composition or biomass) or processes (e.g. carbon stabilization or nitrogen mineralization) over varying time scales in ways that influence allocation to extracellular enzymes. Taken together, we found little evidence for reductions in soil extracellular enzyme activity and temperature sensitivity in response to warming that would moderate the carbon decomposition response to warming.

Table 3-1. Extracellular enzymes measured, their function, and concentrations. Table adapted from (Stone et al. 2012).

Enzyme abbreviation	Enzyme	EC number	Enzyme function	Substrate	Substrate concentrations
α-GLU	α-Glucosidase	3.2.1.20	Glucose from starch	4-MUB α-*D*-glucopyranoside	2 - 200 μM
β-GLU	β-Glucosidase	3.2.1.21	Glucose from cellulose	4-MUB β-*D*-glucopyranoside	2 - 200 μM
CBH	Cellobiohydrolase	3.2.1.91	Disaccharides from cellulose	4-MUB β-*D*-cellobioside	1 - 100 μM
NAG	*N*-acetyl glucosaminidase	3.2.1.1.14	*N*-acetyl glucosamine from chitin	4-MUB *N*-acetyl-β-*D*-glucosaminide	4 - 400 μM

Table 3-2. Significance of statistical analyses (repeated measures mixed-model ANOVA) comparing warming, site, canopy, and season effects on extracellular enzyme kinetics. Analysis temperature (i.e. "temp") was a covariate in the model, used to normalize other effects across the four measurement temperatures (i.e. 4, 12, 20, 30°C). Analyses were restricted to 3-way interactions only. Response variables were ln-transformed for these analyses.

Effects	*α Glucosidase*		*β Glucosidase*		*Cellbiohydrolase*		*N-Acetyl-b-D-glucosaminidase*	
Michaelis-Menten parameters	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*
Warming							*	
Site		****	*	***		*	***	
Season	*		**	***	***		*	
Temp	****	****	****	**	****	**	****	
Warming x Site		†				†	***	†
Warming x Season			***	**	****	****	*	***
Site x Season	*				*			
Warming x Site x Season	****	†	**		****		****	

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001

Table 3-3. Significance of statistical analyses (repeated measures mixed-model ANOVA) comparing warming, site, canopy, and season effects on the temperature sensitivity of extracellular enzyme kinetics. Analyses were restricted to 3-way interactions only.

Effects	*α Glucosidase*		*β Glucosidase*		*Cellbiohydrolase*		*N-Acetyl-b-D-glucosaminidase*	
Arrhenius fit of V_{max}	*A*	*Ea*	*A*	*Ea*	*A*	*Ea*	*A*	*Ea*
Warming							**	*
Site							*	†
Season								
Warming x Site								
Warming x Season								
Site x Season								
Warming x Site x Season					**	*		
Linear fit of K_m	*C_{Km}*	*M_{Km}*	*C_{Km}*	*M_{Km}*	*C_{Km}*	*M_{Km}*	*C_{Km}*	*M_{Km}*
Warming								
Site	†							
Season			†					
Warming x Site								
Warming x Season								
Site x Season						†		
Warming x Site x Season						*		

†P≤0.10, *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001

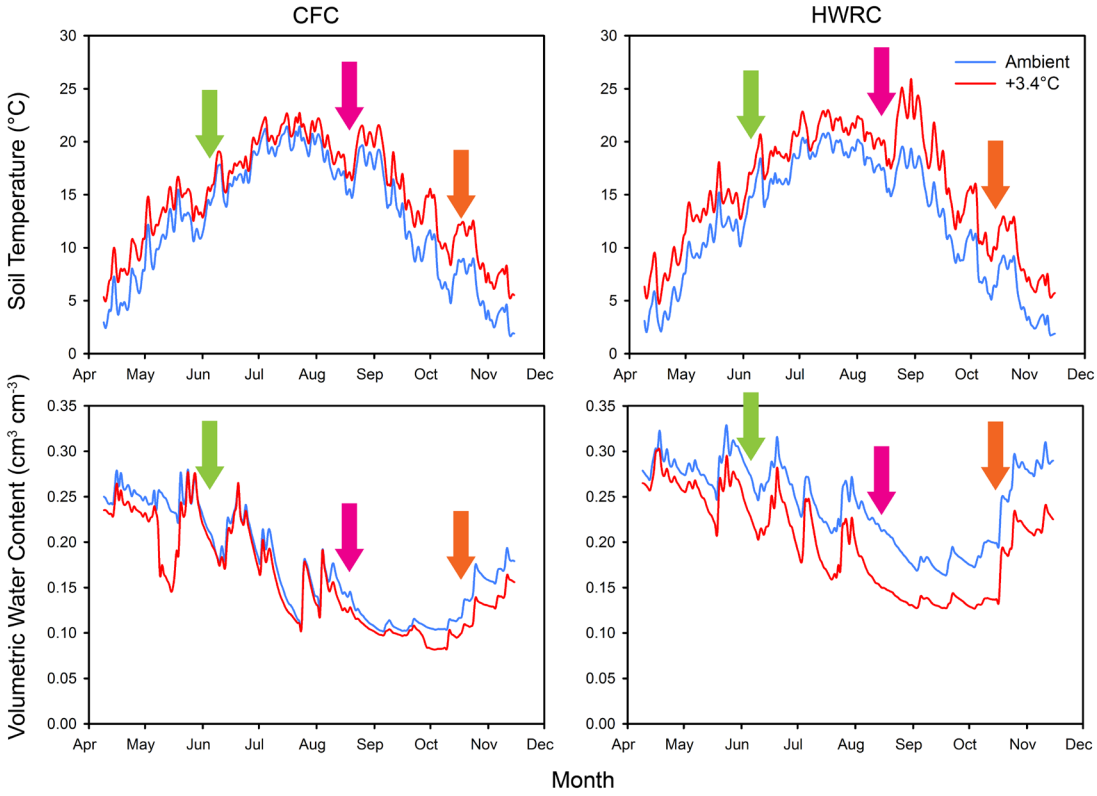

Figure 3-1. Mean daily soil temperature (top) and soil volumetric water content (bottom) for the southern (left) and northern (right) sites at the B4WarmED south boreal forest warming experiment during 2012. Soil temperature was measured continously using thermocouples at 10-cm depth while volumetric water content was mreasured using time-domain reflectometers (0-22.5-cm). The three arrows indicate when soil was sampled for enzymes analyses in June, August, and October 2012

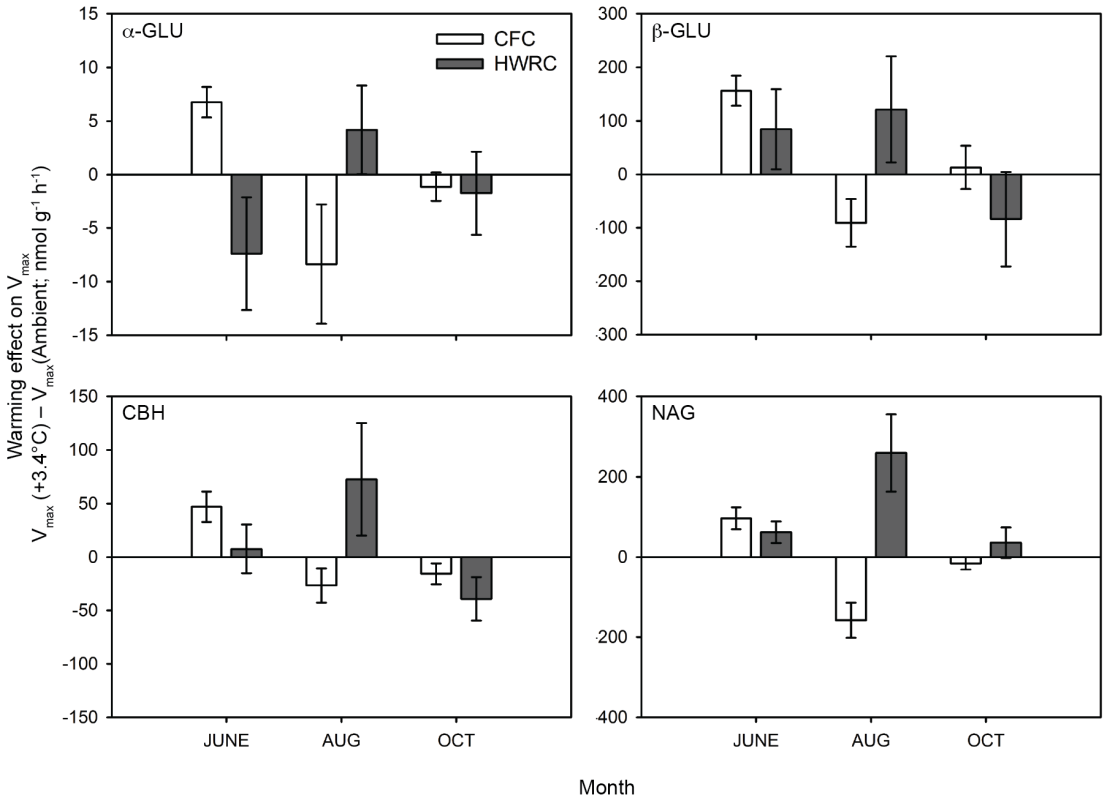

Figure 3-2. Warming treatment effects on enzyme activity (V_{max}) by site and month for the four enzymes measured. A positive value indicates that the mean warming effect on V_{max} was positive, while a negative value indicates that the mean warming effect was negative. α-GLU = α-glucosidase, β-GLU = β-glucosidase, CBH = cellobiohydrolase, and NAG = *N*-acetyl glucosaminidase. Error bars are ± 1 SE

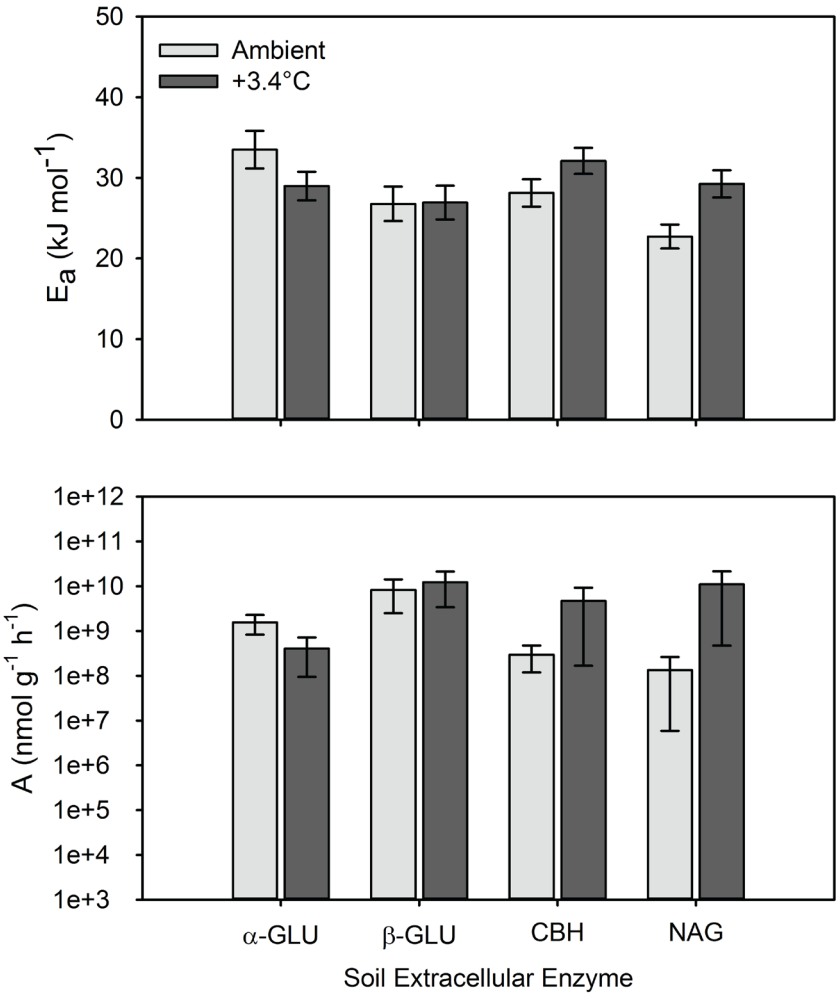

Figure 3-3. Warming effects on the Arrhenius fit of V_{max}, showing activation energy (E_a, top) and the pre-exponential factor (A, bottom) for the four extracellular enzymes measured. E_a is a measure of the temperature sensitivity of the enzyme, while A is a measure of the basal rate of the enzyme.α-GLU = α-glucosidase, β-GLU = β-glucosidase, CBH = cellobiohydrolase, and NAG = *N*-acetyl glucosaminidase. Error bars are ± 1 SE.

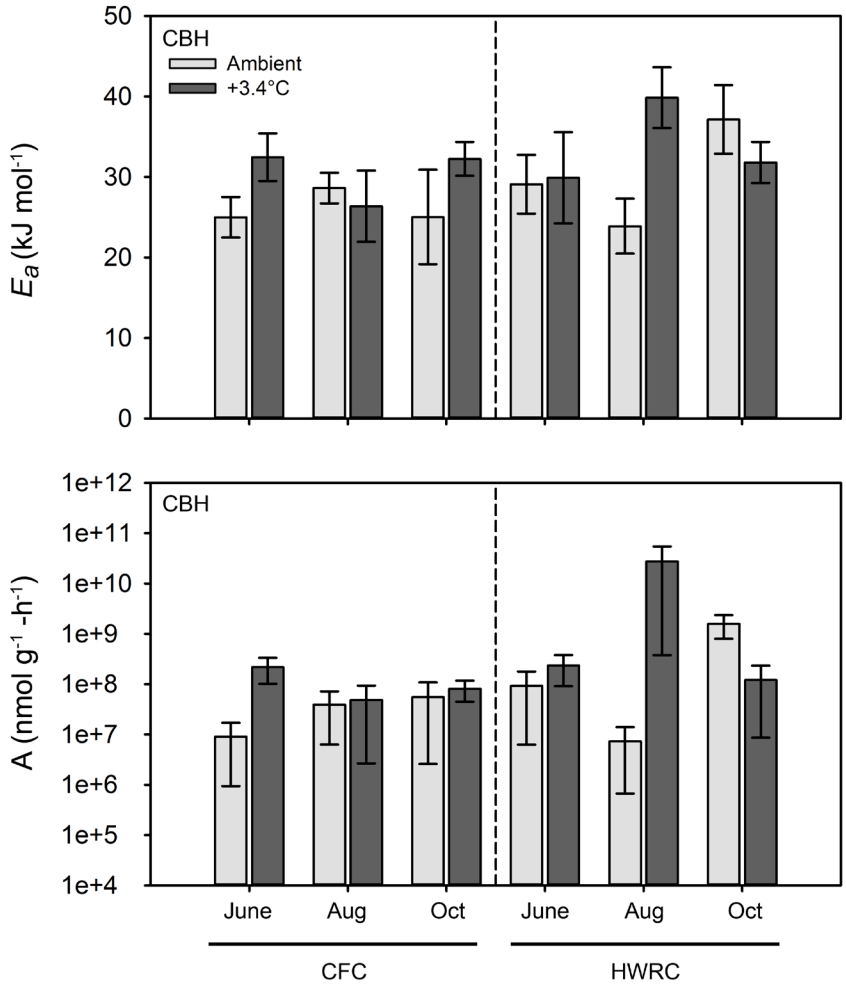

Figure 3-4. Warming effects on the Arrhenius fit of V_{max}, showing activation energy (E_a; above) and pre-exponential factor (A; below) by site and month for cellobiohydrolase. E_a is a measure of the temperature sensitivity, while A is a measure of the basal rate of the enzyme. Error bars are ± 1 SE

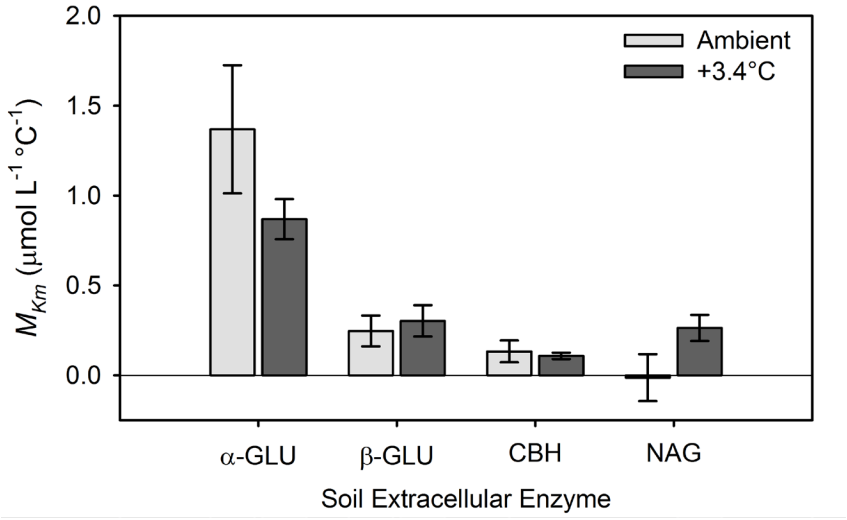

Figure 3-5. Warming effects on the temperature sensitivity of K_m. Error bars are ± 1 SE.

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Appendix A – *In situ* soil respiration measurements at B4warmED by site, date, and warming treatment

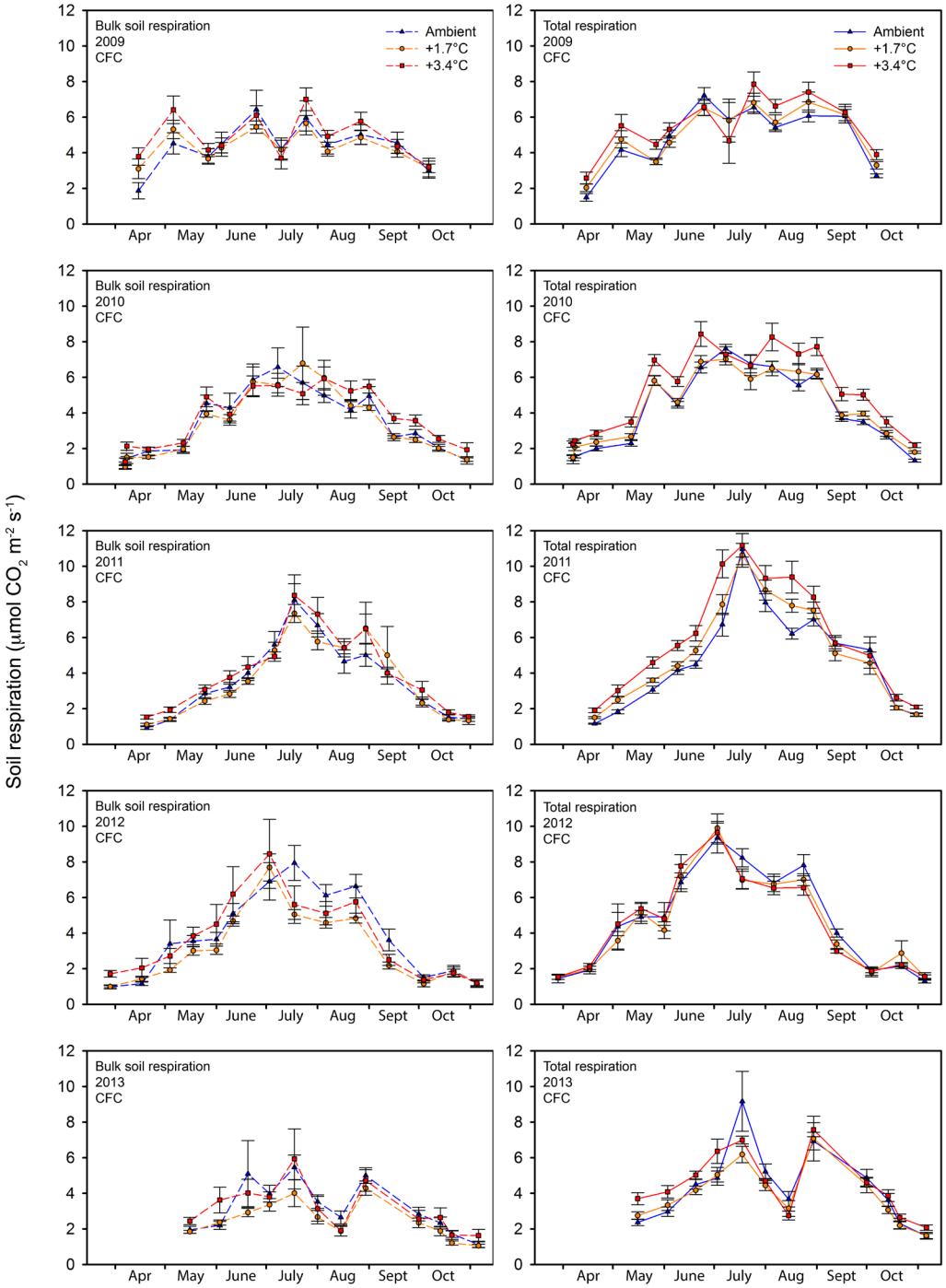

Figure A-1. Warming effects on bulk soil (left column) and total soil (right column) respiration at the southern site (Cloquet Forestry Center, CFC) shown by measurement day across the five years of experimental measurements (2009 - 2013).

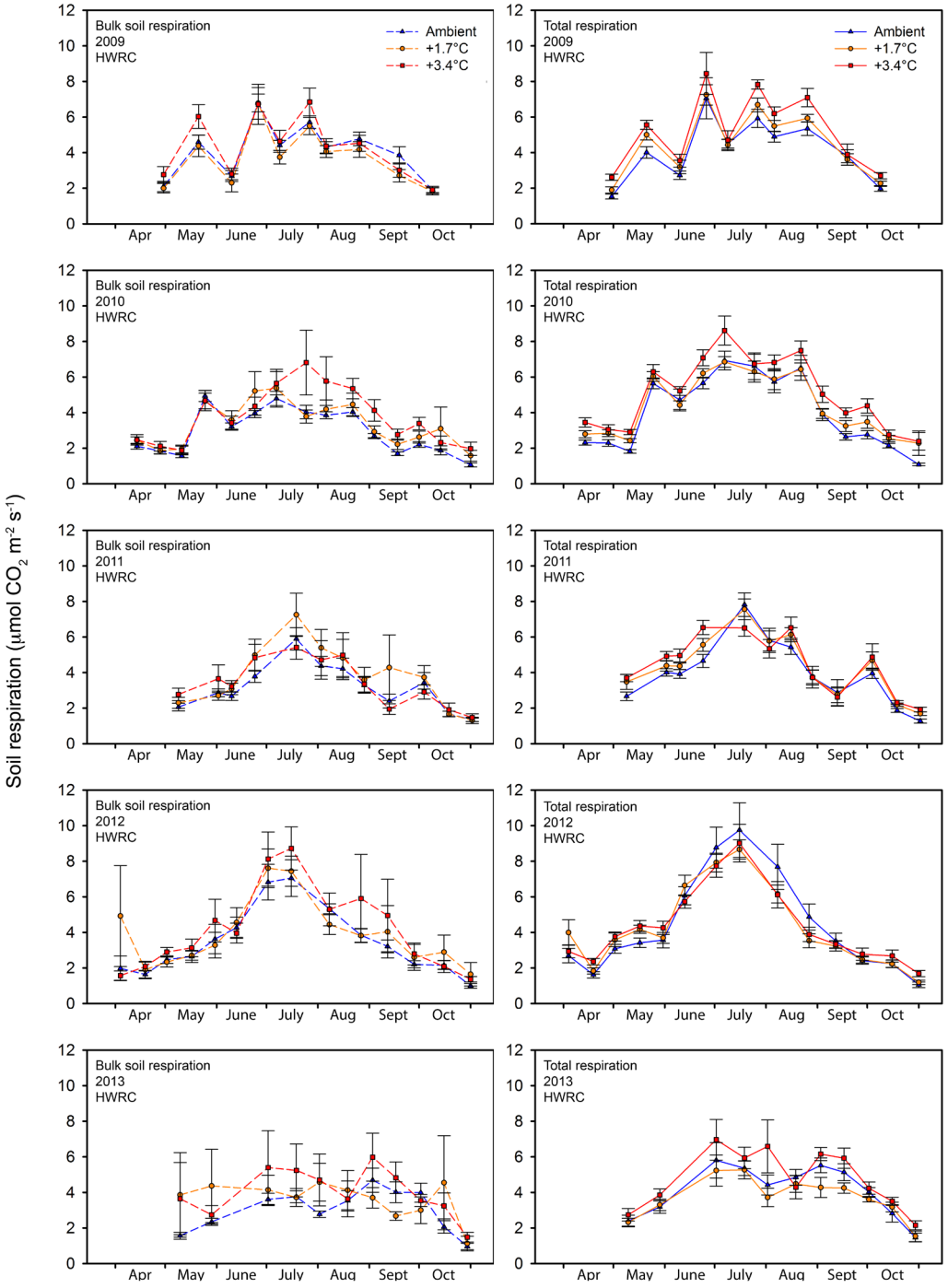

Figure A-2. Warming effects on bulk soil (left column) and total soil (right column) respiration at the southern site (Hubachek Wildnerness Research Center, HWRC) shown by measurement day across the five years of experimental measurements (2009 - 2013).

Appendix B – Actual by predicted plots for decomposition models.

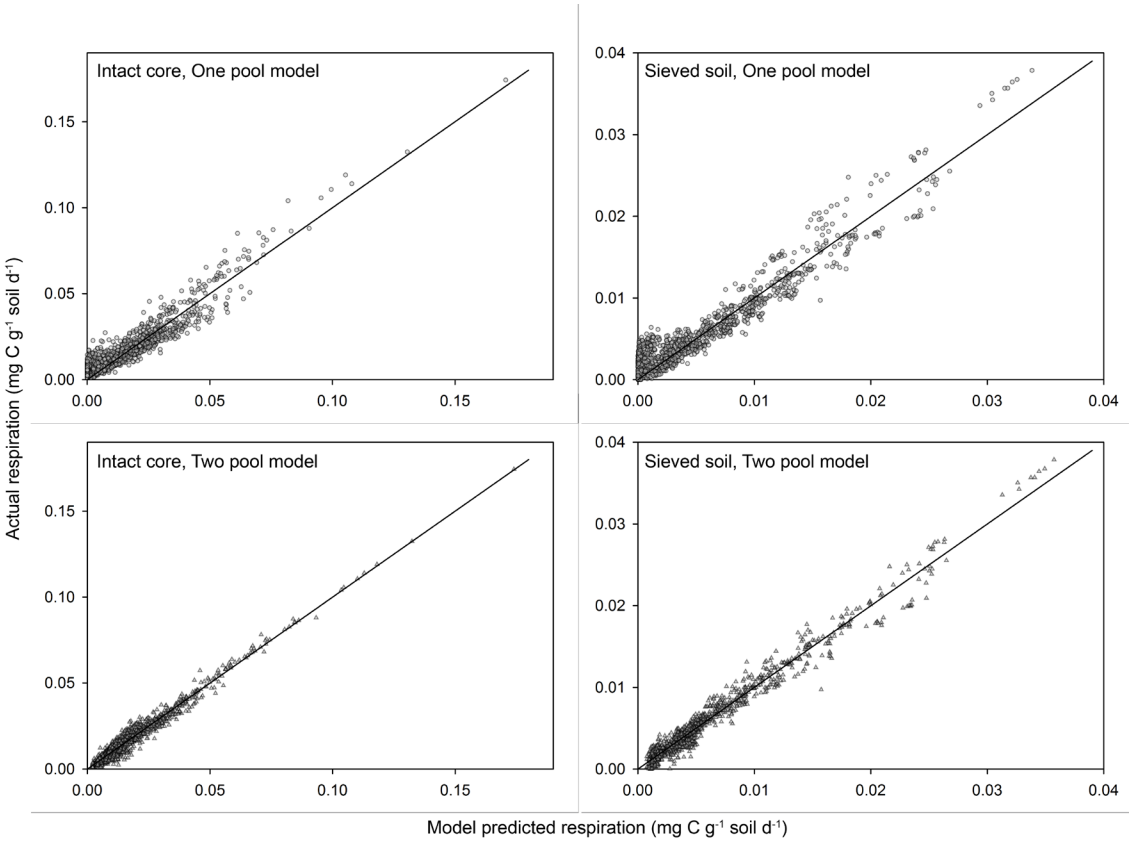

Figure B-1. Actual by predicted plots for decomposition models for one (top) and two pool (bottom) decomposition models for both incubations. Line is 1:1.

Appendix C – Equifinality of decomposition models

Equifinality occurs when multiple parameters sets produce equally acceptable representation of system behavior. We tested for equifinality in the model fits of two models of carbon decomposition. To do this, we generated at least 50,000 random parameter combinations for each model (see below) from a parameter space thought to contain all likely parameter values. R^2 was used to determine goodness-of-fit for each parameter set.

The two models are:

(1). One-pool decomposition model

$$C_{resp}(t)=k_f[C_f e^{-k_f t}]$$

(2). Two-pool decomposition model

$$C_{resp}(t)=k_f[C_f e^{-k_f t}] + k_s[(C_t - C_f)e^{-k_s t}]$$

where C_{resp} is the daily respiration rate (mg C g soil^{-1}day^{-1}), C_f is the fast-cycling C pool (mg C g soil^{-1}), C_t is total C (mg C g soil^{-1}), k_f is the decomposition rate of the fast-cycling (day^{-1}) and k_s is the decomposition rate of the slow-cycling pool (day^{-1}), and t is time in days.

For the sieved soil incubation, k_f was randomly chosen from the range 0 - 0.2 and k_s from the range 0 - 0.001. Random values were chosen for C_f from the range zero to an estimate of the cumulative respiration (calculated as the mean daily respiration for each incubation multiplied by 388-d). For the intact core incubation, estimated parameters occupied a larger space than for the sieved soil incubation, thus we narrowed the random parameters to range from 0.33x to 3x of the maximum likelihood estimated parameter

values.

Every microcosm in both the intact core (*n*-144) and sieved soil (n=96) incubations were tested for equifinality. The two figures in this appendix are examples of two such plots, one from each incubation (Figures C1 & C2). For these, we fit 1,000,000 random parameter combinations, and from a parameter space that allowed for the best graphical characterization of model goodness of fit in the parameter space. .

Several lines of evidence suggest that equifinality is not occurring in these models. First, maximum likelihood estimation (MLE) produced model fits that maximized the model R^2. In every case, the R^2 of the random parameter models approached, but never exceeded that of MLE estimation. Second, parameters for the ten best fitting randomly generated models always clustered around the parameters estimates from maximum likelihood. Finally, we identified only one parameter region where models fit best.

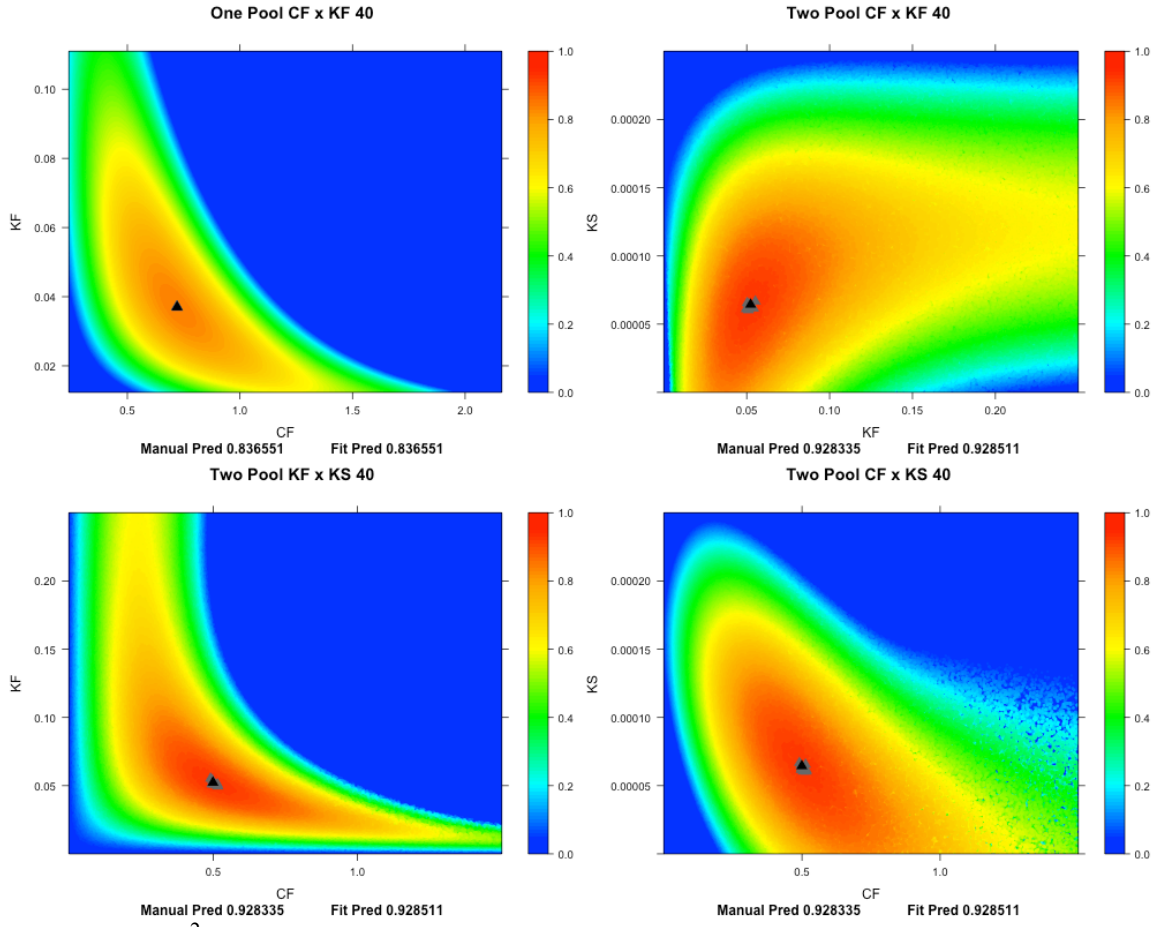

Figure C-1. R^2 obtained by randomly generated parameters for one- (top-left plot) and two-pool (remaining three plots) decomposition models for one intact core incubation (ID = 40) . R^2 is indicated by color scale. C_f is the fast-cycling C pool (mg C g soil^{-1}), C_t is total C (mg C g soil^{-1}), k_f is the decomposition rate of the fast-cycling (day^{-1}) and k_s is the decomposition rate of the slow-cycling pool (day^{-1}), and t is time in days. Two pool models require three parameters. Thus, X and Y axes for these plots show two out of three parameters, while best-fitting values at each point are used for the third parameter. "Manual Pred" is the best R^2 value generated from the fit of random parameter. "Fit Pred" is the MLE R^2 value. Black triangle is the location of parameters from MLE. Grey triangles represent the ten best fitting models in the randomly generated parameter space. These models cluster near around the MLE model, and for the one pool model (top-left) the black triangle representing the MLE model obscures the grey triangles from the randomly generated models.

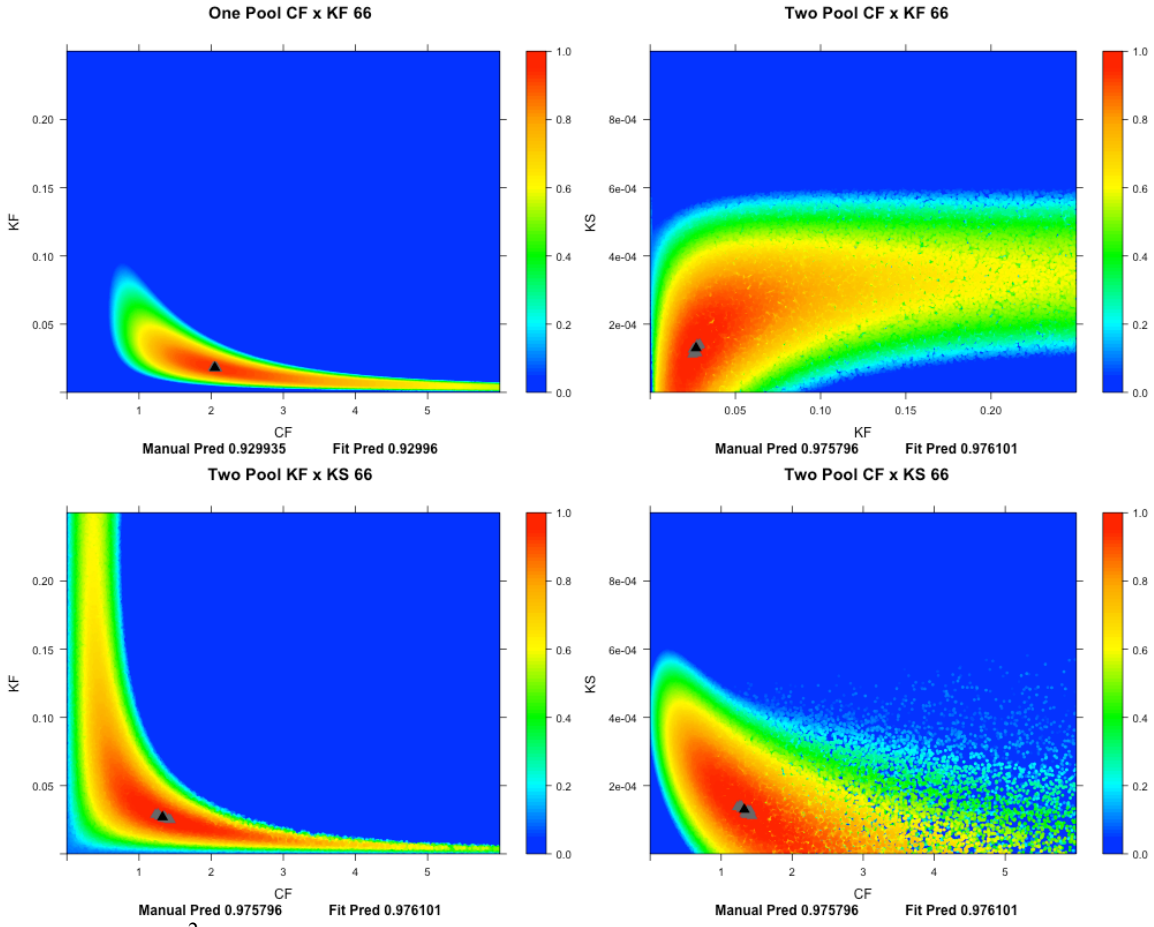

Figure C-2. R^2 obtained by randomly generated parameters for one- (top-left plot) and two-pool (remaining three plots) decomposition models for one sieved soil incubation . R^2 is indicated by color scale. C_f is the fast-cycling C pool (mg C g soil^{-1}), C_t is total C (mg C g soil^{-1}), k_f is the decomposition rate of the fast-cycling (day^{-1}) and k_s is the decomposition rate of the slow-cycling pool (day^{-1}), and t is time in days. Two pool models require three parameters. Thus, X and Y axes for these plots show two out of three parameters, while best-fitting values at each point are used for the third parameter. "Manual Pred" is the best R^2 value generated from the fit of random parameter sets. "Fit Pred" is the MLE R^2 value. Black triangle is the location of parameters from MLE. Grey triangles represent the ten best fitting models in the randomly generated parameter space. These models cluster near around the MLE model, and for the one pool model (top-left) the black triangle representing the MLE model obscures the grey triangles from the randomly generated models.

Appendix D – Choosing a temperature response model

We fit three models temperature response models to soil respiration data.

(1) A simple exponential (i.e. the van 't Hoff) equation (Lloyd and Taylor 1994, Fierer et al. 2005, Davidson et al. 2006, Fierer et al. 2006):

$$C_{resp} = Be^{kT}$$

where C_{resp} is the daily respiration rate (mg C g soil^{-1}day^{-1}), T is temperature in degrees Celsius, B is a fitted parameter describing the carbon substrate quality and bioavailability (Fierer et al. 2005) , and k is a fitted parameter describing the temperature sensitivity of the reaction.

(2) The Arrhenius function:

$$C_{resp} = \alpha e^{-E_a/RT}$$

where R is the universal gas constant, K is temperature in degrees Kelvin, α and E_a are fitted parameters representing a scaling factor and the activation energy, respectively.

(3) A log-polynomial equation:

$$C_{resp} = e^{(a+bT+cT^2)}$$

where a, b, and c are fitted parameters.

Because of their shared structure, the van 't Hoff and Arrhenius models fit the soil respiration data similarly. In comparing these two models only, AIC values that differed by more than three (indicating a significantly better fit for the model with lower AIC value) for 71 of 839 fits for the intact core incubation, and for 36 of 516 fits for the sieved

soil incubation. The van ‘t Hoff equation fit better, in that of the 109 fits where one model was significantly better, 89 favored the van ‘t Hoff equation.

The log-polynomial model has an additional fit parameter, and utilizes a polynomial within an exponent to gain additional freedom to vary beyond the scaled exponential models of the van ‘t Hoff and Arrhenius functions. For these reasons, when we consider all three models, the log-polynomial model was by far the best fitting model, having the lowest AIC values for 903 out of the 1355 fits, while the van ‘t Hoff had the lowest AIC values for 315/1355, and Arrhenius for 137/1355. For only 1 out of the 1355 was either the van ‘t Hoff or Arrhenius significantly better than the log-polynomial model.

Still, we chose to fit the van ‘t Hoff temperature response model for several reasons. First, while the log-polynomial model minimized AIC values, all models fit the data well. Acknowledging that poor fits ($R^2 < 0.60$) were excluded, the mean R^2 for van’t Hoff models were 0.960 and 0.944, for the intact core and sieved soil incubations, respectively, while for the log-polynomial models mean R^2 were 0.988 and 0.968. Next, the van ‘t Hoff model is relatively simple, and generates biologically meaningful parameters. The parameter *B* in the van ‘t Hoff fit model describes the basal respiration rate, related to carbon substrate quality and bioavailability (Fierer et al. 2005), while the parameter *k* describes the temperature sensitivity of the reaction. Moreover, this model has been widely used to describe the temperature sensitivity of soil respiration (e.g. (Fierer et al. 2005, 2006, Suseela et al. 2011) allowing comparison between multiple

studies. Finally, each model is a fit of respiration across only four temperature points. The log-polynomial model tends to overfit these data.

Appendix E – Michael-Menten kinetics and temperature response parameters for four soil extracellular enzymes

Data are shown on following two pages.

Table E-1. Mean Michaelis-Menten kinetics (V_{max} and K_m) by month, site and warming, for the four assay temperatures. V_{max} is in nmol g^{-1} h^{-1} and K_m is in μmol L^{-1}. Means are shown with ± 1 SE shown in parentheses.

Michaelis-Menten parameter			V_{max}				K_m			
Assay Temperature			*4°C*	*12°C*	*20°C*	*30°C*	*4°C*	*12°C*	*20°C*	*30°C*
Month	Site	Warming	α-glucosidase							
June	CFC	Control	7.7 (1.0)	12.4 (2.1)	16.2 (2.1)	25.8 (0.9)	2.7 (0.3)	4.6 (0.5)	10.7 (0.2)	19.0 (3.8)
		+3.4°C	10.9 (1.5)	21.4 (5.0)	23.9 (0.3)	33.0 (3.9)	6.9 (2.1)	19.6 (7.6)	23.9 (7.3)	27.4 (4.9)
	HWRC	Control	9.2 (1.0)	22.8 (2.5)	41.4 (15.9)	36.5 (2.3)	12.3 (2.8)	37.1 (12.2)	112.9 (86.9)	53.0 (21.6)
		+3.4°C	8.4 (1.3)	15.1 (0.5)	22.9 (3.7)	36.2 (7.8)	16.4 (11.4)	25.4 (7.7)	33.2 (8.4)	40.9 (5.4)
August	CFC	Control	10 (2.5)	16.3 (4.3)	21.1 (4.8)	51.4 (21.4)	8.2 (4.0)	11.7 (5.4)	18.1 (8)	67.8 (47.8)
		+3.4°C	8.9 (1.4)	14.3 (1.2)	18.2 (3.2)	23.9 (3.2)	5.9 (2.1)	10.6 (2.4)	14.0 (2.8)	23.2 (2.6)
	HWRC	Control	7.8 (0.8)	14.7 (1.9)	24.4 (6.3)	21.1 (3.2)	12.0 (2.5)	16.2 (3.1)	45.2 (14.4)	28.4 (0.7)
		+3.4°C	8.1 (1.4)	13.7 (0.8)	26.7 (7.4)	41.6 (13.5)	11.1 (3.7)	14.8 (3.2)	54.8 (31.2)	34.4 (15.2)
October	CFC	Control	11.5 (2.6)	15.2 (1.0)	19.5 (1.0)	32.6 (6.2)	9.2 (6.1)	7.8 (2.2)	12.4 (1.8)	27.0 (9.1)
		+3.4°C	9.0 (0.9)	14.7 (1.0)	23.0 (3.3)	27.6 (4.1)	4.7 (1.8)	8.4 (2.8)	17.8 (2.2)	23.5 (5.5)
	HWRC	Control	7.8 (1.3)	15.3 (3.4)	25.6 (4.9)	26.1 (8.0)	9.8 (1.5)	19.0 (3.9)	50.7 (15.4)	34.8 (17.1)
		+3.4°C	6.2 (2.1)	1.0 (2.0)	17.8 (3.8)	37.0 (10.2)	7.8 (3.8)	14.7 (4.2)	28.1 (9.7)	41.0 (9.5)
Month	Site	Warming	β-glucosidase							
June	CFC	Control	291 (33)	386 (48)	579 (74)	671 (77)	27.0 (3.0)	24.1 (2.5)	32.2 (0.8)	23.8 (2.7)
		+3.4°C	352 (13)	542 (9)	777 (61)	881 (21)	23.2 (4.1)	25.8 (1.4)	35.0 (5.4)	27.4 (1.1)
	HWRC	Control	344 (51)	459 (25)	743 (51)	789 (62)	28.0 (2.7)	25.2 (5.2)	35.2 (1.3)	28.7 (0.7)
		+3.4°C	366 (36)	540 (111)	758 (62)	1078 (328)	29.8 (2.0)	31.4 (5.6)	39.0 (3.4)	35.1 (6.8)
August	CFC	Control	273 (64)	509 (102)	591 (144)	937 (66)	19.7 (4.2)	28.2 (5.8)	24.3 (5.0)	26.8 (3.7)
		+3.4°C	259 (57)	430 (62)	567 (91)	689 (90)	15.9 (0.7)	21.9 (2.4)	26.5 (6.9)	23.7 (1.5)
	HWRC	Control	331 (22)	508 (28)	734 (118)	733 (30)	24.2 (1.4)	25.7 (4.2)	31.9 (2.6)	27.3 (1.5)
		+3.4°C	318 (46)	543 (45)	810 (147)	1255 (358)	22.7 (3.2)	27.7 (4.3)	33.4 (4.9)	28.9 (9.4)
October	CFC	Control	270 (7)	432 (38)	556 (47)	751 (79)	24.0 (1.2)	23.5 (2.4)	26.7 (2.0)	27.9 (2.5)
		+3.4°C	288 (58)	413 (50)	615 (87)	745 (75)	21.0 (3.8)	19.9 (1.0)	21.8 (1.5)	22.4 (1.7)
	HWRC	Control	356 (35)	516 (64)	761 (112)	749 (83)	29.3 (2.2)	31.8 (5.0)	43.5 (4.9)	33.0 (6.6)
		+3.4°C	218 (66)	368 (103)	587 (91)	979 (280)	22.1 (2.6)	23.1 (3.4)	31.8 (6.7)	25.6 (4.3)
Month	Site	Warming	Cellobiohydrolase							
June	CFC	Control	37 (8)	78 (15)	103 (18)	128 (28)	5.0 (1.0)	8.3 (1.6)	8.1 (0.9)	6.9 (0.7)
		+3.4°C	50 (3)	104 (10)	161 (8)	220 (23)	7.5 (0.7)	9.6 (0.3)	10.7 (0.9)	10.7 (0.6)
	HWRC	Control	56 (15)	94 (28)	129 (5)	180 (27)	10.4 (2.0)	11.0 (1.8)	12.3 (2.7)	12.0 (1.8)
		+3.4°C	41 (13)	104 (26)	148 (28)	196 (70)	7.4 (2.4)	17.8 (6.6)	13.9 (2.9)	11.1 (3.7)
August	CFC	Control	45 (6)	101 (15)	149 (32)	185 (39)	5.6 (0.7)	8.9 (1.4)	12.1 (2.7)	11.3 (2.6)
		+3.4°C	36 (8)	78 (9)	121 (25)	138 (37)	4.9 (1.4)	7.6 (1.6)	9.2 (1.9)	8.1 (1.8)
	HWRC	Control	45 (9)	93 (24)	122 (23)	138 (15)	8.4 (1.0)	11.0 (0.3)	9.4 (0.4)	9.1 (1.5)
		+3.4°C	49 (5)	98 (31)	168 (32)	373 (177)	9.0 (1.5)	10.1 (3.1)	12.4 (3.8)	11.3 (0.9)
October	CFC	Control	45 (10)	102 (15)	114 (8)	163 (6)	9.1 (3.8)	13.6 (7.0)	9.6 (0.1)	8.8 (0.6)
		+3.4°C	35 (10)	65 (10)	126 (22)	146 (21)	3.7 (1.5)	5.7 (1.5)	9.8 (1.2)	7.8 (1.5)
	HWRC	Control	43 (8)	71 (18)	137 (26)	186 (23)	8.2 (0.6)	9.8 (1.3)	12.6 (2.6)	20.7 (5.1)
		+3.4°C	28 (10)	51 (17)	87 (31)	114 (39)	7.1 (2.6)	6.3 (2.1)	7.6 (3.2)	7.9 (1.7)
Month	Site	Warming	*N*-acetyl glucosaminidase							
June	CFC	Control	84 (12)	149 (25)	186 (33)	213 (45)	29.0 (2.0)	37.3 (6.4)	38.9 (5.7)	29.6 (0.7)
		+3.4°C	120 (20)	171 (10)	372 (92)	356 (5)	65.7 (22.1)	41.5 (3.4)	119.8 (54.1)	50.8 (4.4)
	HWRC	Control	116 (2)	174 (13)	239 (20)	368 (60)	45.6 (2.3)	43.1 (1.6)	42.5 (2.0)	46.1 (4.7)
		+3.4°C	132 (16)	250 (37)	319 (47)	446 (28)	43.0 (3.9)	50.8 (5.4)	51.7 (11.4)	51.5 (1.2)
August	CFC	Control	173 (72)	290 (83)	381 (118)	427 (128)	39.2 (7.1)	59.4 (22.1)	55.9 (14.0)	47.1 (6.6)
		+3.4°C	70 (10)	141 (2)	198 (9)	230 (27)	31.1 (2.3)	49.7 (7.6)	50.3 (7.1)	38.0 (4.9)
	HWRC	Control	110 (23)	150 (30)	212 (38)	267 (62)	60.3 (12.0)	41.7 (5.4)	52.2 (6.8)	43.8 (2.8)
		+3.4°C	176 (35)	357 (69)	413 (63)	831 (282)	34.7 (5.8)	48.2 (13.3)	41.4 (10.1)	41.8 (10.3)
October	CFC	Control	97 (14)	182 (34)	237 (15)	238 (14)	38.0 (2.1)	42.5 (12.0)	43.0 (3.1)	40.0 (9.0)
		+3.4°C	84 (9)	142 (23)	193 (24)	273 (36)	38.8 (9.7)	35.1 (2.8)	36.8 (8.6)	49.4 (7.1)
	HWRC	Control	106 (23)	206 (58)	288 (57)	333 (68)	41.1 (7.8)	60.0 (4.7)	59.1 (18.1)	44.8 (7.1)
		+3.4°C	123 (18)	211 (27)	330 (51)	414 (54)	44.3 (10.2)	43.5 (8.7)	40.8 (8.1)	51.9 (14.1)

Table E-2. Mean temperature response parameter for (V_{max} and K_m) by month, site and warming. A is in nmol g^{-1} h^{-1}, E_a is in kJ mol^{-1}, C_{Km} is in µmol L^{-1}, and M_{Km} in µmol L^{-1} °C^{-1}. Means and ± 1 SE shown.

Michaelis-Menten parameter			V_{max}				K_m			
Temperature response parameter			LnA - Mean	LnA - SE	E_a - Mean	E_a - SE	C_{Km} - Mean	C_{Km} - SE	M_{Km} - Mean	M_{Km} - SE
Month	Site	Warming	α-glucosidase							
June	CFC	Control	16.17	1.72	32.58	4.44	-1.38	1.05	0.645	0.128
		+3.4°C	13.17	0.69	24.37	1.78	7.06	2.68	0.753	0.106
	HWRC	Control	16.94	3.21	32.66	7.25	18.18	5.91	1.070	0.764
		+3.4°C	16.19	1.18	32.03	2.59	14.39	10.47	0.843	0.203
August	CFC	Control	19.57	2.38	39.98	4.93	-10.22	12.01	2.221	1.644
		+3.4°C	12.42	1.31	23.32	3.02	2.74	2.93	0.647	0.148
	HWRC	Control	15.70	3.25	30.90	7.28	2.50	10.26	1.772	1.142
		+3.4°C	16.86	2.32	33.61	5.30	9.37	4.85	1.149	0.501
October	CFC	Control	15.29	3.04	29.86	7.25	2.65	4.02	0.694	0.225
		+3.4°C	13.63	0.93	25.93	1.99	1.06	0.78	0.760	0.126
	HWRC	Control	17.45	2.89	35.01	6.42	3.96	4.87	1.713	0.678
		+3.4°C	17.07	2.85	34.67	6.77	3.61	4.82	1.062	0.446
Month	Site	Warming	β-glucosidase							
June	CFC	Control	15.12	0.12	21.65	0.01	27.36	1.80	-0.036	0.110
		+3.4°C	15.47	0.27	21.78	0.65	24.02	4.12	0.231	0.146
	HWRC	Control	18.25	3.58	28.58	8.53	25.76	5.12	0.277	0.287
		+3.4°C	17.87	1.34	27.52	3.09	28.97	3.73	0.316	0.190
August	CFC	Control	19.46	2.06	31.81	5.43	21.41	6.03	0.202	0.170
		+3.4°C	15.52	0.47	22.61	1.19	16.88	2.19	0.309	0.065
	HWRC	Control	17.80	3.14	27.49	7.25	22.84	3.54	0.341	0.239
		+3.4°C	18.57	2.50	29.20	5.88	23.23	3.13	0.323	0.210
October	CFC	Control	16.44	1.16	24.74	2.70	22.70	1.64	0.171	0.048
		+3.4°C	16.22	0.90	24.15	2.32	20.06	2.38	0.073	0.058
	HWRC	Control	17.38	2.79	26.42	6.42	27.71	4.18	0.525	0.325
		+3.4°C	21.22	4.03	36.39	9.96	18.79	4.92	0.565	0.464
Month	Site	Warming	Cellobiohydrolase							
June	CFC	Control	14.76	1.18	25.01	2.51	6.35	0.74	0.048	0.045
		+3.4°C	18.30	1.27	32.47	2.96	7.61	0.64	0.122	0.034
	HWRC	Control	16.74	1.39	29.11	3.65	10.30	2.44	0.068	0.143
		+3.4°C	17.09	2.51	29.92	5.66	11.35	3.89	0.071	0.010
August	CFC	Control	16.59	0.94	28.64	1.89	5.69	0.61	0.228	0.081
		+3.4°C	15.40	1.96	26.37	4.42	5.40	1.19	0.124	0.021
	HWRC	Control	14.45	1.24	23.89	3.42	9.44	0.58	0.003	0.052
		+3.4°C	21.49	1.84	39.86	3.76	9.03	0.63	0.103	0.062
October	CFC	Control	14.99	2.40	25.04	5.87	10.98	5.52	-0.028	0.175
		+3.4°C	17.82	0.73	32.26	2.11	3.72	1.64	0.183	0.046
	HWRC	Control	19.98	1.60	37.15	4.26	4.95	1.37	0.478	0.189
		+3.4°C	17.20	1.23	31.81	2.56	6.45	2.76	0.047	0.048
Month	Site	Warming	N-acetyl glucosaminidase							
June	CFC	Control	13.12	1.17	19.53	2.54	33.46	0.82	0.014	0.088
		+3.4°C	16.63	1.24	26.86	3.30	65.16	20.42	0.259	0.190
	HWRC	Control	17.84	1.91	30.13	4.47	44.05	3.53	0.017	0.270
		+3.4°C	17.48	1.12	28.66	3.01	44.35	5.57	0.297	0.126
August	CFC	Control	14.24	0.89	20.75	2.19	47.12	12.35	0.199	0.395
		+3.4°C	15.42	1.14	25.04	2.68	38.91	0.86	0.206	0.159
	HWRC	Control	14.30	1.49	22.06	3.28	56.93	11.08	-0.450	0.499
		+3.4°C	21.20	2.55	36.77	5.76	38.96	9.57	0.156	0.329
October	CFC	Control	13.16	2.11	19.16	5.25	39.78	8.35	0.065	0.457
		+3.4°C	17.01	0.90	28.75	2.16	33.24	7.43	0.412	0.155
	HWRC	Control	15.64	0.36	24.73	1.19	50.00	3.73	0.075	0.215
		+3.4°C	17.78	2.55	29.53	6.26	40.97	8.76	0.253	0.195