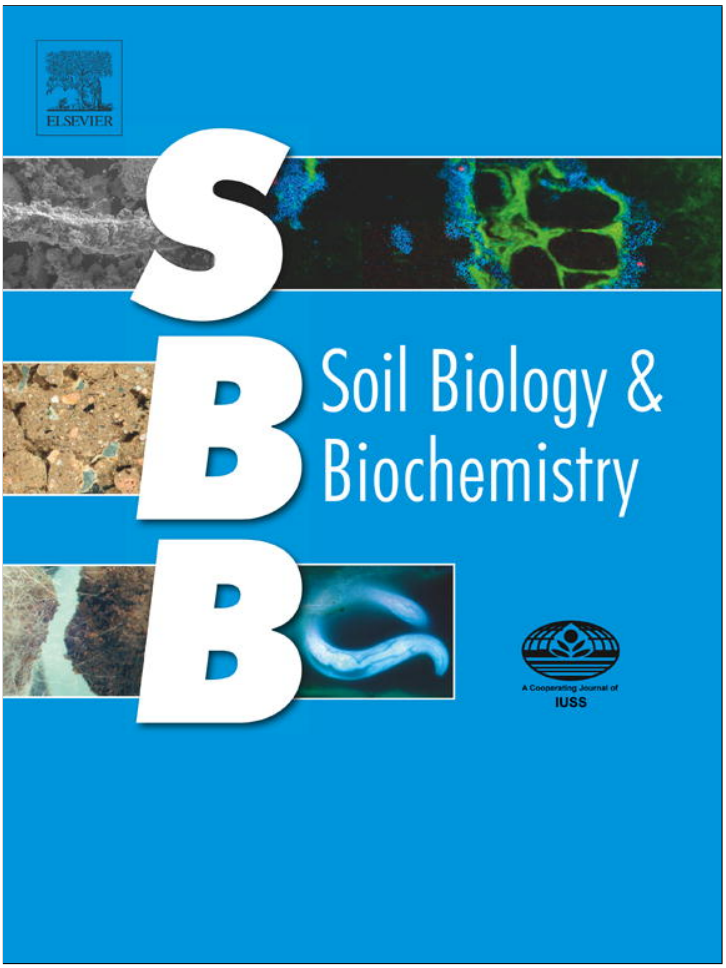


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# Native temperature regime influences soil response to simulated warming

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ABSTRACT

Anthropogenic climate change is expected to increase global temperatures and potentially increase soil carbon (C) mineralization, which could lead to a positive feedback between global warming and soil respiration. However the magnitude and spatial variability of belowground responses to warming are not yet fully understood. Some of the variability may depend on the native temperature regimes of soils. Soils from low temperature climates may release more C than will soils from high temperature climates because soils in cold climates are often C-rich and may experience more warming. We investigated whether soils from low native temperatures respired more than did soils from high native temperatures. We collected intact soil cores from three elevational transects along a latitudinal gradient in the forests of southern Appalachian Mountains. Soil cores were incubated for 292 days at low, medium, and high temperatures (separated by 3 °C each) with diurnal temperature and light regimes that simulated realistic temperature changes likely to occur within the next century. The native temperature regimes of soils negatively influenced soil respiration, such that soils from cold climates respired more in response to experimental warming than did soils from warm climates. Conversely, soils from warm climates mineralized the largest proportion of available soil C and available soil nitrogen in response to warming. Across all soils, modest experimental warming increased soil respiration, the proportion of available soil C that was being respired (respiration/soil C), and the proportion of soil nitrogen that was mineralized (N min/soil N). Taken together, these data suggest that soils from low native temperatures have a greater potential to release C in response to climate warming because the C stocks are larger and respiration rates will be higher than those in soils from high native temperatures.



## 1. Introduction

Forest ecosystems account for approximately half of the Earth's terrestrial surface and understanding their responses to increased global temperature will be vital toward predicting future climate change feedbacks (Dixon et al., 1994). The amount of carbon dioxide ($CO_2$) respired from soils is over 11 times larger than the amount of $CO_2$ released into the atmosphere via anthropogenic processes (Bader and Korner, 2010), and forests account for approximately 40% of global soil C (Dixon et al., 1994). Increasing global temperatures can induce greater soil respiration (Bond-Lamberty and Thomson, 2010), and the presence of a positive feedback between soil carbon (C) release and temperature remains unclear (Campbell et al., 2009; Bader and Korner, 2010). The spatial distribution of C stored in soils will also affect soil C mineralization since climate change is variable at regional scales (CCSP, 2007; Christensen et al., 2007). Our goal was to determine whether the temperature dependent responses of belowground processes are influenced by regional and local variation in the native temperature regimes.

Historical climate has influenced the size and quality of C pools in soils as the biological activity that drives C turnover is temperature dependent (Bottner et al., 2000; Pendall et al., 2004). Soil C pools and turnover rates are also a product of the quantity and quality of inputs from plant litter (Berg, 2000). Aboveground inputs to belowground systems are comprised of simple, easily-decomposed substrates (labile C) as well as complex, structural molecules that are not easily degraded (recalcitrant C), and theoretical studies indicate that these substrates should have different temperature sensitivities (Sierra, 2012). The difference between labile and recalcitrant C pools has important C cycling implications, as many models partition soil C into two or three pools, with varying turnover times ranging from years to millennia (Paustian et al., 1997; Falloon et al., 1998; Tague and Band, 2004; Zhang et al., 2007). In natural systems there is a continuum of soil C recalcitrance, and the proportion of recalcitrant C increases over the course of decomposition during the process of humus formation (Berg, 2000). Humus accumulation is often associated with

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cold climates, such that these ecosystems accumulate C in recalcitrant pools (Hobbie et al., 2000). Since increases in latitude/elevation generally decrease mean annual temperature (Komatsu et al., 2010), forests at high elevations/latitudes contain more stored soil C than do forests at low elevation/latitudes, and this can have important consequences for how soils respond to elevated temperatures (Dixon et al., 1994; Garten and Hanson, 2006; Griffiths et al., 2009).

How soils respond to increasing temperatures will be an important driver of potential carbon-climate feedbacks, but there has been a lack of consensus concerning the temperature sensitivity of soil C cycling. An early meta-analysis suggested that the $Q_{10}$ for soil organic matter decomposition, a change in reaction rate standardized to a 10 °C increase, was the greatest at low temperatures and decreased exponentially with increasing incubation temperature (Kirschbaum, 1995). However, others have also reported that decomposition of a variety of substrates is relatively insensitive to temperature (Katterer et al., 1998), time (Fang et al., 2005), or mean annual temperature (Giardina and Ryan, 2000). In addition, recent work indicates that the intrinsic temperature sensitivity of ecosystem level respiration is uniformly low across all mean annual temperatures (Mahecha et al., 2010). Some of the discrepancies among studies may be due to various experimental designs and protocols that influence soil disturbance, especially when soils are sieved and/or divided into fractions (Thomson et al., 2010). Kinetic theory suggests that temperature sensitivity should increase with soil C recalcitrance, which could limit studies that did not include different soil C pools (Davidson and Janssens, 2006; Sierra, 2012). The incubation time of soils in the laboratory can shift soil C toward recalcitrant stocks as labile C sources are catabolized, and the temperature sensitivity of heterotrophic respiration often increases with soil C recalcitrance (Conant et al., 2008; Hartley and Ineson, 2008). Finally, some mechanistic studies reveal important trends in belowground processes, but may be unrealistic because the magnitude of the experimental warming is often far beyond what soils are expected to experience based on climate change predictions for the next 100 years.

We investigated how variation in the native temperature regimes of soils influences the temperature sensitivity of belowground processes. We approximated field conditions by using relatively large intact microcosms, including the leaf litter layer, and simulating realistic temperature increases. We tracked soil microbial respiration, microbial extracellular enzymatic potential, litter decomposition, and N mineralization. We were able to address regional scale variation in native soil temperatures by collecting soil cores from a latitudinal and elevational range. Our goal was to determine whether rates of C and N cycling in soils from different native temperature regimes, with varying substrate qualities, responded differently to realistic global warming.

## 2. Methods

### 2.1. Site description

We sampled soils along three elevational transects in the southern Appalachian Mountains of North Carolina spanning a 135 km north–south range. Three sites were chosen along each transect, with differences in aspect minimized within each transect. The southernmost site was located at the Coweeta Hydrologic Laboratory in Otto, NC (USDA – USFS), and contained the greatest elevation range (~700 m, Table 1). The next largest range (~450 m Table 1) was located in Pisgah National Forest in Avery County, NC, and the final and northernmost site was on Appalachian State University's Gilley Field Station in Watauga County, NC, and contained the smallest elevation range (~ 200 m, Table 1). Soil texture and taxonomy were fairly consistent within sites but varied among sites (Table 1).

### 2.2. Field collection

PVC tubes 10.3 cm in diameter were inserted into the ground to a depth of 15 cm. Due to the brittle nature of the PVC cores, we necessarily avoided large roots or rocks. We allowed severed fine roots to remain within cores, as fine roots do not vary with elevation in our study region (Davis et al., 2004). Soil cores were carefully excavated from below in order to retain intact soil cores and capped at both ends for transport to the laboratory. Six cores were randomly excavated from each elevation at each site, for a total of 54 cores. Variation in soil characteristics at each site was unavoidable with intact soil cores, but allowed for a better approximation of *in situ* soil response to warming. We chose to use intact soil cores to avoid disturbing soil profiles, as sieving or homogenization can lead to large flushes of microbial activity by altering substrate availability (Thomson et al., 2010). We measured bulk density at three locations per site and monitored soil temperature at 10 cm deep using Hobo data loggers (Onset Computer Corp., Bourne, MA, USA). Soil temperature data were used to characterize the native temperature regimes of each collection site.

In order to standardize starting conditions for microcosm incubations, cores were stored at 4 °C for up to 14 days until all cores were collected. We standardized leaf litter mass among all cores to a mean value of approximately 0.7 g to normalize C inputs into soils during the incubation period. Litter type varied among sites, but all were from mature mixed deciduous stands. We tracked litter decomposition by measuring the initial and final litter mass remaining at the end of the incubation period.

### 2.3. Laboratory incubation

Three incubators (I-36LL, Percival Scientific Inc., Perry, IA, USA) were programmed with three different diurnal light and

**Table 1**
Soil collection site parameters.

| | Elevation (masl) | Coordinates | Soil texture[a] | Soil taxonomy[a] |
|---|---|---|---|---|
| Coweeta | 1381 | 35.0320°N, 83.4654°W | Fine-loamy | mesic Humic Dystrudepts |
| | 1189 | 35.0402°N, 83.4603°W | Fine-loamy | mesic Typic Hapludults |
| | 702 | 35.0563°N, 83.4324°W | Fine-loamy | mesic Typic Hapludults |
| Pisgah | 1146 | 35.9190°N, 81.8888°W | Coarse-loamy | mesic Lithic Dystrudepts |
| | 917 | 35.9180°N, 81.8956°W | Coarse-loamy | mesic Typic Dystrudepts |
| | 701 | 35.9141°N, 81.9016°W | Coarse-loamy | mesic Typic Dystrudepts |
| Gilley | 1025 | 36.2907°N, 81.5865°W | Fine-loamy | mesic Typic Hapludults |
| | 973 | 36.2909°N, 81.5844°W | Coarse-loamy | mesic Typic Dystrudepts |
| | 897 | 36.2914°N, 81.5828°W | Coarse-loamy | mesic Typic Dystrudepts |

[a] Information taken from USDA SSURGO database (Soil Survey Staff, 2010).

temperature regimes. The low temperature incubation treatment was programmed with light regimes that mimicked field conditions for day length from the National Oceanic and Atmospheric Administration (NOAA) and for temperature from soil data loggers at the Pisgah site (mid-latitude, average of elevations). We simulated moderate and high warming scenario for the next century (IPCC, 2007) by incrementally adding three degrees to the low temperature treatment. Two replicates of each site-elevation combination were randomly assigned to each temperature treatment; low, medium (med), and high. Soils were kept moist with DI-$H_2O$ evenly among all cores to approximately maintain field moist conditions. Soil moisture was monitored using a Hydrosense probe (Campbell Scientific, Inc. Logan, Utah, USA) with 10 cm long probes. Within incubators, microcosms were shifted by one row and column after each watering, and incubators were rotated monthly to avoid block effects. After six months (182 days) at the initial temperatures ("fall" temperatures: 10, 13 and 16 °C), incubators were warmed by 14 °C ("summer" temperatures: 24, 27 and 30 °C) for an additional four months, ten months total. Diurnal temperature and light cycles were also adjusted to summer conditions according to NOAA. A winter temperature incubation period was not simulated because portions of the upper 15 cm of soils are intermittently frozen for nearly two months mid-winter at our field sites. At three times, after 0, 124, and 304 days of incubation, subsamples of the upper 10 cm were collected with a 1 cm diameter soil corer. The corer was passed through the litter layer and the resulting leaf disks were separated from the soils for separate analyses. Soil and litter samples were freeze-dried and ground to a fine powder for C and N determination by flash combustion in a Flash EA 1112 NC analyzer (Thermo Fischer Scientific, Delft, The Netherlands).

### 2.4. Respiration and decomposition measurements

Soil $CO_2$ respiration was measured with a Li-8100 soil $CO_2$ flux system with a 10 cm chamber (LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken three times per week initially, and then incrementally reduced to once per month as microbial respiration in microcosms declines exponentially with time (Bradford et al., 2008). We resumed the initial measurement frequency after incubation temperatures were increased from fall to summer incubation treatments, but data presented here are the average of all observations in a given treatment. On days when respiration measurements were taken, cores were not watered until after the respiration was measured because wetting events can cause flushes of heterotrophic respiration in soils (Chatterjee and Jenerette, 2011). We analyzed respiration relative to soil %C (respiration/soil C) as soil C content varies among sites and respiration generally increases with soil C (Garten and Hanson, 2006).

### 2.5. Microbial enzyme activity potentials

We measured microbial extracellular enzyme activity (EEA) by colorimetric reactions based on Madritch et al. (2007). We assayed three enzymes, cellobiohydrolase (CB), β-glucosidase (BG), and leucine aminopeptidase (LA). These enzymes degrade cellulose (CB and BG) and amino acids (LA) and their activity can reflect microbial allocation to C and N acquisition, respectively (Allison et al., 2008). Briefly, we extracted ~1 g freeze dried soil samples in 15 mL of 5 mM acetate buffer pH 5, and duplicate aliquots of 400 μL of extract were given 100 μL of 5 mM 4-pNP-β-D-cellobioside for CB, 4 mM pNP-β-glucopyranoside for BG, 2.5 mM leucine p-nitroanilide for LA (see Madritch et al., 2007). We determined enzyme activity after two hours by fitting results to a p-nitrophenol standard curve. We conducted assays using final soil samples to determine whether the incubation temperatures affected EEA. We also measured N mineralization by comparing ammonium concentration of the soils in the initial and final soil samples using the sodium salicylate/sodium dichloroisocyanurate method as described in Madritch et al. (2007). We used estimates of N mineralization and soil N to indicate whether soils were N-limited or saturated, as more limited systems will tightly cycle available N (Bonito et al., 2003). We also analyzed the ratio of N mineralization to soil %N (N min/soil N) to account for heterogeneity in soil N among sites that may confound mineralization rates (Powers, 1990). N min/soil N can be used as an index of soil N availability and has been employed to compare N mineralization among sites with difference substrate qualities (Knoepp et al., 2008).

### 2.6. Statistical analysis

We calculated the Moran's I statistic using ArcGIS (v10. ESRI Redlands, CA, USA) to determine if response variables were spatially autocorrelated. To describe the initial variation among the soils and litter layers sampled, we performed simple linear regressions of initial characteristics with the native temperature of the origin site. We calculated the native temperature for all nine sites as the average temperature for the month of May at the end of the data logger record, as summer months have the greatest soil respiration rates annually (Garten and Hanson, 2006; Hart, 2006). We performed the same analyses with elevation because several important environmental variables other than temperature often co-vary with elevation. We analyzed response variables using an ANCOVA with native temperature and incubation treatment (low, med, high) as factors. To determine the effect of fall and summer incubation conditions on soil respiration, we included season in our ANCOVA model of respiration. All statistical analyses were performed in SAS JMP (v10.0.0 SAS Institute Inc., Cary, NC, USA), and relationships were considered significant at $p < 0.05$. We tested for interactions among dependent variables when verifying the assumption of homogeneity of regressions for the ANCOVA model (Sokal and Rohlf, 2001). For response variables that significantly varied with incubation temperature, we performed a Tukey test to compare amongst groups. We transformed data as necessary to meet the assumptions of normality. Untransformed data are presented in figures; however, the interpretations and conclusions are based on statistical results.

## 3. Results

Soil and leaf C and N concentrations declined with increasing native temperature regime, and C:N ratios were weakly related to soil's native temperature regime (Fig. 1). Soils varied widely in total C, probably due to variation in organic horizon depth both among sites and among cores collected at each site, and such variation is an important component of how soils from varying temperature regimes will respond to future warming. Soil and leaf C and N concentrations did not vary with elevation, with the exception of soil % N which increased slightly with elevation ($r^2 = 0.13$, $p = 0.01$, data not shown). Soil bulk density increased with the native temperature of the soils, which is likely associated with the low %C of the soils at high native temperature (Fig. 1d). In general, the native temperature regime explained more variation in belowground C and N than did the elevation of collection sites. Consequently, we omit elevation from in-depth analyses or discussion.

Response variables were not spatially autocorrelated (Table S1). Consequently, location was not included as a covariate in our model. All response variables satisfied the homogeneity of regression assumption of the ANCOVA model; none of the independent variables had significant interaction terms (Table S2). Soil respiration was greatest for low native temperature soils regardless of

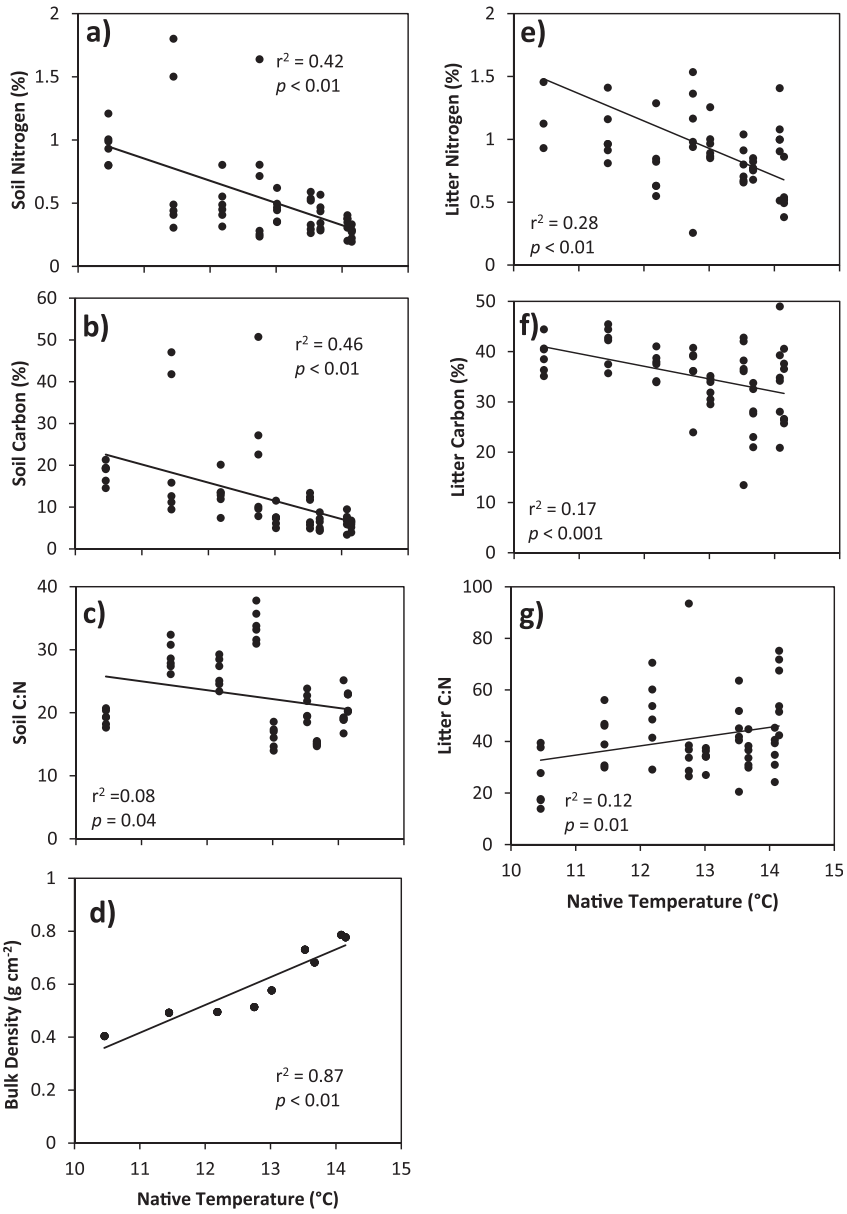


**Fig. 1.** Regressions of native temperature regime of the collection site with soil edaphic factors of nitrogen of upper 10 cm of soil (a), carbon of upper 10 cm of soil (b), soil C:N ratio (c), bulk density (d), litter nitrogen (e), litter carbon (f), and litter C:N ratio (g). Regression lines are shown if $p < 0.05$ ($n = 54$, except for panel d $n = 9$).

incubation treatment (Fig. 2a). In addition to higher basal respiration for low native temperatures, warming increased respiration across all native temperatures, and increased respiration was most apparent in the high temperature treatment (Table 2, Fig. 2a). Respiration/soil C was positively correlated with native temperature and accounting for variation in soil C among cores improved the model fit relative to raw respiration (Table 2, Fig. 2b).

Soil microbial processes that drive soil C turnover were also influenced by the native temperatures of the collection sites. Leaf litter, generally considered to be a fast turnover C pool, decomposed more quickly in microcosms taken from high native temperature sites than those taken from low native temperature sites (Fig. 3). Medium and high temperature incubations both accelerated leaf mass loss, but the amount of warming did not significantly affect the rate of litter decomposition (Table 2, Fig. 3). Soil N transformations likely interact with soil respiration, but absolute rates of N mineralization did not co-vary with native temperature or respond to warming treatments (Table 2). However, rates of N min/soil N, which can be used as an index of N availability (Knoepp et al., 2008), increased with the native temperature of the collection site (Fig. 4). N min/soil N increased with warming but was not sensitive to the degree of warming (med or high temperature), similar to the response of litter decomposition to warming.

We employed extracellular enzyme activity (EEA) potentials to complement our soil respiration measurements in order to better describe the functional response of the soil microbial communities. All three enzymes were negatively related to native temperature regime of the soil (Table 2, Fig. 5a–c). However EEA potentials did not respond to temperature treatments (Table 2).

## 4. Discussion

The variability of belowground responses to elevated temperatures will have important consequences on climate-induced feedbacks with the terrestrial C cycle. Soils from historically cold climates have slow C turnover and have accumulated more C than

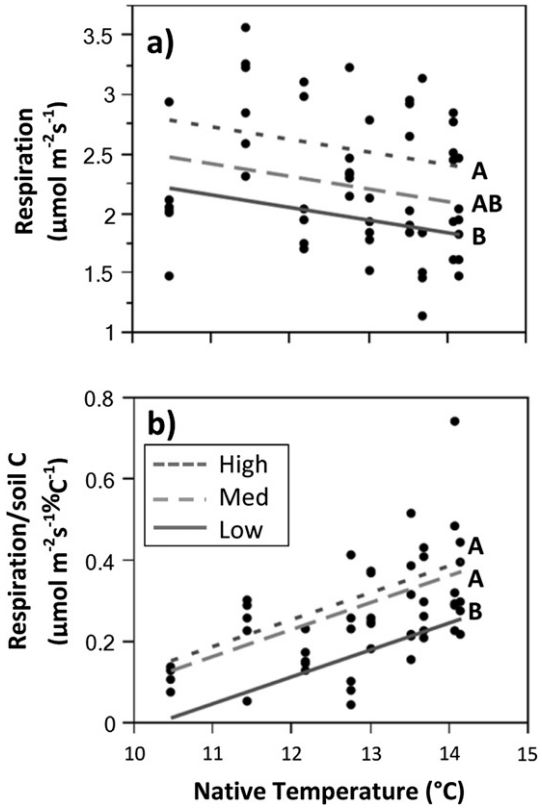


**Fig. 2.** Respiration (a) and respiration/soil C (b) (each point represents the average of 24 observations) varied with native temperature and incubation treatment (low, med, high). Regression lines are shown if $p < 0.05$ ($n = 54$) and upper-case letters indicate Tukey groupings of incubation treatments. See Table 2 for ANCOVA results.

have soils from warm climates (Hobbie et al., 2000). Our initial soil C results show a similar trend in soils from a relatively small, ~4 °C, native temperature gradient. Kinetic theory and empirical studies also predict that soils from cooler climates will increase decomposition rates more than will soils from warmer climates under projected warming (Davidson and Janssens, 2006). The combination of high C density and predicted high temperature sensitivity make soils from cooler climates a potential contributor to positive feedbacks between global warming and soil respiration.

We found that soils from low native temperature soils responded differently than did soils from high native temperatures under moderate warming, such that soil respiration was highest in soils collected from low native temperatures and respiration increased with experimental warming regardless of season. This does not necessarily indicate that recalcitrant C is more or less sensitive to warming as has been previously shown (Knorr et al., 2005; Davidson and Janssens, 2006; Bonan, 2008; Conant et al., 2008; Hartley and Ineson, 2008), but it may indicate that historic native temperature regimes may have more influence on C cycling than does the degree of warming. Because of differences in soil organic horizon depth, soils from cool native temperatures had large organic horizons, as indicated by very high soil %C (Fig. 1b). Our incubations likely missed some of the mineralization of recalcitrant C stocks stored in the mineral horizons because the depth of the organic horizon varied across collection sites. Nonetheless, it is likely that recalcitrant C stored in humified organic layers was at least partially responsible for the higher respiration rates of the low native temperature soils in our study, as temperature sensitivity should increase with soil C recalcitrance (Davidson and Janssens, 2006; Sierra, 2012). For instance, Li et al. (2012) demonstrated that soils from a cooler site respired a greater proportion of $CO_2$ from recalcitrant stores than did soils from the warmer site. They also showed that the switch to more recalcitrant substrates is more pronounced with warming (Li et al. 2012). In addition, litter decomposition, a relatively labile C source, increased with the native temperature of soils, suggesting that labile C pools were not responsible for the overall negative relationship found between soil respiration and native temperature regime. Given the extended duration of our incubation, the increased respiration that accompanied experimental warming was likely derived, in part, from recalcitrant C stocks.

Respiration/soil C is an important metric that that describes the proportion of soil C mineralized, and can normalize respiration because respiration rates increase with C supply (Garten and Hanson, 2006). Respiration/soil C increased with native temperature of soils (Fig. 2) as well as incubation temperature (Table 2) due, at least in part, to recalcitrant C mineralization because the duration of our incubation allowed us to deplete more labile substrates over time. As high temperature soils increasingly catabolize relatively limited C stores, detrital communities may become more C-limited with increased warming, while soils from low temperature sites may not be C-limited. In addition, soil C cycling rates may not increase linearly with warming, as the medium and high incubations were not significantly different from each other. While it appears that recalcitrant C is sensitive to warming, and that soils from warmer climates may respire a larger proportion of recalcitrant C in the short term, the larger recalcitrant C stores in soils from cooler climates will likely sustain high respiration rates and have a large influence on long-term soil C flux.

Several previous laboratory studies conducted with soils from different environmental gradients have not found an effect of soil origin (Niklinska and Klimek, 2007; Schindlbacher et al., 2010) or native temperature regime (Giardina and Ryan, 2000) on decomposition or respiration to artificial warming. Discrepancies among studies may be due to different methodological approaches. For instance, past work has generally focused on relatively short incubation periods of a few days or weeks (Niklinska and Klimek, 2007; Schindlbacher et al., 2010). In addition, previous studies

**Table 2**
Results of analysis of covariance (ANCOVA) of soil responses to native temperature, incubation treatment (low, med, high), and season (fall or summer, for respiration only).

| | Whole model | | | Native temperature | | | Incubation treatment | | | Season | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | *F* ratio | *p*-value | df | *F* ratio | *p*-value | df | *F* ratio | *p*-value | df | *F* ratio | *p*-value |
| Respiration | 4 | **3.74** | 0.01 | 1 | **6.82** | 0.01 | 2 | **3.79** | 0.03 | 1 | 0.53 | 0.47 |
| Respiration/soil C | 4 | **32.68** | <0.01 | 1 | **81.75** | <0.01 | 2 | **24.33** | <0.01 | 1 | 0.30 | 0.59 |
| Litter decomposition | 3 | **12.14** | <0.01 | 1 | **9.12** | <0.01 | 2 | **13.65** | <0.01 | | – | – |
| N mineralization | 3 | 0.53 | 0.66 | 1 | 0.09 | 0.77 | 2 | 0.76 | 0.47 | | – | – |
| N min/soil N | 3 | **8.88** | <0.01 | 1 | **16.26** | <0.01 | 2 | **5.20** | <0.01 | | – | – |
| CB activity | 3 | **25.96** | <0.01 | 1 | **76.56** | <0.01 | 2 | 2.76 | 0.07 | | – | – |
| BG activity | 3 | **20.35** | <0.01 | 1 | **59.66** | <0.01 | 2 | 2.35 | 0.10 | | – | – |
| LA activity | 3 | **13.67** | <0.01 | 1 | **40.64** | <0.01 | 2 | 1.33 | 0.27 | | – | – |

Bold *F*-ratios represent significant effects at $\alpha = 0.05$ level. Carbon (C), Nitrogen (N), Mineralization (min), Cellobiohydrolase (CB), β-glucosidase (BG), Leucine aminopeptidase (LA).

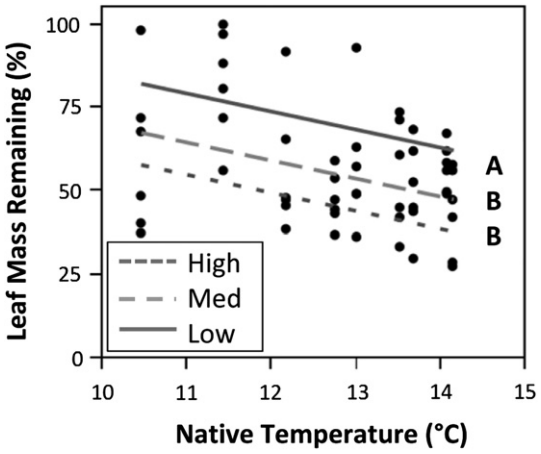


**Fig. 3.** Leaf mass remaining (% initial weight) varied with native temperature and incubation treatment (low, med, high). Regression lines are shown if $p < 0.05$ ($n = 54$) for native temperature, and upper-case letters indicate Tukey groupings of incubation treatments. See Table 2 for ANCOVA results.

have typically used soils that were sieved and separated which alters substrate availability by disrupting water filled pore space, soil aggregates, and bulk density (Hartley et al., 2007). Although the potential variation between samples is increased by employing intact microcosms, incubating intact soil profiles for long periods of time may provide a more realistic response of heterotrophic soil respiration to artificial warming than would well-mixed soils that are incubated for short periods of time.

Terrestrial C flux to the atmosphere may also depend on C sequestration into above- and belowground-biomass that is influenced by N availability (Bonan, 2008). N mineralization rates were not influenced by either the native temperature regime of the soil or our moderate temperature increases. However, similar to respiration/soil C, and in agreement with Powers (1990) and Knoepp et al. (2008), N min/soil N rates did increase with the soil's native temperature regime. At high native temperatures, high respiration/soil C and rapid litter mass loss are both indicators of fast nutrient-cycling soils that support high microbial biomass (Allison et al., 2007; Sinsabaugh et al., 2008). High N min/soil N, at high native temperature sites may also indicate high N availability and thereby enable C sequestration into plant biomass. Tree growth and physiology at low native temperature sites are primarily limited by lower temperatures and the length of growing season (Bresson

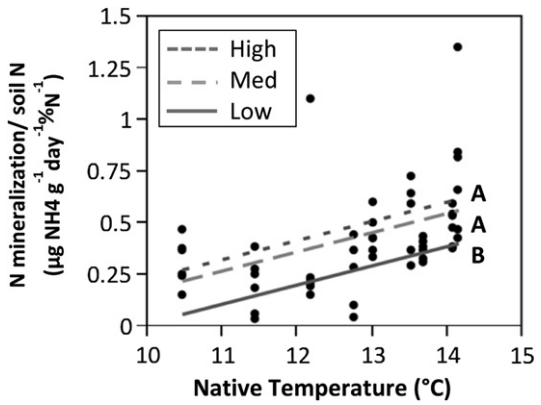


**Fig. 4.** Nitrogen mineralization/soil %N (N min/soil N) varied with native temperature and incubation treatment (low, med, high). Regression lines are shown if $p < 0.05$ ($n = 54$) for native temperature, and upper-case letters indicate Tukey groupings of incubation treatments. See Table 2 for ANCOVA results.

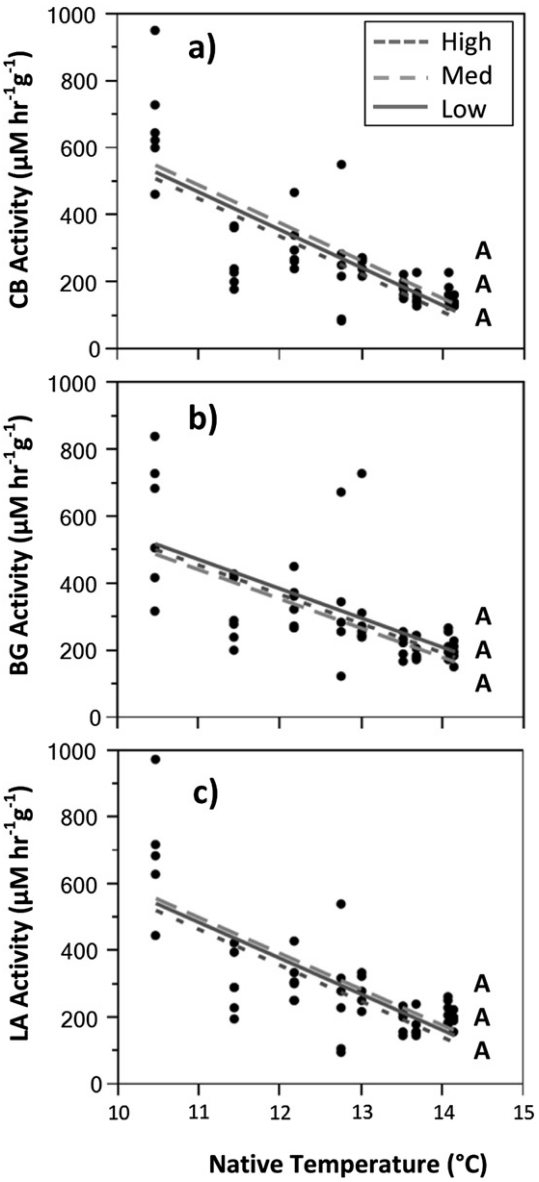


**Fig. 5.** Cellobiohydrolase (CB) activity (a), β-glucosidase (BG) activity (b), and leucine aminopeptidase (LA) activity (c) varied with native temperature, but not incubation treatment (low, med, high). Regression lines are shown if $p < 0.05$ ($n = 54$) for native temperature, and upper-case letters indicate Tukey groupings of incubation treatments. See Table 2 for ANCOVA results.

et al., 2011), which are predicted to increase under warming scenarios (Campbell et al., 2009). Since we did not incorporate plant uptake into our experimental design, it is difficult to determine whether the increased C loss from soils in cold climates would be offset by increased primary production afforded by increased nitrogen availability in the soil. Nonetheless, we have conflicting results indicating that colder soils have more total N present than do warmer soils, but proportionately less nitrogen may be available in colder soils than in warmer soils as N min/soil N declines with the native temperature. Multi-factor experiments that incorporate soil–plant interactions with warming may be able to resolve whether or not plant growth could mitigate soil C loss to the atmosphere.

The quality of substrates and the availability of limiting nutrients along the native temperature gradient likely influenced potential microbial extracellular enzyme activities (EEA). High potential EEA

for low native temperature soils is consistent with the resource allocation theory, where enzyme production is up-regulated when additional nutrients, represented by high soil C and N, are readily available (Allison and Vitousek, 2005). Respiration rates and soil EEA showed similar responses to native temperature regimes because enzyme activity is directly linked to microbial C acquisition and subsequent mineralization (Allison and Vitousek, 2005). However, microbial respiration increased with warming whereas potential EEA did not, and the disconnect may be associated with different microbial biomass or community composition (Bradford et al., 2008). In addition, our respiration measurements were of actual respiration, whereas EEA measurements may not directly reflect *in situ* activity, but rather potential activity (German et al., 2011). Increased respiration/soil C with warming may indicate that microbes are less efficient at incorporating substrate C into biomass at higher temperatures, as suggested by previous studies that demonstrate carbon use efficiency is lower at higher temperatures regardless of substrate quality or incubation duration (Steinweg et al., 2008; Wallenstein et al., 2011). In alpine soils, the temperature-dependency of EEA varies with season (Koch et al., 2007), and our analyses did not capture intra-annual dynamics. Although linkages between potential EEA and soil C cycling are difficult to interpret, our data suggest that low native temperature regimes increase the potential for high extracellular enzyme activity.

At regional scales, historical temperature regimes may determine the magnitude of temperature-induced soil microbial responses to climate change. Carbon stores were catabolized more in low native temperature soils than in high native temperature soils in response to simulated warming. Temperature-induced changes in N min/soil N may mitigate some increased heterotrophic respiration by releasing plants and microbes from N-limitation. However, it is difficult to determine if stimulated growth from increased N availability would occur in soils from low native temperatures because of higher amounts of soil N concurrent with lower rates of N min/soil N. High soil respiration rates combined with large recalcitrant C stores make historically colder soils a large potential source of C release to the atmosphere, which could contribute to positive feedbacks in response to atmospheric warming.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.01.014.

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