ABSTRACT

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Effects of temperature, fertilization, and carbon supply on mass-specific respiration of soil microbes
(Under the Direction of MARK A. BRADFORD)

Heterotrophic soil microorganisms decompose soil organic carbon and release carbon dioxide to the atmosphere. Although microbial respiration rates increase in response to short-term temperature increases, effects of long-term temperature increases remain unclear. Due to mechanisms such as evolutionary trade-offs in enzyme function and shifting community structure, mass-specific respiration (R_{mass}) rates are expected to decrease as communities adapt to higher temperatures. To test this potential, we used a laboratory microcosm approach to impose two thermal regimes (constant 12°C or 28°C) for 84 days on 12 soil samples treated with nitrogen and/or phosphorus. Carbon was added weekly as glucose to one replicate of each soil to account for possible substrate limitation. Carbon dioxide produced by each sample was measured using assay methods similar to those used in animal, plant, and microbial thermal adaptation studies. At intermediate assay temperatures, R_{mass} rates were expected to be greatest for 12°C experimentally incubated soils and lowest for 28°C soils, indicating thermal adaptation. Nitrogen and phosphorus additions were not expected to significantly affect respiration rates. Interestingly, although R_{mass} rates for soils without carbon additions followed predictions for microbial adaptation, those for glucose-treated soils did not, suggesting that substrate limitation and biomass differences contributed to decreased potential respiration rates of warmer soils. Nitrogen and phosphorus additions were significant, indicating that nutrient levels are important in determining microbe response to temperature regimes. This research is necessary to more fully understand microbial respiration responses to changing temperatures and varying nutrient additions and to more accurately predict possible feedbacks between microbial respiration and climate change.

INDEX WORDS: Adaptation, Fertilization, Microbial Community, Nitrogen, Phosphorus, Soil Respiration, Substrate Limitation, Temperature

EFFECTS OF TEMPERATURE, FERTILIZATION, AND CARBON SUPPLY ON MASS-SPECIFIC RESPIRATION OF SOIL MICROBES

by

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CHAPTER 1
INTRODUCTION

Heterotrophic soil microorganisms decompose stores of soil organic carbon and either assimilate the carbon into biomass or release carbon dioxide into the atmosphere through respiration. Soil respiration rates can be affected by multiple mechanisms, including biomass quantities, carbon supplies, or changes in mass-specific respiration rates. Although in the short-term soil respiration rates increase in response to increasing temperatures (Kirshbaum 2006), the effect of long-term temperature increases on respiration rates remains uncertain (Denman *et al.* 2007). A relatively brief increase in soil respiration rates in response to prolonged exposure to warmer temperatures has been observed in field experiments (Jarvis & Linder 2000, Oechel *et al*. 2000; Luo *et al.* 2001; Rustad *et al.* 2001; Melillo *et al.* 2002; Eliasson *et al.* 2005). This can be explained by substrate depletion, in which fast-cycling soil organic carbon pools are quickly consumed, or by thermal adaptation to increased temperatures. Although substrate depletion has often been used to explain the decrease in soil respiration after a short period of increased respiration, the thermal adaptation response is consistent with evolutionary trade-offs in enzyme function and shifting community structure. For example, cold-adapted enzymes have greater conformational flexibility, and thus greater catalytic rates, than do warm-adapted enzymes. If the activity of cold-adapted enzymes and warm-adapted enzymes are compared at an intermediate temperature, the catalytic rates of the cold-adapted enzymes will be greater than those of the warm-adapted enzymes (Hochachka & Somero 2002). Based on this trade-off, it is expected that mass-specific respiration (R_{mass}) rates for cold-adapted soil microorganisms will be

greater than R_{mass} rates for warm-adapted organisms when compared at an intermediate temperature.

Nutrient levels in soils may also affect R_{mass} rates. Generally, soil microorganisms face harsh competition for available nitrogen resources, although some field warming studies have shown that soil nitrogen mineralization rates increase in response to warming, producing an indirect fertilization effect (Rustad *et al.* 2001; Melillo *et al.* 2002). If there is not enough available nitrogen to support microbial growth, soil respiration may decrease in response to lower soil microorganism biomass (Brady and Weil 2000). This relationship seems to indicate that nitrogen additions to soil will increase heterotrophic soil respiration, and this has been shown in some experiments (Gallardo and Schlesinger 1994). However, many studies on the effects of added nitrogen have found that soil respiration is often depressed by nitrogen fertilization (Amador and Jones 1993; Bowden *et al.* 2004; Jin and Zhou 2008; Mo *et al.* 2008; Wilson and Al-Kaisi 2008), while other experiments have shown that soil respiration rates are not significantly altered by increased amounts of nitrogen in the soil (Keith *et al.* 1997; Illeris *et al.* 2003; Allison *et al.* 2008; Kim 2008). Data on the effects of phosphorus additions on soil respiration are similarly inconclusive. While the addition of phosphorus increases soil respiration and/or biomass in some cases (Amador and Jones 1993; Gallardo and Schlesinger 1994; Cleveland *et al.* 2002), other experiments have shown that increases in phosphorus content contribute to reduced soil respiration rates or have no effect on the amount of carbon dioxide released to the atmosphere (Keith *et al.* 1997; Kim 2008). This lack of a clear soil respiration response to nutrient additions shows that other variables, such as seasonal variations, soil type, plant cover, temperature, and moisture heavily influence microbial responses to additions of

nitrogen and phosphorus (Gallardo and Schlesinger 1994; Keith *et al.* 1997, Illeris and Jonasson 1999; Illeris *et al.* 2003; Lee and Jose 2003). It is also possible that background levels of soil nutrients affect how soil microbes respond to fertilization; that is, microbes in nitrogen-poor soils are more likely to be responsive to nitrogen additions than microbes in soils with a higher nitrogen concentration.

It is important to continue to research factors that affect soil respiration because of the relationship between terrestrial ecosystem emissions and climate change. The most recent climate change projections released by the Intergovernmental Panel on Climate Change (IPCC) take into account a positive feedback loop between warming soils and an increased concentration of atmospheric carbon dioxide (Denman *et al.* 2007). However, these models fail to address the possibility that heterotrophic soil microorganisms, which consume carbon-based substrates and release carbon dioxide into the atmosphere, may adapt to long-term temperature increases and respire less than the models suggest. Additionally, agricultural fertilizers, as well as natural nutrient levels in soils, may have an effect on heterotrophic soil microorganism respiration responses to temperature differences. In order to more fully understand the effects of nutrient levels and varying temperature regimes on soil respiration, it is necessary to test respiratory responses to these variables.

The objectives of this research are two-fold. First, by imposing 84 day temperature regimes of 12°C and 28°C on soils, microbial respiration responses to the two temperatures can be measured and analyzed. Second, by using soils that were previously part of a nitrogen-phosphorus factorial experiment, the effect of soil nitrogen and phosphorus levels on respiration can be examined. It is expected that soils incubated at 28°C will have lower mass-specific

respiration (R_{mass}) rates than soils incubated at 12°C because of thermal adaptation to a warmer temperature regime. Since there is little conclusive knowledge about the effects of fertilization on heterotrophic soil respiration, the addition of nitrogen and/or phosphorus is not expected to significantly affect R_{mass} rates.

These hypotheses were tested by incubating twelve soils at 12°C or 28°C for 84 days with weekly additions of water or a glucose solution. Based on previous laboratory experiments, the 84 day incubation is classified as a medium-term incubation, as opposed to a short-term incubation of approximately 10 days or a long-term incubation of 500 to 600 days. At the end of the incubation period, assays were performed to determine potential soil respiration (Soil *R*) rates, soil respiration rates with the alleviation of substrate limitation (Substrate *R*), and the biomass present in each soil sample in order to separate mechanisms that could affect R_{mass} rates. R_{mass} data were compared to examine soil respiration responses to different temperature regimes and nutrient availabilities.

CHAPTER 2
MATERIALS AND METHODS

Study Soils

Soils used in this experiment were collected from an old field, multiple species herbaceous community grassland located in the southern Appalachian Mountains of North Carolina at the U.S.D.A. Forest Service's Coweeta Hydrological Laboratory. A previous factorial nitrogen-phosphorus experiment had divided the sample site into experimental blocks composed of four plots each. In each block, one plot was left untreated while one was fertilized with nitrogen, one with phosphorus, and one with nitrogen and phosphorus. Twelve surface mineral soil samples were obtained by taking samples from the top 10 cm of each of the four plots in three experimental blocks. After passing through a 2 mm sieve to remove roots and stones, soils were transported on ice to the University of Georgia. They were placed in sealed plastic bags and stored in a refrigerator at 5°C prior to this experiment.

Experimental Incubations

In the laboratory, 30 g dry weight of each of the twelve soils were measured into four pre-labeled and pre-weighed 50 mL centrifuge tubes, resulting in a total of 48 tubes (i.e. 12 soils × 4 tubes per soil). The waterholding capacity (WHC) of each soil was determined by thoroughly saturating a small sub-sample of soil in a Whatmann #1 filter paper placed in a glass funnel. Water was allowed to drain through the soil and out of the funnel for two hours before determining the gravimetric soil moisture content at 100% WHC by collecting the sub-samples and oven-drying them at 105°C for 24 hours. Using the gravimetric soil moisture content data, it

was possible to calculate the moisture content of each soil. De-ionized water was added to each tube to standardize the moisture content of all samples to 50% WHC. Two uncapped tubes of each soil were placed in incubators at 12°C and 28°C. A plastic bag was wrapped around each tube rack to help to retain moisture.

The moisture content of each sample was readjusted to 50% WHC every seven days. Because the amount of soil in each tube did not permit active mixing, the proper amount of de-ionized water was added to each tube by injection with a syringe to ensure even distribution of moisture throughout the soil sample. To test the effects of substrate limitation in the experiment, a glucose solution with the amount of 2 mg glucose per g dry weight soil was added to one tube of each soil at each incubation temperature. Glucose was added because it is often a component of fast-cycling soil organic carbon pools that provide fuel for many heterotrophic soil organisms (Gu *et al.* 2004; van Hees *et al.* 2005).

Respiration and Microbial Assays

Thermal adaptation was measured by conducting an assay similar to those used in plant, animal, and cultured microbe adaptation studies (e.g. Hochachka & Somero 2002; Atkin & Tjoelker 2003; Malcolm *et al.* 2008; Tjoelker *et al.* 2008). The short-term nature of the assay allowed measurement of R_{mass} rates before the heterotrophic soil communities could adapt to the assay temperature. The assay was conducted after 84 days of incubation and was performed at 20°C, a temperature intermediate to the experimental incubation temperatures of 12°C and 28°C. The equivalent of 2 g dry weight of each soil was measured into pre-labeled 50 mL centrifuge tubes. Water or glucose was added to the tubes and evenly distributed throughout the sample by mixing with dissecting needles, bringing the soil moisture content of each sample to 65% WHC.

Glucose was added to assay soils in the amount of 42.5 mg per g dry weight soil (in excess of heterotrophic microorganismal demand), to avoid the confounding effect of substrate limitation. The tubes were sealed with air-tight caps modified for gas analysis and flushed for 2 minutes with carbon dioxide-free air to eliminate all carbon dioxide from the tube headspace. All tubes were placed in a 20°C incubator for 24 hours.

Using a gas-tight syringe (SGE, Victoria, Australia), headspaces of the tubes were determined (5 mL over-pressurization followed by a 5 mL sample). The carbon dioxide concentration of each sample was measured using infra-red gas analysis (IRGA). Each gas sample was injected into a 1 mL sample loop and then transferred to the IRGA (LI-7000, LI-COR, Lincoln, USA) using a gas sample valve (VICI Valco Instruments Co. Inc., Houston, USA) connected to an air stream free of carbon dioxide. Headspace concentrations were determined by comparing peak areas with a carbon dioxide-in-air reference standard (1990 uL L^{-1}, Air Liquide America Specialty Gases LLC, Plumsteadville, USA). Assays were performed in duplicate, resulting in 192 tubes for measurements on Day 84 of the incubations; that is, 12 soils × 2 substrate treatments × 2 incubation temperatures × 2 solution types × 2 repeats = 192. This assay method enabled the collection of soil respiration rate data (water-added assays) and data on soil respiration rates without glucose substrate limitation (glucose-added assays). These different expressions of soil respiration will subsequently be referred to as Soil *R* and Substrate *R*, respectively.

A modified substrate-induced respiration (SIR) technique was used to calculate an estimate of microbial biomass in each sample (Fierer *et al.* 2003). 2 g dry weight equivalent of each sample was measured into pre-labelled 50 mL centrifuge tubes. The tubes were placed in a

20°C incubator overnight to condition the soil prior to the addition of a yeast solution. A 2 mL autolysed yeast solution containing 0.012 g yeast per g dry soil was added to each tube and mixed using a dissecting needle. The tubes were lightly capped and placed in a 20°C shaking incubator for 1 hour. The tubes were then tightly capped, flushed for 2 minutes with carbon dioxide-free air, and replaced in the 20°C shaking incubator for 5 hours. Headspace and carbon dioxide concentration measurements were performed using the IRGA method described for the assays. SIR biomass values were calculated as the maximum soil plus substrate-derived carbon dioxide production rate without the use of conversion factors. All SIR assays were performed in duplicate, resulting in a total of 96 tubes (12 soils × 2 incubation temperatures × 2 substrate treatments × 2 repeats = 96 tubes). The microbial biomass estimates obtained through the SIR assays can be used to adjust Substrate *R* for differences in microbial biomass among soils. Hereafter, this mass-specific expression of soil respiration will be called R_{mass}.

Statistical Analyses

Data were analyzed using linear mixed-effects modeling to test for incubation temperature, substrate addition, and fertilization treatment effects on respiration rates (Soil *R*, Substrate *R*, and R_{mass}). Fixed effects were Incubation Temperature (12, 28 °C), Substrate Treatment (water, glucose solution), and Fertilization Treatment (control, nitrogen, phosphorus, nitrogen and phosphorus). Plot (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), and Block (1, 2, 3) were included in analyses as random effects to identify the spatial unit of repeat. Since R_{mass} is a ratio of Substrate *R* to microbial biomass, a covariate approach was used to evaluate treatment effects (Jasienski & Bazzaz 1999). This method used microbial biomass as the covariate and Substrate *R* as the dependent variable. The more complex model (different slopes, different intercepts)

was not an improvement over simpler models, so the simplest covariate model (single slope, different intercepts) was used. Significance values reported for R_{mass} are derived from the simple covariate model structure.

Preliminary statistical tests showed that Block may significantly affect respiration rates. For this reason, data were tested by two models, one with Block classified as a random effect and the other with Block as a fixed effect. If Block was not found to be significant, the analysis from the model with Block as a random effect was retained.

Statistical significance was determined using an α-level of 0.05. All statistical analyses were performed using S-Plus 8.0 (Insightful Corporation, Seattle, WA, US). Respiration values were log-transformed to meet assumptions of linearity, normality, and homogeneity of variance.

CHAPTER 3
RESULTS

Assays were designed in order to permit the assessment of three different response variables. First, assays in which only water was added to soil samples enabled the measurement of potential soil respiration (Soil *R*) rates. Variation in Soil *R* rates among samples could be caused by substrate limitation, differences in microbial biomass, and/or thermal adaptation. A significant interaction was observed between incubation temperature and substrate regime, with Soil *R* rates greater for 12°C incubated soils than 28°C incubated soils and for soils with glucose additions than soils with water-only additions ($P<0.0001$, Figure 1). Also, the interaction between substrate regime and nitrogen fertilization was found to be significant. Specifically, Soil *R* rates in glucose-treated soils with nitrogen fertilizer were higher than in soils without nitrogen fertilizer. Soil *R* rates for soils with water-only additions were very similar between substrate regimes, with soils receiving nitrogen fertilization exhibiting slightly lower Soil *R* rates than soils without nitrogen fertilization ($P=0.0390$, Figure 2). Finally, phosphorus fertilization emerged as a marginally significant main effect. Soils treated with phosphorus exhibited lower Soil *R* rates compared to soils without phosphorus ($P=0.0523$, Figure 3).

Substrate respiration (Substrate *R*), the second response variable, is determined after the alleviation of substrate limitation by the addition of a glucose solution during the assay. Any treatment effects are therefore assumed to be caused by differences in microbial biomass and/or thermal adaptation. As in the Soil *R* assays, the incubation temperature by substrate regime interaction was significant. Substrate *R* rates of soils treated with a glucose solution were higher

than Substrate R rates of soils treated with water across both incubation temperatures. Additionally, Substrate *R* rates for colder glucose-treated soils were lower than those for warmer glucose-treated soils. However, the opposite is true for water-treated soils, with colder soils displaying higher Substrate *R* rates than warmer soils ($P<0.0001$, Figure 4). The interaction between incubation temperature and phosphorus fertilization was also significant. The presence of added phosphorus in soils depresses Substrate *R* rates as it did Soil *R* rates. Substrate *R* rates of soils treated with phosphorus are very similar, while soils without the addition of phosphorus exhibit greater Substrate *R* rates under the warmer incubation temperature ($P=0.0233$, Figure 5). The addition of nitrogen was insignificant ($P=0.3127$, data not shown).

Microbial biomass, as measured by substrate-induced respiration, was significantly affected by the interaction between incubation temperature and substrate regime. Biomass was higher in glucose-treated soils than in water-treated soils across both incubation temperatures. Soils incubated at 12°C and treated with glucose had less microbial biomass than glucose-treated soils incubated at 28°C, while water-treated soils had more microbial biomass at 12°C than at 28°C ($P<0.0001$, Figure 6). Additions of nitrogen and phosphorus were insignificant ($P=0.1915$ and 0.3423 respectively, data not shown).

The third response variable, R_{mass}, or mass-specific respiration, is calculated by dividing substrate respiration by microbial biomass. Thus, effects of substrate limitation and differences in microbial biomass among samples have been eliminated, leaving thermal adaptation as the cause of any treatment effects. The interaction of incubation temperature by substrate regime was again found to be significant, with higher R_{mass} rates for glucose-treated soils than the R_{mass} rates of soils with water additions. R_{mass} rates for samples treated with water were similar across

incubation temperatures, while 12°C soils treated with glucose had lower R_{mass} rates than glucose-treated soils incubated at 28°C (P=0.0005, Figure 7). Additionally, incubation temperature interacted significantly with phosphorus fertilization. As observed for Substrate *R* and Soil *R*, the presence of added phosphorus in soils depressed R_{mass} rates. R_{mass} rates of soils treated with phosphorus are very similar, while soils without the addition of phosphorus exhibit greater R_{mass} rates under the warmer incubation temperature (P=0.0244, Figure 8). These patterns are analogous to those detected in Substrate *R* measurements (Figure 5). Also, similar to the effects observed for Substrate *R* measurements, nitrogen additions were insignificant (P=0.9209, data not shown).

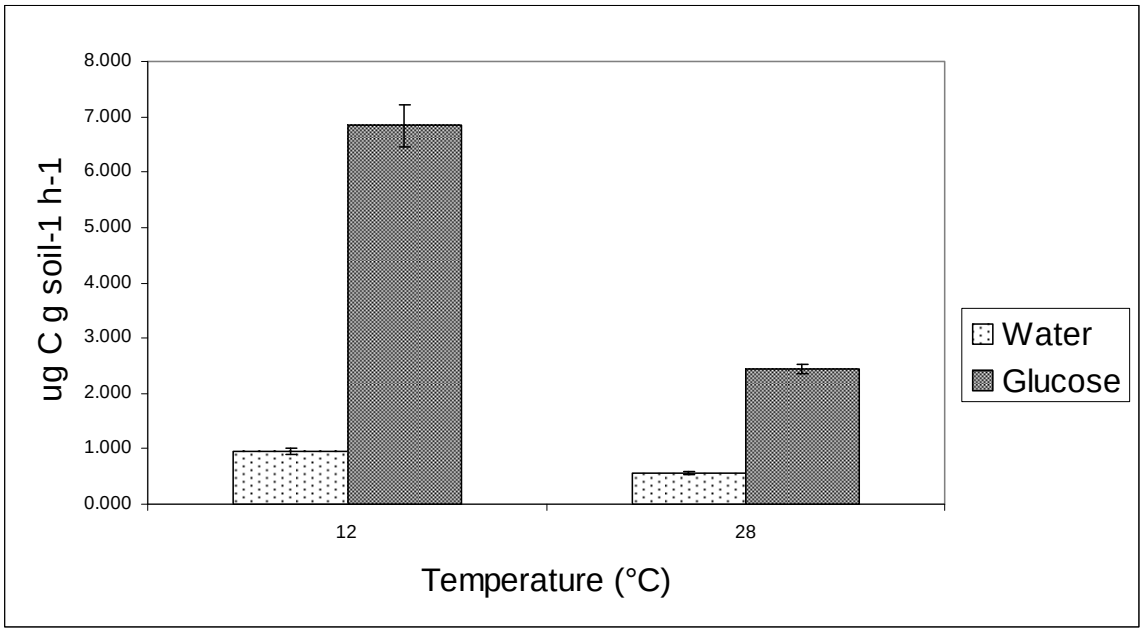

Figure 1. Potential soil respiration rates, substrate regime by incubation temperature interaction. Soils were incubated at 12°C or 28°C and were treated with a weekly addition of glucose solution or water in the laboratory. These results are in accordance with predictions of response to a long term temperature increase, but could be caused by substrate limitation or differential biomass in addition to thermal adaptation. Values are means ± 1 standard error (P<0.0001).

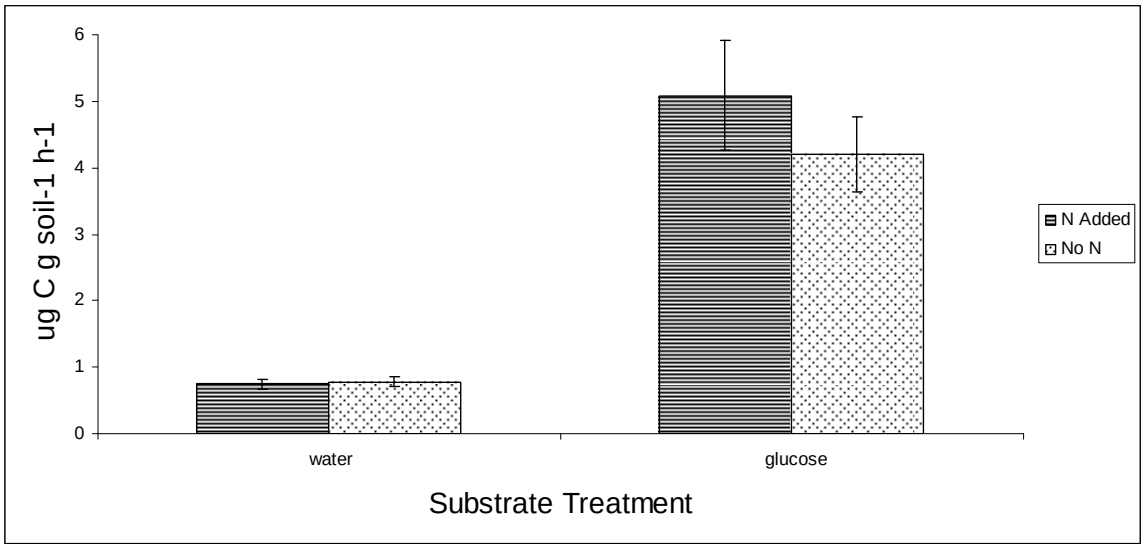

Figure 2. Potential soil respiration rates, substrate regime by nitrogen fertilizer history interaction. Soils were part of a factorial nitrogen-phosphorus experiment in the field and were treated with a weekly addition of glucose solution or water in the laboratory. Note how the glucose-treated soils have a higher mean respiration rate in response to nitrogen additions, perhaps suggesting that microbes are able to more efficiently utilize slower carbon pools in the presence of sufficient nitrogen. Values are means ± 1 standard error (P=0.0390).

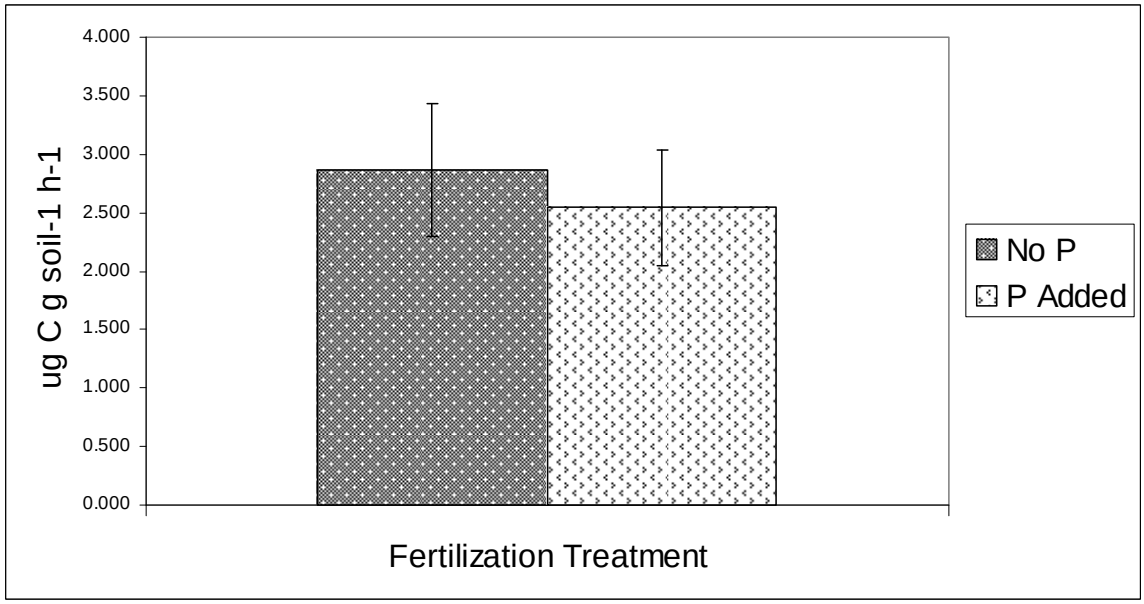

Figure 3. Potential soil respiration rates, effect of phosphorus fertilization. Soils were part of a factorial nitrogen-phosphorus experiment in the field. Note that the presence of phosphorus depresses respiration rates. Values are means ± 1 standard error (P=0.0523).

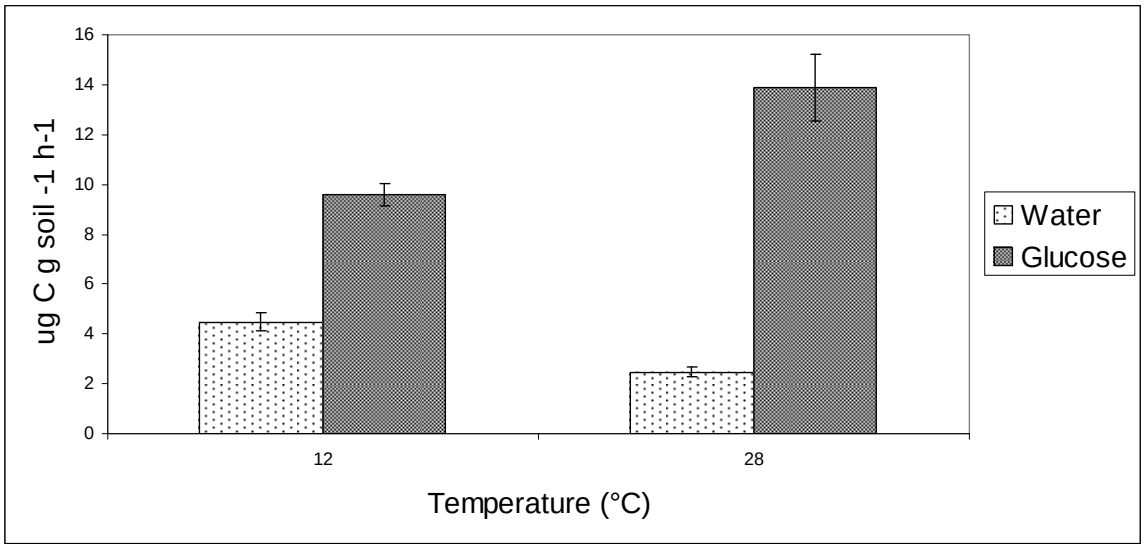

Figure 4. Substrate respiration rates, incubation temperature by substrate regime interaction. Soils were incubated at 12°C or 28°C in the laboratory with weekly additions of water or glucose solution. The increase in respiration of the 28°C soils after the alleviation of substrate limitation indicates that the depressed Soil *R* rates were at least partially caused by substrate depletion. Values are means ± 1 standard error (P<0.0001).

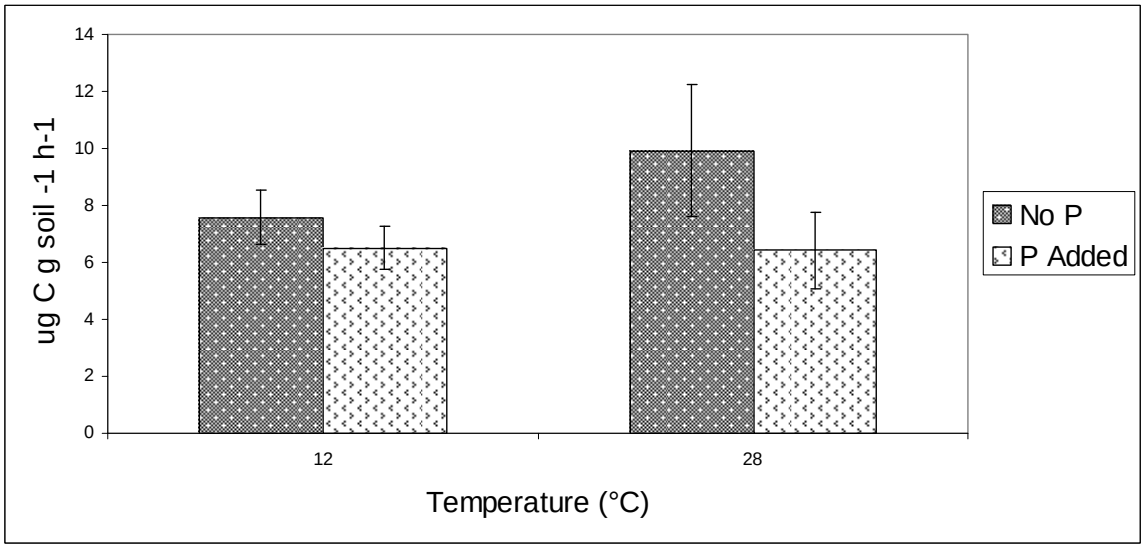

Figure 5. Substrate respiration rates, substrate regime by phosphorus fertilizer history interaction. Soils were part of a factorial nitrogen-phosphorus experiment in the field and were incubated at 12°C or 28°C in the laboratory. Note that the presence of phosphorus depresses respiration rates and seems to negate the effect of incubation temperature. Values are means ± 1 standard error (P=0.0233).

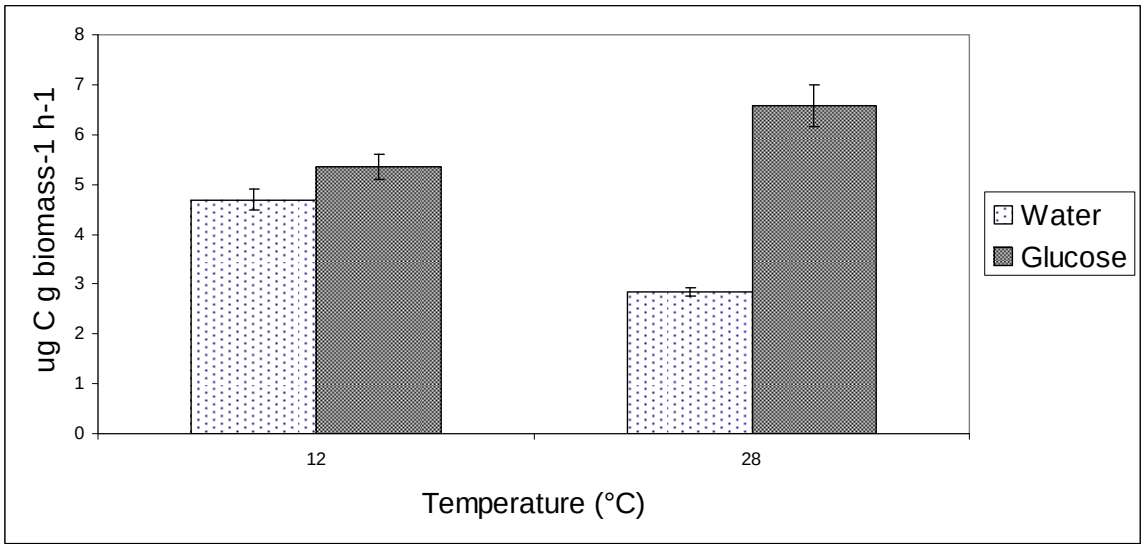

Figure 6. Substrate-induced respiration estimates of microbial biomass, incubation temperature by substrate regime. Notice the low biomass of water-only soils incubated at 28°C, probably caused by substrate limitation, while soils incubated at 28°C with glucose additions have the largest microbial biomass. Values are means ± 1 standard error (P<0.0001).

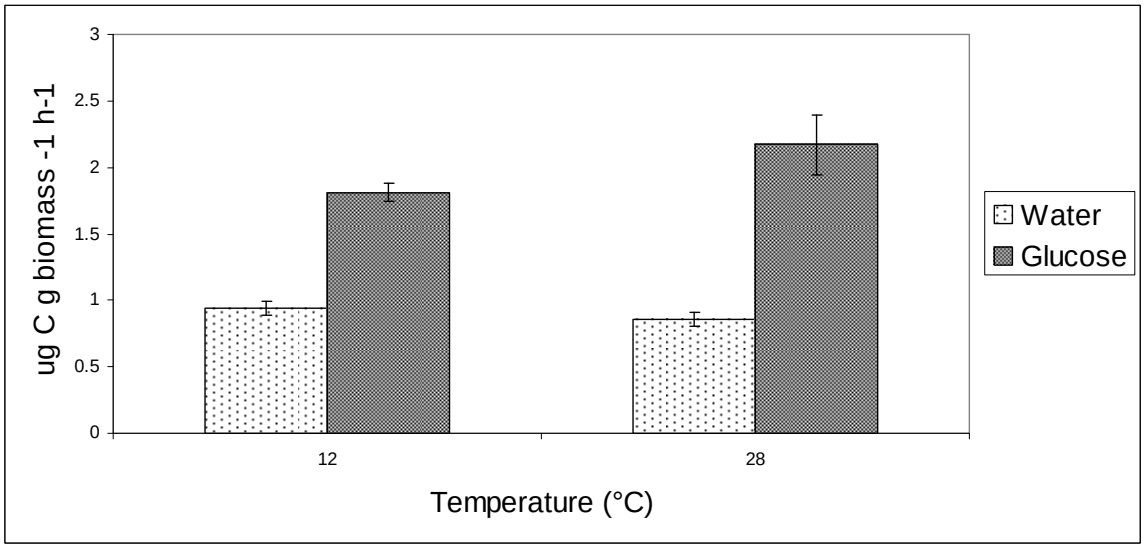

Figure 7. Mass-specific respiration rates, incubation temperature by substrate regime interaction. Soils were incubated at 12°C or 28°C in the laboratory with weekly additions of water or glucose. Note that the mass-specific respiration response for water-treated soils suggests microbial adaptation, while the glucose-treated soils do not follow that pattern. This indicates that substrate limitation and/or microbial biomass differences influenced lower Soil *R* rates among 28°C soils. Values are means ± 1 standard error (P=0.0005).

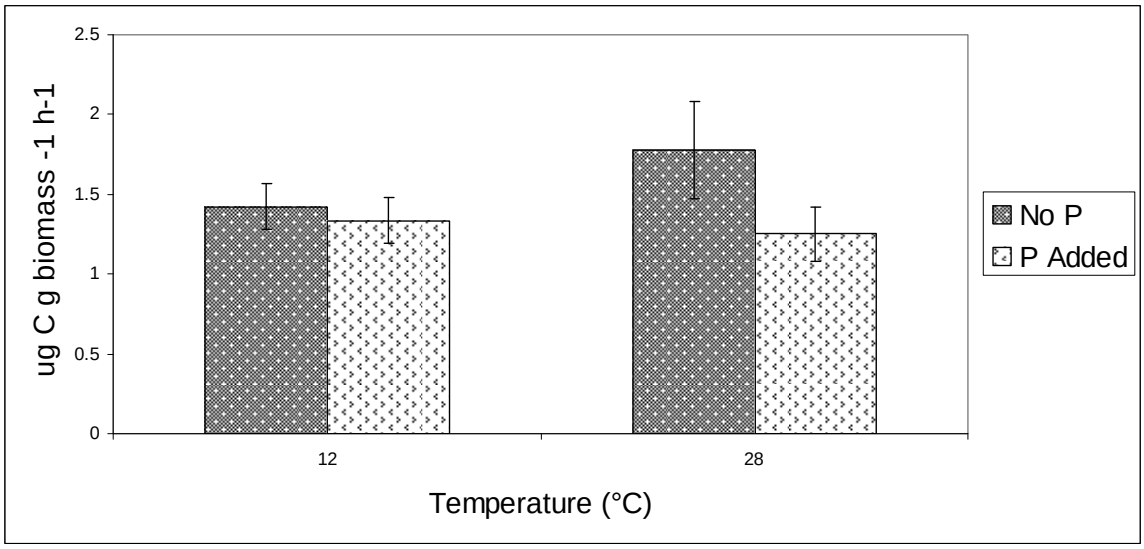

Figure 8. Mass-specific respiration rates, incubation temperature by phosphorus fertilizer history interaction. Soils were part of a factorial nitrogen-phosphorus experiment in the field and were incubated at 12°C or 28°C. Note that the presence of phosphorus depresses respiration rates and seems to negate the effect of incubation temperature. Values are means ± 1 standard error (P=0.0244).

CHAPTER 4
DISCUSSION

The objective of this experiment was to test the respiration response of heterotrophic soil microorganisms to a medium-term increase in temperature. Additionally, data obtained from these tests can be used to determine the effects of nitrogen and phosphorus fertilizers on soil respiration under varying temperature regimes with the presence or absence of substrate limitation. Following established evolutionary trade-offs of adaptation to higher temperatures, it was expected that R_{mass} rates for soils incubated at 28°C would be lower than R_{mass} rates for soils incubated at 12°C, indicating thermal adaptation of soil microbes to a warmer temperature regime. Respiration effects caused by the addition of fertilizer were expected to be insignificant.

After 84 days of incubation at 12°C and 28°C, R_{mass} rates were slightly higher for water-only soils incubated at 12°C than for water-only soils incubated at 28°C, following predictions of thermal adaptation and confirming data from previous experiments (Bradford, unpublished data). However, R_{mass} rates for soils treated with weekly glucose additions and incubated at 12°C were lower than R_{mass} rates of glucose-treated soils incubated at 28°C, which does not fit with the hypothesis of thermal adaptation. These data suggest that factors other than thermal adaptation to a warmer temperature, such as substrate limitation and differences in biomass, are responsible for the measured potential soil respiration (Soil *R*) response to warm and cold temperature regimes. Interestingly, soils used in this experiment that had been treated with both nitrogen and phosphorus fertilizer did respond to the imposed temperature regimes in accordance with the hypothesis; that is, mass-specific respiration rates were lower in soils incubated at 28°C than

soils incubated at 12°C (Figure 9). This observation suggests that nutrient availability plays an important role in determining R_{mass} responses to different temperature regimes.

In fact, nutrient availability had a much greater effect on soil respiration rates than was expected. Rather than the predicted insignificance of fertilization treatments, phosphorus additions were found to significantly decrease Soil *R* rates, Substrate *R* rates, and R_{mass} rates; that is, phosphorus fertilization depressed soil respiration rates regardless of substrate availability or biomass. Additionally, R_{mass} rates for soils with phosphorus mirrored respiration patterns predicted for thermally adapted microbe communities, while soils without phosphorus additions exhibited higher R_{mass} rates for 28°C soils than 12°C soils. This again points to the importance of nutrient availability in heterotrophic respiration responses. One possible mechanism causing this response is that a readily available supply of phosphorus eliminates the microbial necessity of mining soil organic carbon in order to obtain phosphorus, resulting in less carbon released to the atmosphere.

Phosphorus fertilization also served to lessen the impact of incubation temperature on soil respiration rates. While soils without phosphorus fertilization exhibited increased respiration when incubated at 28°C, soils with phosphorus fertilization displayed similar respiration rates across incubation temperatures. Although the cause, or causes, of this effect are unclear, it may arise from the fact that the addition of phosphorus improves the carbon-use efficiency of microbes; that is, available phosphorus in the soil enables microbes to incorporate more of the carbon they consume into biomass rather than releasing it through respiration as carbon dioxide.

Nitrogen had less of an overarching impact on soil respiration rates. The fact that the presence of nitrogen fertilization affected only Soil *R* rates indicates that nitrogen availability

may have the largest effect on potential soil respiration rates when substrate availability is a limiting factor. An abundant nitrogen supply resulted in glucose-treated soils displaying a higher respiration rate than soils with water-only additions, as would be expected for any soil because of higher biomass. However, the fact that the addition of nitrogen increased Soil *R* rates of soils treated with glucose indicates that perhaps increased availability of nitrogen allows heterotrophic microorganisms to more efficiently utilize slower carbon pools after the labile carbon has been consumed from the soil.

A potential shortcoming of this research is derived from the fact that glucose was added as a means of alleviating substrate limitation. In general, bacteria respond more rapidly to simple carbohydrates, such as glucose, than fungi, which prefer carbon substrates such as cellulose (Brady and Weil 2000). Since glucose was added weekly to half the soil samples while the other half received only water, it is possible that the heterotrophic community in the glucose-treated soils became more heavily comprised of bacteria while the water-only soil community became dominated by fungi. Fungi tend to exhibit greater carbon-use efficiency than bacteria, assimilating more of the carbon they consume into biomass rather than releasing it as carbon dioxide (Brady and Weil 2000). If the two communities did develop in this way, this leads to community divergence that cannot be differentiated from species-specific adaptations when explaining soil respiratory responses to various treatments.

Additionally, this research is limited in that results obtained through experiments in a laboratory microcosm cannot necessarily be extrapolated to soil communities in the field. Calculations of R_{mass} were performed under the assumption that all substrate limitation had been alleviated, which is rarely the case outside of the laboratory (Schimel & Weintraub 2003). Also,

many of the mechanisms for the phenomena observed through different respiration rates are unclear, making it difficult to predict how and why microbes in other soil samples will react to certain stimuli. Soil organic carbon fractionations of soil samples used in this experiment may shed light on nutrient levels and substrate availability in the soils. Only through further research and continued exploration of the mechanisms behind heterotrophic soil respiration responses to varying temperature regimes will it be possible to understand the true nature of the feedback loop between increased temperature and soil respiration.

These findings indicate the need for further research regarding the role of nutrient availability in heterotrophic soil respiration under varying temperature regimes. Although at the present time IPCC climate change models allow for changes in soil respiration rates caused by warmer temperatures, these models do not account for varying nutrient levels and their effects on the temperature response of heterotrophic soil microorganisms. The data discussed here indicate that nutrient availability in soils has a significant effect on the amount of carbon dioxide released to the atmosphere through heterotrophic soil respiration. In addition to amending models to account for the possibility of thermal adaptation in response to warmer temperatures, climate change scientists should consider how soils with different nutrient levels will respond to sustained warming. A particularly interesting issue these data raise is the potential effect nitrogen and phosphorus additions to soils may have on the carbon flux between the atmosphere and the terrestrial ecosystem. The data presented here indicate that phosphorus additions decrease soil respiration, thus increasing the potential for soils to act as a carbon sink. However, because of the varied effects of nutrient additions to soils, further research is necessary before this variable can be accurately incorporated into predictions of soil respiration rates.

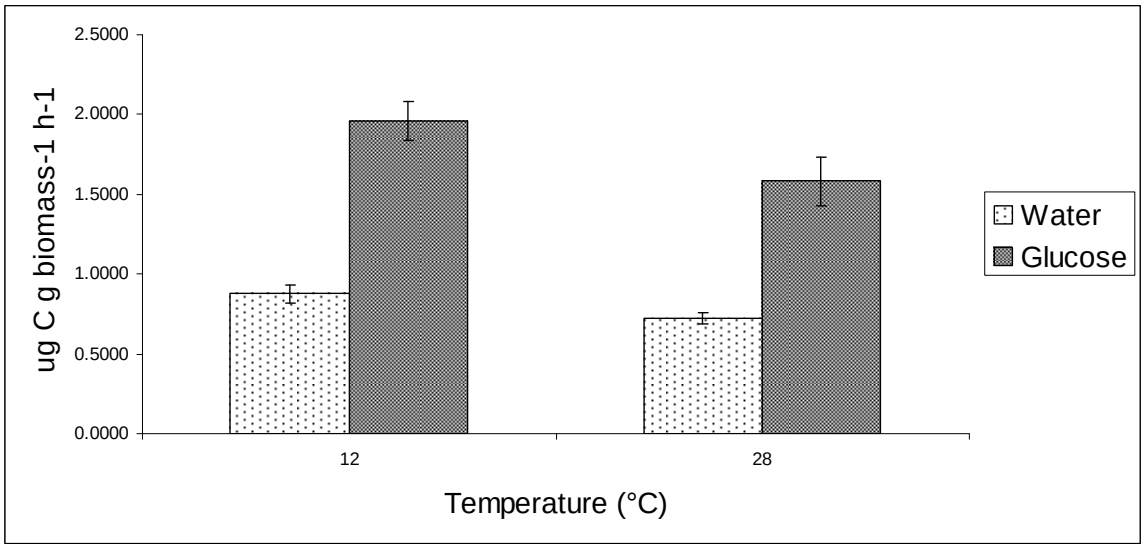

Figure 9. Mass-specific respiration rates, incubation temperature by substrate regime interaction of nitrogen and phosphorus fertilized soils only. Soils were incubated at 12°C or 28°C and treated with water or glucose additions in the laboratory. Note that the respiration rates depicted here are in accordance with the hypothesis of thermal adaptation, indicating that nutrient levels play a part in determining microbial response to variations in temperature. Values are means ± 1 standard error.

WORKS CITED

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

Amador, J. A., and R. D. Jones. 1993. Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. Soil Biology & Biochemistry **25**: 793-801.

Atkin, O. K., and M. G. Tjoelker. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Science **8**: 343-351.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management **196**: 43-56.

Brady, N. C., and R. R. Weil. 2000. Elements of the Nature and Properties of Soils. Prentice-Hall, Inc., Upper Saddle River, New Jersey, USA.

Cleveland, C. C., A. R. Townsend, and S. K. Schmidt. 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems **5**: 680-691.

Denman, K. L., G. Brasseur, A. Chidthaisong, P. Ciais, P. M. Cox, R. E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P. L. da Silva Dias, S. C. Wofsy, and X. Zhang. 2007. Couplings between changes in the climate system and biogeochemistry. Pages 499-587 *in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Eliasson, P. E., R. E. McMurtrie, D. A. Pepper, M. Stromgren, S. Linder, and G. I. Agren. 2005. The response of heterotrophic CO_2 flux to soil warming. Global Change Biology **11**: 167-181.

Fierer, N., J. P. Schimel, and P. A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry **35**: 167-176.

Gallardo, A., and W. H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology & Biochemistry **26**: 1409-1415.

Gu, L. H., W. M. Post, and A. W. King. 2004. Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: A model analysis. Global Biogeochemical Cycles **18**: GB1022.

Hochachka, P. W., and G. N. Somero. 2002. Biochemical adaptation: Mechanism and process in physiological evolution. Oxford University Press, New York, New York, USA.

Illeris, L., A. Michelsen, and S. Jonasson. 2003. Soil plus root respiration and microbial biomass following water, nitrogen, and phosphorus application at a high arctic semi desert. Biogeochemistry **65**: 15-29.

Illeris, L., and S. Jonasson. 1999. Soil and plant CO_2 emisson in response to variations in soil moisture and temperature and to amendment with nitrogen, phosphorus, and carbon in northern Scandinavia. Arctic, Antarctic, and Alpine Research **31**: 264-271.

Jarvis, P. and S. Linder. 2000. Constraints to growth of boreal forests. Nature **405**: 904-905.

Jasienski, M., and F. A. Bazzaz. 1999. The fallacy of rations and the testability of models in biology. Oikos **84**: 321-326.

Jin, X., S. Wang, and Y. Zhou. 2008. Microbial CO_2 production from surface and subsurface soil as affected by temperature, moisture, and nitrogen fertilization. Australian Journal of Soil Research **46**: 273-280.

Keith, H., K. L. Jacobsen, and R. J. Raison. 1997. Effects of soil phosphorus availability, temperature and moisture on soil respiration in *Eucalyptus pauciflora* forest. Plant and Soil **190**: 127-141.

Kim, C. 2008. Soil carbon storage, litterfall and CO_2 efflux in fertilized and unfertilized larch (*Larix leptolepis*) plantations. Ecol Res **23**: 757-763.

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

Lee, K., and S. Jose. 2003. Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management **185**: 263-273.

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

Malcolm, G. M., J. C. Lopez-Gutierrez, R. T. Koide, and D. M. Eissenstat. 2008. Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. Global Change Biology **14**: 1169-1180.

Melillo, J. M., P. A. Steudler, J. D. Aber, K. Newkirk, H. Lux, F. P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morriseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science **298**: 2173-2176.

Mo, J., W. Zhang, W. Zhu, P. Gundersen, Y. Fang, D. Li, and H. Wang. 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology **14**: 403-412.

Oechel, W. C., G. L. Vourlitis, S. J. Hastings, R. C. Zulueta, L. Hinzman, and D. Kane. 2000. Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. Nature **406**: 978-981.

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

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

Tjoelker, M. G., J. Oleksyn, P. B. Reich, and R. Zytkowiak. 2008. Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in *Pinus banksiana* across wide-ranging sites and populations. Global Change Biology **14**: 782-797.

van Hees, P. A. W., D. L. Jones, R. Finlay, D. L. Godbold, and U. S. Lundstrom. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology & Biochemistry **37**: 1-13.

Wilson, H. M., and M. M. Al-Kaisi. 2008. Crop rotation and nitrogen fertilization effect on soil CO_2 emissions in central Iowa. Applied Soil Ecology **39**: 264-270.