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METABOLIC RESPONSE TO TEMPERATURE BY SOIL MICROORGANISMS

A Thesis in

Ecology

by

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ABSTRACT

Because of quick changes in temperature in forest litter between and within seasons and the anticipated continued increase in global surface temperatures, it is important to better understand the metabolic response to temperature by soil microorganisms. Notably, researchers have revealed that different soil dwellers may not metabolically respond to temperature in similar manners. In the case of ectomycorrhizal fungi, their respiration comprises a significant portion of total soil respiration, which is a flux ten times greater in size than that from fossil fuel emissions. Concern exists that a positive feedback may occur between soil respiration and temperature. If ectomycorrhizal fungi are capable of adjusting their metabolic response to temperature, they might partially ameliorate that feedback. In the case of decomposer communities, their activity digests organic soil carbon pools, a large amount of which is found in temperate and boreal forests across the globe. Small proportional changes in the concentration of carbon in soil may cause forest soils to be a large source or sink of carbon dioxide to the atmosphere. Again, the metabolic response of decomposer communities to temperature might determine the size of their carbon demand on forest soils, or put another way, how much CO_2 they return to the atmosphere. The goal of my research was, therefore, to assess the metabolic response to temperature by ectomycorrhizal fungi and decomposer microorganisms at a variety of time scales.

On a shorter time scale, I examined whether ectomycorrhizal fungi acclimated their respiration to three different incubator temperatures over the course of one week. Out of 12 ectomycorrhizal fungal isolates, *Suillus intermedius*, *Cenococcum geophilum,*

and *Lactarius* cf. *pubescens* exhibited significant acclimation to temperature. Ectomycorrhizal fungal isolates also displayed significant differences in temperature sensitivity, or Q_{10}. As the earth warms, those ectomycorrhizal fungi that acclimate to temperature will demand less carbon from their host plant and will add less carbon to the atmospheric carbon dioxide pool than those that do not. The fact that variation occurs among ECM fungal species in their ability to acclimate and in their sensitivity to temperature indicates that the response of the ectomycorrhizal fungal community as a whole will be determined by the structure of that community.

On an evolutionary time scale, I investigated whether ectomycorrhizal fungi collected from contrasting latitudes vary in their respiratory response and sensitivity to temperature. Respiration by ectomycorrhizal fungi from Alaska was higher than that of fungi from Pennsylvania across measurement temperatures, when compared at incubation temperatures that reflected their environment of origin or at common incubator temperatures. Estimated growth rate and temperature sensitivity were also lower for ectomycorrhizal fugal isolates from Alaska. These pieces of evidence suggest that ectomycorrhizal fungi are thermally adapted to the thermal regime of their latitudes of origin. Presumably, this allows ectomycorrhizal fungi from different latitudes of origin to have similar carbon demands from their hosts.

In contrast with the abilities of many plants and some ectomycorrhizal fungi, decomposer communities show very little acclimation ability over the course of a week. The ability of decomposer communities to acclimate may influence decomposition and soil carbon content. At different times during the year, however, decomposer

communities were able to change their respiration as prevailing temperatures changed but not at all time points. This suggests that more than just temperature is important in affecting respiration at different times of year. Decomposer community structure, substrate availability, and moisture might potentially change at different times of year, all of which have the potential to affect respiration.

In summary, my results suggest that soil microorganisms display some differences in metabolic response to temperature. Some ectomycorrhizal fungi acclimated their respiration while, for the most part, decomposer communities largely did not acclimate their respiration. In the one instance (May 2007) where decomposers significantly acclimated their respiration, the reduction in respiration across measurement temperatures was small in comparison with ectomycorrhizal fungi when they acclimated. Notably, the sensitivity to temperature by both ectomycorrhizal fungi and decomposer communities from the same red pine plantation in Pennsylvania was quite similar, which indicates that they might proportionally change their respiration rate in the same manner. When ectomycorrhizal fungi from Alaska and Pennsylvania were compared, our results indicate that their respiratory responses to temperature appear to be adapted to their thermal regime from their latitude of origin. The experiments in my dissertation highlight the facts that 1) much variability exists between different soil microorganisms in their metabolic response to temperature, 2) much variability also exists within a group of soil microorganisms (i.e. only 25% of ectomycorrhizal fungal isolates acclimated), and 3) different time scales can be quite important when examining metabolic responses to temperature.

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Chapter 1

Introduction

This thesis summarizes my doctoral studies regarding the respiration response to temperature by soil microorganisms, including ectomycorrhizal fungi and decomposer microorganisms. While much research has been devoted to the exploration of the effect of climate change on photosynthesis, comparatively little is known about the effects of climate change on respiration. Soil respiration, which contributes a large portion of CO_2 from terrestrial ecosystems to the atmosphere, is particularly poorly understood, so our ability to predict the effects of climate change on its rate is currently very low. Roots, mycorrhizal fungi, and decomposer microorganisms all contribute to soil respiration. It remains unclear whether respiration by roots, mycorrhizal fungi, and decomposer microorganisms respond to temperature in a similar manner. In order to create mechanistic models of soil respiratory responses to climate change, we must explore the temperature responses of the individual components to soil respiration rather than measuring total soil CO_2 efflux responses to temperature as is typical. In different portions of this thesis, the temperature responses of respiration by soil microorganisms were examined at both the level of physiological adjustment (acclimation) and at the level of evolutionary adjustment (adaptation). The core objectives of my thesis work were to 1) build a temperature-controlled gas-analyzer for measuring respiration of cultured microorganisms, 2) determine if respiration by different isolates of

ectomycorrhizal fungi acclimated to temperature, 3) explore the potential for respiratory adaptation to temperature by ectomycorrhizal fungi collected along a latitude gradient, and 4) determine if respiration by decomposer microorganisms in pine litter acclimates to temperature at different times of year. In addition to its relevance to climate-change related issues, objectives 2-4 in this thesis also generate classic Autecology questions that have yet to be examined for soil microorganisms.

Chapter 2 presents a detailed description of the temperature-controlled gas-exchange instrument along with numerous tests and optimizations. In Chapter 3, I explore the ability of respiration by 12 ectomycorrhizal fungal isolates to acclimate to three different incubator temperatures and how long it took respiration to acclimate. I also examine whether there is a connection between acclimation, incubator temperature, and the temperature sensitivity (Q_{10}) of respiration. Chapter 4 addresses whether ectomycorrhizal fungi display latitudinal variation in their respiration response to temperature; this finding would suggest that they are adapted to their latitude of origin. I used four paired-genera contrasts of ectomycorrhizal fungi from Alaska and Pennsylvania in this series of experiments. I compared estimated growth rates and Q_{10} values for ectomycorrhizal fungi from Alaska and Pennsylvania. In Chapter 5, I measured the capacity to which respiration by decomposer microorganisms was able to acclimate to different incubator temperatures. These samples were collected at 6 different time points over the course of two years. I also compared their respiration response to temperature at different times of year to see if decomposer microorganisms adjust their respiration temporally. The last chapter is followed by a short conclusion where I summarize my

work and discuss the findings in light of their relevance to climate change perspectives and to ecological theory.

Chapter 2

Temperature-controlled gas-exchange instrument methodology

Abstract

The separate measurement of respiration by decomposer microorganisms, mycorrhizal fungi, and roots is important in ecosystem carbon cycling studies because each uses different sources of carbon and, therefore, each has different effects on soil carbon storage. Moreover, each may respond differently to temperature. We have developed a system, based on infrared analysis of CO_2, for assessing temperature dependence of respiration by decomposer microorganisms in litter as well as any microorganisms in culture, including ectomycorrhizal fungi. The system was extensively tested to insure that steady-state rates of respiration were determined with ectomycorrhizal fungi. In order to demonstrate the utility of this system, we assessed the temperature dependence of respiration of different isolates of ectomycorrhizal fungi. Measured respiration rates agreed well with those assessed by CO_2 absorption and with other, published values. This system will allow us to determine, separately from roots, whether for important groups of soil microorganisms physiological acclimation to temperature, or genetic adaptation to temperature such as across latitudinal or altitudinal gradients occurs.

Introduction

Understanding the metabolic responses to temperature by soil-dwelling organisms is important in ecosystem C balance studies because soil respiration is a large flux in the global C cycle, second only to gross primary productivity (Raich & Schlesinger, 1992; Rustad *et al.*, 2001). In terrestrial ecosystems, the carbon balance is largely temperature driven, with the possibility of a positive feedback between global surface temperature and carbon dioxide evolution from soil (Kirschbaum, 1995; Cox *et al.*, 2000; Kirschbaum, 2000). Because of the immensity of the soil respiration flux, even relatively small proportional changes associated with shifts in temperature could have large consequences for atmospheric CO_2 concentration.

Mycorrhizal fungi, decomposer microorganisms, and plant roots contribute significantly to soil CO_2 efflux via their respiration. Rather than measuring total soil CO_2 efflux, it is extremely important to measure the individual respiration responses to temperature by soil microorganisms and roots for a few reasons. First, respiration by different soil dwellers may respond differently to variation in temperature (Boone *et al.*, 1998; Bhupinderpal-Singh *et al.*, 2003). Additionally, different soil dwellers utilize different sources of energy and thus have different effects on soil C storage. Decomposer microorganisms that are purely saprotrophic respire C from organic sources in the soil. Their activities, therefore, reduce the C stored in the soil. Roots and many ectomycorrhizal fungi respire recently fixed carbon (Högberg *et al.*, 2001), and their activities such as exudation and death add to the C stored in the soil. However, there is significant variability among ectomycorrhizal fungal species with respect to their C

economies with some obtaining substantial C from soil organic matter (Durall *et al.*, 1994; Colpaert & van Laere, 1996; Colpaert & van Tichelen, 1996; Hobbie *et al.*, 1999), such that they also reduce C stored in the soil. Ectomycorrhizal fungi, therefore, probably have an effect on soil C storage somewhat intermediate to roots and decomposers. Lastly, the contribution to soil respiration by decomposers and mycorrhizal fungi is not trivial compared to roots. ECM fungi contribute between 20 and 30 % of total soil respiration (Söderstrom & Read, 1987; Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003) and decomposer microorganisms account for up to 35% of total soil respiration (Bhupinderpal-Singh *et al.*, 2003).

Effective methods have been developed to measure the effects of temperature on respiration by roots (Bryla *et al.*, 2001; Cooper, 2004; Huang *et al.*, 2005). We have built a temperature-controlled gas exchange instrument that allows us to rapidly measure the respiration of soil microorganisms in culture at a variety of measurement temperatures. For the purpose of optimizing the temperature-controlled gas exchange (TCGE) instrument, we used cultures of ectomycorrhizal fungi for our tests. Preliminary data, which demonstrates the ability of our gas-exchange instrument to obtain fast and reliable results, are presented. Respiration rates at several temperatures obtained from our gas-exchange instrument and from a corollary CO_2 absorption technique are compared. Ultimately, our temperature-controlled gas exchange instrument will allow us to answer important, as yet unanswered, questions regarding the plasticity of ectomycorrhizal fungal respiration to temperature (see Chapter 3 & 4), as well as that of other culturable microorganisms (decomposers; see Chapter 5).

Materials and Methods

Collecting and Maintaining ECM Fungal Specimens:

Several ectomycorrhizal fungi were collected from an approximately 55-yr-old red pine (*Pinus resinosa* Ait.) plantation located in State College, Centre County, PA, USA in 2002. Specimens that were used in testing the Temperature-Controlled Gas-Exchange (TCGE) instrument included the ectomycorrhizal fungal species, *Amanita pantherina* [SC004], *Suillus intermedius* [BX007], *Lactarius yazooensis* [SC001], *Boletus projectillus* [BX005], *Amanita rubescens* [SC047], and *Lactarius sp.* [SC003]. For the comparison between respiration rates attained with our TCGE instrument and with an alkali absorptive method only, an Alaskan ECM fungal isolate, *Suillus grevillei* [AK014], was used.

Each specimen was sterilely cultured onto the growth medium, ½ potato dextrose agar + ammonium (PDAN), in 100 x 15 mm Petri dishes (Falcon brand, Becton Dickinson & Co.). PDAN media was made using 19.5 g powdered potato dextrose agar (Difco: Benton, Dickinson & Co., Sparks, MD, U.S.A.), 7.5 g Bacto agar (Difco) and 0.375 g NH_4Cl per liter of water. Fungal cultures were maintained in incubators set to temperatures reflective of those experienced by the fungi during the growing season at their environment of origin: 17^{o}C for Pennsylvanian fungi and 11^{o}C for the Alaskan fungus. These values were chosen based on litter temperatures monitored in the State College *Pinus resinosa* plantation from 1 June to 31 October 2005 (mean 16.4^{o}C, Malcolm and López-Gutiérrez, unpublished), and in four *Picea mariana* forests of the

Bonanza Creek long-term ecological research station, near Fairbanks, Alaska from 21 July to 21 August, mean 11.5^{o}C (Vogel & Valentine, 2006).

The Temperature-Controlled Gas-Exchange Instrument:

The TCGE instrument's design (see Fig. **2.1** for schematic) was modified for cultured microorganisms from systems constructed to measure root respiration (Bryla et al. 2001). The system included a temperature-controlled steel surface on which are affixed eight-polycarbonate gas exchange chambers. Sample chambers were constructed from 11.1 cm diam. Polycarbonate, wide-mouth jars with polypropylene screw-on lids (Nalge Nunc, International, Rochester, NY, USA). The bottoms of each jar were removed to a final jar height of 4.5 cm before being affixed to the metal surface with glue. Petri dishes containing samples to be measured were covered with their lids to reduce water loss from the sample and placed directly on the steel plate, the temperature of which was regulated by a circulating water bath. All of the sample chambers were insulated from the air in the laboratory by covering them with a thick blanket.

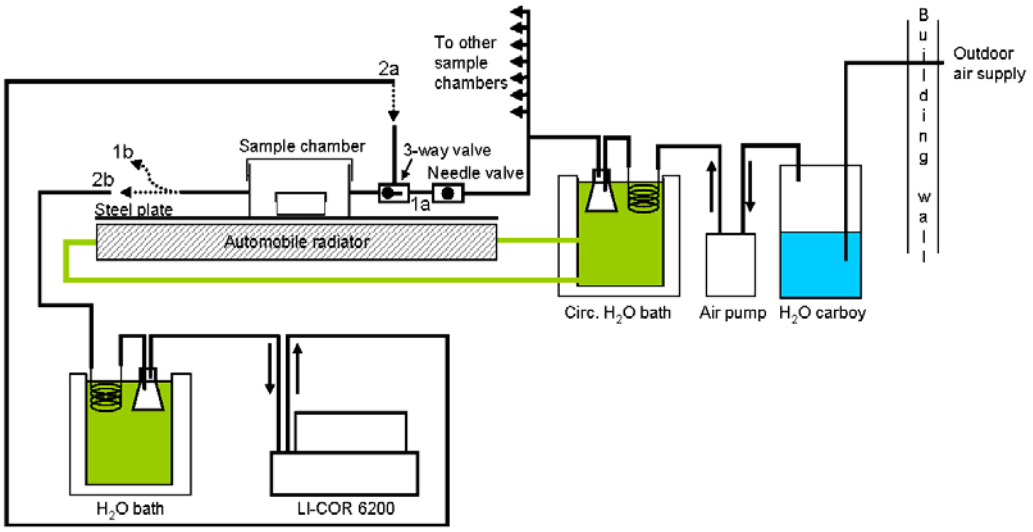

Fig. **2.1**: Schematic drawing of the gas exchange and temperature control apparatus. In the open flow path (1a and 1b), air from the outdoors is humidified and then brought to the temperature of the chamber. After it enters the chamber it passes out into the laboratory. In the closed flow path (2a and 2b) used for measurement of respiration, the chamber is placed in line with the LI-COR 6200 gas analyzer. Temperature control of fungal cultures is achieved by the automobile radiator atop which sits the sample chambers. The radiator temperature is maintained by the circulating water bath.

Each chamber could be individually placed in line with a LI-6200 gas-exchange system (LI-COR Biosciences, Lincoln, NE, USA) to determine the rate of change of CO_2 concentration (flow path 2a, 2b). When not in line with the LI-6200, all chambers received humidified, outside air supplied by a diaphragm pump (model DOA-O704-AA, Gast Manufacturing, Inc., Benton Harbor, MI, U.S.A.) and the air from all chambers was vented into the laboratory (flow path 1a and 1b). The airflow was maintained at the same rate as in the LI-6200 path using needle valves (model A-06393-70, Cole Parmer, Vernon Hills, IL, USA). A three-way nylon miniature ball valve (McMaster Carr, New Brunswick, NJ, USA) was used to switch between open (outside air) and closed (LI-

6200) flow paths. Bev-a-line tubing (0.32 cm i.d., Thermoplastic Processing, Stirling, NJ, USA) was used for carrying air downstream from 3-way valves, while tubing upstream was polyvinyl chloride (PVC). The total volume of the closed-loop pathway, including the LI-6200, was 978 cm^3. The dewpoint of the air was controlled by passing it through a copper coil (0.5 cm i.d.) submerged in a circulating water bath and held at the selected experimental temperature in order to maintain saturation vapor pressure in the sample chambers. This prevented excess moisture on the samples, which could reduce gas exchange.

Individual cultures were initially placed in gas exchange chambers set to the temperature corresponding to their incubator. The temperatures of the samples were monitored using fine-wire copper-constantan thermocouples fed through a small hole in the lid of each sample chamber and through a small hole in the lid of each Petri dish. Holes were plugged with caulking material. Following attainment of steady state (see "Testing the system: Attaining steady state"), the gas analyzer was zeroed and spanned daily. For the LI-6200, the desiccant and SCRUB switches were in the "OFF" position. The measurement of CO_2 concentration was initiated 1 minute after turning on the analyzer pump. The LI-6200 was programmed to calculate a respiration rate during four consecutive 1 - 4 $\mu l\ l^{-1}$ CO_2 changes, depending on time required to achieve the change. Preliminary testing demonstrated that humidity remained constant throughout a measurement. Thus, in making the calculation to determine CO_2 exchange, we had no need to consider water vapor concentrations.

After finishing all measurements, fungal tissues were separated from the agar by melting in a boiling water bath and filtering through a tea strainer. Tissue

weights were determined following drying (60^{o}C) until constant weight was achieved. Respiration rates are expressed as CO_2 efflux divided by tissue dry weight ($\mu mol\ s^{-1}\ mg^{-1}$).

Testing the System

Achieving Steady State:

On several occasions, ECM fungal isolates (including: *Lactarius* sp. [SC003] and *Amanita rubescens* [SC047]) were taken from their incubator and placed inside of the respiration chambers to determine when steady state conditions had been reached. Respiration at a common temperature was measured every hour for a 12 hour period and then again at 24 hours. Respiration rates at a common temperature were stable after 8 hours on average and remained so for up to 24 hours (Fig. **2.2**: for *Amanita rubescens*).

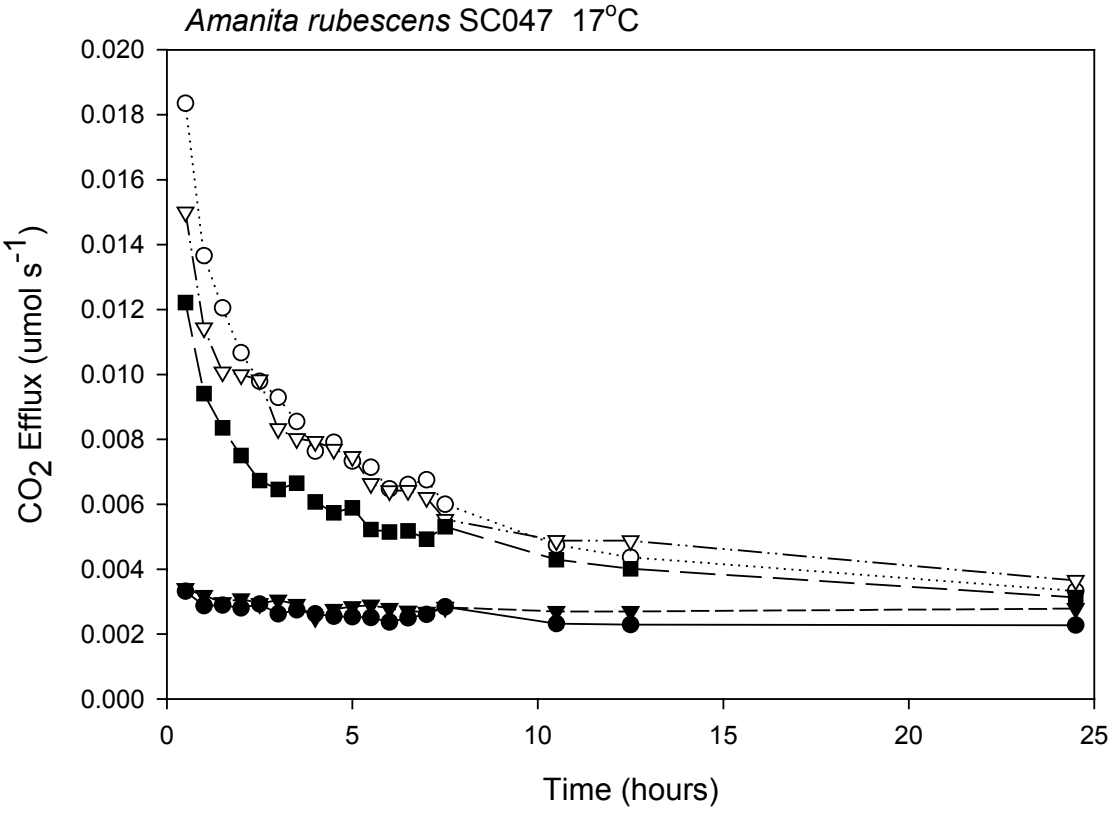

Fig. **2.2**: Replicated cultures (shown with different symbols) of *Amanita rubescens* were grown at 17°C. CO_2 efflux was measured periodically over the course of twenty-four hours to determine when steady-state was achieved inside of the gas-exchange instrument.

In a separate experiment, we also measured respiration at 17°C upon *Amanita rubescens* but when growing in solid media and when excised from the solid media. When the fungus was present on the solid media, CO_2 efflux declined to a steady state value over a five-hour period. When the fungus was excised from the solid media, CO_2 efflux declined to zero within 24 hours, suggesting that the media harbored CO_2 in an alternate equilibrium in the incubators as compared to when in the air flow path in the gas-exchange apparatus (Fig. **2.3**). Thus, cultures containing ECM fungi were always placed in the chambers overnight, for at least 8 hours, in order for steady state to be

achieved. In order to keep experiments running efficiently, we placed our cultures in the TCGE instrument overnight so that measurements could begin first thing in the morning.

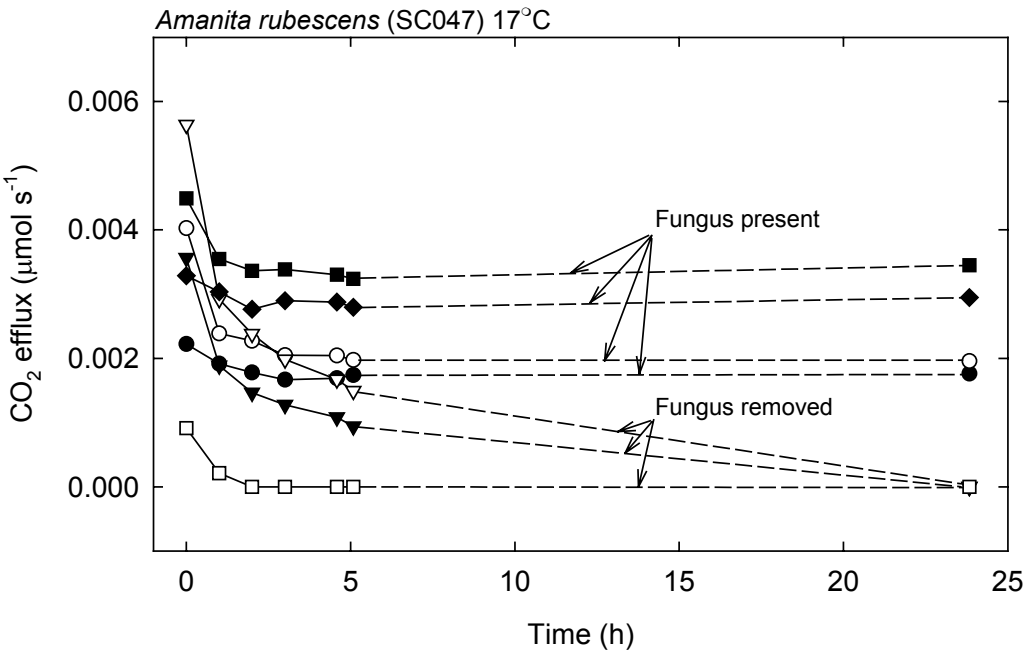

Fig. **2.3**: Rate of CO_2 efflux from gels with (closed symbols) and without (open symbols) *Amanita rubescens* present over the course of time. Different symbols represent replicate fungal cultures. The fungi had been grown in a 17ºC incubator. Average measurement temperature was 18ºC.

Changing Temperature:

After reaching an initial steady state value as described above, less time was needed to reach steady state again for each measurement temperature change. In order to determine how quickly following temperature change a new steady-state respiration was attained, five replicates of the ECM fungus, *Amanita rubescens* [SC047], were used.

ECM fungal cultures were allowed to reach an initial steady state CO_2 efflux at 17°C and then changed to 25°C and 30°C. From the point when the water bath temperature was changed, ECM fungal tissue attained the desired temperature in 50-60 minutes. At each temperature shift, CO_2 efflux was measured as soon as the cultures reached the desired temperature and twice afterward (approximately one half hour and one hour later) before the next temperature change. CO_2 efflux was essentially stable immediately following each of the completed temperature changes (Fig. **2.4**).

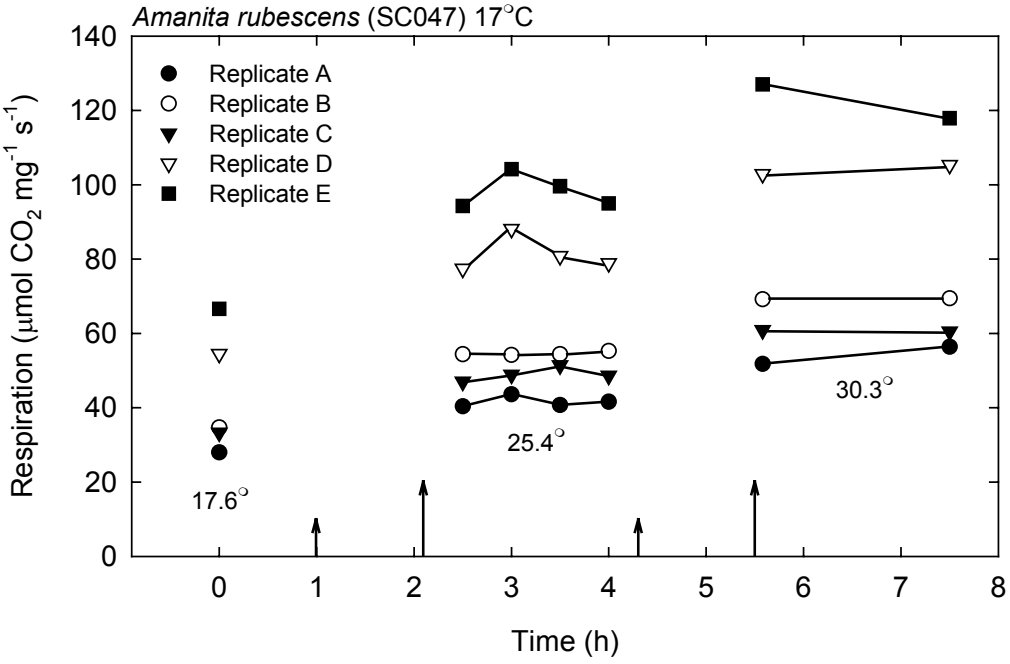

Fig. **2.4**: Repeated measurements of respiration rates of cultures of *Amanita rubescens*, grown in an incubator at 17ºC, following shifts in temperature from 17.6ºC to 25.4ºC and from 25.4ºC to 30.3ºC. Small arrows are placed at the times when the water bath temperatures were altered. Large arrows are placed at the times when the cultures achieved their new stable temperatures.

Two goals when measuring respiration are that the reported measurements are repeatable and that they are reversible after short incubations at other temperatures. To demonstrate that a measurement at a given measurement was repeatable after short incubations at other temperatures, ECM fungal cultures of *Boletus projectillus* [BX005] were first allowed to attain steady-state respiration rates at 20°C. Respiration was measured at approximately 20°C, 15°C, and 10°C, and then returned to 15°C and 20°C for consecutive respiration measurements. The rates of respiration were essentially unchanged the first and second time they were measured at a particular temperature (Fig. **2.5**).

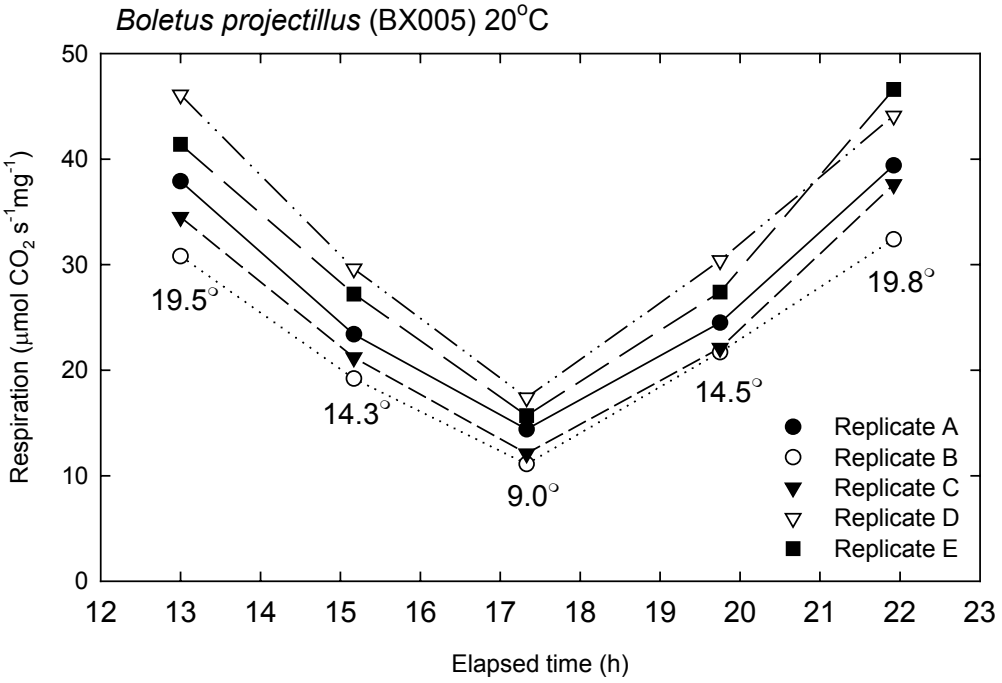

Fig. **2.5**: Stable respiration rates of cultures of *Boletus projectellus*, grown at 20ºC, at increasingly lower and then increasingly higher measurement temperatures (19.5, 14.3, 9.0, 14.5 and 19.8ºC).

To demonstrate that we can detect when respiration at a given temperature is irreversible, or that the fungal cultures are not physiologically changed or damaged, ECM fungal cultures of *Suillus intermedius* [BX007] were allowed to attain steady-state respiration rates at 20°C. Respiration was measured three times, at 30-minute intervals, after reaching stable temperatures of approximately 25°C, 30°C, 35°C, and 40°C. Stable respiration rates were obtained for *S. intermedius* until 40°C, when respiration rates declined by more than 50% by the second measurement at that temperature (data not shown), indicating we had exceeded the temperature at which the fungus could stably respire. Thus, exceeding the thermal tolerance of an ECM fungus, results in detectable, irreversible respiration measurements, displayed by temporal instability.

Comparison of CO_2 Absorption and CO_2 Analyzer-based Gas Exchange Methods:

Since limited data exists in the literature for respiration rates by mycorrhizal fungi standardized on a dry tissue mass basis, we compared respiration rates by an ECM fungus with our gas-exchange instrument and with a modified CO_2 absorption technique (Robertson *et al.*, 1999). Replicate cultures of *Suillus grevillei* [AK014] were grown at 20°C; half were used in order to attain steady-state respiration measurements with the gas-exchange instrument, while the other half were used to attain measurements with a CO_2 absorptive method. Respiration measurements were made with the gas-exchange instrument at approximately 12, 17, and 25°C, five dishes per temperature on separate days. After which, the five fungal cultures from a particular day were placed inside polycarbonate wide-mouth Nalgene jars along with another Petri dish containing 20 ml of

0.05 M NaOH. The lids were screwed on tightly, and the chambers were placed in incubators of corresponding temperatures, 12, 17 and 25^{o}C for ten hours. Three control chambers containing NaOH but no fungal cultures were also incubated at each temperature. Finally, after each of the three incubations, standard back titrations with 0.05 M HCl and the indicator, phenolphthalein, were performed in order to attain the amount of CO_2 absorbed during the incubation.

In both methods, respiration rates by the ECM fungus increased with temperature (Fig. **2.6**). However, respiration rates became less similar as temperature increased, with slightly higher rates from the gas-exchange instrument as compared to the CO_2 absorptive method (Fig. **2.6**). Rates from the infrared gas analysis method were 6% higher at 12^{o}C, 18% higher at 17^{o}C, and 23% higher at 25^{o}C than those given by the CO_2 absorption method.

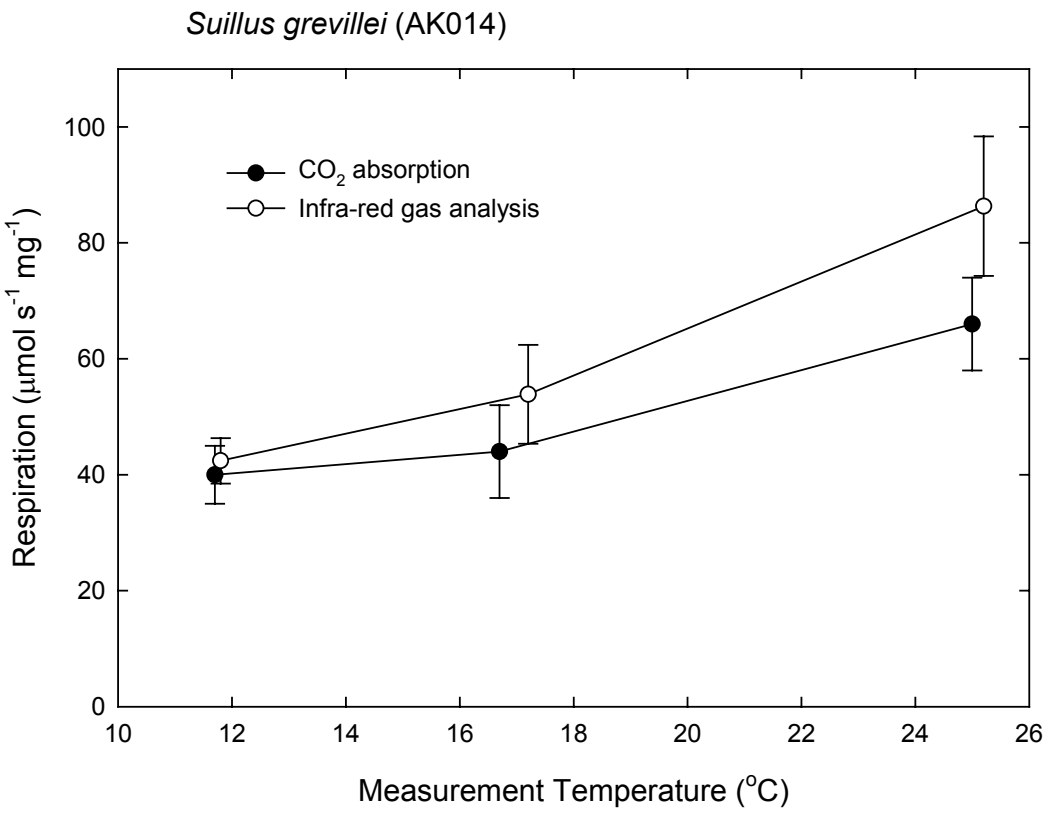

Fig. **2.6**: Respiration rates of *Suillus grevillei* (AK014) grown at 20ºC, determined using two methods including the CO_2 absorption method employing NaOH traps and the proposed method based on infra-red gas analysis.

Temperature-Respiration Curves:

Respiration rates by several ECM fungi, collected in a red pine plantation in State College, were measured at a variety of temperatures to obtain their respiration responses to temperature. Replicate cultures of *Amanita pantherina* [SC004], *Suillus intermedius* [BX007], *Lactarius yazooensis* [SC001], and *Boletus projectillus* [BX005] were allowed to reach steady-state respiration rates in the gas-exchange instrument at 17°C, each species on a different day. Respiration rates were measured first at 17°C, then at

temperatures below that to as low as 9^{o}C, again at 17^{o}C to check for damage, then at several temperatures above that to as high as 30^{o}C. Stable respiration measurements have been measured on the gas-exchange instrument to as low as 5^{o}C and as high as 40^{o}C (data not shown); this varied by species ability to maintain stable respiration rates at the extreme ends of the temperature curves.

In general, respiration rates by *A. pantherina, S. intermedius, L. yazooensis,* and *B. projectillus* showed temperature dependence (Fig. **2.7**). Within each species (i.e. replicate cultures), different variability around the means was shown for respiration rates at particular temperatures.

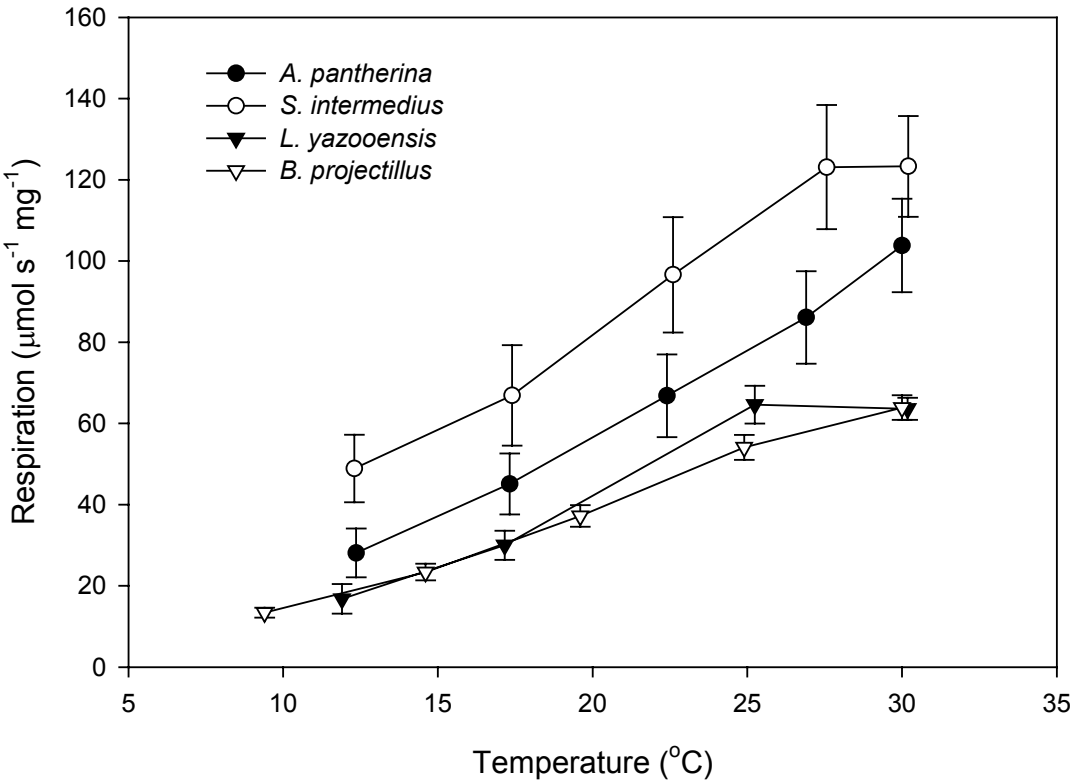

Fig. **2.7**: Average respiration (+/- SE) by PA-ECM fungi in culture was measured at a variety of temperatures up to 30°C. Measurements became unstable at temperatures greater than 30°C, and are thus omitted.

The respiration responses to temperature by decomposer microorganisms can be viewed in Chapter 5.

Discussion

We thoroughly tested our microbial gas exchange instrument with several different species of ectomycorrhizal fungi. We are confident that we could detect when we were measuring steady state, reversible rates of respiration on separate sets of ECM

fungal cultures. We also determined the duration of time needed in order to change temperature 5^{o}C and again attain steady-state respiration values. Finally, respiration rates obtained with our microbial gas-exchange instrument displayed a similar response to temperature as those attained by a CO_2 absorptive method. The rates deviated some as temperature increased, but this is most likely due to the fact that the gas-exchange respiration values are from a dynamic method, while the CO_2 absorptive respiration values are from a static method. Other studies have found that dynamic respiration measurements are generally higher than those made with CO_2 traps (Nay *et al.*, 1994; Bryla *et al.*, 2001); this is probably due to the fact that the absorption by NaOH is rarely in equilibrium with the rate of CO_2 efflux from respiring tissues. Typically, obtaining respiration rates of microorganisms from a single set of cultures at several temperatures on our gas exchange instrument would take less than five hours after steady-state conditions are attained. In this alternate case, the representative CO_2 absorption-based technique chosen stretched the experiment into a three-day affair and forced us to measure respiration on separate batches of mycorrhizal fungal cultures.

The ability to measure decomposer microorganisms and ectomycorrhizal fungi separately from each other and separately from roots is likely to be important because they may have distinct effects on the soil C pool, and because they may respond to temperature in different ways. Respiration by mycelia of mycorrhizal fungi in symbiosis with host plants have been determined from CO2 efflux from root-free hyphal chambers (Söderstrom & Read, 1987; Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003) but these methods were not used to study the effects of temperature on respiration. There may still be some concern about the relevance of respiration rates of ectomycorrhizal

fungi measured when grown apart from their natural host plants. Since some ectomycorrhizal fungi have been shown to have saprotrophic capacity, the temperature response of mycorrhizal fungi when growing apart from host plants is an important consideration. Additionally, substrate availability can affect respiration rates (Atkin *et al.*, 2000; Davidson & Janssens, 2006) by microorganisms. Examining respiration by cultured ectomycorrhizal fungi allowed us to avoid a potential confound with preferential host transfer of carbon to certain species of ectomycorrhizal fungi over others.

Our ECM fungal respiration measurements appear relevant to what might be measured when in symbiosis. Measures of respiration using *in vitro* cultures of ectomycorrhizal fungi were in the range of magnitude of our reported respiration rates: 10 to 16 μmol CO_2 mg^{-1} dry weight s^{-1} at 31°C (Taber & Taber, 1987); 50 to 70 μmol O_2 mg^{-1} dry weight s^{-1} at 25°C (Souto *et al.*, 2000). Our measured *in vitro* rates of respiration also matched closely with those from the few studies that have measured respiration on mycorrhizal roots. Respiration by fungal mycelium in compartmentalized microcosm experiments measured by Söderstrom & Read (1987) and Bååth & Wallander (2003) do not standardize their rates by fungal biomass and were compared on a relative basis only. But, the respiration rates of *Hebeloma crustuliniforme* attached to living roots of *Pinus ponderosa* grown in microcosms as measured by Rygiewicz & Andersen (1994) and Andersen & Rygiewicz (1995) were in the range of 11 to 16 μmol CO_2 mg^{-1} dry weight s^{-1} at 24°C, which agrees with rates we have reported.

It is much more difficult to measure mycorrhizal respiration in the field. But, we can crudely determine whether our reported values of respiration seem reasonable for ectomycorrhizal fungi in the field. Assuming that for respiration the ratio of

ectomycorrhizal mycelium to root plus mycorrhizal fungus is 0.242 (the mean values reported by Rygiewicz and Andersen, 1994 and Söderstrom and Read, 1987) and that the root plus mycorrhizal fungus respiration rate in boreal forest is 70 mg C m^{-2} h^{-1} (Bhupinderpal-Singh *et al.*, 2003), the ectomycorrhizal mycelium respiration rate would be 16.9 mg C m^{-2} h^{-1} (0.242 x 70). If we then assume that the total living biomass of ectomycorrhizal fungal hyphae in boreal forest is 163 kg ha-1 (the mean of 125 and 200 kg ha-1, estimates of yearly production of ectomycorrhizal hyphae, (Wallander *et al.*, 2001), then the ectomycorrhizal fungal respiration rate by hyphae would be 24 μmol CO_2 mg^{-1} dry weight s^{-1}. This agrees well with the values we reported.

If you compare respiration by ectomycorrhizal fungi to that by plant roots, the rates by ECM fungi are within the same order of magnitude. Huang et al. (2005) reported respiration rates between 10 and 40 μmol CO_2 mg^{-1} dry weight s^{-1} between 10 and 30 ^{o}C for Concord grape (*Vitus labruscana* Bailey) roots. Similarly, Cooper (2004) measured respiration rates between 5 and 25 μmol CO_2 mg^{-1} dry weight s^{-1} between 5 and 15 ^{o}C for several different species of arctic buttercups in the genus *Ranunculus*. Bryla et al. (2001) made a comparison between different root stocks of citrus that were acclimated or not acclimated to temperature and found respiration rates between 4 and 10 μmol CO_2 mg^{-1} dry weight s^{-1} between 5 and 40 ^{o}C for acclimated roots and respiration rates between 4 and 23 μmol CO_2 mg^{-1} dry weight s^{-1} between 5 and 40 ^{o}C for non-acclimated roots.

Many researchers have looked for evidence of adaptation to temperature in terms of the respiratory activity of leaves among different plant species (Mooney & Billings, 1961; Larigauderie & Korner, 1995; Arnone & Korner, 1997; Oleksyn *et al.*, 1998; Sommer & Portner, 2004). Tests of adaptation of soil microorganisms generally have not

been made, but this system could be used for such a purpose (see Chapter 4). Hacskaylo *et al.* (1965) found that different species of ectomycorrhizal fungi exhibited their maximum respiration rates at different temperatures, suggesting that there are genetically based differences in response to temperature.

Many researchers have also demonstrated acclimation, or physiological, reversible changes, in response to temperature of leaf respiration of a single plant species (Bryla *et al.*, 1997; Tjoelker *et al.*, 1999; Atkin *et al.*, 2000; Covey-Crump *et al.*, 2002; Atkin & Tjoelker, 2003; Bolstad *et al.*, 2003; Loveys *et al.*, 2003; Cooper, 2004). There is some evidence that soil microorganisms responsible for decomposition also acclimate to temperature, but the evidence is based on bulk soil respiration without the ability to distinguish among roots, mycorrhizal fungi, and decomposer microorganisms (Luo *et al.*, 2001). The ability to distinguish between these will help us better predict the effects of soil warming on the size of the soil C pool.

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Chapter 3

Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi

Abstract

Ectomycorrhizal (ECM) fungi contribute significantly to ecosystem respiration, but little research has addressed the effect of temperature on ECM fungal respiration. Plants have the ability to acclimate to temperature such that long-term exposure to warmer conditions slows respiration at a given measurement temperature and long-term exposure to colder conditions increases respiration at a given measurement temperature. We examined acclimation to temperature and temperature sensitivity (Q_{10}) of respiration by ECM fungi by incubating them for a week at one of three temperatures and measuring respiration over a range of temperatures. Out of 12 ECM fungal isolates, *Suillus intermedius*, *Cenococcum geophilum,* and *Lactarius* cf. *pubescens* exhibited significant acclimation to temperature exhibiting an average reduction in respiration of 20-45% by 23^{o}C -incubated cultures as compared to 11 or 17^{o}C-incubated cultures. ECM fungal isolates differed significantly in their Q_{10} values, which ranged from 1.67 to 2.56. We also found that half of the isolates significantly increased Q_{10} with an increase in incubator temperature by and average of 15%. We conclude that substantial variation exists among ECM fungal isolates in their ability to acclimate to temperature and in their sensitivity to temperature. As soil temperatures increase, ECM fungi that acclimate may require less carbon from their host plants than fungi that do not acclimate. The ability of

some ECM fungi to acclimate may partially ameliorate the anticipated positive feedback between soil respiration and temperature.

Introduction

Ectomycorrhizal fungi (ECM) form a mutualistic relationship with forest trees in boreal, temperate, and tropical ecosystems over a large portion of the earth's land surface (Smith & Read, 1997). Although some ECM fungi can access soil carbon from organic sources (Dighton *et al.*, 1987; Colpaert & van Tichelen, 1996; Hobbie *et al.*, 1999; Read & Perez-Moreno, 2003), most are most are considered biotrophic (Smith & Read, 1997; Wallander *et al.*, 2006) as evidenced by the fact that following host tree girdling fungal sporocarp production is essentially eliminated (Högberg et al. 2001). A large proportion of photosynthate is allocated belowground to roots and their associated symbionts, including mycorrhizal fungi (Lambers *et al.*, 1996; Smith & Read, 1997). The carbon demand by mycorrhizal fungi may be quite large. In some ecosystems their mycelia may comprise one-third of total microbial biomass (Högberg & Högberg, 2002), approximately the same biomass as fine roots (Wallander *et al.*, 2001), and input of C into the soil by ectomycorrhizal mycelia may exceed that by leaf litter and fine root turnover (Godbold *et al.*, 2006). Respiration by ectomycorrhizal fungi, needed for both growth and maintenance, can therefore be substantial (Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003).

Global surface temperatures are predicted to increase between 1.8-3.6^{o}C by the year 2100, driven by an increase in atmospheric CO_2 derived from natural and

anthropogenic sources (IPCC, 2007). In terrestrial ecosystems there is some concern that increasing atmospheric CO_2 concentrations will positively feedback through warming temperatures if respiration is more sensitive to temperature than photosynthesis (Allen & Amthor, 1995; Boone *et al.*, 1998; Davidson & Janssens, 2006; Trumbore, 2006). The major contributors to terrestrial ecosystem respiration are plants, mycorrhizal fungi, and decomposer microorganisms. Under warming scenarios, respiration is assumed to increase with temperature (Cox *et al.*, 2000; Rustad *et al.*, 2001; Atkin *et al.*, 2005), but the sensitivity to temperature change may not be equivalent for different contributors to ecosystem respiration (Boone *et al.*, 1998; Bhupinderpal-Singh *et al.*, 2003). This suggests that understanding the response of respiration to temperature by individual contributors is essential if we will ever be capable of predicting climate-induced change in respiration in different ecosystems. Ectomycorrhizal fungi make large contributions to ecosystem respiration (Söderstrom & Read, 1987; Rygiewicz & Andersen, 1994; Högberg & Högberg, 2002; Bååth & Wallander, 2003), but little is known regarding their respiratory responses to temperature (Hacskaylo *et al.*, 1965).

Temperature acclimation of respiration has been well documented for plants (Mooney, 1963; Chapin & Oechel, 1983; Arnone & Korner, 1997; Bryla *et al.*, 1997; Atkin *et al.*, 2000; Covey-Crump *et al.*, 2002; Loveys *et al.*, 2003; Cooper, 2004). Typically, warm-acclimated plants exhibit slower rates of respiration than cool-acclimated plants at a common measurement temperature (Atkin & Tjoelker, 2003). Whether or not ECM fungi acclimate to temperature is unknown, but their capacity to do so would influence both their contribution to atmospheric CO_2 via respiration and their demand for carbon from individual hosts. Temperature sensitivity (Q_{10}) of respiration

has been shown to differ among plant species, among plant parts (i.e. leaves vs. roots) and in response to differences in growth conditions and/or physiological status of tissues (Azcon-Bieto, 1992; Larigauderie & Korner, 1995; Atkin *et al.*, 2000; Tjoelker *et al.*, 2001; Covey-Crump *et al.*, 2002). We do not know the extent to which temperature sensitivity of respiration by ECM fungi differs among species or in response to temperature acclimation. Our goal, therefore, was to determine whether isolates of ECM fungi varied in their ability to acclimate to temperature and in their sensitivity to temperature change. We compared respiration rates over a range of measurement temperatures of ECM fungal cultures that were held at different temperatures for one week. If temperature acclimation were to occur, we would expect it to happen within a week because plants have typically exhibited acclimation within 1-3 days (Bryla *et al.*, 2001; Atkin & Tjoelker, 2003; Bolstad *et al.*, 2003). There is considerable controversy whether Q_{10} changes with acclimation in plants (Covey-Crump *et al.*, 2002; Atkin & Tjoelker, 2003; Cooper, 2004) and, thus, we are interested in assessing whether or not there is a Q_{10} shift change for ECM fungi. To our knowledge, this is the first study to address whether ECM fungi are capable of acclimation to temperature and how acclimation to temperature might affect the temperature sensitivity of respiration.

Differences in substrate availability can affect an organism's ability to acclimate and its temperature sensitivity (Atkin & Tjoelker, 2003; Davidson & Janssens, 2006). *In symbio*, ECM fungal isolates may differ one from another in carbon supply because of the potential for preferential carbon transfer from certain hosts to certain fungal isolates. Moreover, growth in natural soils could also lead to variability in substrate availability for the fungi because of potential variability among isolates in their ability to acquire C

from organic sources in the soil (Dighton *et al.*, 1987; Colpaert & van Tichelen, 1996; Hobbie *et al.*, 1999; Read & Perez-Moreno, 2003). Therefore, in this study we utilized ECM fungi growing in axenic cultures.

Methods

Biological material:

Sporocarps of *Amanita muscaria* var. *muscaria* (AK001), *Lactarius* cf. *pubescens* (AK010), and *Suillus* cf. *grevillei* (AK014), *Leccinum* cf. *alaskanum* (GL005), and *Amanita muscaria* var. *muscaria* (GL015) were collected in the summer of 2002 near Fairbanks AK (**64.5 N, 147.5 W**). Sporocarps of *Suillus intermedius* (BX007), *Amanita muscaria* var. *formosa* (BX008), *Lactarius sp.* (SC003), *Leccinum aurantiacum* (SC014), *Lactarius chrysorrheus* (SC016), and *Amanita citrina* (SC070) were collected in the summer of 2002 in State College, PA (**40.8 N, 77.9 W**). *Cenococcum geophilum* (SC032) was isolated from a sclerotium from the same location in PA. Fungal isolate designations, specific locations of collections, and the vegetation are given in Table **3.1**. Fungal cultures were isolated and maintained on a modified potato dextrose agar (19.5 g potato dextrose agar, Difco: Benton, Dickinson & Co., Sparks, MD, USA, 7.5 g Bacto agar, Difco, and 0.375 g NH_4Cl per liter of water).

Table **3.1**: Details of collections of the 12 isolates of ectomycorrhizal fungi. The family Russulaceae contains isolates: AK010, SC003, and SC016. The family Amanitaceae contains isolates: AK001, GL015, BX008, and SC070. The family Boletaceae contains isolates: AK014, GL005, BX007, and SC014. The family Elaphomycetaceae contains the isolate: SC032.

ECM Fungal Species	**Isolate**	**Collected**	**Vegetation**
Amanita muscaria var. *muscaria*	AK001	Chatanika, AK	*Betula papyrifera, Populus balsamifera*
Lactarius cf. *pubescens*	AK010	Chatanika, AK	*B. papyrifera, P. balsamifera*
Suillus cf. *grevillei*	AK014	Banana Creek, AK	*Picea mariana*
Leccinum cf. *alaskanum*	GL005	South Fairbanks, AK	*B. papyrifera, P. balsamifera, Picea glauca*
Amanita muscaria var. *muscaria*	GL015	South Fairbanks, AK	*B. papyrifera, P. balsamifera, Picea glauca*
Suillus intermedius	BX007	State College, PA	*Pinus resinosa*
Amanita muscaria var. *formosa*	BX008	State College, PA	*Pinus resinosa*
Lactarius sp.	SC003	State College, PA	*Quercus alba, Q. rubra*
Leccinum aurantiacum	SC014	State College, PA	*Pinus resinosa*
Lactarius chrysorrheus	SC016	State College, PA	*Quercus alba, Q. rubra*
Cenococcum geophilum	SC032	State College, PA	*Pinus resinosa*
Amanita citrina	SC070	State College, PA	*Pinus resinosa*

Incubator temperature shifts:

Isolates were subcultured into 15 separate 100 × 15 mm Petri dishes containing modified potato dextrose agar. Four fungal plugs were placed into each Petri dish in order to increase the rate of new growth. All isolates from Alaska and Pennsylvania were initially maintained in incubators at 11 ºC and 17 ^{o}C, respectively, which are representative of the average temperatures of litter during the fungal fruiting season. Between 21 July and 21 August 2002, the mean temperature for the litter under *Picea mariana* in the Bonanza Creek LTER site was 11.5^{o}C (Vogel & Valentine, 2006).

Between 1 June and 31 October 2002, the mean temperature for the litter under *Pinus resinosa* in State College was 16.4^{o}C (Malcolm *et al*., unpublished data). We waited a variable amount of time (from one to three weeks) for each plug to increase in diameter approximately 2-4 mm before initiating the temperature shift. For each isolate and irrespective of the original temperature of growth, five Petri dishes each were placed into three separate incubators set to 11, 17 and 23 ºC for a period of seven days. Following the temperature shifts, respiration was measured on each isolate over a range of measurement temperatures.

Measuring respiration rates:

We constructed a temperature-controlled gas-exchange system with which to measure respiration rates. The system (Fig. **3.1**) included a temperature-controlled steel surface on which are affixed eight-polycarbonate gas exchange chambers. Each chamber could be individually placed in line with a LI-6200 gas-exchange system (LI-COR Biosciences, Lincoln, NE, USA) to determine the rate of change of CO_2 concentration (flow path 2a, 2b). Preliminary testing demonstrated that humidity remained constant throughout a measurement. Thus, in making the calculation to determine CO_2 exchange, we had no need to consider water vapor concentrations. When not in line with the LI-6200, all chambers received humidified, outside air supplied by a diaphragm pump (model DOA-O704-AA, Gast Manufacturing, Inc., Benton Harbor, MI, U.S.A.) and the air from all chambers was vented into the laboratory (flow path 1a and 1b). This airflow was maintained at the same rate as in the LI-6200 path using needle valves (model A-

06393-70, Cole Parmer, Vernon Hills, IL, USA). Bev-a-line tubing (0.32 cm i.d., Thermoplastic Processing, Stirling, NJ, USA) was used throughout. A three-way nylon miniature ball valve (McMaster Carr, New Brunswick, NJ, USA) was used to switch between open (outside air) and closed (LI-6200) flow paths.

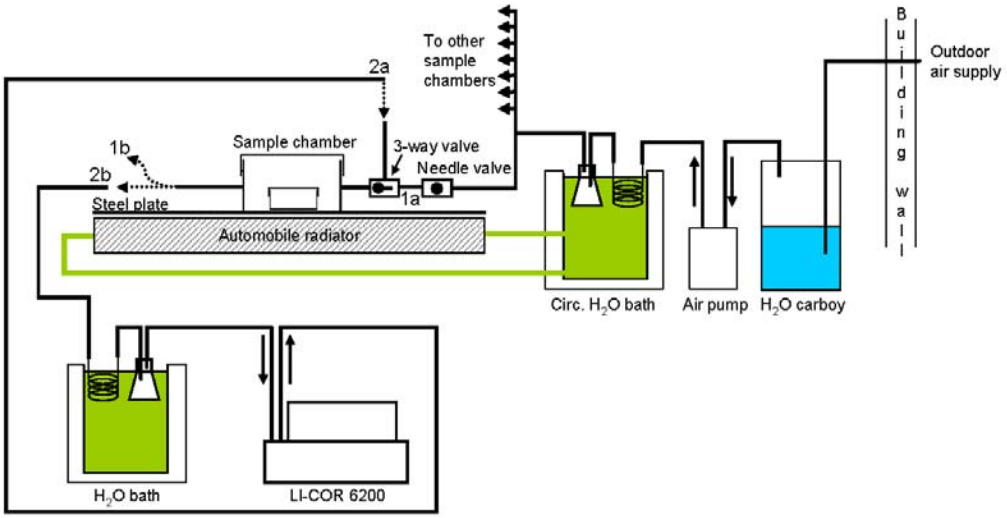


Fig. **3.1**: Schematic drawing of the gas exchange and temperature control apparatus (adapted from Huang *et al.*, 2005). In the open flow path (1a and 1b), air from the outdoors is humidified and then brought to the temperature of the chambers. After it enters the chambers it passes out into the laboratory. In the closed flow path (2a and 2b) used for measurement of respiration, the chambers are placed in line with the LI-COR 6200 gas analyzer. Temperature control of fungal cultures is achieved by the automobile radiator atop which sit the sample chambers. The radiator temperature is maintained by the circulating water bath.

Individual cultures were initially placed in gas exchange chambers set to the temperature corresponding to their incubator. Preliminary testing revealed that because of absorption of CO_2 by the gel in the dish while in the incubators, at least an 8-hour period was needed at the start of a series of respiration measurements to attain steady-state CO_2 efflux rates. Following attainment of steady state, the LI-6200 was

programmed to calculate a respiration rate during four consecutive 1 - 4 µl l^{-1} CO_2 changes, depending on the rate of respiration. Following measurement of respiration rates at the initial temperature, the temperature of the gas exchange chambers was altered by 6°C and the fungi were allowed a period of 50 minutes to attain new steady state respiration rates before respiration was measured again. As a check for hysteresis, for each Petri dish we determined whether the initial respiration rate could be duplicated when the temperature was restored to the initial value. If it could not be duplicated, we considered the tissue to have been irreversibly damaged and the data at the damaging temperatures were not used.

Following respiration measurements, fungal tissues were separated from the agar by melting it in a boiling water bath and filtering out the tissue. Tissue weights were determined following drying (60°C) until constant weight was achieved. Respiration rates are expressed as CO_2 flux divided by tissue dry weight.

Acclimation time course:

We subcultured *Suillus intermedius* (BX007) into numerous Petri dishes and incubated them at 17°C for 9 days. All but three of the dishes were shifted into the 23°C incubator. For those three dishes we measured respiration rates at 17ºC and, 50 min later, at 23°C. Each day for one week after the initial respiration measurements were made, we measured respiration at 23°C on a distinct subset of three Petri dishes that were being incubated at 23ºC. We determined dry weights for each subset following the respiration

measurements. We then fit a third order polynomial to the plot of respiration vs. days to characterize the time course of acclimation.

Temperature Sensitivity (Q_{10}):

Q_{10} values were calculated using the following equation: $Q_{10} = 10^{(10 \times \text{regression slope})}$, where the regression slope is that from a plot of $\log_{10}$ (Respiration) vs. temperature (Atkin *et al.*, 2000). Q_{10} may be temperature-dependent, particularly at low temperatures (Atkin & Tjoelker, 2003). We found Q_{10} to be nearly constant within the 11-23 ^{o}C-range; the regression slope in that range was essentially linear. Q_{10} values were calculated from individual measurement temperature respiration response curves (n = 5 per isolate per incubator).

Statistical Analyses:

Because we measured respiration rates on the same set of cultures at a variety of measurement temperatures for each ECM fungal isolate, we analyzed respiration rate using a Repeated Measures Proc Mixed ANOVA model in SAS (SAS Institute Inc. Version 9.1, 2002-2003). We used the Compound Symmetric (CS) covariance structure for the repeated measures procedure in SAS, which resulted in the lowest values for the Akaike information and Bayesian information criteria. The model for average respiration for all ECM fungal isolates included as factors, isolate, incubator temperature, and measurement temperature. Models for individual ECM fungal species included incubator

and measurement temperature as factors. Least square means for all respiration rates were estimated by the statistical model and used in all subsequent analyses. A significant incubator temperature effect indicated that respiration acclimated to different incubator temperatures when respiration rates were lower for warm-exposed fungi than for cool-exposed fungi. Proc GLM procedures in SAS were used for comparisons among incubator temperatures for Q_{10} values.

Results

Respiration - Measurement Temperature Response Curves:

The respiration rates for *Leccinum* cf. *alaskanum* (GL005) and *Amanita muscaria* var. *muscaria* (AK001) demonstrated hysteresis and/or abnormal growth when incubation occurred at 23 ºC. Therefore, the respiration rates for those isolates at that incubator temperature were not considered further. When all 12 isolates were analyzed together, we found a significant isolate × incubator temperature × measurement temperature interaction (Table **3.2**). We therefore analyzed each isolate separately. The respiration rates of all isolates were significantly increased by measurement temperature (Fig. **3.2**, Table **3.3**). Three of the twelve isolates exhibited acclimation to temperature as evidenced by significantly higher rates of respiration for colder-incubated fungi compared to warmer-incubated fungi. In the case of *L.* cf. *pubescens* (AK010), the respiration rates for 23ºC-incubated cultures were significantly lower (Tukey Honest Significant Difference test) by an average of 20 % compared to either 11º- or 17ºC-incubated cultures (Fig. **3.2**). In the case of *S. intermedius* (BX007), the respiration rates were significantly lower by an average of 48 % when incubated at 23ºC than at 11^{o}C, but not significantly different from when incubated at 17^{o}C (Fig. **3.2**). The difference in respiration rate when incubated at different temperatures increased with measurement temperature, explaining the significant interaction between incubator temperature and measurement temperature (Table **3.3**). In the case of *C. geophilum* (SC032), there was a

significant interaction between measurement and incubator temperature (Table **3.3**, Fig. **3.2**) such that the expected difference in respiration rate between warmer- and colder-incubated fungi (evidence for acclimation) occurred only at the highest measurement temperature, as shown by a 31 % average reduction in respiration from the 11 and 17^{o}C-incubated cultures compared to the 23^{o}C-incubated cultures. None of the other isolates exhibited significantly higher rates of respiration for colder-incubated fungi compared to warmer-incubated fungi.

Table **3.2**: Results of analysis of variance for respiration rate as affected by incubator temperature, measurement temperature, and ECM fungal isolate (n = 5).

Effects	**Num. d.f.**	**Den. d.f.**	**F-value**	**P-value**
Incubator T	2	139	8.14	0.0005
Measurement T	2	278	5754.62	<0.0001
Isolate	11	139	37.51	<0.0001
Incubator T × Measurement T	4	278	1.54	0.1902
Isolate × Measurement T	22	278	58.13	<0.0001
Isolate × Incubator T	22	139	1.42	0.1162
Isolate × Incubator T × Measurement T	44	278	2.98	<0.0001

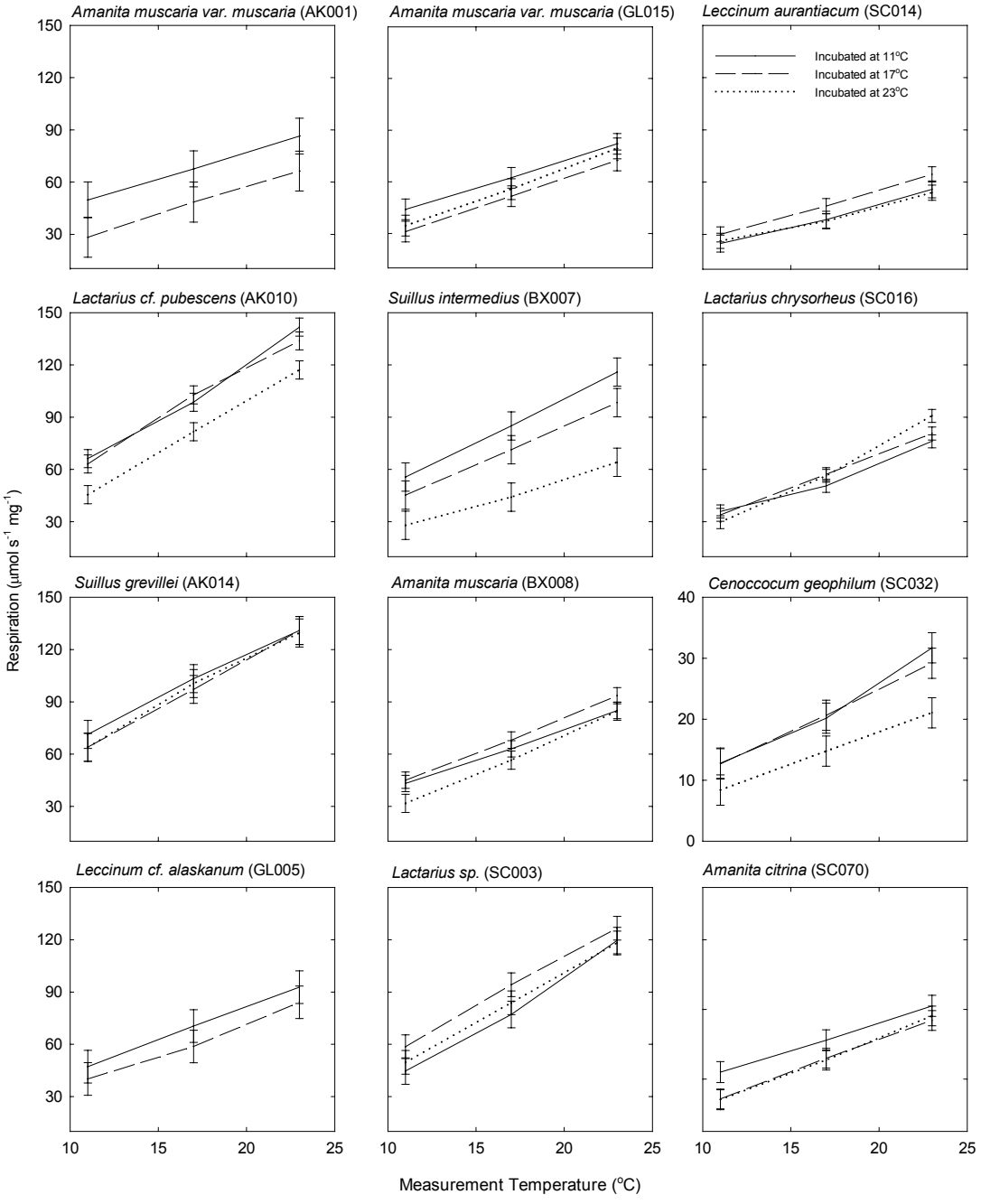


Fig. **3.2**: Mean (±1 s.e.) ECM fungal respiration rates measured at temperatures between 11 and 23 °C after being grown at three incubation temperatures.

Table **3.3**: Results of analysis of variance for respiration rate for each ECM fungal isolate as affected by incubator temperature and measurement temperature (n = 5).

ECM Fungal Species (Isolate)	Effects	Num. d.f.	Den. d.f.	F-value	P-value
A. muscaria var. *muscaria*	Incubator T	1	7	1.75	0.2774
(AK001)	Measurement T	2	14	213.96	<0.0001
	Incubator T × Measurement T	2	14	0.23	0.1700
L. cf. *pubescens*	Incubator T	2	12	5.09	0.0250
(AK010)	Measurement T	2	24	1769.82	<0.0001
	Incubator T × Measurement T	4	24	4.34	0.0080
S. cf. *grevillei*	Incubator T	2	12	0.10	0.9091
(AK014)	Measurement T	2	24	821.20	<0.0001
	Incubator T × Measurement T	4	24	1.42	0.2564
L. cf. *alaskanum*	Incubator T	1	8	0.49	0.5029
(GL005)	Measurement T	2	16	331.16	<0.0001
	Incubator T × Measurement T	2	16	0.91	0.4220
A. muscaria var. *muscaria*	Incubator T	2	12	0.92	0.4224
(GL015)	Measurement T	2	24	339.50	<0.0001
	Incubator T × Measurement T	4	24	0.80	0.5383
S. intermedius	Incubator T	2	12	6.66	0.0114
(BX007)	Measurement T	2	24	364.25	<0.0001
	Incubator T × Measurement T	4	24	7.44	0.0005
A. muscaria var. *formosa*	Incubator T	2	11	1.44	0.2774
(BX008)	Measurement T	2	22	396.43	<0.0001
	Incubator T × Measurement T	4	22	1.77	0.1700
Lactarius sp.	Incubator T	2	11	1.30	0.3123
(SC003)	Measurement T	2	22	717.23	<0.0001
	Incubator T × Measurement T	4	22	2.85	0.0484

L. aurantiacum (SC014)	Incubator T	2	11	1.00	0.3996
	Measurement T	2	11	433.28	<0.0001
	Incubator T × Measurement T	4	22	2.02	0.1274
L. chrysorrheus (SC016)	Incubator T	2	12	0.46	0.6445
	Measurement T	2	12	1164.26	<0.0001
	Incubator T × Measurement T	4	24	19.55	<0.0001
C. geophilum (SC032)	Incubator T	2	12	2.48	0.1254
	Measurement T	2	12	253.23	<0.0001
	Incubator T × Measurement T	4	24	3.82	0.0154
A. citrina (SC070)	Incubator T	2	12	2.42	0.1311
	Measurement T	2	12	227.39	<0.0001
	Incubator T × Measurement T	4	24	0.25	0.9087

Acclimation time course

Suillus intermedius (BX007) acclimated over the course of several days, reaching full acclimation by day six, as shown by the slowing of respiration at the 23^{o}C measurement temperature over time (Fig. **3.3**).

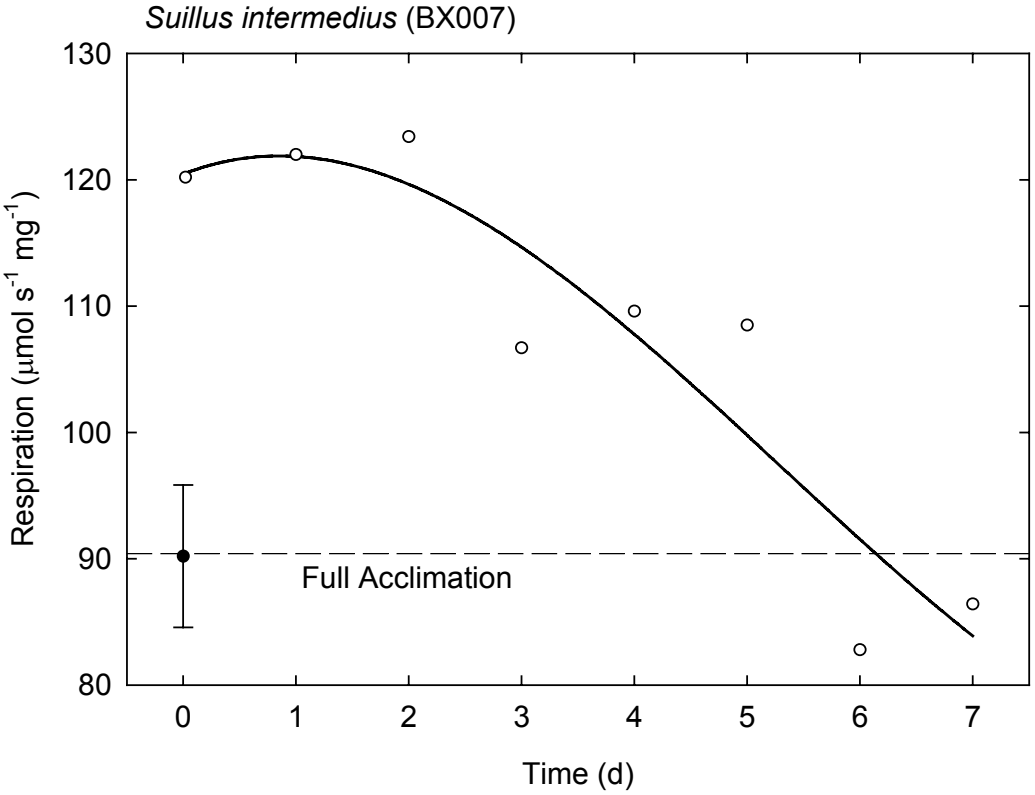


Fig. **3.3**: Modeled ECM fungal respiration rates ($0.1398(x^3) - 2.222(x^2) + 3.479(x) + 120.4$; $r^2 = 0.86$) measured at 23°C following a shift in incubator temperature from 17° to 23°C over the course of a week (n = 3). The solid circle with standard error bars is the mean respiration rate at 17°C on the initial day, and serves as a reference for full acclimation. The open circles represent the actual average respiration rate on separate batches of cultures every 24 hours for a week.

Q_{10}

The significant isolate × incubator temperature interaction (Table **3**.**4**) revealed variability among isolates in the response of Q_{10} to incubator temperature. For the six isolates exhibiting a significant change in Q_{10} with incubator temperature, the average Q_{10} increased by 14 %, 16 %, and 16 % as incubator temperature increased from 11 to 17^{o}C, 17^{o}C to 23^{o}C, and 11 to 23^{o}C, respectively (Table **3**.**5**). Additionally, within an incubator temperature, ECM fungal isolates showed significant variability in Q_{10} (Fig. **3**.**4** A, B, C), ranging from 1.67 to 2.56.

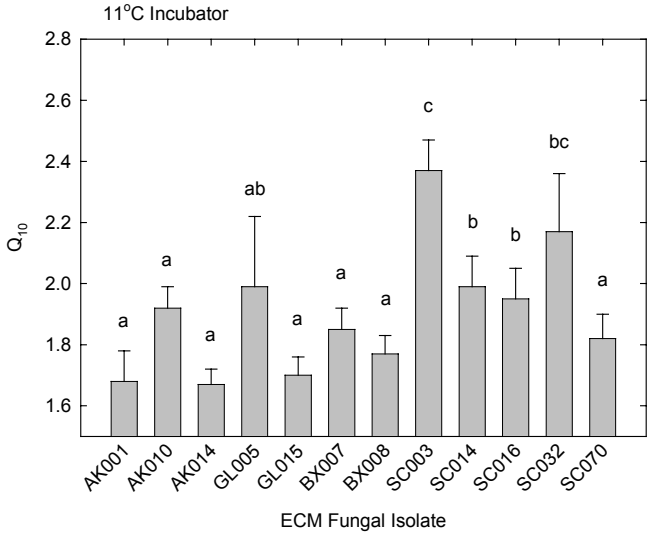
Table **3.4**: Result of analysis of variance for Q_{10} values as affected by incubator temperature and isolate (n = 5). Q_{10} values were calculated for the 11-23^{o}C-measurement temperature range.

Effects	**d.f.**	**Sum of squares**	**F-value**	**P-value**
Incubator T	2	3.02	4.65	<0.0001
Isolate	11	1.67	14.16	<0.0001
Isolate x Incubator T	22	2.87	2.21	0.0031
Error	139	8.22	~	~

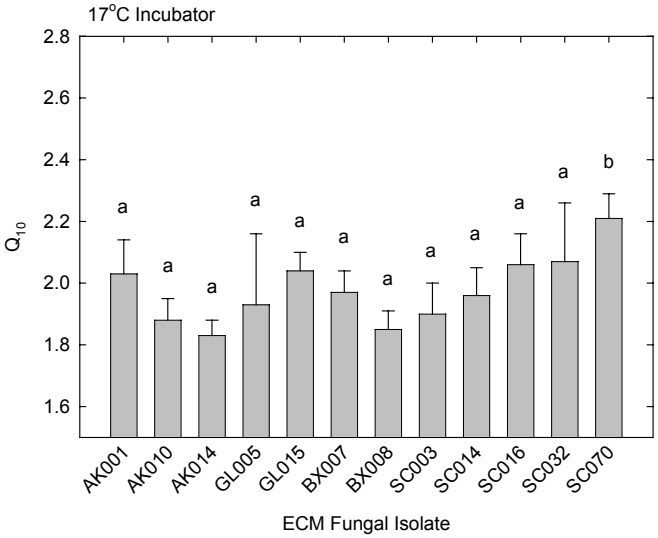
Table **3.5**: Mean Q_{10} (s.e.) for each of three incubator temperatures for each ECM fungal isolate are reported. Significantly different Q_{10} values for incubators for each species are denoted with different letters ($p < 0.05$), taken from a post-hoc Tukey's Honest Significant Different Test.

Isolate	Incub. temp.	Q_{10}
A. muscaria var. *muscaria* (AK001)	11	**1.68** (0.10)
	17	**2.03** (0.11)
	23	
L. cf. *pubescens* (AK010)	11	**1.92** (0.07) ***a***
	17	**1.88** (0.07) ***a***
	23	**2.21** (0.07) ***b***
S. cf. *grevillei* (AK014)	11	**1.67** (0.05)
	17	**1.83** (0.05)
	23	**1.82** (0.05)
L. cf. *alaskanum* (GL005)	11	**1.99** (0.23)
	17	**1.93** (0.23)
	23	
A. muscaria var. *muscaria* (GL015)	11	**1.70** (0.06) ***a***
	17	**2.04** (0.06) ***b***
	23	**2.02** (0.06) ***b***
S. intermedius (BX007)	11	**1.85** (0.07)
	17	**1.97** (0.07)
	23	**2.00** (0.07)
A. muscaria var. *formosa* (BX008)	11	**1.77** (0.06) ***a***
	17	**1.85** (0.06) ***b***
	23	**2.27** (0.06) ***c***
Lactarius sp.(SC003)	11	**2.37** (0.10) ***b***
	17	**1.90** (0.10) ***a***
	23	**2.39** (0.10) ***bc***
L. aurantiacum (SC014)	11	**1.99** (0.10)
	17	**1.96** (0.09)
	23	**1.82** (0.09)
L. chrysorrheus (SC016)	11	**1.95** (0.10) ***a***
	17	**2.06** (0.10) ***a***
	23	**2.56** (0.10) ***b***
C. geophilum (SC032)	11	**2.17** (0.19)
	17	**2.07** (0.19)
	23	**2.28** (0.19)
A. citrina (SC070)	11	**1.82** (0.08) ***a***
	17	**2.21** (0.08) ***b***
	23	**2.31** (0.08) ***b***

A)


11°C Incubator
Q10
2.8
2.6
2.4
2.2
2.0
1.8
1.6
a
a
a
ab
a
a
a
c
b
b
bc
a
AK001
AK010
AK014
GL005
GL015
BX007
BX008
SC003
SC014
SC016
SC032
SC070
ECM Fungal Isolate

B)


17°C Incubator
Q10
2.8
2.6
2.4
2.2
2.0
1.8
1.6
a
a
a
a
a
a
a
a
a
a
a
b
AK001
AK010
AK014
GL005
GL015
BX007
BX008
SC003
SC014
SC016
SC032
SC070
ECM Fungal Isolate

C)

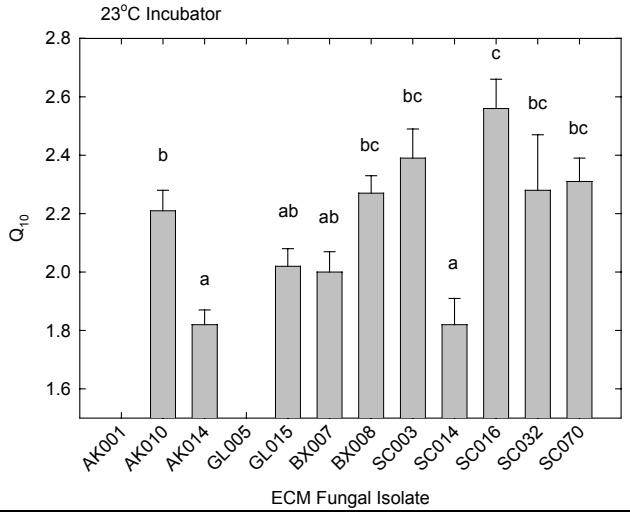


Fig. **3.4**: Mean Q_{10} (s.e.) for each ECM fungal isolate at incubator temperatures of 11°C (a), 17°C (b), and 23°C (c). Q_{10} values were calculated for the 11-23°C-measurement temperature range. Significantly different Q_{10} values for a given incubator temperature for each species are denoted with different letters ($p < 0.05$), determined from a post-hoc Tukey's Honest Significant Different Test.

Discussion

Hacskaylo et al. (1965) previously demonstrated the dependence of respiration on measurement temperature by ECM fungi but, as far as we are aware, this is the first study to address whether or not acclimation to temperature occurs. Our data show that significant variation exists among isolates of ECM fungi in their ability to acclimate to temperature. Three out of twelve ECM fungal isolates (*S. intermedius* and *C. geophilum* from PA and *L.* cf. *pubescens* from AK) exhibited significant acclimation. Acclimation to temperature will tend to ameliorate the effect of warming on soil respiration (Luo *et*

al., 2001), to which ECM fungi contribute significantly (Bååth and Wallander 2003; Rygiewicz and Andersen 1994). The fact that variation occurs among ECM fungal species in their ability to acclimate indicates that the response of the ectomycorrhizal fungal community as a whole will be determined by the structure of that community. Therefore, the many environmental factors that influence the composition of ECM fungal communities, such as soil type (Gehring *et al.*, 1998), soil depth (Dickie *et al.*, 2002; Rosling *et al.*, 2003), nitrogen concentration (Treseder & Allen, 2000; Peter *et al.*, 2001; Lilleskov *et al.*, 2002), and time (Koide *et al.*, 2007) will each have an effect on the respiratory response of soil to warming and the degree of positive feedback between temperature and soil respiration. Whether or not particular ECM fungal isolates in communities acclimate to temperature can greatly influence the respiratory response of the community as a whole because it has often been shown that several ECM fungal species dominate particular forest communities. For instance, *Cenococcum geophilum*, an ECM fungus that dominates our red pine (*Pinus resinosa*) plantation study site, represents one of the ECM fungal isolates for which we documented acclimation (Koide et al. 2007).

Of those isolates that did acclimate in this study, one was from Alaska and two were from Pennsylvania. Thus, there was no evidence that latitude of origin had a significant influence on ability to acclimate. We cannot rule out the possibility that seven days was not enough time to permit acclimation in all isolates of ECM fungi. However, our seven-day time-course with *S. intermedius* (BX007) revealed that six days resulted in full acclimation for this isolate, and we allowed seven days for all isolates. Moreover, plants have typically exhibited acclimation within 1-3 days (Bryla *et al.,* 2001; Atkin &

Tjoelker, 2003; Bolstad *et al.*, 2003). We also exposed ECM fungal isolates to different incubators set to constant temperatures, rather than fluctuating day/night temperatures as might be seen in a field setting. It is unclear whether this might influence the ability by ECM fungal isolates to acclimate.

Acclimation can occur for organisms in response to exposure to colder or warmer temperature environments. While it would have been interesting to assess whether Alaska vs. Pennsylvania ECM fungi were more able to acclimate in a certain temperature direction, our experimental design limited us. Many of the ECM fungal isolates were unable to grow well at temperatures below 11^{o}C or above 23^{o}C. Thus, Alaskan ECM fungal isolates were maintained in the 11^{o}C incubator and shifted into two warmer incubators – 17 and 23^{o}C. The Pennsylvanian ECM fungal isolates were maintained in the 17oC incubator and shifted to an 11^{o}C and a 23^{o}C incubator. For the two ECM fungal isolates from Pennsylvania, one acclimated in both temperature directions while one acclimated only to the warmer incubator temperature.

In studies of photosynthetic acclimation to temperature, the plant species that experienced variable temperature regimes during their lives were more likely to exhibit acclimation than those species experiencing more stable temperature regimes (Björkman, 1981). Different species of ECM fungi occupy different niches in space and in time (Dickie *et al.*, 2002; Rosling *et al.*, 2003; Koide *et al.*, 2007). We wonder whether ECM fungi exhibit a similar pattern to plants in that those which experience large variation in temperature, such as those living in surface soil layers or those that are active during a large portion of the year in temperate climates, are also those that are more likely to

exhibit temperature acclimation. Unfortunately, the temporal and spatial partitioning studies have not yet been performed at sufficient resolution to test this hypothesis.

We found significant variability among fungal isolates in sensitivity to temperature change (Q_{10}). Because of variation among isolates in Q_{10}, the temperature sensitivity of respiration by the ECM fungal community as a whole would be determined by the structure of the community. Therefore, the many sources of variation determining ECM fungal community structure (see above) will influence overall community sensitivity to temperature. Community composition will therefore influence the extent to which demand for host carbon from host plants increases with temperature, as described by Q_{10}.

Respiration rates in this study were measured from *in vitro* cultures. In previous studies of ECM fungal respiration, reported rates were in the same range that we have reported: 10 to 16 μmol CO_2 s^{-1} mg^{-1} dry weight at 31 ^oC (Taber & Taber, 1987), and 50 to 70 μmol O_2 s^{-1} mg^{-1} dry weight at 25 ^oC (Souto *et al.*, 2000). The respiration rates of *Hebeloma crustuliniforme* attached to living roots of *Pinus ponderosa* were also in the range of 11 to 16 μmol CO_2 s^{-1} mg^{-1} dry weight at 24 ^oC (Rygiewicz & Andersen, 1994), which suggests that our measured values on fungi cultured *in vitro* were realistic. It is much more difficult to measure mycorrhizal respiration in the field. However, it is possible to assess whether our reported values of respiration seem reasonable for ectomycorrhizal fungi in the field. Assuming that for respiration the ratio of ectomycorrhizal mycelium to root plus mycorrhizal fungus is 0.242 (the mean values reported by (Söderstrom & Read, 1987; Rygiewicz & Andersen, 1994) and that the root plus mycorrhizal fungus respiration rate in boreal forest is 70 mg C m^{-2} h^{-1}

(Bhupinderpal-Singh *et al.*, 2003), the ectomycorrhizal mycelium respiration rate would be 16.9 mg C m^{-2} h^{-1} (0.242 x 70). If we then assume that the total living biomass of ectomycorrhizal fungal hyphae in boreal forest is 163 kg ha^{-1} (the mean of 125 and 200 kg ha^{-1}, estimates of yearly production of ectomycorrhizal hyphae, (Wallander *et al.*, 2001)), then the ectomycorrhizal fungal respiration rate by hyphae would be 24 μmol CO_2 mg^{-1} dry weight s^{-1}. This agrees well with the values we reported.

Eleven of the twelve ECM fungal isolates respired between 20 and 150 μmol CO_2 s^{-1} mg^{-1} dry weight over the 11-23°C-measurement temperature range. *C. geophilum*, however, had respiration rates that were approximately four times lower than the other species, between 5 and 35 μmol CO_2 s^{-1} mg^{-1} dry weight over the same range. *C. geophilum* is an Ascomycete, while the other eleven isolates are Basidiomycetes. Further respiration data on other Ascomycetes would be needed to assess if the difference in the magnitude of respiration rates stems from this. It is possible that a relatively large fraction of the tissue of *C. geophilum* in the petri dishes was dead, which is consistent with the difficulty we have had in extracting DNA from *Cenococcum*-colonized root tips (unpublished data). We wonder, therefore, whether *Cenococcum* acclimates primarily by producing new tissues that are physiologically distinct from old tissues, while the basidiomycetes acclimate at least partly by physiologically altering pre-existing tissues.

For half of ECM fungal isolates, we found a significant increase in Q_{10} with an increase in incubator temperature. There was no clear relationship between change in Q_{10} and acclimation. This suggests that physiological change in response to incubator temperature is possible without acclimation. In plants, acclimation to warmer temperatures, shown by a reduction in respiration rates at given measurement

temperatures, can occur either with a change in Q_{10} (Type I acclimation; Atkin et al. 2003) or without a change (Type II acclimation; Atkin et al. 2003). Several researchers have similarly found that there is no relationship between Q_{10} and growth temperature (Atkin *et al.*, 2000; Tjoelker *et al.*, 2001; Loveys et al. 2003).

Q_{10} values for ectomycorrhizal fungi ranged from 1.67-2.56 across incubator temperatures, which is similar to the values found for roots in a variety of plant species (Pregitzer *et al.*, 2000 *and references therein*) and to values found for decomposer microorganisms (Luo *et al.*, 2001; Janssens & Pilegaard, 2003) with some exceptions noted in the literature (Larigauderie & Körner, 1995; Davidson *et al.*, 1998). In one microcosm study, Q_{10} values were shown to be approximately 2.0 and the same for roots and their fungal symbiont and decomposer microorganisms (Bååth & Wallander, 2003), which suggests that different belowground contributors to ecosystem respiration may have similar sensitivities to temperature on average. We caution, however, that since Q_{10} can be a temperature dependent process, once outside of our experimental temperature range (11-23 ^{o}C), a non-static Q_{10} may be more appropriate when applied to climate change models (Wythers *et al.*, 2005).

We cannot be certain that when living *in symbio* these isolates of ECM fungi would exhibit the same propensity to acclimate or the same temperature sensitivity as when growing *in vitro*. Acclimation to temperature may occur as a consequence of either altered activity of metabolic enzymes when metabolism is limited by enzyme activity (Klikoff, 1966; Miroslavov & Kravkina, 1991; Atkin & Tjoelker, 2003; Sommer & Portner, 2004), or as a result of a change in the rate of transport of substrate to mitochondria when substrate availability limits metabolism (Covey-Crump *et al.*, 2002).

Because we do not know the extent to which either substrate availability or enzyme activity limits metabolism for fungi living either *in vitro* or *in symbio*, we cannot predict whether acclimation is more or less likely for fungi living *in symbio* than when living *in vitro*. More research is necessary to address these important questions. In the field, autotrophic respiration (roots + mycorrhizal fungi) is separated from heterotrophic respiration (decomposer microorganims) via girldling trees and trenching soil plots (Hanson *et al.* 2000 *and references therein*), with the assumption that mycorrhizal fungi are mostly biotrophic, and thus, making it difficult to study mycorrhizal fungi separately from plant hosts.

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Chapter 4

Variation in respiratory response to temperature by ectomycorrhizal fungi from contrasting latitudes

Abstract

Ectomycorrhizal fungi can be a large sink for photosynthate and a large contributor to soil microbial biomass. They are, therefore, important contributors to soil respiration, but little is known about their respiratory response to temperature. Our objective was to determine whether ectomycorrhizal fungi collected from contrasting latitudes vary in their respiratory response and sensitivity to temperature. Respiration of ectomycorrhizal fungi from Alaska (latitude = 65^{o} 5' N) was greater than that of fungi from Pennsylvania (latitude = 40^{o} 8' N) across a range of measurement temperatures whether the fungi were compared at incubation temperatures reflective of their environments of origin, or at common incubation temperatures. Thus, respiratory responses to temperature appeared to be adapted to the thermal regime of the latitudes of origin such that fungi from both latitudes have similar carbon demands from their hosts. Estimated growth rate and respiration sensitivity (Q_{10}) were lower for ectomycorrhizal fungi from Alaska than for ectomycorrhizal fungi from Pennsylvania. With increasing temperature, therefore, the proportional increase in respiration rate may be lower for ectomycorrhizal fungi from high latitude than for those from lower latitude.

Introduction

A considerable proportion of photosynthesis is used for the growth and maintenance of root systems and their associated symbiotic, mycorrhizal fungi (Lambers et al. 1996; Smith and Read 1997). While some ectomycorrhizal fungi may have limited capacity to acquire carbon from soil organic matter (Lindeberg 1948; Rawald 1962; Lyr 1963; Ritter 1964; Ferry and Das 1968; Palmer and Hacskaylo 1970; Lamb 1974; Trojanowski et al. 1984; Dahm et al. 1987; Haselwandter et al. 1990; Durall et al. 1994), most are considered to be largely biotrophic (Smith and Read 1997; Wallander et al. 2004) as evidenced by a nearly complete cessation of fungal reproduction following the girdling of host trees (Högberg et al. 2001). The carbon demand on hosts by ectomycorrhizal fungi can be substantial. In some ecosystems their mycelia may comprise one-third of total microbial biomass (Högberg and Högberg 2002), approximately the same biomass as fine roots (Wallander et al. 2001), and input of C into the soil by ectomycorrhizal mycelia may exceed that by leaf litter and fine root turnover (Godbold et al. 2006). Respiration by ectomycorrhizal fungi, needed for both growth and maintenance, can therefore be substantial (Rygiewicz and Andersen 1994; Bååth and Wallander 2003).

At the level of the ecosystem, some concern exists that climate warming could cause an increase in the atmospheric CO_2 concentration due to an imbalance between the assimilation of CO_2 by photosynthesis and the release of CO_2 by respiration (Atkin et al. 2006; Boone et al. 1998; Davidson et al. 2006; Trumbore 2006). In a forest ecosystem the major contributors to ecosystem respiration are plants, their associated mycorrhizal

fungi, and soil decomposer microorganisms. When modeling carbon cycling under a warming scenario, ecosystem respiration is often assumed to increase with temperature (Cox et al. 2000; Rustad et al. 2001 Atkin et al. 2005), but the sensitivity to temperature change may vary among the different contributors to respiration (Bhupinderpal-Singh et al. 2003; Pietikainen et al. 2005). Therefore, it may be necessary to understand how each component responds to temperature change separately. Little is known concerning respiratory responses of ectomycorrhizal fungi to temperature change (Hacskaylo et al. 1965).

Several factors are known to influence respiration rates in plants including adaptation to elevation and latitude. For example, respiration rates at given measurement temperatures were higher for plant ecotypes from northern latitudes and higher elevations than for those from southern latitudes and lower elevations (Sowell and Spomer 1986; Mariko and Koizumi 1993; Reich et al. 1996). If ectomycorrhizal fungi respond to latitude as plants do, then at common measurement temperatures fungi from more northern latitudes will exert a greater carbon demand on their hosts than those from more southern latitudes. Moreover, in the face of a warming global climate, the sensitivity of organisms to temperature change becomes important. In two studies on plants, Q_{10} did not change with elevational ecotypes (Sowell & Spomer 1986, Mariko & Koizumi 1993), but nothing is currently known about the sensitivity of ectomycorrhizal fungi to temperature change.

Despite the fact that ectomycorrhizal fungi represent a large source of soil respiration and that they are a very significant demand on their host plants for carbon, nothing is known about either their respiration as a function of latitude or the sensitivity

of their respiration to temperature. The objective of this study, therefore, was to determine whether latitudinal variation exists among ectomycorrhizal fungi and, further, whether latitude influences their sensitivity to temperature. The fact that host specificity exists among ectomycorrhizal fungi and potential hosts (Molina et al. 1992) suggests that host – fungus combination may influence carbon-supply to the fungus. In order to avoid the confounding effects of potentially differential C allocation to the various isolates of ectomycorrhizal fungi from a single plant host species, we examined the respiratory response of ectomycorrhizal fungi in culture.

Materials and Methods

Fungal collection and culturing:

Ectomycorrhizal fungi from four genera and three families: Amanitaceae (*Amanita* spp), Russulaceae (*Lactarius* spp) and Boletaceae (*Leccinum* spp and *Suillus* spp) were collected from both Alaska (latitude = 64.5 N, longitude = 147.5 W) and Pennsylvania (latitude = 40.8 N, longitude = 77.9 W). Sporocarps of *Amanita muscaria var. muscaria* (GL015) and *Leccinum alaskanum* (GL005) were collected in late July or early August of 2003 under mixed *Betula papyrifera*, *Populus balsamifera* and *Picea glauca* vegetation just south of Fairbanks, Alaska. Sporocarps of *Suillus grevillei* (AK014) were also collected at about the same time and location but under *Picea mariana*. *Lactarius cf pubescens* (AK010) sporocarps were collected at about the same time in Chatanika, north of Fairbanks, Alaska under *Betula papyrifera* and *Populus balsamifera* vegetation. Sporocarps were shipped to State College, Pennsylvania, where they were isolated and maintained on a growth medium consisting of 19.5 g of potato dextrose agar (Difco: Becton, Dickinson & Co., Sparks, MD, USA), 7.5 g Bacto Agar (Difco) and 0.375g NH_4Cl per liter of water. *Amanita citrina* (SC070), *Leccinum aurantiacum* (SC014), *Suillus intermedius* (BX007) and *Lactarius chrysorrheus* (SC016) sporocarps were collected from a *Pinus resinosa* plantation in State College, Pennsylvania, and isolated and maintained using the same growing medium. Fungal cultures were maintained in incubators set to temperatures reflective of those experienced

by the fungi during the growing season at their environment of origin: 17°C for Pennsylvanian fungi and 11°C for Alaskan fungi. These values were chosen based on litter temperatures monitored in the State College *Pinus resinosa* plantation from 1 June to 31 October 2005 (mean 16.4°C, Malcolm and López-Gutiérrez, unpublished), and in four *Picea mariana* forests of the Bonanza Creek long-term ecological research station, near Fairbanks, Alaska from 21 July to 21 August 2002, mean 11.5°C (Vogel and Valentine 2006). Before each experiment, fungal cultures were propagated in 100 × 20 mm Petri dishes and grown in their respective incubators, 11°C for Alaskan fungi and 17°C for Pennsylvanian fungi, for 2 to 3 weeks until they had attained sufficient biomass (approximately doubled in size) to yield respiration rates detectable in our respiration system. Cultures were then divided into two subsets. One subset was kept in the same incubator; the second subset was shifted to the 17°C incubator (if they originated from Alaska) and to the 11°C incubator (if they originated from Pennsylvania) for one week before temperature response of respiration was assessed.

Respiration rates:

We measured respiration rates at fixed measurement temperatures with a custom-built gas exchange system. Petri dishes were placed in individual polycarbonate chambers sitting atop a smooth, steel sheet, the temperature of which was controlled by an underlying automobile radiator in line with a temperature-controlled water bath. Each chamber received a constant flow of humidified and temperature-regulated outdoor air supplied by a diaphragm air pump, and each chamber could be isolated and connected to

a LI-6200 photosynthesis system (LI-COR, Lincoln, NE, USA) to measure CO_2 efflux. Because of the natural accumulation of CO_2 in the gel of each Petri dish while in the incubators, the dishes required at least 8 hours of equilibration in the flowing air of the gas exchange chambers prior to measurement of steady state respiration rates (data not shown). Thus, for a given set of measurements five individual Petri dishes from one of the incubators were placed in the gas exchange system the night before measurements were to be made. The temperature of the water bath was set to match that of the incubator and the airflow was set to match that of the LI-6200 pump. For each chamber, a respiration measurement consisted of 4 consecutive set changes in CO_2 concentration, which ranged from 1-4 ppm depending on the rate of change. Respiration was determined from 5 to 29^{o}C at 6^{o}C intervals. We allowed 50 minutes at each temperature before making measurements to attain steady state CO_2 effluxes. In some cases exposing the cultures to 5 or 29^{o}C apparently caused physiological damage resulting in hysteresis at those temperatures. We therefore only report respiration rates in the temperature range in which stable values were possible for all isolates (11-23^{o}C).

Fungal tissue was separated from agar by melting the agar in test tubes held in boiling water. The tissues were placed in a drying oven (60ºC) until constant weight was achieved. Estimated absolute fungal growth rates were calculated by dividing final biomass by the number of days elapsed between culturing and harvesting, assuming the initial fungal biomass to be negligible.

Q_{10} and E_a:

The proportional change in respiration rate over a 10^{o}C interval (Q_{10}) was calculated by plotting the log transformed respiration rates versus measurement temperatures, obtaining the slopes of the linear regression and using them in the following equation:

$Q_{10}=10^{(10 * slope)}$ (Atkin et al. 2000a).

Arrhenius plots were constructed by plotting the natural log transformed respiration rates versus the reciprocal of the absolute temperature. After fitting a linear regression to obtain the slope of the line, the apparent energy of activation (E_a) was calculated using the following equation:

E_a= - slope / R (Lloyd and Taylor 1994),

where R is the universal gas constant (8.314 J mol^{-1} $^{o}K^{-1}$).

We plotted all E_a values versus the corresponding natural log transformed Q_{10} values obtained from each respiration response curve generated in this study to obtain a regression line relating both variables.

Statistical analysis:

Since respiration rates were obtained across a range of measurement temperatures on the same set of individual cultures, we used Repeated Measures Proc Mixed ANOVA Procedure in SAS (SAS Institute Inc. Version 9.1. 2002-2003) to analyze the respiration responses to temperature. We analyzed the effects of genus, latitude, measurement temperature and the interaction between latitude and measurement temperature on respiration rate when: a) fungi from Alaska were incubated at 11^{o}C and fungi from Pennsylvania were incubated at 17^{o}C, b) all fungi were incubated at a common 11^{o}C temperature and c) all fungi were incubated at a common 17^{o}C temperature. Compound Symmetry (CS) was used as the covariate structure that best fit our data according to the Akaike and the Bayesian information criteria. To analyze estimated absolute growth rate and Q_{10} we used Proc GLM ANOVA Procedure in SAS with latitude and genus as main effects. Differences were analyzed in the same cases as above. Because we were limited by the ectomycorrhizal fungi that we were able to collect, propagate and maintain in culture, we compared variation across latitudes using a congeneric contrast in which each genus served as a replicate (Burt 1989). In all cases genus was included in the analysis as a blocking effect to remove variability from that source. All values reported here are adjusted means, i.e. least-squared means (LSMEANS), and standard errors (s.e.) obtained from the different statistical models with n = 4 genera. There were five replicate petri dishes for each genus × incubator temperature combination.

Results

Respiration response to temperature across latitudes:

Latitude of origin was a significant factor determining respiration rate when fungi from Alaska and Pennsylvania were incubated at temperatures reflective of their environment of origin (Table **4.1**). The average respiration rate of Alaskan species incubated at 11^{o}C was significantly greater than that of the Pennsylvanian species incubated at 17^{o}C over the 11-23^{o}C measurement temperature range (Fig. **4.1**). Respiration rate also increased with measurement temperature (Table **4.1**, Fig. **4.1**). There was a significant interaction between latitude of origin and measurement temperature reflecting the fact that as measurement temperature increased, the difference in respiration between latitudes also increased (Table **4.1**, Fig. **4.1**).

Table **4.1**: Analysis of variance of respiration of ectomycorrhizal fungi incubated at temperatures reflective of the litter of their latitude of origin during the growing season (11^{o}C for Alaskan isolates and 17^{o}C for Pennsylvanian isolates). There were three measurement temperatures and four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. n = 4 genera.

Effect	Num. d.f.	Den. d.f.	F-Value	P-value
Latitude	1	35	23.98	<0.0001
Genus	3	35	7.14	0.0007
Measurement temperature	2	76	481.82	<0.0001
Latitude × Measurement temperature	2	76	4.76	0.0113

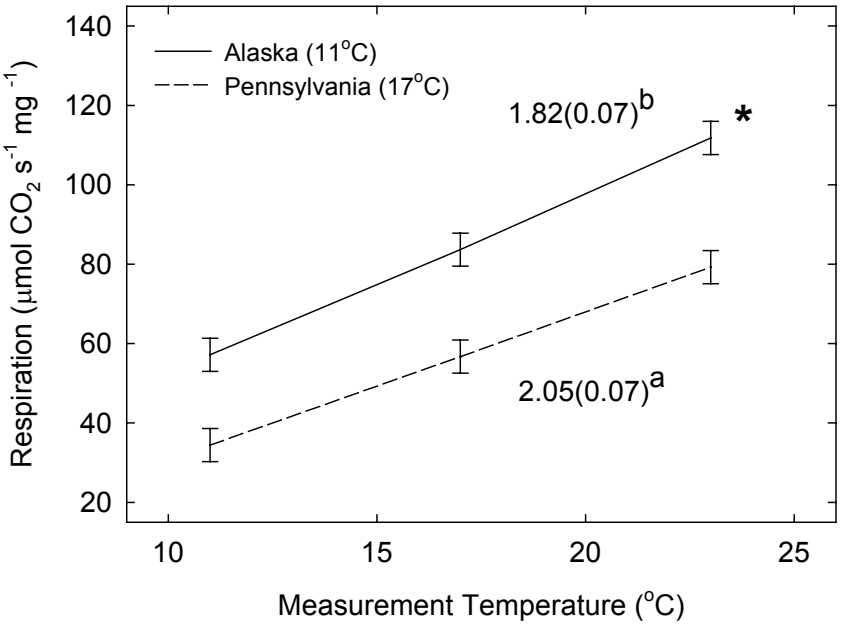


Fig. **4.1**: Mean (±1 s.e.m.) of respiration at three measurement temperatures of ectomycorrhizal fungi originating from Alaska (incubated at 11°C) and Pennsylvania (incubated at 17°C). An asterisk indicates a statistically significant difference between latitudes, averaged over measurement temperature. Numbers are mean (s.e.m.) Q_{10} values for each latitude of origin for the temperature interval 11 - 23ºC. Q_{10} values followed by different letters are significantly different. E_a values are 42.0 and 50.3 kJ mol^{-1} for fungi originating from Alaska and Pennsylvania, respectively. n = 4 genera.

Respiration rate was also significantly influenced by latitude of origin when the comparison was made with fungi from both latitudes incubated at 11°C (Table **4.2**, Fig. **4.2**A) and with fungi from both latitudes incubated at 17°C (Table **4.3**, Fig. **4.2**B). In both cases, the average respiration rate of Alaskan species was significantly higher than that of Pennsylvanian species over the 11 - 23°C measurement temperature range. Also, in both cases, measurement temperature significantly influenced respiration, and there was a significant interaction between latitude of origin and measurement temperature (Tables **4.2**, **4.3**). The significant interaction is reflective of the increased difference in

respiration rate between latitudes with an increase in measurement temperature (Fig. **4.2** A, B).

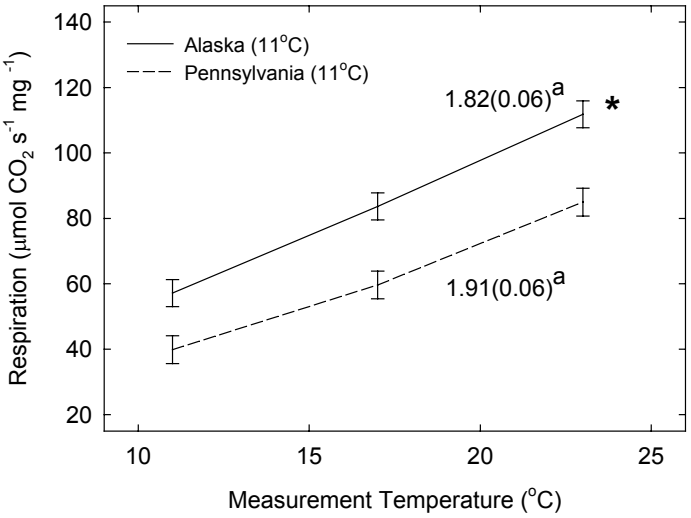
Table **4.2**: Analysis of variance of respiration of ectomycorrhizal fungi incubated at 11°C, which reflects the temperature of litter at the Alaska site. There were three measurement temperatures and four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. n = 4 genera.

Effect	**Num. d.f.**	**Den. d.f.**	**F-Value**	**P-value**
Latitude	1	34	16.48	0.0003
Genus	3	34	8.87	0.0002
Measurement temperature	2	74	453.41	<0.0001
Latitude × Measurement temperature	2	74	4.4	0.0156

Table **4.3**: Analysis of variance of respiration of ectomycorrhizal fungi incubated at 17°C, which reflects the temperature of litter at the Pennsylvania site. There were three measurement temperatures and four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. n = 4 genera.

Effect	**Num. d.f.**	**Den. d.f.**	**F-Value**	**P-Value**
Latitude	1	35	14.7	0.0005
Genus	3	35	9.74	<0.0001
Measurement temperature	2	76	521.43	<0.0001
Latitude × Measurement temperature	2	76	6.15	0.0033

A)


Alaska (11°C)
Pennsylvania (11°C)
1.82(0.06)a
*
1.91(0.06)a
Respiration (μmol CO2 s-1 mg-1)
Measurement Temperature (°C)
140
120
100
80
60
40
20
10
15
20
25

B)

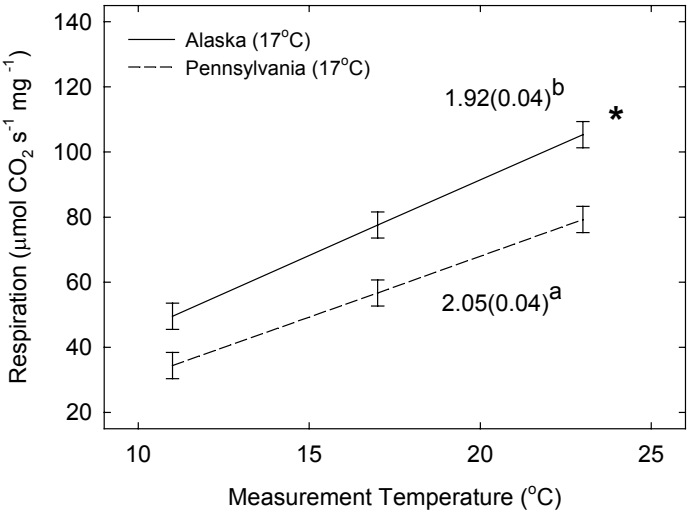

Alaska (17°C)
Pennsylvania (17°C)
1.92(0.04)b
*
2.05(0.04)a
Respiration (μmol CO2 s-1 mg-1)
Measurement Temperature (°C)
140
120
100
80
60
40
20
10
15
20
25

Fig. **4.2**: Means (±1 s.e.m.) of respiration at three measurement temperatures of ectomycorrhizal fungi originating from Alaska and Pennsylvania and incubated at A)11^{o}C and B)17^{o}C. An asterisk indicates a statistically significant difference between latitudes, averaged over measurement temperature. Numbers are mean (s.e.m.) Q_{10} values for each latitude of origin for the temperature interval 11 - 23ºC. Q_{10} values followed by different letters are significantly different. For A) E_a values are 42.0 and 45.3 kJ mol^{-1} and for B) E_a values are 45.71 and 50.30 kJ mol^{-1}, for fungi originating from Alaska and Pennsylvania, respectively. For A) and B), n = 4 genera.

A significant effect of latitude on respiration occurred for three of the four genera (and two of the three families). The genus Amanita was exceptional (Fig. **4.3** A-C).

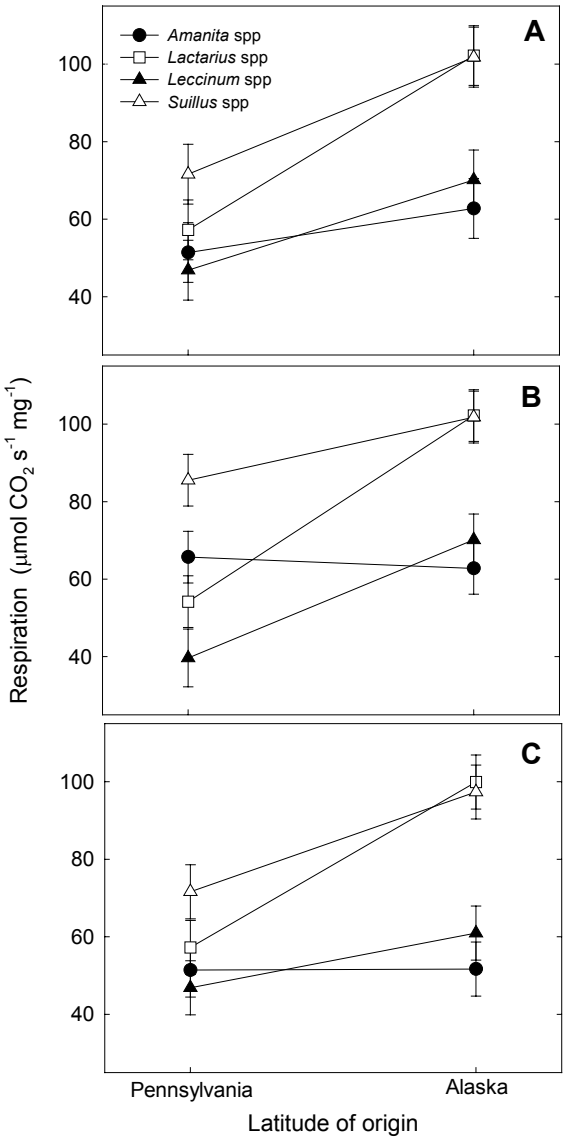


Fig. **4.3**: Mean (±1 s.e.m.) of respiration, averaged over measurement temperatures 11, 17 and 23°C, of each of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. Fungi were either incubated at A) separate temperatures (11°C for Alaska isolates and 17°C for Pennsylvania isolates), or at common temperatures of either B) 11°C or C) 17°C . (in each case, n = 5 dishes per genus × incubator temperature combination).

Estimated fungal growth rates:

Latitude of origin had a significant effect on estimated absolute growth rate when fungi from Alaska and Pennsylvania were incubated at temperatures reflective of their environments of origin (Table **4.4**). Estimated absolute growth rate was lower for Alaskan species incubated at 11°C than for Pennsylvanian species incubated at 17°C (Fig. **4.4** A). The effect of latitude of origin on estimated absolute growth rate was also significant when fungi from both latitudes were incubated at either 11°C (Table **4.4**, Fig **4.4** B) or 17°C (Table **4.4**, Fig **4.4** C).

Table **4.4**: Analysis of variance for the estimated absolute growth rate of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. Fungi were either incubated at separate temperatures: 11°C (Alaska isolates) and 17°C (Pennsylvania isolates), or at common temperatures of either 11°C or 17°C . (n = 4 genera).

Treatment	**Effect**	**d.f.**	**Sum of Squares**	**F-Value**	**P-Value**
Alaska (11°C)	Latitude	1	39.66	40.64	<0.0001
Pennsylvania (17°C)	Genus	3	48.49	16.56	<0.0001
	Error	35	34.16	~	
Alaska (11°C)	Latitude	1	24.12	36.41	<0.0001
Pennsylvania (11°C)	Genus	3	37.03	18.63	<0.0001
	Error	34	22.52	~	
Alaska (17°C)	Latitude	1	27.3	25.46	<0.0001
Pennsylvania (17°C)	Genus	3	48.36	15.04	<0.0001
	Error	35	37.52	~	

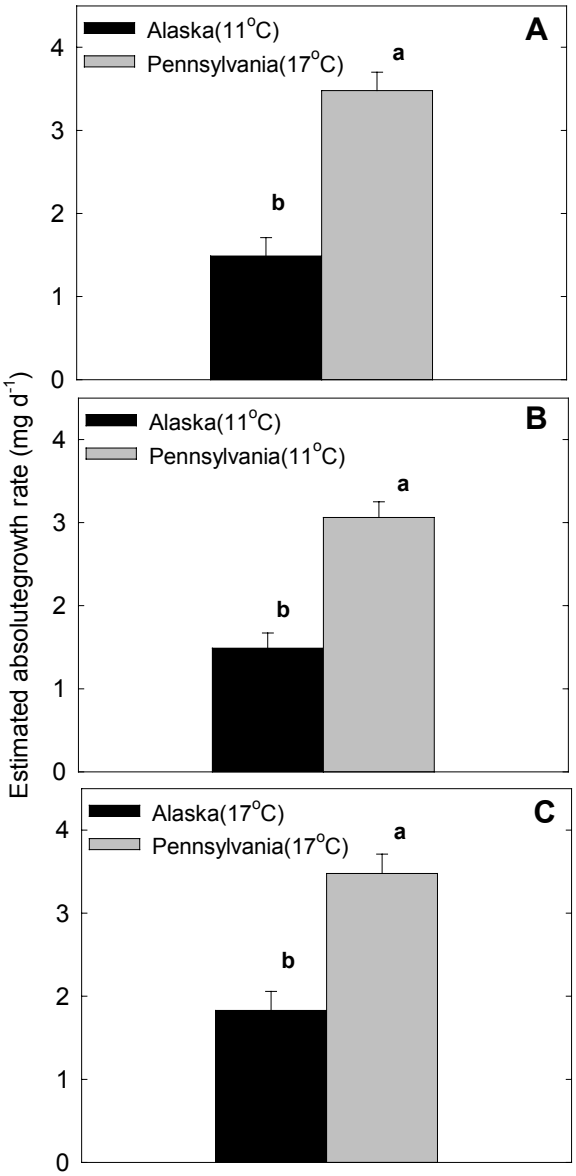


Fig. **4.4**: Means (±1 s.e.m.) of the estimated absolute growth rates of ectomycorrhizal fungi originating from Alaska and Pennsylvania Fungi were either incubated at A) separate temperatures (11°C for Alaska isolates and 17°C for Pennsylvania isolates), or at common temperatures of either B) 11°C or C) 17°C . (in each case, n = 4 genera). Different letters indicate statistically significant differences between latitudes.

Q_{10} and E_a:

In general, Alaskan fungi had a significantly lower Q_{10} value than Pennsylvanian fungi. This was true when the fungi were incubated at temperatures reflective of their environment of origin (Table **4.5**, Fig. **4.1**), and when all fungi were incubated at a temperature representative of Pennsylvania (17ºC, Table **4.5**, Fig. **4.2** B). Latitude was not significant when all fungi were incubated at a temperature representative of Alaska (11ºC, Table **4.5**, Fig. **4.2** A). The log of respiration vs. measurement temperature was essentially linear between 11 and 23°C, thus Q_{10} was essentially constant over that range of measurement temperatures (Atkin et al. 2000a). The Arrhenius plots also formed a straight line that could be described well by a first order polynomial, with a single slope, resulting in a single E_a over the range of measurement temperatures (data not shown).

Table **4.5**: Analysis of variance for the respiration Q_{10} value of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. Fungi were either incubated at separate temperatures: 11°C (Alaska isolates) and 17°C (Pennsylvania isolates), or at common temperatures of either 11°C or 17°C . (n = 4 genera).

Treatment	Effect	d.f.	Sum of Squares	F-Value	P-Value
Alaska (11°C)	Latitude	1	0.53	5.61	0.0235
Pennsylvania (17°C)	Genus	3	0.18	0.64	0.5949
	Error	35	3.28	~	
Alaska (11°C)	Latitude	1	0.07	0.86	0.36
Pennsylvania (11°C)	Genus	3	0.41	1.76	0.1736
	Error	34	2.67	~	
Alaska (17°C)	Latitude	1	0.17	4.99	0.032
Pennsylvania (17°C)	Genus	3	0.29	2.78	0.0557

	Error	35	1.21	~

Discussion

When the fungi were incubated at temperatures reflective of their environments of origin (Alaskan fungi at 11°C and Pennsylvanian fungi at 17°C), the respiration rate at a given measurement temperature was significantly higher for fungi from Alaska than for fungi from Pennsylvania. However, because it is possible for acclimation to occur to new temperatures, at least in plants (Atkin et al. 2000b; Loveys et al. 2003; Cooper 2004), we also made comparisons of latitude when fungi were incubated at common temperatures for one week. For plants, acclimation to temperature has been shown to occur within 1-3 days (Atkin et al. 2000b; Bolstad et al. 2003; Loveys et al. 2003), thus we estimated that a week would be adequate for acclimation to occur in these fungi. In other tests, 6 days was shown to be an adequate duration for acclimation of ectomycorrhizal fungi (Malcolm *et al.,* 2007). Even at a common incubation temperature of either 11°C or 17°C, there was a significant effect of latitude on respiration. Therefore, the variation in respiration between the ectomycorrhizal fungi from two latitudes suggests that their metabolism is adapted to the climates of their latitudes of origin.

Some authors have argued that organisms growing in colder environments possess higher respiration rates due to higher maintenance costs associated with growth under colder conditions (Mariko and Koizumi 1993; Oleksyn et al. 1998). Others argue that higher respiration rates are linked to higher growth rates to compensate for shorter growing seasons in colder environments (Barclay and Crawford 1984). In previous studies there were no significant differences in ectomycorrhizal fungal growth in culture

across latitude gradients (Samson and Fortin 1986; Cline et al. 1987). In the current study, estimated growth rates of Alaskan fungi were approximately half those of Pennsylvanian fungi (Fig. 5) while respiration rates of Alaskan fungi at a measurement temperature of 11ºC were very close to respiration rates of Pennsylvanian fungi at a measurement temperature of 17ºC, irrespective of incubator temperature (Figs. 1-3). Thus, our data do not suggest that higher respiration rates are associated with faster growth. Instead, our data are consistent with the observation that organisms from colder environments maintain higher respiration rates because of increased energy allocation to maintenance (Körner 1989; Reich et al. 1996).

This phenomenon of latitudinal variation in inherent respiration rates of ectomycorrhizal fungi, reported here for the first time, has important implications. In terms of the carbon economy of a single plant, our results might suggest that the carbon costs of supporting ectomycorrhizal fungi could be proportionally greater for host plants growing at higher latitudes than at lower latitudes at a common temperature. However, the average fungal fruiting season temperature of the litter at the Alaskan collection site (11.5ºC) was approximately 5ºC lower than the average at the Pennsylvanian collection site (16.4ºC), and the respiration rate for Alaskan fungi at 11ºC was approximately the same as the respiration rate for Pennsylvania fungi at 17ºC. Thus, under ambient conditions, demand for carbon from host plants, as measured by respiration, would be approximately the same at the two latitudes if there exists no differences in supply of carbon to the fungi. This is evidence for a remarkable physiological homeostasis in carbon demand by the fungi despite large differences in temperature at the two latitudes, and thus large expected differences in respiration rates.

We have shown, however, that latitude influenced the sensitivity to temperature of ectomycorrhizal fungi. Overall, values of Q_{10} were significantly lower for fungi from Alaska than fungi from Pennsylvania. This suggests that as global temperatures increase, proportional increases in respiration for a given temperature shift could be lower for the fungi at high latitude than for those at lower latitude. No comparable data exist for ectomycorrhizal fungi, but Sowell and Spomer (1986) and Mariko and Koizumi (1993) indicated that no significant variation in Q_{10} was seen among plant ecotypes from different elevations (Sowell and Spomer 1986; Mariko and Koizumi 1993).

The Q_{10} values reported for the fungi in this study, between 1.82 and 2.05 for measurement temperatures between 11 and 23ºC, were close to the often-assumed value of 2.0 for most biological systems (Cox et al. 2000; Potter et al. 2001; Atkin et al. 2005). The Q_{10} values were not very different from Q_{10} values reported for many surface soils (Kirschbaum 1995; Rey et al. 2002; Kirschbaum 2006; Pavelka et al. 2007) and roots (Atkin et al. 2000b; Rey et al. 2002; Cooper 2004) although there are notable exceptions (Larigauderie and Korner 1995; Davidson et al. 1998; Bryla et al. 2001; Epron et al. 2001; Burton et al. 2002; Huang et al. 2005). Thus, it would appear that at least in some instances the major components of soil respiration have similar temperature sensitivities, a conclusion that was drawn by Bååth and Wallander (2003). However we must be cautious in this interpretation as Q_{10} can be temperature dependant (Tjoelker et al. 2001; Atkin and Tjoelker 2003). The accuracy of carbon cycling models may be affected by the assumption of a fixed Q_{10} value (Wythers et al. 2005).

In this study we have utilized fungi from four genera, an isolate of each collected from both Alaska and Pennsylvania. We have assumed that the two collections are

representative of the two latitudes and, therefore, of two climates that vary markedly in temperature. We have further assumed that latitudinal variation may be expressed in terms of adaptation to distinct thermal regimes, at least in part. In other studies of organisms collected along latitudinal gradients, similar assumptions have been made . Of course we realize that the climates of Fairbanks, Alaska and State College, Pennsylvania, differ from each other in more ways than in merely temperature. Nevertheless, we found that fungi from the higher latitude had higher respiration rates at given measurement temperatures than fungi from lower latitude, a result that is consistent with studies on plants (Reich et al. 1996).

In order to avoid the confounding effects of potentially differential C allocation to the various isolates of ectomycorrhizal fungi from a single plant host species, we examined the respiratory response of ectomycorrhizal fungi in culture. Obviously we will need to confirm whether latitude influences respiration rate or sensitivity to temperature of ectomycorrhizal fungi *in symbio*.

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Chapter 5

Respiratory acclimation to temperature by decomposer communities in red pine (*Pinus resinosa*) litter at different times of year

Abstract

A large amount of carbon is found in temperate and boreal forests across the globe. Small proportional changes in the concentration of carbon in soil may cause forest soils to be a large source or sink of carbon dioxide to the atmosphere. Because of quick changes in temperature in forest litter between and within seasons, it is important to understand the metabolic response of decomposer communities to temperature. If decomposer communities acclimate to warmer temperatures, or compensate for the change in temperature, they would require less carbon from organic soil pools than when not acclimating. Over the course of several days, we found that decomposer communities displayed limited ability to acclimate their respiration. On a longer time scale (month), decomposer communities showed some ability to change their respiration at different times of year (i.e. cool and warm parts of the growing season), but not at all time points. This suggests that on a longer time scale, more than temperature impacts respiration of decomposer communities. It is likely that from month to month, decomposer community structure, substrate availability, and moisture change. Thus, a better understanding of the cellular regulation of the metabolism of decomposer communities is needed in order to make predictions regarding whether particular forest soils will be a source or sink of carbon to the atmosphere.

Introduction

Relative to other terrestrial habitats, the soils of temperate and boreal forests contain high concentrations of carbon. Temperate and boreal forests also comprise a large fraction of terrestrial ecosystems (Post *et al.*, 1982; Jenkinson *et al.*, 1991; Schimel, 1995). The amount of carbon in the soils of temperate and boreal forests is, therefore, globally significant: even small proportional changes in the concentration of carbon in forest soils may cause them to act as either large sources or large sinks for atmospheric CO_2 (Kirschbaum, 2000; Friedlingstein *et al.*, 2001; Jones *et al.*, 2003; Zeng *et al.*, 2004).

The temperature of forest soils near the surface can change on a relatively short time scale. Significant warming can occur in the shift from spring to summer, and significant cooling can occur in the shift from summer to fall in the course of just several days. Even within a single season relatively rapid changes in soil temperature are possible (Bhupinderpal-Singh *et al.*, 2003; Lee *et al.*, 2003; Davidson *et al.*, 2006). At the field site of this study, we have documented rapid changes in temperature in the litter occur throughout the year.

Because of such rapid shifts in temperature, we need to have a better understanding of the metabolic response to temperature by decomposer microorganisms in forested ecosystems (Fang *et al.*, 2005; Ryan & Law, 2005; Davidson & Janssens, 2006) if we are to understand the effects of temperature on organic matter decomposition. Thus, whether or not that community physiologically acclimates to temperature will influence its demand for carbon and thus the potential for decomposition. Acclimation to warmer temperatures is manifested as a lower respiration rate at a given measurement

temperature and is sometimes accompanied by a change in temperature sensitivity, as described by Q_{10} (Atkin & Tjoelker, 2003). A community of decomposer microorganisms that physiologically acclimates to temperature will require less carbon from organic matter in the soil as the temperature warms than would a community that does not acclimate. Accrued over longer time scales, the difference between acclimating and non-acclimating communities may make a significant difference in decomposition and thus soil carbon content.

Little is known about whether decomposer communities acclimate or if their sensitivity to temperature changes when they do. The concept of physiological acclimation is usually applied to a single organism, not to a community of organisms. Of necessity, however, we treat the community of decomposer microorganisms as if it were a single organism with respect to physiological acclimation. What we observe, then, is the net effect of the physiological changes of the community of microorganisms. Our expectation is that decomposer microorganisms will acclimate to temperature because many other organisms have shown evidence for acclimation (Guderly & Johnston, 1996; Sustr & Block, 1998; Barbariol & Razouls, 2000; Atkin & Tjoelker, 2003), including some ectomycorrhizal fungi (Malcolm *et al.*, 2007; see Chapter 3), species of which belong to the same fungal families as decomposer fungi.

On shorter time scales (days), there may not be large changes in decomposer community composition. However, on longer time scales (months), it is likely that decomposer communities will change (Bossio *et al.*, 1998; Smit *et al.*, 2001). Ectomycorrhizal fungi, comprising the same fungal families as decomposer fungi, have shown temporal variation in community structure (Koide *et al.*, 2007). Moreover, the

soil microorganism community changed in response to warming experiments in tall grass prairie and hardwood ecosystems (Zogg *et al.*, 1997; Zhang *et al.*, 2005). Little is known about whether different decomposer communities possess different capacities to acclimate. Therefore, in our assessment of acclimation, we studied litter collected at different times of year.

Methods

Field Site:

The study site, an approximated 30-hectare stand of red pine (*Pinus resinosa* Ait.) plantation, is located in State College, Centre County PA, USA. The trees were planted approximately 55 years ago. The site is characterized by a well-developed O-horizon (litter), a fermentation (F) layer, and a thin humified (H) layer, which overlies the mineral soil (Morrison sandy loam). The mineral soil consists of a thin eluviated A-horizon over a well-defined sandy B-horizon. Little to no vegetation exists in the portion of the stand sampled (~2000 m^2 in area). At the edges of the plantation, saplings of red pine and hardwoods, including *Acer rubrum* and *Quercus alba*, and *Vaccinium* spp. shrubs exist in small quantities.

Soil temperature and precipitation:

Soil temperatures were monitored constantly by a LI-1000 data logger buried in the soil. Four soil temperature probes were placed above the F-layer in order to capture

the area with slightly decomposed pine needles, hereafter referred to as lower litter. We report the means of the daily mean temperatures for the week prior to the litter-sampling period. Historical daily rainfall data for University Park, PA (within ~18 km from the field site) for 2005-2007 were obtained from Accuweather (http://www.accuweather.com/; zip code = 16801).

Litter collection and incubation:

Eight samples from the lower litter layer were collected from near the same eight randomly chosen red pine trees in the fall (9 September 2005, 26 October 2006), spring (27 April 2006, 9 May 2007), and summer (21 June 2006, 30 August 2006). Hereafter, the collections dates are referred to as 'Fall 05', 'Fall 06', 'Spring 06', 'Spring 07', 'Summer 06a', and 'Summer 06b,' respectively. Samples were brought to the lab, where all root material was removed by hand. Because moisture levels influence decomposition rates (Davidson *et al.*, 1998), the litter samples were also wetted to constant water concentrations (72.4 % ± 2.9% w/w) by gently spraying them with distilled water until they reached field capacity. Each sample of lower litter was gently homogenized before separating into three subsamples (~5-8 g dry wt.) that were placed in 100 x 15 mm disposable Petri dishes. The Petri dishes were placed in plastic containers with damp paper towels and loose fitting lids prior to placing them in incubators. After incubation for one week in this manner, samples lost less than 1% of their moisture.

Subsamples were incubated for one week as suggested by Robertson et al. (1999)

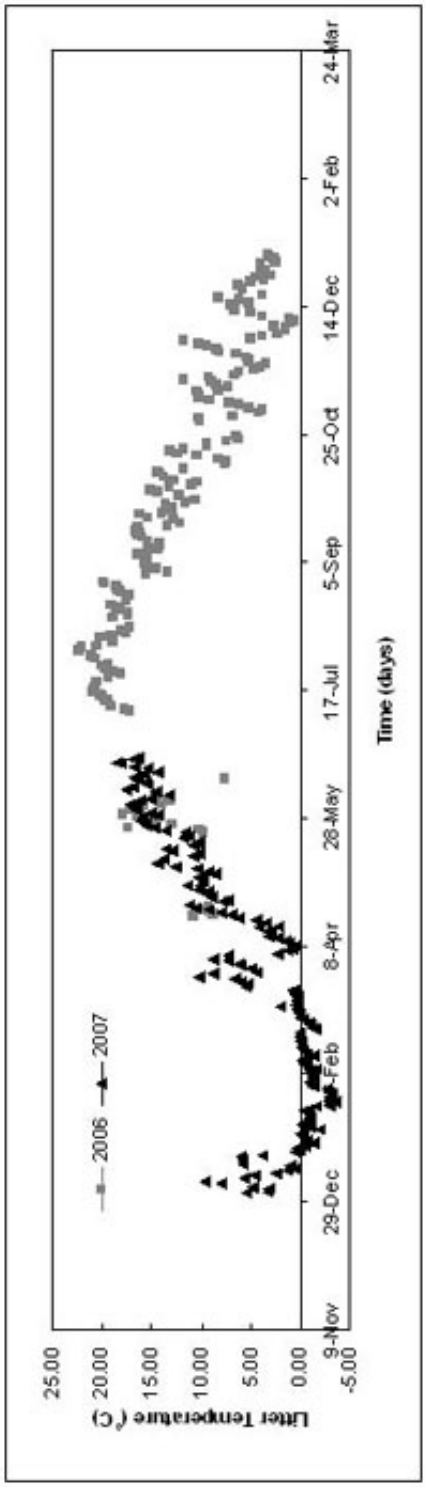

Fig. **5.1**: Mean daily field temperatures from 21 April 2006 through 21 June 2007 (n = 4). Missing data in 2006 resulted from a malfunction of the LI-6100 data logger, which was corrected once found.

in each of three incubators to reduce the potential effects of handling. Also, we expected the decomposer microorganisms to acclimate within a week as seen for plants and ectomycorrhizal fungi (Bryla *et al.*, 1997; Atkin & Tjoelker, 2003; Bolstad *et al.*, 2003; Malcolm *et al.*, 2007). Spring and fall samples were incubated at 5, 11, and 17^{o}C, while summer samples were incubated at 11, 17, and 23^{o}C. The middle temperatures reflect temperatures close to the average daily litter temperatures for the week prior to sample collections (Fig. **5.1**, Fig. **5.2**). This temperature shift regime is relevant because average litter temperatures change by at least ~6^{o}C from spring to summer and from summer to fall (Fig. **5.1**, Fig. **5.2**).

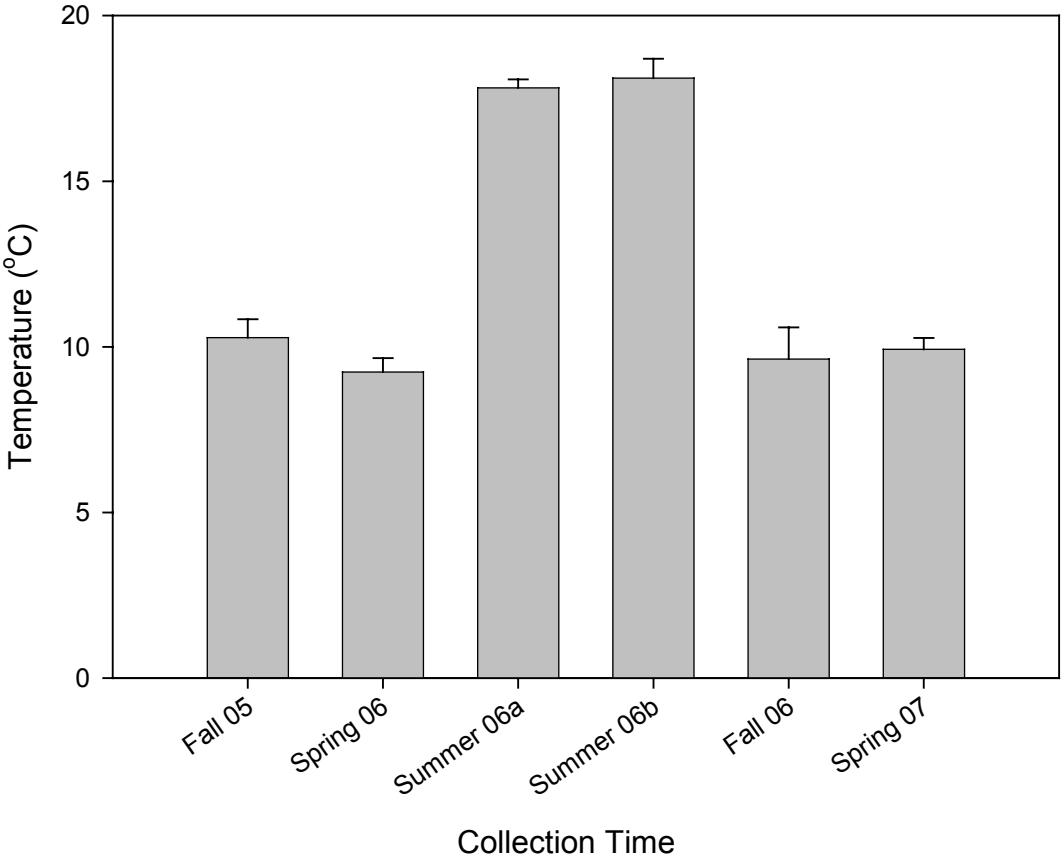

Fig. **5.2**: Mean (± 1 s.e.) daily field temperatures in red pine litter the week prior to sample collection (n = 4).

Litter Respiration Measurements:

CO_2 efflux by decomposer microorganisms was measured at 5, 11, 17, 23, and 29°C using an apparatus we custom-fabricated for this purpose (see Chapter 2). Briefly, the litter subsamples were placed in temperature-controlled chambers that were inline with a LI-COR 6200 gas analyzer. After the subsamples attained steady-state rates of CO_2 efflux at the incubator temperature from which the subsamples were taken,

respiration measurements were initiated. CO_2 effluxes were only reported at those measurement temperatures for which no damage had occurred to the decomposer microorganisms. We assessed when damage occurred to the microorganisms by measuring respiration at an initial temperature, a different temperature, and the initial temperature again. If respiration was different at the initial temperature before and after the temperature change, it was assumed that damage had occurred to the microorganisms.

We standardized respiration rates by microbial-N (MBN) content rather than dry weight of the litter subsamples. MBN content is a more sensitive indicator of the amount of microbial biomass in pine litter (Brookes *et al.*, 1985). Indeed, we found that MBN content varied approximately three times more than litter dry weight did, suggesting that for a given litter dry weight, microbial biomass may be quite variable among samples (Fig. **5.3**). Additionally, MBN-concentrations varied significantly at different times of year (Fig. **5.4**), suggesting that microbial biomass varies temporally. MBN contents were determined following chloroform fumigation and extraction (Robertson *et al.*, 1999). Briefly, after completing a respiration-temperature curve, each lower litter sample was split into three approximately equal parts, one for chloroform fumigation, one for a control, and one for a dry weight measure. The chloroform fumigation portions were exposed to chloroform for 24 hours in a container sealed with grease and evacuated to a certain vacuum. The control portions were extracted with 100ml K_2SO_4 for one hour on a shaker. At the end of the 24-hour fumigation, the chloroform-fumigated samples were also extracted in the same manner. The portions of lower litter used to attain dry masses were maintained at 60^{o}C in a drying oven until a constant mass was attained. Total dissolved-N was attained by heating the fumigation and control litter samples at 400^{o}C

with a strong acid solution that consisted of concentrated sulfuric acid and 30% hydrogen peroxide in a 1:1 ratio. The digestion was followed by colorimetric N determination (Nessler method, Jensen 1962). MBN contents were calculated using an efficiency of extraction factor (k_{en} = 0.54, (Joergensen & Mueller, 1996).

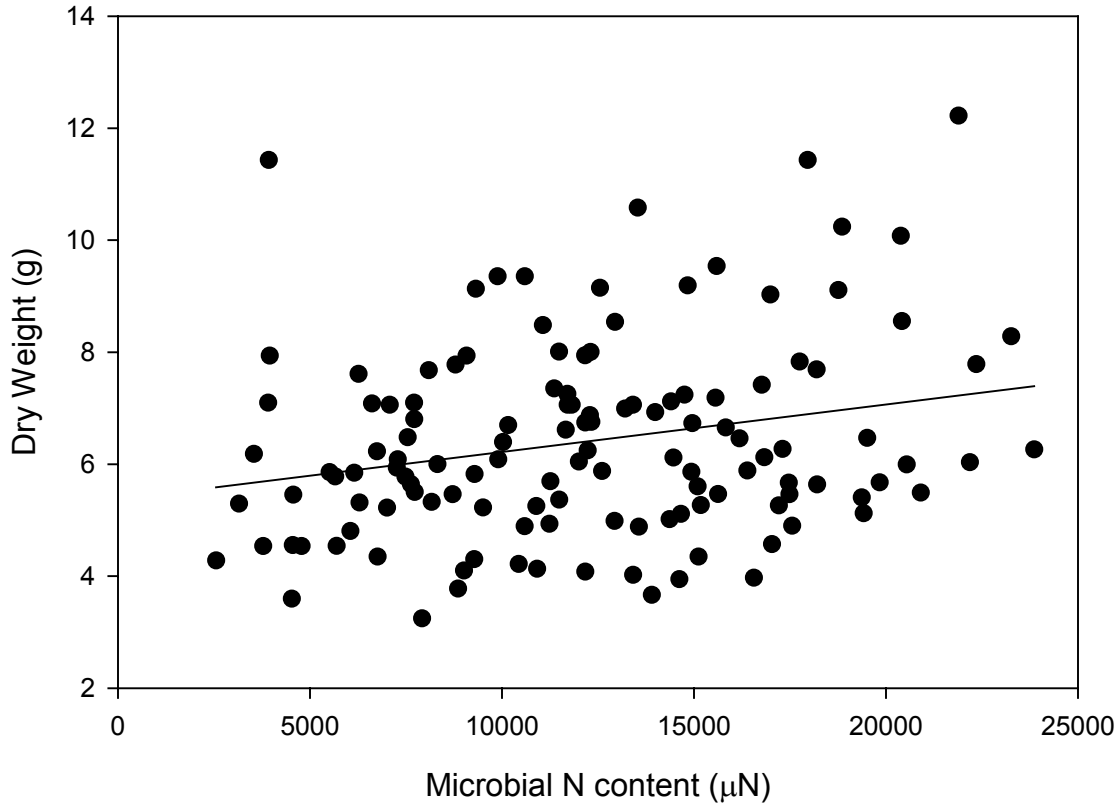

Fig. **5.3**: Correlation plot between dry weight of each pine litter sample and corresponding microbial N contents ($p = 0.006$; $r^2 = 0.0591$). The equation for the linear regression is 8.46×10^{-5} (x) + 5.3703.

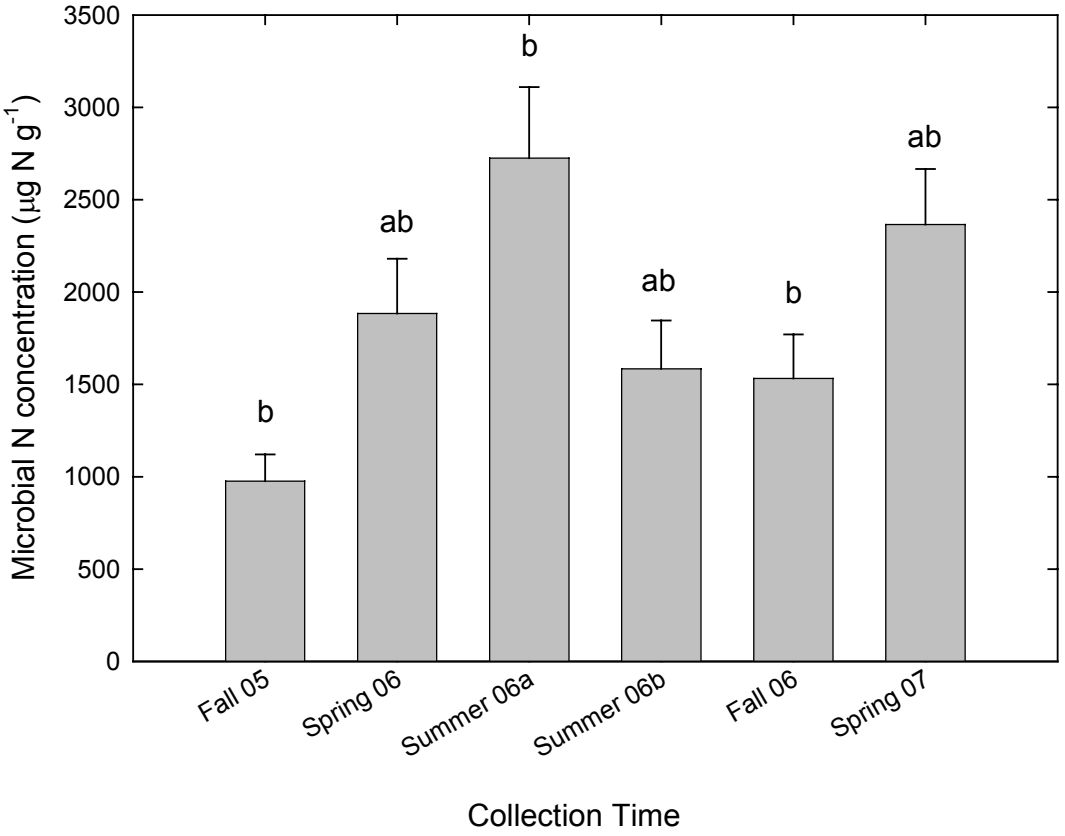

Fig. **5.4**: Mean (± 1 s.e.) microbial N concentrations for decomposer microorganisms collected at six different times are displayed (n = 8).

Temperature Sensitivity: Q_{10}

We calculated temperature quotient (Q_{10}) values for respiration rates at different temperatures using the following equation: $Q_{10} = 10^{(10 \text{ x regression slope})}$, where the regression slope is the slope of a plot of $\log_{10}$ (respiration) against temperature (Atkin *et al.*, 2000). Since the log respiration plots of respiration against measurement temperature

were linear, we calculated one Q_{10} value for each Petri dish of litter per incubator treatment from 5-23^{o}C measurement temperatures.

Statistical Analysis:

Because we measured respiration rates of each litter sample at a variety of measurement temperatures, we analyzed respiration rate using a Repeated Measures Proc Mixed ANOVA model in SAS (SAS Institute Inc. Version 9.1, 2002-2003). We used AR(1) covariance structure for the repeated measures procedure in SAS, which resulted in the lowest values for the Akaike information and Bayesian information criteria. All statistical analyses were done on respiration data that were log transformed. The model for assessing whether respiration rates differed temporally included time and measurement temperatures as factors for respiration rates measured at the incubator temperature that reflected field temperatures at the collection time. The model for assessing acclimation for each individual set of litter samples included incubator temperature and measurement temperature as factors. A significant incubator temperature effect indicated that respiration acclimated to different incubator temperatures only when respiration rates were lower for warm-exposed litter decomposer microorganisms than for cool-exposed litter decomposer microorganisms. Least square means were estimated for all respiration rates and used in all subsequent analyses. Proc Mixed procedures in SAS were used for comparisons among incubator temperatures for Q_{10} values and among collection times for microbial N biomass concentrations.

Results

Acclimation To Temperature By Decomposer Microorganisms:

Only one out of the six collections of litter exhibited acclimation to temperature by decomposer microorganisms, as evidenced by significantly higher rates of respiration for colder-incubated samples compared to warmer-incubated samples. In the single case of Spring 2007, the respiration rates for the 11 and 17^{o}C incubators were significantly lower (Tukey's Honest Significant Difference (HSD) Test) by an average of 2.7 % compared to the 5^{o}C incubator (Fig. **5.5**). The difference in respiration rate when incubated at different temperatures increased with measurement temperature, explaining the significant interaction between measurement and incubator temperature (Fig. **5.5**, Table **5.1**). Respiration at other collection times showed no evidence of temperature acclimation (Fig. **5.5**, Table **5.1**).

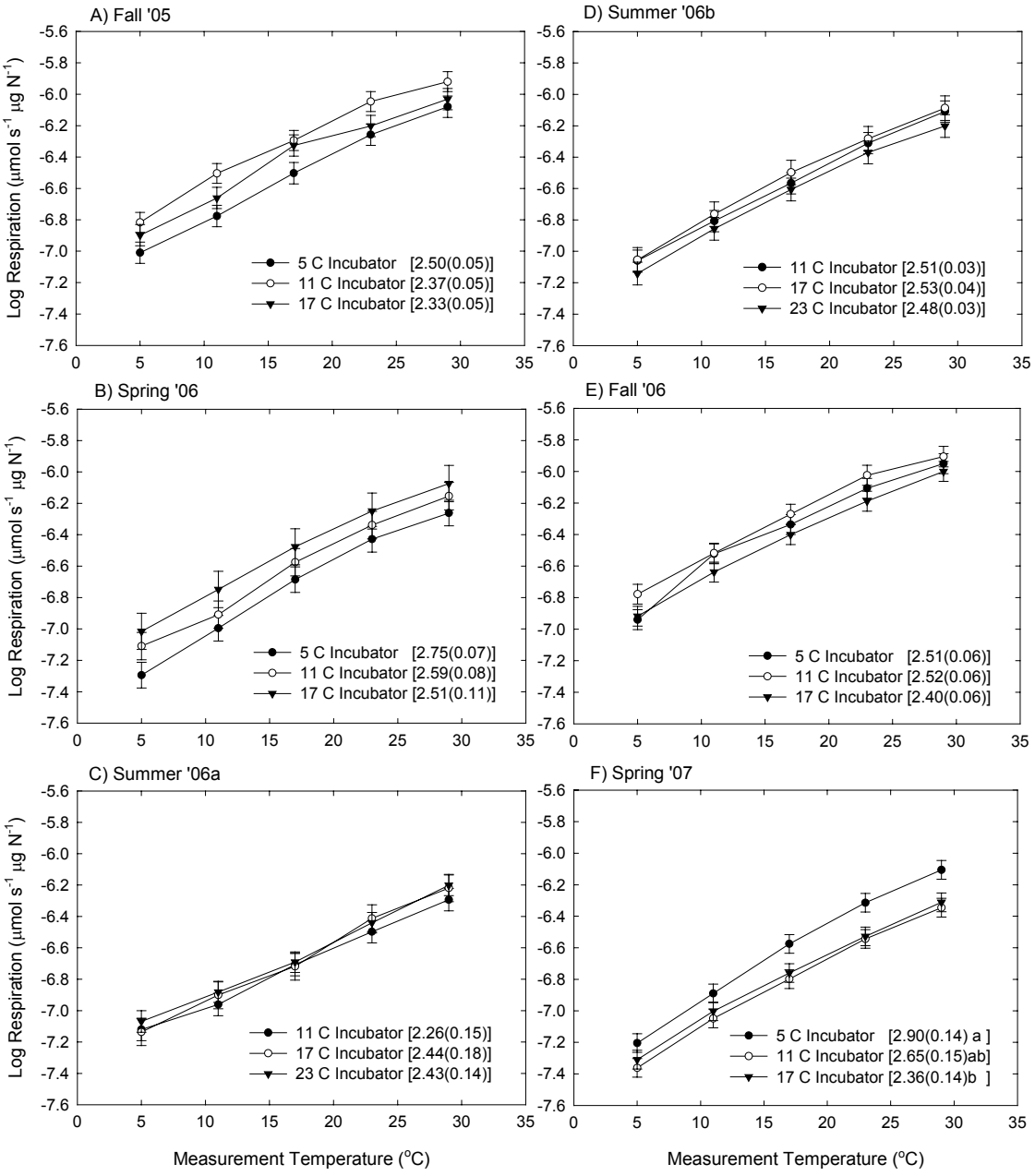

Fig. **5.5**: Mean (± 1 s.e.) decomposer microorganism respiration rates collected at six different times (A-F) and measured at temperatures between 5 and 29°C after exposure to three incubation temperatures. See Table 5.1 for results from the statistical analysis. In the legends, mean Q_{10} values (s.e.) are displayed for each incubator temperature. Significantly different Q_{10} values for incubators for each collection time are denoted with different letters ($p < 0.05$), determined from a post-hoc Tukey's HSD Test.

Table **5.1**: Results of analysis of variance for log respiration rate for decomposer microorganisms at each collection time as affected by incubator temperature and measurement temperature (n = 8).

Collection Time (month/day)	**Effects**	**Num. d.f.**	**Den. d.f.**	**F-value**	**P-value**
Fall '05	Incubator T	2	20	2.69	0.0926
(11/9)	Measurement T	4	76	502.59	<0.0001
	Incubator T × Measurement T	8	76	8.82	<0.0001
Spring '06	Incubator T	2	16	1.38	0.2806
(4/27)	Measurement T	4	64	264.31	<0.0001
	Incubator T × Measurement T	8	64	1.47	0.1842
Summer '06a	Incubator T	2	14	0.21	0.8145
(6/21)	Measurement T	4	53	206.44	<0.0001
	Incubator T × Measurement T	8	53	1.88	0.0834
Summer '06b	Incubator T	2	18	0.45	0.6428
(8/30)	Measurement T	4	72	1838.66	<0.0001
	Incubator T × Measurement T	8	72	3.53	0.0017
Fall '06	Incubator T	2	18	1.10	0.3537
(10/26)	Measurement T	4	68	885.31	<0.0001
	Incubator T × Measurement T	8	68	12.26	<0.0001
Spring '07	Incubator T	2	18	3.42	0.0546
(5/9)	Measurement T	4	72	1344.66	<0.0001
	Incubator T × Measurement T	8	72	12.26	0.006

Temporal Respiration Response of Decomposer Microorganisms To Temperature:

Respiration increased with measurement temperature and varied temporally, as evidenced by significant effects of measurement temperature and time, respectively (Table 2). Average respiration rates in Fall 2005 and Fall 2006 were significantly higher

than average respiration rates at the other collection times, as revealed by Tukey's HSD Test (Fig. **5.6** Fig. 6). Spring 2007 produced the lowest average respiration rates, while Spring 2006 and both Summer 2006 had intermediate average respiration rates (Fig. 6), between those in Spring 2007 and Fall 2005/2006 samples.

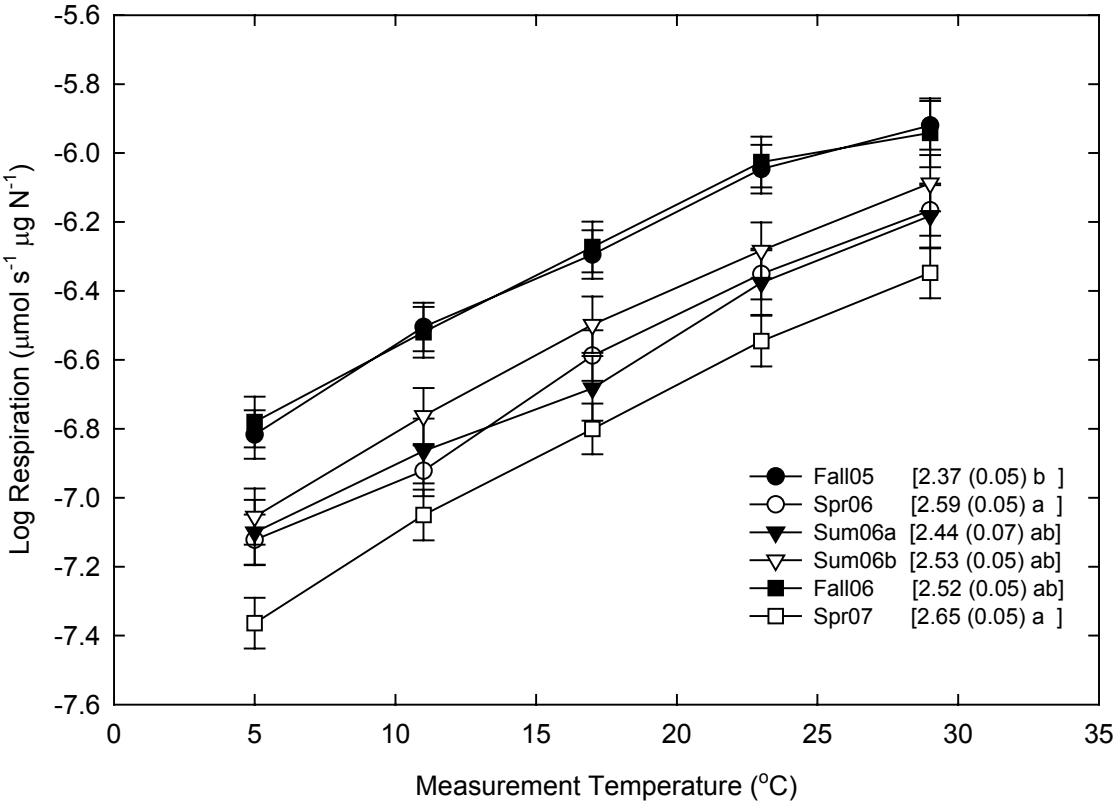

Fig. **5.6**: Mean (± 1 s.e.) decomposer microorganism respiration rates collected at six different times and measured at temperatures between 5 and 29°C after exposure to incubators set to "field temperature," as described in the methods section. In the legend, mean Q_{10} values (s.e.) are displayed for each collection time. Significantly different Q_{10} values for each collection time are denoted with different letters ($p < 0.05$), determined from a post-hoc Tukey's HSD Test.

Q_{10} values:

Q_{10} from Spring 2007 displayed a significant inverse relationship with incubator temperature (Fig. **5.5**). Q_{10} values for decomposer microorganisms from the other collection times displayed no significant relationship between temperature sensitivity and incubator temperature (Fig. **5.5**).

Respiratory Q_{10} values varied significantly for decomposer microorganisms from the different collection times. Q_{10} values were highest for Spring 2006 and Spring 2007, lowest for Fall 2005, and intermediate for Fall 2006 and for both collections times in Summer 2006, as revealed by Tukey's HSD Test (Fig. 5.6).

Discussion

In Spring 2007, we observed evidence of physiological acclimation to temperature, which resulted in a small decrease in respiration at common measurement temperatures as incubator temperature increased. It was only when respiration acclimated that we observed a decrease in Q_{10} as incubator temperature increased. Overall, Q_{10} values ranged between 2.36 and 2.90, which correspond well with those obtained in other studies from roots, ectomycorrhizal fungi, and decomposer microorganisms (Katterer *et al.*, 1998 and references therein; Bryla *et al.,* 2001; Bååth & Wallander, 2003; Malcolm *et al.,* 2007). At the other five collection times, however, we did not observe any evidence of physiological acclimation to temperature or of a change in the sensitivity of respiration (Q_{10}). Thus, the decomposer microorganisms in the litter from this plantation did not collectively make a net, short-term, reversible physiological

adjustment in response to changes in temperature. The result is unexpected given what has been observed in plants, many of which readily acclimate to temperature (Arnone & Korner, 1997; Bryla *et al.*, 1997; Tjoelker *et al.*, 1999; Atkin *et al.*, 2000; Atkin & Tjoelker, 2003; Bolstad *et al.*, 2003; Loveys *et al.*, 2003; Cooper, 2004), although there are some exceptions (Weger & Guy, 1991; Zogg *et al.*, 1996). A small fraction of ectomycorrhizal fungi acclimated to temperature, but when they did, the degree of acclimation was ten times greater than observed for the litter of the May 2007 collection (Malcolm *et al.,* 2007; see Chapter 3). The limited ability of decomposers to acclimate suggests that for short-term fluctuations in temperature (days), changes in the rate of decomposition associated with temperature shifts, will not be ameliorated by the process of acclimation.

Of course we cannot rule out the possibility that the seven days we allowed after temperature shifts of the litter was insufficient to permit acclimation by the resident decomposer microorganisms. However, several isolates of ectomycorrhizal fungi displayed the ability to acclimate to temperature within a week (Malcolm *et al*., 2007; see Chapter 3). Moreover, plants have typically exhibited acclimation within 1-3 days (Bryla *et al.*, 2001; Atkin & Tjoelker, 2003; Bolstad *et al.*, 2003). By using weeklong, short-term incubations, we were able allow sufficient time for handling effects to disappear (Robertson *et al.*, 1999), but presumably not for substrate limitations (Davidson & Janssens, 2006) or substantial community shifts (Panikov, 1999; Avrahami *et al.*, 2003) to occur, all of which can affect respiration.

Since the decomposer community on the whole appeared not to acclimate, the question as to whether different communities possess different capacities to acclimate is

easily answered. We did, however, find that respiration by decomposers changed temporally when compared at common measurement temperatures. Luo et al. (2001) found respiration decreased at common field temperatures when they warmed soils for one year by +2^{o}C. In the fall (cooler time of year) we found higher rates of respiration than in summer (warmer time of year) at common measurement temperatures. Yet in spring (another cooler timer of year), we found respiration rates at common measurement temperatures to be similar to those found in summer. This suggests that something other than temperature may have been influencing respiration by decomposer communities at different times of year. One possibility is a shift in the composition of the decomposer community.

We did not examine whether the structure of decomposer communities varied at different times of year at our field site. Long-term alterations of temperature have been shown to shift microbial community structure and to induce changes in the respiration of certain groups within the microbial community (Zogg *et al.*, 1997; Panikov, 1999; Avrahami *et al.*, 2003; Zhang *et al.*, 2005). It could be that differences in decomposer community structure may partly explain the variation in respiration we saw at different times of year.

Other influences on respiration include seasonal differences in substrate availability and seasonal differences in litter moisture. We found the highest respiration rates in both autumn samples. A greater availability of readily decomposable pine litter at that time of year could partially explain the higher rates of respiration. Numerous studies have revealed a positive relationship between litter quality, quantity, and decomposition rates (Couteaux *et al.*, 1995; Hobbie, 1996; Trofymow *et al.*, 2002; Smith

& Bradford, 2003; Fierer *et al.*, 2005). Although not known specifically for red pine, other pine forests have shown seasonal variability in substrate quality (Kim *et al.,* 1996; Gunadi *et al.,* 1998).

Although we brought all litter samples to a constant field capacity, it is possible that recent rainfall history could influence the size of the decomposer populations and thus respiration rate. Therefore, we also examined average daily rainfall for the week and month prior to our collection dates. Significantly higher respiration rates have been observed directly after heavy rainfall events (Lee *et al.*, 2002; Rey *et al.*, 2002; Lee *et al.*, 2003). However, we observed no obvious patterns of respiration with rainfall that could explain the temporal variation in respiration rates we observed. Clearly, more research is needed to understand the regulation of respiration by soil microorganisms, including the effects of substrate supply, temperature, and water availability on respiration (Davidson *et al.* 2006).

This is one of the first reports that addresses whether decomposer microorganisms can acclimate to temperature. Obviously, more studies in more plant community types need to be performed before general conclusions can be drawn. However, because short-term physiological acclimation to temperature did not occur, our results suggest that Q_{10} values calculated from short-term temperature response curves are relevant, at least with respect to short-term fluctuations in temperature that so often occur in forest soils. Moreover, because seasonal shifts in respiration rate and in Q_{10} occurred, a single Q_{10} may not be a meaningful description of the response to temperature by the decomposer community.

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Chapter 6

Conclusion

Summary

In Chapter 2, I showed that the temperature-controlled gas-exchange instrument, built to measure respiration by soil microorganisms in culture, was fast and reliable. Respiration rates attained were reversible, such that no damage had occurred to the microorganisms, and were taken at steady state. Respiration rates of ectomycorrhizal fungi were comparable to those obtained using an alkali-absorptive method and corresponded well with respiration rates in the literature for mycorrhizal fungi in culture and in microcosms.

In Chapter 3, I revealed that only 25% of the ectomycorrhizal fungal isolates acclimated to temperature and when *Suillus intermedius* acclimated its respiration to temperature, it took approximately 6 days. The ability of some ECM fungi to acclimate may partially ameliorate the anticipated positive feedback between soil respiration and temperature. Also, as soil temperatures increase, ECM fungi that acclimate may require less carbon from their host plants than fungi that do not acclimate. Much variation existed in the temperature sensitivity (Q_{10}) of respiration by the fungal isolates. The fact that variation occurs among ECM fungal species in their ability to acclimate and in the temperature sensitivity (Q_{10}) of respiration indicates that the response of the ectomycorrhizal fungal community as a whole, which contributes significantly to soil

respiration, will be determined by the structure of that community. Lastly, numerous classic Autecology studies have documented a wide range of acclimation ability for plants and other organisms. This study is the first to examine the thermal acclimation ability of respiration by ectomycorrhizal fungi.

In Chapter 4, I reported strong evidence for latitude variation in respiration response to temperature by four paired genera contrasts of ectomycorrhizal fungi from Alaska and Pennsylvania, which suggests that ectomycorrhizal fungi are adapted to the thermal regime of their latitude of origin. Thus, ectomycorrhizal fungi from differing latitudes should have similar carbon demands from their hosts. Ectomycorrhizal fungi from Alaska had lower estimated growth rates and Q_{10} values in response to temperature as compared to ectomycorrhizal fungi from Pennsylvania. With increasing temperature, therefore, the proportional increase in respiration may be lower for ectomycorrhizal fungi from a high latitude than those from a lower latitude. Numerous classic Autecology studies have documented evidence for latitude variation in the physiology of plants and other organisms, but to the best of my knowledge, this study is the first to document the same variation for ectomycorrhizal fungi.

In Chapter 5, I concluded that there is limited evidence for acclimation by decomposer microorganisms. In fact, out of six different times of year, decomposers from one collection, May 2007, displayed acclimation ability. The temperature sensitivity of the decomposer microorganisms from May 2007 decreased as they acclimated to warmer temperatures. Between different points in the season, some decomposer microorganisms appeared to make full adjustment to their respiration rates such that they were equal even when prevailing temperatures in the field differed by $6^{o}C$.

Between different seasons, however, decomposer microorganisms did not appear to adjust their respiration rates in response to the change in prevailing temperature. Again, this highlights a wide variability in the respiration response to temperature by decomposer microorganisms. Other factors, including substrate availability, moisture levels, and community structure, might also affect their respiration rates. Therefore, the respiration response to temperature by decomposer microorganisms may vary widely depending upon the time of year and the particular forest ecosystem in which they are found.

Limitations

For all experiments, I worked with soil microorganisms in culture. While this obviously does not represent the natural condition, it represents a start for exploring the metabolic responses to temperature by soil microorganisms. By working with ectomycorrhizal fungi in culture, I was able to avoid confounds, such as the potential for preferential carbon transport from certain hosts to certain fungal isolates. Substrate availability has been shown to influence both an organism's ability to acclimate to temperature and its temperature sensitivity. Notably, the respiration rates may not be a reflection of absolute values found in the field. The patterns found between respiration and temperature among the ectomycorrhizal fungal isolates, however, should hold true. By working with litter decomposer microorganisms in culture, I was able to maintain an environment of constant moisture across all of the incubator temperature treatments. Low levels of moisture have been shown to reduce soil respiration rates when limiting,

masking any effects temperature may have had on respiration. In all experiments, respiration rates were within the same order of magnitude as examples in the literature from culture, microcosm, and some field studies.

Future Research

Very few studies have explored the physiological plasticity of mycorrhizal fungi, so my thesis represents a novel contribution to this field. Thus, those portions of my thesis were more interesting to me. For the ectomycorrhizal fungal studies, a logical next step would be to explore the same questions I have outlined here, but at the level of the symbiosis. Additionally, classic Autecology studies have revealed that plants with different life histories, life spans, etc tend to have a range of abilities to acclimate. For instance, plants that experienced a more variable temperature regime during their lifetime were more apt to acclimate than plants that experienced a stable temperature regime. It would be quite interesting to see if ectomycorrhizal fungi that tend to be found in surface soil layers versus those in deeper soil layers, for instance, might be more apt to acclimate due to the potentially more variable temperature regime found there.

Concluding Remarks

In my dissertation, I borrowed classic Autecology questions from plant ecology literature and applied them to ectomycorrhizal fungi. It seemed necessary since while much is known regarding the physiological plasticity of plants, little is known for their

symbiont, mycorrhizal fungi. By participating in the Biosphere Atmosphere Research and Training (BART) program (which is part of a National Science Foundation (NSF) Interdiscliplinary Graduate Education and Research Training (IGERT) grant), I began considering how mycorrhizal fungi interacted with the atmosphere. It also became apparent that there was a lack of knowledge regarding how ectomycorrhizal fungi fit into the terrestrial carbon cycle and what we might expect of them under global warming scenarios.

The strength of these series of experiments lies in the classic ecological questions that allowed me to find general patterns among different soil microorganisms. My research also fills in gaps in knowledge regarding the physiological plasticity of soil microorganisms. Although I began by seeking general ecological patterns, I find myself much more interested in the variability among microorganisms in their responses to temperature and how knowledge like this might be scaled up to the level of ecosystems.

VITA

Glenna Marjorie Malcolm

I was born in Pittsfield, Massachusetts in the year one thousand nine hundred and eighty. Fortunately for me, while growing up, a vast swath of forest lay at the back of my yard. I spent my childhood forging trails, camping, and building forts with my brother and the other neighborhood kids. I was considered the "brain" and my brother was considered the "jock". In high school, however, I found my sport – trail running. I blame all of this for my continued interest in nature throughout my schooling.

I attended Union College from 1998-2002. My undergraduate Biology advisor, Dr. Steven Rice, allowed me to pursue my short-lived idea of becoming a science journalist in exchange for doing research for him in his lab for the summer. At the end of the summer, I realized that I had a passion for Ecology and designed an Honor's thesis project where I examined the effects of removing the nitrogen-fixing black locust on growth of native prairie grasses.

My two years of research experience in Dr. Rice's lab allowed me to enter directly into the Ecology program at PSU where I am at the finishing stage of my doctoral degree. In the future, I look forward to teaching at a small college, with some research on the side in order that other students might have similar opportunities as I did while an undergraduate.