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Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi

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Abstract

Ectomycorrhizal (ECM) fungi contribute significantly to ecosystem respiration, but little research has addressed the effect of temperature on ECM fungal respiration. Some 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 cooler 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. Among the 12 ECM fungal isolates that were tested, *Suillus intermedius*, *Cenococcum geophilum*, and *Lactarius* cf. *pubescens* exhibited significant acclimation to temperature, exhibiting an average reduction in respiration of 20–45% when incubated at 23 °C compared with when incubated at 11 or 17 °C. The 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 an 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.

Keywords: acclimation, carbon cycling, climate change, ectomycorrhizal fungi, global warming, Q_{10}, respiration, temperature

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Introduction

Ectomycorrhizal (ECM) fungi form mutualistic relationships 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 organic soil carbon (Dighton *et al*., 1987; Colpaert & van Tichelen, 1996; Hobbie *et al*., 1999; Read & Perez-Moreno, 2003), most are considered to be primarily 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 carbon 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 and 3.6 °C by the year 2100, driven by an increase in atmospheric CO_2 derived from natural and anthropogenic sources (IPCC, 2007). In terrestrial

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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 are to predict climate-induced change in respiration in different ecosystems. Yet, 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*., 2000a, b; 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*., 2000a, b; 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 isolates that were held at different temperatures for 1 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 also assessed whether or not there was a shift in Q_{10} associated with temperature acclimation for these isolates of ECM fungi. To our knowledge, this is the first study to address whether ECM fungi are capable of acclimation to temperature and whether temperature sensitivity of respiration is affected by acclimation.

Differences in substrate availability can affect an organism's ability to acclimate to temperature 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 carbon 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. We realize, of course, that while general ecophysiological patterns may emerge from the use of axenic cultures, respiration rates of ECM fungi *in symbio* may differ due to the effect of host plant on carbohydrate supply.

Methods

Biological material

Sporocarps of *Amanita muscaria* var. *muscaria* (AK001), *Lactarius* cf. *pubescens* (AK010), and *Suillus* cf. *grevillei* (AK014), *Leccinum* cf. *alaskanum* (GL005), and *A. 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), *A. 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. ECM fungal isolates were chosen based on our desire to have broad representation from different fungal clades. Fungal isolate designations, specific locations of collections, and the vegetation at those locations are given in Table 1. Fungal cultures were 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 $NH_4Cl\,L^{-1}$ of water).

Incubator temperature shifts

Each isolate was subcultured in 15 separate 100 mm × 15 mm Petri dishes containing modified potato dextrose agar (above). 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

Table 1 Details of collections of the 12 isolates of ectomycorrhizal fungi

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, LTER, 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	*P. 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*

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.

maintained in incubators at 11 and 17 °C, respectively, which are representative of the average temperatures of forest litter during the fungal fruiting season at their sites of origin. Many ectomycorrhizal fungal species have been found to subsist in the litter layer of forest soils (Dickie *et al*., 2002). Between July 21 and August 21, 2002, the mean temperature for the forest litter under *Picea mariana* in the Bonanza Creek LTER site was 11.5 °C (Vogel & Valentine, 2006). Between June 1 and October 31, 2002, the mean temperature for the forest litter under *Pinus resinosa* in State College was 16.4 °C (G. M. Malcolm, unpublished data). We waited a variable amount of time (from 1 to 3 weeks) for each plug to increase in diameter approximately 2–4 mm before initiating temperature shifts. At that time, five Petri dishes of each isolate were placed into each of three separate incubators set to 11, 17, and 23 °C for a period of 7 days. Following these temperature shifts, respiration from the fungal colony in each Petri dish for each isolate was measured over a range of temperatures.

Measuring respiration rates

We constructed a temperature-controlled gas-exchange system with which to measure respiration rates. The system (Fig. 1) included a temperature-controlled steel surface on which were 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, USA) 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.

Individual Petri dishes 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-h 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 $\mu L\,L^{-1}$ 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 min to attain new steady state respiration rates before respiration was measured again. As a check for hysteresis, we determined for each Petri dish whether the respiration rate at the initial measurement temperature could be duplicated when the temperature was restored to the initial value following measurement at higher or lower

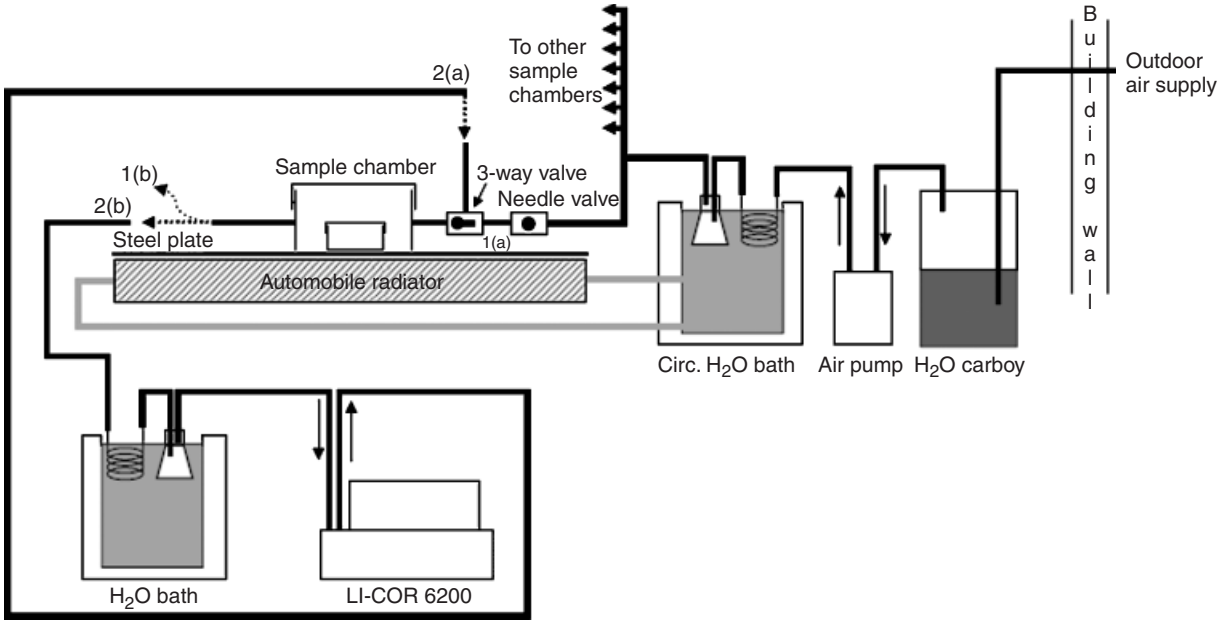

Fig. 1 Schematic drawing of the gas exchange and temperature control apparatus [adapted from Huang *et al.* (2005)]. In the open flow path (1a and 1b), air from the outdoors is humidified and then brought to the temperature of the chambers. After it enters the chambers it passes out into the laboratory. 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.

measurement temperatures. If the initial respiration rate could not be reattained, we considered the tissue to have been irreversibly damaged by the temperature extreme and the data at the damaging temperatures were not used in further analyses.

Following respiration measurements, fungal tissues were separated from the agar by melting it in a boiling water bath and filtering 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 *S. intermedius* (BX007) into numerous Petri dishes and incubated them at 17 °C for 9 days. All but four of the dishes were shifted into the 23 °C incubator. For those four dishes, we measured respiration rates at 17 °C and, 50 min later, at 23 °C. Each day for 1 week after the initial respiration measurements were made, we measured respiration at 23 °C on a distinct subset of four Petri dishes that were being incubated at 23 °C. We determined dry weights for each subset following the respiration measurements. Our goal was to characterize the length of time that it took respiration by *S. intermedius* to reduce respiration rates measured at 23 °C to a lower stable value.

Q_{10}

Q_{10} values ($n = 5$ isolate^{-1} incubator^{-1}) were calculated using the following equation: $Q_{10} = 10^{(10 \times \text{regression slope})}$ (Atkin *et al.*, 2000a, b), where in our case the regression slope is that from a plot of $\log_{10}$ (Respiration) vs. measurement temperature. 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 °C range; in that range, the regression slopes were essentially linear.

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 effect of incubator temperature or incubator × measurement temperature interaction indicated that respiration acclimated to temperature when respiration rates were significantly lower for warmer-incubated fungi than for cooler-incubated fungi, with further detail provided by Tukey's Honest Significant Difference *post hoc* tests for each fungal isolate. 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 *L.* cf. *alaskanum* (GL005) and *A. muscaria* var. *muscaria* (AK001) demonstrated hysteresis and/or abnormal growth when incubation occurred at 23 °C. Therefore, the respiration rates for those isolates at that incubator temperature were not included in the analyses. When all 12 isolates were analyzed together, we found a significant isolate × incubator temperature - measurement temperature interaction (Table 2). We therefore analyzed each isolate separately. The respiration rates of all isolates were significantly increased by measurement temperature (Table 3, Fig. 2). Three of the 12 isolates exhibited acclimation to temperature as evidenced by significantly higher rates of respiration for cooler-incubated fungi compared with warmer-incubated fungi. In the case of *L.* cf. *pubescens* (AK010), the respiration rates for 23 °C-incubated cultures were significantly lower (Tukey's Honest Significant Difference test) by an average of 20% compared with either 11- or 17 °C-incubated cultures (Fig. 2). In the case of *S. intermedius* (BX007), the respiration rates were significantly lower by an average of 48% when incubated at 23 than at 11 °C, but not significantly different when incubated at 17 °C (Fig. 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). In the case of *C. geophilum* (SC032), there was a significant interaction between measurement and incubator temperature (Table 3, Fig. 2) such that the expected difference in respiration rate between warmer- and cooler-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 °C-incubated cultures compared with the 23 °C-incubated cultures. None of the other isolates exhibited significantly higher rates of respiration for colder-incubated fungi compared with warmer-incubated fungi.

Table 2 Results of analysis of variance for respiration rate as affected by incubator temperature, measurement temperature, and ECM fungal isolate ($n = 5$)

Effects	Num. df	Den. df	*F*-value	*P*-value
Incubator temperature	2	139	8.14	0.001
Measurement temperature	2	278	5750	< 0.0001
Isolate	11	139	37.5	< 0.0001
Incubator temperature × measurement temperature	4	278	1.54	0.190
Isolate × measurement temperature	22	278	58.1	< 0.0001
Isolate × incubator temperature	22	139	1.42	0.116
Isolate × incubator temperature × measurement temperature	44	278	2.98	< 0.0001

Acclimation time course

S. intermedius (BX007) acclimated over the course of several days, reaching lower respiration rates that closely matched those measured at 17 °C. The reduction in respiration occurred slowly over the course of about 6 days (Fig. 3).

Q_{10}

The significant isolate × incubator temperature interaction (Table 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, 17 to 23, and 11 to 23 °C, respectively (Table 5). Additionally, within an incubator temperature, ECM fungal isolates showed significant variability in Q_{10} (Fig. 4a–c), ranging from 1.67 to 2.56.

Discussion

Hacskaylo *et al*. (1965) previously demonstrated the dependence of respiration on temperature by ECM fungi but, as far as we are aware, this is the first study to address whether or not ECM fungi acclimate to temperature. Our study indicates that significant variation exists among isolates of ECM fungi in their ability to acclimate to temperature. Three out of 12 ECM fungal isolates (*S. intermedius* and *C. geophilum* from PA and *L.* cf. *pubescens* from AK) exhibited significant acclimation.

Table 3 Results of analysis of variance for respiration rate for each ectomycorrhizal (ECM) fungal isolate as affected by incubator temperature and measurement temperature ($n = 5$)

ECM fungal species (isolate)	Effects	Num. df	Den. df	*F*-value	*P*-value
Amanita muscaria var. *muscaria*	Incubator temperature	1	7	1.75	0.277
(AK001)	Measurement temperature	2	14	214	<0.0001
	Incubator temperature × measurement temperature	2	14	0.23	0.170
Lactarius cf. *pubescens*	Incubator temperature	2	12	5.09	0.0250
(AK010)	Measurement temperature	2	24	1770	<0.0001
	Incubator temperature × measurement temperature	4	24	4.34	0.0080
Suillus cf. *grevillei*	Incubator temperature	2	12	0.10	0.909
(AK014)	Measurement temperature	2	24	821	<0.0001
	Incubator temperature × measurement temperature	4	24	1.42	0.256
L. cf. *alaskanum*	Incubator temperature	1	8	0.49	0.503
(GL005)	Measurement temperature	2	16	331	<0.0001
	Incubator temperature × measurement temperature	2	16	0.91	0.422
A. muscaria var. *muscaria*	Incubator temperature	2	12	0.92	0.422
(GL015)	Measurement temperature	2	24	339	<0.0001
	Incubator temperature × measurement temperature	4	24	0.80	0.538
S. intermedius	Incubator temperature	2	12	6.66	0.0114
(BX007)	Measurement temperature	2	24	364	<0.0001
	Incubator temperature × measurement temperature	4	24	7.44	0.001
A. muscaria var. *formosa*	Incubator temperature	2	11	1.44	0.277
(BX008)	Measurement temperature	2	22	396	<0.0001
	Incubator temperature × measurement temperature	4	22	1.77	0.170
Lactarius sp.	Incubator temperature	2	11	1.30	0.312
(SC003)	Measurement temperature	2	22	717	<0.0001
	Incubator temperature × measurement temperature	4	22	2.85	0.0484
L. aurantiacum	Incubator temperature	2	11	1.00	0.399
(SC014)	Measurement temperature	2	11	433	<0.0001
	Incubator temperature × measurement temperature	4	22	2.02	0.127
L. chrysorrheus	Incubator temperature	2	12	0.46	0.644
(SC016)	Measurement temperature	2	12	1160	<0.0001
	Incubator temperature × measurement temperature	4	24	19.5	<0.0001
Cenococcum geophilum	Incubator temperature	2	12	2.48	0.125
(SC032)	Measurement temperature	2	12	253	<0.0001
	Incubator temperature × measurement temperature	4	24	3.82	0.0154
A. citrina	Incubator temperature	2	12	2.42	0.131
(SC070)	Measurement temperature	2	12	227	<0.0001
	Incubator temperature × measurement temperature	4	24	0.25	0.909

Acclimation to temperature could ameliorate the effect of warming on soil respiration (Luo *et al*., 2001), to which ECM fungi contribute significantly (Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003). The fact that variation occurs among ECM fungal species in their ability to acclimate indicates that the response of the ECM 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 availability (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 the ECM fungal community to warming and the degree of positive feedback between temperature and respiration. However, not all species of an ECM fungal community are predicted to influence community responses to temperature to the same extent. It is often the case that one or a few species of ECM fungi dominate a particular community. For example, *C. geophilum* is dominant in our red pine (*P. resinosa*) plantation study site (Koide *et al*., 2007). Because the isolate of *C. geophilum* was one that exhibited temperature acclimation, it is possible that the ECM fungal community as a whole will exhibit temperature acclimation despite only a fraction of the component species doing so.

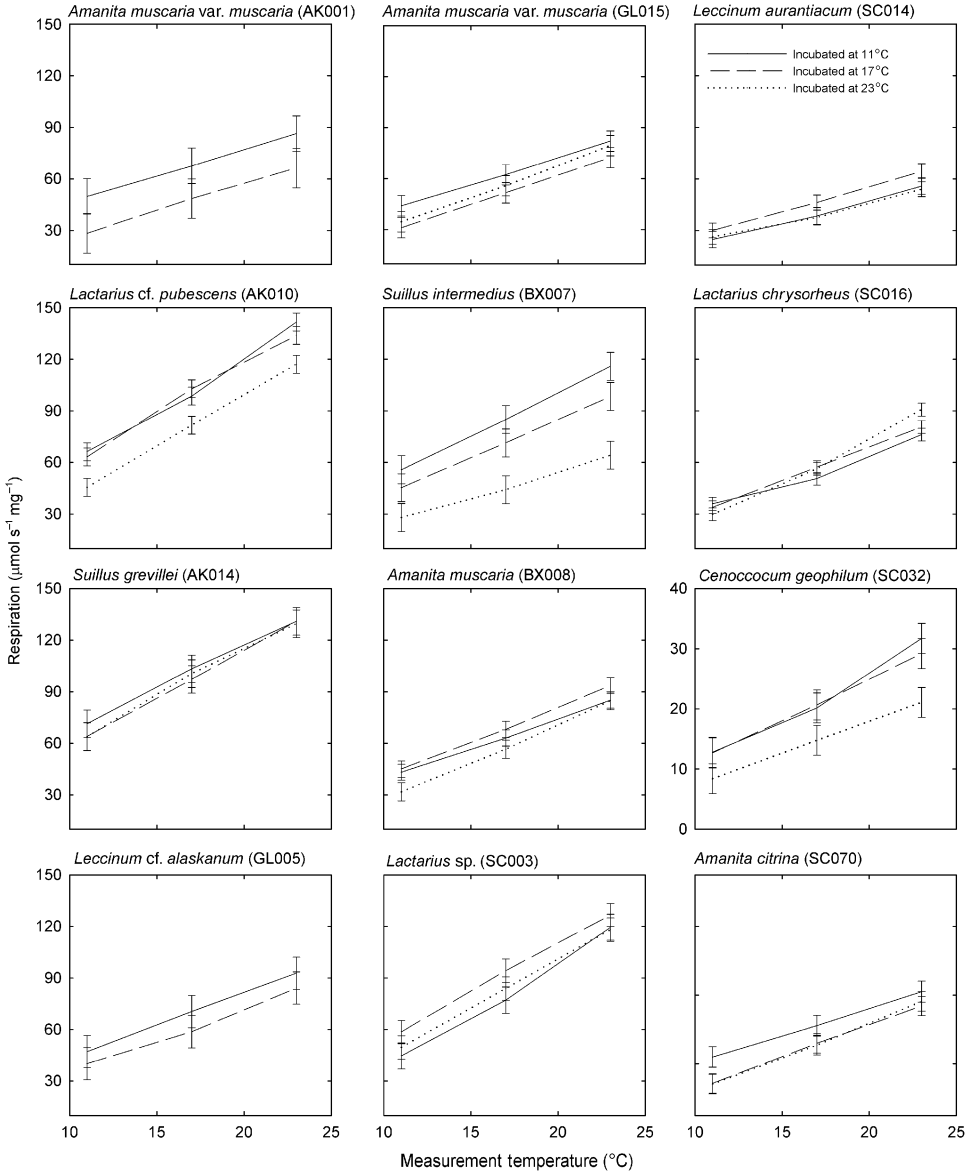

Fig. 2 Mean (±1 SE) ECM fungal respiration rates measured at temperatures between 11 and 23 °C after being grown at three incubation temperatures. ECM, ectomycorrhizal.

While a 7-day incubation was sufficient to reveal temperature acclimation in three of the isolates, we cannot rule out the possibility that 7 days was insufficient to permit acclimation in all isolates of ECM fungi. However, our respiration time course for *S. intermedius* (BX007) revealed that 6 days resulted in a lowering of respiration to the extent that respiration when the fungal isolate was incubated at 17 °C and measured at 17 °C was similar to when the fungal isolate was incubated at 23 °C and measured at 23 °C. Moreover, plants typically exhibit temperature acclimation within 1–3 days (Atkin & Tjoelker, 2003; Bolstad *et al*., 2003). There was also a methodological concern with longer incubation periods. It is possible that as fungal cultures grow, older tissues become less active, even to the point of having cytoplasm retracted from them. Thus, respiration rates calculated using dry weights would become increasingly error-prone as the ratio of active to inactive mycelium declines with time. Our preliminary results were consistent with that hypothesis. In order to avoid that potential error, we incubated cultures for only 7 days.

Global warming is obviously expected to occur over much longer time scales than the 7 days during which we incubated the ECM fungal cultures. Moreover, change in temperature may interact with change in water availability in ways that are not yet predictable

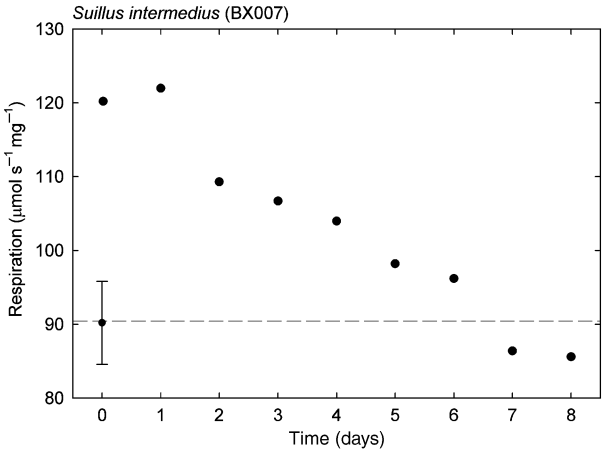

Fig. 3 ECM fungal respiration rates measured at 23 °C following a shift in incubator temperature from 17 to 23 °C over the course of a week ($n = 5$). The solid circle with standard error bars is the mean respiration rate at 17 °C on the initial day, and serves as a reference for respiration reduction due to acclimation. The other solid circles represent the actual average respiration rate every 24 h for 8 days. ECM, ectomycorrhizal.

Table 4 Result of analysis of variance for Q_{10} values as affected by incubator temperature and isolate ($n = 5$)

Effects	df	Sum of squares	*F*-value	*P*-value
Incubator temperature	2	3.02	4.65	<0.0001
Isolate	11	1.67	14.1	<0.0001
Isolate × incubator temperature	22	2.87	2.21	0.0031
Error	139	8.22	~	~

Q_{10} values were calculated for the 11–23 °C-measurement temperature range.

in particular locations. Nevertheless, our results suggest that for the ECM fungi that persist in the community over long periods of time, significantly less than expected respiratory losses may result from warming as a consequence of acclimation. The capacity to acclimate will also influence the physiology of organisms on shorter time scales. For example, diurnal and seasonal shifts in soil temperatures will have less of an effect on ECM fungal respiration for species that acclimate. These species will thus require less carbohydrate from hosts than expected at elevated temperatures, and more carbohydrate than expected at cooler temperatures.

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

Table 5 Mean Q_{10} (SE) for each of three incubator temperatures for each ectomycorrhizal (ECM) fungal isolate are reported. Q_{10} values were calculated for the 11–23 °C-measurement temperature range

Isolate	Incubation temperature	Q_{10}
Amanita muscaria var. *muscaria* (AK001)	11	1.68 (0.10)
	17	2.03 (0.11)
	23	
Lactarius cf. *pubescens* (AK010)	11	1.92 (0.07) a
	17	1.88 (0.07) a
	23	2.21 (0.07) b
Suillus 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
Cenococcum 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

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 Difference test.

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 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.

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: 10–16 μmol CO_2 s^{-1} mg^{-1} dry weight at 31 °C (Taber & Taber, 1987), and 50–70 μmol O_2 s^{-1} mg^{-1} dry weight at 25 °C (Souto *et al*., 2000). The respiration rates of *Hebeloma crustuliniforme* attached to living roots of *P. ponderosa* were also in the range of 11–16 μmol CO_2 s^{-1} mg^{-1} dry weight at 24 °C (Rygiewicz & Andersen, 1994), which suggests that our measured values of respiration on fungi cultured *in vitro* were similar to those for fungi *in symbio*. As a check, we assessed whether our reported values of respiration were reasonable for ECM fungi in the field. Assuming that for respiration the ratio of ECM mycelium to root plus ECM mycelium is 0.242 (the mean values reported by Söderstrom & Read, 1987 and Rygiewicz & Andersen, 1994) and that the root plus ECM mycelium respiration rate in boreal forest is 70 mg C mg^{-1} h^{-1} (Bhupinderpal-Singh *et al*., 2003), the ECM mycelium respiration rate would be 16.9 mg C m^{-2} h^{-1} (0.242 × 70). If we then assume that the total living biomass of ECM fungal mycelium in boreal forest is 163 kg ha^{-1} (the mean of 125 and 200 kg ha^{-1}, estimates of yearly production of ECM hyphae, Wallander *et al*., 2001), then the ECM fungal respiration rate by hyphae would be 24 μmol CO_2 mg^{-1} dry weight s^{-1}. This value is somewhat lower than the values we reported. This could be due to the fact that in the Petri dishes the conditions for growth (water, carbohydrate, and mineral availabilities) were closer to ideal than in the field. Although we are confident in the general conclusion that variation exists among isolates of ECM fungi in their respiratory responses to temperature, we caution readers in the use of the absolute respiration rates reported here for scaling respiration to the level of the ecosystem. Further respiration-temperature studies of ECM fungi under more varied environmental conditions and in symbiosis with plant hosts need to be conducted.

Eleven of the twelve ECM fungal isolates respired between 20 and 150 μmol CO_2 mg^{-1} dry weight s^{-1}

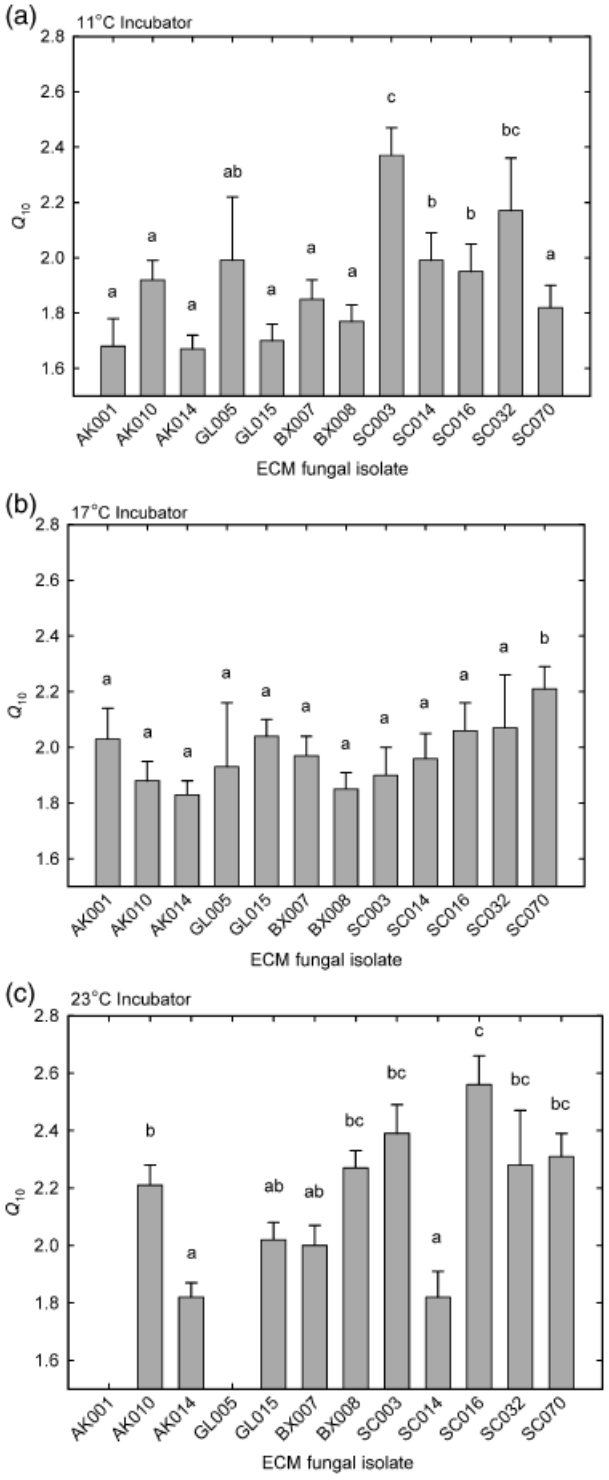

Fig. 4 Mean Q_{10} (SE) for each ECM fungal isolate at incubator temperatures of 11 °C (a), 17 °C (b), and 23 °C (c). Q_{10} values were calculated for the 11–23 °C-measurement temperature range. Significantly different Q_{10} values for incubators for each species are denoted with different letters ($P < 0.05$), determined from a *post hoc* Tukey's Honest Significant Difference test. ECM, ectomycorrhizal.

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 mg^{-1} dry weight s^{-1} over the same range. *C. geophilum* is an Ascomycete, while the other 11 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.

For half of the 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 some physiological changes in response to incubator temperature are 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 & Tjoelker, 2003) or without a change (Type II acclimation; Atkin & Tjoelker, 2003). Several researchers have similarly found that there is no relationship between Q_{10} and growth temperature (Atkin *et al.*, 2000a, b; Tjoelker *et al.*, 2001; Loveys *et al.*, 2003).

Q_{10} values for ectomycorrhizal fungi ranged from 1.67 to 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 because Q_{10} can be a temperature dependent process, once outside of our experimental temperature range (11–23 °C), a nonstatic 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.

We have not adequately addressed in this study whether latitude has an effect on acclimation to temperature. Some of the isolates originated from Alaska and others from Pennsylvania, but only two isolates from Pennsylvania and a single isolate from Alaska exhibited acclimation, and this sample size was too small to adequately test the latitude hypothesis. Future work encompassing many more isolates would be necessary to test this hypothesis.

Conclusions

We present some of the first data describing respiration acclimation to temperature and sensitivity to changes in temperature for isolates of ectomycorrhizal fungi. As global temperatures increase, the ability to acclimate respiration to temperature by ECM fungi could partially ameliorate the positive feedback between soil respiration and temperature and reduce the carbon demand by ECM fungi upon their plant hosts. 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, which contributes significantly to soil respiration (Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003), will be determined by the structure of that community. Similarly, due to variation among isolates in Q_{10}, the temperature sensitivity of respiration by the ECM fungal community would be determined by structure of the community. Substrate availability can influence acclimation and sensitivity to temperature (Atkin & Tjoelker, 2003; Davidson *et al.*, 2006). Since little is known regarding plant hosts' abilities to potentially control the substrate supply to mycorrhizal fungi, future experiments that examine acclimation of respiration by mycorrhizal plant roots are essential. Additionally, little is known regarding the potential environmental controls on the plasticity of fungal respiration in response to temperature.

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