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Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants

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Abstract. Biotic interactions can affect the distribution of species across environmental gradients, and as air and soil temperatures increase, plant community response may depend on interactions with symbionts. We measured the effect of elevated soil temperatures on mycorrhizal function and on the response of both plant and fungal symbionts, using fungal inoculum isolated from either high-temperature thermal or nonthermal grassland soils. Our source for thermal soils was Yellowstone National Park, USA, where plants experience rooting zone temperatures of 45°C or more. In the greenhouse, we grew three plant species (*Dichanthelium lanuginosum*, *Agrostis scabra*, and *Mimulus guttatus*) with three arbuscular mycorrhizal fungal (AMF) treatments (no AMF, nonthermal AMF, thermal AMF) and two soil temperatures (ambient, elevated). Biomass of the facultative thermal plants *Agrostis scabra* and *Mimulus guttatus* decreased by 50% in elevated-temperature soils, and AMF had no effect on measured plant traits. In contrast, the biomass and total root length of the obligate thermal plant *Dichanthelium lanuginosum* were greater at elevated soil temperatures, but only when mycorrhizal. Both mycorrhizal colonization levels and length of extraradical hyphae (ERH) increased with soil temperature across all host species. The source of the AMF inoculum, on the other hand, did not affect colonization level, ERH length, host plant biomass, or flowering for all host species in either temperature treatment, suggesting that AMF from thermal soils are not specifically adapted to higher temperatures. In the field we collected soil cores to measure in situ depth distributions of *D. lanuginosum* roots and ERH, and to determine which AMF species were active in plants growing in thermal soils. Roots were limited to soils with an average temperature $\leq$30°C, while ERH existed in the hottest soils we sampled, averaging 35°C. Molecular analyses of roots indicated that thermal AMF communities were composed of both generalist and possibly unique fungal species. The increase in host plant allocation to AMF, apparent lack of temperature adaptation by AMF, and differential host response to AMF suggest that AMF could be significant drivers of plant community response to increased soil temperature associated with global change.

Key words: Agrostis scabra; *arbuscular mycorrhizae;* Dichanthelium lanuginosum; *extraradical hyphae; extreme environments; heat stress;* Mimulus guttatus; *root growth; soil temperature; Yellowstone National Park, Montana and Wyoming, USA.*

Introduction

Temperature stress affects plants across the globe (Boyer 1982), and relatively high soil temperatures have been documented in alpine, tropical, and desert habitats (Hadley 1970, Chambers 1997, George et al. 2002, Pearson et al. 2002), forested sites that have burned (Iverson and Hutchinson 2002), and thermal soils in geologically active areas (Stout et al. 1997). Because global air temperatures are expected to increase by about 3°C over the next century (Intergovernmental Panel on Climate Change 2007), and because soil temperature tracks air temperature (Pregitzer et al. 2000), understanding plant response to rising soil temperatures is critical. Specifically, plant roots are directly affected by increased soil temperatures, with changes in morphology (Smith and Roncadori 1986, Haugen and Smith 1992), growth rate, and longevity (Forbes et al. 1997). These adjustments can decrease root surface area, volume of soil explored, and root life span, ultimately reducing root function.

Arbuscular mycorrhizae, which form symbiotically between the roots of the majority of terrestrial plants (Smith and Read 1997) and fungi of the phylum Glomeromycota (Schüßler et al. 2001), can decrease environmental stress for the host plant (Entry et al. 2002, Meharg 2003, Baumann et al. 2005). For example, if fungi are more heat tolerant than plant roots, as has been suggested in agricultural systems (Tungate et al. 2007), arbuscular mycorrhizal fungi (AMF) could increase host plants' access to water and nutrients by extending the growth of extraradical hyphae (ERH) into soils where elevated temperatures prohibit root growth, expanding the plants' available habitat.

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In fact, plant carbon allocation to arbuscular mycorrhizal fungi increases with increasing temperatures (Gavito et al. 2003), but the advantages to the host plant of exploring additional soil habitat could be negated by the carbon cost of increased fungal respiration at high temperatures (Hawkes et al. 2008). Host plant response to AMF is dynamic, in terms of cost and benefits (Johnson et al. 1997), varying with plant life stage (Sanders and Fitter 1992), plant species (Hetrick et al. 1988, Wilson and Hartnett 1998, Johnson et al. 2005), fungal species (van der Heijden et al. 2003, Johnson et al. 2005), and abiotic stresses (Titus and del Moral 1998, Johnson et al. 2006). Based on this, our first objective was to research the overall effect of elevated soil temperatures on the mycorrhizal symbiosis, including the response of both the plant and fungal symbionts to elevated temperatures.

Because symbiosis function is dependent upon the response of both partners to the environmental stress, adaptation by either symbiont could alter the nature of the symbiosis. With the high degree of genetic variability within AMF taxa (Corradi et al. 2007, Rosendahl 2008) extreme environments may exert a strong selection pressure for fungal traits that confer survival to both host plants (Oliveira et al. 2005, Marulanda et al. 2007) and the fungal symbiont (Weissenhorn et al. 1993, del Val et al. 1999). There is some evidence that isolates of AMF perform best at their temperatures of origin (Schenck 1975, Siqueira et al. 1985, Grey 1991), but little is known about heat tolerance in AMF. Likewise, plants isolated from high-temperature soils show lower respiration rates and higher total carbohydrate reserves relative to related plants from ambient soils when both are grown at high temperatures (Rachmilevitch et al. 2006, Lyons et al. 2007). Our second objective, therefore, was to determine whether mycorrhizal response to high-temperature soils varied with AMF inoculum source (thermal or nonthermal grassland soils).

To address these two objectives, we conducted a greenhouse experiment with thermal and nonthermal AMF inoculum, and three host plants, including an obligate thermal plant and two facultative thermal plants. All fungal–host plant combinations were grown at either ambient or elevated soil temperatures. Our source for thermal inoculum was high-temperature thermal soils in volcanically active areas of Yellowstone National Park (YNP), Montana and Wyoming, USA, where rooting zone temperatures reach 45°C or more (Stout et al. 1997), and arbuscular mycorrhizae are widespread in multiple host plants (Bunn and Zabinski 2003). If AMF communities in thermal soils are specifically adapted to high-temperature stress, we expect higher functional response by the fungus in terms of host plant colonization levels and soil hyphae production, and a host plant response that could be either positive or negative, depending on whether or not the AMF community is composed of good mutualists (Johnson et al. 1997, Bever 2002).

Our final objective was to relate our findings from the greenhouse to field conditions in two ways. We measured the distribution of roots and mycorrhizal soil hyphae (extraradical hyphae [ERH]) over a temperature gradient in thermal soils of YNP. If AMF possess a greater heat tolerance than plant roots, hyphae would be present at higher soil temperatures than roots. Finally, to determine whether AMF taxa in thermal soils are generalists or possibly unique as a result of the extreme environment, we used molecular tools to characterize the community composition of AMF taxa present within plants growing in thermal soils.

Materials and Methods

Field site

Thermal sites in Yellowstone National Park (YNP) are located on a 6500-km^2 volcanic region that has been intermittently active for 2.2 million years. Groundwater circulates though fractures and tectonic faults, surfacing in the form of thermal pools, geysers, thermal springs, boiling mud pots, fumaroles, and other features unique to thermal areas (Christiansen 1984). Plants growing in these sites experience elevated soil temperatures, with rooting zone temperatures of 45°C or more along with typical diurnal and annual fluctuations (Stout et al. 1997, Stout and Al-Niemi 2002, Bunn and Zabinski 2003, Germino and Wraith 2003, Tercek and Whitbeck 2004). Because temperature increases with depth in thermal soils, rooting density is highest in the surface layers.

Greenhouse experiment: experimental design

To measure plant response to high-temperature soils with or without arbuscular mycorrhizal fungae (AMF), we conducted a greenhouse experiment from December 2003 to March 2004 with three fixed factors: soil temperature (ambient or elevated), AMF treatment (no AMF, thermal AMF, or nonthermal AMF), and plant species (two grasses, *Agrostis scabra* Willd. and *Dichanthelium lanuginosum* [Schmoll] Spellenb.; an annual forb, *Mimulus guttatus* DC). The temperature was controlled with heat blankets that provided a source of heat beneath the pots. The AMF treatment was a whole-soil inoculum, which included spores, extraradical hyphae (ERH), and colonized root fragments, collected from colonized field plants. The three plant species used in this experiment occur in thermal areas of YNP. While *D. lanuginosum* is only present on thermal soils, *A. scabra* and *M. guttatus* are also found on nonthermal soils. The experiment was a split-plot design where the whole-plot treatment was temperature, which was replicated six times, using either six heat blankets or six unheated surfaces. The subplot treatments were a completely randomized factorial design of AMF treatment and plant species. Each AMF $\times$ host plant combination was replicated three times within each whole plot, for a total

of 27 plants per plot and an experiment total of 324 plants.

Temperature treatments were applied to pots on either a heat blanket (BH Thermal, Columbus, Ohio, USA) or an unheated Styrofoam sheet for elevated and ambient treatments, respectively. Twenty-seven pots were located randomly in each plot, and plots were positioned randomly on the bench. Plants were given a 10-d acclimation period following transplanting, after which temperatures were gradually raised to the final treatment temperature. Elevated temperatures were monitored hourly using a CR23X micrologger (Campbell Scientific, Logan, Utah, USA) via copper-constantan thermocouples placed at 0, 5, and 10 cm from the surface in three elevated-temperature plots (three pots total). Average soil temperatures in these pots ranged from 30°C at the surface to 50°C at the base of the pot. Ambient temperatures were monitored weekly with a Taylor temperature probe (Model 9841; Oak Brook, Illinois, USA). Temperatures were taken 10 cm from the bottom of one pot in each ambient-temperature plot (six pots total) at 11:00 hours and averaged 16.5°C. A diurnal variation of 5°C was observed in both temperature treatments.

Soils for inoculum for the thermal AMF treatment were collected from beneath *A. scabra*, *D. lanuginosum*, and *M. guttatus* in Rabbit Creek Basin (44°30′50″ N, 110°48′40″ W), a thermal drainage area located within Midway Geyser Basin, YNP. The temperature of thermal soils varies spatially but consistently increases with depth. Midday soil temperatures during sampling at inoculum collection sites 5 cm below the surface ranged from 30.2° to 46.2°C. Nonthermal AMF inoculum was collected from a recently disturbed field in the Gallatin Valley, Montana, USA. Both inoculum soils were homogenized, sieved through a 9.5-mm mesh, and stored at 4°C until use.

Seeds from greenhouse-grown *D. lanuginosum* plants originally from YNP thermal areas were scarified and the seed coat removed by hand, while *A. scabra* and *M. guttatus* seeds were collected from plants near Rabbit Creek and hand cleaned. Seeds were direct seeded into trays of soil (equal parts by volume of loam soil, concrete sand, and Canadian sphagnum peat moss with AquaGro 2000 G wetting agent (Scotts Miracle-Gro, Marysville, Ohio, USA) blended in at 0.59 kg/m^3 of soil mix; soil was pasteurized by aerated steam (twice at 80°C for 45 minutes with a 24-h resting period). At the two-leaf stage, germinants were transplanted into pots (7.5 cm × 7.5 cm × 11.5 cm deep) containing 700 mL of pasteurized 2:1 (v/v) soil : sand mix (soil component was the same; sand was 30-grit industrial quartz) and 30 mL of the appropriate AMF inoculum (none for non-inoculated plants). A volume of 5 mL of microbial wash (prepared by filtering a 1:5 [v/v] mixed inoculum soil : distilled water slurry through an 11-μm filter; modified from Johnson 1993) was added to each pot receiving AMF and 10 mL to each pot receiving no AMF. The control received twice the amount of microbial wash to compensate for the microbial community present in the inoculum of the two AMF treatments.

Plants were thinned to one plant per pot two days after the final treatment temperature was reached. Artificial light was used to achieve a 16-h photoperiod. Photosynthetically active radiation (LI-250 with LI-190SA Quantum sensor; LICOR, Lincoln, Nebraska, USA) was measured on a weekly basis in each block at 11:00 hours, and reached 180 μmol·m^{-2}·s^{-1}. To reduce differences in water availability between temperature treatments, plants receiving the elevated-temperature treatment were watered to container capacity twice per day, while plants receiving the ambient-temperature treatment were watered to container capacity on alternate days. Pot position in each plot was changed in alternate weeks. Harvest occurred at 60, 70, and 80 d after the final treatment temperature was reached for *M. guttatus*, *A. scabra*, and *D. lanuginosum*, respectively, to allow plants to reach a similar stage of maturity.

Greenhouse experiment: plant analysis

Plants were harvested and shoot tissue was dried at 60°C for 48 h and weighed, while roots were stored in distilled water at 4°C until root analysis. The number of flowers was measured for *M. guttatus* immediately prior to harvesting. Root morphology was assessed by scanning at 400 dots per inch (dpi) with an Epson Expression 1680 scanner (Epson America, San Jose, California, USA). Both *A. scabra* and *D. lanuginosum* were scanned via Lagarde's method to improve detection of fine roots. Root morphology measurements were completed using WinRhizo Pro, Version 2002c, and XLRhizo, Version 2003a (Regent Instruments, Montréal, Quebec, Canada) software. Measurements included total root length and average root diameter. Immediately following root scanning, tissue was dried and weighed.

Greenhouse experiment: AMF analysis

Measured fungal responses included length density of ERH (m ERH/g dry soil) and intraradical colonization. ERH measurements were obtained from 108 plants (consisting of a single replicate of each AMF × host plant combination on each of the 12 whole-plot temperature treatments). During plant harvest, soil from the entire pot was homogenized and a 3-g sample was removed. The sample was suspended in a 4.66 g/L sodium hexametaphosphate solution, and then serially diluted until 50 mg of soil were captured on a 2.1-cm^2, 20-μm nylon filter. The filter was stained with Trypan blue for 1 h, and ERH were collected on a 1.2-μm cellulose nitrate filter (modified method of R. M. Miller, *unpublished manuscript*). Total hyphal length was determined using the gridline intersect method (Tennant 1975) at 200× magnification. AMF were identified by aseptate hyphae with branching angles <90°, character-

istically knobby walls, and a lack of clamp connections (Brundrett et al. 1996, Rillig et al. 1998).

AMF colonization of roots was assessed on the same plants for which ERH measurements were made. Because of the poor growth of *D. lanuginosum* in ambient temperatures, only three plots could be sampled (instead of six). Five nonthermal and three thermal mycorrhizal plants were analyzed for a total of eight samples of ambient-temperature *D. lanuginosum*. Following weighing, roots were rehydrated and cleared in 2.5% (w/v) KOH and stained with Trypan blue (modified method of Phillips and Hayman 1970). AMF colonization was quantified using the magnified-intersections method (McGonigle et al. 1990). For plants with root sizes inadequate to obtain 96 intersections, samples include more than one plant from the same treatment combination and plot.

Because other fungal symbionts, including the endophytic *Curvularia* sp., may be important for conferring tolerance to high-temperature soils (Redman et al. 2002, Marquez et al. 2007), intersections with melanized hyphae were also tallied.

Field sampling for root and hyphae distribution

Field sampling for hyphae and root distribution and for AMF community characterization was done in October 2005 from a thermal area in the Upper Geyser Basin (44°25′89″ N, 110°48′81″ W). The soil is sandy, with a pH of 4.8 and elevated Fe but lower levels of K, Mg, and Ca compared to local nonthermal soils. Soil temperatures in the rooting zone ranged from the midteens during the winter months to >50°C in July during 2005 and 2006 (Y. Lekberg, D. Redecker, and C. Zabinski, *unpublished data*). We recorded the temperature at the midpoint of the following depth intervals below the ground surface: 0–2.5 cm, 2.5–5 cm, 5–7.5 cm, 7.5–10 cm, and 10–12.5 cm. Three 1.5 cm diameter soil cores were extracted around eight *D. lanuginosum* plants for analysis of hyphal and root distribution, separated into the same depth intervals, and composited. ERH extractions were according to Jakobsen et al. (1992).

Molecular identification of AMF in roots

The AMF community was characterized from eight *D. lanuginosum* plants that were excavated using a trowel and processed in the lab within 24 hours. The intact root system (containing rhizosphere and bulk soil) was cut at 2.5, 5, 7.5, and 10 cm below the stem base. Root subsamples were collected from each depth interval for molecular analyses to ensure that we characterized AMF across temperature and depth gradients. Roots were washed, blotted dry, and further dried with a desiccant (Drierite; W. A. Hammond Drierite Company, Xenia, Ohio, USA) before storage at −20°C to await molecular analyses. Quantification of arbuscular mycorrhizal colonization was conducted on separate subsamples, prepared as described.

Fungal DNA was extracted from approximately 10 mg of dried frozen root tissue using the Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, California, USA) according to Koide et al. (2005), and amplified using a nested polymerase chain reaction (PCR) protocol described by Redecker et al. (2000). This procedure uses universal eukaryote primers NS5 and ITS4 (White et al. 1990) in the first PCR, followed by a second PCR on a 1:50 water dilution of PCR1 products with the five separate primer pairs ARCH1311AB/ITS4i, PARA1313/ITS4i, ACAU1661/ITS4i, GIGA1313/GIGA5.8R, and GLOM1310/ITS4i (Redecker et al. 2000, Redecker 2002), targeting members of the Archaeosporaceae, Paraglomeraceae, Acaulosporaceae, Gigasporaceae, and Glomus group A within the Glomeraceae, respectively. We did not use primers that amplify other Glomus groups because a preliminary sampling in this area (Y. Lekberg, D. Redecker, and C. Zabinski, *unpublished data*) and extensive sampling in neighboring geothermal areas (Appoloni et al. 2008) indicated that they were absent or in very low abundance.

We used a hot-start Taq (ABgene House Surrey, KT, UK) and the following thermocycling parameters for both PCR reactions: 15 min at 95°C, 31 cycles of 30 s at 95°C, 30 s of 51°C, and 2 min at 72°C. The final extension phase was 5 min at 72°C. Studies from neighboring thermal areas show that positive PCR products seldom contain more than one phylotype (Appoloni et al. 2008). Therefore, we selected samples that displayed unique restriction types after digestions with *Hin*fI and *Mbo*I (New England Biolabs, Beverly, Massachusetts, USA), and a minimum of two representatives of each restriction type was cloned into a pGEM-T vector (Promega Corporation, Madison, Wisconsin, USA). Inserts were reamplified and digested with *Hin*fI and *Mbo*I to confirm the restriction type and then sequenced in both directions. PCR products were purified using a High Pure Kit (Hoffman LaRoche, Basel, Switzerland). A BigDye Terminator Cycle Sequencing Kit 3.1 (ABI, Foster City, California, USA) was used for labeling, and samples were run on an ABI 310 capillary sequencer. Sequences were deposited in the European Molecular Biology Laboratory database (AM 905249–AM 905256; AM947672–AM947675).

The glomeromycotan origin of the sequences was verified by BLAST (Altschul et al. 1997) and aligned to previously published sequences in PAUP*4b10 (Swofford 2001) and AMF sequences from neighboring thermal areas (Appoloni et al. 2008). Sequence phylotypes were defined as consistently separated monophyletic groups in the phylogenetic trees. Only those clades were used that were supported by neighbor-joining bootstrap analyses and also present in the respective maximum likelihood tree. The presence/absence of phylotypes in each root sample over depth was consolidated, and a sampling effort curve was constructed in EstimateS 7.5 (Colwell 2005) using each plant as a replicate.

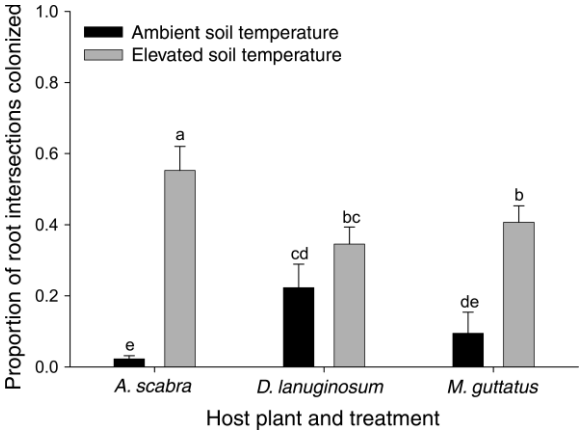

FIG. 1. The proportion (mean + SE) of root intersections colonized by arbuscular mycorrhizal fungi (AMF), for three host plants (*Dichanthelium lanuginosum*, *Agrostis scabra*, and *Mimulus guttatus*) grown in the greenhouse at ambient and elevated soil temperatures. Because colonization levels were the same between AMF inoculum sources, data are pooled over AMF sources. Different lowercase letters indicate significant differences between least-squares means ($P < 0.05$). From left to right, $n = 12, 12, 8, 14, 12, 13$ plants for species × temperature combinations. The number of intersections counted for each treatment combination ranged from 93 to 114, except for *A. scabra* grown at elevated temperature and *D. lanuginosum* grown at ambient temperature where, because of minimal root production, the numbers of intersections examined were 79 and 26, respectively.

RESULTS

Mycorrhizal response to high-temperature soils

Mycorrhizal fungi responded positively to high-temperature soils. Colonization of plants receiving arbuscular mycorrhizal fungal (AMF) inoculum was affected by temperature (three-way, mixed-model, split-plot ANOVA: $F_{1,11} = 45.0$, $P < 0.01$) and species × temperature interactions ($F_{2,55} = 7.5$, $P < 0.01$). Colonization was generally higher at elevated temperatures, although in *Dichanthelium lanuginosum* the difference was not significant (Fig. 1). Colonization of *Agrostis scabra* in ambient temperatures did not differ significantly from zero (least squares mean estimate of 2.2%, $t_{46.8} = 0.42$, $P = 0.68$), while colonization in elevated temperatures was 55%, which was greater than in other species (Fig. 1). Differences in colonization levels were not just the result of host plant root growth, as there was no correlation between arbuscular mycorrhizal colonization levels and root length for plants grown on either ambient- ($r_{32} = 0.116$, $P = 0.53$) or elevated-temperature soils ($r_{39} = 0.017$, $P = 0.92$). Plants without AMF had 1.3% ± 0.8% (mean ± SE) of intersections colonized with AMF-like hyphae, and neither vesicles nor arbuscules were observed in these plants.

AMF produced hyphae external to the root only in high soil temperatures (Fig. 2). An average background extraradical hyphae (ERH) length density of 6.9 m/g was measured in the no-AMF treatments, representing nonviable hyphae and fungal hyphae indistinguishable from AMF present in the pasteurized soil mix. When grown at ambient temperatures, hyphal length densities were the same between no-AMF and AMF-inoculated treatments (Fig. 2). In contrast, with elevated soil temperatures, the inoculated plants had greater ERH length densities than no-AMF plants (temperature × AMF interaction: $F_{2,68} = 4.0$, $P = 0.02$). While the pattern of ERH effects is consistent across plant species, a priori contrasts between no-AMF and AMF plants showed significantly higher ERH levels for *A. scabra* and *Mimulus guttatus* at elevated temperatures, but not for *D. lanuginosum*. ERH length was correlated with internal colonization levels ($r_{17} = 0.53$, $P = 0.01$).

In our study, the fungal endophyte *Curvularia* sp. was not important for host plant response to high-temperature soils. *Curvularia* is a melanized fungus, and we observed melanized hyphae in two *A. scabra* samples and one *M. guttatus* sample at colonization levels <2%.

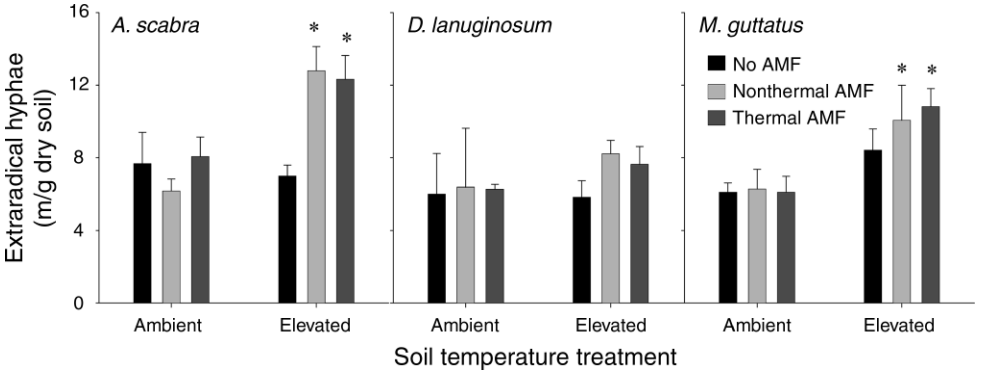

FIG. 2. Total extraradical hyphae (ERH) length density (mean + SE) for three host plants grown in the greenhouse at ambient and elevated soil temperatures, with one of three arbuscular mycorrhizal fungal (AMF) treatments: no AMF, AMF inoculum from a thermal source, and AMF from a nonthermal source. The no-AMF treatment represents the background level of hyphae from pasteurized soil. Asterisks indicate ERH length density significantly higher than in the no-AMF treatment, using a priori contrasts ($P < 0.05$). For all factor combinations, $n = 6$ replicate plants per species and treatment, except *D. lanuginosum* at ambient temperatures, for which $n = 2$.

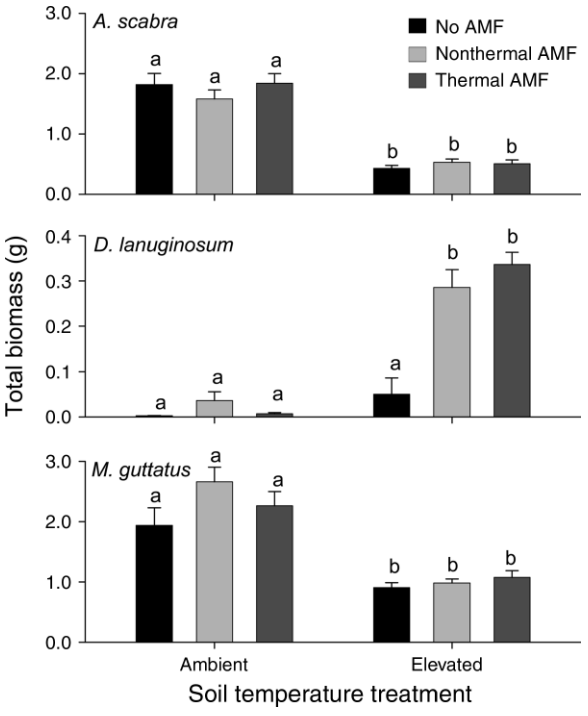

FIG. 3. Plant biomass (mean + SE) for three host species grown in the greenhouse at elevated and ambient soil temperatures with one of three arbuscular mycorrhizal fungal (AMF) treatments: no AMF, AMF inoculum from thermal soils, or AMF from nonthermal soils. Different lowercase letters represent significant differences ($P < 0.05$) between least-squares means of temperature × AMF treatments within each species. For all factor combinations, $n = 18$ replicate plants per species and treatment, except for *D. lanuginosum* in ambient temperatures, where $n = 10$, 10, and 8 for no AMF, nonthermal AMF, and thermal AMF, respectively. Note the different biomass scale for *D. lanuginosum*.

This represents the maximum colonization by *Curvularia* sp. in fine roots, as not all melanized hyphae are *Curvularia* sp.

Plant response to high-temperature soils differed according to whether the host plants are facultative or obligate thermal plants. The two species that occur both on and off thermal soils grew poorly in high-temperature soils in the greenhouse, whereas *D. lanuginosum*, found only on thermal soils, required a high temperature to grow (Fig. 3; two-way, mixed-model, split-plot ANOVA, temperature effect: *A. scabra* $F_{1,10} = 80.5$, $P < 0.01$; *M. guttatus* $F_{1,48} = 44.3$, $P < 0.01$; *D. lanuginosum* $F_{1,35} = 81.1$, $P < 0.01$). Both *A. scabra* and *M. guttatus* were twice the size when growing in ambient- vs. elevated-temperature soils, while the biomass of *D. lanuginosum* plants approached zero when growing at ambient temperatures (Fig. 3). Total root length reflected the same patterns across host plants as total biomass (Table 1; two-way, mixed-model, split-plot ANOVA, temperature effect: *A. scabra* $F_{1,10} = 12.7$, $P < 0.01$; *D. lanuginosum* $F_{1,17} = 10.2$, $P < 0.01$; *M. guttatus* $F_{1,10} = 45.6$, $P < 0.01$) and was correlated with root mass ($r_{290} = 0.85$, $P < 0.001$). Average root diameter was affected by temperature for *A. scabra* ($F_{1,102} = 56.3$, $P < 0.01$) and *M. guttatus* ($F_{1,10} = 57.3$ $P < 0.01$), with coarser roots in ambient temperatures (Table 1).

The effects of AMF on host plant biomass varied with plant species (Fig. 3); neither of the facultative thermal host plants differed in biomass when growing with AMF (AMF treatment effect: *A. scabra* $F_{2,41.1} = 1.26$, $P = 0.30$; *M. guttatus* $F_{2,48} = 1.10$, $P = 0.34$). In contrast, *D. lanuginosum* plants were more than three times as large when grown with AMF, but only on high-temperature soils (*D. lanuginosum* soil temperature × AMF interaction: $F_{1,35} = 14.7$, $P < 0.01$).

The reproductive response of all three host plants either remained constant or increased with elevated soil temperature and was not affected by AMF (Table 2). Flower production for *A. scabra* and *M. guttatus* was high and consistent across temperature and AMF treatments. The number of days to first flower was significantly shorter in high-temperature soils for both *A. scabra* (Table 2; temperature effect: $F_{1,9} = 4.7$, $P = 0.06$) and *M. guttatus* ($F_{1,10} = 12.5$, $P < 0.01$). For *D. lanuginosum*, no plants flowered in ambient tempera-

TABLE 1. Root length and root diameter of three host plant species grown at ambient and elevated soil temperatures with one of three arbuscular mycorrhizal fungal (AMF) treatments.

Soil temperature and AMF treatment	Root length (cm)			Root diameter (mm)		
	A. scabra	*D. lanuginosum*	*M. guttatus*	*A. scabra*	*D. lanuginosum*	*M. guttatus*
Ambient temperature						
No AMF	$9200^{ab} \pm 1700$	$71^{b} \pm 7.1$	$3700^{b} \pm 550$	$0.27^{a} \pm 0.01$	$0.19^{b} \pm 0.00$	$0.35^{a} \pm 0.01$
Nonthermal AMF	$6100^{bc} \pm 840$	$480^{b} \pm 170$	$5800^{a} \pm 760$	$0.26^{a} \pm 0.01$	$0.24^{a} \pm 0.02$	$0.35^{a} \pm 0.01$
Thermal AMF	$9800^{a} \pm 1800$	$130^{b} \pm 23$	$4300^{b} \pm 620$	$0.27^{a} \pm 0.00$	$0.22^{a} \pm 0.01$	$0.34^{a} \pm 0.01$
40°C						
No AMF	$3500^{cd} \pm 470$	$1800^{b} \pm 1500$	$1800^{c} \pm 390$	$0.22^{b} \pm 0.00$	$0.19^{b} \pm 0.00$	$0.27^{b} \pm 0.01$
Nonthermal AMF	$2500^{cd} \pm 380$	$4800^{a} \pm 800$	$1300^{c} \pm 190$	$0.22^{b} \pm 0.00$	$0.23^{a} \pm 0.00$	$0.27^{b} \pm 0.02$
Thermal AMF	$1600^{d} \pm 200$	$4300^{a} \pm 460$	$1300^{c} \pm 260$	$0.24^{b} \pm 0.00$	$0.22^{a} \pm 0.00$	$0.27^{b} \pm 0.02$

Notes: Data are means ± SE for *Agrostis scabra*, *Dichanthelium lanuginosum*, and *Mimulus guttatus*. Superscript letters represent significant differences ($P < 0.05$) between least-squares means of temperature × AMF treatments within each species. The number of samples is 18 plants per species per treatment for all except *D. lanuginosum* in ambient temperatures, for which sample sizes were 7, 8, and 7 for no AMF, nonthermal AMF, and thermal AMF, respectively.

TABLE 2. The number of days to flowering and the proportion of plants flowering for three host plants grown at ambient and elevated soil temperature, with one of three arbuscular mycorrhizal fungal (AMF) treatments.

Soil temperature and AMF treatment	Days to first flower			Proportion of plants flowering		
	A. scabra	*D. lanuginosum*	*M. guttatus*	*A. scabra*	*D. lanuginosum*	*M. guttatus*
Ambient temperature	95[a] ± 1.2	0 ± 0.0	92[a] ± 1.4			
No AMF				0.83 ± 0.09	0 ± 0.00	1.0 ± 0.00
Nonthermal AMF				0.94 ± 0.06	0 ± 0.00	1.0 ± 0.00
Thermal AMF				0.89 ± 0.08	0 ± 0.00	1.0 ± 0.00
40°C	90[b] ± 1.5	109 ± 1.3	86[b] ± 1.3			
No AMF				0.94 ± 0.06	0.06 ± 0.06	1.0 ± 0.00
Nonthermal AMF				0.83 ± 0.09	0.61 ± 0.12	1.0 ± 0.00
Thermal AMF				0.83 ± 0.09	0.72 ± 0.11	1.0 ± 0.00

Notes: Because the AMF treatment had no effect on the number of days to first flower, just the means of each temperature treatment are presented. Values are means ± SE. Letters represent significant differences ($P < 0.06$) between least-squares means of temperature treatments within each species. The number of samples for ambient and elevated temperatures is 48, 47 for *A. scabra*; 54, 54 for *M. guttatus*; and 0, 25 for *D. lanuginosum*. The number of samples is 18 plants per species per treatment for all except *D. lanuginosum* in ambient temperatures, where samples sizes are 10, 10, and 9 for no AMF, nonthermal AMF, and thermal AMF, respectively.

tures, and only one no-AMF plant grown in elevated soil temperatures flowered (Table 2).

Inoculum source effects on AMF function

We found very little evidence that AMF from thermal soils function differently than AMF from nonthermal soils. Across all variables measured for both fungi and host plants in this study, there was only one variable that differed between thermal and nonthermal AMF inoculum treatments: root length in ambient-temperature soils for *A. scabra* and *M. guttatus* (Table 1). For both species, root length of thermal AMF-inoculated plants was the same as root length of no-AMF plants. Plants with nonthermal AMF inoculum had a longer root length for *A. scabra* and a shorter root length for *M. guttatus* than plants with either thermal AMF inoculum or no-AMF inoculum.

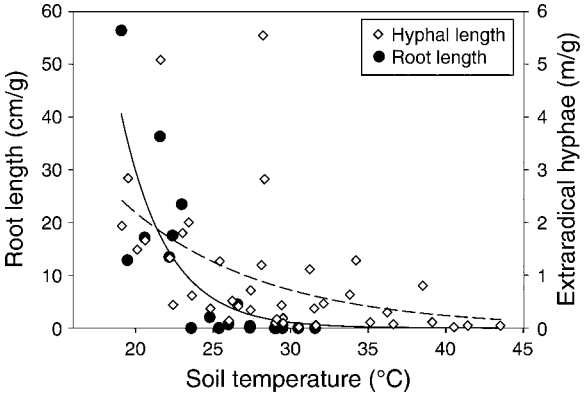

FIG. 4. The declining distribution of *D. lanuginosum* roots and extraradical hyphae (ERH) length density in Yellowstone National Park field soils with increasing soil temperature. Soils were sampled in October 2005. Lines represent two-parameter exponential decay for root length (solid line) and ERH (dashed line). Fungi are present in higher soil temperatures than are roots.

Field root and ERH distribution

Both root and ERH abundance declined with soil depth, and thus with increasing temperature, with a more rapid decline for roots than for ERH (Fig. 4). For the eight plants sampled, the temperature at the soil surface at the time of collection ranged from 19.1° to 28.2°C and increased with depth an average of 9.9° ± 0.9°C (mean ± SE) between 0–2.5 cm and 7.5–10 cm below the surface (Appendix A). While plant roots never extended beyond the 7–10 cm depth (average temperature 29.7°C), ERH were detected in the 10–12.5 cm depth interval in all sites (average temperature 34.9°C). Thus ERH were repeatedly present at higher soil temperatures than were plant roots (one-tailed t test; $t_{10} = 2.4$, $P = 0.02$). Because of diurnal and seasonal fluctuations, the temperatures recorded at the time of sample collection are not the highest temperature experienced at that site, but are a relative comparison of sites within a short time frame on a single day. Mycorrhizal colonization in field-collected roots was moderate, averaging 23% ± 2.8% (mean ± SE), but showed no significant correlation with soil temperature ($r_{19} = 0.08$, $P = 0.72$).

Molecular identification of AMF in roots

Because plants differed in maximum rooting depth, DNA was extracted for molecular analyses from eight samples at the 0–2.5 cm and 2.5–5 cm depths, from five samples at the 5–7.5 cm depth, and from three samples at the 7.5–10 cm depth. No roots were found in the 10–12.5 cm depth. PCR products were amplified in 20 of the 24 root samples, and between zero and five AMF phylotypes were detected in each sample among the five primer pairs (Appendix A). After consolidating samples over depth for each plant, the sampling effort curve indicated that eight plants were sufficient to characterize most AMF phylotypes that were active at the time of sampling in the selected thermal area (Appendix B). In all, we identified eight phylotypes, which consist of a

subset of previously characterized phylotypes from neighboring thermal areas (phylogenetic trees are in Appoloni et al. 2008).

The most common phylotype was Arch-5 (occurring in seven of the eight plants), which belongs to a major clade that is only distantly related to known morphospecies. To date, Arch-5 has only been found in YNP. The second-most common phylotype was *Scutellospora pellucida* (occurring in five of the eight plants). The remaining six phylotypes were less common and included *Glomus intraradices* (Glom-A1, occurring in only one plant), *Glomus diaphanum* (Glom-A13, occurring in three plants), *Acaulospora morrowiae* (Acau-6, occurring in three plants), and three *Paraglomus* phylotypes (Para-1, Para-2, and Para-3, occurring in two, four, and three plants, respectively). Of the *Paraglomus* phylotypes, Para-2 and Para-3 fell within the morphospecies *Paraglomus occultum*, whereas Para-1 corresponded to a putative new species previously detected in a number of environments (e.g., Hijri et al. 2006).

Discussion

Our first objective was to measure the overall effect of elevated soil temperatures on the mycorrhizal symbiosis, including the response of both plant and fungal symbionts. The fungal response to high temperature was consistently positive across all three host plants, as evidenced by an increase in arbuscular mycorrhizal fungal (AMF) structures both internal and external to the roots. Significant extraradical hyphae (ERH) production was found only when plants were grown at elevated temperature. This increase in ERH with soil temperature has been previously observed, albeit across a lower temperature range (Gavito et al. 2003, Heinemeyer and Fitter 2004). Our research suggests that increased carbon allocation to fungal symbionts should be taken into account when modeling plant response to climate change.

The plant response to high-temperature soil, on the other hand, was dependent on whether the species was obligate or facultatively thermal. The two host plants that grow both on and off thermal soils, *Agrostis scabra* and *Mimulus guttatus*, exhibited signs of stress when growing in high-temperature soil, with decreased total biomass, total root length, and root diameter. This response is consistent with morphological changes observed in other plants grown in elevated temperatures (Smith and Roncadori 1986, Haugen and Smith 1992, Forbes et al. 1997). Additionally, flowering began earlier in elevated than ambient temperatures for these two species, potentially indicating either stress evasion or a decrease in time to acquire thermal units required for flowering. These species may avoid stress associated with high-temperature soil by flowering and setting seed in early spring before soil temperatures become limiting in thermal areas (Tercek and Whitbeck 2004).

In contrast, the thermophilic species *Dichanthelium lanuginosum* had the opposite response to the temperature treatments. It showed low survivorship at ambient temperatures and greater biomass and total root length when growing at elevated temperatures, further evidence that this host plant is adapted to high-temperature soils (Stout et al. 1997, Stout and Al-Niemi 2002). This positive response, however, occurred only in mycorrhizal plants. For *D. lanuginosum* and potentially for other thermophilic host species, AMF clearly increase host plant fitness, as measured by biomass and flowering, in high-temperature soil environments. One mechanism whereby AMF could confer an advantage in high-temperature soils could be greater heat tolerance than plant roots (Tungate et al. 2007). Our field survey showed that ERH were present in soils that were on average 5°C warmer than the warmest soil where roots were found. Greater heat tolerance by AMF may facilitate host plant access to soil resources in areas inaccessible to roots.

Neither of the facultatively thermal species benefited from inoculations with AMF in either ambient or elevated-temperature soils. The lack of host plant response to the symbiosis at ambient temperatures is perhaps more easily understood. AMF colonization was relatively low, and there was no ERH production, hence no possibility to extract additional soil nutrients. In high-temperature soils, the carbon costs to the host plant increase for two reasons: an increase in AMF structures both internal and external to the roots, and the carbon cost of fungal respiration, which can be especially significant in higher-temperature soil (Koch et al. 2007, Hawkes et al. 2008). For host plants not particularly adapted to high-temperature stress, as has been indicated for *M. guttatus* at the cellular and biochemical level (Delmer 1974), the host plant may be less able to regulate the symbiosis, and AMF become a carbon sink without concurrent benefits for growth and reproduction. *A. scabra*, however, has increased respiratory efficiency at high temperatures relative to other species (Rachmilevitch et al. 2006), and understanding symbiosis costs and benefits across temperature gradients will require assessment of the respiratory demands of both symbionts at elevated temperatures, along with mechanisms of carbon delivery to the fungal symbiont. This may be especially relevant across light gradients, which are generally not limiting in thermal soil environments but are significantly lower in the greenhouse.

Our second objective, to determine whether AMF function varied with inoculum source, was based on the high genetic (Kuhn et al. 2001, Koch et al. 2004) and functional (Cuenca et al. 2001, Munkvold et al. 2004, Johnson et al. 2005) variability that has been observed within and between AMF taxa, and the potential for selection in an extreme environment. We found no evidence that the AMF community from Yellowstone's thermal areas was uniquely capable of colonizing host plants, producing ERH, and benefiting host plants in high-temperature soils. The similar response between

thermal and nonthermal AMF suggests that these fungi possess a broad tolerance to high soil temperature, in contrast to other studies that suggest that some isolates of AMF perform best at their temperatures of origin (Schenck 1975, Grey 1991). Because our AMF inoculum included a fungal community and not necessarily thermal and nonthermal isolates of the same species, our conclusions regarding adaptation are relevant for the soil community and not single AMF species.

Regarding our objective to relate experimental results to field data, our survey of active AMF taxa in thermal soils here and in previous studies (Appoloni et al. 2008) support this assertion of a broad ecological range by AMF, and indicate that thermal AMF communities are composed of both generalist and possibly unique fungal taxa. For example, *Glomus intraradices* found in thermal soils is commonly found in a wide range of environments (Öpik et al. 2006), reflecting either a wide tolerance to environmental factors or the existence of multiple cryptic species. In contrast, the phylotype Arch-5 currently lacks known morphospecies and may be endemic to Yellowstone National Park (YNP). Research on symbiosis function with these taxa, and whether AMF isolated from thermal soils perform different functions than the same taxa isolated from other environments in high-temperature soils, is currently being investigated using thermal isolates from YNP.

The lack of plant benefits from the higher ERH and internal arbuscular mycorrhizal colonization in the facultatively thermal host plants grown in high-temperature soils indicates that common metrics to estimate fungal abundance are not necessarily related to the benefit conferred. For example, *D. lanuginosum* had lower ERH densities and root colonization than either *M. guttatus* or *A. scabra*, but was the only species to show a positive response to mycorrhizae. Symbiosis function is not directly measurable based on mycorrhizal structures present at a point in time.

Conclusions

With elevated soil temperatures, AMF benefited the plant that occurs exclusively in thermal soils but not those that avoid rather than tolerate high temperatures, suggesting that the AMF effects may depend on the plant's tolerance to heat. However, even plants that showed reduced growth in high-temperature soils allocated more C to fungal structures, and AMF showed an increase in both ERH and root colonization in high-temperature soils. AMF appear to have a greater heat tolerance than plant roots, as evidenced by the hyphal and root distributions in the field. The nonthermal and thermal AMF behaved in a similar manner in our greenhouse experiment, suggesting that AMF possess a broad tolerance to high temperature. The increase in host plant allocation to AMF, lack of evidence of temperature adaptation by AMF, and differential host response to AMF suggest that AMF could be significant drivers of plant community response to increased soil temperature associated with global climate change.

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APPENDIX A

PCR summary and phylotype distribution for eight *Dichanthelium lanuginosum* plants (*Ecological Archives* E090-090-A1).

APPENDIX B

Sampling effort curve of eight *Dichanthelium lanuginosum* plants (*Ecological Archives* E090-090-A2).