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Mycorrhizal status influences the rate but not the temperature sensitivity of soil respiration

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Abstract

Mycorrhizal fungi, which can produce a large portion of total soil respiration, respond strongly to global changes such as elevated CO_2, N-deposition, and land-use change. Predictions of future ecosystem C sequestration hinge on respiration budgets, but the mycorrhizal influence on total soil respiration remains unknown. In this study, sunflowers (*Helianthus annuus*) were subjected to various mycorrhizal treatments, and their root and soil systems were enclosed in chambers that continuously monitored belowground (root + mycorrhizal + heterotrophic) CO_2 production during plant growth, death, and decomposition. Rhizocosms with high mycorrhizal colonization exhibited higher soil respiration rates as plants matured, an increase that was in proportion to the mycorrhizal stimulation of plant growth. Living mycorrhizal plants behaved like nonmycorrhizal ones in that total rhizocosm respiration had the same relationship to plant mass and the same temperature sensitivity as nonmycorrhizal plants. Upon removal of the shoots though, mycorrhizal plants exhibited the largest relative reduction in respiration resulting in a unique relationship of soil respiration with plant mass. The mycorrhizal influence on heterotrophic respiration merits as much attention from experimenters and modelers as the mycorrhizal contribution to autotrophic respiration.

Introduction

Soil respiration (R_S) is the most uncertain component of many climate change models (Jones et al., 2003). Laboratory studies have shown how soil temperature and moisture influence R_S (Kirschbaum, 1995), but the effect of biological variables on respiration and the interaction of biotic and abiotic factors remain poorly understood. Measurements of R_S exhibit great variability among ecosystems, even those with similar abiotic properties (Raich and Tufekcioglu, 2000). Plants contribute to R_S with their own root respiration (R_R) and by providing substrate for heterotrophic respiration (R_H). Mycorrhizal fungi, which are widespread but highly variable in type and abundance among ecosystems, may influence both R_R and R_H and could drive variability in R_S among ecosystems.

Colonization of roots by arbuscular mycorrhizal (AM) fungi imparts several important changes on host plant physiology and soil ecology. AM colonization affects the autotrophic portion of C cycling by stimulating photosynthetic rate at both the whole plant and leaf level and increasing photosynthate demand belowground (Smith and Read, 1997). The specific respiration rate of mycorrhizal hyphae, estimated from compartmentalizing fungi artificial laboratory systems (Ek, 1997), is greater than that of roots. Mycorrhizal respiration is often considered autotrophic (e.g. Högberg et al., 2001) because mycorrhizal fungi have intimate access to

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photosynthate, and the respiration of roots is empirically indistinguishable from that of the associated fungi in an intact root system in the field. AM fungi also influence heterotrophic C cycling rates by translocating photosynthate away from the immediate rhizosphere into the soil in the form of fungal exudates and dead hyphae. These mycorrhizal products are ultimately degraded by heterotrophs. AM exudates are thought to decompose slowly compared to other soil pools (Steinberg and Rillig, 2003), but there is much debate as to how rapidly AM hyphae degrade (Staddon et al., 2003; Zhu and Miller, 2003). The net outcome of mycorrhizal colonization on soil carbon (C) cycling rates depends on how mycorrhizae influence the decomposability of roots, exudates, hyphae, and existing soil organic matter (Langley and Hungate, 2003).

Temperature sensitivity of R_S is another critical component of many ecosystem models (Raich and Schlesinger, 1992) and holds particular importance in the context of climatic warming (IPCC, 2001) where small changes in global temperature could have a large influence on atmospheric C balance (Valentini et al., 2000). At present, many ecosystem models use an exponential relationship (most often expressed as Q_{10}, the factor by which a rate increases with a 10 °C increase in temperature) to describe how respiration responds to temperature. The Q_{10} of soil respiration decreases with increasing base temperature (Kirschbaum, 1995) and decreases with extremes in soil moisture (Borken et al., 1999; Davidson et al., 1998; Xu and Qi, 2001). Biological influence on temperature sensitivity of respiration remains largely unexamined but may be responsible for the frequent disagreement of laboratory and field estimates of Q_{10}. It has been hypothesized that live mycorrhizal fungi, being prevalent in nature but difficult to simulate in vials, have a high Q_{10} and drive the higher temperature sensitivity observed in the intact rhizosphere (Boone et al. 1998). This hypothesis has been refuted in a recent split-pot study (Bååth and Wallander, 2003), but has never been examined in a realistic, intact rhizosphere with controlled AM inoculation.

To estimate the mycorrhizal effect on total soil respiration, we measured respiration of sunflower rhizocosms (air tight chambers enclosing roots, mycorrhizae, hyphae, and soil) under four controlled conditions: a pure culture of *Glomus intraradices* (Gi), a pure culture of *Gigaspora gigantea* (Gg), a suite of desert grassland AM fungal species (DG), and a nonmycorrhizal treatment (NM). The automated gas sampling system provided high temporal resolution of respiration rates, and the rhizocosms were free of thermal gradients that typically confound Q_{10} estimates. We compared the rates and Q_{10} of R_S among mycorrhizal treatments before and after plants were killed to examine the influence of AM colonization on total soil respiration in the presence of autotrophic respiration and with heterotrophic respiration only.

Materials and methods

Soil and plant preparation

One part desert grassland soil (collected 30 km north of Flagstaff, AZ) was mixed with two parts sand and homogenized. The resulting mix was steam sterilized twice to eliminate existing mycorrhizal inoculum. Two hundred 0.5-l potting cones (ConeTainers, mfg.) were filled halfway with the sterile soil mixture. Four mycorrhizal conditions were established: monocultures of *Glomus intraradices* (Gi), and *Gigaspora gigantea* (Gg), (each kindly provided by J. Klironomos, Guelph University), native desert grassland inoculum present in nonsterile soil (DG), and nonmycorrhizal controls (NM). The two pure cultures of fungus we used have been shown to differ in that *Gi. gigantea* produces more extrametrical hyphae in the surrounding soil and *Gl. intraradices* produces more internal root structures (Hart and Reader, 2002). Previous spore identifications of soils from the desert grassland indicate that the AM fungi present in the DG inocula were dominated by the genus *Glomus* (N.C. Johnson, unpublished data). On top of the sterile soil we placed 25 ml of AM inoculum (pure inoculum in perlite for Gi and Gg; unsterile soil mixed in perlite for DG, sterile perlite for NM) and covered it with 5 cm of sterile soil. Seeds of *Helianthus annuus* (Hopi Black Dye a variety native to Arizona, Native Seed Search, Tucson, AZ) were placed on top, covered with 3 cm of the sterile soil mix, watered, and allowed to germinate.

Automated respiration monitoring system

Two days after germination, plants were transferred to 4-l pots (10 cm^2 surface area × 40 cm tall) of the sterilized desert grassland soil mix (described above) and allowed to acclimate for 2 days. They were then placed in air-tight, white PVC cylinders (50 cm tall × 13 cm diameter; Figure 1a) and randomly assigned to a position in the greenhouse. A split rubber stopper (5-cm diameter) with a 1-cm diameter center hole was placed around each stem and pressed into a circular hole in the top of each chamber. A gas-impermeable putty (Quibitac, Qubit Systems, Kingston, Ontario) sealed the gap between the stem and stopper. A total of 160 respiration chambers were constructed, 64 of which were monitored for soil respiration (Figure 1b). Twelve pots from each of five treatments (Gi, Gg, DG, and NM plants, as well as unplanted soils) and four completely empty pots were each assigned to one of the 64 automated respiration sampling channels. The remaining 96 pots were subjected to the exact same conditions and were used to measure treatment effects on other plant and soil parameters. A program written and run in LabView (National Instruments, Austin, Texas) controlled a Hewlett-Packard data acquisition system (HP3495) that managed air handling and signal acquisition. An infrared gas analyzer (model 6262 LiCor, Lincoln, NE) measured the [CO_2] in the air drawn from outside the greenhouse and delivered to the inlet port on each rhizocosm container (reference [CO_2]). The computer-controlled solenoids rotated the output line from one of eight incoming lines at each bench manifold so that air from only one pot at each bench reached the master manifold at a given time. This master manifold cycled in the same way so that air from one bench reached the IRGA. We constricted flows on all manifold vents from pots that were not being sampled in order to maintain a consistent flow-rate for each pot at all times. During each measurement cycle, the IRGA also measured the [CO_2] of the air exiting the rhizocosm container (sample [CO_2]). A mass flow meter (Model 820, Sierra Instruments, Monterrey, CA) measured the flow rate through each container (c. 400–500 ml min^{-1}). Respiratory CO_2 flux from rhizocosms was calculated as: flow rate × (sample [CO_2]– reference [CO_2]), for each of the 64 pots 9–10 times daily. To check for disequilibrium due to possible flow rate change during sampling, we monitored the real-time [CO_2] during the 2.5 min measurement cycle and found that a new equilibrium [CO_2] was reached for a pot in 30–40 s, after which there was no consistent directional trend. Water vapor was also measured by the IRGA, and evaporation from the enclosed soil surface was calculated in the same manner as respiration. Evaporation measurements were used to index and maintain consistent soil moisture among pots.

Harvest scheme

Roots and shoots of plants in the rhizocosms monitored for respiration were harvested at weeks 4, 6 and 13 to describe treatment effects on growth ($n = 8$ replicates × 4 treatment × 3

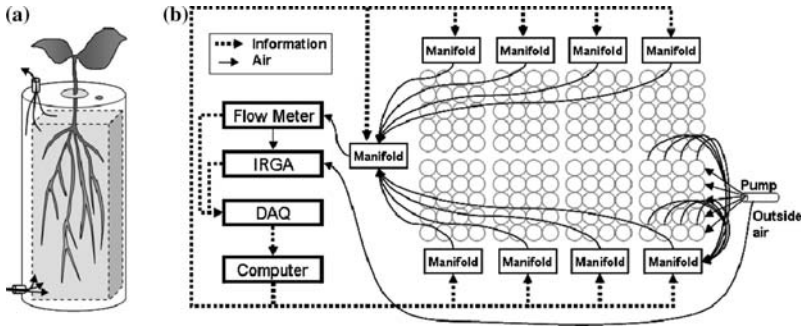

Figure 1. Schematic of a single rhizocosm (a) and the respiration monitoring system (b). Outside air is pumped through each rhizocosm and continuously monitored for [CO_2]. Solenoid-controlled manifolds channel outgoing air from each of 64 rhizocosms to the flowmeter and IRGA 10 times daily to calculate respiration rate.

harvests). Shoots from 12 monitored rhizocosms per treatment were also harvested at week 13, after which the pots were resealed, and respiration of the undisturbed root/soil system was monitored for another 7 weeks. Autotrophic respiration from roots of herbaceous annuals declines sharply hours after the photosynthetic source is removed (Bloom and Caldwell 1988). Thereafter, we were able to isolate the effects of mycorrhizal colonization on purely heterotrophic soil respiration.

Sample processing

Shoots of harvested plants were separated into leaves and stems, dried at 60 °C, and weighed. Roots were removed from the soil, and a 0.2-g subsample was haphazardly taken to stain for fungal colonization. The roots were cleared in 5% KOH for 12 h, and stained in Trypan Blue for 4 h. Thirty 1-cm segments from each sample were mounted on a slide, and colonization was quantified as the number of root segments with aseptate hyphae, arbuscules, or vesicles per thirty segments. Leaves and roots were dried for 24 h at 60 °C and ground. At harvest, soil cores (15 cm deep × 1 cm diameter) were taken to represent the vertical profile. Roots were handpicked out of soil from cores, and those soils were dried at 60 °C for 72 h. Leaf and root tissues, as well as soils, were analyzed for carbon and nitrogen with a Carlo Erba elemental analyzer (Milan, Italy) coupled to a Finnegan Delta Plus isotopic ratio mass spectrometer (Finnegan, Germany) at the Colorado Plateau Stable Isotope Laboratory (http://www4.nau.edu/cpsil/). These samples were analyzed for total P using a modified micro-Kjeldahl digestion (Parkinson and Allen, 1975) followed by colorimetric analysis on a Lachat flow-injection analyzer (Lachat Instruments 1992).

Temperature sensitivity of soil respiration

To estimate an average Q_{10} for a rate, R, using many data points over an interval of time, we estimated a least-square linear relationship by:

$$\ln(R) = aT + b$$

where a and b are fitted constants and T is temperature. Q_{10} is calculated as:

$$Q_{10} = e^{(10a)}$$

Soil temperature was measured with thermocouples buried halfway down (20 cm depth) in the middle (5 cm from each pot wall) of four soil pots, representing each corner bench in the greenhouse. We assumed that if the most insulated part of the soil column equilibrated within 1 h, then that one temperature could be ascribed to the entire soil profile within the pot and used for Q_{10} analyses.

To test temperature equilibration, after the main experiment we placed thermocouples at four positions within one soil pot, the top, bottom, middle and side. We recorded hourly temperatures for a 1 week period, over which daily air temperatures and insolation varied widely. Throughout the test, temperatures from the extreme positions of the soil column varied by no more than 0.3 °C. The average correlation coefficient among the four thermocouples was 0.985 with no consistent lag effect among the four positions. The small differences fall within the margin of error created by correlating asynchronous measurements. This result validates the assumption that the entire pot of soil effectively equilibrated with respect to temperature.

Respiration and temperature readings for each rhizocosm were taken frequently but asynchronously. We used a spline fit model to estimate the soil temperature that corresponded to particular R_S measurements. We calculated a Q_{10} for each pot for each week of R_S measurements for which we had accurate T measurements (from week 8 on). We reasoned that a week was long enough to provide statistical robustness (50–60 observations), but short enough to exclude significant changes in confounding abiotic variables such as soil moisture and biotic variables such as plant growth (Jannsens and Pilegaard, 2003). The mean r^2 for the linear relationships ($\ln(R_S) \times T$) was 0.64.

Statistical analyses

Mycorrhizal effects on plant growth and nutrient composition were analyzed using one-way ANOVAs. The mycorrhizal treatment effect on respiration was assessed with a repeated measures MANOVA (JMP 4.0.4) by collapsing individual

bihourly measurements into weekly means for each pot. Because the weekly mean respiration rates were right skewed within each treatment, they were log transformed. Weekly Q_{10}'s were averaged to one Q_{10} for each pot for the live plant phase and dead plant phase individually. These Q_{10} values were analyzed using a repeated measures MANOVA.

To distinguish the influence on respiration of mycorrhizal status from that of plant size we analyzed the relationships of dependent variables (mean live-phase respiration, mean dead-phase respiration) with plant mass and plant N by treatment. Where linear relationships were significant within treatments, we compared slope estimates among treatments with *t*-tests.

Results and discussion

Colonization

Sunflowers inoculated with *Gl. intraradices* (Gi) had significantly higher AM colonization than sunflowers inoculated with the desert grassland inoculum (DG), and both Gi and DG were significantly higher those inoculated with *Gi. gigantea* (Gg) and nonmycorrhizal (NM) plants (Table 1). Colonization of Gg roots was not statistically different from NM, and neither differed from zero. We expected higher colonization with Gi inoculum because *Gl. intraradices* tends to produce profligate intra-radical structures while *Gi. gigantea* tends to produce more extra-radical structures (Hart and Reader, 2002). Still, we expected a higher colonization frequency than we found in Gg indicating that the inoculation may not have taken in that treatment. The two most highly mycorrhizal treatments (Gi and DG) also produced the greatest shoot and root biomass (Table 1). Those treatments also tended to have higher [P] in root and shoot biomass (summarized in Table 1). Again, the Gg treatment was indistinguishable from the NM control regarding plant growth and nutrition. However, other experiments have shown that these same Gg and Gi inocula may yield opposite effects on plant growth (Johnson et al., in press).

To allow respiration measurements of undisturbed root systems throughout sunflower development and death, we harvested roots only from unmonitored chambers. Root parameters (mass, chemistry and colonization) reflect that subset of plants. In this subset we measured AM colonization and it explained 67% of variation in shoot [P] (Figure 2). The mean [P] of Gg treatment was as low as NM (Table 1), which corroborates the evidence that Gg treatment may have experienced little AM colonization.

Table 1. Summary of mycorrhizal treatment effect on sunflower mass and chemistry. Values are means ± standard error at final harvest (week 13) for shoots ($n = 20$) and roots ($n = 8$). Gi = *Glomus intraradices*, Gg = *Gigaspora gigantea*, DG = desert grassland suite, NM = nonmycorrhizal plants. *P*-values are from one-way ANOVAs and letters denote differences among individual treatments as determined by Tukey's post hoc tests

		Mycorrhizal Treatment				
		Gi	Gg	DG	NM	*P*
Shoot	Mass (g)	2.66 ± 0.33^{a}	2.07 ± 0.18^{ab}	2.71 ± 0.30^{ab}	2.03 ± 0.26^{b}	0.026
Foliar	%C	34.71 ± 0.51^{a}	36.52 ± 0.49^{b}	37.29 ± 0.44^{ac}	35.50 ± 0.41^{bc}	< 0.001
	%N	3.35 ± 0.13	3.20 ± 0.10	3.08 ± 0.13	3.32 ± 0.09	NS
	C/N	10.59 ± 0.39^{a}	12.49 ± 0.63^{b}	10.80 ± 0.31^{ac}	11.63 ± 0.46^{abc}	0.035
	P (mg g^{-1})	1.80 ± 0.18^{a}	0.91 ± 0.24^{b}	1.57 ± 0.26^{a}	0.86 ± 0.07^{b}	< 0.001
Root	Mass (g)	0.50 ± 0.05^{a}	0.34 ± 0.03^{ab}	0.53 ± 0.07^{a}	0.28 ± 0.03^{b}	0.033
	%C	33.47 ± 0.64	34.35 ± 0.77	33.75 ± 0.85	33.83 ± 0.98	NS
	%N	1.88 ± 0.07^{a}	1.52 ± 0.08^{b}	1.63 ± 0.10^{b}	1.77 ± 0.08^{ab}	0.042
	C/N	17.97 ± 0.76^{a}	23.10 ± 1.53^{ab}	21.13 ± 1.14^{a}	19.45 ± 1.20^{b}	0.035
	P (mg g^{-1})	1.97 ± 0.53^{a}	0.79 ± 0.16^{b}	1.49 ± 0.26^{a}	0.94 ± 0.18^{b}	< 0.001
	%AM	82.50 ± 5.37^{a}	13.75 ± 4.91^{b}	53.75 ± 10.61^{c}	4.76 ± 1.70^{b}	< 0.001
Root:Shoot ratio (g g^{-1})		0.185 ± 0.018	0.163 ± 0.015	0.183 ± 0.023	0.147 ± 0.014	NS
Soil	%C	0.760 ± 0.018	0.786 ± 0.022	0.800 ± 0.019	0.720 ± 0.020	0.097

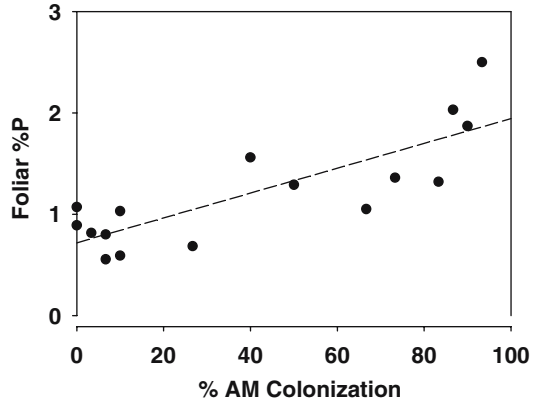

Figure 2. Relationship between AM colonization and foliar P concentration. $y = 0.012x + 0.719$; $r^2 = 0.67$.

Respiration

Mycorrhizal inoculation increased overall rhizocosm respiration ($P = 0.032$; repeated measures, between-treatment *F*-test). The strongest differences arose during weeks 10, 11, and 13 (Figure 3), which was the period of greatest respiration rates. After shoots were removed, variation in respiration among treatments approached significance only in week 15 ($P = 0.078$). At this time, higher respiration in the mycorrhizal pots could have arisen simply from plants being larger in those treatments. To assess the effect of mycorrhizal status on respiration independently of plant size, we analyzed the relationship of respiration with plant mass for the two weekly periods before (live phase) and after death (dead phase). We pooled the two treatments with high AM colonization (Gi and DG) and compared them to the NM treatment. Each group had a positive and significant relationship with plant mass (Figure 4). When the plants were alive, mycorrhizal status did not affect the relationship between soil respiration and plant mass, but the relationships for mycorrhizal and nonmycorrhizal plants diverged after shoots were killed (comparison of slopes, $P < 0.05$).

Mycorrhizal abundance is known to respond to global change perturbations (Treseder, 2004) and so, how mycorrhizas affect soil C processing could provide insight into the future global C cycle. AM colonization appears to direct more photosynthate belowground in the form of roots and root-associated products. First, Gi and DG plants in our study had roughly twice as much root mass as NM plants (Table 1). Second, analyses of root-free soil revealed a trend of greater soil C in AM treatments than in the NM treatment at the end of the experiment (one-way

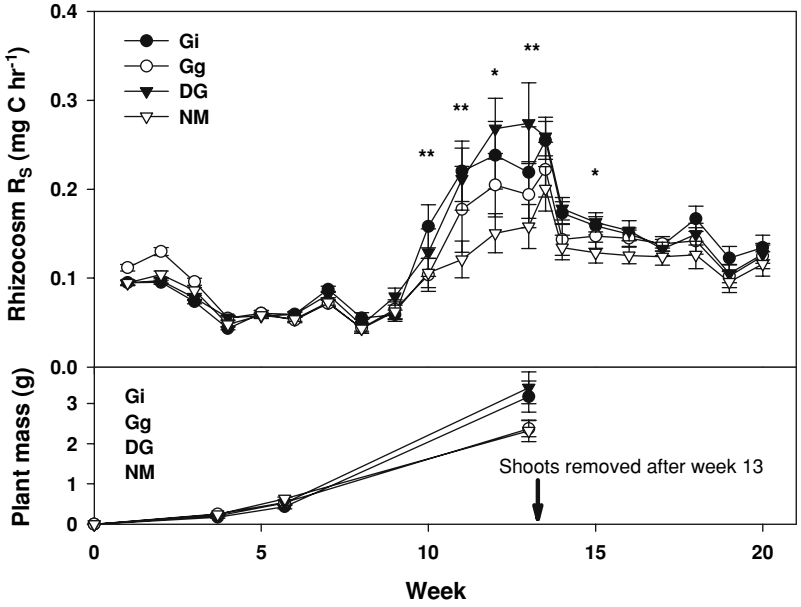

Figure 3. Mean soil respiration rates and plant masses throughout 20 weeks of experiment. Sunflower shoots were removed after week 13. Bars indicate standard errors. Differences among treatments for individual weekly means are indicated as $^{**}P < 0.01$; $^{*}P < 0.1$ according to a repeated measures MANOVA. Treatment differences were driven by Gi and DG being significantly higher than the Gg and NM treatments ($P < 0.05$, post-hoc contrasts on repeated measures treatment effect).

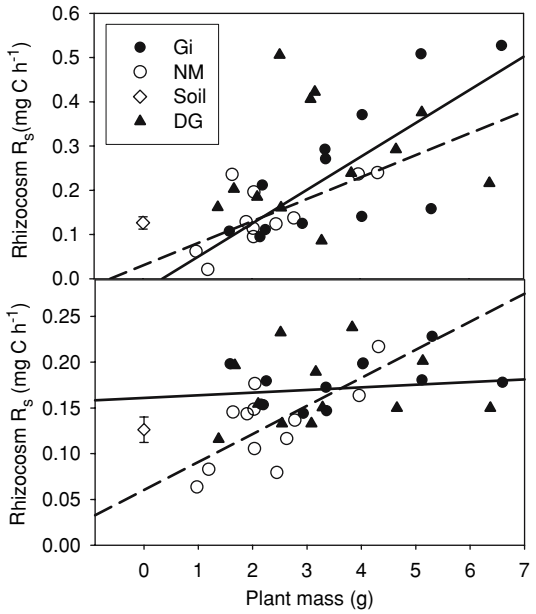

Figure 4. Relationship of soil respiration when plants were alive (top panel) and dead (bottom) with final harvest plant mass. Respiration of rhizocosms with no plants (plant mass = 0) are represented by open triangles with standard error bars. Dark symbols represent AM plants (from Gi and DG treatments). Gg was excluded from this analysis due to uncertain AM colonization. Live plants, AM: $y = 0.106 + 0.044x$, $r^2 = 0.21$, NM: $y = 0.032 + 0.052x$, $r^2 = 0.45$; Dead plants, AM: $y = 0.157 + 0.005x$, $r^2 = 0.05$, NM: $y = 0.060 + 0.031x$, $r^2 = 0.48$.

ANOVA, $P = 0.097$, Table 1). Detecting changes in bulk soil C over short-term experiments is difficult because masses of inputs are small compared to the mass of C in existing soil organic matter (Hungate et al., 1996). That we found consistent trends in that direction, may indicate that mycorrhizal plants are depositing more C in the rhizosphere perhaps in the form of hyphae and hyphal products. How mycorrhizal C pools decompose remains unknown. Our measurements did not allow us to directly calculate a residence time for new photosynthate due to a lack of precise estimates of the quantity of new C belowground and source of respired C.

A mycorrhizal alteration of soil C residence time could arise from two compatible mechanisms: (1) AM fungi directly change the decomposability of C allocated belowground, or (2) AM fungi sequester soil nutrients, thereby indirectly suppressing free-living heterotrophs. Mycorrhizal fungal hyphae have higher nutrient concentrations (Langley and Hungate, 2003) and higher live respiration rates (Bååth and Wallander, 2003) but may alter overall belowground litter quality than NM roots because of fundamentally different carbon chemistry. Moreover, mycorrhizal colonization may induce allocation tradeoffs between labile C pools (e.g. root exudation) and more stable ones (e.g. mycorrhizal tissue). Though some of the C in mycorrhizal hyphae is respired quickly (Staddon et al., 2003), chitin, the structural backbone of fungal tissue, is known to resist decomposition (Swift, 1979). Further, AM fungi produce large quantities of glomalin, which is extremely recalcitrant (Steinberg and Rillig, 2003). Alternatively, mycorrhizas can sequester nutrients in the plant or in their own tissue thereby rendering free-living heterotrophs more nutrient limited and suppressing their respiration (Gadgil and Gadgil, 1971). In our study, AM fungi increased N mass contained in plants by 50%, which likely decreased N availability to heterotrophs in this nutrient-poor soil. Further analyses are required to more directly determine how mycorrhizal fungi affect C residence time and to decipher the precise mechanism.

Temperature sensitivity of respiration

Temperature sensitivity of soil respiration was not affected by mycorrhizal treatment ($P = 0.492$, repeated measures, Figure 5) but did significantly increase when plants were killed ($P < 0.001$, F-test). R_S includes an autotrophic component, root respiration (R_R), and heterotrophic respiration (R_H). The Q_{10} of R_R typically is estimated by excising roots and measuring CO_2 evolution at different temperatures in the laboratory (e.g. Pregitzer et al., 1998). To determine R_H, researchers have similarly removed soil from the field and incubated it at varying temperatures and measured respiration rate. In isolation in the lab, R_H has a much wider range of respiratory Q_{10} (from 1 to 12, Kirschbaum, 1995) than does root respiration (from 1.1 to 2.9, Atkin et al., 2000). Temperature sensitivity of total R_S ranges from 1.3 to 3.3 with a mean of 2.4 (Raich and Schlesinger, 1992). Our Q_{10} estimates for total soil respiration averaged 2.8 for the live phase of the experiment and then elevated to 3.7 following plant death. The presence of respiring roots

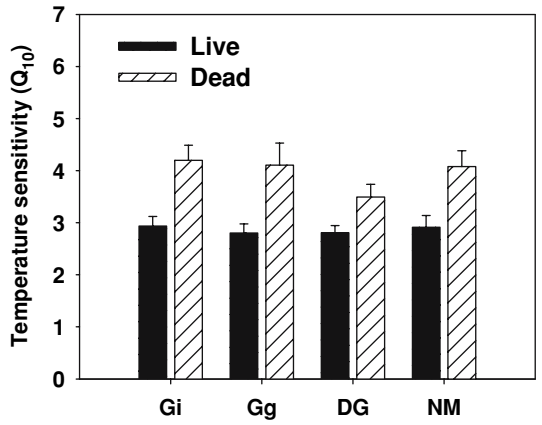

Figure 5. Mean temperature sensitivity of soil respiration for rhizocosms in live and dead plant phases. There were no treatment effects, but killing the plants significantly increased Q_{10}. Bars indicate standard error.

governed the temperature sensitivity of total R_S while plants were alive, possibly indicating that root respiration is limited by the rate that C is made available to the root system (Atkin et al., 2000), ultimately limited by photosynthesis (Kuzyakov and Cheng, 2004). Nonmycorrhizal soil heterotrophs thus appear to have a higher Q_{10} than roots and mycorrhizae. Modeling exercises have shown that availability of high quality substrate may drive heterotrophic respiratory Q_{10} (Eliasson et al., 2005). Winkler et al. (1996) found much stronger temperature sensitivity in relatively rich A-horizon soil than E- or B- horizon soils which had much lower C content and lower C quality. If the temperature sensitivity of R_H depends on substrate quality, the flush of labile plant and mycorrhizal litter made available by sunflower death in our study may have elevated heterotrophic temperature sensitivity thereafter. However, more recent soil incubation studies have found that neither soil C quality, nor the presence of root litter, appear to influence temperature sensitivity of respiration Boone et al. (1998), (Fang et al. 2005).) inferred that the Q_{10} of live mycorrhizal respiration was over four. They reasoned that the Q_{10} of mycorrhizal respiration must be extremely high to explain the differences between laboratory measurements and field estimates of the Q_{10} of R_S. If mycorrhizae had an appreciable influence on the Q_{10} of R_S we would have expected a mycorrhizal treatment effect in the rhizocosms. However, the only trend was slightly lower Q_{10} in DG rhizocosms after death. The discrepancy between field and lab estimates of Q_{10} may arise from methodological artifacts rather than biological interactions. Temperature sensitivity of soil respiration has most commonly been evaluated over seasonal temperature cycles, introducing other biological variables, such as plant phenology (Curiel Yuste et al., 2003) and nutrient availability that vary seasonally (Jannsens and Pilegaard, 2003). The resulting Q_{10} values ascribe the effects of other factors to temperature and may be inappropriate for predictions about the response of soil CO_2 efflux to climate change.

Xu and Qi (2001) correlated R_S with temperatures measured at 6 depths and found that the most appropriate depth (that with highest correlation coefficient) was 20 cm on one plot and 10 cm deep on another plot on the same soil only 40 m away. In field studies, soil temperature has often been measured at only one depth (ranging from 2 to 20 cm; e.g. Borken et al., 2002; Curiel Yuste et al., 2003; Jannsens and Pilegaard, 2003) which may not correspond to the actively respiring soil profile, skewing Q_{10} estimates. For instance, choosing too great a depth at which to measure temperature leads one to underestimate the temperature fluctuation experienced at the site of respiration, thereby causing an overestimation of Q_{10}.

In rhizocosms used in this study, we can evaluate temperature sensitivity of R_S in soil monoliths that are large enough to simulate realistic soil-plant interactions but small enough to measure temperature of the whole soil profile accurately (i.e. the entire soil profile equilibrates uniformly), and controlled enough to maintain consistent soil moisture among replicates and treatments. Using diurnal variation in temperature may introduce undesirable artifacts as well. First, light covaries with temperature and may influence root respiration via light control of photosynthesis. Second, organisms under a regime of cyclic, diurnal warming may not be subject to thermal acclimation as are organisms exposed to a continuous warming treatment (Knorr et al., 2005). The Q_{10} estimates resulting from diurnal temperature variation may not, therefore, be appropriately extrapolated to predict R_S rates under global warming. These

estimates do yield physiological information valuable for explaining patterns of temperature sensitivity.

Conclusion

Colonization by AM fungi increased belowground respiration rates in sunflower rhizocosms. The increase in respiration appeared to be mediated through enhanced nutrition in mycorrhizal plants. The mycorrhizal stimulation of soil respiration was in close proportion to the mycorrhizal increase of plant mass which suggests that AM colonization did not affect respiration independently of its effects on plant growth. Though fungi have higher specific respiration rates, the balance of allocation among roots, mycorrhizal, and exudates netted no effect on the relationship of rhizosphere respiration with plant mass. However, when the plants were killed, R_S in the most mycorrhizal systems tended to decrease more than in nonmycorrhizal ones. These findings indicate that the afterlife effects of mycorrhizal residues may be more important than living mycorrhizal physiology for explaining differences among ecosystems and for predicting future C budgets in a changing environment.

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