

Resource limitation is a driver of local adaptation in mycorrhizal symbioses

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Symbioses may be important mechanisms of plant adaptation to their environment. We conducted a reciprocal inoculation experiment to test the hypothesis that soil fertility is a key driver of local adaptation in arbuscular mycorrhizal (AM) symbioses. Ecotypes of *Andropogon gerardii* from phosphorus-limited and nitrogen-limited grasslands were grown with all possible “home and away” combinations of soils and AM fungal communities. Our results indicate that *Andropogon* ecotypes adapt to their local soil and indigenous AM fungal communities such that mycorrhizal exchange of the most limiting resource is maximized. Grasses grown in home soil and inoculated with home AM fungi produced more arbuscules (symbiotic exchange structures) in their roots than those grown in away combinations. Also, regardless of the host ecotype, AM fungi produced more extraradical hyphae in their home soil, and locally adapted AM fungi were, therefore, able to sequester more carbon compared with nonlocal fungi. Locally adapted mycorrhizal associations were more mutualistic in the two phosphorus-limited sites and less parasitic at the nitrogen-limited site compared with novel combinations of plants, fungi, and soils. To our knowledge, these findings provide the strongest evidence to date that resource availability generates evolved geographic structure in symbioses among plants and soil organisms. Thus, edaphic origin of AM fungi should be considered when managing for their benefits in agriculture, ecosystem restoration, and soil-carbon sequestration.

coevolution | geographic mosaics | mutualism | parasitism

Symbiotic organisms adapt to one another and their abiotic environment. This process varies spatially among populations such that evolutionary mosaics of symbiotic function can arise from geographic differences in the environment (1). The degree to which geographic mosaics of symbioses are caused by variation in the biotic or abiotic environment is not well-understood, but this knowledge is critical for development of a mechanistic understanding of their function within ecosystems (2, 3). Soil fertility mediates the mutualistic function of mycorrhizal symbioses (4, 5); consequently, mycorrhizas from grasslands with varying soil-nutrient environments provide an ideal system to uncouple the importance of biotic (plant and fungal) and abiotic (soil) variation to adaptation by symbionts. Arbuscular mycorrhizal (AM) fungi (Glomeromycota) are obligate biotrophs with vascular plants, and they facilitate plant acquisition of limiting soil resources. Thus, plants and AM fungi may exert reciprocal selective forces on one another. These fungi form arbuscules inside plant roots through which mineral nutrients are exchanged for host photosynthates, and they form extensive networks of extraradical hyphae in the soil through which they forage for mineral nutrients and water. In this way, host plants may benefit from the nutrient-harvesting abilities of their fungal symbionts. The relationship between plant and AM fungus is more likely to be mutualistic when soil-nutrient supply rate is low and fungal access to essential nutrients is beneficial to host growth (6). Parasitic mycorrhizas can develop in fertilized soils if carbon costs of the symbiosis do not outweigh the mineral benefits (4, 5, 7). Glomeromycotan fungi have also been shown to improve soil

structure and are important carbon and nutrient sinks in many ecosystems (8–10). Thus, understanding the factors that control their abundance and symbiotic function can help guide their management so that their desirable community and ecosystem functions can be maximized.

Mutual cooperation is more likely to develop in stable long-term partnerships than in novel combinations of plants and AM fungi (11). Consequently, we looked for evidence of local adaptation and coadaptation in undisturbed or restored grasslands where ecotypes of plants and AM fungi are expected to have a long history of symbiosis in their local soil environment (12). *Andropogon gerardii* Vitm. is the dominant native grass in many North American tallgrass prairies, and mycorrhizas seem to be critical to the maintenance of this dominance. Whole-community experiments show that removal of AM fungi using fungicides reduces *A. gerardii* biomass with a concomitant increase in the biomass of less mycotrophic grasses and forbs (13, 14). Soil-phosphorus availability has been linked to differences in the formation of mycorrhizas among regional ecotypes of *A. gerardii* such that ecotypes from phosphorus-limited sites have coarser roots and benefit more from mycorrhizal symbioses than those from phosphorus-rich sites (15). Enriching soil with chemical fertilizers has been shown to change the species composition and abundance of AM fungi (16) and nearly eliminate populations of *A. gerardii* (17, 18). Plant communities influence the composition of communities of soil organisms (19), and in turn, these organisms may have beneficial, detrimental, or neutral effects on plant growth and thus, generate positive or negative feedbacks (20). Studies show a preponderance of negative feedbacks in early successional plant species and positive feedbacks in late successional species (21).

For a highly mycotrophic, late successional species like *A. gerardii*, we hypothesize that adaptation of plants to their biotic and abiotic soil environment may structure AM symbioses such that mutualistic function between plant genotypes and AM fungal communities will be maximized in nutrient-limited sites. This selection is not expected to occur in nutrient-rich sites, because mycorrhizas are not likely to increase plant fitness if mycorrhizal delivery of nutrients provides little benefit to compensate for the carbon cost of the symbiosis (4, 6). Many studies have linked AM function to phosphorus availability (5, 22). Although AM fungi also deliver nitrogen to their host plants (23, 24), the importance of this to plant fitness is not well-established (25, 26). The purpose of our study was to test three hypotheses:

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H₁: Mycorrhizas improve plant fitness by increasing the uptake of either phosphorus or nitrogen, whichever nutrient is most limiting to plant growth.

H₂: The fitness of plants and biomass of associated AM fungi is greater in coadapted symbionts grown in their local soil compared with those grown in novel combinations.

H₃: Coadaptation in AM symbioses can maximize mutualism in low-fertility soil or minimize parasitism in high-fertility soil.

We tested these hypotheses using a reciprocal-inoculation experiment with local ecotypes of *A. gerardii*, AM fungal communities, and soils from two native prairie remnants, Cedar Creek in Minnesota and Konza Prairie in Kansas, and one restored prairie at Fermi National Laboratory Environmental Research Park in Illinois. All available evidence suggests that the soils at Cedar Creek and Konza prairies have been relatively undisturbed since the last glaciation 11,000 years ago and probably much longer in the case of Konza. The reconstructed prairie at Fermi was established 28 years ago using locally collected seeds from nearby prairie remnants on similar soil types. The Fermi prairie was previously cultivated and managed with conventional agricultural methods from the late 1840s through 1975. The soils at the three sites are very different. Available soil phosphorus is very low at Fermi and Konza and very high at Cedar Creek; soil nitrogen is very low at Cedar Creek and higher at the other sites (Table 1). Therefore, we predict that mycorrhizal benefits should be related to phosphorus uptake at Fermi and Konza and related to nitrogen uptake at Cedar Creek.

Quantifying adaptation within mycorrhizas requires measurements of the influence of the symbiosis on the fitness of the host plant and the fungal biomass as well as some measure of symbiotic exchange. In this study, we used total plant biomass and inflorescence biomass as surrogate measures of plant fitness, the density of extraradical AM hyphae as a measure of fungal biomass, and arbuscular colonization as a measure of symbiotic exchange. We quantified the tissue nitrogen and phosphorus content of plants to test the prediction that AM colonization should be positively associated with phosphorus uptake in Fermi and Konza soils, and it should be positively associated with nitrogen uptake in Cedar Creek soil. Our experimental design allowed us to determine the degree to which plants and AM fungi adapt to each other and their abiotic soil environment. If local adaptation occurs, then the biomass of AM fungi and/or plant fitness should be highest in their home soil. If coadaptation occurs, then plant fitness, fungal biomass, and symbiotic exchange should be greater in “fully home combinations” of plants, fungi, and soils than in “away combinations.”

Results and Discussion

In mesic grasslands, plant tissue N:P < 14 generally indicates nitrogen limitation, N:P > 16 indicates phosphorus limitation, and ratios between 14 and 16 indicate that the two elements are

likely to be colimited (27). Liebig's Law of the Minimum suggests that plant productivity can be maximized when nitrogen and phosphorus are equally limited (27). Mycorrhizas had a strong impact on the tissue N:P of *A. gerardii* in our reciprocal inoculation experiment. Nonmycorrhizal plants grown in Fermi and Konza soils were severely phosphorus-limited (N:P = 26.6 ± 0.91 SE and 24.8 ± 0.58, respectively; *n* = 6), and nonmycorrhizal plants grown in Cedar Creek soil were severely nitrogen-limited (N:P = 6.3 ± 0.54). In contrast, mycorrhizal plants grown in Fermi and Konza soils had tissue-nutrient concentrations indicative of equal limitation (N:P = 14.5 ± 0.21 and 14.1 ± 0.20, respectively), and those grown in Cedar Creek soil suggest minor nitrogen limitation (N:P = 12.5 ± 0.21). The role of AM fungi in optimizing plant N:P ratios is strongly supported by linear relationships between plant tissue-nutrient concentrations and extraradical (in the soil) and intraradical (inside roots) fungal structures (Figs. 1 and 2). Extraradical AM hyphae and intraradical arbuscules are strongly positively related to tissue nitrogen in plants grown in nitrogen-limited Cedar Creek soil (Fig. 1 *E* and *F*) but weakly related in plants grown in phosphorus-limited Fermi (Fig. 1 *A* and *B*) and Konza (Fig. 1 *C* and *D*) soils. In contrast, extraradical AM hyphae and arbuscules are strongly related to tissue phosphorus in plants grown in Fermi (Fig. 2 *A* and *B*) and Konza (Fig. 2 *C* and *D*) soils but not in those grown in Cedar Creek soil (Fig. 2 *E* and *F*). These results clearly indicate that AM fungi increase uptake of the nutrient that is most limiting in the soil. However, our results only partially support the hypothesis that AM symbioses improve plant fitness by increasing the uptake of the most limiting nutrient. Extraradical AM hyphae and arbuscular colonization are positively related to plant biomass in Fermi (Fig. 3 *A* and *B*) and Konza soils (Fig. 3 *C* and *D*) but negatively associated with plant biomass in Cedar Creek soil (Fig. 3 *E* and *F*). Similar positive relationships among inflorescence biomass and AM fungal biomass are observed in Fermi ($R^2_{106} = 0.40$; $P < 0.0001$) and Konza ($R^2_{104} = 0.29$; $P < 0.0001$) soils, and negative relationships are observed in Cedar Creek soil ($R^2_{104} = 0.21$; $P < 0.0001$). Thus, although mycorrhizas improve nitrogen uptake in the nitrogen-limited Cedar Creek soil, they do not increase plant fitness.

Why are AM symbioses mutualistic in Fermi and Konza soils but commensal or parasitic in Cedar Creek soil? We propose that the reason is related to the balance of trade between plants and fungi. Plants exchange carbon for fungal phosphorus and nitrogen (22, 26). Tissue N:P ratios in our experiment indicate that AM symbioses completely satisfy the phosphorus requirements of *A. gerardii* grown in Fermi and Konza soil, but they do not completely satisfy the nitrogen requirements of plants grown in Cedar Creek soil. Consequently, plants grown in this nitrogen-impooverished soil will continue to provision AM fungi with carbon in exchange for nitrogen, and this will increase the biomass of the fungus but not the biomass of the plant. This mechanism accounts for the extremely strong positive correlation between plant nitrogen concentration and extraradical AM

Table 1. Location, climate, and soil characteristics at the three prairie sites

Site	Latitude and longitude	Mean annual precipitation (mm)	Mean January/July temperature (°C)	Soil type	Soil texture	Soil pH	Soil organic matter %	Bray-1 PO ₄ , μg g ⁻¹	NH ₄ -N, μg g ⁻¹	NO ₃ -N, μg g ⁻¹	Soil N:P
Fermi	41°50' N 88°15' W	882	-6.5/22.5	Mesic udollic endoaqualfs	Silt loam	7.6	3.3	11.5	7.2	5.8	1.1
Konza	39°05' N 96°18' W	835	-2.7/26.2	Mesic argiustolls	Silty clay loam	6.2	5.7	18.5	13.4	8.2	1.1
Cedar Creek	45°24' N 93°12' W	660	-10.0/22.2	Typic udipsamments	Sandy loam	5.3	1.4	46.5	5.1	4.9	0.2

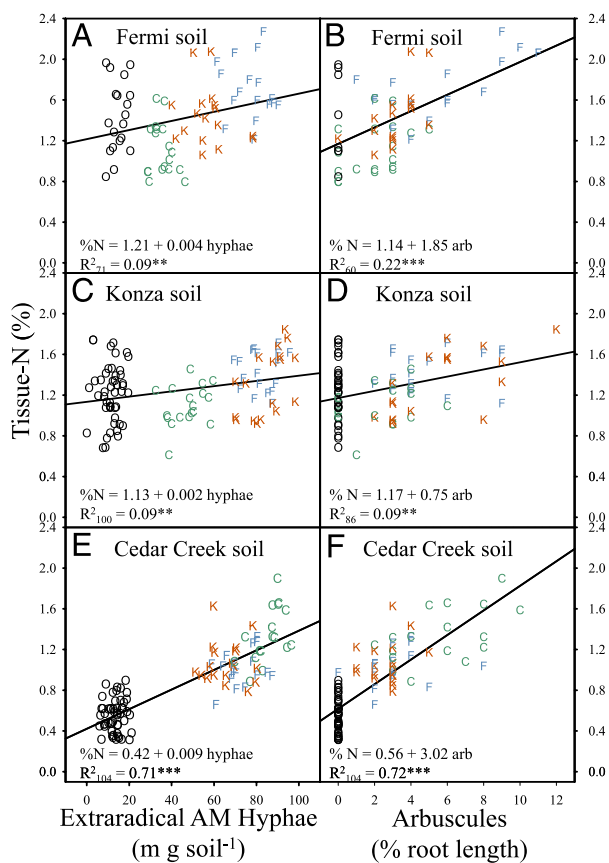


Fig. 1. The relationship between tissue nitrogen and extraradical hyphal density and the proportion of root length colonized by arbuscules in Fermi, Konza, and Cedar Creek soils. Symbols represent treatment-inoculum source (blue F, Fermi; red K, Konza; green C, Cedar Creek; black O, nonmycorrhizal controls). Arbuscule-colonization data were arcsine square root transformed before statistical analysis. R^2 subscript indicates the number of samples included in the regression. Significance of the regression equations are indicated as ** $P < 0.01 > 0.001$ and *** $P < 0.0001$.

hyphae and arbuscules (Fig. 1), despite the negative relationship with plant biomass (Fig. 3) in Cedar Creek soil.

Our findings provide solid evidence that the fitness of plants and their associated AM fungi is greater with coadapted symbionts grown in their local soil compared with those grown in novel combinations. The abundance of home points on the right-hand sides of the scatter plot axes in Figs. 1–3 clearly illustrates a propensity for home-soil advantage in the formation and function of AM symbioses, and the importance of plant genotype origin for arbuscule formation is further illustrated in Fig. 4A. Across all three sites, arbuscule production was highest when fungi were matched with all-home combinations of host plants and soils (letter h in Fig. 4). Recent studies show that AM fungi release signaling molecules, which trigger a series of symbiotic plant genes; this actively prepares the intracellular root environment for colonization and arbuscule formation inside the root cortex (28–30). Our results indicate that the genetic program of local plant ecotypes responds best to the molecular signals sent by coadapted AM fungal communities. Soils at Fermi and Konza were more similar to each other than to soil at Cedar Creek; nevertheless, the superiority of home-soil inoculum is apparent in soil from Fermi, the most phosphorus-limited site (Figs. 1–3).

Production of extraradical hyphae by AM fungi from Cedar Creek and Konza, the two remnant native prairies, also showed strong home-soil fidelity, but hyphal growth was insensitive to host ecotype origin (Fig. 4B). This pattern was not observed for

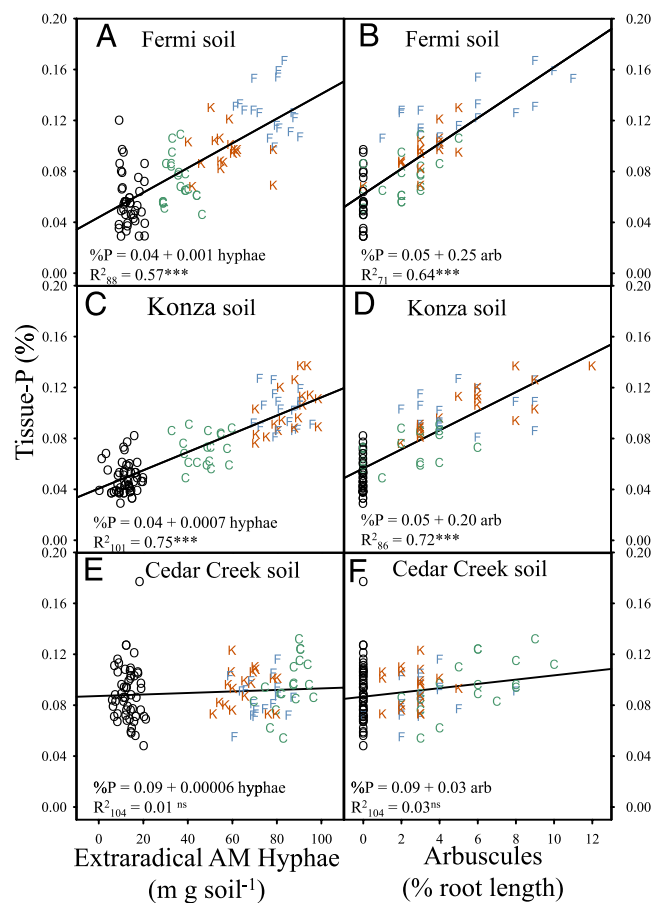


Fig. 2. The relationship between tissue phosphorus and extraradical hyphal density and the proportion of root length colonized by arbuscules in Fermi, Konza, and Cedar Creek soils. Symbols represent treatment-inoculum source (blue F, Fermi; red K, Konza; green C, Cedar Creek; black O, nonmycorrhizal controls). Arbuscule-colonization data were arcsine square root transformed before statistical analysis. R^2 subscript indicates the number of samples included in the regression. Significance of the regression equations are indicated as *** $P < 0.0001$ (ns, not significant).

fungi from the reconstructed prairie at Fermi, where the fungi produced equal amounts of extraradical hyphae across all soil types. The absence of home-soil preference of AM fungi at Fermi corresponds to a shorter time for adaptation (28 years versus millennia). Perhaps Fermi AM fungi have lower home-soil fidelity, because the recent agricultural history of this site generated a community of fungi that could forage equally well on a greater diversity of soil conditions.

Another important finding was that tissue N:P was significantly greater in plants inoculated with soil organisms from nitrogen-limited Cedar Creek (N:P = 14.9 ± 0.21) compared with those inoculated with soil organisms from Konza (N:P = 13.0 ± 0.20) or Fermi (N:P = 13.1 ± 0.21). This indicates that the community of AM fungi and other soil organisms in Cedar Creek soil has a superior ability to acquire nitrogen compared with the communities of soil organisms from the phosphorus-limited sites. As with AM hyphal production, this response seems to be entirely driven by soil origin ($F_{2,156} = 24.6$; $P < 0.001$), inoculum origin ($F_{2,156} = 24.8$; $P < 0.001$), and an interaction of the two ($F_{4,156} = 6.9$; $P < 0.001$), whereas plant genotype had no significant effect ($F_{2,156} = 0.69$; $P = 0.50$).

Our results indicate that coadaptation in AM symbioses can maximize mutualism in phosphorus-limited soil and minimize parasitism in phosphorus-rich soil. Response of inflorescence

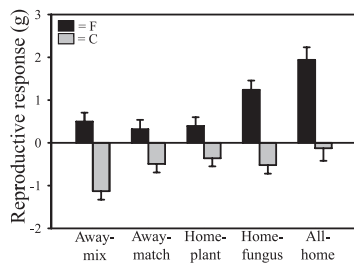


Fig. 5. Reproductive response measured as the difference in total inflorescence biomass of *A. gerardii* grown with and without AM fungi and associated soil organisms. Biomass differences are shown for five mutually exclusive categories of home and away treatment combinations of plants and inoculum from Fermi (black bars) and Cedar Creek (gray bars). Away-mix, soil, plants, and inoculum originated from three different sites; away-match, plants and fungi originated from the same site but from a different location than the soil in which they were grown; home-plant, plants and soil came from the same location (different from the fungi); home-fungus, fungi and soil came from the same location (different from the plants); all-home, soil, plants, and fungi all originated from the same location. Negative values indicate that the nonmycorrhizal plants have larger inflorescences than those inoculated with AM fungi. Values are means \pm SE ($n = 6$).

surrogate measure of fungal biomass, was positively related to plant fitness in the two phosphorus-limited soils but not in the nitrogen-limited Cedar Creek soil (Fig. 3). Plant and fungal stoichiometry are key factors in the balance of trade in mycorrhizas (26, 36), and they may help explain the differences in mycorrhizal function among the soil types. Most organisms require $\sim 10\times$ more nitrogen than phosphorus, and fungi have relatively higher nitrogen requirements than plants (37); therefore, it is possible that the high-phosphorus, low-nitrogen soil at Cedar Creek creates a mycorrhizal marketplace in which host plants can barely break even when exchanging carbon for nitrogen with their AM fungal symbionts.

More research is necessary to explore the importance of selection pressures other than availability of nitrogen and phosphorus, such as the possibility that mycorrhizas at Cedar Creek are selected more for their roles in water relations (38) or protection from pathogens (39) than for their role in nitrogen uptake. Because of the lower water-retention capacity of the Cedar Creek soil (sandy texture and low organic-matter content), it is possible that mycorrhizas at Cedar Creek are an important mechanism for increasing plant drought tolerance through an improved transpiration stream (38). There is evidence that coadapted mycorrhizas may improve plant tolerance to water stress; within the same species of AM fungi, ecotypes isolated from xeric ecosystems have been shown to improve the water relations and drought tolerance of their host plants more than those isolated from mesic environments (40, 41).

This study provides solid evidence that the availability of essential soil resources is an important force in the formation of geographic mosaics of symbiotic function in mycorrhizas. Our reciprocal study design allows us to identify the relative importance of plant genotype, fungal community composition, and soil type in generating a full range of symbiotic function from mutualism to parasitism. Geographic variation among plant genotype was an important factor in the formation of arbuscules, the site for carbon and phosphorus exchange, but it was not important to the formation of extraradical hyphae, the foraging structures of AM fungi. These results combined with recent insights about the evolutionary ecology (42) and population structure (43) of Glomeromycota indicate that coadapted complexes of plants, AM fungi, and other associated soil organisms develop over time such that the fitness of both plants and fungi is maximized under local soil conditions. Selection pressures differ for AM fungi and plants. It behooves AM fungi to be generalist foragers (42), and it behooves plant hosts to be more selective in

the fungi with which they establish trading partnerships (28–30). Nutritional mutualisms are environmentally stable interactions when soil phosphorus is strongly limiting to plant growth. In contrast, when soil-resource availability makes mycorrhizal trading partnerships moot, an evolutionary arms race may occur so that plants minimize mycorrhizal parasitism. These findings may represent general mechanisms of evolutionary processes that occur among communities of plants and soil organisms, and they may help define the phenotypes of communities (44).

Our results have direct relevance to sustainable land management and ecosystem-restoration practices, because they stress the importance of the preservation or establishment of edaphic complexes of communities of locally adapted ecotypes of plants and soil microorganisms (45). The current practice of introducing novel genotypes of AM fungi in commercial inoculum may not have the desired outcomes (46) if the soil properties and host genotypes in the inoculated system are very dissimilar to those under which the inoculant fungi were originally selected. Also, our observation that, in the two native prairie sites, AM fungi produced up to 87% more hyphae in their home soil (Fig. 4B) implies that locally adapted fungi have the potential to sequester more carbon and nutrients in their extraradical hyphae than nonadapted fungi (9, 10). In addition to AM-hyphal biomass inputs to the soil-carbon pool, the filamentous hyphae help create a physical framework for stabilizing primary soil particles into larger, relatively stable soil aggregates. By generating these aggregated soil structures, extraradical hyphae increase soil stability and create conditions that favor the protection of detrital inputs (8, 9). Although the contributions of AM hyphae to this mechanism are important for both Fermi and Konza soils, they may play an even more crucial role in the more coarse-textured sandy loam at Cedar Creek (8).

The reduced hyphal densities found in the away-soil combinations emphasize the need for a better understanding of the adaptive processes that contribute to fungal exploitation of the soil environment. A worldwide increase in the cost of chemical fertilizer, as well as a need to decrease chemical-fertilizer runoff, makes it highly desirable to manage AM symbioses to naturally improve or sustain yields of crops and biofuel feedstocks (47). Because of the development of new carbon-negative biofuels, great advancement has been made in identifying and characterizing aboveground traits in prairie grasses relevant to the needs of biofuel feedstocks (48). However, relatively few studies have ventured below ground, primarily because feedstock interests end at the soil surface. This study identifies the importance of local adaption for biomass allocation to mycorrhizal fungal symbionts and suggests that it may be important to identify belowground traits and soil factors that favor symbiotic processes.

Materials and Methods

Seeds of local *A. gerardii* ecotypes from Cedar Creek, Fermi, and Konza were pregerminated, and 14-day-old seedlings were transplanted into large plastic pots (14 cm tall by 11 cm wide) and filled with 1 kg of sterilized soils from the three prairies. Soils were steam-pasteurized at 80 °C for 2 h and allowed to cool for 72 h to eliminate biotic communities but retain abiotic soil traits. Communities of AM fungi and other soil organisms were added back in a controlled manner by inoculating pots with 20 g of living soil from each of the sites in a complete reciprocal design. The living-soil inoculum was added directly below the seedling roots during transplantation. Differences in fertility among the three types of soil inocula were eliminated by adding 20 g of additional sterilized soil from the two sites that were not used in the AM treatment. Additional pots were established using only sterile soil to create nonmycorrhizal controls for each plant and soil combination. Each of the 54 combinations of treatments and controls were replicated six times for a total of 324 pots. All pots were amended with 60 mL of nonsterile soil sievate, 20 mL from each of the three sites. The sievates were prepared by blending soil and water in a 1:2 ratio and passing the slurry through a 25- μ m sieve. The relatively large AM fungal spores were trapped on the sieve, whereas smaller organisms pass through it; this allows for the addition of

the majority of soil microbes while excluding AM fungi (34). Plants were grown in a glasshouse at Kansas State University, Manhattan, KS.

Plants were grown during the summer with full sunlight. At the end of 14 weeks, plants were harvested, roots were washed free of soil, and biomass was oven dried at 60 °C for 48 h. Shoot, root, and inflorescence dry mass were measured. Plant reproductive response to mycorrhizas was calculated as the difference between inflorescence biomass in AM colonized plants and the mean of the matching control plants. Extraradical AM hyphae were extracted from 5 g of soil, quantified by the gridline-intercept method, and converted to hyphal lengths (49). Roots were stained with trypan blue and quantified for intraradical arbuscular colonization using the magnified gridline-intersect method (50). Shoot tissue N and P concentrations were determined after wet digestion with sulfuric acid and hydrogen peroxide of dried plant material was ground through a 20- μ m mesh. Phosphorus concentrations were determined on the digest using the molybdate-blue method (51), and nitrogen concentrations were determined by a Kjeldahl method with a rapid-flow autoanalyzer.

The relationship between plant-response variables (tissue nitrogen, tissue phosphorus, and shoot dry mass) and fungal-predictor variables (extraradical hyphal density and the proportion of root length colonized by arbuscules)

was measured using simple linear regression. Arbuscule colonization was arcsine square root transformed before statistical analysis. Three-factor ANOVA and Tukey HSD tests were applied to the tissue N:P data to assess the amount of variance that can be accounted for by soil origin, AM fungal origin, and host-plant genotype as main effects and all possible interactions. Nonmycorrhizal control plants were analyzed separately from the mycorrhizal plants to assess the importance of mycorrhizas to tissue-nutrient content. Statistical analyses were performed using JMP version 4.0 (52).

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