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POPULATION ECOLOGY – ORIGINAL RESEARCH

Local adaptation to mycorrhizal fungi in geographically close *Lobelia siphilitica* populations

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Abstract

Mutualism between plants and arbuscular mycorrhizal (AM) fungi is common, and plant populations are expected to have adapted to the AM fungal communities occupying their roots. Tests of this hypothesis have frequently been done with plant populations that are tens to hundreds of kilometers apart. However, because AM fungal community composition differs at scales < 1 km, local adaptation of plant populations to AM fungi may occur at small spatial scales, but this prediction has not been tested. Furthermore, prior experiments do not often experimentally identify whether adaptation is related to specific mycorrhizal functions. To test for plant adaptation to AM fungal communities at small spatial scales, and whether adaptation is associated with the nutritional benefits that AM fungi provide to plants, we grew *Lobelia siphilitica* plants from two geographically close populations (1.4 km apart) in a greenhouse reciprocal transplant experiment with soil biota that either included (whole soil) or excluded AM fungi (microbial wash) at both low and high soil phosphorus availability. Though both plant populations responded positively to the presence of AM fungi in the whole soil biota treatment relative to the microbial wash treatment, the average growth response of plant populations to mycorrhizal fungi was highest when local populations were grown with local AM fungi. In addition, local adaptation was only observed in the presence of AM fungi at low phosphorus levels. Thus, local adaptation of plant populations to AM fungi is present at spatial scales that are much smaller than previously demonstrated and occurred primarily to enhance phosphorus acquisition.

Keywords Adaptation · Arbuscular mycorrhizal fungi · Mutualism · Phosphorus · Soil biota

Introduction

Mutualistic interactions, in which different species exchange resources and services for mutual benefit, are widespread in nature, occurring in nearly every biome and domain of life (Bronstein 2015). Because they are common, mutualisms are expected to influence the evolution of each partner. For example, the diversification of form and function in flowers and their pollinators is hypothesized to have arisen in part through reciprocal natural selection by each partner (Darwin 1862; Harder and Johnson 2009). Despite the general acceptance of mutualism as an important cause of adaptation, evolution in response to natural selection caused by mutualism is generally less well studied than other causes, such as competition, herbivory and climatic variation (Hoeksema and Forde 2008; Leimu and Fischer 2008; Hereford 2009; Baskett and Schemske 2015).

One widespread and ecologically important mutualism takes place between plants and root inhabiting arbuscular mycorrhizal (AM) fungi. The mutualism occurs in at least 70% of seed plants (Brundrett 2009; Maherali et al. 2016), with plants providing sugars to their fungal partners in exchange for greater access to soil resources such as phosphorus, increased protection from pathogens, and improved tolerance of drought stress, among other benefits (Newsham et al. 1995; Smith and Read 2008; Delavaux et al. 2017). The ubiquity of the interaction and the potential benefits that plants obtain from AM fungi suggest that

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plant populations should adapt to specialize on the communities or genotypes of AM fungi that they associate with (Schultz et al. 2001). However, direct tests of plant adaptation to the mycorrhizal mutualism are rare relative to studies of plant adaptation to other aspects of the soil environment (Johnson et al. 2010; Rúa et al. 2016).

The classic test for adaptation is the reciprocal transplant experiment, in which multiple populations are reciprocally grown in each of their environments (Clausen et al. 1948; Leimu and Fischer 2008; Hereford 2009). A population is deemed to be locally adapted if (1) the local population outperforms the foreign population in the home site environment (local vs. foreign comparison) or (2) if a population has higher performance in its home environment relative to the non-home or 'away' environment (home vs. away comparison) (Kawecki and Ebert 2004). The local vs. foreign comparison is considered the most reliable test for local adaptation because the home vs. away comparison can be biased by abiotic resource differences between the home and away environments if these differences are maintained in experimental growth conditions (Kawecki and Ebert 2004; Hereford 2009). In a recent meta-analysis, Rúa et al. (2016) tested for local adaptation of plants to mycorrhizal fungi by comparing the difference in plant biomass response to sympatric combinations of plants and fungi relative to allopatric combinations. They found that there was no difference between the sympatric and allopatric combinations when averaged across studies, and thus no evidence for local adaptation of plants to AM fungi. However, because the sympatric vs. allopatric comparison combines the effects of both local vs. foreign and home vs. away comparisons, it is not known if a lack of difference was caused by the absence of local adaptation, or because of potential differences in resource availability between home and away comparisons (e.g., Schultz et al. 2001).

One important uncertainty in studying local adaptation of plant populations to AM fungi is the spatial scale at which adaptation occurs. For example, previous tests of local adaptation of plants to AM fungi have been carried out using plant populations that are tens to hundreds of kilometers apart (Schultz et al. 2001; Johnson et al. 2010; Pánková et al. 2014a). Though this type of design is advantageous because it minimizes the effects of gene flow among populations, which can overcome local adaptation (Hereford 2009), it ignores whether local adaptation occurs at small spatial scales (Sherrard and Maherali 2012). Because both the genetic composition of AM fungal populations and the species composition of AM fungal communities can differ at very small spatial scales (< 100 m) (Koch et al. 2004; Davison et al. 2012), plant adaptation to AM fungi could be apparent even when populations are geographically close, but this hypothesis has not been formally tested.

A second source of uncertainty in local adaptation studies of plant populations to AM fungi is that the specific resource or service that plant populations gain from AM fungi through adaptation can be difficult to identify if that factor is not experimentally manipulated. If, for example, the benefit obtained from AM fungi is nutritional, then local adaptation is expected to be strongest under experimental conditions where soil nutrients are limited (Schultz et al. 2001; Johnson 2010; Pánková et al. 2014b; Rúa et al. 2016). However, AM fungi provide other services to plants, including protection from other microbial pathogens and parasites (Newsham et al. 1995). If plant growth benefits derived from AM fungi are related to ameliorating the negative effects of non-AM fungal microbes, then local adaptation of plant populations to AM fungi should be observed in experimental conditions where soil nutrients are non-limited and which also contain pathogenic and parasitic microbes (Pregitzer et al. 2010).

To test the hypothesis that plant populations are locally adapted to AM fungi found in their home soils, and whether such adaptation occurs at small spatial scales, we grew two geographically close populations of the native perennial plant *Lobelia siphilitica* (Lobeliaceae) in a reciprocal transplant experiment. *L. siphilitica* is a short-lived native herbaceous perennial wildflower inhabiting upland and bottomland old fields, grassland and forest sites (Johnston 1991; Caruso et al. 2003). It is extensively colonized by AM fungi in the wild and associates with distinct microbial communities throughout its geographic range (Hovatter et al. 2011; Hovatter et al. 2013), suggesting that it is likely to adapt to its AM fungal mutualists. We tested for local adaptation using both local vs. foreign and home vs. away comparisons. To assess whether AM fungi are specifically responsible for local adaptation, we tested for adaptation to whole soil biota, which includes the AM fungal component, versus a soil treatment in which AM fungi were removed but other soil biota were retained. If there was local adaptation to AM fungi, we expected it to occur only in conditions including whole soil biota and not in the non-AM soil biota treatment. By contrast, if plants adapt to non-AM fungal microbes, evidence for local adaptation should be present in the non-AM soil biota treatment. To test whether the level of local adaptation was associated with AM fungal-mediated uptake of phosphorus, plants were grown in conditions where only minimal soil phosphorus was added versus a treatment that was fertilized with higher levels of phosphorus. If local adaptation to soil biota is influenced primarily by AM fungal enhancement of phosphorus acquisition, we expected to find evidence for local adaptation only in treatments where both AM fungi were present and plants received minimal phosphorus fertilization.

Materials and methods

To test the hypotheses that *L. siphilitica* populations are locally adapted to their local AM fungal communities, we used a reciprocal common garden experiment with two plant populations, their respective soil biota either with the AM fungal component (whole soil) or without the AM fungal component (microbial wash), and two soil phosphorus levels. The design was fully factorial, with 12 replicates per treatment combination, for a total of 192 plants (2 *L. siphilitica* populations × 2 soil biota origins × 2 soil biota treatments (with and without AM fungi) × 2 soil phosphorus levels × 12 replicates). The same 12 maternal families in each plant population were used in the experiment (1 replicate plant per plant family per treatment combination).

To obtain plant materials for the experiment, we utilized information from a previous survey of *L. siphilitica* populations in southern Ontario by Caruso et al. (2015). These authors identified five populations in their survey (Table 1 in Caruso et al. 2015) and we collected seeds and soil inoculum for our experiment from the two populations in the closest geographic proximity. These populations were (1) Ancaster Lions pool (LIO) in Ancaster, Ontario (43°13′05″N, 79°59′58″W) and (2) Martin Road (MAR) in Ancaster, Ontario (43°13′43″N, 80°00′36″W) and were approximately 1.4 km apart (Caruso et al. 2015). The LIO population was situated in a small clearing in a deciduous forest adjacent to moderately used hiking trails. The MAR population was found in a similar forest with hiking trails, but situated in a slightly larger and more disturbed clearing under electrical lines. *L. siphilitica* flowers from early August until early October with fruits dehiscing from mid September through November (Caruso 2012). Therefore, seed collections were made in late September and mid October 2013 and we collected three to five fruits per maternal plant for ~ 100 plants at each site.

To determine nutrient availability of soils for each *L. siphilitica* population, we obtained soil cores at 30 randomly chosen locations at each site in June 2013. Soil cores were 20 cm deep × 2 cm diameter (~ 125 mL each). Soil samples were pooled and homogenized within each population and 500 g was analyzed to obtain mean site level concentration of NH_4^+, NO_3^- and extractable P (Bray) (University of Guelph Laboratory Services; http://afl.uoguelph.ca/soil-testing-services). Mean NH_4^+ and NO_3^- were similar between sites, but the LIO site contained more P per unit dry soil mass than the MAR site. For LIO, nutrient levels were 1.48 mg NH_4^+ per kg of dry soil, 5.15 mg NO_3^- per kg of dry soil and 25 mg extractable P per kg dry soil. For MAR, nutrient levels were 0.968 mg NH_4^+ per kg of dry soil, 5.09 mg NO_3^- per kg of dry soil and 3 mg extractable P per kg of dry soil.

To minimize the effects of soil nutrient differences between sites on the outcome of home vs. away comparisons, plants were grown in a common background soil to which a small amount of inoculum from each site was added. To establish the experiment, we collected approximately 200 L of soil from an old field site in the Long-Term Mycorrhizal Research Site (LTMRS) located in the University of Guelph Arboretum in Guelph, ON, in May 2014. Soil from this site was used because it contains low levels of plant-available nitrogen and phosphorus (Sherrard and Maherali 2012), allowing us to manipulate nutrient availability by fertilization. The top 30 cm of soil was collected from a freshly dug pit, and the soil was sieved with a 3/4 inch (19 mm) mesh to remove organic matter and other such debris, homogenized and mixed with approximately 460 L of silica sand (Hutcheson Sand and Mixes, Huntsville, ON) to produce 70:30 sand-to-soil mix. The final mix contained 4.74 mg NH_4^+ per kg dry soil, 1.12 mg NO_3^- per kg dry soil and 1.0 mg extractable P per kg dry soil. Thus, nutrient levels in the experimental soils contained higher NH_4^+, but lower NO_3^- and extractable P than soil from either the MAR or LIO population. Prior to the experiment, the soil mix was sterilized in an autoclave for 90 min at 121 °C to remove any soil biota. Each 1.67 L pot (Model ITML® STD06000, Myers Industries Inc.; Akron, OH) used for the experiment was filled with 2 kg of this background soil mix. To decrease the possibility of light competition between nearby plants, pots were arranged in random order approximately 15 cm apart in all directions on greenhouse benches in the University of Guelph Phytotron.

To create the soil biota treatments used to inoculate the sterilized soil–sand mix, we collected four, 125 mL soil cores from 25 evenly located sampling points within each *L. siphilitica* population in June 2014. Soil cores were pooled and homogenized within each population and the 96 pots in the whole soil treatment received 60 mL of the homogenized soil mix (Koide and Li 1989, Johnson et al. 2010). To isolate the effects of AM fungi relative to other soil microbes, 60 mL of non-mycorrhizal microbial wash, obtained from the living inoculum, was added to the remaining 96 pots. This wash contains many components of the soil microbial community, except for AM fungi, which allows isolation of the mycorrhizal effect on plant growth (Ames et al. 1987; Bever 1994). To isolate the non-mycorrhizal microbes, 4 L of live soil was mixed with deionized water (1:2 ratio) and suspended for 24 h with occasional mixing, and then passed through a 25 µm sieve, which excluded AM fungal spores (30–400 µm) (Daniels et al. 1981; Koide and Li 1989; Johnson et al. 2010), but allowed smaller microbes to pass. Following application of either live soil or microbial wash, pots were topped up with additional sterilized soil mix so that each pot contained 1.3 L of growing medium. Because soil cores were pooled within each site to create the soil

inoculum treatments, this design tests the response of each plant population to the mean soil microbial composition and density at each site and does not account for spatial heterogeneity of soil microbes within each site (Reinhart and Rinella 2016; Cahill et al. 2017).

After inoculation with soil biota or microbial wash, each pot received 25 seeds from one of 12 randomly chosen maternal families from each population. Half of the pots in each soil biota treatment received LIO seeds and the other half received MAR seeds. To break dormancy, *L. siphilitica* seeds were cold stratified in a refrigerator at 4 °C for 2 months prior to the beginning of the experiment. Pots were watered to saturation prior to planting and emerging seedlings were watered via light misting. Once established, plants were thinned to one per pot after approximately 3 weeks. For the duration of the experiment, plants were watered using drip irrigation, with each plant receiving approximately 93 mL of water per day. Pot position on greenhouse benches was randomized, and plants were grown for approximately 16 weeks from 10 June 2014 to 27 September 2014. The greenhouse temperature was maintained at 25.5 °C during the day and at 20 °C at night. Supplemental lighting was used to ensure a 15-h photoperiod from 5:00 AM to 8:00 PM and when ambient light dropped below 300 µmol m^{-2} s^{-1}.

To establish the low soil phosphorus treatment, we fertilized pots with 2.0 mg of P per kg of dry soil, and to create the high phosphorus treatment, we added 24.0 mg of P per kg of dry soil (e.g., Schweiger et al. 1995). To ensure that plants were limited only by phosphorus, nitrogen was added to all replicates at 32 mg of N per kg of dry soil. Phosphorus was provided using Multi-MPK 0-52-34 fertilizer (0.227 mg of P per mg fertilizer, Haifa Chemicals Ltd.; Haifa Bay, Israel) and supplemental N was provided using Plant Prod® 14-0-14 Balance fertilizer (0.140 mg of N per mg fertilizer, Plant Products Co. Ltd.; Ancaster, ON). Both fertilizers were water soluble and were therefore delivered to plants in solution. Plants were fertilized prior to planting, on 7 June 2014 and 8 June 2014, and again after plants had become established, on 14 June 2014 and 15 June 2014.

Plants were harvested in random order from 27 September 2014 to 3 October 2014 and separated into aboveground and belowground components. Aboveground tissue was dried to constant mass in an oven at 60 °C for a minimum of 72 h. Roots were washed thoroughly in distilled water and stored in 120 mL plastic specimen vials in a 1:1 solution of 95% ethanol and deionized water. Dried aboveground biomass was used as the primary indicator of plant performance, because other fitness correlates such as seed production were not possible to measure in the pollinator-free greenhouse. Biomass is a reasonable fitness proxy in *L. siphilitica* because it is under strong positive selection in wild populations (Caruso et al. 2003, 2006). To determine whether plant acquisition of phosphorus differed among treatments, we quantified it in aboveground tissue for a subset of individuals in the experiment (4 per treatment combination for a total of 64 plants). All aboveground tissues were homogenized and a subsample analyzed to determine the percentage of phosphorus (%P) (University of Guelph Laboratory Services; http://afl.uoguelph.ca/plant-health-diagnostics).

To determine whether treatments differed in the level of AM fungal colonization of roots and to verify that plants in the soil wash treatment were not colonized, we measured the proportion of roots colonized by AM fungi. Root systems were cut into 1 cm segments and 15 of these were randomly selected for clearing and staining for the presence of AM fungal structures. Roots were cleared using a 10% KOH solution for 25 min at 90 °C (Brundrett 1994; Sherrard and Maherali 2012) and then stained with a 5% ink and vinegar solution using Black Sheaffer Skrip ink (Sheaffer Pen and Art Supply Co./A.T. Cross Company, Providence, RI), as described by Vierheilig et al. (1998). Following clearing and staining, root segments were mounted onto microscope slides and fixed with a polyvinyl alcohol mounting medium (Koske and Tessier 1983). Because arbuscules are diagnostic of a functional mycorrhizal symbiosis, we measured % arbuscular colonization quantified as the presence or absence of well-stained structures at 45 randomly selected intersections per slide (McGonigle et al. 1990).

To determine whether aboveground biomass and %P in tissues were influenced by the soil biota treatment (i.e., including or not including AM fungi), soil biota origin (LIO or MAR soil), soil-P level and population origin (LIO or MAR plants) and their interactions, we used a four-way ANOVA, with all variables as fixed factors. Data were log-transformed to meet homogeneity of variance assumptions for ANOVA. To test specific local vs. foreign and home vs. away predictions of local adaptation, we used planned orthogonal single degree of freedom contrasts (Sokal and Rohlf 1995). For the local versus foreign criterion, the local population is expected to have higher performance than the foreign population in the local soil biota habitat. For the home vs. away criterion, a population is expected to have higher performance in its home soil biota than in a non-home (i.e., away) soil biota habitat. We expected that local adaptation would be present using both criteria only in soils containing whole soil biota as inoculum and grown under low P conditions. To test whether the proportion of arbuscule colonization differed among treatment combinations, we used a three-way ANOVA with soil biota origin, soil-P level and population origin as fixed factors. Because there was no mycorrhizal colonization of roots in the microbial wash treatment, this treatment was dropped from the statistical analysis. Arbuscule colonization data were arcsine square-root transformed to meet normality and homogeneity of variance assumptions.

To quantify plant growth response to inoculation by AM fungi for each maternal family within each population, we calculated the mycorrhizal growth response (MGR) as the log response ratio, $MGR = \ln[X_M/X_W]$, where X_M is the inoculated (whole soil treatment) biomass and X_w is the microbial wash biomass of individual plants from the same maternal family (Hoeksema et al. 2010). Eight plants died before the end of the experiment, two plants in the low P treatment were accidentally fertilized with high P fertilizer, and one plant from the high P treatment accidentally received the low P fertilizer, and so these individuals were omitted from the analysis. Because corresponding plants from the same maternal family were required to calculate MGR, these plants were also excluded from the analysis. We evaluated whether MGR was influenced by soil biota source, population source and phosphorus fertilization using a three-way ANOVA. We also evaluated local vs. foreign and home vs. away criteria of local adaptation using MGR with planned orthogonal single degree of freedom contrasts. We expected that MGR of local plants would be higher than that of foreign plants in their local soil biota, and that MGR would be higher in plants grown in home soil biota relative to away soil biota. Statistical analyses were done in SPSS (version 22.0, SPSS Inc., Chicago, IL).

Results

Plants inoculated with whole soil biota were 88% larger than plants that received the microbial wash treatment (Fig. 1, Table 1). Plants were also 296% larger in the high phosphorus relative to low phosphorus treatment. In addition, the effect of soil biota treatment depended on phosphorus level; plants inoculated with whole soil biota were 240% larger than plants inoculated with microbial wash at low phosphorus, but there was no difference between soil biota treatments at high phosphorus. Though average aboveground biomass did not differ between populations, the effect of plant population on aboveground biomass depended on the type of soil biota added and the origin of soil biota (soil biota treatment × soil biota origin × population interaction, Table 1). This significant interaction implies that there was local adaptation to soil biota, but statistically weaker higher-order interactions that also included phosphorus (Table 1) imply that local adaptation was less likely to be

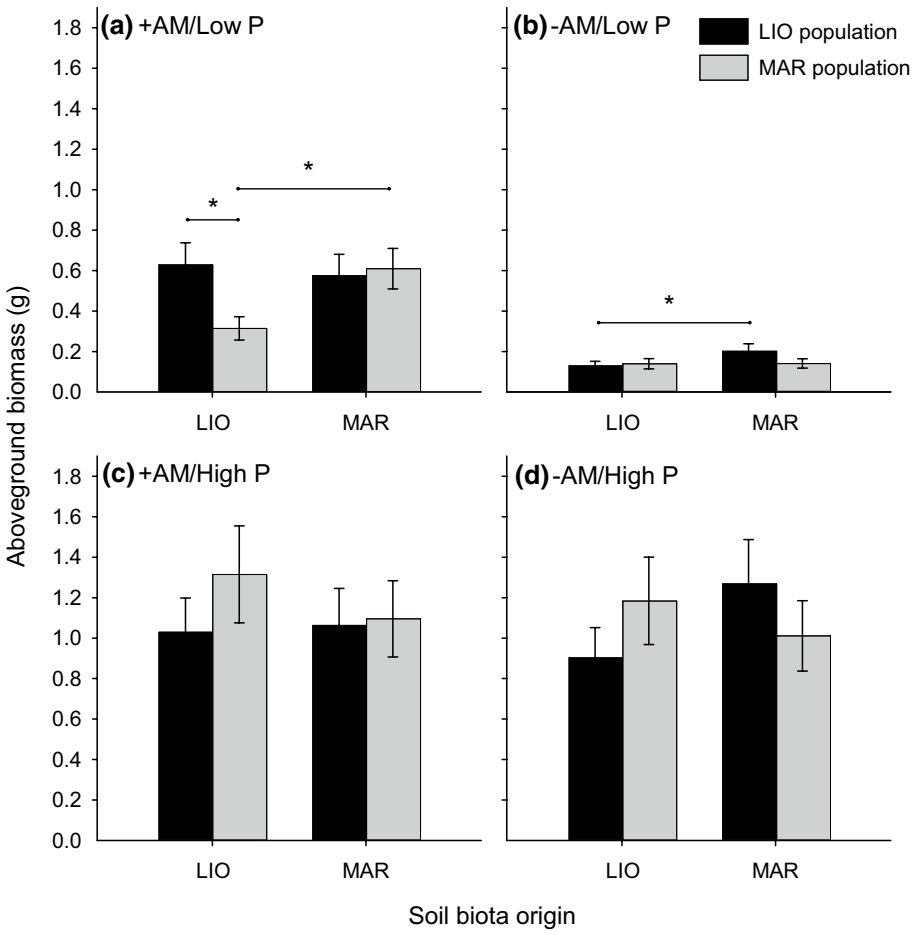

Fig. 1 Aboveground biomass for Ancaster Lions pool (LIO) and Martin Road (MAR) *L. siphilitica* populations grown reciprocally with **a** whole soil biota (+AM) from LIO and MAR soils at low P, **b** microbial wash (−AM) from LIO and MAR soils at low P, **c** whole soil biota from LIO and MAR soils at high P, and **d** microbial wash from LIO and MAR soils with high P. Bars are means (± 1 SEM) and statistically significant contrasts indicated with horizontal lines and asterisks

Table 1 Four-way ANOVA describing the effect of soil biota treatment, phosphorus level, biota origin, and population origin on aboveground biomass

Source	*df*	MS	*F*	*p*
Soil biota treatment	1	17.193	69.833	< 0.001
Phosphorus level	1	81.974	332.951	< 0.001
Soil biota origin	1	0.753	3.059	0.082
Population	1	0.242	0.983	0.323
Soil biota treatment × phosphorus level	1	15.345	62.325	< 0.001
Soil biota treatment × soil biota origin	1	0.031	0.124	0.725
Soil biota treatment × population	1	0.010	0.041	0.841
Phosphorus level × soil biota origin	1	0.671	2.725	0.101
Phosphorus level × population	1	1.035	4.206	0.042
Soil biota origin × population	1	0.107	0.434	0.511
Soil biota treatment × phosphorus level × soil biota origin	1	0.140	0.570	0.451
Soil biota treatment × phosphorus level × population	1	0.231	0.938	0.334
Soil biota treatment × soil biota origin × population	1	1.459	5.924	0.016
Phosphorus level × soil biota origin × population	1	0.718	2.915	0.090
Soil biota treatment × phosphorus level × soil biota origin × population	1	0.551	2.240	0.136
Error	158	0.246		

Degrees of freedom (*df*), mean squares (MS), *F* ratio (*F*) and *p* values are reported

influenced by the level of phosphorus fertilization. However, pre-planned 1-*df* contrasts for specific local vs. foreign and home vs. away predictions confirmed that local adaptation criteria were met only when plants were grown at low phosphorus levels (Table 2). Specifically, within the whole soil biota treatment at low phosphorus, the local LIO population had 100% higher biomass than the foreign MAR population when grown with LIO whole soil biota (Fig. 1a, Table 2). In addition, the home MAR population had 94% greater biomass when grown with home soil biota than when grown with away soil biota (Fig. 1a, Table 2). Thus, the lower growth of the MAR population in LIO soil was responsible for each of the significant local vs. foreign and home vs. away contrasts. We also found that the LIO population had 56% higher biomass when grown in away versus home microbial wash treatment (Fig. 1b, Table 2), but there were no other statistically significant contrasts for microbial wash-treated soils at low phosphorus. In addition, there were no local vs. foreign or home vs. away differences for either the whole soil or microbial wash treatments at high phosphorus (Fig. 1c, d).

Mycorrhizal growth response (MGR) was strongly influenced by phosphorus fertilization; MGR was 35 times higher in the low versus high phosphorus treatment (Table 3, Fig. 2). There were no other statistically significant main effects on MGR, but predictions of local adaptation were partially supported for MGR. For example, when pooled across phosphorus treatments, plant populations grown in their local soil biotic communities had 82% greater MGR than foreign populations (significant soil biota

Table 2 Planned orthogonal single degree of freedom contrasts testing for local adaptation for aboveground biomass using (1) local vs. foreign population and (2) home vs. away criteria in each phosphorus treatment

Soil biota treatment and phosphorus level	Local vs. foreign origin contrast	*F*	*p*	Home vs. away soil environment contrast	*F*	*p*
Whole soil biota at low P	LIO vs. MAR in LIO soil biota	10.225	0.002	LIO home vs. away	0.168	0.683
	MAR vs. LIO in MAR soil biota	0.074	0.786	MAR home vs. away	9.719	0.002
Whole soil biota at high P	LIO vs. MAR in LIO soil biota	1.335	0.250	LIO home vs. away	0.023	0.879
	MAR vs. LIO in MAR soil biota	0.020	0.887	MAR home vs. away	0.719	0.398
Microbial wash at low P	LIO vs. MAR in LIO microbial wash	0.123	0.726	LIO home vs. away	4.183	0.042
	MAR vs. LIO in MAR microbial wash	2.849	0.093	MAR home vs. away	0.002	0.967
Microbial wash at high P	LIO vs. MAR in LIO microbial wash	1.629	0.204	LIO home vs. away	2.696	0.103
	MAR vs. LIO in MAR microbial wash	1.152	0.285	MAR home vs. away	0.533	0.467

F ratio (*F*) and *p* values are reported. Populations are Ancaster Lions pool (LIO) and Martin Road (MAR)

Table 3 Three-way ANOVA describing the effect phosphorus level, soil biota origin, and population origin on mycorrhizal growth response (MGR)

Source	*df*	MS	*F*	*p*
Phosphorus level	1	30.689	59.237	<0.001
Soil biota origin	1	0.061	0.118	0.732
Population	1	0.020	0.039	0.845
Phosphorus level × population	1	0.462	0.891	0.348
Phosphorus level × soil biota origin	1	0.281	0.542	0.464
Soil biota origin × population	1	2.917	5.631	0.020
Phosphorus level × soil biota origin × population	1	1.103	2.129	0.149
Error	79	0.518		

Degrees of freedom (*df*), mean squares (MS), *F* ratio (*F*) and *p* values are reported. Populations are Ancaster Lions pool (LIO) and Martin Road (MAR)

origin × population term, Table 3). At low phosphorus, the LIO population had 95% greater MGR than the MAR population when grown with LIO soil biota ($p = 0.017$; Table 4, Fig. 2a). Though the MAR population had 40% higher MGR than the LIO population when grown in MAR soil biota, this difference was not statistically significant (Table 4). Home vs. away predictions of local adaptation for MGR were also partially supported. The LIO population had 51% higher MGR in its home vs. away soil biota, but this difference was not statistically significant (Table 4). By contrast, the MAR population had 80% higher MGR in its home vs. away soil biota, and this effect was statistically significant ($p = 0.037$, Table 4, Fig. 2a). There were no differences between local vs. foreign or home vs. away MGR values at high phosphorus (Fig. 2b).

Tissue phosphorus content (%P) was strongly influenced by inoculation with soil biota and by P fertilization (Online Resource Table s1, Fig. 3). Plants had 50% more tissue %P in the whole soil biota treatment compared to the microbial wash and had 70% more tissue %P when fertilized with high versus low phosphorus. The effect of soil biota type on tissue %P was stronger at low than at high phosphorus. Specifically, plants had 121% higher %P in the whole soil relative to the microbial wash inoculum at low phosphorus, whereas there was only a 21% difference between whole soil and microbial wash treatments at high phosphorus. Plant population responses to soil biota type varied with phosphorus fertilization (Online Resource Table s1). For example, at low phosphorus, the LIO and MAR populations had 145% and 97% higher tissue %P in the whole soil treatment than in the microbial wash treatment (Fig. 3a). By contrast, at high phosphorus, soil biota type had no effect on %P in the LIO population, but whole soil increased %P by 43% in the MAR population (Fig. 3b). Tissue %P was generally similar

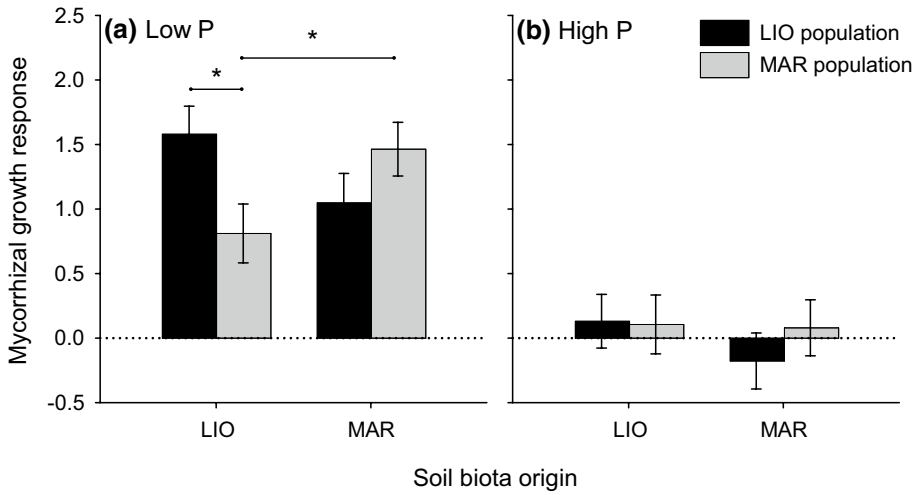

Fig. 2 Mycorrhizal growth response (MGR) for Ancaster Lions pool (LIO) and Martin Road (MAR) *L. siphilitica* populations grown reciprocally in **a** whole soil biota from LIO and MAR soils at low P, and **b** microbial wash from LIO and MAR soils at high P. Bars are means (± 1 SEM) and statistically significant contrasts indicated with horizontal lines and asterisks

Table 4 Planned orthogonal single degree of freedom contrasts for mycorrhizal growth response (MGR) using (1) local vs. foreign population and (2) home vs. away criteria in each phosphorus treatment

Phosphorus treatment	Local vs. foreign origin contrast	*F*	*p*	Home vs. away soil environment contrast	*F*	*p*
Low P	LIO vs. MAR in LIO soil	5.985	0.017	LIO home vs. away	2.864	0.095
	MAR vs. LIO in MAR soil	1.826	0.180	MAR home vs. away	4.498	0.037
High P	LIO vs. MAR in LIO soil	0.007	0.934	LIO home vs. away	1.054	0.308
	MAR vs. LIO in MAR soil	0.702	0.405	MAR home vs. away	0.007	0.935

F ratio (*F*) and *p* values are reported. Populations are Ancaster Lions pool (LIO) and Martin Road (MAR)

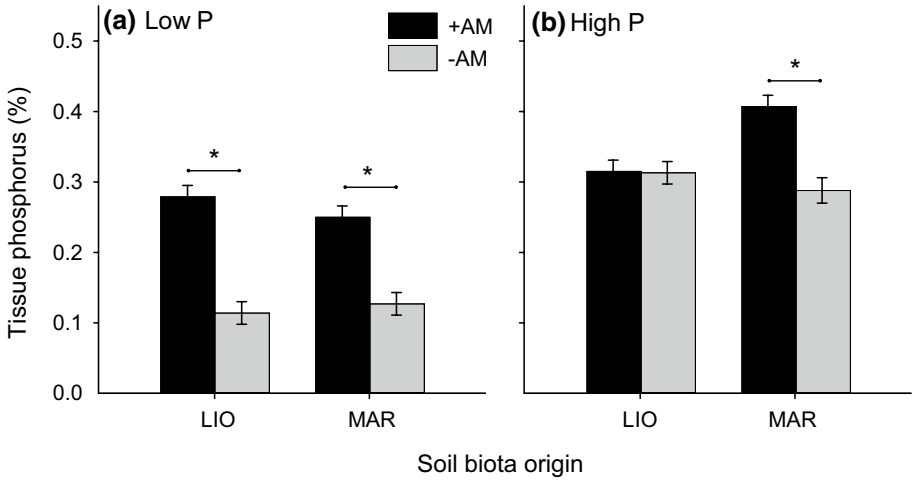

Fig. 3 Tissue phosphorus content (%P) for Ancaster Lions pool (LIO) and Martin Road (MAR) *L. siphilitica* populations grown with whole soil biota (+AM) or with microbial wash (−AM) at **a** low P fertilization and at **b** high P fertilization. Bars are means (±1 SEM) and statistically significant contrasts indicated with horizontal lines and asterisks

between populations, regardless of soil biota origin (Online Resource Fig. s1).

Root colonization by arbuscules did not differ among phosphorus, population origin or soil biota origin treatments or any of their interactions (Fig. 4, Online Resource Table s2). No colonization by fungal structures was observed on roots in the microbial wash treatment.

Discussion

Our results supported the hypothesis that plant populations are locally adapted to the AM fungi, but not other soil microbes found in their home soils. There was evidence for local adaptation using both local vs. foreign and home vs. away criteria in *L. siphilitica* populations when they were grown with AM fungi under conditions where phosphorus was limited (Figs. 1a, 2a). By contrast, we found no evidence for local adaptation to other aspects of soil biota that were contained in the microbial wash treatment (Fig. 1b), or when plants were grown at high phosphorus levels (Figs. 1c, d, 2b). Because performance differences between *L. siphilitica* populations in the directions predicted by local adaptation criteria were only apparent in treatments that contained AM fungi and where phosphorus was limited, our results suggest that local adaptation to AM fungi occurs to facilitate enhanced phosphorus acquisition. If AM fungi were providing other services that enhanced plant performance, such as pathogen protection, local adaptation should also have been observed at high phosphorus, but this was not the case.

Measurements of tissue %P content also supported the hypothesis that plants adapt to local communities of AM fungi to acquire soil phosphorus. For example, under the low phosphorus treatment, plants grown with whole soil inoculum (which contained AM fungi) had significantly higher tissue %P than plants that were inoculated with AM-free microbial wash (Fig. 3a), suggesting that the presence of AM fungi increased plant acquisition of phosphorus. We note, however, that plant populations inoculated with different sources of soil biota did not differ in tissue %P (Online Resource Fig. s1), which might imply that local AM fungal communities did not enhance phosphorus acquisition

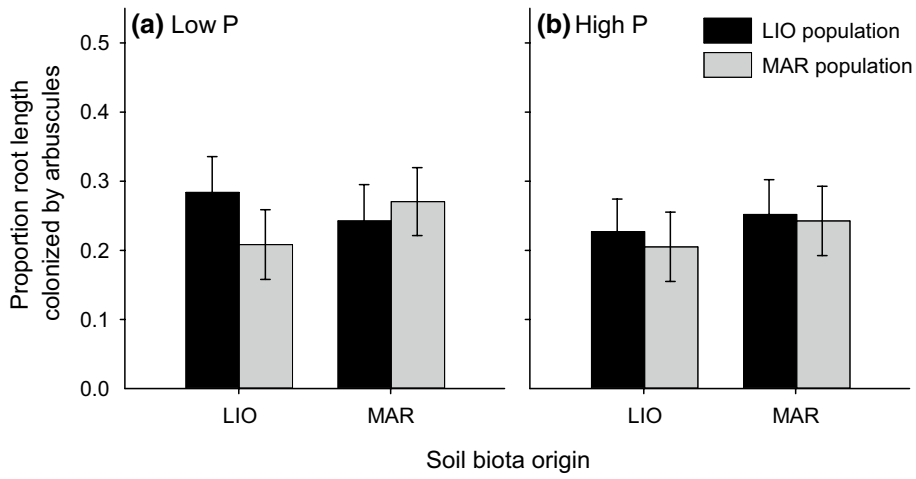

Fig. 4 Proportion of root length colonized by arbuscules for Ancaster Lions pool (LIO) and Martin Road (MAR) *L. siphilitica* populations grown reciprocally in **a** whole soil biota from LIO and MAR soil at low P and **b** whole soil biota from LIO and MAR soil at high P. Bars are means (±1 SEM)

relative to foreign AM fungal communities. Nevertheless, the similarity of %P in inoculated plants still means that larger plants acquired more phosphorus per plant. Thus, a locally adapted *L. siphilitica* population, owing to its larger mass, would still have had higher total phosphorus than the foreign population when grown with local AM fungi.

Previous studies of local adaptation to soil biota have observed that populations from phosphorus rich soils are less likely to display benefits from inoculation by AM fungi (Schultz et al. 2001; Johnson et al. 2010), but this did not occur in our study. Specifically, the extractable phosphorus from soil in the LIO *L. siphilitica* population was ~ 8 times greater than in soil from the MAR site (see Methods), yet both the LIO and MAR plant populations were similarly responsive to their local AM fungi, as measured by mycorrhizal growth response (MGR) (Fig. 2a). One explanation for the evolution of high MGR of the LIO population is that despite the high soil phosphorus availability of its local soil, nitrate levels were relatively low (~ 5 mg NO_3^- per kg dry soil), which could have prevented exploitation of excess phosphorus. If plant growth was limited primarily by nitrogen in the LIO *L. siphilitica* population, natural selection to reduce dependence on AM fungi for phosphorus acquisition may not have occurred. Instead, our findings imply that natural selection could have favored greater dependence on AM fungi for phosphorus acquisition in both populations. Our findings could also be the result of the experimental design, where all plants were fertilized with excess nitrogen, which would have increased phosphorus demand and the likelihood of detecting greater plant growth dependence on mycorrhizal fungi (Hoeksema et al. 2010; Johnson 2010).

Evolutionary theory predicts that once a population has adapted to a particular environment, it will incur a fitness cost when paired with a novel environment, resulting in a trade-off in performance between the home and away environment (Futuyma and Moreno 1988; Hereford 2009). Thus, in situations where there are no differences in abiotic resource availability between growth environments, as was the case for our reciprocal transplant experiment, both the local vs. foreign criterion and the home vs. away criterion for local adaptation should be met (Kawecki and Ebert 2004). Nevertheless, this pattern did not occur in the present study. Specifically, the LIO population outperformed the MAR population when grown in LIO soil biota, but did not have reduced growth when grown in MAR soil biota (Fig. 1a). These findings imply that adaptation of the LIO population to its local AM fungal environment occurred without a fitness cost, which is not unprecedented in the literature. For example, a meta-analysis of local adaptation indicated that even though the local vs. foreign criterion for local adaptation was supported in 71% of cases, home vs. away fitness trade-offs were observed in only 48% of cases (Hereford 2009). The presence of a fitness trade-off for the MAR population without also outperforming the LIO population in MAR soil biota is more difficult to explain. One possibility is that the MAR population has not yet fully adapted to its local environment and may outperform foreign populations in its local soil biota in future generations.

Even though there was evidence for local adaptation to AM fungi in *L. siphilitica*, differences in plant performance across AM inoculum sources were not reflected in the degree of root colonization by AM fungi. Specifically, the percent of root length colonized by arbuscules, where nutrient and carbon exchange occurs, was similar among populations (Fig. 4). Though this finding differs from previous studies showing that AM fungal root colonization is correlated with plant performance in reciprocal transplant experiments (Schultz et al. 2001; Johnson et al. 2010), it is consistent with other studies showing that plant performance is not necessarily correlated with fungal colonization of roots (Ravnskov and Jakobsen 1995; Smith et al. 2004). Therefore, an alternate explanation for our results is that locally adapted plants obtain more phosphorus via AM fungi through enhanced transport rate, rather than through a higher number of arbuscules (Smith et al. 2004). We also observed that the degree of arbuscular root colonization did not differ between P treatments, which contradicts previous work indicating that P fertilization and AM fungal colonization of roots are inversely correlated (Kahiluoto et al. 2001). However, as obligate symbionts, AM fungi may not necessarily reduce root colonization at elevated phosphorus because this could have also reduced carbon transfer from the plant. Such a response may be more likely in situations where plant photosynthesis is not carbon limited, as was observed in a recent study showing that fungal colonization of roots is insensitive to fertilization if plants are grown in high light conditions (Schreiner and Scagel 2016). Because *L. siphilitica* plants were grown in high light conditions in our experiment, the absence of carbon limitation could be responsible for a lack of difference in the level of arbuscular colonization between low and high phosphorus treatments.

Our results show that local adaptation to AM fungi can occur at smaller spatial scales than previously reported. The two *L. siphilitica* populations were 1.4 km apart, a much shorter distance than has typically been used in previous studies of local adaptation to AM fungi, where populations could be several hundred kilometers apart (Schultz et al. 2001; Johnson et al. 2010; Pánková et al. 2014a). Though we did not characterize AM fungal genotypic or community composition between study sites, differences in natural selection on *L. siphilitica* populations may be caused by divergence in either of these characteristics. Differences in both fungal genotypic variation and community composition are known to occur at small spatial scales. For example, AM fungal genotype composition can differ at the scale of meters within a hectare sized field (Koch et al. 2004), and

AM fungal community composition can differ at distances as short as 30 m (Davison et al. 2012). If functionally important differences in AM fungal genetic structure and community composition occur at such fine spatial scales, local adaptation of plant populations to AM fungi may be common in geographically close plant populations.

Our results also provide insight into the magnitude of AM fungal mediated natural selection on plant populations. Adaptive differentiation between populations depends on the balance of homogenizing gene flow and divergent local selection (Slatkin 1987; Garant et al. 2007). *L. siphilitica* flowers are bumble bee (*Bombus* spp.) pollinated (Johnston 1991) and these pollinators typically travel ~ 1 km and as far as 2.5 km while foraging (Hagen et al. 2011). We did not assess levels of gene flow between our populations, but the foraging behavior of bees and the geographical proximity of our sites suggest gene flow was more likely to have occurred between our populations than between those in previous studies of local adaptation to AM fungi (Schultz et al. 2001; Johnson et al. 2010; Pánková et al. 2014b). Observing evidence for local adaptation to AM fungi despite the high likelihood of gene flow implies that natural selection by AM fungi on *L. siphilitica* populations is strong.

In conclusion, we show that plant populations can adapt to AM fungi on relatively fine spatial scales, and that adaptation to AM fungi occurs to facilitate increased acquisition of soil phosphorus, rather than other potential mycorrhizal functions. This result has implications for quantifying the magnitude of plant growth responses to AM fungi, as well as the ecological consequences of such variation. Though the biomass of both *L. siphilitica* populations increased when colonized by AM fungi in whole soil versus microbial wash treatments, average MGR was consistently higher for local vs. foreign plant populations (Fig. 2). This pattern suggests that the magnitude of MGR could be underestimated when non-local isolates of AM fungi are used to quantify it (e.g., Anacker et al. 2014). If a more positive MGR increases the likelihood that a plant population persists in a given community (Umbanhowar and McCann 2005; Lin et al. 2015; Stanescu and Maherali 2017), then the positive effect of local adaptation on MGR has implications for conservation management. For example, it is often recommended that land managers use locally adapted genotypes in ecological restoration because provenance trials show that plant populations are often adapted to abiotic factors such as climate (Bucharova et al. 2018). Our results suggest that local adaptation to soil biota within a climatic zone should also be considered when sourcing seeds for restoration efforts (e.g., Bucharova 2017). The number of studies that have tested for local adaptation to AM fungal communities is still relatively small (Rúa et al. 2016), particularly at small spatial scales within abiotic climate zones (Raabová et al. 2011). Additional studies which test for local adaptation to soil biota, in general, and AM fungi, in particular, are required to determine whether the effects we observed are common, as well as to determine the relative importance of soil biota as cause of adaptation relative to other causes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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