The effect of arbuscular mycorrhizal fungi and soil phosphorus level on selection for shoot phosphorus content and photosynthetic rate in *Lobelia siphilitica*.

by

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ABSTRACT

THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL PHOSPHORUS LEVEL ON SELECTION FOR SHOOT PHOSPHORUS CONTENT AND PHOTOSYNTHETIC RATE IN *LOBELIA SIPHILITICA*.

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Adaptation occurs by natural selection favoring traits that maximize fitness in a given environment. Interactions between species may influence selection, and the influence may depend on environmental conditions. The interaction between arbuscular mycorrhizal (AM) fungi and plants is likely to influence selection on resource-related traits as it involves fungi providing nutrients for the plant in exchange for sugars from photosynthesis. AM fungi are most beneficial to plants in low nutrient soils and so their influence on selection may be strongest in this context. I tested for the effect of inoculation with AM fungi and soil phosphorus level on selection for shoot phosphorus and photosynthesis by growing *Lobelia siphilitica* with and without AM fungi in low and high phosphorus soils. The results indicate that AM fungi increase plant fitness most in poor nutrient conditions, and that despite important trait responses the effect of the symbiosis on trait selection is minimal.

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INTRODUCTION

Species interactions can influence the evolution of traits in the interacting partners (Paterson et al. 2010, Thompson 1999). Different types of species interactions influence selection in different ways (Geber and Griffen 2003, Kingsolver et al. 2001) and are likely to influence selection on traits that are closely related to the interaction (Hoeksema 2010). For instance, plant traits like flower colour (Stanton et al. 1986) and flower size (Thompson 2001) attract more pollinators, and so can be selected in pollinator-plant mutualisms (Stanton et al. 1986, Vanhoenacker et al. 2010, Bartkowska and Johnston 2012). Similarly, damage to plant tissue by herbivores can drive selection for traits involved in damage recovery, like early flowering time and increased branch production (Juenger and Bergelson 2000), and in herbivore avoidance, like chemical and mechanical defense mechanisms (Mauricio 1998, Mauricio and Rausher 1997, respectively). These examples illustrate the influence of both positive and negative species interactions on the evolution and diversification of plant form and function.

The symbiosis between arbuscular-mycorrhizal (AM) fungi and plants is also likely to have influenced selection on plant traits. This symbiosis is one of resource trading, where the fungal partner attaches to the roots of a host plant and increases acquisition of water and limiting nutrients like phosphorus from the soil for the plant in exchange for sugar from the plant (Smith and Smith 2011, Allen et al. 2003, Smith and Read 2008). AM fungi have been involved in plant resource acquisition since plants transitioned to terrestrial environments (Brundrett 2002) and they have been coevolving for over 400 million years (Brundrett 2008). The interaction occurs in 74 percent of land plants (Brundrett 2009) and is the most common adaptation to acquiring nutrients in low nutrient soils (Vance et al. 2003, Smith and Read 2008). The well-established

relationship between AM fungi and plants makes AM fungi likely to have influenced selection on plant traits, and AM fungi is likely to influence selection on plant functions associated with resource acquisition. However, there is no evidence demonstrating AM fungi affect selection for host plant traits, as the symbiosis is understudied relative to other biotic interactions (Hoeksema 2010).

AM fungi could influence the evolution of plant function through natural selection on mechanisms of phosphorus acquisition. Phosphorus is often limiting to plants and is a key component of many macromolecules and cellular-level processes (reviewed in Schachtman et al. 1998). Plants have adapted to low phosphorus soil through the evolution of root traits (Bates and Lynch 2000, Lynch and Brown 2001, Keerthisinghe et al. 1998), including root fineness (Wissuwa 2003), root growth, and root hairs (Rao et al. 1999), all of which increase phosphorus acquisition by increasing root surface area to volume ratio. Chronic phosphorus limitation may also strengthen selection on traits that facilitate phosphorus acquisition, as has been demonstrated for other limiting nutrients, such as nitrogen (Chapin 1991; Donovan et al. 2009). Because AM fungi provide phosphorus to host plants as part of the resource exchange (Smith and Read 2008, Lambers et al. 2008), natural selection may favour a stronger association with fungi over plant traits that increase phosphorus acquisition in low phosphorus soils. The hypothesis that selection may favour association with a fungal partner in low phosphorus soils is supported by the observation that root colonization by AM fungi is typically highest in low phosphorus soils (Mortimer et al. 2008, Black et al. 2000, Fay et al. 1996) and lowest in high phosphorus soils (Paradi et al. 2003), suggesting that low phosphorus soil may strengthen selection for increased colonization by nutrient-providing fungi. While it is difficult to differentiate between phosphorus taken up by AM fungi versus roots, shoot phosphorus levels

are indicative of how much phosphorus is acquired by the plant overall (Donovan et al. 2009, Caron et al. 1986, Cui and Caldwell 1996) and can be used as a proxy of phosphorus acquisition. Despite the potential for shoot phosphorus, and thus phosphorus acquisition, to be under selection, and for AM fungi to influence this selection, the effect of fungal presence and absence on selection for phosphorus uptake has not been tested.

AM fungi need sugars for their own metabolism, and so may also influence selection on photosynthetic capacity of their host plants. Since sugars are required for plant growth, metabolism, and reproduction (Lambers et al. 2008), natural selection is expected to favor high rates of photosynthesis (Arntz et al. 2000 a,b) in the absence of fungi. The increase in photosynthetic rate that occurs when plants associate with AM fungi (Koch et al. 1997, Koide and Schreiner 1994, Huat et al. 2002) may be the result of AM fungi increasing carbon demand in the plant, since AM fungi are heterotrophic and take between 4 and 20 percent of the plant's photosynthate (Lambers et al. 2008, Smith and Read 2008, Kaschuk et al. 2009). Since the plant can allocate this carbon to the fungi at minimal cost (Wright et al. 1998 a,b) and since AM fungi provide nutrient benefits to the plant, natural selection may favour higher rates of photosynthesis when associating with a fungal partner. In other words, plants with higher photosynthesis may have higher fitness because the cost of allocating sugar to the fungal partner is outweighed by the nutrient benefits provided by the fungi. However, higher photosynthetic rate would only increase plant fitness when carbon is allocated to a beneficial fungal partner in low nutrient soils, where fungi increase plant fitness relative to non-mycorrhizal plants (Paradi et al. 2003, Freeden and Terry 1988, Fitter 1991). By contrast, AM fungi do not increase plant fitness in nutrient rich soils (Paradi et al. 2003, Freeden and Terry 1998, Fitter 1991), and there may be weaker selection for high photosynthetic rate to avoid losing carbon to a fungal partner that does not

provide any fitness benefits. The potential for AM fungi to influence selection on photosynthesis in different nutrient levels has not been examined.

In this study, I tested how shoot phosphorus content and photosynthesis traits responded to inoculation with AM fungi at different levels of soil phosphorus. Furthermore, I tested whether the direction and strength of selection on shoot phosphorus content and photosynthesis traits differed among AM fungi and soil phosphorus treatments. I manipulated the presence of AM fungi and soil phosphorus to isolate their effect on the response of each trait and their effect on selection for each trait (Conner and Hartl 2006). I measured the response of each trait to inoculation with AM fungi and soil phosphorus level by comparing mean trait values among plants inoculated with AM fungi and non-inoculated control plants growing in low and high phosphorus soils. I measured selection as phenotypic selection, or the covariance between variation in a trait and variation in fitness (Lande and Arnold 1983), and compared the strength of selection among treatment groups. Isolating the effects of inoculation with AM fungi and soil phosphorus level provides a more accurate understanding of how plant traits and selection on plant traits respond to specific biotic and abiotic components of the environment.

To determine whether there was a response of each trait and fitness measurement to inoculation with AM fungi and soil phosphorus level, I compared mean trait and fitness values among plants inoculated with AM fungi and non-inoculated control plants in high and low phosphorus soils. If AM fungi provide more nutrients to their host plant in low phosphorus soil, plants growing in low phosphorus soil will be more highly colonized by AM fungi than plants growing in high phosphorus soil. If AM fungi increase nutrient availability for their host plant in low phosphorus soil, inoculated plants will have higher fitness, shoot phosphorus content, and photosynthetic rate in low phosphorus soil compared to non-inoculated control plants in low

phosphorus soil. If AM fungi do not increase nutrient uptake relative to non-inoculated control plants in high phosphorus soils, but still take sugar from the host plant, inoculated plants will have lower fitness and photosynthetic rate in high phosphorus soils compared to non-inoculated control plants.

To determine whether there was selection on each trait I estimated the covariance between trait variation and fitness, and to determine whether selection was influenced by inoculation with AM fungi and soil phosphorus level, I compared covariance for each trait among inoculated and control plants in high and low phosphorus soils. To determine whether AM fungi influence selection on shoot phosphorus content I first determined whether shoot phosphorus content is under selection in the absence of AM fungi, where a trait is under selection if it has a nonzero covariance with fitness. If natural selection for higher shoot phosphorus is stronger in low versus high phosphorus environments, the covariance between shoot phosphorus and fitness will be stronger in low relative to high phosphorus soils in the absence of AM fungi. To determine whether AM fungi influence selection on shoot phosphorus I compared selection between inoculated and non-inoculated control treatments. If AM fungi have the same effect as high soil phosphorus level on selection for shoot phosphorus, the strength of selection for shoot phosphorus will be weaker in non-inoculated control, high soil phosphorus treatments and inoculated, low soil phosphorus treatments relative to selection in the non-inoculated control, low soil phosphorus treatment.

To determine whether AM fungi influence selection for high photosynthetic rate, and whether this influence depends on soil phosphorus level, I compared selection for high photosynthetic rate among plants inoculated with AM fungi and non-inoculated control plants in high and low soil phosphorus treatments. If natural selection generally favors plants with higher

photosynthetic rate to feed fungal partners, selection for high photosynthetic rate will be stronger in inoculated relative to control treatments in both low and high phosphorus soils. If AM fungi only provide benefits in low nutrient soils then in low phosphorus soil, selection for high photosynthetic rate will be stronger in inoculated compared to non-inoculated control plants, but in high phosphorus soils selection for high photosynthetic rate will not differ between inoculated and non-inoculated treatments or will be stronger in non-inoculated control plants compared to inoculated plants.

METHODS

To examine selection on plant traits by AM fungi I used *Lobelia siphilitica* and *Glomus intraradices* as my host plant and AM fungi species, respectively. *Lobelia siphilitica* is an perennial wildflower whose range extends across northeastern North America (Johnston 1991). It occurs naturally in a range of soil conditions and can grow both with AM fungi (Hovatter et al. 2011) and without (Caruso et al. 2006, Caruso et al. 2010, Johnston 1992) making it ideal for studying the effects of differing nutrient levels and inoculation. *Glomus intraradices* is a generalist species of AM fungi that can colonize a variety of host plant species (Dixon et al. 1994, Aguilera-Gomez et al. 1999, Berg et al. 2001, Davies et al. 2001, Caravaca et al. 2003, Hajiboland et al. 2010) in a variety of habitats (summarized in Borstler et al. 2008) around the globe (Opik et al. 2006). This generalist nature combined with its frequent occurrence in Ontario soil (Guelph Arboretum, Maherali and Klironomos 2012) makes *G. intraradices* likely to colonize *L. siphilitica*. Use of *G. intraradices* increases the likelihood of detecting an influence of AM fungi on trait responses and selection, since *L. siphilitica* is less likely to have adapted to this commercial inoculum than to its local soil biota. *Glomus intraradices* specifically is known to increase the uptake of phosphorus for its host plant (Viereck et al. 2004), so it is likely to affect nutrient acquisition. Finally, *G. intraradices* can colonize host plants in green house conditions (Sykovora et al. 2007), colonizes plants quickly (Martin et al. 2008), and is frequently used in commercial inoculum (Corkidi et al. 2004), all of which facilitated its use in this study. I used commercially obtained *G. intraradices* spores (Myke Pro Greenhouse G and WP) because they are known to colonize *L. siphilitica* in green house conditions (S. Hensen, unpublished data).

To examine the effect of AM fungi and soil phosphorus level on selection in *L. siphilitica,* I

manipulated the presence of these two factors in a fully factorial design. The experiment consisted of 80 replicates, each replicate consisting of one plant in each of four treatment groups, for a total of 320 plants. Each replicate represented a maternal family, which consisted of seeds collected from the same plant. Using maternal families as replicates eliminated any effect of variation in genetic structure between treatments.

To generate the experimental plant population I collected and planted seeds from a *L. siphilitica* population near Martin Road, Ancaster, Ontario (43.226" N, 80.007" W; Caruso et al. 2015). This Ancaster population had the most mature fruits available for seed collection of all the Ontario populations sampled for seed in October, 2013. Additionally, the plants from this site grow naturally in low phosphorus soil (3 mg P/kg soil, Table 1) which increases the likelihood that they benefit from associating with AM fungi (Johnson 2010). I collected 3-5 mature fruits from each encountered plant to ensure enough seed for experimental replicates, dried collected fruits in silica, and broke seed dormancy using a cold-stratify method (Johnston 1992). I stratified ~100 seeds from each of 106 maternal families to increase the likelihood of 80 final replicates germinating successfully.

To isolate the effects of AM fungi and soil phosphorus level on selection for plant traits, all seeds were planted in identical potting medium and pots. To provide plants with natural soil elements while facilitating root extraction, potting medium was a 30/70 mixture (v/v) of field soil and sand (Hutcheson Dry Topdressing sand, Huntsville Ontario). Field soil was collected from a formerly cultivated (late 1960s) old field in the Guelph Arboretum because it is low in phosphorus (Table 1), facilitating the creation of two phosphorus levels; and because *G. intraradices* occurs naturally in Arboretum soil (Maherali and Klironomos 2012), increasing the likelihood of commercial *G. intraradices* spores establishing in this potting medium. To remove

any soil biota (Klironomos et al. 2004) or contaminants that may affect plant or fungal growth, potting medium was autoclaved (121°C for 90 minutes) then washed of autoclave contaminants in sterilized tubs (L3-2214-C4-BMIST, 61x40.6x22.2 cm, Rubbermaid Canada). One liter of potting medium was placed in each of 320, 1.67 L pots (DLNSTD06000B66C420, Dillen Products Inc., Middlefield OH). To prevent contamination that may affect plant or fungal growth, pots were sterilized prior to potting medium addition. To prevent leaks of potting medium, pots were lined with sterilized mesh (New York Wire Clear Advantage, Saint-Gobain ADFORS America Inc.).

To establish the AM fungi inoculation treatment, I inoculated pots with active *G. intraradices* spores in two forms of inocula. First, I inoculated pots in the inoculated treatment with 390 spores of *G. intraradices* carried on 10-20 mesh perlite (Myke Pro Greenhouse G, 390 spores = 100 mL =26 g at 15 spores/g) to provide enough spores for successful colonization (Bildusas et al. 1986, S. Hensen, unpublished data) without over-supplementing the volume of the potting medium. To ensure that roots came in contact with the spores as early as possible this inoculum was mixed with an additional 100 mL of soil/sand mixture and added as the top 200 mL layer of potting medium. Second, I inoculated pots in the inoculated treatment with 800 spores of concentrated *G. intraradices* inoculum (Myke Pro Greenhouse WP, 800 spores = 0.5 mL = 1 g) sprinkled on top of the first inoculum layer. To establish the non-inoculated control treatment I used autoclaved perlite (121°C for 90 minutes), sieved to the same size as the inoculum particles, in place of active inoculum. Since active inoculum consists primarily of perlite by volume, autoclaved perlite eliminated any effect of adding inoculum volume to the pots while avoiding contamination by active fungal spores. Non-inoculated control pots were not inoculated with inactive WP inoculum because the volume added to the inoculated treatment

pots was negligible. To minimize disturbance to inocula, all pots were covered with a final, thin layer (~50 mL) of soil/sand mixture before seeds were planted. To decrease the chances of spores inactivating over time, inoculum was mixed into potting medium immediately prior to planting (9, 4, and 2 days before planting for perlite, G inoculum, and WP inoculum, respectively).

To establish the soil phosphorus treatment I provided pots with mono potassium phosphate (MKP, Rotem Amfert Negev LTD) dissolved in 100mL of water. The soil phosphorus level was 2 mg P/kg dry soil for the low phosphorus treatment and 26 mg P/kg dry soil for the high phosphorus treatment. These values were based on the range of soil phosphorus levels in which Ontario *L. siphilitica* populations naturally occur (Table 1) and on the amount of phosphorus already in the potting medium (Table 2). Applications occurred once prior to planting, and again approximately 3 and 7 weeks after germination to avoid over-fertilizing the low phosphorus plants (S. Hensen, unpublished data).

To eliminate variation in nutrient levels other than phosphorus across the experiment, and to provide plants with an array of nutrients, pots were provided with phosphorus free, full spectrum fertilizer (14-0-14, Balance Plant Products Ltd. Co. 2012) dissolved in 100 mL of water. Fertilizer application was calibrated to nitrate, which is essential for plant growth (Lambers et al. 2008). To match the maximum naturally occurring ratio of N:P in plant tissues (N:P = 16:1, Knecht and Goransson 2004), nitrate fertilizer was added as 16 times the amount of phosphorus in the low phosphorus treatment, resulting in an application of 32 mg N/kg dry soil for all treatments. Applications occurred three times, 48 hours away from each MPK application to ensure uptake of the provided nutrients.

To establish the experiment, I planted and grew seeds in identical conditions. I planted 20

seeds per pot to account for the 30% minimum germination rate in this potting medium (S. Hensen, unpublished data). Once planted, pots were positioned randomly on three green house benches. Seedlings were thinned to one plant/pot by five weeks after germination. To maintain identical and adequate moisture levels throughout the growth season, all pots were watered equally using the same misting schedule and automated irrigation system (Zwart Systems, Beamsville, Ontario). Irrigation was adjusted throughout germination until soil remained moist all day without water pooling in the pots (6 sessions each day for 20 seconds, drip rate = 0.56 mL/second/pot). A 14-hour photoperiod was maintained throughout the experiment using a combination of natural sunlight and artificial light. Artificial lights came on in the early morning, late evening, or on cloudy days to maintain a minimum light intensity of 300 umol/m^2s. Greenhouse temperatures were set to 24°C during the day and 20°C at night. Plants were harvested in random order at the end of the growth season, between September 29th and October 3rd 2014. Plant shoots were dried (60°C for 72 hours) for subsequent trait and fitness analyses. Plant roots were washed of potting medium and stored in 50/50 (v/v) ethanol and DI-water solution for subsequent colonization analyses.

To examine shoot phosphorus content I measured the ratio of total shoot nitrogen to total shoot phosphorus in a randomly selected subset of 60 plants from each treatment combination. Shoot nutrient levels are indicative of uptake by the plant (Donovan et al. 2009, Caron et al. 1986, Cui and Caldwell 1996) even in the presence of AM fungi (Jia et al. 2004, Mortimer et al. 2008, Mensah et al. 2015). I used the ratio of shoot nitrogen to phosphorus in all analyses because AM fungi should increase phosphorus acquisition relative to nitrogen acquisition (Jia et al. 2004) and because shoot N:P can be compared across treatment groups and across studies (Gusewell 2004). I analyzed whole shoots to eliminate any effect of nutrient reallocation to

different structures within each plant. Shoot nutrient content was analyzed on dry tissue from plant harvest using high temperature dry oxidation and hydrochloric acid analysis (Agriculture and Food Laboratory at University of Guelph, following Hanson et al. 1998).

To examine photosynthesis I measured the rate of the light reactions of photosynthesis and the relative level of chlorophyll in leaves. To measure the light reactions of photosynthesis I used a portable chlorophyll fluorometer (PAM-2500, Heinz Walz GmbH, Germany) and recorded the average yield of photosystem two (Y(II)) (Maxwell and Johnson 2000) on all plants in each treatment combination. Y(II) for each plant was recorded as the average Y(II) of the three youngest, fully-expanded leaves. To avoid fluctuations in photosynthetic rate, Y(II) was measured in random order and all plants were measured within 5 days. Measurements occurred between 8 am and 12 pm each day (following Caruso et al. 2005 and Caruso et al. 2006) to minimize variation in photosynthetic rate that occurs throughout the day (Lambers et al. 2008). Reported Y(II) values represent Y(II) averaged over three measurement dates to minimize the effect of life cycle position on photosynthetic traits. To further infer the amount of photosynthetic machinery available in each plant, and to further infer levels of nitrogen, I measured the relative level of chlorophyll (Chl) in three randomly selected leaves on each plant using a SPAD (Minolta SPAD-502, ©1989 Minolta Co., Ltd.). Reported Chl values represent the relative chlorophyll index averaged over three mid-season measurement dates.

To estimate plant individual fitness I measured dry shoot biomass and counted flower number at the time of plant harvest. Biomass is a measure of plant performance that has been used in previous studies to measure selection on plant traits (Geber and Griffen 2003). Selection can be measured using performance as a proxy for fitness if the performance trait is ecologically meaningful and linked with fitness (Arnold 1983). Biomass is an ecologically meaningful

measure of performance because it is used regularly to quantify plant responses to environmental changes (e.g. Tilman et al. 1997, De Deyn and Van der Putten 2005), including plant response to colonization by AM fungi (e.g. Johnson 2010, van der Heijden et al. 1998). Biomass is linked to reproductive fitness in *L. siphilitica* because it is a good predictor of flower number (Table 4C), and flower number in turn is a good predictor of seed production, and therefore female reproductive fitness, in *L. siphilitica* (Caruso et al. 2003). As a result, it has been used in previous studies of selection on plant function in *L. siphilitica* in previous work (Caruso et al. 2006). I recorded biomass as the mass of dry shoot samples after harvest added to the mass of dry leaves that had fallen off each plant during the growth season. Since flower number is a good predictor of female reproductive fitness in *L. siphilitica* I used flower number as a second measure of fitness. I recorded flower number as the total number of flowering buds (buds, un-opened flowers, and opened flowers) on each plant at the time of harvest.

Selection is covariance between fitness and traits, and the accuracy of selection estimates for each trait increases as more traits are added to a selection model (Conner and Hartl 2006). In order to increase the accuracy of selection estimates for both shoot phosphorus and photosynthesis I incorporated plant size measurements into the selection models. Plant size is related to aspects of fitness including pollinator visitation (Johnston 1991) and reproductive output (Kelly 1992, Andersson 1996). Since plant size is linked to fitness, as well as nutrient uptake (Bates and Lynch 2000) and photosynthetic rate (Artnz et al. 2000), and is an estimate of mid-season plant performance, it is likely to influence selection in *L. siphilitica* in the current study. I measured size as the geometric volume of each plant shoot, using shoot height and rosette diameter, as soon as all pots had been thinned down to one seedling per pot. Since plant size affects plant fitness, and early plant performance can lead to increased plant fitness (Geber

and Griffen 2003), plant growth rate may also affect fitness in *L. siphilitica*. I measured shoot growth rate per day as the difference between two geometric volume measurements divided by the number of days between measurements: the set of geometric volume measurements included in trait and selection analyses, and a second a set of geometric volume measurements recorded 15 days after the first set.

To verify that plants in the inoculated treatment were colonized by AM fungi and that non-inoculated control plants were uncolonized I analyzed root tissue from all inoculated plants and from a 15-plant subsample of each non-inoculated control treatment group. I randomly selected 15 to 20, 2-cm root segments from each root sample, cleared roots with 10% KOH at 90°C for 25 minutes to remove all root content that might pick up stain (Vierheilig et al. 1998) and stained AM fungi using a modified vinegar-and-ink staining method (following Rowe et al. 2007). Stained roots were mounted on glass microscope slides using a cover-slip and polyvinyl alcohol-lactic acid-glycerol slide medium. I estimated colonization using presence/absence scoring with a gridline intersect method (McGonigle et al. 1990) and reported percent colonization by each of three fungal structures: hyphae, arbuscules, and vesicles.

To examine the responses of all traits, colonization, and fitness measures to inoculation by AM fungi and soil phosphorus addition I used a 2-way ANOVA. All data were transformed using a log 10 transformation to fit equal variance of error assumptions, except colonization estimates which were arcsin square root transformed. Before transforming I added 1 to all flower number and growth rate data to eliminate zeros. The eleven growth rate values that were negative due to changes in rosette shape were changed to zero before adding one to all values. Once transformed, the assumption of equal variance was confirmed using visual comparison of standard deviation and variance estimates across treatment groups and Levene's test of equal

variances. The assumption of normality was confirmed using visual inspection of histograms and Shapiro-Wilk tests. Differences in group means for each trait (shoot N:P, Y(II), Chl, geometric volume, growth rate), colonization measurement (proportion of root colonized by hyphae, arbuscules, and vesicles), and fitness measurement (biomass and flower number) were assessed using 95% confidence interval overlap.

To examine selection on all traits and colonization, and the influence of inoculation by AM fungi and soil phosphorus addition on selection, I used phenotypic selection analysis, in which the slope of the regression of a trait against fitness is equal to the selection coefficient (Lande and Arnold 1983). I used standardized trait values and relative fitness calculated from untransformed data in all models in order to compare standardized selection coefficients across traits, treatment groups, and with other experiments (Kingsolver et al. 2001). I calculated both selection differentials and selection gradients. Selection differentials, or total selection, combine the direct effects of traits on fitness with the indirect effects on fitness due to correlations among traits. Selection gradients, or direct selection, account for trait correlations and identify traits with a direct effect on fitness (Conner and Hartl 2006). While selection gradients indicate which traits are direct targets of selection, selection differentials indicate how selection would act on all correlated traits in nature. Both selection coefficients are therefore useful in assessing how natural selection acts on traits. To calculate selection differentials I used ordinary least squares regressions of each trait (shoot N:P, Y(II), Chl, geometric volume of the shoot, and shoot growth rate) against each fitness measurement (biomass and flower number) within each treatment group. Whereas biomass met the assumptions of normality and homogeneity of variances, variance for flower number differed among treatment groups, and this heterogeneity could not be corrected with data transformation. As a result, estimates of selection and their statistical

significance using flower number as a fitness measure may be incorrect. Though attempts to meet assumptions of normality and homogeneity of variances for flower number data were unsuccessful, the similarity of results calculated with flower number as a fitness proxy and results with biomass as a fitness proxy suggests that the assumption violations did not necessarily bias estimates of selection. Simple regression was not used for colonization data because colonization by hyphae, arbuscules, and vesicles is so highly interrelated that correlations among them should be considered when assessing their effects on fitness. I compared the strength of selection on each trait between and within treatment groups using 95% confidence interval overlaps. Selection coefficients with non-overlapping confidence intervals were considered significantly different from each other.

To calculate selection gradients I used multiple regressions of all measured traits (shoot N:P, Y(II), Chl, geometric volume of the shoot, and shoot growth rate) against each fitness measurement (biomass and flower number) within each treatment group. I used two sets of multiple regressions, one including nutrient data and one without, because nutrients were only measured in a sub-sample of plants. I compared the strength of selection on each trait between and within treatment groups using 95% confidence interval overlaps. To avoid any effect of the reduced sample size of nutrient data on confidence interval comparisons, I compared confidence intervals between shoot N:P and other traits using parameters from the nutrient multiple regression, and compared confidence intervals among all non-nutrient traits using parameters from the non-nutrient multiple regression. I estimated the effects of multi-collinearity among traits included in the analyses by examining the variance inflation factor (VIF). VIF for all multiple regressions was below 10, suggesting that all models fulfilled the assumption of no collinearity (Neter et al. 1990). To account for any differences between total and direct selection

estimates that may be caused by trait correlations I performed Pearson's correlations on traits included in the multiple regressions. Since colonization data were available only for inoculated treatments, I used another multiple regression to regress each fitness measure on colonization by hyphae, arbuscules, and vesicles.

The use of maternal families as replicates across treatment groups enables examination of whether genotype performance was correlated across phosphorus levels and inoculation treatments. To test for this correlation, I regressed the performance (biomass) of members of the same maternal family across all treatment groups. All statistical analyses were performed using SPSS (IBM Corp. 2013).

Table 1: Extractable phosphorus levels in soil samples from *Lobelia siphilitica* populations in Ontario that had the lowest and highest levels of phosphorus. Data extracted from AFL soil report 13-040397 made on June 27, 2013.

Population	Extractable phosphorus (mg P/kg dry soil)
Ancaster 1 (Martin Road)	3.0
Stratford	27.0

Table 2: Phosphorus levels in soil/sand potting medium after autoclaving, both before washing and after washing to remove autoclave contaminants. Data from AFL soil report 14-035844 made on May 23, 2014.

Autoclaved soil/sand mixture	Phosphorus (mg P/kg dry soil)	N (ammonium) (mg N/kg dry soil)	N (nitrates) (mg N/kg dry soil)
washed	1.0	4.79	0.649
unwashed	0.9	4.74	1.12

RESULTS

Trait means, trait correlations, and performance correlations

Performance of *L. siphilitica* was increased by inoculation with AM fungi or by high soil phosphorus level, however the effect of inoculation with AM fungi on performance depended on soil phosphorus level (Table 3, Figure 1). In high soil phosphorus there was no difference in final biomass or flower number between inoculated and control treatment groups. By contrast, in low phosphorus soil the inoculated treatment group had 25% higher biomass and 85% more flowers than control plants. These two measures of fitness were positively correlated with each other in each treatment group (Table 4). Similarly, mid-season size and growth were influenced by an interaction between inoculation and soil phosphorus level (Table 3, Figure 2). In high soil phosphorus there was no difference in either geometric volume or growth rate between inoculated and control treatment groups. By contrast, in low soil phosphorus the inoculated treatment group had 103% larger geometric volume and 80% higher growth rate compared to the control treatment group. Mid-season size and growth were positively correlated with each other in each treatment group (Table 4).

Shoot N:P was decreased by inoculation with AM fungi or increased soil phosphorus level (Figure 3), however the effect of inoculation with AM fungi on shoot N:P depended on soil phosphorus (Table 3). In high phosphorus soil there was no difference in shoot N:P between inoculated and control treatment groups. By contrast, in low phosphorus soils inoculated plants had 31% lower shoot N:P than control plants. Shoot N:P was not consistently correlated with any other trait in any treatment group (Table 4B).

Photosynthesis and chlorophyll concentration decreased in response to inoculation with AM

fungi or increased soil phosphorus level, however the effect of inoculation with AM fungi on physiological traits depended on soil phosphorus level (Table 3, Figure 2). In high phosphorus soil photosynthetic rate did not differ between inoculated and control treatment groups. By contrast, in low phosphorus soil photosynthetic rate was 7% lower in inoculated compared to control treatment groups. Similarly, leaf chlorophyll content was influenced by an interaction between inoculation with AM fungi and soil phosphorus level. In high phosphorus soil chlorophyll content did not differ between inoculated and control treatment groups. By contrast, in low phosphorus soils chlorophyll content was 15% lower in inoculated compared to control treatment groups. Photosynthetic rate and chlorophyll content were positively correlated with each other in all treatment groups (Table 4). Both of these traits were negatively correlated with both geometric volume and growth rate (Table 4).

The proportion of root colonized by AM fungi was not affected by soil phosphorus level regardless of whether measured by hyphal, arbuscule, or vesicle structures (Table 3, Figure 4). High soil phosphorus resulted in a 4% decrease in the proportion of root colonized by hyphae, but had no effect on colonization by vesicles or arbuscules. All 30 sub-sampled non-inoculated control roots were completely uncolonized by AM fungi.

Performance, measured as biomass, was only correlated across two treatment groups (Table 5). Biomass in the inoculated high soil phosphorus group was positively correlated with biomass in the control high soil phosphorus group (slope = 0.262, $P = 0.004$). Biomass was not correlated across treatment groups when soil phosphorus was low or when soil phosphorus differed, regardless of inoculation treatment.

Selection Differentials

Selection for reduced shoot N:P was only detected in two treatment groups (Table 6). With biomass as a fitness proxy, there was selection for reduced shoot N:P in the inoculated high phosphorus treatment and the non-inoculated control high phosphorus treatment (Table 6). With flower number as a fitness proxy, there was selection for reduced shoot N:P only in the inoculated high phosphorus treatment. Selection for reduced shoot N:P was stronger in the inoculated high phosphorus group than in the control low phosphorus group, but only with biomass as a fitness proxy (Table 6 superscripts). The relative strength of selection on shoot N:P did not differ from the strength of selection acting on other traits within each treatment group (Table 6 underlines).

Selection for reduced photosynthesis was detected in most treatment groups. With either fitness proxy, there was selection for reduced leaf chlorophyll content in all treatment groups (Table 6). With biomass as a fitness proxy there was selection for reduced photosynthetic rate in the inoculated high phosphorus treatment and in the control low phosphorus treatment. With flower number as a fitness proxy there was selection for reduced photosynthetic rate in all treatment groups except the inoculated low phosphorus treatment. Selection for reduced photosynthetic rate was strongest in the control low phosphorus group but only with flower number as a fitness proxy (Table 6 superscripts). Selection on leaf chlorophyll content did not differ between treatment groups. The relative strength of selection on relative chlorophyll content and photosynthetic rate did not differ from the strength of selection on other traits within each treatment group (Table 6 underlines).

Selection for increased mid-season size, measured as geometric volume and growth rate, was detected in all treatment groups with both fitness proxies (Table 6). With either fitness proxy,

selection for increased mid-season size was stronger in both low phosphorus groups than in both high phosphorus groups (Table 6 superscripts). With flower number as a fitness proxy, selection for increased mid-season size in low phosphorus was stronger in the non-inoculated control than in the inoculated treatment (Table 6 superscripts). Within the inoculated low phosphorus treatment group, selection for increased geometric volume was stronger than selection on any other trait in that treatment group (Table 6 underlines).

Selection Gradients

Selection gradients for reduced shoot N:P were detected in even fewer treatment groups than selection differentials. Selection for reduced shoot N:P was only detected in the inoculated high phosphorus group with flower number as a fitness proxy (Table 7B). Selection for reduced shoot N:P did not differ in strength between treatment groups. Within treatment groups, the relative strength of selection on shoot N:P did not differ from selection on other traits (Table 7B underlines).

Selection gradients for photosynthetic rate differed in direction from selection differentials, while gradients and differentials for relative chlorophyll level were similar. Selection for increased photosynthetic rate was only detected in the inoculated high phosphorus group, and only with flower number as a fitness proxy. Selection for reduced chlorophyll content was detected in both inoculated and non-inoculated control high phosphorus groups. Selection on both chlorophyll content and photosynthetic rate did not differ between treatment groups. Within treatment groups, the relative strength of selection on chlorophyll content and photosynthetic rate did not differ from selection on other traits (Table 7A underlines).

Similar to selection differentials, selection gradients for increased mid-season size were

detected in most treatment groups with both fitness proxies (Table 7A). Selection on mid-season size did not differ between treatment groups. Within the inoculated low phosphorus group, selection on geometric volume was stronger than selection on all other traits with biomass as a fitness proxy. Within the inoculated high phosphorus group selection on growth rate was stronger than selection on other traits with flower number as a fitness proxy (Table 7A underlines).

Shoot N:P was included in a separate multiple regression to avoid using decreased sample sizes when calculating direct selection on all other traits. When shoot N:P was included in the multiple regression, the influence of inoculation and soil phosphorus on selection did not change (Table 7B). There was selection for reduced shoot N:P in the inoculated high soil P treatment group with flower number as a fitness proxy. Selection on shoot N:P did not differ between treatment groups. Within treatment groups, the strength of selection on all traits was similar when N:P was included in the analysis.

There was no selection on the proportion of root colonized by hyphae, arbuscules, or vesicles with either fitness proxy (Table 8).

Table 3: 2-way ANOVA on log10 transformed trait means for fitness and trait measures using two factors: inoculation with AM fungi (M) and soil phosphorus level (P). Table includes degrees of freedom (df), mean square (MS), F-statistic (*F*), and significance (*P*). Colonization measures were arcsine transformed and had soil phosphorus level (P) as a factor.

Trait	Biomass				Flower number				Y(II)			
Term	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
M	1	.926	21.167	6.116×10^{-06}	1	4.107	14.772	1.468×10^{-04}	1	.028	11.536	7.737×10^{-04}
P	1	12.189	278.517	3.307×10^{-45}	1	47.891	172.242	1.143×10^{-31}	1	.093	38.248	2.014×10^{-09}
M*P	1	.621	14.179	1.984×10^{-04}	1	2.190	7.875	5.325×10^{-03}	1	.013	5.465	0.020
Error	314	.044			314	.278			303	.002		

Trait	Chl				Geometric volume				Growth rate			
Term	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
M	1	.077	12.404	4.921×10^{-04}	1	6.210	21.933	4.200×10^{-06}	1	4.871	16.931	4.955×10^{-05}
P	1	.352	56.397	6.230×10^{-13}	1	55.158	194.803	8.042×10^{-35}	1	13.174	45.788	6.432×10^{-11}
M*P	1	.086	13.789	2.420×10^{-04}	1	3.652	12.899	3.811×10^{-04}	1	1.167	4.054	0.045
Error	313	.006			316	.283			315	.288		

Trait	total N				total P				total N:P			
Term	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
M	1	.051	3.316	6.989×10^{-02}	1	1.043	48.790	2.881×10^{-11}	1	.476	76.284	5.245×10^{-16}
P	1	4.610	301.191	1.249×10^{-43}	1	24.664	1153.787	7.777×10^{-93}	1	6.888	1104.923	1.367×10^{-89}
M*P	1	.034	2.192	0.140	1	.387	18.111	3.008×10^{-05}	1	.115	18.426	2.608×10^{-05}
Error	229	.015			236	.021			229	.006		

Trait	proportion hyphae				proportion arbuscules				proportion vesicles			
Term	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
P	1	.115	3.856	.051	1	.008	.679	.411	1	.026	1.712	.193
Error	157	.030			157	.012			157	.015		

Table 4A: 2-tailed Pearson's correlation (*r*) coefficients between all traits used in multiple regression (photosynthetic rate [Y(II)], leaf chlorophyll level [Chl], geometric volume of shoot, and shoot growth rate) in each treatment group (inoculated high soil P, inoculated low soil P, control high soil P, control low soil P). ** $P < 0.01$, * $P < 0.05$.

Inoculated High soil P		Chl	Y(II)	Growth rate	Geometric volume
Chl	*r*	1	.657**	-.217	-.419**
	N	80	80	80	80
Y(II)	*r*		1	-.263*	-.348**
	N		80	80	80
Growth rate	*r*			1	.562**
	N			80	80

Inoculated Low soil P		Chl	Y(II)	Growth rate	Geometric volume
Chl	*r*	1	.499**	-.343**	-.492**
	N	79	77	79	79
Y(II)	*r*		1	-.157	-.338**
	N		77	77	77
Growth rate	*r*			1	.520**
	N			80	80

Control High soil P		Chl	Y(II)	Growth rate	Geometric volume
Chl	*r*	1	.574**	-.365**	-.575**
	N	80	80	80	80
Y(II)	*r*		1	-.326**	-.396**
	N		80	80	80
Growth rate	*r*			1	.468**
	N			80	80

Control Low soil P		Chl	Y(II)	Growth rate	Geometric volume
Chl	*r*	1	.597**	-.535**	-.393**
	N	78	70	78	78
Y(II)	*r*		1	-.614**	-.555**
	N		70	70	70
Growth rate	*r*			1	.675**
	N			79	79

Table 4B: 2-tailed Pearson's correlation (*r*) coefficients between all traits used in the nutrient multiple regression (photosynthetic rate [Y(II)], leaf chlorophyll level [Chl], geometric volume of shoot, shoot growth rate, and shoot N:P) in each treatment group (inoculated high soil P, inoculated low soil P, control high soil P, control low soil P). ** $P < 0.01$, * $P < 0.05$.

Inoculated High soil P		Y(II)	Chl	Geometric volume	Growth rate	N:P
Y(II)	*r*	1	.657**	-.348**	-.263*	.235
	N	80	80	80	80	60
Chl	*r*		1	-.419**	-.217	.245
	N		80	80	80	60
Geometric volume	*r*			1	.562**	-.319*
	N			80	80	60
Growth rate	*r*				1	-.280*
	N				80	60

Inoculated Low soil P		Y(II)	Chl	Geometric volume	Growth rate	N:P
Y(II)	*r*	1	.499**	-.338**	-.157	.169
	N	77	77	77	77	57
Chl	*r*		1	-.492**	-.343**	.398**
	N		79	79	79	59
Geometric volume	*r*			1	.520**	-.004
	N			80	80	59
Growth rate	*r*				1	-.081
	N				80	59

Control High soil P		Y(II)	Chl	Geometric volume	Growth rate	N:P
Y(II)	*r*	1	.574**	-.396**	-.326**	.099
	N	80	80	80	80	60
Chl	*r*		1	-.575**	-.365**	.260*
	N		80	80	80	60
Geometric volume	*r*			1	.468**	-.312*
	N			80	80	60
Growth rate	*r*				1	-.065
	N				80	60

Control Low soil P		Y(II)	Chl	Geometric volume	Growth rate	N:P
Y(II)	*r*	1	.597**	-.555**	-.614**	-.137
	N	70	70	70	70	53
Chl	*r*		1	-.393**	-.535**	.063
	N		78	78	78	54
Geometric volume	*r*			1	.675**	.160
	N			80	79	54
Growth rate	*r*				1	.156
	N				79	54

Table 4C: 2-tailed Pearson's correlation (*r*) coefficients between final dry shoot biomass and flower number in each treatment group (inoculated high soil P, inoculated low soil P, control high soil P, control low soil P). ** $P < 0.01$.

	Treatment group			
	Inoculated High soil P	Inoculated Low soil P	Control High soil P	Control Low soil P
r	.641**	.799**	.705**	.619**
N	80	79	80	79

Table 5: Regression parameters for correlation of performance (biomass) across different treatment groups. None of the slopes (m) are significantly different from each other, using 95% confidence interval overlap of the regression parameters. The regression parameter that differs significantly from zero is bolded.

Regression variables		Regression parameters							
Dependent	Independent	m	SE	t	r^2	Adjusted r^2	*P*	df	*F*
Inoculated Low soil P	Control Low soil P	.136	.127	1.071	0.015	0.002	.288	1,76	1.147
Inoculated High soil P	Control High soil P	**.262**	**.089**	**2.948**	**0.100**	**0.089**	**.004**	**1,78**	**8.694**
Inoculated Low soil P	Inoculated High soil P	.178	.100	1.791	0.040	0.028	.077	1,77	3.206
Control Low soil P	Control High soil P	.049	.076	.651	0.005	0.007	.517	1,77	.423

Table 6: Directional selection differentials (S), standard errors (SE), and sample sizes (N) from simple regression of each trait (photosynthetic rate [Y(II)], leaf chlorophyll level [Chl], geometric volume of shoot [GV], shoot growth rate [GR], and shoot N:P) against each fitness measure (biomass and flower number) in each treatment group from general linear model regression of standardized traits against relative fitness. *P*-value indicates whether selection differential is significantly different from zero. Differences in selection for a trait between treatment groups (superscripts) and between the same traits within treatment groups (underlines) were calculated using 95% confidence intervals of regression slopes.

		Inoculated High soil P				Inoculated Low soil P				Control High soil P				Control Low soil P			
Fitness	Trait	S	SE	*P*	N	S	SE	*P*	N	S	SE	*P*	N	S	SE	*P*	N
Biomass	Y(II)	-.078[a]	.030	0.012	80	-.086[a]	.053	0.107	77	-.057[a]	.039	0.147	80	-.142[a]	.068	0.040	70
	Chl	-.088[a]	.030	4.51×10^{-03}	80	-.196[a]	.049	1.23×10^{-04}	79	-.124[a]	.037	1.15×10^{-03}	80	-.230[a]	.063	5.17×10^{-04}	78
	GV	.163[a]	.026	1.58×10^{-08}	80	.375[b]	.032	7.30×10^{-19}	79	.216[a]	.031	7.44×10^{-10}	80	.380[b]	.053	4.64×10^{-10}	79
	GR	.165[a]	.026	8.73×10^{-09}	80	.300[b]	.041	2.56×10^{-10}	79	.173[a]	.034	2.59×10^{-06}	80	.399[b]	.052	3.57×10^{-11}	79
	N:P	-.114[a]	.034	1.37×10^{-03}	60	.025[ab]	.056	0.655	59	-.111[ab]	.045	0.016	60	.124[b]	.075	0.107	54
Flower number	Y(II)	-.182[a]	.055	1.46×10^{-03}	80	-.164[a]	.138	0.237	77	-.196[a]	.064	3.19×10^{-03}	80	-1.133[b]	.242	1.43×10^{-05}	70
	Chl	-.249[a]	.052	7.36×10^{-06}	80	-.512[a]	.124	8.74×10^{-05}	79	-.338[a]	.056	5.60×10^{-08}	80	-.956[a]	.227	7.14×10^{-05}	78
	GV	.232[a]	.053	3.29×10^{-05}	80	.856[b]	.096	1.50×10^{-13}	79	.379[a]	.053	3.50×10^{-10}	80	1.781[c]	.144	5.06×10^{-20}	79
	GR	.309[a]	.047	6.20×10^{-09}	80	.704[b]	.111	1.44×10^{-08}	79	.382[a]	.053	2.31×10^{-10}	80	1.627[c]	.167	4.46×10^{-15}	79
	N:P	-.234[a]	.063	4.20×10^{-04}	60	-.084[a]	.134	0.531	59	-.112[a]	.075	0.140	60	.350[a]	.326	0.288	54

Table 7A: Directional selection gradients (β) and standard error (SE) from multiple regression of all traits (photosynthetic rate [Y(II)], leaf chlorophyll level [Chl], geometric volume of shoot [GV], and shoot growth rate [GR]) against each fitness measure (biomass and flower number) in each treatment group. *P*-value indicates whether the selection gradient is significantly different from zero. Differences in selection were calculated using 95% confidence intervals of regression slopes. Between treatment groups, gradients for the same trait did not differ. Within treatment groups, differences in selection among different traits are indicated with underlines.

		Inoculated High soil P (N = 80)			Inoculated Low soil P (N = 77)			Control High soil P (N = 80)			Control Low soil P (N = 70)		
Fitness	Trait	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
Biomass	Y(II)	-.002	.032	0.953	.048	.034	0.172	.057	.036	0.123	.208	.072	5.418×10^{-03}
	Chl	-.025	.033	0.450	-.014	.038	0.722	-.015	.041	0.718	-.053	.067	0.436
	GV	.091	.031	4.915×10^{-03}	.311	.037	3.904×10^{-12}	.183	.038	8.801×10^{-06}	.254	.066	2.487×10^{-04}
	GR	.108	.029	4.313×10^{-04}	.140	.035	1.258×10^{-04}	.100	.034	4.126×10^{-03}	.280	.073	2.857×10^{-04}
Flower number	Y(II)	.024	.058	0.675	.200	.106	0.063	.059	.055	0.285	.309	.209	0.144
	Chl	-.205	.060	1.008×10^{-03}	-.175	.118	0.142	-.174	.061	5.801×10^{-03}	-.256	.195	0.193
	GV	.004	.056	0.946	.669	.115	1.407×10^{-07}	.185	.058	2.008×10^{-03}	1.324	.190	1.890×10^{-09}
	GR	.269	.053	2.447×10^{-06}	.337	.106	2.153×10^{-03}	.251	.051	4.998×10^{-06}	.861	.211	1.233×10^{-04}

Table 7B: Directional selection gradients (β) and standard error (SE) from multiple regression of nutrient data (shoot N:P) in addition to all other traits (photosynthetic rate [Y(II)], leaf chlorophyll level [Chl], geometric volume of shoot [GV], and shoot growth rate [GR]) against each fitness measure (biomass and flower number) in each treatment group. *P*-value indicates whether the gradient is significantly different from zero. Differences in selection were calculated using 95% confidence intervals of regression slopes. Between treatment groups, differences in selection on the same trait are indicated with superscripts. Within treatment groups, all traits experienced similar selection strength.

		Inoculated High soil P (N = 60)			Inoculated Low soil P (N = 59)			Control High soil P (N = 60)			Control Low soil P (N = 54)		
Fitness	trait	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
Biomass	Y(II)	-.004[a]	.033	.907	.033[ab]	.045	.471	.072[ab]	.042	.091	.281[b]	.086	.002
	Chl	-.028[a]	.035	.432	-.039[a]	.058	.508	-.007[a]	.050	.883	-.015[a]	.088	.869
	GV	.086[a]	.036	.023	.287[b]	.056	5.120×10^{-06}	.166[ab]	.051	.002	.262[ab]	.073	.001
	GR	.095[a]	.032	.004	.107[a]	.050	.035	.117[a]	.040	.005	.304[a]	.088	.001
	N:P	-.050[a]	.028	.084	.059[a]	.042	.163	-.058[a]	.037	.130	.073[a]	.060	.226
Flower number	Y(II)	.006[a]	.064	.921	.101[a]	.121	.410	.070[a]	.062	.261	.498[a]	.265	.066
	Chl	-.163[a]	.067	.019	-.272[a]	.156	.087	-.158[a]	.074	.038	-.555[a]	.273	.048
	GV	-.021[a]	.070	.765	.617[b]	.150	1.454×10^{-04}	.170[ab]	.075	.027	1.445[c]	.226	6.908×10^{-08}
	GR	.231[a]	.061	4.196×10^{-04}	.159[ab]	.132	.233	.243[a]	.060	1.494×10^{-04}	.913[b]	.272	.002
	N:P	-.130[a]	.055	.021	.003[a]	.111	.981	-.010[a]	.055	.863	.080[a]	.185	.666

Table 8: Directional selection gradients (β) and standard error (SE) from the regression of three colonization traits (proportion of root colonized by hyphae, arbuscules, and vesicles) against each fitness measure (biomass and flower number) in both inoculated treatment groups. Selection gradients do not differ between treatment groups or between traits within treatment groups, calculated using 95% confidence intervals of regression slopes.

		Inoculated low soil P			Inoculated high soil P		
Fitness	Fungal structure	β	SE	P	β	SE	P
Biomass	hyphae	.064	.058	.268	-.044	.036	.227
	arbuscules	-.050	.053	.355	-.006	.034	.852
	vesicles	.034	.058	.559	-.010	.037	.782
Flower number	hyphae	-.111	.067	.099	.129	.147	.382
	arbuscules	.047	.063	.461	-.095	.135	.486
	vesicles	.039	.068	.567	.197	.146	.181

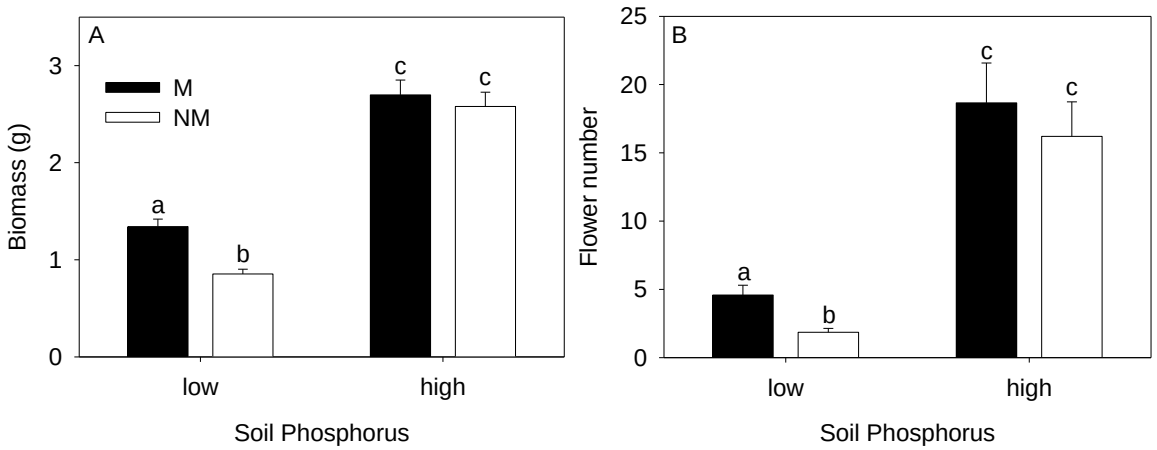

Figure 1: Mean biomass (A) and mean flower number (B) in low and high phosphorous soils (x-axis) in inoculated (black) and non-inoculated control (white) treatments. ANOVAs were performed on log10 transformed data, though values in figure were back-transformed. Letters indicate differences in group means, analyzed using overlapping 95% confidence intervals of the parameters. Error bars represent one standard error. Sample sizes were the same for both biomass and flower number: N inoculated high soil P = 80; inoculated low soil P = 79; control high soil P = 80; control low soil P = 79.

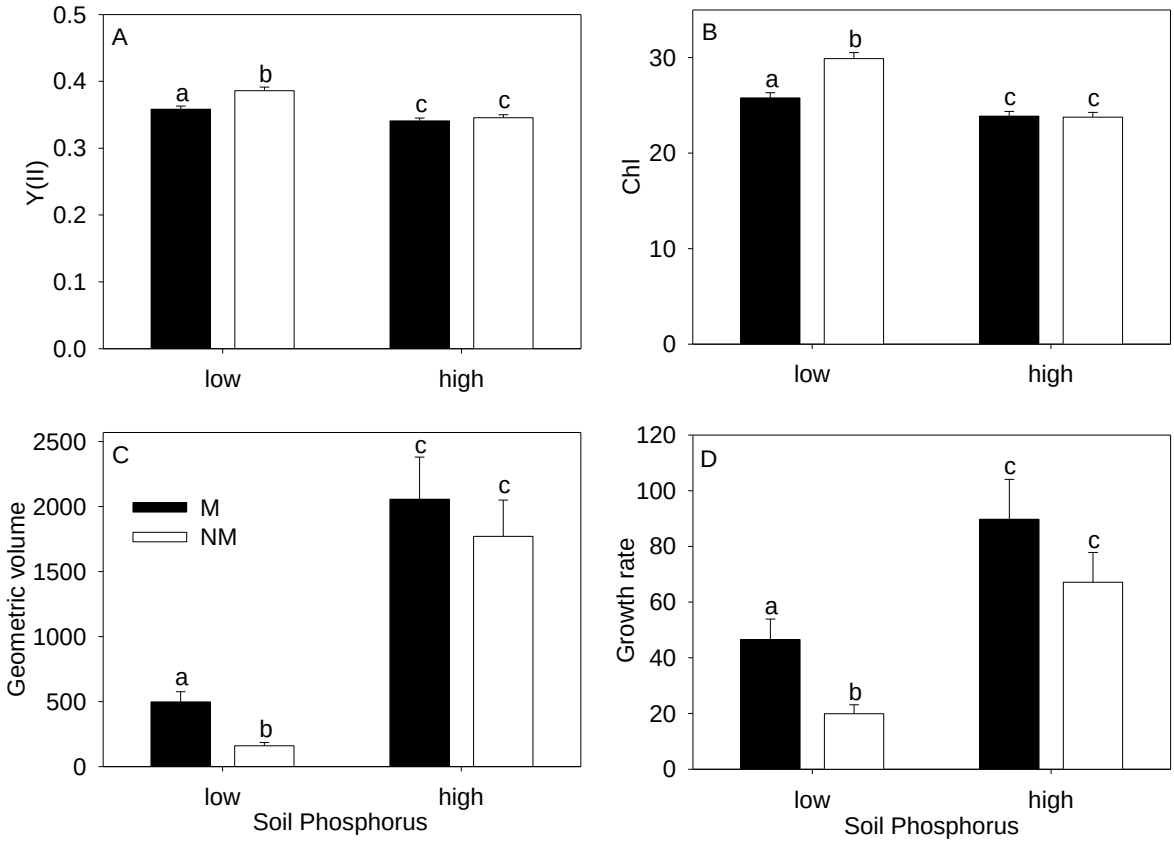

Figure 2: Mean photosynthetic rate (Y(II), A), leaf chlorophyll level (Chl, B), geometric volume of shoot (GV, C), and shoot growth rate (GR, D) in low and high phosphorous soils (x-axis) in inoculated (black) and non-inoculated control (white) treatments. ANOVAs were performed on log10 transformed data, though values in figure were back-transformed. Letters indicate differences in group means, analyzed using overlapping 95% confidence intervals of the parameters. Error bars represent one standard error. Sample sizes for inoculated high soil P, inoculated low soil P, control high soil P, and control low soil P are 80, 77, 80, 70 for Y(II); 80, 79, 80, 78 for Chl; 80, 80, 80, 80 for GV; and 80, 80, 80, 79 for GR, respectively.

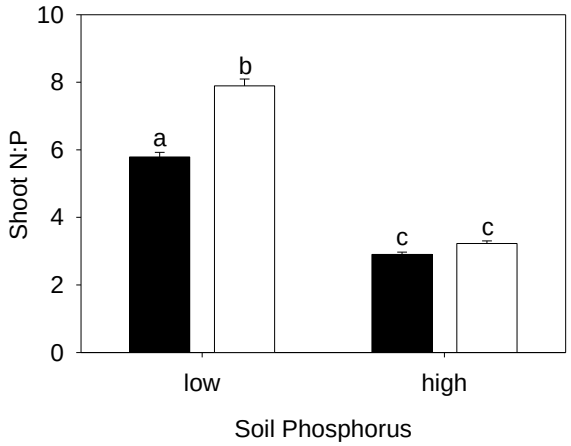

Figure 3: Mean shoot N:P in low and high phosphorous soils (x-axis) in inoculated (black) and non-inoculated control (white) treatments. ANOVA was performed on log10 transformed data, though values in figure were back-transformed. Letters indicate differences in group means, analyzed using overlapping 95% confidence intervals of the parameters. Error bars represent one standard error. Sample size for shoot N:P is 60 for inoculated high soil P, 59 for inoculated low soil P, 60 for control high soil P, and 54 for control low soil P.

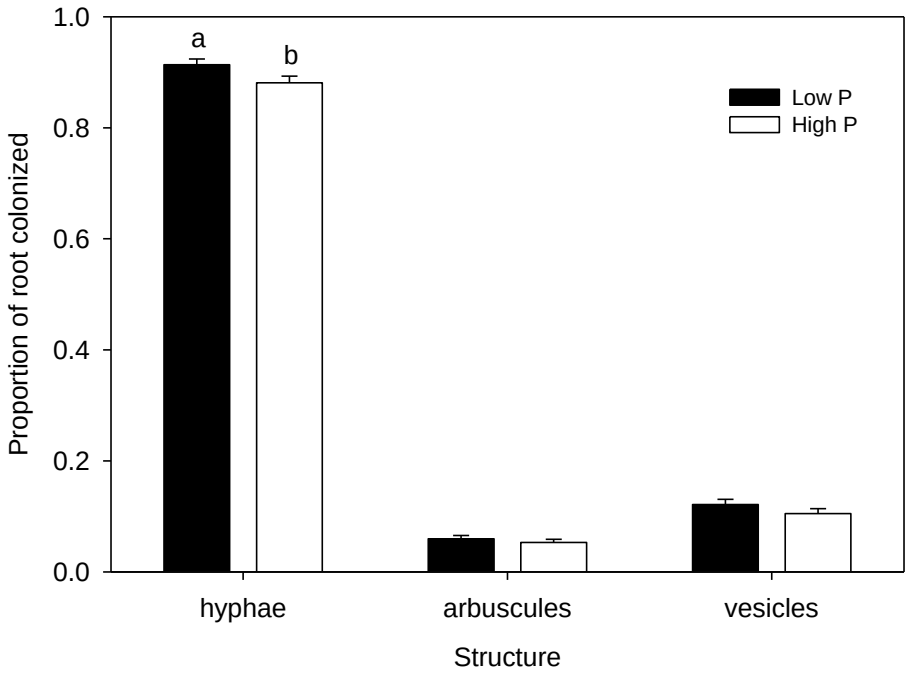

Figure 4: Mean proportion of root colonized by hyphae, arbuscules, and vesicles in low (black) and high (white) phosphorus soils in all inoculated plants. ANOVAs were performed on arcsin square root transformed data, though values in figure were back-transformed. Letters indicate differences in group means, analyzed 95% confidence interval overlap of the parameters. Error bars represent one standard error. Sample size for low and high phosphorus is 79 and 80, respectively. None of the sub-sampled non-inoculated control plants were colonized by AM fungi.

DISCUSSION

Inoculation with *G. intraradices* and fertilization with phosphorus increased performance and phosphorus acquisition in *L. siphilitica* but did not influence direct selection for phosphorus acquisition or photosynthesis. Performance and shoot phosphorus content increased in response to inoculation with AM fungi in low phosphorus soil (Figure 1, 3), while photosynthesis decreased in response to inoculation in low phosphorus soil (Figure 2). Although performance, shoot phosphorus content, and photosynthesis all responded to inoculation with AM fungi, direct selection on shoot phosphorus content and photosynthesis was minimal or not affected in a consistent manner by inoculation with AM fungi or soil phosphorus availability (Table 7A, B). Total selection for shoot phosphorus content and photosynthesis was sometimes influenced in a consistent manner by a combination of AM fungal inoculation and soil phosphorus availability (Table 6), indicating that AM fungi and soil phosphorus level may influence correlations and total selection more than direct selection on plant traits.

The responses of performance and shoot N:P to inoculation with AM fungi in low phosphorus soil confirm that AM fungi are most beneficial to plants when soil nutrients are limiting. Specifically, biomass, flower number, and mid-season size traits increased with inoculation with AM fungi in low phosphorus soils (Figure 1, 2). Inoculation with AM fungi also increased shoot phosphorus content relative to nitrogen in nutrient poor soils, as indicated by lower shoot N:P in plants inoculated with AM fungi compared to non-inoculated control plants in low phosphorus soil (Figure 3). Thus inoculation with AM fungi increased plant fitness in low phosphorus soil by increasing acquisition of phosphorus, the nutrient most limiting to plant growth (Johnson 2010).

Performance and nutrient acquisition in high phosphorus soil do not support the hypothesis

that inoculation with AM fungi would be costly to the host plant when nutrients are abundant. Biomass, flower number, mid-season size, and shoot N:P did not differ between plants inoculated with AM fungi and non-inoculated control plants in high phosphorus soil (Figure 1, 2, 3), suggesting that AM fungi do not negatively affect the fitness of their host plant in high nutrient environments, despite taking photosynthate (Lambers et al. 2008) without providing any nutrient benefits. The lack of cost associated with AM fungi may be the result of decreased root production in plants inoculated with AM fungi. The cost of producing roots and AM fungi can be measured in carbon (Fitter 1991) since both structures require photosynthate for their metabolism (Brundrett 2002). AM fungi are a fraction the size of roots and therefore likely require an order of magnitude less carbon to produce than roots (Harley 1989, Helgason and Fitter 2009). Since AM fungi may be cheaper, and can be the primary method of acquiring phosphorus despite high soil phosphorus levels (Smith et al. 2009), plants may favor associating with AM fungi over producing roots. If AM fungi decreased root production, then a trade-off between associating with AM fungi and producing roots may have resulted in a similar below-ground carbon cost among colonized roots with smaller root biomass and uncolonized roots with larger root biomass. Similar cost of below ground biomass between treatment groups implies that resource availability for above ground performance was similar, which would explain why inoculation with AM fungi did not incur a cost in above ground performance to the host plant. Despite the potential for AM fungi to decrease root production, the design of the current study did not allow for measuring root biomass.

Reduced photosynthesis in response to inoculation with AM fungi in low phosphorus soil does not support the hypothesis that a beneficial fungal partner stimulates carbon assimilation. Both photosynthetic rate and leaf chlorophyll content decreased with inoculation in low

phosphorus soil (Figure 3), which contradicts the positive effect of AM fungi on photosynthesis reported in the literature (Koide and Schreiner 1994, Black et al. 2000, Auge 2001, Fay et al. 1996, Adolfsson 2015). An increase in photosynthesis following inoculation by AM fungi has been explained by the presence of heterotrophic fungi causing an increase in carbon demand in the plant (Wright et al. 1998 a,b, Miller et al. 2002, Lambers et al. 2008) and a compensatory increase in the uptake of limiting nutrients by the fungal partner (Rao and Terry 1989, deGroot et al. 2000). In the current study, inoculation by AM fungi increased shoot phosphorus content (Figure 3) and high colonization of roots by AM fungi (Figure 4) likely increased carbon demand in the plant (Miller et al. 2002). A decrease in photosynthesis has been explained by an attempt by the host plant to deter a costly fungal partner (Bildusas et al. 1986), however this was not the case in the current study as inoculation with AM fungi did not decrease plant fitness (Figure 1).

The observed decrease in photosynthesis may have been caused by a trade-off in nitrogen allocation between leaves and reproductive structures. Nitrogen is essential for forming reproductive structures like flowers, and reallocation occurs at a cost to vegetative structures (Reekie and Bazzaz 2005). Since photosynthesis is limited by nitrogen (de Groot et al. 2000) nitrogen reallocation can reduce leaf photosynthesis during reproduction (Maherali et al. 2009, Reekie et al. 2002). Because flower production differed between treatment groups, the reallocation of nitrogen may explain the observed response of photosynthesis to inoculation with AM fungi and to the addition of phosphorus in the soil. Specifically, treatment groups with the most flowers (both high phosphorus soil groups, and the inoculated low phosphorus soil group, Figure 1) also had the lowest photosynthetic rates and leaf nitrogen content (as indicated by leaf chlorophyll content; Figure 2). Thus the increased supply of phosphorus by AM fungi (Figure 3) and subsequent increase in flower production (Figure 1) may have caused reallocation of

nitrogen away from leaves, and thus a decrease in photosynthesis. The observed decrease in photosynthesis likely differs from the increase typically reported in the literature because the aforementioned studies measured photosynthesis prior reproduction (Koide and Schreiner 1994, Black et al. 2000, Auge 2001, Fay et al. 1996, Adolfsson 2015), and thus prior to the reallocation of nitrogen.

The proportion of root colonized by AM fungi did not likely cause trait responses to inoculation by AM fungi to occur in low, but not high, phosphorus treatments. This is because colonization by AM fungi did not differ between high and low phosphorus treatments (Figure 4). The lack of an association between the amount of root colonized by AM fungi and plant performance contradicts previous observations of a positive correlation between colonization and performance (Treseder 2013). High colonization by AM fungi that was similar among low and high soil phosphorus treatments may be explained by nitrogen limitation in the high phosphorus treatment. High colonization can be maintained in abundant soil phosphorus if another nutrient, like nitrogen, becomes limiting (Nouri et al. 2014). Since AM fungi can take up limiting nitrogen independently of phosphorus uptake (Mensah et al. 2015, Toussaint et al. 2003), plants may favor associating with a fungal partner despite high phosphorus levels if plant growth becomes limited by nitrogen. Plants in the high phosphorus treatment group were likely nitrogen-limited, since they had more flowers (Figure 1) and thus a greater demand for nitrogen (Reekie and Bazzaz 2005). Thus colonization by AM fungi was likely high in both low and high soil phosphorus treatments because plant growth was always limited by one of phosphorus or nitrogen.

The results do not support the hypothesis that inoculation with AM fungi and increased phosphorus available in the soil would weaken selection for phosphorus acquisition. While

shoot phosphorus content, which is indicative of phosphorus acquisition, increased in response to inoculation with AM fungi and soil phosphorus addition (as indicated by reduced shoot N:P, Figure 3), neither inoculation with AM fungi nor soil phosphorus addition influenced selection on shoot N:P (Table 6, 7B). In addition, minimal direct selection on shoot N:P suggests that shoot phosphorus content is not an important predictor of plant fitness. Total selection for reduced shoot N:P suggests that high shoot phosphorus content increases fitness indirectly through trait correlations (Conner and Hartl 2006). However, it is difficult to determine which trait correlations contributed to the indirect effects that caused a negative selection coefficient for shoot N:P, as there were no consistent patterns in trait correlations (Table 4B). While selection on shoot phosphorus content may be affected indirectly, similar selection coefficients for shoot N:P among treatment groups implies the relationship between soil phosphorus availability and selection for shoot phosphorus content is not as strong as was originally predicted.

The results do not support the hypothesis that inoculation with AM fungi would influence direct selection on photosynthesis differently depending on soil phosphorus level. Selection gradients for relative chlorophyll content and photosynthetic rate did not differ between treatment groups (Table 7), suggesting that AM fungi do not affect selection for photosynthesis, regardless of soil phosphorus availability. In addition, the minimal direct selection detected on both chlorophyll content and photosynthetic rate suggests photosynthesis does not directly affect plant fitness. Total selection for reduced photosynthesis (Table 6) suggests that photosynthesis is affected by selection indirectly through trait correlations (Conner and Hartl 2006). The existence of this indirect relationship may be the result of an abundance of correlations with other traits including mid-season size, leaf chlorophyll level, and shoot nutrients (Table 4, Ackerley et al. 2000) that then caused the opposing direction of selection gradients and differentials (Table 6, 7).

Previous work on the indirect effects of photosynthesis on plant fitness have also suggested that selection acts on suites of correlated physiological traits instead of directly targeting photosynthesis (Artnz and Delph 2001).

There was no support for the hypothesis that total selection for higher rates of photosynthesis would be influenced differently by inoculation with AM fungi depending on soil phosphorus availability. The hypothesis that selection would favor higher rates of photosynthesis did not account for the reallocation of nitrogen that occurs during the reproductive phase (Reekie and Bazzaz 2005), which may have resulted in the negative relationship between photosynthesis and fitness. Since both photosynthesis and fitness are limited by nutrients, this trade-off may be stronger in nutrient-limited plants. A negative relationship between photosynthetic rate (Y(II)) and fitness that is strengthened by nutrient limitation is illustrated in the results, where the treatment group with the fewest nutrients had the strongest selection for reduced photosynthesis (non-inoculated low phosphorus, Table 6). Reduced nutrients likely increased the negative effect of allocating scarce nitrogen away from photosynthesis, strengthening selection to reduce photosynthesis. In contrast, treatment groups with increased available nutrients had weaker selection for reduced photosynthesis (inoculated low phosphorus, and both high phosphorus treatment groups, Table 6). Increased nutrient acquisition, through a fungal partner or addition of soil phosphorus, likely reduced the cost of a plant to allocate nitrogen to photosynthesis instead of to reproductive structures, minimizing the negative association between photosynthesis and fitness. Selection differentials differed between treatment groups only with flower number as a fitness proxy (Table 6), emphasizing the effect of photosynthesis and nutrient limitations on reproductive fitness specifically.

The inability to detect differences in direct selection between treatment groups did not likely

arise from limited variation in traits. Limited trait variation reduces the ability of selection to act on traits (Conner 1988), which in turn reduces the ability to detect differences in selection. To determine whether trait variation limited selection I compared the coefficient of variance (CV) for traits that experienced significant selection and traits that did not using an independent t-test. Traits that experienced selection included mid-season size traits, and traits that did not experience selection included photosynthetic rate, leaf chlorophyll content, and shoot N:P. I calculated the coefficient of variance using standardized trait values to eliminate the influence of magnitude on CVs. Variation was equal for all traits in each treatment group, regardless of whether they were under strong selection (Table 9), suggesting that trait variation did not limit the ability to detect selection.

The inability to detect selection did not likely arise from limited variation in fitness. Natural selection requires variation in fitness, so reduced variation in fitness limits the opportunity for selection (Conner 1988). Adjusted r^2 values from phenotypic selection analyses are indicative of the variation in fitness (Conner 1988). For example, a low r^2 value implies that variation in fitness is high. I determined whether variation in fitness limited selection by regressing adjusted r^2 values from selection differential analyses against the absolute value of the differentials within each treatment group. Regression slopes were positive in all treatment groups (Table 10), suggesting that weak selection coefficients were associated with a lower adjusted r^2 value, or ample variation in fitness. Ample variation in fitness suggests there was high opportunity for selection, and that variation in fitness did not limit the ability to detect selection.

Selection on geometric volume and growth rate indicate that natural selection favours increased mid-season size when nutrients are scarce. Direct selection for increased mid-season size supports previous work on the association between high early performance and greater final

fitness (Geber and Griffen 2003, Verdu and Traveset 2005, Mungia-Rosas et al. 2011, Forrest 2014). Direct selection on mid-season size was strengthened by lower levels of phosphorus in the soil (Table 7B superscripts), suggesting that soil nutrient abundance can target selection for increased early performance. Total selection for increased mid-season size was weakened by both soil phosphorus addition and inoculation with AM fungi (Table 6 superscripts). Similar to total selection on photosynthesis, the influence of inoculation by AM fungi on total selection and not direct selection suggests that AM fungi influence selection through trait correlations.

The results suggest that there are no genetic trade-offs in being adapted to a particular environment. Maternal family performance was not correlated across environments in three out of four comparisons (Table 8). Since maternal families represent genotypes, the lack of correlations suggests that having a genotype that is well suited to a particular environment is neither costly nor beneficial to performance in another environment. While *L. siphilitica* genotypes were not adapted to specific nutrient environments, there is evidence of adaptation to different nutrient environments across species. For example, trade-offs in resource allocation strategies may help different species adapt to different nutrient environments (Aerts and Chapin 1999). Slow-growing species adapted to retain nutrients in nutrient poor environments do not do well in nutrient rich environments, while fast-growing species adapted to allocating biomass to shoots do not do well in nutrient poor environments (Chapin 1991). These adaptive strategies may occur more between than within species, which may explain why no trade-offs were observed in this single-species study.

While AM fungi did not affect selection on shoot N:P or photosynthesis, the symbiosis may be targeting other, unmeasured traits associated with the symbiosis. For example, leaf surface area is a trait that is limited by phosphorus and therefore increases following inoculation with

AM fungi (Freeden et al. 1989, Koide and Schreiner 1994). Increased leaf surface area has also been employed to explain an increase in overall photosynthesis, rather than an increase in photosynthetic rate, that helps the plant provide carbon for beneficial fungal partners (Freeden and Terry 1988, Wright et al. 1998 a,b, Adolfsson 2015). Since inoculation with AM fungi increased shoot phosphorus (Figure 3) and leaf surface area affects fitness in *Lobelia* plants (Caruso et al. 2006), inoculation with AM fungi may influence selection acting on leaf surface area.

AM fungi may not affect selection on plant traits because there is selection for the ability to form the symbiosis instead of selection by the symbiosis on plant traits. There is likely selection for the symbiosis because associating with a fungal partner increased nutrient acquisition (Figure 3) and fitness (Figure 1). If the symbiosis is more beneficial in low nutrient soil, selection for the symbiosis may be stronger in environments where nutrients are limiting. The hypothesis that selection favors the ability to associate with AM fungi in low nutrient environments is supported by the evolutionary loss of this ability in plant lineages typically found in high nutrient environments (Wang and Qiu 2006). If the symbiosis is maintained through natural selection because AM fungi provide nutrient and fitness benefits to their host plant, then the symbiosis may facilitate the transition of plants into novel environments. Thus the symbiosis between AM fungi and plants may contribute to the evolution of plant traits not directly, but by introducing plants to new environments and the variety of selective pressures therein.

Table 9:Independent t-test for equality of means of coefficient of variance for traits that experienced significant selection (geometric volume and growth rate) and traits that did not experience significant selection (Y(II), Chl, shoot N:P). Comparisons were made within each treatment group.

	Equal variance between groups		t-test for equality of means				
Treatment group	F	*P*	t	df	*P*	Mean difference	Standard error of difference
Inoculated High soil P	4.769	0.117	5.324	3.000	0.013	0.290	0.055
Inoculated Low soil P	172.131	0.001	0.819	1.041	0.559	0.256	0.313
Control High soil P	285.686	0.000	0.065	1.020	0.958	0.015	0.232
Control Low soil P	20.047	0.021	2.502	1.103	0.223	0.797	0.319

Table 10: Parameters from linear regression of adjusted r^2 values from selection differentials against the absolute value of selection differentials. Regressions were done within each treatment group for biomass and flower number separately.

Fitness Measure	Treatment Group	Regression slope	SE	*P*	Adjusted r^2
Biomass	Inoculated High soil P	3.130	.156	.000	.985
	Inoculated Low soil P	1.891	.223	.000	.922
	Control High soil P	2.453	.187	.000	.966
	Control Low soil P	1.582	.107	.000	.973
Flower number	Inoculated High soil P	1.215	.160	.001	.904
	Inoculated Low soil P	.661	.068	.000	.940
	Control High soil P	1.375	.082	.000	.979
	Control Low soil P	.483	.053	.000	.932

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