The Effects of Mycorrhizae and Soil Biota Feedback on the Outcome of Plant Competition

By

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ABSTRACT

THE EFFECTS OF MYCORRHIZAE AND SOIL BIOTA FEEDBACK ON DETERMINING THE OUTCOME OF PLANT COMPETITION

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University of Guelph, 2012

Advisor:
Professor H. Maherali

The difference in the ability of plants to obtain resources has been used to predict the competition outcomes. Competitive interactions between plants can be influenced by trophic interactions. If mycorrhizae increase the growth of inferior competitors, then it can prevent competitive exclusion. I examined the effect of mycorrhizae on competitive interactions and found that inferior competitors had slow growth in the absence of mycorrhizae, but greater growth with mycorrhizae. By providing greater growth responses to inferior versus superior competitors, mycorrhizae promoted coexistence. In a separate experiment, I looked at the effects of whole soil biota on competitive ability. Coexistence can occur if dominant competitors experience negative feedback and or if inferior competitors experience positive feedback. I examined *Plantago lanceolata* L. specific feedback effects on competitive ability of 21 co-occurring species. I found that feedback effects did not affect hierarchies, and that positive feedback did not improve species competitive ability.

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CHAPTER I: THE EFFECTS OF MYCORRHIZAE ON DETERMINING THE OUTCOME OF PLANT COMPETITION

ABSTRACT

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Competition is an often studied interaction between plants because of its implications for species coexistence. The difference in the ability of plants to obtain resources has been used to predict the outcome of competition. However, resource partitioning as a determinant of the outcome of competition has received mixed support. If mycorrhizae increase the growth of inferior competitors, then it can prevent competitive exclusion. In a greenhouse experiment, I examined competitive interactions between 22 species grown with or without mycorrhizae. I found that mycorrhizae changed hierarchies built on competitive ability by increasing growth of inferior competitors. Inferior competitors had slow growth in the absence of mycorrhizae, but greater growth in the presence of mycorrhizae. Superior competitors did not benefit from mycorrhizae. By providing greater growth responses to inferior versus superior competitors, mycorrhizae promoted coexistence. In the absence of mycorrhizae, nutrient uptake and root traits did not determine competitive ability.

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Introduction

Competitive interactions between plants are often studied because of their potential to shape species patterns and abundance (Tansley, 1917; Goldberg and Barton, 1992; Aplet and Laven, 1993; Howard and Goldberg, 2001; Mayfield and Levine, 2010). Competition between plants is relatively frequent (Goldberg and Barton, 1992) and has a negative effect on biomass (Gurevitch *et al.*, 1992). For example, the removal of competitively dominant species from a community encourages the growth of species initially excluded from that community (Goldberg and Barton, 1992). The difference in the ability of plants to obtain resources, such as light, water and nutrients, has been used to predict the outcome of competition (Tilman, 1982; Harpole and Tilman, 2006; Fargione and Tilman, 2006; Johnson, 2010). However, the partitioning of abiotic resources as a sole determinant of the outcome of competition has received mixed support (Miller *et al.*, 2005; Bever *et al.*, 2010). The outcome of competition is also mediated by a plant's interaction with other trophic levels, such as herbivores, mutualists and pathogens (van der Putten and Peters, 1997; Umbanhowar and McCann, 2005; Gurevitch *et al.*, 2006; Gross *et al.*, 2010).

Trophic interactions that affect resource uptake ability or consume plant tissue can alter competitive interactions between plants (Umbanhowar and McCann, 2005; van der Putten and Peters, 1997). Herbivores can cause a plant to switch from being light limited to being soil resource limited by consuming plant tissue (Wise and Abrahamson, 2005; Bagchi and Ritchie, 2011). Furthermore, both herbivores and pathogens can mediate competition by preferentially consuming or infecting one species and reducing its competitive ability (van der Putten and Peters, 1997; Bagchi and Ritchie, 2011). Predator mediated coexistence can occur if a predator preferentially consumes a dominant competitior (Gause, 1934; Paine, 1966; Holt *et al.*, 1994). If a species is an inferior competitor in terms of resource uptake but is better at withstanding herbivory, then it will be able to coexist with a stronger competitor for resources that experiences more severe herbivory (Holt *et al.*, 1994). By negatively affecting a dominant competitor, trophic interactions can promote coexistence between strong and weak competitors (Bever *et al.*, 2010).

One way in which trophic effects can directly affect resource uptake ability in plants is through interactions with root inhabiting soil fungi. Arbuscular mycorrhizal (AM) fungi form symbioses with 73% of all vascular plant species (Brundrett, 2009). Mycorrhizae can provide plants with limiting nutrients because their hyphae extend past the resource depletion area of roots (Hetrick, 1991). Plants receive phosphorus, nitrogen and other micronutrients in exchange for photosynthate (Hartnett and Wilson, 1999; Smith and Read, 2008; Johnson, 2010). By providing plants with limiting resources, mycorrhizal symbioses have the potential increase competitive ability for soil resources (Bever *et al.*, 2010; Johnson, 2010). However, whether a mycorrhizal association is beneficial or not depends on the identity of the plant and its fungal symbiont (Bever, 2002; Klironomos, 2003). Species that are superior competitors in the absence of mycorrhizae will experience lower growth response from symbiosis with because mycorrhizae will not provide enough nutrients to exceed the carbon cost (van der Heijden *et al.*, 1998; Umbanhowar and McCann, 2005). Thus, coexistence will occur if dominant non-mycorrhizal competitors fare poorly when in competition with mycorrhizal-dependent competitors (Umbanhowar and McCann, 2005; Gross *et al.*, 2010).

How mycorrhizae interact with their hosts depends in part on the plants' root morphology and architecture (Hetrick, 1991; Zangaro *et al.*, 2005). Morphological traits can also be used to understand the mechanisms involved in plant competition (Goldberg, 1996; Wang *et al.*, 2010). Competition for resources will depend on traits related to nutrient acquisition, and these traits can predict the outcome of competition (Venterink and Güsewell, 2010). Root and leaf traits will affect the nutrient acquisition, availability and economy of a plant (Hetrick, 1991; Fargione and Tilman, 2006; Gross *et al.*, 2010). For example, plants that are superior competitors for below-ground resources tend to allocate more biomass to roots and have higher root to shoot ratio, increased root fineness, increased specific root length and more abundant root hairs. Traits such as these confer plants a competitive advantage in nutrient acquisition, and species with these traits do not usually rely on mycorrhizal symbioses for nutrient uptake (Hetrick, 1991; Zangaro *et al.*, 2007). Conversely, plants that allocate less biomass to root structures and have lower root to shoot ratios, coarser roots and lower specific root length are inferior competitors in terms of nutrient

acquisition. Their root architecture is conducive to mycorrhizal colonization, and these species often have positive responses to mycorrhizae (Hetrick, 1991).

Current theory suggests that mycorrhizae can mediate competitive interactions between plants by mediating resource acquisition (Umbanhowar and McCann, 2005). However, most studies have examined plant competition in different nutrient environments (Venterink and Güsewell, 2010; Wang *et al.*, 2010), or the effect of mycorrhizae on plant competition and abundance (Moora and Zobel, 1996; Hartnett and Wilson, 1999; Zangaro *et al.*, 2007), but not the effect of mycorrhizae on both competition and nutrient uptake. I hypothesized that if mycorrhizae affect coexistence between plants by mediating competitive interactions, then species that have the greatest positive growth response to mycorrhizae will be inferior competitors in the absence of the symbiosis. Without mycorrhizae, species that deplete most resources from the soil should be the strongest competitors (Tilman, 1982). Thus, mycorrhizal association should change the hierarchy of competitive performance of species by providing greater benefits to inferior competitors. If mycorrhizae increase the growth of inferior competitors, then these species should take up more nutrients when in associated with mycorrhizae. If the uptake of below-ground resources is important in determining the competitive performance of species, then superior competitors will possess finer roots and invest more in root biomass, which are traits that are associated with greater nutrient uptake. These species will rely less of mycorrhizal association for nutrient uptake.

Materials and Methods

Study System

I tested competitive interactions between 22 species commonly found in old fields of Southern Ontario, Canada (Table 1). The seeds of these species were collected from the Long Term Mycorrhizal Research Site (LTMRS) in Guelph, Ontario, from August- October 2010 or ordered from Richter's Herbs (Goodwood, Ontario). These study species were chosen based on seed availability and germination success in the greenhouse. LTMRS (43° 32' 3.5", -80° 12' 41.8") is an old field that is phosphorus limited (Sherrard, 2010; Maherali and Klironomos, 2012). This site had been cultivated until 1967

(Maherali and Klironomos, 2012). Because the soil from this field is resource-poor, mycorrhizal symbioses are more likely to affect the fitness of plants in this environment by providing limiting nutrients to their hosts (Johnson, 2010).

Experimental Design

To determine the effects of mycorrhizae and competition affect the hierarchy of competitive performance of my target species, I grew each species with or without competition from a phytometer (or indicator), *Plantago lanceolata* L., in mycorrhizal and non-mycorrhizal soil. I chose *P. lanceolata* as the phytometer species because previous studies have shown that mycorrhizae affect its competitive ability (Bever, 2002). The above treatments resulted in 22 species * 2 competition conditions * 2 soil treatments were arranged in a randomized complete block design. There were a total of three blocks, each containing two replicates, resulting in a total 528 experimental units (pots). The replicates were split into three blocks due to the large number of plants that needed to be transplanted. Each set of replicates were planted per week, one week apart, and were arranged in a completely randomized fashion on one greenhouse bench each in the Guelph Phytotron (Phytotron, University of Guelph, Guelph, Ontario). Thus, each replicate represented a block in time and space. Each set of replicates was grown for 10 weeks, and the experiment ran for a total of 12 weeks.

I used the phytometer method to quantify competitive interactions between my study species because it requires fewer treatments than comparing pair wise interactions (Keddy *et al.,* 2002). . Hierarchies of competitive ability are positively correlated with species abundance, which suggests that competition is important in determining community structure (Aplet and Laven, 1993; Howard and Goldberg, 2001; Fargione and Tilman, 2006). Mapping competitive interactions onto hierarchies requires testing species pair-wise interactions. However, when testing interactions between large numbers of species, exploring all possible pair-wise outcomes will result in a very large experiment that only uses a few species. For example, six competition pairs will result from testing pair-wise interactions between four species, whereas 190 pairs will result from testing twenty species. The phytometer method has been

used to simplify this procedure: competitive hierarchies are ranked according to the mean competitive ability of species tested against a phytometer (or indicator) species (Keddy *et al.*, 1994; Keddy *et al.*, 2002, Wang *et al.*, 2010). This method allows to simultaneously test numerous species interactions in a common environment (Keddy *et al.*, 2002).

In order to have a soil background of similar resource background to that in the field, I collected 400 L of native soil for the treatments from 15 sampling locations in LTMRS. The soil was collected in May of 2011. I sieved then sterilized this soil by autoclaving for two hours. The final soil for the treatments contained two thirds of the sterilized LTMRS soil and one third sand that was also sterilized by autoclaving for two hours.

For the mycorrhizal treatment, I cultured the mycorrhizal fungi, *Glomus intraradices*, on leek (*Alium ampeloprasum* L.). The roots of leek can be heavily colonized by many species of mycorrhizae, making an appropriate inoculum medium (Snellgrove *et al.*, 1982). The mycorrhizal treatment received chopped leek roots colonized by *G. intraradices*, whereas the non-mycorrhizal treatment received colonized leek roots that were sterilized by autoclaving for 4 hours (avg. 22 grams of inoculum per pot). This treatment was used as a control for the mycorrhizal treatment. To determine if the colonization was present in the mycorrhizal treatment and absent in the non-mycorrhizal treatment, I assessed the percent colonization of 176 root samples. I randomly selected four root samples of each species in each soil treatment, two for the each species when grown alone and two when grown with *P. lanceolata*. I used the gridline intersection method to quantify the presence or absence of non-septated hyphae, vesicles and arbuscules at 50 intersections per root sample (McGonicle *et al.*, 1990). The roots were cleared using a 10% solution of potassium hydroxide then stained with a 5% ink and vinegar solution using Black Shaeffer ink (Cult Pens, The SQL Workshop, Unit 5 Tiverton Trade Centre, Lowman Way, Tiverton, EX16 6SR, UK). After staining, the roots were rinsed with tap water and acidified with a few drops of vinegar (Vierheilig *et al.*, 1998).

In order to quantify the mechanisms of competition, I measured early growth biomass and root architecture traits (root diameter, Specific Root Length and root to shoot ratio) in a separate experiment. I chose these specific root traits because Hetrick (1991) has shown that these traits influence the mycorrhizal growth response of plants. Ten replicates of each species were grown in an autoclaved mixture of 2/3 silica sand and 1/3 topsoil. I used a greater concentration of sand so I could remove the roots without damaging them. The sand allows the soil to easily wash off roots. Because the seeds for all species were allowed to germinate at the same time in plug trays, seedlings were transplanted to larger pots once they were large enough to handle. The pots were completely randomized on a greenhouse bench in the Phytotron. The plants were allowed to grow for three weeks from transplant, such that the roots did not become pot-bound. I harvested the roots after the growing period and stored them in 50% ethanol, and I dried the shoots at 60°C for 72 hours. To analyze root architecture, I dyed the roots with 0.05% Toluidine Blue O solution and scanned their image into WinRhizo (version 2009a; Régent Instruments, 2009). I calculated specific Root Length (SRL) as the total root length (cm) per gram of root biomass (which was dried and weighed after scanning). In addition, I calculated the root to shoot ratio as the total root biomass divided by the total shoot biomass. WinRhizo directly calculated values average root diameter that did not require further transformation.

To determine if competition for below-ground resources was important in predicting which species are superior competitors, I measured the amount of nutrients each species used. The resource competition theory predicts that species that can decrease resource levels in soil and survive at those levels are superior competitors. This level of resource has been termed R*; the best competitor for a resource will have the lowest R* value for that resource (Tilman, 1982; Miller *et al.,* 2005; Johnson, 2010). I calculated the difference in nitrate, phosphorus, ammonium, magnesium and potassium content of the LTMRS soil prior to planting and after harvest for the mycorrhizal and non-mycorrhizal soil treatments. The 300g soil sample analyzed per treatment prior to planting included 100g samples from each block. Each 100g from each block was obtained after inoculum was added and the soil and inoculum was thoroughly mixed. For the soil collected after harvest, I used soil from the roots of each species

grown alone pooled across all 3 blocks, ranging from 4 to 6 samples per species, per soil treatment. Thus, for each species, I had a pooled soil sample for the mycorrhizal soil treatment and pooled sample for the non-mycorrhizal soil treatment. The soil was removed from roots by sieving the roots out with 4mm and 2mm sieves. The difference between soil resource content before transplant and after harvest will hereafter be referred to as R*, modified from Tilman (1982). The soil samples were analyzed by University of Guelph Laboratory Services.

Statistical Analysis

To determine the effects of competition, soil treatment and species identity for their effects on above-ground biomass for target species I used a three-way ANOVA with Type III sum of squares (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). To determine the effects of soil treatment and species identity on the biomass of the phytometer, *Plantago lanceolata*, I used a two-way ANOVA with Type III sum of squares. This analysis was two-way because there was only one competition treatment for *Plantago lanceolata* . In order to fulfill the assumptions of ANOVA, I performed Levene's Test to test for homogeneity of variances between groups of target species biomass grown with or without mycorrhizae and grown with or without the phytometer. I found that the variances between target species biomass between growing with or without mycorrhizae were not significantly different ($p=0.667$). However, variances differed between target species biomass grown with or without the phytometer ($p=0.003$). I also tested homogeneity of variance for the biomass of *P. lanceolata* between growing with or without mycorrhizae and found that the variances differed between these groups ($p=0.029$). Thus, some of the assumption of ANOVA were violated, creating artificially low p values, and increasing Type-I error. I analyzed the significance of main and interaction effects using the Tukey's HSD (Honestly Significant Difference) post-hoc comparison.

To quantify competitive ability, I measured the competitive effect and competitive response values. A species' competitive performance can be described in terms of its ability to suppress the growth of neighbours (competitive effect) and the ability to withstand suppression from neighbours (competitive

response)(Goldberg and Landa, 1991; Wang *et al*, 2010). Competitive effect has been associated with a suite of morphological traits, whereas competitive response has been linked to a species' ability to tolerate competition and environmental conditions (Wang *et al.*, 2010). However, I measured both competitive effect and response because these values are usually positively correlated and consistent in the same environment (Goldberg, 1996; but see Keddy *et al.*, 1998). I calculated competitive effect of target species (CE) by taking the natural log of the mean dried weight of the phytometer grown alone (P) divided by the phytometer grown with a target species (P_T) (Eq.1). Similarly, I calculated competitive response (CRS) as the natural log of mean biomass of target species when grown with the phytometer (T_P) divided by the mean biomass of target species grown alone (T) (Eq. 2).

$$CE = \ln\left(\frac{P}{P_T}\right) \qquad \text{(Eq. 1)}$$

$$CRS = \ln\left(\frac{T_P}{T}\right) \qquad \text{(Eq. 2)}$$

Higher values of competitive effect (CE) represent species that are better competitors against the phytometer. Higher values of competitive response (CRS) represent species that are better able to withstand competition from the phytometer. Greater values of the competitive ability of *P. lanceolata* (CA) represent greater competitive effect of the phytometer on target species, which is the inverse measure of target species competitive effect. To determine which traits were associated with competitive effect and response, I regressed traits against competitive effect and response for each soil treatment. I used ANCOVAs to determine whether these relationships were different in mycorrhizal versus non-mycorrhizal soil treatments. Prior to running the regressions, I removed 10 outlier data points from the traits data using the Bonferroni outlier tests. Bonferroni outlier test reports the p-value of the most extreme observations which are considered outliers if the p-value is less than 0.05. The Bonferroni adjustment multiplies the usual two-sided p-value by the number of observations (R, R Development Core Team, 2012, car package, Fox and Weisberg, 2011). Before removing outliers, species values for Specific Root Length and average root diameter were positively correlated. However, because these two

traits are both measures of root fineness, they should be negatively correlated (i.e. finer roots have lower average root diameter and higher Specific Root Length) (Hetrick, 1991). Outliers were removed on the basis of having a significant Bonferroni adjusted p value for linear regressions between (1) early growth biomass and root biomass, (2) Specific Root Length and root biomass and (3) Specific Root Length and average root diameter. After the removal of 5 outliers from the 3rd set of data, average root diameter and SRL were negatively correlated. The regressions between competitive effect values and traits (see Appendix A, Tables 1&2), and competitive response values and traits (see Appendix A, Tables 3&4) were not significantly changed by the removal of outliers.

To test whether hierarchies for competitive effect and competitive response changed in the in response to mycorrhizal colonization, I ran regressions to test whether competitive effect and response were correlated between the mycorrhizal and non-mycorrhizal soil treatments. To determine if species response to mycorrhizae was correlated with their competitive ability, I ran regressions between competitive effect and response in either soil treatment with mycorrhizal response. To determine if morphological traits influenced mycorrhizal response, I ran regressions between SRL, average root diameter, root to shoot ratio and early growth biomass with mycorrhizal response. I calculated mycorrhizal response as the natural log of the biomass of target species in mycorrhizal soil divided by the biomass of target species in non-mycorrhizal soil (Hoekesema *et al.*, 2010)(Eq. 3):

$$mycorrhizal\ response = \ln\left(\frac{mycorrhizal\ biomass}{non-mycorrhizal\ biomass}\right) \text{ (Eq. 3)}$$

To determine whether competitive performance or response to mycorrhizal symbiosis could predict the abundance of species in the field, I used a Pearson correlation between competitive effect, competitive response and mycorrhizal response with species abundance data (Table 2). The species abundance was determined by noting presence or absence of a species at 100 randomly placed one meter squared plots at LTMRS (Klironomos, 2002).

To determine whether R* values for ammonium, nitrate, phosphorus, magnesium and potassium influenced a species competitive ability, I ran regressions between the R* values for each nutrient with

competitive effect and response. To determine whether morphological traits were correlated with nutrient uptake ability, I ran regressions between R* values for each soil nutrient and species values for SRL, Root to Shoot ratio, average root diameter and early growth biomass. I used ANCOVAs to test if there were differences in these relationships between the two soil treatments. I performed all statistical analyses in R (R Development Core Team, 2012) and SPSS because data manipulation was more flexible to perform in R whereas the methodology for some statistical analyses was easier to understand in SPSS.

Results

I found that mycorrhizal colonization occurred, thus I could analyze the effect of mycorrhizae on plant competition. In the mycorrhizal soil treatment, species had an average of 68.24% (SE=3.86) colonization by mycorrhizae. In the non-mycorrhizal soil treatment, species had an average of 0.76% (SE=0.38) colonization. Mycorrhizal colonization was significantly different between the two soil treatments (significant AM presence effect, $F_{(1, 80)}$=239.44, p=0.000), but did not differ between species (non significant Species effect $F_{(21, 80)}$=0.832, p=0.667).

Growth with *P. lanceolata* had a greater overall impact on the aboveground biomass of target species in the mycorrhizal soil treatment compared to the non-mycorrhizal soil treatment (significant AM presence*Competition interaction, Table 2, Fig. 1). Across all soil treatments, target species had 48% lower biomass when grown with the phytometer, *Plantago lanceolata*, than when grown alone (significant Competition effect Table 2, Fig. 1). Target species biomass was 44% greater in the mycorrhizal soil treatment compared to the non-mycorrhizal soil treatment (significant AM presence effect, Table 2, Fig. 1.)In the mycorrhizal soil treatment, target species biomass was 23% lower when grown with *P. lanceolata* (P<0.05, Tukey HSD) than when grown alone, whereas a non-significant difference of 17% (P>0.05, Tukey HSD) was observed in the non-mycorrhizal soil treatment (Fig. 1). In

the mycorrhizal soil, the growth of *Trifolium pratense* was 59% lower when grown with *P. lanceolata* than when grown alone. Conversely, *Clinopodium vulgare* L. had 18% greater biomass when grown with *P. lanceolata* than when grown alone (Fig. 2.A). In the non-mycorrhizal soil treatment, *Bromus inermis*, *Poa compressa* L. and *Solidago canadensis* L. had greater biomass when grown with *Plantago lanceolata* than when grown alone (Fig 2.B). Conversely, the biomass of *Hieracium pilosella* L. was 179% lower when it was grown with *Plantago lanceolata* than when grown alone (Fig. 2.B).

The phytometer, *Plantago lanceolata*, had greater biomass in the presence of mycorrhizae, but only when in competition with target species. Although the biomass of *P. lanceolata* was 40% greater in the mycorrhizal soil when grown alone, this result was not statistically significant ($p=0.22$, Fig 3). The biomass of *P. lanceolata* was lower when grown with target species in the absence of mycorrhizae. When grown with target species, *P. lanceolata* biomass was 89% greater in the mycorrhizal soil treatment compared to the non-mycorrhizal soil treatment (significant AM presence effect, Table 3, Fig. 3). *P. lanceolata* biomass varied when grown with different target species (significant Species effect, Table 3), however, this variation did not change between soil treatments (non-significant AM presence*Species interaction, Table 3).

Species with greater competitive effect values had a lower biomass response when grown in the presence of mycorrhizae. In addition, these species also had greater early growth. The mycorrhizal response of species when grown alone was negatively correlated with their competitive effect size in the non-mycorrhizal soil (Fig. 4.A). However, there was no relationship between mycorrhizal response values and competitive effect values in the mycorrhizal soil treatment (Fig. 4.A). Conversely, the mycorrhizal response was not correlated with species competitive response values in either soil treatments (Fig. 4.B). Species that had lower early growth had greater response to mycorrhizae (Fig. 5.D). However, root traits including Specific Root Length (SRL), root to shoot ratio and average root diameter were not correlated with species mycorrhizal response (Fig. 5.A-C). Moreover, the competitive ability of *P. lanceolata* was negatively correlated with the competitive response of species in the mycorrhizal and non-

mycorrhizal soil treatment, but was not correlated with competitive response in the non-mycorrhizal soil treatment (Table 9).

The presence of mycorrhizae altered the competitive effect and competitive response values of species. The competitive effect sizes of species in the mycorrhizal soil treatment were not correlated with competitive effect sizes in the non-mycorrhizal soil treatment (Fig 6.A) and were significantly different (paired t test, $p<0.05$). The competitive response sizes of species in the mycorrhizal soil treatment were not correlated with the competitive response sizes in the non-mycorrhizal soil treatment (Fig. 6.B) and were significantly different between the two soil treatments (paired t test, $p<0.05$). In addition, both the competitive effect and response values in the mycorrhizal soil treatment were significantly lower than the competitive effect and response values in the non-mycorrhizal soil treatment (paired t tests, $p<0.05$).

Only some root traits predicted the competitive performance of species in the absence of mycorrhizae. SRL, root to shoot ratio and average root diameter were not correlated with species competitive effect values in the non-mycorrhizal soil treatment (Table 7, Fig. 7). Species competitive response values were also not predicted by SRL and root to shoot ratios in non-mycorrhizal soil (Table 7, Fig. 8). In the non-mycorrhizal soil species with greater average root diameters had lower values of competitive response (Table 7, Fig. 8). However, this relationship depends on a single out-lying data point with high leverage: if the data point is removed, the relationship becomes non-significant. The difference between slopes for the regressions between competitive response and average root diameter in mycorrhizal versus the non-mycorrhizal soils was significantly different (ANCOVA, $p<0.05$, Table 7). In the mycorrhizal soil treatment, species with greater root to shoot ratios had both greater competitive effect and competitive response values (Table 6, Figs. 8 & 9).

Root traits did not predict resource uptake or competitive performance in the absence of mycorrhizae. Species competitive effect and competitive response values were not correlated with uptake of ammonium (NH_4), nitrate (NO_3), phosphorus (P) or potassium (K) in non-mycorrhizal soil (Table 5)..

Only some root traits were associated with resource uptake in non-mycorrhizal soil. Species with greater SRL values took up more ammonium and less phosphorus in non-mycorrhizal soil (Table 8). The regression slopes between SRL and ammonium uptake were significantly different between the two soil treatments (ANCOVA, Table 8). Species with greater root to shoot ratios took up more nitrate in non-mycorrhizal soil (Table 8). In the mycorrhizal soil, species with greater competitive effect and response values were correlated with reduced magnesium (Mg) uptake in the mycorrhizal soil (Table 4). In this soil treatment species with greater early growth biomass took up more nitrate and less magnesium (Mg) in the mycorrhizal soil treatment (Table 8).

The response to mycorrhizae and the competitive performance of species did not predict species abundance in the field. The competitive effect and competitive response of species in both the mycorrhizal and the non-mycorrhizal soil treatments were not correlated their abundance in the field (Table 6). Similarly, mycorrhizal response was also not correlated to the abundance of species in the field (Table 6).

Discussion

I found that mycorrhizae changed the hierarchies of competitive effect by providing greater benefits to inferior competitors. The competitive effect of species in mycorrhizal soil treatment was not correlated with competitive effect in the non-mycorrhizal soil (Fig. 6.A). Thus, species ability to suppress *P. lanceolata* was altered by the presence of mycorrhizae. Mycorrhizae changed the hierarchy of competitive performance by providing different growth responses to each species. Species that were weak competitors against *P. lanceolata* in the absence of mycorrhizae had the greatest mycorrhizal response when grown alone (Fig. 4.A). Similarly, species that were strong competitors against *P. lanceolata* had the lowest mycorrhizal response. Thus, by improving the competitive performance of inferior compared to superior competitors, mycorrhizae evened out the competitive performance of species (Fig 4.A). In

doing so, mycorrhizae can promote coexistence by preventing competitive exclusion This finding is consistent with theory which predicts that mycorrhizae can promote coexistence between superior and inferior competitors when a trade-off exists between plant and fungal benefit (Hart *et al.*, 2003; Umbanhowar and McCann, 2005; Bever *et al.* 2010).

Only some studies have found that competitive hierarchies are consistent between environments (Goldberg, 1996). If competitive hierarchies are not consistent between environments, then these hierarchies are not determined by specific plant traits (assuming that hierarchies are consistent within an environment and that competitive ability is a property of a particular taxon, rather than a combination of taxa) (Goldberg, 1996; Gurevitch *et al*., 2006). In my study, mycorrhizae altered competitive hierarchies, suggesting that benefit from mycorrhizae rather than plant traits were important in determining species competitive effects. However, this trend only applied to the competitive effect sizes of species. Although the competitive response values in the mycorrhizal soil treatment were not correlated with competitive response values in the non-mycorrhizal soil, this was not due to the presence of mycorrhizae: competitive response values were not correlated with species mycorrhizal response in either soil treatments (Fig 4.B). Thus, mycorrhizal association changed a species' ability to suppress *P. lanceolata* but did not change the ability to withstand competition from *P. lanceolata*.

One way that mycorrhizae can promote coexistence is by providing greater growth response to slower growing species compared to faster growing species. Plant biomass is a good predictor of a plant's competitive ability (Gaudet and Keddy, 1995) and larger neighbours tend to have disproportionately negative effects on the biomass of smaller neighbouring plants (Moora and Zobel, 1996; Gurevitch *et al.*, 2006). Moreover, fast growing species tend to take up nutrients faster (Aerts, 1999). I found that slower growing species had greater growth responses from mycorrhizal association when grown alone (Fig. 5). Moreover, slower growing species were inferior competitors, but only in the absence of mycorrhizae (Fig. 7.D). Thus mycorrhizal symbiosis can promote coexistence between faster and slower growing species by

providing greater benefits to slower-growing species. Although mycorrhizae increased the overall magnitude of competition, it also improved the competitive performance of slower-growing species.

I found that measures of competitive effect and competitive response provided different insights on how mycorrhizae affected competitive ability. Some studies have found that species that had greater values of competitive effect also had greater values of competitive response (Novoplansky and Goldberg, 2001; Wang *et al.*, 2010), but I found this to only be the case in the presence of mycorrhizae (Table 9). Competitive effect values were negatively correlated with mycorrhizal response in the absence of mycorrhizae (Fig. 4.A) whereas competitive response values were not (Fig. 4.B) In addition competitive effect values were correlated with early growth biomass in the absence of mycorrhizae (Fig. 7.D), whereas competitive response values were not (Fig. 8.D). Thus, only competitive effect values represented a species overall competitive ability. This is consistent with previous studies that have found that competitive response values are representative of a species' tolerance of environmental conditions, and fundamentally different from a species' competitive effect (Keddy *et al.*, 1998; Wang *et al.*, 2010). Thus, target species suppressed, but were not suppressed by *P. lanceolata* in the non-mycorrhizal treatment.

The competitive performance of species in the absence of mycorrhizae was not successfully predicted by root traits associated with nutrient uptake. Contrary to my prediction, competitive effect sizes were not correlated with measures of Specific Root Length, root to shoot ratio and average root diameter in the absence of mycorrhizae (Fig. 7.A-C). Other studies have found that competitive effect is correlated with size traits in high nutrient environments and root traits in low nutrient environments (Aerts, 1999; Wang *et al.*, 2010). Since I found that competitive effect was correlated with fast growth but not root traits, it would suggest that early fast growth was more important than root traits in determining competitive effect in the absence of mycorrhizae. Furthermore, competitive response sizes of species were negatively correlated with greater average root diameter values in the non-mycorrhizal soil treatment (Fig. 8.C). Thus, species with thicker roots did not resist suppression from growing with *P.*

lanceolata in the absence of mycorrhizae. In the absence of mycorrhizae, species respond to low nutrient availability by developing finer roots (increased specific root length and decreased root diameter) (Zangaro *et al.,* 2007). I found that both measures of root fineness (specific root length and average root diameter) did not predict species competitive effect or response sizes in the absence of mycorrhizae (Fig. 7.A & C). These findings are not consistent with other studies that have found that species with thicker roots tend to be poorer competitors and rely more on mycorrhizal symbiosis for acquiring limiting soil resources (Hetrick, 1991; Garnett *et al.,* 2009) .

Mycorrhizal association increased the competitive performance of species with greater root to shoot ratios. Although increased root to shoot ratios are generally considered an alternative strategy that plants adopt in nutrient poor soils in the absence of mycorrhizae (Hetrick, 1991; Aerts, 1999; Johnson, 2010), I found that species with greater values of root to shoot ratio had the greatest growth response from mycorrhizae, which increased their competitive effect and competitive response. These results are consistent with those of Zangaro *et al.,* (2005) that found that mycorrhizal response was positively correlated with root to shoot ratios. Furthermore, I found that when grown in the absence of mycorrhizae, species with greater root to shoot ratios took up more nitrate (NO_3) (Table 8), although this did not increase species ability to suppress the growth of- or resist suppression from- *P. lanceolata*. Mycorrhizal symbiosis provided greater benefits to species that had low growth rate and greater root to shoot ratios. However, growth rate and root to shoot ratio values were not correlated ($F_{(1,19)}$ =1.83, p=0.19, b=0.16, R^2=0.08), suggesting that species that benefited from mycorrhizal symbiosis either had low growth rates or high root to shoot ratios, but not both.

The hierarchies of competitive effect in the absence of mycorrhizae values did not predict species abundance in the field (Table 6), suggesting species competitive ability in the absence of mycorrhizae (i.e. ability to take up resources) did not determine abundance. This is consistent with studies that have found that competitive effect values derived from adult growth in pair-wise species comparisons did not predict hierarchy of abundance in the field (Howard, 2001; Engel and Weltzin, 2007), although

other studies have found a positive correlation between hierarchies and species abundance (Aplet and Laven, 1993; Howard and Goldberg, 2001; Fargione and Tilman, 2006). In addition, competitive effect values in the mycorrhizal soil also did not predict species abundance in the field. This can be due to the fact that hierarchies of competitive performance derived from experiments that test plant responses in the presence or absence of mycorrhizae are better predictors of how plants assemble in early successional communities, where the soil is disturbed and mycorrhizae are usually patchy (Hetrick *et al.*, 1989; Hartnett and Wilson, 1999; Hart *et al.*, 2003). This also suggests that species abundance in the field is also affected by organisms other than mycorrhizae, such as pathogens, as found by Klironomos (2002).

Resource uptake was not a mechanism for species competitive performance in either the presence or absence of mycorrhizae. Contrary to my predictions, species that were superior competitors against *P. lanceolata* did not take up more nutrients when grown alone in the absence of mycorrhizae, thus nutrient uptake did not confer a competitive advantage to target species. However, the presence of *P. lanceolata* had negative effects on the biomass of target species, suggesting that competition occurred, although not for resources measured in this experiment. However, I measured resources used at the end of the experiment, but the best predictor of competitive ability early fast growth, which occurred at the beginning of the experiment. This suggests that I did not capture resource uptake during the time period in which it was most critical in determining competitive ability, i.e., in the first few weeks of growth. This finding is not consistent with the resource competition theory that suggests that the species that take up more resources are superior competitors for that resource (Tilman, 1982; Miller *et al.*, 2005; Johnson, 2010). Other studies suggest that plant traits related to nutrient retention, such as nitrogen use efficiency, are more important in determining competitive performance in resource poor environments than those that increase resource uptake (Aerts, 1997; Aerts, 1999). Thus, it is possible that nutrient uptake traits are important in determining competitive ability during early growth, whereas nutrient retention traits can be important during the remainder of a plant's lifetime, once nutrient depletion has occurred in the plant's immediate surroundings. This would be consistent with the fact that plants experience trade-offs between

ability to take up nutrients and ability to retain them (Aerts, 1999). If different traits are more important at determining competitive ability during different life stages of a plant, then future research should investigate whether mycorrhizae affect these traits.

Mycorrhizal symbiosis increased magnesium uptake for species that were poorer competitors. In the presence of mycorrhizae, species that took up more magnesium when grown alone had smaller values for competitive effect and competitive response. So far, no other studies have confirmed that mycorrhizae can improve magnesium nutrition in plants (Marschner and Dell, 1994; Karasawa *et al.*, 2012). Magnesium is an important component of chlorophyll and acts as a cofactor in many enzymatic reactions. Thus, a deficiency in magnesium can affect the metabolic processes of plants (Marschner, 1995; Chou *et al.*, 2011). Plants generally benefit from mycorrhizal symbiosis through improved phosphorus and nitrogen nutrition in exchange for carbon (Hartnett and Wilson, 1999; Yoshida and Allen, 2001; Smith and Read, 2008; Johnson, 2010), however, the presence of mycorrhizae did not increase uptake of phosphorus and nitrogen in this study.

The results of this study suggest that mycorrhizae can affect competitive interactions between species by changing hierarchies of competitive ability. Changes in competitive hierarchies between environments can provide insight on whether the environment or intrinsic plant traits are important in influencing competitive interactions between plants (Goldberg, 1996; Gurevitch *et al.*, 2006). A literature review indicates that not all studies of hierarchies between different environments are consistent (Goldberg, 1996). For example, Wang *et al.*, (2010) found that hierarchies of competitive effect were consistent between different fertility environments, whereas I found that hierarchies of both competitive effect and response were not consistent between soils with the presence and absence of mycorrhizae. Moreover, Zanagaro *et al.*, (2007) show that a plant's response to mycorrhizae rather than soil fertility is more important to plant establishment. I also found that species response to mycorrhizae predicted their competitive effect ability. The correlation between mycorrhizal response and competitive effect, taken together with the change in competitive hierarchy in the presence of mycorrhizae, suggests that species

response to mycorrhizae was an important factor in determining competitive interactions between plants. In addition, mycorrhizal response was greatest for slow growing species, which improved their competitive ability, since they were inferior competitors in the absence of mycorrhizae. Fast-growing species were superior competitors in the absence of mycorrhizae and received fewer benefits from mycorrhizal symbiosis. Thus, mycorrhizae changed hierarchies of competitive ability by providing greater benefits to poor competitors in terms of growth rate, suggesting that mycorrhizae can promote coexistence between inferior and superior competitors. This can occur from a potential trade-off between mycorrhizal benefit and growth rate. Other studies have shown that there can be significant metabolic costs of maintaining symbiosis with mycorrhizae (Lynch and Ho, 2005; Zangaro *et al.*, 2007). Thus future research should explore whether trade-offs exist between plant growth and mycorrhizal benefit in more detail, and if mycorrhizal benefit is correlated to life-history traits.

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Table 1. Family names, species name and common name of 22 species and their abundance data as measured at the Long Term Mycorrhizal Research Site (LTMRS), in Guelph, Ontario. Species abundance data collected by noting the presence or absence of each species at 100 randomly placed one square meter plots at LTMRS (Klironomos, 2003). * - the seeds of these plants were ordered from Richter's Herbs.

Family	Species Name	Common Name	Life History	Abundance
Asteraceae	*Achillea millefolium* L.	Yarrow	Perennial	55
Poaceae	*Bromus inermis* Leyss	Smooth brome	Perennial	81
Asteraceae	*Centaurea jacea* L.	Brown Knapweed	Perennial	29
Asteraceae	*Cichorium intybus* L.*	Chicory	Perennial	1
Lamiaceae	*Clinopodium vulgare* L.*	Wild Basil	Perennial	N/A
Fabaceae	*Securigera varia* (L.) Lassen*	Crown Vetch	Perennial	11
Asteraceae	*Leucanthemum vulgare* Lam.*	Ox-eye Daisy	Perennial	72
Apiaceae	*Daucus carota* L.	Queen Anne's Lace	Biennial	42
Boraginaceae	*Echium vulagre* L.*	Viper's Bugloss	Biennial/Perennial	15
Asteraceae	*Hieracium pilosella* L.*	Mouse Ear Hawkweed	Perennial	14
Clusiaceae	*Hypericum perforatum* L.*	Common St. John's Wort	Perennial	31
Plantaginaceae	*Plantago lanceolata* L.	English Plantain	Biennial/Perennial	30
Poaceae	*Poa compressa* L.	Wire grass	Perennial	68
Poaceae	*Poa pratensis* L.	June Grass	Perennial	52
Rosaceae	*Potentilla recta* L.*	Rough-fruited Cinquefoil	Perennial	13
Lamiaceae	*Prunella vulgaris* L.*	Selfheal	Perennial	63
Asteraceae	*Rudbeckia hirta* L.	Black-Eyed Susan	Biennial/ Perennial	51
Asteraceae	*Solidago canadensis* L.	Canada Goldenrod	Perennial	88
Asteraceae	*Solidago graminifolia* (L.) Salisb.	Flat-Topped Goldenrod	Perennial	67
Asteraceae	*Taraxacum officinale* F.H. Wigg*	Common Dandelion	Perennial	5
Fabaceae	*Trifolium pratense* L.	Red Clover	Biennial/Perennial	N/A
Scrophulariaceae	*Veronica officinalis* L.*	Common Speedwell	Perennial	3

Table 2. Three way analysis of variance (ANOVA) table for the effects of target species identity, *P. lanceolata* competition, presence of AM fungi and their interactions on target species biomass.

	Target Species Biomass				
Source of Variation	Type III Sum of Squares	df	Mean Square	F value	Sig
AM presence	3.005	1	3.005	53.250	**1.5E-12**
Competition	3.445	1	3.445	61.038	**4.6E-14**
Species	26.052	20	1.303	23.082	**2.9E-55**
AM presence * Competition	1.537	1	1.537	27.236	**2.8E-7**
AM presence * Species	5.349	20	.267	4.739	**2.2E-10**
Competition * Species	1.066	20	.053	.944	0.531
AM presence * Competition * Species	.822	20	.041	.729	0.797
Block	2.013	2	1.006	17.832	**3.7E-8**
Error	23.476	416	.056		

Table 3. Two way analysis of variance (ANOVA) table for the effects of target species identity, presence of AM fungi and their interactions on the biomass of the phytometer, *P. lanceolata*.

	Phytometer Biomass				
Source of Variation	Type III Sum of Squares	df	Mean Square	F value	Sig
AM presence	9.887	1	9.887	64.281	**7.9E-14**
Species	6.448	20	.322	2.096	**0.005**
AM presence * Species	3.829	20	.191	1.245	0.221
Block	9.046	2	4.523	29.406	**5.8E-12**
Error	31.684	206	.154		

Table 4. Regressions between mycorrhizal response and competitive effect, and between mycorrhizal response and competitive response in the mycorrhizal (M) and non-mycorrhizal (NM) soil treatments.

			Regression Parameters		
y	x	AM Presence	b	R^2	p
CE	Mycorrhizal Response	M	0.0089	0.0004	0.9251
	Mycorrhizal Response	NM	**-0.3359**	**0.198**	**0.0379**
CRS	Mycorrhizal Response	M	-0.0286	0.003	0.79
	Mycorrhizal Response	NM	0.0855	0.007	0.708

Table 5. Regressions between competitive effect (CE) and competitive response (CRS) in mycorrhizal (M) and non-mycorrhizal soil (NM) with uptake of soil nutrients (R*) for ammonium (NH_4), nitrate (NO_3), phosphorus (P), magnesium (Mg) and potassium (K). P values marked with † are not significant after sequential Bonferroni adjustment.

			Regression parameters		
y	x	AM presence	b	R^2	p
CE	R*NH_4	M	-0.1081	0.03473	0.4063
		NM	0.14509	0.1286	0.1013
	R*NO_3	M	0.06716	0.1037	0.1438
		NM	0.07279	0.04963	0.319
	R*P	M	0.05077	0.05763	0.2819
		NM	0.09829	0.01932	0.5373
	R*Mg	M	**-0.007845**	**0.2091**	**0.03239**†
		NM	0.00162	0.01267	0.6179
	R*K	M	-0.04702	0.1081	0.1352
		NM	0.004603	0.00381	0.7849
CRS	R*NH_4	M	-0.2677	0.1641	0.06144
		NM	-0.01581	0.0008381	0.8982
	R*NO_3	M	0.07486	0.09942	0.1529
		NM	0.08542	0.0375	0.3879
	R*P	M	0.0554	0.05292	0.303
		NM	0.042	0.001936	0.8458
	R*Mg	M	**-0.009284**	**0.2258**	**0.02542**†
		NM	0.006544	0.1135	0.1252
	R*K	M	-0.03597	0.0488	0.3232
		NM	0.03192	0.1006	0.1504

Table 6. Pearson's correlations between competitive effect (CE), competitive response (CRS) in mycorrhizal (M) and non-mycorrhizal soil (NM) and mycorrhizal response with species abundance in the field.

			Correlation Parameters	
y	x	AM presence	Pearson's Correlation	p
Abundance	CE	M	-0.341	0.141
		NM	0.168	0.480
	CRS	M	-0.173	0.466
		NM	0.265	0.260
	Mycorrhizal Response		0.089	0.710

Table 7. Regressions between competitive effect (CE) and competitive response (CRS) with specific root length (SRL), Root to Shoot Ratio, Average Root Diameter and Early Growth Biomass in the mycorrhizal (M) and non-mycorrhizal soil (NM). ANCOVAs compare the difference between the two soil treatments. Significant results are bolded and starred. P values marked with † are not significant after sequential Bonferroni adjustment.

			Regression parameters			ANCOVA
y	x	AM presence	b	R^2	p	p
CE	SRL	M	-0.0000049	0.07649	0.2249	0.5446
		NM	-0.00001	0.0877	0.1924	
	Root to Shoot Ratio	M	**1.1451**	**0.329**	**0.006556**†	0.5257
		NM	0.5405	0.02008	0.54	
	Avg. Root Diameter	M	0.03732	0.0000087	0. 9679	0.1945
		NM	-2.4615	0.1042	0.1535	
	Early Growth Biomass	M	1.2017	0.1104	0.1411	0.1341
		NM	**3.5953**	**0.2708**	**0.01559**†	
CRS	SRL	M	-0.000003	0.02044	0. 5364	0.2061
		NM	0.0000114	0.06244	0.2747	
	Root to Shoot Ratio	M	**1.2486**	**0.2754**	**0.01457**†	0.5021
		NM	2.0407	0.16	0.0724	
	Avg. Root Diameter	M	-0.1849	0.001512	0.8671	**0.007893**
		NM	**-6.2188**	**0.3718**	**0.00334**	
	Early Growth Biomass	M	**1.9649**	**0.2078**	**0.03781**†	0.4621
		NM	0.2597	0.0007895	0.9038	

Table 8. Regressions between species resource uptake ability (R*) for ammonia (NH_4), nitrate (NO_3), phosphorus (P), magnesium (Mg) and potassium (K) with species values for specific root length (SRL), Root to Shoot Ratio, Average Root Diameter and Early Growth Biomass in the mycorrhizal (M) and non-mycorrhizal soil (NM). ANCOVAs compare the difference between the two soil treatments. Significant results are bolded and starred. P values marked with † are not significant after sequential Bonferroni adjustment.

			Regression parameters			ANCOVA
y	x	AM presence	b	R^2	p	p
R*NH_4	SRL	M	0.007167	0.0495	0.3196	**0.02415**
		NM	**0.03549**	**0.1826**	**0.04732**†	
	Root to Shoot Ratio	M	-0.7421	0.0491	0.3217	0.4794
		NM	0.7274	0.007093	0.7094	
	Avg. Root Diameter	M	-1.966	0.0725	0.2256	0.3785
		NM	2.016	0.01147	0.6353	
	Early Growth Biomass	M	-0.0005133	0.007578	0.7001	0.3279
		NM	0.003034	0.03981	0.3733	
R*NO_3	SRL	M	-0.01487	0.02753	0.4605	0.6379
		NM	-0.0004891	0.000022	0.9832	
	Root to Shoot Ratio	M	1.6218	0.03028	0.4387	0.1621
		NM	**5.723**	**0.2864**	**0.01027**†	
	Avg. Root Diameter	M	-1.1881	0.003419	0.796	0.7159
		NM	-3.7027	0.02522	0.4803	
	Early Growth Biomass	M	**0.008361**	**0.2596**	**0.01543**†	0.9088
		NM	0.007789	0.1711	0.05567	
R*P	SRL	M	-0.00008913	0.000001	0.9964	0.3428
		NM	**-0.021149**	**0.198**	**0.03799**†	
	Root to Shoot Ratio	M	0.1597	0.000063	0.7314	0.652
		NM	1.7558	0.1262	0.1047	
	Avg. Root Diameter	M	30.71	0.01537	0.9719	0.5378
		NM	3.2875	0.0931	0.1673	
	Early Growth Biomass	M	0.003133	0.03749	0.3879	0.7708
		NM	0.001952	0.05034	0.3155	
R*Mg	SRL	M	0.4173	0.1468	0.07843	0.2327
		NM	-0.2681	0.01317	0.6111	
	Root to Shoot Ratio	M	-23.107	0.04162	0.3624	0.9313
		NM	-17.9	0.005455	0.7439	
	Avg. Root Diameter	M	30.75	0.0155	0.5809	0.8618
		NM	7.881	0.000221	0.9476	
	Early Growth Biomass	M	**-0.12049**	**0.3651**	**0.00289**	0.2314
		NM	-0.00346	0.000065	0.9715	
R*K	SRL	M	0.02972	0.05172	0.3088	0.9396
		NM	0.03768	0.00699	0.7114	
	Root to Shoot Ratio	M	-3.6949	0.07394	0.2209	0.5014
		NM	3.664	0.006114	0.7294	
	Avg. Root Diameter	M	5.047	0.02903	0.4484	0.635
		NM	-6.298	0.0038	0.7852	
	Early Growth Biomass	M	-0.002269	0.008998	0.6746	0.7603
		NM	-0.008142	0.009738	0.6622	

Table 9. Regressions between competitive effect (CE) and competitive response (CRS) of plants in mycorrhizal (M) and non-mycorrhizal (NM) soil.

			Regression parameters			ANCOVA
y	x	AM presence	b	R^2	p	p
CE	CRS	M	**0.83290**	**0.4885**	**0.0004238***	0.3913
CE	CRS	NM	0.4251	0.101	0.1604	

Figure 1. Mean biomass of target species in the mycorrhizal and non-mycorrhizal soil treatments for each competition treatment. The error bars represent one standard deviation, the asterisk represents a within soil treatment significant difference, and the cross represents between soil treatments significant difference as per Tukey's HSD post-hoc comparison.

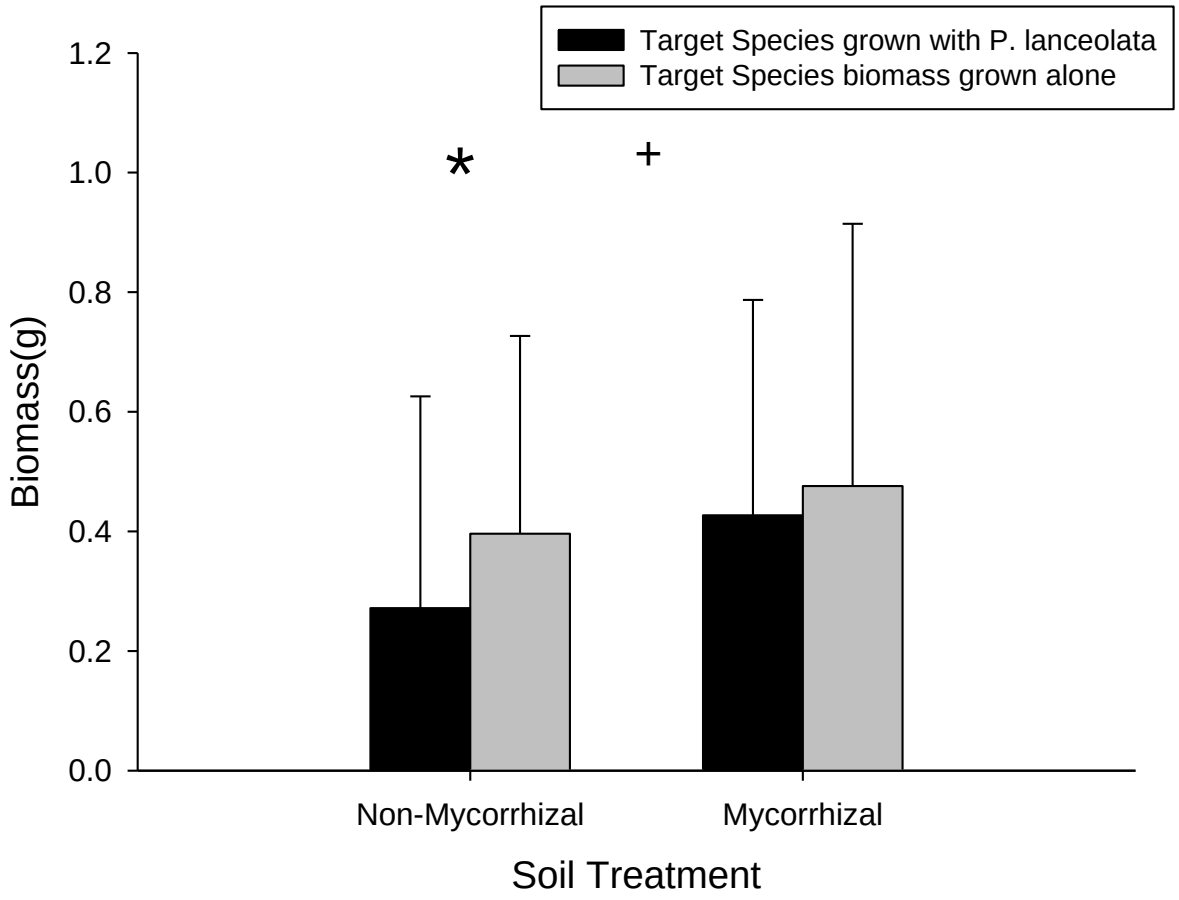


Figure 2. Target species biomass and their overall average when grown with or without *P. lanceolata* in (A) mycorrhizal and (B) non-mycorrhizal soil treatments. The error bars are one standard deviation, and the asterisk represents a significant difference between the competition treatments using Tukey HSD test.

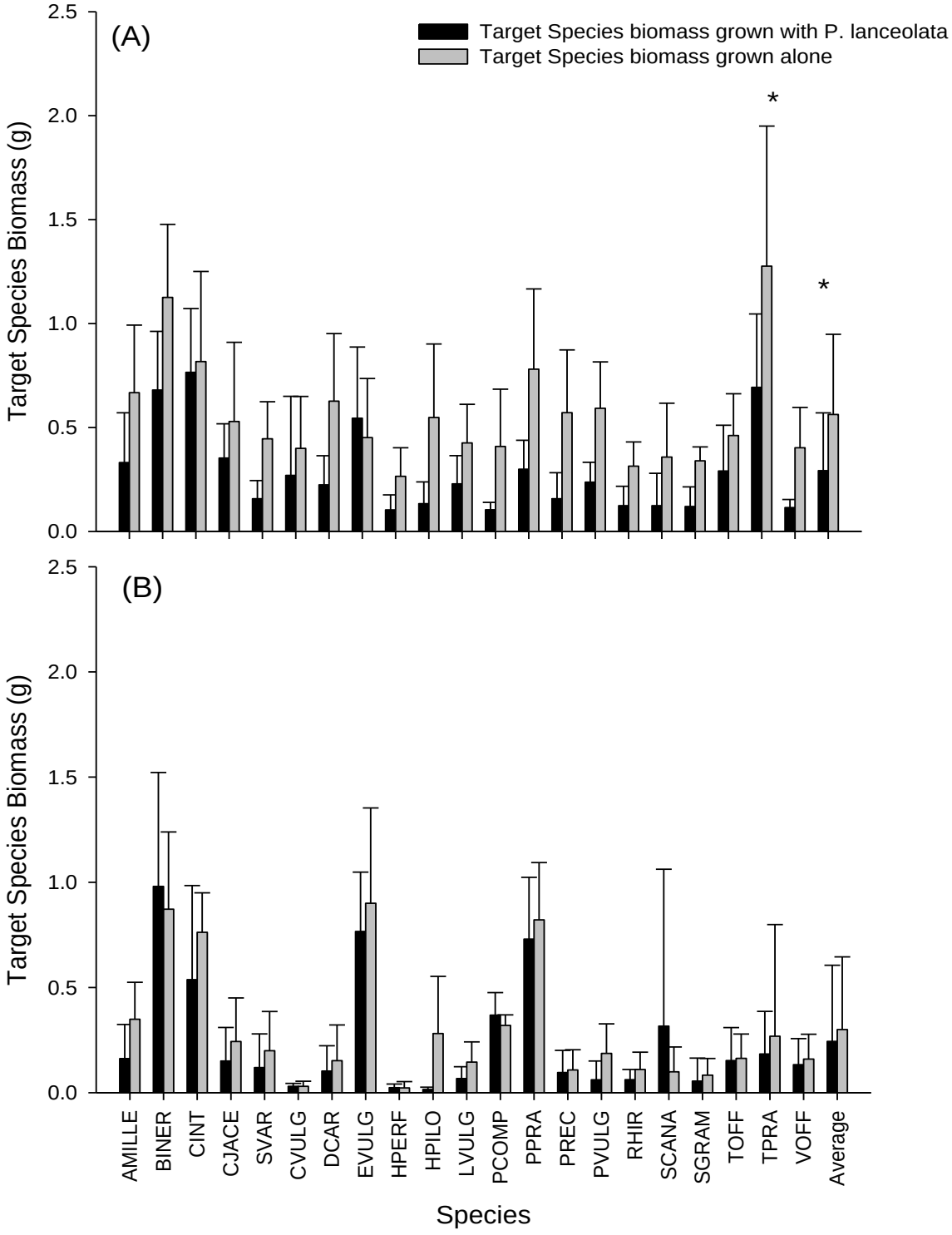


Figure 3. Biomass of *P. lanceolata* when grown with each target species and grown alone and it's average biomass in each soil treatment. Error bars represent one SD, and the asterisk represents a significant difference between the means using Tukey's HSD post-hoc comparison.

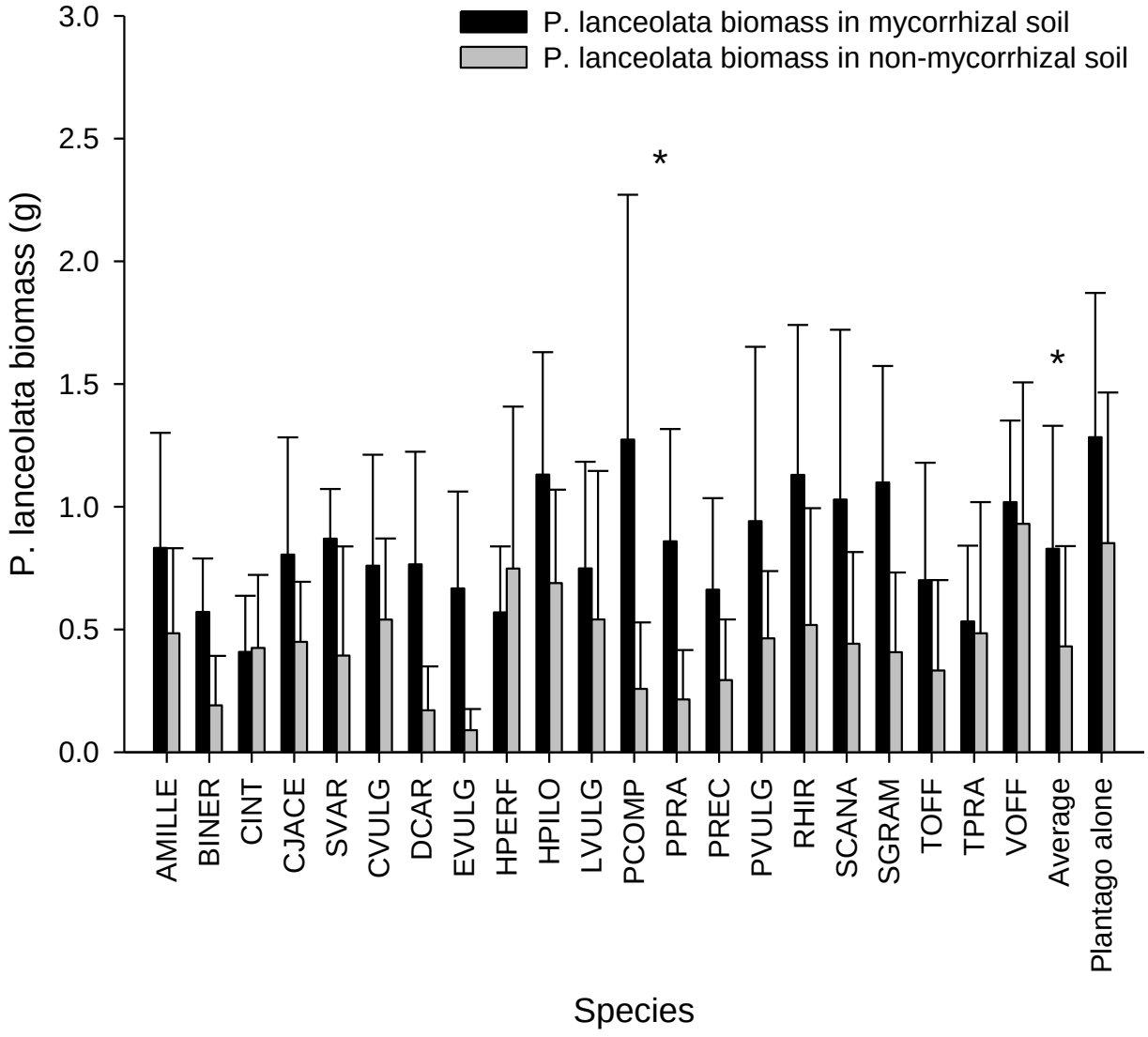
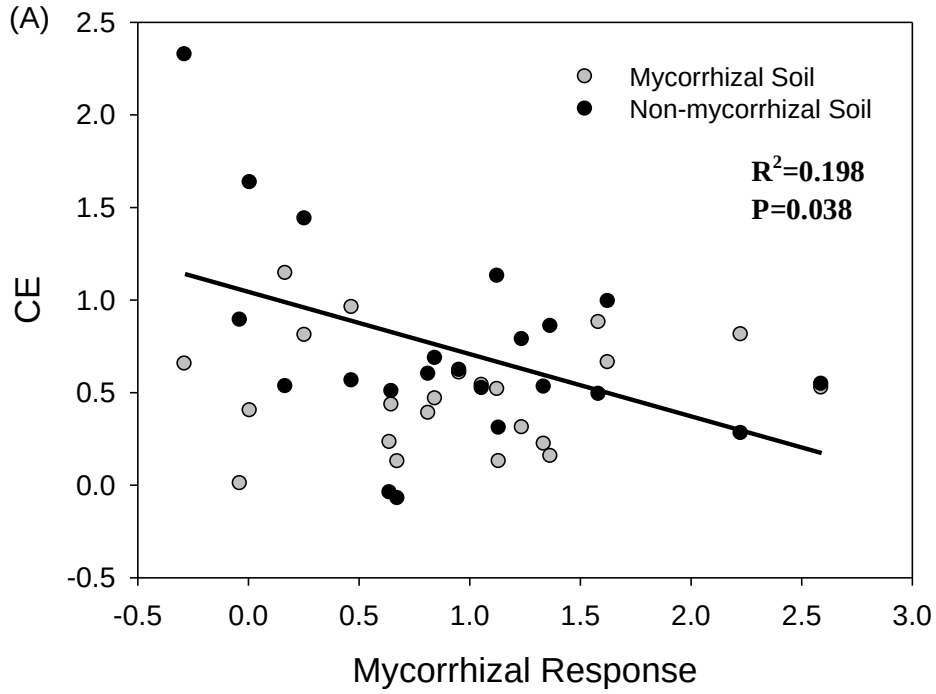


Figure 4. Relationships between species mycorrhizal response and (A) competitive effect and (B) competitive response in mycorrhizal and non-mycorrhizal soil. Regression equations can be found in Table 4.



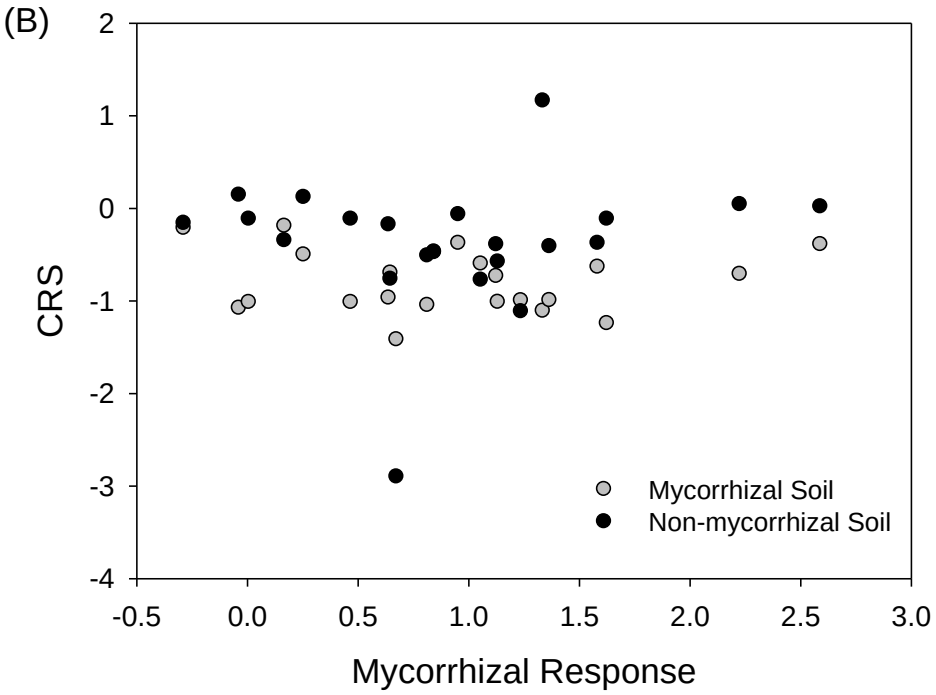


Figure 5. Relationships between mycorrhizal response and species traits: (A) Specific Root Length, (B) Root to Shoot Ratio, (C) Average Root Diameter and (C) Early growth biomass.

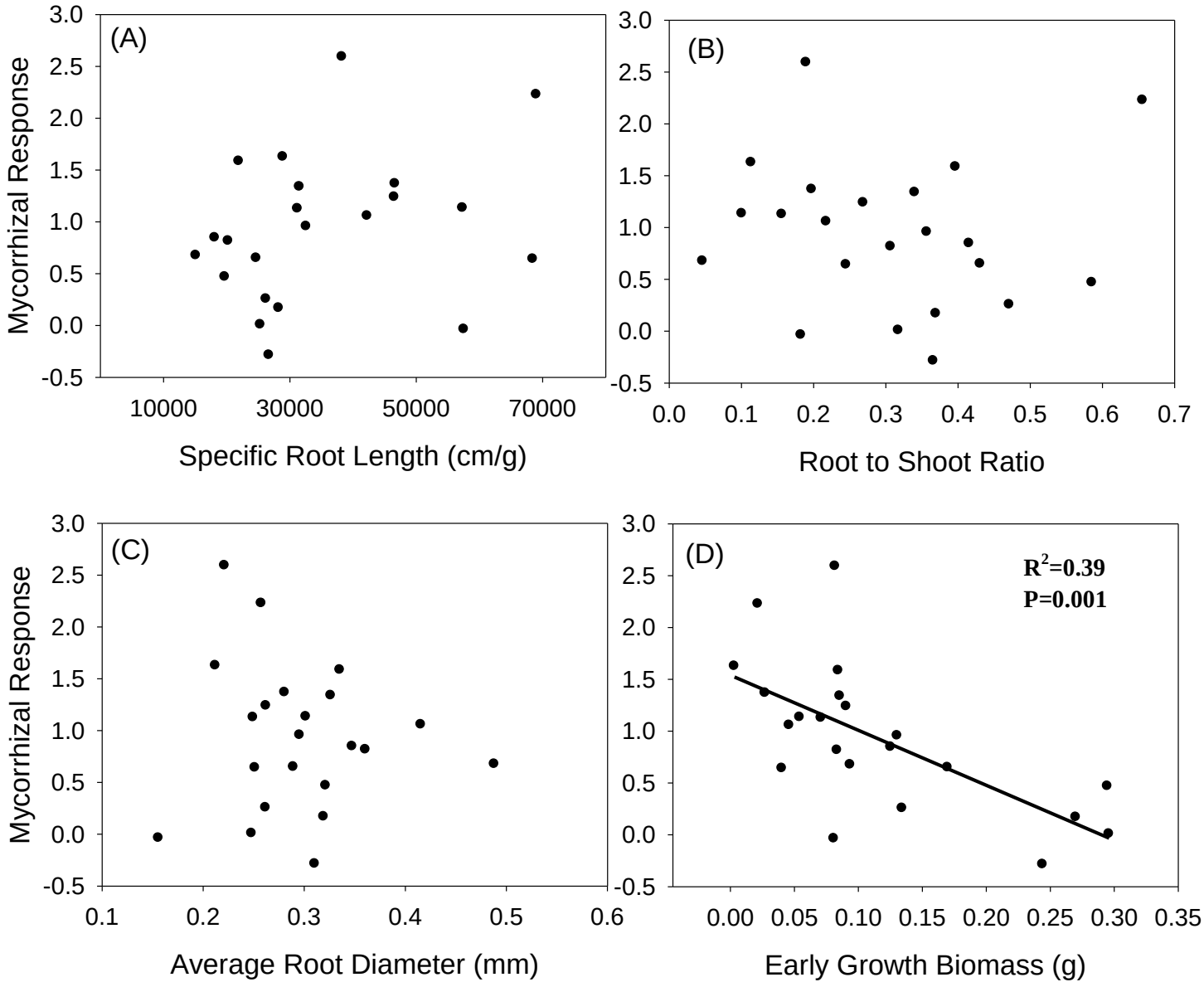
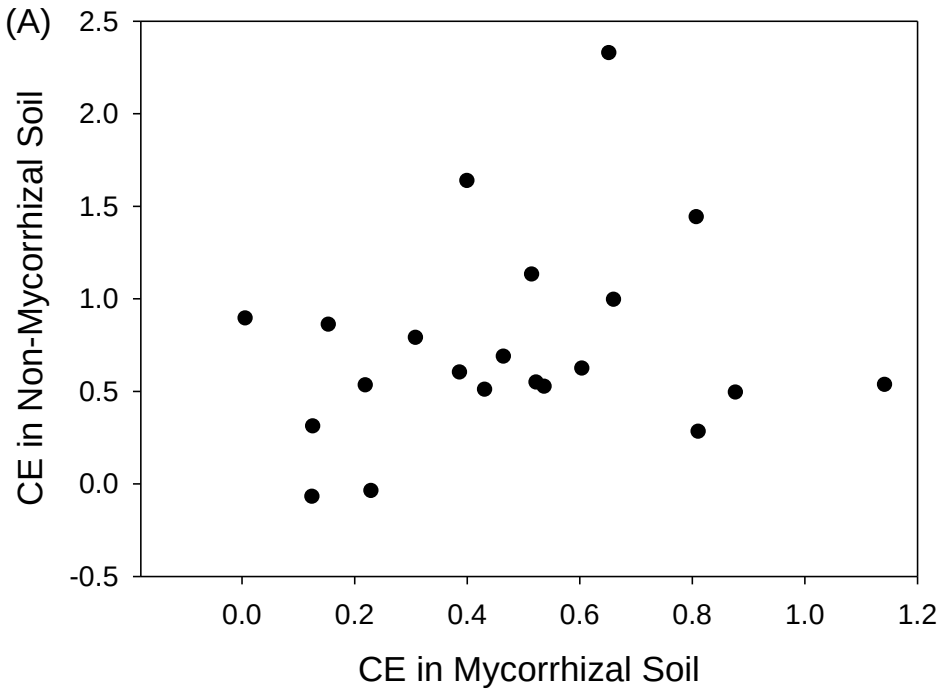


Figure 6. Relationships between (A) competitive effect in mycorrhizal and non-mycorrhizal soil and (B) competitive response in mycorrhizal and non-mycorrhizal soil. Regression equations can be found in Table 9.



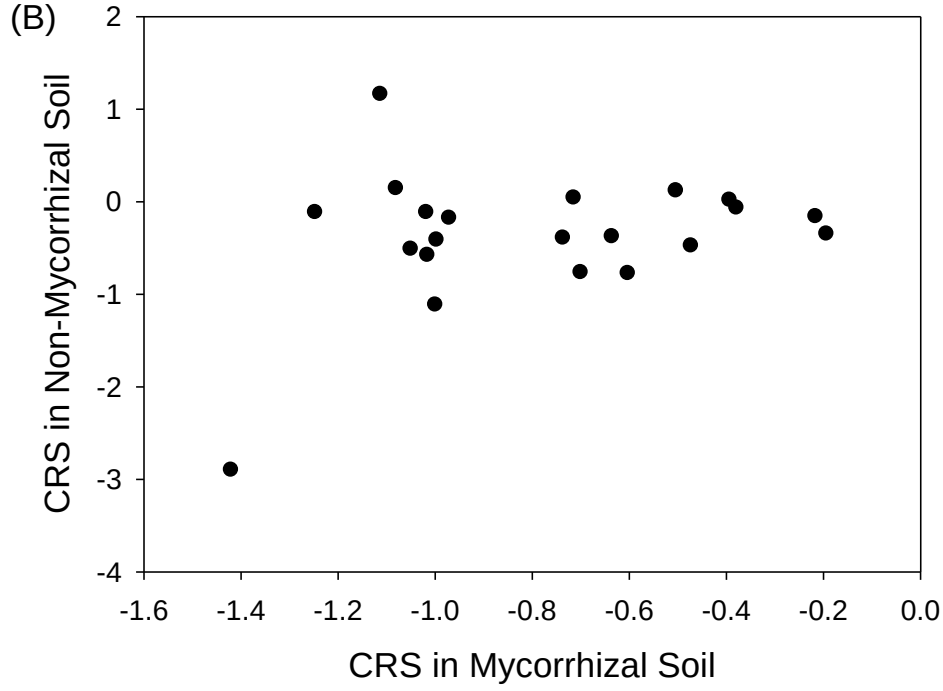


Figure 7. Relationships between competitive effect (CE) and (A) Specific Root Length, (B) Root to Shoot Ratio, (C) Average Root Diameter and (D) Early Growth Biomass in mycorrhizal and non-mycorrhizal soil. Regression equations can be found in Table 7.

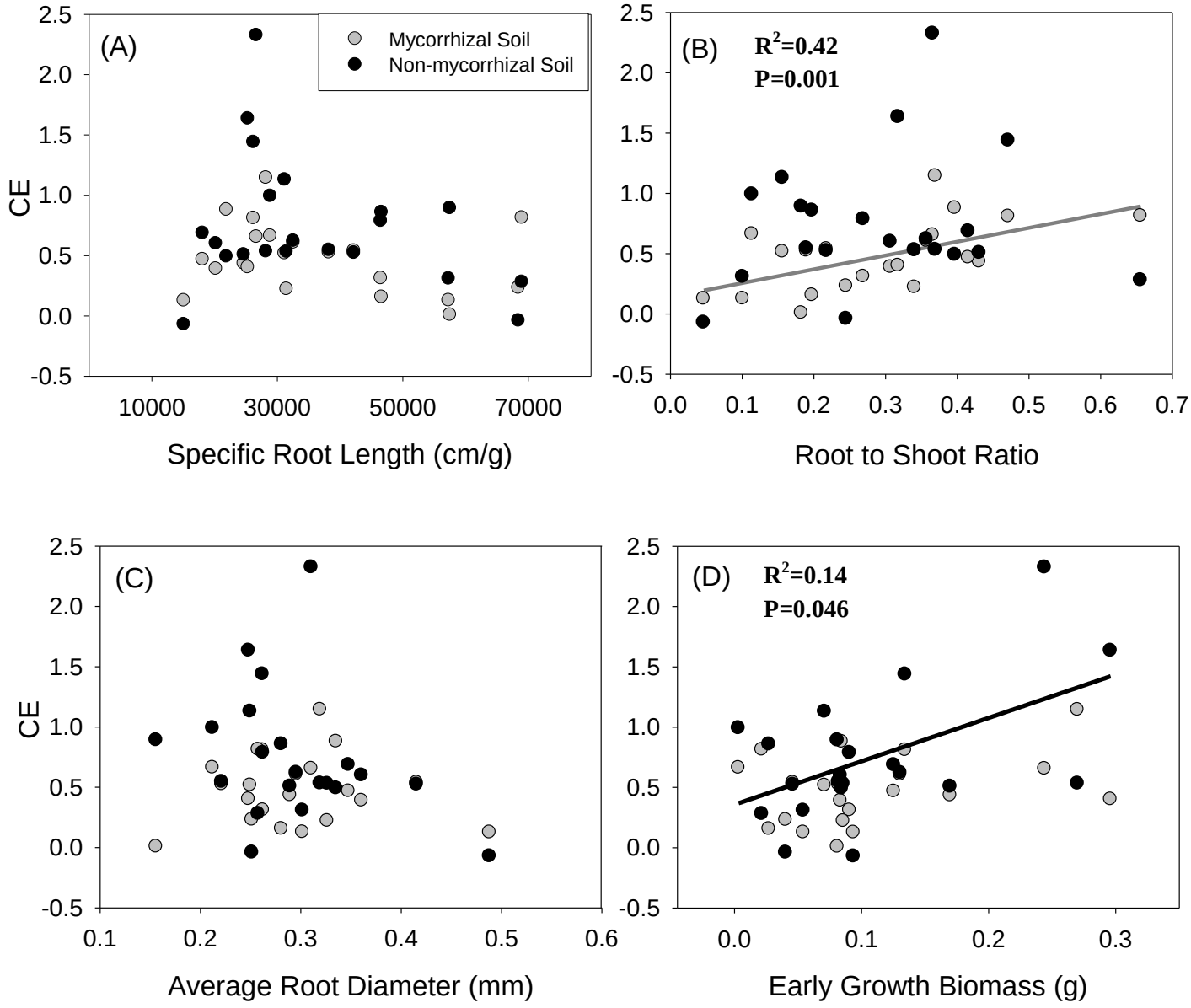
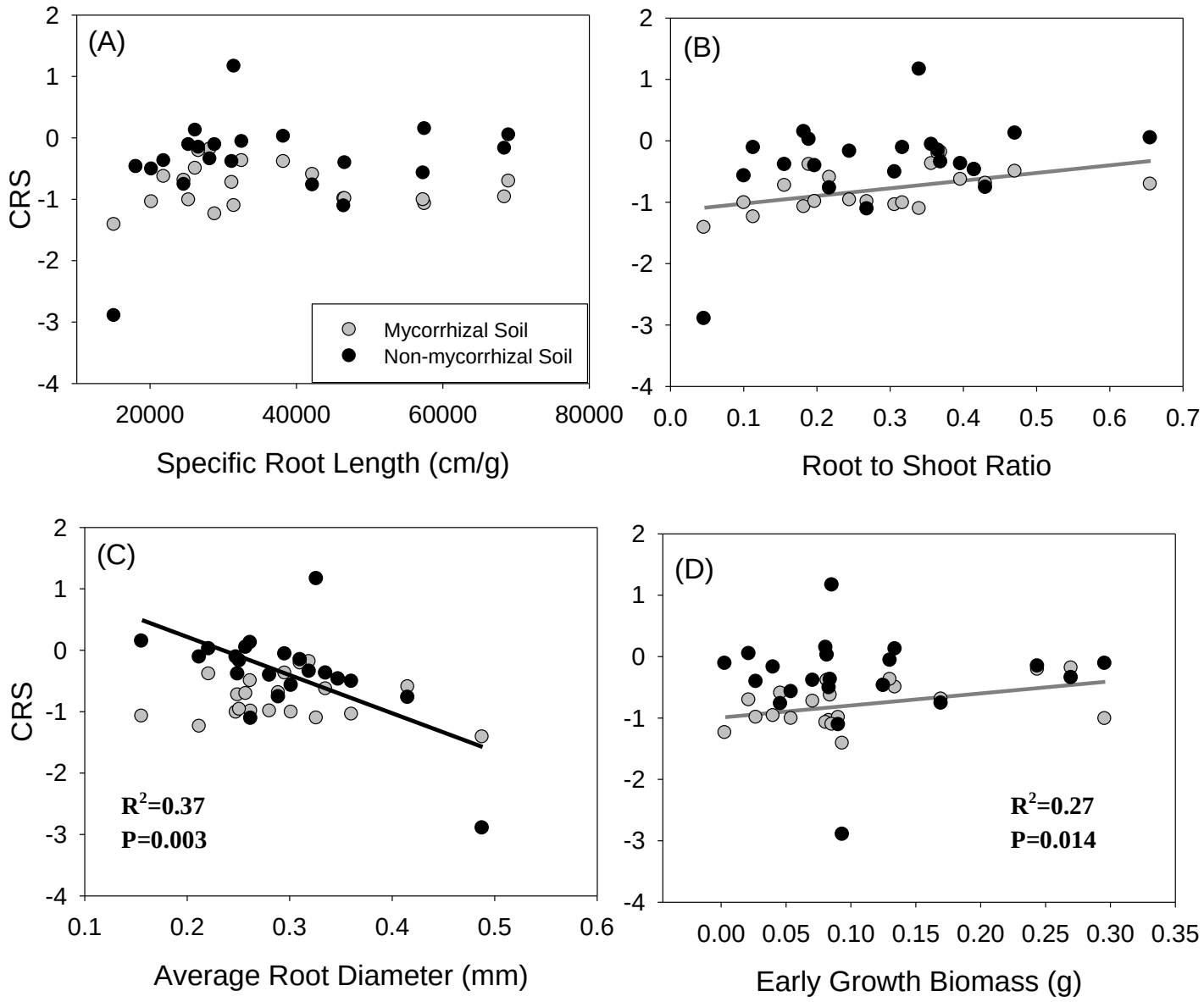


Figure 8. Relationships between competitive response (CRS) and (A) Specific Root Length, (B) Root to Shoot Ratio, (C) Average Root Diameter and (D) Early Growth Biomass in mycorrhizal and non-mycorrhizal soil. Regression equations can be found in Table 7.



CHAPTER II: THE EFFECTS OF PLANT-SOIL FEEDBACK ON THE COMPETITIVE ABILITY OF *PLANTAGO LANCEOLATA* L.

ABSTRACT

THE EFFECTS OF PLANT-SOIL FEEDBACK ON THE COMPETITIVE ABILITY OF *PLANTAGO LANCEOLATA* L.

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Plant-soil feedback, the response of plant growth to the microbial soil community, can affect competitive interactions between plants. If feedback effects influence coexistence, then hierarchies of competitive ability will change due to feedback effects. Positive and negative feedback should lead increased or decreased competitive ability, respectively. I compared species-specific feedback effects on competitive hierarchies between *Plantago lanceolata* and 21 target species growing in soil with *Plantago* soil community, *Plantago* microbial community, and in sterile soil. Target species had greater growth responses to the whole soil community compared to *Plantago*. The whole soil community did not affect the growth of *Plantago* and or change hierarchies of competitive effect compared to the sterile soil. Although target species experienced positive growth from the whole soil, they had low competitive performance when growing with *Plantago*. This suggests that *Plantago* feedback does not influence its ability to compete with other species, or change competitive hierarchies.

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Introduction

Feedback effects from the microbial soil community can influence competitive interactions between plants, and therefore community composition (van der Putten and Peters, 1997; Bever, 2002; Bever *et al.,* 2010). Plant-soil feedback effects result from the interactions between plants and the microbial soil community. Plant-soil feedback is a two step process: first, the plant community influences the composition of the microbial soil community; second, the microbial community differentially changes the growth of individual species (Bever, 2003; Kardol *et al.,* 2007; Bever *et al.,* 2010; Brinkman *et al.,* 2010). This occurs due to the heterogeneous nature of the microbial community that can change its composition rapidly in response to the plant community (Bever *et al.,* 2010; Brinkman *et al.,* 2010). In addition, plants can also affect their own performance by changing the composition of their soil communities (van der Putten *et al.,* 1993; Bever, 1994; Bever *et al.,* 2009). Thus, the dynamic interactions between plants and their soil community can affect the long term coexistence of species (Bever *et al.,* 1997; Bever, 2002; Bever *et al.,* 2010; Brinkman *et al.,* 2010; MacDougall *et al.,* 2011).

Negative feedback can promote coexistence within a community, whereas positive feedback can lead to monodominance of species (Kulmatiski *et al.,* 2008; Bever *et al.,* 2010). Feedback effects are considered negative if the resulting change in the microbial community decreases the growth of a host plant, whereas positive feedback effects result in an increase the growth of host plants. Decreased growth from negative feedback occurs due to an accumulation of host specific pathogens or parasites (Bever, 1994; Klironomos, 2002). Pathogenic fungi for example, can degenerate roots (van der Putten and Peters, 1997), thus have direct negative effect to the host plant. In addition, conspecifics will also experience negative feedback when growing together (Klironmos, 2002; MacDougall *et al.,* 2011). Coexistence can occur when species are limited by growing with their own soil community (Bever, 2003; Bever *et al.,* 2010; Kulmatiski *et al.,* 2008; MacDougall *et al.,* 2011).

Symbiosis with arbuscular mycorrhizal fungi can lead to either positive or negative feedback effects. The positive feedback of mycorrhizae on plant growth can result from two distinct processes:

improved nutrition and improved defence against pathogens. Mycorrhizae provide plants limiting nutrients, such as phosphorus and nitrogen, in exchange for carbon (Hartnett and Wilson, 1999; Smith and Read, 2008; Johnson, 2010). Limiting nutrients can be either allocated to greater growth or defence (Vanette and Hunter, 2011). Moreover, arbuscular mycorrhizae can have a positive effect on plant competitive ability by preventing infection from pathogenic fungi by outcompeting these fungi for infections sites, resources etc., in a host plant's roots (van der Putten *et al.,* 2003; Wehner *et al.,* 2009). In addition to receiving benefits from mycorrhizal symbiosis, plants can influence the degree of feedback by selectively allocating more photosynthate to mycorrhizae that are most beneficial, allowing these mycorrhizae to proliferate on the host's roots (Bever *et al.,* 2009). However, the specificity of mycorrhizae for a particular host can also lead to negative feedback effects. The species that grows best with particular host may not provide the best benefit to its host, compared to mycorrhizae that are specific to a different host species (Bever, 2002). Thus, by increasing the growth of individual species or populations, mycorrhizae can lead to reduced diversity in a community through positive soil feedback effects (Hartnett *et al.,* 1993; Bever *et al.,* 1997; Gross *et al.,* 2010) or promote coexistence through specificity of host preference (Bever, 2002).

Because feedback effects are hypothesized to influence dominance, they can be studied by constructing competitive hierarchies. Hierarchies based on competitive ability are positively correlated with species abundance, thus examining hierarchies can give insights into species coexistence (Aplet and Laven, 1993; Howard and Goldberg, 2001; Fargione and Tilman, 2006). Hierarchies of competitive ability are built by testing species pair-wise interactions, which require $n(n-1)/2$ treatments (where n is the number of species) (Keddy *et al.,* 2002). In order to test feedback effects using this method, each species must culture its own soil community, and competitive interactions for each species pair are measured twice: once in each species' cultured soil (Bever, 1994; Klironomos, 2002; Brinkman, *et al.,* 2010), resulting in $n(n-1)$ treatments. Thus, to construct a competitive hierarchy for 20 species, the pair-wise test method would require 190 treatments, and to test feedback effects it would result in 380 treatments. However, the phytometer method is used to build hierarchies based on the mean competitive

ability of target species tested against a phytometer (or indicator) species (Keddy *et al.*, 1994; Keddy *et al.*, 2002, Wang *et al.*, 2010). In this case, species-specific biota only needs to be cultured for the indicator species. By using the phytometer method, building a hierarchy of competitive interactions for 20 species would require 40 treatments, and to test feedback effects it would require 80 treatments. Thus, using the phytometer method, one can analyze a larger array feedback effects experienced by the indicator species when in competition with numerous target species.

In addition, the phytometer method makes it possible to examine the direction and magnitude of feedback effects that target species experience from biota cultured by an indicator species, and how these plant-soil feedbacks affect competitive interactions between the indicator and target species. Feedback effects from the soil community can have different implications for competitive interactions within (conspecific) and between (heterospecific) species. Usually, species experience more negative conspecific compared to heterospecific effects (Bever, 1994; Bever *et al.*, 1997; Klironomos, 2002; Casper and Castelli, 2007; MacDougall *et al.*, 2011), which can reduce their competitive ability (van der Putten and Peters, 1997; Casper and Castelli, 2007). Indeed, some studies have found links between conspecific feedback effects and abundance (Klironomos, 2002; Comita *et al.*, 2010; MacDougall *et al.*, 2011) and competitive ability (Casper and Castelli, 2007). However, conspecific effects can be more negative even if heterospecific effects are neutral, and the inverse pattern may not apply. In other words, whether species experience positive feedback from growing in the soils of heterospecifics increases their competitive ability is implied (Bever *et al.*, 2010) but not known.

In this chapter, I tested if feedback effects from soil biota cultured by an indicator species affected hierarchies of competitive ability between the indicator and target species. Comparing the growth of target species in sterile soil compared to soil cultured by an indicator species (i.e. heterospecific effects) can provide insight on the magnitude and direction of feedback effects that the target species experience from the biota cultured by the indicator species. If heterospecific feedback effects from biota cultured by the indicator species on target species influence competitive hierarchies, then these effects will be correlated with the competitive ability of the target and indicator species. Target species that have

lower heterospecific effects (i.e. experience negative feedback from the soil community) should be inferior competitors when grown with the indicator species. In addition, if hierarchies are influenced by feedback effects from the soil cultured by the indicator, then these hierarchies of competitive effect built in the cultured soil will differ from hierarchies in the sterile soil.

Materials and Methods

Experimental Design

To determine the effects of plant-soil feedback on plant competition, I ran a phytometer experiment in which I examined the effect of the soil community cultured by an indicator species on the competitive interactions between the indicator and target species. To determine the magnitude and direction of feedback effects, and I used *Plantago lanceolata* as an indicator species, and 21 target species commonly found in old fields of Southern Ontario, Canada. The seeds for the study species were either collected from Long Term Mycorrhizal Research Site (LTMRS) (43° 32’ 3.5”, -80° 12’ 41.8”) or ordered from Richters Herbs (Goodwood, Ontario) (Table 1). Target species were selected based on seed availability and germination success in the greenhouse. *P. lanceolata* was selected as an indicator species because previous studies show that it experiences negative feedback from the soil community (Bever, 2002). Feedback experiments that are best at examining the strength and sign of feedback effects compare plant growth between a sterilized control and the inoculated soil (Brinkman *et al.*, 2010). Thus, I grew *P. lanceolata* with or without each target species (including *P. lanceolata*), in sterile soil, whole soil cultured by *P. lanceolata* and non-mycorrhizal *P. lanceolata* whole soil (from now referred to as microbial wash soil). In order to culture a realistic *P. lanceolata* soil community, I collected 400 L of soil for the treatments from 15 sampling locations in LTMRS, where my study species naturally occur. LTMRS is old field that has been abandoned from agriculture for over 40 years (Maherali and Klironomos, 2012). In order to have the same background soil prior to adding inocula, I sieved then sterilized the field soil by

autoclaving for two hours. The final soil for the treatments contained two thirds of the sterilized LTMRS soil and one third sand that was also sterilized by autoclaving for two hours.

To generate *P. lanceolata* specific soil feedback, I cultured the soil biota community that is commonly found on the presence of *P. lanceolata* plants in the field. I collected individual *P. lanceolata* plants (including above ground structures and the root ball) from LTMRS. I removed the above-ground biomass and finely chopped the roots and soil. In order to condition the soil community to be *P. lanceolata* specific, it was cultured in a *P. lanceolata* only environment. To do so, I mixed the soil and roots obtained from the field *P. lanceolata* plants with a one to one ratio of volume of soil to sterilized silica sand. I placed this mixture in 15.24 cm pots and seeded each pot with *P. lanceolata* seeds collected from LTMRS in 2006. The plants were allowed to grow for 4 months in the Guelph Phytotron (University of Guelph, Guelph, Ontario). After the growth period, I let the soil dry order to generate mycorrhizal spores (INVAM). I chopped the roots and soil of *P. lanceolata* to use as inocula for the whole soil treatment. To isolate the microbial portion of *P. lanceolata* specific soil for the microbial wash treatment, I suspended the whole soil in a wash and passed the supernatant through a 37 micron sieve, which excluded mycorrhizal fungal spores (Koide and Li, 1989; Bever, 1994), but allowed bacteria and other pathogens to pass through. The *P. lanceolata* specific whole soil treatment (from now on referred to as the whole soil treatment) received chopped *P. lanceolata* roots and soil, and the microbial wash soil treatment received the sieved microbial wash, and soil and roots that did not pass through the 37 micron sieve that were sterilized by autoclaving for four hours. The sterilized soil treatment received chopped roots of leek (*Allium ampeloprasum* L.) colonized by *Glomus intraradices* that were sterilized by autoclaving for four hours.

The above treatments and their controls resulted in 22 species * 2 competition conditions * 3 soil treatments were replicated six times for a total 792 pots. Due to the large number of plants that needed to be transplanted, one third of replicates were planted per week, one week apart. Each third of the replicates were completely randomized and occupied one greenhouse bench (Phytotron, University of Guelph,

Guelph, Ontario). Thus, each replicate represented a block in time and space. Each set of replicates was grown for 10 weeks, and the experiment ran for a total of 12 weeks.

To determine the effects of competition and soil treatment on the biomass of the indicator and target species, the replicates in each block were harvested sequentially after a 10 week growth period. Each block was harvested over the course of 5 days. The above-ground biomass each individual plant was clipped, then stored in a paper bag. The biomass was dried at 60°C for 72 hours, then weighed to obtain above-ground biomass measurements. Root samples were taken from 264 pots to be used for colonization assessment which were stored in a solution of 50% volume of 95% ethanol and 50% deionized water.

To determine if the colonization was present in the whole soil treatments and absent in the microbial wash and sterile treatments, I assessed the percent colonization of 264 root samples representative of each species, soil treatment and competition treatment. I randomly selected four root samples of each species in each soil treatment, two for the each species when grown alone and two when grown with *P. lanceolata*. I used the gridline intersection method to quantify the presence or absence of non-septated hyphae, vesicles and arbuscules at 50 intersections per root sample (McGonicle *et al.*, 1990). The roots were cleared using a 10% solution of potassium hydroxide then stained with a 5% ink and vinegar solution using Black Shaeffer ink. After staining, the roots were rinsed with tap water and acidified with a few drops of vinegar (Vierheilig *et al.*, 1998).

Statistical Analysis

To determine the effects of competition and feedback on above-ground biomass of *P. lanceolata*, I used two-way ANOVAs with Type III sum of squares for species identity and soil treatments, with a randomized block design. The treatments within each block (3 blocks in total) were completely randomized and separated by space (3 different benches) and time (3 different planting weeks). To determine the effects of soil treatment on the growth of *P. lanceolata* when grown alone, I used one-way ANOVAs for soil treatments, with a randomized block design as above. The soil treatments in the ANOVA analyses were the sterile soil, microbial wash soil and whole soil. I included the data for the

sterile soil in order to calculate feedback effects relative to a sterile environment (Brinkman *et al.,* 2010). I analyzed the significance of the main and interaction effects using the Tukey's HSD (Honestly Significant Difference) post-hoc comparison.

To quantify competitive ability, I measured the competitive effect and competitive response values. A species' competitive performance can be quantified in terms of its ability to suppress the growth of neighbours (competitive effect) and the ability to withstand suppression from neighbours (competitive response)(Goldberg and Landa, 1991; Wang *et al*, 2010). I calculated competitive effect of target species (CE) by taking the natural log of the mean dried weight of the phytometer grown alone (P) divided by the phytometer grown with a target species (P_T) (Eq.1). Similarly, I calculated competitive response (CRS) as the natural log of mean biomass of target species when grown with the phytometer (T_P) divided by the mean biomass of target species grown alone (T) (Eq. 2):

$$CE = \ln\left(\frac{P}{P_T}\right) \qquad \text{(Eq. 1)}$$

$$CRS = \ln\left(\frac{T_P}{T}\right) \qquad \text{(Eq. 2)}$$

Higher values of competitive effect (CE) represent greater ability of target species to suppress the growth of *P. lanceolata*, whereas lower values represent greater ability of *P. lanceolata* to suppress target species. Higher values of competitive response represent species ability to withstand suppression from growing with *P. lanceolata*, whereas lower values represent greater ability of *P. lanceolata* to suppress target species.

To determine the effect of the whole soil community and the microbial portion of the soil community cultured by *P. lanceolata* on target species, I calculated heterospecific effects, modified from Macdougall *et al.,* (2011) as the natural log of the ratio of biomass of target species grown alone in the *P. lanceolata* cultured whole soil compared to the sterilized soil (Eq. 3) and the ratio of the biomass of target species grown in the microbial wash soil compared to the sterilized soil (Eq. 4). Positive values of heterospecific effects indicate that target species experienced positive feedback from the soil community

of *Plantago lanceolata*, whereas negative values indicate that target species experienced negative feedback from the soil community of *P. lanceolata*:

$$HE_{Whole} = ln\frac{Biomass_{Whole\ Soil}}{Biomass_{Sterilized\ Soil}} \quad \text{(Eq. 3)}$$

$$HE_{Wash} = ln\frac{Biomass_{Microbial\ Wash\ Soil}}{Biomass_{Sterilized\ Soil}} \quad \text{(Eq. 4)}$$

To determine whether feedback effects from the whole soil community affected competitive ability in that treatment, I ran regressions between heterospecific effects in the whole soil with competitive effect and response values in the whole soil. To determine if feedback effects from the microbial community influenced competitive ability in the whole soil, I ran regressions between heterospecific effects in the microbial wash and competitive effect and response in the whole soil. In addition, I also ran regressions between heterospecific effects in the microbial wash soil with competitive effect and response in the microbial wash soil to determine if feedback effects from the microbial community influenced competitive ability in the microbial wash soil.

I also calculated heterospecific effects from the whole soil and microbial wash soil communities for the biomass of target species when grown with *P. lanceolata* in order to determine whether these effects change in the presence of competition. I ran regressions between heterospecific effects in the whole soil treatment and competitive effect and competitive response in the whole soil treatment to determine if the whole soil community influenced target species performance in that treatment. I also ran regressions between heterospecific effects in the microbial soil treatment with competitive effect and response in the whole soil treatment to determine whether the microbial soil community affected competitive performance in the whole soil. I also ran regressions between heterospecific effects in the microbial soil treatment and competitive effect and response in the microbial soil treatment to determine if the microbial soil community influenced competitive performance in that treatment.

In order to assess if hierarchies of competitive effect differed between soil treatments, I compared competitive effect values from each soil treatment using Pearson correlations (graphs can be found in the Appendix B). Pearson correlations have previously been used to compare biomass ratio data for

competitive effect and response hierarchies (Wang *et al.*, 2010). Although ranked data for competitive effect and response have also been used to compare hierarchies (Gaudet and Keddy, 1995), Pearson correlations were appropriate because the data for competitive effect in all soil treatments was normally distributed (Shapiro-Wilk tests, $p>0.05$), thus ranking the data (and using a Spearman correlation) was not necessary. In order to compare the competitive effect measures from each soil treatment for the above correlation analysis, I standardized them to have a mean of 0 and a standard deviation of 1 using SPSS. To determine if feedback effects changed hierarchies of competitive effect, I compared the competitive effects values in sterile soil to competitive effect values in whole soil and in microbial wash soil. To determine if mycorrhizae important at determining competitive effect in the whole soil treatment or microbial wash treatment, I compared the hierarchies of competitive effect in the mycorrhizal soil treatment to hierarchies in the whole soil and microbial wash treatments. Data for the mycorrhizal soil treatment was included to see if mycorrhizae contribute to feedback effects. This data was obtained from a previous experiment (Stanescu, 2012).

Results

Mycorrhizal colonization differed between the three soil treatments (ANOVA, significant soil treatment effect, $F_{(2, 126)}=682.27$, $p=0.000$), but did not vary between species (ANOVA, non-significant Species effect $F_{(21, 126)}=1.98$, $p=0.316$). For both the indicator and target species, roots in the whole soil treatment had 69.54% (SE=3.42) root mycorrhizal colonization. Roots of plants grown in the sterile soil and the microbial wash soil had 0.77% (SE=0.38) and 0.79% (SE=0.57) colonization rates respectively.

I found that the growth of *Plantago lanceolata* was influenced by growing with its own soil community when grown alone. *P. lanceolata* had the highest biomass in the sterile soil, the next highest biomass in the whole soil and the lowest biomass in the microbial wash soil (Fig. 1). The biomass of *P.*

lanceolata was the 74% greater when grown in the whole soil compared to the microbial wash (significant Whole Soil Biota effect, Table 2, Fig. 1). Its biomass was 87% greater in the sterile soil compared to the microbial wash soil and 15% greater compared to the whole soil treatment (significant Whole Soil Biota effect, Table 2, Fig. 1). However, the difference in biomass was only significantly different between the sterile soil and the microbial wash soil (Tukey's HSD, $p<0.05$).

When grown in competition with target species, *P. lanceolata* biomass differed between all the soil treatments (significant Whole Soil Biota effect, Table 3). *P. lanceolata* had the highest biomass in the whole soil treatment, the second highest biomass in the sterile soil treatment and the lowest biomass in the microbial treatment. The biomass of *P. lanceolata* was 99% greater in the whole soil treatment compared to the microbial wash treatment, 74% greater in the sterile soil compared to the microbial wash and 31% greater in the whole soil compared to the sterile soil (significant Whole Soil Biota effect, Table 3, Fig. 2). The biomass of *P. lanceolata* between all soil treatments was significantly different (Tukey's HSD test, $p<0.05$). The identity of target species did not influence the growth of *P. lanceolata* differently between soil treatments (non-significant Whole Soil Biota*Species interaction, Table 3). In all three soil treatments, the biomass of *P. lanceolata* was lowest when growing with *Echium vulgare* L.. In the sterile soil, the biomass of *P. lanceolata* was greatest when grown with *Hieracium pilosella* L.. In the microbial wash, *P. lanceolata* biomass was greatest when growing with *Bromus inermis* Leyss., and in the whole soil, its biomass was greatest when growing with *Trifolium pratense* L. (Fig. 2).

I found that target species had the highest biomass in the whole soil, second highest biomass in the sterile soil, and the lowest biomass in the microbial wash soil (significant Whole oil Biota effect, Table 4, Fig. 3.A). Target species had 88% greater biomass in the whole soil compared to the microbial wash soil, 40% greater in the whole soil compared to the sterile soil, and 52% greater in the sterile soil compared to the microbial wash soil. The differences in target species biomass between all the soil treatments were significantly different (Tukey's HSD, $p<0.01$). Overall, the heterospecific effect values in the whole soil treatment were significantly greater than those in the microbial wash soil (paired t-test,

t=6.381, df=21, p<0.01, Fig. 3.B). Only *Echium vulgare* and *Rudbeckia hirta* L. had greater heterospecific effect values in the microbial wash compared to the whole soil (Fig. 3.B). Four species, *Cichorium intybus* L., *Echium vulgare, Poa compressa* L. and *Poa pratensis* L. had negative heterospecific effect values in the whole soil treatment, whereas the remaining 17 target species had positive heterospecific effects in the whole soil (Fig. 3.B). By contrast, only three species, *Hypericum perforatum* L., *Potentilla recta* L., and *Solidago canadensis* L., had positive heterospecific effects in the microbial wash soil, whereas the remaining 18 species had negative heterospecific effects.

Hierarchies of competitive effect values between sterile, microbial wash and whole soil treatments mostly correlated between the soil treatments. The hierarchy of the competitive effect values in the sterile soil were positively correlated with competitive effect values in the whole soil and in the microbial wash soil (Table 5). However, the competitive effect values in the sterile soil were not correlated to those in the mycorrhizal soil (Table 5). Moreover, competitive effect values in the mycorrhizal soil treatment were positively correlated with those in the whole soil treatment. In addition, competitive effect values in the whole soil and sterile soil were lower than those in the microbial wash soil (paired t tests, sterile vs microbial: t=-3.21, df=21, p=0.002; whole soil vs microbial: t=-4.04, df=21, p=0.000).

Target species heterospecific effects differed when target species were grown with or without *P. lanceolata*. Target species heterospecific effects when grown alone in the whole soil and in the microbial wash soil were not correlated to their heterospecific effects when grown with *P. lanceolata* (Table 7). Target species that had more positive heterospecific effects in the whole soil treatment had lower values of competitive effect and competitive response when grown with *P. lanceolata* in the whole soil treatment (Table 6, Figure 4.A & D). However, target species heterospecific effects in the microbial wash soil were not correlated with their competitive effect or response values in the whole soil (Table 6, Figure 4.B & E). Heterospecific effects in the microbial wash soil were not correlated with either the competitive effect or competitive response values in the microbial wash soil (Fig, 4.C&F). When grown with *P. lanceolata*,

target species heterospecific effects in the whole soil were not correlated with competitive effect or response in that soil treatment (Table 8). Target species heterospecific effects in the microbial soil in competition were also not correlated with competitive effect and response in the whole soil treatment (Table 8). However, heterospecific effects in the microbial wash soil in competition were positive correlated with competitive effect values in that treatment, but not correlated with competitive response values in that treatment (Table 8). In addition, mycorrhizal response was positively correlated with heterospecific effects in the whole soil, but not correlated with heterospecific effects in the microbial wash soil (Table 9).

Discussion

I found that although feedback effects from the soil community cultured by *Plantago lanceolata* predicted the competitive ability of target species, the pattern was contrary to my prediction: target species that experienced more positive heterospecific effects were poorer competitors when grown with *P. lanceolata* in the whole soil (Fig. 4.A & D). However, target species heterospecific effects in the microbial wash soil were not correlated with competitive effect and response values in that treatment (Fig. 4.C & F). This suggests that the mycorrhizal rather than the microbial community present in the whole soil treatment may be the factor driving this relationship. Indeed the mycorrhizal growth response of target species is positively correlated with their heterospecific effect in the whole soil treatment (Table 9). In addition, target species experienced negative feedback from the microbial soil community when grown alone, however, this feedback effect did not affect their competitive ability. I also found that heterospecific effects of target species when grown with *P. lanceolata* were not correlated with their competitive effect values in the whole soil treatment (Table 8). These findings are consistent with those of Casper and Castelli (2007) who found that the effects of feedback for the soil community on *Sorghastrum nutans* when grown alone disappeared in the presence of competition. Furthermore, and heterospecific

effects were not correlated for target species when they were grown alone compared to when grown with *P. lanceolata* (Table 7). Thus, heterospecific effects from *P. lanceolata* biota did not determine competitive interactions between target species and *P.lanceolata*.

Mycorrhizae in the whole soil community may influence the pattern between heterospecific effects and competitive effect values in the whole soil treatment. Results from the previous chapter show that mycorrhizal response values were negatively correlated with species competitive effect values in the sterile soil treatment, which suggested that inferior competitors benefited most from mycorrhizal association (Stanescu, 2012). Mycorrhizal response was calculated as the difference in biomass between sterile soil and soil inoculated with generic mycorrhizal fungi, *Glomus intraradices*. Similar to heterospecific effects, mycorrhizal response values are also negatively correlated with competitive effect values in the whole soil treatment (Table 9). In fact, heterospecific effects in the whole soil treatment are positively correlated with mycorrhizal response values (Table 9). Thus, a species growth response to growing with *G. intraradices* was closely linked to its growth response to from *P. lanceolata* specific biota. In addition, competitive effect of target species in the mycorrhizal soil were positively correlated with competitive effect values in the whole soil (Table 5). This suggests that the positive heterospecific effects in the whole soil treatment are a result of the mycorrhizal community.

The growth of *P. lanceolata* was not influenced by the presence of *P. lanceolata* specific soil community. The biomass of *P. lanceolata* was the lowest in the microbial wash treatment both when grown alone (Fig. 1) and when grown with target species (Fig. 2) compared to the biomass in the sterile soil. In addition, target species had greater competitive effect values against *P. lanceolata* when grown the microbial wash soil compared to the sterile soil. This suggests that *P. lanceolata* was more severely suppressed by growth with target species in the microbial wash soil. However, the biomass of *P. lanceolata* did not differ between the sterile and the whole soil treatments when it was grown alone, suggesting that whole soil treatment had a neutral effect on the growth of *P. lanceolata*. Thus, mycorrhizae may compensate the negative effects of the microbial community in the whole soil

treatment. This finding is consistent with those in the previous chapter, where I found that the biomass of *P. lanceolata* was significantly greater when grown with a single species of fungi, *G. intraradices*, compared to when grown in sterile soil (Stanescu, 2012). In addition, Klironomos (2002) found that most plants only experience positive feedback from the mycorrhizal soil community if there is no negative feedback from the pathogen community. This indicates that in the absence of pathogens, *P. lanceolata* would benefit from mycorrhizal association. However, the finding that *P. lanceolata* experiences neutral feedback is not consistent with Bever (2002), who found that *P. lanceolata* experienced negative feedback from its own mycorrhizal soil community. In addition, Bever *et al.*, (2009) found that species can allocate more photosynthate to beneficial fungi, but in mixed fungal communities, both beneficial and less beneficial mycorrhizae can proliferate in host roots. Thus, the neutral feedback experienced by *P. lanceolata* can be due to an accumulation of fungi that do not provide a significant increase in growth. Moreover, Wagg *et al.*, (2011) show that increased plant productivity can occur due to either one beneficial fungi or a larger diversity to fungi depending on soil characteristics. Thus, neutral feedback can occur in the whole soil community where both beneficial and pathogenic fungi can colonize the roots of *P. lanceolata* or because in the soil used for this experiment, *G. intraradices* was more beneficial for the growth of *P. lanceolata*.

Target species experienced positive feedback from growing with whole soil biota cultured by *P. lanceolata*, and negative feedback from the microbial portion of the soil community. Although target species had more negative heterospecific effects in the microbial wash treatment compared to the whole soil treatment (Fig. 3 B.), they had greater competitive effect values in the microbial wash treatment. This suggests that although target species experienced poorer growth in the microbial wash soil, *P. lanceolata* was a much poorer competitor in this treatment. In addition, when target species were grown alone, they had significantly greater biomass in the whole soil treatment compared to the sterile soil treatment (Fig. 2.A), whereas the biomass of *P. lanceolata* when grown alone did not differ between the sterile and whole soil treatments. These results are consistent with other studies that have found that plants grow better in

soils that have biota cultured by other species (Bever, 2002; Klironomos, 2002; MacDougall *et al.*, 2011). This can be due to host specificity of the soil biota, particularly from mycorrhizae (Bever, 2002). Because the mycorrhizal community cultured by *P. lanceolata* was less beneficial for *P. lanceolata* compared to target species suggests that *P. lanceolata* experienced neutral feedback from its own soil because of the specificity of the mycorrhizal community, rather than the soil characteristics of this experiment. In addition, target species had greater competitive effect values in the microbial soil community compared to *P. lanceolata*, but still experienced overall negative effects from the soil community. This suggests that the microbial soil biota can reduce the growth of both conspecific and heterospecific hosts. Since target species experienced positive feedback and *P. lanceolata* experienced neutral feedback in the whole soil community also suggests that the mycorrhizal community cultured by *P. lanceolata* determined the direction and magnitude to feedback effects.

I found that feedback effects from biota cultured by *Plantago lanceolata* did not change the hierarchy of competitive ability of *P. lanceolata* and target species. The fact that competitive hierarchies were correlated between the sterile and whole soil treatment (Table 5) indicates that the effects of the whole soil treatment did not change competitive interactions between the target species and *P. lanceolata* compared to the interactions in the absence of the soil community. In addition, in the whole soil treatment, target species experienced positive feedback, suggesting that positive feedback effects do not influence competitive interactions. Furthermore, since competitive hierarchies were correlated between the sterile soil and microbial wash soil suggests that the negative feedback effect from the microbial community also did not change how target species interacted with *P. lanceolata*. Only the hierarchy of competitive effect in the mycorrhizal soil was not correlated with the hierarchy in the sterile soil. These results suggest that the effect of generic mycorrhizae changed competitive interactions, whereas mycorrhizae and pathogens specific to *P. lanceolata* did not change competitive interactions. This suggests that the host specificity of soil biota cultured by *P. lanceolata* did not affect how target species and *P. lanceolata* interacted. These results are not consistent with other studies that have found that

cultured soil biota pathogens influenced interactions between plants (van der Putten and Peters, 1997; Bever, 2002). This may be the case because the soil biota cultured by *P. lanceolata* may only affect the establishment and survival of conspecifics, since most studies have found that negative conspecific effects, rather than heterospecific effects influence plant abundance (Klironomos, 2002; MacDougall *et al.*, 2011). Since *P. lanceolata* experienced neutral feedbacks from its own soil community and that these feedbacks did not change the hierarchies of competitive effect suggests that feedback effects from the natural soil community may be weak and have small effects on competitive abilities.

These findings suggest that feedback effects on individual plants may not have implications for how species interact with neighbours. I found that positive feedback effects were negatively correlated with competitive ability in the whole soil, and negative feedback effects were not correlated with competitive ability in the microbial soil. This finding is opposite of the suggested model that positive and negative feedback should result in greater and lesser competitive ability respectively of species that experience it (Bever 2003; Bever *et al.*, 2010). In fact, heterospecific effects of target species when grown alone were not correlated to heterospecific effects when grown with *P. lanceolata*, and the link between heterospecific effects and competitive ability was not present when target species were growing with a competitor (Table 8). This finding is consistent with Casper and Castelli (2007), who found that the effects of feedback on a grassland species disappeared in the presence of competition. In addition, because the negative relationship between heterospecific effects and competitive ability disappears in the presence of competition suggests that feedback effects are weak and do not influence competitive interactions. In addition, since hierarchies of competitive effect did not change between the sterile soil treatment and soil cultured by *P. lanceolata*, and the fact that *P. lanceolata* experienced neutral feedback from its soil community suggests that feedback effects may not affect the abundance of *P. lanceolata* in the field. Thus, the phytometer method revealed that unlike conspecific effects, heterospecific effects do not predict competitive ability of the species that experience it, are most likely not linked to the abundance of species in the field. On the other hand, heterospecific effects may have implications for

species establishment. Plants experience the greatest mortality rates from pathogens from seeds to the seedling stage (Augspurger, 1984; Packer and Clay, 2000; Gurevitch *et al.,* 2006). Thus, if seedlings experience positive heterospecific effects, then the soil community can promote heterospecific establishment even if conspecific effects are not negative to prevent conspecific establishment. Indeed, Farrer and Goldberg (2011) found that the relative strength of conspecific and heterospecific effects differed between life-history stages. Thus future research should examine whether the strength feedback effects also change between life-history stages.

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Table 1. Family names, species name and common name of 22 species and their abundance data as measured at the Long Term Mycorrhizal Research Site (LTMRS), in Guelph, Ontario.

* - the seeds of these plants were ordered from Richter's Herbs.

Family	Species Name	Common Name	Life History
Asteraceae	*Achillea millefolium* L.	Yarrow	Perennial
Poaceae	*Bromus inermis* Leyss	Smooth brome	Perennial
Asteraceae	*Centaurea jacea* L	Brown Knapweed	Perennial
Asteraceae	*Cichorium intybus* L*	Chicory	Perennial
Lamiaceae	*Clinopodium vulgare* L*	Wild Basil	Perennial
Fabaceae	*Securigera varia* (L.) Lassen*	Crown Vetch	Perennial
Asteraceae	*Leucanthemum vulgare* Lam.*	Ox-eye Daisy	Perennial
Apiaceae	*Daucus carota* L.	Queen Anne's Lace	Biennial
Boraginaceae	*Echium vulagre* L.*	Viper's Bugloss	Biennial/Perennial
Asteraceae	*Hieracium pilosella* L.*	Mouse Ear Hawkweed	Perennial
Clusiaceae	*Hypericum perforatum* L.*	Common St. John's Wort	Perennial
Plantaginaceae	*Plantago lanceolata* L.	English Plantain	Biennial/Perennial
Poaceae	*Poa compressa* L.	Wire grass	Perennial
Poaceae	*Poa pratensis* L.	June Grass	Perennial
Rosaceae	*Potentilla recta* L.*	Rough-fruited Cinquefoil	Perennial
Lamiaceae	*Prunella vulgaris* L.*	Selfheal	Perennial
Asteraceae	*Rudbeckia hirta* L.	Black-Eyed Susan	Biennial/ Perennial
Asteraceae	*Solidago canadensis* L.	Canada Goldenrod	Perennial
Asteraceae	*Solidago graminifolia* (L.) Salisb	Flat-Topped Goldenrod	Perennial
Asteraceae	*Taraxacum officinale* F.H. Wigg*	Common Dandelion	Perennial
Fabaceae	*Trifolium pratense* L.	Red Clover	Biennial/Perennial
Scrophulariaceae	*Veronica officinalis* L.*	Common Speedwell	Perennial

Table 2. One way analysis of variance (ANOVA) table for the effects of soil treatments on the biomass of *P. lanceolata* when grown alone in the sterilized soil, whole soil and microbial wash soil.

Plantago lanceolata Biomass					
Source of Variation	Type III Sum of Squares	df	Mean Square	F value	Sig.
Whole Soil Biota	0.996	2	0.498	4.738	**0.024***
Block	0.624	2	0.312	2.968	0.080
Error	1.682	16	0.105		

Table 3. Two way analysis of variance (ANOVA) table for the effects of target species identity, soil treatments and their interactions on the biomass of the phytometer, *P. lanceolata* in the sterilized soil, whole soil and microbial wash soil.

Plantago lanceolata Biomass					
Source of Variation	Type III Sum of Squares	df	Mean Square	F values	Sig
Whole Soil Biota	9.486	1	4.743	59.617	**9.6E-23**
Species	5.363	21	0.255	3.210	**4.5E-6**
Whole soil biota*Species	4.123	42	0.098	1.234	0.162
Block	3.942	2	1.971	24.771	**9.9E-11**
Error	25.539	321	0.080		

Table 4. Two way analysis of variance (ANOVA) table for the effects of target species identity, soil treatments and their interactions on the biomass of target species in the sterilized soil, whole soil and microbial wash soil.

Target Species Biomass					
Source of Variation	Type III Sum of Squares	df	Mean Square	F values	Sig
Whole Soil Biota	6.135	2	3.067	84.795	**2.2E-30**
Species	17.677	21	0.842	23.269	**1.4E-62**
Whole soil biota*Species	10.577	42	0.252	6.962	**4.2E-26**
Block	1.181	2	0.591	16.330	**1.7E-7**
Error	11.793	326	0.036		

Table 5. Pearson correlation matrix for the z-scores of competitive effect values (CE) in whole soil (WS), sterile soil (S), mycorrhizal soil (M), and microbial wash soil (MW). Significant correlations are bolded. P values marked with † are not significant after sequential Bonferroni adjustment.

		CE-S	CE-WS	CE-MW	CE-M
CE-S	Correlation Coefficient	1.000	**0.612**	**0.480**	0.172
	Sig.	.	**0.002**	**0.024**†	0.443
	N	22	22	22	22
CE-WS	Correlation Coefficient	**0.612**	1.000	**0.523**	**0.427**
	Sig.	**0.002**	.	**0.013**	**0.047**†
	N	22	22	22	22
CE-MW	Correlation Coefficient	**0.480**	**0.523**	1.000	-0.181
	Sig.	**0.024**	**0.013**	.	0.420
	N	22	22	22	22
CE-M	Correlation Coefficient	0.172	**0.427**	-0.181	1.000
	Sig.	0.443	**0.047**	0.420	
	N	22	22	22	22

Table 6. Regressions between target species heterospecific effects in whole soil (WS) and microbial wash soil (MW) with their competitive effect (CE) ability and competitive response ability (CRS) in the respective soil treatments against *P. lanceolata*.

		Regression parameters		
y	x	b	R^2	p
CE - WS	HE -WS	**-0.30166**	**0.4331**	**0.001883**
	HE - MW	-0.02124	0.001473	0.8688
CE - MW	HE - MW	-0.06312	0.003922	0.7874
CRS-WS	HE -WS	**-0.4146**	**0.3388**	**0.005636**
	HE - MW	-0.1308	0.02313	0.5105
CRS-MW	HE - MW	-0.002032	0.1346	0.1019

Table 7. Regressions between heterospecific effects of target species when grown alone (HE_A) and heterospecific effects when grown with *Plantago lanceolata* (HE_P) in the whole soil (WS) and microbial wash soil (MW).

		Regression parameters		
y	x	b	R^2	p
HE_P-WS	HE_A-WS	0.3042	0.0935	0.1176
HE_P-MW	HE_A-MW	-0.0482	0.0024	0.8322

Table 8. Regressions between target species competitive effect (CE) and competitive response (CRS) values in the whole soil (WS) and microbial wash soil (MW) and heterospecific effects when grown in the competition treatment. P values marked with † are not significant after sequential Bonferroni adjustment.

		Regression parameters		
y	x	b	R^2	p
CE-WS	HE-WS	-0.1026	0.0463	0.3488
	HE-MW	0.1413	0.0692	0.2492
CRS-WS	HE-WS	-0.0950	0.0166	0.5772
	HE-MW	0.0480	0.0033	0.8030
CE-MW	HE-MW	**0.4369**	**0.2019**	**0.0409**†
CRS-MW	HE-MW	0.0620	0.0031	0.8105

Table 9. Regressions between target species mycorrhizal response and heterospecific effects (HE) in the whole soil (WS) and in the microbial wash soil (MW) and between mycorrhizal response and competitive effect (CE) and competitive response (CRS). P values marked with † are not significant after sequential Bonferroni adjustment.

		Regression parameters		
y	x	b	R^2	p
HE-WS	Mycorrhizal Response	**0.8725**	**0.6**	**2.318E-5**
HE-MW	Mycorrhizal Response	0.0008	8.046E-7	0.9968
CE-WS	Mycorrhizal Response	**-0.2925**	**0.3212**	**0.0059**
CRS-WS	Mycorrhizal Response	**-0.3650**	**0.1991**	**0.0373**†

Figure 1. *Plantago lanceolata* biomass when grown alone in the sterile (S), whole soil (WS) microbial wash soil (MW). The error bars represent one SD, the asterisk represents a significant difference using Tukey's HSD test ($p<0.05$) in the biomass of *P. lanceolata* in the sterile soil (S) versus the microbial wash soil (MW).

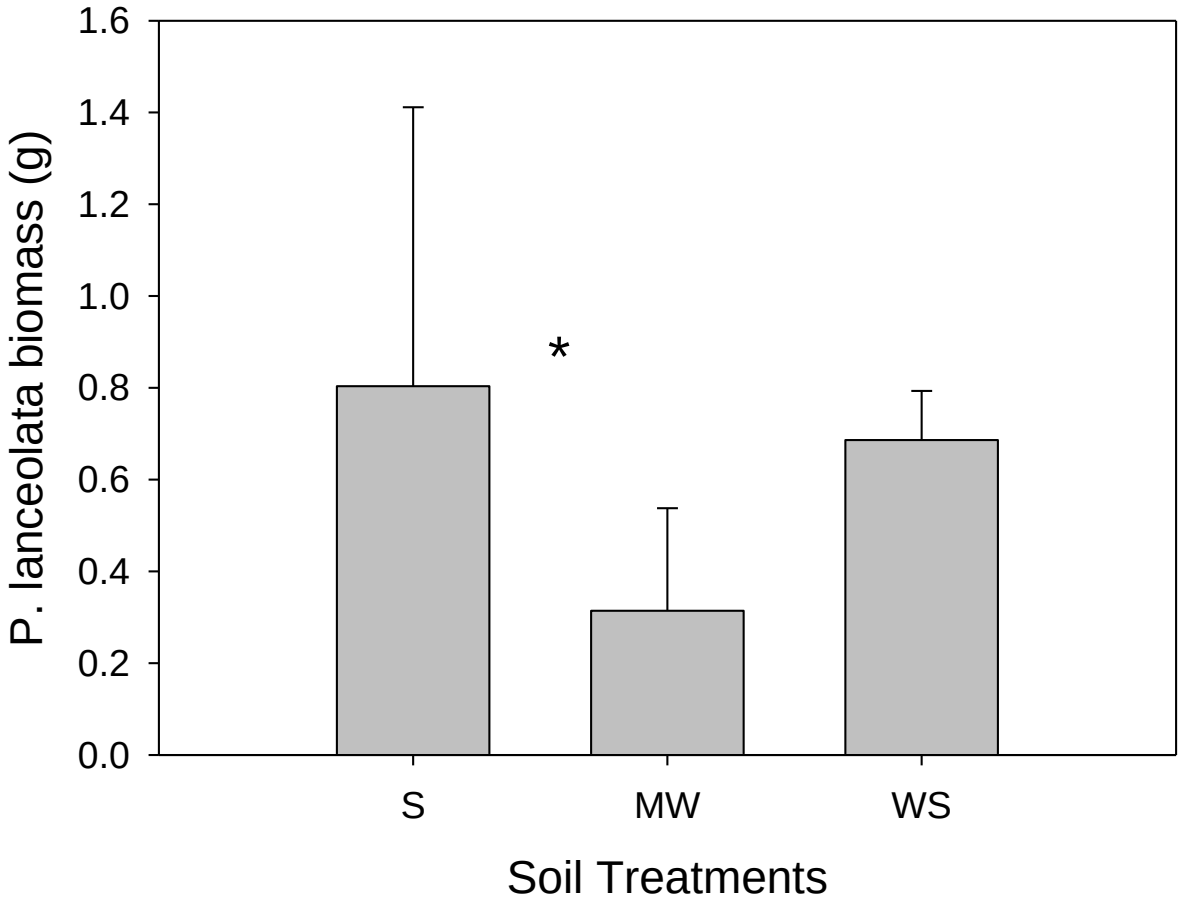


Figure 2. Reaction norms for *Plantago lanceolata* biomass when grown alone, with itself and with each target species in sterile soil (S), microbial wash (MW) and whole soil (WS).

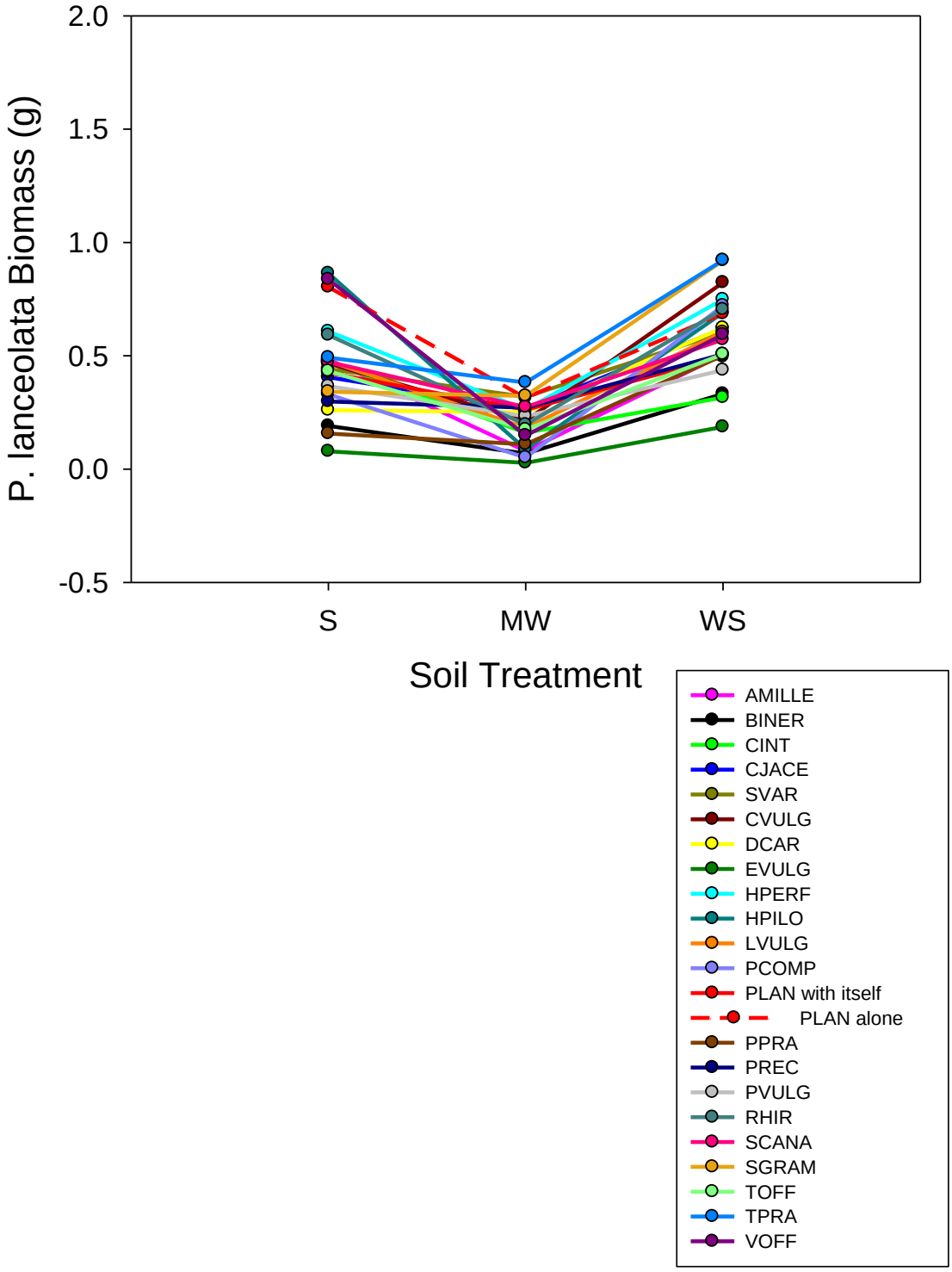


Figure 3. Reaction norms for target species biomass in the sterile soil (S), microbial wash (MW) and whole soil (WS) (A) and target species heterospecific effects (HE) in whole soil versus microbial wash soil.

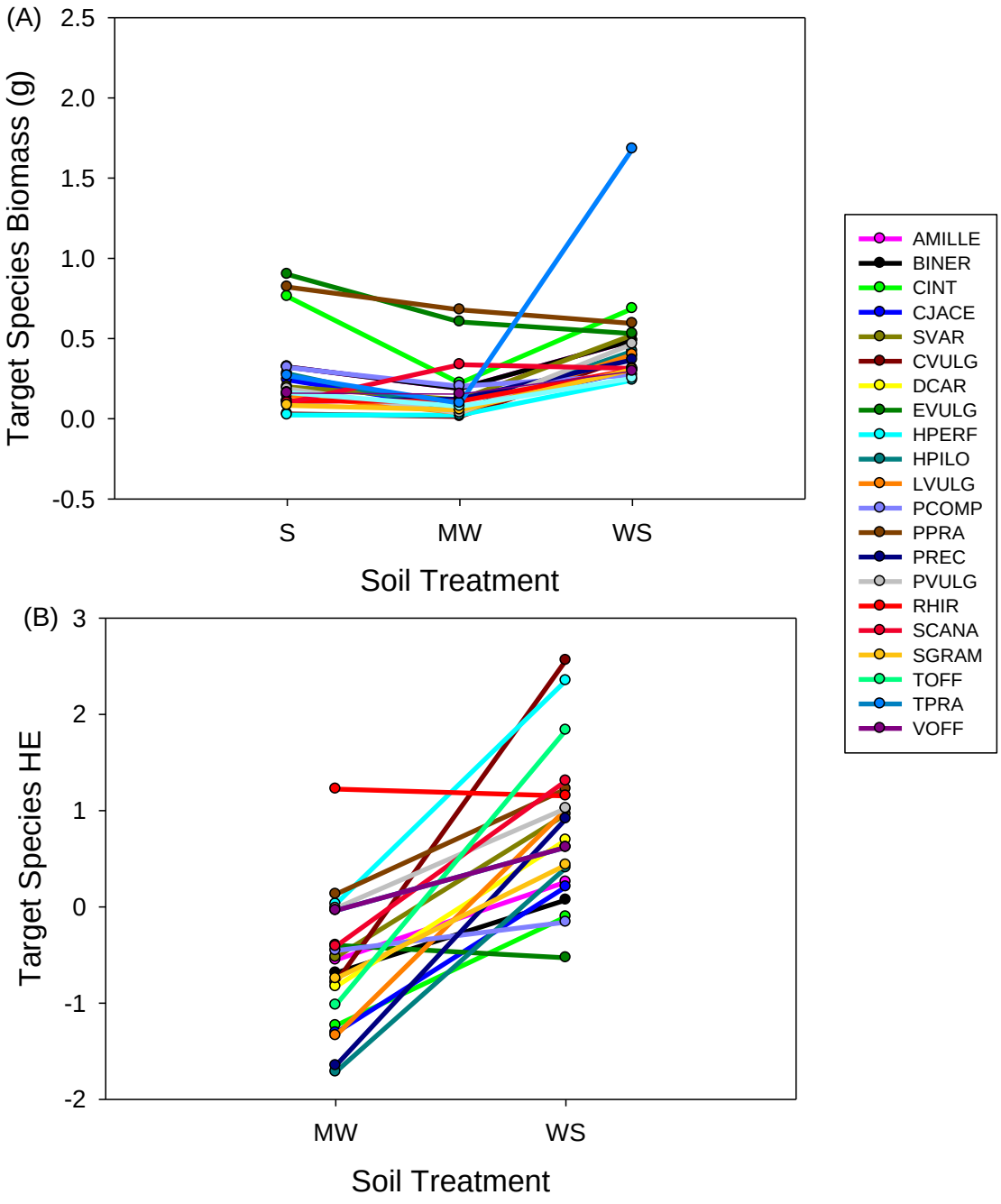
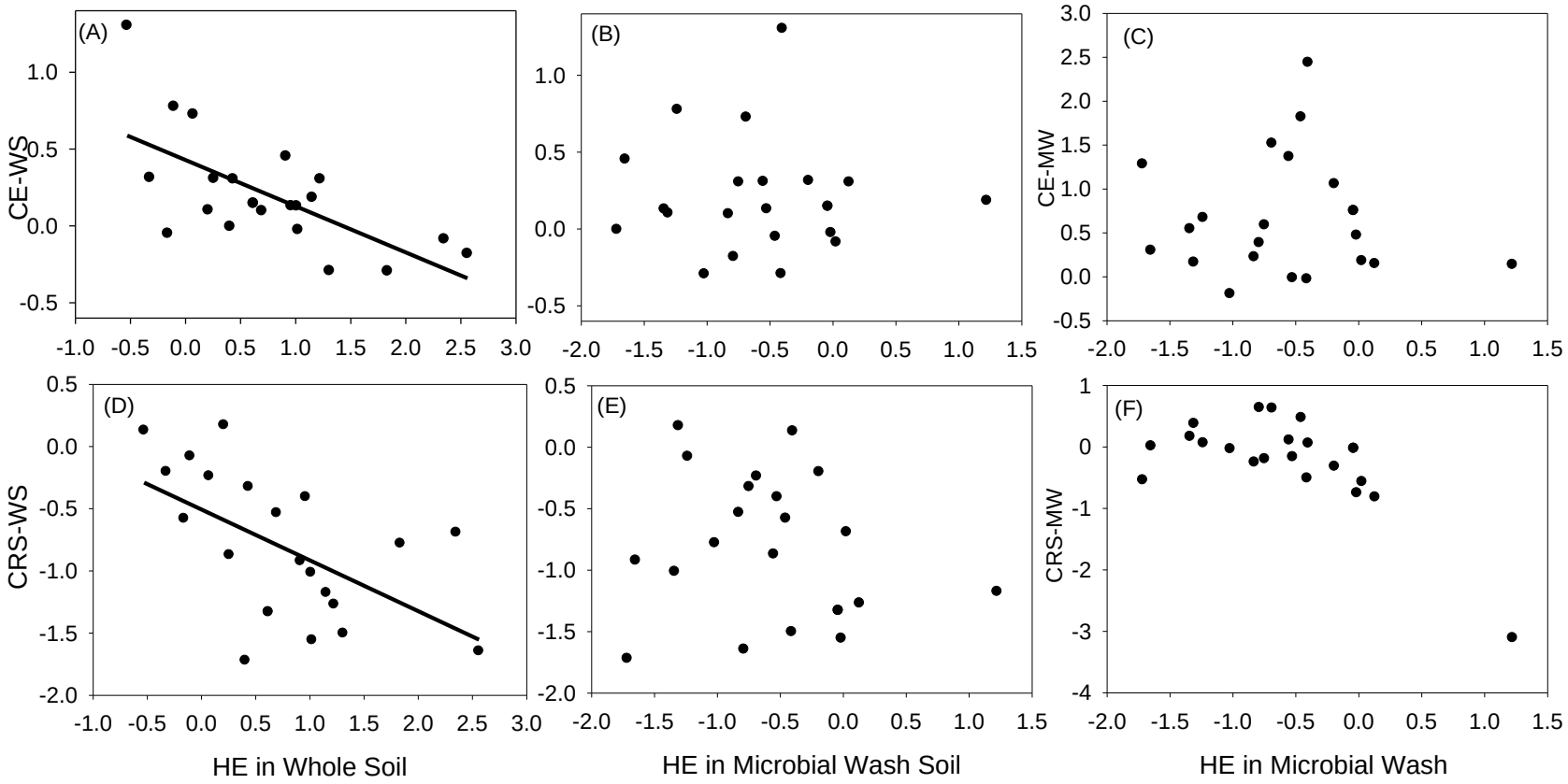


Figure 4. Regressions between competitive effect (CE) values in whole soil (A-B) and microbial wash soil (C) and competitive response (CRS) values (D-E) in whole soil and in microbial wash soil (F) with species heterospecific effect values in whole soil (left panel) and heterospecific effect values in the microbial wash soil (two right panels). Regression equations can be found in Table 6.



Appendix A – Regressions between Root Traits and Competitive Effect and Response Before and After Outlier Removal for Chapter I

		CE-M			CE-NM		
Traits	Regression parameters	b	R^2	p	b	R^2	p
SRL		-0.000002	0.07649	0.2249	-0.000004	0.0877	0.1924
Root to Shoot Ratio		**0.49731**	**0.329**	**0.00655***	0.2347	0.02008	0.54
Avg. Diameter		0.01621	0.000008	0. 9679	-1.0690	0.1042	0.1535
Early Growth biomass		0.52189	0.1104	0.1411	**1.56144**	**0.2708**	**0.01559***

Table 1: Regression parameters between competitive effect in mycorrhizal (CE-M) and non-mycorrhizal (CE-NM) soil treatments prior to outlier exclusion.

		CE-M			CE-NM		
Traits	Regression parameters	b	R^2	p	b	R^2	p
SRL		-0.000002	0.1825	0.05343	-0.000002	0.05816	0.2923
Root to Shoot Ratio		**0.54030**	**0.4213**	**0.00145***	0.2142	0.01814	0.5605
Avg. Diameter		-0.1243	0.006465	0.729	-1.0763	0.1328	0.1043
Early Growth biomass		0.48327	0.102	0.1582	**1.40514**	**0.2362**	**0.02549***

Table 2: Regression parameters between competitive effect in mycorrhizal (CE-M) and non-mycorrhizal (CE-NM) soil treatments after outlier exclusion.

		CRS-M			CRS-NM		
Traits	Regression parameters	b	R^2	p	b	R^2	p
SRL		-0.000003	0.05354	0.3129	0.000005	0.02643	0.4814
Root to Shoot Ratio		**1.2792**	**0.3136**	**0.00828***	1.8873	0.1485	0.08455
Avg. Diameter		-0.3561	0.007049	0.7175	**-5.1949**	**0.3261**	**0.00685***
Early Growth biomass		**1.9447**	**0.2193**	**0.03225***	0.2787	0.000979	0.8929

Table 3: Regression parameters between competitive response in mycorrhizal (CRS-M) and non-mycorrhizal (CRS-NM) soil treatments prior to outlier exclusion.

		CRS-M			CRS-NM		
Traits	Regression parameters	b	R^2	p	b	R^2	p
SRL		-0.000003	0.02044	0.5364	0.00001	0.06244	0.2747
Root to Shoot Ratio		**1.2486**	**0.2754**	**0.01457***	2.0407	0.16	0.0724
Avg. Diameter		-0.1849	0.001512	0.8671	**-6.2188**	**0.3718**	**0.00334***
Early Growth biomass		**1.9646**	**0.2078**	**0.03781***	0.2597	0.000789	0.9038

Table 4: Regression parameters between competitive effect in mycorrhizal (CRS-M) and non-mycorrhizal (CRS-NM) soil treatments after outlier exclusion.

Appendix B – Multiple Pearson Correlation Graphs between Competitive Effect Values in all Soil Treatments for Chapter II

Figure 1. Correlation graphs between competitive effect values (CE) in the sterile soil (S), whole soil (WS), microbial wash soil (MW) and mycorrhizal soil (M) treatments. Correlation coefficients can be found in Table 5 of Chapter II.