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Gary T Poon, Hafiz Maherali

The novel weapons hypothesis posits that biochemical compounds secreted by an invasive species facilitate its success by reducing the performance and survival of other species. This mechanism has been proposed to explain the widespread invasion of the biennial plant, *Alliaria petiolata*, in North America. Root exudates produced by *A. petiolata*, a nonmycorrhizal plant, suppress the growth of mycorrhizal fungi, which is expected to strengthen its competitive ability relative to plant species that rely on mycorrhizal fungi for nutrient uptake services. To test this hypothesis, we grew 27 mycorrhizal tree, forb and grass species that are representative of invaded habitats in the absence or presence of competition with *A. petiolata* in soils with and without a legacy of the invader. A legacy of *A. petiolata* in soil reduced mycorrhizal colonization of competitor species by >50%. Contrary to expectations, competition between *A. petiolata* and other species was stronger in control than legacy soil. The invader suppressed the biomass of 19 of 27 competitor species in control soil but only 7 species in legacy soil. This pattern may have been caused by a stronger negative effect of legacy soil on *A. petiolata* biomass relative to competitor species. The legacy treatment reduced plant available nitrogen by >50% relative to control soil and reduced *A. petiolata* biomass by 56%, whereas the average biomass of competitor species was reduced by 15%. Our results suggest that despite effective suppression of mycorrhizal fungi, a legacy of *A. petiolata* in soil does not increase its competitive advantage against other species. Instead, the negative effect of nutrient depletion on *A. petiolata* was stronger than the negative effect of suppressing mycorrhizal colonization on competitor species. Therefore, the potential for *A. petiolata* to suppress mycorrhizal plant species through allelopathic effects on mycorrhizal fungi may be weaker than previously expected.

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Abstract

The novel weapons hypothesis posits that biochemical compounds secreted by an invasive species facilitate its success by reducing the performance and survival of other species. This mechanism has been proposed to explain the widespread invasion of the biennial plant, *Alliaria petiolata*, in North America. Root exudates produced by *A. petiolata*, a nonmycorrhizal plant, suppress the growth of mycorrhizal fungi, which is expected to strengthen its competitive ability relative to plant species that rely on mycorrhizal fungi for nutrient uptake services. To test this hypothesis, we grew 27 mycorrhizal tree, forb and grass species that are representative of invaded habitats in the absence or presence of competition with *A. petiolata* in soils with and without a legacy of the invader. A legacy of *A. petiolata* in soil reduced mycorrhizal colonization of competitor species by >50%. Contrary to expectations, competition between *A. petiolata* and other species was stronger in control than legacy soil. The invader suppressed the biomass of 19 of 27 competitor species in control soil but only 7 species in legacy soil. This pattern may have been caused by a stronger negative effect of legacy soil on *A. petiolata* biomass relative to competitor species. The legacy treatment reduced plant available nitrogen by >50% relative to control soil and reduced *A. petiolata* biomass by 56%, whereas the average biomass of competitor species was reduced by 15%. Our results suggest that despite effective suppression of mycorrhizal fungi, a legacy of *A. petiolata* in soil does not increase its competitive advantage against other species. Instead, the negative effect of nutrient depletion on *A. petiolata* was stronger than the negative effect of suppressing mycorrhizal colonization on competitor species. Therefore, the potential for *A. petiolata* to suppress mycorrhizal plant species through allelopathic effects on mycorrhizal fungi may be weaker than previously expected.

Key words - competition, competitive effect, functional traits, garlic mustard, nutrient depletion, soil feedback, species invasion

Introduction

Invasions by exotic species are common and can negatively influence the structure and function of invaded communities and ecosystems (Pimental et al., 2000). Designing effective control and eradication programs to limit the spread of an invasive species, however, requires identifying the specific mechanism that facilitated invasion (Mack et al., 2000). Numerous mechanisms have been identified to explain successful invasions (Catford et al., 2009; Gurevitch et al., 2011). For example, successful invaders may have high propagule production (Colautti et al., 2006), possess or evolve superior competitive ability for limiting resources (Blossey & Notzold, 1995), be released from specialist antagonists in their native range (Callaway et al., 2004), possess the ability to acclimate to a wide variety of conditions (Parker et al., 2003), or secrete novel biochemical compounds that reduce the performance and survival of native inhabitants (Callaway & Ridenour, 2004), among other mechanisms (Catford et al., 2009).

Recent reviews suggest that successful invasions cannot be easily explained by a single mechanism (Catford et al., 2009; Gurevitch et al., 2011), which has led to the proposal that multiple mechanisms may interact synergistically to influence invasion (Gurevitch et al., 2011; Lau & Schultheis, 2015). For example, the allelopathic effects of novel biochemical weapons are expected to directly inhibit the germination, growth and survival of other species (Callaway & Aschehoug, 2000; Callaway & Ridenour, 2004; Stinson et al., 2006), but also enhance the intrinsic competitive ability of the invader (Lau & Schultheis, 2015). Similarly, functional aspects of species in the invaded community, such as a poor ability to acquire soil resources or resist herbivory may interact with novel weapons to increase susceptibility to invasion (Lau & Schultheis, 2015). Empirical assessments of interactions or synergies between different mechanisms of invasion, however, are infrequent (Zheng et al., 2015).

The novel weapons hypothesis has been proposed to explain the widespread invasion of *Alliaria petiolata* ((M. Bieb.) Cavara & Grandem, Brassicaceae), a biennial species native to Europe that was introduced to North America in the late 19th century (Cavers et al., 1979). Allelopathic phytochemicals in this species are present in leaf litter and released as root exudates (Cipollini et al.,

2005; Cipollini & Gruner, 2007; Rodgers et al., 2008), but have limited direct negative effects on neighbouring plant species (McCarthy & Hanson 1998; Roberts & Anderson, 2001; Prati & Bossdorf, 2004; Cipollini et al., 2008). Instead, these phytochemicals tend to suppress the growth of mycorrhizal fungi (Roberts & Anderson, 2001; Stinson et al., 2006; Callaway et al., 2008; Rodgers et al., 2008; Wolfe et al., 2008; Cantor et al., 2011), though the effect is variable (Burke, 2008; Lankau et al., 2009; Lankau, 2011). Because *A. petiolata* is non-mycorrhizal, whereas most plant species rely on mycorrhiza for nutrient uptake services (Wang & Qiu, 2006; Brundrett, 2009), the suppression of mycorrhizal fungi is expected advantage *A. petiolata* relative to other species (Stinson et al., 2006; Callaway et al., 2008; Hale & Kalisz, 2012).

The successful invasion of *A. petiolata* may be explained by an interaction between novel weapons and competitive ability (Blossey & Notzold, 1995). Resource competition theory suggests that the strongest competitors are species that deplete limiting resources to the lowest level (Tilman, 1988; Tilman & Wedin, 1991). If *A. petiolata* is a strong competitor, it should deplete soil nutrients below tolerable limits for other species, suppressing other species more than itself. The potential for *A. petiolata* to deplete soil nutrients more than other species, while simultaneously suppressing mycorrhizal fungi, should result in stronger suppression of mycorrhizal competitors in soils with a legacy of *A. petiolata* than soils without such a legacy. This is because legacy soils would have reduced nutrients and lack the symbionts which assist mycorrhizal plants in nutrient uptake (Bever et al., 2010). Despite the potential for novel weapons to strengthen the competitive ability of *A. petiolata*, direct competition between *A. petiolata* and other species has been examined in relatively few studies (Meekins & McCarthy, 1999; Rodgers et al., 2008; Lankau, 2010; Leicht-Young et al., 2012; Smith & Reynolds, 2014). These studies suggest that *A. petiolata* is a weaker competitor than some species, but stronger than others (Rodgers et al., 2008). However, no studies have examined if a legacy of *A. petiolata* in soils enhances its competitive ability against mycorrhizal plant species.

Even though the combination of novel weapons and competitive ability could increase the success of *A. petiolata*, resident species may still be able to resist invasion. The ability of resident species to resist invasion could depend on the morphological and physiological traits that influence

acquisition of soil nutrients and light, which are often most limiting to plant growth (Grime, 1977; Gaudet & Keddy, 1988; Goldberg & Landa, 1991; Wardle et al., 1998). The depletion of soil nutrients and the suppression of mycorrhizal fungi by *A. petiolata* suggests that species which resist *A. petiolata* invasion should have root systems which are efficient at acquiring nutrients. For example, plants with thin roots maximize absorptive root surface area for resource uptake while minimizing energetic investment (Goldberg, 1996; Casper & Jackson, 1997). In addition, species that successfully resist invasion by *A. petiolata* may be those which are effective at acquiring light (Stinson & Seidler, 2014), particularly by accelerated height growth, which would allow them to overtop neighbors, and by having high photosynthetic light use efficiency (Gaudet & Keddy, 1988; Goldberg & Landa, 1991; Rosch et al., 1997; Keddy et al., 2002; Wang et al., 2010).

To study the influence of soil legacy on competitive interactions between *A. petiolota* and other species, we grew *A. petiolata* with and without potential competitor species in field soils that either been left intact or had previously been planted with *A. petiolata* (e.g., Callaway et al., 2008). The soil legacy treatment included the combined effects of nutrient depletion and suppression of mycorrhizal fungi by *A. petiolata*. Because *A. petiolata* occurs in a wide variety of habitats, including old fields, road sides, forest edges and forest understories (Cavers et al., 1979; Stinson & Seidler, 2014; Smith & Reynolds, 2014; Biswas et al., 2015), we quantified competition between *A. petiolata* and 27 native and non-native mycorrhizal competitor species that represent these different habitats (e.g., Cavers et al., 1979). We predicted that *A. petiolata* soil legacy inhibits mycorrhizal plant species, making it more likely for *A. petiolata* to both suppress the growth of, and resist growth suppression by, competitor species. We predicted that competitor species with finer roots, greater height extension, and higher photosynthetic efficiency would be more likely to resist competition against *A. petiolata*.

Materials and Methods

To determine if *A. petiolata* is a strong competitor for resources, we grew 27 target species in competition against *A. petiolata* (Table 1). Competitor species included forest trees, forest understory herbs, old field herbs and grasses that are commonly found in areas typically invaded by *A. petiolata* in

126 southern Ontario (e.g., Biswas et al., 2015). *Alliaria petiolata* seeds were bulk collected from the Wild
127 Goose Woods, a mixed hardwood forest in the University of Guelph Arboretum (43° 32'N, 80° 12'W)
128 in July 2009. *Alliaria petiolata* can be found in dense patches along the periphery of the forest
129 throughout this site. Seeds for each competitor species were harvested within the Guelph Arboretum as
130 well as purchased from suppliers [Acorus Restoration, Walsingham, ON; Angelgrove Seed Company,
131 Harbour Grace, NL, Ontario Tree Seed Facility, Angus, ON; Richter's Herbs, Goodwood, ON (Table
132 1)].

133 To simulate a soil environment that *A. petiolata* is likely to encounter upon invasion, we grew
134 plants in a forest soil without a history of *A. petiolata.* Soil was collected from a mixed deciduous
135 forest dominated by *Acer saccharum* in the Koffler Scientific Reserve (44° 03' N, 79° 29' W,
136 Newmarket, ON). Soil was sieved to remove roots and stones and placed into 30, 35L tubs (Roughneck
137 Storage Box #2214, Newell Rubbermaid Inc, Atlanta, GA). Tubs had holes drilled in the bottom to
138 facilitate drainage. To experimentally create a legacy of *A. petiolata* invasion, we grew *A. petiolata*
139 plants in half of the field collected soil (e.g., Callaway et al., 2008). *A. petiolata* seedlings, germinated
140 from seeds that were cold stratified at 4°C for 120 days, were transplanted into 15 randomly selected
141 tubs. After 6 weeks, seedlings were thinned to 80 plants/m^2, which approximates the upper end of *A.*
142 *petiolata* density in field populations (Meekins & McCarthy, 2002). Tubs were randomly arranged on
143 the greenhouse bench and watered to maintain field capacity. Seedlings were transplanted into tubs in
144 January 2010, and harvested after 5 months. After harvest, soil was sieved to remove roots and
145 homogenized within each soil treatment. To determine ~~if~~ the effect of *A. petiolata* legacy on soil
146 nutrients, we sampled 500 mL of soil from each homogenized mixture and analyzed it for NO_3^-, NH_4^+,
147 P, Mg, and K (University of Guelph Laboratory Services;
148 www.guelphlabservices.com/AFL/plants.aspx).

149 To study the effects of soil legacy, interspecific competition and competitive species identity on
150 the growth of either *A. petiolata* or the competitor, we used a three-factor design. To quantify
151 competition, we grew each competitor species in the presence and absence of an *A. petiolata* individual
152 in the same pot (e.g., Gaudet & Keddy, 1988; Wang et al., 2010). Each treatment combination [(27

153 species + *A. petiolata*) × 2 soil treatments × 2 competition treatments] was replicated 6 times for a total
154 of 744 pots. Pots were randomly arranged in a checkerboard pattern across 53 trays (57 N25T, Stuewe
155 and Sons, Inc., Tangent, OR) to minimize competition for light between pots. To induce germination
156 all seeds were cold stratified for 30-120 days based on information provided by seed suppliers. Cold
157 stratification times were staggered to ensure all species germinated at the same time. After
158 vernalization, seeds were moved to the University of Guelph Phytotron greenhouse and germinated in a
159 medium of 2/3 top soil and 1/3 silica sand. Seedlings were transplanted into 650 mL pots (6.4 cm wide
160 × 25 cm deep; D40 R, Stuewe and Sons Inc., Tangent, OR) filled with either *A. petiolata* legacy or
161 control field soil. Because of slow germination in some species, they were planted in two groups
162 separated by two weeks. All plants were grown for the same number of days and were completely
163 randomized across the greenhouse benches. Because soil with a legacy of *A. petiolata* had very low
164 nutrient levels, 100 mL of ¼ strength 18-9-18 N:P:K fertilizer (Plant Products, Leamington, ON) was
165 added once to all pots to promote seedling establishment but still maintain nutrient differences between
166 the soil treatments. After 63 days, the aboveground parts of plants were harvested, separated according
167 to species, dried at 60°C for 48 hours and weighed.

168 To determine if *A. petiolata* soil legacy suppressed arbuscular mycorrhizal (AM) fungi, we
169 harvested the roots of a subset of competitor species when grown alone in both legacy and control soil.
170 Because of time limitations for colonization measurements, we selected 8 species that represented the
171 range of growth forms in the experiment. Root cell contents were cleared with potassium hydroxide
172 and AM fungi were stained with Chlorazol black E (Brundrett et al., 1984). Samples were mounted on
173 glass slides and viewed under a compound microscope at 250× magnification. To quantify fungal
174 colonization by AM hyphae, arbuscules, and vesicles, we used the gridline intersection method
175 (McGonigle et al., 1990). Colonization was quantified as the presence or absence of well-stained
176 structures at 50 intersections per root sample.

177 To determine whether morphological and physiological traits could explain the ability of
178 competitive species to either resist suppression by, or suppress *A. petiolata*, we measured aboveground
179 traits on all plant species when grown alone in both soil treatments. Plant traits were measured in the

absence of competition. We measured leaf chlorophyll concentration and photosynthetic efficiency of up to 6 individuals from each species in each soil treatment at five and nine weeks growth. Height at five weeks on these individuals was recorded as the vertical distance from the soil surface to the tip of the tallest leaf. We measured chlorophyll concentration on the three youngest fully expanded leaves per plant using a portable chlorophyll meter (SPAD 502, Minolta, Inc., Ramsey, NJ), and calculated an average value per plant. We measured photosynthetic efficiency as instantaneous fluorescence yield under saturating light conditions (1500 μmol m^{-2} s^{-1}), a measure of the efficiency of photosystem II in converting light energy for photochemistry (Maxwell & Johnson, 2000). The three youngest fully expanded leaves per plant were measured using a light-adapted fluorometer (PAM-2500, Heinz Walz APbH, Effeltrich, Germany) and an average value per plant was calculated.

To determine if root traits influence competitive ability, we grew 5 replicates of all plant species in a separate experiment in a sterilized mixture of 2/3 silica sand and 1/3 topsoil for 35 days. Plants were grown individually in 650 mL pots (D40 R, Stuewe and Sons Inc., Tangent, Oregon, USA). The shorter growing period and silica sand-topsoil mixture prevented plant roots from becoming pot bound and facilitated the harvest of intact root systems. At harvest, roots were cleaned and preserved in 50% ethanol. For analysis, roots were stained with 0.05% Toluidine Blue O to improve the visibility of fine roots, spread out in water to minimize overlap and photographed with a high resolution (600 dpi) scanner (Epson V700, Epson Canada Limited, Markham, ON). Root images were analyzed with WinRhizo software (version 2009a; Regent Instruments 2009, Quebec City, QC) using the automatic pixel classification setting to assess the length and average root diameter of each root system. After scanning, roots were dried at 60°C for 48 hours and weighed. In addition to average root diameter, we also calculated specific root length (SRL), or the ratio of root length to root mass is indicative of the amount of surface area available for nutrient absorption (Craine et al., 2001).

To assess the magnitude and variation in resistance of competitor species to *A. petiolata* competition and whether the magnitude of resistance is influenced by soil legacy, we analyzed aboveground biomass of competitor species with a three way analysis of variance (ANOVA) with competition, soil legacy and competitor species identity and all interactions as factors. Planned

orthogonal single degree of freedom (1-df) contrasts were used to determine whether each competitor species biomass differed between competition treatments within each soil legacy treatment. We also used 1-df contrasts to test whether growth forms (trees, forbs, grasses) differed as a whole between competition treatments in each soil legacy treatment.

To assess the magnitude and variation in the ability of competitor species to influence *A. petiolata* aboveground biomass, and whether this species effect was influenced by soil legacy, we used a two-way ANOVA with competitor species identity, soil legacy and their interaction as factors. To test whether growth with a competitor species suppressed the biomass of *A. petiolata* in each soil legacy treatment, we used planned orthogonal 1-df contrasts to compare the biomass of *A. petiolata* grown alone relative to its growth i) with each competitor, ii) with each growth form in aggregate, and iii) across all competitor species in aggregate. The effect of soil treatment on fungal colonization of roots was determined with a 2 way ANOVA with soil and species as factors, and 1-df contrasts were used to test for soil effects on colonization for each species. The effect of growth form and soil treatment on plant traits was tested with a 2 way ANOVA using species means for each trait as the replicate. Differences among growth forms were determined by comparing the 95% confidence intervals for each growth form for overlap following a significant main effect. All ANOVAs and 1-df contrasts were done with SPSS 22.0 (IBM Corp., Armonk, NY).

To quantify variation in the ability of competitor species to either resist either resist suppression by *A. petiolata* or suppress *A. petiolata*, we calculated two indices of competition. The ability of a competitor species to resist suppression is defined as competitive response (CR, Wang et al., 2010), and was quantified as ln(biomass under competition/biomass alone). The ability of each competitor species to suppress *A. petiolata* is defined as competitive effect (CE, Gaudet & Keddy, 1988; Wang et al., 2010), and was quantified as –ln(*A. petiolata* biomass under competition/*A. petiolata* biomass alone). When calculated this way, greater values reflect stronger competitive ability.

To determine whether morphological and physiological traits of competitor plants were associated with competitive ability, we used phylogenetic generalized least squares (PGLS) multiple regression, with competitive ability as the dependent variable and traits as independent variables.

Growth form of plants was used as a covariate in the analysis. Because root traits were assessed in a different experiment, multiple regression analyses were run separately for aboveground and belowground traits. To analyze data, we used the time calibrated phylogenetic tree from Davies et al. (2004) in Phylomatic (Webb et al., 2008), pruned to include the competitor species. In PGLS regression, the phylogenetic variance-covariance matrix is incorporated into the calculation of coefficients (β) for either a univariate or multiple regression model (Martins & Hansen, 1997; Pagel, 1999). To calculate the magnitude of phylogenetic effects on the regression, maximum likelihood is used to estimate λ, an index which varies from 0, indicating complete independence between variation in the regression residuals and phylogeny, and 1, indicating complete dependence between residual variation with Brownian model of evolution (Freckleton et al., 2002). When $\lambda = 0$, the PGLS regression is identical to ordinary least squares regression. PGLS regression and estimates of λ were done in R version 3.12 (R Core Team, 2015) using the 'pgls' command in the package caper, version 0.5.2 (Orme et al., 2013).

Results

The average aboveground biomass of competitor species was reduced by the presence of *A. petiolata* compared to when they were grown alone (significant competition main effect, Table 2). However, competition was weaker in legacy relative to control soils (significant soil legacy × competition interaction, Table 2, Fig. 1). The average biomass of competitor species was reduced by 59% in control soil compared to 27% in *A. petiolata* legacy soil. The influence of soil legacy on competition also varied among species (significant species × soil legacy × competition interaction, Table 2, Fig.1). For example, the aboveground biomass of 19 species (13/18 native, 6/9 introduced) was suppressed by competition in control soil whereas only 7 species (4/18 native, 3/9 introduced) were suppressed by *A. petiolata* competition in legacy soil. On a growth form basis, trees (-38%, $P = 0.01$), forbs (-62%, $P < 0.000001$) and grasses (-56%, $P < 0.000001$) were all suppressed by *A. petiolata* in control soil, whereas the biomass of forbs (-32%, $P < 0.000001$) and grasses (-14%, $P =$

0.007), but not trees ($P = 0.228$), was significantly reduced by the presence of *A. petiolata* in legacy soil (Fig.1, insets).

The average biomass of *A. petiolata* in competition was not significantly different from its average biomass when grown alone in either soil treatment ($P_{legacy} = 0.284$, $P_{control} = 0.602$; Fig. 2). *Alliaria petiolata* biomass varied in response to competition with different competitor species (significant species effect, Table 3, Fig. 2). In most cases, these species effects were not consistent between control and legacy soils (significant species × soil legacy interaction, Table 3). For example, relative to its biomass when alone, *A. petiolata* was significantly smaller in competition with *He. matronalis, B. inermis, E. canadensis, E. riparius,* and *E. virginicus* in control soil but significantly smaller in competition with *Q. macrocarpa, He. matronalis* a*E. canadensis* in legacy soil (Fig. 2). In some cases, *A. petiolata* biomass was higher when grown with a competitor species than when grown alone. This response occurred with *Pi. strobus* and *Th. occidentalis* in control soil and *Hy. perforatum* in legacy soil. *A. petiolata* biomass response to competition also varied with growth form, and this effect differed between soil treatments (Fig. 2, insets). In control soil, *A. petiolata* biomass was 27% higher ($P = 0.041$) when grown with trees than when grown alone, 43% lower ($P = 0.001$) when grown with grasses than when grown alone, and not influenced by forbs ($P = 0.61$). In legacy soil, *A. petiolata* biomass was not affected by competition with trees ($P = 0.96$) or forbs ($P = 0.38$), but was 62% lower ($P = 0.033$) when grown with grasses than when grown alone.

On average, competitor species grown alone in soil with a legacy of *A. petiolata* were 15% smaller than plants grown in control soil ($F_{1,261} = 21.991$, $P = 0.000004$, Fig. 3). Competitor species also differed in their response to *A. petiolata* soil legacy ($F_{26,261} = 3.042$, $P = 0.000003$), though a majority showed no significant difference between treatments. Significant negative effects of soil legacy were found for *Q. macrocarpa* (-37%, $P = 0.041$), *He. matronalis* (-29%, $P = 0.041$), *R. hirta* (-43%, $P < 0.000001$), *E. riparius* (-20%, $P = 0.048$), and *Pa. virgatum* (-33%, $P < 0.000001$). The strongest negative response to soil legacy was observed for *A. petiolata*, whose biomass was 56% lower in legacy than in control soil ($P = 0.004$).

Plants grown in soils with a legacy of *A. petiolata* had reduced levels of arbuscular mycorrhizal colonization of roots (Fig. 4). On average, plants in the soil legacy treatment had 57% reduced hyphal colonization ($F_{1,60} = 20.47$, $P = 0.000029$), 53% reduced arbuscular colonization ($F_{1,60} = 4.97$, $P = 0.029$), and 57% reduced vesicular colonization ($F_{1,60} = 4.95$, $P = 0.030$) than plants grown in control soils. These effects were strongest in *Q. macrocarpa*, *F. virginiana* and *E. canadensis* for hyphae (Fig. 4a), *Hy. perforatum* for arbuscles (Fig. 4b) and *F. virginiana* for vesicles (Fig 4c). We found that growing *A. petiolata* in field soil depleted soil nutrients. Field collected soil contained 160 mg/kg NO_3^- ,18.3 mg/kg NH_4^+, 23 mg/L P, 77 mg/L Mg, and 52 mg/L K. In legacy soil, these amounts were reduced to 29.2 mg/kg for NO_3^- (-82%), 8.56 mg/kg for NH_4^+ (-53%), 19 mg/L for P (-17%), 53 mg/L for Mg (-31%) and 40 mg/L for K (-23%).

Morphological and physiological traits of competitor plants grown alone differed among growth forms, but were not generally affected by growing in soil with a legacy of *A. petiolata* (Fig. 5). Quantum yield of photosystem II [Y(II)] measured at week 5 was significantly higher in herbs and grasses relative to trees. In week 9, Y(II) was significantly higher in herbs compared to grasses and trees (Fig 5a). The yield of photosystem II did not differ between soil treatments in week 5, but was lower in legacy soil than control soil in week 9. Chlorophyll concentration in week 5 was significantly higher in herbs and grasses than trees, but did not differ among growth forms in week 9, and did not differ between soil legacy treatments (Fig. 5b). At week 5, grasses were significantly taller than trees and herbs, but height was not influenced by soil legacy treatment (Fig. 5c). Trees had significantly larger root diameter than either herbs or grasses, which did not differ significantly from each other. Trees also had significantly lower SRL than herbs, whereas grasses had intermediate SRL that did not differ significantly from that of trees and herbs (Fig. 5d & 5e).

Though above and belowground functional traits varied among growth forms, these traits were generally not associated with their ability to compete in either soil environment, measured as either the ability to resist suppression from (competitive response, CR) or suppress (competitive effect, CE) *A. petiolata* (Table 4). The only exception to this pattern was the nearly significant ($P = 0.06$) positive relationship between Y(II) @ 5 weeks and competitive response in legacy soil. In addition, even

though species varied in their response to soil legacy, this variation was also not correlated with competitive ability. The ln response ratio of growth in legacy versus control soils (Fig. 3) was not associated with either competitive response ($F_{1,25} = 0.07$, $r^2 = 0.003$, $P = 0.79$, $\lambda = 0.978$) or competitive effect ($F_{1,25} = 0.57$, $r^2 = 0.022$, $P = 0.45$, $\lambda = 0.266$).

Metrics of competitive ability were not strongly correlated across soil treatments. Specifically, CR in control soil only explained 5.7% of the variation in CR in legacy soil, and CE in control soil only explained 12% of the variation in CE in legacy soil (Fig. 6). CR and CE were not correlated with each other either in legacy soil ($F_{1,25} = 2.73$, $r^2 = 0.098$, $P = 0.11$, $\lambda = 0$) but were positively correlated in control soil ($F_{1,25} = 7.44$, $r^2 = 0.229$, $P = 0.01$, $\lambda = 1$).

Discussion

Our results indicate that though multiple mechanisms can interact to influence the performance of *A. petiolata*, these interactions are unlikely to facilitate a successful invasion. Though *A. petiolata* was a strong competitor when tested against a range of common mycorrhizal old field and forest species in uninvaded soil, growth in soil with a legacy of *A. petiolata* weakened its competitive ability. In uninvaded control soil, for example, *A. petiolata* suppressed the biomass of a majority of competitor plant species by an average effect size that exceeded 50% (Fig. 1). By contrast, the suppression of competitor species' biomass by *A. petiolata* was weaker in legacy soil, with an effect size that was less than half of that observed in control soil. Moreover, only a minority of species responded negatively to the presence of *A. petiolata* in legacy soil. These findings suggest that newly introduced *A. petiolata* may displace competitor species in previously uninvaded sites in the short term, but modification of the soil environment by invasion may not enhance the longer term persistence of the invader.

The weaker competitive effect of *A. petiolata* on other species in legacy soil occurred despite relatively strong suppression of mycorrhizal fungi. Mycorrhizal colonization in legacy soil (Fig. 4) was suppressed at levels comparable to that observed in the field (e.g., Barto et al., 2011), with concomitant reductions in competitor plant growth (Fig. 3). However, competitor species were still better able to resist competition from *A. petiolata* in legacy than control soils. Our findings conflict with previous

studies that show that *A. petiolata* tends to inhibit the growth of other species more than itself when grown in legacy soils (Klironomos, 2002; Callaway et al., 2008; reviewed in Hale & Kalisz, 2012). However, these studies did not include the combined effects of mycorrhizal suppression and nutrient depletion in the legacy soil treatment. In our study, where legacy soils had both lower mycorrhizal colonization potential and depleted nutrients, particularly NO_3^- and NH_4^+, the opposite pattern occurred: the biomass of *A. petiolata* was more inhibited than competitor species in legacy versus control soil (Fig. 3). The weaker competitive ability of *A. petiolata* in legacy soils, therefore, most likely occurred because the negative effect of nutrient depletion on *A. petiolata* was stronger than the negative effect of suppressing mycorrhizal colonization on competitor species. The observation that competition was weaker overall in legacy soil is also consistent with the hypothesis that when plant growth is suppressed by environmental stress or low fertility, limited overall demand for resource uptake reduces the strength of competition (Grime 1977; Lamb et al. 2007). Weak competition in legacy soils also suggests that the potential for *A. petiolata* to suppress other species through allelopathic effects on mycorrhizae may have been overestimated.

Despite its ability to suppress mycorrhizal growth and reproduction (Stinson et al., 2006; Hale & Kalisz, 2012), several other studies also show that *A. petiolata* is not a uniformly strong competitor (Meekins & McCarthy, 1999; Bossdorf et al., 2004; Herold et al., 2011; Leicht-Young et al., 2012; Davis et al., 2012; Phillips-Mao et al., 2014). For example, Smith & Reynolds (2014) found that *A. petiolata* did not suppress the community biomass of a suite of native species found in temperate forest and forest edge habitats. Many of the genera used in their study (*Acer*, *Quercus*, *Lobelia*, *Elymus*) overlapped with ours (Table 1), suggesting that our findings are representative of temperate communities. Smith & Reynolds (2014) hypothesized that the weak effects of *A. petiolata* competition on other species may have been caused by not carrying out their study in legacy soils. However, our findings do not support this hypothesis because growth in soils with a legacy of *A. petiolata* weakened the competitive ability of the invader. Thus, the weak community effects of competition with *A. petiolata* reported in prior studies are likely robust to the inclusion of soil legacy effects.

One explanation for *A. petiolata*'s inconsistent ability to suppress competitors is that it is more likely to experience intra-specific than inter-specific competition, a pattern that has been established by experiments that manipulate both competitor identity and density (Meekins & McCarthy, 1999; Leicht-Young et al., 2012). These experiments are supported by demographic analyses showing that in situations where other biotic factors such as herbivory are excluded, established *A. petiolata* populations decline towards extinction (Kalisz et al., 2014). The propensity for *A. petiolata* to draw down soil nutrients to a level that more detrimentally affects its own growth relative to other species, as was observed in the present study, could explain previous observations of density dependent population regulation in this species. Self-limitation in legacy soil also implies that *A. petiolata* is not a strong competitor from the perspective of resource competition theory (Tilman, 1988, Tilman & Wedin, 1991). If, following establishment, *A. petiolata* is self-limiting and a relatively weak competitor for resources, then the successful invasion of this species is more likely to depend on other mechanisms. Recent studies suggest, for example, that the unpalatability of *A. petiolata* to deer relative to other plant species is a primary determinant of its persistence in temperate North American forests (Knight et al., 2009; Kalisz et al., 2014).

Growth form could be the best predictor of the ability of competitor species to either resist or suppress *A. petiolata*, but this effect varied with soil legacy and competition metric. For example, *A. petiolata* suppressed the growth of all three growth forms in control soil, but this effect was much more modest in legacy soil. By contrast, grasses suppressed invader biomass in both soil treatments, whereas forbs had no effect, and trees appeared to facilitate the growth of *A. petiolata* in control soils. The ability of grasses to suppress *A. petiolata* may arise because they were taller than other growth forms at a young age, which would increase light acquisition (Grime 1977; Gaudet & Keddy, 1988; Goldberg & Landa, 1991; Rosch et al., 1997; Keddy et al. 2002; Wang et al. 2010). Grasses also had relatively fine roots, which would increase nutrient uptake capacity (Aerts et al., 1991; Goldberg, 1996; Casper & Jackson, 1997). Nonetheless, height may be the most important factor because grasses and forbs had similar photosynthetic capacity and root architecture, yet forbs did not suppress *A. petiolata* biomass. Meekins and McCarthy (1999) also found that *A. petiolata* was a weaker competitor against tall

relative to short species. The ability of the invader to grow larger when paired with tree species in control soil was unexpected. To our knowledge, there are no hypotheses that predict this outcome. However, the effect may be due to aspects that were unique to the two tree species, *Pi. strobus* and *Th. occidentalis*, that had the strongest beneficial effect on *A. petiolata*. These species were the only conifers in the sample and also ranked lowest in terms of growth rate (Fig 1). The relatively strong growth form effects of competitor species on *A. petiolata* we report here may not be universal however. Other studies suggest that trees can be strong competitors (Meekins & McCarthy, 1999; Smith & Reynolds, 2014) and grasses can be weak competitors (Smith & Reynolds, 2014) against *A. petiolata*.

Aside from differences associated with growth forms, functional traits did not predict either the ability of competitor species to resist suppression by, or their ability to suppress, *A. petiolata*. When growth form was included in multiple regressions between traits and competitive response or competitive effect, no significant relationships were found, regardless of soil treatment (Table 4). There was also limited trait plasticity in response to *A. petiolata* legacy in soil (Fig. 5), despite strong effects on plant biomass. The absence of coordination between trait values and biomass responses across soil treatments reinforces the conclusion that trait values do not influence competitive response or competitive effect independently of growth form. These findings are consistent with those of Wang et al. (2010), who also reported weak relationships between trait values and competitive ability. The inability to detect specific relationships between traits and competitive ability could be caused by the possibility that competitive ability depends on combinations of several traits or traits that were not measured (Wardle et al., 1998; Wang et al., 2010), or because functionally alternate strategies, such as efficient resource acquisition or resource storage, can result in similar competitive abilities (Grime, 1977).

Our findings have implications for recent hypotheses about how competitive response and competitive effect should be correlated across environments (Keddy et al., 1994; Keddy et al., 2002; Wang et al., 2010). Specifically, competitive response is expected to be context specific, varying with resource availability or other ecological and environmental factors, and is not expected to be correlated

420 across environments. By contrast, competitive effect is expected to be a general property of a species,
421 such that it is positively correlated across environments (Wang et al., 2010). Our results are generally
422 consistent with these predictions (Fig. 6), but the relationship between competitive effect in control and
423 legacy soils was weaker than (i.e., $r^2 = 0.12$, Fig. 6b) found in other studies (Keddy et al., 1994; Keddy
424 et al., 2002; Wang et al., 2010). Observing such context dependency in the competitive effect of *A.*
425 *petiolata* was not unique to our study. For example, Smith & Reynolds (2014) found that *A. petiolata*
426 could suppress other species under high light conditions, but had much weaker effects in the shade.
427 Our findings suggest the ability of competitor species to either resist suppression by, or suppress, *A.*
428 *petiolata* cannot be confidently predicted from one ecological context to another.
429 In conclusion, our findings show that though *A. petiolata* has the potential to displace resident
430 species in a community upon initial invasion via a relatively strong competitive ability, its competitive
431 ability is weakened, rather than strengthened, by soil legacy effects. Like previous studies, we observed
432 that soil with a legacy of *A. petiolata* reduces the ability of mycorrhizal fungi to colonize the roots of
433 competitor species. However, this negative novel weapons effect on mycorrhizal plant species could
434 not overcome the negative legacy effects of soil nutrient depletion on *A. petiolata*. The tendency for
435 soil legacy to negatively affect its own growth and competitive ability suggest that the inhibitory
436 potential of *A. petiolata* on competitor species via mycorrhizal suppression is likely to have been
437 overestimated. As a result, eradication or control measures based on minimizing novel weapons effects
438 are less likely to be successful than other approaches. As suggested by other studies, reducing
439 propagule pressure (Phillips-Mao et al., 2014) and browsing by deer (Kalisz et al., 2014) could be more
440 effective strategies to counteract the successful invasion of *A. petiolata* in North America.
441

442 **Acknowledgements**
443 We thank A. Benoit, E. Bothwell, C.M. Caruso, E. Pacey, P. Rekret and R. Rivkin, for comments on
444 the manuscript.

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607 **Figure Legends**

608 Figure 1. Biomass of competitor species in response to competition with *A. petiolata* in control (A) or
609 soil with a legacy of *A. petiolata* (B). Biomass within each growth form are shown in the insets.
610 Statistically significant differences were determined using planned orthogonal 1-df contrasts, and are
611 indicated with an asterisk.

612

613 Figure 2. Biomass of *A. petiolata* alone or in response to competition with other species in control (A)
614 or soil with a legacy of *A. petiolata* (B). Biomass of *A. petiolata* alone versus in competition with
615 members of different growth forms are shown in the insets. Statistically significant differences were
616 determined using planned orthogonal 1-df contrasts, and are indicated with an asterisk.

617

618 Figure 3. The log response ratio of plant biomass, without competition, in legacy versus control soils.
619 Statistically significant differences between soil treatments were determined using planned orthogonal
620 1-df contrasts, and are indicated with an asterisk.

621

622 Figure 4. The effect of a legacy of *A. petiolata* on the colonization of roots by arbuscular mycorrhizal
623 (AM) hyphae (A) AM arbuscules (B), and vesicles (C). Statistically significant differences between
624 soil treatments were determined using planned orthogonal 1-df contrasts, and are indicated with an
625 asterisk.

626

627 Figure 5. The effect of growth form and *A. petiolata* soil legacy on quantum yield of PSII at weeks 5
628 and 9 (A), leaf chlorophyll concentration at weeks 5 and 9 (B) and plant height at week 5 (C). The
629 effect of growth form on root diameter (D) and specific root length (E). Different letters above bars,
630 when present, represent statistically significant differences ($P<0.05$) among groups within each
631 treatment, as determined by a comparison of 95% confidence limits among groups.
632 Figure 6. Relationships between competitive response (A) or competitive effect (B) across control and
633 legacy soils.

634 Table 1. List of competitor species used in the study, along with information on their plant family
635 affiliation, growth form, status in North America (18 native, 9 introduced), and whether plants are
636 [1]arbuscular mycorrhizal, [2]ecto-mycorrhizal, or [3]ambiguous (both mycorrhizal and non-mycorrhizal
637 states reported in the literature). Mycorrhizal state was determined from Wang & Qiu (2006). Seeds
638 were obtained from [A]Acorus Restoration, [B]Angelgrove Seed Company, [C]Ontario Tree Seed Facility
639 [D]Richters Herbs, or field collections from the [E]University of Guelph Arboretum.
640

Latin name	Family	Growth Form	Status
[1]*Acer saccharum* L.	Aceraceae	Tree	Native[C]
[1]*Juglans nigra* L.	Juglandaceae	Tree	Native[C]
[2]*Pinus strobus* L.	Pinaceae	Tree	Native[C]
[1]*Prunus virginiana* L.	Rosaceae	Tree	Native[C]
[2]*Quercus macrocarpa* Michx.	Fagaceae	Tree	Native[C]
[1]*Thuja occidentalis* L.	Cupressaceae	Tree	Native[C]
[1]*Achillea millefolium* L.	Asteraceae	Perennial Forb	Native[D]
[1]*Aquilegia vulgaris* L.	Ranunculaceae	Perennial Forb	Introduced[D]
[1]*Aster umbellatus* Miller	Asteraceae	Perennial Forb	Native[D]
[1]*Daucus carota* L.	Apiaceae	Biennial Forb	Introduced[E]
[1]*Fragaria virginiana* Miller.	Rosaceae	Perennial Forb	Native[B]
[3]*Hesperis matronalis* L.	Brassicaceae	Biennial Forb	Introduced[A]
[1]*Hypericum perforatum* L.	Clusiaceae	Perennial Forb	Introduced[A]
[1]*Leucanthemum vulgare* Lam.	Asteraceae	Perennial Forb	Introduced[A]
[1]*Lobelia siphilitica* L.	Campanulaceae	Perennial Forb	Native[A]
[1]*Plantago lanceolata* L.	Plantaginaceae	Perennial Forb	Introduced[D]
[1]*Prunella vulgaris* L.	Lamiaceae	Perennial Forb	Native[B]
[1]*Rudbeckia hirta* L.	Asteraceae	Perennial Forb	Native[B]
[3]*Sambucus nigra spp. canadensis* L.	Caprifoliaceae	Perennial Forb	Native[A]
[1]*Solidago canadensis* L.	Asteraceae	Perennial Forb	Native[E]
[1]*Taraxacum officinale* F.H. Wigg.	Asteraceae	Perennial Forb	Introduced[B]
[1]*Trifolium pratense* L.	Fabaceae	Biennial Forb	Introduced[E]
[1]*Bromus inermis* Leyss.	Poaceae	Perennial Grass	Introduced[E]
[1]*Elymus canadensis* L.	Poaceae	Perennial Grass	Native[B]
[1]*Elymus riparius* Wiegand.	Poaceae	Perennial Grass	Native[B]
[1]*Elymus virginicus* L.	Poaceae	Perennial Grass	Native[B]
[1]*Panicum virgatum* L.	Poaceae	Perennial Grass	Native[A]

642 Table 2. A three way ANOVA table describing the effects of species identity, competition with *A.*

643 *petiolata*, soil legacy and their interactions on dry mass of competitor species.

644

Source	Type III Sums of Squares	df	Mean Square	*F*	*P*
Species	1141.51	26	43.90	66.78	5.29×10^{-144}
Soil legacy	1.41	1	1.41	2.14	0.144
Competition	209.97	1	209.97	319.38	1.57×10^{-55}
Species * Soil legacy	32.20	26	1.24	1.88	0.006
Species * Competition	141.18	26	5.43	8.26	1.76×10^{-25}
Soil legacy* Competition	45.14	1	45.14	68.65	1.10×10^{-15}
Species * Soil legacy* Competition	68.44	26	2.63	4.00	4.42×10^{-10}
Error	326.75	497	0.66		

645

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646 Table 3. A two way ANOVA table showing the effects of competitor species identity, soil legacy and
647 their interaction on the dry mass of *A. petiolata*.

648

Source	Type III Sums of Squares	df	Mean Square	*F*	*P*
Species	92.4	26	3.55	6.76	3.74×10^{-17}
Soil legacy	179.59	1	179.59	341.39	9.51×10^{-48}
Species * Soil legacy	40.37	26	1.55	2.95	7.30×10^{-06}
Error	124.15	236	0.53		

649

650

651

652 Table 4 Partial correlation coefficients (*β*) indicating relationships between competitive response (CR) or competitive effect (CE) in control
653 or legacy soil, and plant functional traits, including height at 5 weeks, quantum yield of PS II in the light [Y(II)] at 5 and 9 weeks, leaf
654 chlorophyll content at 5 and 9 weeks, mean root diameter and specific root length (SRL). Because traits differed between trees, forbs and
655 grasses, plant growth form was included as a covariate in the analysis, but only *β* and significance values for traits are shown. The degree to
656 which residuals from the multiple regression were correlated with phylogeny is indicated by λ.

Dependent variable	**Trait**	***β***	***P***	**Dependent variable**	**Trait**	***β***	***P***
CR control soil	Height @ 5 wks	-0.12	0.59	CR legacy soil	Height @ 5 wks	-0.09	0.70
λ = 0	Y(II) @ 5 wks	0.023	0.92	λ = 1	Y(II) @ 5 wks	0.42	0.06
	Y(II) @ 9 wks	0.18	0.44		Y(II) @ 9 wks	0.082	0.72
	Chl @ 5 wks	-0.040	0.86		Chl @ 5 wks	0.080	0.73
	Chl @ 9 wks	0.053	0.82		Chl @ 9 wks	-0.16	0.49
CE control soil	Height @ 5 wks	-0.02	0.93	CE legacy soil	Height @ 5 wks	0.064	0.78
λ = 0	Y(II) @ 5 wks	0.27	0.24	λ = 0	Y(II) @ 5 wks	0.063	0.79
	Y(II) @ 9 wks	-0.11	0.64		Y(II) @ 9 wks	0.050	0.83
	Chl @ 5 wks	0.061	0.79		Chl @ 5 wks	-0.12	0.61
	Chl @ 9 wks	0.11	0.63		Chl @ 9 wks	0.25	0.27
CR control soil	Root diameter	-0.23	0.28	CR legacy soil	Root diameter	-0.11	0.61
λ = 0	SRL	0.09	0.67	λ = 0.981	SRL	-0.13	0.57
CE control soil	Root diameter	-0.35	0.11	CE legacy soil	Root diameter	-0.22	0.32
λ = 1	SRL	-0.10	0.64	λ = 0	SRL	-0.23	0.30

657

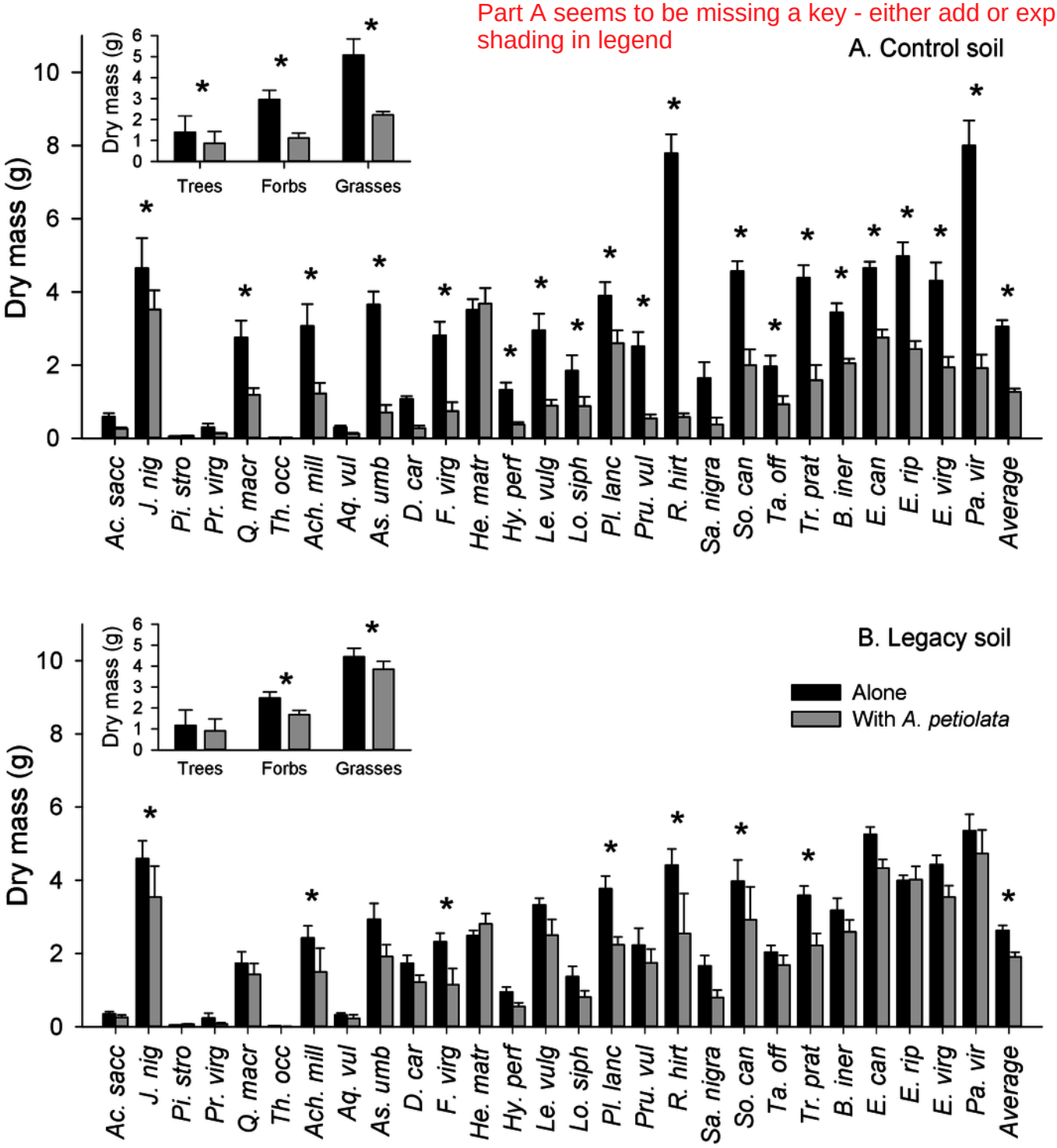
658

1

Figure 1

Figure 1. Biomass of competitor species in response to competition with A. petiolata in control (A) or soil with a legacy of A. petiolata (B). Biomass within each growth form are shown in the insets. Statistically significant differences were determined using planned orthogonal 1-df contrasts, and are indicated with an asterisk.

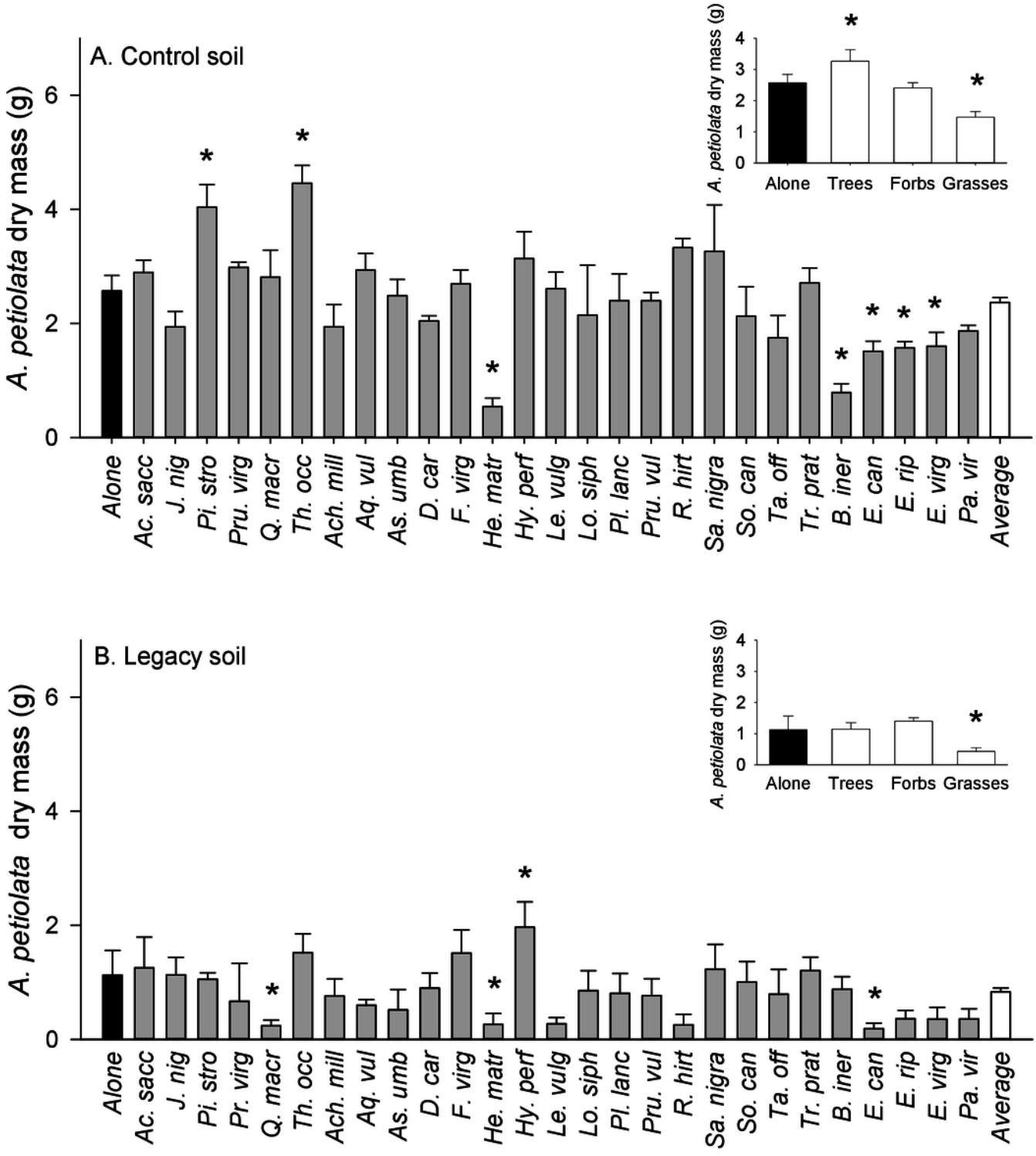
Part A seems to be missing a key - either add or explain shading in legend



2

Figure 2

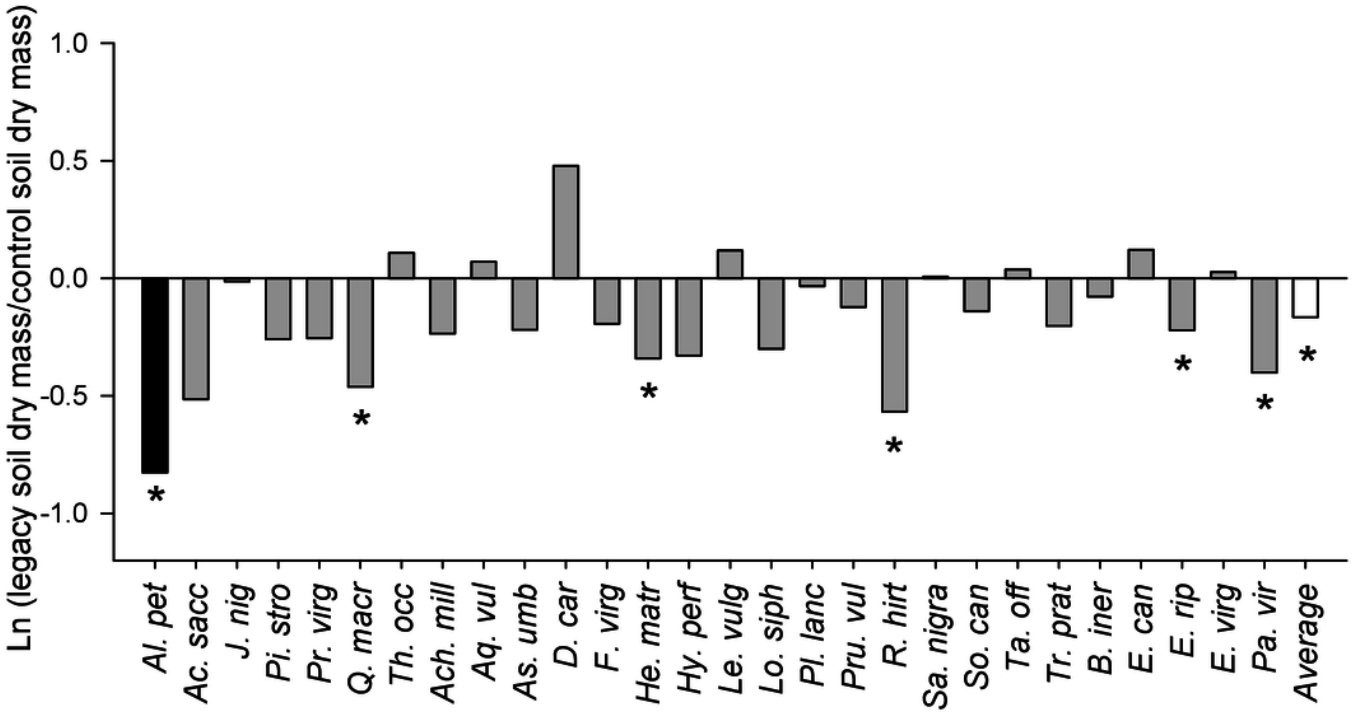
Figure 2. Biomass of A. petiolata alone or in response to competition with other species in control (A) or soil with a legacy of A. petiolata (B). Biomass of A. petiolata alone versus in competition with members of different growth forms are shown in the insets. Statistically significant differences were determined using planned orthogonal 1-df contrasts, and are indicated with an asterisk.


A. Control soil
A. petiolata dry mass (g)
0
2
4
6
Alone
Ac. sacc
J. nig
Pi. stro
Pru. virg
Q. macr
Th. occ
Ach. mill
Aq. vul
As. umb
D. car
F. virg
He. matr
Hy. perf
Le. vulg
Lo. siph
Pl. lanc
Pru. vul
R. hirt
Sa. nigra
So. can
Ta. off
Tr. prat
B. iner
E. can
E. rip
E. virg
Pa. vir
Average
A. petiolata dry mass (g)
0
1
2
3
4
Alone
Trees
Forbs
Grasses
B. Legacy soil
A. petiolata dry mass (g)
0
2
4
6
Alone
Ac. sacc
J. nig
Pi. stro
Pr. virg
Q. macr
Th. occ
Ach. mill
Aq. vul
As. umb
D. car
F. virg
He. matr
Hy. perf
Le. vulg
Lo. siph
Pl. lanc
Pru. vul
R. hirt
Sa. nigra
So. can
Ta. off
Tr. prat
B. iner
E. can
E. rip
E. virg
Pa. vir
Average
A. petiolata dry mass (g)
0
1
2
3
4
Alone
Trees
Forbs
Grasses

3

Figure 3

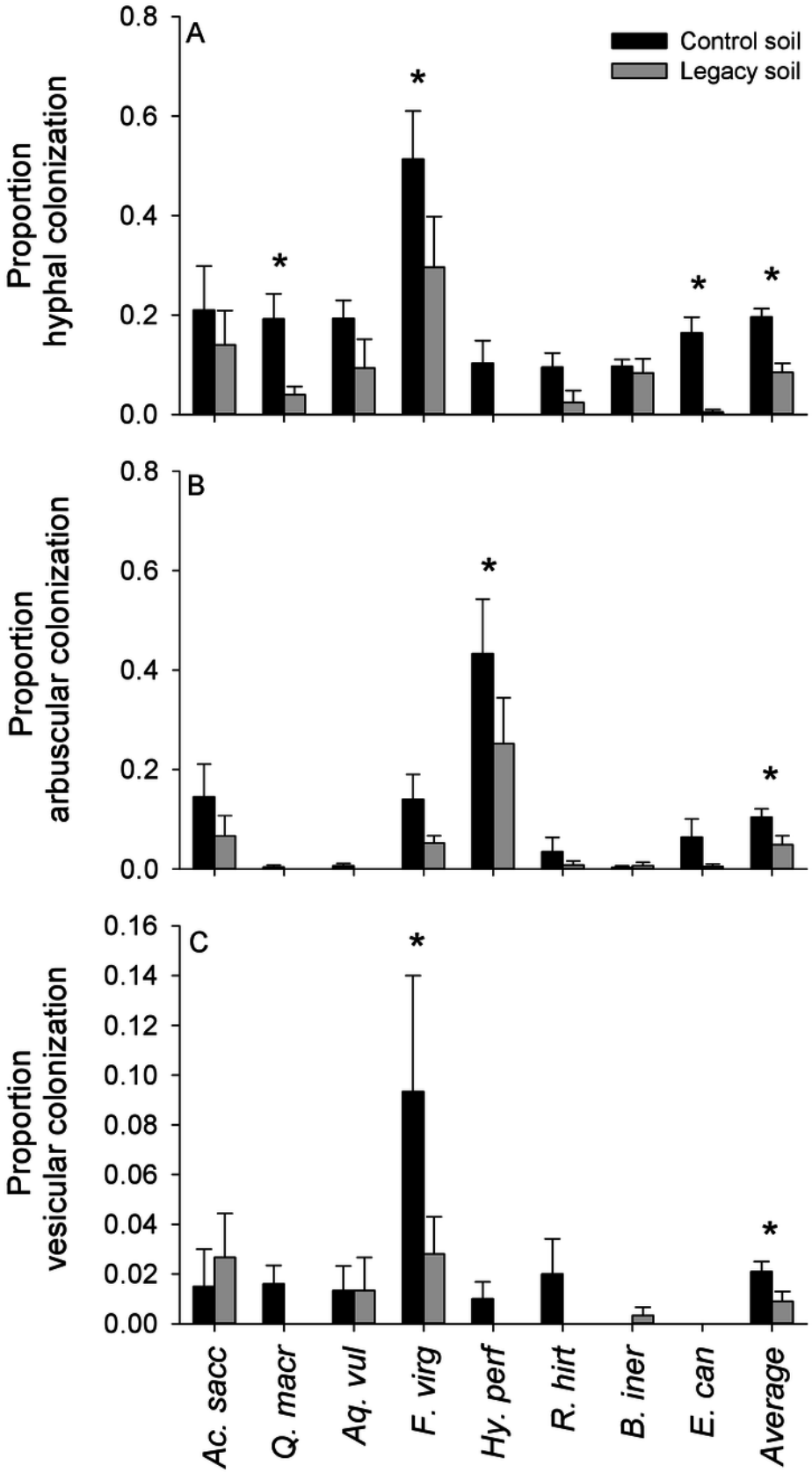
Figure 3. The log response ratio of plant biomass, without competition, in legacy versus control soils. Statistically significant differences between soil treatments were determined using planned orthogonal 1-df contrasts, and are indicated with an asterisk.


Ln (legacy soil dry mass/control soil dry mass)
1.0
0.5
0.0
-0.5
-1.0
Al. pet
Ac. sacc
J. nig
Pi. stro
Pr. virg
Q. macr
Th. occ
Ach. mill
Aq. vul
As. umb
D. car
F. virg
He. matr
Hy. perf
Le. vulg
Lo. siph
Pl. lanc
Pru. vul
R. hirt
Sa. nigra
So. can
Ta. off
Tr. prat
B. iner
E. can
E. rip
E. virg
Pa. vir
Average

4

Figure 4

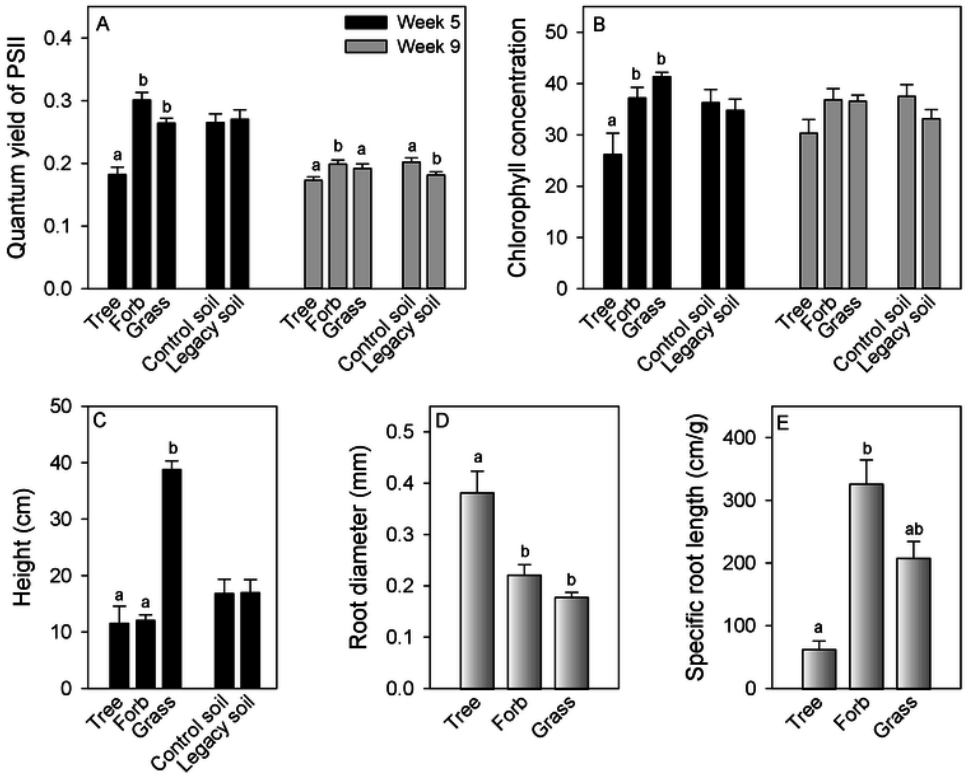
Figure 4. The effect of a legacy of A. petiolata on the colonization of roots by arbuscular mycorrhizal (AM) hyphae (A) AM arbuscules (B), and vesicles (C). Statistically significant differences between soil treatments were determined using planned orthogonal 1-df contrasts, and are indicated with an asterisk.


A
B
C
Control soil
Legacy soil
Proportion hyphal colonization
Proportion arbuscular colonization
Proportion vesicular colonization
0.0
0.2
0.4
0.6
0.8
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
*
Ac. sacc
Q. macr
Aq. vul
F. virg
Hy. perf
R. hirt
B. iner
E. can
Average

5

Figure 5

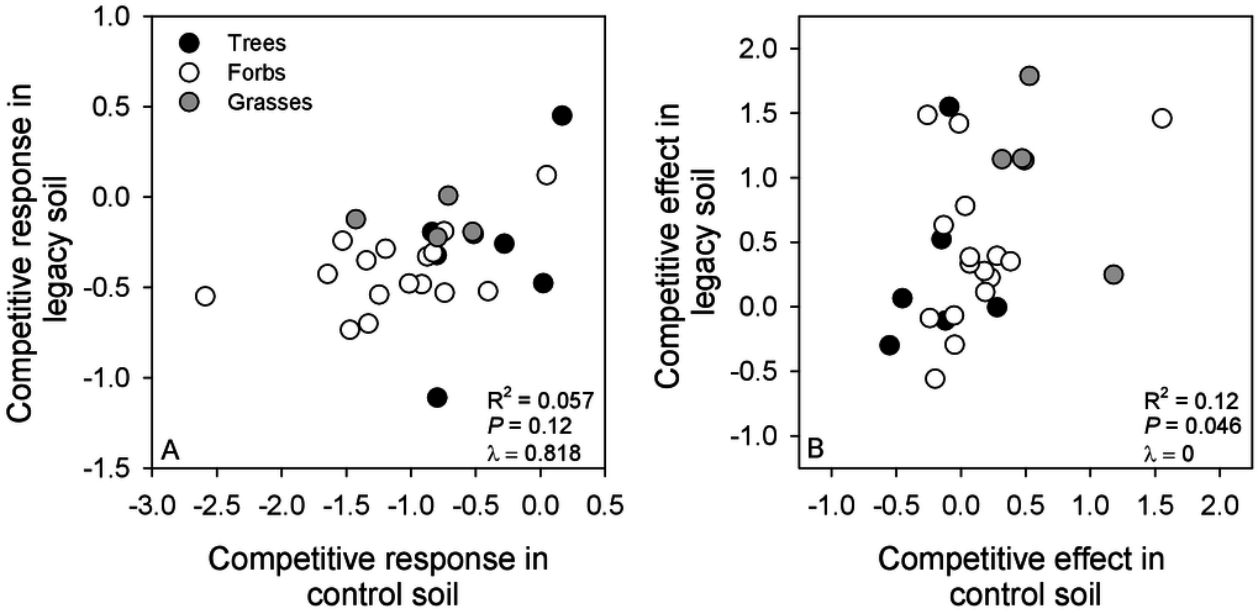
Figure 5. The effect of growth form and A. petiolata soil legacy on quantum yield of PSII at weeks 5 and 9 (A), leaf chlorophyll concentration at weeks 5 and 9 (B) and plant height at week 5 (C). The effect of growth form on root diameter (D) and specific root length (E). Different letters above bars, when present, represent statistically significant differences ($P<0.05$) among groups within each treatment, as determined by a comparison of 95% confidence limits among groups.



6

Figure 6

Figure 6. Relationships between competitive response (A) or competitive effect (B) across control and legacy soils.


Trees
Forbs
Grasses
Competitive response in legacy soil
Competitive response in control soil
R^2 = 0.057
P = 0.12
λ = 0.818
A
Competitive effect in legacy soil
Competitive effect in control soil
R^2 = 0.12
P = 0.046
λ = 0
B