Plant phylogeny and life history predict AM fungal species and genetic composition, but only life history and genetic composition predict feedback

Short title: AM fungal feedback across phylogeny and life history

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Abstract

Symbioses exert strong influences on host phenotypes; however, benefits from symbioses can increase or degrade over time. Understanding the context-dependence of reinforcing or degrading dynamics is pivotal to predicting stability of symbiotic benefits. Host phylogenetic relationships and host life history traits are two candidate axes that have been proposed to structure symbioses. However, the relative influence of host evolutionary history and life history on symbiont composition, and whether changes in symbiont composition translate into stronger mutualistic benefits is unknown. We tested the influence of plant phylogenetic relationships and plant life history on the composition of Arbuscular Mycorrhizal (AM) fungi, perhaps the most ancestral and influential of plant symbionts, and then tested whether AM fungal differentiation resulted in improved mutualism. We constructed mycobiomes composed of seven AM fungal isolates derived from tallgrass prairie and grew them for two growing seasons with 38 grassland plant species. We found that host phylogenetic structure was a significant predictor of the composition of AM fungal communities and the genetic composition of AM fungal species, patterns consistent with phylosymbiosis. However, the phylogenetic structure of AM fungi failed to translate to improved benefit to their host. While AM fungi generally improved plant growth and mycorrhizal feedback was generally positive, the strength of feedback was not predicted by plant phylogenetic distance. The composition of the AM fungal community and genetic composition within AM fungal species were also significantly influenced by plant life history. In addition, feedbacks between early and late successional species were generally positive. Interestingly, changes in species composition did not predict feedback, while changes in genetic composition of the two most abundant AM fungal species predicted positive mycorrhizal feedback. These

reinforcing mycorrhizal feedbacks across life history can mediate plant species turnover during succession and suggests that consideration of mycorrhizal dynamics could improve restoration.

Introduction

Symbioses exert strong influences on phenotypes. Symbionts are particularly important to plants, with 80% of all terrestrial plant species, including most crop plants, associating with arbuscular mycorrhizal (AM) fungi [1]. This symbiosis has been identified as ancestral to all root symbioses [2] with arbuscules predating the origin of roots [3,4]. AM fungi mediate host acquisition of soil resources, particularly phosphorus, and can have a large influence on plant community dynamics [5–9]. Together, these aspects of AM fungi illustrate the critical importance of symbionts on host evolution and ecology, the understanding of which is a central question in biology.

Host phylogeny has been posited as an important factor structuring symbiont composition and function [10]. Phylogenetic structure of symbiont composition, either of symbiont species or genetic variants within symbiont species, has been called phylosymbiosis and has been observed in many systems including: the internal microbiomes of insects (*Drosophila* flies, mosquitoes, and *Nasonia* wasps) [11–13], the gut microbiota of animals (*Peromyscus* deer mice, pack rats, house mice, cranes, decapod shrimps) [11,14–16], and the bacterial communities of corals [17,18], providing a basis for generalizing across host-symbiont interactions. To date, there has been no comprehensive test of plant phylogenetic influence on AM fungal fitness and composition.

Inference on symbiont function from observations of phylogenetic structure in symbiont composition are challenging as they depend upon differential influence of symbionts on host fitness. While it is tempting to assume that differentiation in symbiont composition results in improved symbiont functioning for their hosts, fitness effects from host-specific differentiation of symbionts can result in positive or negative feedback [19] which correspond to strengthening or

deteriorating host fitness with phylosymbiosis. Several studies have found evidence of reinforcing coadaptation of symbionts on their hosts, including within *Nasonia* wasp species [12,13] and within some *Peromyscus* mouse species, but not others [11]. The benefit of AM fungi to their hosts has been shown to decline [20,21] or increase [22,23] with host-specific differentiation of symbionts. Whether host phylogenetics influence the likelihood of positive, reinforcing feedback remains an open question.

It is also possible that host traits unrelated to phylogeny are primary factors structuring symbiont differentiation on hosts. While the candidate host traits driving symbiont differentiation are many, major axis of plant trait variation has been shown to align with the fast-slow/acquisitive-conservative continuum of life history strategies [24,25]. Plant life history has also been shown to be important for plant-AM fungal interactions. Specifically, slow growing late successional plant species have been shown to be more responsive to AM fungi [26,27], to be more sensitive to AM fungal identity [28], and to benefit particularly from AM fungi found in undisturbed sites dominated by late successional plant species [29,30], potentially generating positive feedback [23]. Such trait mediated AM fungal feedbacks could have important consequences for community coexistence and turnover during succession.

Plants acquire their AM fungal symbionts from the environment each generation—there is no horizontal inheritance of this symbiosis. While AM fungal species have been shown to be functionally variable [8,31], variants within AM fungal species can also vary in their ecology [32,33]. AM fungi are multinucleated, and different nuclei within AM fungal individuals can have substantially different genetic content [34–36]. Parent AM fungal cells can segregate nuclei, passing different nuclei to different offspring, and these segregants have been shown to differ in their impacts on host plants [32,33,37–39]. To date, we do not know the relative importance of changes in species composition vs genetic composition within species in driving functional consequences of AM fungal dynamics.

91 To test how plant host identity, plant host phylogenetic relationships, and plant life history
92 characteristics shape symbiotic AM fungal communities, we grew a common community of AM
93 fungi, a constructed synthetic mycobiome, with 38 individual plant species (Fig. 1a) representing
94 a phylogenetically diverse group of early and late successional host prairie plants (S1 Fig and S2
95 Table). All plants were grown in a common greenhouse environment to remove potential
96 confounding environmental effects [10]. We monitored the changes in AM fungal composition
97 over two growing seasons using LSU rDNA amplicon sequencing [40–42]. As rDNA of AM fungi
98 are highly polymorphic [43,44], we grouped amplicon sequence variants (ASVs) into species to
99 permit assessment of changes in AM fungal species composition and changes in genetic
100 composition of individual AM fungal species (Fig. 1b). We tested for consistent effects across
101 plant phylogeny and across plant life history categories with two approaches. PerMANOVA
102 controls for non-independence of amplicons in individual samples. Phylogenetic mixed models
103 explicitly test for host phylogeny influence on AM fungal composition and simultaneously control
104 for phylogenetic non-independence. Both test for effects of host life history on AM fungal
105 composition (Fig. 1c).
106 We used multiple approaches to assess the ecological impact of changes in AM fungal
107 composition (Fig 1). As our constructed mycobiomes included isolates that have been
108 characterized in their impacts on host growth across plant family and across plant life history
109 [28,45], we test for correlations of AM fungal fitness responses to hosts with AM fungal impacts
110 on host growth (Fig. 1d,e,). A positive correlation of host and symbiont fitness impacts predicts
111 positive, reinforcing feedback that would generate co-adaptation [19,46]. Secondly, we directly
112 tested the functional consequences of host-specific differentiation in AM mycobiome composition
113 using a feedback assay (Fig. 1f) in which plant species were grown with the differentiated
114 mycobiomes in full factorial combinations [47,48]. We then tested whether the strength of
115 feedback is positively correlated with phylogenetic distance, as expected by co-adapted

116 phylosymbiosis, and whether AM fungal feedback varies consistently with plant life history, as
117 expected from mycorrhizal dynamics influencing species turnover during succession (Fig. 1g).
118 Finally, we tested whether changes in AM fungal species or genetic composition within AM fungal
119 species predicted resulting feedbacks (Fig. 1h).

120 **Fig 1: Conceptual Figure of the Experimental Design**

121 A common AM fungal community composed of mixtures of characterized isolates was grown with
122 38 plant species for two growing seasons (a). Changes in relative abundance of AM fungi were
123 monitored using amplicon sequencing (b) and used to test for plant phylogenetic and life history
124 impacts on AM fungal species and genetic composition (c). The effect of the AM fungal inoculum
125 strains used in this experiment on plant species was characterized using meta-analyses of prior
126 studies (d). Correlations of AM fungal relative fitness and fungal impacts (e) on host phylogenetic
127 and life history groupings were tested as one measure: mycorrhizal feedback [19]. Feedback was
128 also measured directly by testing growth responses of different plant species to trained AM fungal
129 communities (f). Pairwise feedbacks, as represented by interaction coefficients (Bever et al.
130 1997), were calculated and used to test patterns of strength of feedback across host phylogeny
131 and life history (g). We also tested whether the observed changes in AM fungal species
132 composition or changes in genetic composition within species predicted strength of mycorrhizal
133 feedback (h).

134 Results

135 We found significant influence of plant phylogeny and plant life history on the species and genetic
136 composition of the AM mycobiome. This holds true whether controlling for the multivariate nature
137 of the amplicon data using PerMANOVA or whether testing for and controlling for phylogenetic

relationships of host plants using phylogenetic generalized mixed models (PGLMMs). As these results are complementary, but redundant, we present the PGLMM results which provide a direct test of phylosymbiosis below and the PerMANOVA within the appendix (S3 and S4 Tables).

Host phylogeny impacts on AM Mycobiome

Grouping ASV counts by individual AM fungal species, we found significant changes in AM fungal composition and density with plant phylogeny. Host phylogeny significantly affected AM fungal diversity and density in year 1 ($p<0.01$, $p<0.001$) and the relative proportion of *Ce. pellucida* in year 1 ($p<0.05$), *R. fulgida* in year 2 ($p<0.001$), and *E. infrequens* in both years ($p<0.001$, $p<0.05$, S5 Table, Fig 2). This suggests that phylogenetically proximate plant species have more similar AM fungal composition, reflecting more similar impacts of host on AM fungal relative fitness, than more phylogenetically distant plant species. *Ce. pellucida* had high relative growth rates with plants in Apocynaceae, *E. infrequens* had low relative fitness with grasses, and *R. fulgida* tended to perform best with grass species (Fig 2).

Fig 2: AM Fungal Species Relative Abundances Across Plant Phylogeny

AM fungal relative abundances for *Cl. lamellosum*, *Cl. claroideum*, *E. infrequens*, *F. mosseae*, *R. fulgida*, *Ce. pellucida*, and *A. spinosa* are presented alongside estimated abundance (proportion of ASVs identified as inoculated over total AM fungal ASVs) and Shannon's Diversity. Results are by host plant species arranged phylogenetically. Significance phylogenetic effects are calculated with a Likelihood Ratio Test using the Chi square distribution. Year 1 and Year 2 data are presented as grouped columns with year one being the lighter shaded top bar.

158 # Host phylogeny impacts on genetic composition of AM
159 fungal species

160 We also tested the influence of host phylogeny on the genetic composition of individual AM fungal
161 species. ASV's identified for each species were tested in order of decreasing overall abundance.
162 We found significant differences in ASV composition due to plant phylogenetic relatedness for the
163 most abundant *E. infrequens* ASVs for both years ($p<0.01$, Fig 3). The most abundant ASV of *Cl.*
164 *lamellosum* was marginally affected by plant phylogeny in year 1 ($p>0.1$). *F. mosseae's*, *Cl.*
165 *claroideum's,* and *Ce. pellucida's* third most abundant ASV had a significant effect of host plant
166 phylogenetic distance in year 1 ($p<0.01$, $p<0.001$, $p<0.001$). Finally, *R. fulgida* was marginally
167 affected by phylogeny for its seventh most abundant ASV in year 1 ($p>0.1$). There were also
168 significant effects of phylogeny for its most abundant ASV in year 2 ($p>0.001$).

169 **Fig 3: AM Fungal Genetic Variation Across Plant Phylogeny**
170 AM fungal genetic variation (ASV relative abundances) were tested for significant effects of
171 phylogenetic distance within *Cl. lamellosum*, *Cl. claroideum*, *E. infrequens*, *F. mosseae*, *R.*
172 *fulgida*, *Ce. pellucida*, and *A. spinosa*. The significance of each phylogenetic effect is calculated
173 with a Likelihood Ratio Test using the Chi square distribution. Results are reported for the most
174 common ASV with a significant result. The rank of the most common significant ASV is given as a
175 superscript next to the significance symbols.

176 # Plant life history impacts on AM mycobiome

177 PGLMMs of total ASV counts grouped by individual AM fungal species showed significant
178 changes in AM fungal composition with plant life history. The relative abundance of *F. mosseae*

179 and *R. fulgida* were marginally significantly higher when associated with late successional plants
180 in year 2, with *Cl. claroideum* being significantly higher ($p<0.1$, $p<0.1$, $p<0.05$, S5 Table, Fig 2).
181 Late successional plant species had more diverse AM fungal communities than early
182 successional plant species (Shannon index, year 2 $p\leq0.05$, S6 Fig). Estimated AM fungal density
183 was not significantly affected by host plant life history in either year ($p>0.1$).

184 Plant life history impacts on genetic composition of AM 185 fungal species

186 Host plant life history significantly impacted the genetic composition of several AM fungal species.
187 PGLMM analysis found the relative abundance of the second most abundant ASV of *Cl.*
188 *lamellosum*, *F. mosseae*, and *Ce. pellucida* were each affected by host plant life history in year 1
189 ($p<0.1$, $p>0.05$, $p>0.1$, Fig 3). In year 2, the most abundant *F. mosseae* ASV and fourth most
190 abundant *Ce. pellucida* ASV were significantly affected by host plant life history ($p<0.05$, $p>0.05$).
191 Finally, the most abundant *Cl. claroideum* ASV had a marginal response to host plant life in year
192 2 only, and the fourth most abundant *F. mosseae* ASV had a significant response, ($p<0.1$,
193 $p<0.05$).

194 Correlation of host and symbiont relative fitness

195 Previous work with the AM fungal isolates used in this experiment [28,45] allowed us to test for
196 general patterns across four family groups: Asters (Asteraceae), Legumes (Fabaceae), Lillies
197 (Amaryllidaceae and Commelinaceae), and Grasses (Poaceae). We were particularly interested
198 in the differential impacts on plant families and their correlation with the differential fitness
199 responses to these families. We found that *E. infrequens*, *Cl. lamellosum*, and *Cl. claroideum*

200 confer greater benefits to plants in the Asters relative to plants in the Grasses group (S7B Fig).
201 We also saw that *E. infrequens*, *Cl. lamellosum*, *Cl. claroideum, F. mosseae, A. spinosa,* and *Ce.*
202 *pellucida* confer greater benefits to plants in the Lilly group compared to the Grasses. These
203 effects are weakly positively correlated with the relative fitness responses to these plant families
204 (Fig 4). This provided weak evidence of mycorrhizal positive feedback particularly between plants
205 in the comparison of the phylogenetic groups Asters and Grasses (R^2_{yr1}=0.32, p=0.187,
206 R^2_{yr2}=0.48, p=0.127) as compared to Legumes – Grasses (R^2_{yr1}=0.32, p=0.188, R^2_{yr2}=0.49,
207 p=0.183) and Lillies – Grasses (R^2_{yr1}=0.03, p=0.702, R^2_{yr2}=0.01, p=0.877). This may suggest a
208 strengthening of host fitness with phylosymbiosis, though none of these correlations were
209 significant.

210 **Fig 4: Predicted Feedbacks Between Phylogenetic Groups**

211 We analyzed the correlation of meta-analysis results of host plant response to AM fungal species
212 associated with host plants of different phylogenetic groups, with the measured differential AM
213 fungal response to host plant phylogenetic group. R^2 and p values corresponded to linear
214 correlations weighted by AM fungal relative abundance. There were no significant correlations at
215 the p≤0.1 level.

216 We also found the growth promotion by AM fungal isolates differed in early vs. late
217 successional plant species (S7A Fig). Moreover, there was a negative correlation between AM
218 fungal growth and benefits to plant hosts, with late successional plants growing less well with the
219 AM fungal isolates grown on other late successional hosts (R^2_{yr1} =0.23, p=0.274, R^2_{yr2}=0.94,
220 p=0.001, Fig 5). This pattern is consistent with changes in AM fungal composition generating
221 negative feedback between plants of different life history strategies. Given that AM fungal species
222 diversity increased with late successional plant species in the second year of AM mycobiome
223 training (Fig 2), it is possible that the change in AM fungal diversity feedback on fitness of late

successional plant species. However, when analyzing two studies comparing early and late successional plant species in response to single and mixed inocula, we did not find a significant difference in response to AM fungal diversity between plant life history stages (p= 0.748, S8 Fig), casting doubt on feedback through changes in mycorrhizal diversity.

Fig 5: Predicted Feedbacks Between Life History Categories

We analyzed the correlation of meta-analysis results of host plant response to AM fungal species associated with host plants of different life history categories to the measured differential AM fungal response to host plant life history category. R^2 and p values correspond to linear correlations weighted by AM fungal relative abundance. There is a significant positive correlation in year 2 (p=0.001).

AM fungal feedbacks: tests of patterns

AM fungal feedback between pairs of plant species varied from significantly positive to significantly negative (Fig 6). Across all pairs of plants, median (p = 0.003) and mean (t = 3.9472, p≤ 0.001) AM mycobiome feedback was generally positive (S9 Fig). However, there was no relationship between strength of feedback and plant phylogenetic distance. The best fit was a marginal quadratic fit (x: p=0.167, x^2: p=0.085, Fig 6A), as closely related species and most distantly related species tended to have zero or negative feedbacks. Together this indicates that the observed plant phylogenetic influence on AM mycobiome composition did not consistently result in improved host fitness.

Fig 6: Measured Feedback Strength Between AM fungi and Plant Hosts

Mycorrhizal feedback, measured as pairwise interaction coefficients, are plotted on the Y-axis in all plots. Phylogenetic distance (A) had only a marginally significant quadratic relationship with

fitness (p=0.085). (B) shows feedbacks broken out by both early, both late, and early and late host plant parings. Both early (p=0.011) and early-late (0.003) parings had significantly positive feedbacks. Both panels show individual pairwise interaction values plotted as shaded dots, with grey for insignificant interaction coefficients and black for significant interactions (p>0.05).

When analyzing feedback effects between plant families (restricted to the three best represented families: grasses, asters, and legumes). We tended to find mean positive feedback between legume species (t_{13}=2.233, p=0.058), negative feedback between Aster–Grass pairings (t_{18}=-2.236, p=0.029) and Grass–Grass pairings (t_6=-2.082, p=0.082). Other comparisons were not consistently different from zero (S10 Fig). Median Legume-Legume pairings remained marginally positive (p-value = 0.078), and Aster-Grass remained significantly negative (p=0.014). Grass–Grass pairings did not have significant negative median feedbacks (p=0.109).

In comparing within and between plant life history categories, the median pairwise feedbacks between pairs of early successional species tended to be positive (p=0.069), and the mean feedback was significantly positive (t_{41}=2.654, p=0.01, Fig 6B). Comparing feedback between early and late successional plant species, both the median (p=0.009) and mean values (t_{68}=3.055, p=0.003) were significantly positive. When both plant species were late successional neither the median (p=0.488) nor the mean (t_{14}=-0.009, p=0.993) feedback were significantly different from zero.

AM fungal feedbacks: community and/or genetic drivers

Differences in AM fungal species composition and/or differences in genetic composition within AM fungal species could drive mycorrhizal feedbacks. Changes in AM fungal species composition, as represented by dissimilarity between plant species pairs, failed to predict feedback strength (Fig 7a, R^2=0, F= 0.2922, p=0.59). However, changes in genetic composition

269 of individual AM fungal species did predict mycorrhizal feedback. Specifically, positive pairwise
270 feedback was significantly predicted by dissimilarity in genetic composition of the two most
271 common AM fungal species: *E. infrequens* (Fig 7b, R^2=0.13, F= 17.78, p<0.001) and *Cl.*
272 *lamellosum* (Fig 7c, R^2=0.1, F=13.27, p<0.001). Genetic dissimilarity of other AM fungal species
273 did not predict pairwise feedback (S11 Fig).

274 **Fig 7: Mycorrhizal Feedback Strength Predicted by Genetic Dissimilarity of AM fungi**
275 Regressions of strength of pairwise feedback against measures of AM fungal dissimilarity. AM
276 fungal species composition, as measured by pairwise dissimilarity of total species counts
277 (Aitchison distance) did not predict feedbacks (p=0.59). Pairwise feedback was significantly
278 predicted by genetic dissimilarity of two AM fungal species, *E. infrequens* (p<0.001) and *Cl.*
279 *lamellosum* (p<0.001).

280 Discussion

281 Using 38 plant species, we present a uniquely robust test of plant phylogeny and life history
282 impacts on the composition and symbiotic function of arbuscular mycorrhizal (AM) fungi. We
283 found strong phylosymbiosis, as phylogenetic distance was an important predictor of the
284 differences in AM fungal diversity, density, and community composition across plant host species.
285 In addition, we detected significant differences in genetic composition of individual AM fungal
286 species across plant host species. These differences are often predicted by plant phylogenetic
287 distance, indicating that AM fungal species can evolve rapidly in response to their host. However,
288 while AM fungi generally improved host growth, we found only weak evidence that the
289 phylogenetically structured divergence of symbiont community and genetic composition
290 consistently altered symbiont impacts on their hosts. After controlling for the effect of plant
291 phylogeny, we also found that AM fungal density, community diversity, community composition,

292 and genetic composition within species changed with plant life history, as represented by early vs
293 late successional stage categories. In this case, the shift in symbiont composition with plant life
294 history differentially benefited plants of the same life history generating positive mycorrhizal
295 feedback. Differential positive feedbacks that vary between plants of different with life history
296 characteristics could contribute to plant species turn over during succession. Interestingly, our
297 results suggest that the genetic change within AM fungal species, rather than changes in relative
298 abundance of AM fungal species, generated the observed positive mycorrhizal feedback.
299 Together, our results indicate that both plant phylogeny and plant life history traits govern the
300 associated AM fungal community and genetic composition, but only plant life history traits have
301 strong evidence for consistent reinforcing positive feedbacks on plant fitness.
302 We were able to confirm that plant host identity and phylogenetic relationships were
303 important in shaping the AM fungal community composition. Previous studies have demonstrated
304 that AM fungal growth rates varied between individual plant species [48,49]. We affirm that this
305 result is general across plant species, as AM fungal composition diverged significantly across the
306 38 plant species studied. Moreover, host-specific differentiation of AM fungal composition had a
307 strong phylogenetic signature. That is, the growth rates of individual AM fungal species tended to
308 be similar on phylogenetically similar host plants. This phylogenetic influence was evident in the
309 differential abundance of *E. infrequens, R. fulgida*, and *Ce. pellucida* (Fig 2). Phylogenetic
310 distance also predicted measures of AM fungal diversity. While previous work has shown
311 phylogenetic signature to plant species specific differentiation of soil bacterial and fungal
312 communities [50], this study provides clear evidence of phylogenetic structure to host-specific
313 differentiation of AM fungal communities.
314 We note that the influence of host identity on AM fungal composition does not appear to
315 result from differential exclusion of AM fungal species from roots, as would be expected from
316 incompatibilities of plant-AM fungal signaling. Rather, inoculated AM fungi were found with all

317 plant species. Given that inoculated AM fungi had equivalent densities across each plant species
318 at the beginning of the experiment, the change in AM fungal composition is due to host-specific
319 differences in AM fungal growth rates (i.e., fitness). The observation of substantial influence of
320 host phylogeny on AM fungal composition may reflect that plant influence on AM fungal relative
321 fitness can be moderated by phylogenetically conserved plant traits, such as root architecture [51]
322 or secondary chemical composition [52].

323 Our first measure of host phylogenetic influence on mycobiome composition solely
324 examined species level changes, and this may underestimate the impact of host phylogeny which
325 could operate on genetic changes within each species. AM fungal species and isolates have
326 highly variable rDNA genes [43,53], and we observed changes in rDNA composition with plant
327 host species across most AM fungal species included in this study. Using both PerMANOVA and
328 PGLMM methods, we detected unique host family/phylogenetic structure of the genetic
329 composition of all our AM fungal species with the exception of *A. spinosa*, a species that had very
330 low relative abundances. Our most abundant AM fungal species, *E. infequens,* exhibited host
331 phylogenetic influence on its genetic composition for both years, while *Cl. lammelosum*, *Cl.*
332 *claroideum, Ce. pellucida*, *R. fulgida*, and *F. mosseae* exhibited phylogenetic responses in
333 genetic composition in one year regardless of the analysis used. In addition, host plant life history
334 showed at least a marginal effect on AM fungal genetic composition for all species except *E.*
335 *infrequens* and *A. spinosa*. Previous work had shown that genetic composition of AM fungal
336 species can change rapidly with environment and host plant species [33,54,55]. These rapid
337 changes could be due to differential segregation of nuclei in multi-nucleate cells [32,33,38]. We
338 provide evidence that such genetic change can occur widely across AM fungal taxa and that the
339 host-specific effects on AM fungal genetic composition are structured by plant phylogeny.

340 We find host-specific changes in AM fungi composition generate feedback on plant
341 growth that is, on average, positive and ranges from significantly negative to strongly positive for

342 individual plant species pairs. This is consistent with previous studies [20,22,23]. Our study
343 provides the first attempt to generalize the patterns of AM fungal feedback, specifically testing
344 whether AM fungal feedback is structured by plant phylogeny or plant life history. We test these
345 patterns by testing correlations of AM fungal differentiation with growth impacts of these fungi
346 observed in previous growth assays with our AM fungal isolates [28,45]. In addition to this
347 correlational approach, we also measured AM fungal feedback effects by analyzing patterns
348 observed among feedbacks directly measured using an independent greenhouse assay [48].
349 While the correlation of plant and AM fungal fitness responses only considers the impacts of
350 changes in AM fungal species composition, the direct measurements of AM fungal feedbacks
351 integrates across changes in AM fungal species and genetic composition.

352 Both correlations of relative plant and fungal fitness (Fig 4) and patterns of AM fungal
353 feedbacks (Fig 6C) show weak responses to plant phylogenetic structure. That is, the AM fungi
354 with the highest growth rates on a particular group of phylogenetically-related plants do not
355 generally promote the growth of these phylogenetically-related plants better than other AM fungi.
356 Interestingly, the best fit of AM fungal feedbacks to plant phylogenetic relatedness was
357 curvilinear, with regions of phylogenetic distance over which plants tended to benefit more from
358 AM fungal communities cultivated by more distantly related host plants and regions where plants
359 tended to benefit more from AM fungal communities cultivated by more phylogenetically similar
360 host plants (Fig 6C). Given that this is the first test of such relationships, more work is necessary
361 in the plant-AM fungal systems as well as other host-symbiont interactions.

362 In contrast to plant phylogeny, AM fungal feedbacks are structured by plant life history.
363 AM fungal feedback, as measured by direct greenhouse assays, were consistently positive
364 between early and late successional plant species (Fig 6b). This result is consistent with
365 observations greater responsiveness of late successional species to mycorrhizal fungi [28,45],
366 and of positive mycorrhizal feedback observed in mesocosms [23] and field inoculations with

367 prairie AM fungi [31,56,57]. Positive mycorrhizal feedback could play an important role in plant
368 dynamics during succession and restoration, potentially accelerating turnover during succession,
369 or contribute to alternative stable states [19]. Mycorrhizal feedback between pairs of late
370 successional plant species were not generally different from zero, consistent with all late
371 successional species benefiting equivalently from changes in mycorrhizal composition, while
372 feedback between pairs of early successional species tended to be positive, suggesting that this
373 life-history category includes plants with substantial variation in relationships with AM fungi.

374 The change in AM fungal species composition was not sufficient to explain the positive
375 AM fungal feedback between early and late successional plant species (Fig. 5). In fact, across all
376 the data, the change in AM fungal species composition did not predict observed patterns of
377 mycorrhizal feedback (Fig. 7a). AM fungal species diversity also increased with late successional
378 plant species, and AM fungal diversity can influence host response [58] potentially generating
379 positive AM fungal feedbacks. However, this hypothesis was not supported by meta-analysis of
380 differential benefits to late successional plant species from AM fungal diversity (S8 Fig).

381 The observed patterns of mycorrhizal feedback appear to have resulted from changes in
382 genetic composition within individual AM fungal species. This hypothesis is directly supported as
383 the genetic change within the two most common and beneficial AM fungal species, *E. infrequens*
384 and *Cl. Lamellosum*, significantly predicted the strength of positive mycorrhizal feedback (Fig.
385 7b,c). Our results are consistent with previous observation of host-driven genetic changes within
386 AM fungal isolates altering AM fungal impacts on plant fitness [32,38], and of patterns consistent
387 with a role of genetic variation within AM fungal species generating mycorrhizal feedback [21].
388 Our work illustrates that rapid genetic change within AM fungal species can have functional
389 consequences, even exceeding those of changes in AM fungal species composition. Our result
390 highlights limitations to efforts to understand AM fungal ecology using patterns of species level
391 morphological traits [59,60] as we find that mycorrhizal feedback, the product of variation in

growth responses to hosts and impacts on hosts, meaningfully varied between genotypes within AM fungal species rather than between AM fungal species.

We find both ecological (i.e. changes in relative abundance of AM fungal species) and evolutionary (i.e changes in genetic composition within AM fungal species) dynamics operating concurrently within the plant – AM fungal symbiosis. We find strong evidence that individual AM fungal species, and genotypes within species, vary in their response to plant species, and that patterns of AM fungal divergence were structured by both plant phylogeny and plant traits governing life history variation. AM fungal composition generally improved host fitness, i.e. mycorrhizal feedbacks were generally positive. However, there was substantial variation in strength of mycorrhizal feedbacks, with variation in plant life history predicting stronger positive feedbacks, while variation due to plant phylogeny did not. Moreover, evolutionary changes within individual AM fungal species were the major driver of positive feedback rather than ecological changes in AM fungal species composition. Given the evidence that AM fungal diversity and composition can be critical to plant community and terrestrial ecosystem function [5–9], our work suggests that improved understanding of both evolutionary and ecological dynamics of AM fungi are necessary for optimal management of terrestrial ecosystems and projections of ecosystem response to anthropogenic disturbance.

Materials and Methods

A common community of AM fungi made up of seven cultured isolates derived from tallgrass prairie (*Claroideoglomus lamellosum, Claroideoglomus claroideum, Entrophospora infrequens, Funneliformis mosseae, Racocetra fulgida, Cetraspora pellucida, Acaulospora spinosa*) was distributed into replicate mesocosms. The mesocosms were planted in the greenhouse with one of 38 grassland plant species from the prairie region chosen to provide a high degree of both

phylogenetic diversity as well as diversity in life history traits associated with plant successional stage (S1 and S2 Figs). AM fungal composition in mesocosms was allowed to differentiate in response to host species over two growing seasons (2017 and 2018) and the composition of the AM fungal community was sampled after each growing season. In both years plants were grown in the greenhouse for the entire growing season (March to September) and were harvested in the fall. Plants were grown in two-gallon pots with four individuals of a species to a pot in the first year. After the first year, half of the soil in each pot was retained as inocula for the same host species and same replicate in year 2. Soil samples were taken after each growing season for determination of AM fungal composition using next-gen Illumina™ sequencing. We assumed over-representation of sequences as indicative of positive fitness impacts in plant treatments and under-representation indicative of negative fitness impacts. In year 3 we conducted a greenhouse feedback experiment where each soil community, trained by association with specific plant species, was used as inocula for other plants in the experiment. These were grown with a single host plant individual in 1-liter deep pots and inoculated with 100 ml of soil from the year 2 mesocosms. From the plant biomass data we were able to calculate feedback coefficients.

Meta-analysis Methods

The seven AM fungi isolates used in this experiment were used previously in three separate studies testing their impact on early and late successional plant species: one reported in [45] and two in [28]. We conducted a meta-analysis of these studies to obtain estimates of the growth promotion for each isolate to plant species of different plant phylogenetic groups (grass, asters, and legumes) and from different life histories/origin groups (early successional native, non-native, and late successional native plants). We calculated the log mycorrhizal responsiveness (LRR) using equation (1) where $\underline{x}_{inoc}$ and $\underline{x}_{ctrl}$ are mean plant biomass for the inoculated treatments

and sterile controls, respectively. The sampling variance ($\grave{\sigma}_2$) of LRR was estimated using equation (2) where SD_{inoc}^2 and SD_{ctrl}^2 are the standard deviations and n_{inoc} and n_{ctrl} are the sample sizes of the treatment and controls respectively (Hedges et al., 1999; Hoeksema et al., 2018).

$$1)\quad LRR = ln\left[\frac{\underline{x}_{inoc}}{\underline{x}_{ctrl}}\right]$$

$$2)\quad \grave{\sigma}_2 = \frac{SD_{inoc}^2}{n_{inoc} \times \grave{x}_{inoc}^2} + \frac{SD_{ctrl}^2}{n_{ctrl} \times \grave{x}_{ctrl}^2}$$

The analysis was conducted using the metafor package in R [61]. Moderators were the host plant life history, host plant phylogenetic group, the AM fungal isolate used, and the interaction of the isolate used and plant life history and phylogenic group. Random effects included individual experiments and plant host species. The model was estimated using the restricted maximum-likelihood ("REML") method. Marginal means were estimated for the interaction terms using the emmeans package [62]. The metafor model was converted to a reference grid for compatibility with the emmeans package using the qdrg function.

We also tested whether plant life history predicted plant response to differences in AM fungal diversity of inocula. Only the two studies in Cheeke et al. (2019) included multi-species with single AM fungal species inoculations. Following the approach developed in Magnoli and Bever (2023), we calculated LRR in this analysis as the ratio of multiple species inocula mean plant biomass $\underline{x}_{multi}$ to the mean plant biomass averaged across all single inocula $\underline{x}_{mean}$. We also calculated the ratio of $\underline{x}_{multi}$ to the mean plant biomass of the single most beneficial AM fungal inocula $\underline{x}_{most}$. For each of these tests we constructed measures of variance by modifying equation 2.

459 # Bioinformatics Methods

460 To measure the changes in relative abundances of AM fungal species 0.2 g of soil was retained
461 from each pot for Illumina™ sequencing. Extraction was performed on 311 samples using the
462 Qiagen DNeasy Powersoil Kit. One sample of the 311 was lost during storage in both years. DNA
463 was amplified using the LROR and FLR2 primers [63,64]. Volumes of 12.5μL of Phusion® Hot
464 Start Flex DNA Polymerase master mix, 0.5μL of each primer, and 10.5μL of MilliporeSigma™
465 Direct-Q™ 3 type I water per sample were combined with 1μL of extracted DNA. PCR was
466 conducted using one 5 minute cycle at 94.0 C, thirty-five cycles alternating between 94.0 C for 30
467 seconds, 48.0 C for 30 secs, 72.0C for 30 seconds and 72.0 C for 10 minutes.

468 Bioinformatics was conducted using the Dada2 and Qiime 2 pipelines as described in
469 [40–42]. Primers were removed using Cutadapt. Using Dada2 forward reads were truncated to a
470 length where 95% of base pair Phred quality scores (Q scores) had a greater than 99% base call
471 accuracy, with forward reads truncated to a length of 190bp and reverse reads to a length of
472 140bp. Forward and reverse reads were merged using the "justconcatonate" flag in Dada2 and
473 chimeras were removed. Taxonomy was assigned by building a phylogenetic tree of the amplicon
474 sequence variants (ASVs) using RAxML together with an included library of known AM fungi
475 species [40,41,43,65,66]. All AM fungi identified sequences were blasted against the reference
476 library. Several ASVs that were placed inside AM fungi had unacceptably long branch lengths
477 and were suspected of being non homologous gene regions that were erroneously being placed
478 in the phylogeny [40]. Those ASVs were excluded from the analysis. All background soil was
479 sterilized. Therefore, the ASVs that were not identified as one of the seven inoculated species
480 were assumed likely to be legacy DNA from dead organisms in the background soil and were
481 also not included in the statistical analysis. ASVs were assigned a species taxonomy if they
482 clustered within known species sequences from the reference library.

483 # Plant Phylogenetic Methods

484 A phylogeny was constructed for all plants in the study to determine phylogenetic distance.
485 Sequences were obtained for the rbcL gene for all 38 species of plants from the NCBI GenBank
486 database. A constraint tree was obtained using the Phylomatic "Tree of Trees" software using the
487 slik megatree [67,68]. Tree construction was conducted using RaXML [66].

488 # Statistical Methods

489 ASV abundances for inoculated AM fungal species were centered log transformed and a
490 PerMANOVA analysis was conducted using the adonis function in the R package vegan [69]. The
491 predictors used were host plant life history, host plant phylogenetic group, and the interaction of
492 host plant life history with host plant phylogenetic group. Phylogenetic groups are characterized
493 as lilies (Amaryllidaceae and Commelinaceae), grasses (Poaceae), legumes (Fabacea),
494 milkweeds (Apocynaceae), mints (Lamiaceae and Plantaginaceae), and asters (Apiaceae and
495 Asteraceae).

496 Using a categorical factor, such as phylogenetic group, to analyze the effect of
497 phylogenetic relationships is a limited approach. We used a method to include phylogenetic
498 distances as a random effect to preserve the information included in the full phylogenetic tree.
499 When visualizing the phylogenetic relationships and AM fungal composition, there are hints at
500 relationships that would be difficult to test using more traditional statistical methods (Fig 2). A
501 phylogenetic generalized mixed model (PGLM) was fitted using the phyr R package [70,71]. Host
502 plant successional status, and the interaction between them were modeled as fixed effects. Block
503 and the log transformed sequences were included alongside phylogenetic distance and species
504 as random effects. As a univariate analysis each AM fungal species' ASVs were grouped, and

their abundances logit transformed. We also assessed Shannon's Diversity and the logit-transformed proportion of the inoculated AM fungi over the total number of ASVs. The reason for analyzing this proportion is that as the legacy background DNA would not be growing in response to the treatments, the proportion of inoculated AM fungi could be used as a proxy for changes in fungal density.

We purposely included full factorial tests of plant species and training plant species for subsets of the 38 plant species. Each subset was treated as a block, and we attempted to maximize comparisons across the phylogeny, while maintaining a relatively even number of both early and late successional plants (S12 Fig). Within these sub-set pairs of plant species, we estimated pairwise feedbacks that govern the influence of mycorrhizal fungal dynamics on plant-plant interactions. Pairwise feedbacks are measured by the interaction coefficient (I), as derived from the models of interguild frequency dependence and host-microbiome feedback. It is calculated according to equation (3) [19,47]. Where $F_{A\alpha}$ is the fitness of plant A in soil α, $F_{A\beta}$ the fitness of Plant A in soil β, $F_{B\alpha}$ the fitness of plant B in soil α, and $F_{B\beta}$ the fitness of plant B in soil β . In this experiment we used plant biomass as our measure of plant fitness. If this coefficient is positive then the plants are experiencing positive feedback, and if the coefficient is negative the plants are experiencing negative feedback. We used a type III ANOVA analysis to test the effect on the interaction coefficient from host plant and training plant life history and the interaction between them.

$$3) \quad I = F_{A\alpha} - F_{A\beta} - F_{B\alpha} + F_{B\beta}$$

Dissimilarity between species compositions and genetic compositions within each AM fungal species was calculated using Aitchison distances (Euclidean distances based on center log transformed counts). For species composition all ASVs assigned to a single AM fungal species were first summed. For genetic change dissimilarity, separate dissimilarity matrices were calculated for all ASVs characterized as belonging to each AM fungal species. These

dissimilarities were used in the PerMANOVA analyses. The list of pairwise dissimilarities derived from these matrices were used in regression analysis against feedback strength (interaction coefficients)

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539 # References

540 1. Smith SE, Read D. Mycorrhizal Symbiosis. Elsevier; 2008. doi:10.1016/B978-0-12-370526-
541 6.X5001-6
542 2. Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev
543 Microbiol. 2008;6: 763–775. doi:10.1038/nrmicro1987
544 3. Simon L, Bousquet J, Lévesque RC, Lalonde M. Origin and diversification of
545 endomycorrhizal fungi and coincidence with vascular land plants. Nature. 1993;363: 67–69.
546 doi:10.1038/363067a0
547 4. Redecker D, Kodner R, Graham LE. Glomalean Fungi from the Ordovician. Science.
548 2000;289: 1920–1921. doi:10.1126/science.289.5486.1920
549 5. Wilson GWT, Hartnett DC. Effects of mycorrhizae on plant growth and Dynamics in
550 Experimental Tallgrass Prairie Microcosms. American Journal of Botany. 1997;84: 478–
551 482. doi:10.2307/2446024
552 6. Heijden MGA van der, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, et
553 al. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and
554 productivity. Nature. 1998;396: 69–72. doi:10.1038/23932
555 7. Hartnett DC, Wilson GWT. Mycorrhizae Influence Plant Community Structure and Diversity
556 in Tallgrass Prairie. Ecology. 1999;80: 1187–1195. doi:10.1890/0012-
557 9658(1999)080[1187:MIPCSA]2.0.CO;2
558 8. Vogelsang KM, Reynolds HL, Bever JD. Mycorrhizal fungal identity and richness determine
559 the diversity and productivity of a tallgrass prairie system. New Phytologist. 2006;172: 554–
560 562. doi:10.1111/j.1469-8137.2006.01854.x

561 9. Bauer JT, Kleczewski NM, Bever JD, Clay K, Reynolds HL. Nitrogen-fixing bacteria,
562 arbuscular mycorrhizal fungi, and the productivity and structure of prairie grassland
563 communities. Oecologia. 2012;170: 1089–1098. doi:10.1007/s00442-012-2363-3
564 10. Lim SJ, Bordenstein SR. An introduction to phylosymbiosis. Proceedings of the Royal
565 Society B: Biological Sciences. 2020;287: 20192900. doi:10.1098/rspb.2019.2900
566 11. Brooks AW, Kohl KD, Brucker RM, Opstal EJ van, Bordenstein SR. Phylosymbiosis:
567 Relationships and Functional Effects of Microbial Communities across Host Evolutionary
568 History. PLOS Biology. 2016;14: e2000225. doi:10.1371/journal.pbio.2000225
569 12. van Opstal EJ, Bordenstein SR. Phylosymbiosis Impacts Adaptive Traits in Nasonia Wasps.
570 mBio. 2019;10: 10.1128/mbio.00887-19. doi:10.1128/mbio.00887-19
571 13. Brucker RM, Bordenstein SR. The Hologenomic Basis of Speciation: Gut Bacteria Cause
572 Hybrid Lethality in the Genus Nasonia. Science. 2013;341: 667–669.
573 14. Trevelline BK, Sosa J, Hartup BK, Kohl KD. A bird's-eye view of phylosymbiosis: weak
574 signatures of phylosymbiosis among all 15 species of cranes. Proceedings of the Royal
575 Society B: Biological Sciences. 2020;287: 20192988. doi:10.1098/rspb.2019.2988
576 15. Kohl KD, Dearing MD, Bordenstein SR. Microbial communities exhibit host species
577 distinguishability and phylosymbiosis along the length of the gastrointestinal tract. Molecular
578 Ecology. 2018;27: 1874–1883. doi:10.1111/mec.14460
579 16. Tang Y, Ma KY, Cheung MK, Yang C-H, Wang Y, Hu X, et al. Gut Microbiota in Decapod
580 Shrimps: Evidence of Phylosymbiosis. Microb Ecol. 2021;82: 994–1007.
581 doi:10.1007/s00248-021-01720-z
582 17. Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, et al. Coral-associated
583 bacteria demonstrate phylosymbiosis and cophylogeny. Nat Commun. 2018;9: 4921.
584 doi:10.1038/s41467-018-07275-x

18. O'Brien PA, Tan S, Yang C, Frade PR, Andreakis N, Smith HA, et al. Diverse coral reef invertebrates exhibit patterns of phylosymbiosis. The ISME Journal. 2020;14: 2211–2222. doi:10.1038/s41396-020-0671-x

19. Bever JD. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. Ecology Letters. 1999;2: 52–61. doi:10.1046/j.1461-0248.1999.21050.x

20. Bever JD. Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society of London Series B: Biological Sciences. 2002;269: 2595–2601. doi:10.1098/rspb.2002.2162

21. Castelli JP, Casper BB. Intraspecific Am Fungal Variation Contributes to Plant–Fungal Feedback in a Serpentine Grassland. Ecology. 2003;84: 323–336. doi:10.1890/0012-9658(2003)084[0323:IAFVCT]2.0.CO;2

22. Mangan SA, Herre EA, Bever JD. Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback. Ecology. 2010;91: 2594–2603. doi:10.1890/09-0396.1

23. Koziol L, Bever JD. Mycorrhizal feedbacks generate positive frequency dependence accelerating grassland succession. Journal of Ecology. 2019;107: 622–632. doi:10.1111/1365-2745.13063

24. Adler PB, Salguero-Gómez R, Compagnoni A, Hsu JS, Ray-Mukherjee J, Mbeau-Ache C, et al. Functional traits explain variation in plant life history strategies. Proceedings of the National Academy of Sciences. 2014;111: 740–745. doi:10.1073/pnas.1315179111

25. Reich PB. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. Journal of Ecology. 2014;102: 275–301. doi:10.1111/1365-2745.12211

26. Koziol L, Bever JD. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology. 2015;96: 1768–1774. doi:10.1890/14-2208.1

610 27. Bauer JT, Mack KML, Bever JD. Plant-soil feedbacks as drivers of succession: evidence
611 from remnant and restored tallgrass prairies. Ecosphere. 2015;6: art158. doi:10.1890/ES14-
612 00480.1
613 28. Cheeke TE, Zheng C, Koziol L, Gurholt CR, Bever JD. Sensitivity to AMF species is greater
614 in late-successional than early-successional native or nonnative grassland plants. Ecology.
615 2019;100: e02855. doi:10.1002/ecy.2855
616 29. Koziol L, Schultz PA, Parsons S, Bever JD. Native mycorrhizal fungi improve milkweed
617 growth, latex, and establishment while some commercial fungi may inhibit them. Ecosphere.
618 2022;13: e4052. doi:10.1002/ecs2.4052
619 30. Koziol L, McKenna TP, Crews TE, Bever JD. Native arbuscular mycorrhizal fungi promote
620 native grassland diversity and suppress weeds 4 years following inoculation. Restoration
621 Ecology. 2023;31: e13772. doi:10.1111/rec.13772
622 31. Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, Henning JA, et al. Locally
623 adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native
624 prairie plant species. Ecosphere. 2015;6: 1–16. doi:10.1890/ES15-00152.1
625 32. Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR. Segregation in a Mycorrhizal
626 Fungus Alters Rice Growth and Symbiosis-Specific Gene Transcription. Current Biology.
627 2010;20: 1216–1221. doi:10.1016/j.cub.2010.05.031
628 33. Angelard C, Tanner CJ, Fontanillas P, Niculita-Hirzel H, Masclaux F, Sanders IR. Rapid
629 genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and
630 enhanced by segregation. ISME J. 2014;8: 284–294. doi:10.1038/ismej.2013.154
631 34. Pringle A, Moncalvo J-M, Vilgalys R. High Levels of Variation in Ribosomal DNA
632 Sequences within and among Spores of a Natural Population of the Arbuscular Mycorrhizal
633 Fungus Acaulospora colossica. Mycologia. 2000;92: 259–268. doi:10.2307/3761559

634 35. Kuhn G, Hijri M, Sanders IR. Evidence for the evolution of multiple genomes in arbuscular
635 mycorrhizal fungi. Nature. 2001;414: 745–748. doi:10.1038/414745a
636 36. Rodriguez A, Clapp JP, Dodd JC. Ribosomal RNA gene sequence diversity in arbuscular
637 mycorrhizal fungi (Glomeromycota). Journal of Ecology. 2004;92: 986–989.
638 doi:10.1111/j.1365-2745.2004.00935.x
639 37. Hijri M, Sanders IR. Low gene copy number shows that arbuscular mycorrhizal fungi inherit
640 genetically different nuclei. Nature. 2005;433: 160–164. doi:10.1038/nature03069
641 38. Angelard C, Sanders IR. Effect of segregation and genetic exchange on arbuscular
642 mycorrhizal fungi in colonization of roots. New Phytologist. 2011;189: 652–657.
643 doi:10.1111/j.1469-8137.2010.03602.x
644 39. Bever JD, Wang M. Arbuscular mycorrhizal fungi: Hyphal fusion and multigenomic
645 structure. Nature. 2005;433: E3–E3. doi:10.1038/nature03294
646 40. Delavaux CS, Sturmer SL, Wagner MR, Schütte U, Morton JB, Bever JD. Utility of large
647 subunit for environmental sequencing of arbuscular mycorrhizal fungi: a new reference
648 database and pipeline. New Phytologist. 2021;229: 3048–3052. doi:10.1111/nph.17080
649 41. Delavaux CS, Ramos RJ, Sturmer SL, Bever JD. Environmental identification of arbuscular
650 mycorrhizal fungi using the LSU rDNA gene region: an expanded database and improved
651 pipeline. Mycorrhiza. 2022 [cited 18 Feb 2022]. doi:10.1007/s00572-022-01068-3
652 42. Delavaux CS, Ramos RJ, Stürmer SL, Bever JD. An updated LSU database and pipeline
653 for environmental DNA identification of arbuscular mycorrhizal fungi. Mycorrhiza. 2024;34:
654 369–373. doi:10.1007/s00572-024-01159-3
655 43. House GL, Ekanayake S, Ruan Y, Schütte UME, Kaonongbua W, Fox G, et al.
656 Phylogenetically Structured Differences in rRNA Gene Sequence Variation among Species
657 of Arbuscular Mycorrhizal Fungi and Their Implications for Sequence Clustering. Appl
658 Environ Microbiol. 2016;82: 4921–4930. doi:10.1128/AEM.00816-16

659 44. Pawlowska TE. Genetic processes in arbuscular mycorrhizal fungi. FEMS Microbiology
660 Letters. 2005;251: 185–192. doi:10.1016/j.femsle.2005.08.007
661 45. Koziol L, Bever JD. AMF, phylogeny, and succession: specificity of response to mycorrhizal
662 fungi increases for late-successional plants. Ecosphere. 2016;7: e01555.
663 doi:10.1002/ecs2.1555
664 46. Abbott KC, Eppinga MB, Umbanhowar J, Baudena M, Bever JD. Microbiome influence on
665 host community dynamics: Conceptual integration of microbiome feedback with classical
666 host–microbe theory. Ecology Letters. 2021;24: 2796–2811. doi:10.1111/ele.13891
667 47. Bever JD, Westover KM, Antonovics J. Incorporating the Soil Community into Plant
668 Population Dynamics: The Utility of the Feedback Approach. Journal of Ecology. 1997;85:
669 561–573. doi:10.2307/2960528
670 48. Bever JD. Host-specificity of AM fungal population growth rates can generate feedback on
671 plant growth. Plant and Soil. 2002;244: 281–290. doi:10.1023/A:1020221609080
672 49. Burrill HM, Magnoli SM, Bever JD. Soil microbiome composition is highly responsive to
673 precipitation and plant composition manipulations in a field biodiversity experiment. Front
674 Microbiomes. 2025;4: 1460319. doi:10.3389/frmbi.2025.1460319
675 50. Kaplan I, Bokulich NA, Caporaso JG, Enders LS, Ghanem W, Ingerslew KS. Phylogenetic
676 farming: Can evolutionary history predict crop rotation via the soil microbiome? Evolutionary
677 Applications. 2020;13: 1984–1999. doi:10.1111/eva.12956
678 51. Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB. A worldview of root traits:
679 the influence of ancestry, growth form, climate and mycorrhizal association on the functional
680 trait variation of fine-root tissues in seed plants. The New Phytologist. 2017;215: 1562–
681 1573.
682 52. Formenti L, Iwanycki Ahlstrand N, Hassemer G, Glauser G, van den Hoogen J, Rønsted N,
683 et al. Macroevolutionary decline in mycorrhizal colonization and chemical defense

684 responsiveness to mycorrhization. iScience. 2023;26: 106632.
685 doi:10.1016/j.isci.2023.106632
686 53. Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet J, Sanders IR. High genetic
687 variability and low local diversity in a population of arbuscular mycorrhizal fungi.
688 Proceedings of the National Academy of Sciences. 2004;101: 2369–2374.
689 doi:10.1073/pnas.0306441101
690 54. Kokkoris V, Chagnon P-L, Yildirir G, Clarke K, Goh D, MacLean AM, et al. Host identity
691 influences nuclear dynamics in arbuscular mycorrhizal fungi. Current Biology. 2021;31:
692 1531-1538.e6. doi:10.1016/j.cub.2021.01.035
693 55. Ehinger M, Koch AM, Sanders IR. Changes in arbuscular mycorrhizal fungal phenotypes
694 and genotypes in response to plant species identity and phosphorus concentration. New
695 Phytologist. 2009;184: 412–423. doi:10.1111/j.1469-8137.2009.02983.x
696 56. Koziol L, Bever JD. The missing link in grassland restoration: arbuscular mycorrhizal fungi
697 inoculation increases plant diversity and accelerates succession. Journal of Applied
698 Ecology. 2017;54: 1301–1309. doi:10.1111/1365-2664.12843
699 57. Middleton EL, Bever JD. Inoculation with a Native Soil Community Advances Succession in
700 a Grassland Restoration. Restoration Ecology. 2012;20: 218–226. doi:10.1111/j.1526-
701 100X.2010.00752.x
702 58. Magnoli SM, Bever JD. Plant productivity response to inter- and intra-symbiont diversity:
703 Mechanisms, manifestations and meta-analyses. Ecology Letters. 2023;26: 1614–1628.
704 doi:10.1111/ele.14274
705 59. Chaudhary VB, Holland EP, Charman-Anderson S, Guzman A, Bell-Dereske L, Cheeke TE,
706 et al. What are mycorrhizal traits? Trends in Ecology & Evolution. 2022;37: 573–581.
707 doi:10.1016/j.tree.2022.04.003

60. Antunes PM, Stürmer SL, Bever JD, Chagnon P-L, Chaudhary VB, Deveautour C, et al. Enhancing consistency in arbuscular mycorrhizal trait-based research to improve predictions of function. Mycorrhiza. 2025;35: 14. doi:10.1007/s00572-025-01187-7

61. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. Journal of Statistical Software. 2010;36: 1–48. doi:10.18637/jss.v036.i03

62. Lenth RV, Buerkner P, Herve M, Love J, Riebl H, Singmann H. emmeans: Estimated Marginal Means, aka Least-Squares Means. 2021. Available: https://CRAN.R-project.org/package=emmeans

63. Trouvelot S, van Tuinen D, Hijri M, Gianinazzi-Pearson V. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. Mycorrhiza. 1999;8: 203–206. doi:10.1007/s005720050235

64. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology. 1990;172: 4238–4246. doi:10.1128/jb.172.8.4238-4246.1990

65. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13: 581–583. doi:10.1038/nmeth.3869

66. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30: 1312–1313. doi:10.1093/bioinformatics/btu033

67. Slik JWF, Franklin J, Arroyo-Rodríguez V, Field R, Aguilar S, Aguirre N, et al. Phylogenetic classification of the world's tropical forests. PNAS. 2018;115: 1837–1842. doi:10.1073/pnas.1714977115

68. Webb CO, Donoghue MJ. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes. 2005;5: 181–183. doi:https://doi.org/10.1111/j.1471-8286.2004.00829.x

69. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. 2020. Available: https://CRAN.R-project.org/package=vegan

70. Ives A, Dinnage R, Nell LA, Helmus M, Li [aut D, cre. phyr: Model Based Phylogenetic Analysis. 2020. Available: https://CRAN.R-project.org/package=phyr

71. Ives AR. Mixed and Phylogenetic Models: A Conceptual Introduction to Correlated Data. Leanpub; 2018. Available: https://leanpub.com/correlateddata

Supporting information

S1 Fig. Host Plant Characteristics. Plant species were selected to represent a phylogenetically diverse group of early and late successional plants. Plants were selected from 5 large phylogenetic groups; grasses (Poaceae), legumes (Fabaceae), asters (Asteraceae and Apiaceae), mints (Lamiaceae and Plantaginaceae), milkweeds (Apocynaceae and Asclepiadaceae), and lilies (Liliaceae and Commelinaceae).

S2 Table. Plant Species Used.

S3 Table. PerMANOVA Results for AM Fungi Species. To test for overall differences in AM fungal composition with plant life history and plant family, we conducted a PerMANOVA on centered log ratio (CLR) transformed counts of ASVs grouped by AM fungal species. This analysis reveals statistically significant effects on AM fungal species composition of plant phylogenetic group in both years ($p \leq 0.001$, $p \leq 0.001$). We also tested for overall differences in AM fungal composition with plant life history and plant family using the PerMANOVA approach. This analysis reveals statistically significant effects on AM fungal species composition of plant life history on AM fungal species composition in year two ($p < 0.001$)

S4 Table. PerMANOVA Results for AMF ASVs Variation within Species. As AM fungi are known to have substantial variability in rDNA composition within individual multinucleate cells, we also test for changes in ASV composition within individual AM fungal species using a PerMANOVA approach. For E. infrequens and Cl. claroideum we find evidence of change in genetic composition with plant phylogenetic group in both year one ($p \leq 0.001$, $p \leq 0.01$) and year two ($p \leq 0.1$, 0.01). Genetic composition of Cl. lamellosum and Ce. pellucida showed significant responses of plant phylogenetic group in year 1 ($p \leq 0.001$, $p \leq 0.001$), while genetic composition of F. mosseae and R. Fulgida had significant effects in year 2 ($p \leq 0.01$, $p \leq 0.001$). The genetic

762 composition of F. mosseae, Cl. claroidium, and R. fulgida showed an effect of host plant life
763 history on intraspecies ASV variation in year 2 ($p<0.001$, $p<0.01$, $p<0.05$).
764 **S5 Table. PGLMM Results for AM Fungi Species.** Phylogenetic generalized linear mixed model
765 (PGLMM) results for relative proportions of each AM fungal species (combined ASV counts), as
766 well as Shannon Diversity and Logit transformed density estimates.
767 **S6 Fig. Marginal Means of Relative Abundance by Plant Life History.** These are the
768 estimated marginal means of AM fungal relative abundances when host plants were early or late
769 successional. AM fungal species are arranged top to bottom in order of most to least beneficial.
770 S7 Fig. Meta-Analysis Results. The marginal means for the log response ratio's (LRT) of each of
771 the seven AM fungal species used in this study. The LRT is the natural log of the ratio between
772 how large plant species grew with AM fungal inoculation versus how they grew in a sterile control.
773 These are derived from a meta-analysis of three previous experiments using these same
774 cultures. (A) shows late successional plant benefits when grown in association with E. infrequens,
775 Cl. lamellosum, F. mosseae, and Cl. claroideum. (B) shows the marginal means for the log
776 response ratio's (LRT) of each of the seven AM fungal species used in this study by host plant
777 phylogenetic group. Asteraceae experienced significantly greater growth than Poaceae in
778 association with all Claroideoglomus species (including E. infrequens). We also saw significant
779 differential greater growth in Liliaceae over Poaceae as well as Liliaceae over Asteraceae in
780 several AM fungal species.
781 **S8 Fig. LRR by Life History of AM Fungal Mixtures to Single Simbiont.** These are the log
782 response ratios of the mean plant biomass for plants inoculated with a mixture of AM fungi to the
783 mean plant biomass representative of the average single symbiont and to the most effective
784 single symbiont respectively. Each panel breaks our results depending on if the host plant was an
785 early or late successional.

S9 Fig. Histogram of mycorrhizal feedback measured as pairwise interaction coefficients. This shows counts of all paired comparisons with both the median and mean being significantly positive ($p = 0.003$, $p \leq 0.001$).

S10 Fig: Feedbacks by Plant Phylogenetic Group. Mycorrhizal feedback, as measured by the pairwise interaction coefficient, compared across phylogenetic group pairings. Feedback between individual pairs of plant species are indicated with dots, with pairwise feedback that was significantly different from zero indicated in black. We note that pairwise mycorrhizal feedbacks ranged from significantly negative to significantly positive. On average, we observed significantly positive mycorrhizal feedback between two legume species ($p \leq 0.01$) and significant negative mycorrhizal feedback between aster and grass species ($p \leq 0.05$).

S11 Fig: Mycorrhizal Feedback Strength Predicted by Genetic Dissimilarity of AM fungi for All AM fungal Species

Regressions of strength of pairwise feedback against measures of AM fungal genetic dissimilarity. Pairwise feedback was significantly predicted by genetic dissimilarity of two AM fungal species, *E. infrequens* ($p<0.001$) and *Cl. lamellosum* ($p<0.001$).

S12 Fig. Feedback Experimental Design. The experimental design for the feedback experiment included 5 subset groups of plants where all plant pairings were made in a fully factorial design. Each group also included early and late successional plants. When these pairings are arranged phylogenetically it becomes clearer that we also have a good representation of species pairs across the plant phylogeny. This allows us to test pairwise feedbacks, host plant characteristics, and plant phylogeny effects in a single experiment.

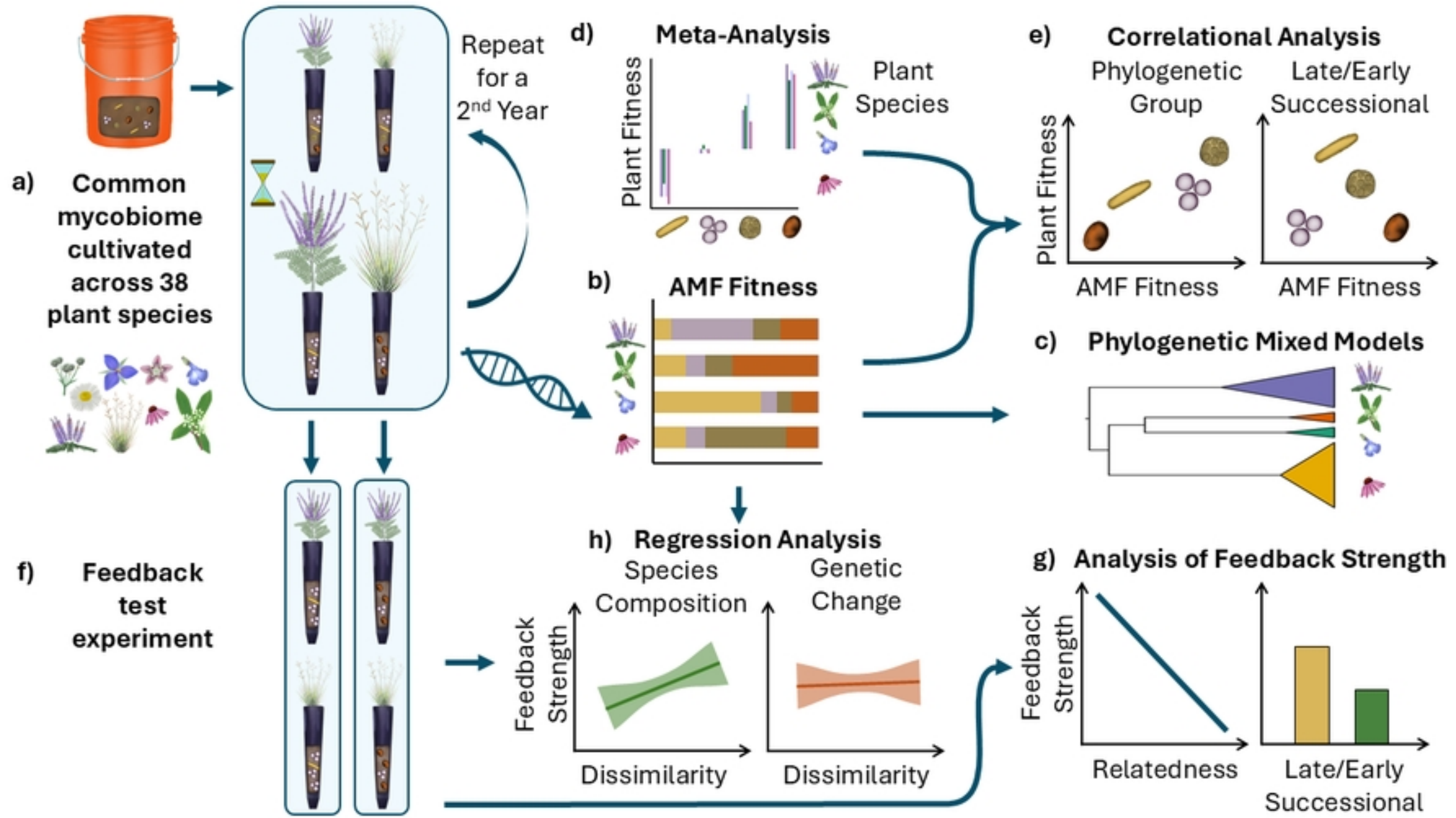
a)
Common mycobiome cultivated across 38 plant species
Repeat for a 2nd Year
d)
Meta-Analysis
Plant Fitness
Plant Species
e)
Correlational Analysis
Phylogenetic Group
Late/Early Successional
Plant Fitness
AMF Fitness
AMF Fitness
b)
AMF Fitness
c)
Phylogenetic Mixed Models
f)
Feedback test experiment
h)
Regression Analysis
Species Composition
Genetic Change
Feedback Strength
Dissimilarity
Dissimilarity
g)
Analysis of Feedback Strength
Feedback Strength
Relatedness
Late/Early Successional

Figure 1

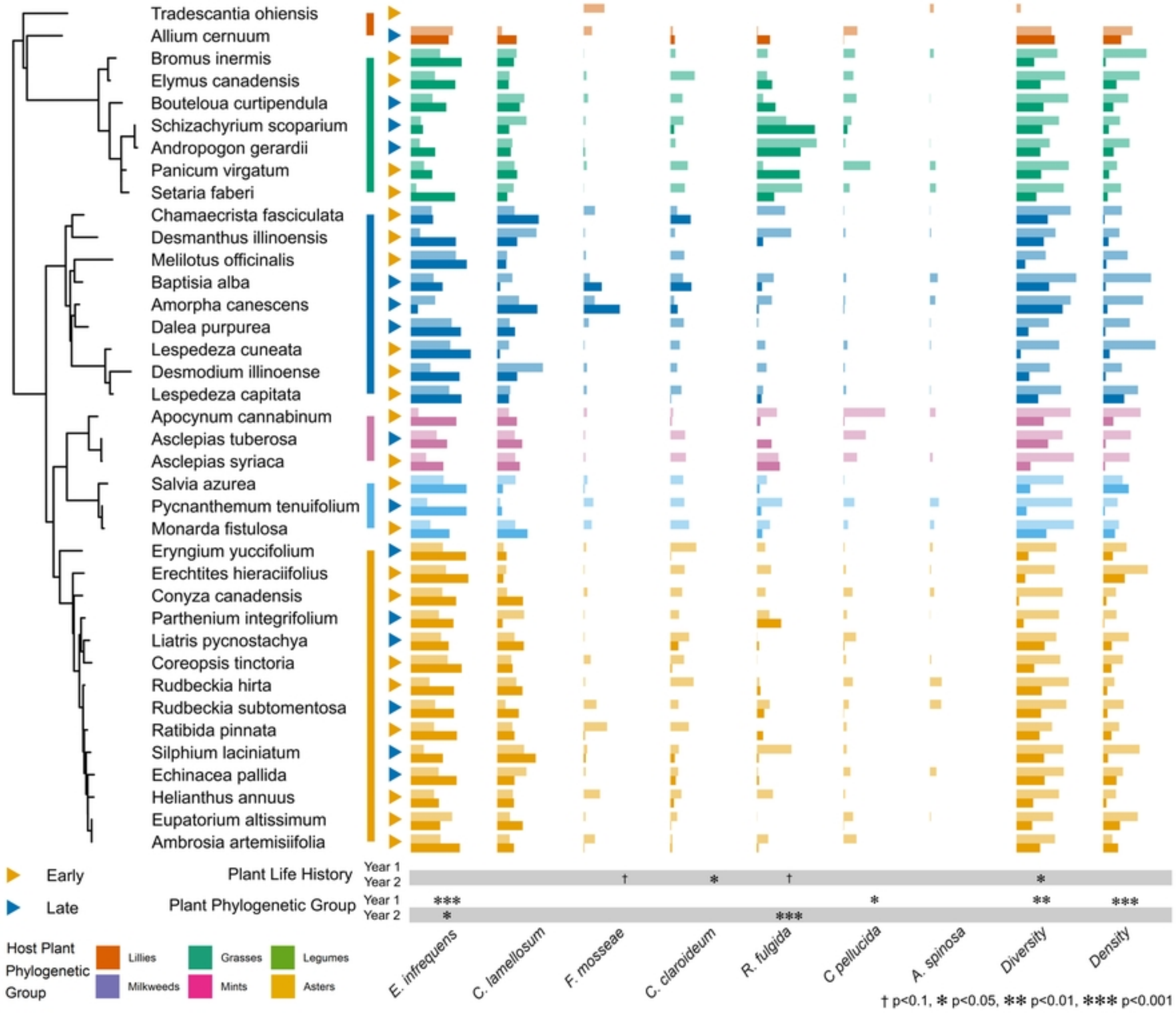
Tradescantia ohiensis
Allium cernuum
Bromus inermis
Elymus canadensis
Bouteloua curtipendula
Schizachyrium scoparium
Andropogon gerardii
Panicum virgatum
Setaria faberi
Chamaecrista fasciculata
Desmanthus illinoensis
Melilotus officinalis
Baptisia alba
Amorpha canescens
Dalea purpurea
Lespedeza cuneata
Desmodium illinoense
Lespedeza capitata
Apocynum cannabinum
Asclepias tuberosa
Asclepias syriaca
Salvia azurea
Pycnanthemum tenuifolium
Monarda fistulosa
Eryngium yuccifolium
Erechtites hieraciifolius
Conyza canadensis
Parthenium integrifolium
Liatris pycnostachya
Coreopsis tinctoria
Rudbeckia hirta
Rudbeckia subtomentosa
Ratibida pinnata
Silphium laciniatum
Echinacea pallida
Helianthus annuus
Eupatorium altissimum
Ambrosia artemisiifolia
Early
Late
Plant Life History
Year 1
Year 2
Plant Phylogenetic Group
Year 1
Year 2
E. infrequens
C. lamellosum
F. mosseae
C. claroideum
R. fulgida
C pellucida
A. spinosa
Diversity
Density
† p<0.1, * p<0.05, ** p<0.01, *** p<0.001
Host Plant Phylogenetic Group
Lillies
Grasses
Legumes
Milkweeds
Mints
Asters

Figure 2

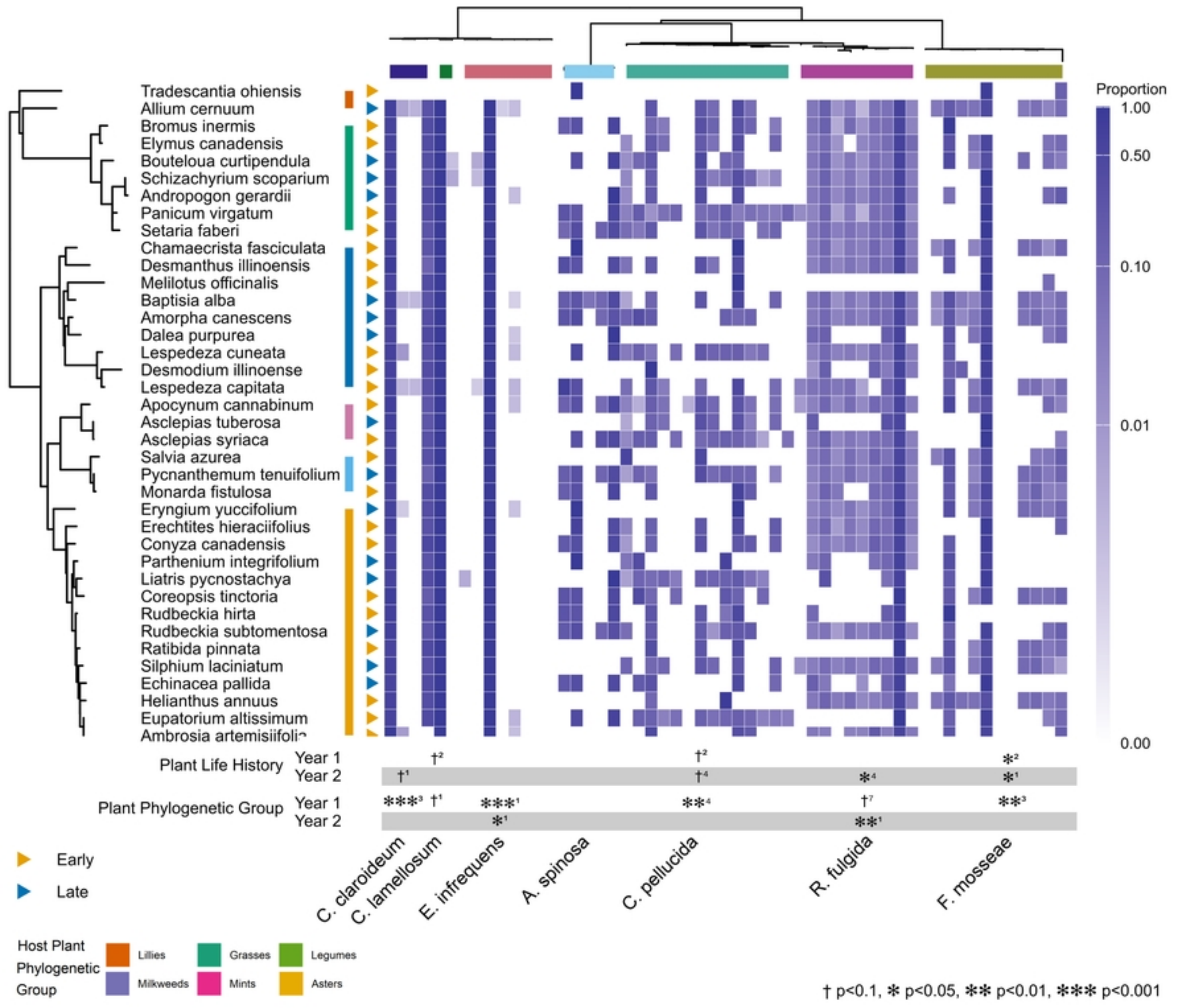
Tradescantia ohiensis
Allium cernuum
Bromus inermis
Elymus canadensis
Bouteloua curtipendula
Schizachyrium scoparium
Andropogon gerardii
Panicum virgatum
Setaria faberi
Chamaecrista fasciculata
Desmanthus illinoensis
Melilotus officinalis
Baptisia alba
Amorpha canescens
Dalea purpurea
Lespedeza cuneata
Desmodium illinoense
Lespedeza capitata
Apocynum cannabinum
Asclepias tuberosa
Asclepias syriaca
Salvia azurea
Pycnanthemum tenuifolium
Monarda fistulosa
Eryngium yuccifolium
Erechtites hieraciifolius
Conyza canadensis
Parthenium integrifolium
Liatris pycnostachya
Coreopsis tinctoria
Rudbeckia hirta
Rudbeckia subtomentosa
Ratibida pinnata
Silphium laciniatum
Echinacea pallida
Helianthus annuus
Eupatorium altissimum
Ambrosia artemisiifolia
Proportion
1.00
0.50
0.10
0.01
0.00
Plant Life History
Year 1
Year 2
Plant Phylogenetic Group
Year 1
Year 2
C. claroideum
C. lamellosum
E. infrequens
A. spinosa
C. pellucida
R. fulgida
F. mosseae
Early
Late
Host Plant Phylogenetic Group
Lilies
Milkweeds
Grasses
Mints
Legumes
Asters
† p<0.1, * p<0.05, ** p<0.01, *** p<0.001

Figure 3

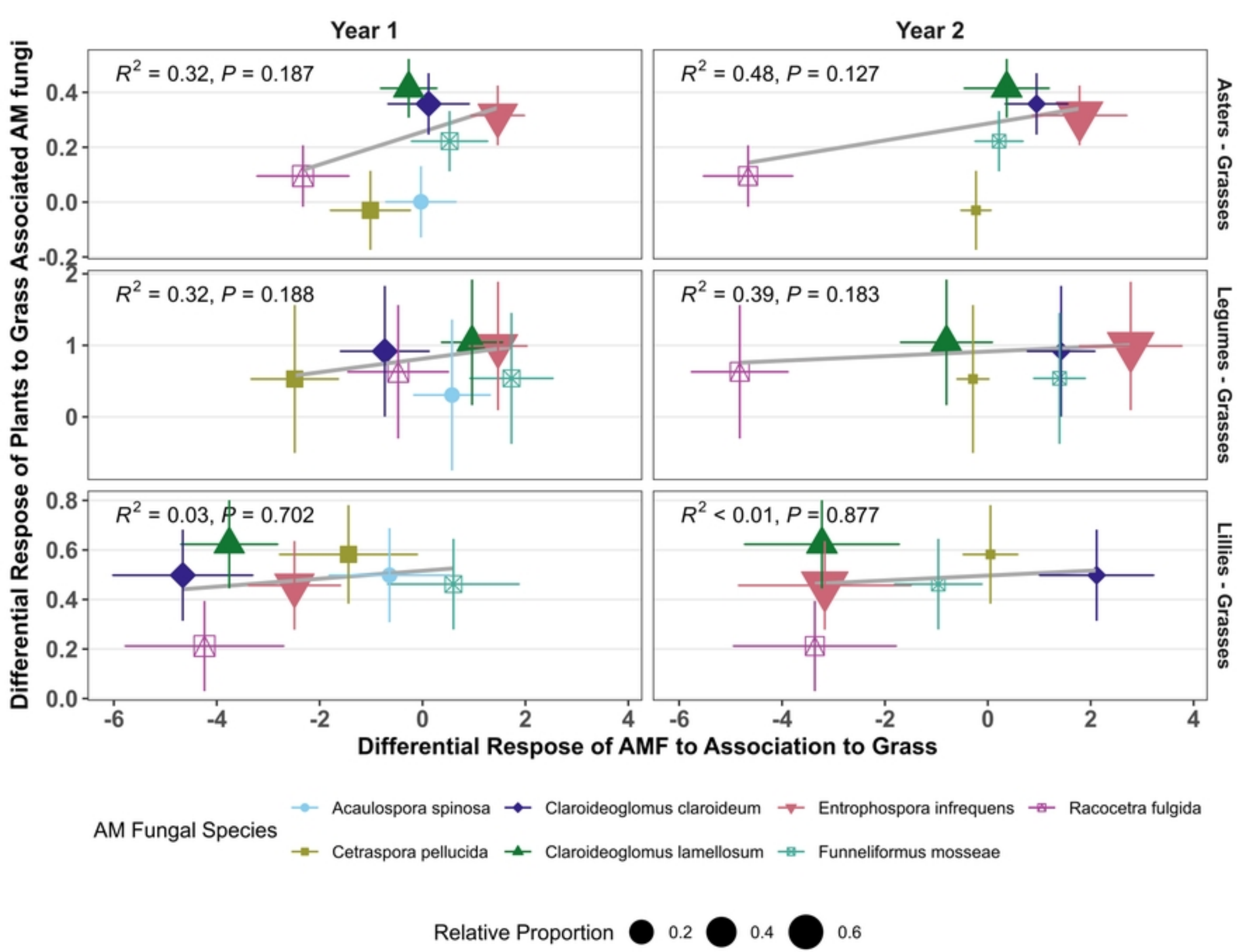
Year 1
Year 2
Asters - Grasses
Legumes - Grasses
Lillies - Grasses
R^2 = 0.32, P = 0.187
R^2 = 0.48, P = 0.127
R^2 = 0.32, P = 0.188
R^2 = 0.39, P = 0.183
R^2 = 0.03, P = 0.702
R^2 < 0.01, P = 0.877
Differential Response of Plants to Grass Associated AM fungi
Differential Respose of AMF to Association to Grass
AM Fungal Species
Acaulospora spinosa
Claroideoglomus claroideum
Entrophospora infrequens
Racocetra fulgida
Cetraspora pellucida
Claroideoglomus lamellosum
Funneliformus mosseae
Relative Proportion
0.2
0.4
0.6

Figure 4

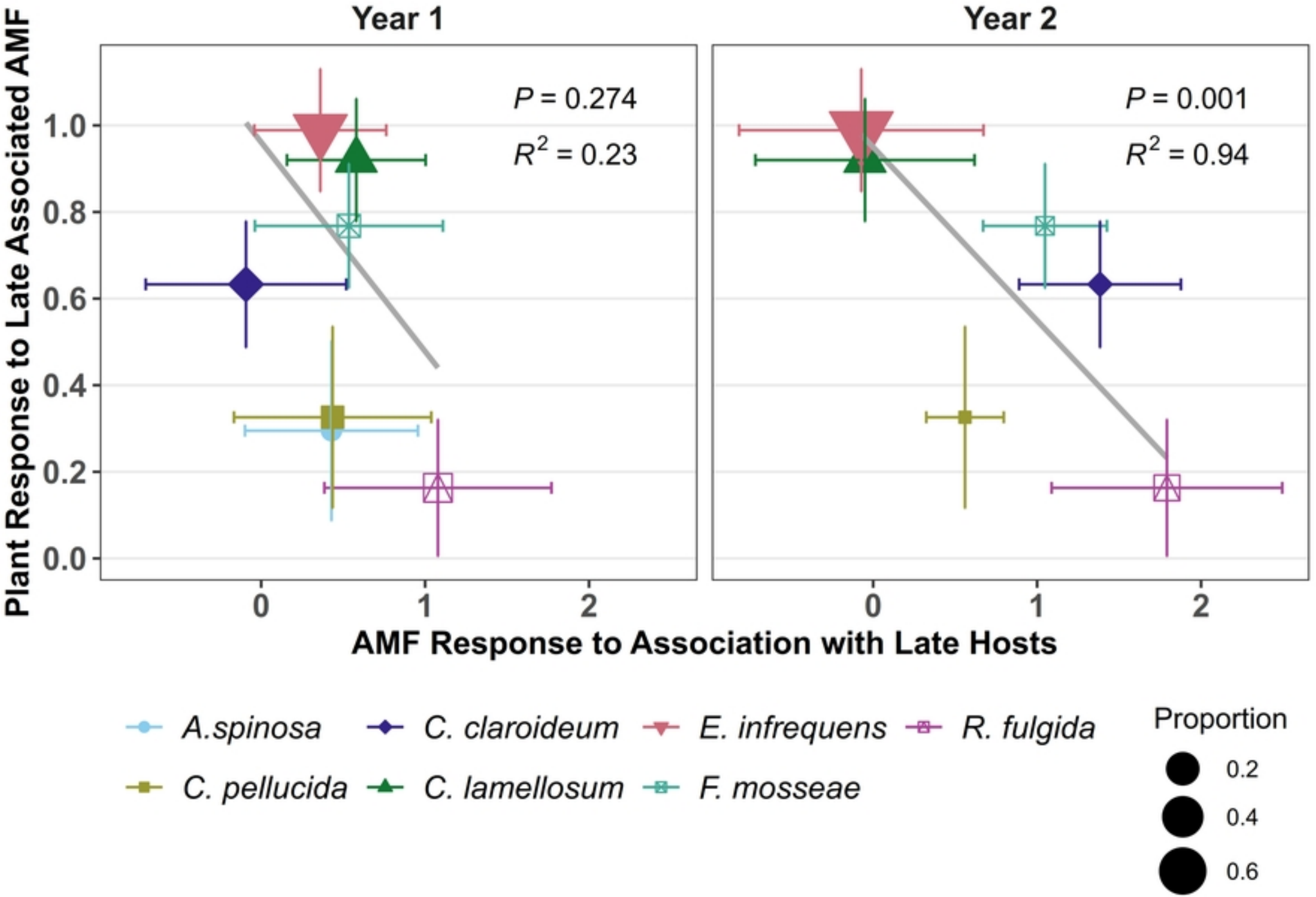
Year 1
Year 2
P = 0.274
R2 = 0.23
P = 0.001
R2 = 0.94
Plant Response to Late Associated AMF
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
AMF Response to Association with Late Hosts
A.spinosa
C. claroideum
E. infrequens
R. fulgida
C. pellucida
C. lamellosum
F. mosseae
Proportion
0.2
0.4
0.6

Figure 5

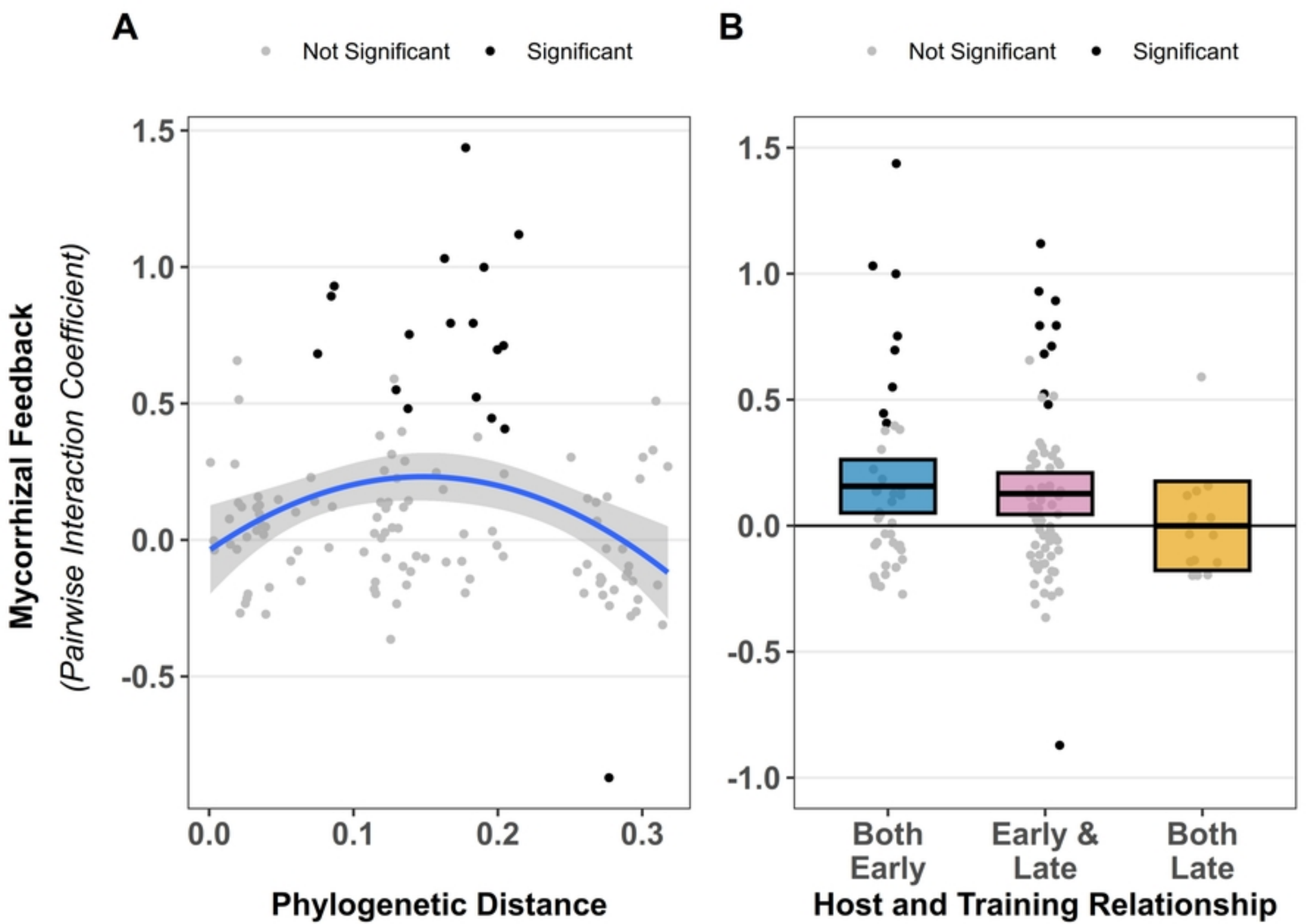
A
Not Significant
Significant
Mycorrhizal Feedback
(Pairwise Interaction Coefficient)
1.5
1.0
0.5
0.0
-0.5
0.0
0.1
0.2
0.3
Phylogenetic Distance
B
Not Significant
Significant
1.5
1.0
0.5
0.0
-0.5
-1.0
Both Early
Early & Late
Both Late
Host and Training Relationship

Figure 6

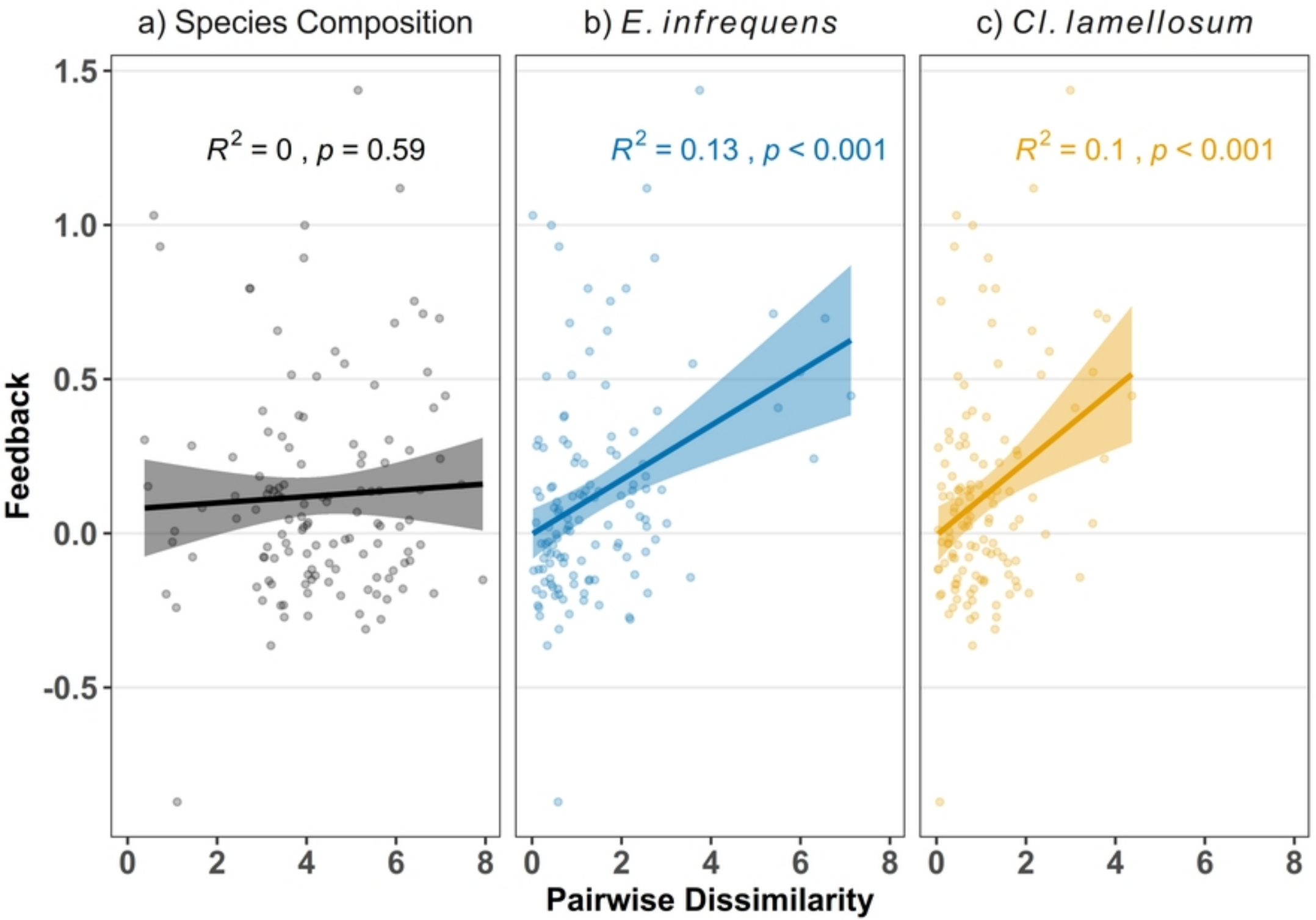
a) Species Composition
b) E. infrequens
c) Cl. lamellosum
$R^2 = 0$, $p = 0.59$
$R^2 = 0.13$, $p < 0.001$
$R^2 = 0.1$, $p < 0.001$
Feedback
1.5
1.0
0.5
0.0
-0.5
0
2
4
6
8
Pairwise Dissimilarity

Figure 7