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Investigating Plant Physiological Responses to Global Phylogenetic Diversity of Glomeromycotina

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INVESTIGATING PLANT PHYSIOLOGICAL RESPONSES TO GLOBAL PHYLOGENETIC DIVERSITY OF GLOMEROMYCOTINA

by

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Abstract

Arbuscular mycorrhizal (AM) fungi are ubiquitous symbionts of terrestrial plant species with associations predominantly characterized as mutualistic. In addition to well-documented enhancement of host growth response, more recent analyses have demonstrated the conferral of host benefits under numerous biotic and abiotic stressors. However, much of the established evidence originates from studies involving limited AM fungal diversity. Accordingly, this study sought to evaluate the potential effects of inoculation on plant host physiological traits within a growth chamber environment, investigate potential correlations between host trait responses, & assess the degree of phylogenetic signal observed in trait responses due to the presence of AM fungi. Overall, inoculation did not result in meaningfully different effects in host trait responses relative to controls. The effects of unique inoculum identity were also not meaningfully different from one another, although some instances of deviation from this trend were observed. Trait correlations were also largely absent after accounting for species relatedness. Further, model selection criteria tended to endorse an effect of unique inoculum identity but was not suggestive of effects due to evolutionary history. The presently described experimental implementation of AM phylogenetic diversity, comprising 36 taxa across 8 families, contributes to a greater contextual understanding of the AM symbiosis and offers an approach suitable for future studies.

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Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs engaged in the largely mutualistic association with root systems of an estimated 72-80% of terrestrial plant species (Brundrett & Tedersoo, 2018). Distinct from other mycorrhizal classifications, AMF have been conventionally characterized by the intraradical development of haustoria-like structures known as arbuscules (Bonfante, 2018). Although recently challenged as the definitive diagnostic feature of AMF (Field & Pressel, 2018), these structures and the enveloping peri-arbuscular membrane are well-established as the symbiotic interface between plant and AMF partners (Hause & Fester, 2005). Like other mycorrhizal associations, AMF extend the root systems of host plants beyond depletion zones where scavenging and hyphal uptake of nutrients facilitates improved nutrient acquisition of hosts (Helgason & Fritter, 2009). Given the order of magnitude difference between physical diameters of hyphae and root structures, AMF associations offer an alternative plant nutrient uptake strategy where the metabolic costs of fine root construction are far higher than the investment of carbon reserves into symbiotic partnership (Bergmann et al., 2020).

Phylogenetically, AMF comprise the subphylum Glomeromycotina, one of three subphyla within the larger phylum of Mucoromycota (Spatafora et al., 2016). Although ancestry of Glomeromycotina has been considered the evolutionary origin of most modern fungal-plant associations (Chang, et al. 2015), recent evidence suggests that such origination more likely occurred in the most recent common ancestor of Mucoromycotina and Glomeromycotina (Spatafora et al. 2017). Accordingly, each of these groups retain critical importance to contemporary explanations regarding the early terrestrialization of plants (Strullu-Derrien, et al. 2018), wherein AMF are believed to have a single evolutionary origin (Berbee et al. 2017). The

ubiquity and antiquity of AM symbiosis would appear to support such explanations; 92% of plant families are reported to demonstrate at least facultative AM symbiosis (Wang & Qiu, 2006), while the presence of AMF has been estimated to range from 400 million years ago in ancient root fossil records of the Devonian (Remy et al. 1994; Taylor et al. 1995) to 460 million years ago in spore fossils of the Ordovician (Redecker et al. 2000).

Although principally regarded as a nutritional mutualism, contemporary investigations have enforced the notion that AM symbioses confer multi-faceted benefits to plant hosts. Beyond the well-documented improvements of host nutrient and growth response (Smith & Read, 2008), AM symbioses have demonstrated increased host tolerance to biotic stressors through enhanced immune response (Cameron et al. 2013), heightened secondary-metabolite defensive capabilities (Korenblum & Aharoni, 2019; De Deyn et al., 2009) and antagonist protection (Vannette & Rasmann, 2012). Likewise, AM associations also improve host responses to abiotic stressors such as drought (Auge 2001), metal toxicity (Audet & Charest 2007) and salinity (Chandrasekaran et al. 2014). Broader still, AM symbioses have emergent importance in the enhancement of plant species diversity and composition (van der Heijden 1998), improved soil aggregation (Wilson et al. 2009), and the mediation of critical nutrient cycling and global carbon sequestration by nature of source-sink dynamics (Field & Pressel, 2018). Accordingly, these benefits have motivated a long-standing interest in leveraging the application of AM symbiosis towards efforts of ecological restoration of native soils (Koch, et al. 2006) and implementation of AMF as targeted bioinoculants within agricultural/horticultural systems (Herrera-Estrella & Lopez, 2016).

The AM symbiosis exhibits complex spatio-temporal dynamics, as well as considerable variability on account of symbiont identities, genotype-by-genotype, and genotype-by-

environment interactions. These complexities have hampered efforts to establish necessary predictive models of AMF functioning in agricultural settings (Rodriguez and Sanders, 2015). While concepts such as shared life history strategies (Chagnon et al. 2013), phylogenetic conservation of functional attributes (Koch et al. 2017), and molecular trait indicators (Gamper et al. 2010) have been examined as approaches to achieve greater systematic understanding, the implementation of AM science into agriculture remains stalled.

In addition to the wealth of studies exploring how AM symbioses impact plant host response to biotic and abiotic stressors, efforts have also been made to explore ways in which symbiotic performance may vary according to the taxonomic identity of respective symbionts (see Soudzilovskaia et al. 2020). Overall, investigations show that benefits in host plant growth response are generally observed (for review see Hoeksama et al. 2018) despite considerable context dependence (Hoeksama et al. 2010). However, the variable results observed across studies illustrate the difficulty of predicting symbiotic performance, particularly regarding host growth and nutrient response. For instance, there remains a critical lack of resolution as to the relative importance of inter- and intraspecies variation upon observed outcomes of mycorrhizal growth response (MGR), among other AM functional attributes (Ehinger et al., 2009; de Novais, et al. 2014). Significant variation in MGR among isolates of various species is well-documented in numerous studies, employing various plant host and AMF genotypes (for review see van Geel et al. 2016; see also Sale et al., 2021; Watts-Williams et al. 2019; Taylor & Harrier, 2000). Other investigations have shown that variation in observed host growth response among isolates within a single species may be comparable, if not greater than that observed between isolates of different species (Munkvold, et al., 2004; Mensah et al. 2015; Koch et al. 2017; Koch et al. 2006; Ehinger et al. 2009). Such results support that substantial variation in AM functional traits exists

within species (Ehinger et al. 2009) and that, consequently, the underpinnings of predictive frameworks regarding AM symbiosis might reasonably require specificity regarding the particular functional attribute in question, as suggested by Munkvold et al. (2004) with regard to linkage between phylogeny and AMF functioning.

The value of phylogenetic analyses for understanding variation in functional attributes among species provides a degree of predictive capability towards the outcome of species interactions (Cavender-Bares et al. 2009). Reinhart et al. (2012) recognized and advocated the need to develop systematic predictions of which plant species might benefit from AMF. In their efforts, the researchers found that host phylogeny, among other variables, could be utilized to partially predict mycorrhizal growth response (MGR) and arbuscular mycorrhizal colonization of roots (RC) of plant species. Here, we sought to approach the need for systematic understanding in an alternative manner, where differences in physiological responses of a single generalist host species would be observed and surveyed for the presence or absence of phylogenetic signal across a global representation of AMF diversity. Accordingly, variation in AM functional attributes could be examined and a more comprehensive assessment of symbiotic performance may be made through the inclusion of greater phylogenetic representation of AMF.

Potential AMF phylogenetic signal has been invoked and explored in previous evaluations of symbiotic performance. In their review, Hoeksema et al. (2018) report an absence of evidence that phylogenetic diversification in AMF explains patterns of plant responsiveness. Rather, recent diversification among plants provided a greater account of variation in outcomes of AM symbiosis (Hoeksema et al. 2018). However, investigation by Sale et al. (2021) found that plant benefit could indeed be linked to fungal identity and phylogeny, with marked differences in plant performance observed among 18 species with representatives from 5 AMF

orders, 13 genera, and 8 families. Accordingly, the researchers suggest that, apart from contextual influence, the commonly limited representation of AMF diversity found in prior investigations may explain such differences in conclusions (Sale et al. 2021). Notably, Koch et al. (2017) implemented a study with 56 isolates of AMF, comprising 17 genera and 6 families to investigate the link between AMF phylogeny and host physiological response and reported results consistent with those outlined by Hoeksema et al. (2018). Considering most variance in growth response occurring among isolates within AMF species, Koch et al. (2017) endorse greater focus upon population and species level quantitative and functional genomic approaches to determine evolutionary and ecological effects of AMF on plants (Gamper et al., 2010).

Although calls for increasing adoption of genomic approaches are warranted, further study incorporating greater breadth of AMF diversity is required to reach more accurate conclusions regarding the role of AMF phylogeny as a predictor of host physiological response. Here, we sought to satisfy the need for increased diversity and taxonomic representation of Glomeromycotina through the implementation of 36 AMF species comprising 16 genera across all orders of AMF phylogeny. Accordingly, a central aim of this study was to investigate phylogenetic relationships between AMF inoculation and subsequent host physiological responses with the inclusion of more comprehensive phylogenetic representation. Plant performance under symbiosis was assessed based on total plant biomass (equivalent to MGR in this context) biomass allocation according to shoot mass and root mass fractions, leaf photosynthetic pigment concentrations as a proxy for net assimilation rate (NAR), and specific leaf area (SLA). These traits individually provided proxies for each of the components of relative growth rate (RGR):

$$RGR = SLA \times LMF \times NA\mathrm{R}$$

Methods

We undertook a controlled-environment, growth chamber experiment to assess growth and nutrient responses of a single accession of Sudangrass (*Sorghum x drummondii*) to 36 taxa of AMF. Recorded data for functional traits and overall plant growth were then employed in subsequent analysis to examine potential phylogenetic relationships between AMF diversity and host physiological responses.

Growth Conditions, Randomization & Replication

The experiment was conducted within a climate-controlled growth chamber (Darwin Chambers, St. Louis, MO), using 13 shelving racks with suspended banks of white LED plant growth lights. Each light bank was set to approximately 163 watts (1.3 amps) with recorded PPFD 6" from light source measured at roughly 700 (+/- 100) µmol m^{-2} s^{-1}. Daytime growth chamber conditions were characterized by a 16-hour photoperiod (6:00 am - 10:00pm), sustained temperature of 28°C, and 70% relative humidity (RH). Nighttime conditions (10:00 pm-6:00 am) were characterized by 8-hour periods of 22°C and 50% RH.

Spatial randomization determined the distribution of trays containing each respective species of AMF inoculum. Trays were utilized to prevent potential cross contamination of fungal species within racks. Two trays of 4 replicate pots were assigned to each respective species of AMF, resulting in a total of 8 replicate plants per AMF species. Four trays of 4 replicate pots were devoted to controls. Each tray of 4 replicates per AMF species, as well as controls, were spatially interspersed through randomization among 2 sets of 6 shelves available in the growth chamber, where each rack contained up to 6 trays with each tray placed directly below a light-emitting diode. Three AMF species belonging to the genus *Rhizophagus* (*R. Clarus, R.*

intraradices, and R. sinosus) were spatially segregated from all other pots to avoid cross-contamination given the known 'weediness' of these taxa due to early and heavy sporulation. Trays containing these taxa were sub-randomized onto a separate shelf on the far end of the growth chamber, under otherwise identical light and temperature conditions (See S1 for spatial design). In total, 288 inoculated and 16 control pots were distributed throughout the growth chamber.

Soil Media Preparation, Seed Sowing & Seedling Thinning

Pots of roughly 400 mL holding capacity were filled with a 1:1 (v/v) sterilized mix of blended sand and commercial potting soil. Sterilization occurred in an electric soil sterilizer (Pro-Grow: Electric Soil Sterilizer, Model SST-15) at 93.33°C over two one-hour periods with an intermittent 6-8 hour cooldown and agitation of media (Habte & Osorio, 2001). Prior to pot filling, pots were sterilized in a 10% bleach solution for 30 minutes and thoroughly rinsed with DI water. Seeds of commercially obtained *Sorghum* × *drummondii* (Piper Sudangrass, Johnny's Seeds, Product ID 2858, Fairfield, ME) were surface-sterilized with 10% bleach solution for 10 minutes, subsequently rinsed with deionized (DI) water, and air-dried before placement into a similarly sterilized air-tight, glass container. Four seeds were then sown roughly 2 cm below the soil surface of each pot. The holes in which seeds were placed were dug with a sterilized scoopula. Six days after seeding, pots were thinned to the single, healthiest appearing seedling utilizing sterilized tweezers. Removed seedlings were subsequently discarded.

Inoculation, Watering, & Soil Amendments

Inoculation was undertaken 6 days after seeding, immediately following thinning of seedlings. 1mL of crude inoculum, obtained directly from the International Vesicular and Arbuscular Mycorrhizal Culture Collection at West Virginia University (see Supplement S1 for full culture list), was dispensed for each replicate. Crude inoculum was comprised of mycorrhizal root fragments, spores, and extraradical mycelium. Inoculum was deposited into 3-4cm holes made in each pot. These shallow holes were situated diagonal from the healthy seedling in each pot, dug with a sterile scoopula, and promptly covered with excess sterilized potting media.

DI water was manually dispensed three times per week into the bottom of pot-containing trays, allowing for a temporarily thin layer of standing water. Visible signs of nutrient deficiency prompted fertilization during the 6^{th} week of the growth period. Adherence to the minimal use of additional fertilizer was accomplished by applying approximately 0.5 g/1 level tsp. of standard release 15-9-12 resin-coated fertilizer per plant (Osmocote Plus, Scotts, Maryville, OH).

Data Collection

Pre-harvest phenotypic data was recorded for leaf hyperspectral reflectance. Reflectance measurements were taken on the most recently fully expanded leaf (MRFEL) using CI-710s SpectraVue units (CID Bioscience, Camas, WA), providing leaf reflectance, absorbance, and transmittance data across wavelengths from 200nm-1300nm. Given the length of Sudangrass leaves, 3 matched measurements were taken at the base of the leaf, the tip of the leaf, and the midpoint of the leaf. All measurements were centered to the midrib of the leaves. Total chlorophyll content was determined according to Parry et al. (2014) utilizing absorbances at 663nm and 645nm:

$$CPHLT = (8.2 \times A663) + (20.2 \times A645)$$

After 7 weeks of growth, plants were destructively harvested for data collection. For each plant, the MRFEL was removed and scanned with a standard flatbed scanner at 300 dpi. Images were processed using ImageJ (Schneider et al., 2012) to determine leaf area, and MRFELS were dried at 60°C for 3 days until constant mass in a forced air-drying oven to determine dry mass. Specific leaf area was determined according to dry leaf mass relative to scanned areas of MRFELs. Total plant shoot biomass was then harvested by cutting the stem of each plant at the soil surface, and belowground biomass was obtained by gently washing soil from roots. Both shoot and root biomass were separately dried at 60°C for 3 days and then weighed. From root and shoot biomass values, total plant biomass as well as shoot mass fraction and root mass fraction were calculated.

Data Analysis

Assessment of trait response variation was achieved through the construction of 84% confidence intervals. Implementation of this threshold was based upon the reasonable fulfillment of the assumption of homoscedasticity and closer approximation of an $\alpha = 0.05$ test (Payton, et al. 2003). Constructed intervals were calculated for each group through multiplication of standard errors by the corresponding z-score for 84 percent confidence. Results were visualized in R version 4.2.1. utilizing the *ggplot2* package version 3.4.1.

Due to shared evolutionary history between experimental taxa, linear mixed models were utilized to correct for non-independence of data and assess trait correlations. Models were implemented using the R packages *Rphylopars* version 4.2.2 (Goolsby et al. 2017), *ape* version

4.2.2, and *phytools* version 4.2.2. The recent pan-AMF phylogeny of Delavaux et al. (2022) was employed to conduct phylogenetic comparative methods. Tips of the tree were pruned to coincide with the 36 experimental taxa and inclusion of *A. thaliana* as outgroup. The following modifications were made for complete experimental coverage: *Acaulospora mellea* was added as a sister taxa to *Acaulospora morrowiae* (Trejo-Aguilar, et al. 2015), *Dentiscutata erythropus* was changed to its synonym *Dentiscutata erythropa*, *Glomus microaggregaturm* was substituted in place of FR750203 Glomus sp., *Rhizophagus sinuosus* was added as sister to *Rhizophagus intraradices* and *Rhizophagus clarus* as a polytomy, *Racocetra gregaria* was substituted in place of *Racocetra verrucosa*, *Claroideoglomus luteum* was added as sister to *Claroideoglomus claroideum* (Crossay, 2018). The tree was converted to an ultrametric tree using *treePL* (Smith, SA., & O'meara, BC., 2012, Sanderson, MJ., 2002).

Trait evolution was simulated under either Pagel's λ, star, or Brownian motion evolutionary models. For a given pairwise correlation of trait values, models were ranked according to Akaike information criterion (AIC) with the lowest returned value indicative of the best supported model. Additionally, models enabled the partitioning of variance at the level(s) of within and/or among species variation. Correlations between traits were therefore assessed with model characteristics further describing the presence or absence of phylogenetic signal and relative contributions of intraspecies and interspecies variation to overall variance in trait responses.

Results

Assessing Variation in Host Trait Responses

Under the present conditions, no meaningful differences were observed in host total biomass due to the presence or absence of AMF. Figure 1 demonstrates this visually as no single inoculum deviates meaningfully from the control response. Furthermore, the confidence intervals of most unique inoculum overlap with one another (Figure 1, Panel A). *Archaeospora schenkii* demonstrates a lower response relative to: *Am. gerdemannii, E. infrequens, G. microaggregatum, R. sinuosus, D. eburnea, De. heterogama, Gi. Margarita,* and *Gi. gigantea*. The biomass responses of *Am. leptoticha* and *F. mosseae* are similarly lower than those of *G. microaggregatum, R. sinuosus, De. heterogama,* and *Gi. margarita.* Finally, *R. intraradices* exhibits lower biomass response relative to *G. microaggregatum* and *De. heterogama*. Overall, however, the predominant trend indicates that estimated group means are largely indistinguishable from one another.

The results described for observed host responses in biomass based on unique inoculum identity are also evident for responses in SLA, LMF, and total chlorophyll content. SLA and total chlorophyll content exhibit complete overlap of estimated means across groups (Fig 1, Panels B & D). LMF, in contrast, exhibits exceptions as hosts inoculated with *C. pellucida* register lower LMF under inoculation relative to: *G. microaggregatum, R. intraradices, Sg. constrictum, D. eburnea, A. colombiana, Gi. margarita,* and *Gi. gigantea* (Figure 1, Panel C). There is an absence of meaningful difference for response values between non-inoculated control and inoculated groups in any of the measured physiological variables.

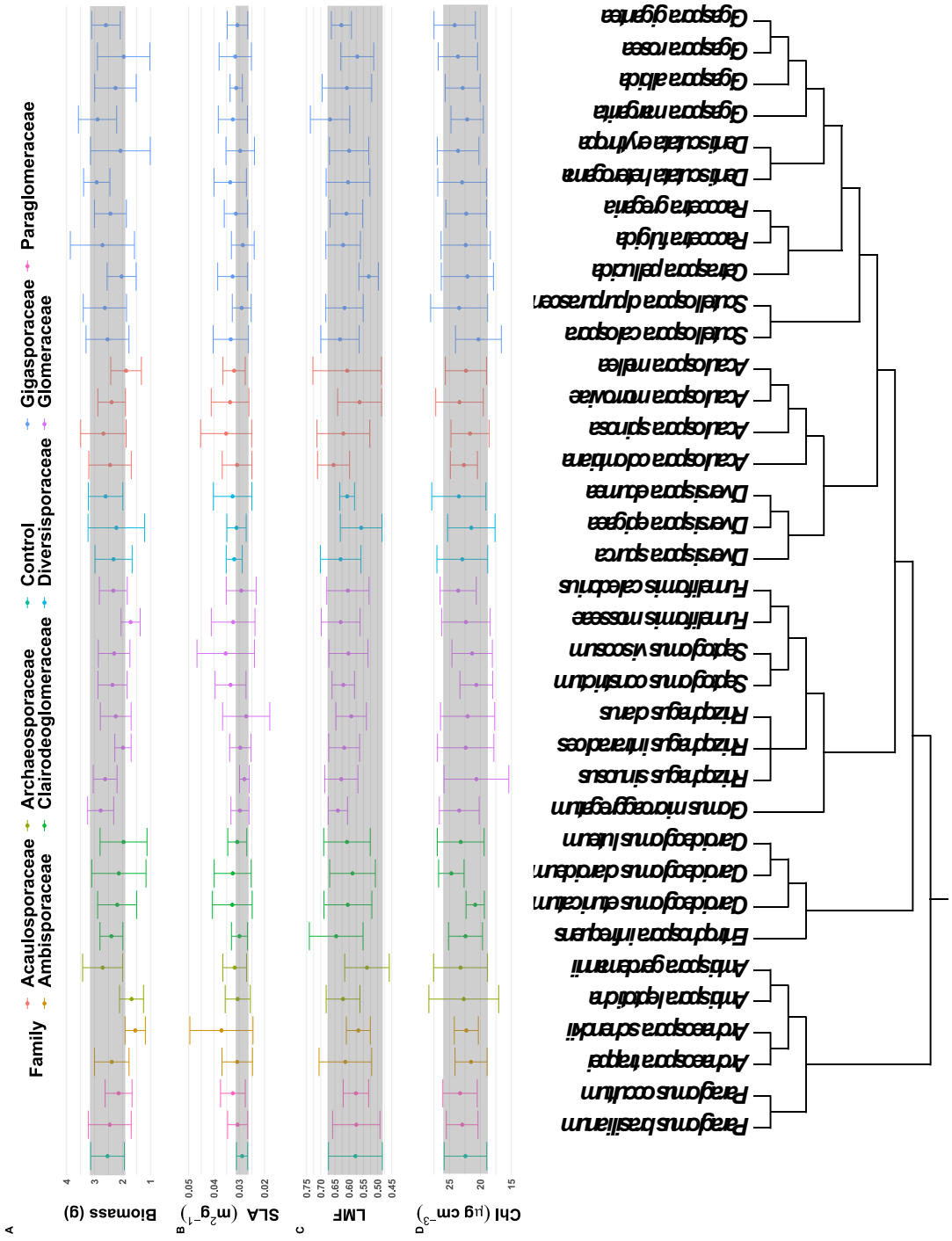

Figure 1. Estimated Trait Values by Group

Ordering of species along the horizontal axis coincides with the ordering of species along the lower-located phylogeny. Vertical axes designate trait values for biomass (Panel A), SLA (Panel B), LMF (Panel C), and Chlorophyll Content (Panel D). Species' trait values are colored according to respective familial classification and designated by the upper-located legend. Circles denote species means while error bars signify 84% CI. Gray bars are included for convenient comparison relative to control values.

Evaluation of Adjusted Trait Correlations and Degree of Phylogenetic Signal

After accounting for autocorrelation of data due to shared evolutionary history, pairwise correlations were evaluated among total, aboveground, and belowground host biomass responses and variables of LMF, SLA, and total chlorophyll content. Overall biomass was not meaningfully correlated with any underlying predictor variable according to the best supported model. No more than approximately 3% of variation in total biomass was explained by corresponding variation in either LMF ($R^2 = 0.03$), SLA ($R^2 = 0.04$), or total chlorophyll content ($R^2 = 0.02$).

Adjusted correlations in aboveground biomass responses were similarly negligible in relation to SLA ($R^2 = 0.0363$) and total chlorophyll content ($R^2 = 0.0238$). However, phylogenetically corrected correlation of host aboveground biomass and LMF demonstrated a meaningful relationship (r = 0.4979, $R^2 = 0.2479$). Partitioning of variance further illustrates that among species correlation contributed to ~30.5% of total correlation, whereas within species correlation contributed to ~69.5%. Corresponding model characteristics were indicative of a detectable treatment effect of species identity but an absence of phylogenetic signal.

Adjusted correlations in belowground biomass responses were additionally negligible when coupled with SLA ($R^2 = 0.0187$) and total chlorophyll content ($R^2 = 0.0124$). In contrast, phylogenetically corrected correlation of host belowground biomass and LMF demonstrated a moderate, negative relationship ($r = -0.3459,\ R^2 = 0.1196$). Corresponding model characteristic were suggestive of a detectable effect of inoculum identity but an absence of signature for evolutionary relatedness. The proportion of explained variance was descriptive for within but not among species relationships.

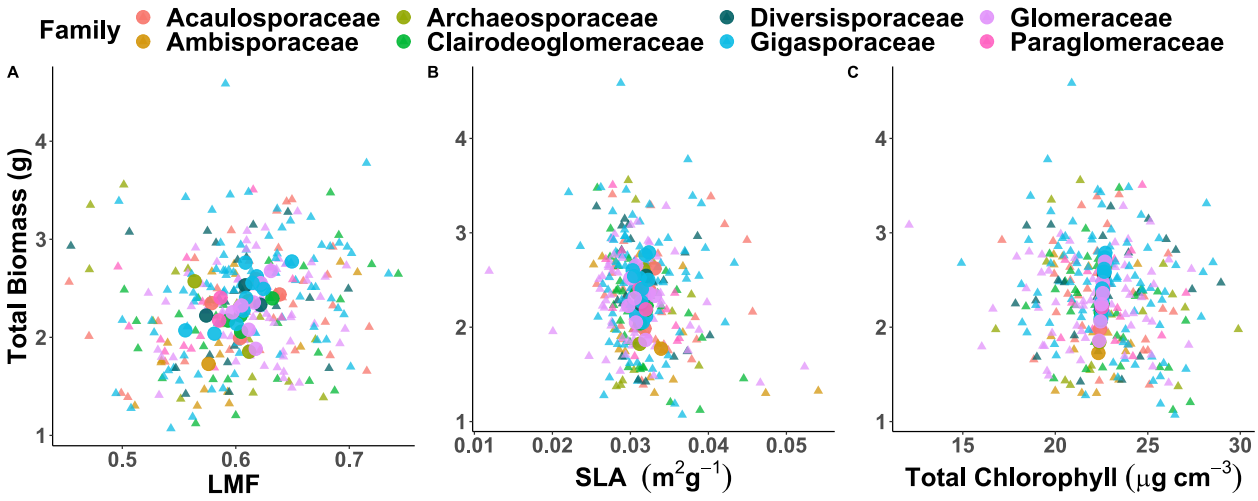

Figure 2. Matrix of Scatterplots for Variables of Interest Against Total Biomass

Triangular points represent individual replicate values for leaf mass fraction (Panel A), specific leaf area (Panel B), or chlorophyll content (Panel C) scaled along horizontal axes whereas biomass scales along the vertical axis. Larger circles are pooled means for replicates of each species. Points are colorized according to respective families and listed within the upper-located legend. Panels were produced and assembled into a common figure utilizing the packages "ggplot2" version 3.4.1 and "multi_plot" version 1.0.4 in R version 4.2.1.

Discussion

Biomass Responses and Relative Differences Among Groups

Overall, estimation of group means suggests that there was an absence of meaningful difference between control and inoculated groups for biomass responses. Such a result fails to exemplify the consensus of AM partnership resulting in enhancement of host growth (Bennet & Groten, 2022). However, the symbiosis between plant and AM partners has been shown to be highly contextual with numerous influential conditions, including pot & greenhouse experimentation (Qin, et al., 2022). Under Biological Market Theory (Noë & Hammerstein, 1994), the current observations in host growth response may be considered such that early investment of C by hosts did not see reciprocal payoff by AM fungal partners.

Interestingly, variation in response among groups failed to clearly reflect classification of AM functional groups such as those described under the CSR framework of life history strategies (Chagnon, et al. 2013, Grime, 1974). Accordingly, competitive fungi able to rapidly increase host P might be reasonably expected to result in higher host growth response. Functionally, members of Gigasporaceae are believed to exemplify the "competitive" strategy due to conservation of functional traits (Powell, et al. 2009, Weber, et al. 2019). Among the limited differences in host biomass observed, *De. heterogama, Gi. margarita,* and *Gi. gigantea* did exceed MGR values for the four lowest registering groups (see Figure 1). However, higher MGR was not unique to members of the Gigasporaceae, as various species in other families also exhibited relatively high MGR.

Nutrient availability and stoichiometry are important drivers of plant growth, including under AM colonization (Johnson, 2010). Although conservative, fertilization may have resulted in conditions amenable to plant growth, such that hosts would benefit from foregoing or

minimizing investment of photosynthetic products into AM partnership. Such outcomes for high nutrient availability have been documented (Wipf, et al. 2019). Consequently, the establishment of a more nutrient stressed environment may have resulted in conditions conducive to greater AM partnership and resulted in larger differences among AM species treatments.

The present lack of variation between unique species identities is notably similar to the results presented by Marro et al. (2022). This global meta-analysis examined the benefits of individual AM species and taxonomic groups on plant growth and nutrition. 25 AM species were represented in biomass evaluation across 4 taxonomic groups. For both host plant biomass and additional host response metrics, confidence intervals for estimated effect sizes due to unique species inoculation were found to be largely overlapping. However, effect sizes were indicative of positive response relative to non-inoculated controls. This indicates that the presence of AM partners improved host growth, but that AM species were largely equivalent partners.

Recent meta-analysis by Qin et al. (2022) offers even further insight into the results of our study. In their analysis, the authors found that experimental duration, the ratio of plant root to shoot biomass, and pot size were the three most important influences that determine the effects AM fungi exert on plant shoot, root, and overall biomass. Taken into consideration, these results would suggest that a growth period limited to approximately 7 weeks may have been insufficient to register more commonly observed outcomes in host responses to AM fungi. Furthermore, the implemented pot size of less than 1 kilogram of soil media may have adversely limited resultant outcomes in biomass.

Correlations of Physiological Variables to Biomass Response

Relationships among changes in trait responses largely demonstrate a lack of meaningful correlation after accounting for shared evolutionary history. Notably, none of the underlying variables dictating changes in relative growth rates (RGR) (Evans, 1972, Lambers, et al. 2008) were detected to meaningfully explain variation in overall biomass. Such lack of variation may be explained by the presence of masking conditions such as high nutrient availability or insufficient study length. Further, the possibility of measurement error for one or more variables could have potentially confounded estimations of trait values. Finally, assessment of NAR as a component of RGR was conducted through a proxy in total chlorophyll (Chl a & b) content according to El-Hendawy, et al. (2022). Although relevant to NAR, total chlorophyll content may have failed to sufficiently describe the desired component of RGR.

While the use of total chlorophyll content as a proxy for NAR has limitations, evaluation of chlorophyll content is common within studies of AM partnership. Inoculation with AM fungi has been demonstrated to increase chlorophyll content in models such as banana (Sahodaran, et al. 2019) and pepper (Sensoy, et al. 2007). However, these studies implemented more limited AM representation and corresponding authors reported the enhancement of chlorophyll in hosts inoculated with *Gi. margarita*, *G. intraradices,* or *F. mosseae*. Such effects were not observed in our results.

In our study, plants typically contained 50-70% shoot biomass, a scenario with a low root-to-shoot ration (R/S). Optimal partitioning theory (Johnson & Thornley, 1987) suggests that this pattern of allocation in biomass investment would be reflective of conditions in which aboveground resources (e.g., light) are more limiting than those found belowground (e.g., water, nutrients). Qin et al. (2022) found that higher R/S values coincided with greater increases of both

shoot and total biomass under AM inoculation. Therefore, limitation of AM function may have occurred due to constraints of low R/S in our study, which may result from pot size, plant age, nutrient supply, or other factors.

Evolutionary Relatedness as a Predictor of Host Response

Differences in growth outcomes among AM partner species were not supported by shared evolutionary history. Qualitatively, this is suggested by the lack of meaningful differences between estimated host response values due to unique inoculum identity (Figure 1). However, more robust support is illustrated under reported model selection characteristics.

Where LMF explained a meaningful proportion of variance in host aboveground biomass responses, model criteria indicated the absence of phylogenetic signal alongside a detectable effect of species identity. Therefore, trait evolution was best simulated by a large polytomy (the star model). The star model was also best supported for the other significant relationship detected, between changes in host belowground biomass responses and LMF.

Numerous studies have examined mycorrhizal growth response with comparisons among AM taxa. However, much of AM taxonomic representation has been biased towards members within Glomeraceae, specifically *R. intraradices* & *F. mosseae*. Recent meta-analysis by Marro et al. (2022) tested whether AMF taxonomic groups benefited hosts differentially under both stressed and non-stressed conditions. Considering biomass and nutrient responses, the authors found that Diversisporales were most beneficial to non-stressed hosts, whereas Gigasporales conferred the greatest host benefits under stress. Our results do not suggest such differences by taxonomy, though may differ due to study context and the presence of reasonably non-stressed conditions.

In a similar implementation of PGLS regression, Koch et al. (2017) reported that observed responses in plant biomass due to inoculation by AM fungi could not be predicted by AM species identity. This common garden experiment, implementing 56 AM isolates and 3 unique plant host species, sought to determine whether variation in AM fungal morphology and growth correlated with AM fungal effects on plant growth. Our results align with this finding and reinforce the inability of interspecies relationships to account for adequate levels of variation in host biomass. In fact, because this study used multiple unique isolates within AM fungal species, the authors were able to determine that host responses were strongly affected by intraspecies isolate identity and not AM fungal species. This suggests that genetic and functional trait variation within AM taxa may strongly influence host plant responses, indicating high evolutionary lability of AM functional traits at the microevolutionary level (among isolates) in parallel to that detected in our study at the macroevolutionary level (among species).

In conjunction with Koch et al. (2017), our results support the findings of the broader meta-analysis conducted by Hoeksema et al. (2018) and subsequent conclusion that phylogenetic diversification of AM fungi does not reliably explain observations of inoculated host responses. This likely results from high evolutionary lability of AM functional traits that results in low phylogenetic signal for host plant responses. As noted by Hoeksema et al. (2018), studies that report low phylogenetic signal for host plant responses often suffer from comparatively limited AM fungal diversity. Accordingly, with small methodological adjustments to duration and pot size, the approach used in this thesis is suitable to assess AM species effects on host trait responses across a representative cross-section of the global diversity of Glomeromycotinian fungi.

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