Received: 25 March 2024 | Accepted: 1 May 2024

DOI: 10.1002/ajb2.16369

RESEARCH ARTICLE

American Journal of Botany

Measuring leaf and root functional traits uncovers multidimensionality of plant responses to arbuscular mycorrhizal fungi

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Abstract

Premise: While many studies have measured the aboveground responses of plants to mycorrhizal fungi at a single time point, little is known about how plants respond belowground or across time to mycorrhizal symbiosis. By measuring belowground responses and growth over time in many plant species, we create a more complete picture of how mycorrhizal fungi benefit their hosts.

Methods: We grew 26 prairie plant species with and without mycorrhizal fungi and measured 14 functional traits to assess above- and belowground tissue quality and quantity responses and changes in resource allocation. We used function-valued trait (FVT) modeling to characterize changes in species growth rate when colonized.

Results: While aboveground biomass responses were positive, the response of traits belowground were much more variable. Changes in aboveground biomass accounted for 60.8% of the variation in mycorrhizal responses, supporting the use of aboveground biomass response as the primary response trait. Responses belowground were not associated with aboveground responses and accounted for 18.3% of the variation. Growth responses over time were highly variable across species. Interestingly, none of the measured responses were phylogenetically conserved.

Conclusions: Mycorrhizal fungi increase plant growth in most scenarios, but the effects of these fungi belowground and across time are more complicated. This study highlights how differences in plant allocation priorities might affect how they utilize the benefits from mycorrhizal fungi. Identifying and characterizing these differences is a key step to understanding the effects of mycorrhizal mutualisms on whole plant physiology.

KEYWORDS

arbuscular mycorrhizal (AM) fungi, function-valued traits, functional trade-offs, fungal collaboration gradient, mycorrhizal response, plant functional traits, root economics spectrum, trait variation

Mycorrhizal fungi play a critical role in the successful growth of many plant species. Plants receive growth-limiting soil nutrients from the mycorrhizal fungi by providing sugars to the fungi, which increases growth and provides other secondary benefits to the plant (Delavaux et al., 2017). Within this context, mycorrhizal growth response (MGR), defined as the log-response ratio of plant aboveground biomass to mycorrhizal inoculation, is the most useful metric to quantify the benefits that plants receive through their associations with mycorrhizal fungi (Hetrick et al., 1992).

Stahlhut, K. N., D. G. Neupert, J. E. Laing, L. J. Witt, and J. T. Bauer. Measuring leaf and root functional traits uncovers multidimensionality of plant responses to arbuscular mycorrhizal fungi. *American Journal of Botany* 111: XXXX.

This metric captures aboveground growth promotion, one of the most notable benefits mycorrhizal fungi provide to plants and is simple and cost-efficient to measure. As a plant functional trait, researchers have used MGR to assess whether plant benefits from mycorrhizal fungi are phylogenetically and environmentally conserved (Reinhart et al., 2017; Hoeksema et al., 2018; Maherali, 2019) and as a focal trait for selection in plant breeding (Ellouze et al., 2016; Lehnert et al., 2018; Thirkell et al., 2022). Within an ecological context, MGR seems to be positively correlated with late successional plant species and slow life history strategies, indicating that this trait may have significant predictive power on plant community successional dynamics for some ecosystems (Koziol and Bever, 2015; Bauer et al., 2018; Cheeke et al., 2019).

Despite the many benefits of its use, MGR may not describe functionally meaningful variation in plant species for mycorrhizal response (Maherali, 2019). This metric varies across the plant and fungal phylogenies, as well as within species, making it difficult to generalize which plant clades and species are the most responsive (Hoeksema et al., 2018; Stahlhut et al., 2023). Other benefits to plants such as water uptake, pathogen resistance, and soil aggregation are also not reflected in MGR, and these secondary benefits of symbiosis may be more important to plant species in some environments (Delavaux et al., 2017). The complexity of appropriately measuring plant response to mycorrhizal fungi is likely why many synthesis studies have found inconsistent or no evidence linking MGR to geographic distribution, phylogeny, or plant traits (Maherali, 2014; Reinhart et al., 2017; Hoeksema et al., 2018; Maherali, 2019). By measuring more diverse trait responses to mycorrhizal colonization beyond growth response, we could better understand how plants utilize mycorrhizal symbiosis to alleviate environmental stress and maximize growth and fitness.

Plant, leaf, and root functional traits are often used to represent physiological constraints as plants grow and balance trade-offs in above- and belowground resource acquisition. The leaf economic spectrum is one of the most-studied coordinated trade-off spectra, running from inexpensive, short-lived leaves to expensive, long-lived leaves (Wright et al., 2004). A lesser-studied root economic spectrum has been defined, but unlike the single trade-off axis of the leaf economic spectrum, it is multidimensional (Bergmann et al., 2020). One axis of this multidimensional root economic spectrum is well aligned with the leaf economic spectrum: slow-growing plants generally have roots with high root tissue density and low nitrogen content, similar to expectations of the leaf economic spectrum. The other major axis is defined independently by specific root length, a belowground trait analogous to specific leaf area, which is one of the major functional traits defining the leaf economic spectrum (Kramer-Walter et al., 2016). The leaf and root economic spectra identify functional traits that are associated with important plant physiological constraints and provide a framework to assess how mycorrhizal symbiosis may affect correlated plant functional traits.

Given the links between plant functional traits and nutrient acquisition strategies, mycorrhizal response should be associated with plant functional traits, but the connection between these traits has not been clearly identified. The first root trait axis has been identified as potentially important for mycorrhizal symbiosis, such that plants with high specific root length adopt a "do-it-yourself" strategy, while plants with low specific root length outsource nutrient uptake to mycorrhizal mutualists (Bergmann et al., 2020). Many researchers have hypothesized that plants that respond strongly to mycorrhizal fungi will have larger roots with lower total root length because mycorrhizal fungi directly increase the area of nutrient uptake in a plant (Smith and Read, 2008). Therefore, a common plant response to mycorrhizal fungi should be to create root systems that have roots with larger diameters and lower specific root lengths, while simultaneously reducing the relative allocation to roots. In this case, species with high morphological trait plasticity would receive the most aboveground benefits from mycorrhizal fungi (Kumar et al., 2019). We believe many plant species that exhibit a positive aboveground response to mycorrhizal fungi will have shifts from fibrous root systems to coarse root systems because the plants decrease allocation to foraging roots in exchange for increased allocation to mycorrhizal partners. However, we do not believe that this shift is a necessary precursor to aboveground mycorrhizal response, and the opposite root response can occur in some species due to the alleviation of nutrient stress, which would disproportionately affect plant species that are dependent on mycorrhizal fungi (Janos, 2007; Lopez et al., 2023). Therefore, instead of a single axis that defines mycorrhizal response of plant traits, we expect that there will be at least two that separate out the aboveground and belowground responses plants have to mycorrhizal fungi.

Mycorrhizal response metrics are often limited because measurements to calculate MGR require destructive harvest, preventing the longitudinal measurement of plant response throughout growth. Given the potential for functional variation in plant response and species-specific differences in allocation priorities during growth, mycorrhizal symbioses might have different effects on plant growth curves across the plant kingdom. For example, the increase in nutrients provided to the plant by mycorrhizal fungi could lead to either more growth at the end of the growing period or cause the plant to mature quicker than competitors. Either outcome could increase plant fitness, but estimates of MGR may discount temporal benefits of mycorrhizal symbiosis. By parameterizing growth metrics using function-valued trait (FVT) modeling, we can capture the potential effects of mycorrhizal symbiosis on total growth rate and total plant size (Baker et al., 2018; Baker and Wang, 2021). The response of some of these parameters to mycorrhizal symbiosis is likely species-dependent, but for species that have low MGR, changes to growth rate might be

an important response that is not captured in traditional metrics.

Multidimensionality of plant benefits to mycorrhizal fungi may obscure trends between MGR and other plant traits. In this paper, we used a diverse selection of herbaceous tallgrass prairie plant species to assess how mycorrhizal fungi affect aboveground and belowground plant functional traits. By comparing the plant functional traits of plant individuals grown with and without mycorrhizal fungi, we were able to characterize above- and belowground functional trait responses to mycorrhizal fungi. In doing so, we built an understanding of how mycorrhizal fungi affect plant growth beyond aboveground biomass accumulation and determine trade-offs in plant responses to mycorrhizal fungi. We also measured plant growth during the growing period to detect species differences in when and how mycorrhizal aboveground growth benefits are being received by plants.

MATERIALS AND METHODS

Source material and planting

To understand the effects of mycorrhizal fungi on plant traits across the plant kingdom, we inoculated 26 prairie plant species (Table 1) with a mix of AM fungi containing *Claroideoglomus claroideum* (= *Glomus claroideum*), *Funneliformus mosseae* (= *Glomus mosseae*), *Cetraspora pellucida*, *Claroideoglomus lamellosum*, *Acaulospora spinosa*, *Racocetra fulgida* and *Entrophospora infrequens* (Myco-Bloom LLC, Lawrence, Kansas, USA). The plant species we chose for this experiment represent most of the functional and phylogenetic diversity present in the Midwest prairie ecosystem. The mycorrhizal inoculum was initially cultured from Midwest prairie soils and contains species that provide diverse benefits to hosts (Koziol and Bever, 2016). Before inoculation, we extracted spores from the inoculum to confirm spore counts and viability.

For each focal species, we scarified and cold-stratified seeds for a month, then moved these seeds to the greenhouse for an additional month so that plants could germinate and grow robust enough to withstand transplantation into test pots. We filled each test pot with 1:1 sterilized soil–sand medium and maintained soil moisture at consistent levels with drip irrigation. Right before transplanting seedlings, we added mycorrhizal inoculum to each inoculated treatment pot. We chose the healthiest seedlings from each species and randomly planted two seedlings in each test pot to account for potential mortality due to transplantation and after 2 weeks of growth, the smallest of the two seedlings was removed. We used two sets of plants to measure root and leaf traits. To collect root trait data, we grew plants for 4 weeks and harvested the plants before the root systems were too large to accurately measure root traits. Plants that were used for measuring leaf traits were grown for 8 weeks so that we could track differentiation in growth over time for each species in the control and inoculated soils. Data from both sets of plants were used to calculate trait responses to mycorrhizal fungi and for a principal component analysis. For each treatment group of each species, we had four replicate pots, for a total of 16 plants per species.

TABLE 1 The 26 plant species used in the analysis of plant trait response to mycorrhizal fungi. Plant species with an asterisk were included in the function-valued trait (FVT) analysis of mycorrhizal effects on plant growth rate, which was conducted by fitting logistic curves to growth data. Species without the asterisk were dropped because they either had no growth after transplant or continued to grow exponentially throughout the study, which prevented growth curves from being fitted.

Species ID	Plant species	Family
Achmil*	*Achillea millefolium*	Asteraceae
Agrgry	*Agrimonia gryposepala*	Rosaceae
Amocan	*Amorpha canadensis*	Fabaceae
Andger*	*Andropogon gerardii*	Poaceae
Ascsyr	*Asclepias syriaca*	Apocynaceae
Asctub	*Asclepias tuberosa*	Apocynaceae
Concan*	*Erigeron canadensis*	Poaceae
Dalpur*	*Dalea purpurea*	Fabaceae
Echpal	*Echinacea pallida*	Asteraceae
Eutmac	*Eutrochium maculatum*	Asteraceae
Helhel	*Heliopsis helianthoides*	Asteraceae
Lescap	*Lespedeza capitata*	Fabaceae
Monfis*	*Monarda fistulosa*	Lamiaceae
Oenbie*	*Oenothera biennis*	Onagraceae
Panvir*	*Panicum virgatum*	Poaceae
Pendig*	*Penstemon digitalis*	Plantaginaceae
Plalan*	*Plantago lanceolata*	Plantaginaceae
Ratpin	*Ratibida pinnata*	Asteraceae
Rudhir	*Rudbeckia hirta*	Asteraceae
Schsco	*Schizachyrium scoparium*	Poaceae
Silint	*Silphium integrifolium*	Asteraceae
Solcan*	*Solidago canadensis*	Asteraceae
Sornut	*Sorghastrum nutans*	Poaceae
Symnov*	*Symphyotrichum novae-angliae*	Asteraceae
Sympil*	*Symphyotrichum pilosum*	Asteraceae
Verfas*	*Vernonia fasciculata*	Asteraceae

Measuring plant functional traits

We measured 14 focal functional traits in this experiment to represent five types of plant tissue response to mycorrhizal

fungi: (1) change in quantity of aboveground tissues, (2) change in quality of aboveground tissues, (3) change in quantity of belowground tissues, (4) change in quality of belowground tissues, and (5) change in proportion of biomass allocation to plant tissues. We measured dried aboveground biomass (AGB), height or longest leaf in species without stems (H), and leaf area (LA) to assess aboveground tissue quantity responses to mycorrhizal fungi. To assess aboveground tissue quality responses to mycorrhizal fungi, we measured specific leaf area (SLA; leaf area/leaf dry mass), leaf dry matter content (LDMC; dry leaf mass/fresh leaf mass), and leaf lifespan (LL; calculated as the proportion of living leaves at final time point to total number of leaves throughout growing period). We used SLA and LDMC as predictors of species' placement on the leaf economic spectrum (Wright et al., 2004). We used both SLA and LDMC because, while SLA was initially described as a key element of this spectrum, the trait varies across different nutrient and light environments. Leaf dry matter content depends less on environmental conditions, so it has also been proposed as a better predictor variable for this spectrum (Hodgson et al., 2011). By measuring LL, we were able to capture the degree of photosynthetic organ turnover, which is also associated with the leaf economic spectrum (Edwards et al., 2014). To assess belowground tissue quantity response, we measured belowground dried biomass (BGB), root length (RL), root system volume (RV), and average root diameter (RD). These four metrics capture the biomass allocation belowground and the size of individual roots. We measured specific root length (SRL; total root length/dry root mass), root tissue density (RTD; dry root mass/root volume), and root dry matter content (RDMC; dry root mass/fresh root mass) to assess species belowground tissue quality responses. The root traits RTD and SRL represent the trade-offs on two axes of the proposed root economic spectrum (Bergmann et al., 2020). We used RDMC as a directly analogous trait to aboveground tissue quality trait LDMC. Finally, we used RSR to assess the relative investment to roots and shoots in each plant (Qi et al., 2019). Throughout the growing period, we also measured plant height measurements (or longest leaf for plants without clearly defined stems) to track plant aboveground growth over time.

To measure changes in root traits, we grew inoculated and non-inoculated seedlings with a 1:1 sand–soil mix in 164 mL pots (cone-tainers, Stuewe and Sons, Tangent, OR, USA) for 4 weeks. The roots were placed on a 31 × 21 cm water-filled tray and light scanned using an Expression 10000XL Pro scanner (Seiko Epson, Nagano, Japan). Root length and average root diameter was measured using WinRhizo software 2019a (Regent Instruments, Québec City, Quebec, Canada). We measured root volume by measuring water displacement in a graduated cylinder, which is more accurate for roots with heterogeneous diameter classes than measurements with WinRhizo (Rose, 2017). We oven dried and weighed the root and shoot tissues of these plants to calculate root to shoot ratio (RSR). We used the dried root biomass, total root length, and volume of the roots to calculate specific root length (SRL) and root tissue density (RTD). To assess aboveground traits responses to mycorrhizal fungi, inoculated and non-inoculated seedlings were grown for 8 weeks in 656-mL pots (Deepots, Stuewe and Sons,). We weighed and scanned all leaves during harvest and used ImageJ (v. 1.53) to calculate the total leaf surface area for each plant. The aboveground biomass of the plants was oven-dried and weighed. We collected a subsample of roots from inoculated and non-inoculated plants and stained them to test the efficacy of the mycorrhizal inoculation treatments.

Data analyses

We extracted phylogenetic relationships from a larger, published phylogeny of seed plants (Smith and Brown, 2018). This phylogeny was constructed using both genetic data from GenBank and phylogenetic data from the Open Tree of Life to create an inclusive, dated phylogeny of seed plants. Some species in our data set were not represented in this phylogeny, so we chose a closely related species to represent these species instead. We used the function multi2di in the R package ape (v. 5.7.1) (Paradis and Scliep, 2019) in R version 4.2.0 (R Core Team, 2023) to convert all polytomies in this tree to dichotomies.

To understand how plant functional traits are affected by mycorrhizal fungi, we assessed the correlations between the traits for inoculated and non-inoculated plants separately. Mycorrhizal and non-mycorrhizal traits were log- or square-root-transformed when necessary to reduce skew. To test whether our chosen functional traits exhibited phylogenetic signal across plant species, we used the function phylosig in the R package phytools (v. 1.5.1) (Revell, 2012) to assess Pagel's λ and Blomberg's K. Because most of the traits exhibited no phylogenetic signal (Appendix S1), we chose to not use phylogenetic comparative methods in subsequent analyses.

To test the effect of mycorrhizal fungi on these plant traits, we created a separate set of variables that were calculated as the natural logarithm of a species trait mean when grown with mycorrhizal fungi divided by the mean when grown in sterile conditions. With these metrics, positive values indicate that the trait mean increases when the species is grown with mycorrhizal fungi compared to sterile conditions. One goal of this study was to determine whether there were coordinated responses to mycorrhizal fungi, so we ran a standard PCA with the 14 response traits. The PCA was based on a variance–covariance matrix because the traits for this analysis were already transformed to equivalent scales (log-response ratios), meaning that plant functional traits that have larger degrees of change between mycorrhizal and non-mycorrhizal treatments are more strongly weighted in the PCA. Due to high plant mortality, primarily in the control treatments, there were unequal sample sizes across the 14 traits measured (Appendix S2). To account for uneven sample sizes due to plant mortality, we

multiplied each value in the response trait dataframe by the square root of the smallest sample size used to calculate each trait. This method preserves the direction of response but allows for data points with more confidence to be weighted stronger in the analyses. Ultimately, the weighted PCA and unweighted PCA yielded similar results, so we present the results of the weighted PCA.

We used either plant height or longest leaf in plants without stems to create function-valued trait (FVT) models. In short, this method used time series data on plant growth to plot an average logistic growth curve for each species, and we calculated the response ratio of the average curve parameters to describe changes in growth over time. We dropped all individuals that did not fit logistic growth models from further analyses. We were able to fit logistic growth curves to 23 species when plants were grown with mycorrhizal fungi, compared to 13 species grown in sterile conditions. For comparison of growth parameters, we could only include species that had logistic growth in both the inoculated and control groups. The species that were not included in this analysis were generally dropped because the growth of the control plants did not fit a logistic model due to lack of growth. We used the remaining species to calculate how response to mycorrhizal fungi changed over time by calculating maximum growth rate, time until growth curve inflection, and asymptote. Changes in growth rate indicate that mycorrhizal fungi change the rate at which the plant is able to create aboveground tissue. Decreases in inflection point when plants are colonized by mycorrhizal fungi indicate that plants reach their maximum growth rate earlier than when they were colonized by mycorrhizal fungi, whereas increases in inflection point indicate that plants are able to grow exponentially for a longer time. Changes in asymptote are directly analogous to changes in final size.

RESULTS

Colonization by mycorrhizal fungi generally increased aboveground plant growth responses while having very little effect on aboveground tissue quality (Figure 1; Appendix S3). Relative allocation to roots generally decreased when plants were colonized by mycorrhizal fungi, as measured using RSR, and the amount of aboveground tissue, measured using biomass (AGB), height (H), and leaf area (LA), increased when colonized. The natural log response for these three traits varied widely across the species included in this analysis, ranging from −0.15 to 3.73 for AGB, −0.15 to 1.40 for H, and 0.07 to 4.03 for LA ($N = 26$). Leaf quality response traits varied less than leaf quantity response traits, but the direction of response for these traits was also relatively conserved across species. Leaf dry matter content (LDMC) decreased and specific leaf area (SLA) and leaf lifespan (LL) increased for the majority of plant species, a shift that is consistent with more slow acquisition strategies on the leaf economic spectrum (Wright et al., 2004).

Root trait responses to mycorrhizal fungi varied in both magnitude and direction. While belowground biomass

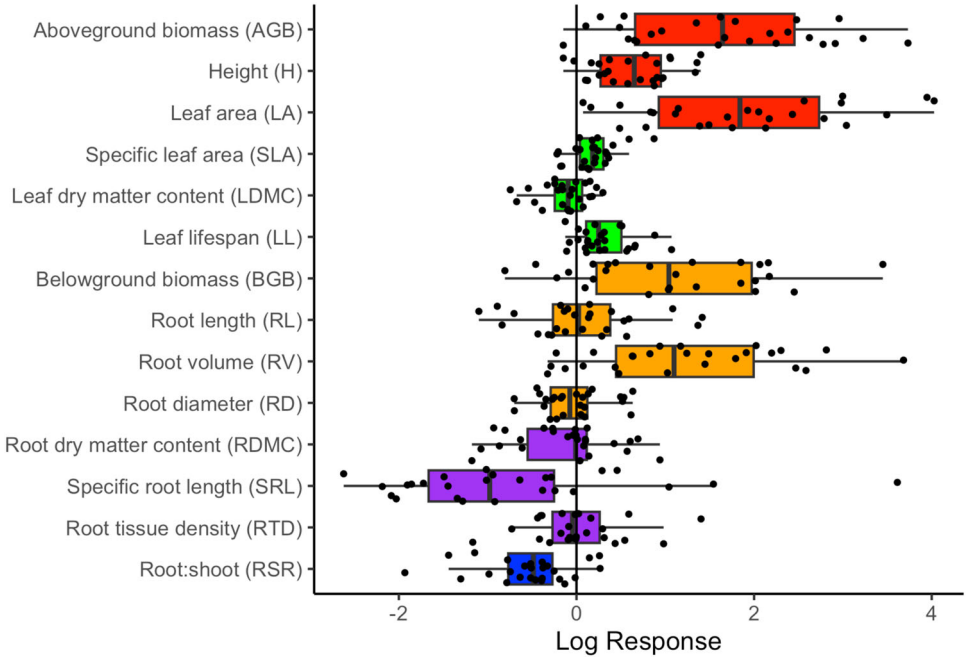

FIGURE 1 Responses of 14 plant traits to colonization of mycorrhizal fungi. Trait responses are calculated as the log of the average trait value of mycorrhizal individuals over control individuals. Each point represents a species response for the trait ($N = 26$). Red boxes represent traits that measure aboveground tissue quantity, green boxes represent traits that measure aboveground tissue quality, orange boxes represent traits that measure belowground tissue quantity, purple boxes represent traits that measure belowground tissue quality, and blue boxes represent traits that measure allocation. Boxes represent first and third quantiles, and whiskers extend to 1.5× interquartile range.

(BGB) and root volume (RV) increased for most species, the direction of change in root diameter (RD) and root length (RL) when plants were colonized by mycorrhizal fungi varied by plant species. Specific root length (SRL) and root dry matter content (RDMC) decreased, and root tissue density (RTD) increased for the majority of the species analyzed in this study. Species with high SRL in control plants were more likely to have negative SRL responses (i.e., root systems shifted from fine without mycorrhizal fungi to coarse with mycorrhizal fungi), while plants with low SRL when grown in control conditions were more likely to have positive SRL responses ($r = -0.46$, $df = 24$, $P = 0.018$) (Appendix S4). None of the response traits were phylogenetically conserved, as measured using Pagel's λ and Blomberg's K (Appendix S1).

The response of measured functional traits to mycorrhizal fungi could be adequately represented by two trait dimensions (Figure 2, Table 2). These two trait dimensions represented 60.9% and 18.3% of the variation in response traits, with 79.2% cumulative variation represented. The species that loaded positively on the first principal component (PC1) generally had large increases in aboveground and belowground biomass associated traits, such as ABG, BGB, LA, and RV. The second principal component (PC2) is primarily described by changes in SRL, and to a lesser extent RSR, RDMC, and RTD. Plant species that loaded positively on this axis did not shift biomass allocation away from roots when colonized by mycorrhizal fungi, but instead invested in larger root systems. This axis was not associated with changes in aboveground functional traits, but plant species that had positive or neutral SRL responses (i.e., creating more finely branched root systems in response to mycorrhizal colonization) did not achieve positive aboveground biomass responses as high as those species that had negative SRL responses ($r = -0.40$, $df = 24$, $P = 0.041$).

Thirteen species had sufficient growth to calculate changes in plant growth variables due to mycorrhizal inoculation. The other 13 plant species that were not included in this analysis because some individuals in the control groups had almost no growth during the experiment or the logistic models poorly fit growth rate. In both of these cases, logistic growth curves with meaningful growth parameters could not be fit for both control and inoculated individuals, so growth curve responses could not be calculated. Most of the individuals from these species produced true leaves early in development before being transplanted into experimental pots. Uninoculated individuals from these species generally did not produce more aboveground plant tissue after being moved into a more nutrient-poor growing medium, while inoculated individuals continued to grow. While we were unable to include them in further FVT analyses, the limited growth of individuals in the sterile conditions suggests that half of the species we used in this study depend on mycorrhizal fungi for growth.

TABLE 2 Results of the standard PCA for plant trait responses to mycorrhizal fungi. The eigenvalues, proportion of variation explained, and eigenvalues for each trait are included for the first two PCs. Eigenvectors with absolute values greater than 0.20 are bolded for each PC.

Traits	PC1	PC2
Aboveground biomass (AGB)	**0.47**	−0.15
Height (H)	0.12	−0.04
Leaf area (LA)	**0.48**	−0.20
Specific leaf area (SLA)	−0.03	−0.02
Leaf dry matter content (LDMC)	−0.03	0.02
Leaf lifespan (LL)	0.11	−0.04
Belowground biomass (BGB)	**0.44**	0.11
Root length (RL)	0.14	**−0.22**
Root volume (RV)	**0.44**	−0.15
Root diameter (RD)	0.06	−0.11
Root dry matter content (RDMC)	−0.11	0.19
Specific root length (SRL)	**−0.32**	**−0.84**
Root tissue density (RTD)	−0.01	**0.21**
Root shoot ratio (RSR)	−0.06	**0.23**
Eigenvalues	3.88	**2.12**
Proportion of variance	60.9	**18.3**

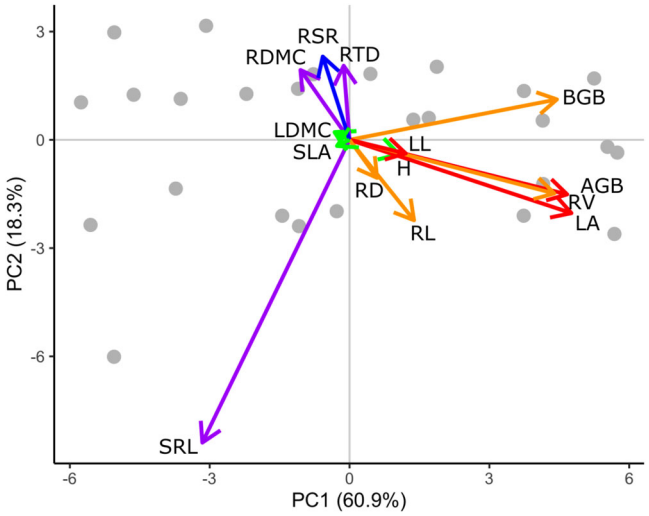

FIGURE 2 Two dimensional plots of the standard PCA ordination results of plant response to mycorrhizal fungi. Points represent the values for each of the 26 species included in the analysis. Red arrows represent traits that measure aboveground tissue quantity, green arrows represent traits that measure aboveground tissue quality, orange arrows represent traits that measure belowground tissue quantity, purple arrows represent traits that measure belowground tissue quality, and blue arrows represent traits that measure allocation. Arrows represent loadings for each trait response: AGB, aboveground biomass; H, height; LA, leaf area; SLA, specific leaf area; LDMC; leaf dry matter content; LL, leaf lifespan; BGB, belowground biomass; RL, root length; RV, root volume; RD, root diameter; RDMC, root dry matter content; RSR, root shoot ratio; RTD, root tissue density; SRL, specific root length. See Table 2 for associated eigenvalues and eigenvectors.

For the species with uninoculated individuals that did grow after being transplanted, mycorrhizal fungi had an effect on plant growth over time, but the effects varied across species (Figure 3). For all species, the asymptote of the growth curve increased with mycorrhizal colonization, with an average response of 0.58 (SD = 0.43, min = 0.04, max = 1.28). For most species, the time to reach the inflection point increased when colonized by mycorrhizal fungi (mean = 0.34, SD = 0.49, min = −0.41, max = 1.15), while only three of 13 species (*Achillea millefolium*, *Oenothera biennis*, and *Solidago canadensis*) had decreases when colonized by mycorrhizal fungi. The effect of mycorrhizal colonization on growth rate was mixed, with an average response rate of 0.09 (SD = 0.39, min = −0.52, max = 0.76).

DISCUSSION

Mycorrhizal fungi had a neutral or positive impact on aboveground biomass production in all plant species included in this analysis. The impact of mycorrhizal fungi on leaf quality traits (SLA and LDMC) was minimal, but generally reflected allocation to leaves that were less resource intensive. Belowground biomass generally increased when plants were colonized by mycorrhizal fungi, while the responses of other traits associated with root system size were more variable. We predicted that total root length and volume would decrease and that root diameter would increase when plants were colonized by mycorrhizal fungi, because these shifts in root traits maximize the benefits they receive from mycorrhizal fungi (Bergmann et al., 2020). However, the opposite might also occur in species that are extremely nutrient limited without mycorrhizal fungi (Lopez et al., 2023). Given the diversity of species used in this study, both shifts in root traits were likely occurring across species. However, traits associated with root quality (root tissue density [RTD], specific root length [SRL], and root dry matter content [RDMC]) generally shifted so that root tissues were longer lived and matched a slower growth strategy. This shift suggests that plants deprioritize allocation to fast growing, foraging roots when colonized by mycorrhizal fungi, instead allowing mycorrhizal fungi to do more of the work of fine, absorptive roots. Finally, when plants were colonized by mycorrhizal fungi, the relative investment of plants to belowground tissues generally decreased. The effects of mycorrhizal fungi on aboveground and allocational plant traits are similar to the effects of different soil nutritional environments, where root mass fraction of plants grown in nutrient-poor soils were higher than those grown in nutrient-rich soils (Freschet et al., 2015). Ultimately, many of the hypothesized shifts in traits across plant species when colonized by mycorrhizal fungi were supported.

Despite the differences in belowground responses to mycorrhizal fungi, not all changes in root system traits were associated with tissue quantity responses aboveground. The first axis we identified was primarily explained by increases in plant tissue quantities above- and belowground. The first principal component captured a shift in plant root strategy; species that load positively on this axis had large increases in plant biomass and a shift to more coarse root systems when colonized with mycorrhizal fungi. The second axis was described by differences in root trait responses across plant species, where positive values represent shifts to coarser roots and negative values represent shifts to finer roots.

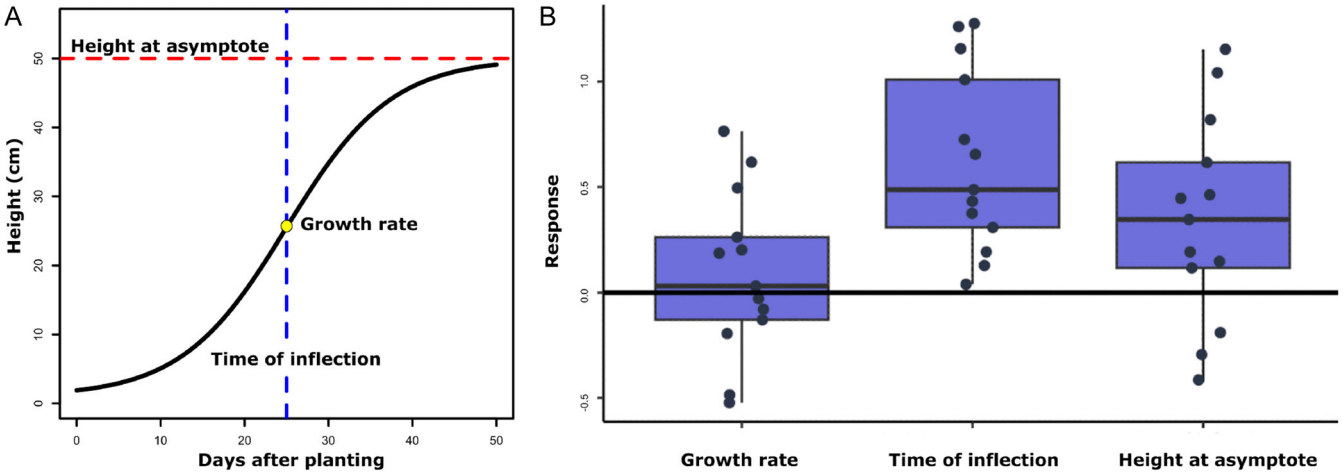

FIGURE 3 (A) Example plot of the parameters estimated from individual plant growth curves. We estimated the time of inflection (blue dashed line), height at asymptote (red dashed line), and maximum growth rate (yellow point) using a logistic growth model. Responses of function-valued trait (FVT) responses of 13 plant species to mycorrhizal fungi. We fit logistic growth curves to time-series measurements of height (or longest leaf in plants without stems), and the parameters of these curves were used as traits to describe plant growth. Trait responses were calculated as the log of the average trait value of mycorrhizal individuals over control individuals. Each point represents a species response for the trait ($N = 13$). Growth rate is the slope of the line at the inflection point of the curve, or the maximum growth rate. Height is the actual or predicted height of plants at the asymptote. Time of inflection is the time at which the inflection point occurred (or when plants stopped exponential growth). Boxes represent first and third quantiles and whiskers extend to 1.5 × interquartile range.

Species in the top right quandrant were characterized by increased biomass and coarser root systems when colonized by mycorrhizal fungi; species in the top left quadrant had similar root responses with lower biomass responses. Species in the bottom right quadrant had low biomass responses, but unlike species in the upper quadrants, plants colonized by mycorrhizal fungi had finer root systems; the positive SRL response in these species was likely due to the alleviation of nutrient stress when plants are colonized by mycorrhizal fungi, which highlights the species' inability to acquire an adequate amount of soil nutrients for their growth without mycorrhizal fungi.

Root plasticity is an important facet of optimizing response to mycorrhizal fungi. In our data set, plants species were unable to achieve a high aboveground response while also maintaining a similar SRL to non-colonized individuals, and plants that responded had a high aboveground response to mycorrhizal fungi and appeared to do so at the cost of roots with high SRL. Thus, species that responded the most aboveground to mycorrhizal fungi were those that had greater root trait plasticity to accommodate mycorrhizal fungi, which was a phenomenon originally described in mycorrhizally responsive warm-season grasses and non-responsive cold-season grasses (Hetrick et al., 1991). It is possible that this second PC axis is informative for the distinction between mycorrhiza-responsive and mycorrhiza-dependent plants (Janos, 2007; Maherali, 2014). Interestingly, the results of this study suggest that, while the root traits of the plant species that have the largest aboveground biomass response are highly plastic, other plant species exhibit less root trait plasticity but still benefit a fair amount from mycorrhizal fungi. In previous studies, these plants have been labeled as less responsive to mycorrhizal fungi because the log biomass response ratio was lower than for other species, but these species might rely more on mycorrhizal symbiosis for survival than other species with greater biomass response do (Janos, 2007). These differences in responses could also reflect life history trade-offs of these species. Many of the species that are difficult to reestablish in prairie restorations (e.g., *Amorpha canescens*, *Dalea purpurea*) were moderately responsive aboveground but had a belowground response that loaded negatively on the second PC response axis, while many of the more generalist species included in this analysis loaded more positively.

Function-valued trait modeling identified diverse effects of mycorrhizal symbiosis on plant growth. The three traits measured in our models were growth rate, time to inflection, and height at asymptote, and responses of all three traits were highly variable across the species included in this analysis. Unsurprisingly, height at asymptote generally increased when plants were colonized by mycorrhizal fungi, but the effects of AM fungi colonization on growth rate were more variable. Mycorrhizal fungi increased the growth rate of some species, likely due to an alleviation of nutritional limitations that allowed the plant to increase growth rate, which quickly increased their growth relative to control plants. Other plant species seemed to be initially hampered by their associations with mycorrhizal fungi, likely due to the initial investment into mycorrhizal fungi, but the growth of mycorrhizal plants outpaced that of sterile plants by the end of the study. The differential effects of growth across species could either be explained by differences in investment strategies across plant species or by differences in the mycorrhizal species recruited by the plant. Either case suggests that plant associations with mycorrhizal fungi can give rise to additional functional diversity within plant communities (Jespersen et al., 2019). The differences across the growth rate and inflection point parameters across the species that we used in this portion of the analysis points to different roles that mycorrhizal fungi might play for plants—some species might rely on mycorrhizal fungi early in development to gain nutrients necessary before full root development, while others might utilize mycorrhizal fungi later in their growth to acquire more nutrients than neighbors. Interestingly, the species that could not be included in this analysis generally had more negative loadings on the second PC axis than those that were. This difference was primarily due to the fact that when these species were colonized by mycorrhizal fungi, the plant was able to create more fine roots with lower root tissue density, likely because the mycorrhizal fungi alleviated nutrient limitations that affect root development. While our FVT models could not capture the effect of mycorrhizal fungi on these plants, the data supports that mycorrhizal fungi are a critical component of successful growth early in development for half of the plants in our study.

We could not measure changes in the root systems over time for this particular study, but we believe that comparing the growth curves of aboveground and belowground growth could be an important next step for understanding how plants chose to allocate resources early in their growth with and without mycorrhizal fungi. Mycorrhizal growth response measures the responsiveness of plant species to mycorrhizal fungi by using aboveground biomass growth as a proxy for benefits a plant receives from mycorrhizal fungi. Interestingly, this metric of plant response to mycorrhizal fungi, and the others used in this study, were not phylogenetically conserved across the plant species studied. This finding provides more evidence to the claim that root architecture and root strategies do not limit the variation of growth responses to mycorrhizal fungi (Hoeksema et al., 2010; Maherali, 2014; Valverde-Barrantes et al., 2017). While mycorrhizal growth response is not limited by particular root allocation strategies, there may be upper bounds of mycorrhizal growth response, at least in the short term, that may only be achieved by specific allocation priorities of a plant, as indicated by the identified trade-off between aboveground biomass and SRL response. It is also possible that the allocation priorities identified here that increase mycorrhizal growth response in the short term may not provide the most benefits to the plant in the long term. It is also possible that particular shifts in functional traits are associated with different fungal communities that provide different benefits to the plants, further complicating measures of MGR (López-García et al., 2017; Davison et al., 2020).

In conclusion, we find that changes in aboveground growth captures much of the variation in plant response to mycorrhizal colonization. However, other plant traits, particularly those belowground, respond independently from aboveground growth response, which provides some insights into the complexity of plant responses to AM fungi. Changes in root shoot ratio, root tissue density, and specific root length vary by plant species, suggesting species-specific changes in root quality and quantity when colonized by AM fungi. Additionally, changes in plant growth over time uncover diverse growth benefits across plant species, including not only increased nutrient uptake and growth, but also increased growth rate. Ultimately, our results support the use of growth response as an important metric to measure plant interactions with AM fungi, but the exclusive use of this metric obscures potentially important and diverse functional responses to the mutualism. Continued study of the functional responses included in this study and others, paint a more complete picture of the interactions between plants and their mycorrhizal partners.

AUTHOR CONTRIBUTIONS

K.N.S., J.E.L., L.J.W., and J.T.B. conceived and planned the initial project. K.N.S., J.E.L., and L.J.W. carried out experiments, and J.E.L. and L.J.W. measured all traits. K.N.S., with the assistance of J.E.L. and L.J.W., analyzed data on functional trait responses. D.G.N. conducted FVT analyses on growth data. K.N.S. led manuscript preparation, and all authors contributed to writing.

ACKNOWLEDGMENTS

The authors thank E. Goolsby for his advice and assistance on phylogenetic analyses, R. Baker and A. Ascanio Moreno for assistance on FVT modeling, H. Stevens and M. O'Connell for advice and assistance on data analyses, E. Galloway for assessing root colonization, and the reviewers for valuable feedback that increased the impact of this paper. This work was supported by the National Science Foundation (GRFP-2037786, DBI-2150197, DEB-1927696) and Miami University Undergraduate Summer Scholars program.

DATA AVAILABILITY STATEMENT

The data and code used for the analyses can be found on the Dryad digital repository: https://doi.org/10.5061/dryad.2z34tmpv1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Pagel's λ, Blomberg's K, and associated P-values for traits measured in plants grown with mycorrhizal fungi, plants grown without mycorrhizal fungi, and plant trait response to mycorrhizal fungi.

Appendix S2. Sample sizes for functional trait (see Table 2) averages for each treatment (mycorrhizal (M), control (C)) and species (see Table 1).

Appendix S3. The PCA loadings, functional trait responses, average trait values under mycorrhizal conditions, and average trait values under control conditions for each plant species.

Appendix S4. Correlation between a species average specific root length of in non-mycorrhizal conditions and overall specific root length (SRL) response.

How to cite this article: Stahlhut, K. N., D. G. Neupert, J. E. Laing, L. J. Witt, and J. T. Bauer. 2024. Measuring leaf and root functional traits uncovers multidimensionality of plant responses to arbuscular mycorrhizal fungi. *American Journal of Botany* 111(7): e16369. https://doi.org/10.1002/ajb2.16369