New Phytologist

Forum

Letter

Herbivory-driven shifts in arbuscular mycorrhizal fungal community assembly: increased fungal competition and plant phosphorus benefits

Introduction

In terrestrial ecosystems, arbuscular mycorrhizal (AM) fungi engage in symbiosis with >70% of terrestrial plants (Brundrett & Tedersoo, 2018). These fungi occupy the soil where their hyphae grow and forage for resources such as phosphorus (P). Their ability to access such resources is fundamental to their obligate symbiotic relationship with plants, as supply of soil nutrients is exchanged for carbon within the host roots (Smith & Read, 2008). Thus, AM fungi occupy a dual habitat, inhabiting the soil but also plant root systems in which they often form complex and dynamic communities (Öpik *et al.*, 2006). While the symbiosis is often characterised by the transfer of nutrients and carbon, the ecological roles of AM fungi extend beyond the exchanging of resources. They can significantly support plant resilience against various stresses, such as drought, and are important for soil structure, nutrient cycling, and carbon cycling (Powell & Rillig, 2018). Although research into the AM symbiosis advances, key knowledge gaps remain regarding the factors that shape the diversity and community assembly of these fungi within plant roots, especially in the context of other plant biotic interactions.

The majority of plants which AM fungi associate with are subject to attack from insect herbivores (Price *et al.*, 2011). For an estimated 350 million years, this relationship has exerted substantial influence on the evolution and diversification of plants (Agrawal *et al.*, 2012). Herbivory is expected to have significant impacts on the AM symbiosis and AM fungi because of the sizable effects on plant carbon budgets along with potential shifts in the needs and allocation of resources of the host plant (Orians *et al.*, 2011). The carbon-limitation hypothesis posits that removal of photosynthetic tissue by insect herbivores would have a negative impact on the AM symbiosis due to diminished carbon availability for the fungi (Gehring & Whitham, 1994). Indeed, this was demonstrated in recent works where aboveground insect herbivory reduced plant carbon allocation to AM fungi within the roots (Charters *et al.*, 2020; Durant *et al.*, 2023). In this way, aboveground herbivory can alter the host plant quality for the fungi as their carbon resources are diminished.

It is well-established in ecology that resource availability is a critical determinant of community assembly (Weiher & Keddy, 2001; Tilman, 2004). Decreased availability of a potentially limiting resource is expected to intensify competition between individuals (Johnson, 2010), resulting in shifts in community composition. For AM fungi, their access to carbon from the host has been shown to be related to the nutrient benefit they provide, with potential for adjustments in trade depending on demand (Noë & Kiers, 2018). However, it is important to note these interactions are more nuanced and complex when broader ecological interactions and context are taken into account (Bennett & Groten, 2022). Yet, if the carbon availability for the fungi is decreased, then less competitive taxa are expected to be outcompeted by taxa that can grow and survive with less resources, or those who are more cost-effective for the host when it comes to P delivery (i.e. provide a level of benefit per unit of carbon). Assuming that traits are phylogenetically conserved in AM fungi (Cahill *et al.*, 2008; Powell *et al.*, 2009), competitive exclusion among closely related fungal taxa might be expected to lead to a community in which species are less closely related to each other (i.e. phylogenetic overdispersion; Violle *et al.*, 2011). Should carbon allocation to AM fungi be reduced under herbivory, AM fungal communities would contain taxa that are more distantly related to each other when compared to communities inhabiting herbivore-free plant hosts. That said, increased competition may also result in more closely related communities (phylogenetic clustering) if certain phenotypes, such as those associated with low resource requirements, are associated with competitive dominance (Kraft *et al.*, 2015). In this instance, community assembly may have outcomes analogous to environmental filtering, being based on particular suite of traits that permit community membership (Pausas & Verdú, 2010).

Previous research has studied the impact of soil nutrient status on AM fungal community assembly (Liu *et al.*, 2015), yet the role of insect herbivory remains unexplored. While it is expected that aboveground herbivory would significantly affect the community assembly and diversity of AM fungi within plant roots, we have limited data to provide insight on this. Recent work on belowground insect herbivory found root herbivores can significantly reduce species richness and alter community structure of root-colonising AM fungi (Frew, 2022). Studies on other forms of herbivory, such as grazing, have found variable impacts on soil AM fungal communities where intense grazing decreases diversity (Ba *et al.*, 2012) while moderate or light grazing has little effect, or may even increase diversity (Ba *et al.*, 2012; van der Heyde *et al.*, 2017). Other forms of defoliation and damage, such as mechanical mowing in managed systems, are often found to have limited impacts on AM fungal diversity (Zubek *et al.*, 2022), but can still influence interactions between AM fungi and plant communities (Qin *et al.*, 2022). We are aware of only one study which has empirically examined the impacts of aboveground insect herbivory

on root AM fungal diversity and composition (Wilkinson *et al.*, 2019) where the authors found no changes in the richness of AM fungi in plant roots, but did observe an increase in community evenness in plants with insect herbivores. The scarcity of studies here highlights a knowledge gap which continues to be overlooked despite its importance, particularly considering the ubiquity and ecological significance of the interactions between insect herbivory and AM fungi.

The composition of an AM fungal community within a root system, as a result of assembly processes, is a fundamental determinant of the functional outcome of mycorrhizal symbiosis for the host plant. AM fungal taxa are functionally diverse, with different taxa being more or less associated with particular functions for their host such as P delivery or enhanced plant defence (Hart & Reader, 2002; Sikes *et al.*, 2009; Chagnon *et al.*, 2013). A number of studies have shown how different AM fungal taxa, combinations of taxa or communities can have distinct plant phenotypic outcomes relating to plant growth, nutrient status, and stress tolerance (van der Heijden *et al.*, 1998; Bennett & Bever, 2007; Frew, 2019). Thus, changes in root-dwelling fungal communities will have direct consequences for host plant performance and affect plant productivity and ecosystem functioning (van der Heijden *et al.*, 2008; Bardgett & van der Putten, 2014).

We explored how aboveground insect herbivory impacts the taxonomic and phylogenetic diversity and composition of root-colonising AM fungal communities to infer the associated community assembly processes. We hypothesised that herbivory would reduce richness of AM fungi in plant roots, potentially indicating increased competition for carbon resources. By examining the phylogenetic structure of the fungal communities, we expected the herbivory-driven increase in competition for carbon would either (1) increase phylogenetic overdispersion as a result of increased competitive exclusion or (2) increase phylogenetic clustering if competition selects for a particular set of AM fungal traits which confer a competitive advantage (Kraft *et al.*, 2015).

Materials and Methods

We performed a factorial glasshouse study using *Sorghum bicolor* L. Moench cv 'MR Taurus'. Our experiment had two treatment factors: herbivory (presence or absence) and AM fungi (presence or absence). Each of the four treatment combinations was replicated 13 times, totalling 52 *S. bicolor* plants at the experiment initiation. Seeds were surface sterilised with 10% diluted commercial bleach (comprised of 4% sodium hypochlorite), germinated in Petri dishes for 6 d, and then transplanted as individual seedlings into 3.7 l pots. These pots were filled with an autoclave-sterilised 40 : 60 sand/soil mix (Supporting Information Table S1 for soil nutrient data), and plants received an initial dose of the low-P fertiliser Osmocote Native Controlled Release Fertiliser (The Scotts Co. LLC) at the initiation of the experiment. Plants in the 'with AM fungi' treatment were inoculated by potting with 150 g of sieved, air-dried field soil inoculum combined with the sterile sand/soil mixture. One week before initiation of the experiment, the inoculum was sourced from the top 20 cm of soil alongside an organically managed arable field in southern Queensland, Australia (−27.4326°, 152.3495°), a site previously verified to harbour a diverse AM fungal community (Ng *et al.*, 2023). Conversely, the 'no AM fungi' treatment used 150 g of autoclaved field soil inoculum. All pots were given 300 ml of microbial filtrate, derived from washed field soil passed through a series of sieves (to the smallest aperture of 20 µm to exclude all AM fungal spores; Aguilar-Trigueros *et al.*, 2019), to standardise the non-AM fungal microbial community (Koide & Li, 1989). All plants were grown in the glasshouse and watered *ad libitum* with tap water, and soil moisture was checked every few weeks with a soil moisture metre to ensure similar moisture status across pots. Plants were grown with *c.* 13-h day length and day : night temperatures of 28°C : 18°C, while average daylight in the glasshouse for the period was 750 µmol m^{-2} s^{-1}. Pots were rearranged randomly within the glasshouse chamber every 2 wk.

After 8 wk, half of the pots were introduced to five fourth-instar *Helicoverpa punctigera* larvae each. These larvae had been fed as per the diet medium detailed by Teakle & Jensen (1985) and were sourced from CSIRO Agriculture & Food, Narrabri, Australia. To retain the larvae within their designated pots, all pots (including those without herbivores) were placed into individual enclosures of fine nylon mesh (Bugdorm enclosures; Megaview Science Co. Ltd). At this stage, one replicate plant (with AM fungi, with herbivory) was lost from the experiment. All insects were weighed before being introduced to the plants and again at harvest to obtain a mean insect growth rate per pot. These data represent herbivore performance, a proxy for herbivore treatment intensity across replicates.

Plants were harvested 12 wk from germination at which point their roots were separated from the aboveground tissues. After washing the roots, 1 g of fresh roots was collected from each plant by sampling from multiple distinct areas of the outer root system for evaluating mycorrhizal fungal colonisation. The residual plant tissue was dried in an oven at 38°C and subsequently weighed. The aboveground tissue was ground for subsequent chemical analyses, while homogenised dried root samples from the AM fungi plants were reserved for DNA metabarcoding.

To measure the phosphorus benefits to the plant from the AM symbiosis, we assessed foliar phosphorus concentration using inductively coupled plasma (ICP) spectroscopy. This was done postdigestion of dried plant material with hydrogen peroxide and nitric acid, as described by Rayment & Lyons (2011). Root carbon concentration was measured using the high-temperature combustion method (LECO elemental analyser) on dried and ground root material. Mycorrhizal fungal colonisation was evaluated in the AM fungal treatment roots, while its absence was confirmed in the 'No AM fungi' treatment roots. Freshly harvested root samples were cleared using 10% KOH at 90°C for 15 min and stained with 5% ink-vinegar (Vierheilig *et al.*, 1998). The stained roots were then placed on microscope slides as 5 cm fragments to be examined using the intersect method (McGonigle *et al.*, 1990) across at least 100 intersections per sample at ×200 magnification.

DNA was extracted from 70 mg of dried root samples using a DNeasy Powersoil Pro Kit (Qiagen, GmBH) following the manufacturer's instructions with the modification that 0.5 mm fragmented dried roots were added to extraction tubes, rather than

soil, which were ground in a FastPrep-24™ (MP Biomedical, Irvine, CA, USA) for 30 s before downstream processing. Sequencing was conducted at Western Sydney University's next-generation sequencing facility, following their protocols. Briefly, the DNA was purified using Agencourt AMPure XP Beads (Beckman Coulter, Brea, CA, USA) and quality-verified using Quant-iT™ PicoGreen fluorescence-based analysis (ThermoFisher Scientific, Waltham, MA, USA). The purified DNA then underwent amplification using polymerase chain reaction (PCR) using the small-subunit (SSU) ribosomal RNA gene with AM fungal-specific primers, WANDA (Dumbrell *et al.*, 2011) and AML2 (Lee *et al.*, 2008). Sequencing was performed on the Illumina MiSeq platform using the Illumina MiSeq reagent kit v3 2 × 300 bp paired-end chemistry as per the manufacturer's instructions.

Bioinformatics processing was done using the graphical downstream analysis tool (gDAT; Vasar *et al.*, 2021). From the total 2 × 1952 318 raw reads, cleaning and demultiplexing procedures retained 2 × 1438 308 cleaned reads. This involved checking double barcodes, verifying correct primer sequences, and ensuring an average quality of at least 30. Chimeric sequences (0.1% of cleaned reads) were eliminated using VSEARCH v.2.15.0. The resulting sequences were identified and assigned to virtual taxa (VT) using the MAARJAM database (Öpik *et al.*, 2010) with a 97% sequence identity and 95% alignment thresholds using BLAST+ (v.2.7.1). The top-scoring representative sequences for each VT were selected and aligned using CLUSTALW. A neighbour-joining phylogenetic tree (Fig. S1) of these sequences was constructed with MEGA based on the maximum composite likelihood method (Tamura *et al.*, 2021).

For all regression analyses, data exploration was carried out following the protocol described in Zuur *et al.* (2010). To counteract bias from differences in sequencing depth, we used the variance-stabilising transformation from the DESEQ2 R package (Love *et al.*, 2014; McMurdie & Holmes, 2014) before downstream analyses. The effect of the herbivore treatment on AM fungal VT richness and Shannon diversity was analysed by fitting linear models using *lm* and then applying the ANOVA function from the R package CAR. Dissimilarity in community composition and structure of the root-colonising AM fungal communities were visualised using principal coordinate analysis (PCoA, package PHYLOSEQ; McMurdie & Holmes, 2014) based on Bray–Curtis dissimilarity. To statistically test the effects of the herbivore treatment on the observed changes in community dissimilarity, we used permutational multivariate ANOVA (perMANOVA) using the *adonis2* function from the R package VEGAN (Oksanen *et al.*, 2013).

To investigate potential differences in the community assembly processes under herbivory, we calculated the phylogenetic diversity and structure of AM fungal communities. We used (1) Faith's phylogenetic diversity (Faith, 1992) which is equivalent to the sum phylogenetic distance within a community; (2) the mean pairwise distance (MPD), which measures the mean phylogenetic distance between all VT pairs in a community, and the (3) mean nearest taxon distance (MNTD) to measure the distance between each VT and its nearest relative, all employing the PICANTE package (Webb *et al.*, 2002; Kembel *et al.*, 2010). We further calculated standardised effect sizes (SES) for these metrics by way of the *ses.pd*, *ses.mpd* and *ses.mntd* functions in the PICANTE package (Kembel *et al.*, 2010). For the two latter metrics, higher values suggest phylogenetic overdispersion due to competitive exclusion, whereas lower values imply phylogenetic clustering, likely from environmental filtering (Pausas & Verdú, 2010). We employed linear models and the ANOVA function from the R package CAR to determine the effects of herbivory on these metrics (Fox & Weisberg, 2011).

To determine how herbivory influenced plant responses to AM fungi, we calculated the mycorrhizal growth response (MGR) and the mycorrhizal P response (MPR). These plant mycorrhizal responses (%) were calculated as ((plant response with AM fungi—mean plant response with No AM fungi)/mean plant response with no AM fungi) × 100, where the plant response was either the total biomass or tissue P concentration. To determine the effects of herbivory on these plant mycorrhizal responses, as well as on AM fungal root colonisation, plant biomass, root : shoot ratios, root C concentration, aboveground P concentrations and content, we fitted general linear models using *lm* and then applied ANOVA from the CAR package. A general linear model using *lm* was also fitted to assess the effects of AM fungi on the mean change in mass of the insect herbivores. Response variables which did not meet the assumptions of the model were log transformed to reduce heteroscedasticity and ensure normality of residual distribution.

All statistical analyses and data visualisations were carried out using R v.4.0.5 and RSTUDIO v.2022.07.2.

Results and Discussion

Our results demonstrate that aboveground insect herbivory reduces alpha diversity and affects the composition and structure of root-colonising AM fungal communities (Fig. 1). Herbivory reduced root carbon concentrations and increased AM fungal phylogenetic diversity while communities became more phylogenetically overdispersed (Fig. 2), which suggests competitive exclusion is a greater driver of community assembly under herbivory, relative to the control treatment. Furthermore, under herbivory, AM fungi provided a greater phosphorus benefit to their host plants (Fig. 3d).

Plants exposed to insect herbivory hosted AM fungal communities within their roots that were different to herbivore-free plant fungal communities (Fig. 1a). Overall, we identified 26 AM fungal VT across all plants, seven of which were unique to plants without herbivory, 10 VT were unique to plants with insect herbivores, and nine were present in plants from both treatments. Specifically, herbivory caused a reduction in AM fungal VT richness (Fig. 1b; Table S2) and alpha diversity (Fig. 1c; Table S2), by 33.2% and 31.6%, respectively. Such an outcome may be expected in circumstances when competition intensity increases, in this case as plants lose photosynthetic tissue and potentially alter resource allocation. Herbivory-induced reductions in AM fungal alpha diversity have been observed in studies which have looked at vertebrate grazing or belowground insect herbivory (Ba *et al.*, 2012; Frew, 2022). In addition to shifts in alpha diversity, herbivory also altered AM fungal community structure and composition (Fig. 1d; Table S2). In the roots of herbivore-free plants, the communities

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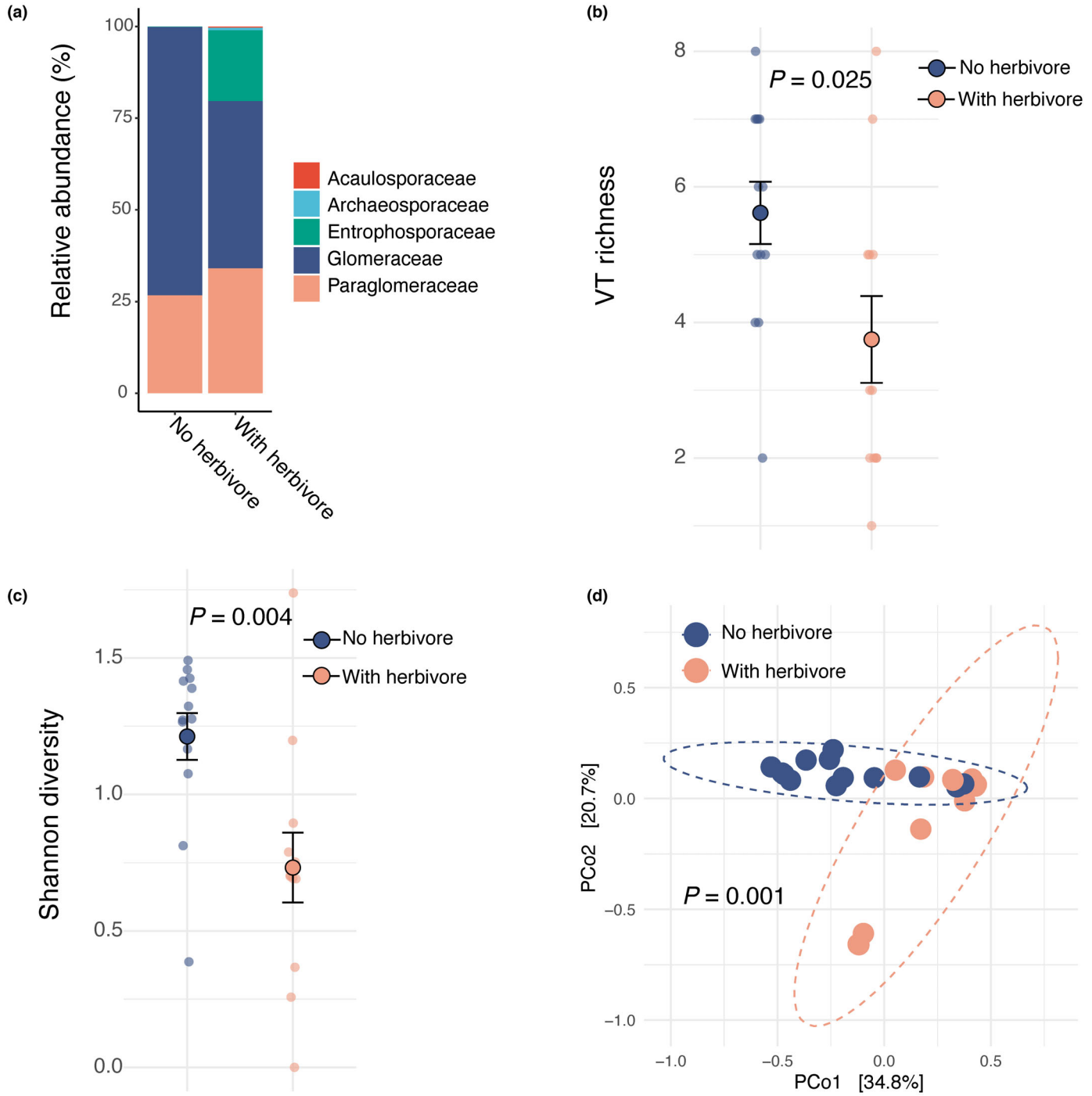

Fig. 1 Effects of herbivory on root-colonising arbuscular mycorrhizal (AM) fungal communities. (a) Relative abundance of AM fungal families, (b) AM fungal virtual taxon (VT) richness, and (c) the Shannon alpha diversity of AM fungi in the roots of *Sorghum bicolor*. Solid points and error bars represent the mean ± SE which are overlaid on top of the raw data points. *P*-values reported are results from fitting general linear models and an ANOVA. (d) Principal coordinate analysis (PCoA) of beta diversity (Bray–Curtis dissimilarity) comparing the structure of AM fungal communities in the roots of plants either with or without aboveground insect herbivory (*Helicoverpa punctigera*). Each point on the ordination represents a community of AM fungi in the roots of a plant, the associated ellipses represent 95% confidence intervals, and proportions of variation explained by each axis (PCo1 and PCo2) are also shown. Reported *P*-value is from PERMANOVA.

consisted solely of VT from the Glomeraceae and Paraglomeraceae families. Fungi of these families are all considered to be rhizophilic, that is, taxa that allocate higher biomass to intraradical hyphae than extraradical hyphae (Weber *et al.*, 2019). In contrast to this, with herbivory, the communities also included taxa from the Entrophosporaceae (previously Claroideoglomeraceae; Błaszkowski *et al.*, 2022), Archaeosporaceae, and Acaulosporaceae families. Though the Entrophosporaceae are also rhizophilic, Archaeosporaceae

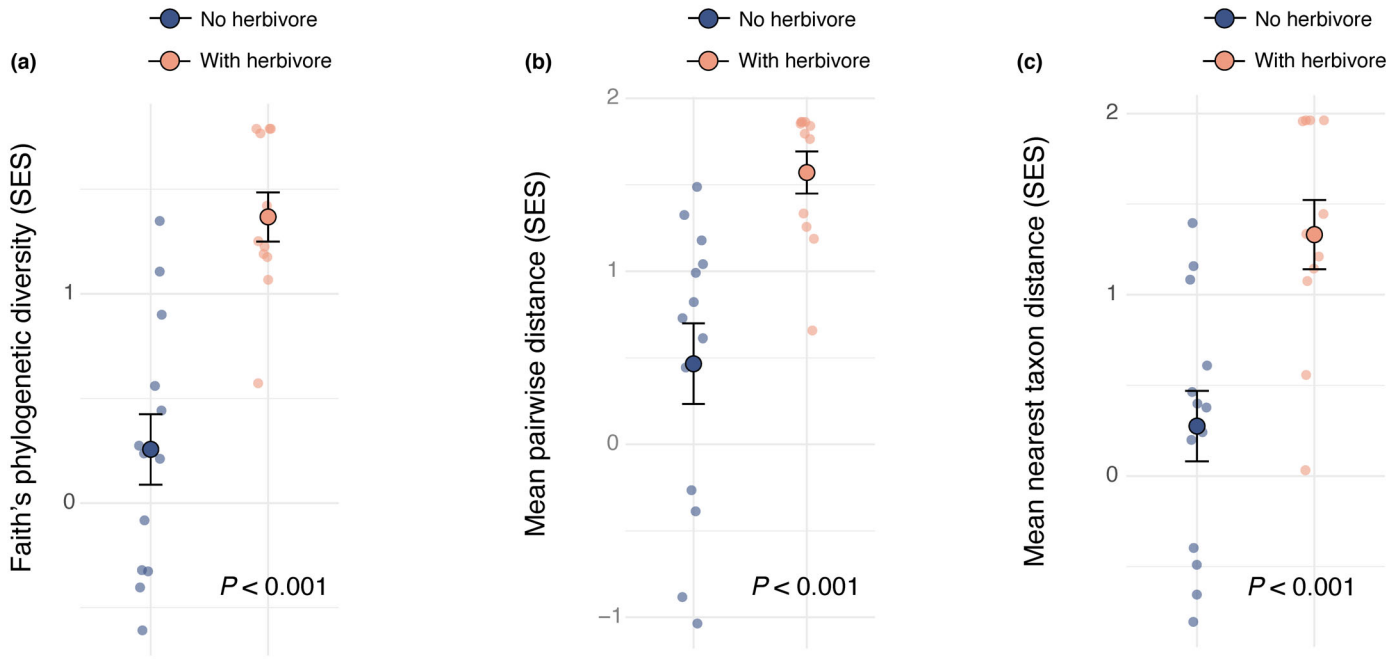

Fig. 2 Effects of herbivory on the phylogenetic diversity and structure of root-colonising arbuscular mycorrhizal (AM) fungi. The effects of aboveground insect herbivory (*Helicoverpa punctigera*) on the standardised effect sizes (SES) for (a) phylogenetic diversity (Faith's), (b) mean pairwise distance, and (c) mean nearest taxon distance of root-colonising AM fungal communities in *Sorghum bicolor* roots. Solid points and error bars represent the mean ± SE which are overlaid on top of the raw data points. *P*-values reported are results from fitting general linear models and then ANOVAs.

and Acaulosporaceae are classified to fall within the 'ancestral' guild. While fungi within this guild do not exhibit biomass allocation preferences, they tend to have lower fungal biomass overall. If these fungi represent less biomass, they may be more competitive when carbon resources from the host are low, as their limited biomass production means their carbon needs are comparatively lower than other AM fungal groups.

It is common for the AM symbiosis to reduce insect herbivore performance, particularly for chewing insects (Koricheva *et al.*, 2009). In this instance, however, we observed no difference between the growth of insects feeding on plants with and without AM fungi (Fig. S2). Although many studies have demonstrated AM fungal-enhanced plant resistance, there can be significant variability even between AM fungal isolates of the same species (Roger *et al.*, 2013). Additionally, the scope of research on the effects of AM fungi on plant resistance to insects is mostly limited to a select minority of AM fungal taxa. Thus, we currently have a narrow understanding of the broader implications of AM fungal diversity on plant–herbivore interactions (Frew *et al.*, 2022).

While AM fungal taxonomic diversity decreased under herbivory, phylogenetic diversity increased (Fig. 2a) and fungal communities became more phylogenetically overdispersed compared with the AM fungal communities inhabiting roots of herbivore-free plants (Fig. 2b,c). This is typically attributed to heightened competitive exclusion in community assembly (Webb *et al.*, 2002; Pausas & Verdú, 2010). In line with this, the carbon concentration of plant roots was reduced in herbivory-treated plants by 3.5% (Fig. S3). Our findings therefore support the hypothesis that aboveground herbivory intensifies competition between AM fungal taxa, likely due to decreased carbon availability, thereby increasing the role of competitive exclusion in community assembly, characterised by AM fungal communities composed of more distantly related taxa. While herbivore-induced reductions in carbon allocation to fungi have been shown (Charters *et al.*, 2020; Durant *et al.*, 2023), the consequences of this for AM fungal communities have been unexplored. The resultant scarcity of available carbon likely exacerbated competition among fungal taxa within the roots looking to occupy the same habitat with an increasingly limited shared resource. As such, this increased pressure from competitive exclusion reduced VT richness and the remaining members of these communities were more distantly related to each other.

Competition between AM fungal taxa is recognised to be strongly influenced by phylogenetically conserved traits (Powell *et al.*, 2009). While we might expect that the coexisting taxa under herbivory may share an ability to grow under low carbon conditions, their coexistence suggests sufficient differences in their characteristics (or traits) that minimise competition when it comes to resource use. The respective traits to consider may relate to differences in their preferential allocation of hyphae to soil or roots (Weber *et al.*, 2019), differences in spatial and/or temporal occupancy of the root system, hyphal turnover rates, or hyphal growth rates (Chagnon *et al.*, 2013; Chaudhary *et al.*, 2022). On this occasion, the more phylogenetically dispersed communities provided 116% greater P benefit to their hosts compared with the communities assembled in roots of the herbivore-free plants (Fig. 3d). Thus, while a more simplistic interpretation might be that competition only selected for more 'beneficial' fungal taxa in terms of their ability to acquire and/or deliver P, this is probably less likely. Rather, we suspect that competition led to greater functional

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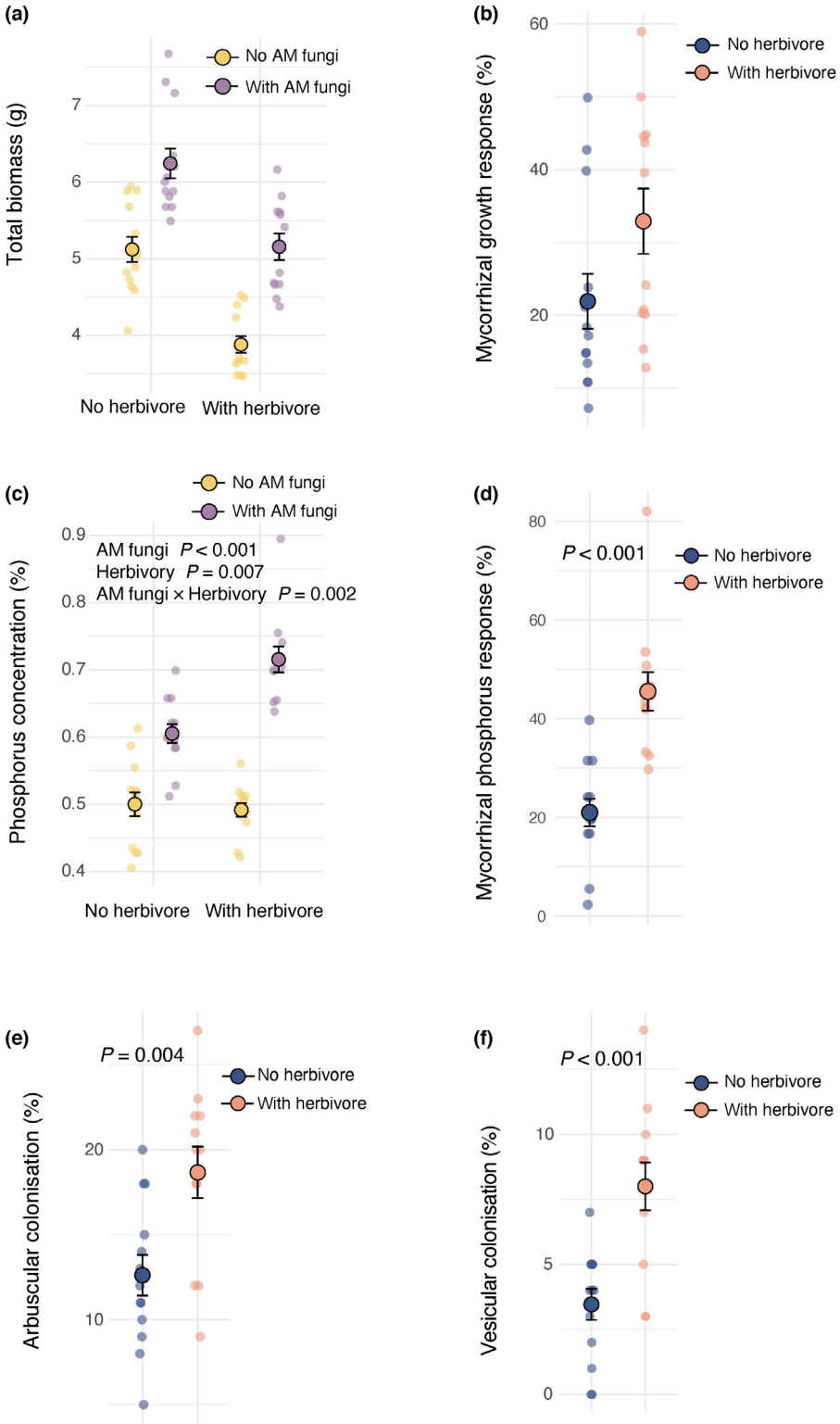

Fig. 3 Plant (*Sorghum bicolor*) responses to arbuscular mycorrhizal (AM) fungi and aboveground herbivory from *Helicoverpa punctigera*. Effects of herbivory on the (a) total biomass (g) and (c) phosphorus concentration (%) of *S. bicolor* grown with or without an arbuscular mycorrhizal (AM) fungal community. Also shown are the effects of herbivory treatment on the (b) mycorrhizal growth response (%), (d) mycorrhizal phosphorus response (%), (e) arbuscular colonisation (%), and (f) vesicular colonisation (%) of *S. bicolor*. Solid points and error bars represent the mean ± SE, which are overlaid on top of the raw data points. Reported *P*-values are from fitting general linear models then ANOVAs.

complementarity that manifested in enhanced P benefit to the host overall (Jansa *et al.*, 2008). This enhanced P status of the host may also serve to benefit defence in some instances, particularly for plants which rely more on tolerance-based defences against herbivory over resistance-based defences, such as many C_4 plants (Heckathorn *et al.*, 1999; Strauss & Agrawal, 1999).

In contrast to the mycorrhizal P response, the mycorrhizal growth response was not significantly affected by herbivory (Fig. 3a). However, the root : shoot ratios significantly shifted under herbivory where, unsurprisingly, plants with herbivores had 22% higher root : shoot ratios, likely due to aboveground tissue loss. Contrastingly, plants with AM fungi had lower root : shoot

ratios (Fig. S3), which is not an uncommon phenomenon when comparing plants with and without AM fungi (Veresoglou *et al.*, 2012), ostensibly as plants with AM fungi are able to invest less in root mass to forage for soil-based resources. While the relative total colonisation of plant roots by AM fungi was unaffected by herbivory (Fig. S3), both arbuscular and vesicular colonisation was higher (by 48% and 131%, respectively) in herbivore-treated plants (Fig. 3e,f). An increase in the presence of arbuscules suggests an increase in the resource exchange between fungi and plants, which may be the case as AM fungi provided greater P benefit for their hosts under herbivory. Vesicles are primarily recognised as storage structures, and their formation can be characteristic of particular AM fungal taxa (Smith & Read, 2008). While they represent a significant amount of carbon, their presence may be indicative of a slow growth strategy, where the fungi allocate carbon to storage rather than their own growth or metabolism. Such slow growth strategy is commonly found in other organisms suited to low resource or 'stressful' environments, such as desert plants or in bulb and tuber forming plants, which invest more heavily in storage organs than abundant soft vegetative tissue to deal with low resources or seasonal resource availability (De Deyn *et al.*, 2008; Májeková *et al.*, 2014). Indeed, it is important to be cognisant of the turnover and transient nature of arbuscules, and that our colonisation assessment here was confined to one time point. As such, the variability in colonisation by arbuscules and vesicles is likely to be more nuanced and dynamic than what our data here are able to capture.

While our understanding of the diversity and community assembly processes of AM fungi is currently advancing (Moora *et al.*, 2014; Vályi *et al.*, 2016; Davison *et al.*, 2021; Vasar *et al.*, 2022), important knowledge gaps remain. Our study shows, for the first time, that aboveground insect herbivory can significantly shape the diversity and phylogenetic structure of root-colonising AM fungal communities and the concomitant outcomes for plant growth and P status. Our results highlight that greater efforts should go towards understanding these ubiquitous tripartite interactions and how they can regulate AM fungal community ecology.

Acknowledgements

The authors acknowledge the Traditional Owners of the lands on which this work was conducted. This work was supported by an Australian Research Council Discovery Early Career Researcher Award (DE220100479) awarded to AF. CAA-T was supported by a Feodor-Lynen Fellowship (Alexander von Humboldt Foundation). JO, IH, MÖ and TV are supported by Estonian Research Council (grants nos. 1789, 1170, and 784). The authors would like to thank the editor and three anonymous reviewers for their comments. Open access publishing facilitated by Western Sydney University, as part of the Wiley - Western Sydney University agreement via the Council of Australian University Librarians.

Competing interests

None declared.

Author contributions

AF conceived and designed the experiments which were developed from discussions with MÖ, JO and TV. AF conducted the experiment and performed the lab work with advice from JO. AF and CAA-T analysed and interpreted the data with insights MÖ, TV and IH. AF and CAA-T led the writing of the initial manuscript draft with support from TV and IH. All authors contributed critically to the final draft and provided approval for publication.

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Data availability

Data that support this study are available from the figshare repository at the following doi: 10.6084/m9.figshare.24240046. Raw DNA sequencing data are available under NCBI BioProject accession no. PRJNA1023676.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Neighbour-joining phylogenetic tree of arbuscular mycorrhizal (AM) fungal virtual taxa.

Fig. S2 Effects of AM fungi on the mass change of the insect herbivore.

Fig. S3 Effects of herbivory and AM fungi on root carbon, foliar phosphorus, and root: shoot ratio, and the effects of herbivory on total AM fungal root colonisation.

Table S1 Soil nutrient analysis data.

Table S2 Model results looking at the effect of herbivory and AM fungi on plant responses as well as fungal colonisation, diversity, and phylogenetic structure.

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Key words: arbuscular mycorrhizal fungal diversity, competition, insect herbivores, microbial communities, phylogenetic diversity.

Received, 5 October 2023; accepted, 26 November 2023.