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RESEARCH ARTICLE

Host filtering, not competitive exclusion, may be the main driver of arbuscular mycorrhizal fungal community assembly under high phosphorus

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Abstract

1. A major goal in ecology is understanding the factors which determine the diversity and distribution of organisms. The outcome of the symbiotic relationship between plants and arbuscular mycorrhizal (AM) fungi is strongly influenced by soil phosphorus (P) availability. Despite this knowledge, there is still much to uncover about how soil P status can shape the taxonomic and phylogenetic assembly of root-colonising AM fungi. Additionally, there is a paucity of understanding about the implications of these changes for the outcome of the AM symbiosis in terms of plant growth, nutrient status and defence traits.
2. We conducted a factorial pot experiment where sorghum (*Sorghum bicolor*) was grown under three different P treatments (low, medium and high), in the presence or absence of a natural AM fungal community. By analysing the diversity and community structure of the fungal community colonising roots, we aimed to determine if and how soil P influences the relatedness of these communities and whether competitive exclusion or environmental filtering play a more significant role in their assembly. Additionally, we evaluated the concomitant outcomes for plant growth, nutrient acquisition and defensive chemistry (phenolics).
3. Increasing P availability reduced AM fungal richness and increased community evenness. Root-colonising AM fungal communities under the high P treatment had significantly reduced phylogenetic diversity and comparatively lower mean pairwise distances among all treatments. This indicated that AM fungal communities became more closely related (phylogenetically clustered) with increasing soil P. The mycorrhizal growth and mycorrhizal P responses of plants were positive under low and medium P, but this was lost under high P, however, plant phenolics were increased.
4. Our results suggest that under high P conditions, environmental filtering plays an important role in AM fungal community assembly as host plants alter their selectivity of fungal functional groups prioritising those associated with enhancing plant stress resistance and defences, rather than nutrient acquisition. Here we

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demonstrated how soil P status can shape taxonomic and phylogenetic assembly of AM fungi and the associated functional outcomes for the host.

KEYWORDS
arbuscular mycorrhizal fungi, community assembly, phylogenetic diversity, plant defence, plant phosphorus

1 | INTRODUCTION

The functioning of the arbuscular mycorrhizal (AM) symbiosis is heavily influenced by the fertility of the soil (Smith & Read, 2008b). As part of this relationship, plants provide carbon to the fungi while the fungi supply nutrients, particularly phosphorus, to the plants. The availability of these resources can determine the ultimate outcome for both the host plant and the fungus, the so called mycorrhizal phenotype (Johnson et al., 2015). AM fungi, with their extensive mycelial networks, can access soil phosphorus more effectively than their plant hosts. It is widely accepted that plants growing in phosphorus-limited environments can benefit significantly from their association with AM fungi, helping them acquire necessary nutrients (Smith & Read, 2008a).

Many studies have shown that manipulating nutrient availability can impact the growth of plants associating with AM fungi compared to those that are not (Klironomos, 2003), termed the mycorrhizal growth response (MGR). The exchange of resources, including phosphorus (P) and carbon (C), between the plant and fungus plays a key role in determining plant MGR. When P is limiting, plants often exhibit a positive MGR because they benefit from the fungi's ability to provide additional P. However, when nutrients are not limiting, plants may exhibit neutral or even negative MGRs. In these cases, economic models suggest that there is limited reward for the host plant to provide additional C to the AM fungi without receiving sufficient benefits in return (Johnson et al., 2015; Wipf et al., 2019; Wyatt et al., 2014). Research shows that increasing plant P availability can reduce root colonisation by AM fungi (Ova et al., 2015; Yazici et al., 2021), and reduce C allocation from plant to fungus (Konvalinková et al., 2017). Further to this, evidence suggests plants allocate more C to the more beneficial fungal partners that provide more P (Fellbaum et al., 2014; Kiers et al., 2011), but this preferential allocation can depend on various factors such as light availability or soil fertility (Zheng et al., 2015).

The functionality of the AM symbiosis goes beyond P uptake, affecting tolerance to abiotic stress as well as plant defences against herbivores and pathogens (Frew et al., 2022; Wehner et al., 2010). Although the symbiosis may be based upon nutrient acquisition, there are several mechanisms by which AM fungi can improve plant fitness. As such, different AM fungal taxon groups are known for particular characteristics, or symbiotic functional traits (Chaudhary et al., 2022). For example, the Glomeraceae are often associated with an increase in plant resistance to pests and herbivory, while other groups, such as Gigasporaceae and Diversisporaceae, are more associated with nutrient uptake and efficient resource use (Maherali & Klironomos, 2007; Sikes et al., 2009). Considering their functional diversity and the dynamic nature of the plant–fungal symbiosis, it then follows that changes to nutrient availability will shift diversity and community composition of AM fungi, with functional consequences for the host.

The responses of soil-dwelling AM fungal communities to P addition can be variable (Beauregard et al., 2010; Chen et al., 2014; da Silva et al., 2021; Hammer et al., 2011), and are affected by various factors including the edaphic environment and local host plant identities (Sepp et al., 2019). Overall, however, species richness of AM fungal communities can be expected to decline under high P availability, including in agro-ecosystems (Ma et al., 2021). This decrease in species richness has also been observed in AM fungal communities colonising plant roots, although the extent of this effect can vary (Higo et al., 2020).

While it is expected that an increase in P availability would lead to a decrease in AM fungal species richness, the community assembly mechanisms behind this relationship are not fully understood. When plants have access to sufficient P, they benefit less from their AM fungal partner for nutrient supply, which may lead to lower allocation of carbon to fungi. The AM fungal community structure response to these conditions could be concurrently driven by two community assembly mechanisms. On the one hand, low availability of total C from the host could intensify competition among potential fungal partners (Johnson, 2010; Johnson et al., 2015) where competitive taxa may be more efficient at acquiring carbon from the host plant or simply have low carbon demands (Chagnon et al., 2013; Grover, 1991). These taxa may provide a cost-effective benefit to the plant by providing nutrient benefits, even when environmental P is high (i.e. only the species that can deliver a high amount of P relative to C invested from the plant; Kiers et al., 2011; Konvalinková et al., 2017). According to the competition-relatedness hypothesis (Cahill et al., 2008) closely related taxa are more likely to have intense competition compared to taxa that are more distantly related, which limits their ability to coexist. As such, when environmental P is high and C availability to fungi is low, the taxa within a root-colonising AM fungal community will be more distantly related to each other, that is, phylogenetically dispersed (Violle et al., 2011). Indeed, intense competition has been recorded among closely related AM fungal species in empirical studies (Engelmoer et al., 2014).

However, other mechanisms can also have a strong influence on AM fungal community assembly. When plant available P is

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high then plant hosts may no longer invest in their fungal partners primarily for nutritional gains, but still allocate carbon to fungal partners that provide other key beneficial functions offered by the symbiosis (Delavaux et al., 2017; Powell et al., 2009; Powell & Rillig, 2018), such as herbivore and pathogen defence (Frew et al., 2022; Wehner et al., 2010). Thus, instead of competitive exclusion, host selection for AM fungal partners that provide protection from biotic stressors may become the dominating community assembly process (Chagnon et al., 2015). In symbiotic relationships, host selection is a component of environmental filtering in community assembly, where filtering can be driven by various factors such as temperature, pH and soil type, among many others. Community membership would be restricted to a particular functional type (Maherali & Klironomos, 2012) as hosts select for these functional traits (Chaudhary et al., 2022; Kiers et al., 2011). Assuming niche-related traits of AM fungi are similar among closely related lineages (Maherali & Klironomos, 2012; Powell et al., 2009), filtering by the host plant will lead to communities that are more phylogenetically clustered (taxa are more closely related to one another). As these taxa may not be competitive in terms of resource use or nutrient delivery, growth or nutrient benefits for the host are likely to be limited. However, plants will benefit from an upregulation of defence-associated traits, such as phenolics, which are often associated with plant resistance to biotic stress such as herbivory or pathogens (Mithöfer & Boland, 2012). Underlying this is the functional diversity of AM fungi, with particular taxa being more associated with particular symbiotic outcomes such as enhancing plant nutrient uptake, or enhancing plant defences (Sikes et al., 2009). Additionally, it has already been shown that phenolics are, at least partially, regulated by AM fungal community composition (Frew, 2020; Frew & Wilson, 2021), where certain AM fungal communities will upregulate phenolic-based plant defences, while other communities do not.

In order to determine which of these two processes have a greater relative influence on the community assembly of AM fungi under high P conditions, we experimentally manipulated soil P availability and measured its effects on taxonomic diversity, phylogenetic structure and the functioning of AM symbiosis in sorghum (*Sorghum bicolor*) in terms of nutrient-related benefits and defence-related benefits. The economic significance of sorghum, its responsiveness to the AM symbiosis, and the history of research on the crop provided a good model with which to test our hypotheses. We grew plants either with or without a natural AM fungal community under low, medium or high P conditions. We identified root-colonising AM fungal communities using DNA metabarcoding then measured their taxonomic and phylogenetic diversity and structure alongside plant growth, nutrient and phenolic responses to AM fungi and their root colonisation. Specifically, we used this experimental set-up to test (Figure 1):

A *Community assembly dominated by competitive exclusion*. According to this hypothesis, we predicted that with increasing P availability, competition between AM fungi would increase for the low amounts of C allocated from the host, leading to species poor community but with high phylogenetic diversity (i.e. communities becoming increasingly phylogenetically dispersed). While plants may still experience positive growth or phosphorus responses from the AM symbiosis, it is unlikely that defence traits such as plant phenolics would be affected.

B *Community assembly dominated by host filtering*. Under this hypothesis, with increasing P availability, plants would become more selective towards certain mycorrhizal functional groups that confer stress tolerance and defence benefits, rather than nutrient acquisition. This filtering process leads to a species poor community that is phylogenetically clustered. As a result, plants may not experience positive growth or nutrient responses from AM fungi, but defence-associated traits, such as phenolics, will be enhanced.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

We conducted a factorial greenhouse experiment with *Sorghum bicolor* L. Moench cv. 'MR Taurus' and two treatment factors: a 'phosphorus' treatment (with three levels of low, medium and high) and 'AM fungi' treatment (with two levels, with or without AM fungal communities), each treatment combination was replicated seven times. Thus, 42 *S. bicolor* were grown from seeds that were surface sterilised using 10% sodium hypochlorite solution before being germinated in Petri dishes for 6 days at which point individual seedlings were transplanted into 3.7 L pots. These pots contained a 40:60 sand/soil mixture that was sterilised by autoclaving (Table S1).

Plants grown 'with AM fungi' were grown in pots within which 150 g of sieved and air-dried field soil inoculum was mixed with the sterile sand/soil mixture, while the 'no AM fungi' pots received 150 g of autoclaved field soil inoculum. Pots also received 300 mL of microbial filtrate from washed field soil filtered through a 38 μm sieve to standardise the non-AM fungal microbial community (Koide & Li, 1989). The field soil inoculum was taken from the top 0–20 cm of soil from a strip of soil adjacent to an organically managed arable field. Soil from this site had previously been sampled and sequenced for prior studies, thus was known to harbour a diverse AM fungal community (Ng et al., 2023). The site was identified to harbour 63 virtual taxa (VT) of AM fungi (Frew, 2022) 1 month prior to sampling to make the inoculum. Phosphorus treatments were achieved via supplementation with KH_2PO_4 where pots under the low phosphorus treatment received 1 mg P kg^{-1}, the medium phosphorus treatment received 18 mg P kg^{-1}, while the high phosphorus treatment received 40 mg P kg^{-1}. These treatments resulted in plant available (Colwell) P concentrations of 5.11 mg kg^{-1} (±1.1), 16 mg P kg^{-1} (±0.92) and 29 mg P kg^{-1} (±3.2), in the low, medium and high P treatments respectively. Thus the low

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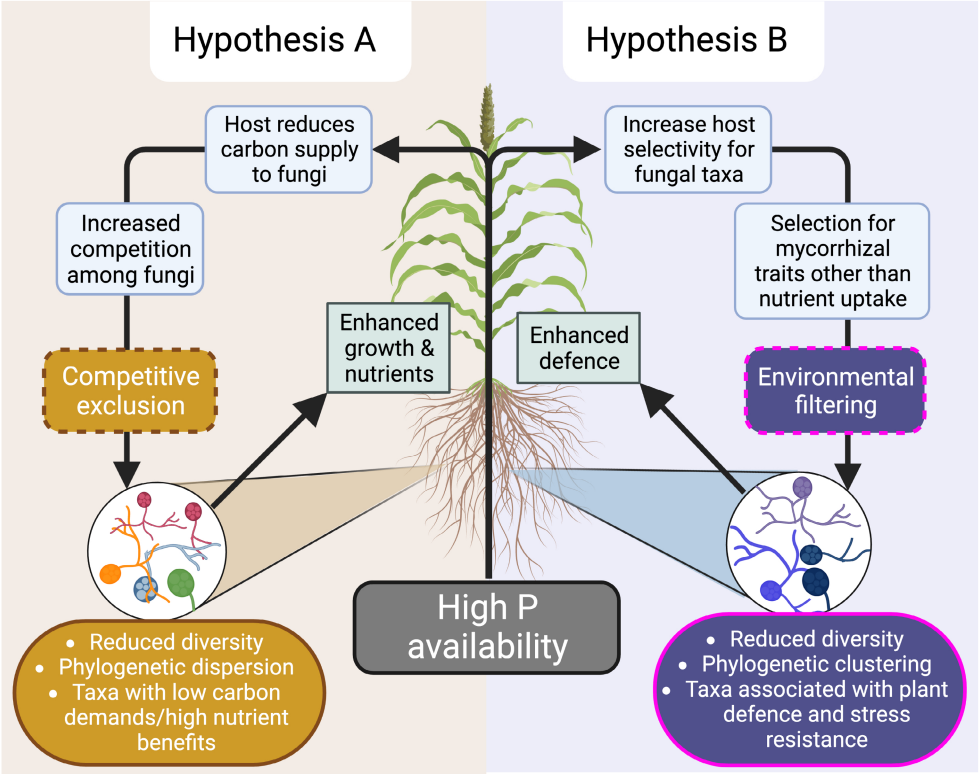

FIGURE 1 Hypothetical assembly of root-colonising arbuscular mycorrhizal (AM) fungi. In hypothesis A, high soil phosphorus (P) availability results in reduced allocation of plant carbon towards fungi as the host plant is able to acquire P without a carbon cost being allocated to the fungi. This reduction in available carbon resources for the fungi increases competition and competitive exclusion becomes the primary driver of community assembly resulting in reduced diversity and phylogenetic dispersion. Thus, only the most competitive fungal taxa remain successful in the community. These taxa have low carbon requirements and/or are able to deliver additional P benefit at a low 'cost' to the plant. In hypothesis B, high P availability results in increased host selection for AM fungal taxa with functional benefits other than P delivery. This selectivity acts as an environmental filter for communities which provide stress resistance to hosts (e.g. defence against antagonists). Assuming niche conservatism, fungal taxa with similar functions are more closely related, thus environmental filtering for more closely related AM fungi results in phylogenetic clustering. Figure created with BioRender.

P treatment was below the critical P values for sorghum (15–22 mg P kg^{-1}), while the medium P treatment fell within these values and the high P treatment resulted in P availability that was above these values (Peverill et al., 1999; Watts-Williams et al., 2021). All plants were grown in the glasshouse, watered ad libitum with tap water for 12 weeks with 11 h day length and day/night temperatures of 28°C/18°C, while average daylight in the glasshouse for this period was 750 μmol m^{-2} s^{-1}. Pots were rearranged within the glasshouse chamber weekly.

At harvest, plants were removed from pots and roots and above-ground tissues were separated. Roots were washed and a 1 g subsample of fresh roots were taken from each plant for mycorrhizal fungal colonisation assessment. A subsample of above-ground tissue taken and freeze dried for total phenolic assay, while all remaining plant tissue was oven dried at 38°C then weighed. Above-ground plant tissue was ground to powder for subsequent chemical analyses, and homogenised dried root samples from plants with AM fungi were taken for molecular analyses. A small subsample of roots from five plants from the 'no AM fungi' treatment were also taken for molecular analyses to confirm the absence of AM fungal VT.

2.2 | Chemical analysis and DNA metabarcoding of AM fungi

In order to determine nutritional- and defence-related benefits from the AM symbiosis to the plant, we measured foliar nitrogen, phosphorus and phenolic concentrations. To measure nitrogen, dried and ground foliar material was analysed using the high-temperature combustion method (LECO analyser) where samples are loaded into a combustion tube and flushed with oxygen. Gases generated from this process are then measured using a thermal conductivity cell. Tissue phosphorus was measured by inductively coupled plasma (ICP) spectroscopy after digestion of the dried plant material with hydrogen peroxide and nitric acid (Rayment & Lyons, 2011). Total phenolics in leaves were determined using a Folin–Ciocalteu assay with gallic acid (Sigma-Aldrich) as the quantification standard (Salminen & Karonen, 2011).

DNA was extracted from 70 mg of the dried root samples using a DNeasy Powersoil Pro Kit (Qiagen, GmBH) according to the manufacturer's instructions, with the modification that dried root material cut into small 0.5 mm fragments is added to extraction tubes. One sample (from low phosphorus treatment)

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failed to yield sufficient DNA and thus was not included for sequencing. Sequencing was processed through the Western Sydney University's Next-Generation Sequencing Facility's liquid handling pipeline using in-house optimised protocols. The DNA was purified using the Agencourt AMPure XP Beads (Beckman Coulter), followed by a quality assessment using the Qunat-iT™ PicoGreen fluorescence-based analysis (ThermoFisher Scientific). The purified DNA then underwent amplification using polymerase chain reaction (PCR) targeting the small-subunit (SSU) ribosomal RNA gene using AM fungal-specific primers (Lekberg et al., 2018): 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 bps paired-end chemistry as per manufacturer's instructions.

Bioinformatic data analysis and processing was conducted using the graphical downstream analysis tool (gDAT) for analysing rDNA sequences (Vasar et al., 2021). Raw reads (2×815,160 reads in total) were demultiplexed and cleaned using a series of bioinformatic steps (see Vasar et al., 2017, 2021). In short, reads were demultiplexed by checking double barcodes, allowing one mismatch for both reads. Reads were retained if they carried the correct primer sequences (WANDA and AML2; allowing one mismatch for each) and had an average quality of at least 30, and orphan reads were removed (leaving 2× 658,132 cleaned reads). Putative chimeric sequences (6952; 1.1% of cleaned reads) were identified and removed using *vsearch* v2.15.0 (Rognes et al., 2016) with the default parameters in reference database mode against the Maarj*AM* database (status July 2021; Öpik et al., 2010). Cleaned and chimera-free sequences were assigned to VT using BLAST+ (v2.7.1, Camacho et al., 2009) referencing the Maarj*AM* database (Öpik et al., 2010) with at least 97% identity and 95% alignment thresholds. Representative sequences of each VT were then picked selecting those with the highest scores as described in Vasar et al. (2021) for conducting phylogenetic analysis. Picked sequences were aligned with *ClustalW* (Thompson et al., 2003) and a neighbour-joining phylogenetic tree (Figure S1) of representative sequences was constructed using MEGA11 using maximum composite likelihood method (Tamura et al., 2021).

2.3 | AM fungal colonisation

To confirm colonisation of roots from plants inoculated with AM fungi and the absence of colonisation in the plants under the no AM fungi treatment, root subsamples were cleared with 10% potassium hydroxide heated to 80°C for 20 min and then stained with 5% ink vinegar (Vierheilig et al., 1998). The cleared and stained roots were mounted on glass slides with glycerine under a cover slip and scored for the presence of AM fungi using the intersect method (McGonigle et al., 1990). To conservatively quantify colonisation, hyphae were counted as AM fungal so long as AM structures (i.e. arbuscule, vesicle, spore) were also present within the root segment under assessment (5 cm).

2.4 | Statistical analyses

All analyses were carried out using R v4.0.5 and RStudio v2022.07.2.

To counteract bias from differences in sequencing depth, all samples were rarefied to the minimum number of sequences per sample (762 sequences) by means of the *rarefy_even_depth* function from the R package 'Phyloseq' (McMurdie & Holmes, 2013).

The effects of the P treatments on AM fungal virtual taxon (VT) richness were analysed by Kruskal–Wallis test (this response variable violated assumptions of residual normality and heteroscedasticity) using *kruskal.test* from the 'stats' package in R (R Core Team, 2017). Community evenness (measured with Pielou's evenness index) response to P treatments was analysed by fitting a linear model using *lm* then applying *Anova* function from the R package 'car' (Fox & Weisberg, 2011). Dissimilarity in community composition and structure of the root-colonising AM fungal communities were visualised using principle coordinate analysis (PCoA, package 'Phyloseq'; McMurdie & Holmes, 2013) based on Bray–Curtis dissimilarity. To statistically test the effects of the phosphorus treatments on the observed changes in community dissimilarity, we used permutational multivariate ANOVA (perMANOVA) using the *adonis* function from the R package 'vegan' (Oksanen et al., 2015). To explore preferential treatment-taxa associations, we used indicator species analysis from the R package 'indicspecies' which provides an association index indicating the level of association between a taxon and a particular group (Cáceres & Legendre, 2009).

To determine the community assembly process behind increases in P availability, we calculated phylogenetic diversity and structure of AM fungal communities using three metrics: (i) Faith's phylogenetic diversity (Faith, 1992) which measures the sum total phylogenetic distance of the community (using the *pd* function from the 'picante' package; Kembel et al., 2010); (ii) mean pairwise distance, which measures the mean phylogenetic distance between all pairs of VT within a community (using the *mpd* function from 'picante'); and (iii) mean nearest taxon distance which measures the mean phylogenetic distance between each VT and its closest relative within the community (using function *mntd* from 'picante'; Webb et al., 2002). We further calculated standardised effect sizes (SES) for these metrics by way of the *ses.mpd* and *ses.mntd* functions in the 'picante' package (Kembel et al., 2010). Positive values of these SES metrics indicate communities that are more distantly related (phylogenetic overdispersion) corresponding to competitive exclusion, while negative values indicate that members of the community are more closely related (phylogenetic clustering), ostensibly due to environmental filtering (Pausas & Verdú, 2010). To statistically test the effect of increasing level of P on these metrics, we fitted linear models using *lm* and *Anova* functions (Fox & Weisberg, 2011) to assess the differences between P treatments.

To determine how increasing levels of P shifted the outcome of the AM symbiosis between nutritional- and defence-based benefits, we fitted standard linear models for total biomass, above-ground and below-ground biomass, root:shoot, P, N, total phenolic concentrations, C:N and N:P using *lm* and the *Anova* function from

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'car'. We also calculated the MGR, mycorrhizal P response (MPR) and the mycorrhizal phenolic response (MPhenR) under each of the P treatments. These plant mycorrhizal responses (%) were calculated as ([*plant response – mean plant responses with No AM fungi*]/*mean plant responses with no AM fungi*) x *100*, where the plant response was either the total biomass, P concentration or total phenolic concentration of plants with AM fungi. To determine the effects of the P treatments on these plant mycorrhizal responses, we fitted standard linear models using *lm* and then applying *Anova* from the 'car' package (Fox & Weisberg, 2011). We used the same approach to determine the effects of P treatments on the total colonisation, arbuscular and vesicular colonisation of plant roots. To investigate if diversity was associated with fungal colonisation, we fitted linear models using *lm*, for each of the fungal colonisation metrics as a function of Faith's phylogenetic diversity.

3 | RESULTS

A total of 60 VT were recorded across all samples (Table S2). The five most dominant taxa in the dataset made up 55.5% of all AM fungal sequences (Figure 2a) and were members of the genera *Glomus* (VT342, 28%; VT108, 7.9%; VT384, 7.8%) and *Claroideoglomus* (VT57, 6.5%; VT56, 5.2%). Across the three phosphorus treatments there were a total of 41 VT identified in high phosphorus, 37 in the medium and 48 in the low phosphorus (Figure 2c).

Indicator species analysis revealed certain VT were indicative of particular phosphorus treatments (Figure 2b). Specifically, one VT was an indicator of low phosphorus treatment while 23 VT were shared indicators of both low and medium phosphorus treatments, and 11 VT were strongly associated with the high phosphorus treatment (Table S4).

Diversity and community structure of root-colonising AM fungi were significantly affected by the phosphorus treatments. As expected, the AM fungal VT richness decreased as phosphorus availability increased (Table S3) where richness in the high phosphorus treatment was 30% and 31% lower than in the low and medium phosphorus treatments respectively (Figure 3a). Meanwhile, community evenness was greatest under the high phosphorus treatment (Table S3), which was 12% and 23% higher compared with the low and medium treatments respectively (Figure 3b). Furthermore, the root-colonising fungal community under the high phosphorus treatment was particularly distinct (Figure 3c). Phylogenetic diversity (Faith's) was also significantly affected by phosphorus availability (Table S3) where communities under high phosphorus had 27% and 32% lower phylogenetic diversity compared with the low and medium phosphorus treatments respectively (Figure 4a). Additionally, the AM fungal communities under high phosphorus also had distinctly lower mean pairwise distances (SES) compared to the communities under low and medium phosphorus (Figure 4b). In contrast, mean nearest taxon distances (SES) were unaffected by phosphorus in this instance (Figure 4c).

Plant total biomass increased with phosphorus availability and was also promoted by AM fungi (Figure 5a). This effect of AM fungi on biomass, however, was lost under the high phosphorus treatment. This response was also observed in the above-ground biomass which increased as phosphorus availability increased and with AM fungi (Figure S2a). Furthermore, plant root:shoot ratios decreased as phosphorus availability increased (Figure S2c), an effect mostly driven by the phosphorus-driven increases in above-ground biomass. These responses were also clear in the MGRs where plant growth responses to AM fungi were on average 24% and 30% under low or medium phosphorus, respectively, but MGR was −4% under high phosphorus (Figure 5b). Plant phosphorus concentration followed a similar response pattern to treatments, where phosphorus was

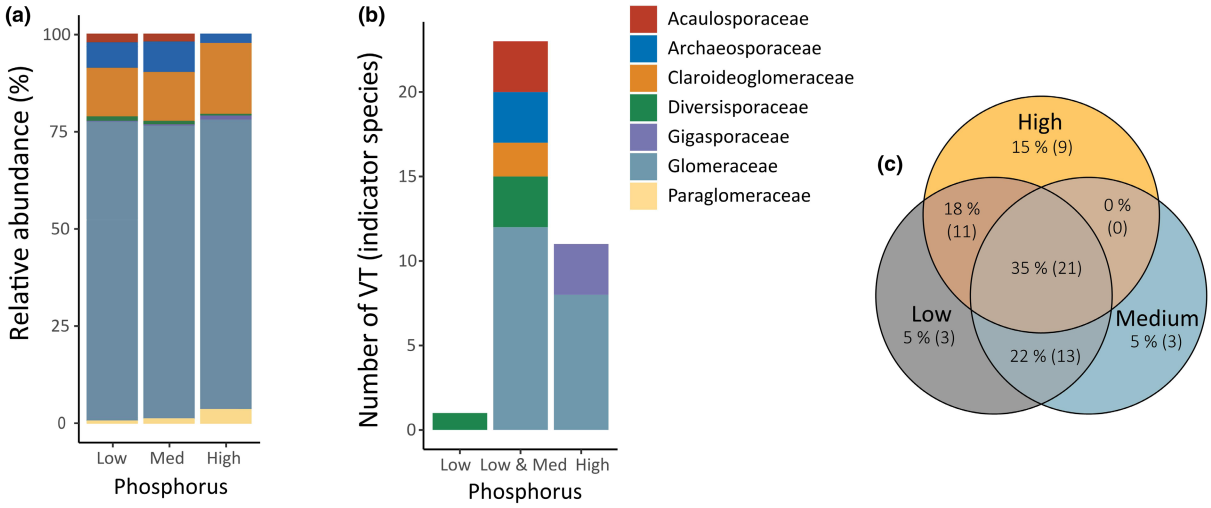

FIGURE 2 (a) Relative abundance of arbuscular mycorrhizal (AM) fungal families in roots of *Sorghum bicolor* under three phosphorus treatments, (b) number of AM fungal virtual taxa (VT) which are indicator species closely associated with either low, low and medium, or high phosphorus treatments. (c) Venn diagram showing the number and proportion (%) of AM fungal VT shared and unique between the three phosphorus treatments.

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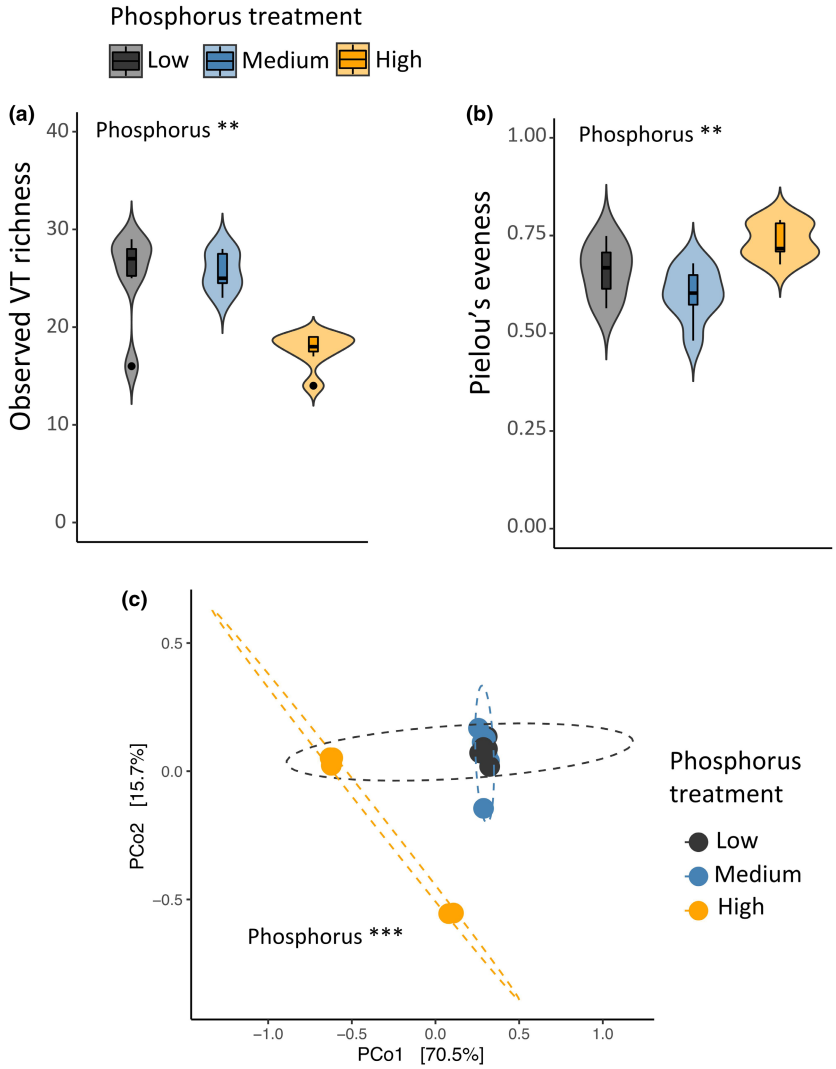

FIGURE 3 Violin plots showing the effects of the three phosphorus treatments on the (a) virtual taxon (VT) richness and (b) community evenness (Pielou's evenness index) of root-colonising arbuscular mycorrhizal (AM) fungal communities. The width of the violin represents data densities while the embedded boxplot represents the median, 25% and 75% quantiles. (c) Principal coordinate analysis (PCoA) of beta diversity (Bray–Curtis dissimilarity) comparing the structure of AM fungal communities under the three phosphorus treatments, associated ellipses represent 95% confidence intervals. Significant effects of phosphorus are shown $^{**}p<0.01$, $^{***}p<0.001$.

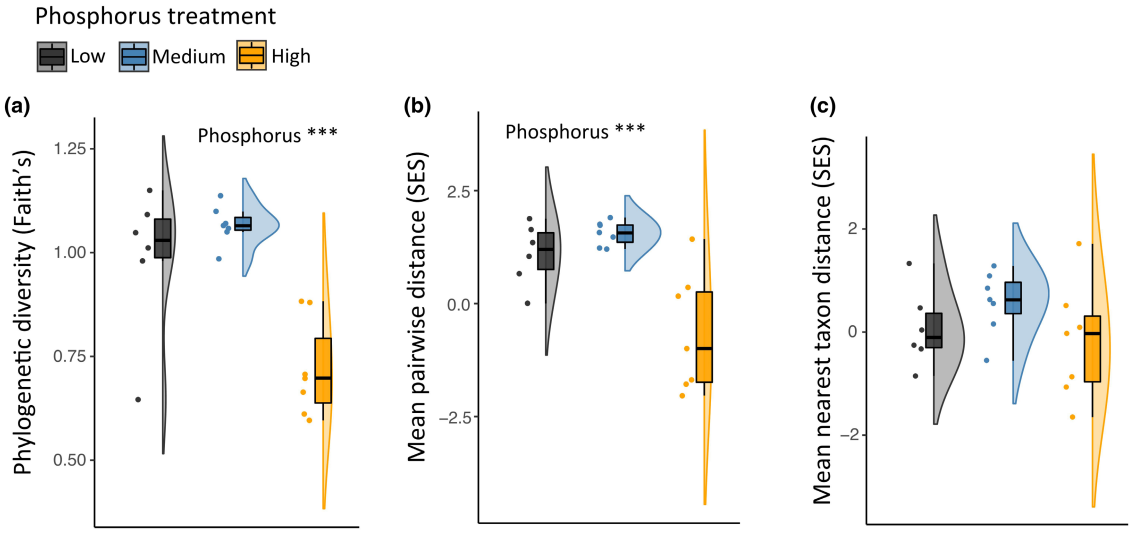

FIGURE 4 Raincloud plots showing the effects of the phosphorus treatments on the (a) phylogenetic diversity (Faith's), (b) mean pairwise distance (standardised effect sizes) and (c) mean nearest taxon distance (standardised effect sizes) of root-colonising arbuscular mycorrhizal fungal communities of *Sorghum bicolor* roots. The width of the violin represents data densities while the boxplots represent the median, 25% and 75% quantiles. Significant effects are shown $^{***}p<0.001$.

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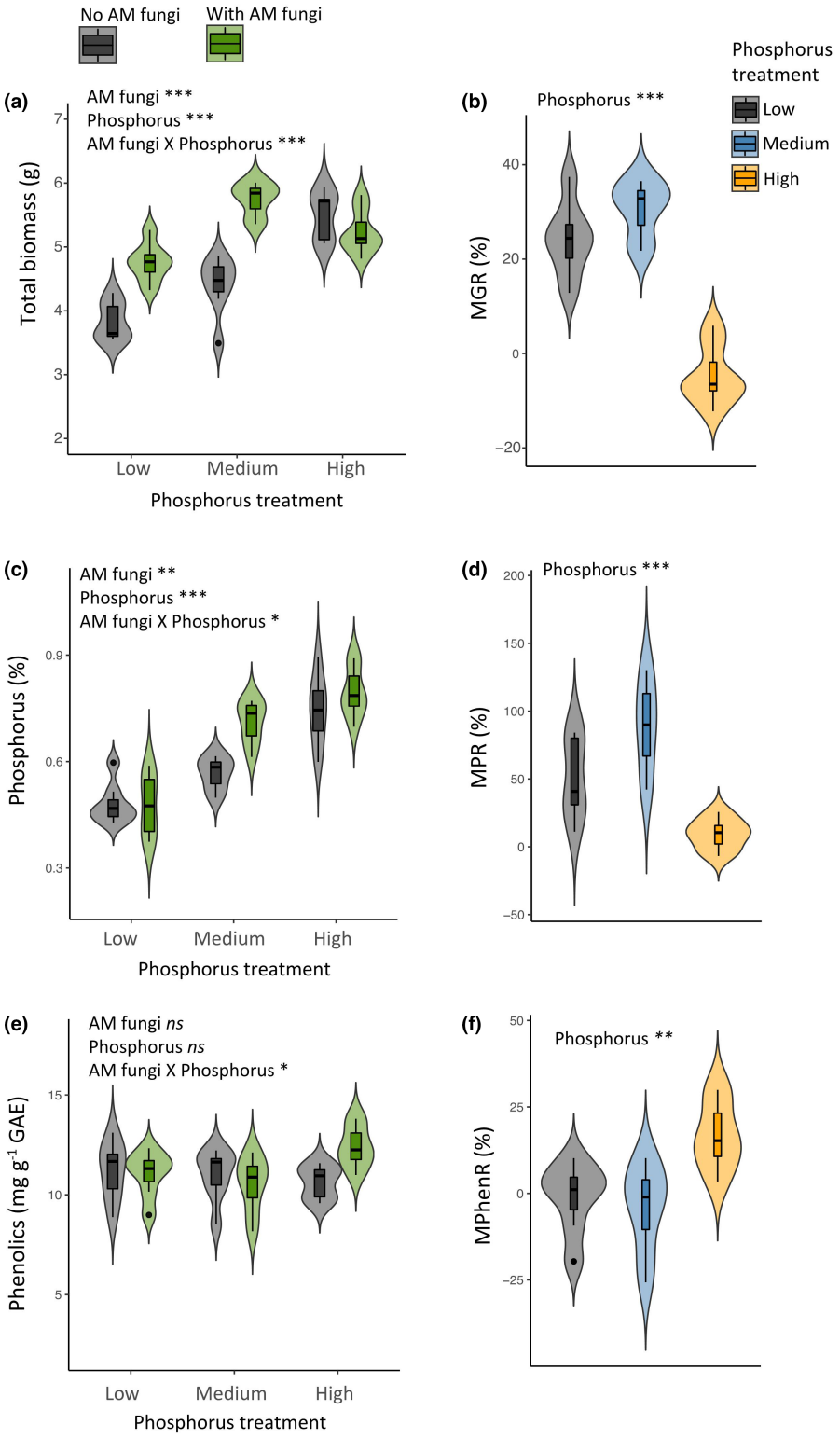

FIGURE 5 Violin plots showing the effects of phosphorus treatments on the (a) total biomass (g), (c) foliar phosphorus concentration (%), and (e) foliar phenolic concentrations (mg g^{-1} gallic acid equivalent) of *Sorghum bicolor* grown with or without an AM fungal community. Violin plots also showing the effects of the phosphorus treatments on the (b) mycorrhizal growth responses (%), (d) mycorrhizal phosphorus responses (%) and (f) mycorrhizal phenolic responses (%). The width of the violin represents data densities while the boxplots represent the median, 25% and 75% quantiles. Significant effects are shown $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

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promoted by both phosphorus availability and AM fungi (Figure 5c). The mycorrhizal phosphorus responses (MPRs) were 82% and 90% lower under high phosphorus treatment, compared to plants with low or medium phosphorus respectively (Figure 5d). There were no significant main effects of AM fungi or phosphorus on plant total phenolic concentrations, however, phenolics increased in response to AM fungi, but only under high phosphorus (Figure 5e). This effect was particularly apparent in mycorrhizal phenolic response (Figure 5f), where AM fungi increased phenolics by more than 16% under high phosphorus.

Total AM fungal colonisation by AM fungi in the AM fungal treated plants ranged from 34% to 68% (Figure S4a), while the plants without AM fungi were entirely absent of any AM fungal colonisation. The total colonisation of roots by AM fungi was significantly affected by phosphorus treatments. Interestingly, overall colonisation (comprising hyphal, arbuscular and vesicular) was greatest under high phosphorus, while it was 65% and 24% lower under the low and medium phosphorus treatments respectively (Figure S4). Furthermore, total colonisation decreased with phylogenetic diversity (Figure 6a), while vesicular colonisation tended to decrease, this was not significant (Figure 6c). In contrast, arbuscular colonisation significantly increased as phylogenetic diversity of the fungal communities increased (Figure 6b).

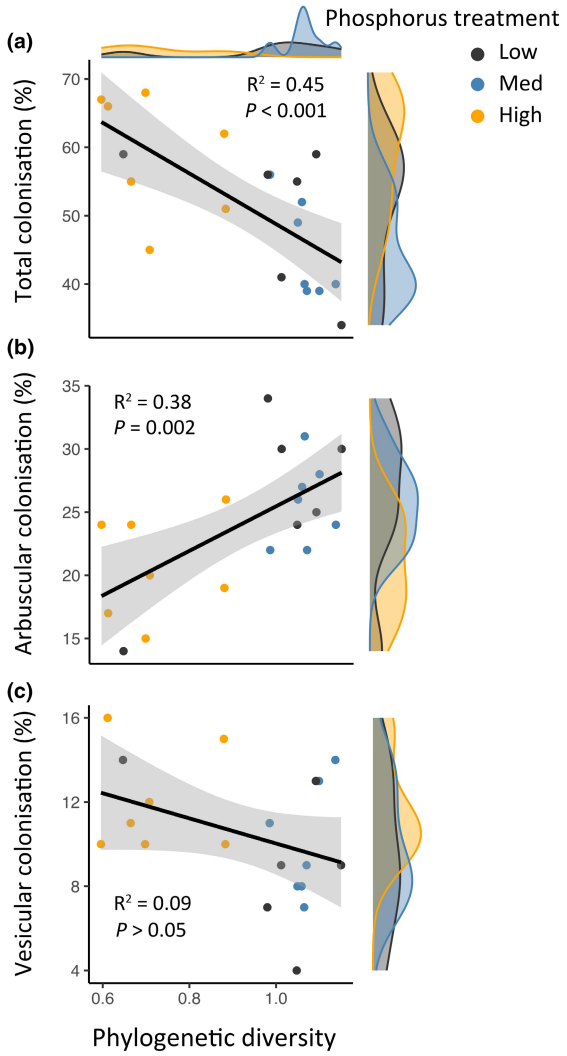

FIGURE 6 Relationship between phylogenetic diversity (Faith's) and (a) total colonisation (%), (b) arbuscular colonisation (%) and (c) vesicular colonisation by arbuscular mycorrhizal fungi of *Sorghum bicolor* under three different phosphorus treatments. Solid lines represent linear regression through all points and shaded areas represent 95% confidence intervals, density plots indicate data densities of the *x*-axis (phylogenetic diversity) and *y*-axis (colonisation). Coefficient of determination (R^2) and *p* values are shown.

4 | DISCUSSION

Our findings are consistent with a community assembly process dominated by host filtering towards AM fungal communities that provide defence-related benefits as P availability increases (hypothesis B). We observed that the fungal communities in plant roots became less diverse and more closely related (phylogenetically clustered) under high P conditions. This effect was coupled with a reduction in arbuscular colonisation and a concurrent loss of any mycorrhizal growth and P benefits for the host plant. At the same time, we also observed an increase in total AM fungal colonisation of roots under high P along with an increase in defence-associated chemistry, phenolics. These patterns suggests that, while plants may lose some of the benefits provided by AM fungi in terms of growth and nutrient uptake under high P, they still maintain symbioses with fungal taxa that may provide other functions such as defence and stress resistance.

Our results revealed that altering P availability leads to significant changes in the diversity of AM fungi in plant roots. In most cases, fungal communities under high P were distinct, while the fungal communities under low and medium phosphorus were comparatively similar. It is typical that an increase in P availability will decrease plant reliance on the AM symbiosis (Johnson et al., 2015; Smith & Read, 2008b), however, the threshold for such effects on root-colonising communities remains uncertain and is likely to be highly specific to the host plant and environmental context (e.g. light conditions, soil pH, temperature, soil nutrient profile). Under high P there was a reduction in VT richness and an increase in community evenness such that most taxa were represented in relatively similar abundances. In contrast, communities under low and medium P harboured more VT with relatively low abundances.

The distinct nature of the AM fungal communities under high P treatment was also reflected in the phylogenetic diversity and structure. Faith's phylogenetic diversity was reduced and mean pairwise distances were significantly lower compared to the communities under the other P treatments. This suggests that AM fungal species in the high P treatment were more closely related to each other (clustered) on average than the fungal taxa within communities under the other treatments. Typically, this community clustering is understood to indicate that environmental filtering is a dominant driver of community assembly, as opposed to competitive exclusion

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which is expected to increase dispersion (Pausas & Verdú, 2010; Webb et al., 2002). In this case, in support of our hypothesis B, the environmental filter driving community clustering under high P is likely to be host selectivity for AM fungi, as the plants possibly maintained symbioses with fungi based on function. This low contribution of interspecific AM fungal competition relative to host effects in driving community assembly of AM fungi is congruent with empirical studies that show that the intensity of competition among AM fungal species is not influenced by P availability to the host (Engelmoer et al., 2014).

Most theories on community assembly processes have been tailored to free-living organisms where there is clearer distinction between abiotic environmental factors and biotic interactions driven by competition (Aguilar-Trigueros et al., 2017). However, in the case of symbiotic species such as AM fungi, this distinction is blurred by host responses. Among free-living organisms changes in resource supply (like in our case for P) directly determine the occurrence and outcome of competition (Goldberg et al., 2017). In contrast, for the symbiotic AM fungi, the effect of the host is more important than resource supply alone. Our study stresses the importance of considering host responses rather than abiotic factors alone in explaining shifts in AM fungal communities (Vályi et al., 2016), and the same should extend to other symbiotic interactions.

While the overall relative abundances of fungal families across all three treatments were heavily dominated by Glomeraceae, there were distinct differences in particular groups, as highlighted by the different indicator taxa for each of the treatments. Even though only one AM fungal VT was found to be an indicator of the low P treatment alone, 23 indicator VT shared their strong association with both the low and medium P treatments. These VT comprised of five fungal families, on the other hand, indicator VT for the high P treatment were restricted to the Glomeraceae and Gigasporaceae. Glomeraceae are typically considered ruderal, with high hyphal turnover and low biomass allocation to extraradical hyphae (Alguacil et al., 2010; Verbruggen & Kiers, 2010). Furthermore, taxa from this family are also considered to be particularly effective at enhancing plant resistance (Frew et al., 2022; Sikes et al., 2009). Indeed, some indicator taxa of the high P treatment (VT248 and VT125) have previously been associated with stress resistance (Downie et al., 2008; Wang et al., 2021; Yamato et al., 2009). This too would support the hypothesis that hosts partner with AM fungi where the cost–benefit of the partnership remains advantageous within the specific environmental context. In this instance, plants are likely to have selected AM fungi that were effective at providing functions not necessarily related to P uptake.

It is also worth noting that we also found Gigasporaceae taxa to be indicative of high P, even though their overall relative abundance was comparatively low. These fungi are typically considered to be competitive AM fungi, efficient at acquiring plant carbon, exhibiting slow growth, delayed sporulation and high investment in extraradical hyphae to acquire nutritional resources (Chagnon et al., 2013; Hart & Reader, 2002, 2005; Maherali & Klironomos, 2007; Staddon et al., 2003). As such, we might have expected Gigasporaceae to be indicative of high P based on our hypothesis A. That said, our two hypotheses are not necessarily mutually exclusive, as competitive taxa may still persist and host plants may associate with them due to their efficiency as partners providing nutrients. While we presented two alternative hypotheses, aspects of both theories may be simultaneously true in nature, where a host may select for functional types while competition is active within communities. The relative importance of these processes of assembly will also be dynamic, and shift with time and environmental context.

Plant biomass allocation above-ground increased with P availability, while the root:shoot ratios decreased, suggesting a shift in biomass investment from below-ground to above-ground as soil P became more abundant. Under the low and medium P treatments, plants acquired growth and P uptake benefits from the AM fungal associations. However, these benefits were entirely lost in high P environments, where taxonomic and phylogenetic diversity were lowest and negatively correlated with arbuscular colonisation of plant roots. Arbuscules are typically considered to be the key sites for resource exchange between host plant and the fungi, thus this may be related to the outcomes we observed in terms of plant growth and nutrient responses to AM fungi. Perhaps counterintuitively, the total fungal colonisation (inclusive of hyphae, arbuscules and vesicles) was greatest under the high P treatment. This may be associated with the AM fungal-induced increase in phenolic compounds we observed. Several studies across different systems have demonstrated that AM fungi can increase plant phenolics (Ceccarelli et al., 2010; Jung et al., 2012; Schweiger & Müller, 2015) and how this effect can vary according to fungal composition (Frew & Wilson, 2021). While phenolic compounds are diverse in their functions, many are important plant defences including flavonoids and tannins (Mithöfer & Boland, 2012; Schweiger & Müller, 2015). Thus, it is probable that the increase in phenolics we observed in high P conditions was an outcome of the distinct fungal community which had colonised plant roots. This community produced less nutrient exchange-related structures (i.e. arbuscules), but increased hyphal colonisation, possibly indicative of their nonnutrient-related functions. Mycorrhiza-induced resistance is expected to be more pronounced when plants are challenged by a herbivore or pathogen (Cameron et al., 2013), therefore, it would be revealing to build on our findings here and experimentally examine how P-driven changes in AM fungal community assembly shape mycorrhiza-induced resistance to herbivore attack.

A further point to consider is the role of other soil microbes in the outcomes of the AM symbiosis for host plants, something which is becoming increasingly recognised (Jiang et al., 2021; Xu et al., 2023). In our case, although the non-AM fungal microbial communities were standardised at the initiation of the experiment, these microbes are likely to have complex interactions with the AM fungal communities. Such interactions may influence the community assembly of AM fungi in plant roots, and, consequently, their functioning in plant nutrient uptake and defence chemistry. Thus, to obtain a more comprehensive understanding of the ecology of

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AM fungal community assembly, future work should look to evaluate the influence of other soil microbial communities in these processes.

5 | CONCLUSIONS

As we delve deeper into the complexities of the relationship between AM fungi and the ecosystems they inhabit, both natural and cultivated, one of the main obstacles we face is understanding not only how these communities are assembled, but also the consequences of these assemblies. Our study found that high levels of soil P led to a reduction in the diversity and a phylogenetic clustering of AM fungal communities, resulting in a loss of benefits relating to plant growth and P uptake. However, this decrease in diversity was coupled with an increase in the chemical defences. Our findings suggest that under high P conditions, the host plant alters selectivity of fungal functional groups, prioritising those associated with enhancing plant stress resistance and defences. Our research illustrates the way in which soil P status can shape the diversity, both taxonomically and phylogenetically, and community assembly of AM fungal communities and their corresponding impact on the symbiotic relationship.

AUTHOR CONTRIBUTIONS

Adam Frew designed the study and collected the data. Adam Frew and Carlos A. Aguilar-Trigueros analysed the data. Adam Frew, Carlos A. Aguilar-Trigueros and Meike Katharina Heuck interpreted the data and outcomes. Adam Frew wrote the first draft of the article, Meike Katharina Heuck and Carlos A. Aguilar-Trigueros revised the article, and all authors contributed critically to final draft and provided approval for publication.

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CONFLICT OF INTEREST STATEMENT

Adam Frew is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper. The authors confirm there is no other conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available from the FigShare repository at https://doi.org/10.6084/m9.figshare.22085435. Raw DNA sequencing data are available under the NCBI accession number PRJNA932842.

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SUPPORTING INFORMATION

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

Figure S1. Neighbour-joining phylogenetic tree of arbuscular mycorrhizal fungal virtual taxa detected in roots across all phosphorus treatments.

Figure S2. Violin plots showing the effects of the three phosphorus treatments on the (a) aboveground biomass (g), the (b) belowground biomass (g), and the root:shoot ratio of *Sorghum bicolor* grown with or without arbuscular mycorrhizal fungi.

Figure S3. Violin plots showing the effects of the three phosphorus treatments on the (a) carbon:nitrogen ratio and the (b) nitrogen:phosphorus ratio of *Sorghum bicolor* grown with or without arbuscular mycorrhizal fungi.

Figure S4. Violin plots showing the effects of three phosphorus treatments on the (a) total mycorrhizal fungal colonisation (b) arbuscular colonisation, and the (c) vesicular colonisation of *Sorghum bicolor* roots.

Table S1. Nutrient analysis of source soil/sand substrate. Analysis by CSBP Soil & Plant Analysis Laboratory, WA, Australia.

Table S2. List of virtual taxa (VT) of arbuscular mycorrhizal fungi showing total sequence abundance (after rarefaction) of each VT across treatments.

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Table S3. Model results for the effects of arbuscular mycorrhizal fungi and phosphorus treatments, and their interaction, on plant total biomass, aboveground biomass, belowground biomass, root:shoot ratios, along with phosphorus, nitrogen, and phenolic concentration, C:N and N:P.

Table S4. Indicator virtual taxa of arbuscular mycorrhizal fungi in plants under one of three phosphorus treatments of 'low', 'medium', or 'high'.

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