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RESEARCH ARTICLE

Causal determinism by plant host identity in arbuscular mycorrhizal fungal community assembly

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Abstract

1. An assumption in ecology is that plant identity plays a central role in the assembly of root-colonising arbuscular mycorrhizal (AM) fungal communities. While numerous correlational studies support this notion, with evidence of host selectivity among fungal taxa and host-specific responses to different AM fungi, empirical demonstrations of host-driven AM fungal community assembly remain surprisingly limited.
2. We conducted a factorial experiment growing two globally significant crop species, wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*), with a common pool of AM fungal species, or without AM fungi. We hypothesised strong differences in AM fungal community structure between the two species driven by strong habitat filtering. Plants were harvested at two time points in which we analysed the community structure of AM fungi in the roots, the phylogenetic diversity, and interactions with plant physiological responses.
3. As we expected, there were distinct trajectories in both the composition and phylogenetic diversity of AM fungal communities between the two host plants through time. However, the effect of habitat filtering during community assembly differed between the two species. In sorghum roots, AM fungal communities exhibited increased richness and became more phylogenetically clustered over time. This shift suggests that community assembly was primarily driven by habitat filtering, or selectivity, imposed by the host which was accompanied by significant increases in plant mycorrhizal growth (from 11.83% to 43.67%) and phosphorus responses (from −0.6% to 43.3%). In contrast, AM fungal communities in wheat displayed little change in diversity, remained phylogenetically unstructured, and

provided minimal benefits to the host, indicating a more stochastic assembly process with a stronger influence of competitive interactions.
4. As the field looks to understand what determines the distribution of AM fungi and their community composition while simultaneously seeking to utilise AM fungi for ecosystem benefits, it is important to know the extent to which host identity can influence fungal assembly within plant roots. Our results provide empirical support of host-determinism in AM fungal community assembly and suggest that this determinism is associated with the growth and nutrient benefits provided by the symbiosis to plants.

KEYWORDS
arbuscular mycorrhiza, environmental filtering, fungal community, host selection, plant roots, symbiosis

1 | INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are essential components of terrestrial ecosystems, establishing symbiotic relationships with the roots of most plant species and playing a pivotal role in plant productivity, nutrient cycling and soil structure (Powell & Rillig, 2018; Smith & Read, 2008). The composition and structure of AM fungal communities are influenced by the plant communities they inhabit, which in turn have strong effects on plant health and ecosystem functionality (Helgason et al., 2007; Wagg et al., 2011, 2014). Although the mutual influences between plant hosts and AM fungal communities are well-recognised, the importance and magnitude of these interactions during community assembly—the temporal process by which ecological factors shape the species present in a local community—remain poorly defined (Zobel & Öpik, 2014). On the one hand, AM fungi are generalists and, as such, the same AM fungal taxa are found across multiple host species (Davison et al., 2015; Smith & Read, 2008). On the other hand, a central assumption in their ecology is that the identity of the host plant acts as a major selective force in the assembly of AM fungal communities within plant roots (Davison et al., 2011; Martínez-García et al., 2015; Zobel & Öpik, 2014).

Researchers in plant and mycorrhizal ecology typically expect a strong effect of plant identity—whether determined by genotype, species or functional group—on the composition of AM fungal communities within the roots. This expectation is based on multiple lines of evidence including many studies showing host-specific responses to AM fungi (e.g. Cheeke et al., 2019; Frew, 2019), seminal studies on host-specific effects on AM fungal sporulation (Bever, 2002), differential carbon allocation from a host to different AM fungal species (Kiers et al., 2011) and from different hosts to an AM fungus (Elliott et al., 2021), specific effects on soil microbial community structure in plant–soil-feedback experiments (Revillini et al., 2016), and observational field studies (Davison et al., 2011; Martínez-García et al., 2015). Different degrees of plant species selectivity have been demonstrated using trap-cultures (Řezáčová et al., 2016), as well as comprehensive sampling campaigns (Sepp et al., 2019). Yet, experiments explicitly testing if and to what extent host species trigger distinct assembly processes on root-colonising AM fungal communities are lacking.

Determining the role of host identity on AM fungal community assembly is complex given the multiple evolutionary and ecological pressures at play (Koch et al., 2017). As obligate symbionts, AM fungi rely exclusively on host plants for carbon access, provided in the forms of sugars and lipids (Smith & Read, 2008). However, the degree of plant dependency on the AM fungal symbionts varies significantly among host taxa and environmental conditions, such as soil fertility, pH and water availability (Hoeksema et al., 2010). As the total amount of carbon that plants allocate to AM fungi can be substantial (Hawkins et al., 2023), we might expect evolutionary pressures on plants to develop selectivity towards AM fungal taxa that return the highest benefits, depending on the environmental context. Such selectivity, however, is at odds with evolutionary pressures on AM fungi, which would benefit from maintaining generalist traits to derive carbon from a wide array of potential hosts. This preferential partnering or allocation of resources has been explored at various scales (Magkourilou et al., 2024). In vitro experiments have shown that plants can preferentially allocate carbon to fungi that deliver more nutrients under specific conditions (Kiers et al., 2011). Pot studies on crop plants, such as sorghum, have found AM fungal communities become increasingly dominated by closely related taxa through time (Frew & Aguilar-Trigueros, 2024), suggesting a degree of selectivity. Furthermore, the nitrogen and phosphorus benefits a host receives from the AM symbiosis can be fungal species-specific (de Souza Campos et al., 2021; Walder et al., 2012) and different crop cultivars have been found to allocate varying amounts of carbon to their AM fungal partners (Elliott et al., 2021).

In natural systems, factors such as dispersal mechanisms and environmental variations significantly influence the extent that plant hosts dictate the community structure of AM fungi (Van Geel et al., 2018). For instance, certain AM fungal species may

be absent from otherwise suitable hosts due to constraints in dispersal ability (Chaudhary et al., 2020; García de León et al., 2016), limiting the diversity of AM fungi accessible to those hosts. Conversely, AM fungi may establish in a plant principally due to efficient and effective dispersal from spatially adjacent hosts. Additionally, environmental conditions alter the mutualistic benefits derived by hosts, such as when soil fertility is high (Riley et al., 2019), consequently this may reduce the host's selectivity towards these symbiotic partners.

Considering these complexities, our strong reliance on correlational observations to understand host identity effects on AM fungal community assembly in roots presents certain challenges. There is no doubt that such studies offer valuable insights and do so often by using advanced inferential statistical methods, yet their ability to separate host identity effects from dispersal effects or other environmental filtering factors remains limited (König et al., 2010; Lekberg & Waller, 2016; Šmilauer et al., 2020; Van Geel et al., 2018).

Given the fundamental nature of this topic, we require more experimental data from explicit studies focussed on inferring AM fungal community assembly in plant roots to validate many assumptions in the field. Building on recent observations of strong phylogenetic clustering in root-colonising fungal communities (Frew & Aguilar-Trigueros, 2024), we aimed at quantifying the importance of host plant species identity during the assembly of root-colonising AM fungal communities, controlling for dispersal and other factors. For this, we used two host plants of global agricultural significance, wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*), two of the top five most important cereal crops (FAO, 2024). Specifically, we track the trajectory of the same pool of AM fungal taxa exposed to the two contrasting hosts through time. This temporal aspect is crucial because community assembly is not instantaneous but a time-dependent phenomenon (HilleRisLambers et al., 2012). We hypothesised that, at earlier stages, AM fungal communities in roots will resemble each other regardless of host identity, as the symbiosis may not yet have stabilised with hosts yet to discriminate which AM fungal taxa best fit their metabolic needs. At later stages, we expect higher fungal community dissimilarity between the two hosts as a result of strong host-driven habitat filtering. Finally, we hypothesised that, despite differences in the resulting community structures, the host-selected communities will confer a nutritional benefit to each host.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

A factorial greenhouse experiment was conducted to explore the assembly of root-colonising AM fungal communities in two plant species, wheat (*Triticum aestivum* L. cv. 'Raider') and sorghum (*Sorghum bicolor* L. Moench cv. 'MR Taurus'). The experiment comprised of three factors, each with two levels: host plant species (wheat or sorghum), AM fungi (with or without) and time of harvest ('early' at 4 weeks or 'late' at 9 weeks post germination).

Plants were grown in a homogenised mix of sandy loam soil and washed river sand (30: 70) that underwent gamma irradiation (50 kGy). For plants grown with an AM fungal community, this soil: sand mix was then fully mixed with a soil inoculum (10%). This inoculum was composed of field soils collected from agricultural and non-agricultural sites in Queensland and New South Wales, Australia, over a 12-month period. The soils were air-dried and sieved after field collection, they were then stored with silica gel until use. The soil inoculum was generated from a mix of four of these soils that were previously observed to harbour high AM fungal diversity to maximise the pool of potential root-colonising AM fungal taxa. The diversity and composition of the AM fungal community in this soil inoculum (Figure 1a) was characterised by high-throughput amplicon sequencing as described below. For the "without AM fungi" treatment, the sterile soil:sand mix was fully mixed with autoclaved soil inoculum (10%). All pots were supplemented with 200 mL of microbial liquid filtrate derived from washing the soil inoculum filtered through sieves down to 20 μm to standardise the non-AM fungal microbial community across all pots. An initial 10 g dose of low-phosphorus fertiliser, Osmocote Native Controlled Release Fertiliser (The Scotts Company LLC), was mixed through for all pots. Seeds of both wheat and sorghum were surface sterilised with a 10% diluted commercial bleach solution (4% sodium hypochlorite), germinated for 6 days in sterile Petri dishes, and then transplanted into 3.7 L pots filled with the respective soils as described (either with or without AM fungi).

At the start of the experiment, for both early and late harvests, wheat and sorghum without AM fungi had 11 replicates each, and those with AM fungi had 13 replicates. The eventual number of replicates among treatments varied due to differences in germination success and early seedling losses. For plants without AM fungi, both wheat and sorghum had 11 replicates for early and late harvests. For plants with AM fungi: sorghum had nine replicates at the early harvest and 12 at the late harvest; wheat had 13 replicates at the early harvest and 10 at the late harvest. Plants were watered as required and soil moisture was monitored every 3 days with a soil moisture meter (PMS-714, Lutron Electronics, PA USA), to maintain consistent conditions across all pots. The greenhouse had day/night temperatures of 27 and 16°C, respectively, with a 13 h daylength. Pots were randomly rearranged within the greenhouse every 2 weeks to mitigate any spatial effects.

Plants were harvested at the designated early (4 weeks) and late (9 weeks) harvest timepoints. These time points were selected to provide insight at an early and late stage of vegetative development for both crops, prior to either plant flowering. Root samples were processed where a 1 g subsample was taken and stored in 50% ethanol prior to mycorrhizal fungal colonisation assessment, and the remaining above- and below-ground plant tissues were all dried at 38°C (Hart et al., 2015). Biomass was recorded and all above-ground tissue was ground and homogenised prior to any chemical analyses, while dried root tissue was retained for downstream DNA extractions and sequencing.

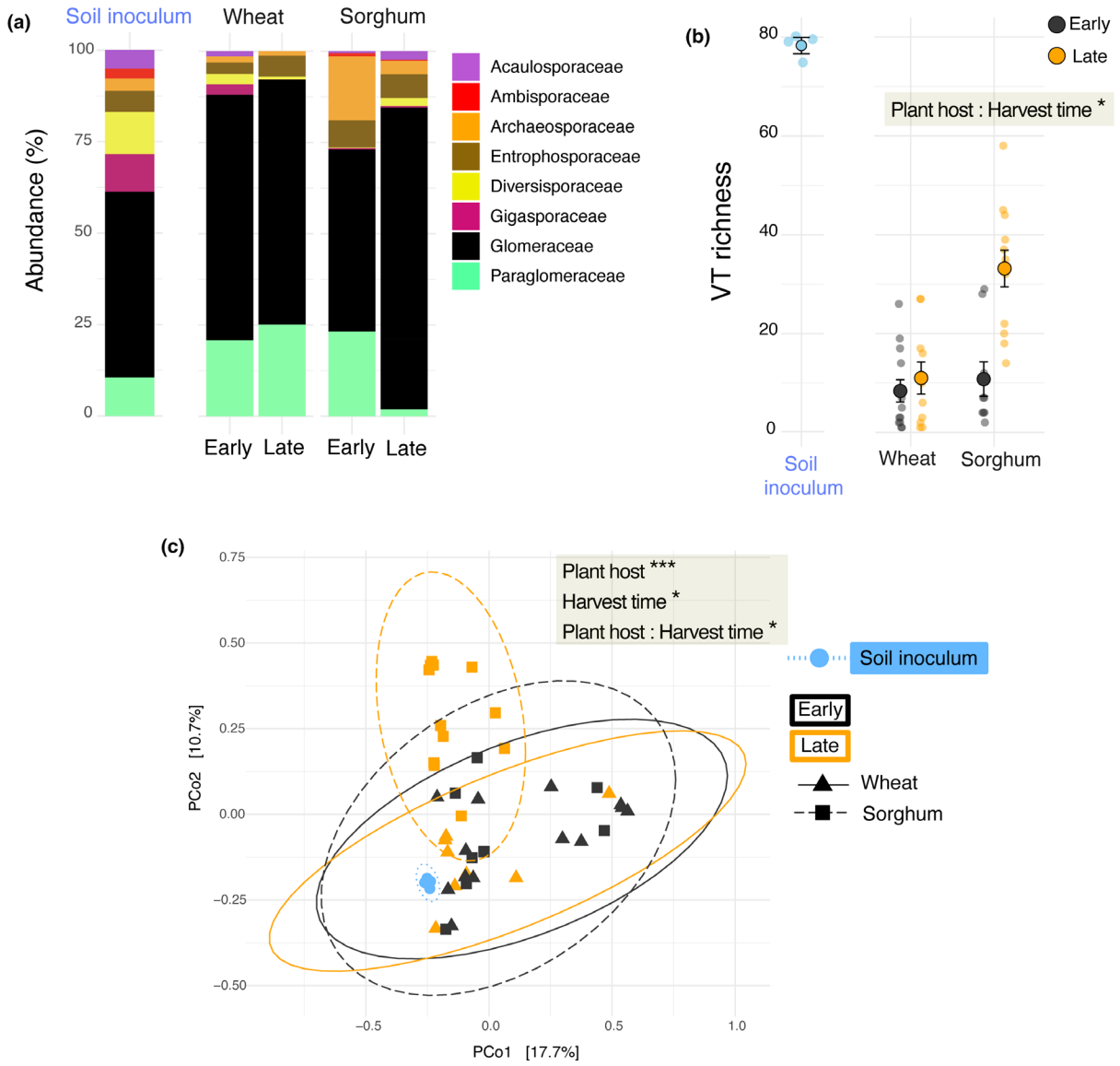

FIGURE 1 Communities of arbuscular mycorrhizal (AM) fungi in the soil inoculum, and in roots of wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*). Communities in the soil inoculum are shown for visual contrast purposes but were not included in statistical analyses. (a) Relative abundances (%) of AM fungal families and the (b) AM fungal virtual taxa (VT) richness in the soil inoculum, and in the roots of wheat and sorghum at the early and late harvest times, the solid points and error bars represent the mean ± SE, which are overlaid on top of the raw data points. Significant effect shown $^{*}p < 0.05$. (c) Principal coordinate analysis (PCoA) of beta diversity (Bray–Curtis dissimilarity) comparing AM fungal communities in the initial inoculum, and in wheat and sorghum at the early and late harvest times. Each point on the PCoA represents a community of AM fungi, the associated ellipses represent 95% confidence intervals and the proportions of variation explained by each axis (PCo1 and PCo2) are shown. Significant effects are shown $^{*}p < 0.05$, $^{***}p < 0.001$.

2.2 | Chemical analyses and fungal colonisation

Phosphorus (P) concentrations in plants were assessed via inductively coupled plasma (ICP) spectroscopy following digestions with nitric acid (Zarcinas et al., 1987). Carbon (C) and nitrogen (N) concentrations were determined using the high-temperature combustion method on a LECO elemental analyser. For assessing mycorrhizal fungal colonisation, ethanol-stored root samples were cleared with 10% KOH at 90°C for 15 min, stained with 5% ink-vinegar at 90°C for 20 min. Root fragments were mounted on glass slides with glycerine under a cover slip and microscopically examined using the intersect method at 200× magnification for 100 intersections per sample (McGonigle et al., 1990).

2.3 | Amplicon sequencing and bioinformatics

DNA was extracted from 70 mg of dried root samples using the DNeasy Powersoil Pro Kit (Qiagen, GmbH). Sequencing was processed through the Western Sydney University's Next-Generation Sequencing Facility's liquid handling pipeline. The DNA was purified using the Agencourt AMPure XP Beads (Beckman

Coulter), followed by a quality assessment using the Quant-iT™ PicoGreen fluorescence-based analysis (ThermoFisher Scientific). The purified DNA then underwent amplification using polymerase chain reaction targeting the small-subunit ribosomal RNA gene (Lekberg et al., 2018) using AM fungal-specific primers WANDA and AML2 (Dumbrell et al., 2011; Lee et al., 2008).

Raw reads (2×1,894,886 reads in total) were demultiplexed and cleaned using a series of bioinformatic steps (see Vasar et al., 2017, 2021). Briefly, 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 2×1,003,029 cleaned reads). Putative chimeric sequences (12,214; 1.2% of cleaned reads) were identified and removed using *vsearch* v2.15.0 (Rognes et al., 2016) with the default parameters referencing against the Maarj*AM* database (2021 release; Öpik et al., 2010). Cleaned and chimera-free AM fungal sequences, and their taxonomies, were identified using BLAST+ (v2.7.1, Camacho et al., 2009) where each individual read was identified in comparison to the Maarj*AM* database (Öpik et al., 2010) as a reference using the nucleotide local pairwise alignment *BLASTN* algorithm. This identified sequences to phylogenetically defined taxonomic units called virtual taxa (VT) 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).

2.4 | Statistics

All analyses were conducted using R version 4.3.3 (R Core Team, 2024). For our 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) prior to downstream analyses. To assess differences in VT richness in plant roots between the two plant species and the two harvests, we fitted linear models using *lm* from base R, and *Anova* (type = III) from the 'car' R package. Dissimilarity in the composition and structure of root-colonising AM fungal communities were visualised by principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity of communities using the *ordinate* function from the 'Phyloseq' package (McMurdie & Holmes, 2013). To test for the effects of plant host and the time of harvest on Bray-Curtis dissimilarities, we used permutational multivariate ANOVA (perMANOVA) using *adonis2* from the R package 'vegan' (Oksanen et al., 2015).

To investigate potential differences in the community assembly processes between the two hosts, we calculated the phylogenetic diversity and structure of AM fungal communities. We used the mean pairwise distance (using the *mpd* function), measuring the mean phylogenetic distance between all VT pairs in a community, and the mean nearest taxon distance (using the *mntd* function) to measure the distance between each VT and its nearest relative, all employing the 'picante' package (Kembel et al., 2010; Webb et al., 2002). We further calculated standardised effect sizes (SES), accounting only for VT presence and also abundance-weighted, by way of the *ses.mpd* and *ses.mntd* functions (Kembel et al., 2010). Higher values suggest phylogenetic overdispersion, whereas lower values imply phylogenetic clustering (Pausas & Verdú, 2010). We employed linear models and the *Anova* function from the 'car' package to determine how these metrics differed between plants and harvests (Fox & Weisberg, 2011).

To examine AM fungal associations with the host plant species across the two harvests we used the joint species distribution Bayesian framework, Hierarchical Modelling of Species Communities (HMSC: Tikhonov et al., 2020). This allowed us to model multiple VT simultaneously and account for phylogenetic relationships, interactions and hierarchical data structures. We applied a hurdle model, consisting of one occurrence model (Bernoulli distribution with probit link function) on the data truncated to presence-absence (non-zeros set to one, and zeros kept as zeros), and an abundance model (normal distribution) that modelled AM fungal VT abundances conditional on their presence. Only VT that were present in two or more samples were included. Our fixed predictors were host plant species and harvest time (both categorical with two levels), and their interaction, as well as the log-transformed sequencing depth (continuous variable). To account for the study design, we also included the random effect of pot number/sample. Model fitting was with the 'Hmsc' R package and the *Hmsc* function, assuming the default prior distributions (Ovaskainen & Abrego, 2020). We sampled the posterior distribution using four Markov Chain Monte Carlo (MCMC) chains, each producing 250 samples after a thinning of 1000 iterations. Each chain performed a total of 300,000 iterations, which includes a burn-in of 50,000 iterations per chain, culminating in 1,200,000 iterations across all chains. We examined the MCMC convergence by examining the potential scale reduction factors of model parameter (Figure S2; Gelman & Rubin, 1992). We applied variance partitioning to calculate the proportion of explained variance in VT occurrences attributed by each of our model predictors. We also evaluated posterior support for the beta parameters that describe individual AM fungal VT-level responses to each predictor in the models. Due to the interaction between host and harvest time predictors we fitted additional occurrence models for each host separately to more directly examine how much variation was attributable to harvest time in explaining AM fungal assembly between the two host plants.

To explore the effects of AM symbiosis on host plants at two time points, we fitted models for each plant response variable, assessing the fixed effects of host species, harvest time, and their interaction. We calculated the plant mycorrhizal growth response (MGR) and plant mycorrhizal P responses (MPR). These plant mycorrhizal responses were calculated as ([*plant response – mean plant responses with No AM*

fungi]/mean plant responses with no AM fungi) × *100*, where the plant response was either the total biomass or P concentration. MGR responses were analysed using permutation tests for linear models from the R package 'lmPerm' to handle the non-normal distribution of residuals (Wheeler & Torchiano, 2016). MPR was analysed using *lm* and *Anova* (type = III) from the 'car' package in R. Plant N:P ratios were calculated by dividing leaf nitrogen (N) by phosphorus (P) concentration. To explore the plant N:P responses to the AM fungal treatment and the plant hosts across the two time points, data were also analysed using *lm* and *Anova* (type = III) from the 'car' package in R.

3 | RESULTS

Communities of AM fungi across both plant hosts and harvest times were dominated by Glomeraceae taxon abundance, which was also the case for the communities in the initial soil inoculum (Figure 1a). Notably, this dominance increased in sorghum from the early to the late harvest time, coinciding with a decline in other fungal families such as Paraglomeraceae and Archaeosporaceae. Compared to the soil inoculum, plant root communities of AM fungi had lower richness. Among the AM fungal communities in roots, we observed high variation in VT richness across plants at both harvest times. The VT richness in wheat and sorghum were similar at the early harvest, and in wheat, this did not change by the late harvest. In contrast, the richness of communities in sorghum more than tripled from the early to the late harvest time (Figure 1b). Beta diversity exhibited a similar pattern where, initially, at the early harvest time, the structure of the root-colonising AM fungal communities in wheat and sorghum were similar; however, by the late harvest, significant shifts were observed but only in sorghum (Figure 1c; Table S2).

Similar distinct responses between sorghum and wheat were observed in terms of the AM fungal phylogenetic beta diversity. In the sorghum-colonising AM fungal communities, the SES for mean pairwise distance and nearest taxon distances exhibited significant decreases from the early to the late harvest times (Figure 2a,b). This same pattern was observed in the abundance-weighted mean pairwise distance (Figure 2c). In contrast to this, no significant changes in phylogenetic beta diversity were observed in the AM fungal communities in wheat roots (Figure 2).

Our HMSC models evaluated both occurrence (presence-absence) and abundance (conditional on presence) of AM fungal VT. The occurrence model demonstrated very good discrimination ability overall (Figure S3; mean area under the curve = 0.88, range 0.5–1.00), indicating it could accurately separate instances where taxa are likely to occur from those where they are not, based on our predictor variables (Fielding & Bell, 1997). The model attributed 46.3% of the explained variation to read depth, the interaction between plant host identity and harvest time explained 26.5%, while plant host alone explained 8.3% and harvest time explained 5.8% (Figure 3a). We found strong support for AM fungal VT specific responses, with the majority of VT present in roots having significantly positive associations towards sorghum at the late harvest time (Figure 3b). This was particularly evident for Glomeraceae and Acaulosporaceae taxa but was absent among Archeosporaceae and Paraglomeraceae VT. Indeed, these responses were significantly related to their phylogeny, with a moderate to high phylogenetic signal ($\rho = 0.57 \pm 0.16$). Our abundance model also had good support, with strong explanatory power (Figure S3; mean $R^2 = 0.83$, range 0.29–1.00). Harvest time alone explained 23.3% of the variation in AM fungal abundance, while the interaction between plant host identity and harvest time explained 13.1%, and host identity alone explained 6.6% (Figure S4). Our post-hoc occurrence models for individual host plants further supported the clear difference in AM fungal community assembly between the two hosts. Specifically, when modelling only the wheat AM fungal communities, harvest time accounted for 10% of explained variation, compared to 54.2% in the model for sorghum (Figure S5). Similarly, no AM fungal VT were significantly associated with the late harvest time in wheat, compared to 47 VT in sorghum (Figure S5).

Both plant hosts exhibited positive plant MGRs across both harvest times but this varied between hosts and harvest times (Figure 4a). Wheat showed consistent responses between harvest times, while sorghum exhibited increases of 11.83% to 43.67% in MGR and −0.6% to 43.3% in phosphorus mycorrhizal response from the early to late harvest (Figure 4a,b). Regarding the foliar N:P ratio responses, the presence of AM fungi reduced N:P in sorghum, particularly by the late harvest timepoint where we observed a 35.8% reduction in N:P in sorghum with AM fungi, compared to sorghum without AM fungi (Figure 4c). In contrast, wheat N:P was not affected by AM fungi, nor did it exhibit shifts between the two harvest times. There was also an associated shift in the allocation of plant biomass to below- and above-ground tissue where the root:shoot ratios of sorghum plants with AM fungi declined at the late harvest time (Figure S6). This was strongly driven by changes in the below-ground (root) biomass (Figure S6). In terms of the AM fungal colonisation of plant roots, wheat and sorghum at the early harvest had 14% and 18% colonisation, respectively. By the late harvest timepoint, this had increased to 24.9% in wheat and 53.5% in sorghum (Figure S6). No colonisation was observed in the plants without AM fungi.

4 | DISCUSSION

Community assembly begins with a regional species pool, often defined as the species that potentially could inhabit a local site or community (Zobel, 1997). Dispersal, abiotic factors and biotic interactions act as 'filters' that AM fungi must navigate to gain membership to the root colonising 'local' community. The potential for host identity to influence this assembly was well-known even prior to the widespread availability of amplicon sequencing (Bever et al., 1996). Accordingly, it is surprising that there are little experimental data quantifying the host identity effects on AM fungal community structure and the community assembly process behind those effects. Our study provides a novel experimental demonstration of differences in AM fungal community assembly through time between two globally significant crop species controlling for other environmental factors, and the associated plant phenotypic outcomes.

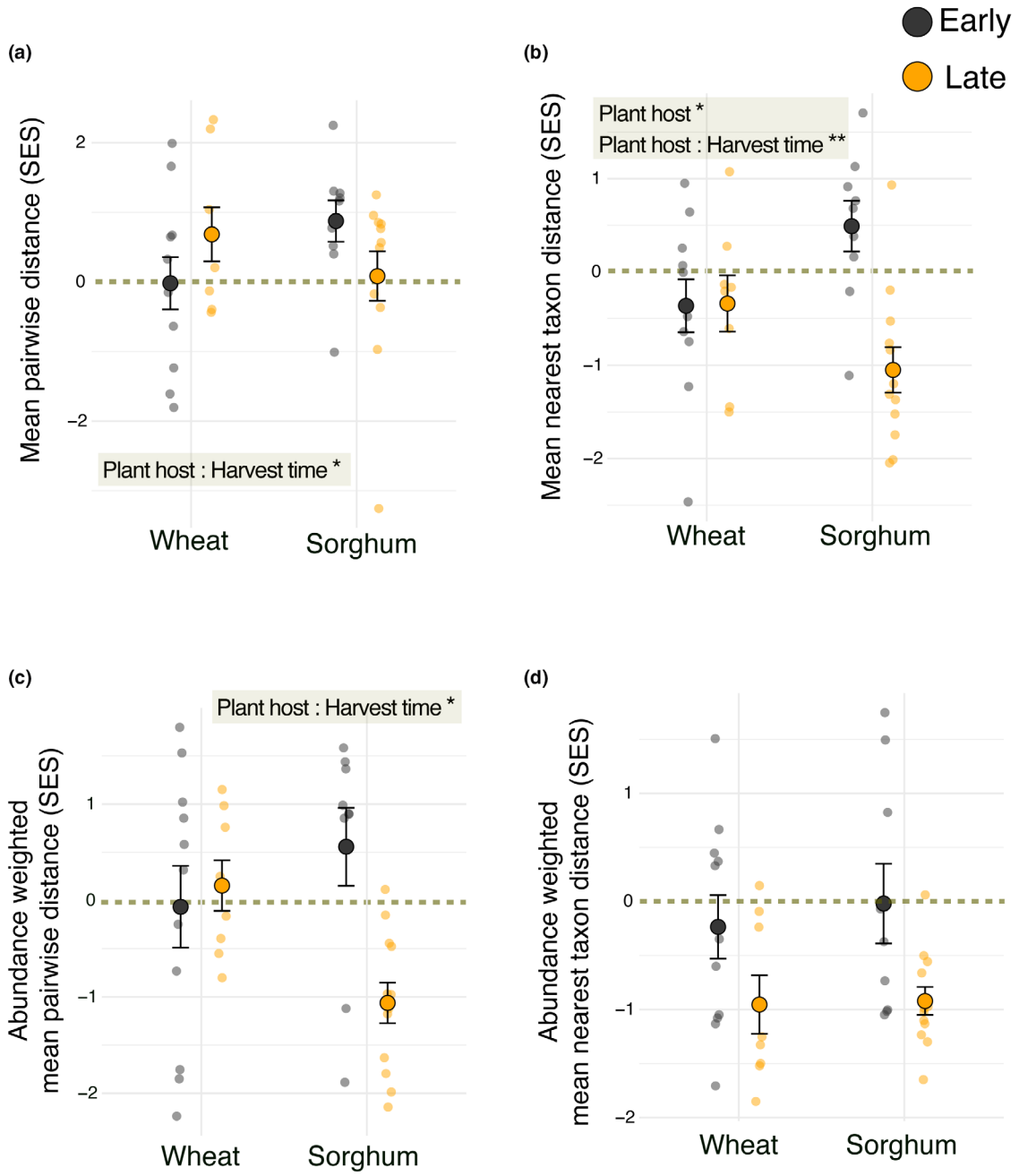

FIGURE 2 Phylogenetic diversity of arbuscular mycorrhizal (AM) fungal communities in the roots of wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*). The standardised effect sizes (SES) for (a) mean pairwise distances, (b) mean nearest taxon distances, the (c) abundance-weighted mean pairwise distances and (d) abundance-weighted mean nearest taxon distances are shown for AM fungal communities in wheat and sorghum at the early and late harvest times. Solid points and error bars represent the mean ± SE, which are overlaid on top of the data points. Significance indicated $^{*}p < 0.05$, $^{**}p < 0.01$.

4.1 | Community assembly of AM fungi differs between hosts and is phylogenetically structured

As we hypothesised, host-specific effects on community assembly were stronger by the later harvest time point, but the mechanism behind those effects differed between hosts. We identified a strong temporal shift in both VT richness and community structure of the root-colonising AM fungal communities in sorghum only. The distinct assembly in the two hosts was further corroborated by our HMSC occurrence model (Figure 3), as well as the individual occurrence models for wheat and sorghum (Figure S5). These responses were phylogenetically structured, that is, that closely related AM fungal VT responded similarly to the effect of sorghum through time. Our phylogenetic diversity analyses also aligned with this as both the mean pairwise distances (including abundance-weighted) and mean nearest taxon distances became significantly lower in sorghum between the early and late harvests, but again, this was not observed in wheat. This suggests that, by the late

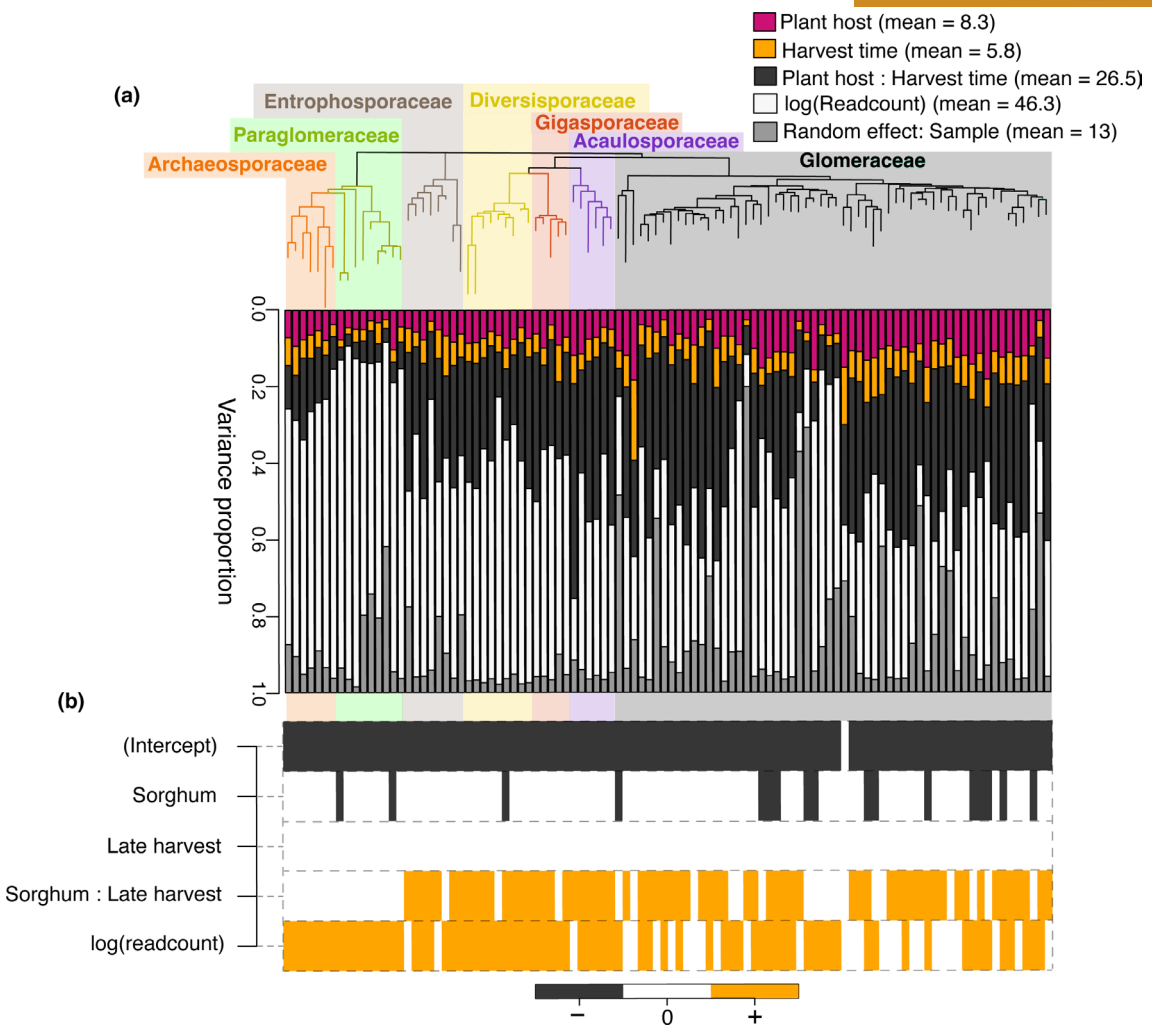

FIGURE 3 (a) The proportion of explained variation in arbuscular mycorrhizal (AM) fungal virtual taxon occurrence (presence-absence) by plant host identity, time of harvest sampling, interaction between plant host and harvest time, the sampling depth (read count), and the random effect of sample identity. The mean variance explained by each explanatory variable is shown. (b) Beta coefficients indicating a positive (orange), negative (black) or no significant relationship (blank/white) in the occurrence of AM fungal virtual taxa (VT) (with at least a posterior probability of 0.95) between hosts, in time, the interaction host and time, and with sampling depth as log(readcount). In this case, the beta-coefficient of wheat corresponds to the intercept. The AM fungal VT are sorted horizontally according to their phylogenetic relatedness.

harvest, the communities of AM fungi inhabiting sorghum roots were composed of more closely related taxa, that is phylogenetically clustered (Pausas & Verdú, 2010). This clustering was particularly apparent in the abundance-weighted mean pairwise distances, which is consistent with the compositional increase in the relative abundances of Glomeraceae we observed in sorghum by the late harvest (Figure 1a).

Phylogenetic clustering can arise from various processes that structure communities (Cadotte & Tucker, 2017), but is frequently attributed to environmental filtering (Pausas & Verdú, 2010). This is relevant to AM fungi, which exhibit niche conservatism, meaning that closely related taxa share similar traits, leading to trait-based structuring of communities (Maherali & Klironomos, 2007). In the context of our study, however, this filtering is mostly represented by the host plant. In this case, all plants were exposed to the same initial pool of potential AM fungal symbionts in pots, in close physical proximity to roots. Host-identity effects on community assembly can be driven by several potential mechanisms. For instance, contrasting host traits, such as variations in host physiology, likely create divergent ecological habitats for AM fungi that are then occupied by distinct taxa. In our study, sorghum had more root mass overall (Figure S6) compared to the wheat plants, potentially offering more 'real estate' for occupation by potential AM fungal partners. As wheat would then have comparatively less available habitat for AM fungi than sorghum, the first fungi to colonise roots would more rapidly occupy the potential habitat space in wheat than in sorghum. Could priority effects, therefore, be stronger or more persistent in wheat? Possibly, particularly considering the similarity in the early colonising communities of wheat and sorghum observed at the first harvest.

With more efficient photosynthesis, C_4 plants are thought to be limited by nutrients more often than C_3 species

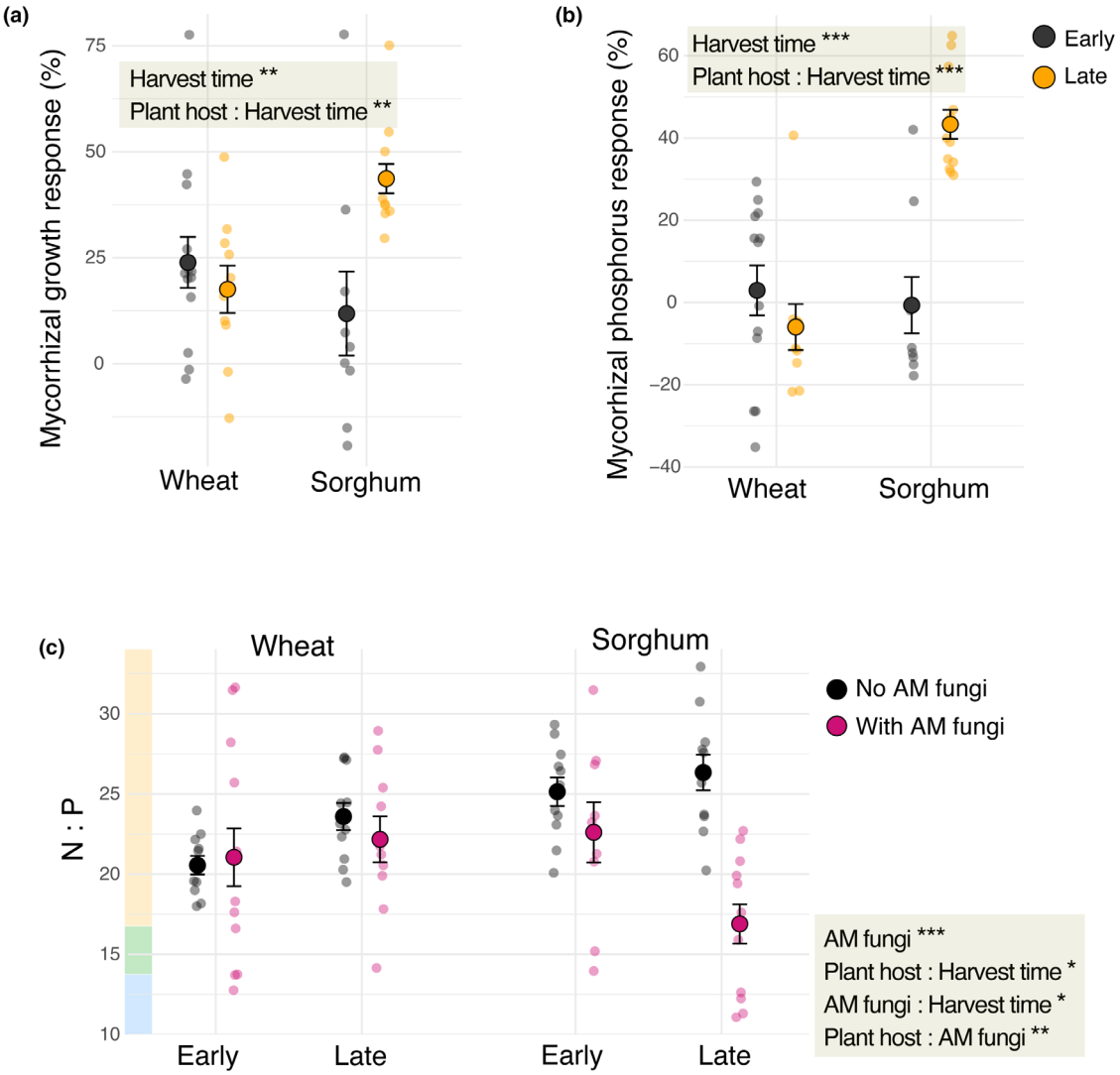

FIGURE 4 The (a) mycorrhizal growth responses (%) and (b) mycorrhizal phosphorus responses (%) of wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*) at early and late harvest times. (c) Also shown are the foliar nitrogen (N):Phosphorus (P) ratios of wheat and sorghum at the early and late harvest times, grown with or without arbuscular mycorrhizal (AM) fungi. Colouration on the *y*-axis represents a general indication of plant nutrient limitation by P (yellow, N:P >16) or limited by N (blue, N:P <14). Solid points and error bars represent the mean ± SE, which are overlaid on top of the raw data points. Significance indicated $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

(Hoeksema et al., 2010). As such, most C_4 plants, like sorghum, are thought to rely more on the AM symbiosis than C_3 hosts, such as wheat. This notion is also supported by studies that observe C_4 and C_3 hosts associate with distinct communities (Davison et al., 2020) and that have found sorghum attains more growth and nutrient benefits from the AM symbiosis than wheat (Frew, 2019). In our study, the lack of support for phylogenetic clustering in wheat communities suggests that their assembly was not driven by a strong host filtering effect, and instead, biotic interactions or stochastic effects play a more prominent role (Frew et al., 2023). Host determinism in community assembly may also be driven by active selection by a plant for specific taxa by rewarding carbon to the fungi that provide the most benefit under a given environmental context. Any such mechanisms would result in phylogenetically clustered fungal communities if both adaptation to host traits and benefit are conserved in evolutionary history. To test this hypothesis, future controlled experiments should examine a series of plant species representing a range of responsiveness to arbuscular mycorrhizas, potentially across functional groups (e.g. C_3 or C_4).

4.2 | Community assembly is associated with plant mycorrhizal responses

Our results showed that the phylogenetically structured assembly of AM fungi in sorghum coincided with significant growth and nutritional benefits from the symbiosis, something not observed in wheat. Specifically, we found a significant increase in both MGRs and mycorrhizal phosphorus responses in sorghum between the two timepoints. The distinct phenotypic outcomes for the late

harvested sorghum was also evident in their foliar N:P ratios. As the availability of P in the soil was intentionally restricted (Table S1), it was unsurprising that most plants in the experiment had N:P ratios between 20.5–26.4, suggesting P limitation (Elser et al., 2010; Güsewell, 2004), which was possibly alleviated in sorghum by the late harvest. This, along with the concomitant decrease in root:shoot ratios (Figure S6c), presents strong evidence that the assembly of AM fungi in sorghum, driven by host selection, alleviated the plant P limitation. The growth and nutrient benefits sorghum derived from its AM fungal communities suggest that selection for AM fungal taxa based on the benefits they might provide for the host would be stronger in plants more able to reap such benefits.

4.3 | Influence of experimental conditions and further implications

In our manipulative pot experiment, we have inevitably selected for fungal taxa capable of withstanding the disturbances associated with generating the soil inoculum and the conditions imposed by the pot experiment. Certain fungal taxa may have persisted in the soil inoculum but were unable to tolerate the limited space and resources within the pot. Consequently, the AM fungal richness in plant roots was substantially lower than in the initial soil inoculum (Figure 1b). This observation suggests that the AM fungi identified in the different plant roots, compared to the soil, were influenced not only by host selection but also by environmental filtering favouring disturbance-tolerant taxa (Chagnon et al., 2013). Would it be reasonable to interpret our findings as applicable to a particular subset or functional group of AM fungi? This is a plausible interpretation, as is often the case in pot experiments involving AM fungi (Ohsowski et al., 2014). However, given that both hosts in our study are crops that would be grown in agroecosystems subject to frequent disturbance, the outcomes are likely to be more ecologically relevant than if we had assessed assembly in two native plant species.

Our results raise important questions about the ecological and evolutionary mechanisms driving mycorrhizal dependency and partner selection in different plant species. Phylogenetically structured community assembly along with associated growth and nutrient benefits was only observed in the more typically mycorrhizal dependent host plant. Why does sorghum support a more distinctly structured fungal community in this instance? Could higher mycorrhizal dependency drive stronger selective pressures that shape fungal communities? Although logical, consistent support for this is yet to be established. While future studies should certainly explore whether stronger host selection is linked to traits such as mycorrhizal growth or nutrient responses, we encourage researchers to also account for mycorrhizal functional benefits like enhanced defence or stress tolerance, which have been mostly ignored in the context of AM fungal community assembly (Delavaux et al., 2017; Frew, Weinberger, et al., 2024).

5 | CONCLUSIONS

The influence of host plant identity on root-colonising AM fungal communities has been shown to varying degrees, sometimes using extensive datasets (Davison et al., 2011, 2016; Öpik et al., 2010, 2013). Here we provide a novel experimental demonstration that a given species pool of AM fungi can exhibit vastly contrasting community trajectories over time entirely driven by the identity of the hosts. Given the same pool of potential fungal partners in equal environmental conditions, the two host plant species initially harboured relatively similar communities, however by the later timepoint, the resultant communities departed from each other. For sorghum, the fungal community from the late harvest consisted of closely related AM fungal taxa that provided substantial nutritional and growth benefits. In contrast, the communities in wheat were comparatively more phylogenetically dispersed and provided less nutritional benefit. Given we compared only two crop species, future research should examine host-driven assembly across a range of plant hosts to robustly assess the generality of these findings. All in all, our results not only show that host identity can shape AM fungal community assembly, but suggest that hosts capable of garnering significant benefits from the symbiosis may exert the most deterministic effects on AM fungal community assembly.

AUTHOR CONTRIBUTIONS

Adam Frew designed the experiment and collected the data. The data analyses were led by Adam Frew with support from Yuxiong Zheng and Carlos A. Aguilar-Trigueros, and results were interpreted with additional input from Yanrong Fu and Zhenyu Wang. Adam Frew wrote the initial manuscript draft alongside Carlos A. Aguilar-Trigueros and Yuxiong Zheng, and all authors then contributed critically to developing the final manuscript and provided approval for publication.

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CONFLICT OF INTEREST STATEMENT

Adam Frew is an associate editor for Functional Ecology, and took no part in the peer review and decision-making process for this paper. All other authors confirm that there are no other conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data that support this study are openly available from the *figshare* repository at the following https://doi.org/10.6084/m9.figshare.25988335 (Frew, Zheng, et al., 2024). Raw DNA sequencing data are available under NCBI BioPoject accession number PRJNA1120940.

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REFERENCES

Bever, J. D. (2002). Negative feedback within a mutualism: Host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*(1509), 2595–2601. https://doi.org/10.1098/rspb.2002.2162

Bever, J. D., Morton, J. B., Antonovics, J., & Schultz, P. A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology*, *84*, 71–82.

Cadotte, M. W., & Tucker, C. M. (2017). Should environmental filtering be abandoned? *Trends in Ecology & Evolution*, *32*(6), 429–437. https://doi.org/10.1016/j.tree.2017.03.004

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*(1), 421. https://doi.org/10.1186/1471-2105-10-421

Chagnon, P.-L., Bradley, R. L., Maherali, H., & Klironomos, J. N. (2013). A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, *18*(9), 484–491. https://doi.org/10.1016/j.tplants.2013.05.001

Chaudhary, V. B., Nolimal, S., Sosa-Hernández, M. A., Egan, C., & Kastens, J. (2020). Trait-based aerial dispersal of arbuscular mycorrhizal fungi. *New Phytologist*, *228*(1), 238–252. https://doi.org/10.1111/nph.16667

Cheeke, T. E., Zheng, C., Koziol, L., Gurholt, C. R., & Bever, J. D. (2019). Sensitivity to AMF species is greater in late-successional than early-successional native or nonnative grassland plants. *Ecology*, *100*(12), e02855. https://doi.org/10.1002/ecy.2855

Davison, J., León, D., Zobel, M., Moora, M., Bueno, C. G., Barceló, M., Gerz, M., León, D., Meng, Y., Pillar, V. D., Sepp, S.-K., Soudzilovaskaia, N. A., Tedersoo, L., Vaessen, S., Vahter, T., Winck, B., & Öpik, M. (2020). Plant functional groups associate with distinct arbuscular mycorrhizal fungal communities. *New Phytologist*, *226*(4), 1117–1128. https://doi.org/10.1111/nph.16423

Davison, J., Moora, M., Jairus, T., Vasar, M., Öpik, M., & Zobel, M. (2016). Hierarchical assembly rules in arbuscular mycorrhizal (AM) fungal communities. *Soil Biology and Biochemistry*, *97*, 63–70. https://doi.org/10.1016/j.soilbio.2016.03.003

Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A. G., Hiiesalu, I., Jairus, T., Johnson, N. C., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., … Zobel, M. (2015). Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science*, *349*(6251), 970–973.

Davison, J., Öpik, M., Daniell, T. J., Moora, M., & Zobel, M. (2011). Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology*, *78*(1), 103–115. https://doi.org/10.1111/j.1574-6941.2011.01103.x

de Souza Campos, P. M., Borie, F., Cornejo, P., Meier, S., López-Ráez, J. A., López-Garcia, Á., & Seguel, A. (2021). Wheat root trait plasticity, nutrient acquisition and growth responses are dependent on specific arbuscular mycorrhizal fungus and plant genotype interactions. *Journal of Plant Physiology*, *256*, 153297. https://doi.org/10.1016/j.jplph.2020.153297

Delavaux, C. S., Smith-Ramesh, L. M., & Kuebbing, S. E. (2017). Beyond nutrients: A meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology*, *98*(8), 2111–2119. https://doi.org/10.1002/ecy.1892

Dumbrell, A. J., Ashton, P. D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A. H., & Helgason, T. (2011). Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist*, *190*(3), 794–804. https://doi.org/10.1111/j.1469-8137.2010.03636.x

Elliott, A. J., Daniell, T. J., Cameron, D. D., & Field, K. J. (2021). A commercial arbuscular mycorrhizal inoculum increases root colonization across wheat cultivars but does not increase assimilation of mycorrhiza-acquired nutrients. *Plants, People, Planet*, *3*(5), 588–599. https://doi.org/10.1002/ppp3.10094

Elser, J. J., Fagan, W. F., Kerkhoff, A. J., Swenson, N. G., & Enquist, B. J. (2010). Biological stoichiometry of plant production: Metabolism, scaling and ecological response to global change. *New Phytologist*, *186*(3), 593–608. https://doi.org/10.1111/j.1469-8137.2010.03214.x

FAO. (2024). *The state of food security and nutrition in the world 2024*. FAO; IFAD; UNICEF; WFP; WHO.

Fielding, A. H., & Bell, J. F. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, *24*(1), 38–49. https://doi.org/10.1017/S0376892997000088

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage Publications.

Frew, A. (2019). Arbuscular mycorrhizal fungal diversity increases growth and phosphorus uptake in C_3 and C_4 crop plants. *Soil Biology and Biochemistry*, *135*, 248–250. https://doi.org/10.1016/j.soilbio.2019.05.015

Frew, A., & Aguilar-Trigueros, C. A. (2024). Increasing phylogenetic clustering of arbuscular mycorrhizal fungal communities in roots explains enhanced plant growth and phosphorus uptake. *Microbial Ecology*, *87*(1), 139. https://doi.org/10.1007/s00248-024-02457-1

Frew, A., Heuck, M. K., & Aguilar-Trigueros, C. A. (2023). Host filtering, not competitive exclusion, may be the main driver of arbuscular mycorrhizal fungal community assembly under high phosphorus. *Functional Ecology*, *37*(7), 1856–1869. https://doi.org/10.1111/1365-2435.14349

Frew, A., Weinberger, N., Powell, J. R., Watts-Williams, S. J., & Aguilar-Trigueros, C. A. (2024). Community assembly of root-colonising arbuscular mycorrhizal fungi: Beyond carbon and into defence? *The ISME Journal*, *18*, wrae007. https://doi.org/10.1093/ismejo/wrae007

Frew, A., Zheng, Y., Wang, Z., Fu, Y., & Aguilar-Trigueros, C. A. (2024). Data from: Causal determinism by plant host identity in arbuscular mycorrhizal fungal community assembly [Dataset]. *Figshare Digital Repository*. https://doi.org/10.6084/m9.figshare.25988335

García de León, D., Moora, M., Öpik, M., Jairus, T., Neuenkamp, L., Vasar, M., Bueno, C. G., Gerz, M., Davison, J., & Zobel, M. (2016). Dispersal of arbuscular mycorrhizal fungi and plants during succession. *Acta Oecologica*, *77*, 128–135. https://doi.org/10.1016/j.actao.2016.10.006

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science*, *7*(4), 457–472. https://doi.org/10.1214/ss/1177011136

Güsewell, S. (2004). N: P ratios in terrestrial plants: Variation and functional significance. *New Phytologist*, *164*(2), 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Hart, M. M., Aleklett, K., Chagnon, P.-L., Egan, C., Ghignone, S., Helgason, T., Lekberg, Y., Öpik, M., Pickles, B. J., & Waller, L. (2015). Navigating the labyrinth: A guide to sequence-based, community ecology of arbuscular mycorrhizal fungi. *New Phytologist*, *207*(1), 235–247. https://doi.org/10.1111/nph.13340

Hawkins, H.-J., Cargill, R. I. M., Van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., Soudzilovskaia, N. A., & Kiers, E. T. (2023). Mycorrhizal mycelium as a global carbon pool. *Current Biology*, *33*(11), R560–R573. https://doi.org/10.1016/j.cub.2023.02.027

Helgason, T., Merryweather, J. W., Young, J. P. W., & Fitter, A. H. (2007). Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *Journal of Ecology*, *95*, 623–630.

HilleRisLambers, J., Adler, P. B., Harpole, W. S., Levine, J. M., & Mayfield, M. M. (2012). Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 227–248. https://doi.org/10.1146/annurev-ecolsys-110411-160411

Hoeksema, J. D., Chaudhary, V. B., Gehring, C. A., Johnson, N. C., Karst, J., Koide, R. T., Pringle, A., Zabinski, C., Bever, J. D., Moore, J. C., Wilson, G. W. T., Klironomos, J. N., & Umbanhowar, J. (2010). A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, *13*(3), 394–407. https://doi.org/10.1111/j.1461-0248.2009.01430.x

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*, 1463–1464.

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., & Bücking, H. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, *333*(6044), 880–882. https://doi.org/10.1126/science.1208473

Koch, A. M., Antunes, P. M., Maherali, H., Hart, M. M., & Klironomos, J. N. (2017). Evolutionary asymmetry in the arbuscular mycorrhizal symbiosis: Conservatism in fungal morphology does not predict host plant growth. *New Phytologist*, *214*(3), 1330–1337. https://doi.org/10.1111/nph.14465

König, S., Wubet, T., Dormann, C. F., Hempel, S., Renker, C., & Buscot, F. (2010). TaqMan real-time PCR assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: Effects of soil characteristics, plant species richness, and functional traits. *Applied and Environmental Microbiology*, *76*(12), 3765–3775. https://doi.org/10.1128/AEM.02951-09

Lee, J., Lee, S., & Young, J. P. W. (2008). Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology*, *65*(2), 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x

Lekberg, Y., Vasar, M., Bullington, L. S., Sepp, S.-K., Antunes, P. M., Bunn, R., Larkin, B. G., & Öpik, M. (2018). More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? *New Phytologist*, *220*(4), 971–976. https://doi.org/10.1111/nph.15035

Lekberg, Y., & Waller, L. P. (2016). What drives differences in arbuscular mycorrhizal fungal communities among plant species? *Fungal Ecology*, *24*, 135–138. https://doi.org/10.1016/j.funeco.2016.05.012

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Magkourilou, E., Bell, C. A., Daniell, T. J., & Field, K. J. (2024). The functionality of arbuscular mycorrhizal networks across scales of experimental complexity and ecological relevance. *Functional Ecology*. https://doi.org/10.1111/1365-2435.14618

Maherali, H., & Klironomos, J. N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, *316*(5832), 1746–1748. https://doi.org/10.1126/science.1143082

Martínez-García, L. B., Richardson, S. J., Tylianakis, J. M., Peltzer, D. A., & Dickie, I. A. (2015). Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. *New Phytologist*, *205*(4), 1565–1576. https://doi.org/10.1111/nph.13226

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytologist*, *115*(3), 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*(4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Ohsowski, B. M., Zaitsoff, P. D., Öpik, M., & Hart, M. M. (2014). Where the wild things are: Looking for uncultured Glomeromycota. *New Phytologist*, *204*(1), 171–179. https://doi.org/10.1111/nph.12894

Oksanen, J., Blanchet, F. G., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2015). *vegan: Community ecology package*. http://CRAN.R-project.org/package=vegan

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., Reier, Ü., & Zobel, M. (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist*, *188*(1), 223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

Öpik, M., Zobel, M., Cantero, J. J., Davison, J., Facelli, J. M., Hiiesalu, I., Jairus, T., Kalwij, J. M., Koorem, K., Leal, M. E., Liira, J., Metsis, M., Neshataeva, V., Paal, J., Phosri, C., Põlme, S., Reier, Ü., Saks, Ü., Schimann, H., … Moora, M. (2013). Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, *23*(5), 411–430. https://doi.org/10.1007/s00572-013-0482-2

Ovaskainen, O., & Abrego, N. (2020). *Joint species distribution modelling: With applications in R*. Cambridge University Press.

Pausas, J. G., & Verdú, M. (2010). The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. *Bioscience*, *60*(8), 614–625. https://doi.org/10.1525/bio.2010.60.8.7

Powell, J. R., & Rillig, M. C. (2018). Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist*, *220*(4), 1059–1075. https://doi.org/10.1111/nph.15119

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Revillini, D., Gehring, C. A., & Johnson, N. C. (2016). The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. *Functional Ecology*, *30*(7), 1086–1098. https://doi.org/10.1111/1365-2435.12668

Řezáčová, V., Gryndler, M., Bukovská, P., Šmilauer, P., & Jansa, J. (2016). Molecular community analysis of arbuscular mycorrhizal fungi—Contributions of PCR primer and host plant selectivity to the detected community profiles. *Pedobiologia*, *59*(4), 179–187. https://doi.org/10.1016/j.pedobi.2016.04.002

Riley, R. C., Cavagnaro, T. R., Brien, C., Smith, F. A., Smith, S. E., Berger, B., Garnett, T., Stonor, R., Schilling, R. K., Chen, Z.-H., & Powell, J. R. (2019). Resource allocation to growth or luxury consumption drives mycorrhizal responses. *Ecology Letters*, *22*, 1757–1766. https://doi.org/10.1111/ele.13353

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Sepp, S.-K., Davison, J., Jairus, T., Vasar, M., Moora, M., Zobel, M., & Öpik, M. (2019). Non-random association patterns in a plant–mycorrhizal

fungal network reveal host–symbiont specificity. *Molecular Ecology*, *28*(2), 365–378. https://doi.org/10.1111/mec.14924

Šmilauer, P., Košnar, J., Kotilínek, M., & Šmilauerová, M. (2020). Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. *New Phytologist*, *225*(1), 461–473. https://doi.org/10.1111/nph.16112

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis*. Academic Press.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2003). Multiple sequence alignment using ClustalW and ClustalX. *Current Protocols in Bioinformatics*. https://doi.org/10.1002/0471250953.bi0203s00

Tikhonov, G., Opedal, Ø. H., Abrego, N., Lehikoinen, A., de Jonge, M. M. J., Oksanen, J., & Ovaskainen, O. (2020). Joint species distribution modelling with the r-package Hmsc. *Methods in Ecology and Evolution*, *11*(3), 442–447. https://doi.org/10.1111/2041-210X.13345

Van Geel, M., Jacquemyn, H., Plue, J., Saar, L., Kasari, L., Peeters, G., van Acker, K., Honnay, O., & Ceulemans, T. (2018). Abiotic rather than biotic filtering shapes the arbuscular mycorrhizal fungal communities of European seminatural grasslands. *New Phytologist*, *220*(4), 1262–1272. https://doi.org/10.1111/nph.14947

Vasar, M., Andreson, R., Davison, J., Jairus, T., Moora, M., Remm, M., Young, J. P. W., Zobel, M., & Öpik, M. (2017). Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, *27*(8), 761–773. https://doi.org/10.1007/s00572-017-0791-y

Vasar, M., Davison, J., Neuenkamp, L., Sepp, S.-K., Young, J. P. W., Moora, M., & Öpik, M. (2021). User-friendly bioinformatics pipeline gDAT (graphical downstream analysis tool) for analysing rDNA sequences. *Molecular Ecology Resources*, *21*, 1380–1392. https://doi.org/10.1111/1755-0998.13340

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wagg, C., Jansa, J., Stadler, M., Schmid, B., & Heijden, M. G. A. (2011). Mycorrhizal fungal identity and diversity relaxes plant–plant competition. *Ecology*, *92*(6), 1303–1313. https://doi.org/10.1890/10-1915.1

Walder, F., Niemann, H., Natarajan, M., Lehmann, M. F., Boller, T., & Wiemken, A. (2012). Mycorrhizal networks: Common goods of plants shared under unequal terms of trade. *Plant Physiology*, *159*(2), 789–797. https://doi.org/10.1104/pp.112.195727

Webb, C. O., Ackerly, D. D., McPeek, M. A., & Donoghue, M. J. (2002). Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, *33*, 475–505.

Wheeler, B., & Torchiano, M. (2016). *lmPerm: Permutation tests for linear models*. R package Version 2.1.0. https://CRAN.R-project.org/package=lmPerm

Zarcinas, B. A., Cartwright, B., & Spouncer, L. R. (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, *18*(1), 131–146. https://doi.org/10.1080/00103628709367806

Zobel, M. (1997). The relative of species pools in determining plant species richness: An alternative explanation of species coexistence? *Trends in Ecology & Evolution*, *12*(7), 266–269. https://doi.org/10.1016/S0169-5347(97)01096-3

Zobel, M., & Öpik, M. (2014). Plant and arbuscular mycorrhizal fungal (AMF) communities—Which drives which? *Journal of Vegetation Science*, *25*(5), 1133–1140. https://doi.org/10.1111/jvs.12191

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

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 root-colonising arbuscular mycorrhizal fungal virtual taxa.

Figure S2. Distributions of potential scale reduction factors for Markov Chain Monte Carlo chains.

Figure S3. Explanatory power of the joint species distribution models.

Figure S4. Variance partitioning and beta coefficients for the abundance Hierarchical Modelling of Species Communities models of arbuscular mycorrhizal fungal virtual taxa.

Figure S5. Variance partitioning and beta coefficients for occurrence Hierarchical Modelling of Species Communities models of arbuscular mycorrhizal fungal virtual taxa for separate wheat and sorghum models.

Figure S6. Root:shoot ratios of plants and colonisation of plant roots by arbuscular mycorrhizal fungi.

Table S1. Soil nutrient profile.

Table S2. Model results on the effects of plant host identity, harvest time, and AM fungi on plant responses, fungal colonisation, diversity, and phylogenetic diversity.

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