Agricultural land-use favours Mucoromycotinian, but not Glomeromycotinian, arbuscular mycorrhizal fungi across ten biomes

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Summary

1. Globally, agricultural land-use negatively affects soil biota that contribute to ecosystem functions such as nutrient cycling. Yet, arbuscular mycorrhizal fungi (AMF) are promoted as essential components of agroecosystems. AMF include Glomeromycotinian AMF (G-AMF) and the arbuscule-producing fine root endophytes, recently re-classified into the Endogonales order within Mucoromycotina. The correct classification of Mucoromycotinian AMF (M-AMF) and the availability of new molecular tools can guide research to better the understanding of their diversity and ecology.
2. To investigate the impact on G-AMF and M-AMF of agricultural land-use at a continental scale, we sampled DNA from paired farm and native sites across ten Australian biomes.
3. G-AMF were present in both native and farm sites in all biomes. Putative M-AMF were favoured by farm sites, rare or absent in native sites, and almost entirely absent in tropical biomes. Temperature, rainfall, and soil pH were strong drivers of richness and community composition of both groups with plant richness an important mediator.
4. Both fungal groups occupy different, but overlapping, ecological niches, with M-AMF thriving in temperate agricultural landscapes. Our findings invite exploration of the origin and spread of M-AMF and continued efforts to resolve the phylogeny of this newly reclassified group of AMF.

Keywords: arbuscular mycorrhizal fungi; *Endogonales*; fine root endophytes; Glomeromycotina; *Glomus tenue*; Mucoromycotina; *Planticonsortium*

Introduction

Conversion of land to industrialised farming has been invoked, globally, as one of the main causes of native ecosystem degradation and biodiversity loss (Chaudhary *et al.*, 2016; Tilman *et al.*, 2017). The consequences of land transformation on ecosystem function and diversity of plants and animals have been extensively studied, but the effects on the biodiversity and functionality of microbial communities, such as those of arbuscular mycorrhizal fungi (AMF), are less well understood (Bowles *et al.*, 2017; Wood *et al.*, 2017; Guerra *et al.*, 2020; Guzman *et al.*, 2021). Glomeromycotinian AMF (G-AMF) are ubiquitous worldwide (Kivlin *et al.*, 2011; Tedersoo *et al.*, 2014) and, while many questions remain to be answered, they are considered important for ecosystem functioning due to their role in plant health, soil aggregation, and nutrient cycling (Powell & Rillig, 2018). Recently, the arbuscule-forming fine root endophytes (Endogonales order within subphylum Mucoromycotina, including the genus *Planticonsortium* (Walker *et al.*, 2018)) were classified as a group of AMF distinct from G-AMF (Orchard *et al.*, 2017a). Since fine root endophytes have been historically misplaced within Glomeromycotina, we know little about their ecology (Orchard *et al.*, 2017b). Here we refer to fine root endophytes as Mucoromycotinian AMF (M-AMF) since they form arbuscules and current evidence suggests they provide similar benefit to their hosts as G-AMF, at least in liverworts and early divergent vascular plants (Orchard *et al.*, 2017b; Hoysted *et al.*, 2019).

Distribution and abundance of G-AMF can be driven by climate, vegetation, and soil type (Tedersoo *et al.*, 2014; Pärtel *et al.*, 2017; Garcia de Leon *et al.*, 2018). Anthropogenic land-use can alter some of these interactions. Industrialised conversion of landscapes from native ecosystem-dominated to farmland reduces diversity of plant hosts as vegetation is cleared and replaced with a single or few exotic species (Turner & Meyer, 1991). Agricultural land-use is also characterised by relatively high soil disturbance, and increased occurrence of bare soil due to cultivation or high densities of hoofed ruminants or periods of bare fallow (Turner & Meyer, 1991). Additionally, repeated biomass and nutrient removal via cropping, grazing, and logging, along with external inputs (e.g. fertilisers, irrigation, pesticides) can alter nutrient cycles and soil chemistry (Turner & Meyer, 1991; Penuelas *et al.*, 2020). Unfortunately, most studies evaluating effects of agricultural land-use on AMF (both Glomeromycotinian and Mucoromycotinian) do not include a comparison to untransformed native ecosystems (Oehl *et al.*, 2010; Vályi *et al.*, 2015; Trejo *et al.*, 2016); the studies that do

are restricted to a single biome or ecoregion (González-Cortés *et al.*, 2012; Dai *et al.*, 2013; Oehl *et al.*, 2017; Sepp *et al.*, 2018). That said, a global study conducted by Stürmer et al. (2018) showed that diversity of G-AMF increased with proximity to tropical biomes, while Pärtel et al. (2017) showed diversity of G-AMF decreased with proximity to human disturbance. The handful of studies on M-AMF suggest their diversity and abundance can be promoted by lower temperatures (Albornoz *et al.*, 2020b), agricultural land-use (Orchard *et al.*, 2017b), or presence of exotic plant species (Hilbig & Allen, 2015). Furthermore, a global study showed Mucoromycotina (including M-AMF) were more diverse in the tundra biome while their abundance was lowest in the tropical dry forest biome, but this study only included native ecosystems (Tedersoo *et al.*, 2014). Recently, Albornoz *et al.* (2020b) showed contrasting responses of M- and G-AMF to temperature and edaphic conditions across southern Australia, with higher temperatures and soil pH decreasing richness of M-AMF but increasing richness of G-AMF. However, this study was limited to a single exotic pasture legume species within temperate pastures. Indeed, most research to date on M-AMF is highly biased towards temperate biomes and is limited to measures of root colonisation (Orchard *et al.*, 2017b; Albornoz *et al.*, 2020b). Hence, whether M-AMF are as ubiquitous or diverse as G-AMF, and whether their responses differ to the interacting effects of land-use and climate, are open questions.

Globally, Australia has some of the oldest soils, yet some of the youngest anthropogenic soils. Extensive agricultural land-use commenced in Australia in the 1800s with the arrival of European settlers. However, some native landscapes in Australia have been converted to industrialised agriculture as recently as the 1980s (Kasel & Bennett, 2007). Prior to European settlement, the reports of early European explorers suggest that the indigenous communities practiced low-impact, low intensity, sustainable agriculture utilising native species, with cultivation methods that involved minimal soil disturbance and little modification of the native nutrient cycles; there were no hard-hoofed ruminants (Pascoe, 2018; Paterson, 2018). In contrast, Europe and Asia have a long (centuries) history of land-use for industrialised agriculture (Goldewijk & Ramankutty, 2004). In Australia, the diversity of climates ranging from arid to tropical to cool montane (Kottek *et al.*, 2006), and the gradient of soil fertility from nutrient-poor to nutrient-rich, has resulted in a diversity of both agricultural systems and native ecosystems (Johnston *et al.*, 2003). The native biomes include the oldest tropical rainforest in the world (i.e. Daintree rainforest) and the world's most diverse shrubland (i.e.

kwongkan) (Cowling *et al.*, 1996). Because of this, Australia offers a unique opportunity to evaluate the effects of land-use across large climatic, soil, and vegetation gradients.

Here, we seek to understand the effects of industrialised agriculture on the distribution and richness of M-AMF and G-AMF. To do so, we surveyed fungi associated with roots at agricultural (hereafter 'farm') sites and native sites in a paired design across 10 distinctive biomes of Australia (Fig. 1). We take a conservative approach and do not assume all Endogonales found are M-AMF, and hence refer to them simply as Endogonales. Given the predilection of M-AMF for cold environments (Tedersoo *et al.*, 2014), and agricultural land (Orchard *et al.*, 2017b), and the predilection of G-AMF for warmer climates, native ecosystems, and less acidic soils (Pärtel *et al.*, 2017; Albornoz *et al.*, 2020b), we hypothesised that: 1) communities of both Endogonales and G-AMF would be strongly driven by biome and system (i.e. native or farm); 2) the effects of biome on community structure of both fungal groups would be mainly due to changes in temperature and rainfall among biomes, while the effects of system would be due to changes in soil chemistry and plant richness; and 3) the relative abundance and richness of Endogonales would be higher in colder and wetter biomes and farm sites, while that of G-AMF would be higher in tropical biomes and native sites.

Materials and methods

Site selection

In total 52 sites were sampled from ten biomes across Australia. Biomes selected represent a wide range of climatic, soil and vegetation variables (Fig. 1). Within each biome, we sampled native and farm systems, with one-to-three pairs of native and farm sites in each biome (Table 1). Native sites were selected from the Biomes of Australian Soil Environment project (Bissett *et al.*, 2016) and were considered representative of each biome. Native sites included: rainforest, shrubland, woodland, savannah, and grassland (Fig. 1; Table S1). Farm sites were selected for proximity to native sites to match, as much as possible, the native site soil type, topography and climate, as well as incorporating, deliberately, a variety of farm management practices (Fig. 1). The need for permission from landowners to conduct research on their farms also impacted farm site choice. On average, distance between paired native and farm sites was 14 ± 3 km (mean ± standard error). Farm sites included: pastures used for livestock

grazing (native plant-based and sown/weedy exotic species of both annuals and perennials), annual crops (maize, wheat, canola), orchards (avocado, mango, rambutan, apples; most with weeds or sown pasture species between rows), silvicultural sites (exotic pine and native eucalypts), and others (e.g. truffle farm) (Fig. 1; Table S1).

Sample collection and processing

Sampling occurred between March and November 2019. To avoid confounding effects of sampling season on AMF, each biome was sampled during its plant growing season (Rabatin, 1979; Dodd & Jeffries, 1986; Kowal *et al.,* 2020). At each site, samples were taken 20 m apart at five points along a 100 m transect. Transect direction was subject to site dimensions. At each point, vascular plant species richness was recorded within a 2 m radius (12.56 m^2 area), and two adjacent soil cores (5 cm diameter) were obtained from the top 15 cm of soil. Roots were immediately separated from soil and stored in 98% ethanol pending processing for fungi. The two soil cores at each point were also immediately mixed, homogenised, and stored for chemical analyses. Equipment was cleaned between samples by removing all soil particles with water, then spraying with 10% bleach, and then with 98% ethanol. A dummy core was taken and discarded at each new sampling point. All samples were stored in dry ice and remained frozen during transport by airfreight or vehicle overnight to laboratories for processing. Upon arrival, soil and root samples were stored at -20°C. Root samples were washed with deionised water to remove all debris, and fine roots of < 2 mm were retained, thoroughly cleaned, cut into 5 mm pieces, homogenised, and stored at -80°C for two hours. Clean roots were freeze-dried for one week and ground. A total of 260 soil and 260 root samples were collected. Location was used to extract temperature and rainfall information from the Australian Bureau of Meteorology (Bureau of Meteorology 2018) and soil type (nationalmap.gov.au, accessed on 16/07/2021). Climate data obtained were mean annual rainfall (hereafter rainfall) and mean annual minimum temperature (hereafter temperature).

Evidence linking Endogonales sequences to fine root endophyte morphology is based on correlative analysis (Orchard *et al.,* 2017a), and the phylogenetic affiliation of the recently described M-AMF *Planticonsortium tenue* comb. nov. (Walker *et al*., 2018) has not been determined, with morphological characteristics alone used to define the species. Therefore, to include a DNA sequence of *Planticonsortium tenue* in our phylogenetic analysis, we also

obtained fresh roots of *Plantago lanceolata* from the *Planticonsortium tenue* culture described by Walker et al. (2018). This culture originated in New Zealand in 1974 and was maintained as culture BEG249 at the International Bank for the Glomales, with a sub-culture maintained at the University of Reading, UK, from which samples for the Walker et al. (2018) and the current study were obtained. Mark Tibbett and Chris Walker provided roots and visual confirmation of root colonisation by *P. tenue*. Roots were washed free of sand before DNA extraction and sequencing, as described for field-collected root samples.

Soil chemistry

Frozen soil samples were sent to CSBP Laboratories (Bibra Lake, Western Australia) for nutrient analyses. Plant-available phosphorus (P) and potassium (K) were measured using the Colwell test (Colwell, 1963; Rayment & Higginson, 1992). Available sulfur (S) was analysed according to Blair et al. (1991), and organic carbon (OC) was determined according to Walkley et al. (1934). Ammonium-nitrogen and nitrate-nitrogen were measured as per Searle (1984). Soil pH was measured in $CaCl_2$ in a solution ratio of 1:5 (Rayment & Lyons, 2012).

Sequencing and bioinformatic analyses

DNA was extracted from 20 mg of ground root material using the DNeasy Plant Mini kit (Qiagen, Carlsbad, USA) following the manufacturer's protocol. For each DNA sample, 15 ng DNA was used to amplify approximately 260 bp of the 18S rRNA gene using the AMF primer set AMV4.5NF and AMDGR (Sato *et al.*, 2005). We used these primers as they accurately retrieve a wide range of taxa of G-AMF, as well as Mucoromycotina (Orchard *et al.* 2017a; Albornoz *et al.* 2020b). PCR reactions were performed in a reaction volume of 25 µl, containing Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs) and 0.5 µM of each primer. Thermocycling consisted of an initial denaturation at 98℃ for 30 s followed by 35 cycles of 98℃ for 10 s, 60℃ for 15 s and 72℃ for 20 s. The final extension was at 72℃ for 5 min. Following PCR, the DNA amplicon was purified using Agencourt AMPure XP beads (Beckman Coulter, United States) according to the manufacturer's instructions. The adapted amplicon was then modified by attaching indices and Illumina sequencing adapters using the Nextera XT Index Kit v2 by PCR as described in the

manufacturer's protocol. DNA amplicon was then purified and normalized using the SequalPrepTM Normalization Plate (96) Kit (Invitrogen) and quantitatively assessed using a Qubit 2.0 Fluorometer (Life Technologies, United States). The final concentration of the library was 4 nM. The library was sequenced using the MiSeq Reagent Kit v3 600-cycle (Illumina) at The University of Warwick, UK.

Following sequencing, adapters were trimmed with a maximum error rate of 0.1 using 'cutadapt' (Martin, 2011). Only sequences that contained primers were retained. After trimming, all other quality checks and sequence processing were done in VSEARCH v2.14.1 with default parameters unless otherwise stated (Rognes *et al.*, 2016). Trimmed paired-end sequences were merged with a minimum of 10 bp overlap. Merged sequences were filtered using a maximum error rate of 0.1 and a minimum length of 200 bp. On average, 83% of sequences were retained after trimming, merging, and quality checks. Filtered sequences were dereplicated at 100% identity and chimera detection was done *denovo*. Singletons were discarded. Remaining sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold and consensus OTUs were queried against the SILVA 18S rRNA database v138 (Quast *et al.*, 2013). Taxonomy was assigned to OTUs with a threshold of > 95% match and query cover of > 90%. To our knowledge, this is the first study to describe the abundance and richness of Endogonales across biomes since taxonomic revision of the arbuscule-forming fine root endophytes and their placement within Mucoromycotina rather than Glomeromycotina (Orchard *et al.*, 2017a). Hence, we took a conservative approach throughout the Results section, and did not assume OTUs matching Endogonales sequences were M-AMF. In the discussion section, we infer putative M-AMF based on phylogenetic analyses and sequence match to known M-AMF sequences (Orchard *et al.*, 2017a; Walker *et al.*, 2018). We classified as G-AMF any sequence that best matched a Glomeromycotina reference.

Phylogenetic analysis

A phylogenetic tree of the Endogonales OTUs was produced, which included reference sequences previously classified as M-AMF, as well as other Endogonales, such as the genera *Endogone* and *Jimgerdemannia* (with the accession numbers) from NCBI GenBank. We

included the sequence obtained from *P. tenue* (Results S1). We also included *Mortierella multidivaricata* as an outgroup for a total of 40 reference sequences.

Sequence alignments were generated using MUSCLE alignment tool with default parameters (Edgar, 2004). The evolutionary history was inferred using the maximum likelihood method and Tamura 3-parameter model with 1000 bootstrap replicates (Tamura, 1992). Initial trees for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among nucleotide sites. All positions with less than 95% site coverage were eliminated. There was a total of 212 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

Statistical analyses

To avoid sequencing depth bias and standardise sampling effort, we rarefied the entire raw dataset to the smallest sequencing depth (10,484 sequences, excluding samples that contained no sequences of Endogonales or G-AMF) (Fig. S1) (Dickie, 2010). Hereafter, we refer to rarefied richness and relative sequence abundance simply as richness and abundance, respectively. We consider sequence abundance a likely proxy for the traditionally-used metric of abundance, percentage of root length colonised, based on results of a previous study of co-occurring Endogonales and G-AMF using the same primers (Orchard *et al.*, 2017b). Authors of this study found a linear positive correlation between root colonisation and sequence abundance of both fungal groups. Unlike fungal richness, sampling effort of plant richness was the same for all sampling points (12.56 m^2 area), hence we did not rarefy these data. We acknowledge this small area might generate more accurate estimates of plant richness for sites with herbaceous and homogeneous vegetation than for sites with woody and heterogeneous vegetation. However, Endogonales were absent from most sites with large vegetation. All variables were scaled and centred on their means to correct for large differences in scales among variables. Due to multicollinearity, a composite variable of 'available nutrients' was created using the first axis of a PCA between available P and ammonium-nitrogen (hereafter 'available NP'). Nitrate-nitrogen was not included in analyses

since it was not selected for any model during model selection steps. Including nitrate-nitrogen in the available NP variable did not improve models. This axis explained 80% of the variation in the PCA. 'System' was treated as a binomial variable where 0 and 1 represent native and farm sites, respectively.

To test whether farm sites, when compared with native sites, harbour more or less unique OTUs than expected by chance, we used the 'permatswap' function of the *vegan* package (Oksanen *et al.*, 2017) to create a null expectation. Based on Monte Carlo iterations, we created a total of 999 random simulated matrices keeping total number of OTUs per sample and total number of sequences of each OTU constant. Most samples contained no DNA sequences of Endogonales (n = 182), so further community composition analyses for this group were conducted only on samples where Endogonales were present (n = 78). To visualise variation in community composition of both fungal groups among biomes and systems (i.e. native vs farm sites), we used non-metric multidimensional scaling (NMDS). To test for statistical differences in fungal community composition among biomes and systems, we used permutational multivariate analysis of variance 'adonis2' with the *vegan* package (Oksanen *et al.*, 2017) using both variables in the same model. To test for potential environmental factors driving fungal community composition, we first selected the best explanatory variables for community composition of both fungal groups. We included all climatic, vegetation, and soil data. Collinearity among explanatory variables was inspected using the *vif* function (Fox & Weisberg, 2019). Variables with highest vif index were sequentially removed until all explanatory variables showed a vif index < 10. Then, variable selection was done by permutation tests with forward selection. We used CCA (Ter Braak, 1986) to determine the relative importance of each selected variable in explaining patterns of community composition. A biplot was drawn on the ordination to display the relationship between the explanatory variables and the ordination axes, where the angle and length of the line indicate the direction and strength of the relationship. To identify OTUs with a disproportionate influence on community patterns we used indicator species analysis with 9,999 permutations using the *indicspecies* package (De Caceres *et al.*, 2016).

To test for potential drivers of abundance and richness of fungi, we built a piecewise structural equation model (SEM). First, individual models were fitted with 'biome' as a random effect, and residuals were inspected for assumption violations. Individual models were fitted using the *nlme* package (Pinheiro *et al.*, 2017). Then, path models were fitted with

the *piecewiseSEM* package (Lefcheck *et al.*, 2016). Due to the high number of samples containing no Endogonales sequences, two SEM models were built to analyse fungal abundance and richness: (1) a binomial model using all samples evaluating presence/absence of fungi (n = 260), and (2) a linear model on abundance and richness of fungi in samples in which Endogonales were present (n = 78). Nitrate-nitrogen, K, OC, and S were not selected by the best models and were removed from further analyses. For simplicity, figures only show significant pathways. All data were analysed, and figures were created, in R (R Core Team, 2016).

Results

Fungal community composition

In total, there were 97 and 927 OTUs of Endogonales and G-AMF, respectively, with remaining 2,558 OTUs matching other taxa. Almost all Endogonales sequences best-matched an uncultured sequence previously classified as putative M-AMF, while only 6% best-matched described non-AM fungal *Endogone* species. Only 30% of root samples contained sequences of Endogonales, while 57% contained sequences of G-AMF and 30% of samples contained both. Most samples that did not contain either M- or G-AMF belonged to native sites of kwongkan, montane forest, and Mediterranean and temperate woodland biomes, as well as farm sites dominated by plants typically not colonised by AMF like *Lupin*, *Eucalyptus*, and *Pinus*. No Endogonales were found in tropical rainforests or woodlands in either system (Fig. S2). In the kwongkan and temperate woodland biomes, Endogonales were found only in farm sites (Fig. S2). In contrast, G-AMF were present in all biomes and systems except for the kwongkan and temperate woodland biomes, where G-AMF were found only in farm sites (Fig. S2).

Roots from farm sites were associated with almost four times more unique OTUs of Endogonales than native sites, with only 13% being present in both (Fig. 2a). Unique OTUs found in either system were twice as many as expected by random sampling (Fig. 2a). This was confirmed by indicator species analysis where 10 OTUs were selected as indicators of farm sites, while only two were selected as indicators of native sites (Fig. S3). The three most abundant OTUs (i.e. OTU_2, OTU_43 and OTU_170) represented 73% of all sequences of Endogonales, and all three were selected as indicator species of farm sites. These three OTUs

best matched sequences of uncultured M-AMF (Table S2), and the most abundant OTU, OTU_2, was an identical match to *P. tenue*. The fourth and seventh most abundant OTUs (i.e. OTU_93 and OTU_112) represented only 6% of all sequences and were selected as indicator species of native sites. These OTUs best-matched *Endogone* sp. and an uncultured Mucoromycotina, respectively (Table S2). Each of the top 15 most abundant OTUs of Endogonales showed a clear preference for either system, with 10 out of the 15 being found almost exclusively in farm sites (Fig. S4). The only exception was OTU_966, which was only found in the tropical savannah biome, with no clear preference for a system (Fig. S4).

Each system showed a relatively equal number of unique OTUs of G-AMF, with 43% of them present in both (Fig. 2b). However, shared OTUs between systems was 1.5 times lower than expected by random sampling (Fig. 2b). Indicator species analysis selected 109 OTUs of G-AMF as indicators of native sites, and 57 of farm sites (Fig. S3). The most abundant OTU of G-AMF, OTU_9, represented 11% of all sequences of G-AMF and best-matched *Rhizophagus* sp. This OTU was selected as an indicator of farm sites. The third most abundant OTU of G-AMF, OTU_10, best-matched *Rhizophagus intraradices* and represented only 5% of all sequences of G-AMF. This OTU was selected as an indicator of native sites. Of the 15 most abundant OTUs of G-AMF, none were found exclusively in a system (Fig. S5). However, four of these OTUs showed a clear preference for farm sites, and three for native sites (Fig. S5).

Community composition of Endogonales differed among biomes (adonis2 $R^2 = 0.24$, $P < 0.0001$), and to a lesser extent between systems (adonis2 $R^2 = 0.06$, $P < 0.0001$) (Fig. 3a). System, rainfall, temperature, plant richness, available nutrients, and soil pH explained 19% of the variation in community composition of Endogonales (Permutation test $\chi^2 = 2.16$; d.f. = 6; $P < 0.001$; Fig. 4a). From these, system and soil pH were the strongest drivers on the X axis (i.e. variation between systems) while rainfall, temperature, plant richness and available nutrients explained variation along the Y axis (i.e. variation within systems) (Fig. 4a).

Community composition of G-AMF also differed among biomes (adonis2 $R^2 = 0.24$, $P < 0.0001$) and between systems (adonis2 $R^2 = 0.04$, $P < 0.0001$) (Fig. 3b). System, rainfall, temperature, plant richness, available nutrients, and soil pH explained 13% of the variation in community composition of G-AMF (Permutation test $\chi^2 = 2.67$; d.f. = 6; $P < 0.001$; Fig. 4b). From these, soil pH and system were the strongest drivers on the diagonal between the X and

Y axes while temperature and available nutrients on the diagonal between the X and Y axes (Fig. 4b). For G-AMF, both axes contributed to the variation between and within systems.

Fungal abundance and richness

Sequence abundance of Endogonales was lower (58% of samples) or equal (41% of samples) to that of G-AMF across samples where both were present, except for three samples from the farm site within the arid shrubland biome. Sequence abundance of Endogonales was 2.6 times higher in farm sites than in native sites (Fig. S6). Tropical savannah showed the lowest sequence abundance of Endogonales when present (Fig. S2a). Farm sites of cold biomes, such as montane grasslands and forests, showed the highest sequence abundance of Endogonales (Fig. S2a). The only non-temperate site with high abundance of Endogonales was the wheat farm associated with the arid shrubland biome, which showed the highest abundance of Endogonales (Fig. S2a). Of the native sites, arid shrubland and one of the Mediterranean woodland sites showed the highest sequence abundance of Endogonales.

Sequence abundance of G-AMF did not differ between systems (Fig. S6). When present, G-AMF showed the lowest abundance in native sites of montane forests and highest abundance in farm sites of the tropical woodland biome (Fig. S2a). The site with the highest sequence abundance of G-AMF was the golf course in the montane forest biome, and the pasture and mango plantation in the tropical savannah biome. The site showing the lowest sequence abundance of G-AMF, when present, was the same wheat farm in the arid shrubland biome that harboured the highest sequence abundance of M-AMF.

Richness of Endogonales was lower (56% of samples) or equal (44% of samples) to that of G-AMF. Richness of Endogonales was higher in farm sites than native sites (Fig. S6), except for arid shrubland, where it was higher in the native site than in the farm site (Fig. S2b). Farm sites within temperate forest and montane grassland biomes showed the highest richness of Endogonales, while native sites of the same biomes were among the lowest values, when present (Fig. S2b). Richness of G-AMF did not differ between systems (Fig. S6), with the exceptions of the arid shrubland biome where it was higher in the native site, and montane and temperate forest biomes, where it was higher in farm sites (Fig. S2b). Richness of G-AMF was highest in tropical biomes and lowest in arid shrublands and Mediterranean-climate woodlands (Fig. S2b).

the multivariate *piecewise* SEM linking climate, system, and soil chemistry with presence of fungi was well supported by the data (χ^2 = 0.19; d.f. = 4; P = 0.99). Given that χ^2 represents the discrepancies between observed and modelled variance-covariance matrices, obtaining a p-value > 0.05 means discrepancies are not different from zero, and the model is a good fit. The best model selected system as the only explanatory variable for presence of Endogonales (i.e. higher probability of finding Endogonales in a farm than in a native site) (Fig. S7). In contrast, increasing rainfall indirectly reduced the probability of finding G-AMF, mediated by a decrease in soil pH, while higher temperature and system indirectly increased the probability of finding G-AMF, mediated by an increase in soil pH (Fig. S7).

The multivariate piecewise SEM linking climate, system, plant richness, and soil chemistry with fungal sequence abundance and richness was also well supported by the data (χ^2 = 5.8; d.f. = 6; P = 0.44). The best model showed that sequence abundance and OTU richness of Endogonales and G-AMF were directly and indirectly driven by environmental factors with plant richness as one of the main mediators (Fig. 5). There was a strong positive total effect of system on both abundance and richness of Endogonales (i.e. higher abundance and richness in farm sites) (Fig. S6), however the indirect effects were complex and almost entirely mediated by soil pH, available nutrients and plant richness (Fig. 5a). In detail, there was a negative indirect effect of system on abundance of Endogonales, mediated by decreased plant richness, but a positive indirect effect mediated by increased soil pH which increased plant richness (Fig. 5a). That said, total effect of pH on abundance of Endogonales was negative albeit marginally non-significant (β = -0.26; P = 0.09). Increasing temperature had a strong indirect negative effect on both abundance and richness, mediated by decreased available nutrients, while a small indirect positive effect mediated by increased plant richness because of a decrease in available nutrients (Fig. 5a). Increasing rainfall had a positive indirect effect on Endogonales through an increase in available nutrients, but a small negative effect mediated by decreased plant richness (Fig. 5a).

There was no total direct effect of system on G-AMF (Fig. S6), but there were indirect effects. In detail, system showed indirect positive and negative effects on abundance and richness of G-AMF mediated by increased soil pH and decreasing plant richness; while increasing rainfall showed a negative effect on abundance of G-AMF mediated by decreased soil pH (Fig. 5b). Increasing temperature was the strongest driver of richness of G-AMF, having both a direct and indirect positive effect mediated by decreased available nutrients

(Fig. 5b). Finally, increasing rainfall had a negative effect on richness of G-AMF mediated by decreased soil pH and increased available nutrients, both increasing and decreasing plant richness, respectively (Fig. 5b).

Phylogenetic diversity of Endogonales

Phylogenetic analyses identified four clades within Endogonales, with each clade populated by OTUs identified in this study and verified by reference sequences (Fig. S8). However, support for each node was poor (i.e. < 50% of bootstrap replicates) for most branches. Most OTUs (69%) were placed within clade A1 with known and putative M-AMF, including *P. tenue*. Another 13% were placed in clade A2 with unknown Mucoromycotina sequences; 7% in clade B1 with other putative M-AMF; and 11% in clade B2 with non-AMF Endogonales such as *E. pisiformis* and *J. flammicorona*. From the 15 most abundant OTUs of Endogonales, the ML analysis placed the 10 that showed a clear preference for farm sites in clade A1; of the four that showed a preference for native sites, two were placed in clade B2 (i.e. OTU_93 and OTU_237) and two were placed in the most distant branch of clade A2 (i.e. OTU_112 and OTU_223) (Fig. S8).

Discussion

This is the first study to directly compare the abundance and richness of the two groups of arbuscule-forming fungi, M-AMF and G-AMF, across a range of contrasting biomes. Our phylogenetic analysis suggests most Endogonales sequences we sampled were putative M-AMF. However, overall support for the phylogenetic tree was poor and we suggest caution when interpreting these results. Our results, from across the Australian continent, show putative M-AMF are not as ubiquitous as G-AMF, with these two groups of AMF having different but overlapping ecological niches. Our findings also suggest putative M-AMF are less speciose and more specialised, by environment and potentially host plants, than G-AMF. Communities of both fungal groups were structured by biome and, to a lesser degree, system, supporting our first hypothesis. Both fungal groups showed a contrasting response to temperature, rainfall, and soil pH, but responded in a similar way to plant richness, hence only partially supporting our second hypothesis. Putative M-AMF thrived in agricultural

land, while only community structure of G-AMF was affected by land-use, hence partially supporting our third hypothesis. Overall, our study supports the hypothesis proposed by previous authors that cold and wet biomes with acidic soils favour M-AMF (Tedersoo *et al.*, 2014; Orchard *et al.*, 2017b; Albornoz *et al.*, 2020b). The importance of plant diversity, soil pH, rainfall, and temperature has been previously addressed for G-AMF but not for M-AMF (Brundrett & Tedersoo, 2018; Weber *et al.*, 2019; Davison *et al.*, 2021).

We found a preference of putative M-AMF for agricultural soils as previously suggested by Orchard *et al.* (2017b). The geographic origin of M-AMF is unknown, as are the ecosystems for which they are native. However, the high abundance and richness of putative M-AMF in farm sites, coupled with their almost complete absence from most native sites, suggest M-AMF might benefit from agricultural land-use. This hypothesis is further supported by the fact that the most abundant Endogonales favoured by native sites best-matched *Endogone* species thought to be ectomycorrhizal fungi (Desirò *et al.*, 2017; Yamamoto *et al.*, 2017). These OTUs are probably not M-AMF, but rather undescribed species of ectomycorrhizal fungi. Therefore, putative M-AMF occur almost exclusively in farm sites and thus anthropogenic land-use may be promoting diversity and abundance of M-AMF (Sjödin *et al.*, 2018). How can land-use promote diversity of M-AMF is beyond this study but the advances in molecular tools, such as full genome sequencing, offer the potential to explore this hypothesis and perhaps determine the geographic origin of the M-AMF thriving in Australian agricultural landscapes.

We found M-AMF thrived in open herbaceous ecosystems, consistent with previous studies (Öpik *et al.*, 2006; Moora *et al.*, 2014). Within farm sites, putative M-AMF were most abundant in pastures and wheat farms, while being absent or in low abundance in tree plantations and orchards, except for the apple farm, which featured one of the highest weed diversities of all the farm sites. Following Grime's CSR framework (Grime, 1979), annual grass and forb plant species can be roughly classified as 'ruderal', meaning they are fast growing and capable of colonising recently disturbed environments, like most invasive weeds (Alexander *et al.*, 2016). All farm sites in non-tropical biomes harboured a relatively diverse understory of weedy annuals, except for the pine and eucalypt plantations (Fig. 1; FEA, personal observations 01/09/2019-20/11/2019). Taken together, these data and observations suggest that M-AMF can adopt a 'ruderal' life strategy as previously proposed (Chagnon *et al.*, 2013). However, given their persistence in extreme environments such as acidic soils and

cold biomes, a “stress-tolerator” life strategy is also plausible. A compatible CSR life strategy between symbiont and host is likely allowing M-AMF to thrive in agricultural systems.

Soil pH had a complex effect on Endogonales and G-AMF. Richness of both fungal groups was either directly or indirectly increased by increasing soil pH. This was not the case for their relative abundance, where that of Endogonales decreased while that of G-AMF increased with increasing pH. Previous studies suggest that lower richness but higher abundance of putative M-AMF in acidic soils is due to environmental filtering favouring only species capable of thriving in such soils (Albornoz *et al.*, 2020b). It is worth noting that Australian soils are generally very acidic, with the range studied here being pH 3.1–7 (measured in $CaCl_2$). Hence, the higher richness and abundance of G-AMF with increasing soil pH is not related to a preference for alkaline soils, but rather for less acidic soils, where P is more available to plants (Lambers *et al.*, 2008). Our findings of ecological segregation between Endogonales and G-AMF supports previous research (Orchard *et al.*, 2017b; Albornoz *et al.*, 2020b), but here we provide evidence of it across a larger environmental and climatic gradient, as well as across a diverse range of host and farm managements.

Putative M-AMF showed a preference for biomes with lower temperatures and higher rainfall, in addition to acidic soils. This agrees with previous research of root colonisation by M-AMF and G-AMF (Albornoz *et al.*, 2020b), and is consistent with research showing M-AMF to have a greater tolerance for waterlogged environments than G-AMF (Orchard *et al.*, 2016). Here, we evaluated the effects of temperature and rainfall on diversity of Endogonales, showing that while rainfall increased their abundance and richness, temperature acted as an environmental filter selecting for a subset of species that can exist in warmer climates. This, together with the fact that putative M-AMF were almost entirely absent from tropical biomes (Brundrett & Ashwath, 2013), suggests a preference for cold and wet climates (Tedersoo *et al.*, 2014). Glomeromycotinian AMF, in contrast, were ubiquitous with a slight preference for tropical biomes and warmer temperatures, the latter consistent with previous findings (Tedersoo *et al.*, 2014; Pärtel *et al.*, 2017; Albornoz *et al.*, 2020b).

Increased soil fertility, associated with farm sites, promoted abundance of Endogonales and had no direct effect on G-AMF abundance. This contradicts previous findings where increased soil P availability decreased root colonisation of *Trifolium spp.* by both M-AMF and G-AMF in pastures across temperate Australia (Ryan *et al.*, 2000; Albornoz *et al.*, 2020b). Due to their roles in plant nutrition, we expected to see a decline in relative

abundance with increasing soil fertility of both fungal groups, particularly in farm systems where inputs of P can be high (Ryan *et al.*, 2000; Ryan & Angus, 2003). However, emerging evidence questions the expectation of decline in abundance of AMF with increasing soil P availability, at least for large-scale Australian field studies like ours (Krüger *et al.*, 2015). Perhaps the relationship holds for individual fungal species and their plant hosts but is less evident for multiple fungal species across multiple hosts. Also, a negative correlation between increasing P availability and G-AMF only occurs at intermediate P levels, with suppression of G-AMF at high and low P supply (Abbott *et al.*, 1984; Bolan *et al.*, 1984; Bolan *et al.*, 1987; Reddell *et al.*, 1997). While inferences about the nutritional benefits of M-AMF to hosts is beyond the scope of this study, our findings provide important context for future research on this topic.

The indirect effects of soil pH and fertility mentioned above were strongly mediated by plant richness for both fungal groups. While plant richness acted as mediator between some environmental variables and abundance and richness of M-AMF, plant richness mediated all indirect effects for G-AMF. The only exceptions were temperature and soil pH which had a direct effect on richness and abundance, respectively, in addition to indirect effects. The role of host specificity or aboveground host diversity on G-AMF has been vastly studied but remains unresolved, with studies showing no-to-strong effects (e.g. Martínez-García *et al.*, 2015; Weber *et al.*, 2019; Toussaint *et al.*, 2020), while the role of plant richness on M-AMF has not been previously studied. Our findings suggest the importance of plant richness for mediating abiotic effects on both fungal groups. Including these data in future studies may help to resolve causal relationships among biotic and biotic predictors and the distribution and abundance of M-AMF and G-AMF.

Conclusions

Our study provides empirical evidence that putative M-AMF and G-AMF have distinct, but overlapping, ecological niches for temperature, rainfall, and soil pH at a large geographic scale and across a steep environmental gradient. These findings are pivotal given that M-AMF, as members of Endogonales, have been excluded until recently from molecular studies of G-AMF owing to their misclassification within Glomeromycotina. Thus, these findings contribute to a growing understanding of the biology, phylogeny, and ecology of M-AMF

(Orchard *et al.*, 2017b; Hoysted *et al.*, 2018; Albornoz *et al.*, 2020b) and reinforce the validity of the hypothesis that M-AMF are adapted to extreme environments. Our findings also highlight the importance of environmental context when studying ecological responses to land-use. Experimental designs limited to one or two ecosystems or environmental factors likely will not capture the large variability in responses of these two fungal groups (Garcia de Leon *et al.*, 2018). Coupled with their preference for agricultural land, we suggest putative M-AMF adopt strategies of 'stress-tolerators' or 'ruderals' but not 'competitors' (Chagnon *et al.*, 2013). Mucoromycotinian-AMF are likely globally distributed, given many of our sequences of putative M-AMF showed homology to M-AMF from other continents, including the northern hemisphere. This suggests a role of humans in either the introduction or expansion of M-AMF that begs further investigation. Our short reads (~260 bp) did not allow for a robust phylogenetic analysis and hence, their phylogeny remains unresolved. A better taxonomic resolution will help us understand the true diversity of M-AMF. Finally, while putative M-AMF prefer farms to native sites, their role in sustainable agriculture should not be assumed until tested in an agronomically-relevant manner (Ryan & Graham, 2018).

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Author contribution

M.H.R, R.J.S, G.D.B, and I.A.D developed the project and obtained funding. F.E.A., M.H.R, R.J.S, and G.D.B conducted sampling and data collection. G.D.B and S.H. conducted DNA molecular work. F.E.A. conducted bioinformatics work and data analysis. F.E.A lead the manuscript preparation, with all authors contributed to the writing and providing conceptual advice. D.B.G and I.A.D. provided statistical advice.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Sequences are available at NCBI (BioProject PRJNA745025).

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Tables

Table 1 Summary table of environmental and vegetation variables used in this study. Variables were mean annual minimum temperature, mean annual rainfall, plant richness around a 2 m radius from sample (12.56 m^2 area), nitrogen (N) as ammonium (NH_4-N) and nitrate (NO_3-N), available phosphorus (P), potassium (K), and sulfur (S), soil pH (in $CaCl_2$), and organic carbon (OC). There were five samples collected from each site and *n* represent the number of sites from each system and biome combination. Values are mean ± standard error of the five samples per site and then averaged for *n* sites.

Biome	State	System	*n*	Temperature (°C)	Rainfall (mm)	Plant Richness (12.56 m^2)	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	S (mg kg^{-1})	pH ($CaCl_2$)	OC (%)
Tropical	Northern	Farm	3	21 ± 0	905 ± 8	6 ± 1	5 ± 1	1.1 ± 0.4	4 ± 1	30 ± 5	2.5 ± 0.4	4.8 ± 0.1	1.6 ± 0.2
savannah	Territory	Native	3	22 ± 0	1340 ± 59	10 ± 0	5 ± 0	0.4 ± 0.1	3 ± 0	111 ± 19	1.3 ± 0.1	5.0 ± 0.1	0.9 ± 0.1
Tropical	Queensland	Farm	2	20 ± 1	2471 ± 401	5 ± 0	36 ± 5	9.1 ± 2.1	76 ± 15	110 ± 8	8.0 ± 0.9	5.2 ± 0.1	3.2 ± 0.3
forest		Native	3	19 ± 1	2205 ± 287	11 ± 0	39 ± 4	3.0 ± 0.7	15 ± 1	162 ± 18	9.7 ± 0.9	4.5 ± 0.1	3.9 ± 0.3
Tropical	Queensland	Farm	2	17 ± 0	823 ± 0	3 ± 0	4 ± 1	4.7 ± 0.7	29 ± 1	530 ± 67	4.9 ± 1.1	5.3 ± 0.1	1.6 ± 0.2
woodland		Native	1	17 ± 0	823 ± 0	11 ± 0	10 ± 2	0.9 ± 0.3	62 ± 20	398 ± 76	3.3 ± 0.6	5.4 ± 0.2	3.5 ± 0.3
Montane	Tasmania	Farm	3	6 ± 0	1306 ± 236	7 ± 0	17 ± 3	3.6 ± 0.7	128 ± 34	365 ± 78	9.7 ± 0.9	5.3 ± 0.2	3.3 ± 0.4
forest		Native	3	6 ± 0	1450 ± 209	5 ± 1	16 ± 1	0.6 ± 0.1	20 ± 2	155 ± 18	10.8 ± 1.3	3.9 ± 0.1	4.5 ± 0.3
Montane	Tasmania	Farm	3	4 ± 1	1163 ± 172	8 ± 1	28 ± 3	1.8 ± 0.3	33 ± 7	549 ± 107	7.2 ± 0.7	4.7 ± 0.1	4.8 ± 0.2
grassland		Native	3	4 ± 1	1366 ± 225	7 ± 0	31 ± 5	1.1 ± 0.2	24 ± 1	166 ± 20	14.2 ± 1.2	4.4 ± 0.2	5.6 ± 0.2
Temperate	Tasmania	Farm	3	7 ± 1	1621 ± 241	8 ± 0	27 ± 5	3.3 ± 0.5	65 ± 10	306 ± 41	7.3 ± 0.8	4.8 ± 0.1	4.4 ± 0.2
forest		Native	3	7 ± 1	1373 ± 149	4 ± 0	23 ± 4	1.3 ± 0.4	27 ± 3	156 ± 17	16.8 ± 1.5	3.7 ± 0.1	4.9 ± 0.2
Arid	Western	Farm	1	11 ± 0	218 ± 0	2 ± 0	7 ± 1	30.8 ± 3.8	16 ± 2	99 ± 18	26.9 ± 3.6	5.0 ± 0.3	0.5 ± 0.1
shrubland	Australia	Native	1	14 ± 0	153 ± 0	7 ± 0	2 ± 0	1.2 ± 0.3	1 ± 0	94 ± 7	6.5 ± 1.7	4.6 ± 0.2	0.6 ± 0.1

Kwongkan	Western Australia	Farm	3	13 ± 0	365 ± 0	4 ± 0	1 ± 0	1.5 ± 0.35	7 ± 1	35 ± 6	6.9 ± 2.4	5.7 ± 0.1	1.2 ± 0.1
		Native	3	13 ± 0	365 ± 0	16 ± 0	1 ± 0	0.6 ± 0.1	1 ± 0	12 ± 1	1.9 ± 0.3	5.5 ± 0.1	0.7 ± 0.1
Mediterranean woodland	Western Australia	Farm	3	13 ± 0	199 ± 12	2 ± 0	1.1 ± 0	2.4 ± 0.72	32 ± 2	54 ± 5	6.2 ± 1.1	5.7 ± 0.1	0.7 ± 0.1
		Native	3	13 ± 1	199 ± 12	7 ± 1	2.4 ± 1	0.63 ± 0.1	1 ± 0	80 ± 5	5.6 ± 2.4	5.3 ± 0.1	1.0 ± 0.4
Temperate woodland	Western Australia	Farm	3	10 ± 0	1026 ± 10	6 ± 1	4.3 ± 1	0.67 ± 0.17	11 ± 2	30 ± 4	3.9 ± 0.6	5.2 ± 0.2	2.0 ± 0.3
		Native	3	10 ± 0	1055 ± 0	17 ± 1	1.2 ± 0	0.5 ± 0	2 ± 0	13 ± 1	2.4 ± 0.2	4.1 ± 0.1	1.4 ± 0.2

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Figures

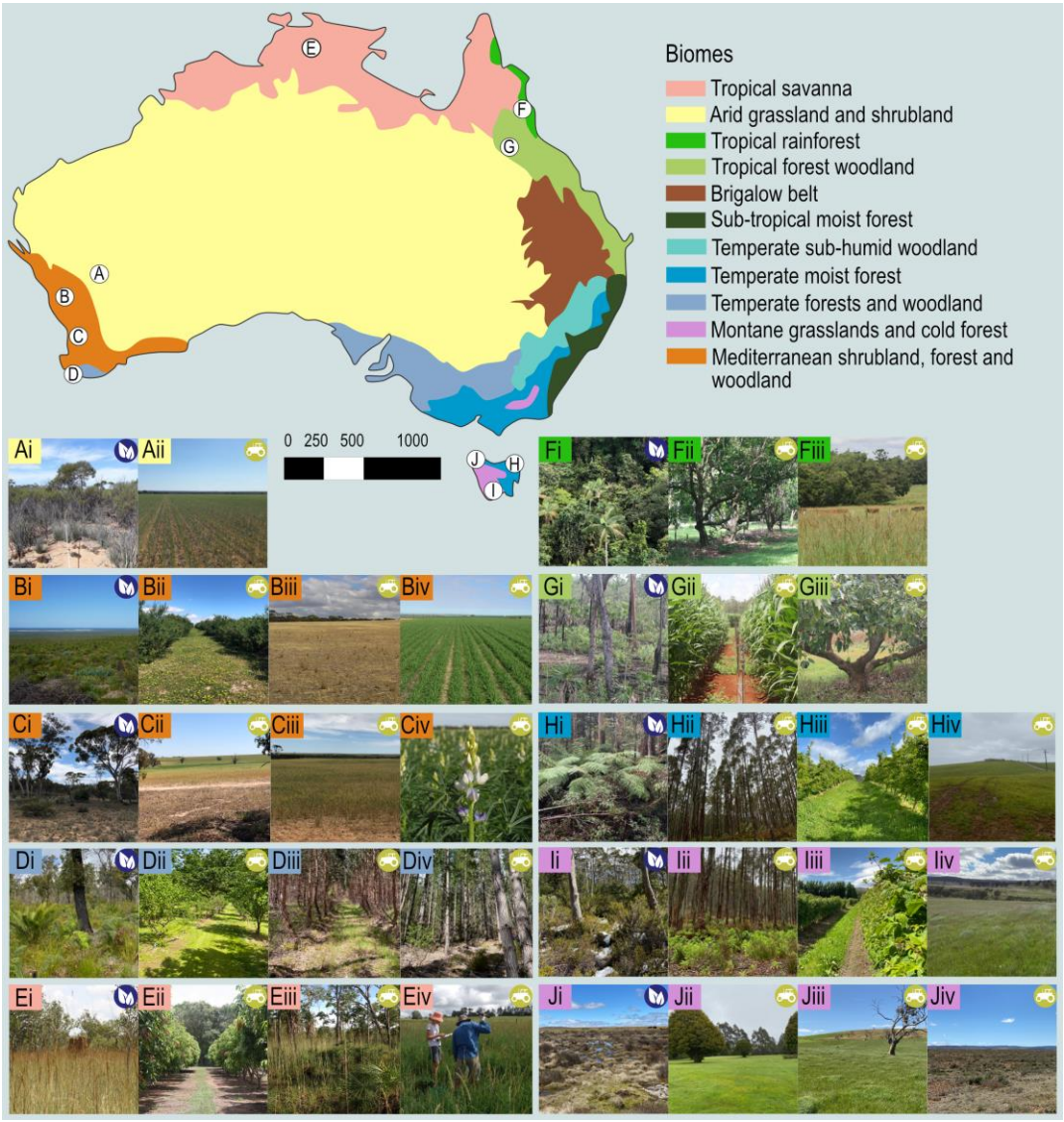
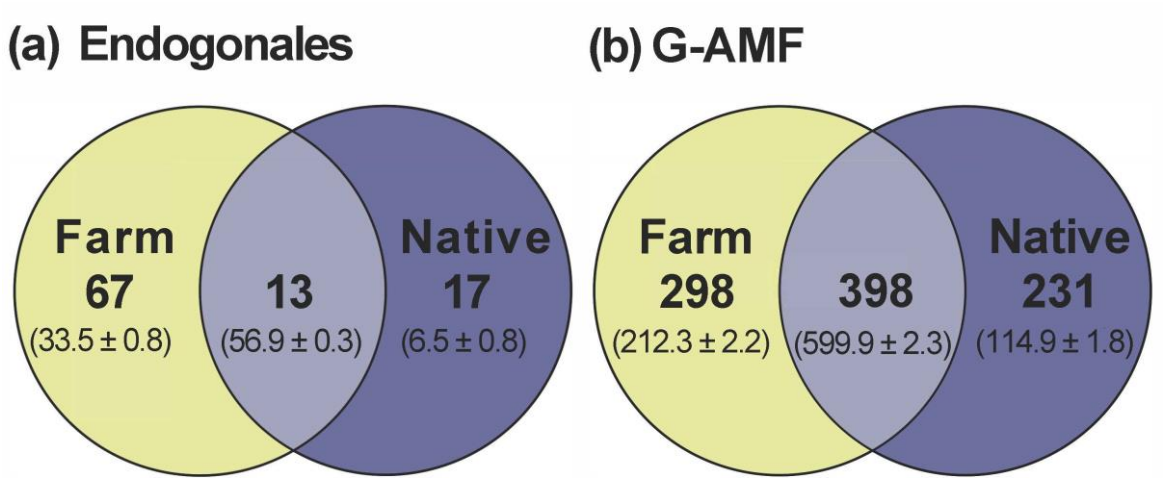


Figure 1. Map of biomes within Australia. Biomes sampled in this study were: arid shrubland (A), Mediterranean shrubland (B), Mediterranean woodland (C), temperate woodland (D), tropical savannah (E), tropical forest (F), tropical woodland (G), temperate forest (H), montane cold forest (I), and montane grassland (J). Each biome is represented with a colour and a letter on the map except for Mediterranean shrubland and forest, and temperate forest and woodland, which were grouped together, respectively, due to their mosaic distribution. Within a biome, the left panel shows the native system sampled (blue leaf symbol), while the right panel shows the farm system sampled (yellow tractor symbol).



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820 **Figure 2**. Observed and expected number of operational taxonomic units (OTUs) of **(a)**
821 Endogonales and **(b)** Glomeromycotinian arbuscular mycorrhizal fungi (G-AMF) between
822 farm (yellow) and native systems (blue). Expected number of OTUs were based on Monte
823 Carlo random sampling (999 iterations) and are displayed in brackets (mean ± 95%
824 confidence intervals).

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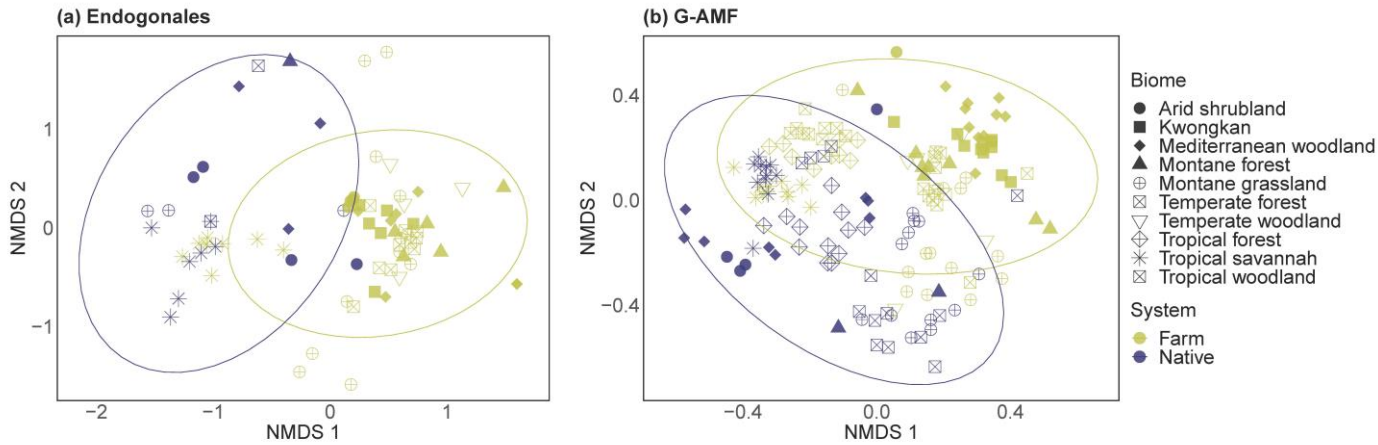

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834 **Figure 3** Nonmetric multidimensional scaling (NMDS) ordination plot showing differences in
835 community composition of **(a)** Endogonales and **(b)** Glomeromycotinian arbuscular mycorrhizal fungi
836 (G-AMF) in roots across ten biomes in Australia. Biomes are indicated by symbol shape and systems
837 by symbol colour. Ellipses represent 95% confidence area around the mean centroid for each site
838 using Bray-Curtis dissimilarity values (plot stress = 0.18 and 0.24 for Endogonales and G-AMF,
839 respectively).

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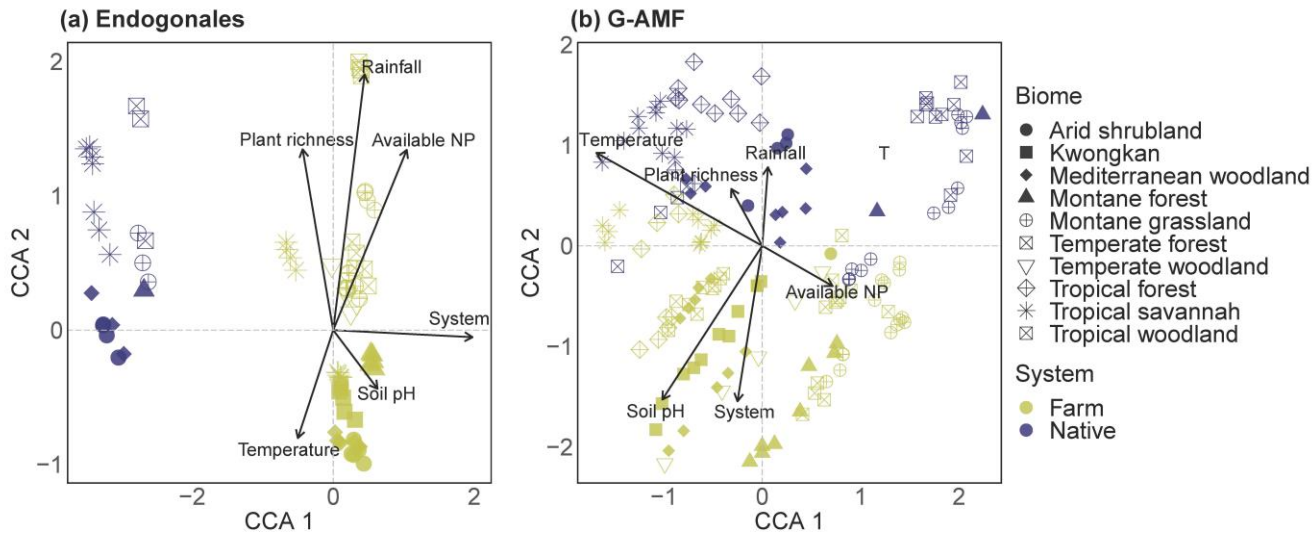

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848 **Figure 4**. Canonical correspondence analysis of environmental factors structuring
849 communities of **(a)** Endogonales and **(b)** Glomeromycotinian arbuscular mycorrhizal fungi
850 (G-AMF). The system categorical variable was transformed to binomial (native = 0; farm =
851 1). Explanatory variables shown were selected by permutation test with forward selection
852 using 999 permutations. These variables explained 19% and 13% of the community variation
853 of Endogonales (Permutation test χ^2 = 2.16; d.f. = 6; P < 0.001) and G-AMF (Permutation
854 test χ^2 = 2.67; d.f. = 6; P < 0.001), respectively. Length of arrows is proportional to the effect
855 on community composition.

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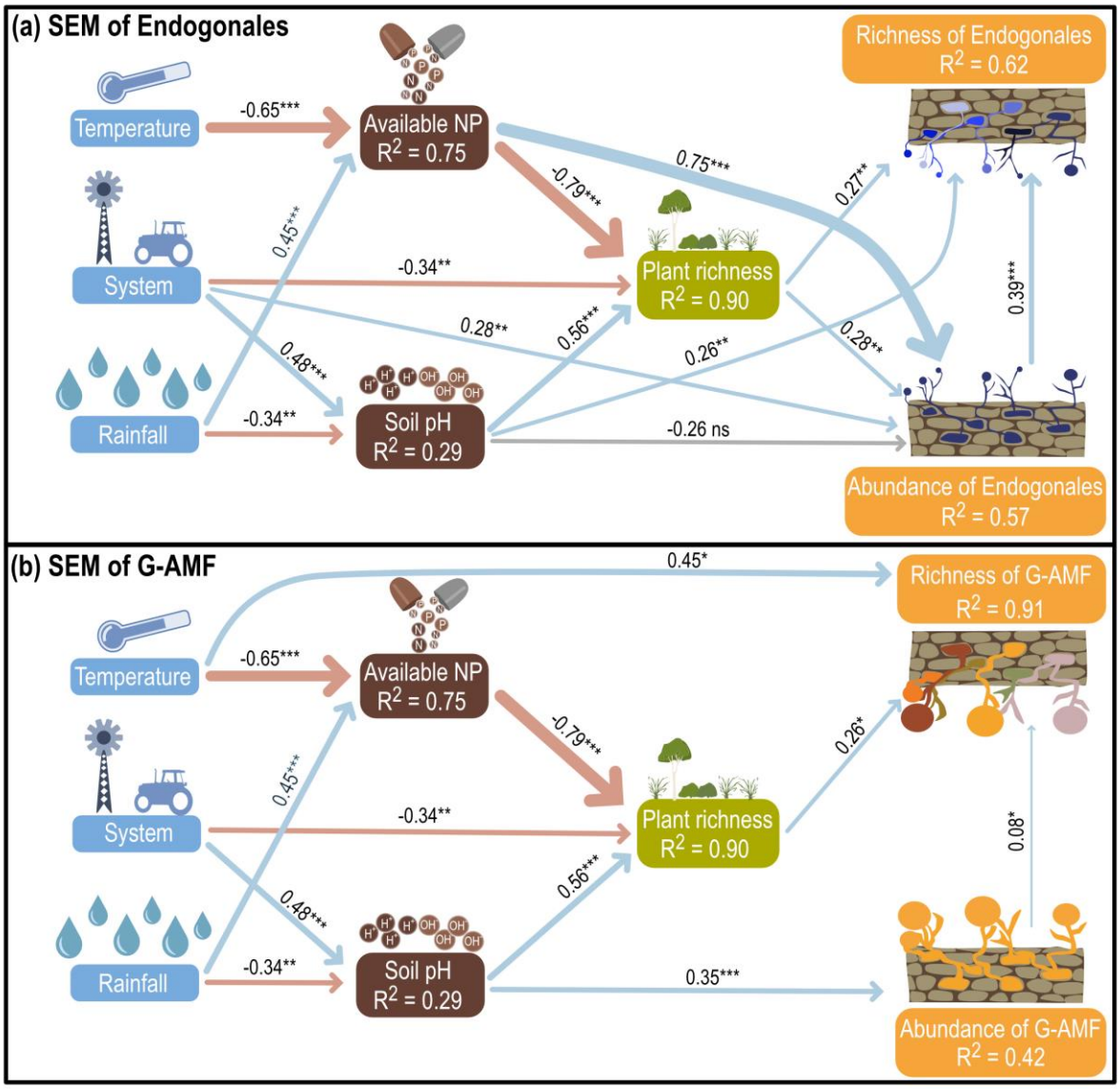

Figure 5. Multilevel pathway model showing the direct and indirect pathways by which environment influences abundance and richness of **(a)** Endogonales and **(b)** Glomeromycotinian arbuscular mycorrhizal fungi (G-AMF). Boxes represent climatic variables and systems (native = 0; farm =1) (blue), soil chemistry (brown), plant richness (green), and fungal variables (orange). The model was well supported by the data (χ^2 = 5.8; d.f. = 6; P = 0.44). Arrows represent positive (blue), negative (pink), and marginally non-significant (grey) effects. For simplicity, non-significant effects are not shown (P > 0.1). Width of arrows are proportional to standardised path coefficients. Unstandardised path coefficients are shown for each arrow. The amount of variation explained by each variable in the model (conditional R^2) is shown within each box.