Title: Divergent responses between lineages of arbuscular mycorrhizal fungi to soil phosphorus and nitrogen availability

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Running title: Arbuscular mycorrhizas and soil fertility

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Highlights

- Arbuscular mycorrhizal fungi exhibit diverse growth and colonisation strategies
- Families of these fungi differed in their responses to soil fertility
- Relationships were stronger with soil phosphorus than with soil nitrogen
- Gigasporaceae decreased and Glomeraceae increased as soil phosphorus increased
- Relationships were stronger in woodlands than in forests and grasslands

Abstract

Arbuscular mycorrhizal (AM) associations are multifunctional. Two important functions they perform are facilitating nutrient uptake in host plants and protecting plants from biotic stress, among other functions. AM fungal taxa vary in how capably they perform these functions and can also respond differently to environmental selection. Therefore, there is a need to better understand how particular environmental variables might alter the response of AM fungal communities. Here, we analysed data from a DNA-based survey of fungal communities in soils collected throughout Australia to observe relationships among soil fertility and the abundance of two AM fungal taxa that reportedly vary in function – the Gigasporaceae (putatively more important for nutrient uptake) and Glomeraceae (putatively more important for biotic stress). Relationships were assessed in three vegetation types – grasslands, forests and woodlands – to assess whether associations with soil fertility varied depending on carbon availability for AM fungi. Fungi from the Gigasporaceae decreased in frequency as available phosphorus increased, while those from the Glomeraceae increased or were unresponsive as available phosphorus increased. Similar patterns were observed for nitrate availability, although only in woodlands. These patterns are consistent with expectations that AM fungi from the Gigasporaceae, in general, are better suited to alleviate nutrient limitation in hosts as soil fertility decreases. This knowledge may aid in implementing optimal strategies involving AM fungal inoculum best suited to the local conditions of future land management and agricultural projects.

Keywords

Illumina MiSeq, Mutualism, Spatial lag model, Symbiosis

Introduction

Communities of arbuscular mycorrhizal (AM) fungi are typically composed of functionally diverse taxa. For example, comparative studies have observed that AM fungi belonging to the Gigasporaceae may acquire and provide more nutrients to their host plants relative to those from the Glomeraceae (Powell et al. 2009; Sikes, Cottenie & Klironomos 2009). As a result, variation in the abundance of these AM fungal taxa may potentially reflect variation in community function. Despite the importance of community structure in determining community function, there are still significant gaps in our understanding of how AM fungal communities assemble (Vályi et al. 2016). Improving our understanding of how environmental factors might select for functionally diverse groups of AM fungi will aid in identifying variables that can be used to predict patterns in AM fungal communities within natural systems, as it has helped do so for fungi in general (e.g., Aguilar-Trigueros et al. 2023, Siciliano et al. 2014). There is evidence of environmental variables such as soil fertility (Teste et al. 2016; Treseder & Allen 2002), aridity (Weber et al. 2019; Staddon et al. 2003) and pH (Yang et al. 2011; Davison et al. 2021) having significant effects on AM fungal abundance. How these effects vary among functionally different AM fungal taxa over large environmental gradients is less clear since previous studies have largely been conducted over relatively small environmental gradients (Treseder & Allen 2002; Bhadalung et al. 2005; Johnson et al. 2003; Camenzind et al. 2016). Comparing the relationships between functionally different AM fungal taxa and environmental variables over a greater spatial scale and variety of vegetation may help better understand just how significant such variables are in the assembly of AM fungal communities.

The abundance of AM fungi may be directly affected by characteristics of the environment that limit their growth. Although primarily known for nutrient trade with their hosts, AM fungi have their own nutritional requirements. This is most prominent in regard to N, which AM fungi require in relatively high amounts (Johnson et al. 2015; Hodge & Fitter 2010), with N content in AM fungal tissue (3-5%) observed to be greater than that for plants (approximately 1%) (Hodge & Fitter 2010). The abundance of AM fungi may also be directly limited by P in soils where it is extremely deficient, although AM fungi are likely more commonly N-limited given the relatively large N-demand required for fungal growth (Hodge & Fitter 2010). Nutritional demand likely varies somewhat between the Gigasporaceae and

Glomeraceae due to variation in where and how much resource is allocated to production of fungal structures: taxa associated with the Gigasporaceae exhibit, on average, larger spores (Aguilar-Trigueros et al. 2019), lower spore density (de Souza et al. 2005), slower growth rates (Powell et al. 2009; Hart & Reader 2002) and a greater proportion of extraradical mycelium relative to root colonisation (Powell et al. 2009; Hart & Reader 2002) compared to the Glomeraceae. Therefore, it would be expected that the relationship between soil fertility and the abundance of taxa associated with these two AM fungal families would vary as a result of direct effects alone. For instance, N and P required for chitin in cell walls and lipids in cell membranes might more strongly limit production by fungi in the Gigasporaceae when nutrient availability is very low.

Additionally, environmental characteristics may also differentially influence the abundance of functionally distinct AM fungal taxa indirectly via altering the AM requirement of local plants. Plants provide their AM fungal partners with photosynthetically-fixed carbon in exchange for nutrients such as P and N (Smith & Read 2008). This host-derived carbon acts as the sole carbon source for AM fungi. Therefore, AM fungi that best improve host fitness should themselves receive a fitness advantage, tracking that of their host (Johnson 2010). This concept is supported by studies that observed hosts actively promoting their most beneficial AM fungal partners, in terms of P and N-trade, with a greater investment of carbon (Kiers et al. 2011; Bever et al. 2009). The availability of this carbon is largely dependent on the local vegetation and conditions that can vary significantly between different environments. For example, canopy cover can greatly reduce the levels of sunlight that reach the understory, limiting photosynthesis in plants that reside in shaded regions beneath these canopies and carbon available for trade between AM fungi and understorey vegetation (Koorem et al. 2017). The average extent of canopy cover significantly varies between grassland, woodland and forest vegetation types. Forest environments are typically characterised as having a denser canopy than woodlands, with significantly less sunlight reaching smaller shrubs and grasses in the understory (Missouri Department of Conservation 2021; Shvidenko et al. 2005). Grasslands are significantly more open than both with relatively little canopy cover restricting sunlight to AM hosts below (Breshears 2006).

While grasslands are typically dominated by AM plant species (Miller, Wilson & Johnson 2012; Treseder & Cross 2006), the greater abundance of woody perennial hosts within

112 woodland and forest environments brings with them a greater diversity in mycorrhizal types
113 and the associated fungi that may influence the carbon pools available to AM fungi (Smith &
114 Read 2008; Breshears 2006; Brundrett 2017). There is significant variation in the dominant
115 mycorrhizal type, generally either AM or ectomycorrhizal (EcM), among forests and
116 woodlands around the globe (Read 1991; Lin et al. 2017; Brundrett & Kendrick 1988;
117 Phillips, Ward & Jones 2014). However, there are a relatively large number of EcM plant
118 species in Australia when compared with the rest of the world (Brundrett 2009, 2017), with
119 EcM tree species typically dominating in Australian woodland and forest vegetation types
120 (Brundrett 2017). For example, trees in the Myrtaceae, which includes *Eucalyptus*, are hosts
121 of EcM fungi (Brundrett 2017). *Eucalyptus* forests are estimated to account for 77% of the
122 total native forest area within Australia (Australian Department of Agriculture and Water
123 Resources 2018). Although many of these taxa are considered dual-mycorrhizal, able to host
124 both AM and EcM fungi, it is proposed that EcM fungi are dominant in adult *Eucalyptus*
125 hosts (Adams et al. 2006; Chen, Brundrett & Dell 2000). As a result, the proportion of host
126 carbon available to AM fungi is expected to be reduced within woodland and forest systems
127 compared to that of grasslands when the dominant trees are not, or are only weakly, AM.
128 Therefore, AM fungi are expected to receive a greater amount of carbon per unit of P and N
129 in grasslands.

130 When the carbon value (i.e., the amount of carbon received by AM fungi per unit of P or N)
131 is greatest, the abundance of AM fungi is expected to be more sensitive to changes in the
132 concentration of soil nutrients. This carbon value is expected to vary between different
133 vegetation types and ultimately, environmental selection based on soil fertility and its
134 influence on AM fungal community assembly is expected to vary accordingly. In addition,
135 AM fungi may have a more significant role in P-trade than N-trade with host plants (Johnson
136 2010). As a result, P-trade likely accounts for a larger degree of the carbon that AM fungi
137 receive directly via nutrient exchange with their hosts. Coupled with the fact that Australian
138 soils are commonly P-limited (Hopper 2009; Rossel & Bui 2016; Kooyman, Laffan & Westoby
139 2017), it could be expected that AM fungal abundance will respond more significantly to
140 changes along a gradient of soil P compared to a gradient of soil N within Australian soils.

141 The first objective of this study was to compare the relationships between the relative
142 abundance of Gigasporaceae and Glomeraceae with available soil P (PO_4^+, phosphate) and N

(NO_3^- and NH_4^+, hereafter referred to as nitrate-N and ammonium-N), using data associated with isolated DNA and soil properties from a continental scale survey of soils across Australia (Bissett et al. 2016). We then assessed whether these relationships varied across three vegetation types (grasslands, woodlands and forests), a proxy for the proportion of available photosynthetically fixed carbon allocated for trade with AM fungi. We hypothesized that the relative abundance of the Gigasporaceae, due to greater mycelial investment, would be more sensitive to changes in N and P availability than that of the Glomeraceae, and that both groups would be more sensitive to changes in soil P compared to N. Of the two forms of N, we hypothesised that AM fungi would be more sensitive to changes in nitrate-N given its relative abundance and mobility compared to that of ammonium-N in most soils (Hodge & Storer 2015).

Methods

Sample and data collection

The data associated with isolated fungal DNA and properties of the soils they were detected within were provided by the Biomes of Australian Soil Environments (BASE; now known as the Australian Microbiome Initiative, https://www.australianmicrobiome.com/) soil microbial diversity database (Bissett et al. 2016). Collection, handling and analysis of the samples from which these data are derived was performed by contributors to the BASE database as described by Bissett et al. (2016) and, where required, details of scientific licenses obtained by sample collectors can be found in the database. Briefly, for each sampling site, between nine and twenty-five soil samples were taken from 25 x 25 m quadrats at two soil depths (0-10 cm and 20-30 cm). For each depth, soil samples were homogenised into a composite sample representative of the plot at that depth. Here we focussed only on topsoil samples (0-10 cm depth); see Table S1 for a list of samples used here. Available P was measured using the Colwell method, while both nitrate-N and ammonium-N were extracted using 1 M potassium chloride at 25° C and measured using colorimetric analyses. These measurements on soil chemistry were generally conducted at CSBP Laboratories (Perth, WA). Data associated with samples where the concentration of P, nitrate-N or ammonium-N were greater than 50 mg/kg were excluded as few samples were available for analyses beyond this amount, which may lead to a few samples having substantially more leverage during model fitting. Both nitrate-N:P and ammonium-N:P were calculated as nitrate-N (mg/kg) or ammonium-N (mg/kg) divided by P (mg/kg).

Fungal DNA was extracted from soils in triplicate using Mobio Powerlyzer PowerSoil DNA Isolation kits, performed following methods employed by the Earth Microbiome Project (https://earthmicrobiome.org/ protocols-and-standards/dna-extraction-protocol/). Following amplification of the fungal ITS region (using primers ITS1F and ITS4; Gardes & Bruns 1993; White et al. 1990), the DNA of each sample was sent to the Australian Genome Research Facility (Melbourne, Australia) and the Ramaciotti Centre for Genomics (Sydney, Australia) for sequencing. The ITS amplicons were sequenced using 300 bp paired end sequencing on an Illumina MiSeq (Illumina, Inc., San Diego, USA). The sequenced region (ITS1-5.8S-ITS2) is 550bp on average in fungi but can be much larger (Nilsson *et al.* 2015);

thus for many reads there was not sufficient (or any) overlap between the forward and reverse pair of each template sequence to merge them into a single sequence read. To ensure that those long fungal ITS1-5.8S-ITS2 sequences were not excluded from our analysis, the ITS1 and ITS2 regions were separately extracted from forward and reverse reads, respectively, using ITSx (Bengtsson-Palme *et al.* 2013) and both regions were processed and summarised independently. Zero-radius operational taxonomic units (zOTUs) were generated from identified ITS regions and frequencies of zOTUs within each sample were determined. Protocols that describe the above methods in further detail can be found on the Australian Microbiome Initiative website (https://www.australianmicrobiome.com/protocols/).

Here we analysed a subset of the fungal ITS data that could be assigned to the two AM fungal families of focus. A potential criticism of this approach is that relatively low amplification of AM fungal sequences has been reported when using general fungal ITS primers (Tedersoo et al. 2015; Tedersoo, Tooming-Klunderud & Anslan 2018; Lekberg et al. 2018) and low read counts due to bias against AM fungal amplification would be misrepresentative when comparing AM fungal relative abundance with other non-AM fungal taxa. However, we argue that they are still useful when comparing relative abundances of AM fungal taxa and have an added benefit that in comparisons across soils from different vegetation types estimates of AM fungal read frequency relative to the total number of fungal reads reflect expected patterns in AM fungal relative abundance (i.e., grassland > woodland > forest). To generate this subset, the representative sequence associated with each zOTU was compared with the UNITE database version 8.2 using BLAST. OTUs were assigned fungal family-level annotations when they produced at least a 92% match (minimum 92% coverage) to at least one database sequence. When more than one match was observed, the taxonomy was assigned if that family made up more than 50% of the database matches. All other zOTUs were considered unassigned and were excluded from further analysis. Counts associated with multiple zOTUs assigned to the same family were summed, resulting in a single count for each family in each sample. The relative abundance of the Gigasporaceae and Glomeraceae in each sample was calculated as the sum of Gigasporaceae or Glomeraceae zOTUs within a sample divided by sequencing depth of the entire sample (all fungal ITS sequences). Sequencing depth varied among samples

within and between vegetation types (Table S2), but normalisation of reads would potentially underestimate the abundance of rarer AM fungal taxa and reduce the power of the modelling approaches employed in this study (see next section).

Following the data processing steps described above, this resulted in a total of 369 samples (83 grassland, 213 woodland and 73 forest) distributed throughout Australia (Figure 1).

Statistical Analyses

All data analyses were performed in R version 4.0.3 (R Core Team 2020). Because of the likelihood of spatial autocorrelation, a spatial linear regression modelling approach was employed to analyse the relationships between the relative abundance of the Gigasporaceae and Glomeraceae with soil fertility across vegetation type. The response variable was the proportion of sequence reads assigned to each AM family relative to all fungal reads, which was $\log_{10}$-transformed after adding a small constant (0.00001, slightly less than the minimum observed non-zero value, to avoid calculating undefined values). For predictor variables, we used AM fungal family (Gigasporaceae and Glomeraceae), vegetation type (grasslands, woodlands, forests), soil P, nitrate-N and ammonium-N (mg/kg), as well as nitrate-N:P and ammonium-N:P. The distribution of data for quantitative variables was skewed and therefore $\log_{10}$-transformed. Initially, both a linear and polynomial regression model were fitted, with the first- and second-degree polynomial function of the log-transformed P, nitrate-N and ammonium-N variables included as predictors in the latter. Using the 'spdep' R package (Bivand 2020), we employed a permutation test for Moran's I statistic (999 permutations) to test for spatial autocorrelation in the residuals of both fitted models and calculated the spatial weight for neighbour lists using the "knn2nb" and "knearneigh" (k = 13) functions. Significant spatial autocorrelation was identified in the residuals of both fitted models (Moran I's = 0.08, p = 0.001). To account for this, the "spdep" R package was again used to fit both a spatial lag and spatial error model. The Akaike information criterion (AIC) was compared between these two models and used to determine that the spatial lag model was the best fit. Subtractive modelling was then used to improve the model fits by testing all combinations of predictor variables and excluding those that weakened the compared linear and polynomial spatial lag models

(Table S3) and interpreting patterns based on the sign, magnitude and significance (relative to a null hypothesis of each slope being equal to zero) of estimated parameter coefficients. Following subtractive modelling, the strongest model included a quadratic component for nitrate-N, thus the polynomial spatial lag model was chosen over the linear spatial lag model.

Results

We found strong evidence that the two AM fungal families responded differently to changes in soil P across vegetation type. In general, the Gigasporaceae declined while the Glomeraceae increased in relative abundance as soil P increased (Table 1; Figure 2) but these relationships varied among vegetation types (Figure 3A; Table 1). AM fungal abundance was observed to be more sensitive to changes in soil P within woodlands, rather than grasslands; while the Gigasporaceae decreased in frequency as soil P increased across all three vegetation types, the Glomeraceae was only observed to increase in frequency as soil P increased within woodlands. The positive trend between the Glomeraceae and soil P in forests was non-significant (*P = 0.14)*. The inclusion of a quadratic term for soil P did not improve the model fit (Table S3), suggesting the rate at which AM fungal relative abundance changed with soil P was relatively consistent along the gradient.

The Gigasporaceae and Glomeraceae also differed significantly in how they responded to changes in soil nitrate-N and, similarly to with soil P, the relationships varied among the three vegetation types (Table 2). Due to the complexity of the models, we interpreted them independently for each vegetation type (Figure 3B-C; Table 2). Relationships with increasing nitrate-N were again strongest in woodlands; in this vegetation type the Gigasporaceae decreased non-linearly and the Glomeraceae increased linearly as nitrate-N increased (Table 2; Figure 3B). Neither family had a significant association with changes in nitrate-N within grasslands and forest systems despite positive trends (Table 2; Figure 3B).

The only significant pattern with ammonium-N was a negative relationship with the frequency of Glomeraceae in woodlands (Table 2). This was a weak relationship and not

apparent without accounting for spatial autocorrelation using the spatial lag model (Figure 3C).

Discussion

This study provides evidence of soil P and N availability differentially affecting the functional composition of AM fungal communities across a diversity of ecosystems. These findings are consistent with the hypothesis that soil fertility, and particularly soil P availability, may act as a selective agent during AM fungal community assembly. The Gigasporaceae were more negatively associated with increased soil P than the Glomeraceae, and this was observed in all three vegetation types. This is consistent with observations of reduced AM benefit and AM fungal colonisation of roots with increasing soil P (Kluber et al. 2012; Lin et al. 2020; Smtih & Read 2008; Stribley, Tinker & Rayner 1980; Williams et al. 2017).

On the other hand, frequencies of the Glomeraceae along gradients of soil P were stable in grasslands and forests, and increasing frequencies of the Glomeraceae with increasing soil P observed in woodlands in particular contrasts with these observations, suggesting the potential for P-limitation of fungi in some soils. Some experiments have noted increases in the abundance of some AM fungal taxa within P-limited soils following P-fertilisation (Treseder & Allen 2002; Alguacil et al. 2010; Camenzind et al. 2016). While Camenzind et al. (2016) reported a general increase and did not differentiate between taxa, both Treseder and Allen (2002) and Alguacil et al. (2010) noted that the Glomeraceae were promoted following P-additions to P-limited soils, particularly so in the latter study. Also apparent in our study, in which we observed that these relationships were linear across the full range of soil [P] in our study, encompassing a large range of natural levels ranging from P-limiting to P-rich, is the lack of a threshold value that would indicate a change in the relationship between abundance and soil P once this threshold was met.

The neutral and positive relationships observed with soil P in this study, in the context of current models of partner promotion in AM associations (Steidinger & Bever 2016; Werner & Kiers 2015), suggest that the abundance of the Glomeraceae are perhaps less tied to plant requirements for P uptake compared to that of the Gigasporaceae, which were instead

consistently negatively associated with increasing soil P. One possibility is that some Glomeraceae taxa are instead selected for increased pathogen protection for hosts (Powell et al. 2009; Sikes, Cottenie & Klironomos 2009), particularly at higher soil fertility. Shifts in the relative abundances of AM fungal taxa may also be exacerbated if increased P availability selects for plants that are less dependent on AM for nutrient uptake, particularly if those plants have root systems that are more susceptible to pathogen infection (Sikes, Cottenie & Klironomos 2009). Future research should attempt to shed light on the significance of pathogen protection in natural systems and the role this function may play in AM fungal community assembly.

Availability of N in either form played less of a role for either group of AM fungi in this study, with relationships being weak (nitrate-N with both groups in woodlands, ammonium-N with Glomeraceae in woodlands), saturating at relatively low levels of availability (nitrate-N with Glomeraceae in woodlands), or not observed (all other cases). There is still substantial evidence to suggest that N availability can influence the abundance of AM fungal taxa (Treseder 2004; Han et al. 2020; Camenzind et al. 2014; Van Diepen et al. 2007; Jiang et al. 2018; Kim et al. 2015), but this is perhaps relatively minor compared to P availability, particularly in environments where P-limitation might be more prevalent as in many Australian soils (Hopper 2009; Rossel & Bui 2016; Kooyman, Laffan & Westoby 2017). This limited responsiveness to N may also be due to the variable and often non-contributions to plant N by AM associations (Johnson 2010; Smith & Smith 2011; Reynolds et al. 2005). While evidence is mounting as to the ability of AM fungi to contribute significant levels of N to their host (Corrêa, Cruz & Ferrol 2015), such observations appear to be highly context dependent and the biological significance of AM-mediated N exchange is unclear (e.g., Riley et al. 2019, Zhang et al. 2020).

It's unclear why relationships between soil fertility and AM fungal abundance were stronger and more frequently observed in woodlands compared with grasslands and forests. The number of woodland samples was more than double that of both grasslands and forests. However, trendlines were generally flat in most of those contexts with few exceptions (e.g., that for the Glomeraceae and soil P in forests, Figure 3A), sample numbers in grasslands and forests were not small and variance estimates were not much different than those for coefficients that were significant. Thus, we suspect that this was not an issue with statistical

power. A characteristic of woodlands is a significantly greater canopy coverage of the understorey compared to grasslands while significantly less than forests (Breshears 2006; Shvidenko et al. 2005; Missouri Department of Conservation 2021), which would limit carbon fixation by AM hosts in the understorey compared with those hosts in grasslands but would facilitate an environment that is more favourable for carbon fixation by hosts in the understorey than in forests. There may also be greater potential for competitive interactions with other microbes, particularly EcM fungi. Many of the tree species in Eucalypt woodlands can be colonised by both EcM and AM fungi (Brundrett 2017; Australian Department of Agriculture and Water Resources 2018) with a tendency for EcM states to be more frequent in mature trees and AM in seedlings and saplings (Adams et al. 2006; Chen, Brundrett & Dell 2000). However, EcM fungal frequencies were relatively insensitive to P availability in woodland systems in this system, instead being negatively associated with nitrate-N (Zhang et al. 2023). That same study observed positive associations between frequencies of putative pathogenic fungi and both available P and nitrate-N, suggesting potential for increased competition between pathogens and fungi in the Gigasporaceae as well as larger demand for pathogen protection by fungi in the Glomeraceae.

A limitation of this study is that DNA sequences were obtained from soil samples rather than roots. Significant variation has been reported between AM fungal taxa detected within roots and surrounding soil (Hempel, Renker & Buscot 2007; Yang et al. 2013; Ji et al. 2020). How the relative abundance of the Gigasporaceae and Glomeraceae vary with soil fertility in roots compared to within soil is not clear and should be determined through similar analyses using AM fungal DNA data collected from root samples. Accounting for host species from which these samples were derived would also add an important element to comparative analyses which could help explain variation in AM fungal responses to changes in soil fertility, particularly across different vegetation types, via host specific interactions.

The context dependency of AM fungal associations with soil fertility has implications for exploitation of AM fungi in managed systems, particularly fertilised systems. Further understanding this context dependency would aide optimisation of using AM fungi to improve nutrient uptake, pathogen protection and ecosystem resiliency in agriculture, horticulture and restoration. For instance, are there fungal species within these taxa that perform better than expected in a particular soil fertility environment, whether they are

365 from the Glomeraceae at low-P or the Gigasporaceae at high-P, that may be better suited
366 for improving management outcomes? Does the apparent carbon limitation for AM fungi in
367 woodland and forest environments affect their capacity to benefit hosts, particularly at very
368 low (for the Gigasporaceae) or high (for the Glomeraceae) levels of soil fertility? Are there
369 threshold levels of soil fertility that are associated with shifts between stages of fungal N-, P-
370 and carbon-limitation but are not apparent in our study design?

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Data availability statement

The raw data on which this study is based are all available via the Australian Microbiome Data Portal (https://data.bioplatforms.com/organization/australian-microbiome). The derived data used in this manuscript are available at figshare (DOI: XXXXXXXXX). R code used for data analyses are available at zenodo (DOI: XXXXXXXX). *<The links in this statement will be updated following review.>*

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Table 1. Slope, standard error and significance values for the relationships between log-transformed relative abundance and log-transformed soil P for the Gigasporaceae and Glomeraceae by vegetation type. Vegetation types include data from all vegetation types or solely from grasslands, woodlands and forests. The interaction *p*-value represents whether the relationship between log P and fungal relative abundance significantly varies between the Gigasporaceae and Glomeraceae. Cells shaded green represent significant effects.

Nutrient Coefficient	Vegetation	Fungal Order	Slope	Std Error	*p*-Value	Interaction *p*-Value
Log P	All	Gigasporaceae	-0.495	0.11	< 0.01	< 0.01
		Glomeraceae	0.282	0.11	0.01	
	Grasslands	Gigasporaceae	-0.73	0.33	0.03	0.07
		Glomeraceae	0.12	0.33	0.73	
	Woodlands	Gigasporaceae	-0.48	0.20	0.02	< 0.01
		Glomeraceae	0.90	0.20	< 0.01	
	Forests	Gigasporaceae	-0.72	0.34	0.03	0.01
		Glomeraceae	0.50	0.34	0.14	

Table 2. Slope, standard error and significance values for the relationships between log-transformed relative abundance and log-transformed soil N. This includes linear and quadratic nitrate-N as well as linear ammonium-N coefficients. Vegetation types include data from all vegetation types or solely from grasslands, woodlands and forests. The interaction *p*-value compares how statistically different observed relationships are between the Gigasporaceae and Glomeraceae. Cells shaded green represent significant effects.

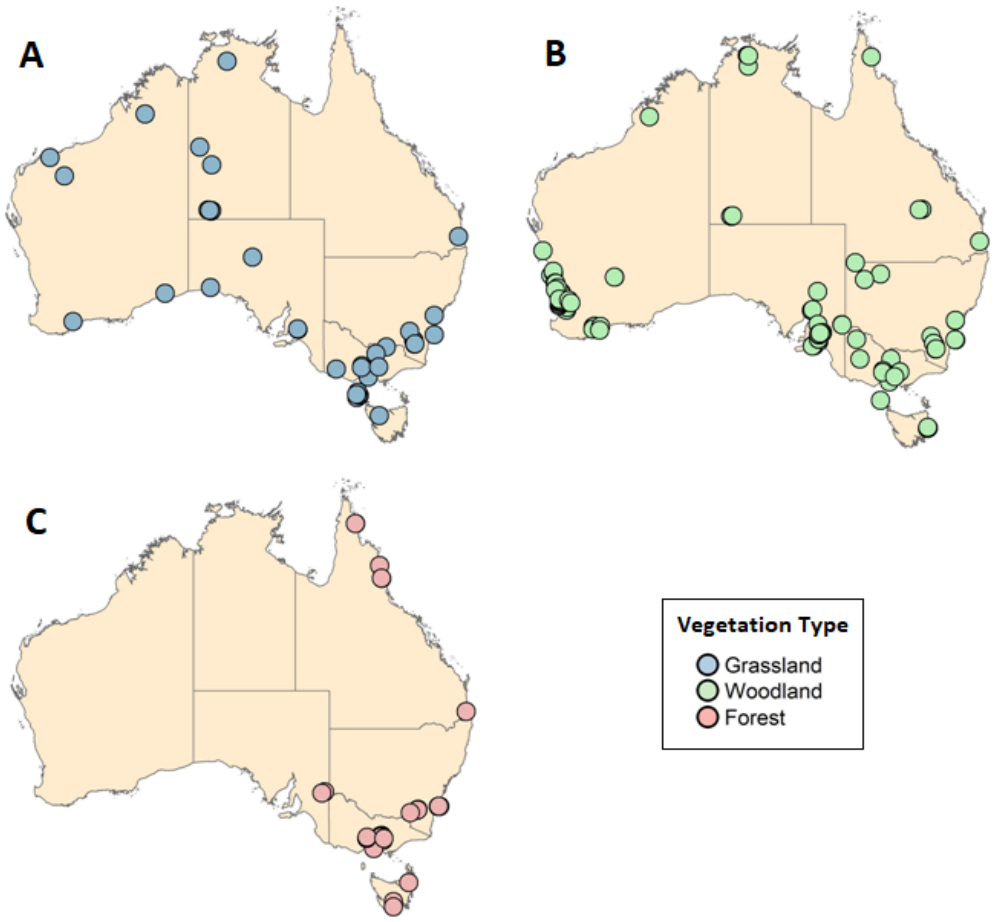
Nutrient Coefficient	Vegetation	Fungal Order	Slope	Std Error	*p*-Value	Interaction *p*-Value
Linear Log NO_3	All	Gigasporaceae	-0.27	0.18	0.14	0.04
		Glomeraceae	0.25	0.18	0.17	
	Grasslands	Gigasporaceae	-0.22	0.59	0.70	0.55
		Glomeraceae	0.27	0.59	0.65	
	Woodlands	Gigasporaceae	-0.76	0.33	0.02	< 0.01
		Glomeraceae	0.86	0.33	0.01	
	Forests	Gigasporaceae	0.71	0.55	0.19	0.06
		Glomeraceae	-0.73	0.55	0.18	
Quadratic Log NO_3	All	Gigasporaceae	0.501	0.17	< 0.01	0.01
		Glomeraceae	-0.09	0.17	0.60	
	Grasslands	Gigasporaceae	0.97	0.54	0.07	0.08
		Glomeraceae	-0.34	0.54	0.53	
	Woodlands	Gigasporaceae	0.65	0.33	0.05	0.01
		Glomeraceae	-0.56	0.33	0.09	
	Forests	Gigasporaceae	0.02	0.44	0.96	0.22
		Glomeraceae	0.80	0.44	0.07	
Log NH_4	All	Gigasporaceae	0.22	0.13	0.09	0.01
		Glomeraceae	-0.26	0.13	0.06	
	Grasslands	Gigasporaceae	0.15	0.40	0.68	0.58
		Glomeraceae	-0.13	0.37	0.72	
	Woodlands	Gigasporaceae	0.24	0.24	0.32	0.03
		Glomeraceae	-0.49	0.24	0.04	
	Forests	Gigasporaceae	-0.05	0.46	0.91	0.98
		Glomeraceae	-0.07	0.46	0.88	

Figure captions

Figure 1. The sites at which grassland (A; *n*=83), woodland (B; *n*=213) and forest (C; *n*=73) samples were collected from across Australia.

Figure 2. The linear relationship between log-transformed relative abundance and log-transformed soil P for both the Gigasporaceae (red) and Glomeraceae (blue) with linear correlation co-efficient values. The figure includes all samples without separating by vegetation type. Each point represents the proportion of fungal reads associated with either the Gigasporaceae or Glomeraceae for a particular soil sample. Ribbons around trend lines represent confidence intervals (95%) for the linear relationship. The proportion of fungal reads associated with the Gigasporaceae decreased as soil P became more available. In contrast, the proportion of fungal reads associated with the Glomeraceae increased. Although these predictions do not account for spatial non-independence of samples, they are qualitatively similar to predictions observed in the spatial lag model (Table 1) and therefore included for visualisation of relationships.

Figure 3. The relative abundance of detected Gigasporaceae (red) and Glomeraceae (blue) reads over gradients of soil P **(A)**, nitrate-N **(B)** and ammonium-N **(C)** across three vegetation types: grasslands, woodlands and forests. The relationship between the relative abundance of AM fungal taxa and both P and ammonium-N was linear, while the relationship with nitrate-N was non-linear and included a quadratic component, although only the corresponding linear coefficients are presented above (blue coefficients for the Glomeraceae, red for the Gigasporaceae). Significant responses are represented by an asterisk beside the corresponding treatment coefficient. Although these predictions do not account for spatial non-independence of samples, they are generally qualitatively similar to predictions observed in the spatial model (Table 1; Table 2) and therefore included for visualisation of relationships. However, there are some inconsistencies as a result, most notably the relationship between the Glomeraceae and ammonium-N in woodlands; while there appears to be a positive trend between the Glomeraceae and ammonium-N in the figure, the relationship was actually significantly negative after accounting for spatial non-independence (Table 2; $p = 0.04$).



658

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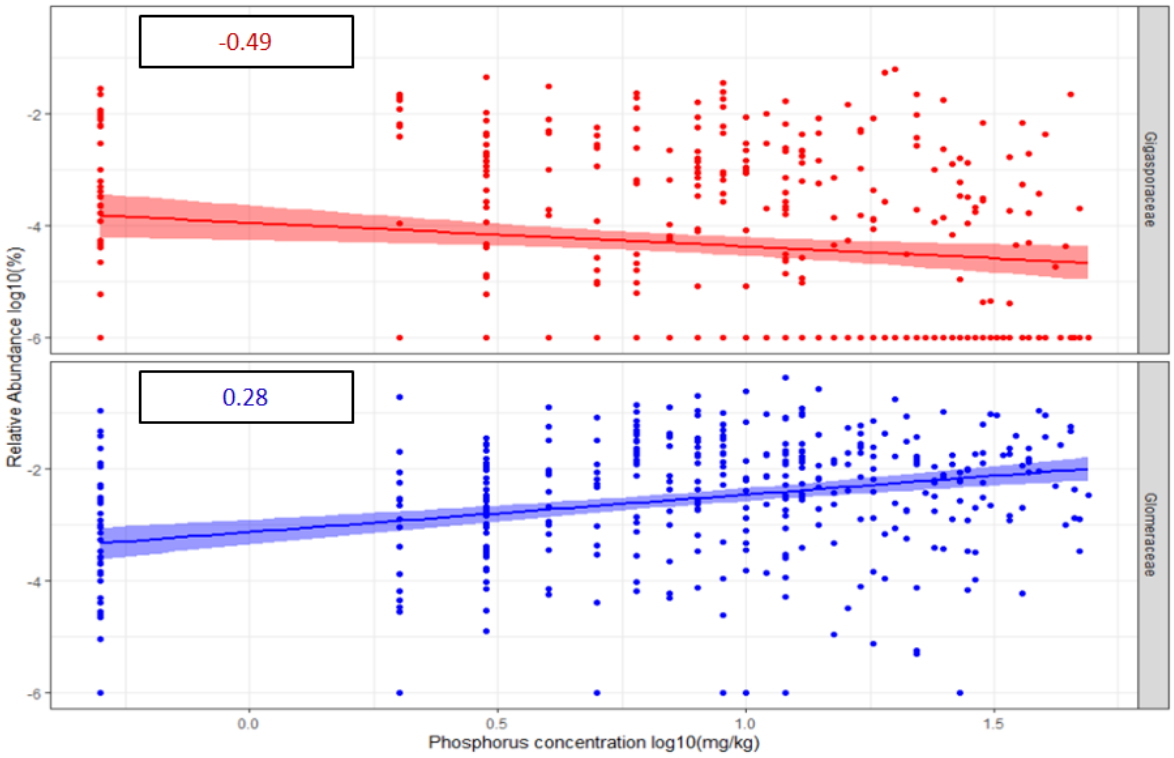

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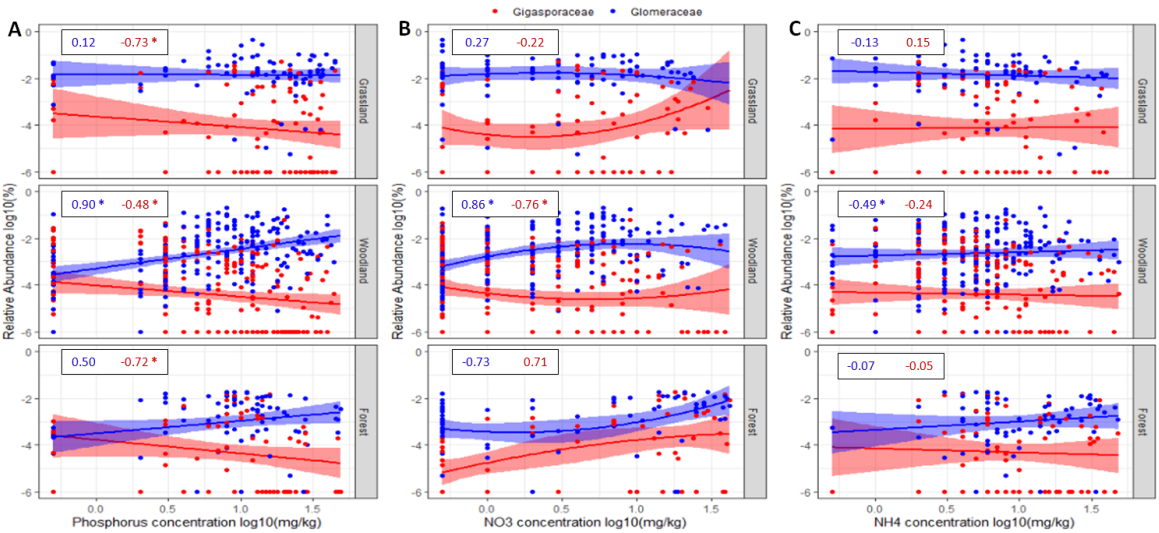

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Supplementary Materials for “Divergent responses between lineages of arbuscular mycorrhizal fungi to soil phosphorus and nitrogen availability”

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Table S1. Samples used in this study. Sample names map to records in the Biomes of Australian Soil Environments (BASE; now known as the Australian Microbiome Initiative, https://www.australianmicrobiome.com/) soil microbial diversity database.

Sample	Vegetation Type	Sample	Vegetation Type	Sample	Vegetation Type	Sample	Vegetation Type
7035	Grassland	9488	Woodland	19245	Woodland	39198	Forest
7049	Grassland	9492	Woodland	19247	Woodland	39202	Forest
7051	Grassland	9494	Woodland	19249	Woodland	39204	Forest
7061	Grassland	9496	Woodland	19251	Woodland	39221	Forest
7063	Woodland	9498	Woodland	19253	Woodland	39222	Forest
7067	Grassland	9502	Woodland	19257	Woodland	39234	Forest
7069	Grassland	9504	Woodland	19259	Woodland	39242	Forest
7083	Woodland	9510	Woodland	19267	Woodland	39254	Forest
7085	Woodland	9522	Woodland	19269	Woodland	39256	Forest
7091	Grassland	9567	Grassland	19281	Woodland	39264	Forest
7093	Grassland	9569	Grassland	19283	Woodland	39270	Forest
7827	Forest	9577	Woodland	19285	Woodland	39272	Forest
7829	Woodland	9579	Grassland	19287	Woodland	39274	Forest
7831	Forest	9581	Woodland	19289	Woodland	39286	Forest
7833	Woodland	9586	Forest	19291	Woodland	42146	Woodland
7837	Forest	9588	Forest	19293	Woodland	42147	Woodland
7839	Forest	10720	Woodland	19295	Woodland	42150	Forest
7843	Forest	12424	Woodland	19297	Woodland	42151	Forest
7845	Forest	12426	Woodland	19299	Woodland	42154	Forest
7847	Forest	12428	Woodland	19301	Woodland	42155	Forest
7849	Grassland	12430	Woodland	19303	Woodland	42158	Forest
7855	Forest	12438	Woodland	19305	Woodland	42159	Forest
7882	Woodland	12447	Forest	19307	Woodland	42163	Forest
7886	Woodland	12459	Woodland	19309	Woodland	42166	Forest
7896	Woodland	12463	Forest	19311	Woodland	42167	Forest
7898	Woodland	12467	Forest	19313	Woodland	42170	Forest
7900	Grassland	12473	Forest	19315	Woodland	42171	Forest
7906	Woodland	12477	Forest	19317	Woodland	42174	Forest
7910	Woodland	12483	Woodland	19319	Woodland	42175	Forest
7916	Woodland	12487	Woodland	19321	Woodland	42178	Forest
7918	Woodland	12489	Woodland	19323	Woodland	42179	Forest
8076	Woodland	12491	Grassland	19325	Woodland	42182	Forest
8078	Woodland	12493	Woodland	19327	Woodland	42183	Forest
8080	Woodland	12495	Grassland	19453	Grassland	42236	Woodland
8082	Woodland	12497	Woodland	19455	Grassland	42238	Woodland
8084	Woodland	12499	Grassland	19457	Grassland	42240	Woodland
8086	Woodland	12558	Forest	19459	Grassland	42242	Woodland
8088	Woodland	12560	Grassland	19461	Grassland	42244	Woodland
8114	Woodland	12562	Woodland	19463	Grassland	42246	Woodland
8124	Woodland	12564	Grassland	19465	Grassland	42248	Woodland
8142	Woodland	12566	Woodland	19467	Grassland	42250	Woodland
8144	Grassland	12568	Grassland	19475	Woodland	42252	Woodland
8146	Woodland	12570	Woodland	19477	Woodland	42254	Woodland
8148	Grassland	12572	Grassland	19479	Woodland	42256	Woodland
8150	Woodland	12574	Woodland	19481	Woodland	42258	Woodland
8152	Grassland	12576	Grassland	19483	Woodland	42260	Woodland
8154	Grassland	12578	Woodland	19485	Grassland	42262	Woodland
8156	Grassland	12580	Grassland	19489	Grassland	42264	Woodland
8158	Grassland	12582	Woodland	19491	Grassland	42266	Woodland
8160	Grassland	12816	Forest	19493	Grassland	42268	Woodland
8162	Grassland	12818	Forest	19495	Grassland	42270	Woodland
8164	Grassland	12819	Forest	19497	Grassland	42272	Woodland
8170	Grassland	12824	Forest	19499	Grassland	42274	Woodland
8172	Grassland	12830	Forest	19501	Woodland	42276	Woodland
8268	Woodland	12834	Woodland	19503	Woodland	42278	Woodland
8276	Woodland	12836	Grassland	19505	Woodland	42280	Woodland
8278	Grassland	12838	Forest	19507	Woodland	42282	Woodland
8280	Grassland	12858	Grassland	19509	Woodland	42284	Woodland
8282	Grassland	12884	Woodland	19511	Woodland	42286	Woodland
8284	Grassland	12886	Grassland	19515	Woodland	42288	Woodland

8286	Grassland	12897	Forest	19517	Woodland	42290	Woodland
8288	Grassland	12899	Forest	39113	Grassland	62120	Forest
8292	Grassland	12901	Woodland	39115	Grassland	62122	Forest
8453	Woodland	12903	Forest	39117	Grassland	62124	Woodland
8455	Grassland	13262	Grassland	39119	Grassland	62126	Grassland
8457	Grassland	13266	Grassland	39121	Grassland	62128	Forest
8459	Grassland	13268	Woodland	39123	Grassland	62130	Woodland
8461	Grassland	13272	Grassland	39125	Woodland	62132	Grassland
8463	Woodland	13276	Grassland	39127	Woodland	62134	Forest
8465	Woodland	13282	Grassland	39129	Woodland	62136	Woodland
8469	Grassland	13286	Grassland	39131	Woodland	62138	Grassland
8487	Woodland	13729	Woodland	39133	Woodland	62142	Woodland
8501	Forest	13731	Woodland	39135	Woodland	62144	Woodland
8503	Forest	13733	Woodland	39137	Woodland	62147	Woodland
8505	Forest	13735	Woodland	39139	Woodland	62148	Grassland
8507	Grassland	13892	Woodland	39141	Woodland	62152	Woodland
8511	Woodland	13893	Woodland	39143	Woodland	62156	Woodland
8529	Woodland	13894	Woodland	39145	Woodland	62158	Woodland
8531	Woodland	13895	Woodland	39147	Woodland	62160	Woodland
9430	Woodland	13896	Woodland	39149	Woodland	62168	Forest
9434	Grassland	13897	Woodland	39151	Woodland	62170	Woodland
9436	Woodland	13898	Woodland	39153	Woodland	62172	Grassland
9438	Forest	13899	Woodland	39155	Woodland	62174	Woodland
9440	Forest	13900	Woodland	39157	Woodland	62176	Woodland
9446	Woodland	13901	Woodland	39159	Woodland		
9448	Forest	13904	Woodland	39167	Woodland		
9450	Forest	13906	Woodland	39169	Woodland		
9452	Forest	14181	Woodland	39171	Woodland		
9458	Forest	14183	Woodland	39173	Woodland		
9460	Forest	19231	Woodland	39175	Woodland		
9462	Forest	19235	Woodland	39179	Woodland		
9464	Grassland	19237	Woodland	39181	Woodland		
9466	Woodland	19239	Woodland	39183	Woodland		
9468	Forest	19241	Woodland	39185	Woodland		
9486	Woodland	19243	Woodland	39187	Woodland		

Table S2. The mimimum and maximum sequencing depths for samples collected in each of the three target vegetation types: woodlands, grasslands and forests.

	Woodland	Grassland	Forest
Minimum Reads	283	20449	14989
Maximum Reads	402402	388139	530864

Table S3. Comparison of model fit (AIC scores) via subtractive modelling. The spatial lag model chosen for subsequent result interpretations was that with the lowest AIC and degrees of freedom (df). This model included a quadratic element for NO_3 only as the removal of this quadratic element from P and NH_4 strengthened the model.

Included Coefficients	df	AIC
All	24	2492
Intercept Only	3	2823.6
order	4	2542.8
order + vegetation_type + vegetation_type:order	8	2527.9
order + vegetation_type + vegetation_type:order + log_p + order:log_p	10	2495.6
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3	12	2491
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4	14	2490
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4 + I(log_no3^2) + order:I(log_no3^2)	16	**2484.4**
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4 + I(log_no3^2) + order:I(log_no3^2) + I(log_p^2) + order:I(log_p^2)	18	2485.5
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4 + I(log_no3^2) + order:I(log_no3^2) + I(log_nh4^2) + order:I(log_nh4^2)	18	2485.6
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4 + I(log_no3^2) + order:I(log_no3^2) + logNO3logPRatio + order:logNO3logPRatio	18	2486.2
order + vegetation_type + vegetation_type:order + log_p + order:log_p + log_no3 + order:log_no3 + log_nh4 + order:log_nh4 + I(log_no3^2) + order:I(log_no3^2) + logNH4logPRatio + order:logNH4logPRatio	18	2487.9