MOLECULAR ECOLOGY

Molecular Ecology (2015) **24**, 4912–4930 doi: 10.1111/mec.13363

The rise and fall of arbuscular mycorrhizal fungal diversity during ecosystem retrogression

MANUELA KRÜGER,* FRANÇOIS P. TESTE,†‡ ETIENNE LALIBERTÉ,†§ HANS LAMBERS,† MEGAN COGHLAN,¶ GRAHAM ZEMUNIK†¶ and MICHAEL BUNCE§

Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, Průhonice CZ-252 43, Czech Republic, †School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley, Perth, WA 6009, Australia, ‡Grupo de Estudios Ambientales, IMASL-CONICET & Universidad Nacional de San Luis, Av. Ejercito de los Andes 950 (5700), San Luis, Argentina, §Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, Montréal, QC H1X 2B2, Canada, ¶Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama, §Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Kent Street, Perth, WA 6845, Australia

Abstract

Ecosystem retrogression following long-term pedogenesis is attributed to phosphorus (P) limitation of primary productivity. Arbuscular mycorrhizal fungi (AMF) enhance P acquisition for most terrestrial plants, but it has been suggested that this strategy becomes less effective in strongly weathered soils with extremely low P availability. Using next generation sequencing of the large subunit ribosomal RNA gene in roots and soil, we compared the composition and diversity of AMF communities in three contrasting stages of a retrogressive >2-million-year dune chronosequence in a global biodiversity hotspot. This chronosequence shows a ~60-fold decline in total soil P concentration, with the oldest stage representing some of the most severely P-impoverished soils found in any terrestrial ecosystem. The richness of AMF operational taxonomic units was low on young (1000's of years), moderately P-rich soils, greatest on relatively old (~120 000 years) low-P soils, and low again on the oldest (>2 000 000 years) soils that were lowest in P availability. A similar decline in AMF phylogenetic diversity on the oldest soils occurred, despite invariant host plant diversity and only small declines in host cover along the chronosequence. Differences in AMF community composition were greatest between the youngest and the two oldest soils, and this was best explained by differences in soil P concentrations. Our results point to a threshold in soil P availability during ecosystem regression below which AMF diversity declines, suggesting environmental filtering of AMF insufficiently adapted to extremely low P availability.

Keywords: community ecology, dune chronosequence, ecosystem development, Glomeromycota, nutrient limitation, phosphorus

Received 12 August 2014; revision received 12 August 2015; accepted 21 August 2015

Introduction

Mycorrhizal fungi play a major role in terrestrial ecosystems, allowing plants to efficiently acquire poorly mobile soil nutrients (Smith & Read 2008). The vast majority of plant families (~92%) and species (>80%) form mycorrhizas (Brundrett 2009). Arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) form the most widespread type of mycorrhizas, found in ~74% of vascular plant species (Brundrett 2009). Arbuscular mycorrhizal fungi are well known to enhance uptake of phosphorus (P), and to some degree nitrogen (N), in plants (Hodge *et al.* 2010; Whiteside *et al.* 2012; Smith

Correspondence: François Teste, Fax: + 61 8 6488 1108;
E-mail: francois.teste@uwa.edu.au
Manuela Krüger and François P. Teste contributed equally to this work.

et al. 2015). As such, AMF are important drivers of plant productivity in terrestrial ecosystems (van der Heijden *et al.* 1998, 2008; Rosendahl 2008).

During long-term ecosystem development (i.e. tens to hundreds of thousands of years), important changes in soil nutrient availability occur. Primary productivity is initially N-limited on young soils, because N enters ecosystems primarily through biological N_2 fixation, but productivity peaks relatively rapidly to maximum levels (Odum 1969). Conversely, P becomes increasingly limiting in older soils as its amount and availability decline through time (Walker & Syers 1976; Vitousek & Farrington 1997; Laliberté *et al.* 2012). Eventually, severe P limitation in old, strongly weathered soils leads to ecosystem retrogression, whereby rates of ecosystem processes decline (Wardle *et al.* 2004; Peltzer *et al.* 2010; Turner *et al.* 2013). These important changes in soil nutrient availability during ecosystem retrogression have been suggested to lead to shifts in the relative importance of different mycorrhizal associations (Read 1993). In particular, it has been proposed that associations with AMF become less important for plant nutrient acquisition on very old and strongly weathered soils (Lambers *et al.* 2008), because AMF are less effective at acquiring P when P availability is very low (Parfitt 1979; Lambers *et al.* 2008, 2015a,b).

Long-term soil chronosequences, or gradients of soil age, are useful model systems to explore how plant–soil interactions depend on the type and strength of nutrient limitation, while holding other important ecosystem state factors (e.g., parent material, climate, topography) relatively constant (Walker *et al.* 2010). For example, soil chronosequences can be used to explore how AMF community composition and diversity vary during ecosystem development and associated declines in P availability (Dickie *et al.* 2013). This is important, as little is known about changes in AMF communities during ecosystem retrogression (Dickie *et al.* 2013). This is particularly true for old landscapes in the Southern Hemisphere, as most studies on AMF communities have been conducted on younger soils or short-term soil chronosequences in sites from the Northern Hemisphere (Dickie *et al.* 2013).

Despite the low number of known AMF species worldwide (~250; www.amf-phylogeny.com), local AMF communities tend to be species-rich, often exceeding the richness of the plant communities hosting them (Hawksworth 2001; Öpik *et al.* 2009). A first step at enlarging the global molecular diversity data set for AMF was recently published by Öpik *et al.* (2013), where 33 new 'virtual taxa' were discovered across six continents and five climatic zones. This indicates that the currently described AMF species are most likely only a proportion of the total global diversity, although virtual taxa are not equivalent to AMF species. Surveys of AMF communities in understudied regions of the Southern Hemisphere are important for improving our understanding of AMF phylogeny and diversity. For example, there are few biodiversity studies of AMF in Australia (Logan *et al.* 1989; Shi *et al.* 2012), and little is known regarding AMF communities in hyperdiverse plant communities of Australia such as kwongkan shrublands (Hopper & Gioia 2004). Important advances in molecular tools (e.g. pyrosequencing) for analysing soil fungal communities are now providing unprecedented opportunities to uncover AMF diversity in all ecosystems (Fierer *et al.* 2013; Talbot *et al.* 2014; Tedersoo *et al.* 2014; van der Heijden *et al.* 2015).

In this study, we sequenced AMF ribosomal DNA from root and soil samples collected along a >2-million-year chronosequence in a biodiversity hotspot (Laliberté *et al.* 2012; Turner & Laliberté 2015). This chronosequence shows strong declines in P availability during retrogression, eventually leading to some of the most P-impoverished soils on Earth (Laliberté *et al.* 2012; Turner & Laliberté 2015) that support species-rich kwongkan shrublands (Zemunik *et al.* 2015). The main objective of our study was to determine the diversity and composition of AMF communities during ecosystem retrogression. We hypothesized that AMF diversity would initially increase with declining P availability during the early stages of ecosystem retrogression, but would eventually decline on very old soils with extremely low P availability. We also hypothesized that there would be little overlap in AMF community composition between the youngest and the oldest soils, due to large differences in soil characteristics such as P availability and pH (Laliberté *et al.* 2012; Turner & Laliberté 2015). Our study provides a comprehensive survey of changes in AMF communities during ecosystem retrogression across a well-characterized soil chronosequence forming an exceptionally strong P availability gradient (Turner & Laliberté 2015).

Our study addresses two critical knowledge gaps in mycorrhizal ecology and evolution (van der Heijden *et al.* 2015), namely the description of AMF communities from understudied, biodiversity hotspots, and how key environmental variables, such as soil nutrient availability, control partner selection in mycorrhizal symbioses. Long-term, retrogressive soil chronosequences provide excellent opportunities to study how strong declines in soil P concentration shape mycorrhizal symbioses, but their potential as model systems has yet to be fully realized (Dickie *et al.* 2013; Martínez-García *et al.* 2015).

Materials and methods

Study system

The study was conducted along the Jurien Bay >2-million-year dune chronosequence (Laliberté *et al.* 2013) located in southwestern Australia (~200 km northwest of Perth). The Jurien Bay dune chronosequence comprises a series of three main pedogenic associations represented by well-defined morphostratigraphic dune systems of marine origin (McArthur & Bettenay 1974). The region is characterized by a strong Mediterranean climate with annual long-term average precipitation of 570 mm, with no precipitation gradient across the chronosequence and an annual average temperature of 25 °C (Australian Bureau of Meteorology, www.bom.gov.au/climate/data). The study area is not forested, due to large annual water and nutrient deficits, but is characterized by shrublands with exceptionally high levels of woody plant biodiversity at all spatial scales (Laliberté *et al.* 2014; Turner & Laliberté 2015).

Selection of chronosequence stages

We selected three distinct chronosequence stages for this study that differ strongly in age, soil nutrient availability, and the strength and type of nutrient limitation, and which represent the retrogressive phase of ecosystem development along this sequence (Laliberté *et al.* 2012).

First, we selected the Quindalup dunes (referred as 'young dunes' hereafter), corresponding to stage 2 in Laliberté *et al.* (2014). These dunes are associated with the Holocene transgression (<7 ka) and have alkaline soils due to their high carbonate content. Stage 2 was selected (Hayes *et al.* 2014; Laliberté *et al.* 2014; Turner & Laliberté 2015) as it shows the highest soil N and plant-available P concentrations along the chronosequence (Turner & Laliberté 2015). Evidence from a nutrient-limitation phytometer bioassay (Laliberté *et al.* 2012), and analyses of foliar nutrient concentrations of native plants along the chronosequence (Hayes *et al.* 2014), point towards N and P colimitation of plant growth at this chronosequence stage.

The second stage we selected, the Spearwood dunes (referred as 'middle-aged dunes' or 'middle dunes' hereafter), corresponds to stage 4 in Laliberté *et al.* (2014). These dunes were formed in the Middle Pleistocene (~120 ka) and have a calcarenite core overlain by siliciclastic sands that have been fully decalcified due to prolonged weathering (Turner & Laliberté 2015). The soils have low total P concentrations (~23 mg/kg) and plant growth is limited by P availability (Laliberté *et al.* 2012; Hayes *et al.* 2014).

The third stage considered is the oldest one (stage 6 in Laliberté *et al.* 2014); the Bassendean dunes (referred as 'old dunes' hereafter) were formed in the Early Pleistocene (≥2 Ma) or possibly Late Pliocene (Kendrick *et al.* 1991). The strongly weathered soils are extremely low in total P (~5 mg/kg), and plant growth is strongly limited by P availability on these soils (Laliberté *et al.* 2012; Hayes *et al.* 2014).

Vegetation sampling

Flora surveys were conducted on 30 permanent plots (10 m × 10 m), 10 for each chronosequence stage. Each plot was surveyed at least once between August 2011 and March 2012. The majority of plant species along this chronosequence have evergreen foliage, but some species (e.g. geophytes, annuals) do not have persistent aboveground structures. Therefore, plots that were first surveyed in drier months (November to March) were resurveyed in September 2012, to ensure that species with nonpersistent aboveground structures were recorded. To estimate canopy cover and number of individuals for each plant species within the 10 m × 10 m plots, seven randomly located 2 m × 2 m subplots were surveyed within each plot. Within each subplot, all vascular plant species were identified and counted and the vertically projected vegetation canopy cover was estimated.

From the 30 plots, five replicate plots per chronosequence stage for the present study were randomly selected for a total of 15 plots and nutrient-acquisition strategies of all collected plant species were determined across the chronosequence (Zemunik *et al.* 2015). The AM status of the host plants that were present on the sampling sites was assessed and reported elsewhere (Zemunik *et al.* 2015). In brief, mycorrhizal status of the host plants was based on published literature (Brundrett 2009) and supplemented with root sampling of plant species with unknown mycorrhizal status that had a substantial canopy cover (>4% relative canopy cover, Zemunik *et al.* 2015). The additional root samples were collected from at least four individuals per plant species; in total, 62 plant species representing 27 families were assessed for mycorrhizal status, using standard protocols (Smith & Read 2008).

Root and soil sampling

In June, July and August 2012, soils were sampled with a sand auger (5 cm diameter, 20 cm deep) in each subplot within all plots. Soil samples were immediately sieved (mesh size 2 mm), and all roots were extracted. In addition, soil was sampled from fungal in-growth cores (Wallander *et al.* 2001) at the edge of each subplot

in April 2012 as part of an ongoing fungal biomass experiment. They were left to settle until June and then sampled, and in July and August, they were sampled again, being in the field for at total of 5 months. The purpose of this sampling was to determine the composition of the AMF community that scavenges over relatively large distances (>3 cm from roots) in these soils via extraradical hyphae and/or is found as spores in soil. Prior to installation of the fungal in-growth cores (7.5 cm diameter; 20 cm height), roots were removed and prevented from growing into the cores with nylon mesh bags or polyvinyl chloride (mesh size 50 μm). The cardinal direction and the order of the cores along the edge of the subplot were randomly assigned. Soil and root samples were stored at −20 °C until further processing. Roots were then thawed, washed, lyophilized for 7 days and finally disrupted with a custom-built bead mill.

Soil nutrient analyses

Soil nutrient analyses were carried out on the same soil samples from which roots were extracted. Sieved soil was homogenized and air-dried prior to chemical analysis. Total soil P concentration ([P]) was measured by ignition (550 °C, 1 h) and extraction in 1 M H_2SO_4. Resin P was extracted by anion-exchange membranes (1 cm × 4 cm; manufactured by BDH, Poole, UK, and distributed by VWR International, West Chester, PA, USA), and phosphate was desorbed from the membranes by shaking for 1 h in 0.25 M H_2SO_4. Phosphate detection was performed by automated online neutralization and molybdate colorimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Total soil N concentration was determined by combustion and gas chromatography on a Thermo Flash 1112 analyser (CE Elantech, Lakewood, NJ, USA). Soil pH was determined in a 1:2 soil to solution ratio in 10 mM $CaCl_2$ using a glass electrode. Relevant site, soil, and vegetation data were deposited in Dryad and Aekos and are available at http://datadryad.org/resource/doi:10.5061/dryad.ps320 and http://portal.aekos.org.au/dataset/193192.

DNA extraction, amplification and gel electrophoresis

DNA was extracted from the disrupted roots using a cetyltrimethylammonium bromide protocol as described in Krüger *et al.* (2009) of up to 30 mg dried homogenized material. Soil was taken from each sampling time point, mixed (10 g in total) and extracted with the -PowerMax Soil DNA Isolation Kit (Mo-Bio Laboratories Inc., Carlsbad, USA) following the instructions of the manufacturer, with the exception of the fourth step, where samples were vortexed for 10 min, then placed in a water bath at 65 °C, and mixed every 3 min. The AMF spores from single spore cultures (*Acaulospora laevis* WUM11, *Scutellospora calospora* WUM12) were used as positive controls in PCR.

We used a nested approach for PCR amplification with the AMF-specific primer set SSUmAf and LSUmAr in the first PCR, followed by a subsequent PCR with the newly designed primer LSU-D2f (5′- GTGAAA TTGTTGAAAGGGAAAC- 3′) modified from FLR3 (Gollotte *et al.* 2004) and the degenerated LSUmBr primer (Table S1, Supporting information) modified from Krüger *et al.* (2009). The primers used here (targeting the LSU) have previously been assessed as a valid approach and to yield the highest AMF diversity from field communities (Krüger *et al.* 2009; Gorzelak *et al.* 2012, Kohout *et al.* 2014). Due to potential PCR inhibition, DNA extracts were diluted 1:200, 1:1000, 1:2000, 1:5000 and 1:10000. In the first PCR, 5 μL of diluted DNA extracts was used as template and 0.2 μL of the first PCR was used in nested PCR. The GoTaq Green Mastermix (Promega, Sydney, Australia) was used in both PCRs with final concentrations of 1× GoTaq Green Mastermix, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 0.5 μM primers. The conditions of the first PCR were as follows: 5 min initial denaturation at 95 °C, 40 cycles of 45 s denaturation at 95 °C, 90 s annealing at 56 °C and 2 min elongation at 72 °C, followed by a final elongation of 10 min. In the nested PCR, multiplex identifier (MID) tagged fusion primers (Table S1, Supporting information) were used, amplifying ~400–550 bp of the LSU rRNA gene covering the variable LSU-D2 rDNA region. The same conditions as in the first PCR were used for the nested PCR except for the annealing temperature, which was 57 °C. In addition, the elongation step was shortened to 1 min at 72 °C, and 35 cycles were carried out. Fusion-tagged amplicon generation was conducted in triplicates for each root and soil sample. All positive PCR amplicons of the same sampling site, roots and soil separately, irrespective of the dilution used in PCR, were pooled and cleaned twice with the Agencourt AMPure XP beads (Beckman Coulter Genomics, MA, USA). The cleaned PCR amplicons were visualized on a 2% (w/v) agarose gel for estimation of DNA concentration for the subsequent equimolar pooling in emulsion PCR. The bead:template ratio for 454 sequencing was determined by quantitative PCR against standards of known molarity (Bunce *et al.* 2012).

Sequencing and bioinformatics

Sequencing of the PCR amplicons was conducted on the Roche GS Junior system (Branford, CT, USA) using Lib-A chemistry. Preprocessing of the resulting 454

sequence reads was performed as described in Coghlan *et al.* (2012) and involved searching for exact MID-tags and primer sequences. Sequencing was performed at the State Agricultural Biotechnology Centre at Murdoch University, Australia. The trimmed and preprocessed sequence reads were checked for chimeras using the *de novo* mode of the UCHIME algorithm (Edgar *et al.* 2011) and the remaining sequence reads were clustered using USEARCH and the UCLUST algorithm (Edgar 2010), with a cut-off of 0.97 sequence similarity. Singletons were removed from the data set. All sequences of one cluster were concatenated to one consensus sequence using the iterative refinement method (L-INS-i) of MAFFT, followed by a second clustering with the same cutoff and final consensus sequences were created.

The final consensus sequences were checked for their identity (taxonomic affinity) using BLAST against the public databases (DDBJ/EMBL/GenBank). All nontarget consensus sequences were omitted for further analyses, and the remaining sequences were aligned to the reference data set published in Krüger *et al.* (2012). The phylogenetic trees were calculated using the RAXML software (RAxML-HPC2 on XSEDE ver. 7.6.3.; Stamatakis *et al.* 2008) on the CIPRES web-portal using the tree interference GTRGAMMA model with 1000 bootstraps (raxmlHPC-HYBRID -T 6 -s infile -n result -p 12345 -× 12345 -N 1000 -f a -m GTRGAMMA). Operational taxonomic units (OTUs) were labelled with taxon identifiers according to their phylogenetic placement for subsequent statistical analyses.

The trimmed sequence reads and the resulting final consensus sequences were deposited in Dryad and are available at http://dx.doi.org/10.5061/dryad.tq0ft.

Statistical analyses

To explore what semi-quantitative signal might exist in the next generation sequencing data (Nguyen *et al.* 2015), the most conservative approach that maintains some quantitativeness to the OTU abundance data was employed, by ranking the abundance and then using the Gower distance metric. We transformed the number of read data into either (i) presence–absence or (ii) rank order prior to multivariate community analyses. Jaccard (for presence–absence data) and Gower (for semi-quantitative data) dissimilarities among plots were subsequently calculated using the 'VEGAN' package (Oksanen *et al.* 2013) in R (R Development Core Team 2013, www.r-project.org). The AMF-OTU richness and plant host species richness was estimated through rarefaction analysis using the function 'rarefy' in the 'VEGAN' package (Oksanen *et al.* 2013). To allow comparisons of AMF richness among samples, rarefaction was based on the sample with the lowest number of AMF sequences. Phylogenetic diversity (PD), net relatedness index (NRI) and nearest taxon index (NTI) of AMF were calculated from the branch length of the OTUs in the phylogenetic trees using PHYLOCOM (Faith 1992; Webb *et al.* 2008) and QIIME (Caporaso *et al.* 2010).

A linear model was used to test for differences in rarefied richness, plant host relative cover, PD, NRI and NTI between chronosequence stages with five replicate plots per stage. Model assumptions (normality and homogeneity of variance of residuals) were assessed graphically. Tukey's honestly significant difference (HSD) error bars and 95% confidence intervals (CIs) were calculated with the 'EFFECTS' package (Fox 2003) and used for statistical inference. In Figs 3, 7 and S5, the CIs are accompanied by Tukey HSD bars ($\alpha = 0.05$) and used as visual multiple-mean comparison tests (Altman *et al.* 2000), providing sufficient information for conducting inferences (Di Stefano *et al.* 2005; Cumming 2008). To quantify relationships between key soil variables (e.g., total P) and AMF-OTU richness, and between key plant host variables (e.g., AM plant host richness) and AMF-OTU richness, we performed a multiple linear regression after selecting candidate variables via stepwise and all subset variable selection approaches (Murtaugh 2009). Then, for the significant variables selected, we calculated the relative importance (Grömping 2006) of each variable (% contribution to the multiple R^2) and performed a simple regression with the most important predictor variable and OTU richness.

Nonmetric multidimensional scaling (NMDS) was performed using the 'metaMDS' function in the 'VEGAN' package (Oksanen *et al.* 2013) to visualize differences in AMF community composition among the three chronosequence stages. Due to visual indication from the NMDS plot (Fig. 4) that the young dunes might show significantly greater multivariate dispersion (i.e. greater beta diversity; Anderson *et al.* 2006) than the other two groups, a distance-based test of homogeneity of within-group (i.e. dune system) dispersions was performed, using the function 'betadisper' in the R package 'VEGAN' (Oksanen *et al.* 2013). Permutational distance-based multivariate analysis of variance (PERMANOVA) was used to test for differences in AMF community composition among chronosequence stages (Anderson *et al.* 2008); only statistics with $P \leq 0.05$ were considered for multiple comparisons. For explanation of variation in AMF community structure, a canonical redundancy analysis (RDA; Legendre & Anderson 1999) using key soil (e.g., total P, N, C, etc.) and host plant variables (AM host relative canopy cover, relative abundance, richness and Simpson diversity) was performed. A forward selection procedure was used to identify the most-important predictor variables. The statistical significance of the RDA models (global and subset of

selected predictor variables), the individual canonical axes and the forward selection procedure was determined with permutation tests in R (Borcard *et al.* 2011; Legendre *et al.* 2011; Oksanen *et al.* 2013), using 9999 permutations. Finally, to assess the statistical significance of the relationship between dominant AMF-OTU occurrence or abundance and chronosequence stages, we conducted indicator species analysis with the 'INDICSPECIES' package (Cáceres & Legendre 2009).

Results

Phylogenetic classification

We identified 78 unique AMF-OTUs from root and soil samples across all plots, from a total of 21681 AMF reads. From the root samples, 66 OTUs (~80%) could be assigned to AMF, while 17 OTUs were from nontarget organisms such as Basidiomycota and Ascomycota (Table 1). In soil, only 12 OTUs (~10%) of 184 in total were of glomeromycotan origin (Table 1). The AMF-OTUs in soil, mainly related to *Diversispora* and *Acaulospora*, were never found in roots and *vice versa* (Table 2), while for the nontarget organisms, there was overlap of soil and root OTUs (Table S2, Supporting information). The uncultured *Rhizophagus* sequences from soil clustered apart from the *Rhizophagus* sequences derived from roots (data not shown). The AMF-OTUs within roots mainly comprised *Rhizophagus* and *Glomus* (Fig. 1; Fig. S2, Supporting information). Only two OTUs clustered within *Claroideoglomus*, two in *Scutellospora* (*sensu* Redecker *et al.* 2013) and one in *Archaeospora* (Fig. 1,

Table 1 Fungal operational taxonomic units (OTUs) along the Jurien Bay dune chronosequence including the Quindalup dunes (Young), the Spearwood dunes (Middle) and the Bassendean dunes (Old) classified according to soil age

Dune system	Plot (site)	Sample type	#OTUs	Non-AMF	AMF	#Reads	#AMF reads	% AMF reads
Young	Q.M.7	R	4	2	2	499	492	98.6
	Q.M.18		7	6	1	222	15	6.8
	Q.M.23		1	0	1	185	185	100.0
	Q.M.25		2	0	2	2170	2170	100.0
	Q.M.26		2	0	2	1328	1328	100.0
Middle	S.W.6	R	29	0	29	1951	1951	100.0
	S.W.11		14	0	14	1749	1749	100.0
	S.W.14		12	0	12	2141	2141	100.0
	S.W.17		24	0	24	1485	1485	100.0
	S.W.26		14	1	13	1224	1221	99.8
Old	B.NL.1	R	11	0	11	3287	3287	100.0
	B.HR.2		2	0	2	299	299	100.0
	B.L.5		6	6	0	3363	0	0
	B.L.6		14	0	14	1952	1952	100.0
	B.L.14		8	3	5	2220	1981	89.2
Young	Q.M.7	S	8	6	2	1128	2	0.2
	Q.M.18		10	10	0	1013	0	0
	Q.M.23		11	9	2	921	179	19.4
	Q.M.25		7	3	4	622	505	81.2
	Q.M.26		9	9	0	413	0	0
Middle	S.W.6	S	6	6	0	1606	0	0
	S.W.11		11	11	0	1153	0	0
	S.W.14		10	10	0	1192	0	0
	S.W.17		19	17	2	1073	562	52.4
	S.W.26		24	22	2	980	132	13.5
Old	B.NL.1	S	15	15	0	1170	0	0
	B.HR.2		14	10	4	1526	45	2.9
	B.L.5		18	18	0	1320	0	0
	B.L.6		14	14	0	1546	0	0
	B.L.14		8	8	0	269	0	0

The DNA was extracted (Sample type) from roots (R) or soil (S). The #OTUs is the number of all OTUs detected at the respective sample site (Plot); nonarbuscular mycorrhizal fungi (non-AMF) shows the number of OTUs assigned to Ascomycota or Basidiomycota; Arbuscular mycorrhizal fungi (AMF) displays the number of OTUs assigned to AMF; #Reads is the number of all sequence reads observed for the respective site; #AMF reads is the number of sequence reads assigned to AMF; % of AMF reads shows the percentage of AMF reads detected at the sampling site.

Table 2 The 10 (or >5% relative sequence abundance) most frequently detected operational taxonomic units (OTUs) for DNA extracted from roots or soil (Sample type) along the Jurien Bay dune chronosequence

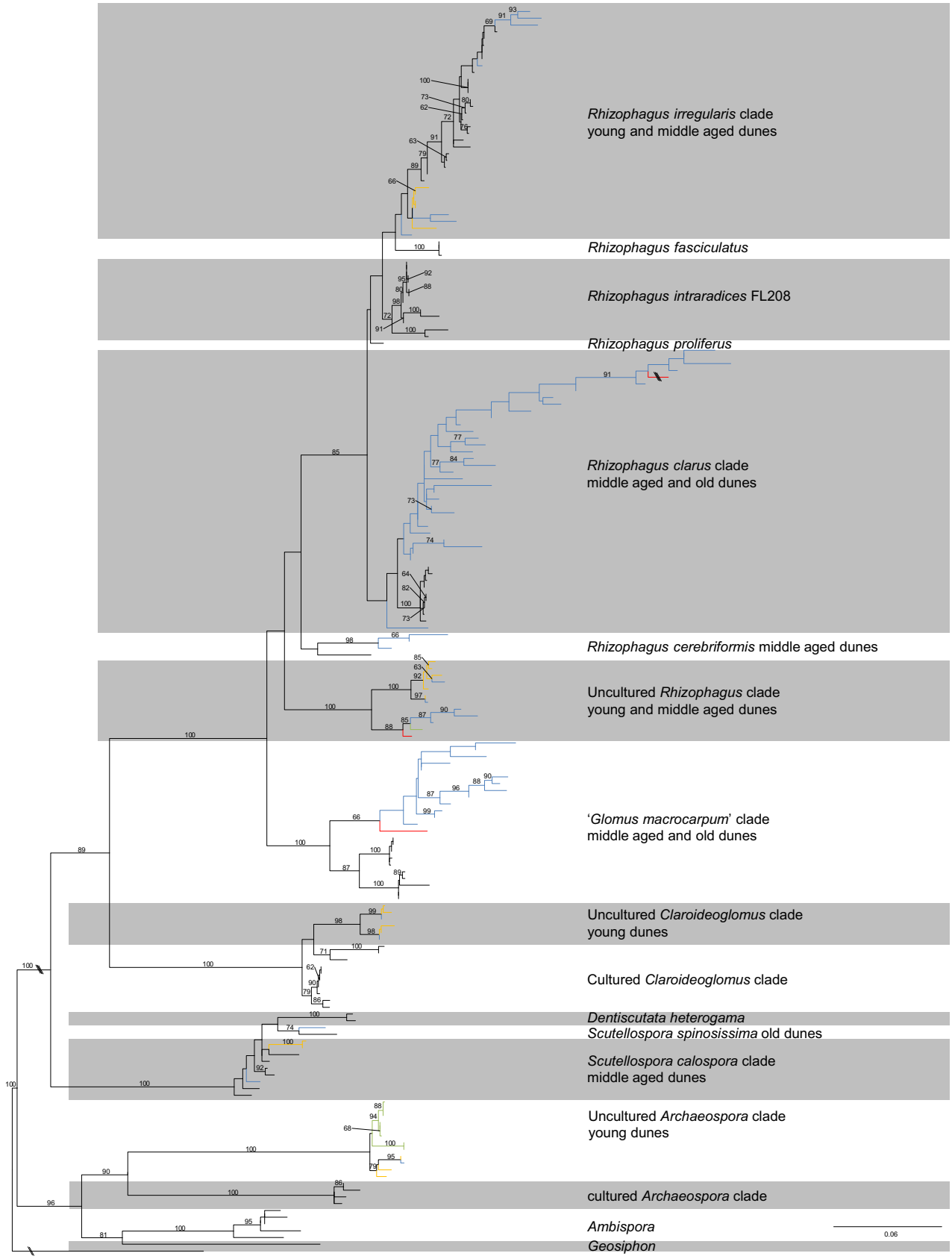
OTU	Taxonomic affinity	Similarity	#Reads	Dune system	Sample type	Plot (site)
CL003	Uncultured *Rhizophagus* 3	96	4758	M, O	Roots	S.W.6, S.W.11, S.W.14, S.W.17, B.NL.1, B.L.6, B.L.14
CL005	Uncultured *Rhizophagus* 3	96	3142	M, O	Roots	S.W.6, S.W.11, S.W.14, S.W.17, B.NL.1, B.H.2, B.L.6, B.L.14
CL010	Uncultured *Rhizophagus* 1	98	2167	Y	Roots	Q.M.25
CL012	Uncultured *Rhizophagus* 6	98	1522	Y, M	Roots	Q.M.26, S.W.11, S.W.17
CL004	Uncultured *Glomus* 1	91	1482	M, O	Roots	S.W.17, S.W.26, B.NL.1, B.L.6
CL016	Uncultured *Rhizophagus* 1	97	954	M	Roots	S.W.6, S.W.14
CL002	Uncultured *Rhizophagus* 3	96	922	M, O	Roots	S.W.6, S.W.11, S.W.14, B.NL.1, B.H.2
CL001	Uncultured *Rhizophagus* 4	94	817	M, O	Roots	S.W.6, S.W.11, S.W.17, B.NL.1
CL015	Uncultured *Rhizophagus* 3	94	788	M, O	Roots	S.W.6, B.NL.1
CL018	Uncultured *Rhizophagus* 3	96	703	M, O	Roots	S.W.6, S.W.11, S.W.14, S.W.17, B.NL.1, B.H.2, B.L.6, B.L.14
CL062	Uncultured *Acaulospora* 1	97	295	M	Soil	S.W.17
CL032	Uncultured *Diversispora* 3	93	281	Y	Soil	Q.M.25
CL029	Uncultured *Acaulospora* 1	99	267	M	Soil	S.W.17
CL078	Uncultured *Diversispora* 3	93	203	Y	Soil	Q.M.25
CL060	Uncultured *Diversispora* 1	93	109	Y, O	Soil	Q.M.7, Q.M.23, B.H.2
CL051	Uncultured *Diversispora* 1	92	94	Y, O	Soil	Q.M.7, Q.M.23, B.H.2
CL071	Uncultured *Diversispora* 2	95	68	M	Soil	S.W.26
CL223	Uncultured *Diversispora* 2	95	64	M	Soil	S.W.26
CL121	Uncultured *Rhizophagus* 5	96	13	O	Soil	B.H.2
CL112	Uncultured *Diversispora* 4	98	12	Y	Soil	Q.M.25

OTU shows the identifier of the most abundant OTUs; Taxonomic affinity displays the phylogenetic assignment shown in Figs 1, 2; Similarity shows the sequence similarity to the closest BLAST hit in percentage (DDBJ/EMBL/GenBank); #Reads is the number of all sequence reads observed for the respective OTU; Dune system shows the chronosequence stage where the OTU was found, the young Quindalup dunes (Y), middle-aged Spearwood dunes (M) and old Bassendean dunes (O).

Fig. S2, Supporting information). No Paraglomerales, and only *Archaeospora* within Archaeosporales, were observed.

Most OTUs (~72%) within roots were associated with *Rhizophagus*, with the largest cluster close to *Rhizophagus clarus* W3776/Att894-7. The '*Rhizophagus irregularis*' clade comprised OTUs from the young and middle-aged dunes. A basal cluster within *Rhizophagus* was formed by the OTUs from the Jurien Bay chronosequence, environmental sequences from Yalgorup National Park (Shi *et al.* 2012), from a Tibetan Plateau, Himalaya, and sequences of an uncultured *Rhizophagus* sp. from the Peruvian Andes (Senés-Guerrero *et al.* 2014). The majority of OTUs within *Rhizophagus* were not assigned to any described *Rhizophagus* species. The remaining OTUs isolated from roots clustered in *Glomus* (13), *Claroideoglomus* (2), *Scutellospora* (2) and *Archaeospora* (1). The OTUs assigned to *Scutellospora*, clustered either within sequences of *S. calospora* (the type species) or with *S. spinosissima*, apart from the Yalgorup sequences. The solely detected *Archaeospora* sp. clustered with three environmental Yalgorup sequences sister to an uncultured *Archaeospora* sp. from the Peruvian Andes. The OTUs derived from soils were mainly related to *Diversispora*, *Acaulospora* and *Rhizophagus fasciculatus* MUCL46100 (Fig. 2). The OTUs isolated from soil of the young dunes were exclusively assigned to *Diversispora*, forming their own clusters or clustered together with

Fig. 1 Truncated maximum-likelihood phylogenetic tree of all operational taxonomic units (OTUs) detected in roots along the Jurien Bay dune chronosequence. Long branches were shortened by 75%, indicated with three diagonal slashes, and the root was reduced by 50% indicated by two diagonal slashes. Bootstrap support (BS) is only shown for genus and/or subgenus level and BS ≥60%. The blue branches show OTU sequences from this study; the orange branches are equivalent to sequences from Shi *et al.* (2012; Yalgorup National Park, part of the Quindalup dunes); the red branches display environmental sequence of the Tibetan Plateau, Himalaya; the green branches belong to environmental sequences from the Peruvian Andes (Senés-Guerrero *et al.* 2014). Paraglomus occultum AFTOL-ID844 was used as outgroup. The alternative shading illustrates the different clades within Glomeromycota *sensu* Redecker *et al.* (2013). The scale bar indicates the number of substitutions per site.



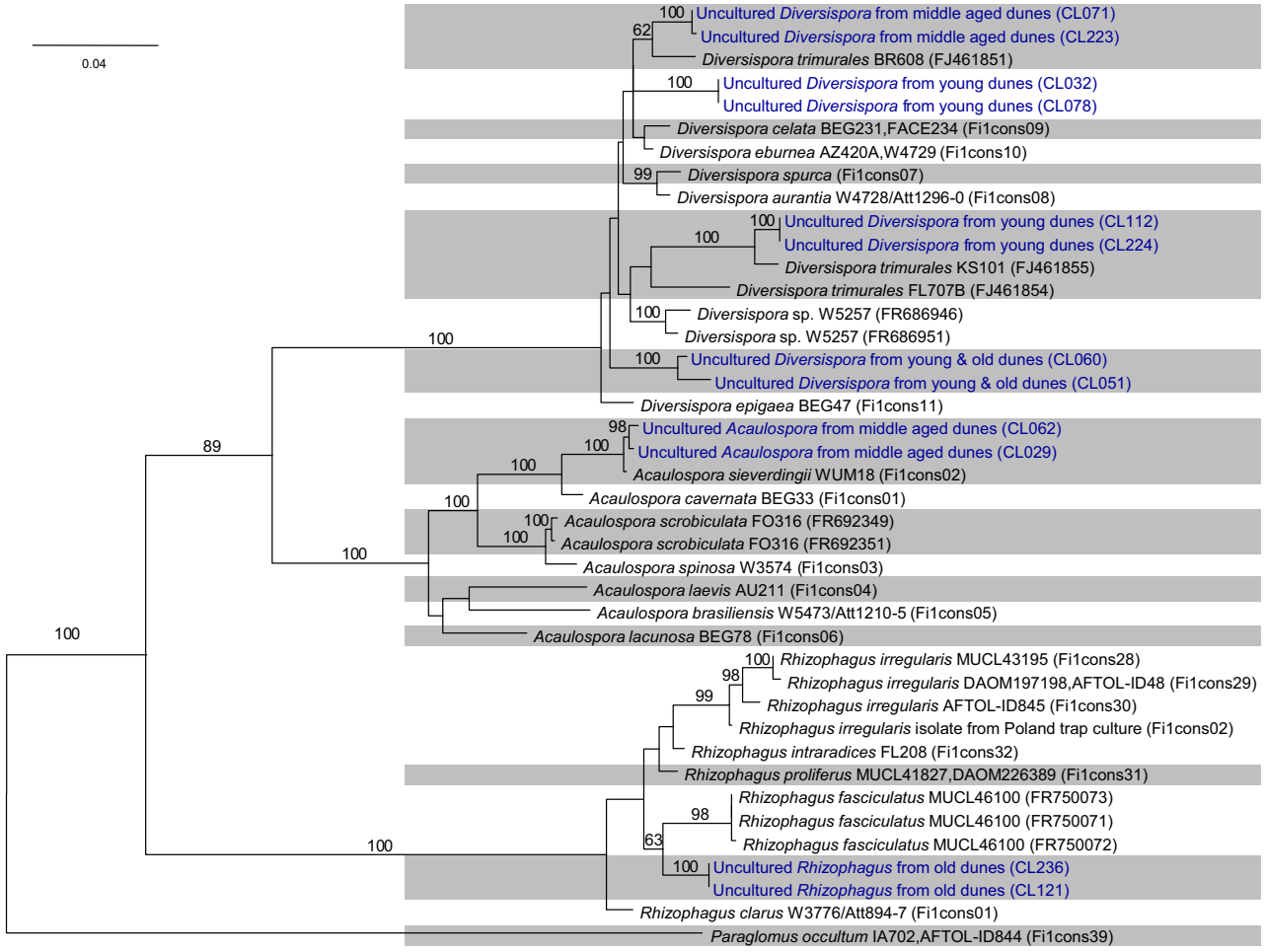


Fig. 2 Maximum-likelihood phylogenetic tree of all operational taxonomic units (OTUs) detected in soil along the Jurien Bay dune chronosequence shown in blue with the respective OTU identifier in parentheses. Bootstrap support (BS) ≥60% is shown. The alternative shading illustrates the different clades within Glomeromycota *sensu* Redecker *et al.* (2013). Paraglomus occultum AFTOL-ID844 was used as outgroup. The scale bar indicates the number of substitutions per site. Terminal nodes marked with 'Fi1cons#' represent consensus sequences as published in Krüger *et al.* (2012).

the old dunes OTUs. In the middle-aged dunes, OTUs could also be assigned to *Acaulospora*, which clustered with *A. sieverdingii* WUM18 originating from Nedlands, Western Australia (collected by L.K. Abbott) and *Diversispora*, clustering close to *Diversispora trimurales* BR608 (Fig. 2).

Richness and diversity of OTUs

The greatest richness and diversity of AMF-OTUs were found within roots of the middle-aged dunes (Fig. 3). These samples showed the highest OTU number, covering all four glomeromycotan genera detected across all root samples, with the highest PD (15.6; NRI 18.9, Table S3b, Supporting information) of all three dunes. These trends were maintained when analyses focused on the dominant *Rhizophagus* spp., as most OTUs clustered within *Rhizophagus* (Fig. 1).

The young and old dunes showed a significantly lower AMF richness and diversity (young only) than the middle dunes (Fig. 3). This was also reflected by lower PD and NRI values (young 5.5 & -1.31; old 6.9 & 9.11, respectively; Table S3b, Supporting information). At the dune level, the old dunes had 19 OTUs compared with only six in the youngest, while the middle-aged dunes had 92 OTUs in roots. The PD values of OTUs in soil from the old dunes, comprising *Diversispora* and *Rhizophagus*, were high compared with those from the other dune systems. The OTUs from soil of the young dunes were exclusively assigned to *Diversispora*, while in the middle dunes, *Diversispora* and *Acaulospora* were detected (Fig. 2). In both the old and the

young dunes, the PD and NRI values for AMF-OTUs in soil were generally higher than those from roots (Table S3a, Supporting information). However, this pattern was reversed in the middle-aged dunes.

Differences in AMF community composition

Comparisons of the AMF communities based on their presence–absence (Jaccard dissimilarity) or rank abundances (Gower dissimilarity) indicated large differences in AMF community composition among chronosequence stages, especially between the youngest and the other two older dunes (Table 3). AMF community composition in the middle dunes was most dissimilar to that in the young dunes, but also different from the oldest, based on Jaccard dissimilarities (Table 3). Non-parametric multidimensional analyses using presence–absence data of all samples (roots and soil) with dune types (young, middle, old) showed some separation between the chronosequence stages (Fig. 4), where AMF community composition in the youngest dunes was most distinct. There were no significant differences in multivariate dispersions among dune systems ($F = 0.50$, $DF_{groups} = 2$, $DF_{residuals} = 11$, $P = 0.618$, data not shown), despite the NMDS plot (Fig. 4) suggesting a possible greater dispersion in the young dunes.

The AMF communities on the old dunes differed significantly in their phylogenetic composition from those on the other two chronosequence stages. For example, no OTU within the '*Rhizophagus irregularis*' clade was detected in the old dunes, while these were present in the young and middle-aged dunes. The OTUs detected in the old dunes were mainly assigned to *Rhizophagus*, close to *R. clarus* (W3776/Att894-7), comprising also OTUs from the middle dunes, *Glomus* and *Scutellospora spinosissima* (W3009/Att664-1). The OTUs derived from soil of the old dunes include a unique *Rhizophagus* species, basally to *R. fasciculatus* MUCL46100. The OTUs related to *R. clarus* were dominant in the old and middle-aged dunes, while absent in the young dunes. This held true for a large cluster within the '*Glomus macrocarpum*' clade (*sensu* Redecker *et al.* 2013). The OTUs from the middle-aged dunes comprised primarily *Rhizophagus*, close to *R. clarus* as in the old, uncultured *Rhizophagus* sp. and '*Rhizophagus irregularis*' clade as in the young dunes and *R. cerebriformis*. OTUs from middle dunes also shared a common clade within *Glomus* with the old dunes OTUs. The only OTUs assigned to *S. calospora, Archaeospora* and *Acaulospora* were found in the middle dunes. Similar to the middle, the young dunes harboured OTUs within the '*Rhizophagus irregularis*' clade, but also had *Claroideoglomus* OTUs that were not detected in the other chronosequence stages. *Diversispora* OTUs found in the young dunes were also present in the oldest chronosequence stage.

Indicator AMF taxon (e.g. taxonomic identifier: OTU or O. species) analysis from roots showed that the 'uncultured *Rhizophagus* sp. 3' was an indicator taxon for the middle and old dunes (Indicator statistic = 0.943, *P*-value = 0.025). Although AMF community composition in the young dunes soil was significantly different from that in the other dunes when presence–absence data were considered, this was not the case when the Gower dissimilarity index was used with the rank-based sequence data (Fig. S3, Supporting information). Accumulation curves of the number of OTUs and sequences show the sampling effort appeared adequate (Fig. S4, Supporting information).

Soil nutrient availability

As expected from previous studies, soil total P concentrations ([P]) were highest (~420 mg/kg) in the young dunes (Laliberté *et al.* 2012; Hayes *et al.* 2014; Turner & Laliberté 2015). There was a sharp decline in soil total P concentration in the middle dunes (~23 mg/kg), and a further decrease (~5 mg/kg) in the oldest chronosequence stage (Fig. S5, Supporting information). The same trend was observed for total N concentrations in the soil for the sampling sites. Soils in the young dunes were alkaline and became progressively more acidic in the middle-aged and old dunes (Fig. S5, Supporting information).

Host plant cover and diversity

Variable selection approaches identified the key host plant and soil variables such as soil total [P] and relative cover of AM host plants as the only variables significantly explaining the variation in AMF-OTU rarified richness in roots (Table S6, Supporting information). Subsequently, nonlinear regression analyses of the most-important variable (total [P]) showed a significant trend between AMF-OTU richness and soil total [P], where lower AMF-OTU richness was significantly correlated with the lowest and highest soil [P] (Fig. 5). Similar trends were found when soil-based OTUs were included in these analyses (Fig. S6, Supporting information). The redundancy analysis (RDA) of the AMF-OTUs from roots with key soil variables (e.g. total [P]) and host plant responses (e.g. richness and diversity) identified total [P] as the most important factor (Fig. 6). Concentrations of Cu did indicate a marginal effect on the AMF communities, in particular in the medium-aged soils (Fig. 6). AM plant host species richness or relative cover/abundance was not selected by the forward selection procedure, suggesting that host abundance or diversity was likely not an important driver of AMF community composition along the chronosequence. On the other hand, soil Cu concentrations explained part of the variation in AMF

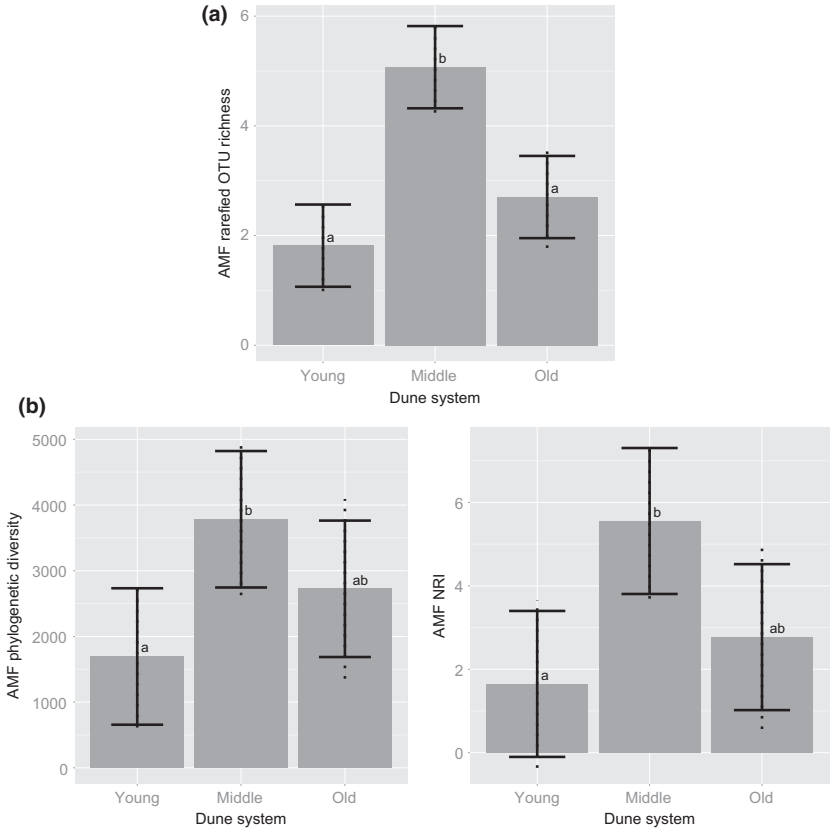


Fig. 3 (a) Rarefied richness and (b) diversity expressed as phylogenetic diversity (PD) and net relatedness index (NRI) of arbuscular mycorrhizal fungal (AMF) communities along the Jurien Bay dune chronosequence based on the number of sequences per operational taxonomic unit (OTU). The NRI value indicates degree of phylogenetic clustering of AMF communities, where a positive value indicates clustered communities and negative values indicate overdispersed communities. The nearest taxon index (NTI) was invariant to chronosequence stages (Table S5, Supporting information). The three most distinct chronosequence stages, the young Quindalup dunes (Young), middle-aged Spearwood dunes (Middle), and old Bassendean dunes (Old) are shown. Values are means with 95% confidence intervals (dotted lines), Tukey's HSD error bars (solid), and different letters to indicate statistically significant differences between means (Tukey HSD tests with $P \leq 0.05$).

community composition although this was mainly driven by a single data point (Fig. 6). The richness of AM–host plants did not increase with soil age along the chronosequence (Fig. 7a). On the other hand, the relative canopy cover of AM host plants was lower in the old dunes, although the difference in relative cover (16% lower in the old vs. middle-aged dunes) was relatively low (Fig. 7b). Furthermore, the richness of all plants was only significantly greater in the old dunes compared with the young dunes (Fig. 7c), while diversity did not increase with soil age along the chronosequence (Fig. 7d).

Discussion

AMF community dynamics with ecosystem development

Our results show that AMF-OTU richness, and to some degree phylogenetic diversity, increased during the early stage of ecosystem retrogression along with an associated decline in P availability, but that AMF richness eventually declined on very old soils where P availability was extremely low. Strikingly, this decline in AMF richness on the oldest soils occurred despite: (i) invariant host plant richness; (ii) a small decline in host relative canopy cover; (iii) only a small increase in plant (all species) richness; and (iv) invariant plant (all species) diversity. The lower AMF richness and diversity observed for the distinct AMF community in the oldest and most P-impoverished soils compared with those in the younger dunes suggest that relatively few AMF species can effectively acquire P under extremely low P availability.

Our correlative analyses of AMF community composition and diversity with potentially important abiotic and biotic factors such as soil chemical properties, host plant richness, relative cover, relative abundance and diversity, suggest that total [P] in these soils

Table 3 Multivariate community analyses using permutation distance-based multivariate ANOVA (PERMANOVA) showing degree of dissimilarity between arbuscular mycorrhizal fungal communities on the three chronosequence stages young Quindalup dunes (Y), middle-aged Spearwood dunes (M) and old Bassendean dunes (O) along the Jurien Bay dune chronosequence

Taxonomic identifier	Data matrix type	Dissimilarity measure	Dune system comparisons	*P*-value
Operational taxonomic units	Presence–absence (roots & soil)	Jaccard	—	**0.007**
			Y vs. M	**0.01**
			Y vs. O	**0.034**
			M vs. O	0.564
	Rank (roots)	Gower	—	0.543
	Rank (soil)	Gower	—	0.396
Operational species-level	Presence–absence (roots & soil)	Jaccard	—	**0.005**
			Y vs. M	**0.023**
			Y vs. O	**0.035**
			M vs. O	**0.025**
	Rank (roots)	Gower	—	**0.044**
			Y vs. M	0.216
			Y vs. O	**0.008**
			M vs. O	0.988
	Rank (soil)	Gower	—	0.359

P-values < 0.05 are shown in bold, nonsignificant ($P > 0.05$) multiple comparison tests are not included. The PERMANOVA F statistic can be interpreted similarly to a classic univariate ANOVA. Only PERMANOVA statistics with $P \leq 0.05$ were considered for multiple comparisons

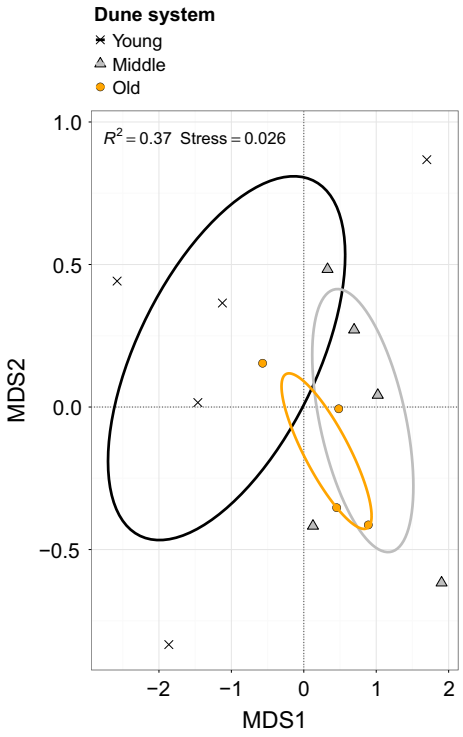


Fig. 4 Nonparametric multidimensional scaling (NMDS) plot of all samples (roots and soil) along the Jurien Bay dune chronosequence with the three chronosequence stages young Quindalup dunes (Young), middle-aged Spearwood dunes (Middle) and old Bassendean dunes (Old). The Jaccard dissimilarity index was used (presence–absence matrix) derived from sequence numbers per operational taxonomic units. This NMDS ordination was performed using the metaMDS function in R via the 'VEGAN' package to visualize the similarity in the AM fungal community between chronosequence stages. Each point symbolizes a single AM fungal community, and ellipses represent 95% confidence intervals around the chronosequence stage centroids. Nonoverlapping ellipses are considered significantly different and can be used to visually estimate the degree of similarity (overlap) or difference (no overlap) in the AM fungal community. R^2 values represent the amount of variation in the data set explained by the chronosequence stages, based on RDA. The stress value is a measure of the disagreement between the rank order in the original data set and that in the NMDS (lower numbers indicate better agreement and rule of thumb: stress >0.3 provides a poor representation).

is the most important factor structuring the AMF communities. Indeed, the lowest levels of soil P were correlated with lower AMF-richness found in the oldest soils. On the other hand, relatively high levels of total P in the young soils were also associated with low AMF-OTU richness, whereas the most diverse AMF communities were associated with soils with intermediate levels of total soil P. In addition, total [P] was the most important factor explaining variation in AMF community structure being significantly different on the older, P-poor soils compared with the younger, relatively P-rich soils.

Recently, Martínez-García *et al.* (2015) were the first to quantify shifts in AMF community composition and diversity during ecosystem retrogression using next generation sequencing. They found that plant host identity was an important driver of AMF community composition, whereas we did not find such a strong effect of host richness or diversity. They used the well-known Franz Josef glacier chronosequence in New Zealand, but the oldest and most P-impoverished sites are relatively young and P-rich compared with the oldest sites along the Jurien Bay dune chronosequence which are among the most P-impoverished soils ever found (Laliberté *et al.* 2012; Turner & Laliberté 2015). In addition, the Jurien Bay dune chronosequence is located in one of the world's biodiversity hotspots (Myers *et al.* 2000), with exceptionally high plant diversity at all spatial scales (Hopper & Gioia 2004; Mucina *et al.* 2014). Importantly, plant species turnover across the Jurien Bay chronosequence is exceptionally high, and such high turnover is directly linked to changes in soil properties during long-term soil formation (Zemunik *et al.*

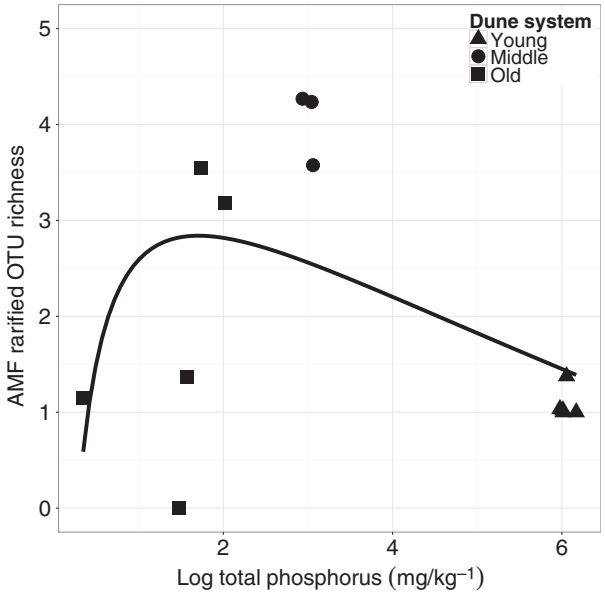


Fig. 5 Regression analyses of rarified AMF-OTU richness on roots and soil total phosphorus concentration along the Jurien Bay dune chronosequence. The three chronosequence stages selected were as follows: young Quindalup dunes (Young), middle-aged Spearwood dunes (Middle) and old Bassendean dunes (Old). Shown here is a second-order polynomial regression fit ($y = 2.52 - 1.66x - 5.49x^2$, Adjusted $R^2 = 0.66$, $P < 0.001$). Similar results were found for all OTUs (roots + soil), see Fig. S6.

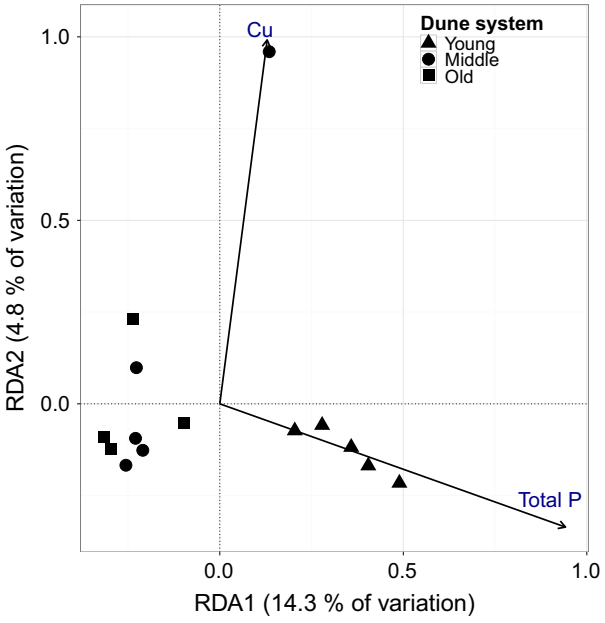


Fig. 6 Redundancy analysis (RDA) biplot with fitted plot and forward-selected soil nutrient variables. Forward selection of soil and host plant factors identified concentrations of total phosphorus (P) and exchangeable copper (Cu) as key drivers of the variation in AMF-OTU composition. The first two canonical axes explained 19.2% ($R^2 = 0.316$; $R^2_{adj} = 0.192$; pseudo-$F = 2.54$, $DF_{model} = 2$, $DF_{residual} = 11$, $P < 0.001$) of the total variation in the community data, where the first axis alone explains 14.3% based on the proportion of R^2_{adj}. Only the first RDA axis was statistically significant when the permutation test was conducted by axis (pseudo-$F = 3.79$, $DF_{model} = 1$, $DF_{residual} = 11$, $P < 0.001$).

2015); the strong covariation between soil properties and plant species composition could partly explain why we found that soil properties (e.g. total soil [P]) are the most important drivers of AMF community structure. Our study is particularly interesting as we found that AMF diversity declined despite invariant host plant diversity, despite earlier predictions that AMF diversity should mirror changes in AM host plant diversity (van der Heijden *et al.* 1998, 2008).

The sharp decline in AMF richness on the oldest dunes was not completely unexpected, because AMF may be less effective in acquiring P (Lambers *et al.* 2008) in strongly P-impoverished soils as many AMF species only enhance plant P acquisition by 'scavenging' inorganic P from the soil solution (Smith & Read 2008). The decrease in potential AM host cover may play an additional role. In soils with extremely low P availability, resource trading within AM involving many AMF species that fail to effectively acquire P may no longer be reciprocal or favourable to both partners (Kiers *et al.* 2011; Lambers & Teste 2013). Our current understanding of the functioning of mycorrhizal fungal communities relies heavily on work done on relatively young soils or short-term chronosequences lacking an important part of long-term ecosystem development, namely ecosystem retrogression (Dickie *et al.* 2013). As such, our survey of AMF communities during ecosystem retrogression is the among most comprehensive to date. Importantly, it highlights, for the first time, that there is a threshold in P availability below which AMF diversity declines, confirming predictions made by some authors (Lambers *et al.* 2008).

Martínez-García *et al.* (2015) found greater beta diversity of AMF communities at young sites, similar to the present trend (although not significant, possibly due to lower statistical power). The authors suggested that stochastic priority effects during community assembly led to greater beta diversity in the young soils, whereas AMF community assembly on their oldest sites was controlled by stronger environmental filtering. Our results partly corroborate the findings of Martínez-García *et al.* (2015), as our NMDS suggests a possible role for environmental filtering in reducing beta diversity in the oldest soils.

One of the strengths of long-term soil chronosequences as soil nutrient availability gradients is that they maximize variation in one factor (in this case, soil

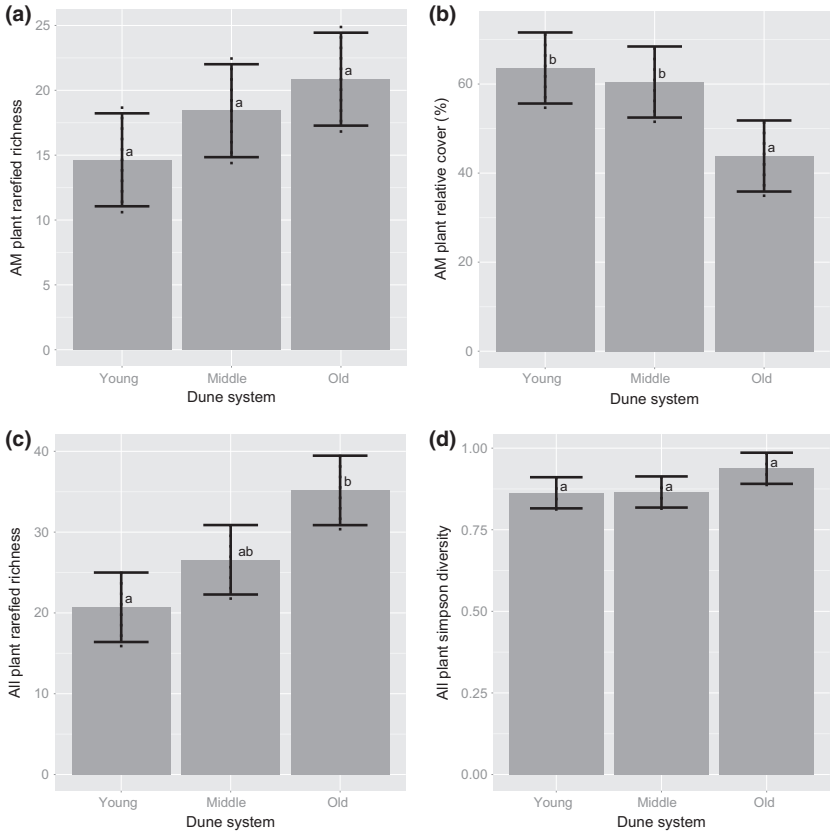


Fig. 7 (a) Rarefied richness of arbuscular mycorrhizal (AM) host-plant communities along the Jurien Bay dune chronosequence based on the number of individuals per plot and (b) relative cover. Also shown is the (c) rarefied richness and (d) diversity of all plant species. The three most distinct chronosequence stages, the young Quindalup dunes (Young), middle-aged Spearwood dunes (Middle), and old Bassendean dunes (Old) are shown. Values are means with 95% confidence intervals (dotted lines), Tukey's HSD error bars (solid), and different letters to indicate statistically significant differences between means (Tukey HSD tests with $P \leq 0.05$).

age, which influences soil nutrient availability through long-term weathering) while minimizing variation in other important factors such as soil type climate and parent material (Walker *et al.* 2010). Previous work along the Jurien Bay dune chronosequence showed that parent material is reasonably consistent, except for the oldest dunes (Bassendean), which probably contained less carbonate at deposition (Laliberté *et al.* 2012, 2013; Turner & Laliberté 2015); however, we do not consider the lower initial carbonate content of the oldest dunes to be problematic in studies of plant–soil interactions, as carbonate loss from these soils is very rapid (Turner & Laliberté 2015). Although older dunes are further from the coast than younger ones, there is negligible change in rainfall across the 15-km wide chronosequence (Laliberté *et al.* 2012; Turner & Laliberté 2015). Salinity does not play a role either, as exchangeable Na concentrations are very low across all dunes and not greater in the younger dunes closest to the coast (Turner & Laliberté 2015). Nevertheless, any 'natural gradient' can contain possible confounding factors, including dispersal limitation of AMF spores.

AMF communities on P-impoverished soils

The observed increase in AMF richness in the middle-aged dunes was due to the persistence of AMF associated with pioneer plant species from the young dunes as well as the appearance of new AM host plants on the middle-aged dunes (Zemunik *et al.* 2015). The much lower soil pH associated with the most diverse AMF community in the middle-aged dunes, when compared with that in the young dunes, suggests that community composition of AMF could be pH-driven (Lekberg *et al.* 2011; Meadow & Zabinski 2012) with varying growth response of AMF species to low pH (van Aarle *et al.* 2002). However, under P limitation, the higher AMF diversity in the middle-aged dunes is more likely driven by greater allocation of plant C to AMF under P limitation. Plants growing in the oldest, severely P-im-

poverished soils use a diverse range of strategies to efficiently acquire and use P (Zemunik *et al.* 2015). They efficiently acquire P by scavenging soil P like the AM and ectomycorrhizal plants (Lambers 2014), release large amounts of carboxylates and phosphatases to mobilize P (Lambers *et al.* 2006), resorb P extremely efficiently from senescing leaves (Hayes *et al.* 2014) and fine roots (Shane *et al.* 2004), and maintain a very high photosynthetic P-use efficiency like the cluster-rooted plants (Lambers *et al.* 2012). However, AM plant species cover was significantly lower in the oldest dunes compared with those in the middle-aged dunes. Therefore, the decline in AMF richness on the oldest soils could be partially explained by the lower relative AM host plant cover. The decline in P availability during ecosystem development can also be accompanied by a shift of bacterial communities (Jangid *et al.* 2013), for example, bacteria promoting mycorrhizal formation, which might play a role during ecosystem retrogression and AM development in the oldest dunes. The lower AMF richness and diversity found in the younger dunes might also be due to low spore abundance, reduced formation of hyphae and the alkaline soil pH (Abbott & Gazey 1994).

Phylogenetic diversity of AMF

The AMF discovered in this long-term chronosequence in southwestern Australia cover all main glomeromycotan lineages, with the exception of Paraglomerales. The absence of Archaeosporales, except *Archaeospora*, is congruent with the results of AMF inhabiting the young dunes (Shi *et al.* 2012), sand dunes in Southern Arabia (Al-Yahya'ei *et al.* 2011), in Portugal (Rodríguez-Echeverría & Freitas 2006), Southern and Northern Brazil (Córdoba *et al.* 2001; da Silva *et al.* 2012), Northern Venezuela (Alarcón & Cuenca 2005) or in southwestern Japan (Yamato *et al.* 2012). The highest number of AMF-OTUs was assigned to Glomeraceae, abundant throughout the dunes (Tables S2 and S4, Supporting information) with the highest phylogenetic diversity (PD and NRI) at the middle-aged dunes (Table S3b, Supporting information). Glomeraceae are globally widespread and found in a variety of environments, including some harsh conditions such as high aridity, salinity and high soil acidity and are reported to be dominant in sandy soils in Brazil, Italy and Poland (Córdoba *et al.* 2001; Turrini *et al.* 2010; Błaszkowski *et al.* 2002). Gigasporaceae were present in the middle and late stage of ecosystem progression due to their potential of adapting to low [P] in soil (Hart & Reader 2002) and were previously reported from coastal sand dunes (Córdoba *et al.* 2001; da Silva *et al.* 2012). *Acaulospora* spp., which are common in the middle-aged dunes, are frequent in early successions and remain so in mature ecosystems (Johnson *et al.* 1991; Sikes *et al.* 2012) and are present in most sandy soils (Al-Yahya'ei *et al.* 2011; de Souza *et al.* 2013). Shi *et al.* (2012) recently analysed part of the young (Quindalup) dunes system in Yalgorup National Park in southwestern Australia, mainly observing *Archaeospora* and *Glomus* species. The AMF-OTUs detected with next generation sequencing are congruent with the traditionally cloning and Sanger sequencing approach of Shi *et al.* (2012). Furthermore, we found new OTUs mainly related to *Diversispora* and *Acaulospora* within the study site. This might be due to the fact that Shi *et al.* (2012) used only roots from trap-cultured plants, and not soil, as we showed that the AMF-OTUs detected in soil were not detected in roots, and vice versa. The lack of overlap of AMF-OTUs from soil and root samples was unexpected, but suggests that long-distance scavenging AMF species were marginal (below the detection level) or absent.

Despite the lower number of replicates for the young dunes compared with those in Shi *et al.* (2012), we observed almost the same AMF across these dunes. The AMF detected in the Jurien Bay dune chronosequence were also reported in studies from the Southern Hemisphere and Northern Hemisphere, and globally found in sandy soils. While Glomeraceae in roots and *Diversispora* in soil were distributed across the chronosequence, *Archaeospora* and Gigasporaceae were restricted to parts of it (Tables S2 and S4, Supporting information), mainly due to the different availability of host plants, AMF–plant preferences and nutrient availability. The latter might be one of the main factors in combination with soil chemistry that drives AMF composition. However, the ecological factors driving AMF distribution in sandy soils are not well understood.

The nested PCR approach with part of the AMF-inclusive primers published in Krüger *et al.* (2009) provided sufficient resolution for AMF community ecology studies in biodiverse ecosystems. The use of primer mixes targeting the LSU rDNA region has been assessed as a good approach to identify a high diversity AMF taxa with sufficient resolution from in-field communities (Krüger *et al.* 2009; Gorzelak *et al.* 2012). However, the lower support of the phylogenetic tree compared with the longer SSUpart-ITS-LSUpart rDNA fragment indicates the need for a sufficiently large reference data set, ideally from the specific study site (Senés-Guerrero *et al.* 2014). Important advances in molecular tools for analysing soil fungal communities are now providing unprecedented opportunities for uncover AMF diversity in all ecosystems (Fierer *et al.* 2013; Talbot *et al.* 2014; Tedersoo *et al.* 2014; van der Heijden *et al.* 2015).

Conclusions

We investigated ecosystem retrogression that included much older and more P-impoverished soils than used in previous studies, which allowed us to expand the understanding of soil factors shaping AMF communities. Our results showed that abiotic factors such as soil nutrient levels were more important than host plant diversity in driving AMF-community composition during ecosystem development that includes a wider range of soil ages; however, host plant community composition and diversity strongly covary with soil properties along the present chronosequence (Zemunik *et al.* 2015), making it difficult to disentangle effects of soil properties from those due to host plants. Our study shows important shifts in AMF diversity and community composition during ecosystem retrogression across a long-term soil chronosequence in a global biodiversity hotspot. The AMF-community OTU richness, phylogenetic diversity and composition were relatively low within the young dunes with moderately P-rich soils, peaked in the older, low-P dunes, and finally declined in the oldest, extremely low-P dunes. The invariant AM host richness and the relatively small declines in relative cover during ecosystem retrogression suggest that declines in AMF diversity are not solely driven by host plant diversity and abundance. Instead, we propose that AM host plants in extremely P-impoverished soils form associations with a restricted number of AMF taxa, possibly because plant carbon costs of the symbiosis exceed nutritional benefits for many AMF taxa under extreme P limitation. Our findings on AMF diversity along this P availability and soil age gradient, including ecosystem retrogression, provide a starting point to develop general principles about long-term feedbacks of AMF communities during long-term pedogenic processes and ecosystem development.

Acknowledgements

We thank the Western Australian Department of Parks and Wildlife for their cooperation and help acquiring permits to sample biota and soil in the national parks. We are grateful to Patrick Hayes, Osmarina Marinho, Davide Abade, Felipe Albornoz, Kenny Png, Thomas Mazet, James Haile and Frances Brigg for help with the field and laboratory work. Funding was provided by The University of Western Australia with a Research and Development Award granted to FT and EL, an Australian Research Council (ARC) Discovery Early Career Researcher Award to EL (DE120100352), and ARC Future Fellowship to MB (FT0991741).

References

van Aarle IM, Olsson PA, Söderström B (2002) Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist*, **155**, 173–182.

Abbott L, Gazey C (1994) An ecological view of the formation of VA mycorrhizas. *Plant and Soil*, **159**, 69–78.

Alarcón C, Cuenca G (2005) Arbuscular mycorrhizas in coastal sand dunes of the Paraguaná Peninsula, Venezuela. *Mycorrhiza*, **16**, 1–9.

Altman DG, Machin D, Bryant TN *et al.* (2000) *Statistics with Confidence, Confidence Intervals and Statistical Guidelines*. British Medical Journal Books, London, UK.

Al-Yahya'ei MN, Oehl F, Vallino M *et al.* (2011) Unique arbuscular mycorrhizal fungal communities uncovered in date palm plantations and surrounding desert habitats of Southern Arabia. *Mycorrhiza*, **21**, 195–209.

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. *Ecology Letters*, **9**, 683–693.

Anderson BT, Ruane AC, Roads JO, Kanamitsu M, Salvucci G (2008) A new metric for estimating local moisture cycling and its influence upon seasonal precipitation rates. *Journal of Hydrometeorology*, **9**, 576–588.

Błaszkowski J, Tadych M, Madej T (2002) Arbuscular mycorrhizal fungi (Glomales, Zygomycota) of the Bledowska Desert, Poland. *Acta Societatis Botanicorum Poloniae*, **71**, 71–85.

Borcard D, Gillet F, Legendre P (2011) *Numerical Ecology with R*. Springer, New York.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants, understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil*, **320**, 37–77.

Bunce M, Oskam CL, Allentoft ME (2012) Quantitative real-time PCR in a DNA research. *Methods in Molecular Biology*, **840**, 121–132.

Cáceres MD, Legendre P (2009) Associations between species and groups of sites, indices and statistical inference. *Ecology*, **90**, 3566–3574.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.

Coghlan ML, Haile J, Houston J *et al.* (2012) Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. *PLoS Genetics*, **8**, e1002657.

Córdoba AS, Mendonça MM, Stürmer SL, Ryglewicz PT (2001) Diversity of arbuscular mycorrhizal fungi along a sand dune stabilization gradient, a case study at Praia de Joaquina, Ilha de Santa Catarina, South Brazil. *Mycoscience*, **42**, 379–387.

Cumming G (2008) Replication and *p* intervals: *p* values predict the future only vaguely, but confidence intervals do much better. *Perspectives on Psychological Science*, **3**, 286–300.

Di Stefano J, Fidler F, Cumming G (2005) Effect size estimates and confidence intervals, an alternative focus for the presentation and interpretation of ecological data. In: *New Trends in Ecology Research*(ed. Burk AR), pp. 71–102. Nova Science, New York City, New York, USA.

Dickie IA, Martínez-García LB, Koele N *et al.* (2013) Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant and Soil*, **367**, 11–39.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460–2461.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194–2200.

Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.

Fierer N, Ladau J, Clemente JC *et al.* (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science*, **342**, 621–624.

Fox J (2003) Effect displays in R for generalised linear models. *Journal of Statistical Software*, **8**, 1–27.

Gollotte A, Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza*, **14**, 111–117.

Grömping U (2006) Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software*, **17**, 1–27.

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, **153**, 335–344.

Hawksworth DL (2001) The magnitude of fungal diversity, the 1.5 million species estimate revisited. *Mycological Research*, **105**, 1422–1432.

Hayes P, Turner BL, Lambers H, Laliberté E (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology*, **102**, 396–410.

van der Heijden MGA, Klironomos JN, Ursic M *et al.* (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69–72.

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority, soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, **205**, 1406–1423.

Hodge A, Helgason T, Fitter A (2010) Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology*, **3**, 267–273.

Hopper SD, Gioia P (2004) The southwest Australian floristic region, evolution and conservation of a global hot spot of biodiversity. *Annual Review of Ecology, Evolution and Systematics*, **35**, 623–650.

Gorzelak MA, Holland TC, Xing X, Hart MM (2012) Molecular approaches for AM fungal community ecology: a primer. *Journal of Microbiological Methods*, **90**, 108–114.

Jangid K, Whitman WB, Condron LM, Turner BL, Williams MA (2013) Soil bacterial community succession during long-term ecosystem development. *Molecular Ecology*, **22**, 3415–3424.

Johnson NC, Zak DR, Tilman D, Pfleger FL (1991) Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia*, **86**, 349–358.

Kendrick GW, Wyrwoll KH, Szabo J (1991) Pliocene-Pleistocene coastal events and history along the western margin of Australia. *Quaternary Science Reviews*, **10**, 419–439.

Kiers ET, Duhamel M, Beesetty Y *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, **333**, 880–882.

Kohout P, Sudová R, Janoušková M *et al.* (2014) Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biology and Biochemistry*, **68**, 482–493.

Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of Glomeromycota, one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist*, **183**, 212–223.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist*, **193**, 970–984.

Laliberté E, Turner BL, Costes T *et al.* (2012) Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. *Journal of Ecology*, **100**, 631–642.

Laliberté E, Grace JB, Huston MA *et al.* (2013) How does pedogenesis drive plant diversity? *Trends in Ecology and Evolution*, **28**, 331–340.

Laliberté E, Zemunik G, Turner BL (2014) Environmental filtering explains variation in plant diversity along resource gradients. *Science*, **345**, 1602–1605.

Lambers H (ed.) (2014) *Plant Life on the Sandplains in Southwest Australia, a Global Biodiversity Hotspot*. University of Western Australia Publishing, Crawley, Western Australia, Australia.

Lambers H, Teste FP (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants, do non-mycorrhizal species at both extremes of nutrient availability play the same game? *Plant Cell & Environment*, **36**, 1911–1915.

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany*, **98**, 693–713.

Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*, **23**, 95–103.

Lambers H, Bishop JG, Hopper SD, Laliberté E, Zúñiga-Feest A (2012) Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. *Annals of Botany*, **110**, 329–348.

Lambers H, Martinoia E, Renton M (2015a) Plant adaptations to severely phosphorus-impoverished soils. *Current Opinions in Plant Biology*, **25**, 23–31.

Lambers H, Clode P, Hawkins HJ *et al.* (2015b) Metabolic adaptations of the non-mycotrophic Proteaceae to soil with a low phosphorus availability. In: *Annual Plant Reviews: Phosphorus Metabolism in Plants* (eds Plaxton WC, Lambers H), vol. 48, pp. 289–335. Wiley-Blackwell, in press.

Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, **69**, 1–24.

Legendre P, Oksanen J, ter Braak CJ (2011) Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution*, **2**, 269–277.

Lekberg Y, Meadow J, Rohr JR, Redecker D, Zabinski CA (2011) Importance of dispersal and thermal environment for mycorrhizal communities, lessons from Yellowstone National Park. *Ecology*, **92**, 1292–1302.

Logan V, Clarke P, Allaway W (1989) Mycorrhizas and root attributes of plants of coastal sand-dunes of New South Wales. *Functional Plant Biology*, **16**, 141–146.

Martínez-García LB, Richardson SJ, Tylianakis JM, Peltzer DA, Dickie IA (2015) Host identity is a dominant driver of myc-

orrhizal fungal community composition during ecosystem development. *New Phytologist*, **205**, 1565–1576.

McArthur WM, Bettenay E (1974) *The Development and Distribution of the Soils of the Swan Coastal Plain*. vol. 16, Soil Publication CSIRO, Australia, 55pp.

Meadow JF, Zabinski CA (2012) Linking symbiont community structures in a model arbuscular mycorrhizal system. *New Phytologist*, **194**, 800–809.

Mucina L, Laliberté E, Thiele KR, Dodson JR, Harvey J (2014) Biogeography of kwongan: origins, diversity, endemism and vegetation patterns. In: *Plant Life on the Sandplains in Southwest Australia, a Global Biodiversity Hotspot*(ed. Lambers H), pp. 35–80. UWA Publishing, Nedlands, Western Australia, Australia.

Murtaugh PA (2009) Performance of several variable-selection methods applied to real ecological data. *Ecology Letters*, **12**, 1061–1068.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nguyen NH, Smith D, Peay K, Kennedy P (2015) Parsing ecological signal from noise in next generation amplicon sequencing. *New Phytologist*, **250**, 1389–1393.

Odum EP (1969) The strategy of ecosystem development. *Science*, **164**, 262–270.

Oksanen J, Blanchet FG, Kindt R *et al*. (2013) vegan, Community ecology package. *R package version 2.0-6*. http//CRAN.R-project.org/package=vegan, 2.0-6.

Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist*, **184**, 424–437.

Öpik M, Zobel M, Cantero J *et al*. (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, **23**, 411–430.

Parfitt RL (1979) The availability of P from phosphate-goethite bridging complexes. Desorption and uptake by ryegrass. *Plant and Soil*, **53**, 55–65.

Peltzer DA, Wardle DA, Allison VJ *et al*. (2010) Understanding ecosystem retrogression. *Ecological Monographs*, **80**, 509–529.

Read DJ (1993) Mycorrhiza in plant communities. *Advances in Plant Pathology*, **9**, 1–31.

Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza*, **23**, 515–531.

Rodríguez-Echeverría S, Freitas H (2006) Diversity of AMF associated with Ammophila arenaria ssp. arundinacea in Portuguese sand dunes. *Mycorrhiza*, **16**, 543–552.

Rosendahl S (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. *New Phytologist*, **178**, 253–266.

Senés-Guerrero C, Torres-Cortés G, Pfeiffer S, Rojas M, Schüßler A (2014) Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. *Mycorrhiza*, **24**, 405–417.

Shane MW, Szota C, Lambers H (2004) A root trait accounting for the extreme phosphorus sensitivity of Hakea prostrata (Proteaceae). *Plant, Cell and Environment*, **27**, 991–1004.

Shi P, Abbott LK, Banning NC, Zhao B (2012) Comparison of morphological and molecular genetic quantification of relative abundance of arbuscular mycorrhizal fungi within roots. *Mycorrhiza*, **22**, 501–513.

Sikes BA, Maherali H, Klironomos JN (2012) Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. *Oikos*, **121**, 1791–1800.

da Silva DKA, Pereira CMR, Souza RG, Silva GA, Oehl F, Maia LC (2012) Diversity of arbuscular mycorrhizal fungi in restinga and dunes areas in Brazilian Northeast. *Biodiversity and Conservation*, **21**, 2361–2373.

Smith SE, Read DJ (2008) *Mycorrhizal Symbiosis*. Academic Press and Elsevier London, New York City, New York, USA.

Smith SE, Anderson IC, Smith AF (2015) Mycorrhizal associations and phosphorus acquisition: from cells to ecosystems. In: *Annual Plant Reviews: Phosphorus Metabolism in Plants* (eds Plaxton W, Lambers H), **48**, 409–439.

de Souza RG, da Silva DKA, de Mello CMA *et al*. (2013) Arbuscular mycorrhizal fungi in revegetated mined dunes. *Land Degradation & Development*, **24**, 147–155.

Stamatakis A, Hoover P, Rougemont J (2008) A fast bootstrapping algorithm for the RAxML web-servers. *Systematic Biology*, **57**, 758–771.

Talbot JM, Bruns TD, Taylor JW *et al*. (2014) Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences*, **111**, 6341–6346.

Tedersoo L, Bahram M, Põlme S *et al*. (2014) Global diversity and geography of soil fungi. *Science*, **346**, 1052–1053.

Turner BL, Laliberté E (2015) Soil development and nutrient availability along a 2 million-year coastal dune chronosequence under species-rich mediterranean shrubland in southwestern Australia. *Ecosystems*, **18**, 287–309.

Turner BL, Lambers H, Condron LM *et al*. (2013) Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant and Soil*, **367**, 225–234.

Turrini A, Sbrana C, Strani P, Pezzarossa B, Risaliti R, Giovannetti M (2010) Arbuscular mycorrhizal fungi of a Mediterranean island (Pianosa), whitin a UNESCO Biosphere Reserve. *Biology and Fertility of Soils*, **46**, 511–520.

Vitousek PM, Farrington H (1997) Nutrient limitation and soil development, experimental test of a biogeochemical theory. *Biogeochemistry*, **37**, 63–75.

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–19.

Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology*, **98**, 725–736.

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, **151**, 753–760.

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science*, **305**, 509–513.

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom, software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, **24**, 2098–2100.

Whiteside MD, Digman MA, Gratton E, Treseder KK (2012) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. *Soil Biology and Biochemistry*, **55**, 7–13.

Yamato M, Yagame T, Yoshimura Y, Iwase K (2012) Effect of environmental gradient in coastal vegetation on communities of arbuscular mycorrhizal fungi associated with *Ixeris repens* (Asteraceae). *Mycorrhiza*, **22**, 623–630.

Zemunik G, Turner BL, Lambers H, Laliberté E (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants*, doi:10.1038/nplants.2015.50.

F.T. and E.L. designed the experiment; F.T., E.L. and G.Z. collected the field data; F.T. performed the experiments; F.T., M.K., M.C. and M.B. performed the laboratory analyses; F.T. and M.K. analysed the data; F.T., M.K., E.L., H.L., M.C., G.Z. and M.B. wrote the manuscript.

Data accessibility

The trimmed sequence reads, the resulting final consensus sequences and all data sets used for statistical analyses or the calculations of phylogenetic trees were deposited in Dryad and are available at http://dx.doi.org/10.5061/dryad.tq0ft (doi:10.5061/dryad.tq0 ft). Other relevant site, soil and vegetation data for the Jurien Bay chronosequence can be accessed at Dryad and Aekos, available at http://datadryad.org/resource/doi:10.5061/dryad.ps320 (Laliberté *et al.* 2014) and http://portal.aekos.org.au/dataset/193192 (Zemunik *et al.* 2015).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Diagram of a typical 10 m × 10 m plot showing the 2 m × 2 m subplots on a given dune along the Jurien Bay dune chronosequence where the roots and soil samples were taken.

Fig. S2 Maximum likelihood phylogenetic tree of all operational taxonomic units (OTUs) detected in roots along the Jurien Bay dune chronosequence.

Fig. S3 Non-parametric multidimensional scaling (NMDS) plot of the root samples with the three main dune types Young (Quindalup), Middle (Spearwood) and Old (Bassendean).

Fig. S4 Operational taxonomic units (OTUs) rarefaction curves from root and soil samples of **a)** the three main dune systems, Young (Quindalup), Middle (Spearwood) and Old (Bassendean) dunes, and **b)** per sampled plot (i.e. sites and see Table 1).

Fig. S5 Operational taxonomic units (OTUs) sampling effort curves of **a)** root and soil samples and **b)** for the three main dune systems, Young (Quindalup), Middle (Spearwood) and Old (Bassendean).

Fig. S6 Key soil chemical properties of the Young (Quindalup), Middle (Spearwood) and Old (Bassendean) dunes along the Jurien Bay chronosequence.

Fig. S7 Regression analyses of AMF rarified OTU richness found on all OTUs (roots + soil) and soil total phosphorus at the Jurien Bay dune chronosequence with the three chronosequence stages Quindalup dunes (Young), Spearwood dunes (Middle) and Bassendean dunes (Old)

Table S1 Fusion Primers used for 454 sequencing with the Roche GS Junior system using Lib-A chemistry (Roche, www.454.com).

Table S2 Detailed list of all detected operational taxonomic units (OTUs) for DNA extracted from roots and soil samples along the Jurien Bay dune chronosequence; fungal operational taxonomic units (OTUs) classified according to soil age including the Quindalup dunes (Young = Y), the Spearwood dunes (Middle = M) and the Bassendean dunes (Old = O).

Table S3 Phylogenetic diversity (PD), net relatedness index (NRI), and nearest taxon index (NTI) calculated with PHYLOCOM (Faith 1992; Webb *et al.* 2008) and QIIME (Caporaso *et al.* 2010) for arbuscular mycorrhizal fungal operation taxonomic units (OTUs) **a)** of the individual soil and root samples for each plot; **b)** of the three different stages (roots and soil together) along the Jurien Bay dune chronosequence.

Table S4 The frequencies (in percentage) of the different OTUs for the 15 plots at the Jurien Bay chronosequence grouped by genera.

Table S5 Analysis of variance (ANOVA) tables and associated Tukey HSD tests for Figs 3 and 7.

Table S6 Summary table of the multiple linear regression analysis **a)** for OTUs found on roots only, and **b)** all OTUs (roots + soil) conducted after variable selection.