MOLECULAR ECOLOGY

Molecular Ecology (2016) **25**, 4919–4929 doi: 10.1111/mec.13778

Changes in ectomycorrhizal fungal community composition and declining diversity along a 2-million-year soil chronosequence

FELIPE E. ALBORNOZ,* FRANÇOIS P. TESTE,*† HANS LAMBERS,* MICHAEL BUNCE,‡ DÁITHÍ C. MURRAY,‡ NICOLE E. WHITE‡ and ETIENNE LALIBERTÉ*§

**School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA 6009, Australia, †Grupo de Estudios Ambientales, IMASL-CONICET, Avenida Ejercito de los Andes 950, San Luis 5700, Argentina, ‡Trace and Environmental DNA (TrEnD) Laboratory, Department of Environment and Agriculture, Curtin University, Perth, WA 6102, Australia, §Institut de Recherche en Biologie Végétale, Département de Sciences Biologiques, Université de Montréal, 4101 Sherbrooke Est, Montréal, QC H1X 2B2, Canada*

Abstract

Ectomycorrhizal (ECM) fungal communities covary with host plant communities along soil fertility gradients, yet it is unclear whether this reflects changes in host composition, fungal edaphic specialization or priority effects during fungal community establishment. We grew two co-occurring ECM plant species (to control for host identity) in soils collected along a 2-million-year chronosequence representing a strong soil fertility gradient and used soil manipulations to disentangle the effects of edaphic properties from those due to fungal inoculum. Ectomycorrhizal fungal community composition changed and richness declined with increasing soil age; these changes were linked to pedogenesis-driven shifts in edaphic properties, particularly pH and resin-exchangeable and organic phosphorus. However, when differences in inoculum potential or soil abiotic properties among soil ages were removed while host identity was held constant, differences in ECM fungal communities and richness among chronosequence stages disappeared. Our results show that ECM fungal communities strongly vary during long-term ecosystem development, even within the same hosts. However, these changes could not be attributed to short-term fungal edaphic specialization or differences in fungal inoculum (i.e. density and composition) alone. Rather, they must reflect longer-term ecosystem-level feedback between soil, vegetation and ECM fungi during pedogenesis.

Keywords: chronosequence, ecosystem development, ectomycorrhizas, fungi, high-throughput sequencing, phosphorus

Received 4 January 2016; revision received 29 June 2016; accepted 16 July 2016

Introduction

Major changes in soil properties occur during long-term soil and ecosystem development, with shifts from nitrogen (N) limitation of plant productivity in young soils to phosphorus (P) limitation in old, strongly weathered soils (Walker & Syers 1976; Wardle *et al.* 2004b; Laliberté *et al.* 2012). These changes in soil properties are associated with significant changes in plant community composition (Kitayama & Mueller-Dombois 1995; Richardson *et al.* 2004) and increasing plant diversity (Wardle *et al.* 2008; Laliberté *et al.* 2014; Zemunik *et al.* 2015). In addition, recent studies have highlighted changes in communities of ecologically important root symbionts such as arbuscular mycorrhizal fungi during long-term soil and ecosystem development (Krüger *et al.* 2015; Martínez-García *et al.* 2015). However, less is known about changes in ectomycorrhizal (ECM) fungi during long-term ecosystem development (Dickie *et al.*

Correspondence: Felipe E. Albornoz, E-mail: felipe.albornozramirez@research.uwa.edu.au

© 2016 John Wiley & Sons Ltd

2013), despite ECM fungi playing their important role in plant N and P acquisition (Smith & Read 2008; Smith *et al.* 2015). Understanding how ECM fungal communities change during long-term soil and ecosystem development also has implications for ecosystem functioning, as ECM fungi act as drivers of soil carbon (C) accumulation (Orwin *et al.* 2011; Clemmensen *et al.* 2013; Averill *et al.* 2014).

Soil abiotic properties such as concentrations of N and P and pH affect ECM fungi (Twieg *et al.* 2009; Navarro-Ródenas *et al.* 2012). Fungal communities covary with host plant communities along soil fertility gradients (Davey *et al.* 2015), and host plant communities can control their root-associated fungal communities (Ishida *et al.* 2007; Gao *et al.* 2015; Martínez-García *et al.* 2015). On the other hand, ECM fungal communities may respond in similar, yet independent ways to the same environmental factors driving host plant communities (Toljander *et al.* 2006; Glassman *et al.* 2015; Tedersoo *et al.* 2015). To determine the extent to which soil properties drive ECM fungal communities, it is valuable to study ECM fungal community changes within the same host plant species across a range of soil properties thereby avoiding 'host identity' as a confounding factor (Hart *et al.* 2014; Davey *et al.* 2015).

Host identity and fungal edaphic specialization are not the only drivers of ECM fungal communities along soil fertility gradients; the composition of root-associated ECM fungal communities may reflect stochastic colonizing events during fungal community assembly (Kennedy & Bruns 2005; Kennedy *et al.* 2009; Fukami *et al.* 2010). Such 'priority effects' reflect the fact that the order of arrival of colonizing ECM fungal species can lead to divergent communities due to competition for limited space (e.g. number of root tips), even under similar soil abiotic conditions (Kennedy & Bruns 2005). Priority effects can persist up to a year after inoculation, as shown for wood-decaying fungi (Dickie *et al.* 2012); however, whether these effects can persist to drive ECM fungal communities along fertility gradients or long-term ecosystem development remains unknown. Therefore, to better understand what drives host–symbiont selection during long-term soil and ecosystem development, we need to disentangle the role of inoculum potential from soil abiotic factors driving root colonization by ECM fungi.

In this study, we hypothesized that ECM fungal communities vary in composition with soil age and that changes are driven by soil properties, rather than inoculum potential, specifically soil P availability, which drives plant (Zemunik *et al.* 2015) and arbuscular mycorrhizal fungal communities (Krüger *et al.* 2015) along this chronosequence. Additionally, we hypothesized that ECM fungal richness increases with soil age, as a reflection of the increase in total and ECM host plant diversity across the chronosequence (Zemunik *et al.* 2015). To test these hypotheses, we evaluated soil fertility and soil inoculum effects on the ECM fungal community composition and richness along a 2-million-year dune chronosequence in southwestern Australia. We conducted a glasshouse experiment with two inoculum treatments to disentangle the effects on root-associated ECM fungal communities of edaphic specialization from those due to inoculum potential (i.e. priority effects). Studying changes in ECM fungal communities along soil fertility gradients within single host plant species helped us better understand environmental drivers structuring ECM fungal communities, independent of host identity. Because these different drivers of ECM fungal communities are difficult to disentangle, studies based on experimental manipulations (i.e. modifying soil properties and inoculum potential) are needed to better understand what drives the composition and structure of ECM fungal communities along soil fertility gradients.

Materials and methods

Study area

The Jurien Bay dune chronosequence, located in southwestern Australia (30.29° S, 115.04° E), spans over two million years of pedogenesis over approximately 10 km with no dispersal barriers among stages. The chronosequence has been described in detail elsewhere (Laliberté *et al.* 2012; Hayes *et al.* 2014; Turner & Laliberté 2015). This chronosequence provides an exceptionally strong natural nutrient availability and stoichiometry gradient driven by long-term pedogenesis (Laliberté *et al.* 2014; Turner & Laliberté 2015; Table 1). We used the same classification of five chronosequence stages as in Hayes *et al.* (2014).

Site selection and soil collection. We used all five chronosequence stages to cover the progressive and retrogressive phases of long-term ecosystem development along this chronosequence (Laliberté *et al.* 2012; Table 1). From each chronosequence stage, we randomly selected five plots (10 × 10 m) from the same set of permanent plots used in earlier studies (Hayes *et al.* 2014; Laliberté *et al.* 2014; Turner & Laliberté 2015; Zemunik *et al.* 2015).

We collected soils in November 2013, and in each plot, we collected 5.3 l of bulk soil from the top 30-cm layer from three randomly positioned points around the plot. We also collected 1.6 l of inoculum soil from the top 20-cm layer of soil from seven randomly positioned points. We used these soil depths to match previous work on soil properties and fungi on this

© 2016 John Wiley & Sons Ltd

Table 1 Soil properties and vegetation variables among chronosequence stages. Soil properties are from Turner & Laliberté (2015), and vegetation properties are taken from Zemunik *et al.* (2015)

	Chronosequence stage				
	1	2	3	4	5
Soil properties					
Total N (g/kg)	0.51 ± 0.01c	1.16 ± 0.01a	0.8 ± 0.01b	0.28 ± 0.01d	0.2 ± 0.01e
Total P (mg/kg)	354 ± 2b	416 ± 14a	289 ± 7c	22 ± 2d	6 ± 1e
Resin P (mg/kg)	1.4 ± 0.1ab	1.9 ± 0.4a	1.4 ± 0.2ab	1.1 ± 0.1b	0.5 ± 0.0c
Organic P (% of total P)	0.3 ± 0.2e	1.5 ± 0.4d	3.3 ± 0.6c	21.6 ± 2b	43.5 ± 1a
pH	9.26 ± 0.03a	8.59 ± 0.04b	8.58 ± 0.05b	6.49 ± 0.06c	5.81 ± 0.13d
Exchangeable K ($cmol_c$/kg)	0.03 ± 0.01b	0.04 ± 0.01b	0.04 ± 0.01b	0.07 ± 0.01a	0.02 ± 0.01b
Exchangeable Mn ($cmol_c$/kg)	0 ± 0a	0 ± 0a	0 ± 0a	0.003 ± 0.001b	0.003 ± 0.002b
Vegetation properties					
Total plant cover (%)	36 ± 7c	71 ± 7a	58 ± 4b	65 ± 5ab	52 ± 4b
ECM plant cover (%)	15.0 ± 6abc	24.3 ± 6a	18 ± 3a	12.0 ± 1b	8 ± 1c
ECM plant relative cover (%)	33 ± 10a	31 ± 5a	31 ± 3a	19 ± 1b	19 ± 3b
Total plant richness	17 ± 2d	29 ± 2c	26 ± 1c	39 ± 3b	50 ± 5a
ECM plant richness	3.6 ± 0.6b	5.0 ± 0.8a	5.6 ± 0.6a	5.4 ± 0.4a	5.6 ± 1.4a
Acacia rostellifera cover (%)	8.8 ± 4.2a	10.3 ± 4.6a	7.7 ± 3.2a	11.9 ± 8.7a	1.9 ± 1.7b
Acacia rostellifera relative cover (%)	28.0 ± 13.2a	13.0 ± 5.2a	13.8 ± 6.5a	15.9 ± 11.9a	2.9 ± 2.4b
Melaleuca systena cover (%)	0.4 ± 0.4c	12.3 ± 1.5a	15.2 ± 2.7a	3.2 ± 1.1b	1.8 ± 1.8bc
Melaleuca systena relative cover (%)	1.4 ± 1.4d	18.6 ± 2.6b	25.4 ± 3.0a	5.2 ± 1.7c	4.3 ± 4.3cd

Different letters indicate significant ($P \leq 0.05$) differences among chronosequence stages based on post hoc Tukey tests.

chronosequence (Laliberté *et al.* 2012; Krüger *et al.* 2015; Turner & Laliberté 2015; Teste *et al.* 2016). The collected soils were then mixed and bulked for each plot, keeping bulk and inoculum soil separate.

In June, July and August of 2012, using a subsample of stages (i.e. stages 2, 4 and 5), we also collected root and soil samples following the same approach used by Krüger *et al.* (2015). Description of the methodology used for these samples can be found in Appendix S1 (Supporting information).

Species selection. We selected two plant species that naturally co-occur across the chronosequence (Zemunik *et al.* 2016; Table 1) to control for host plant identity, and, therefore, focus primarily on the effects of soil properties and changes in fungal inoculum on root-associated ECM fungal communities. These two plant species were *Acacia rostellifera* (Fabaceae) and *Melaleuca systena* (Myrtaceae). Both species form arbuscular mycorrhizal and ECM associations and *A. rostellifera* also produce N_2-fixing nodules (Albornoz *et al.* 2016).

Glasshouse experiment

Soil treatments. To distinguish between changes in root-associated ECM fungal communities due to soil abiotic properties and those due to changes in ECM fungal inoculum, we used three soil treatments (Table 2). First, we used unaltered (i.e. unsterilized) fresh bulk soil from each plot (hereafter referred to as 'field soil') to explore possible differences in ECM fungal community composition and diversity that were due to the combined effects of soil properties and ECM fungal inoculum. Second, we triple-pasteurized bulk soil from each plot, followed by inoculation with 7% (v/v) mixed live soil inoculum from all five chronosequence stages (hereafter referred to as 'pooled inoculum'). This second treatment was used to isolate the effects of soil abiotic properties, while exposing the host plant to the entire ECM fungal regional species pool across the whole chronosequence. Finally, we used triple-pasteurized, mixed bulk soil from all five chronosequence stages (i.e. mixed in equal proportions, on a volume basis), but with addition of 7% (v/v) live soil plot-specific inoculum from each specific stage (hereafter referred to as 'specific inoculum'). This third treatment allowed us to determine whether mycorrhizal fungal inoculum (i.e. composition, density) could be an important factor driving responses in ECM fungal community composition, as this treatment maintained abiotic conditions constant. Using small volumes of inoculum soil requires more time for soil biota communities to fully establish (Brinkman *et al.* 2010), whereas larger volumes provokes confounding local nutrient effects and decreases plant growth (van der Putten *et al.* 1988). We used 7% as a compromise to minimize the chances of these two undesirable outcomes. The bulk soil from all plots for the pooled and specific inoculum treatments was sterilized via triple-steam pasteurization at 80 °C for

© 2016 John Wiley & Sons Ltd

Table 2 Summary table of inoculum treatments used in the glasshouse experiment

Inoculum treatment	Bulk soil	Bulk soil sterilization	ECM fungal inoculum	Aim
Field soil	Each plot separately	None	None	Evaluate natural factors
Pooled inoculum	Each plot separately	Triple-pasteurized	All plots mixed in equal volumes	Isolate abiotic effect
Specific inoculum	All plots mixed in equal volumes	Triple-pasteurized	Each plot separately	Isolate inoculum effect

2 h per day over 7 days, following previous studies (Fang *et al.* 2012; Ryan *et al.* 2012).

Plant growth. We germinated *A. rostellifera* and *M. systena* seed in triple-steam-pasteurized sand at 19 °C with a 12-h photoperiod. Twenty days after germination, healthy seedlings were transplanted into soil-filled pots (November 2013). Seedlings were immediately transplanted into pots and watered to 75% field capacity throughout the duration of the experiment. After five (*A. rostellifera*) and seven months (*M. systena*) of growth in the glasshouse, seedlings were harvested by severing shoots from roots. We harvested *A. rostellifera* first because of its higher growth rate than that of *M. systena*. Soil attached to roots was carefully washed away from root systems over a 1-mm sieve, and clean roots were immediately freeze-dried. Equipment was thoroughly washed and surface-sterilized with 70% (v/v) ethanol between each root system harvested. We subsampled and pooled 20 mg of roots of each species from each soil treatment–plot combination. Finally, we pooled both plant species for each treatment–plot combination to maximize the amount of ECM fungal species colonizing roots, while still controlling for host identity for a total of 73 samples; two samples did not contain enough root material as plants were too small (five replicates per combination of treatments).

DNA extraction, amplification and high-throughput sequencing. A total of 40 mg of pulverized root material was extracted using Qiagen's DNeasy Plant Mini Kit with a modified protocol. Several negative controls were used to assess possible environmental contamination. DNA extracts were then quantified via real-time quantitative PCR (qPCR) to assess for quality and quantity of genomic DNA (gDNA) using the primers ITS3-KYO2/ITS4 (Toju *et al.* 2012). Then, the ITS region was amplified and sequenced (in a single PCR) on an Illumina MiSeq system utilizing modified fusion primers with a unique 8-bp multiplex identifier tag (MID-tag) and custom adaptors for paired-end sequencing on both primers.

The pooled amplicons were purified using Agencourt AMPure XP PCR Purification Kit (Beckman Coulter Genomics, NSW, Australia) following the manufacturer's protocol. Purified amplicons were electrophoresed on 2% agarose (w/v) gel and pooled in approximately equimolar ratios based on ethidium-stained band intensity to form a MID-tagged DNA sequencing library. Illumina MiSeq sequencing was performed using a MISEQ Reagent Kit v3 (600 cycles) 300-bp paired-end protocol as per manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). Details on the DNA extraction, amplification and high-throughput sequencing protocol can be found in Appendix S2 (Supporting information).

Bioinformatics

Deconvolution of amplicon sequences into sample batches followed the method described in Murray *et al.* (2015), using GENEIOUS v8.0.2 (Drummond *et al.* 2011). Sequences were then dereplicated at 100% identity across their full length and clustered at a 97% identity threshold using the UPARSE (Edgar 2013) algorithm implemented in USEARCH (Edgar 2010) to identify operational taxonomic units (OTUs). OTUs were mapped against the original amplicon sequence fasta file from which sequences identified as singletons and/or chimerics were removed. An OTU table was subsequently created using USEARCH identifying the presence or absence of each OTU in each sample. Following OTU identification, representative sequences from each OTU were queried against UNITE, UNITE Envir. or annotated INSD data sets with Mega BLAST (Altschul *et al.* 1990) using MassBLASTer with default parameters (Abarenkov *et al.* 2010). Operational taxonomic units with less than 85% similarity to best match and 90% query were removed from the study. Ectomycorrhizal fungal lineages were assigned according to Tedersoo & Smith (2013).

Statistical analyses

Ectomycorrhizal community composition. To visualize differences in composition of ECM fungal communities among chronosequence stages, we calculated the

© 2016 John Wiley & Sons Ltd

Jaccard dissimilarity among samples and used non-metric multidimensional scaling (NMDS). To test for statistical differences in ECM fungal community composition among stages, we used permutational multivariate analysis of variance (PERMANOVA) and, when appropriate, pairwise comparisons between each pair of chronosequence stages and adjusted *P*-values for multiple comparisons. To explore whether shifts in plant composition were related to shifts in ECM fungal community composition, we performed a Procrustes test (Lisboa *et al.* 2014) between vegetation and ECM fungal OTU matrices. Vegetation data from the same plots were obtained from Laliberté *et al.* (2014).

To evaluate whether changes in ECM fungal community composition were related to other factors (i.e. abiotic soil properties and ECM plant diversity and cover), we performed a canonical correspondence analysis (CCA) to test whether community composition differences were related to soil properties or vegetation. Relevant explanatory environmental variables were selected by permutation tests with forward selection. Soil property data from all of our sites were obtained from Turner & Laliberté (2015), and plant species richness and cover were obtained from Zemunik *et al.* (2015).

Ectomycorrhizal fungal richness. To allow meaningful comparisons of fungal OTU richness among samples while standardizing for number of sequences, we compared richness estimates using individual-based rarefaction (Gotelli & Colwell 2001) based on the minimum number of sequences per sample found across all samples (447 and six sequences for the glasshouse experiment and field-collected samples, respectively). Rarefied ECM OTU richness and its relationship with soil properties among chronosequence stages were tested using generalized least squares models. Residuals were inspected visually to check model assumptions. When models did not meet assumptions (i.e. residuals centred around zero and homoscedasticity), appropriate variance structures were specified in a second model, and both models were compared using the Akaike Information Criterion (AIC) and likelihood ratio tests (Zuur *et al.* 2009). When a main term was significant, *post hoc* Tukey tests were performed.

Finally, to test the robustness of our models from the glasshouse experiment, we calculated richness for all fungal OTUs found in the study to ensure that our results were not purely driven by an overall trend in fungal OTU richness across the chronosequence (Fig. S1a–c, Supporting information). Furthermore, as southwestern Australian ECM fungi are poorly studied, we also calculated ECM OTU richness using a more relaxed criterion (i.e. classifying an OTU as ectomycorrhizal if it belonged to an ECM fungal family, or if the reference sequence was isolated from an ectomycorrhiza; Fig. S1b–d, Supporting information). As both models showed the same trend, we only show results using the more conservative 85% similarity with best match. For the field-collected samples, we used 85% similarity with best match. All statistical analyses were conducted in R (R Core Team 2015). Detailed functions and packages used in this study are listed in Appendix S2 (Supporting information).

Results

Ectomycorrhizal OTUs and lineages

We obtained 532 694 ECM fungal sequences across all root samples from all seedlings in our experiment, representing 128 unique ECM OTUs from 15 ECM fungal lineages. In the field soil treatment, the proportion of OTUs from each lineage varied with chronosequence stage (Fig. 1a). Lineages such as /pulvinula, /terfezia-peziza depressa, and /tomentella-thelephora became less frequent in older stages of the chronosequence ($P \leq 0.01$; Table S1, Supporting information), while /marcelleina-peziza gerardii increased in frequency ($P \leq 0.01$; Table S1, Supporting information), and /cenococcum was found only in seedlings grown in soils from stages 4 and 5 (Fig. 1a). By contrast, /tuber-helvella was found in similar abundance in seedlings grown in soils from all chronosequence stages (Fig. 1a). On the other hand, all chronosequence stages from the pooled and specific inoculum treatments were dominated by the same three lineages ($P \geq 0.2$; Fig. 1b, c), with /terfezia-peziza depressa being the most common one, representing around 76% of all OTUs.

Ectomycorrhizal community composition

Ectomycorrhizal fungal community composition strongly covaried with plant community composition across the chronosequence for seedlings grown in field soil (Procrustes correlation = 0.88; $P \leq 0.0001$), but not for ones in the other soil treatments (Procrustes correlation ≤ 0.3; $P = 0.09$). In seedlings grown in the field soil treatment, ECM fungal communities differed significantly in composition among all chronosequence stages (Fig. 2a; $P \leq 0.05$, after Holm correction for pairwise comparisons), except for stages 2 and 3, which were indistinguishable from each other (Fig. 2a). These changes were explained by pedogenesis-driven shifts in abiotic soil properties: pH, organic P, % organic P of total P and exchangeable manganese (Mn) (Fig. 3a; $R^2_{adj} = 0.33$, $P \leq 0.001$). Ectomycorrhizal plant species richness, cover and relative cover were not retained as

© 2016 John Wiley & Sons Ltd

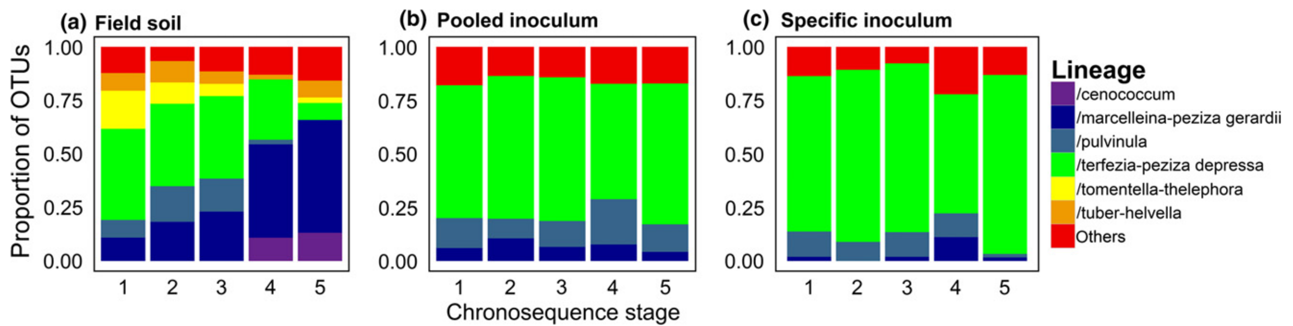

Fig. 1 Proportion of ECM OTUs of all lineages found in roots of *Acacia rostellifera* and *Melaleuca systena* growing in (a) field soil, (b) pooled inoculum or (c) specific inoculum treatments. Lineages with less than three OTUs per chronosequence stage were pooled in 'others'. Each chronosequence stage is represented by five sites.

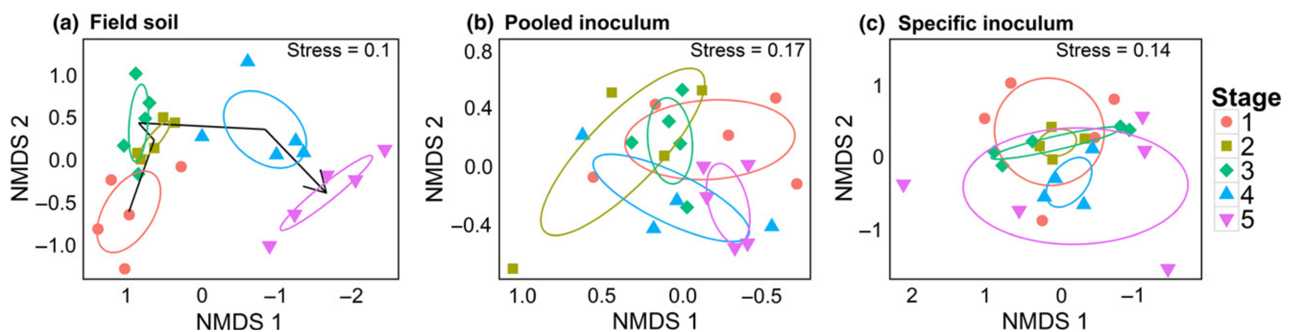

Fig. 2 Nonmetric multidimensional scaling ordination plots showing differences in ECM fungal community composition associated with *Acacia rostellifera* and *Melaleuca systena* growing in soils from all five chronosequence stages for (a) field soil, (b) pooled inoculum treatment and (c) specific inoculum treatment. Ellipses show the 95% confidence intervals around the mean centroid for each chronosequence stage based on Jaccard's dissimilarity scores. Black arrow links centroids and shows the chronological sequence of the chronosequence stages from the youngest (stage 1) to the oldest (stage 5) soils. Statistically significant differences in composition were determined using pairwise comparisons with Holm correction (Holm 1979). For the field soil, composition was different between all pairs of stages ($P \leq 0.001$) except between stages 2 and 3, while there were no differences for the two other treatments.

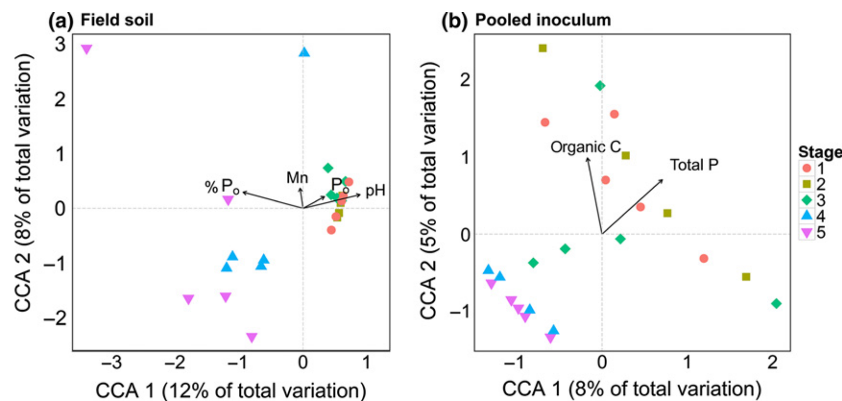

Fig. 3 Canonical correspondence analysis between ECM OTUs community composition on roots from *Acacia rostellifera* and *Melaleuca systena*, and soil properties among the different chronosequence stages between two inoculum treatments. Relevant explanatory environmental variables ($P \leq 0.01$) were selected by permutation test with forward selection using 9999 number of permutations. Organic phosphorus (P_o), percentage of P_o of total P (%P_o), total phosphorus (P), organic carbon (C), exchangeable manganese (Mn) and soil pH were the only variables selected. For the (a) field soil, soil properties explained 33% of total variation ($P \leq 0.001$), while in the (b) pooled inoculum only 13% was explained ($P \leq 0.01$).

© 2016 John Wiley & Sons Ltd

explanatory variables by our variable selection model for all soil treatments.

In contrast to the field soil treatment, there were no significant differences in ECM fungal community composition for seedlings grown in soils from the pooled inoculum and specific inoculum treatments (Fig. 2b, c; $P = 0.13$). However, in the pooled inoculum treatment, differences in ECM fungal community composition could still be partly explained by total P and organic C (Fig. 3b; $R^2_{adj} = 0.13$, $P \leq 0.01$).

Ectomycorrhizal richness

Rarefied ECM OTU richness in seedlings grown in the field soil treatment was lowest in stage 5 and greatest in stage 1 (Fig. 4a; $P \leq 0.04$). Furthermore, ECM OTU richness was strongly associated with a decline in soil pH and resin P (plant-available P), and with an increase in the percentage of total P as organic P, and in exchangeable Mn (Table S5, Supporting information). On the other hand, there were no differences in rarefied ECM OTU richness among chronosequence stages for seedlings grown in soils from the pooled inoculum treatment (Fig. 4b; $P = 0.2$), while in the specific inoculum treatment, rarefied ECM OTU richness was lower in stage 5 compared with stage 2, with no differences among other stages (Fig. 4c; $P \leq 0.05$).

Results from the field-collected samples showed the same trends found in the field soil treatment of the glasshouse experiment (Appendix S3, Supporting information). Indeed, relative abundance of ECM lineages changed with chronosequence stage (Fig. S2a, Supporting information), community composition differed among all chronosequence stages ($P \leq 0.01$; Fig. S2b, Supporting information), and these changes were explained mainly by soil pH and total P (Fig. S2c, Supporting information; $R^2_{adj} = 0.27$, $P \leq 0.001$). Finally, rarefied OTU richness was lower in the oldest stage compared to stage 4 ($P \leq 0.01$; Fig. S2d, Supporting information), but only marginally lower than that of stage 2 ($P = 0.1$; Fig. S2d, Supporting information).

Discussion

Our results show major changes in ECM fungal communities and a decline in richness during long-term soil and ecosystem development, in both the field-collected samples and in the glasshouse where plant host identity was constant. Our results indicate a key role of soil pH and P as drivers of ECM fungal community composition during long-term ecosystem development along this chronosequence. Interestingly, our results also show that changes in ECM fungal communities cannot be independently attributed to short-term fungal edaphic specialization or differences in fungal inoculum, because differences in ECM composition in our glasshouse experiment largely disappeared when seedlings were exposed to the entire fungal species pool or when they were grown under the same abiotic conditions, but exposed to different fungal inoculum. This is likely a result from fungal priority effects when plants have access to a different inoculum pool (Kennedy 2010) or due to an altered competitive balance among ECM fungal species due to soil disturbance (Baar *et al.* 1999; Teste *et al.* 2009). Results from our study suggest that long-term feedback between soil, plants and their root-associated ECM fungi plays a key role in structuring ECM fungal communities along this soil chronosequence.

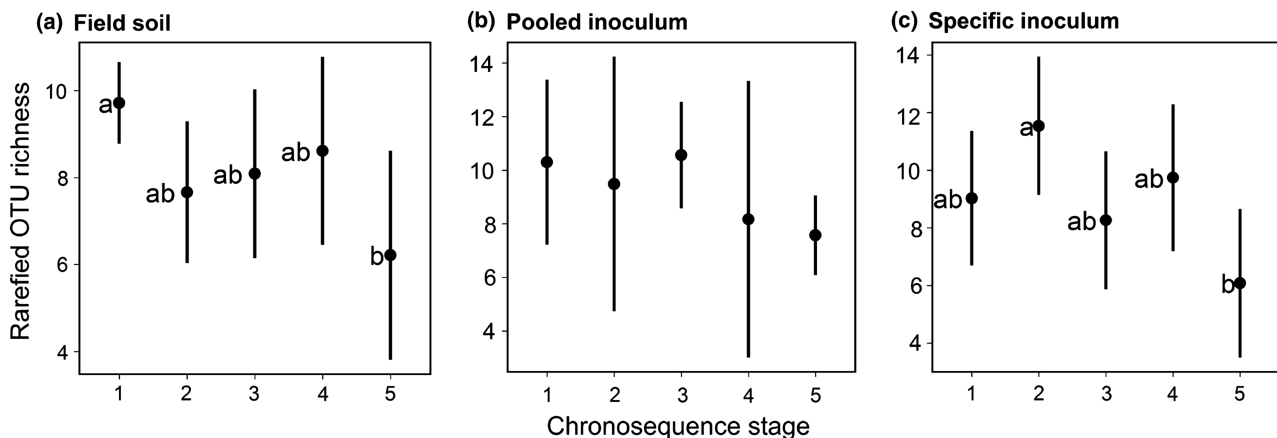

Fig. 4 Rarefied ECM OTU richness from roots of *Acacia rostellifera* and *Melaleuca systena* grown in soils from five chronosequence stages from (a) field soil, (b) pooled inoculum and (c) specific inoculum treatments. Values are means (points) with 95% confidence intervals (bars). Different letters indicate significant ($P \leq 0.05$) differences among soil ages based on *post hoc* Tukey tests.

© 2016 John Wiley & Sons Ltd

Shifts in ectomycorrhizal community composition

The importance of soil abiotic properties in driving changes in ECM fungal communities along a long-term retrogressive chronosequence is consistent with our first hypothesis and with other results from tropical rainforests (Peay *et al.* 2010) and boreal forests (Toljander *et al.* 2006). Indeed, ECM community composition differed strongly among chronosequence stages in both field-collected samples and in the field soil treatment of the glasshouse experiment. Other studies have found that ECM fungal communities vary with soil age along other soil chronosequences (Clemmensen *et al.* 2015; Gao *et al.* 2015). Similar results were obtained for arbuscular mycorrhizas by Martínez-García *et al.* (2015) and Krüger *et al.* (2015). However, Davey *et al.* (2015) found that ECM fungal OTUs follow the same trajectory as its associated vegetation in two short-term glacier chronosequences in Norway. Similarly, composition of ECM fungal communities on seedlings strongly correlated with the composition of whole plant communities along the entire chronosequence in both the field and glasshouse experiment. Host and symbiont communities are presumably strongly interlinked, but this covariation probably results from similar responses of plants and fungi to abiotic soil factors, rather than a direct effect of vegetation composition on fungal communities (Tedersoo *et al.* 2015).

Most ECM fungal lineages showed clear changes among chronosequence stage in the field-collected samples and the field soil treatment. For example, OTUs belonging to the/cenococcum lineage were present solely in the latest stages of the chronosequence, while the/tomentella-thelephora lineage was almost restricted to the youngest stage (absent in stage 4 and only one OTU in stage 5). This is surprising given that *Cenococcum* is thought to be a ubiquitous generalist group (Dickie 2007). Nevertheless, in an alpine forest in southern Germany, Baier *et al.* (2006) found that *Cenococcum* prefers organic soil layers, while *Tomentella* prefers mineral soil horizons, and van der Heijden *et al.* (1999) found in the coastal dunes of the Wadden Isles that the ECM fungal genus *Cenococcum* does not occur in young calcareous soils. Additionally, *Cenococcum* is highly stress-tolerant (Pigott 2006; Fernandez & Koide 2013), which may allow it to persist in these strongly P-impoverished soils. On the other hand, previous studies showed that *Thelephora* species are indicators of young stages of ecosystem development (Jumpponen *et al.* 2002; Dickie *et al.* 2013; Davey *et al.* 2015). These changes in lineages during ecosystem development may be related to the efficiency of acquiring different forms of N and P of each ECM fungal species, given that the composition of N and P forms varies strongly along the chronosequence (Turner & Laliberté 2015). The few discrepancies on the lineages between field-collected samples and glasshouse experiment might be because in the glasshouse we considered only two host plant species, to the low sequencing depth of the 454 sequencing method (field-collected samples; Supporting information), as opposed to Illumina (glasshouse root samples).

A significant fraction of the variation in ECM fungal community with soil age was explained by soil abiotic factors, accounting for 27 and 33% of the total variation in the field-collected samples and field soil treatment, respectively. Soil pH and total P concentration were the strongest drivers for the field-collected samples, while soil pH, organic P concentration and [Mn] were the strongest drivers in field soil treatment. These results support our first hypothesis that ECM fungal communities are partly driven by soil chemical and nutrient availability, and agree with previous studies showing that soil pH (van der Heijden *et al.* 1999) and P (Roy *et al.* 2013) can be important drivers of ECM fungal communities at the local scale. Other studies also showed that soil N is a key factor determining ECM fungal communities (Lilleskov *et al.* 2002; Cox *et al.* 2010; Sterkenburg *et al.* 2015). However, those studies have been carried out mostly in systems where plant productivity is limited by N availability, while P becomes the growth-limiting nutrient along the Jurien Bay chronosequence (Laliberté *et al.* 2012; Hayes *et al.* 2014).

When plants were exposed to the entire regional ECM fungal species pool, the differences in ECM fungal community composition among chronosequence stages found in the field soil treatment largely disappeared, likely as a result of short-term colonizing events (i.e. priority effects; Kennedy & Bruns 2005; Dickie *et al.* 2012). Kennedy *et al.* (2009) experimentally showed that for four species of the ECM genus *Rhizopogon* colonizing *Pinus muricata*, the order of arrival (i.e. priority effect) of ECM fungi strongly impacts ECM fungal colonization dynamics. Conversely, when soil abiotic differences were removed (i.e. specific inoculum treatment), the trend found in the field soil treatment was also not found. This suggests that changes in ECM fungal community composition during ecosystem development cannot be independently attributed to differences in soil abiotic properties or inoculum potential. Rather, we surmise that changes in ECM fungal communities reflect long-term ecosystem-level plant–soil–fungal feedback (Wardle *et al.* 2004a, 2008). However, we cannot discount the possibility that our experimental soil manipulations might have favoured certain ECM fungal species. For example, our use of 7% of inoculum by volume would have diluted the amount of inoculum which may have favoured fast-growing fungal species.

© 2016 John Wiley & Sons Ltd

Such potential biases are unavoidable in experimental manipulations to isolate effects of fungal inoculum, because too large of a volume would have confounded abiotic and biotic effects (Brinkman *et al*. 2010).

Decrease in ectomycorrhizal fungal diversity

Our second hypothesis that ECM fungal OTU richness would increase with increasing soil age was rejected despite increases in ECM host plant diversity (Zemunik *et al.* 2015). In fact, the opposite pattern was found in both field-collected samples and in the glasshouse experiment using unaltered field soils. Other studies have found that ECM fungal diversity increases with soil age (Davey *et al.* 2015), but these studies were conducted on postglacial soils with more available P, and where soils contain more organic matter. The decline in ECM OTU richness might be accounted for by strong environmental filtering favouring ECM fungal species that are adapted to the extremely low soil P availability in older soils along the Jurien Bay dune chronosequence (Laliberté *et al*. 2012; Turner & Laliberté 2015).

Conclusions

We show how changes in soil properties during long-term pedogenesis influence ECM fungal community composition and richness, even when host identity is constant. Priority effect appears to drive ECM fungal community composition in the short term (Kennedy & Bruns 2005; Kennedy *et al.* 2009). However, our results suggest that over time, environmental filtering plays a stronger role, and long-term fungal edaphic specialization becomes an important driver. Hence, we surmise that changes in ECM fungal communities during pedogenesis reflect a synergistic effect of edaphic specialization and changes in fungal inoculum resulting from long-term feedback between soil, vegetation and ECM fungi. As such, our study advances recent field-based studies of mycorrhizal communities along long-term retrogressive chronosequences (Clemmensen *et al*. 2015; Martínez-García *et al.* 2015) and addresses a recent call by Dickie *et al.* (2013) to use chronosequences as model systems to better understand plant–fungal–soil feedback throughout ecosystem development. Further studies are required to elucidate the functional roles that ECM fungal species play during long-term ecosystem development, particularly with regard to plant P nutrition in severely P-impoverished soils.

Acknowledgements

We thank James Taylor for technical assistance with DNA extraction and amplification. We also thank Dr. Ian Dickie for providing constructive comments on an earlier draft of our manuscript. Funding was provided by the Australian Research Council (ARC) through a DECRA (DE120100352), Discovery Project (DP130100016) and Hermon Slade grant to EL and a UWA Research and Development Award granted to FPT. We also acknowledge financial support to FEA through CONICYT BECASCHILE/DOCTORADO (72130286), the ANZ Holsworth Wildlife Research Endowment, and the University of Western Australia. We also thank the Pawsey centre for computing time on their high-performance computing infrastructure.

References

Abarenkov K, Tedersoo L, Nilsson RH *et al.* (2010) Plutof- a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics*, **2010**, 189–196.

Albornoz FE, Lambers H, Turner BL, Teste FP, Laliberté E (2016) Shifts in symbiotic associations in plants capable of forming multiple root symbioses across a long-term soil chronosequence. *Ecology and Evolution*, **6**, 2368–2377.

Altschul S, Gish W, Miller W (1990) Basic local alignment search tool. *Journal of Molecular Biology*, **215**, 403–410.

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, **505**, 543–545.

Baar J, Horton TR, Kretzer A, Bruns TD (1999) Mycorrhizal recolonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist*, **143**, 409–418.

Baier R, Ingenhaag J, Blaschke H, Göttlein A, Agere R (2006) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. *Mycorrhiza*, **16**, 197–206.

Brinkman PE, van der Putten WH, Bakker E-J, Verhoeven KJF (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology*, **98**, 1063–1073.

Clemmensen KE, Bahr A, Ovaskainen O *et al.* (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, **339**, 1615–1618.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, **205**, 1525–1536.

Cox F, Barsoum N, Lilleskov EA, Bidartondo MI (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. *Ecology Letters*, **13**, 1103–1113.

Davey M, Blaalid R, Vik U *et al.* (2015) Primary succession of *Bistorta vivipara* (L.) Delabre (Polygonaceae) root associated fungi mirrors plant succession in two glacial chronosequences. *Environmental Microbiology*, **17**, 2777–2790.

Dickie IA (2007) Host preference, niches and fungal diversity. *New Phytologist*, **174**, 230–233.

Dickie IA, Fukami T, Wilkie JP, Allen RB, Buchanan PK (2012) Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. *Ecology Letters*, **15**, 133–141.

© 2016 John Wiley & Sons Ltd

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

Drummond A, Ashton B, Buxton S *et al.* (2011) Geneious.

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

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, **10**, 996–998.

Fang X, You MP, Barbetti MJ (2012) Reduced severity and impact of Fusarium wilt on strawberry by manipulation of soil pH, soil organic amendments and crop rotation. *European Journal of Plant Pathology*, **134**, 619–629.

Fernandez CW, Koide RT (2013) The function of melanin in the ectomycorrhizal fungus *Cenococcum geophilum* under water stress. *Fungal Ecology*, **6**, 479–486.

Fukami T, Dickie IA, Paula Wilkie J *et al.* (2010) Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters*, **13**, 675–684.

Gao C, Zhang Y, Shi N *et al.* (2015) Community assembly of ectomycorrhizal fungi along a subtropical secondary forest succession. *New Phytologist*, **205**, 771–785.

Glassman SI, Peay KG, Talbot JM *et al.* (2015) A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. *New Phytologist*, **205**, 1619–1631.

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Hart MM, Gorzelak M, Ragone D, Murch SJ (2014) Arbuscular mycorrhizal fungal succession in a long-lived perennial. *Botany-Botanique*, **92**, 313–320.

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 EW, De Vries F, Kuyper TW (1999) Mycorrhizal associations of *Salix repens* L. communities in succession of dune ecosystems. I. Above-ground and below-ground views of ectomycorrhizal fungi in relation to soil chemistry. *Canadian Journal of Botany*, **77**, 1821–1832.

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Ishida TA, Nara K, Hogetsu T (2007) Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist*, **174**, 430–440.

Jumpponen A, Trappe JM, Cázares E (2002) Occurrence of ectomycorrhizal fungi on the forefront of retreating Lyman Glacier (Washington, USA) in relation to time since deglaciation. *Mycorrhiza*, **12**, 43–49.

Kennedy P (2010) Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms, and future research directions. *New Phytologist*, **187**, 895–910.

Kennedy PG, Bruns TD (2005) Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. *New Phytologist*, **166**, 631–638.

Kennedy PG, Peay KG, Bruns TD (2009) Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? *Ecology*, **90**, 2098–2107.

Kitayama K, Mueller-Dombois D (1995) Vegetation changes along gradients of long-term soil development in the Hawaiian montane rainforest zone. *Vegetatio*, **120**, 1–20.

Krüger M, Teste FP, Laliberté E *et al.* (2015) The rise and fall of arbuscular mycorrhizal fungal diversity during ecosystem retrogression. *Molecular Ecology*, **24**, 4912–4930.

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

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Below-ground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology*, **83**, 104–115.

Lisboa FJG, Peres-Neto PR, Chaer GM *et al.* (2014) Much beyond mantel: bringing procrustes association metric to the plant and soil ecologist's toolbox. *PLoS One*, **9**, 1–9.

Martínez-García LB, Richardson SJ, Tylianakis JM, Peltzer DA, Dickie IA (2015) Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. *New Phytologist*, **205**, 1565–1576.

Murray DC, Coughland ML, Bunce M (2015) From benchtop to desktop: important considerations when designing amplicon sequencing workflows. *PLoS One*, **10**, e0124671.

Navarro-Ródenas A, Pérez-Gilabert M, Torrente P, Morte A (2012) The role of phosphorus in the ectendomycorrhiza continuum of desert truffle mycorrhizal plants. *Mycorrhiza*, **22**, 565–575.

Orwin KH, Kirschbaum MUF, St John MG, Dickie IA (2011) Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecology Letters*, **14**, 493–502.

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD (2010) Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. *The New Phytologist*, **185**, 529–542.

Pigott C (2006) Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. *New Phytologist*, **92**, 513–517.

van der Putten WH, Van Dijk C, Troelstra SR (1988) Biotic soil factors affecting the growth and development of *Ammophila arenaria*. *Oecologia*, **76**, 313–320.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria URL http://www.R-project.org/.

Richardson SJ, Peltzer DA, Allen RB, McGlone MS, Parfitt RL (2004) Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia*, **139**, 267–276.

Roy M, Rochet J, Manzi S *et al.* (2013) What determines Alnus-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. *New Phytologist*, **198**, 1228–1238.

Ryan MH, Tibbett M, Edmonds-Tibbett T *et al.* (2012) Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell & Environment*, **35**, 2170–2180.

Smith SD, Read DJ (2008) *Mycorrhizal Symbiosis*. Academic Press, London, UK.

© 2016 John Wiley & Sons Ltd

Smith SE, Anderson IC, Smith FA (2015) Mycorrhizal associations and phosphorus acquisition: from cells to ecosystems. In: *Annual Plant Reviews, Phosphorus Metabolism in Plants*(eds Lambers H, Plaxton WC), pp. 409–440. Wiley-Blackwell Publishing, Chichester, UK.

Sterkenburg E, Bahr A, Brandstr M, Clemmensen KE (2015) Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist*, **204**, 1145–1158.

Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, **27**, 83–99.

Tedersoo L, Bahram M, Cajthaml T *et al.* (2015) Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *ISME Journal*, **10**, 346–362.

Teste FP, Simard SW, Durall DM (2009) Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal Ecology*, **2**, 21–30.

Teste FP, Laliberté E, Lambers H *et al.* (2016) Mycorrhizal fungal biomass and scavenging declines in phosphorus-impoverished soils during ecosystem retrogression. *Soil Biology and Biochemistry*, **92**, 119–132.

Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS One*, **7**, e40863.

Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor FS (2006) Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist*, **170**, 873–884.

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.

Twieg BD, Durall DM, Simard SW, Jones MD (2009) Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. *Mycorrhiza*, **19**, 305–316.

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

Wardle DA, Bardgett RD, Klironomos JN *et al.* (2004a) Ecological linkages between aboveground and belowground biota. *Science*, **304**, 1629–1633.

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

Wardle DA, Bardgett RD, Walker LR, Peltzer DA, Lagerström A (2008) The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. *Oikos*, **117**, 93–103.

Zemunik G, Turner BL, Lambers H, Laliberté E (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants*, **1**, 1–4.

Zemunik G, Turner BL, Lambers H, Laliberté E (2016) Increasing plant species diversity and extreme species turnover accompany declining soil fertility along a long-term chronosequence in a biodiversity hotspot. *Journal of Ecology*, **104**, 611–897.

Zuur AF, Leno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY, USA.

F.T. and E.L. designed the experiment. F.T. and F.A. supervised and maintained the experiment. F.A. conducted DNA extraction and amplification, and bioinformatics work with assistance of M.B., D.M. and N.W. F.A. performed all statistical analyses with assistance from E.L. F.A. wrote the manuscript, and all authors contributed to revisions.

Data accessibility

Data have already been made accessible in GenBank. Accession nos are BankIt1865121: KT948386–KT948513 for the sequences obtained from the glasshouse, and BankIt1881078: KU353449–KU353509 for the sequences obtained from the field-collected samples. Dryad doi:10.5061/dryad.kt38r.

Supporting information

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

Appendix S1 Description of collecting method for field-collected roots.

Appendix S2 Detailed description of DNA amplification, sequencing, bioinformatics and statistical functions and packages used.

Appendix S3 Detailed description of results from field-collected samples.

Fig. S1 Comparison of OTU richness and rarefied OTU richness among chronosequence stages between all fungal and only ECM OTUs.

Fig. S2 Summary of results from field-collected samples.

Table S1 Pairwise comparisons among chronosequence stages for each individual ECM lineage for the field soil treatment.

Table S2 Multivariate relationship between soil properties and ECM OTU richness for the field-collected samples.

Table S3 Multivariate relationship between soil properties and ECM OTU richness for the glasshouse experiment.

Table S4 OTU identification, BLAST matches and sequence abundance among samples for the field-collected samples.

Table S5 OTU identification, BLAST matches and sequence abundance among samples for the glasshouse experiment.

© 2016 John Wiley & Sons Ltd