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Contrasting patterns of plant and microbial diversity during long-term ecosystem development

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Abstract

1. Long-term ecosystem development involves changes in plant community composition and diversity associated with pedogenesis and nutrient availability, but comparable changes in soil microbial communities remain poorly understood. In particular, it is unclear whether the diversity of plants and microbes respond to similar abiotic drivers, or become decoupled as resources change over long time scales.

2. We characterised communities of archaea, bacteria, and fungi in soils along a 2-million-year chronosequence of coastal dunes in a biodiversity hotspot in Western Australia. The chronosequence involves marked changes in soil pH and nutrient availability that drive major shifts in plant community composition and diversity as soils age.

3. Patterns of α-diversity for microbial groups differed along the chronosequence. Bacterial α-diversity was greatest in intermediate-aged soils, archaeal diversity was greater in young alkaline or intermediate-aged soils, while fungal α-diversity – like plant diversity – was greatest in old, strongly weathered soils where phosphorus is the limiting nutrient.

4. Changes in microbial community composition along the chronosequence were explained primarily by the long-term decline in soil pH, with a smaller influence of the relative abundance of plant nutrient-acquisition strategies. However, changes between the prokaryote and fungal communities, and between fungal and plant communities, became increasingly decoupled along the chronosequence, demonstrating that the coordination of change in biological communities by abiotic drivers becomes weaker during long-term ecosystem development.

5. Several bacterial taxa, including DA101 (Verrucomicrobia), "*Candidatus* Solibacter" (Acidobacteria) and *Gaiella* (Actinobacteria), were particularly abundant on the oldest dunes, indicating that they are adapted to acquire phosphorus from extremely infertile soils. However, we cannot disentangle the influence of phosphorus from the long-term decline in soil pH along the chronosequence.

6. *Synthesis:* These results provide evidence for contrasting patterns of plant and microbial community composition and α-diversity in response to acidification and nutrient depletion during long-term pedogenesis.

1. INTRODUCTION

Soils harbour a remarkable diversity of microbes and there is currently considerable interest in understanding how environmental properties influence biogeographical patterns below ground. For example, recent studies have described the distributions of bacterial, archaeal, and fungal taxa in soils worldwide (Tedersoo et al., 2014; Thompson et al., 2017; Delgado-Baquerizo et al., 2018), and there is evidence that microbial community composition varies predictably in relation to environmental parameters such as temperature (Zhou et al., 2016), soil pH (Lauber et al., 2009; Rousk et al., 2010) and fertility (Leff et al., 2015). Of these, soil pH is a particularly important constraint on bacterial diversity, leading to lower diversity in both strongly alkaline (pH > 7.5) and acidic (pH < 5) soils (Fierer, 2017). However, patterns of below ground microbial diversity do not respond consistently to the abiotic

environment (Hendershot et al., 2017), indicating that much remains to be learned about drivers of below ground diversity.

A central question is the extent to which soil microbial diversity is related to plant diversity. The strong functional relationships between above and below ground communities suggest that they should be correlated positively (Kardol & Wardle, 2010). Yet the α-diversity of bacteria, fungi, and archaea appear unrelated to plant diversity in grassland ecosystems (Prober et al., 2015), while a study of old-field succession found that plant community change was associated only with the fungal community, because bacterial community change was determined primarily by abiotic conditions, particularly soil pH (Cline & Zak, 2015). The relationship between plant and fungal α-diversity holds worldwide (Tedersoo et al., 2014) and presumably reflects symbiotic relationships (i.e. mycorrhizal fungi) and the direct dependence of fungi on inputs of plant detritus. We might therefore expect links between above and below ground communities to differ for bacteria and fungi in response to variation in abiotic properties during long-term ecosystem development.

Soil chronosequences offer an important opportunity to assess the influence of soil properties on biological diversity. A chronosequence is a series of soils that differ only in the time since the onset of soil formation, with other soil-forming factors (climate, topography, parent material, organisms) remaining relatively constant (Stevens & Walker, 1970). This allows patterns of soil and ecosystem development to be studied using a space-for-time approach (Walker et al., 2010). Long-term pedogenesis involves the gradual acidification of soil and leads to predictable changes in nutrient availability, with a shift from nitrogen limitation on young soils to phosphorus limitation on old soils (Vitousek & Farrington, 1997; Laliberté et al., 2012; Coomes et al., 2013). This shift drives parallel changes in the biomass (Wardle, Walker & Bardgett, 2004) and diversity (Laliberté et al., 2013; Laliberté, Zemunik & Turner, 2014) of associated plant communities. Below ground responses to long-term ecosystem development have been less frequently studied, but bacterial and fungal communities have been shown to change rapidly during the progressive (Cutler, Chaput & van der Gast, 2014; Castle et al., 2016; Roy-Bolduc et al., 2016) and retrogressive (Tarlera et al., 2008; Jangid et al., 2013; Uroz et al., 2014) stages of ecosystem development. In particular, several studies have examined below ground communities along the Franz Josef post-glacial chronosequence, finding differences related to declining soil pH and phosphorus availability that are most strongly linked to plant community change during the

early progressive stage (Williamson, Wardle & Yeates, 2005; Allison et al., 2007; Jangid et al., 2013; Turner et al., 2017).

The Jurien Bay chronosequence in Western Australia provides an opportunity to examine patterns of plant and soil microbial diversity during long-term ecosystem development. The chronosequence is formed of coastal dunes deposited during interglacial marine high-stands since the Early Pleistocene, approximately two-million years ago (Wyrwoll, Turner & Findlater, 2014; Turner & Laliberté, 2015). Nutrient limitation of plant productivity shifts from nitrogen on young soils to phosphorus on old soils (Laliberté et al., 2012), reflected in a marked increase in the diversity of nutrient acquisition strategies in the plant community as soils age (Zemunik et al., 2015). The chronosequence occurs in a global biodiversity hotspot, and plant diversity increases continually throughout the sequence, driven by environmental filtering from the regional species pool as pH declines during long-term pedogenesis (Laliberté, Zemunik & Turner, 2014). Recent studies have characterised changes in the composition and diversity of specific symbiotic microbial groups along the sequence (Krüger et al., 2015; Albornoz et al., 2016; Birnbaum et al., 2018), but there is so far little information on comparable changes in the broader soil microbial community, including non-symbiotic microbes, although there is a shift from bacterial to fungal energy channels in the below ground food web as soils age (Laliberté et al., 2017). Here, we studied bacterial, archaeal, and fungal communities along the Jurien Bay chronosequence. We aimed to quantify overall patterns of soil microbial diversity along the chronosequence and assess the extent to which the diversity of above and below ground communities are coordinated during long-term ecosystem development.

2. MATERIALS AND METHODS

2.1 The Jurien Bay Chronosequence

The two-million-year Jurien Bay chronosequence is located in Western Australia, approximately 200 km north of Perth (30°01’–30°24’ S, 114°57’–115°11’ E). The climate is Mediterranean, with hot, dry summers and cool, moist winters. The mean annual temperature at Jurien Bay is 19°C. Mean annual rainfall is 533 mm, with a six month dry season between October and April. The chronosequence has been described in detail elsewhere, including the soils (Turner & Laliberté, 2015; Turner, Hayes & Laliberté, 2018) and plant communities (Laliberté et al., 2012; Laliberté, Zemunik & Turner, 2014; Zemunik et al., 2015; 2016). The chronosequence is formed of coastal sand deposits formed by Quaternary sea level fluctuations and three main dune systems are recognized, representing dunes

formed during the Holocene (Quindalup dunes), Middle Pleistocene (Spearwood dunes), and Early Pleistocene, or possible late Pliocene (Bassendean dunes) (Kendrick, Wyrwoll & Szabo, 1991; Laliberté et al., 2012; Wyrwoll, Turner & Findlater, 2014). Detailed maps of the plots, dune formations, and associated soils can be found elsewhere (Turner & Laliberté, 2015; Zemunik et al., 2016).

Soils show a clear pattern of pedogenesis along the chronosequence and have been studied in detail (Turner & Laliberté, 2015; Turner, Hayes & Laliberté, 2018). Holocene soils are formed of calcareous sand and are initially strongly alkaline. As the soil acidifies, carbonate is dissolved and leached from the profile, leaving residual quartz sand over a petrocalcic horizon, with iron oxide coatings giving the sand a characteristic yellow colour. As pedogenesis proceeds, iron oxides are leached from the soil by podzolisation, yielding bleached quartz sand many meters deep on the oldest dunes.

Soil development is accompanied by marked changes in nutrient availability. Young soils are relatively high in phosphorus but contain little nitrogen. As pedogenesis proceeds, nitrogen accumulates rapidly (i.e. in hundreds to thousands of years) and then declines, while phosphorus declines continuously, so that old soils contain extremely low phosphorus concentrations, among the lowest reported worldwide (Turner & Laliberté, 2015). Plant productivity therefore appears limited by nitrogen on young soils and by phosphorus on old soils (Laliberté et al., 2012), reflected in marked shifts in foliar nutrients (Hayes et al., 2014) and plant nutrient acquisition strategies (Zemunik et al., 2015) along the chronosequence.

The chronosequence is located in a global biodiversity hotspot known as the Southwest Australian Floristic Region (Myers et al., 2000). The vegetation is Mediterranean low shrubland known as kwongan (Hopper, 2014), dominated by schlerophyllous shrubs and trees with remarkably high species richness and endemism (Lamont, Hopkins & Hnatiuk, 1984). Along the chronosequence, families such as Fabaceae and Myrtaceae are common on younger dune systems, while Proteaceae become more common on older dunes (Zemunik et al., 2016). There is a marked increase in plant species richness with soil age along the chronosequence (Laliberté, Zemunik & Turner, 2014), a pattern consistent with other long-term retrogressive chronosequences worldwide (Laliberté et al., 2013).

2.2 Soil and plant sampling

Soil samples were collected from five replicate 10 × 10 m plots on each of five stages of the chronosequence spanning approximately 2 million years of ecosystem development. In each plot,

samples were taken from five points from the upper 20 cm of the soil using a 5 cm diameter sand auger, and combined into a single composite sample per plot. For microbial community analysis, soils were stored on ice and then frozen within 6 h. Subsamples for physical and chemical analysis were air-dried for 7 d at ambient room temperature.

The plant communities along the chronosequence have been studied in detail (Laliberté, Zemunik & Turner, 2014; Zemunik et al. 2015; Zemunik et al. 2016). Briefly, all vascular plants rooted in each 10 × 10 m plot were recorded and identified to the species level. Canopy cover and number of individuals for each plant species was estimated in seven randomly-located 2 m × 2 m subplots within each 10 m × 10 m plot. The full data set and detailed methodology is available online (doi: 10.4227/05/56AEB32FC11D4).

2.3 Soil analysis

Soil analysis was described previously (Laliberté et al., 2012; Turner & Laliberté, 2015; Turner, Hayes & Laliberté, 2018), although we performed new analyses using the same methods on the samples taken for this study. Briefly, soil pH was determined in both deionized water and 10 mm $CaCl_2$ in a 1:2 soil to solution ratio. Total carbon and nitrogen were determined simultaneously by automated combustion and gas chromatography using a Thermo Flash 1112 elemental analyser (CE Elantech, Lakewood, NJ, USA). Total phosphorus was determined by ignition (550°C, 1 h) and extraction in 1 M H_2SO_4 (16 hours, 1:50 soil to solution ratio) (Walker & Adams, 1958). Exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ (2 h, 1:30 soil to solution ratio) and detection by inductively-coupled plasma optical-emission spectrometry (ICP–OES) with an Optima 7300 DV (Perkin-Elmer Ltd, Shelton, CT, USA) (Hendershot, Lalande & Duquette, 2008). Carbonate was determined by mass loss after acidification (Loeppert & Inskeep, 1996) and organic C was calculated as the difference between total C and $CaCO_3$-C. Readily-exchangeable phosphate (resin phosphorus) was determined by extraction with anion exchange membranes (Turner & Romero, 2009). Total exchangeable bases (TEB) was calculated as the sum of the charge equivalents of Ca, K, Mg and Na; effective cation exchange capacity (ECEC) was calculated as the sum of the charge equivalents of Al, Ca, Fe, K, Mg, Mn and Na; base saturation was calculated by (TEB / ECEC) × 100.

2.4 DNA extraction and sequencing

We used the MoBio Laboratories PowerMax Soil DNA extraction kit to maximize DNA recovery from these sandy soils. Soil (10 g fresh weight) was extracted following the manufacturer's instructions. The final solutions containing DNA were frozen, lyophilized, and stored at –80°C until they were resuspended in 2 mL Tris-EDTA buffer prior to sequencing. Two samples did not amplify well (one sample from each of the two Holocene dunes) and were omitted from further analysis.

Microbial diversity was assessed using high throughput sequencing to characterize the distribution and abundance of soil archaea, bacteria and fungi. For bacteria and archaea we used the V4 hypervariable region of the 16S RNA gene using the 515F–806R primer pair (Caporaso et al., 2011). For fungi, we sequenced the first internal transcribed spacer (ITS1) region of the rRNA operon, using the primer pair ITS1F and ITS2R (White et al., 1990). The primers included all necessary Illumina adapters with barcodes to distinguish samples. The bacterial 806R primer included a 10 bp barcode sequence, while the ITS1F and ITS2R primers each included an 8 bp barcode sequence, allowing us to multiplex multiple samples. PCR reactions were done in triplicate 25 µl reactions with 5Prime 2X Hot Mastermix. Triplicates were pooled and cleaned by excising the amplified bands from a gel and purifying with the QIAQuick Gel Extraction kit (Qiagen). Samples were then concentrated by ethanol precipitation and quantified on a Qubit. Equal quantities of each sample were then pooled together and the resulting libraries (one 16S, one ITS) concentrated with ethanol precipitation. Final library concentrations were estimated using both a BioAnalyzer and qPCR. Libraries were sequenced on paired end 2x250 bp Illumina Miseq runs at the STRI Ecological and Evolutionary Genomics Laboratory.

2.5 Bioinformatics

Sequence data were analyzed using QIIME 1.9.1 (Caporaso et al., 2010a; Caporaso et al., 2010b), primarily using default parameter values. Sequences were initially filtered for q>20 during the split-libraries step. Chimera checking was done using usearch_61 (Edgar, 2010). We used the Greengenes 13_8 (McDonald et al., 2012) and the Unite databases (January 2016 release; Abarenkov et al., 2010) to assign taxonomy for 16S and ITS rRNA sequences respectively. Raw sequences were mapped to phylotypes at the 97% similarity threshold using the 'usearch' classifier. For fungi, we used the 'dynamic' similarity threshold and the BLAST method (Altschul et al., 1990) for assigning taxonomy; all unidentified OTUs and those corresponding to the Kingdom Protista were removed prior to downstream analysis (<4% of total OTUs). Representative sequences, phylotype abundance tables, and corresponding sample information is available online (doi: 10.6084/m9.figshare.7180388).

2.6 Statistical analyses

2.6.1 Alpha diversity

Rarefaction is well suited for comparing richness among samples (Magurran, 2004), because it samples a fixed number of sequences for all samples and therefore controls for differences in sequencing depth. We therefore used rarefied richness as our primary measures of α-diversity of both plant and microbial communities. We also calculated Simpson's 1/D and the Shannon diversity metric, both commonly used for plant communities, although the Shannon metric is least useful for microbial communities because it relies on the assumption that the community has been completely sampled, which is untrue below ground. Finally, we calculated diversity in microbial communities using the Chao-1 estimator, which attempts to account for the many unmeasured rare species that occur belowground (Chao, 1984).

All statistical analyses were done in R (version 3.3.1). The Shannon's and inverse (1/D) Simpson's diversity indices were calculated for the microbial communities using the 'diversity' function from 'vegan'. Richness was estimated with the non-parametric 'chao' estimator and OTU accumulation curves of the mean richness for all stages were calculated from 100 permutations using the 'estaccumR' function from 'vegan'. Richness per plot was rarefied to the minimum number of sequences across all plots of the chronosequence and was calculated with the 'rarefy' vegan function. Differences between stages were assessed by Tukey's Honest Significant Differences tests.

2.6.2 NMDS ordinations

To investigate relationships between soil properties and biological communities, data for each taxonomic group were ordinated by NMDS using the 'metaMDS' function from 'vegan', with the data standardised by the modified Gower distance (Anderson et al., 2006) and logarithmic bases set to 2. Higher bases give less weight to changes in abundance vs. changes in composition: a base 10 puts the same weight for a change in abundance from 10 to 100 as a change in composition from 0 to 1 (Anderson et al. 2006). For microbial communities, dissimilarities were calculated using the lowest taxonomic level (i.e. OTU) using the 'vegdist' function from vegan. Stress values represent a measure of the distortion in the multi-dimensional scaling, with lower stress indicating a better two-dimensional representation.

2.6.3 Redundancy analysis

To assess the influence of soil properties or nutrient acquisition strategies on biological communities we performed a redundancy analysis (RDA) using the 'rda' function from 'vegan', with the response being either the Hellinger-transformed community (ITS or 16S) data or dissimilarities calculated with 'vegdist' after standardization with the modified Gower distance ('logbase' set to 2 or 10). When the modified Gower distance was used, the 'capscale' function was used to perform the redundancy analysis instead of 'rda'. The threshold for the forward selection (adjR2thresh parameter) was the adjusted R^2 from an RDA run with the full set of soil variables ($pHCaCl_2$, total carbon, total nitrogen, total phosphorus, organic carbon, carbonate, resin phosphorus, Al, Ca, Fe, K, Mg, Mn, Na, ECEC). Significance of the model was assessed with the 'permutest' function. Hellinger-transformation and standardization by the modified Gower distance of the ordination data was done using the 'decostand' function from vegan. The constraining variables for the analysis, soil and nutrient-acquisition strategies, were combined and the set was then reduced to a minimal explanatory set by forward and backward selection using 'ordistep' from vegan. Because there were many nutrient-acquisition strategies, some with several strategies in combination, the set of nutrient-acquisition strategies used in the selection process combined these strategies into larger strategy classes, following Zemunik et al. (2015): arbuscular mycorrhizal (but not ectomycorrhizal), ectomycorrhizal (and possibly others), nitrogen-fixing (and possibly others), ericoid mycorrhizal, parasites (and possibly others), other mycorrhizal, non-mycorrhizal (all types).

2.6.4 Procrustes ANOVA and regressions

To compare patterns of change among the 16S (bacteria and archaea), ITS, and vegetation communities we performed Procrustes analyses on the NMDS ordinations. We used the 'procrustes' function from the vegan R package and determined the significance of the transformations with the 'protest' function. To assess whether differences existed between pairwise combinations of the fungal, 16S and vegetation community data across the different sampling regions, we used the Procrustean association metric (PAM) with an analysis of variance (ANOVA) (Lisboa et al., 2014). In essence, this analysis can help detect differences among groups of sites by using the residuals from the Procrustes rotation, so that pairs of points that are further apart have greater residuals and the ordinations are therefore considered less similar or more decoupled. Because Procrustes rotations require the same dimensionality of the two input matrices, and because it is important to have a minimal quantified amount of the variation explained in the source ordinations, the community data sets were first Hellinger-transformed and then ordinated by principal component analysis (PCA). A minimum number of principal components (PCs) to

represent at least 70% of the variation were used from each PCA ordination to form matrices of the same dimension for each community data set.

The residuals from the Procrustes rotation were then used as the response of a linear regression with the chronosequence stages as the predictor variable. Because the residuals of the regression using the 'lm' function exhibited heteroscedasticity, the 'gls' function from 'nlme' was used with a variance function (varExp) to achieve constant variance of the model residuals. Confidence intervals from the model fit were calculated with the 'effect' function from the 'effects' package.

2.6.5 Community differences due to soil or vegetation

The PAM was combined with PCA ordination, in a manner outlined by Lisboa et al. (2014), to detect community differences due to either soil or the associated vegetation. Similar to the ANOVA using the PAM, the community data sets were Hellinger-transformed and ordinated by PCA. The soil and (Hellinger-transformed) vegetation data sets were also ordinated by PCA. From these ordinations, the PC scores (from a minimum subset of all PCs, chosen to explain at least 70% of the variation) were used in Procrustes rotation, with the fungal and 16S communities being compared against the soil and vegetation (four comparisons in total). The residuals from these Procrustes rotations were then assembled into a matrix (sites as columns and rows containing the residuals from each Procrustes rotation) and the matrix was then ordinated by PCA. This technique allows for visual assessment of differences produced by either the soil or vegetation, although no statistical significance can be ascribed due to the limited sample size (4) of the ordination.

2.6.6 Multivariate regression trees

We used multiple regression trees to further investigate the influence of soil properties or nutrient acquisition strategies on variation in the 16S and ITS communities, which allow prediction of site groupings based on binary decisions. The combined set of soil and nutrient-acquisition strategies (determined as described above) was used as the predictor in the 'mvpart' function from the 'mvpart' package. The response was the Hellinger-transformed 16S (bacteria and archaea) or fungal community data. The 'ggdendro' package was used to produce the binary trees with each branch length corresponding to the amount of variation explained.

3. RESULTS

3.1 Soil properties

Soils were alkaline (pH 8.6) on Holocene dunes and slightly to moderately acidic on Middle Pleistocene (pH 6.4) and Early Pleistocene (pH 6.0) dunes (Table 1). Holocene soils contained carbonate, but this was absent from Pleistocene dunes (the carbonate is dissolved and leached from the profile as soils acidify during pedogenesis). Concentrations of organic carbon, nitrogen, phosphorus, and cations declined throughout the sequence. In particular, resin phosphorus declined from 4.38 ± 2.52 mg P kg^{-1} on the youngest Holocene dunes to < 1 mg P kg^{-1} on Pleistocene dunes, while total phosphorus declined from 429 ± 38 mg P kg^{-1} on the youngest Holocene dunes to 5.2 ± 1.2 mg P kg^{-1} on the oldest Pleistocene dunes (Table 1), thus representing some of the lowest phosphorus soils globally. Soils at all stages were sands (> 95% sand) with high base saturation (≥ 99%).

3.2 Microbial community composition

We obtained almost 5 million sequences for 16S and more than 1.5 million sequences for ITS. Mean numbers of sequences, total OTUs, and observed OTUs for each dune system are shown in Table 2. OTU accumulation curves for 16S and ITS communities are shown in Supplementary Fig. 1.

The 16S community (i.e., bacteria and archaea) was dominated by Actinobacteria (24.7–28.9%), Proteobacteria (29.3–33.5%), and Acidobacteria (10.4–15.4%) across all sites, with smaller proportions of Bacteriodetes (4.0–6.2%), Chloroflexi (1.9–3.1%), Gemmatimonadetes (1.3-5.8%), Planctomycetes (5.3–6.9%), and Verrucomicrobia (3.1–7.0%) (Table 3). Other individual phyla accounted for 1% or fewer of the total OTUs. Archaea constituted 0.4–3.9% of total abundance, predominantly in the ammonia-oxidizing genus *Nitrososphaera* (phylum Crenarchaeota).

The fungal community included a considerable proportion of unidentified fungi (21.6–56.4%). Of the identified phyla, the majority were Ascomycota (26.7–54.8%) and Basidiomycota (9.5–22.8%), with Chytridiomycota, Glomeromycota, Rozellomycota, and Zygomycota each accounting for ≤ 3.0% (Table 3).

Microbial taxa showed contrasting patterns during ecosystem development (Fig. 1, Table 3). Of the dominant bacterial taxa, the abundance of Acidobacteria, Alphaproteobacteria, Planctomycetes, and Verrucomicrobia increased significantly along the chronosequence (Kruskal-Wallis, FDR-p < 0.05), while the Archaea, Chloroflexi, Deltaproteobacteria, Gammaproteobacteria and Gemmatimonadetes all

declined (Table 3). However, there was considerable variation among classes within a phylum, and among orders within a class (e.g. for the Proteobacteria; Fig. 1). For fungi, Ascomycota and Basidiomycota increased in abundance along the sequence, while all other phyla declined (Table 3).

A number of bacterial genera were abundant throughout the chronosequence, including *Rodoplanes* (a phototrophic Alphaproteobacteria in the order Rhizobiales), *Mycobacterium* (Actinobacteria), and *Solirubrobacter* (the only genus in the Actinobacteria family Solirubrobactereaceae) (Kim et al., 2007). Common families were the Chitinophagaceae (Bacteriodetes), Pseudonocardiaceae (Actinobacteria), and the Rhodospirillaceae (purple nonsulfur photosynthetic bacteria in the Alphaproteobacteria), the latter being particularly abundant on the oldest soil.

Soil pH and resin phosphorus were correlated strongly in our dataset, so it is difficult to isolate the influence of these variables on individual taxa (Supplementary Fig. 2). However, several bacterial taxa were most abundant in the older, low phosphorus soils. In particular, the genus DA101 (Verrucomicrobia) was the most abundant single genus in the two oldest soils (Fig. 2a). Other genera that were particularly abundant at low phosphorus were "*Candidatus* Solibacter" (Acidobacteria), *Gaiella* (the only genus in the Actinobacteria family Gaiellaceae) (Albuquerque et al., 2011), and "*Candidatus* Koribacter" (Acidobacteria) (Fig. 2a). Both "*Candidatus* Koribacter" and "*Candidatus* Solibacter", which are closely related, were isolated originally from Australian pasture soil (Sait, Hugenholtz & Janssen, 2002). In addition, the relative abundance of a number of families increased markedly on the oldest, low phosphorus soils, including Acetobacteraceae (Alphaproteobacteria), Acidobacteriaceae (Acidobacteria), Caulobacteraceae (Alphaproteobacteria), and Conexibacteraceae (constituting two species of Gram-positive mesophilic members of the Actinobacteria) (Fig. 2b).

The basidiomycete genera *Cortinarius* and *Sebacina* (an ecto/ericoid-mycorrhizal fungus) were the most abundant fungal genera on the oldest soils. While not common (< 0.5% of total abundance), fungi from the phylum Glomeromycota were more diverse and abundant in the younger soils (Fig. 3a), and the OTUs that were detected tended to differ between the younger and older soils (Fig. 3b). The Glomeromycota became much less variable among plots within a stage during later stages of the chronosequence, despite the decreasing proportion of arbuscular mycorrhizal plants along the chronosequence (Zemunik et al., 2015).

Patterns of bacterial taxa associated with symbiotic nitrogen fixation differed markedly along the chronosequence. Of the families in the Proteobacteria that include a number of nitrogen-fixing genera, the Bradyrhizobiaceae varied little along the chronosequence, while the Hyphomicrobiaceae and Burkholderiaceae increased in abundance (Fig. 3c). Two families declined significantly on old soils (Phyllobacteriaceae and Rhizobiaceae). The relative abundance of genus *Frankia* (Actinobacteria) decreased significantly on older soils (Fig. 3c; FDR $p < 0.05$), while *Rhizobium* (in the Alphaproteobacteria) peaked in intermediate-aged soils (not shown).

3.3 Alpha diversity

Patterns of diversity varied between microbial communities along the chronosequence (Fig. 4). Based on rarefied richness, inverse Simpson, and Shannon indices, α-diversity in the fungal community was lowest in young soils and increased continually along the chronosequence. In contrast, α-diversity in the bacterial community followed a unimodal pattern, being initially low in the youngest soils, increasing to a maximum in intermediate-aged soils, and then declining to lowest values in the oldest soils (although differences were not significant for Simpson's 1/D). For archaea, α-diversity by rarefied richness followed a similar pattern to bacteria, reaching a maximum in intermediate-aged soils. However, by other measures (Simpson, Shannon) archaeal diversity declined continually throughout the chronosequence. Diversity patterns according to the Chao-1 metric were similar to those for rarefied richness, with archaeal and bacterial diversity peaking in intermediate-aged soils, and fungal diversity increasing throughout the chronosequence (Table 2).

As in previous broader assessments of the plant community along the chronosequence (Zemunik et al. 2015; 2016), plant diversity increased continuously along the chronosequence (Fig. 4). This was clearest for rarefied richness, but less so for Shannon or Simpson metrics for the subset of plots studied here. For patterns at the family level, of particular note is that the relative abundance of plants forming cluster-roots, predominantly in the family Proteaceae, increased markedly on the oldest dunes (Table 3).

3.4 NMDS ordinations

Ordination of both bacterial and fungal communities revealed a clear separation of the communities in the younger Holocene soils as opposed to the older soils (Fig. 5a,b). In addition, the oldest (Early Pleistocene) soils were distinct from the two Middle Pleistocene soils for both 16S and ITS. For the

412 vegetation community, there was clear separation of sites from each chronosequence stage, including
413 the youngest Middle Pleistocene soils (Fig. 5c).
414
415 **3.5 Redundancy analysis**
416 The minimal set of soil variables and nutrient acquisition strategies selected for the 16S community data
417 (Fig. 6a) explained far more of the variation than for the fungal community data (Fig. 6b). When the 16S
418 community data were Hellinger transformed, the first constrained axis of the ordination explained 49%
419 of the total variation. For the ITS community, the first constrained axis of the ordination explained only
420 18% of the total variation. For both communities, the first axis was associated primarily with soil pH and
421 total N, while the second axis reflected the abundance of plants associated with particular mycorrhizal
422 communities – arbuscular mycorrhizas for the 16S community, and ectomycorrhizas for the ITS
423 community – although the secondary axes explained only a small proportion (5%) of the total variation.
424
425 **3.6 Procrustes rotations**
426 Procrustes rotations of the NMDS ordinations revealed stronger coordination of change in biological
427 communities on the younger dunes (i.e. vector length increased for older dunes), and overall greater
428 similarity of change in 16S and ITS communities than either of the microbial communities with the
429 vegetation community (Supplementary Fig. 3). Thus, the procrustes rotation for the 16S and ITS
430 communities (logbase 10) produced a greater Procrustes correlation (i.e. greater similarity: $r = 0.959$;
431 Supplementary Fig. 3a) than the rotations of the 16S and vegetation communities ($r = 0.859$;
432 Supplementary Fig. 3b), and the fungal and vegetation communities ($r = 0.856$; Supplementary Fig. 3c).
433
434 **3.7 Procrustes ANOVA**
435 Based on the residuals from the Procrustes rotations, we found significant differences ($p < 0.05$) in
436 changes in the vegetation and ITS communities (Fig. 7a), and between the fungal and 16S communities
437 (Fig. 7b) along the chronosequence, but not between the vegetation and 16S communities (Fig. 7c). As
438 shown by the Procrustes errors (Supplementary Fig. 3), communities from the two Holocene stages
439 were better coordinated than those from all other chronosequence stages (i.e. residuals were smaller
440 on Holocene dunes; Fig. 7), demonstrating increasing decoupling between the fungal community and
441 both the bacterial and vegetation communities on the older stages.
442

3.8 Multivariate regression trees

Trees created with the multivariate regression methodology generally had soil variables at selection nodes (Supplementary Fig. 4). The Holocene soils were always in groups by themselves, but the Middle and Early Pleistocene soils were generally spread across several nodes. Total carbon occupied the first order node for the fungal community (i.e. separating soils with and without carbonate), with subsequent nodes formed by soil pH and exchangeable iron. For bacteria, soil pH was the first order node, with a variety of soil properties occupying subsequent nodes.

4. DISCUSSION

Long-term pedogenesis leads to shifts in plant community composition and diversity, but corresponding patterns below ground remain poorly understood. By studying microbial communities in soils spanning two million years of pedogenesis in a global biodiversity hotspot, we find evidence for contrasting patterns of diversity in archaea, bacteria, fungi, and plants during long-term ecosystem development. In particular, trends in α-diversity differed along the chronosequence, peaking in young or intermediate-aged soils for bacteria and archaea, but increasing continuously for fungi and plants. This general pattern is consistent with a recent study showing that soil food webs shift from bacterial- to fungal-dominated along the Jurien Bay sequence (Laliberté et al., 2017). We also find that although abiotic properties appear to drive these patterns of diversity above and below ground, biological communities respond in different ways and become increasingly decoupled as ecosystem development proceeds.

Of the various abiotic changes along the chronosequence, the long-term change in soil pH appears to be the primary driver of community change for both prokaryotes and eukaryotes, although these groups respond to long-term acidification in different ways. Plant diversity increases continuously during ecosystem development along chronosequences worldwide (Laliberté et al., 2013), including the coastal dunes at Jurien Bay (Laliberté, Zemunik & Turner, 2014; Zemunik et al., 2016), which is best explained by long-term acidification and environmental filtering of the regional species pool dominated by plants adapted to old, acidic, infertile soils (Laliberté, Zemunik & Turner, 2014). Similarly, fungal α-diversity increased continuously along the chronosequence, driven primarily by the long-term decline in soil pH. Soil pH is among the strongest abiotic correlates of fungal richness at the global scale (Tedersoo et al., 2014), while a link between the species richness of plant and fungal communities has been reported elsewhere at local, regional, and global scales (Peay, Baraloto & Fine, 2013; Tedersoo et al., 2014; Roy-Bolduc et al., 2016). Soil pH also drives variation in bacterial community composition and diversity

across the globe, with greatest diversity at neutral to slightly acid pH (approximately pH 6 to 7) and a marked reduction under strongly acidic and alkaline conditions (Lauber et al., 2009; Rousk et al., 2010; Fierer, 2017). In agreement with the global pattern, bacterial α-diversity at Jurien Bay showed a unimodal pattern with soil age, increasing on young soils, reaching a maximum on intermediate-aged soils, and then declining to the lowest values on the old, most acidic soils (although these only reached pH 6). Archaeal diversity followed a similar pattern according to the rarefied richness and Chao-1 indices, although it declined continuously for both the inverse Simpson and Shannon metrics. Acidification associated with long-term pedogenesis therefore provides a unifying control on local diversity of plant and microbial communities during long-term ecosystem development, although prokaryotic and eukaryotic communities respond to soil pH change in different ways.

Despite the consistent response of above and below ground communities to long-term change in soil pH, we found evidence for increasing decoupling of biological communities during the older stages of ecosystem development. In other words, prokaryotic and eukaryotic communities change in a coordinated manner during the early stages of ecosystem development in response to declining soil pH, but become increasingly decoupled through time towards the late, retrogressive stages of ecosystem development. A similar decoupling of change in the communities of plants and soil organisms, including invertebrates, was observed recently in long-term retrogressive chronosequences in Australia and New Zealand (Bokhorst et al., 2017). Although several studies have linked patterns of plant and microbial diversity, there is evidence that bacteria and fungi respond to different drivers (Cline & Zak, 2015), and overall associations remain unclear (Fierer & Jackson, 2006; Hendershot et al., 2017). Our results from the Jurien Bay chronosequence therefore suggest that some of this variation might be linked to the developmental stage of the ecosystem. For microbial communities, changes in the prokaryotic and fungal communities became significantly more decoupled in older soils. The same was true for fungal and vegetation communities, despite their similar patterns of α-diversity through time, demonstrating that simple measures of local-scale diversity can mask a decoupling of community change.

In terms of symbiotic fungi, we found the greatest diversity of Glomeromycota in Middle Pleistocene soils, supporting previous findings for arbuscular mycorrhizal fungi along the Jurien Bay chronosequence that used a different locus to specifically target Glomeromycota (Krüger et al., 2015). However, we found that the relative abundance of OTUs from this group was greater in young Holocene soils, where their community composition differed from Pleistocene soils (Fig 3a,b), further supporting a decoupling

of plant and fungal diversity as ecosystem development proceeds. Although the fungal ITS primer we used might lead to poor amplification of the Glomeromycota, there is support for its use in broad studies of variation in the dominant taxa (Lekberg et al. 2018). For ectomycorrhizal fungi, Albornoz et al. (2016) found that species richness of fungi infecting the roots of two co-occurring host plant species declined along the chronosequence. This seems unlikely to be due to a reduction in potential fungal partners given the increasing fungal diversity we detected here with soil age.

We assessed the influence of abiotic parameters on community composition in a variety of ways, including redundancy analysis, regressions, PCA ordinations and multivariate regression trees, none of which identified the large decline in soil phosphorus as a controlling factor. However, we cannot exclude the possibility that changes in soil phosphorus also play a role in driving community change, because strong co-variation between pH and phosphorus at Jurien Bay means that it is difficult to disentangle the effects of these two properties along the chronosequence (Turner & Laliberté, 2015; Laliberté, Zemunik & Turner, 2014; see below). Indeed, given that plants on the oldest soils at Jurien Bay are growing on some of the lowest phosphorus soils in the world, and exhibit many mechanisms that allow them to efficiently acquire and conserve phosphorus (Lambers et al., 2010; Zemunik et al., 2015), it seems likely that many of the abundant microbes on the oldest soils are also well-adapted to the low-phosphorus concentrations in their environment. Indeed, several studies have linked below-ground community composition with fertility (e.g. Leff et al., 2015), including along retrogressive chronosequences of marine terraces (Uroz et al., 2014) and post-glacial surfaces (Jangid et al., 2013). A number of bacterial taxa increased markedly in abundance along the Jurien Bay chronosequence and reached their maximum abundance on the oldest, most strongly weathered soils, indicating that these taxa are likely to be extremely efficient at either acquiring or recycling phosphorus. As noted above, it is difficult to isolate the influence of individual abiotic variables along the chronosequence, because pH and phosphorus co-vary strongly. However, the soil pH reached only slightly to moderately acidic values and varied relatively little among the older stages of the chronosequence (pH 6.0–6.4), while the old soils are among the lowest phosphorus soils in the world (Yang et al., 2013; Turner & Laliberté, 2015). Indeed, several bacteria increased markedly in abundance along the Jurien Bay sequence at resin phosphorus concentrations < 2 mg P kg^{-1}, which is a threshold that drives variation in above and below ground plant and microbial communities in lowland tropical forests (Sheldrake et al., 2017; Turner, Brenes-Arguedas & Condit, 2018; Yao et al., 2018). A role for phosphorus therefore seems likely, although experimental work is required to isolate this from the overall decline in soil pH along the chronosequence.

The Verrucomicrobia have been identified previously as low fertility specialists (Fierer et al., 2013), in particular the DA101 genus. At Jurien Bay, this organism increased markedly along the chronosequence, eventually representing one of the most abundant bacterial OTU on the oldest soils. Its assignment as a low phosphorus specialist is supported by the surprisingly small genome in members of this genus (Brewer et al., 2016), which allows it to conserve phosphorus in particularly infertile environments. In contrast, "*Candidatus* Solibacter usitatus"*,* a species in the genus *Solibacter* that was also abundant on the oldest soils, has a large genome that enables it to obtain a number of potentially limiting resources, including phosphorus, which might also offer an advantage in low nutrient environments (Ward et al., 2009; Challacombe et al., 2011). Indeed, members of the Solibacterales order in general appear to have abundant genes for phosphatase enzyme synthesis and phosphorus solubilization (Bergkemper et al., 2016). Given the vast diversity of phosphatase genes in microbes in low phosphorus soils (Yao et al., 2018), organisms that are abundant on the old soils at Jurien Bay are likely to be particularly efficient at phosphorus acquisition from extremely infertile environments. This provides important novel insight into the ecology of these soil microbes and identifies them as potential targets for efforts to understand biological adaptation to extreme nutrient stress.

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AUTHORS' CONTRIBUTIONS

DATA ACCESSIBILITY

Data available from Figshare: https://doi.org/10.6084/m9.figshare.7180388 (insert reference + citation)

REFERENCES

Abarenkov, K., Henrik, N. R., Larsson, K. H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U. & Kõljalg, U. (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist*, 186, 281-285.

Albornoz, F. E., Teste, F. P., Lambers, H., Bunce, M., Murray, D. C., White, N. E. & Laliberté, E. (2016) Changes in ectomycorrhizal fungal community composition and declining diversity along a 2-million-year soil chronosequence. *Molecular Ecology*, 25, 4919-4929.

Albuquerque, L., França, L., Rainey, F. A., Schumann, P., Nobre, M. F. & da Costa, M. S. (2011) *Gaiella occulta* gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class *Actinobacteria* and proposal of *Gaiellaceae* fam. nov. and *Gaiellales* ord. nov. *Systematic and Applied Microbiology*, 34, 595-599.

Allison, V. J., Condron, L. M., Peltzer, D. A., Richardson, S. J. & Turner, B. L. (2007) Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biology & Biochemistry*, 39, 1770–1781.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403-410.

Bergkemper, F., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M. & Schulz, S. (2016) Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental Microbiology*, 18, 1988-2000.

Birnbaum, C., Bissett, A., Teste, F. P. & Laliberté, E. (2018) Symbiotic N_2-fixer community composition, but not diversity, shifts in nodules of a single host legume across a 2-million-year dune chronosequence. *Microbial Ecology*, 76, 1009-1020.

Brewer, T. E., Handley, K. M., Carini, P., Gilbert, J. A. & Fierer, N. (2016) Genome reduction in an abundant and ubiquitous soil bacterium '*Candidatus* Udaeobacter copiosus'. *Nature Microbiology*, 2, 16198.

Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L. & Knight, R. (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, 26, 266-267.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J.,

Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010b) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7, 335-336.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. & Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108, 4516-4522.

Castle, S. C., Nemergut, D. R., Grandy, A. S., Leff, J. W., Graham, E. B., Hood, E., Schmidt, S. K., Wickings, K. & Cleveland, C. C. (2016) Biogeochemical drivers of microbial community convergence across actively retreating glaciers. *Soil Biology & Biochemistry*, 101, 74-84.

Challacombe, J. F., Eichorst, S. A., Hauser, L., Land, M., Xie, G. & Kuske, C. R. (2011) Biological consequences of ancient gene acquisition and duplication in the large genome of *Candidatus* Solibacter usitatus Ellin6076. *PLOS ONE*, 6, e24882.

Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 11, 265-270.

Cline, L. C. & Zak, D. R. (2015) Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. *Ecology*, 96, 3374-3385.

Coomes, D., Bentley, W., Tanentzap, A. & Burrows, L. (2013) Soil drainage and phosphorus depletion contribute to retrogressive succession along a New Zealand chronosequence. *Plant and Soil*, 367, 77-91.

Cutler, N. A., Chaput, D. L. & van der Gast, C. J. (2014) Long-term changes in soil microbial communities during primary succession. *Soil Biology & Biochemistry*, 69, 359-370.

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K. & Fierer, N. (2018) A global atlas of the dominant bacteria found in soil. *Science*, 359, 320-325.

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

Fierer, N., Ladau, J., Clemente, J. C., Leff, J. W., Owens, S. M., Pollard, K. S., Knight, R., Gilbert, J. A. & McCulley, R. L. (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science*, 342, 621-624.

Fierer, N. (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, 15, 579-590.

Hayes, P., Turner, B. L., 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.

Hendershot, J. N., Read, Q. D., Henning, J. A., Sanders, N. J. & Classen, A. T. (2017) Consistently inconsistent drivers of microbial diversity and abundance at macroecological scales. *Ecology*, 98, 1757-1763.

Hendershot, W. H., Lalande, H. & Duquette, M. (2008) Chapter 18. Ion exchange and exchangeable cations. *Soil Sampling and Methods of Analysis* (eds M.R. Carter & E. Gregorich), pp. 173-178. Canadian Society of Soil Science and CRC Press, Boca Raton, FL.

Hopper, S. D. (2014) Sandplain and Kwongkan: historical spellings, meanings, synonyms, geography and definition. *Plant life on the sandplains in Southwest Australia, a global biodiversity hotspot* (ed. H. Lambers). UWA Publishing, Crawley, Australia.

Jangid, K., Whitman, W. B., Condron, L. M., Turner, B. L. & Williams, M. A. (2013) Soil bacterial community succession during long-term ecosystem development. *Molecular Ecology*, 22, 3415–3424.

Kardol, P. & Wardle, D. A. (2010) How understanding aboveground–belowground linkages can assist restoration ecology. *Trends in Ecology & Evolution*, 25, 670-679.

Kendrick, G. W., Wyrwoll, K.-H. & Szabo, B. J. (1991) Pliocene-Pleistocene coastal events and history along the western margin of Australia. *Quaternary Science Reviews*, 10, 419-439.

Kim, M. K., Na, J.-R., Lee, T.-H., Im, W.-T., Soung, N.-K. & Yang, D.-C. (2007) *Solirubrobacter soli* sp. nov., isolated from soil of a ginseng field. *International Journal of Systematic and Evolutionary Microbiology*, 57, 1453-1455.

Krüger, M., Teste, F. P., Laliberté, E., Lambers, H., Coghlan, M., Zemunik, G. & Bunce, M. (2015) The rise and fall of arbuscular mycorrhizal fungal diversity during ecosystem retrogression. *Molecular Ecology*, 24, 4912-4930.

Laliberté, E., Grace, J. B., Huston, M. A., Lambers, H., Teste, F. P., Turner, B. L. & Wardle, D. A. (2013) How does pedogenesis drive plant diversity? *Trends in Ecology & Evolution*, 28, 331–340.

Laliberté, E., Kardol, P., Didham, R. K., Teste, F. P., Turner, B. L. & Wardle, D. A. (2017) Soil fertility shapes belowground food webs across a regional climate gradient. *Ecology Letters*, 20, 1273-1284.

Laliberté, E., Turner, B. L., Costes, T., Pearse, S. J., Wyrwoll, K.-H., Zemunik, G. & Lambers, H. (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, B. L. (2014) Environmental filtering explains variation in plant diversity along resource gradients. *Science*, 345, 1602-1605.

Lambers, H., Ahmedi, I., Berkowitz, O., Dunne, C., Finnegan, P. M., St J. Hardy, G. E., Jost, R., Laliberté, E., S.J., P. & Teste, F. P. (2013) Phosphorus nutrition of phosphorus-sensitive Australian native plants: threats to plant communities in a global biodiversity hotspot. *Conservation Physiology*, 1, cot010 (2013). doi: 2010.1093/conphys/cot2010.

Lambers, H., Brundrett, M. C., Raven, J. A. & Hopper, S. D. (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil*, 334, 11-31.

Lamont, B. B., Hopkins, A. J. M. & Hnatiuk, R. J. (1984) The flora - composition, diversity and origins. *Kwongan: Plant Life of the Sandplain* (eds J.S. Pate & J.S. Beard), pp. 27–50. University of Western Australia Press, Nedlands, Western Australia.

Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75, 5111-5120.

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J. & Fierer, N. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, 112, 10967-10972.

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

Lisboa, F. J. G., Peres-Neto, P. R., Chaer, G. M., Jesus, E. d. C., Mitchell, R. J., Chapman, S. J. & Berbara, R. L. L. (2014) Much beyond Mantel: Bringing Procrustes association metric to the plant and soil ecologist's roolbox. *PLOS ONE*, 9, e101238.

Loeppert, R. H. & Inskeep, W. P. (1996) Iron. *Methods of Soil Analysis, Part 3 – Chemical Methods* (eds D.L. Sparks & e. al.), pp. 639–664. Soil Science Society of America, Madison, WI.

Magurran, A.E. (2004) Measuring Biological Diversity. Blackwell Science, Oxford, UK.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R. & Hugenholtz, P. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, 6, 610-618.

695 Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. (2000) Biodiversity
696 hotspots for conservation priorities. *Nature*, 403, 853-858.
697 Peay, K. G., Baraloto, C. & Fine, P. V. A. (2013) Strong coupling of plant and fungal community structure
698 across western Amazonian rainforests. *The ISME Journal*, 7, 1852-1861.
699 Prober, S. M., Leff, J. W., Bates, S. T., Borer, E. T., Firn, J., Harpole, W. S., Lind, E. M., Seabloom, E. W.,
700 Adler, P. B., Bakker, J. D., Cleland, E. E., DeCrappeo, N. M., DeLorenze, E., Hagenah, N., Hautier, Y.,
701 Hofmockel, K. S., Kirkman, K. P., Knops, J. M. H., La Pierre, K. J., MacDougall, A. S., McCulley, R. L.,
702 Mitchell, C. E., Risch, A. C., Schuetz, M., Stevens, C. J., Williams, R. J. & Fierer, N. (2015) Plant diversity
703 predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters*,
704 18, 85-95.
705 Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R. & Fierer, N.
706 (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME Journal*, 4,
707 1340-1351.
708 Roy-Bolduc, A., Laliberté, E., Boudreau, S. & Hijri, M. (2016) Strong linkage between plant and soil fungal
709 communities along a successional coastal dune system. *FEMS Microbiology Ecology*, 92, fiw156,
710 https://doi.org/10.1093/femsec/fiw156
711 Sait, M., Hugenholtz, P. & Janssen, P. H. (2002) Cultivation of globally distributed soil bacteria from
712 phylogenetic lineages previously only detected in cultivation-independent surveys. *Environmental*
713 *Microbiology*, 4, 654-666.
714 Shane, M. W. & Lambers, H. (2005) Cluster roots: A curiosity in context. *Plant and Soil*, 274, 101-125.
715 Sheldrake, M., Rosenstock, N. P., Revillini, D., Olsson, P. A., Wright, S. J. & Turner, B. L. (2017) A
716 phosphorus threshold for mycoheterotrophic plants in tropical forests. *Proceedings of the Royal*
717 *Society B: Biological Sciences*, 284.
718 Stevens, P. R. & Walker, T. W. (1970) The chronosequence concept and soil formation. *The Quarterly*
719 *Review of Biology*, 45, 333-350.
720 Tarlera, S., Jangid, K., Ivester, A. H., Whitman, W. B. & Williams, M. A. (2008) Microbial community
721 succession and bacterial diversity in soils during 77 000 years of ecosystem development. *FEMS*
722 *Microbiol Ecology*, 64, 129-140.
723 Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios,
724 A. M., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T.,
725 Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K.,
726 Otsing, E., Nouhra, E., Njouonkou, A. L., Nilsson, R. H., Morgado, L. N., Mayor, J., May, T. W.,

Majuakim, L., Lodge, D. J., Lee, S. S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T. W., Harend, H., Guo, L.-d., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F. Q., Bonito, G., Anslan, S., Abell, S. & Abarenkov, K. (2014) Global diversity and geography of soil fungi. *Science*, 346.

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Zech Xu, Z., Jiang, L., Haroon, M. F., Kanbar, J., Zhu, Q., Jin Song, S., Kosciolek, T., Bokulich, N. A., Lefler, J., Brislawn, C. J., Humphrey, G., Owens, S. M., Hampton-Marcell, J., Berg-Lyons, D., McKenzie, V., Fierer, N., Fuhrman, J. A., Clauset, A., Stevens, R. L., Shade, A., Pollard, K. S., Goodwin, K. D., Jansson, J. K., Gilbert, J. A., Knight, R. & The Earth Microbiome Project, C. (2017) A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 551, 457.

Turner, B. L., Brenes-Arguedas, T. & Condit, R. (2018) Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature*, 555, 367.

Turner, B. L., Hayes, P. E. & Laliberté, E. (2018) A climosequence of chronosequences in southwestern Australia. *European Journal of Soil Science*, 69, 69-85.

Turner, B. L. & 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, B. L. & Romero, T. E. (2009) Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal*, 73, 1972-1979.

Turner, S., Mikutta, R., Meyer-Stüve, S., Guggenberger, G., Schaarschmidt, F., Lazar, C. S., Dohrmann, R. & Schippers, A. (2017) Microbial community dynamics in soil depth profiles over 120,000 years of ecosystem development. *Frontiers in Microbiology*, 8, https://doi.org/10.3389/fmicb.2017.00874

Uroz, S., Tech, J. J., Sawaya, N. A., Frey-Klett, P. & Leveau, J. H. J. (2014) Structure and function of bacterial communities in ageing soils: Insights from the Mendocino ecological staircase. *Soil Biology & Biochemistry*, 69, 265-274.

Vitousek, P. M. & Farrington, H. (1997) Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry*, 37, 63-75.

Walker, L. R., Wardle, D. A., Bardgett, R. D. & Clarkson, B. D. (2010) The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology*, 98, 725-736.

Walker, T. W. & Adams, A. F. R. (1958) Studies on soil organic matter: I. influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. *Soil Science*, 85, 307-318.

Ward, N. L., Challacombe, J. F., Janssen, P. H., Henrissat, B., Coutinho, P. M., Wu, M., Xie, G., Haft, D. H., Sait, M., Badger, J., Barabote, R. D., Bradley, B., Brettin, T. S., Brinkac, L. M., Bruce, D., Creasy, T., Daugherty, S. C., Davidsen, T. M., DeBoy, R. T., Detter, J. C., Dodson, R. J., Durkin, A. S., Ganapathy, A., Gwinn-Giglio, M., Han, C. S., Khouri, H., Kiss, H., Kothari, S. P., Madupu, R., Nelson, K. E., Nelson, W. C., Paulsen, I., Penn, K., Ren, Q., Rosovitz, M. J., Selengut, J. D., Shrivastava, S., Sullivan, S. A., Tapia, R., Thompson, L. S., Watkins, K. L., Yang, Q., Yu, C., Zafar, N., Zhou, L. & Kuske, C. R. (2009) Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology*, 75, 2046-2056.

Wardle, D. A., Walker, L. R. & Bardgett, R. D. (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science*, 305, 509-513.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications* (eds M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White), pp. 315-322. Academic Press, New York.

Williamson, W. M., Wardle, D. A. & Yeates, G. W. (2005) Changes in soil microbial and nematode communities during ecosystem decline across a long-term chronosequence. *Soil Biology & Biochemistry*, 37, 1289-1301.

Wyrwoll, K.-H., Turner, B. L. & Findlater, P. (2014) On the origins, geomorphology and soils or the sandplains of south-western Australia. *Plant Life on the Sandplains in Southwest Australia, a Global Biodiversity Hotspot* (ed. H. Lambers). University of Western Australia, Crawley, Australia.

Yang, X., Post, W. M., Thornton, P. E. & Jain, A. (2013) The distribution of soil phosphorus for global biogeochemical modeling. *Biogeosciences*, 10, 2525-2537.

Yao, Q., Li, Z., Song, Y., Wright, S. J., Guo, X., Tringe, S. G., Tfaily, M. M., Paša-Tolić, L., Hazen, T. C., Turner, B. L., Mayes, M. A. & Pan, C. (2018) Community proteogenomics reveals the systemic impact of phosphorus availability on microbial functions in tropical soil. *Nature Ecology & Evolution*, 2, 499-509.

Zemunik, G., Turner, B. L., Lambers, H. & Laliberté, E. (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants*, 1, 15050.

788 Zemunik, G., Turner, B. L., Lambers, H. & Laliberté, E. (2016) Increasing plant species diversity and
789 extreme species turnover accompany declining soil fertility along a long-term chronosequence in a
790 biodiversity hotspot. *Journal of Ecology*, 104, 792-805.
791 Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., Voordeckers, J. W.,
792 Nostrand, J. D. V., Buzzard, V., Michaletz, S. T., Enquist, B. J., Weiser, M. D., Kaspari, M., Waide, R.,
793 Yang, Y. & Brown, J. H. (2016) Temperature mediates continental-scale diversity of microbes in forest
794 soils. *Nature Communications*, 7, 12083.

Table 1. Soil properties along the Jurien Bay chronosequence. Values are means ± standard deviation of five plots on each chronosequence stage. ECEC, effective cation exchange capacity; NO_3, nitrate. Chronosequence stages are based on the numbering system established previously (Laliberté *et al.* 2012; Turner & Laliberté 2015).

Chronosequence stage	**2**	**3**	**4**	**5**	**6**
Dune formation	**Quindalup**	**Quindalup**	**Spearwood**	**Spearwood**	**Bassendean**
Geological period	**Holocene**	**Holocene**	**Middle Pleistocene**	**Middle Pleistocene**	**Early Pleistocene**
Approximate age (years)	**100-1000**	**6500**	**120,000**	**200-400,000**	**2,000,000**
Soil pH (water)	8.6 ± 0.1	8.6 ± 0.1	6.4 ± 0.1	6.4 ± 0.3	6.0 ± 0.2
Carbonate (%)	77 ± 7	22 ± 11	0	0	0
Organic C (%)	1.87 ± 0.74	1.16 ± 0.25	0.69 ± 0.14	0.40 ± 0.07	0.53 ± 0.22
Total N (%)	0.115 ± 0.014	0.069 ± 0.010	0.026 ± 0.005	0.011 ± 0.003	0.019 ± 0.010
Total P (mg P kg^{-1})	429 ± 38	187 ± 62	20.2 ± 3.1	8.4 ± 2.2	5.2 ± 1.2
Resin P (mg P kg^{-1})	4.38 ± 2.52	1.67 ± 0.17	0.93 ± 0.17	0.53 ± 0.08	0.73 ± 0.09
Extractable NO_3 (mg N kg^{-1})	4.3 ± 1.5	2.7 ± 1.5	0.9 ± 0.4	0.7 ± 0.5	0.8 ± 0.6
ECEC ($cmol_c$ kg^{-1})	11.4 ± 2.7	10.5 ± 1.7	3.3 ± 0.4	1.9 ± 0.2	2.4 ± 0.6

Table 2. Mean number ± standard deviation of sequences per sample, total OTUs found, and α-diversity calculated by observed OTUs and the Chao-1 estimator for five stages of the Jurien Bay chronosequence. Rarefaction levels for α-diversity calculations using QIIME 1.9.1: Archaea 570, bacteria 58,400, fungi 34,000. Diversity values are mean ± standard deviation of four (stages 2 and 3) or five (stages 4-6) replicate plots at each stage. For Observed OTUs and Chao-1, values with the same letter are not significantly different ($p > 0.05$)

Chronosequence stage	**2**	**3**	**4**	**5**	**6**
Dune formation	**Quindalup**	**Quindalup**	**Spearwood**	**Spearwood**	**Bassendean**
Approximate age (years)	**100-1000**	**6500**	**120,000**	**200-400,000**	**2,000,000**
Archaea (16S)					

Mean No. of Seqs	3522 ± 1524	5687 ± 2878	1437 ± 532	1777 ± 718	1084 ± 430
Total OTUs	101	76	101	88	65
Observed OTUs	20 ± 1 [ac]	22 ± 3 [ac]	29 ± 3 [bc]	23 ± 5 [c]	20 ± 4 [abc]
Chao-1	33 ± 6 [ac]	34 ± 5 [ac]	47 ± 5 [bc]	35 ± 5 [c]	27 ± 6 [ac]
Bacteria (16S)					
Mean No. of Seqs	112,393 ± 56,033	137,966 ± 48,435	235,018 ± 64,155	288,403 ± 72,195	248,106 ± 21,909
Total OTUs	20,254	18,285	32,863	34,796	28,925
Observed OTUs	7478 ± 96 [a]	7688 ± 386 [a]	8265 ± 417 [a]	7749 ± 460 [a]	6918 ±487 [a]
Chao-1	12,100 ± 407 [a]	12,980 ± 914 [a]	13,770 ± 939 [a]	13,181 ± 706 [a]	11,397 ± 599 [a]
Fungi (ITS)					
Mean No. of Seqs	64,263 ± 24,092	53,337± 21,320	77,857 ± 13,379	66,885 ± 13422	71,587 ± 19,950
Total OTUs	2335	2406	6173	6314	6849
Observed OTUs	817 ± 90 [a]	926 ± 81 [ab]	1540 ± 373 [ab]	1626 ± 234 [ab]	1805 ± 104 [b]
Chao-1	1064 ± 107 [ab]	1157 ± 118 [b]	2103 ± 474 [ab]	2186 ± 385 [ab]	2389 ± 157 [a]

Table 3. Biological community change along the Jurien Bay chronosequence. For microbes, only abundant phyla are shown (> 0.5% of total OTUs). Values are mean percent relative abundance ± standard deviation of five plots on each chronosequence stage (four plots on the Holocene Quindalup dunes). Mean relative cover (%) is shown for the seven plant families with greatest canopy cover.

Chronosequence stage	**2**	**3**	**4**	**5**	**6**
Dune formation	**Quindalup**	**Quindalup**	**Spearwood**	**Spearwood**	**Bassendean**
Approximate age (years)	**100-1000**	**6500**	**120,000**	**200-400,000**	**2,000,000**
Archaea (16S)					
Crenarchaeota	3.0 ± 0.6	3.9 ± 0.7	0.6 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
Bacteria (16S)					
Acidobacteria	10.4 ± 1.5	13.4 ± 1.0	13.1 ± 1.8	13.8 ± 1.0	15.4 ± 0.7
Actinobacteria	26.6 ± 1.8	26.4 ± 2.5	28.9 ± 3.9	26.0 ± 3.9	24.7 ± 4.7
Bacteroidetes	6.2 ± 0.7	4.1 ± 0.5	5.2 ± 1.1	4.0 ± 1.2	4.0 ± 0.6
Chloroflexi	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.6	2.8 ± 1.0	1.9 ± 0.6
Firmicutes	1.1 ± 0.4	0.9 ± 0.4	1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
Gemmatimonadetes	5.3 ± 0.7	5.8 ± 1.0	2.8 ± 1.2	2.6 ± 1.4	1.3 ± 0.7
Nitrospirae	0.9 ± 0.1	1.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
Planctomycetes	5.8 ±1.1	5.9 ± 0.7	5.3 ± 1.0	6.8 ± 2.3	6.9 ± 1.0
Proteobacteria	32.0 ± 1.2	29.3 ± 1.0	33.5 ± 1.8	31.1 ± 4.5	32.2 ± 2.2
Verrucomicrobia	3.2 ± 0.5	3.1 ± 0.5	5.9 ± 0.9	6.8 ± 0.8	7.0 ± 1.5

WPS-2	<0.1	<0.1	0.1 ± 0.3	0.3 ± 0.1	1.2 ± 0.8
Fungi (ITS)					
Ascomycota	26.7 ± 5.1	40.7 ± 7.7	54.8 ± 11.2	50.9 ± 15.5	39.6 ± 7.8
Basidiomycota	12.9 ± 6.0	9.5 ± 2.2	22.1 ± 8.5	22.9 ± 14.2	17.2 ± 6.8
Chytridiomycota	1.2 ± 1.6	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.3
Glomeromycota	0.4 ± 0.6	0.4 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Zygomycota	2.2 ± 0.5	3.1 ± 2.9	0.7 ± 0.3	2.0 ± 2.9	1.6 ± 1.3
Unidentified	56.4 ± 11.6	45.9 ± 8.1	21.6 ± 12.5	23.4 ± 7.3	40.4 ± 12.7
Plants					
Cyperaceae	1.4 ± 1.4	5.4 ± 2.0	2.8 ± 1.7	14.4 ± 3.5	7.8 ± 3.5
Dilleniaceae	0	0	20.4 ± 16.3	7.4 ± 4.8	4.3 ± 4.7
Fabaceae	13.8 ± 11.8	13.0 ± 10.4	5.5 ± 4.5	3.7 ± 6.0	20.1 ± 13.6
Haemodoraceae	8.2 ± 4.8	6.5 ± 4.2	4.5 ± 1.4	2.3 ± 2.2	4.5 ± 1.4
Myrtaceae	29.1 ± 9.5	29.9 ± 11.9	20.9 ± 13.4	32.6 ± 24.2	19.4 ± 7.0
Proteaceae	0	0	36.3 ± 24.2	28.6 ± 9.5	28.0 ± 14.9
Restionaceae	12.1 ± 8.6	16.3 ± 12.0	4.0 ± 2.5	3.3 ± 1.2	2.2 ± 0.7

Figure 1. Changes in the relative abundance of selected bacterial taxa along the Jurien Bay chronosequence, showing (a) classes within the phylum Proteobacteria, (b) orders within the class Alphaproteobacteria. Values are means ± standard deviation of five plots on each chronosequence stage (four plots on the Holocene dunes). Approximate soil ages are shown in the *x* axis.

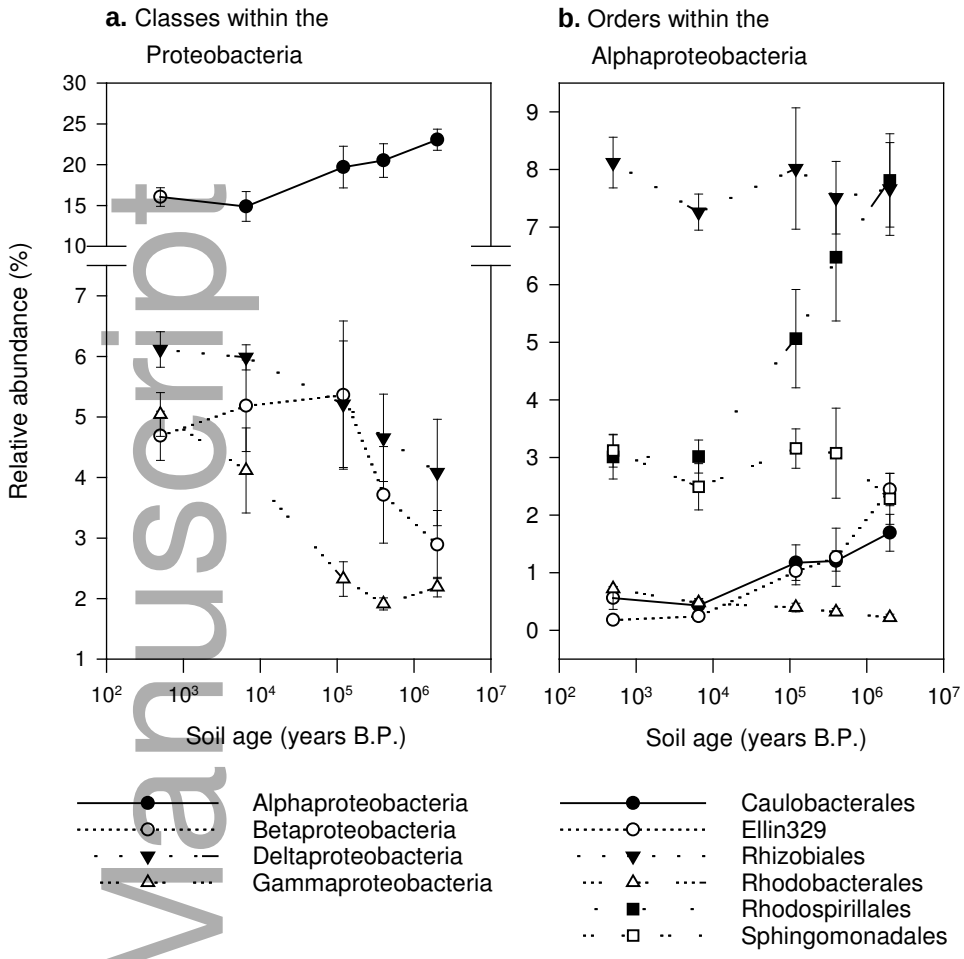

Figure 2. Changes in the relative abundance of (a) genera, and (b) families of bacteria that were more abundant on the old, low phosphorus soils. Values are means ± standard deviation of five plots on each chronosequence stage (four plots on the Holocene dunes).

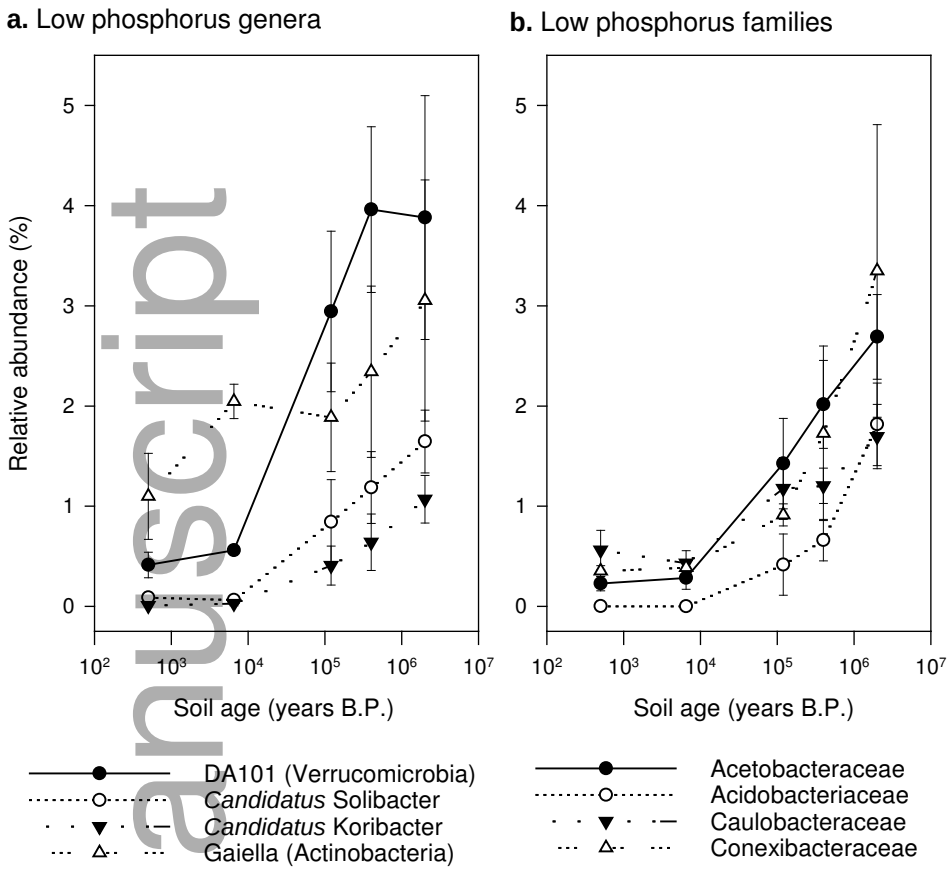

Figure 3. Changes in the relative abundance of (a) total fungal OTUs in the phylum Glomeromycota, (b) two families of fungi in the Glomeromycota exhibiting different patterns along the chronosequence, and (c) families containing bacteria that fix nitrogen. In (c) only families containing a number of nitrogen-fixing genera are shown, in addition to the genus *Frankia*. Values are means ± standard deviation of five plots on each chronosequence stage (four plots on the Holocene dunes).

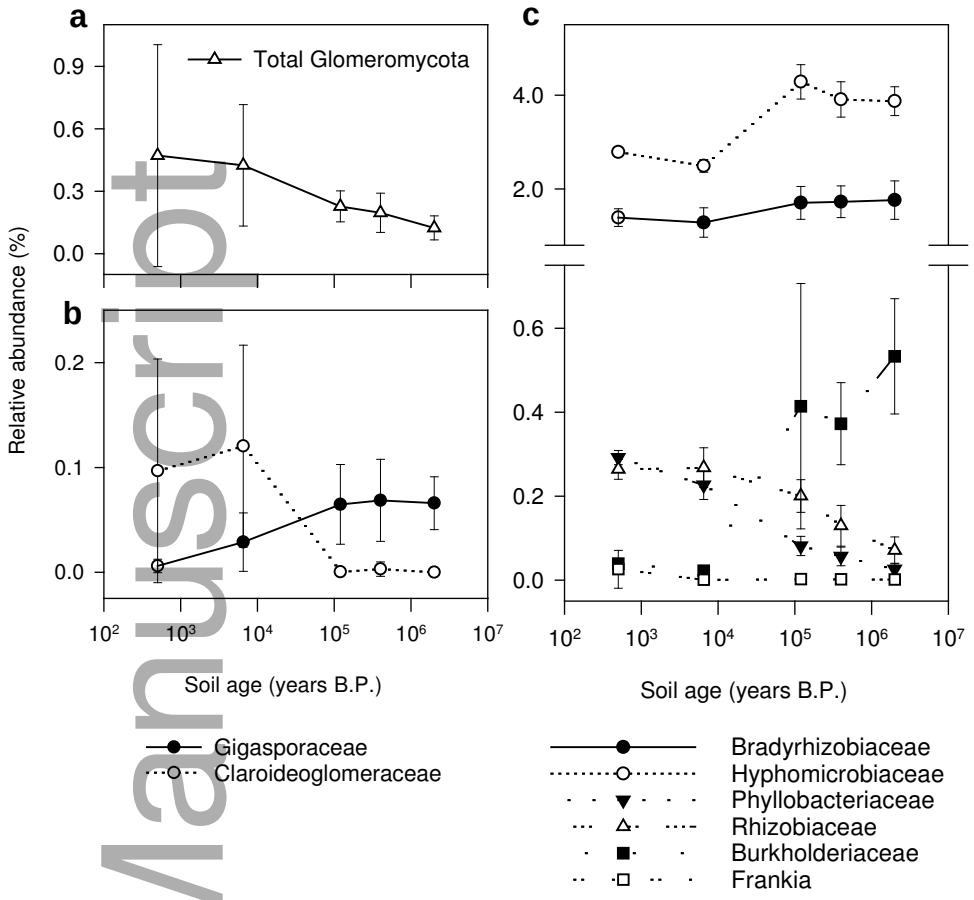

Figure 4. Rarefied richness (a–d), Simpson (1/D) diversity (e–h), and Shannon diversity (i–l) for fungi (a, e, i), bacteria (b, f, j), archaea (c, g, k) and plants (d, h, l) along the Jurien Bay chronosequence. The boxes contain all values within the 25th and 75th percentiles, the whiskers extend to 150% of the interquartile range from the box, and outliers are represented by dots.

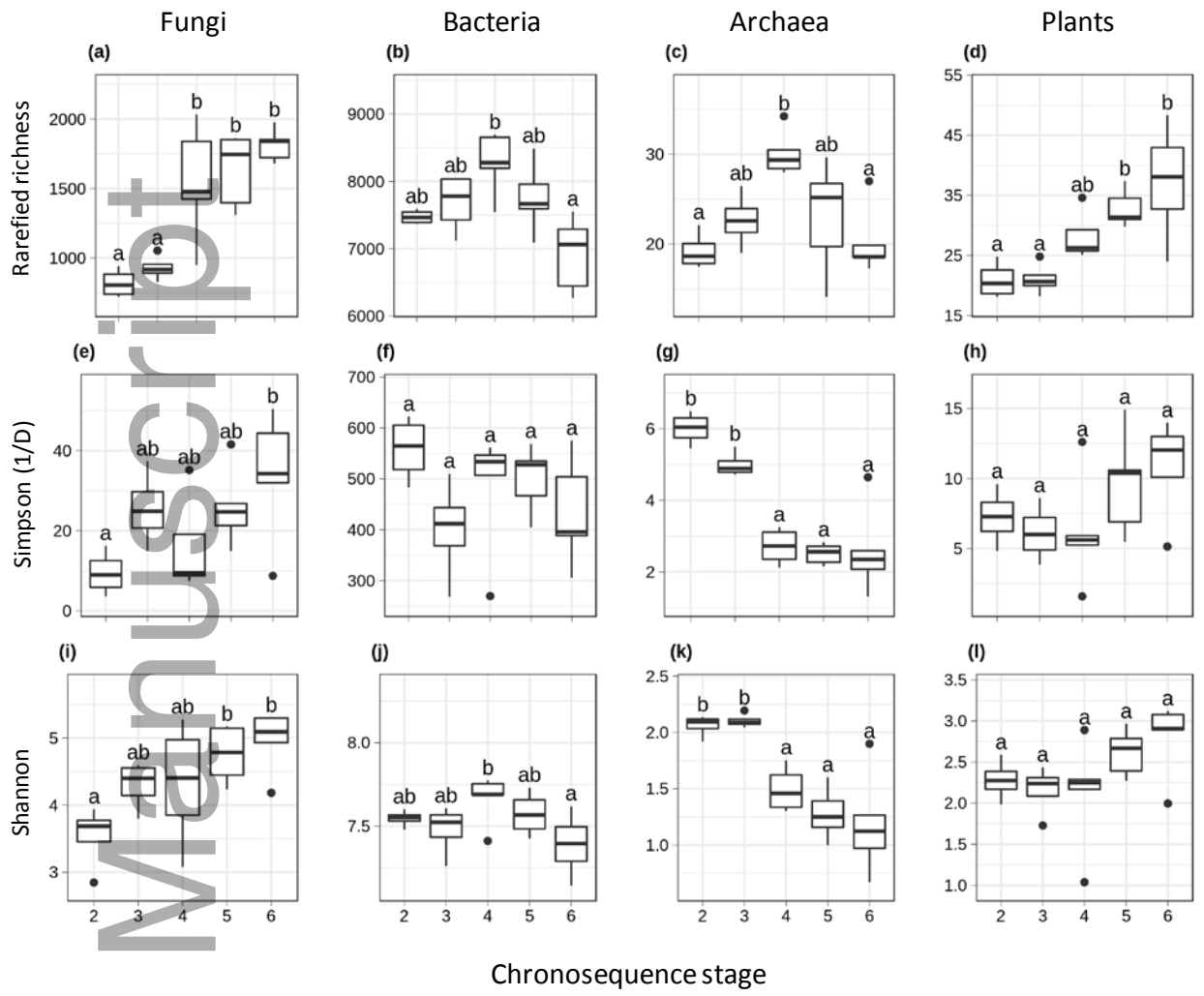

Figure 5. NMDS ordinations of biological communities along the Jurien Bay chronosequence, showing (a) the 16S (bacteria and archaea) community data with logbase of the modified Gower distance set to 2 (stress 0.05), (b), fungal community data with logbase of the modified Gower distance set to 2 (stress 0.11), (c) the vegetation community (relative cover) with logbase of the modified Gower distance set to 2 (stress 0.10).

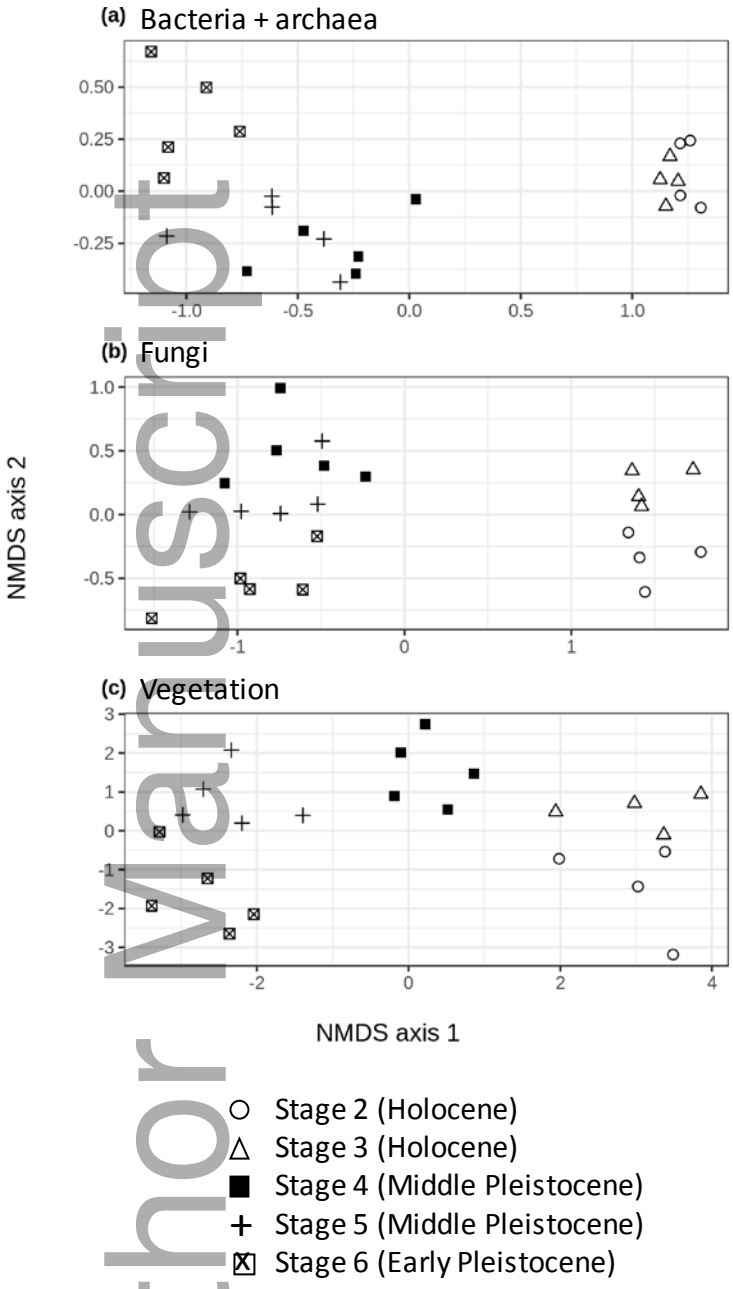

Figure 6. RDA ordination with Hellinger-transformed (a) 16S (bacteria and archaea) and (b) ITS (fungi) community data as the response to a combination of soil variables and nutrient acquisition strategies. Red lines are individual taxa (names omitted for clarity).

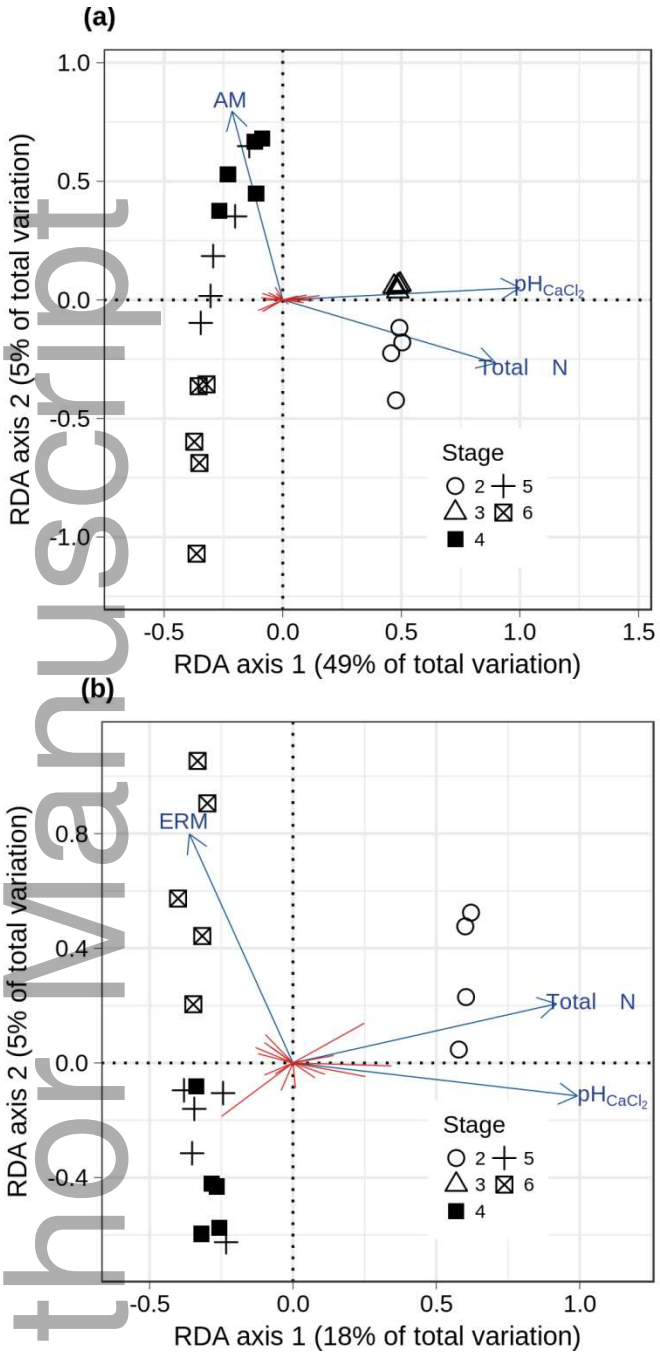

Figure 7. Means and 95% confidence intervals of the Procrustean association metric (PAM; procrustes residuals) against chronosequence stage from (a) the vegetation and ITS community data, (b) the fungal and bacterial community data, and (c) the vegetation and 16S data. In (a) and (b) the procrustes residuals are significantly greater on older soils, indicating increasing decoupling of the communities during long-term ecosystem development.