Mycorrhizal fungal biomass and scavenging declines in phosphorus-impoverished soils during ecosystem retrogression

François P. Teste[1,2], Etienne Laliberté[1,3], Hans Lambers[1], Yasha Auer[4], Susanne Kramer[4], and Ellen Kandeler[4]

Affiliations:

[1]School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA, 6009, Australia

[2]Grupo de Estudios Ambientales, IMASL-CONICET & Universidad Nacional de San Luis, Av. Ejercito de los Andes 950 (5700), San Luis, Argentina

[3]Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, Montréal, QC H1X 2B2, Canada

[4]Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, Emil Wolff Str. 27, 70599 Stuttgart, Germany

Author for correspondence:

François Teste

Email: francois.teste@uwa.edu.au

ABSTRACT

Mycorrhizal fungi enhance plant phosphorus (P) acquisition via their extraradical hyphae (ERH) that scavenge nutrients outside root depletion zones. While soil P availability declines during ecosystem retrogression, how ERH biomass and scavenging vary during ecosystem retrogression remains unknown; it is expected to increase if plants allocate more carbon (C) to mycorrhizal fungi as P availability declines. We measured fungal and bacterial biomass using in-growth cores and lipid biomarkers along a 2-million-year dune chronosequence in an Australian biodiversity hotspot showing a ~60-fold decline in total soil P concentration with increasing soil age. We compared the levels of key fungal biomarkers (ergosterol, NLFA 16:1ω5, and PLFA 18:2ω6,9) between closed, mesh, and open cores during five months (four sampling dates including the wet winter months), thus allowing us to also determine the

dynamics of mycorrhizal fungal scavenging. We found strikingly low and declining biomass of ERH with declining P availability, with minimal long-distance scavenging by ERH. Biomass of ERH was highest in the younger (*c.* 1 ka) soils that were comparatively rich in P and other nutrients. By contrast, the oldest, most P-impoverished soils had the lowest biomass of ERH, despite high mycorrhizal root colonisation, and high abundance and diversity of potential plant hosts. We show that extremely low P availability constrains ERH biomass. Such low mycorrhizal fungal biomass highlights the need for a more 'mycocentric' view of plant-mycorrhizal relationships in old, severely P-impoverished ecosystems.

Keywords: arbuscular mycorrhizal; ecosystem development; ectomycorrhizal; extraradical hyphae; ergosterol; neutral lipid and phospholipid fatty acid (NLFA, PLFA).

1. Introduction

Mycorrhizal fungi form mutualistic associations with plant roots and contribute significantly to plant nutrition by efficiently scavenging poorly-available soil nutrients, especially phosphorus (P) (Smith and Read, 2008). Arbuscular mycorrhizal (AM) fungi colonise roots of the vast majority of plant species (~80%) in most ecosystems (Brundrett, 2009). They improve P uptake (Smith et al., 2015; van der Heijden et al., 2006), and under certain conditions they can also contribute to nitrogen (N) uptake (Hodge et al., 2010; Whiteside et al., 2012). By contrast, ectomycorrhizal (ECM) fungi colonise fewer plant species (mostly woody), but enhance plant acquisition of inorganic and organic forms of N and P and can release carboxylates, explaining their greater carbon (C) costs for the plant compared with that of AM (Cairney, 2011; Smith and Read, 2008). The benefits conferred by mycorrhizal fungi for plant nutrient acquisition are partly attributable to their ability to forage well beyond root depletion zones (Li et al., 1991; Owusu-Bennoah and Wild, 1979). Hence, the most-important component of the mycorrhizal fungi, from a functional perspective, is extraradical hyphae (ERH) exploring the soil matrix (Smith and Read, 2008). Although the ecology of ERH has received some attention (Cairney, 2012; Johnson et al., 2010; Koide and Kabir, 2000), current knowledge is biased towards younger forested ecosystems with relatively high levels of soil P (Vitousek et al., 2010), and where plant growth is generally limited by N rather than P availability (Elser et al., 2007).

Important changes in soil N and P availability occur during long-term (i.e. tens to hundreds of thousands of years) soil and ecosystem development. Plant productivity is initially N-limited on young soils, because most rocks contain negligible amounts of N, and N enters young ecosystems primarily through biological N_2-fixation. By contrast, P becomes increasingly limiting in older soils as its amount and availability decline through time (Laliberté et al., 2012; Vitousek and Farrington, 1997; Walker and Syers, 1976). Eventually, severe P limitation in old, strongly-weathered soils leads to ecosystem retrogression, whereby rates of ecosystem processes such as primary productivity decline (Peltzer et al., 2010; Turner and Condron, 2013; Wardle et al., 2004). These important changes in soil nutrient availability during ecosystem development may also impact the productivity of soil microbiota, but no studies have yet explored how productivity of ERH varies during ecosystem retrogression, despite the great functional importance of ERH for plant nutrition, especially with regard to P.

Long-term soil chronosequences (i.e. soil age gradients) offer great potential to better understand how soil nutrient availability influences the productivity of plant and associated microbiota during ecosystem retrogression (Laliberté et al., 2013b; Peltzer et al., 2010; Walker et al., 2010). This is because soil chronosequences maximise variation in one factor (i.e. soil age and associated changes in nutrient availability) while minimising variation in other important factors such as parent material, climate and topography (Walker et al., 2010). Yet despite their potential as natural soil nutrient availability gradients, well-studied long-term soil chronosequences have rarely been used in studies of mycorrhizal associations (Clemmensen et al., 2015; Dickie et al., 2013; Martínez-García et al., 2014), particularly with regard to functional aspects such as fungal productivity. The production of ERH by mycorrhizal fungi and scavenging is expected to be more prominent in nutrient-poor soils, if plants allocate more carbon (C) to mycorrhizal symbionts under P limitation (Smith and Read, 2008). Yet, these predictions are mostly based on studies conducted in the ecosystems with orders of magnitude higher soil P concentrations than those found in older, strongly-weathered soils, such as those in south-western Australia (Turner and Laliberté, 2015) and some lowland tropical rainforests (Fyllas et al., 2009). Consequently, there is a need for studies of functional aspects of mycorrhizal associations during ecosystem retrogression and associated declines in soil P.

Production of mycorrhizal ERH has been investigated *in situ* using fungal in-growth cores (Hendricks et al., 2006; Wallander, 2006; Wallander et al., 2001). This approach estimates the production of ERH per volume of soil, by including a series of different-size mesh-bags filled with root-free substrate (Wallander et al., 2013). The method relies on the use of fungal-specific biomarkers such as ergosterol and certain phospholipid and neutral-fatty acids (PLFAs and NLFAs) to estimate ECM and AM fungal biomass (Frostegård et al., 2011; Kandeler, 2015; Wallander et al., 2013). Specifically, ergosterol concentrations in soil can represent all soil-borne fungi, whereas the NLFA 16:1ω5 and PLFA 18:2ω6,9 can be used as a proxy to quantify AM and ECM fungal biomass in soils (Kandeler, 2015; Lekberg et al., 2012; Wallander et al., 2013). However, while this method has been successfully used in younger, N-limited ecosystems in the northern hemisphere, we know little about the ecology and functioning of mycorrhizal ERH in old, strongly-weathered soils where P strongly limits plant productivity. Interestingly, the plant communities found on these P-impoverished soils can be very species-rich (Laliberté et al., 2014; Zemunik et al., 2015), such as the biodiverse tropical or Mediterranean ecosystems that contain much of the Earth's plant diversity

(Laliberté et al., 2013a; Myers et al., 2000). Therefore, estimates of the production of ERH are not only needed to better understand ecosystem-level C balance, but could help us better understand the belowground mechanisms maintaining plant diversity in these ecosystems (Laliberté et al., 2013a; Laliberté et al., 2015). For example, only plants hosting mycorrhizal fungi with efficient ERH scavenging strategies may sustain their growth and fitness to remain competitive, since there is a tendency towards giving-up the 'services' of mycorrhizal fungi as exemplified by a plant community more dominated by non-mycorrhizal plants (Lambers et al., 2008; Lambers et al., 2014) and less diverse AM fungal communities in the most impoverished soils (Krüger et al., in press).

Besides ERH productivity and scavenging, we know little about changes in mycorrhiza-associated soil bacteria during ecosystem retrogression. Soil fungal and bacterial communities interact intensively in a fungal-soil interface, called the mycorrhizosphere, where sharp gradients of nutrients occur (Johannsson et al., 2004). Movements of C from plant roots or litter into the mycorrhizosphere represent the main energy source of mycorrhiza-associated bacterial communities. Thus, it is expected that productivity in plant and belowground communities are correlated, unless the productivity of belowground communities is limited more strongly by soil nutrient availability outside the mycorrhizosphere than by C availability. Bacteria-fungi interactions in the mycorrhizosphere can modify P dynamics in soils (Richardson et al., 2011; Warmink and van Elsas, 2008); therefore, these interactions could have important implications for plant nutrient acquisition. There remain some fundamental questions concerning the role of these bacteria in relation to ERH productivity in P-impoverished soils that need to be addressed. For example, are ERH and bacterial biomass correlated or are bacterial communities more responsive to the presence of roots regardless of scavenging ERH?

The overall aim of this study was to better understand the functional ecology of mycorrhizal fungi (e.g., ERH biomass and scavenging) and associated bacteria during ecosystem retrogression. To achieve this, we set-up a field experiment with fungal in-growth cores in a hyperdiverse Mediterranean region in south-western Australia (Hopper and Gioia, 2004) along a well-studied, retrogressive 2 million-year old coastal dune chronosequence (Hayes et al., 2014; Laliberté et al., 2014; Turner and Laliberté, 2015) showing strong and clear shifts from N to P limitation of plant growth with increasing soil age. Specifically, we aimed to: i) quantify mycorrhizal root colonisation and ERH biomass; ii) quantify the extent of

mycorrhizal fungal scavenging ability via fungal in-growth cores; iii) relate the soil nutrient availabilities to the observed mycorrhizal fungal and bacterial biomass; and iv) determine the impact of soil nutrient availability on fungal and bacterial community composition, using lipid biomarkers. Our main hypothesis was that there would be the greatest ERH biomass and scavenging in the oldest, most-severely P-impoverished soils, if plants allocate more C to mycorrhizal fungi under increasingly severe P limitation.

2. Materials and Methods

2.1. Study system

We conducted the study along the Jurien Bay >2-million year dune chronosequence (Laliberté et al., 2012; Laliberté et al., 2013b; Turner and Laliberté, 2015). The Jurien Bay dune chronosequence is located in south-western Australia (~200 km north of Perth), one of the world's 25 biodiversity hotspots (Hopper and Gioia, 2004; Myers et al., 2000). The chronosequence runs roughly parallel to the coast, is approximately 15 km wide, and comprises a series of three main dune systems of marine origin (McArthur and Bettenay, 1974; Turgay and Nonaka, 2002; Wyrwoll and King, 1984). This chronosequence forms an exceptionally strong soil nutrient availability gradient (Laliberté et al., 2012; Turner and Laliberté, 2015), and shows shifts from N to P limitation of plant growth (Hayes et al., 2014; Laliberté et al., 2012) that are expected to occur during long-term pedogenesis (Peltzer et al., 2010; Vitousek and Farrington, 1997; Wardle et al., 2004). The climate is Mediterranean (hot, dry summers and wet, cool winters) with annual precipitation of ~570 mm, of which about 80% falls between May and September. There is no significant precipitation gradient over the entire 15-km chronosequence. Annual average temperature is 25 °C (Australian Bureau of Meteorology, www.bom.gov.au/climate/data). Vegetation is characterised by shrublands with high levels of woody plant diversity at all spatial scales (Laliberté et al., 2014; Zemunik et al., 2015). Fire is the main disturbance to vegetation, with fire-return intervals <30 years (Enright et al., 2005; Griffin and Burbidge, 1990).

2.2. Selection of chronosequence stages

For quantification of ERH biomass, we selected the three most distinct chronosequence stages, in terms of soil nutrient availability, that have been described and used in previous studies (Laliberté et al., 2014; Turner and Laliberté, 2015; Zemunik et al., 2015), out of a total of six dune stages that were outlined. These three distinct stages differ strongly in age, soil nutrient availability, and the strength and type of nutrient limitation (Table 1), and together

represent the retrogressive phase of ecosystem development along this sequence (Laliberté et al., 2012; Turner and Laliberté, 2015). A comprehensive overview of soil characteristics for the entire chronosequence can be found in Turner and Laliberté (2015). Details on plot selection and delineation of the chronosequence stages are given elsewhere (Laliberté et al., 2014; Turner and Laliberté, 2015; Zemunik et al., 2015) and overall rationale for using the Jurien Bay chronosequence, these three stages, and five plots per stage is presented in the Appendix A (Notes S2).

2.3. Flora surveys

Comprehensive flora surveys were conducted on 10 permanent plots per chronosequence stage (each plot = 10 m × 10 m), between August 2011 and March 2012, as described by Laliberté et al. (2014) and Zemunik et al. (2015). In brief, we estimated canopy cover and number of individuals for each plant species within seven randomly-located 2 m × 2 m subplots within each larger 10 m × 10 m plot. Within each subplot, all vascular plants were identified to species level (whenever possible) and counted.

2.4. Root sampling for mycorrhizal colonisation and frequency of cluster roots

All live roots (estimated visually, based on colour and turgescence) were retrieved from the surface soil samples (0-20 cm depth) collected from each 2 m × 2 m subplot after sieving through a 2-mm screen. We collected and bulked roots at the 10 m × 10 m plot level to determine the overall levels (i.e. community-level) of colonisation by AM and ECM fungi, and the frequency of cluster roots. Mycorrhizal colonisation of the bulked root samples was quantified following a vinegar and ink clearing and staining protocol (Vierheilig et al., 1998) optimised for Australian native plants (Teste et al., 2014). Mycorrhizal colonisation of roots was quantified for all six chronosequence stages. Cluster-root frequency and AM fungi root colonisation were determined using the gridline intersect method (Giovannetti and Mosse, 1980). We considered a cluster root as any live root fragment that had >50 rootlets. For ECM fungi root colonisation, we scored all viable root tips as ECM or non-mycorrhizal (Teste et al., 2006).

2.5. Fungal in-growth cores

In April 2012, we randomly selected five replicate plots in each of the three stages described in Table 1 (out of a total of 10 plots per stage). Between April 19 and 24, 2012, fungal in-growth cores Wallander et al. (2001) were installed on the edge of each 2 m × 2 m subplot. A

cardinal direction (N, S, W, E) was randomly assigned to each subplot, and the order of the cores was randomly assigned along the edge of the subplots (Fig. S1, S2). The cores (depth = 20 cm; diameter = 7.5 cm) were made of either: (i) 50 µm nylon mesh bags (mesh with 100 µm thickness) or (ii) polyvinyl chloride (PVC) tubes (Fig. S1, S2). Two control treatments were included, the no core with disturbance (No-CoreD) and the no core undisturbed (No-CoreU) and also see the Appendix A (Methods S1 and Notes S2) for details on the installation protocol.

The objective of installing fungal in-growth cores was to estimate fungal ERH biomass of the mycorrhizal fungi capable of exploring the soil at a distance of >100 µm (thickness of the mesh) away from plant host roots (Berner et al., 2012; Wallander et al., 2013; Wallander et al., 2001). The PVC cores only sustain the growth of saprophytic fungi, because mycorrhizal fungi and roots cannot physically access soil inside the PVC core. The mesh bags allow mycorrhizal and saprophytic fungi to enter and explore soil inside the core, but prevent access by plant roots. Finally, the controls allow all fungi to explore, in the presence of roots. To estimate the contribution of the mycorrhizal fungal component, biomass values from the PVC cores were subtracted from the mesh bag values (mesh – PVC) in some analyses (thus removing the contribution by saprophytic fungi), which assumes that the different treatments have additive effects (Hendricks et al., 2006; Wallander et al., 2013; Wallander et al., 2001) and see Fig. S1.

2.6. Soil and fungal sampling

From April 19 to 24, 2012, we sampled ~80 ml (~15 g) of soil with a mini-corer (depth = 20 cm, diameter = 2.25 cm) from the controls on each subplot (total = ~100 g) prior to and after the core installation procedures (i.e. prior-installation sampling had roots and after-installation sampling was of the sieved soil). During the first weeks of June, July and August (i.e. the wet winter months during which fungal ERH biomass is expected to be maximum in this seasonally-dry Mediterranean climate), we sampled all fungal in-growth cores with the mini-corer at different locations inside the cores. Plastic straws of a similar diameter as the mini-corer were left in holes to avoid repeated sampling in the same location. Soils were immediately placed on ice inside a cooler in the field, and then transferred within 4 hours to a -20 °C freezer until further processing. On September 8, 2012, soils were promptly weighed and sieved (2 mm), then shipped under dry ice to the Institute of Soil Science and Land Evaluation at the University of Hohenheim for biochemical analyses (see details below).

247

248 *2.7. Ergosterol, neutral lipid and phospholipid fatty acid analysis*

249 Ergosterol was extracted using a modification of the method described by Djajakirana et al.
250 (1996). Ergosterol served as a general proxy for biomass of all fungi, although excluding AM
251 fungi (Watzinger et al., 2014). Given the good positive relationship with PLFA 18:2ω6,9 (Fig.
252 S3) in the sandy nutrient-poor soils along this chronosequence we used ergosterol to better
253 indicate all saprotrophic and ECM fungi. Extraction of lipids and separation into neutral lipid
254 fatty acids (NLFA) and phospholipid fatty acids (PLFA) was done after Frostegård et al.
255 (1991). Data are expressed as nmol g^{-1} soil dry weight (dw). The NLFA 16:1ω5 was used as a
256 biomarker for AM fungi and served as a proxy for AM fungal biomass (Hedlund, 2002;
257 Ngosong et al., 2012; Ruess and Chamberlain, 2010) and it has been widely used in various
258 ecosystems (Birgander et al., 2014; Francini et al., 2014; Lekberg et al., 2012; Mechri et al.,
259 2014). The PLFAs i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0 and cy19:0 were considered as
260 bacterial in origin (Frostegård and Bååth, 1996). The branched PLFAs i15:0, a15:0, i16:0 and
261 i17:0 were regarded as gram-positive and cy17:0 and cy19:0 as gram-negative in origin
262 (Zelles, 1999). Lipid 18:2ω6,9 has been assessed as a saprotrophic / ECM fungal biomarker
263 (Frostegård and Bååth, 1996; Kaiser et al., 2010). However, there is evidence that it can serve
264 as a good ECM fungal biomarker (Högberg et al., 2010; Högberg et al., 2007), particularly in
265 nutrient- and/or organic-poor soils (i.e. sands found at Jurien Bay) and if fungal in-growth
266 cores are deployed (Wallander et al., 2013) to account for saprophytic fungi. We used
267 18:2ω6,9 as a proxy for ECM fungal biomass based on previous studies (Högberg et al., 2010;
268 Högberg et al., 2007; Yarwood et al., 2009) and references therein that demonstrate strong
269 correlations between this biomarker and ECM fungi biomass. Furthermore, we also found
270 many ECM fungal taxa in the mesh cores at the same sites (Table S5), thus we have good
271 evidence to suggest that the use of PLFA 18:2ω6,9 as a good proxy for estimating ECM
272 fungal biomass in our study system was justified.

273 Our on-going mycorrhizal fungal community ecology studies (Krüger et al. (in press), Teste et
274 al., unpublished results) using next generation DNA sequencing support the use of NLFA and
275 PLFA as proxies for the estimation of AM and ECM fungal biomass since the soil in the mesh
276 cores had many relatively common AM and ECM fungal taxa (Table S4, S5). It should be
277 noted that if spores represent a substantial component of the fungal biomass in the soils of this
278 Mediterranean ecosystem, the detection of ERH scavenging should not be invalid since the
279 spore biomass signal would simply be 'additive' to the biomass of the ERH according to our
280 experimental design (see above and Notes S2). Finally, the issue of variable amounts of

background spore biomass that can be a concern when aiming to detect ERH biomass (Wallander et al., 2013), was not a concern since this potential variation was removed by our experimental approach given that we pooled and homogenised seven soil subsamples within the same plot (see above and Notes S2).

2.8. Soil analyses

Soil nutrient concentrations were determined by Turner and Laliberté (2015) on the same samples from which roots were extracted for overall mycorrhizal colonisation and cluster-root frequency. All methods are described in Turner and Laliberté (2015) and are summarised in the Appendix A (Methods S1).

2.9. Data analysis

The data were analysed as a partly nested design (i.e. split-plot design) (Quinn and Keough, 2002) with the main factor being soil age (levels: young, middle-aged or old) and the nested factor being the fungal in-growth cores (referred to as 'core' hereafter; levels: PVC, Mesh, No-CoreD, No-CoreU). The 'soil age' and 'core' factors were set as the fixed effects, and sampling plots were set as the random factor. When we included sampling date (month of sampling) in the analyses, data were analysed as a repeated measures partly nested design (Quinn and Keough, 2002). We used a linear mixed-effects model to fit the data to test for differences in fungal biomass with plots set as the random factor, with soil age and core as the fixed effects. Model assumptions (normality and homogeneity of variance of residuals) were assessed graphically in R (R Core Team, 2015) and with ggplot2 (Wickham, 2009). The overall AM or ECM root mycorrhizal colonisation levels and frequency of cluster roots were analysed as proportion data with generalised linear models. Linear and non-linear regressions were used to determine the relationships between fungal biomass and soil chemical properties at the plot level. More details on the data analyses can be found in Appendix A (Methods S1).

3. Results

3.1. Extraradical hyphal biomass

Fungal biomass, expressed as ergosterol concentrations, was significantly greater in the no-core treatments (No-CoreD and No-CoreD) compared with the mesh and PVC (Fig. 1a) across all soil ages. In addition, fungal biomass in the No-CoreU treatment was slightly, but significantly, greater than that in the No-CoreD treatment (Fig. 1a). Differences in AM (NLFA 16:1ω5: Fig. 1b) and ECM fungal biomass (PLFA 18:2ω6,9: Fig. 1c) among core

315 treatments varied with soil age (NLFA 16:1ω5 and PLFA 18:2ω6,9 core treatment × soil age
316 interaction; $P = 0.008$; $P < 0.001$, respectively). There was consistently more AM and ECM
317 fungal biomass in the young soils than in the old soils at all levels of the core treatment except
318 for the PVC cores (Fig. 1b,c). In particular, AM fungal biomass in the old soils, for the two
319 no-core treatments, was consistently lower compared with that in the young and middle-aged
320 soils (Fig. 1b). Finally, ECM fungal biomass in the old soils was the same as that in the
321 middle-aged soils across all core treatments (Fig. 1c). The NLFA 16:1ω5 to PLFA 18:2ω6,9
322 biomarker ratio was significantly higher in the PVC and No-CoreU treatments compared with
323 that in the mesh and No-CoreD, although differences were small (Fig. 1d).
324
325 *3.2. Extraradical hyphal scavenging*
326 We found some evidence for seasonal- and soil-age specific ERH scavenging (mesh-PVC,
327 Fig. 2, Fig. S4). In particular, there was greater AM fungal scavenging in the young soils
328 compared with that in the old soils, but only in August ($t = 3.25$, $P = 0.012$; Fig. 2a, Fig. S4).
329 In addition, there was significant ECM fungal scavenging across all soil ages, but also only in
330 August (Fig. 2b). However, when testing for the main effect of the fungal in-growth cores, we
331 found that there was no overall difference in mycorrhizal fungal biomass between the mesh
332 and PVC treatment (NLFA 16:1ω5: Fig. 1b; PLFA 18:2ω6,9: Fig. 1c), suggesting that there
333 was no long-distance (>100 μm) extraradical hyphae (ERH) scavenging (measured as mesh –
334 PVC). Indeed, even in the oldest, most P-impoverished old soils, AM and ECM fungal
335 biomass did not differ among core treatments (Fig. 1b,c).
336
337 *3.3. Comparison of fungal biomass in soils of other biomes*
338 Overall, fungal biomass was less in the old soils compared with that in the young soils (Fig. 3,
339 Table S3). In particular, AM and ECM fungal biomass showed much lower levels in the old
340 soils compared with that in the young and middle-aged soils (Fig. 3). In general, AM and
341 ECM fungal biomass levels found in other ecosystem types or biomes, particularly boreal
342 forests, were much greater than those found in our study system (Fig. 3).
343
344 *3.4. Fungal biomass and soil nutrient concentrations*
345 We found positive curvilinear relationships between the fungal biomarkers and readily-
346 exchangeable soil P across all treatments (Fig. 4, Fig. S5). These positive relationships were
347 more pronounced in the mesh treatment (Fig. 4). Similar relationships were found only
348 between the NLFA and PLFA biomarkers (i.e. not ergosterol) and soil pH (Fig. S6). We also

found positive relationships between ECM fungal biomass and soil dissolved organic N for all core treatments and when the mesh treatment was analysed separately (Fig. 5). No other fungal biomarkers showed any significant relationship with soil N (data not shown).

3.5. Seasonality of soil biomarkers

Fungal biomass in soil based on ergosterol was low in April, before the start of the wet winter months and remained consistently higher onwards (Fig. 6a). Biomass of AM fungi in soil, estimated with the NLFA 16:1ω5 biomarker, was high in April, peaked in June, at the start of the rainy winter season, and then declined in July (Fig. 6b). The relative abundance of AM fungi, estimated from the ratio of NLFA and ergosterol, varied across the season (i.e. peaked in June), but was not influenced by root and hyphal exclusion, since we found no significant effect of the core treatments on this ratio (Fig. S7). Fungal biomass of ECM fungi in soil, across all the fungal in-growth core treatments, did not vary throughout the sampling months (Fig. 6c).

3.6. Effects of plant composition on fungal biomass

We found a positive linear correlation between fungal biomass estimated from ergosterol and percent ECM plant canopy cover across all core treatments (Fig. S8). A stronger correlation was found between the ECM fungal biomarker PLFA 18:2ω6,9 and ECM plant cover (Fig. S8). By contrast, arbuscular mycorrhizal host plant richness, diversity, or percent canopy cover showed no relationships with AM fungal biomass across the different soil ages (data not shown).

3.7. Mycorrhizal colonisation and frequency of cluster roots along the chronosequence

The highest levels of AM and ECM fungal colonisation were found on roots from the middle-aged soils (Fig. 7a). Percent root colonisation by AM fungi showed a sharp increase in the middle-aged soils, and was significantly greater than levels found in all other soil ages (Fig. 7a). The young soils had the lowest levels of AM fungal colonisation, although these low levels were only significantly lower than those found in the middle-aged and old soils (Fig. 7a). Colonisation by ECM fungi showed a gradual increase with soil age, reaching the highest levels in the middle-aged soils, although this was not statistically different from the older soils (Fig. 7b). The frequency of cluster roots found in these community root samples also showed a gradually increasing trend with soil age, with the highest absolute values found in the older soils (Fig. 7c).

3.8. Bacterial biomass associated with extraradical hyphal scavenging

The bacteria-specific PLFA biomarkers showed a similar interaction (core treatment × soil age interaction; $P < 0.001$) and trend seen with the ECM fungal biomass (Fig. 8, Fig. 1c). Bacterial biomass was greater in the young soils compared with that in the other soil ages (Fig. 8). Furthermore, in the young and middle-aged soils, the no-core treatments had greater bacterial biomass than the PVC or mesh treatments (Fig. 8), whereas in the old soils, the bacterial biomass was similarly low across all fungal in-growth core treatments (Fig. 8). We did not find greater bacterial biomass (Fig. S4) associated with greater ERH scavenging (Fig. 2).

4. Discussion

4.1. Extraradical hyphal scavenging by mycorrhizal fungi during ecosystem retrogression

Our main finding was that declining soil P availability in the oldest soils during ecosystem retrogression constrained mycorrhizal fungal extraradical hyphae (ERH) biomass and scavenging. The ERH scavenging that was recorded was likely of very short distance away from roots and occurred only in specific soil ages and only towards the end of the most favourable time of the year in terms of precipitation (August). This key finding is intriguing, given that plant roots were relatively well colonised by AM and ECM fungi and that the most nutrient-impoverished soils harbour the greatest host plant diversity and significant host plant cover (Zemunik et al., 2015). We acknowledge that the declining soil pH along this chronosequence may have also limited the production of ERH. However since: i) we did not find any relationship between soil pH and ergosterol, ii) ECM fungi are not known to be inhibited by low soil pH (Smith and Read, 2008), iii) plants had relatively high levels of mycorrhizal fungal colonisation, iv) the number of mycorrhizal host plant species and relative cover remain high in the old soil with low pH (Zemunik et al., 2015), we surmise that declining soil pH probably inhibited production of ERH of AM fungi via decreased micronutrient or P availability. Our results did not support our initial hypothesis that there would be the greatest amount of ERH biomass in the most nutrient-impoverished soils, where we had hypothesised that plants would allocate more C to their mycorrhizal symbionts. Based on previous studies (Koide and Kabir, 2000; Smith and Smith, 2011; Wallander et al., 2010), this hypothesis seemed plausible, since the prominence of ERH would allow plants to benefit the most from mutualistic mycorrhizal fungi in these soils in terms of nutrient acquisition (Cairney, 2011). The ERH scavenging found only in August, in these seasonally dry

Mediterranean plant communities, suggests a modest investment in ERH only after some time of favourable wet conditions and/or root development. In our study, two 'no-core treatments' were included and allowed us to measure the ERH normally found in the close presence of roots (i.e. roots were sieved prior to analysis; also see Appendix A (Notes S2). Furthermore, since ERH biomass was greater in the no-cores compared with that in the mesh cores (only allowed hyphal growth), we suggest that ERH scavenging occurred, but was localised around roots.

4.2. Bacterial biomass associated with extraradical hyphae during ecosystem retrogression

The possibility that associated bacteria may have modified P availabilities in the mycorrhizosphere (Brooks et al., 2011; Richardson et al., 2011; Warmink and van Elsas, 2008) could be suggested via analyses of total bacterial-specific biomass and differences in community composition along the different soil ages (also see Appendix A Notes S1). Overall, bacterial biomass followed a similar interactive pattern found with the mycorrhizal fungal biomass; differences between the fungal in-growth cores (differences generally found between the no core and PVC treatments) was only found in the young soils. Furthermore, biomass of bacterial communities as estimated from bacteria-specific PLFAs was greater in the young soil compared to the other soil ages. We expected greater bacterial biomass would be associated with cases where we found evidence for ERH scavenging, but our results did not support this hypothesis. Rapid bacterial production may occur in synchrony with the ERH burst shown in August, but higher-resolution temporal sampling would be required to detect such changes in bacterial production.

Like fungi, some bacteria are very responsive to increased nutrient availability in soil (Kandeler, 2015). Nazir et al. (2010) highlighted different key mechanisms that could drive bacterial selection in the mycosphere such as efficient acquisition of specific released nutrients, the capacity for flagellar movement, or the capability of forming biofilms. For example, ECM fungi influence the taxonomic composition of bacterial communities in the mycorrhizosphere, especially in the close vicinity of ERH (Nurmiaho-Lassila et al., 1997). Our findings suggest that bacterial growth was associated with sites where we found more fungal biomass such as in the no-core treatments and with greater soil nutrient availability. Also, we found that many soil properties such as P availability, total [Mn], organic C, exchangeable Na, Fe and Zn were strongly correlated with the bacterial PLFA concentrations.

451 The three soil ages had distinct bacterial community compositions, where the young soils
452 were most different from the old soils, suggesting that soil nutrient availability is also an
453 important factor shaping bacterial communities. The biomass of these bacteria, like fungi, was
454 likely constrained under extremely nutrient-impoverished conditions.
455
456 *4.3. Effects of soil nutrient availability on fungal and bacterial biomass*
457 Changes in soil P and N availabilities during ecosystem development (e.g., along dune
458 successional stages) can help explain the occurrence, biomass, and function of mycorrhizal
459 fungi (Read, 1989), and there is good evidence that ECM fungi occur and function across a
460 wider range of soil conditions than previously thought (van der Heijden and Vosatka, 1999;
461 van der Heijden et al., 1999). Therefore, we also aimed to determine the impacts of soil
462 abiotic conditions on the biomass of mycorrhizal fungi and associated bacterial communities.
463 The positive relationships between the concentrations of available P and N in soil and
464 mycorrhizal fungal biomass suggest that the scavenging ability of mycorrhizal fungi becomes
465 impaired or significantly altered at very low nutrient availability, perhaps because the growth
466 of mycorrhizal fungi themselves becomes P-limited. The optimum curves show that
467 mycorrhizal fungi in these severely nutrient-impoverished soils appear to operate at the far
468 left of typical P supply vs. fungal biomass relationships found in other studies (Cairney, 2011;
469 Smith and Read, 2008). Soil P concentrations in the oldest soils we sampled are among the
470 lowest ever recorded (Turner and Laliberté, 2015), and our findings point to declining –rather
471 than increasing– AM and ECM fungal ERH growth in such severely P-impoverished soils.
472 From relevant glasshouse studies on AM and ECM fungi, the relationship between root
473 colonisation and ERH growth at the extremely low soil P levels tested (Abbott et al., 1984;
474 Jones et al., 1990) support our findings. Our study is the first to demonstrate these
475 relationships in a natural ecosystem, and declining mycorrhizal fungal biomass and
476 scavenging could be a general feature during ecosystem retrogression in very old, strongly
477 weathered soils when P availability declines to extremely low levels.
478
479 Plants that produce mycorrhizas in severely P-impoverished soils still need to acquire P and N
480 effeciently for adequate growth; hence, we do not rule out the possibility that mycorrhizal
481 fungi, especially in the oldest and poorest soils, possess efficient physiologies and/or have
482 modified their scavenging strategy to perhaps resemble that of competing and frequently
483 encountered cluster roots from the Proteaceae (Lambers et al., 2014), which are short-lived
484 structures. We expected that the greater relative amounts of organic P (proportional to total P)

found in the oldest soils (Turner and Laliberté, 2015; Turner et al., 2013) would increase the relevance of ectomycorrhizas for P acquisition due to their potentially more extensive extramatrical mycelium (Cairney, 2011; Plassard and Dell, 2010; Read and Perez-Moreno, 2003), which could have translated into greater ERH biomass and scavenging; however, our biomarker data suggest greater ERH biomass and scavenging in the younger soils that are characterised by higher N and P availability and lower relative amounts of organic P (Turner and Laliberté, 2015). These findings imply that adequate availability of key nutrients, such as P, appears important for sustained mycorrhizal fungal biomass. Nevertheless, we can't rule out the possibility that plants may also reduce their carbon allocation to their mycorrhizal symbionts in severely P-impoverished soils, which would also be responsible for the lower fungal biomass that we observed. However, our findings point more towards the fungi themselves becoming P limited, given the relatively high mycorrhizal root colonisation levels found on the poorest soils.

4.4. Seasonality of mycorrhizal fungal scavenging and associated bacteria

Seasonality affected ergosterol concentrations in these nutrient-poor soils, indicating, as expected, that favourable moist conditions generally increased fungal production. However, ECM fungal biomass was not affected by seasonality, nevertheless we suspect that the small increase in ergosterol after April may have been driven by active ECM fungal hyphae. For future studies, we propose quantifying free and total ergosterol in conjunction with PLFA 18:2ω6,9 to tease apart the active from recalcitrant ECM fungal fractions that may be relevant to C sequestration (Clemmensen et al., 2013; Koide et al., 2014). During the wet months, some level of mycorrhizal scavenging probably occurred, but likely only close to the roots, functioning much like root hairs or rootlets of cluster roots. Therefore, based on the evidence gathered in this study, we propose that a 'boom-and-bust' ERH scavenging tactic may be favoured in these seasonally-dry ecosystems, following rain events. However, some ECM fungal sporocarps appeared between June and August which provides evidence that ECM fungi were nevertheless active throughout the winter.

4.5. Ectomycorrhizal fungal biomass estimation and effects of N availability

The fungal biomass estimated by ergosterol is mostly represented by Basidiomycota and Ascomycota fungi found in soil (Kandeler, 2015). The fungal-specific PLFA 18:2ω6,9 biomarker is mainly represented by ECM fungi, and has a particularly strong relationship with ergosterol (Frostegård et al., 2011) which was also the case in our system. Ergosterol

concentrations gradually declined from the young to the old soils, while PLFA 18:2ω6,9 corroborated the ergosterol pattern with soil age. The positive relationship found between ECM fungal biomass and ECM plant host cover is not associated with the greater ECM fungal colonisation levels and highest number of ECM host plant species found in the old soils (Zemunik et al., 2015). Indeed, ECM fungal biomass was greatest in the young soils where the greatest ECM cover was found, despite lower ECM host plant richness, indicating that ECM fungi were more active and perhaps scavenged more readily in the younger, more nutrient-rich soils. This evidence in conjunction with the relationship observed between PLFA 18:2ω6,9 and dissolved organic N for the mesh cores suggests that the biomass of ECM fungi in severely nutrient-impoverished soils is also partly driven by readily-available N. By contrast, AM fungal biomass showed no relationship with soil N variables, thus suggesting that reduced AM fungal biomass in this system was more driven by soil P availability.

4.6. Spores of mycorrhizal fungi in Mediterranean ecosystems

Since our PVC cores had detectable amounts of AM and ECM fungal biomass, viable spores likely represented a considerable component of the mycorrhizal fungal biomass in these severely nutrient-impoverished soils. Indeed, detailed lipid analyses suggests that NLFA-rich spores can represent up to 90 % of the external biomass in AM fungi (Horton et al., 1998). The possibility that saprophytic fungi in the PVC cores may have been released from potential mycorrhizal-fungal competition was not supported since ergosterol (biomass of all fungi) showed similar trends to that of the mycorrhizal fungal biomass in the PVC. However, facultative saprotrophy does not appear common for ECM fungi (Lindahl and Tunlid, 2015), therefore the relatively high levels of ECM fungal biomass in the PVC cores suggest that resting bodies such as sclerotia are also important components in these soils. Furthermore, AM fungi were slightly more abundant than ECM fungi in the PVC (Fig. 1d) and the NLFA:ergosterol ratio was considerably greater in April (when roots (Pate and Bell, 1999) and fungi (Fig. 6a) are mostly inactive), thus supporting the hypothesis that a high density of mycorrhizal fungal resting spores are found in these soils. Finally, across all soil ages, the AM and ECM fungi in the PVC core that was likely represented by spores was surprisingly high in April, the driest sampling month before the onset of winter rains; we regard this as evidence for the importance of mycorrhizal spores and dormant structures in these seasonally-dry Mediterranean ecosystems.

The possibility that the biomass due to spores may have 'overwhelmed' the detection of any ERH scavenging was not supported by our data since we found evidence for ERH scavenging but it was context dependent (i.e. only at a given date and soil age). Furthermore, our on-going mycorrhizal fungal community ecology studies suggest there were some common well-known AM and ECM fungal taxa in the mesh cores thus the ERH scavenging detected via the fungal biomarkers and our experimental setup was validated (Table S4, S5). Nevertheless if spores did 'overwhelm' the detection of the ERH biomass at the other sampling dates and soil ages, this simply reinforces the evidence for relatively low ERH biomass in this system.

4.7. Mycorrhizal colonisation levels at Jurien Bay and biome comparisons of fungal biomass

Arbuscular mycorrhizal fungal colonisation increased gradually from the youngest to older soils, and peaked in the middle-aged soils. It declined sharply in the old soils during the later stage of ecosystem retrogression where soil P concentrations become extremely low. These data suggest that some AM fungal species may fail to acquire sufficient P for their host plants and are themselves under extreme P limitation. The ECM fungal colonisation also peaked in the middle-aged soils, but it remained high in the old soils. This trend was likely due to the greater host plant species richness or suggesting that ECM fungi may function more reliably as mutualists in the poorest soils. Also, organic P proportionally increases with increasing soil age along this sequence (Turner and Laliberté, 2015; Turner et al., 2013), thus perhaps giving ECM fungi a competitive advantage over AM fungi since ECM fungi possess more diverse foraging strategies (Cairney, 2011). The Mediterranean Jurien Bay soil chronosequence had, compared with other biomes, very low levels of mycorrhizal fungal biomass in soil. This is particularly intriguing, since this chronosequence harbours an impressive diversity of host plant species (Hayes et al., 2014; Zemunik et al., 2015), including the two major mycorrhizal types (AM and ECM).

5. Conclusion

Our results suggest that the productivity of mycorrhizal fungal ERH and associated bacteria is limited by declining soil P availability, and possibly soil pH (co-limiting AM only) during ecosystem retrogression. Our study highlights the need for a more 'mycocentric' view of plant-mycorrhizal relationships (Treseder and Allen, 2002), whereby mycorrhizal fungal production can be more strongly influenced by soil nutrient availability than by C supply from plant hosts. Seasonal water deficit is likely an additional major interacting factor contributing

to the performance of the mycorrhizal symbioses in these severely nutrient-impoverished soils. Our study supports the model of Lambers et al. (2008) that suggests mycorrhizal nutrient-acquiring strategies become less dominant in severely P-impoverished soils. Specifically, our results suggest that mycorrhizal fungi may remain dormant in roots during most of the year, mostly scavenge at relatively short distances from host roots, and scavenge at long-distances from roots only when soil moisture and C allocation to roots are both favourable. This ephemeral 'boom-and-bust' functioning, perphaps in conjunction with a large proportion of biomass allocated to spores, may be characteristic of mycorrhizal communities in dry severely nutrient-impoverished ecosystems entering the retrogressive phase of ecosystem development.

Acknowledgements

We thank the Western Australia Department of Parks and Wildlife (DPaW) for their cooperation and help acquiring permits to sample biota and soil in the national parks. We are grateful to Patrick Hayes, Osmarina Marinho, Davide Abade, and Thomas Mazet for help with the field and laboratory work. Funding was provided by The University of Western Australia with a Research and Development Award granted to FPT and EL, an Australian Research Council Discovery Early Career Research Award (DE120100352 Laliberté) to EL, Discovery Projects (DP130100016 Laliberté) to EL and FPT, (DP0985685 Lambers) to HL, and with the DFG project KA 1590/12-1 (The importance of fungal-fungal and bacterial-fungal interactions for phosphorus dynamics in forest soils) to EK.

607

608 **References**

609 Abbott, L.K., Robson, A.D., Boer, G.D., 1984. The effect of phosphorus on the formation of
610 hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus*
611 *fasciculatum*. New Phytologist 97, 437-446.
612 Berner, C., Johansson, T., Wallander, H., 2012. Long-term effect of apatite on
613 ectomycorrhizal growth and community structure. Mycorrhiza 22, 615-621.
614 Birgander, J., Rousk, J., Olsson, P.A., 2014. Comparison of fertility and seasonal effects on
615 grassland microbial communities. Soil Biology and Biochemistry 76, 80-89.
616 Brooks, D., Chan, R., Starks, E., Grayston, S., Jones, M., 2011. Ectomycorrhizal hyphae
617 structure components of the soil bacterial community for decreased phosphatase
618 production. FEMS Microbiol Ecol 76, 245–255.
619 Brundrett, M.C., 2009. Mycorrhizal associations and other means of nutrition of vascular
620 plants: understanding the global diversity of host plants by resolving conflicting
621 information and developing reliable means of diagnosis. Plant and Soil 320, 37-
622 77.
623 Cairney, J.W., 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of
624 carbon dynamics in forest soil. Soil Biology and Biochemistry 47, 198-208.
625 Cairney, J.W.G., 2011. Ectomycorrhizal fungi: the symbiotic route to the root for
626 phosphorus in forest soils. Plant and Soil 344, 51-71.
627 Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H.,
628 Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated
629 fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615-
630 1618.
631 Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D., 2015.
632 Carbon sequestration is related to mycorrhizal fungal community shifts during
633 long-term succession in boreal forests. New Phytologist 205, 1525-1536.
634 Dickie, I.A., Martínez-García, L.B., Koele, N., Grelet, G.A., Tylianakis, J.M., Peltzer, D.A.,
635 Richardson, S.J., 2013. Mycorrhizas and mycorrhizal fungal communities
636 throughout ecosystem development. Plant and Soil 367, 11–39.
637 Djajakirana, G., Joergensen, R., Meyer, B., 1996. Ergosterol and microbial biomass
638 relationship in soil. Biology and fertility of soils 22, 299-230.
639 Elser, J., Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT,
640 Seabloom EW, Shurin JB, JE, S., 2007. Global analysis of nitrogen and phosphorus
641 limitation of primary producers in freshwater, marine and terrestrial ecosystems.
642 Ecology Letters 10, 1135–1142.
643 Enright, N., Lamont, B., Miller, B., 2005. Anomalies in grasstree fire history
644 reconstructions for south-western Australian vegetation. Austral Ecology 30,
645 668–673.
646 Francini, G., Mannisto, M., Alaoja, V., Kytoviita, M.M., 2014. Arbuscular mycorrhizal
647 fungal community divergence within a common host plant in two different soils
648 in a subarctic Aeolian sand area. Mycorrhiza 24, 539-550.
649 Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate
650 bacterial and fungal biomass in soil. Biology & Fertility of Soils 22, 59-65.
651 Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils.
652 Soil Biology and Biochemistry 43, 1621-1625.
653 Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid
654 phosphate in soils of different organic content. J Microbiol Methods 14, 151-163.

Fyllas, N., Patino, S., Baker, T., Nardoto, G., Martinelli, L., Quesada, C., Paiva, R., Schwarz, M., Horna, V., Mercado, L., 2009. Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. Biogeosciences 6, 2677–2708.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84, 489-500.

Griffin, E., Burbidge, A., 1990. Description of the Region, In: Burbidge, A., Hopper, S., van Leeuwen, S. (Eds.), Nature Conservation, Landscape and Recreation Values of the Lesueur Area. Environmental Protection Authority, Perth, Western Australia, pp. 15-24.

Hayes, P., Turner, B., 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.

Hedlund, K., 2002. Soil microbial community structure in relation to vegetation management on former agricultural land. Soil Biology and Biochemistry 34, 1299-1307.

Hendricks, J.J., Mitchell, R.J., Kuehn, K.A., Pecot, S.D., Sims, S.E., 2006. Measuring external mycelia production of ectomycorrhizal fungi in the field: the soil matrix matters. New Phytologist 171, 179-186.

Hodge, A., Helgason, T., Fitter, A.H., 2010. Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecology 3, 267-273.

Högberg, M.N., Briones, M.J., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485-493.

Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590-601.

Hopper, S.D., Gioia, P., 2004. The southwest Australian floristic region: evolution and conservation of a global hot spot of biodiversity. Annual Review of Ecology, Evolution, and Systematics 35, 623-650.

Horton, T.R., Cázares, E., Bruns, T.D., 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza 8, 11-18.

Johannsson, J., Paul, L., Finlay, R., 2004. Microbial interactions in the mycorhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48, 1-13.

Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., Miller, R.M., 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci U S A 107, 2093-2098.

Jones, M.D., Durall, D.M., Tinker, P.B., 1990. Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhizas at different soil phosphorus levels. New Phytologist 115, 259-267.

Kaiser, C., Frank, A., Wild, B., Koranda, M., Richter, A., 2010. Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2ω6,9 and 18:1ω9. Soil Biology & Biochemistry 42, 1650-1652.

Kandeler, E., 2015. Physiological and Biochemical Methods for Studying Soil Biota and Their Function, In: Paul, E.A. (Ed.), Soil Microbiology, Ecology and Biochemistry, Fourth edition ed. Elsevier Inc, pp. 187-222.

Koide, R., Kabir, Z., 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. New Phytologist 148, 511-517.

705 Koide, R.T., Fernandez, C., Malcolm, G., 2014. Determining place and process: functional
706 traits of ectomycorrhizal fungi that affect both community structure and
707 ecosystem function. New Phytologist 201, 433-439.
708 Krüger, M., Teste, F.P., Laliberté, E., Lambers, H., Coghlan, M., Zemunik, G., Bunce, M., in
709 press. The rise and fall of arbuscular mycorrhizal fungal diversity during
710 ecosystem retrogression. Mol Ecol MEC-15-0513.R1.
711 Laliberté, E., Grace, J.B., Huston, M.A., Lambers, H., Teste, F.P., Turner, B.L., Wardle, D.A.,
712 2013a. How does pedogenesis drive plant diversity? Trends in Ecology &
713 Evolution 28, 331-340.
714 Laliberté, E., Lambers, H., Burgess, T.I., Wright, S.J., 2015. Phosphorus limitation, soil-
715 borne pathogens and the coexistence of plant species in hyperdiverse forests and
716 shrublands. New Phytologist 206, 507–521.
717 Laliberté, E., Turner, B.L., Costes, T., Pearse, S.J., Wyrwoll, K.H., Zemunik, G., Lambers, H.,
718 2012. Experimental assessment of nutrient limitation along a 2-million-year dune
719 chronosequence in the south-western Australia biodiversity hotspot. Journal of
720 Ecology 100, 631-642.
721 Laliberté, E., Turner, B.L., Zemunik, G., Wyrwoll, K.-H., Pearse, S.J., Lambers, H., Gibson,
722 D., 2013b. Nutrient limitation along the Jurien Bay dune chronosequence:
723 response to Uren & Parsons (). Journal of Ecology 101, 1088-1092.
724 Laliberté, E., Zemunik, G., Turner, B.L., 2014. Environmental filtering explains variation
725 in plant diversity along resource gradients. Science 345, 1602-1605.
726 Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E., 2008. Plant nutrient-acquisition
727 strategies change with soil age. Trends in Ecology & Evolution 23, 95-103.
728 Lambers, H., Shane, M., Laliberté, E., Swarts, N., Teste, F., Zemunik, G., 2014. Plant
729 Mineral Nutrition, In: Lambers, H. (Ed.), Plant Life on the Sandplains in Southwest
730 Australia, a Global Biodiversity Hotspot. UWA Publishing, Crawley, Australia, pp.
731 101-127.
732 Lekberg, Y., Rosendahl, S., Michelsen, A., Olsson, P.A., 2012. Seasonal carbon allocation to
733 arbuscular mycorrhizal fungi assessed by microscopic examination, stable
734 isotope probing and fatty acid analysis. Plant and Soil 368, 547-555.
735 Li, X.l., George, E., Marschner, H., 1991. Phosphorus depletion and pH decrease at the
736 root–soil and hyphae–soil interfaces of VA mycorrhizal white clover fertilized
737 with ammonium. New Phytologist 119, 397-404.
738 Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi - potential organic matter
739 decomposers, yet not saprotrophs. New Phytologist.
740 Martínez-García, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A., Dickie, I.A., 2014.
741 Host identity is a dominant driver of mycorrhizal fungal community composition
742 during ecosystem development. New Phytologist, doi: 10.1111/nph.13226.
743 McArthur, W.M., Bettenay, E., 1974. The development and distribution of the soils of the
744 Swan Coastal Plain. CSIRO, Australia.
745 Mechri, B., Manga, A.G.B., Tekaya, M., Attia, F., Cheheb, H., Meriem, F.B., Braham, M.,
746 Boujnah, D., Hammami, M., 2014. Changes in microbial communities and
747 carbohydrate profiles induced by the mycorrhizal fungus (Glomus intraradices)
748 in rhizosphere of olive trees (Olea europaea L.). Applied Soil Ecology 75, 124-133.
749 Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., Kent, J., 2000.
750 Biodiversity hotspots for conservation priorities. Nature 403, 853-858.
751 Nazir, R., Warmink, J., Boersma, H., van Elsas, J., 2010. Mechanisms that promote
752 bacterial fitness in fungal-affected soil microhabitats. FEMS Microbiol Ecol 71,
753 169-185.

754 Ngosong, C., Gabriel, E., Ruess, L., 2012. Use of the signature Fatty Acid 16:1ω5 as a tool
755 to determine the distribution of arbuscular mycorrhizal fungi in soil. J Lipids
756 2012, 236807.
757 Nurmiaho-Lassila, E.-L., Timonen, S., Haahtela, K., Sen, R., 1997. Bacterial colonization
758 patterns of intact *Pinus sylvestris* mycorrhizospheres in dry pine forest soil: An
759 electron microscopy study. Can J Microbiol 43, 1017-1035.
760 Owusu-Bennoah, E., Wild, A., 1979. Autoradiography of the depletion zone of phosphate
761 around onion roots in the presence of vesicular-arbuscular mycorrhiza. New
762 Phytologist 82, 133-140.
763 Pate, J., Bell, T., 1999. Application of the ecosystem mimic concept to the species-rich
764 Banksia woodlands of Western Australia. Agroforestry systems 45, 303-341.
765 Peltzer, D.A., Wardle, D.A., Allison, V.J., Baisden, W.T., Bardgett, R.D., Chadwick, O.A.,
766 Condron, L.M., Parfitt, R.L., Porder, S., Richardson, S.J., 2010. Understanding
767 ecosystem retrogression. Ecological Monographs 80, 509-529.
768 Plassard, C., Dell, B., 2010. Phosphorus nutrition of mycorrhizal trees. Tree physiology
769 30, 1129-1139.
770 Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists.
771 Cambridge University Press.
772 R Core Team, 2015. R: A language and environment for statistical computing. R
773 Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL
774 http://www.R-project.org/.
775 Read, D., 1989. Mycorrhizas and nutrient cycling in sand dune ecosystems. Proceedings
776 of the Royal Society of Edinburgh B 96, 80-110.
777 Read, D., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems–a
778 journey towards relevance? New Phytologist 157, 475-492.
779 Richardson, A., Lynch, J., Ryan, P., Delhaize, E., Smith, F., Smith, S., Harvey, P., Ryan, M.,
780 Venklaas, E., Lambers, H., Oberson, A., Culvenor, R., Simpson, R., 2011. Plant and
781 microbial strategies to improve the phosphorus efficiency of agriculture. Plant
782 and Soil 349, 121-156.
783 Ruess, L., Chamberlain, P.M., 2010. The fat that matters: Soil food web analysis using
784 fatty acids and their carbon stable isotope signature. Soil Biology and
785 Biochemistry 42, 1898-1910.
786 Smith, S.E., Anderson, I.C., Smith, A.F., 2015. Mycorrhizal associations and phosphorus
787 acquisition: from cells to ecosystems, In: Plaxton, W., Lambers, H. (Eds.), Annual
788 Plant Reviews Volume 48: Phosphorus Metabolism in Plants, First Edition. John
789 Wiley & Sons, Ltd., pp. 409–440.
790 Smith, S.E., Read, D.J., 2008. Mycorrhizal symbiosis. Academic Press, New York, NY, USA.
791 Smith, S.E., Smith, F.A., 2011. Roles of arbuscular mycorrhizas in plant nutrition and
792 growth: new paradigms from cellular to ecosystem scales. Annual review of plant
793 biology 62, 227-250.
794 Teste, F.P., Karst, J., Jones, M.D., Simard, S.W., Durall, D.M., 2006. Methods to control
795 ectomycorrhizal colonization: effectiveness of chemical and physical barriers.
796 Mycorrhiza 17, 51-65.
797 Teste, F.P., Veneklaas, E.J., Dixon, K.W., Lambers, H., 2014. Complementary plant
798 nutrient-acquisition strategies promote growth of neighbour species. Functional
799 Ecology 28, 819-828.
800 Treseder, K.K., Allen, M.F., 2002. Direct nitrogen and phosphorus limitation of arbuscular
801 mycorrhizal fungi: a model and field test. New Phytologist 155, 507-515.
802 Turgay, O.C., Nonaka, M., 2002. Effects of land-use and management practices on soil
803 ergosterol content in andosols. Soil Science and Plant Nutrition 48, 693–699.

804 Turner, B.L., Condron, L.M., 2013. Pedogenesis, nutrient dynamics, and ecosystem
805 development: the legacy of T.W. Walker and J.K. Syers. Plant and Soil 367, 1-10.
806 Turner, B.L., Laliberté, E., 2015. Soil development and nutrient availability along a 2
807 million-year coastal dune chronosequence under species-rich mediterranean
808 shrubland in southwestern Australia. Ecosystems 18, 287-309.
809 Turner, B.L., Lambers, H., Condron, L.M., Cramer, M.D., Leake, J.R., Richardson, A.E.,
810 Smith, S.E., 2013. Soil microbial biomass and the fate of phosphorus during long-
811 term ecosystem development. Plant and Soil 367, 225-234.
812 van der Heijden, E., Vosatka, M., 1999. Mycorrhizal associations of *Salix repens* L.
813 communities in succession of dune ecosystems. II. Mycorrhizal dynamics and
814 interactions of ectomycorrhizal and arbuscular mycorrhizal fungi. Canadian
815 Journal of Botany 77, 1833-1841.
816 van der Heijden, E., Vries, F.d., Kuyper, T.W., 1999. Mycorrhizal associations of *Salix*
817 *repens* L. communities in succession of dune ecosystems. I. Above-ground and
818 below-ground views of ectomycorrhizal fungi in relation to soil chemistry.
819 Canadian Journal of Botany 77, 1821-1832.
820 van der Heijden, M.G., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen,
821 K., Boller, T., Wiemken, A., Sanders, I.R., 2006. The mycorrhizal contribution to
822 plant productivity, plant nutrition and soil structure in experimental grassland.
823 New Phytologist 172, 739-752.
824 Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y., 1998. Ink and vinegar, a simple staining
825 technique for arbuscular-mycorrhizal fungi. Applied and Environmental
826 Microbiology 64, 5004-5007.
827 Vitousek, P., Farrington, H., 1997. Nutrient limitation and soil development:
828 experimental test of a biogeochemical theory. Biogeochemistry 37, 63–75.
829 Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus
830 limitation: mechanisms, implications, and nitrogen-phosphorus interactions.
831 Ecological Applications 20, 5–15.
832 Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of
833 chronosequences in studies of ecological succession and soil development.
834 Journal of Ecology 98, 725-736.
835 Walker, T., Syers, J., 1976. The fate of phosphorus during pedogenesis. Geoderma 15, 1-
836 19.
837 Wallander, H., 2006. External mycorrhizal mycelia–the importance of quantification in
838 natural ecosystems. New Phytologist 171, 240-242.
839 Wallander, H., Ekblad, A., Godbold, D., Johnson, D., Bahr, A., Baldrian, P., Björk, R.,
840 Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Plassard, C., Rudawska, M.,
841 2013. Evaluation of methods to estimate production, biomass and turnover of
842 ectomycorrhizal mycelium in forests soils – A review. Soil Biology & Biochemistry
843 57, 1034-1047.
844 Wallander, H., Johansson, U., Sterkenburg, E., Brandstrom Durling, M., Lindahl, B.D.,
845 2010. Production of ectomycorrhizal mycelium peaks during canopy closure in
846 Norway spruce forests. New Phytologist 187, 1124-1134.
847 Wallander, H., Nilsson, L.O., Hagerberg, D., Bååth, E., 2001. Estimation of the biomass and
848 seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New
849 Phytologist 151, 753-760.
850 Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H.,
851 2004. Ecological linkages between aboveground and belowground biota. Science
852 304, 1629-1633.

853 Warmink, J., van Elsas, J., 2008. Selection of bacterial populations in the mycosphere of
854 Laccaria proxima: is type III secretion involved? . ISME Journal 2, 887-900.
855 Watzinger, A., Feichtmair, S., Kitzler, B., Zehetner, F., Kloss, S., Wimmer, B., Zechmeister-
856 Boltenstern, S., Soja, G., 2014. Soil microbial communities responded to biochar
857 application in temperate soils and slowly metabolized C-13-labelled biochar as
858 revealed by C-13 PLFA analyses: results from a short-term incubation and pot
859 experiment. European Journal of Soil Science 65, 40-51.
860 Whiteside, M.D., Garcia, M.O., Treseder, K.K., 2012. Amino acid uptake in arbuscular
861 mycorrhizal plants. PloS one 7, e47643.
862 Wickham, H., 2009. ggplot2: elegant graphics for data analysis. Springer, New York, USA.
863 Wyrwoll, K.-H., King, P.D., 1984. Acriticismof the proposed regional extent of Late
864 Cenozoic arid zone advances into south-western Australia. Catena 11, 273-288.
865 Yarwood, S.A., Myrold, D.D., Högberg, M.N., 2009. Termination of belowground C
866 allocation by trees alters soil fungal and bacterial communities in a boreal forest.
867 FEMS Microbiol Ecol 70, 151-162.
868 Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the
869 characterisation of microbial communities in soil: A review. Biology & Fertility of
870 Soils 29, 111-129.
871 Zemunik, G., Turner, B.L., Lambers, H., Laliberté, E., 2015. Diversity of plant nutrient-
872 acquisition strategies increases during long-term ecosystem development.
873 Nature Plants 10.1038/nplants.2015.50.
874
875