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

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## 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.

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## 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 (van der Heijden et al., 2006; Smith et al., 2015), 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 (Smith and Read, 2008; Cairney, 2011). The benefits conferred by mycorrhizal fungi for plant nutrient acquisition are partly attributable to their ability to forage well beyond root depletion zones (Owusu-Bennoah and Wild, 1979; Li et al., 1991). 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 (Koide and Kabir, 2000; Johnson et al., 2010; Cairney, 2012), 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

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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 (Walker and Syers, 1976; Vitousek and Farrington, 1997; Laliberté et al., 2012). Eventually, severe P limitation in old, strongly-weathered soils leads to ecosystem retrogression, whereby rates of ecosystem processes such as primary productivity decline (Wardle et al., 2004; Peltzer et al., 2010; Turner and Condron, 2013). 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 (Peltzer et al., 2010; Walker et al., 2010; Laliberté et al., 2013b). 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 (Dickie et al., 2013; Martínez-García et al., 2014; Clemmensen et al., 2015), 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 (Wallander et al., 2001; Hendricks et al., 2006; Wallander, 2006). 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; Wallander et al., 2013; Kandeler, 2015). 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 (Lekberg et al., 2012; Wallander et al., 2013; Kandeler, 2015). 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 (Myers et al., 2000; Laliberté et al., 2013a). 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, 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, 2014) and less diverse AM fungal communities in the most impoverished soils (Krüger et al., 2015).

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 (Warmink and van Elsas, 2008; Richardson et al., 2011); 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, 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 (Myers et al., 2000; Hopper and Gioia, 2004). 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; Wyrwoll and King, 1984; Turgay and Nonaka, 2002). 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 (Laliberté et al., 2012; Hayes et al., 2014) that are expected to occur during long-