



Digging mammals contribute to rhizosphere fungal community composition and seedling growth

Shannon J. Dundas, et al. [full author details at the end of the article]

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Abstract

Bioturbation is an important ecosystem process, and the loss of native digging mammals due to introduced predators and habitat loss may have detrimental consequences for ecosystem health. The mycophagous woylie (*Bettongia penicillata ogilbyi*) was once widespread across the Australian continent and currently exists in a greatly reduced range, while the omnivorous quenda (*Isodon fusciventer*), which once occurred across the southern part of Western Australia (WA), remains common in south west WA over a reduced range. Populations of these two digging marsupials are currently maintained within sanctuaries where they can reach high densities. To assess the influence these digging marsupials have on fungal assemblages, we investigated fungal root associations among seedlings of a key mycorrhizal forest canopy species, *Corymbia calophylla*, R. Br. K. D. Hill and L. A. S. Johnson. Seedlings were grown in soil collected from inside (heavily-dug soil) and outside (minimally-dug soil) two predator-proof sanctuaries. Our results showed that above-ground seedling biomass was significantly greater for seedlings grown in soil collected from inside the sanctuaries. There were no differences in the diversity or species richness of rhizosphere fungal communities isolated from these seedlings; however, the community composition was significantly different. This was most obvious for the predator-proof enclosure that had been in place for 20 years (Karakamia Sanctuary) compared with the more recently-installed Perup Sanctuary (fenced in 2010; 4 years before this study). At Karakamia, there were greater numbers of putatively hypogeous ectomycorrhizal fungi inside the enclosure and four times the number of operational taxonomic units of arbuscular mycorrhizal fungi outside the enclosure. The differences in fungal communities suggest that digging mammals play a pivotal role in ecosystem functioning by influencing the rhizosphere of this key forest canopy species, which has implications for maintaining the health and persistence of forests.

Keywords Bioturbation · Mammal reintroductions · Brush-tailed bettong · Marsupial · Mycorrhiza · Biopedturbation · Rhizosphere fungi

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mammals, it may result in a cascade effect with reduced digging activity, lower rates of scat deposition and therefore potentially reduced colonisation by fungi, which may further exacerbate forest declines.

The woylie (*Bettongia penicillata ogilbyi*; Potoroidae) was once abundant across ~35% of the Australian mainland (Abbott 2008; Shortridge 1909), where it inhabited a broad range of habitats (Finlayson 1958; Start et al. 1998). Today, largely due to the impact of introduced predators, this species occupies less than 1% of its original range in the wild in south Western Australia, reinforced by additional populations within predator-proof sanctuaries (Wayne et al. 2015). Each woylie (~1.3 kg body mass) creates between 38 and 114 diggings per night searching for their main diet of hypogeous fungi, extrapolating to ~4.8 tonnes of soil/year per individual (Garkaklis et al. 2004). The decrease in woylie foraging has had a marked impact on soil turnover, soil dynamics, and nutrient cycling across these landscapes (Garkaklis et al. 2000, 2003).

Quenda (*Isoodon fusciventer*; Peramelidae) are common within south western Australia; however, their historical distribution of has contracted in the last 150 years (Abbott 2008). Quenda inhabit dense vegetation within native forest (Abbott 2008) but are also commonly seen in remnant vegetation within urban areas (Bryant et al. 2017; Hillman and Thompson 2016). The quenda is omnivorous, feeding on insects, plants, fruits, seeds and fungi (Quin 1985). Quenda are active diggers, creating characteristic cone shaped diggings which collect leaf litter and seeds (Valentine et al. 2013). A single quenda (1.1–1.7 kg body mass) can create ~45 digs per day, which equates to 3.9 tonnes of soil moved per year by each animal (Valentine et al. 2013). Their diggings markedly alter water infiltration, nutrient cycling, seedling recruitment (Valentine et al. 2017) and growth (Valentine et al. 2018).

Previous studies have shown that the absence or exclusion of digging mammals influences fungal communities (e.g. Clarke et al. 2015; Gehring et al. 2002), and it is therefore important to identify the impacts this may have on plant growth and health. Mycophagous mammals influence soil properties with their digging activity (Eldridge et al. 2015; Valentine et al. 2017), in addition to being vectors of fungal spores which in turn is likely to influence soil fungi assemblages (Tay et al. 2018). Seedlings grown in the spoil heap of quenda diggings exhibited improved growth compared to those grown in soil from within the digging or in undug soil (Valentine et al. 2018) and seedlings inoculated with quenda scats demonstrate a greater diversity of rhizosphere fungi compared with controls (Tay et al. 2018). The existence of woylies and quenda at high densities within large predator-proof wildlife sanctuaries provides the opportunity to examine their impact on communities of rhizosphere fungi and flow-on effects for seedling establishment.

‘Dug’ soil was collected from within predator-proof sanctuaries where woylies and quenda have been reintroduced and the turnover of soil is considerable. ‘Undug’ soil was collected from immediately outside these sanctuaries where lower densities of animals still dig, but the overall rate of soil turnover is considerably lower compared to within the sanctuary. We compared the rhizosphere fungi for seedlings grown in soil collected from inside and outside two predator-proof sanctuaries, and identified whether dug soils had improved seedling growth. We predicted that the communities of rhizosphere fungi in the presence of greater densities of woylies and quenda (within sanctuaries) would be distinctly different from those in the absence of such high densities (outside sanctuaries). For example, we predicted a greater abundance of hypogeous ectomycorrhizal species associated with seedlings grown on soil from within sanctuaries. We also predicted that seedlings grown in soil from within sanctuaries would have a larger biomass than seedlings grown in soil from outside due to the increased abundance and diversity of beneficial rhizosphere fungi.

rounding area. Current population estimates suggest ~ 1 woylie/ha inside the sanctuary (M. Virgo Department of Biodiversity, Conservation and Attractions, *pers. comm.*). Woylies persist outside the sanctuary but at lower densities compared with inside the fence. Quenda are also present both inside the sanctuary (current estimate ~ 1.42 quenda/ha, M. Virgo Department of Biodiversity, Conservation and Attractions, unpublished data) and outside the sanctuary at lower densities. There are also 10 animal-proof enclosures within the main fence, each 10 × 10 m, constructed in 2010, which are monitored for vegetation changes.

Soil collection

A total of 30 soil cores were collected at each study site; 15 from within the sanctuary and 15 from the surrounding forest outside the sanctuary fence. In addition, 11 soil cores were collected at the Perup site from within six of the 10 vegetation monitoring enclosures. All cores were collected from within the rhizosphere of mature *C. calophylla* trees, located < 1 m from the base of randomly-selected trees.

Dry leaves and sticks were brushed from the surface of the soil, and then soil corers [100 × 270 mm (diameter × length) plastic PVC pipe with a sharpened cutting edge] were hammered into the ground to a depth of ~ 200 mm with a rubber mallet. Decomposed organic matter on the soil surface was collected with the soil core. The intact soil core was then transferred to new 160 × 160 mm (diameter × height) plastic pots. Asepsis was maintained throughout soil collection activities with the corer and digging tools wiped with methylated spirits between samples. Pots were kept covered during transport and efforts were made when handling pots to ensure no cross-contamination occurred.

Seedling biomass

Pots were seeded with *C. calophylla* seeds sourced from the Northern Jarrah Forest bioregion. Three seeds per pot were planted; seedlings were thinned at the cotyledon stage to one per pot. Seedlings were grown in an evaporatively-cooled glasshouse for 4 months from October 2014 until February 2015. Pots were watered automatically for only 30 s, three times a day, in an effort to reduce leaching from the free-draining pots. Pot locations were randomised weekly. In late January, seedlings exhibited symptoms of nutrient deficiency and were consequently treated with 50 mL of a general plant fertiliser (Thrive All Purpose liquid fertiliser; Yates, Padstow NSW) 0.25 g/L solution, twice weekly for 4 weeks before harvest.

Root harvesting and seedling measurements

Seedlings were harvested after 4 months and the roots manually extracted and gently washed to remove all soil. The fine roots (i.e. those most likely to contain mycorrhiza and other fungi of interest) were then stripped off by hand and stored at – 18 °C until analysis by High Throughput Sequencing (HTS). The harvested seedlings were dried at 51 °C for 72 h before being weighed.

close alignment with sequences from a known source (e.g. fruiting body, mycorrhizal root tip) and this information was used as an additional layer to guide putative life history classification. Guilds used in this study included ectomyccorhiza (ECM), arbuscular mycorrhiza (AM), ericoid mycorrhiza (ericoid), saprotroph, endophyte, or pathogen. Where guild membership was ambiguous (i.e. ECM or saprotroph), OTUs were assigned membership in both groups.

Data analysis

Seedling above-ground biomass

We compared the above-ground biomass by two-way factorial ANOVA with sanctuary and location (inside/outside enclosure) as independent factors. Additionally, we carried out a one-way ANOVA for the Perup data only with location (enclosure inside, enclosure outside and enclosure inside) as an independent factor as we had additional samples from enclosures within the sanctuary.

Fungal diversity and species richness

To compare the diversity of fungi present, we calculated the Shannon's Diversity Index (PAST v3) and total number of fungal taxa and compared these data by two-way factorial ANOVA with site (Karakamia and Perup) and location (inside/outside sanctuary) as independent factors. We also carried out one-way ANOVA for the Perup data only as we had additional samples from enclosures within the sanctuary.

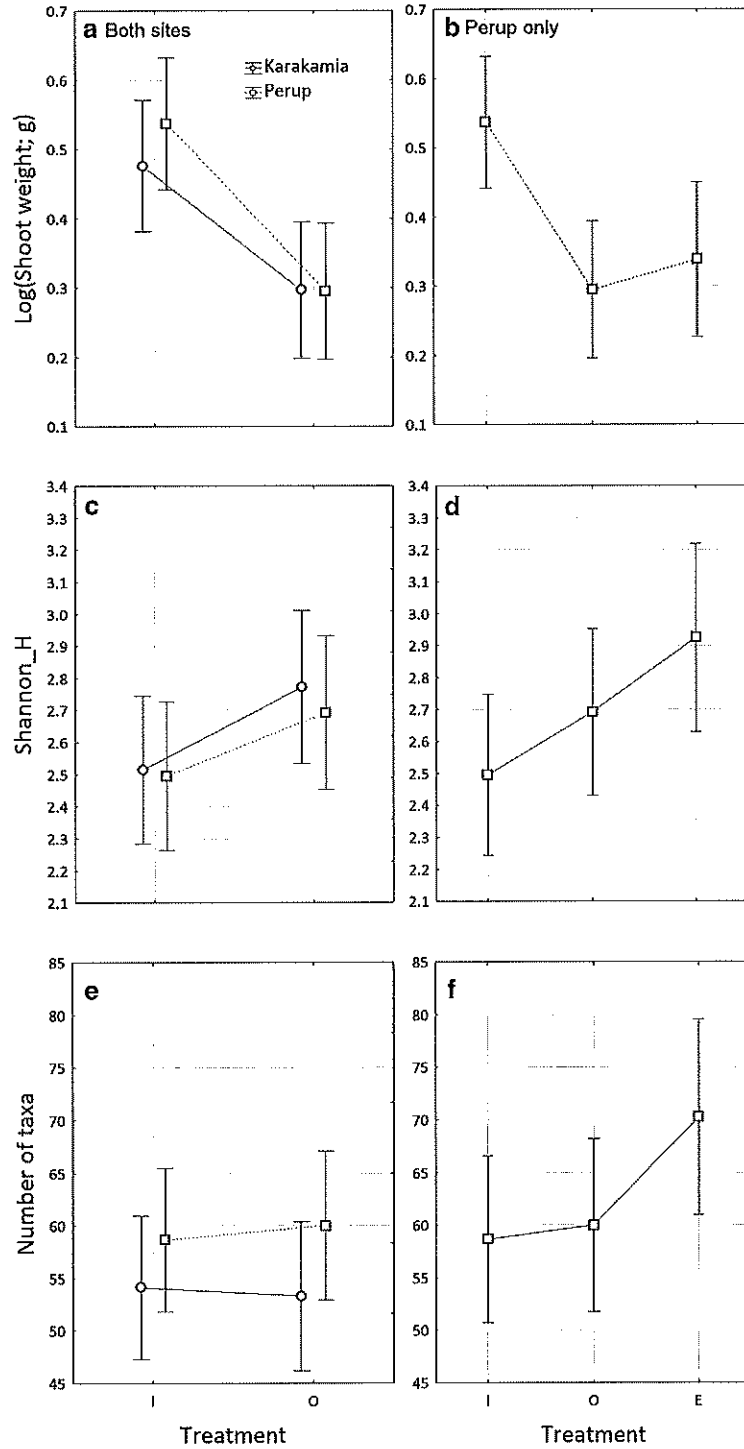
Fungal community composition

To compare the fungal community composition in the root samples, we performed non-metric Multidimensional Scaling (MDS) using Bray–Curtis similarity index (PAST v3) (Hammer and Harper 2013; Hammer et al. 2001) using the relative abundances of each fungal OTU (Nguyen et al. 2017; Ottosson et al. 2015). A two-way PERMANOVA was carried out, comparing sanctuary (Karakamia and Perup) and location (inside and outside the enclosure) as independent factors. For Perup, we also carried out a one-way PERMANOVA comparing fungal community composition by location (inside enclosure, outside enclosure, or within the animal enclosures). Two samples (one from each sanctuary) were marked outliers on the Multidimensional scaling plot (MDS) and were therefore excluded from analysis. These analyses were followed by similarity percentage (SIMPER) analyses to determine which OTUs contributed to the observed differences in fungal communities inside and outside the sanctuaries.

Results

Seedling above-ground biomass

There was a significant effect of location of soil sample on *C. calophylla* seedling growth, with seedlings having significantly higher above-ground biomass (dry weight) when grown in soils taken from within the sanctuaries compared with outside ($F_{1,54} = 16.91$, $p < 0.001$;



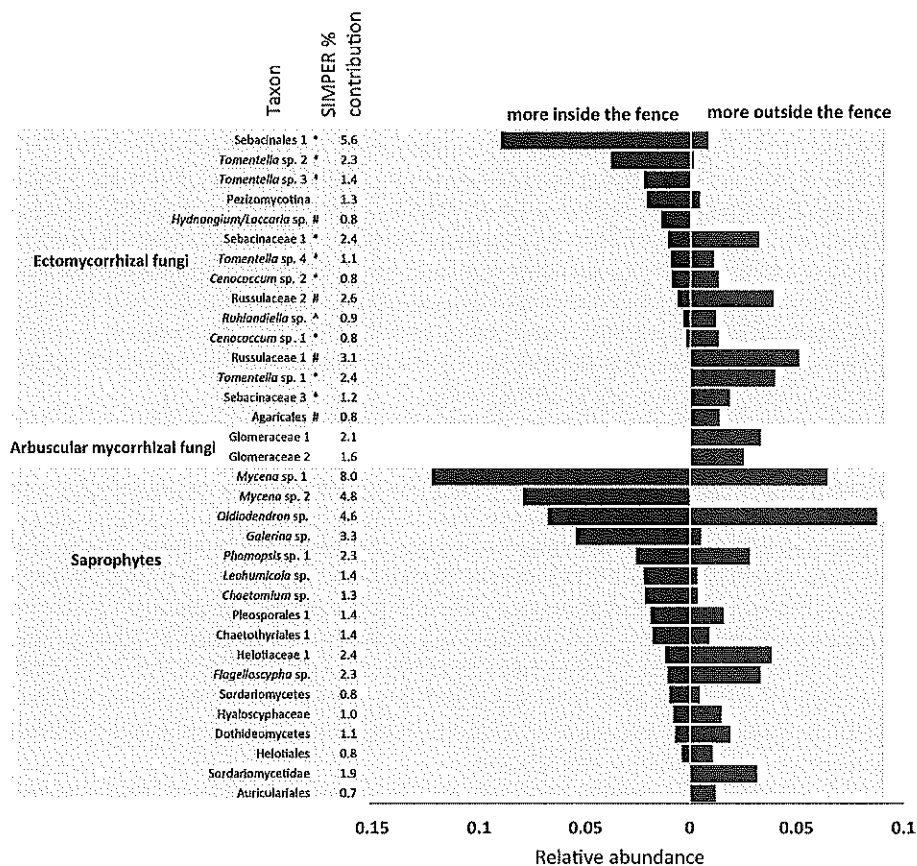


Fig. 4 The difference in relative abundance (inside sanctuary and outside sanctuary) for all identified fungal OTUs from *Corymbia calophylla* seedling roots grown in soils sampled from Karakamia Sanctuary, south western Australia, contributing to the top 70% of a similarity percentage (SIMPER) analysis

study, fungi found both inside and outside the sanctuaries formed fruiting bodies that could be eaten and dispersed by woylies and quenda. There were many more putatively hypogeous fungi exclusively found inside the enclosure at Karakamia. The difference in fungal communities inside and outside the sanctuary and the improved seedling growth in soil collected from within the sanctuaries may be an indirect result of woylie and quenda activity. Increased plant growth associated with digging activities has also been noted by Travers et al. (2012). Our work suggests that digging mammals such as the woylie and quenda play an important part as dispersers of fungi and are important for plant growth in forest ecosystems.

It is likely that the length of time that the sanctuaries had been in existence influences the degree of difference in fungal communities on either side of the fence. The differences in fungal communities were most obvious for Karakamia Sanctuary, which had been in place for 20 years at the time of this study, and has markedly fewer digging mammals outside the sanctuary compared with inside. By contrast, Perup Sanctuary had been fenced only four years before this study and animals were also present outside the fence, albeit at lower densities. Therefore, although both sanctuaries have similar densities of digging

sanctuaries where these digging mammals are present. With the widespread loss of digging mammals across the Australian landscape, it is likely these ecosystems have changed drastically. Improving our knowledge of how digging mammals influence fungal communities and seedling development contributes to a better understanding of ecosystem functioning, revealing how these mammals can play a critical role in maintaining and restoring forests.

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Affiliations

Shannon J. Dundas^{1,2} · Anna J. M. Hopkins^{1,3} · Katinka X. Ruthrof^{1,4} ·
Natasha E. Tay¹ · Treena I. Burgess¹ · Giles E. St. J. Hardy¹ · Patricia A. Fleming¹

✉ Shannon J. Dundas
S.Dundas@murdoch.edu.au

¹ School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA 6152, Australia

² NSW Department of Primary Industries, 1447 Forest Rd, Orange, NSW 2800, Australia

³ Centre for Ecosystem Management, School of Science, Edith Cowan University, 270 Joondalup Drive, Joondalup, WA 6027, Australia