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Native soilborne pathogens equalize differences in competitive ability between plants of contrasting nutrient-acquisition strategies

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Summary

1. Soilborne pathogens can contribute to the maintenance of local plant diversity by reducing differences in competitive ability between co-occurring plant species. It has been hypothesized that efficient phosphorus (P) acquisition by plants in P-impoverished ecosystems might trade off against resistance to root pathogens. This could help explain high plant diversity in severely nutrient-impoverished ecosystems. However, empirical evidence of such a trade-off remains scarce.

2. In hyperdiverse shrublands in south-western Australia, non-mycorrhizal cluster-rooted Proteaceae are very efficient at acquiring P. However, Proteaceae co-occur with many other plant species using other P-acquisition strategies, such as ectomycorrhizal (ECM) associations.

3. In a glasshouse experiment, we grew Proteaceae and ECM plant species from hyperdiverse shrublands alone and in competition with each other, and in the presence or absence of native soilborne pathogens (*Phytophthora* spp.). We hypothesized that native *Phytophthora* species are more detrimental to Proteaceae than co-occurring ECM plants, due to a trade-off between highly efficient P-acquisition and pathogen defence, and that this equalizes differences in competitive ability between these two plant groups.

4. When seedlings were grown alone, biomass of non-mycorrhizal plants was reduced in the presence of *Phytopthora*, while ECM species were unaffected by this pathogen. When non-mycorrhizal and ECM species were planted together, ECM plants grew better in the presence of *Phytophthora* than in its absence, because *Phytophthora* reduced the growth of the non-mycorrhizal competitors.

5. Growth of ECM plants was positively correlated with per cent root colonization by ECM fungi, but this was only significant when ECM plants were grown in the presence of *Phytophthora.*

6. *Synthesis*. Our study shows that native soilborne pathogens equalized differences in competitive ability between seedlings of contrasting nutrient-acquisition strategies, thus supporting the hypothesis proposing a trade-off between highly efficient P-acquisition and resistance against root pathogens. We found that non-mycorrhizal cluster-rooted species may be the most efficient at acquiring the growth-limiting resource, but that co-occurring ECM species are better defended against root pathogens. Our results suggest that native soilborne pathogens and ECM contribute to the maintenance of the plant hyperdiversity in severely P-impoverished ecosystems.

Key-words: cluster roots, determinants of plant community diversity and structure, ectomycorrhizas, phosphorus, *Phytophthora*, Proteaceae, soil nutrient availability

Introduction

Most plant pathogens have detrimental impacts on both natural and managed ecosystems, threatening plant biodiversity

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and productivity in many biomes across the globe (Fisher *et al.* 2012). In many cases, the pathogens causing a decline in plant diversity have been introduced from other regions (Anagnostakis 1987; Brown & Hovmøller 2002). These introduced pathogens can cause significant damage to plants that have not evolved specific defences against those introduced pathogens (Cahill *et al.* 2008). By contrast, very little is known about the ecological role of native soilborne pathogens that have co-evolved with plant species in a given region, although a potential role of pathogens to the maintenance of local plant species diversity is receiving increasing attention in recent years (Gilbert 2002; Bagchi *et al.* 2010b; Laliberté *et al.* 2015).

Plant pathogens can contribute to plant species coexistence and thus promote local plant diversity through different mechanisms (Mills & Bever 1998; Gilbert 2002; Mordecai 2011; Laliberté *et al.* 2015). For example, this can occur through conspecific negative density dependence (Wurst *et al.* 2015), or by reducing differences in competitive ability between co-occurring plant species (Terborgh 2012). Negative density dependence (i.e. Janzen–Connell effect) occurs when an increasing density of conspecific individuals leads to the local accumulation of host-specific pathogens, reducing conspecific seedling survival and growth (Janzen 1970; Connell 1971; Freckleton & Lewis 2006). On the other hand, pathogens with low host specificity can still promote plant species diversity by being more detrimental to (or building up larger populations around) plant species showing higher competitive ability, thus enabling less competitive plant species to persist (Bell, Freckleton & Lewis 2006; Bagchi, Press & Scholes 2010a). These different effects of pathogens are not mutually exclusive and can both contribute to the maintenance of local plant diversity (Gilbert 2002). Determining the ecological role of soilborne pathogens for plant species coexistence should help us understand how highly diverse plant communities are maintained (Laliberté *et al.* 2015).

Highly diverse plant communities such as tropical rain forests and Mediterranean shrublands often occur on old, strongly weathered, very infertile soils that are particularly low in phosphorus (P) (Huston 1994; Laliberté *et al.* 2013). Some of these plant communities exhibit a wide range of nutrient-acquisition strategies (Lambers *et al.* 2014; Zemunik *et al.* 2015). Laliberté *et al.* (2015) surmised that low soil P availability contributes to plant coexistence in these hyperdiverse communities and that this might be related to a trade-off between P-acquisition efficiency and root defences against pathogens. Indeed, roots that are highly efficient at acquiring P tend to be short-lived, poorly lignified, with a thin epidermis, thus making them more susceptible to root pathogens (Newsham, Fitter & Watkinson 1995). Evidence of pathogens contributing to plant species coexistence exists for tropical rain forests (Freckleton & Lewis 2006; Terborgh 2012), which are renowned for their high plant diversity. For example, Bagchi *et al.* (2014) experimentally showed that applying fungicides reduced tree seedling diversity in a tropical forest in Belize, pointing to a role of fungal pathogens in maintaining plant diversity. However, to our knowledge, the ecological role of native pathogens on plant interactions and diversity in other highly diverse ecosystems such as seasonally dry shrublands has not yet been studied.

Soils in the kwongan shrublands in south-western Australia are old, strongly weathered and severely nutrient-impoverished, especially with respect to P (Laliberté *et al.* 2015; Viscarra Rossel & Bui 2016). Plant communities in kwongan are renowned for high plant diversity with contrasting nutrient-acquisition strategies, such as different mycorrhizal associations and non-mycorrhizal strategies such as cluster roots (Lamont, Hopkins & Hnatiuk 1984; Zemunik *et al.* 2015). In particular, non-mycorrhizal, cluster-rooted Proteaceae are particularly successful in these habitats (Lambers *et al.* 2006; Zemunik *et al.* 2015), because this nutrient-acquisition strategy is highly effective at acquiring different forms of P (Lambers *et al.* 2006, 2012). On the other hand, cluster roots are fine short-lived roots (Shane *et al.* 2004; Lambers *et al.* 2006), and thought to be highly susceptible to soilborne pathogens as a trade-off of high efficiency in P-acquisition (Laliberté *et al.* 2015).

Although cluster roots might be the most efficient strategy at acquiring the growth-limiting soil nutrient, P, in these shrublands (Lambers, Martinoia & Renton 2015), many other plant species with contrasting P-acquisition strategies coexist with Proteaceae in hyperdiverse, P-impoverished south-western Australian shrublands (Laliberté, Zemunik & Turner 2014; Zemunik *et al.* 2015). In particular, associations with ectomycorrhizal (ECM) fungi are another common nutrient-acquisition strategy, but considered to be less efficient than cluster roots at acquiring different forms of P in P-impoverished soils (Lambers *et al.* 2008). However, ECM fungi not only contribute to plant nutrient acquisition (Smith, Anderson & Smith 2015), but also confer physical and chemical defences against root pathogens (Marx 1972; Strobel & Sinclair 1991). Therefore, it is possible that soilborne pathogens might promote the coexistence of non-mycorrhizal and mycorrhizal plants in P-impoverished soils, because of a trade-off between efficient P-acquisition and defence against pathogens; however, to our knowledge, this has never been tested experimentally.

Oomycetes are considered to be ecologically important soilborne pathogens in hyperdiverse south-western Australian shrublands (Laliberté *et al.* 2015). On the one hand, the invasive oomycete *Phytophthora cinnamomi* Rands has caused devastating damage to native flora in Australia since it was introduced in the early 1900s (Cahill *et al.* 2008). For example, in the south-west botanical province approximately 40% of the native flora is susceptible (Shearer, Crane & Cochrane 2004). On the other hand, south-western Australia also harbours several native species of *Phytophthora* (Rea *et al.* 2011; Simamora *et al.* 2013), whose ecological roles are unknown. In the present study, we evaluated how native *Phytophthora* species could affect the outcome of interactions between ECM Myrtaceae and non-mycorrhizal Proteaceae in hyperdiverse shrublands by reducing differences in competitive ability among these co-occurring species. Specifically, we aimed to test the following hypotheses: (i) non-mycorrhizal Proteaceae are more severely negatively affected by the

presence of different native *Phytophthora* strains than ECM species; (ii) the presence of native *Phytophthora* will reduce the competitive superiority of non-mycorrhizal Proteaceae over ECM species; and (iii) higher ECM root colonization will offer greater protection against *Phytophthora*, and hence increase growth of ECM plants.

Materials and methods

STUDY AREA AND SITE SELECTION

Our study focused on the oldest chronosequence stage of the Jurien Bay dune chronosequence, located in south-western Australia (30.29° S, 115.04° E), because soils from this oldest chronosequence stage are severely P-impoverished and host the highest plant species and functional diversity (Laliberté, Zemunik & Turner 2014; Turner & Laliberté 2015; Zemunik *et al.* 2015). The Jurien Bay dune chronosequence spans over two million years of pedogenesis over approximately 10 km and has been described in detail elsewhere (Laliberté *et al.* 2012, 2013; Hayes *et al.* 2014; Laliberté, Zemunik & Turner 2014; Turner & Laliberté 2015; Zemunik *et al.* 2015). This chronosequence is located in a global biodiversity hotspot (Myers *et al.* 2000). A detailed description of soil properties along the entire chronosequence can be found in Laliberté *et al.* (2012) and Turner & Laliberté (2015).

SOIL COLLECTION

Using a network of permanent 10 × 10 m plots from earlier studies (Hayes *et al.* 2014; Laliberté, Zemunik & Turner 2014; Turner & Laliberté 2015; Zemunik *et al.* 2015), we collected soils from five plots in the oldest stage (stage 6 in Turner & Laliberté 2015) that were at least 1 km apart. We collected *c.* 10 kg bulk soil from each plot. Soils were collected from the top 30-cm layer at three randomly positioned points within each plot. Soils were air-dried, mixed and sieved through a 2-mm sieve. Then, bulk soil from all plots was sterilized via triple-steam pasteurization at 80 °C for 2 h per day over 7 days, following previous studies (Fang, You & Barbetti 2012; Ryan *et al.* 2012).

SPECIES SELECTION

We selected six plant species for our experiment: three non-mycorrhizal Proteaceae that form cluster roots (*Banksia attenuate* R. Br., *Banksia menziesii* R. Br. and *Hakea ruscifolia* Labill.), and three ECM species from the Myrtaceae (*Calothamnus quadrifidus* R. Br., *Eucalyptus todtiana* F.Muell. and *Eremaea asterocarpa* Hnatiuk). Myrtaceae species are solely ECM with the exception of *E. asterocarpa* which can also form arbuscular mycorrhizal association (Zemunik *et al.* 2015). Seedlings were germinated in triple-steam pasteurized soil, and 1-month-old seedlings were transferred into 1-L pots. At the time of planting, small plastic tubes (2 cm diameter, 10 cm long) were inserted next to seedlings to leave space to insert *Phytophthora* inoculum to allow for infestation of the rhizosphere of growing plants.

INOCULUM WITH ECTOMYCORRHIZAL FUNGI

To ensure ECM species would be colonized by ECM fungi, 20 individuals of each ECM species were germinated and grown in non-sterile soils collected from the field for 4 months. Then, we harvested their roots, cut and mixed them, and used these roots as ECM fungal inoculum. We visually assessed for lesions and damping-off symptoms on roots. Despite the fact that this inoculum containing ECM fungi likely also contained other micro-organisms, no traces of damage by pathogens were observed in these seedlings and their roots. We added 50 mg of inoculum with ECM fungi under each seedling (ECM and Proteaceae) during transplantation into triple-pasteurized soils for all *Phytopthora* inoculation treatments.

PHYTOPHTHORA INOCULUM PREPARATION

We selected five strains of *Phytophthora* representing different native species isolated from kwongan vegetation (Simamora *et al.* 2013, 2015): *Phytophthora arenaria* (CBS 127950) and four less common species isolated from kwongan vegetation during routine surveys, *P.* taxon cooljarloo (CLJO100), *P.* taxon kwongan (TCH009), *P.* aff. *rosacearum* (HSA2350) and *Phytophthora rosacearum* (HSA1658). Inocula were produced as described in Aghighi *et al.* (2015). Briefly, a sterile medium made of vermiculite (with 0.1% of millet seed) wetted with V8 juice was inoculated with actively growing mycelium and left for 8–12 weeks at 20 °C for the mycelium to fully colonize the medium.

EXPERIMENT 1

Colonized media of strains of *Phytopthora*, except *P. arenaria*, were pooled in equal quantities (w/w). After seedlings were transplanted and grown for 2 weeks in 1-L pots with soils with inoculum with ECM fungi, we added 5 g of (0.4% of total soil weight) each of the following treatments: (i) '− *Phytophthora*' (double-autoclaved inocula), (ii) '+ *Phytophthora*' (mix of *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacearum*) or iii) '+ *P. arenaria*'. We used a sample size of 10 seedlings for this experiment. Three days later, pots were watered to field capacity, and then twice weekly to 70% of field capacity. Seedlings were grown in a glasshouse for 4 months and then harvested to avoid root growth becoming pot-bound.

EXPERIMENT 2

Given that *P. arenaria* did not show to be more detrimental to plant growth than the other *Phytophthora* strains in Experiment 1, in this experiment all strains were pooled. Furthermore, *E. asterocarpa* and *H. ruscifolia* were not used in this experiment, due to poor germination. One individual of either ECM species (*C. quadrifidus* or *E. todtiana*) was potted in a 2.7-L pot together with one seedling of *B. menziesii* and one seedling of *Banskia attenuata* for a total of three seedlings per pot. This was done in order to maximize the interaction between Proteaceae and ECM plant species. Each ECM plant was planted with 50 mg of inoculum with ECM fungi as described above. After seedlings were transplanted and grown for 2 weeks in sterile soils with inoculum with ECM fungi, we inoculated each pot with 5 g of either: (i) '− *Phytophthora*' (double-autoclaved inocula) or (ii) '+ *Phytophthora*' (mix of *P. arenaria*, *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacerarum*). We used a sample size of nine seedlings for this experiment. Three days later, pots were watered to field capacity and then twice weekly to 70% to field capacity. Seedlings were grown for 4 months in the glasshouse and then harvested as per experiment 1.

POST-HARVEST ANALYSES

After 4 months of growth in the glasshouse, seedlings were harvested by severing shoots from roots. Roots were carefully washed over a 1-mm sieve immediately after harvesting to remove soil particles. Shoots and roots were oven-dried for 3 days at 60 °C and weighed separately. Later, roots were rehydrated in water at 5 °C for 48 h, and cleared using potassium hydroxide (10%, w/v) for 3 h at 90 °C in a water bath. Following clearing, we used a 5% (v/v) ink–vinegar solution to stain roots (Vierheilig *et al.* 1998). Finally, roots were placed in a 50% (v/v) lactoglycerol mixture for storage.

Root colonization by ECM fungi was determined using the gridline intersect method (Giovannetti & Mosse 1980) at 200× magnification, counting root tips with an ECM mantle and/or Hartig net when the mantle was not conspicuous. At least 150 total root tips were counted for each sample.

STATISTICAL ANALYSES

All analyses were conducted, and figures were drawn in R (R Core Team, 2015). Statistical differences in biomass between species and inoculum treatment were tested using linear mixed-effect models with the function gls() in experiment 1 and lme() in experiment 2 (with 'pot' as random effect) from the 'NLME' package (Pinheiro *et al.* 2012). Residuals were inspected visually to check model assumptions. When a given model did not meet model assumptions (i.e. residuals centred on zero and homoscedasticity), a revised model with an appropriate variance structure was used (Supporting Information). The quality of the new model was evaluated using the Akaike Information Criterion (AIC) and likelihood ratio tests (Zuur *et al.* 2009). When a main term was significant, *post hoc* Tukey tests were performed using the function glht() from the 'multcomp' package (Hothorn, Bretz & Westfall 2008). Relationship between ECM root colonization and seedling biomass was calculated by fitting linear regression models using lm().

Results

EXPERIMENT 1

All seedlings survived during this experiment. The two + *Phytophthora* treatments led to lower seedling biomass in Proteaceae species, compared with those grown in the − *Phytophthora* treatment; by contrast, the biomass of ECM plant species was unaffected by the presence of *Phytopthora* (species × *Phytophthora* treatment interaction; $F_{2,10} = 9.99$; $P \leq 0.0001$; Fig. 1). Indeed, biomass for all three Proteaceae species was reduced by 20–40% when plants exposed to *Phytophthora* were compared with plants grown in the − *Phytophthora* treatment ($P \leq 0.02$; Fig. 1). On the other hand, biomass of ECM species was not observed to be affected by either of the + *Phytophthora* treatments ($P \geq 0.08$; Fig. 1). No differences were found between + *Phytophthora* and the + *P. arenaria* treatments for ECM or Proteaceae species ($P \geq 0.65$; Fig. 1).

Root-to-shoot ratio differed significantly between treatments, but this varied among species (species × *Phytophthora* treatment interaction; $F_{2,10} = 3.32$; $P \leq 0.0001$; Fig. 2). For all three Proteaceae, root:shoot ratio was 40–50% lower in the two + *Phytophthora* treatments compared with that in the − *Phytophthora* treatment ($P \leq 0.01$; Fig. 2). However, no differences were observed between the two + *Phytophthora* treatments ($P \geq 0.7$; Fig. 2). For ECM species, *E. asterocarpa* showed a lower root:shoot ratio in both + *Phytophthora* treatments compared with that in the − *Phytophthora* treatment ($P \leq 0.01$; Fig. 2); while the root:shoot ratio of *C. quadrifidus* was only lower when exposed to *P. arenaria* compared with that in the − *Phytophthora* treatment ($P \leq 0.05$). There was no evidence that the root:shoot ratio of *E. todtiana* was affected by *Phytophthora* treatments (Fig. 2).

We found a positive relationship between total ECM seedling biomass and ECM root colonization in both + *Phytophthora* treatments, although not for the − *Phytophthora* treatment (Fig. 3). Indeed, this relationship was significant for both + *Phytophthora* treatments for *C. quadrifidus* (≤ 0.05; Fig. 3), *E. todtiana* ($P \leq 0.01$; Fig. 3) and *E. asterocarpa* ($P \leq 0.01$; Fig. 3), while it was not significant in the − *Phytophthora* soil for any of these three species ($P \geq 0.18$; Fig. 3).

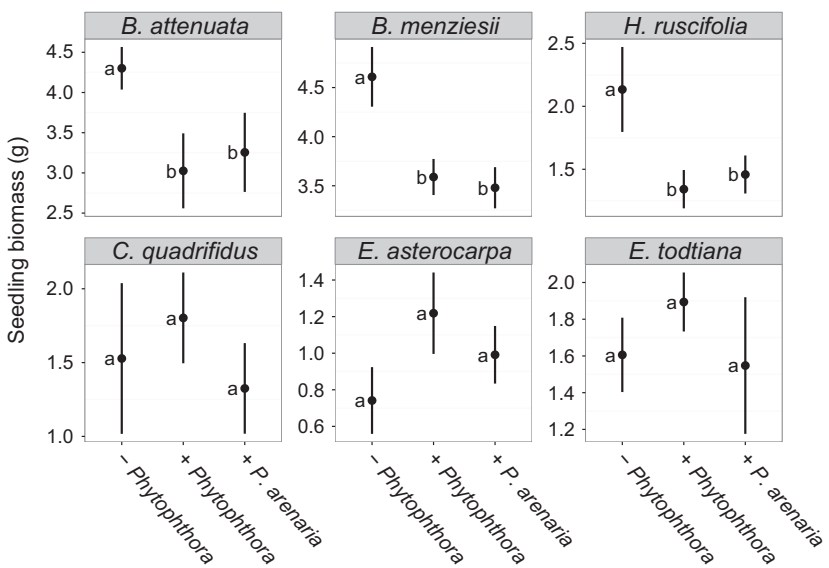

Fig. 1. Final biomass of non-mycorrhizal cluster-rooted (top row; *Banskia attenuata*, *Banksia menziesii* and *Hakea ruscifolia*) and ectomycorrhizal plant species (bottom row; *Calothamnus quadrifidus*, *Eremaea asterocarpa* and *Eucalyptus todtiana*) grown under three inoculum treatments: (i) − *Phytophthora*, (ii) '+ *Phytophthora*' (mix of *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacerarum*) and (iii) + *Phytophthora arenaria*. Different letters indicate significant ($P \leq 0.05$) differences among treatments based on *post hoc* Tukey tests. Means and 95% confidence intervals (CI) are shown.

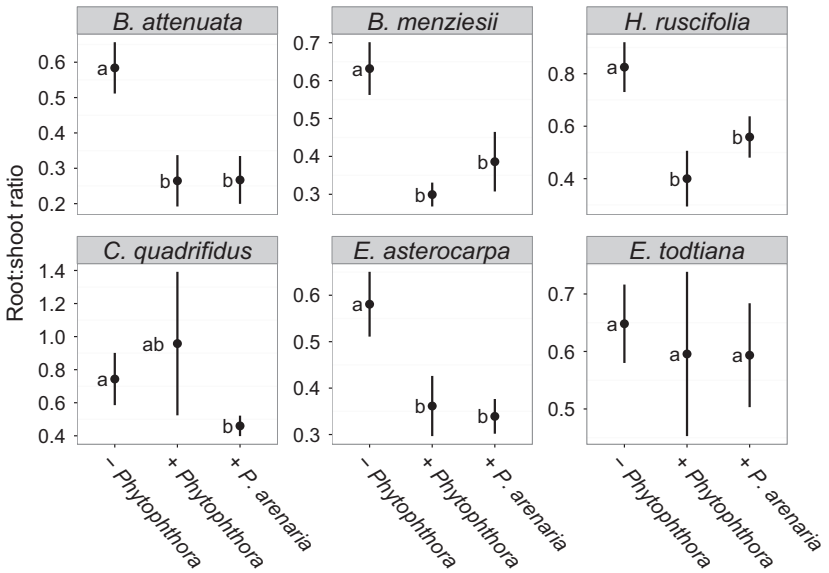

Fig. 2. Root-to-shoot ratio of non-mycorrhizal cluster-rooted (top row; *Banskia attenuata*, *Banksia menziesii* and *Hakea ruscifolia*) and ectomycorrhizal plant species (bottom row; *Calothamnus quadrifidus*, *Eremaea asterocarpa* and *Eucalyptus todtiana*) grown under three inoculum treatments: (i) – *Phytophthora*, (ii) '+ *Phytophthora*' (mix of *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacerarum*) and (iii) + *Phytophthora arenaria*. Different letters indicate significant ($P \leq 0.05$) differences among treatments based on *post hoc* Tukey tests. Means and 95% confidence intervals (CI) are shown.

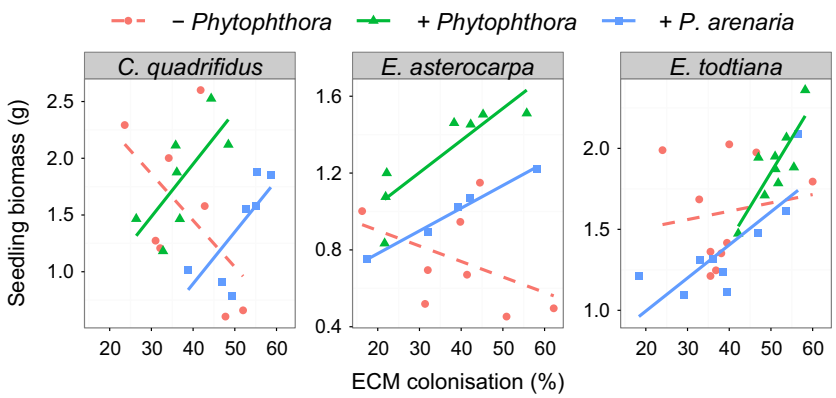

Fig. 3. Relationships between ectomycorrhizal (ECM) root colonization and final seedling biomass in three ECM plant species (*Calothamnus quadrifidus*, *Eremaea asterocarpa* and *Eucalyptus todtiana*). Seedlings were grown with three inoculum treatments: (i) – *Phytophthora* (red circles and dashed line), (ii) '+ *Phytophthora*' (green triangles and solid line; mix of *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacerarum*), and (iii) + *Phytophthora arenaria* (blue squares and solid line). Relationships between ectomycorrhizal (ECM) colonization and biomass were only significant for + *Phytophthora* and + *P. arenaria* treatments for all ECM plant species. For *C. quadrifidus*, R^2 was 0.41 and 0.53, respectively; for *E. todtiana*, R^2 was 0.64 and 0.56, respectively; for *E. asterocarpa*, R^2 was 0.7 and 0.97, respectively. Solid lines indicate significant relationship ($P \leq 0.05$), while dashed line indicates non-significant relationship ($P \geq 0.05$).

EXPERIMENT 2

The effect of *Phytophthora* inoculum treatment on final biomass varied among plant species (species × *Phytophthora* treatment interaction, $F_{1,3} = 17.58$; $P \leq 0.0001$; Fig. 4). Final biomass of both ECM species competing with Proteaceae was greater in the + *Phytophthora* treatment, compared with that in the – *Phytophthora* treatment ($P \leq 0.01$; Fig. 4). Conversely, biomass of *B. menziesii* was less in the + *Phytophthora* treatment compared with that in the – *Phytophthora* treatment ($P \leq 0.05$; Fig. 4); that of *B. attenuata* was not observed to differ among treatments ($P = 0.2$; Fig. 4).

Root-to-shoot ratio varied among species and *Phytophthora* treatments (species × *Phytophthora* treatment interaction, $F_{1,3} = 18.48$; $P \leq 0.0001$; Fig. 5). Root:shoot ratio of *C. quadrifidus* and *E. todtiana* was almost twice as high in the – *Phytophthora* treatment than in the + *Phytophthora* treatment ($P \leq 0.001$; Fig. 5). By contrast, no differences between treatments in root:shoot ratio were found for either of the Proteaceae ($P = 0.1$; Fig. 5).

Finally, both *C. quadrifidus* and *E. todtiana* showed a significant positive relationship between ECM root colonization and seedling biomass in the + *Phytophthora* treatment ($P \leq 0.04$; Fig. 6), while it was not significant in the – *Phytophthora* treatment ($P \geq 0.64$; Fig. 6). There were no statistically significant correlations between ECM root colonization of Myrtaceae and biomass of competing Proteaceae in the different *Phytophthora* treatments ($P \geq 0.8$).

Discussion

Overall, our results show that non-mycorrhizal Proteaceae were more susceptible to native soilborne pathogens than

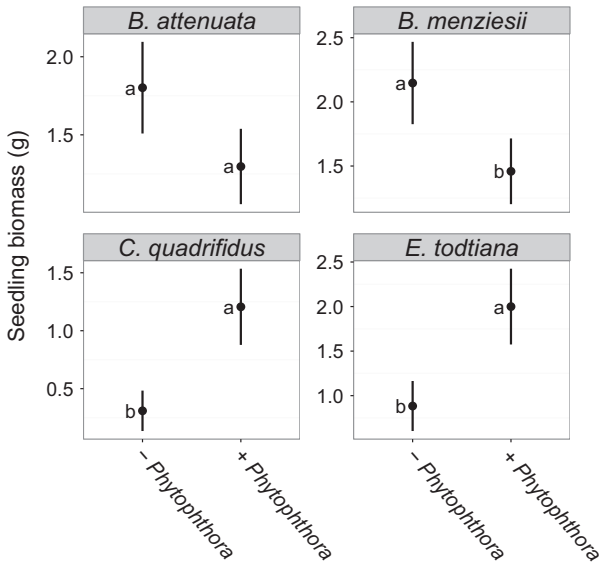

Fig. 4. Final biomass of non-myoccorhizal cluster-rooted (top row; *Banskia attenuata* and *Banksia menziesii*) and ectomycorrhizal (ECM) plant species (bottom row; *Calothamnus quadrifidus* and *Eucalyptus todtiana*) when grown together in competition with each other: one ECM plant species planted with both cluster-rooted species under two inoculum treatments: (i) – *Phytophthora* or (ii) '+ *Phytophthora*' (mix of *Phytophthora arenaria*, *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. *rosacearum* and *P. rosacerarum*). Different letters indicate significant ($P \leq 0.05$) differences among treatments based on *post hoc* Tukey tests. Means and 95% confidence intervals (CI) are shown.

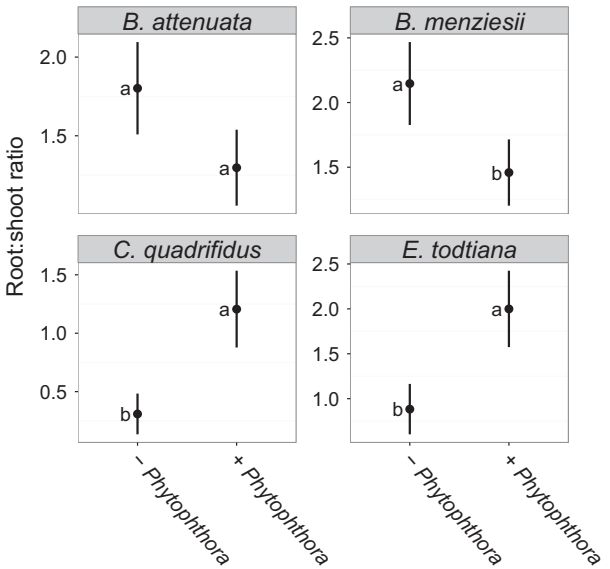

Fig. 5. Root-to-shoot ratio of non-mycorrhizal cluster-rooted (top row; *Banskia attenuata* and *Banksia menziesii*) and ectomycorrhizal (ECM) plant species (bottom row; *Calothamnus quadrifidus* and *Eucalyptus todtiana*) grown together in competition with each other: one ECM plant species planted with both cluster-rooted plant species under two inoculum treatments: (i) – *Phytophthora* and (ii) '+ *Phytophthora*' (mix of *Phytophthora arenaria*, *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. rosacearum and *P. rosacerarum*). Different letters indicate significant ($P \leq 0.05$) differences among treatments based on *post hoc* Tukey tests. Means and 95% confidence intervals (CI) are shown.

ECM plant species, and this translated into a relaxation of competition between species with these two nutrient-acquisition strategies, presumably because non-mycorrhizal Proteaceae species are most effective in acquiring the growth-limiting resource in these soils, P (Lambers, Martinoia & Renton 2015). In agreement with our hypotheses, we found that biomass gain of Proteaceae was reduced by *c.* 26% in the presence of native *Phytophthora* species, while the growth of ECM species was not affected. This supports the contention that non-mycorrhizal cluster-rooted species are more susceptible to soilborne pathogens than ECM species (Laliberté *et al.* 2015). Furthermore, when competing with Proteaceae, ECM species showed higher biomass gain in the presence of native *Phytophthora* species than in their absence, suggesting that the presence of native soilborne pathogens can modulate competitive interactions between ECM and Proteaceae species. Additionally, this increase in ECM plant biomass in the presence of *Phytophthora* was positively correlated with ECM root colonization, suggesting an important role in pathogen defence by ECM fungi. Our study suggests that soilborne pathogens may contribute to the maintenance of highly diverse ecosystems by reducing differences in competitive ability among plant species of contrasting nutrient-acquisition strategies. However, our experimental design does not allow us to quantify how pathogens modulate density-dependent competition among Proteaceae and Myrtaceae (e.g. Gibson *et al.* 1999; Connolly, Wayne & Bazzaz 2001). Quantifying how pathogen-mediated negative density dependence varies among co-occurring plant species of contrasting nutrient-acquisition strategies is an important avenue for future research that will help us better understand mechanisms of plant species coexistence in hyperdiverse vegetation (Laliberté *et al.* 2015).

For the Proteaceae tested here, while not killed by the native *Phytophthora* species, there was a reduction in overall growth. On the other hand, growth of ECM plants was not negatively affected by the presence of these native *Phytophthora* species. Branzanti, Rocca & Pisi (1999) showed that the inoculation of chestnut seedlings by *P. cinamomi* or *Phytophthora cambivora* reduces leaf and root size of non-mycorrhizal chestnut seedlings by 43–48%, while not affecting growth of chestnut seedlings previously inoculated with ECM fungi. Our results show how plants with different nutrient-acquisition can have contrasting responses to the same native pathogen. Results support the trade-off between P-acquisition efficiency and pathogen defence proposed by Laliberté *et al.* (2015). This trade-off could partly explain why Proteaceae do not dominate in severely P-impoverished systems, despite having a more efficient P-acquisition strategy than ECM species (Lambers, Martinoia & Renton 2015). On the other hand, the fact that we did not find differences in growth among Proteaceae species in the + *Phytophthora* treatments suggests that coexistence among Proteaceae is not modulated by soilborne pathogens. In this study, we did not evaluate whether non-mycorrhizal Proteaceae promote the local build-up of

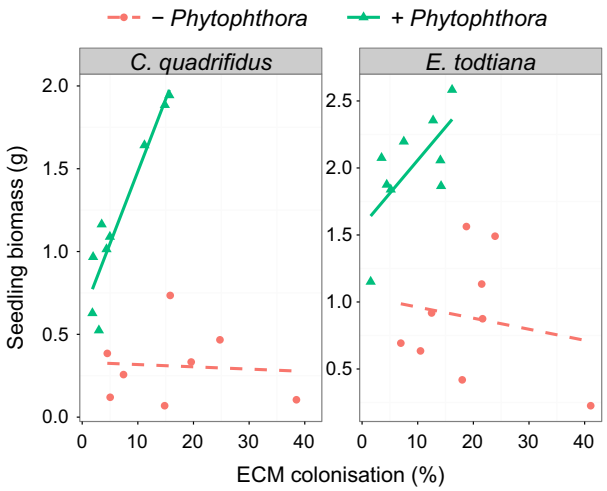

Fig. 6. Relationship between ectomycorrhizal (ECM) root colonization and final seedling biomass of two ECM species (*Calothamnus quadrifidus* and *Eucalyptus todtiana*). Seedlings were grown together with competing non-mycorrhizal cluster-rooted species, and exposed to two inoculum treatments: (i) − *Phytophthora* (red circles and dashed line) and (ii) '+ *Phytophthora*' (green triangles and solid line; mix of *Phytophthora arenaria*, *P.* taxon cooljarloo, *P.* taxon kwongan, *P.* aff. *rosacearum* and *P. rosacerarum*). R^2 values were only significant for the + *Phytophthora* treatment for both plant species and were 0.87 and 0.38 for *C. quadrifidus* and *E. todtiana*, respectively. Solid lines indicate significant relationship ($P \leq 0.05$), while dashed line indicates non-significant relationship ($P \geq 0.05$).

soilborne pathogens to a greater extent than ECM plant species, as has been hypothesized (Laliberté *et al.* 2015). Future studies should evaluate this possibility, as this process could lead to negative plant–soil feedback between Proteaceae and their associated soil biota which might further contribute to plant species coexistence in these ecosystems.

When planted together with Proteaceae, ECM plant biomass gain was greater in the presence of *Phytophthora* compared with that when grown in the absence of pathogens; conversely, biomass of Proteaceae species was lower. Likewise, several studies have shown how ECM fungi offer protection from pathogens to their host by several mechanisms, such as a physical barrier (Marx 1972) and the biosynthesis of fungicides (Duchesne, Peterson & Ellis 1988a,b). Hence, our results suggest that *Phytophthora* can affect growth of non-mycorrhizal plant species while not affecting that of co-occurring ECM species, thus conferring an advantage to ECM species in terms of accessing scarce P resources. Pathogen-mediated plant coexistence has been reported in other ecosystems and with herbaceous plants (Burdons & Chilvers 1974; Mills & Bever 1998), but to our knowledge, this is the first study to show empirical evidence of this for woody plants in a hyperdiverse, seasonally dry shrubland. Our glasshouse experiment used seedlings rather than mature plants, because the studied species are long-lived, slow-growing woody perennial plants. As such, care must be taken when extrapolating our results to longer-term interactions between mature plants. We believe that our results are relevant for mature plants, because plant competition is mainly for below-ground resources in this system, and plant nutrient acquisition primarily takes place in superficial soil layers, where plant nutrients and fine roots of both seedlings and mature plants are concentrated (Dodd *et al.* 1984).

In both experiments, seedling biomass of ECM plant species was positively correlated with ECM root colonization, but only in the presence of *Phytophthora*. This suggests an important role of ECM fungi in pathogen defence, as previously shown (Branzanti, Rocca & Pisi 1999; Whipps 2004). A previous study showed detrimental effects of ECM fungi on two *Phytophthora* species when cultured together on agar plates (Branzanti, Rocca & Zambonelli 1994). On the other hand, no relationship was found between biomass and ECM colonization in the − *Phytophthora* treatment. In a recent study from the same shrublands studied here, Teste *et al.* (2016) showed how external hyphal biomass of mycorrhizal fungi was very low in P-impoverished soils compared with that in younger and P-richer soils, despite mycorrhizal root colonization being high. This, together with our results from the present study, suggests that the main function of ECM fungi in these P-impoverished soils may not be to scavenge nutrients, but to protect ECM plants against root pathogens. This hypothesis deserves further attention as ECM fungi could still enhance nutrient uptake, which might not be reflected in seedling biomass, but in increased leaf nutrient concentrations (Smith, Anderson & Smith 2015).

Finally, our results show that native species of *Phytophthora* were generalist pathogens for both plant families, despite not affecting total biomass gain of ECM plant species. Indeed, root:shoot ratio of not only Proteaceae but also ECM plant species was lower in the presence of *Phytophthora* compared with that of plants grown in soil without *Phytophthora*, except for *E. todtiana*. Oomycetes cause damping-off and root damage (Cohen & Coffey 1986; Bell, Freckleton & Lewis 2006) and hence reduce the root:shoot ratio of their hosts. Many invasive *Phytophthora* species are generalist in Australia (Scott *et al.* 2009; Scott, Burgess & Hardy 2013), yet, until now, there was no information about the host specificity of many native species of *Phytophthora*. However, Rea *et al.* (2011) reported that *P. arenaria* is often associated with non-mycorrhizal, cluster-rooted *Banskia* species. This observation, taken together with our ECM root colonization results, provides some evidence that the resistance of ECM plant species is provided by ECM fungi, rather than an intrinsic defence of the ECM plant species themselves. Notwithstanding, we used fresh roots to inoculate both Proteaceae and Myrtaceae species with ECM fungi. This approach likely introduced micro-organisms other than ECM fungi. Hence, potential contamination by other endophytes or pathogens cannot be discarded. However, other pathogens would have been introduced equally, irrespective of plant species and treatment. Hence, any potential effects on seedlings would not have obscured our results.

In conclusion, our results show how native soilborne pathogens can equalize plant competition among seedlings of contrasting nutrient-acquisition strategies. We surmise that root pathogens may play a key role in coexistence of plants with

different nutrient-acquisition strategies in these hyperdiverse shrublands. Moreover, we provide further evidence for the hypothesis that there is a trade-off between P-acquisition efficiency and pathogen defence (Laliberté *et al.* 2015). We propose that in old, strongly weathered and severely P-impoverished soils, ECM fungi are important for pathogen defence and potentially the persistence of their hosts. Our results highlight the need for considering soil microbiota in studies on plant interactions as well as plant diversity and ecosystem functioning, since pathogens and mycorrhizal fungi may strongly affect the outcome of plant competition.

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Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.515j4 (Albornoz *et al.* 2016).

References

Aghighi, S., Burgess, T.I., Scott, J.K., Calver, M. & Hardy, G.E.S.J. (2015) Isolation and pathogenicity of *Phytophthora* species from declining *Rubus anglocandicans*. *Plant Pathology*, **65**, 451–461.

Albornoz, F.E., Burgess, T.I., Lambers, H., Etchells, H. & Laliberté, E.(2016) Data from: Native soilborne pathogens equalize differences in competitive ability between plants of contrasting nutrient-acquisition strategies. *Dryad Digital Repository,* doi: 10.5061/dryad.515j4.

Anagnostakis, S.L. (1987) Chestnut blight: the classical problem of an introduced pathogen. *Mycologia*, **79**, 23–37.

Bagchi, R., Press, M.C. & Scholes, J.D. (2010a) Evolutionary history and distance dependence control survival of dipterocarp seedlings. *Ecology Letters*, **13**, 51–59.

Bagchi, R., Swinfield, T., Gallery, R.E., Lewis, O.T., Gripenberg, S., Narayan, L. & Freckleton, R.P. (2010b) Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. *Ecology Letters*, **13**, 1262–1269.

Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E., Freckleton, R.P. & Lewis, O.T. (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, **506**, 85–88.

Bell, T., Freckleton, R.P. & Lewis, O.T. (2006) Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, **9**, 569–574.

Branzanti, M.B., Rocca, E. & Pisi, A. (1999) Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza*, **9**, 103–109.

Branzanti, M.B., Rocca, E. & Zambonelli, A. (1994) Effects of ectomycorrhizal fungi on *Phytophthora cambivora* and *Phytophthora cinnamomi*. *Micologia Italiana*, **23**, 47–52.

Brown, J.K.M. & Hovmøller, M.S. (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, **297**, 537–541.

Burdons, J.J. & Chilvers, G.A. (1974) Fungal and insect parasites contributing to niche differentiation in mixed species stands of Eucalypt saplings. *Australian Journal of Botany*, **22**, 103–114.

Cahill, D.M., Rookes, J.E., Wilson, B.A., Gibson, L. & McDougall, K.L. (2008) *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Australian Journal of Botany*, **56**, 279–310.

Cohen, Y. & Coffey, M.D. (1986) Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology*, **24**, 311–338.

Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics of Populations* (eds P.J.D. Boer & G.R. Gradwell), pp. 298–312. Center for Agriculture Publishing and Documentation, Wageningen, the Netherlands.

Connolly, J., Wayne, P. & Bazzaz, F.A. (2001) Interspecific competition in plants: how well do current methods answer fundamental questions? *The American Naturalist*, **157**, 107–125.

Dodd, J., Heddle, E.M., Pate, J.S. & Dixon, K.W. (1984) Rooting patterns of sandplain plants. *Kwongan: Plant Life of the Sandplain* (eds J.S. Pate & J.S. Beard), pp. 146–177. University of Western Australia Press, Nedlands, Australia.

Duchesne, L.C., Peterson, R.L. & Ellis, B.E. (1988a) Pine root exudate stimulates the synthesis of antifungal compounds by the ectomycorrhizal fungus *Paxillus involutus*. *New Phytologist*, **108**, 471–476.

Duchesne, L.C., Peterson, R. & Ellis, B.E. (1988b) Interaction between the ectomycorrhizal fungus *Paxillus involutus* and *Pinus resinosa* induces resistance to *Fusarium oxysporum*. *Canadian Journal of Botany*, **66**, 558–562.

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

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L. & Gurr, S.J. (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature*, **484**, 186–194.

Freckleton, R.P. & Lewis, O.T. (2006) Pathogens, density dependence and the coexistence of tropical trees. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **273**, 2909–2916.

Gibson, D.J., Connolly, J., Hartnett, D.C. & Weidenhamer, J.D. (1999) Designs for greenhouse studies of interactions between plants. *Journal of Ecology*, **87**, 1–16.

Gilbert, G.S. (2002) Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology*, **40**, 13–43.

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

Hayes, P., Turner, B.L., Lambers, H. & Laliberté, E. (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology*, **102**, 396–410.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Huston, M.A. (1994) *Biological Diversity: The Coexistence of Species*. Cambridge University Press, Cambridge.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *The American Naturalist*, **104**, 501–528.

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

Laliberté, E., Turner, B.L., Costes, T., Pearse, S.J., Wyrwoll, K.-H., Zemunik, G. & Lambers, H. (2012) Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. *Journal of Ecology*, **100**, 631–642.

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

Laliberté, E., Lambers, H., Burgess, T.I. & Wright, S.J. (2015) Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist*, **206**, 507–521.

Lambers, H., Martinoia, E. & Renton, M. (2015) Plant adaptations to severely phosphorus-impoverished soils. *Current Opinion in Plant Biology*, **25**, 23–31.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J. & Veneklaas, E.J. (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany*, **98**, 693–713.

Lambers, H., Raven, J.A., Shaver, G.R. & Smith, S.E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*, **23**, 95–103.

Lambers, H., Bishop, J.G., Hopper, S.D., Laliberté, E. & Zúñiga-Feest, A. (2012) Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. *Annals of Botany*, **110**, 329–348.

Lambers, H., Shane, M.W., Laliberté, E., Swarts, N.D., Teste, F.P. & Zemunik, G. (2014) Plant mineral nutrition. *Plant Life on the Sandplains in Southwest Australia, a Global Biodiversity Hotspot* (ed. H. Lambers), pp. 101–127. UWA Publishing, Crawley, UK.

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

Marx, D.H. (1972) Ectomycorrhizae as biological deterrents to pathogenic root infections. *Annual Review of Phytopathology*, **10**, 429–454.

Mills, K.E. & Bever, J.D. (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology*, **79**, 1595–1601.

Mordecai, E.A. (2011) Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs*, **81**, 429–441.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Newsham, K.K., Fitter, A.H. & Watkinson, A.R. (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, **10**, 407–411.

Pinheiro, J.C., Bates, D.M., DebRoy, S., Sarkar, D. & Team, R. (2012) *nlme: Linear and Nonlinear Mixed Effects Models. R Package Version, 3, 103*. The Comprehensive R Archive Network, Vienna, Austria.

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

Rea, A.J., Burgess, T.I., Hardy, G.E.S.J., Stukely, M.J.C. & Jung, T. (2011) Two novel and potentially endemic species of *Phytophthora* associated with episodic dieback of Kwongan vegetation in the south-west of Western Australia. *Plant Pathology*, **60**, 1055–1068.

Ryan, M.H., Tibbett, M., Edmonds-Tibbett, T., Suriyagoda, L.D.B., Lambers, H., Cawthray, G.R. & Pang, J. (2012) Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell & Environment*, **35**, 2170–2180.

Scott, P., Burgess, T. & Hardy, G. (2013) Globalization and Phytophthora. *Phytophthora: A Global Perspective* (ed. K. Lamour), pp. 226–232. CAB International, Boston, MA, USA.

Scott, P.M., Burgess, T.I., Barber, P.A., Shearer, B.L., Stukely, M.J.C., Hardy, G.E.S.J. & Jung, T. (2009) *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia*, **22**, 1–13.

Shane, M.W., Cramer, M.D., Funayama-Noguchi, S., Cawthray, G.R., Millar, A.H., Day, D.A. & Lambers, H. (2004) Developmental physiology of cluster-root carboxylate synthesis and exudation in harsh *Hakea*. Expression of phosphoenolpyruvate carboxylase and the alternative oxidase. *Plant Physiology*, **135**, 549–560.

Shearer, B.L., Crane, C.E. & Cochrane, A. (2004) Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. *Australian Journal of Botany*, **52**, 435–443.

Simamora, A., Hardy, G.E.S., Stukely, M. & Burgess, T.I. (2013) More new *Phytophthora* species from natural ecosystems in Western Australia. 19th Australasian Plant Pathology Conference, 25–28th November. Aukland, New Zealand. pp. 127.

Simamora, A.V., Stukely, M.J.C., Hardy, G.E.S.J. & Burgess, T.I. (2015) *Phytophthora boodjera* sp. nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update on the status of *P. alticola*. *IMA Fungus*, **6**, 319–335.

Smith, S.E., Anderson, I.C. & Smith, F.A. (2015) Mycorrhizal associations and phosphorus acquisition: from cells to ecosystems. *Annual Plant Reviews, Phosphorus Metabolism in Plants* (eds H. Lambers & W.C. Plaxton), pp. 409–440. Wiley-Blackwell Publishing, Chichester, UK.

Strobel, N.E. & Sinclair, W.A. (1991) Role of flavanolic wall infusions in the resistance induced by *Laccaria bicolor* to *Fusarium oxysporum* in primary roots of Douglas-fir. *Phytopathology*, **81**, 420–425.

Terborgh, J. (2012) Enemies maintain hyperdiverse tropical forests. *The American Naturalist*, **179**, 303–314.

Teste, F.P., Laliberté, E., Lambers, H., Auer, Y., Kramer, S. & Kandeler, E. (2016) Mycorrhizal fungal biomass and scavenging declines in phosphorus-impoverished soils during ecosystem retrogression. *Soil Biology and Biochemistry*, **92**, 119–132.

Turner, B.L. & Laliberté, E. (2015) Soil development and nutrient availability along a 2 million-year coastal dune chronosequence under species-rich Mediterranean shrubland in southwestern Australia. *Ecosystems*, **18**, 287–309.

Vierheilig, H., Coughlan, A., Wyss, U. & Piche, Y. (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology*, **64**, 5004–5007.

Viscarra Rossel, R.A. & Bui, E.N. (2016) A new detailed map of total phosphorus stocks in Australian soil. *Science of the Total Environment*, **542**, 1040–1049.

Whipps, J.M. (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*, **82**, 1198–1227.

Wurst, S., Kaiser, N., Nitzsche, S., Haase, J., Auge, H., Rillig, M.C. & Powell, J.R. (2015) Tree diversity modifies distance-dependent effects on seedling emergence but not plant–soil feedbacks of temperate trees. *Ecology*, **96**, 1529–1539.

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

Zuur, A.F., Leno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY, USA.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of models with their respective variance structures used.