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Facilitative and competitive interactions between mycorrhizal and nonmycorrhizal plants in an extremely phosphorus-impoverished environment: role of ectomycorrhizal fungi and native oomycete pathogens in shaping species coexistence

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Summary

- Nonmycorrhizal cluster root-forming species enhance the phosphorus (P) acquisition of mycorrhizal neighbours in P-impoverished megadiverse systems. However, whether mycorrhizal plants facilitate the defence of nonmycorrhizal plants against soil-borne pathogens, in return and via their symbiosis, remains unknown.
- We characterised growth and defence-related compounds in *Banksia menziesii* (nonmycorrhizal) and *Eucalyptus todtiana* (ectomycorrhizal, ECM) seedlings grown either in monoculture or mixture in a multifactorial glasshouse experiment involving ECM fungi and native oomycete pathogens.
- Roots of *B. menziesii* had higher levels of phytohormones (salicylic and jasmonic acids, jasmonoyl-isoleucine and 12-oxo-phytodienoic acid) than *E. todtiana* which further activated a salicylic acid-mediated defence response in roots of *B. menziesii*, but only in the presence of ECM fungi. We also found that *B. menziesii* induced a shift in the defence strategy of *E. todtiana*, from defence-related secondary metabolites (phenolic and flavonoid) towards induced phytohormone response pathways.
- We conclude that ECM fungi play a vital role in the interactions between mycorrhizal and nonmycorrhizal plants in a severely P-impoverished environment, by introducing a competitive component within the facilitation interaction between the two plant species with contrasting nutrient-acquisition strategies. This study sheds light on the interplay between beneficial and detrimental soil microbes that shape plant–plant interaction in severely nutrient-impoverished ecosystems.

Introduction

Severely phosphorus (P)-impoverished environments in south-western Australia exhibit high plant diversity. They contain many species that evolved adaptations to enhance P-acquisition efficiency. Proteaceae, one of the most abundant families in these environments, form cluster roots (CRs), a highly efficient P-acquisition strategy (Shane & Lambers, 2005). Cluster roots are short-lived nonmycorrhizal and groups of densely packed hairy rootlets effectively 'mine' P sorbed onto soil particles by exuding large amounts of carboxylates (Shane *et al.*, 2004). Conversely, mycorrhizas, alternative nutrient-acquisition strategies characterised by an association of fine roots with 'scavenging' fungal hyphae, are less efficient at acquiring nutrients in extremely P-impoverished environments (Abbott *et al.*, 1984; Bolan *et al.*, 1984; Treseder & Allen, 2002; Albornoz *et al.*, 2021). While the abundance of cluster-rooted species increases along a 2-Myr chronosequence with declining soil P availability in south-western Australia, that of both arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) species decreases, but they do not disappear (Zemunik *et al.*, 2015). Mobilisation of P and some micronutrients by cluster-rooted species may benefit neighbouring species with contrasting nutrient-acquisition strategies (Muler *et al.*, 2014; Shen *et al.*, 2024; Staudinger *et al.*, 2024; Yu *et al.*, 2023), and nutrient-impoverished environments are characterised by a prevalence of inter-species facilitation rather than competition (Callaway & Walker, 1997; Brooker *et al.*, 2008; Al-Namazi *et al.*, 2017; Lekberg *et al.*, 2018). The diverse array

of nutrient-acquisition strategies plays a crucial role in shaping community assemblies and maintaining overall plant species diversity, particularly in severely nutrient-impoverished environments (Lambers *et al.*, 2018).

Mycorrhizal fungi, which establish symbiotic associations with *c.* 80% of terrestrial vascular plants, enhance mineral nutrition, water uptake and overall growth of their host plants (Smith & Read, 2008). Ectomycorrhizal fungi also enhance the protection of the host plants against soil-borne pathogens through various mechanisms (Pozo & Azcón-Aguilar, 2007). A trade-off exists between efficient P acquisition and root defence levels, making CRs highly susceptible to pathogens (Albornoz *et al.*, 2017; Lambers *et al.*, 2018). However, whether ECM colonisation confers pathogen tolerance to neighbouring nonmycorrhizal plants is unknown. Investigating plant–plant interactions such as these in severely nutrient-impoverished environments provides valuable insights into the mechanisms that underlie species coexistence.

Introduced pathogens pose a significant threat to plant productivity and diversity – exemplified by the devastating impact of root-rot *Phytophthora cinnamomi* in highly diverse ecosystems (Lambers *et al.*, 2013; Hardham & Blackman, 2018) – but plants have also co-evolved with soil-borne pathogens, including native oomycete species of the *Phytophthora* genus (Ricklefs, 2010; Rea *et al.*, 2011; Albornoz *et al.*, 2017; Sarker *et al.*, 2023). Previous studies have demonstrated the enhanced resistance of both AM- and ECM-colonised plants to *Phytophthora* spp. (Guillemin *et al.*, 1994; Branzanti *et al.*, 1999; Ozgonen & Erkilic, 2007; Pozo & Azcón-Aguilar, 2007). In addition to providing physical protection to the root with a fungal mantle and Hartig net, ECM fungi also release antimicrobial compounds into the rhizosphere, inhibiting the growth of pathogens (Marx, 1972). Mycorrhizal colonisation triggers the induction of pathogenesis-related (PR) proteins and/or production of phenolic compounds, which are associated with pathogen defence (Pozo *et al.*, 1999; Pozo & Azcón-Aguilar, 2007; Ozgonen *et al.*, 2009). In the intricate network of plant-defence responses, some phytohormones also play a crucial role as signalling molecules that coordinate and regulate defence pathways. Small organic molecules like salicylic acid (SA) and jasmonic acid (JA) are examples of these phytohormones that activate defence mechanisms, including the synthesis of defence compounds (Durner *et al.*, 1997; Avanci *et al.*, 2010; War *et al.*, 2011). While mycorrhizal species benefit from their symbiotic association, nonmycorrhizal plants exhibit a greater susceptibility to soil-borne pathogens. The range of susceptibility between mycorrhizal and nonmycorrhizal species potentially contributes to maintaining the megadiversity in nutrient-impoverished environments through diversity-dependent resistance mechanisms (Johnson *et al.*, 2015; Thakur *et al.*, 2021).

Recent evidence suggests that nonhost plants may participate in mycorrhizal networks, expanding the scope of these symbiotic interactions (Wang *et al.*, 2022). Interestingly, two ECM fungi, *Tuber melanosporum* and *T. aestivum*, colonise the roots of nonmycorrhizal neighbouring plants, potentially acting as root endophytes (Schneider-Maunoury *et al.*, 2018, 2020). Arbuscular mycorrhizal fungi establish early signalling interactions with nonhost *Arabidopsis thaliana*, but subsequent colonisation fails to occur (Fernández *et al.*, 2019). In a compatible interaction with *Populus tremula* × *alba*, the ECM fungus *Laccaria bicolor* actively suppresses the induction of the host-defence responses and alters the sensitivity of roots to certain phytohormones, including enhanced and diminished responsiveness to SA and JA, respectively (Basso *et al.*, 2020). Ectomycorrhizal fungi also alter sensitivity of host plants to phytohormones, particularly to JA, during the establishment of the symbiosis (Enebe & Erasmus, 2023). The modulation of plant-defence responses occurs locally in the roots and can extend systemically throughout the plant, resulting in primed defence responses that increase the resistance of mycorrhizal plants to pathogens (Jung *et al.*, 2012; Vlot *et al.*, 2021). Understanding the responses of nonhost plants to ECM fungi and investigating the potential priming effects of these noncompatible interactions on defence responses to pathogens are critical areas for further research.

In this study, we aimed to explore the role of native oomycete pathogens, *Phytophthora* spp., and ECM fungi, in shaping the interactions between two plant species with contrasting P-acquisition strategies: *Banksia menziesii* (Proteaceae), a nonmycorrhizal CR-forming species, and *Eucalyptus todtiana* (Myrtaceae), an ECM species. In a glasshouse experiment, we cultivated *B. menziesii* and *E. todtiana* separately or together, with or without inoculation with ECM fungal spores and native oomycete (*Phytophthora* spp.). We investigated the plant growth and defence responses, and the interactions between the two species. We hypothesised that (1) the presence of native *Phytophthora* spp. will negatively impact the competitive ability of *B. menziesii*, as observed previously (Albornoz *et al.*, 2017); (2) the colonisation of *E. todtiana* roots by ECM fungi will trigger defence responses to *Phytophthora* spp. through increased levels of phytohormones and/or secondary metabolites; and (3) defence mechanisms will also be induced in the roots of *B. menziesii* when roots of *E. todtiana* are colonised by ECM fungi. By examining these interactions, we aimed to enhance our understanding of the intricate dynamics between two plant species with contrasting nutrient-acquisition strategies, native pathogens and ECM fungi, shedding light on the mechanisms underlying the coexistence of key dominant taxa of Proteaceae and Myrtaceae in a severely P-impoverished megadiverse environment.

Materials and Methods

Species selection and experimental design

Banksia menziesii R.Br. (Proteaceae) and *Eucalyptus todtiana* F.Muell. (Myrtaceae) share a similar distribution in southwestern Australia and were selected as representative of their families in kwongan vegetation (Pate & Beard, 1984) with contrasting P-acquisition strategies (i.e. carboxylate-exuding CRs and mycorrhizal associations, respectively).

Seeds of *B. menziesii* and *E. todtiana* were sown and inoculated with ECM fungal spores in seedling trays on 10 August 2021, and allowed to germinate in growth chambers. Seedlings were transferred into 4.5 l pots 7 wk later, on 1 October 2021, and allowed to acclimate in controlled glasshouses, with four

seedlings per pot (Fig. 1). On 31 November 2021, before inoculation with *Phytophthora* spp., six dead plants were removed from six pots. Nine weeks after transferring seedlings into final pots, on 7 December 2021, inoculum of *Phytophthora* spp. was inserted into the pots. Finally, plants were harvested 7 wk after oomycete inoculation, between 24 January and 29 January 2022. In total, there were 120 pots and 480 plants in the experiment (monocultures/mixture (three) × ECM treatment (two) × *Phytophthora* treatment (two) × replication (10); Fig. 1).

Soil preparation

Bulk soil (0–200 mm) was collected from multiple spatially distributed (*c.* 10 m apart within a 100 m radius) sites within the *c.* 2-Myr-old Bassendean dune system, where both species naturally co-occur (30°10′59.4″S, 115°07′55.8″E, *c.* 230 km north of Perth, Western Australia). All sites were located within the Jurien Bay dune chronosequence. Details on the physicochemical properties of soil from Bassendean dunes and the Jurien Bay chronosequence can be found elsewhere (Laliberté *et al.*, 2012; Turner & Laliberté, 2015; Turner *et al.*, 2018). Soil was air-dried, mixed and sieved (2 mm) before being subjected to triple-steam pasteurisation at 80°C for 2 h d^{-1} over 7 d (Albornoz *et al.*, 2017).

Inoculation of seeds with ectomycorrhizal fungi and growth conditions

Basidiocarps of the ECM fungus *Pisolithus* spp. with varied shape (round to ovoid), colour (yellow to brown) and size (30–80 mm long) were collected from different locations in Western Australia: Jurien Bay (30°11′05.8″S 115°06′39.1″E and 30°10′59.6″S 115°07′26.0″E, potential hosts *Melaleuca* sp. and *E. todtiana* trees, respectively); Lesueur National Park (30°09′41.5″S 115°11′59.1″E, potential host *E. todtiana*); Whiteman Park (31°48′59.9″S 115°55′19.2″E, potential host *E. todtiana*); and Bold Park (31°57′05.1″S 115°45′59.0″ E, potential host *E. gomphocephala*). Basidiocarps were air-dried at 25°C for 48 h before the outer layer was manually broken to collect fungal spores and stored in the dark at 20°C until inoculation. Isolates of *Pisolithus* sp. 8 isolate MU98/103 (potential hosts *Eucalyptus* and *Acacia* spp.), *P. albus* isolate MH115 (potential hosts *Eucalyptus* and *Acacia* spp.) and *P. microcarpus* isolate MH97 (potential hosts *Eucalyptus* and *Melaleuca* spp.) were recovered from long-term storage from the mycology herbarium at Murdoch University and used as inoculum. GenBank accession numbers for *Pisolithus* isolates MU98/103, MH115 and MH97 are AF374663, AF374714 and AF374713, respectively (Martin *et al.*, 2002).

Seeds of *B. menziesii* and *E. todtiana* were purchased (Nindethana Seed Co. Albany, WA, Australia) after collection from natural populations in Western Australia. Seeds were surface-sterilised in 1% (w/v) NaClO for 20 s and then in 70% (v/v) ethanol for 20 s and thoroughly rinsed in deionised (DI) water. Surface-sterilised seeds were sown in seedling trays filled with soil. A subset of seeds used in the mycorrhizal treatment of both species was inoculated with *c.* 10 mg of fungal spore inoculum placed around the seeds into the soil during sowing. Seeds were germinated under artificial light (12 h : 12 h, light : dark; 300 µmol photons m^{-2} s^{-1}) at 16°C until the cotyledons emerged, keeping inoculated seedling trays separate from uninoculated ones. The temperature was then increased to 20°C : 16°C, day : night to promote the growth of the seedlings. When seedlings had their second pair of leaves, they were transferred from the seedling trays into 4.5 l pots (190 mm diameter and 180 mm deep), which were sealed with plastic bags and filled with pasteurised Bassendean soil. Four plants were transferred into each pot: four *B. menziesii* (monoculture), four *E. todtiana* (monoculture), or two *B. menziesii* and two *E. todtiana* (mixture; Fig. 1). Pots were arranged in a randomised block design with

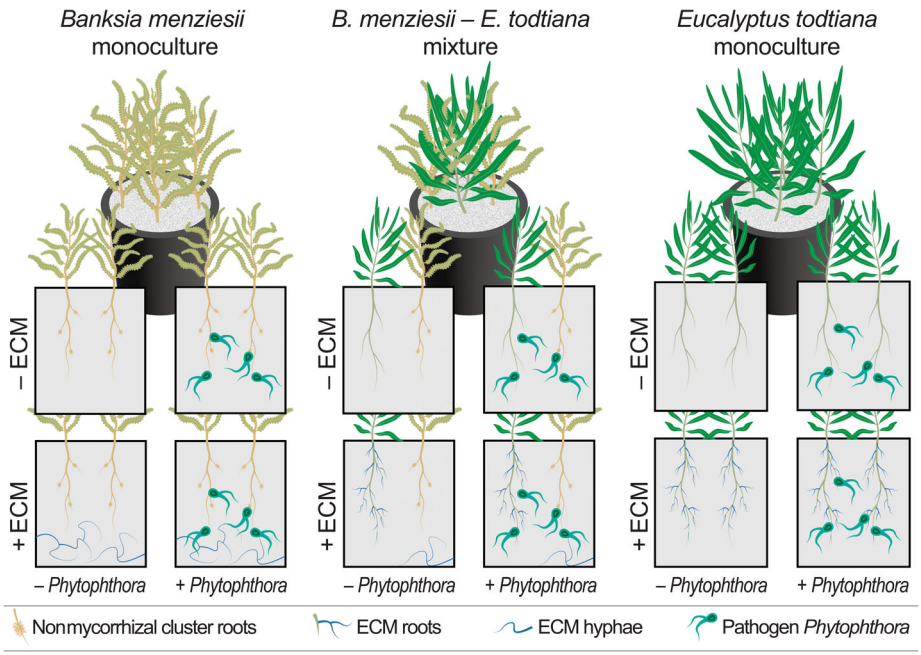

Fig. 1 Experimental design of this study conducted in a glasshouse at the University of Western Australia between August 2021 and January 2022. Seedlings of *Banksia menziesii* and *Eucalyptus todtiana* were grown in monoculture or mixture, with or without oomycete pathogens, *Phytophthora* spp., and with or without ectomycorrhizal (ECM) fungi ($n = 10$).

further randomisation every week, and pots were watered by weight to 75% pot capacity, twice weekly and were not fertilised. Pots were covered with a thick layer (*c.* 10 mm) of white plastic beads to avoid potential cross-contamination between ECM and pathogenic treatments and all material was disinfected between treatments during watering. Pots were placed in a controlled-environment glasshouse under natural light with temperatures of 22°C and 17°C during the day and night, respectively.

Inoculation of seedlings with *Phytophthora* spp.

Five native species of *Phytophthora* previously isolated from kwongan vegetation with reported pathogenicity on *Banksia* spp. were selected (Rea *et al.*, 2011; Simamora *et al.*, 2015; Burgess *et al.*, 2021): *P. arenaria* (CBS 125800), *P. thermophila* (PN 42.13), *P. kwonganina* (CBS 143060), *P. cooljarloo* (CBS 143062) and *P. constricta* (CPSM 21.42). GenBank accession numbers for ITS region sequences for these species are HQ013205, MF593927, JN547636, HQ012957 and OR256249, respectively. Subcultures of each species were prepared according to Belhaj *et al.* (2018), with minor modifications. In brief, subcultures were transferred onto sterile V8 agar medium at 27°C for 2 wk for growth of mycelium and then transferred into Erlenmeyer flasks with growth medium supplemented with sterilised millet seeds in the dark at 27°C for 8 wk. The flasks were sealed and shaken weekly to spread the inoculum evenly. Colonisation of the millet inoculum was confirmed and checked for contamination by plating *c.* 3 g subsamples of each species onto *Phytophthora*-selective NARPH agar medium (Hüberli *et al.*, 2000).

Nine weeks after establishment in 4.5 l pots, seedlings that were allocated to *Phytophthora* treatments were inoculated with 1 g of each millet-seed inoculum (total inoculum 5 g), according to Albornoz *et al.* (2017), with minor modifications. In brief, the inoculum was added into the void where a 15 ml Falcon tube was initially inserted into the soil in the middle of the pot. Pots without *Phytophthora* treatment were inoculated with 5 g of triple autoclaved millet-seed medium (121°C for 20 min on each of three consecutive days). Pots were watered immediately to 100% pot capacity to facilitate colonisation of soil and roots by the pathogens. Watering was then reduced back to 75% pot capacity, twice weekly until harvest.

Harvest and growth-related measurements

Seven weeks after *Phytophthora* inoculation, plants were harvested by severing mature and other leaves (young and senescing) from the stem. All aboveground parts (stem, mature and other leaves) were oven-dried at 70°C to a constant dry weight (DW) and their combined weight was recorded as the aboveground biomass. Mature leaves were ground into a fine powder in a vertical ball-mill grinder using plastic vials and yttrium-stabilised zirconium ceramic beads (GenoGrinder, Spex SamplePrep, Metuchen, NJ, USA). Mature leaf P ([P], mg g^{-1} DW) and Mn ([Mn], mg kg^{-1} DW) concentrations were determined from *c.* 200 mg ground leaf material using inductively coupled plasma optical emission spectroscopy (Optima 5300DV; PerkinElmer, Waltham, MA, USA) after digestion in hot concentrated (6 : 1 v/v) HNO_3 : $HClO_4$ (Zarcinas *et al.*, 1987). Leaf P content (mg) was calculated as mature leaf [P] multiplied by total leaf biomass.

The root systems of each plant in the pots were gently separated by washing off adhering soil with water. Most of the roots remained attached to the primary root and stem and could easily be traced back to the plant to which they belonged. Based on the different root morphology between the two species (i.e. branching pattern and colour), detached roots were attributed to a species to contribute to the average biomass per species per pot (see Statistical analyses). Lateral roots were separated from the primary root and CRs were collected separately for *B. menziesii*. The primary root was oven-dried at 70°C to constant weight. Lateral roots and CR were snap-frozen in liquid nitrogen, freeze-dried for 10 d (VirTis BenchTop Pro 'K' Freeze Dryer; SP Scientific, Warminster, PA, USA), then weighed for biomass and stored at −80°C with silica desiccant, until further analyses. The weights of the primary root, lateral roots, as well as CRs for *B. menziesii*, were combined to give the belowground biomass.

Microbial colonisation

A subsample of four to five cleaned soil-free and branched lateral roots was used for microscopic observation to assess colonisation by ECM fungi and *Phytophthora* spp. in inoculated treatments or lack thereof in noninoculated controls, following Vierheilig *et al.* (1998), with minor modifications. In brief, roots were cleared in 10% (w/v) KOH for 24 h at 60°C. Clearing was continued in fresh 10% (w/v) KOH at room temperature, if required, then the roots were rinsed in deionised water and stained with 3% (v/v) ink/vinegar for 45 min. Stained roots were rinsed with deionised water, de-stained in 1% (v/v) HCl for 35 min and stored in acidified glycerol (1% (v/v) HCl) until microscopic observation (Zeiss Axioskop fitted with Zeiss Axiocam; Zeiss).

In each treatment, three to four plants per species from each treatment were randomly selected and checked, with consistent observations as follows (Supporting Information Fig. S1). Roots of *B. menziesii* never showed any ECM fungi colonisation in either inoculated or noninoculated treatments, while those of *E. todtiana* that were inoculated were heavily colonised (> 80% of fine root tip regions observed, Fig. S1). Roots of *E. todtiana* that were not inoculated with ECM fungi did not show any colonisation. Roots of both species were abundantly colonised by *Phytophthora* oospores only in inoculated treatments.

Total root phenolics and flavonoids

Total root phenolic and flavonoid compounds were extracted from *c.* 80 mg of freeze-dried ground root material using 1.5 ml 75% (v/v) ethanol, shaken at 900 rpm at 20°C for 30 min in a thermomixer (Eppendorf, Hamburg, Germany), then incubated in an ultrasonic bath at 40 kHz for 20 min at 20°C. The extracts were cleared by centrifugation at 845 ***g*** at 20°C for 15 min. The extraction was repeated twice with 1 ml 75% (v/v) ethanol and shaking times of 45 and 90 min, respectively, followed by

sonication (Romo Pérez *et al.*, 2018). The supernatants were pooled and filtered through 0.45 µm filters and kept in the dark at −20°C until quantification.

Total root phenolic compounds were quantified as in Santas *et al.* (2008), with minor modifications. In brief, 200 µl (1 : 1 v/v) ethanol root extract: 75% (v/v) ethanol was mixed with 1.5 ml (1 : 10 v/v) Folin–Ciocalteu : H_2O reagent (Sigma-Aldrich) and 1.5 ml (2% w/v) sodium carbonate and incubated in the dark at 20°C for 2 h. Absorbance was measured at 765 nm using a spectrophotometer against a blank containing 75% (v/v) ethanol. Total phenolic equivalents were determined from a calibration curve prepared from a series of gallic acid (GA; Sigma-Aldrich) standards ranging from 0 to 200 mg l^{-1}. Results were expressed as mg GA equivalents (GAE) g^{-1} dry weight (DW).

Total root flavonoids were quantified by mixing 300 µl of root ethanol extract with 900 µl of 95% (v/v) ethanol, 60 µl of 10% (w/v) aluminium trichloride and 60 µl of 1 M potassium acetate and incubating in the dark at 20°C for 30 min. Absorbance was measured at 415 nm using a spectrophotometer against a blank containing 75% (v/v) ethanol. Total flavonoid equivalents were determined from a calibration curve prepared from a series of quercetin (Sigma-Aldrich) standards ranging from 0 to 200 mg L^{-1}. Results were expressed as mg quercetin equivalents g^{-1} DW.

Root phytohormones

The root phytohormones SA, JA, jasmonoyl-isoleucine (JA-Ile) and 12-oxo-phytodienoic acid (OPDA) were extracted from *c.* 30 mg of freeze-dried ground root material and analysed by UHPLC–MS/MS, using an Acquity UPLC I-Class system (Waters Corp., Milford, MA, USA) connected to a QTRAP 6500+ (SCIEX, Framingham, MA, USA) as in Glauser *et al.* (2014), with minor modifications. In brief, 1 ml of ethyl acetate : formic acid (99.5 : 0.5 v/v), spiked with d_5-JA, $^{13}C_6$-JA-Ile and d_6-SA at 100 ng ml^{-1}, was added to ground dry root powder (25 mg) inside a 2 ml microcentrifuge tube, which also contained three or four glass beads, and was vortexed for 10 s. Hormones were further extracted in a mixer mill (Retsch MM400; Retsch GmbH, Haan, Germany) at 30 Hz for 4 min. Samples were centrifuged at 14 000 ***g*** for 4 min, and the supernatant was transferred to new tubes. The extraction step was repeated with the pellet and 0.5 ml of ethyl acetate : formic acid (99.5 : 0.5 v/v). The supernatants were combined and evaporated to dryness in a centrifugal concentrator (CentriVap Centrifugal Concentrator, Labconco, KS City, MO, USA) at 35°C. The residue was re-suspended in 200 µl 50% (v/v) methanol, and the suspension was transferred to a 0.2 ml microcentrifuge tube and centrifuged for 3 min at 14 000 ***g***. The supernatant was transferred to HPLC glass vials for analysis. For HPLC, 2 µl of extract was injected onto an Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm particle size; Waters Corp., Milford, MA, USA). Mobile phase A consisted of H_2O : formic acid (99.95 : 0.05 v/v) and mobile phase B consisted of acetonitrile : formic acid (99.95 : 0.05 v/v). A gradient of 5–65% B in 6.5 min was applied, followed by column washing with 100% B and equilibration with 5% B for 2 min. The flow rate was set to 0.4 ml min^{-1} and the column temperature to 35°C. The mass spectrometer was operated in electrospray negative ionisation mode with multiple reaction monitoring (MRM). A six-point calibration curve (0.02, 0.1, 0.5, 5, 20 and 100 ng ml^{-1}, containing all isotopically labelled standards at 5 ng ml^{-1}) was used for quantification. Linear regressions weighted by $1/x$ were applied. Analyst v.1.7.1 was used to control the instrument and for data processing.

Statistical analyses

For growth-related measurements, that is aboveground, belowground, CR, non-CR and total biomass, the average of all plants from a given species within a pot constituted a data point. For example, plant total biomass was calculated as the average of the total biomass of four plants in monoculture treatments, and as the average of two plants for each species in mixture treatments. Five to twelve plants and three to five plants from each of the 12 treatments were randomly selected for leaf nutrient and defence-related measurements, respectively.

The differences among treatments were tested in all measured variables using multifactorial linear mixed-effect models, with the block as a random effect, using the nlme package (Pinheiro & Bates, 2000). Models with different variance structures were compared using the Akaike information criterion (AIC). The normality of the residuals of the best model based on the lowest AIC was checked with Shapiro–Wilks test, and log_{10}-transformed data were used to fit models when the assumption was not respected. First, we considered the effect of the other species by comparing 'monoculture' vs 'mixture' for each species, regardless of microbial treatments. We then tested the effect of the inoculation of *Phytophthora* spp. regardless of ECM inoculation. Finally, we tested the effect of ECM inoculation. Ectomycorrhizal treatment was used as the finest level of analysis in regard to the hypotheses of the study, that is ECM colonisation contributes to defence against *Phytophthora* spp. in roots of both mycorrhizal and nonmycorrhizal plants. Pearson correlation analysis was used to analyse the correlation between defence-related compounds. We characterised the defence strategies of the two species in the treatments using a principal component analysis (PCA), considering the following traits: root concentrations of phenolics, flavonoids, SA, JA, JA-Ile and OPDA. The PCA was run with the FactoMineR package on log_{10}-transformed data (Lê *et al.*, 2008). To test whether the defence strategies varied between the two species and in response to the treatments, the scores of each individual plant on the first two dimensions of the PCA explaining 76% and 11% of the variance, respectively, were extracted, and linear mixed-effect models were used to detect significant differences along the defence-strategy space. To compensate for interspecific variation, we characterised and analysed the distribution of each species along the defence-strategy space using species-specific PCAs and linear mixed-effect models, as described previously. Data were analysed using R software (R Core Team, 2023).

Results

Growth-related parameters of plants

Both *B. menziesii* and *E. todtiana* showed a significant effect on the growth of the other species when grown together. On average, the presence of *B. menziesii* increased the total biomass accumulation of *E. todtiana* by 52%, while its own biomass was reduced by 29% in mixture compared with monoculture (Fig. 2a; Table 1). For both species, similar patterns of biomass changes were observed for the above- and belowground biomass considered separately (Fig. S2; Table 1). Furthermore, the presence of *E. todtiana* increased the root-to-shoot ratio, CR biomass and CR-to-non-CR ratio of *B. menziesii* (Figs S2, S3; Table 1). In mixture and in the absence of pathogens, ECM fungi significantly contributed to a reduction in the growth of *B. menziesii* (Table S1). The ECM fungi had a positive effect on the growth of *E. todtiana* only in monoculture and in the absence of pathogens (Fig. 1a). Surprisingly, the presence of *Phytophthora* spp. did not affect the biomass accumulation of either species, regardless of whether they were grown in monoculture or mixture (Figs 1a, S2, S3; Table 1).

There was little variation in mature leaf [P] and [Mn] in both species grown either alone or in mixture (Figs 2b, S4; Table 1). However, inoculation with *Phytophthora* spp. significantly increased leaf [P] of *B. menziesii* in monoculture, while it was reduced in mixture (Fig. 2b; Table 1). Leaf [P] also increased in *E. todtiana* inoculated with *Phytophthora* spp. only in the monoculture (Fig. 2b; Table 1). Similar to plant biomass, leaf P content in both species was significantly impacted by the presence of the other species (Fig. 2c; Table 1). Inoculation with pathogens increased leaf P content in both species in monoculture, while it reduced that of *B. menziesii* and increased that of *E. todtiana* in mixture. Inoculation with ECM fungi reduced leaf P content in both species in mixture only in the absence of pathogens (Fig. 2c; Table 1).

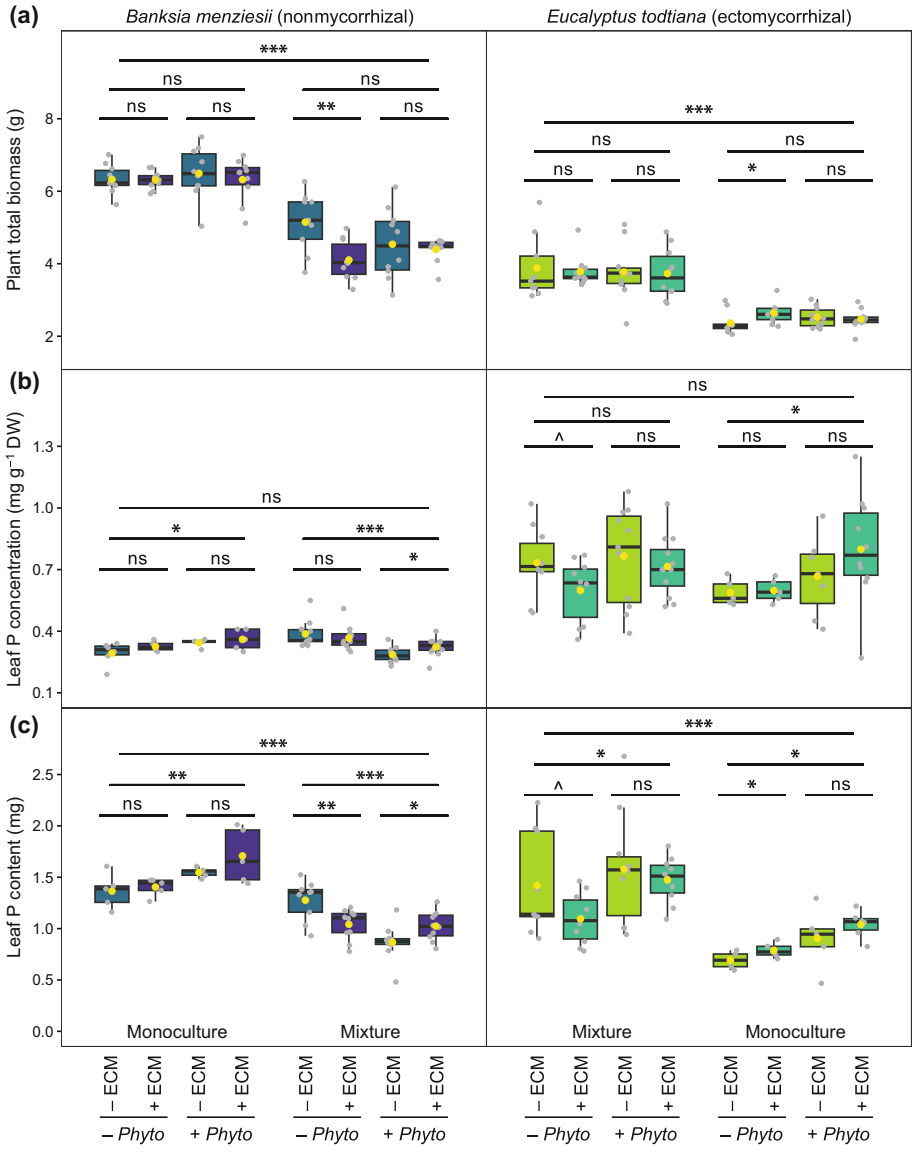

Fig. 2 Total plant biomass (a), leaf phosphorus (P) concentration (b) and leaf P content (c) of *Banksia menziesii* (left panels) and *Eucalyptus todtiana* (right panels) grown in monoculture or in mixture, inoculated with *Phytophthora* pathogens (+ *Phyto*) or not (− *Phyto*) and/or with ectomycorrhizal (ECM) fungi (+ ECM, dark colour) or not (− ECM, light colour). The bottom and top of the box denote the 25th and 75th percentiles, respectively, the central line is the median, and the yellow dot represents the mean ($n = 8$–10). Whiskers extend to the most extreme data points up to a maximum of 1.5 times the lower and upper quartiles. Significant differences between treatments were tested with linear mixed-effect models (^, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant, i.e. $P > 0.1$).

New Phytologist

Table 1 *P*-values of multifactorial linear mixed-effect models for growth-related traits of *Banksia menziesii* and *Eucalyptus todtiana* to test the significance of the effect of all treatments (i.e. monoculture vs mixture > – *Phyto* vs + *Phyto* > – ECM vs + ECM; ^, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

	B. menziesii – monoculture		*B. menziesii* – mixture		*E. todtiana* – mixture		*E. todtiana* – monoculture	
	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM
Total biomass (df = 132)	0.947 0.666 **<0.001***	0.538	**0.001**** 0.530	0.838	0.895 0.637 **<0.001***	0.936	**0.027*** 0.988	0.661
Aboveground biomass (df = 134)	0.422 0.265 **<0.001***	0.727	**0.014*** 0.413	0.921	0.792 0.788 **<0.001***	0.704	**0.011*** 0.409	0.662
Belowground biomass (df = 132)	0.395 0.508 **<0.001***	0.464	**<0.001***** 0.758	0.562	0.599 0.647 **<0.001***	0.797	0.137 0.571	0.984
Root : Shoot ratio (df = 132)	0.270 0.371 **<0.001***	0.493	**0.003**** 0.873	0.689	0.227 0.929 0.319	0.590	0.704 0.407	0.905
Cluster root biomass (df = 62)	0.901 0.254 **0.002****	0.526	**0.017*** 0.888	0.281	n/a			
Cluster root : Root ratio (df = 62)	0.428 0.369 **<0.001***	0.997	0.759 0.969	0.285	n/a			
Leaf [P] (df = 108)	0.211 **0.049*** 0.875	0.634	0.457 **<0.001*****	**0.036***	**0.074^** 0.183 0.774	0.540	0.905 **0.028***	0.163
Leaf P content (df = 94)	0.599 **0.001**** **<0.001***	0.210	**0.006**** **<0.001*****	**0.035***	**0.084^** **0.022*** **<0.001***	0.819	**0.050*** **0.010***	0.305
Leaf [Mn] (df = 108)	0.451 0.906 0.640	0.252	**0.092^** 0.900	**0.078^**	0.831 0.864 0.989	**0.099^**	0.579 0.186	0.952

Significant values ($P < 0.1$) are bolded for visualisation and the degrees of freedom (*df*) are given in brackets. ECM, ectomycorrhizal fungi; *Phyto*, *Phytophthora* spp.

Defence-related compounds in fine roots

Roots of *E. todtiana* had nearly twice the concentration of defence-related secondary metabolites (97 mg GAE g^{-1} DW phenolics, 3.9 mg quercetin equivalents g^{-1} DW flavonoids) compared with those of *B. menziesii* (55 mg GAE g^{-1} DW phenolics, 2.4 mg quercetin equivalents g^{-1} DW flavonoids; Figs 3, S5). The concentrations of these compounds in roots of each species were not affected by the presence of the other species. Interestingly, inoculation of *Phytophthora* pathogens also did not affect total root phenolic and flavonoid concentrations (Figs 3, S5; Table 2). By contrast, the presence of ECM fungi significantly increased the root phenolic concentrations of both species in a mixture and in the presence of pathogens (Fig. 3; Table 2).

In contrast to those of total phenolics and flavonoids, the concentrations of all measured defence-related phytohormones (SA, JA, JA-Ile and OPDA) were higher in roots of *B. menziesii* than in those of *E. todtiana* (Figs 4, S6). The concentrations of all measured phytohormones were greater in roots of *E. todtiana* growing with *B. menziesii* than in those in the monoculture, with 1.3-, 1.8-, 3.4- and 2.3-fold greater concentrations, on average, for SA, JA, JA-Ile and OPDA, respectively (Figs 4, S6; Table 2). Conversely, the concentrations of JA and JA-Ile were lower in roots of *B. menziesii* in the mixture than in those in the monoculture (33% and 40% lower, respectively). The concentration of SA in roots of *B. menziesii* was greater when grown with *E. todtiana* in the presence of ECM fungi and significantly increased with inoculation of pathogens (Fig. 4a; Table 2). The concentration of OPDA also significantly increased in roots of *B. menziesii* in the mixture and inoculated with *Phytophthora* spp. (Fig. S6b; Table 2). The concentrations of JA were marginally lower in roots of *E. todtiana* grown in the mixture, when inoculated with ECM fungi and in the absence of *Phytophthora* spp. (Fig. 4b; Table 2).

The correlations between all defence-related compounds were significant ($P < 0.05$) with concentrations of defence-related secondary metabolites negatively correlated with phytohormone concentrations (Fig. 5a). As expected, JA-related phytohormones (JA, JA-Ile and OPDA) showed a very strong correlation among each other. The negative correlation between constitutive defence-related secondary metabolites and measured phytohormone concentrations in roots of *E. todtiana* and *B. menziesii* was

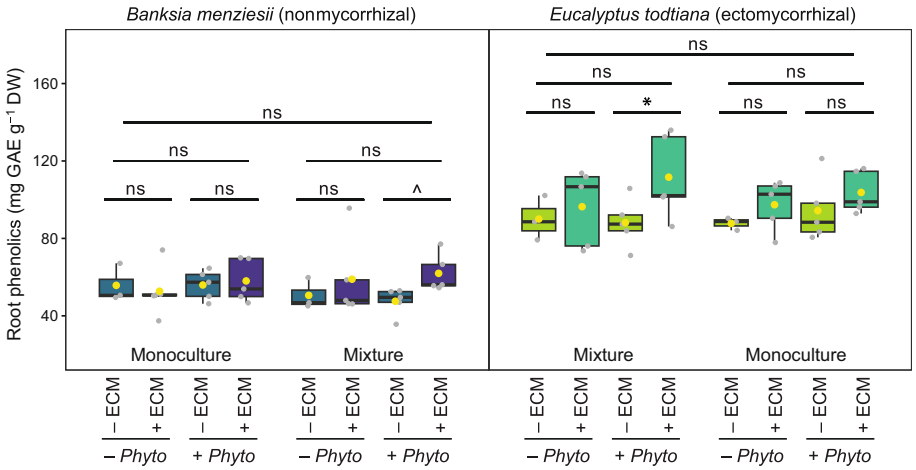

Fig. 3 Root total phenolic concentrations for *Banksia menziesii* (left panels) and *Eucalyptus todtiana* (right panels) grown in monoculture or in mixture, inoculated with *Phytophthora* pathogens (+ *Phyto*) or not (– *Phyto*) and/or with ectomycorrhizal (ECM) fungi (+ ECM, dark colour) or not (– ECM, light colour). The bottom and top of the boxes denote the 25th and 75th percentiles, respectively, and the central line is the median, and the yellow dot represents the mean ($n = 3$–5). Whiskers extend to the most extreme data points up to a maximum of 1.5 times the lower and upper quartiles. Significant differences between treatments were tested with linear mixed-effect models (^, $P < 0.1$; *, $P < 0.05$; ns, not significant, i.e. $P > 0.1$).

Table 2 *P*-values of multifactorial linear mixed-effect models for defence-related traits in roots of *Banksia menziesii* and *Eucalyptus todtiana* to test the statistical significance of the effect of all treatments (i.e. monoculture vs mixture > – *Phyto* vs + *Phyto* > – ECM vs + ECM; ^, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; df = 47).

	Banksia menziesii monoculture		*Banksia menziesii* mixture		*Eucalyptus todtiana* mixture		*Eucalyptus todtiana* monoculture	
	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM	– *Phyto* +/– ECM	+ *Phyto* +/– ECM
Phenolics	0.720	0.779	0.319	**0.053^**	0.572	**0.025***	0.392	0.357
	0.625		0.999		0.382		0.394	
	0.996				0.991			
Flavonoids	0.240	0.633	0.911	0.272	0.180	0.380	0.177	0.205
	0.931		0.127		0.827		0.664	
	0.958				0.526			
Salicylic acid	0.123	**0.060^**	0.196	0.147	0.856	0.587	0.833	0.569
	0.996		**0.039***		0.930		0.672	
	0.967				0.394			
Jasmonic acid	0.499	0.596	0.163	0.185	**0.065^**	0.847	0.911	0.755
	0.957		0.455		0.665		0.167	
	0.059^				**0.005****			
JA-Ile	0.632	0.669	0.162	**0.093^**	0.228	0.574	0.610	0.384
	0.519		0.932		0.761		**0.072^**	
	0.034*				**<0.001*****			
OPDA	0.859	0.696	0.403	0.105	0.188	0.379	0.292	0.135
	0.124		**0.009****		0.596		0.206	
	0.225				**<0.001*****			

Significant values ($P < 0.1$) are bolded for visualisation. ECM, ectomycorrhizal fungi; JA-Ile, jasmonoyl-isoleucine; OPDA, 12-oxo-phytodienoic acid; *Phyto*, *Phytophthora* spp.

reflected in a defence-strategy space along one main component in the PCA for defence-related compounds when considering individuals from both species (Fig. 5b).

The first axis of the PCA (PC1) explained 76% of the observed variance, which reflected that the presence of *B. menziesii* had a significant effect on the position of *E. todtiana* along the defence-strategy space, shifting towards higher phytohormone levels from the defence-related secondary metabolites (Figs 5b, S7). Furthermore, the presence of ECM fungi had a significant effect on the position of *E. todtiana* along PC1 in the defence-strategy space, but only in the presence of *B. menziesii*. Inoculation with *Phytophthora* spp. shifted the defence strategy of *E. todtiana* in the monoculture from defence-related secondary metabolites towards phytohormones (Fig. S7). There was very little variation among groups along PC2, with a significant shift of *B. menziesii* in the mixture and inoculated with *Phytophthora* spp., highlighting differences in concentrations of SA and flavonoids, the two variables with the largest contribution to PC2 (Figs 4a, S5, S7; Table S2).

The presence of the neighbouring species significantly shifted the distribution of both species along PC1 in individual PCAs per species (Fig. 5). In the presence of *E. todtiana*, *B. menziesii* shifted away from JA-related phytohormones (JA, JA-Ile and OPDA), strongly contributing to PC1, while we observed no differences along PC2 represented by secondary metabolites

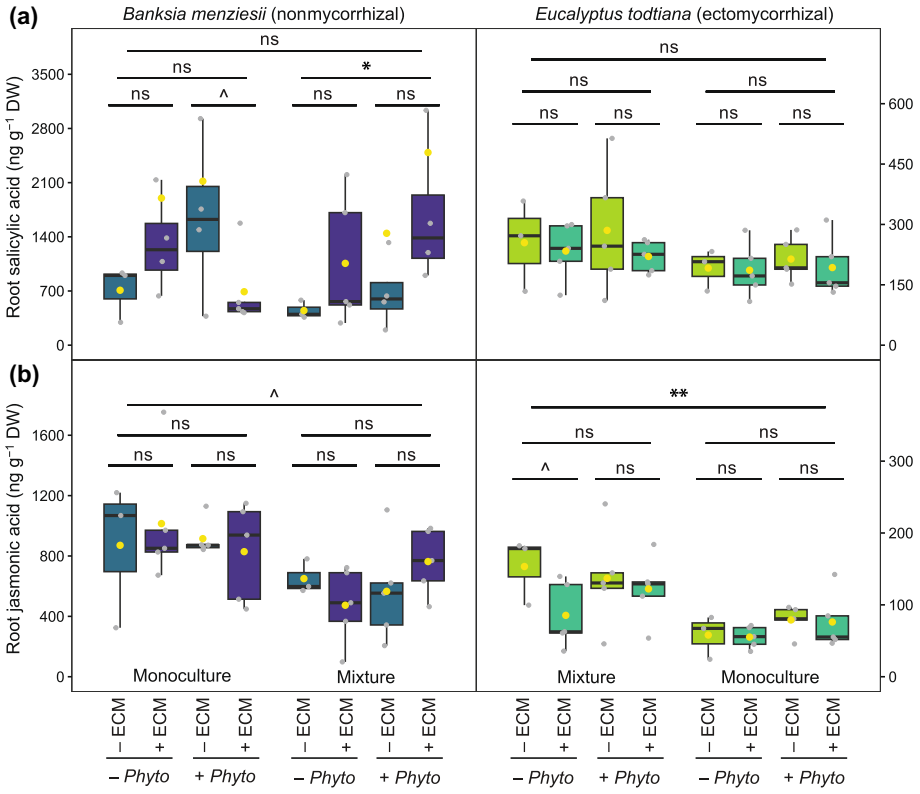

Fig. 4 Root salicylic acid (a) and jasmonic acid (b) concentrations for *Banksia menziesii* (left panels) and *Eucalyptus todtiana* (right panels) grown in monoculture or in mixture, inoculated with *Phytophthora* pathogens (+ *Phyto*) or not (− *Phyto*) and/or with ectomycorrhizal (ECM) fungi (+ ECM, dark colour) or not (− ECM, light colour). The bottom and top of the boxes denote the 25th and 75th percentiles, respectively, and the central line is the median, and the yellow dot represents the mean ($n = 3$–5). Whiskers extend to the most extreme data points up to a maximum of 1.5 times the lower and upper quartiles. Note different *y*-axis range for the two species. Significant differences between treatments were tested with linear mixed-effect models (^, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ns, not significant, i.e. $P > 0.1$).

(Fig. 5c–e; Table S3). Conversely, the distribution of *E. todtiana* shifted towards JA-related phytohormones, both in the monoculture in the presence of pathogens, and in the mixture (Fig. 5f,g; Table S4). Similar to *B. menziesii*, the distribution of *E. todtiana* did not significantly change along PC2 or PC3, explained by other defence-related compounds (Fig. 5h; Table S4).

Discussion

We explored key growth and defence-related traits in two plant species with contrasting P-acquisition strategies naturally occurring in severely P-impoverished environments and challenged by native soil-borne oomycete pathogens. Our results support the hypothesis that *B. menziesii*, a nonmycorrhizal CR-forming Proteaceae, facilitated the P acquisition and growth of *E. todtiana*, a mycorrhizal Myrtaceae that forms mycorrhizal association, but does not produce carboxylate-releasing CRs. However, the study of defence-related traits, that is constitutive defence-related secondary metabolites and signalling phytohormones, contradicted our hypotheses and the species have contrasting strategies resulted in various effects depending on the mixture of the two species, the inoculation with *Phytophthora* spp. and the presence of ECM fungi. We highlight a competitive component within the interaction between these mycorrhizal and nonmycorrhizal plants in an extremely P-impoverished environment involving a balance between the contrasting P-acquisition strategies of the plants and soil-borne microbes. This finding supports our hypothesis that native *Phytophthora* spp. impact the competitive ability of nonmycorrhizal species. This study enhances our understanding of the interactions between mycorrhizal and nonmycorrhizal plants in severely P-impoverished environments, mediated by detrimental and beneficial soil microbes, advancing on previous studies (Albornoz *et al.*, 2017; Lambers *et al.*, 2018).

Facilitative and competitive dynamics between a mycorrhizal and a nonmycorrhizal species

Surprisingly, the facilitation of the growth and P acquisition of *E. todtiana* by *B. menziesii* also involved a competitive component in the interaction. This was evident from the negative impact *E. todtiana* had on the growth and P content of *B. menziesii*. These impacts included reductions in above- and belowground biomass and leaf P content, as well as an increase in root-to-shoot ratio. Inclusion of *E. todtiana* also increased CR production and the CR-to-non-CR ratio in *B. menziesii*. Taken together, these results reflected the greater demand to mobilise P of *B. menziesii* in the mixture when *E. todtiana* competed for some of the mobilised P (Zhao *et al.*, 2021). Conversely, the significant increase in biomass and leaf P content without alteration of root-to-shoot ratio of *E. todtiana* indicated its P status was enhanced by facilitation by the carboxylate-releasing P-mobilising *B. menziesii*. In environments with a very low P availability, CR-forming and other carboxylate-releasing species commonly facilitate P acquisition of mycorrhizal and/or non-CR-forming neighbouring plants (Lambers & Teste, 2013; Muler *et al.*, 2014; Shen *et al.*, 2024; Staudinger *et al.*, 2024; Yu *et al.*, 2023). For instance, in the presence of a P-mobilizing facilitator (*B. attenuata*), *Hibbertia racemosa* (a non-CR-producing mycorrhizal species) adjusts its biomass-allocation pattern

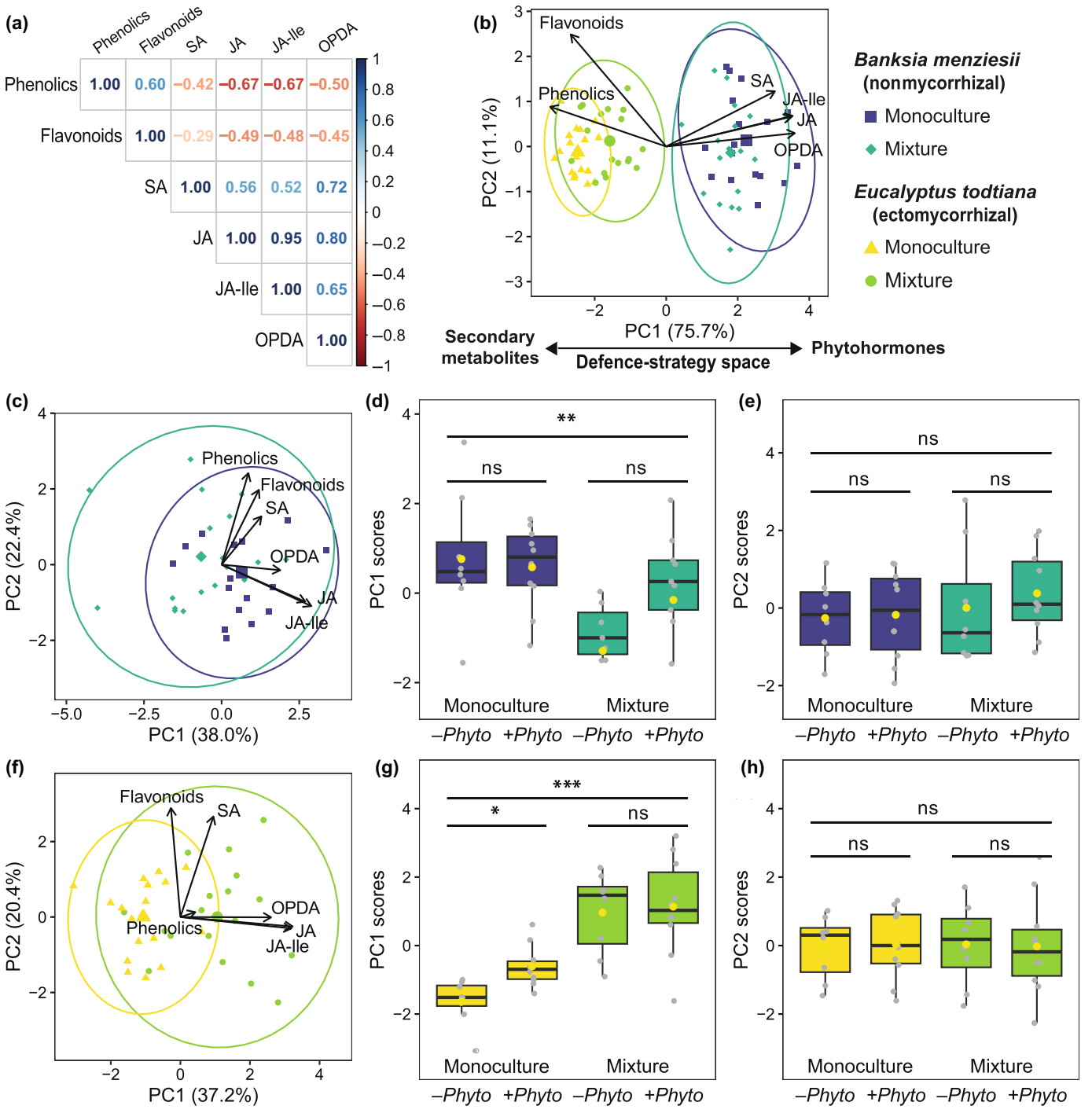

Fig. 5 Correlation matrix with Pearson's *r* coefficient ($P < 0.05$) (a), principal component analysis (PCA) of defence-related compounds in roots of *Banksia menziesii* and *Eucalyptus todtiana* (b); and PCA of defence-related compounds and test of the distribution of both species and pathogen treatments along a defence-strategy space represented by the first and second dimensions of each individual PCA for *B. menziesii* (c–e) and for *E. todtiana* (f–h). In boxplots (d, e, g and h), the bottom and top of the boxes denote the 25th and 75th percentiles, respectively, and the central line is the median, and the yellow dot represents the mean ($n = 3$–5). Whiskers extend to the most extreme data points up to a maximum of 1.5 times the lower and upper quartiles. Significant differences between treatments were tested with linear mixed-effect models (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant, i.e. $P > 0.05$). Outputs of the PCAs are given in Supporting Information Tables S2–S4. JA, jasmonic acid; JA-Ile, jasmonoyl-isoleucine; OPDA, 12-oxo-phytodienoic acid; *Phyto*, *Phytophthora* spp.; SA, salicylic acid.

and root system architecture (de Britto *et al.*, 2021). Here, we show that *E. todtiana* benefited with a net gain in above- and below-ground biomass but did not exhibit a different root-to-shoot ratio when growing with *B. menziesii*. Roots of both species were highly intermingled, particularly in the close proximity of CR of *B. menziesii* where we found many roots of *E. todtiana*. Roots were not

pot-bound, and we surmise that plant density likely affects the degree of facilitation and competition between the two species. We provide insights into the two-way interaction between mycorrhizal and nonmycorrhizal plants in extremely P-impoverished environments. This raises the question of the specific dynamics and mechanisms underlying the competitive effects imposed by ECM fungi that contribute to P acquisition on the present two coexisting species.

In the absence of both ECM fungi and pathogenic *Phytophthora* spp., aboveground and belowground biomass production of *B. menziesii* was reduced, whereas *B. menziesii* had a higher leaf P content in the presence of *E. todtiana*. This highlights the stronger competitive ability of nonmycorrhizal P-mining species in severely P-impoverished environments in the presence of oomycete pathogens. In the absence of these pathogens impacting their growth, however, nonmycorrhizal species strongly compete with mycorrhizal species for the acquisition of limiting P (Albornoz *et al.*, 2017; Lambers *et al.*, 2018). This further supports the view of a trade-off between nutrient-acquisition efficiency and defence against pathogens. The reduced growth of *B. menziesii* in the presence of ECM fungi, exclusively in the presence of *E. todtiana*, suggests that ECM fungi are strongly involved in nutrient acquisition by *E. todtiana*. This underscores the importance of ECM fungi in the interaction between nonmycorrhizal and mycorrhizal species, which exacerbated the competition between the two species. While facilitation of P acquisition in other species enhances their leaf [Mn] (Lambers *et al.*, 2021; Yu *et al.*, 2023), this was not the case in *E. todtiana*, indicating that mycorrhizal fungi were involved in acquiring P mobilised by *B. attenuata*.

Although there was no effect on leaf [P], despite P being the major limiting nutrient in the Bassendean soil used in this experiment (Laliberté *et al.*, 2012; Hayes *et al.*, 2014), the presence of both species significantly affected their respective growth and leaf P content. Phosphorus mobilised by CRs of *B. menziesii* and made available to roots of *E. todtiana* led to more growth in the latter. The absence of an effect on leaf [P] is because at very low P supply, all P that is taken up leads to more growth, rather than higher leaf [P], therefore resulting in higher leaf P content (De Groot *et al.*, 2003; Shane *et al.*, 2003; Shen, 2023). In turn, both ECM fungi and pathogen inoculation affected P the nutrition and growth of both species. While facilitation of P uptake can be proxied by leaf [Mn] (Lambers *et al.*, 2021; Yu *et al.*, 2023), leaf Mn of *E. todtiana* did not vary between the monoculture and mixture. This indicates that mycorrhizas likely contributed more to the P acquisition of *E. todtiana* than its roots did, when grown alongside *B. menziesii*. Phosphorus uptake is tightly regulated in mycorrhizal roots which down-regulate their direct root Pi-uptake pathway and promote fungal hyphal uptake and translocation into the root (Smith *et al.*, 2011). Moreover, Mn did not accumulate in leaves of *E. todtiana* because mycorrhizas mainly facilitate P uptake rather than Mn (Lehmann & Rillig, 2015), and Mn is likely intercepted by the ECM network (Canton *et al.*, 2016). However, the mechanisms by which *Phytophthora* spp. altered P nutrition of both species, in balance with the ECM fungi, remain unclear.

The ECM colonisation had negligible effects on the growth of *E. todtiana*, compared with what is commonly observed in environments with moderate P limitation or when plant growth is limited by other elements (Burgess *et al.*, 1993; Montesinos-Navarro *et al.*, 2019). In severely P-impoverished environments, ECM associations likely contribute other benefits to plants that are complementary to P nutrition, such as protection against root pathogens (Marx, 1972; Standish *et al.*, 2021). Teste *et al.* (2014) showed a synergistic effect between ECM hyphal scavenging and nutrient-mobilising CRs. However, the non-nutritional roles of ectomycorrhizas on neighbouring plants deserve further attention in nutrient-poor environments.

Phytochemical responses and pathways of defence against *Phytophthora* spp.

Eucalyptus todtiana constitutively exhibited higher levels of defence-related secondary metabolites (phenolics and flavonoids) than *B. menziesii*. Furthermore, like other *Eucalyptus* species (e.g. *E. pipularis*; Ashford *et al.*, 1989; Vesk *et al.*, 2000) and most woody species (Brundrett & Tedersoo, 2020), *E. todtiana* most likely possesses a suberised exodermis that acts as a physical barrier to root pathogens (Ranathunge *et al.*, 2008). *Banksia* species lack a suberised exodermis, presumably allowing the release of large amounts of carboxylates to the rhizosphere (Lambers *et al.*, 2018). A recent study demonstrated that the release of carboxylates in *Hakea laurina* (Proteaceae) originates from the root cortex, rather than the epidermis (Hirotsuna Yamada, pers. comm.). This further supports the contention of a trade-off between a high nutrient-acquisition efficiency in *Banksia* species and susceptibility to soil-borne pathogens, although the pathogen inoculation here did not affect biomass accumulation. While the *Phytophthora* spp. were selected to reflect the natural occurrence of native *Phytophthora* species with inoculation rates similar to previous studies (Albornoz *et al.*, 2017), we surmise that the pathogen pressure in the present study was not strong enough to suppress the growth of *B. menziesii* in monoculture, but it was strong enough to induce root phytochemical responses.

The intrinsically contrasting defence strategies observed between the present two species, that is higher levels of phytohormones in *B. menziesii* vs higher concentrations of secondary metabolites (phenolics and flavonoids) in *E. todtiana*, likely reflect divergent evolutionary adaptations of each plant to its environment. While the inoculation of *Phytophthora* spp. had little effect on the biomass of either species, we observed some relevant phytochemical responses, that is JA and JA-Ile concentrations increased in roots of *E. todtiana* inoculated with the pathogen in monoculture. This shows that *E. todtiana* perceived the inoculation of *Phytophthora* and induced a JA-related defence pathway. Interestingly, mycorrhizal associations contribute to priming the defences of their hosts by activating the JA-dependent responses (Pozo & Azcón-Aguilar, 2007). Most species of *Phytophthora* are haemibiotrophs with a variable biotrophy period before switching to necrotrophy (Sarker *et al.*, 2023). The JA pathway is commonly induced in response to necrotrophic pathogens (Glazebrook, 2005), as observed in roots of *E. todtiana* here. In roots of *B. menziesii*, the concentration of OPDA increased

with pathogen inoculation. This phytohormone has previously been linked to the JA-dependent pathway and shown to be involved in defence against pathogens (Gleason *et al.*, 2016), although its role might be extended to jasmonate-independent pathways (Jimenez Aleman *et al.*, 2022). The induction of JA-related phytohormones, including OPDA, commonly leads to a vast array of defence mechanisms, for example production of defence-related secondary metabolites or the reinforcement of root physical barriers (cell-wall thickening, suberisation and lignification). However, we surmise that the basal expression of defence-related secondary metabolism in roots of *E. todtiana*, which might be genetically controlled (Kroymann, 2011), was sufficient to counteract the low pathogenicity of *Phytophthora* spp. in this trial, mimicking that in the natural environment. In the mixture of the two plant species, *Phytophthora* spp. did not trigger phytohormone responses in roots of *E. todtiana*. We surmise that the increase in belowground biomass due to the facilitation by *B. menziesii* contributed to its stronger ability to resist pathogen infection.

Interactions between ectomycorrhizal fungi and *Phytophthora* spp. alter defence responses in the nonmycorrhizal *Banksia menziesii*

The defence responses of *B. menziesii* were affected by *Phytophthora* spp. and involved ECM fungi. Inoculation of *Phytophthora* spp. increased SA and OPDA concentrations in roots of *B. menziesii* in the mixture of the two plant species. The inoculation of ECM fungi also trended towards higher concentrations of SA in roots of *B. menziesii* both with and without pathogen inoculation ($P = 0.147$ and $P = 0.196$, respectively), in marked contrast to no significant effect in the monoculture ($P = 0.996$). The activation of SA-dependent responses can be coordinated by ECM colonisation (Pozo & Azcón-Aguilar, 2007). In the presence of both ECM fungi and *Phytophthora* spp., there was even a potential association between the increase in signalling molecules (SA and JA) and a systemic response with the production of secondary metabolites (i.e. phenolics) for both species in the mixture. Similar results were observed in *Capsicum annuum* when simultaneously inoculated with the oomycete *Phytophthora capsici* and AM fungi (Ozgonen *et al.*, 2009). Ectomycorrhizal fungi inhibit the negative effect of two species of *Phytophthora* in *Castanea sativa* seedlings, likely by providing a physical barrier around the roots (Branzanti *et al.*, 1999). Although there is no evidence of colonisation of roots of Proteaceae like *B. menziesii* by mycorrhizal fungi, the presence of the ECM fungi induced molecular responses in roots of *B. menziesii*. We propose that the higher concentrations of SA in roots of *B. menziesii* in the presence of ECM fungi may result from (1) a negative interaction induced by incompatibility with the roots of *B. menziesii* in the presence of *E. todtiana* (Pozo *et al.*, 2015; Benjamin *et al.*, 2022) or (2) signals emitted by *E. todtiana* or mycorrhizal hyphae to the roots of *B. menziesii* (Babikova *et al.*, 2013; Gorzelak *et al.*, 2015; Johnson & Gilbert, 2015). The ECM fungi may have been perceived as potential pathogens by roots of the nonmycorrhizal *B. menziesii*, but the origin of signals in the presence of mycorrhizal *E. todtiana* remains unknown. Interactions between neighbours do not require physical contact but may involve either water-soluble or volatile signals (Birkett *et al.*, 2001; Bais *et al.*, 2006; Weston & Mathesius, 2013; Sugimoto *et al.*, 2014; Erb, 2018). Whether those putative signals contributed to priming defence responses against *Phytophthora* spp. requires further investigation.

Conclusions

This study highlights the complex and intertwined nature of facilitative and competitive interactions between a mycorrhizal and a nonmycorrhizal species and their responses to soil-borne pathogens in extremely P-impoverished environments. Our results show that ECM fungi contributed to the competitive component of the interaction between *B. menziesii* and *E. todtiana* by inducing phytochemical responses in roots of *B. menziesii*. This highlights the importance of all four players – mycorrhizal and nonmycorrhizal plants, ECM fungi and pathogens – in shaping plant megadiversity (Laliberté *et al.*, 2015). Further investigation is warranted to explore the non-nutritional roles of ectomycorrhizas, particularly their involvement in defence against native *Phytophthora* species. Future investigations, including long-term studies, focusing on different combinations of species or considering other biotic and abiotic factors (e.g. herbivory and microbiome) are needed to fully understand how interactions among plant species shape species diversity in severely nutrient-impoverished environments.

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Competing interests

None declared.

Author contributions

CEG, HL, PMF, KR, PEH and TIB designed the study. CEG performed the experiment and collected the data. GG performed

the phytohormone analyses. CEG and DM performed microscopic observations. CEG, PMF, HL, PEH, KR and FdT analysed the data. CEG, FdT and PD developed the figures. CEG wrote the manuscript. All authors contributed critically to improve the manuscript and gave final approval for publication.

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Data availability

The data that support the findings of this study are freely available at doi: 10.26182/MX6Q-GE51 (DATASET-Facilitative and competitive interactions between mycorrhizal and non-mycorrhizal plants in an extremely phosphorus-impoverished environment: role of ECM fungi and native oomycete pathogens in shaping species coexistence).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Colonisation of roots of *Eucalyptus todtiana* and *Banksia menziesii* by ectomycorrhizal fungi and *Phytophthora* species.

Fig. S2 Below- and aboveground biomass and root-to-shoot ratios of *Banksia menziesii* and *Eucalyptus todtiana* in monoculture and in mixture, subjected to *Phytophthora* spp. and ectomycorrhizal treatments.

Fig. S3 Cluster-root (CR) biomass and CR-to-non-CR ratios of *Banksia menziesii* in monoculture and in mixture with *Eucalyptus todtiana*, subjected to *Phytophthora* spp. and ectomycorrhizal treatments.

Fig. S4 Manganese concentrations in leaves of *Banksia menziesii* and *Eucalyptus todtiana* in monoculture and in mixture, subjected to *Phytophthora* spp. and ectomycorrhizal treatments.

Fig. S5 Total flavonoid concentrations in roots of *Banksia menziesii* and *Eucalyptus todtiana* in monoculture and in mixture, subjected to *Phytophthora* spp. and ectomycorrhizal treatments.

Fig. S6 Jasmonoyl-isoleucine (JA-Ile) and 12-oxo-phytodienoic acid (OPDA) concentrations in roots of *Banksia menziesii* and *Eucalyptus todtiana* in monoculture and in mixture, subjected to *Phytophthora* spp. and ectomycorrhizal treatments.

Fig. S7 Distribution of *Banksia menziesii* and *Eucalyptus todtiana* in monoculture and in mixture, subjected to *Phytophthora* spp. and ectomycorrhizal treatments, along a defence-strategy space.

Table S1 Output of multifactorial linear mixed-effect models for growth-related traits of *Banksia menziesii* in the mixture.

Table S2 Output of the principal component analysis of defence-related compounds in roots of *Banksia menziesii* and *Eucalyptus todtiana*.

Table S3 Output of the principal component analysis of defence-related compounds in roots of *Banksia menziesii*.

Table S4 Output of the principal component analysis of defence-related compounds in roots of *Eucalyptus todtiana*.

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