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Eucalypt species drive rhizosphere bacterial and fungal community assembly but soil phosphorus availability rearranges the microbiome

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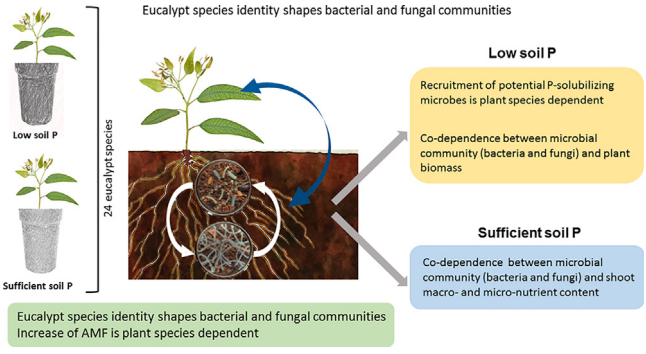
HIGHLIGHTS

- Eucalypt species shape bacterial and fungal community assembly in rhizosphere.
- Soil P availability modules the rhizomicrobiome of specific eucalypt species.
- Sufficient P strenghten the co-dependence between plant nutrient contents and eucalypt rhizomicrobiome.
- Low P intensifies the co-dependence between microbial communities and plant biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil phosphorus (P) availability may limit plant growth and alter root-soil interactions and rhizosphere microbial community composition. The composition of the rhizosphere microbial community can also be shaped by plant genotype. In this study, we examined the rhizosphere microbial communities of young plants of 24 species of eucalypts (22 *Eucalyptus* and two *Corymbia* species) under low or sufficient soil P availability. The taxonomic diversity of the rhizosphere bacterial and fungal communities was assessed by 16S and 18S rRNA gene amplicon sequencing. The taxonomic modifications in response to low P availability were evaluated by principal component analysis, and co-inertia analysis was performed to identify associations between bacterial and fungal community structures and parameters related to plant growth and nutritional status under low and sufficient soil P availability. The sequencing results showed that while both soil P availability and eucalypt species influenced the microbial community assembly, eucalypt species was the stronger determinant. However, when the plants are subjected to low P-availability, the rhizosphere selection became strongest. In response to low P, the bacterial and fungal communities in the rhizosphere of some species showed significant changes, whereas in others remained relatively constant under low and sufficient P. Co-inertia analyses revealed a significant co-dependence between plant nutrient contents and bacterial and fungal community composition only under sufficient P. By contrast, under low P, bacterial community composition was related to plant biomass production. In conclusion, our study shows that eucalypt species identity was the main factor modulating rhizosphere microbial community composition; significant shifts due to P availability were observed only for some eucalypt species.

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1. Introduction

The eucalypt group, family *Myrtaceae*, is economically important in the forestry sector and native to Australia, Papua New Guinea, Timor, Indonesia, Tasmania and the Philippines (Gonçalves et al., 2013). Members of this group are now distributed in diverse regions worldwide, including high-elevation forests, areas with high temperature variations, and humid and dry forests. To adapt to a wide range of edaphoclimatic conditions, eucalypts have evolved strategies to grow in soils poor in resources such as water and nutrients, particularly phosphorus (P) (Beadle, 1954).

Eucalypts vary in their physiological responses to soil P availability (Bahar et al., 2018), including contrasting P use efficiency and responsiveness to P addition (Bulgarelli et al., 2019). Low soil P availability can limit plant productivity and require chemical fertilisation to increase yield and plant performance (da Silva et al., 2016). Phosphorus is absorbed by roots mainly as orthophosphate (PO_4^{3-}), which may be tightly bound to soil iron (Fe) and aluminium (Al) oxides and hydroxides, particularly in tropical and subtropical soils (Friesen et al., 1997). Vance et al. (Vance et al., 2003) estimated that only 20% of applied P is absorbed by roots due to soil retention of phosphate. The P acquisition and use efficiency of plants depend on root system characteristics such as root architecture and morphology and associations with beneficial microorganisms (Valadares et al., 2020a). Beneficial associations with soil microorganisms can improve plant nutrient acquisition, growth, tolerance to stress conditions, and protection against soil pathogens (Richardson and Simpson, 2011; Valadares et al., 2020b). Rhizosphere microbes can also improve P-acquisition efficiency through the exudation of organic acids and enzymes (Richardson and Simpson, 2011). However, the composition and P response of the bacterial and fungal communities in the rhizosphere of eucalypt species have not been described.

Mycorrhizal fungi are soil-borne microorganisms that are directly involved in P acquisition and transfer to host plants. Symbiosis between fungi and roots may be favoured under P scarcity (Smith and Read, 2008). Fungi and bacteria that participate in soil P cycling by hydrolysing organic P and desorbing P bound to soil particles are called phosphate-solubilising microorganisms (PSM) (Richardson and Simpson, 2011). Plants attract PSM to their rhizosphere according to their root structure and physiology, mainly through the compounds they exude (Broeckling et al., 2008). Thus, root exudates can determine the composition of the rhizosphere microbial community and soil microbial assemblage (Sasse et al., 2018). These communities may be challenged by factors influencing root activity, such as plant nutritional demands (Kuramae et al., 2012). Thus, the microbial community in the rhizosphere is defined not only by the quality and quantity of exudates, which depend on plant genotype or species, but on the physiological status and developmental stage of the plant (Schlemper et al., 2018). Studies of maize (Gomes et al., 2018), switchgrass (Sawyer et al., 2019), and soybean (Liu et al., 2019) have indicated that the structure of rhizosphere microbial communities is more closely related to plant genotype than to soil fertility or soil type. In addition, a recent study with eucalypt plants showed that aging increases the diversity of the associated soil bacterial community, as the quality of litter produced by older trees alters soil nutrient dynamics, favouring taxa related to nitrogen and sulphur cycling processes (Qu et al., 2020).

Soil microbial community diversity and plant productivity are linked, and altering this interaction may have consequences for the ecosystem both above- and belowground (Schnitzer et al., 2011). Nutrient addition affects soil microbial community composition directly or indirectly by decreasing oligotrophic bacterial taxa, methanogenic archaea and mycorrhizal fungi (Leff et al., 2015). The diversity of arbuscular mycorrhizal fungi (AMF) belowground depends on plant community composition, as the plant host is the source of fungal carbon, whereas bacterial diversity is more influenced by the quality of soil organic matter and root exudates (Smith and Read, 2008; Millard and Singh, 2010).

In the present study, we examined the assembly of bacterial and fungal communities in the rhizospheres of young plants of 24 eucalypt species grown under low or sufficient soil P availability. We expected that the influence of soil P availability on the rhizosphere microbial community varies according to eucalypt species. We also expected that shifts in the assembly of microbial communities are related to the response of plants to soil P availability. Given these expectations, we hypothesised that under low P, the rhizosphere is enriched in microbial taxa with P-mobilising capabilities.

2. Material and methods

2.1. Experimental design and growth conditions

We previously evaluated the growth efficiency of young plants of 24 eucalypt species under low P conditions and their responsiveness to the addition of P (Bulgarelli et al., 2019). In this study, rhizosphere soil was collected from plants of the same experiment to evaluate the composition of the microbial communities in response to low P availability in the soil. The experimental design consisted of 24 eucalypt species and two P levels (low P and sufficient P), and each treatment had three replicates, giving a total of 144 soil experimental units. The 24 species were *Eucalyptus acmenoides*, *E. amplifolia*, *E. brassiana*, *E. camaldulensis*, *Corymbia citriodora*, *E. deanei*, *E. cladocalyx*, *E. dunnii*, *E. exserta*, *E. globulus*, *E. grandis*, *C. maculata*, *E. microcorys*, *E. pellita*, *C. henryi*, *E. propinqua*, *E. resinifera*, *E. robusta*, *E. saligna*, *E. tereticornis*, *E. urograndis*, *E. urophylla*, *E. crebra* and *E. botryoides*. Seeds were disinfected by immersion in 2% sodium hypochlorite solution for 10 min, washed three times for 1 min each in sterile water and then germinated in a mix of a commercial garden substrate and a sample of a ferrosol soil sample (1:1, v:v). The chemical characteristics of the original soil sample used in this experiment are described in Bulgarelli et al. (2019). The commercial garden substrate (Vida Verde ®) showed the following chemical characteristics: pH_{H2O} 4.8, P 1 mg kg^{-1}, K 56 mg kg^{-1}, organic carbon 306 g kg^{-1} and carbon to nitrogen ratio of 78. When the plantlets had two true leaves, they were transferred to 7-L pots containing the soil-substrate mix with low or sufficient P levels. The substrate used for growing the plants after transplantation was a mix (1:1, v/v) of soil, the same ferrasol sample used for germination, and coarse sand. This substrate showed a concentration of 3,7 mg kg^{-1} of available P (P resin) and considered the "low P" treatment. For "sufficient P" treatments additional phosphate was added to reach a concentration of 7.7 mg kg^{-1}, that is within the adequate range for eucalypt cultivation in Brazil (Raij et al., 1996). P was supplied to the soil as KH_2PO_4 only for soil samples of the sufficient P treatments. Here "available P" refers to the P concentration extracted with anion-exchange resin. The chemical characteristics of the substate are described in Bulgarelli et al. (2019). Plants were maintained for 23 weeks under greenhouse conditions with average maximum and minimum temperatures of 38.9 °C and 19.2 °C, respectively, between September 2017 and June 2018, in Campinas (22°49′10.38"S 47°04′12.88"W), São Paulo, Brazil. The photoperiod ranged between 12/12 and 10/14 h light/dark cycle, with a maximum photon flux density of 1800 μmol m^{-2} s^{-1}. Nitrogen (N) was supplied as aqueous solutions of NH_4NO_3 and KNO_3 on five occasions during the experiment at a total of 125 mg of N per plant (Bulgarelli et al., 2019).

2.2. Soil sampling and total DNA extraction

At harvest, the roots were manually and carefully separated from the soil, and the rhizosphere soil adhered to the roots was collected with the aid of brushes, immediately frozen in liquid nitrogen and stored at −80 °C. A total of 0.25 g of rhizosphere soil from each replicate was used for total DNA extraction and purification with the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and quality were determined in a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.3. 16S and 18S rRNA gene amplification, sequencing, and processing

The microbial community composition was characterised by amplicon sequencing of V4-V5 region of the 16S, and V4 region of the 18S rRNA genes. The 16S rRNA gene was amplified by PCR with the eubacterial

primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′). The V4 region of the 18S rRNA gene was amplified by PCR with the primers FR1 (5′-AICCATTCAATCGGTAIT-3′) and FF390.1 (5′-CGWTAACGAACGAGACCT-3′) based on Verbruggen et al. (2012). Amplicons were sequenced using the Illumina MiSeq platform at the Genome Quebec Innovation Centre (Canada).

Raw sequencing data were assessed using FastQC v11.9 prior to processing (Andrews, 2015), and most sequences showed high Phred quality scores (≥ 25). Residual adapter and primer sequences were trimmed with cutadapt v1.12 (Martin, 2011). All subsequent analyses were conducted using R v4.0.1. The 16S and 18S rRNA gene sequences were processed using the R package dada2 (Callahan et al., 2016) and aligned against the online SILVA rRNA gene database (version 138) (Quast et al., 2013) with a confidence threshold of 80%. Sequences assigned as chloroplast and mitochondrial were removed, and the phyloseq package (McMurdie and Holmes, 2013) was used to generate taxonomic assignments as amplicon sequence variants (ASVs). The table of found taxa in each treatment was generated at the genus level, and only taxa occurring in 10 or more samples were considered in further analyses.

2.4. Statistical analyses

Statistical analyses were conducted in R v3.1.1 using different packages. The Gjam package (Clark et al., 2017) was used with the microbial community data table (at genus level for bacteria and species level for fungi) to estimate the effects of both plant species and P availability on the rhizosphere soil microbiome in the form of regression coefficients. The main advantage of using GJAM package is its capacity to analyze compositional data and avoid potential bias as previously described (Gloor et al., 2017). For model diagnosis, the Markov Chain Monte Carlo (MCMC) method was used to check when the estimated coefficients reached a stable value (after 2000 simulations). Since the experiment consisted of a two-way factorial design, regression coefficients were compared against the following hypotheses: H1 - within each eucalypt species there is a difference between P availabilities; H2 - within each P availability there is a difference between eucalypt species. Therefore, the regression coefficients represent the different treatments in shaping the microbiome. A positive or negative coefficient indicates that the treatment significantly shifted the abundance of a microbe taxon when compared against the global mean that represents our null hypotheses (lack of differences between plant species and/or P availability). The set of all significant regression coefficients constitutes the microbiome profile and represent, in our study, the set of microbes selected by one plant species under a specific condition of P availability. The microbiome profiles for each treatment combination were highlight using a heatmap. To identify similarities between the treatment effect on the microbes, we analysed the similarity between the microbiome profiles via cluster analysis using Ward's algorithm (Euclidean distance) and principal component analysis (PCA). The PCAs of the two P availability levels were merged, and an arrow plot was used to highlight modifications in the composition of microbial communities in the rhizosphere of eucalypt species induced by low soil P availability. In the arrow plot, the PCA data points under low and sufficient P were connected by an arrow, the length of which corresponded to the intensity of the microbiota response.

Co-inertia analysis was used to examine the general associations of bacterial and fungal communities with plant variables [biomass production and macro- and micro-nutrient content across the two P treatments, both data sets extracted from Bulgarelli et al. (2019)]. Co-inertia analysis is a multivariate analysis that describes the relationships between two or more groups of variables via the analysis of their co-variance. For the microbial communities, we accounted for the effects of P availability by subsetting the fungal and bacterial communities according to their response to the plant selection. The fungal communities were subset into those that were significantly affected by eucalypt species (FungiSig) and those that did not show significant changes (FungiNS). The bacterial communities were subset in a similar manner (BactSig and BactNS). Other groups of variables corresponded to plant factors: biomass (leaf, stem, and root biomass production); macronutrient content (ContMacro, e.g., phosphorus, nitrogen, and magnesium content in shoots); and micronutrient content (ContMicro, e.g., copper, iron, zinc, and manganese content in shoots). The plant nutrition and growth dataset were obtained in our previous study (Bulgarelli et al., 2019) from the same plants used to collect the rhizosphere soil studied here. Community abundances were transformed into centred log-ratio (CLR) values (Gloor and Reid, 2016). The significance of the co-variance was checked with a Monte Carlo test (999 permutations) on the sum of values in the co-inertia analysis (Dray et al., 2003). Significance of the coefficients of co-inertia was considered for *p*-values (permutation values) smaller than 0.05, and those *p*-values between 0.05 and 0.10 were considered near-significant.

3. Results

3.1. 16S and 18S rRNA partial gene sequencing

For bacteria, a total of 3,306,493 raw reads from 143 samples (the third replicate of *E. acmenoides* under sufficient P was lost) were generated after paired-end sequencing with a read length of 250 bp. After quality assessment and filtering of chimeric, chloroplast and mitochondrial sequences, 2,462,435 bacterial reads and 4,509,119 fungal reads with a length of 200–240 bp were clustered into 15,591 and 1959 ASVs respectively. After taxonomic classification at the genus level and selection of the taxa that occurred in ten or more samples, 184 bacterial taxa belonging to 15 phyla, 27 classes, 77 orders, 109 families and 135 genera (Supplementary Table 1) and 24 fungal taxa belonging to 4 phyla, 8 classes, 15 orders, 11 families and 5 genera (Supplementary Table 2) were obtained.

3.2. Modulation of bacterial and fungal communities by eucalypt species and P availability

Fig. 1 shows the changes in bacterial (Fig. 1A) and fungal (Fig. 1B) community composition in the rhizosphere in response to P availability. The values of arrow length, which are reported in Supplementary Tables 3 and 4, indicate the intensity of the microbiota response to low P. Eucalypt species was the main factor explaining the differences in the bacterial (Fig. 1A) and fungal (Fig. 1B) rhizosphere communities in response to P availability. The bacterial communities in the rhizosphere of *E. deanei* (3.17), *E. microcorys* (3.13), *E. saligna* (2.84), *E. robusta* (2.55) and *E. grandis* (2.51) underwent the greatest shifts in composition in response to low P, while the rhizosphere bacterial communities of *E. cladocalyx* (0.16), *E. urograndis* (0.49), *E. pellita* (0.55), *E. propinqua* (0.99) and *E. brassiana* (1.04) exhibited the smallest changes in response to low P compared with sufficient P (Fig. 1A and Supplementary Table 3). The composition of the fungal communities in the rhizosphere of *E. robusta* (3.48), *E. deanei* (3.30), *E. citriodora* (2.98), *E. exserta* (2.98) and *E. camaldulensis* (2.87) changed significantly in response to low soil P availability, while those of *E. globulus* (0.07), *E. microcorys* (0.18), *E. grandis* (0.35), *E. saligna* (0.77) and *E. brassiana* (0.95) exhibited small changes in response to low P (Fig. 1B and Supplementary Table 4). PCA of the arrow plot in Fig. 1 revealed that for both axes, the distance between species was larger than the difference in P availability conditions within the same eucalypt species. For example, under sufficient P (base of the arrow), the distance between *E. acmenoides* and *E. grandis* is larger than the arrow length of each of these species (representing the P response). Therefore, the rhizosphere bacterial communities of these two eucalypt species differed greatly in composition. Interestingly, under low P conditions, the arrows representing *E. acmenoides* and *E. grandis* converged to the same region in the PCA plot, suggesting that these two species had similar rhizosphere bacterial community compositions under low P (Fig. 1A). Similarly, the rhizosphere fungal communities of *E. resinifera* and *E. exserta*, for example, differed significantly in composition under sufficient P but converged to more similar compositions under low P (Fig. 1B).

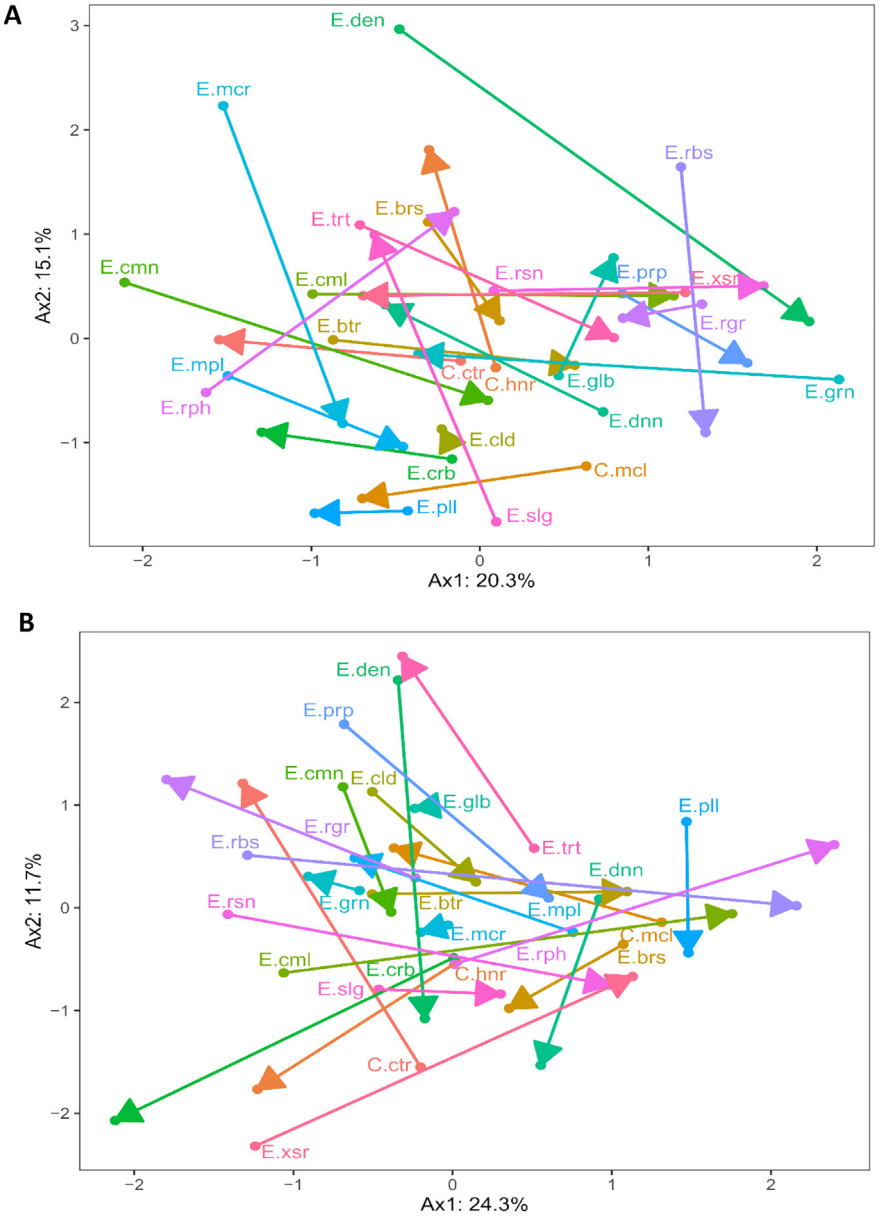

Fig. 1. Arrow plot showing the changes in the bacterial (A) and fungal (B) rhizosphere communities of 24 eucalypt species in response to low P availability. Arrow length corresponds to the intensity with which the microbiome responded to low P conditions (head of the arrow) compared with sufficient P (base of the arrow). *Eucalyptus acmenoides* (E.cmn), *E. amplifolia* (E.mpl), *E. brassiana* (E.brs), *E. camaldulensis* (E.cml), *Corymbia citriodora* (C.ctr), *E. deanei* (E.den), *E. cladocalyx* (E.cld), *E. dunnii* (E.dnn), *E. exserta* (E.xsr), *E. globulus* (E.glb), *E. grandis* (E.grn), *C. maculata* (C.mcl), *E. microcorys* (E.mcr), *E. pellita* (E.pll), *C. henryi* (C.hnr), *E. propinqua* (E.prp), *E. resinifera* (E.rsn), *E. robusta* (E.rbs), *E. saligna* (E.slg), *E. tereticornis* (E.trt), *E. urograndis* (E.rgr), *E. urophylla* (E.rph), *E. crebra* (E.crb) and *E. botryoides* (E.btr).

3.3. Bacterial community assembly in the rhizosphere of eucalypt species

The main differences in eucalypt rhizosphere bacterial composition under sufficient and low soil P are illustrated as significant positive and negative shifts in the relative abundances of bacterial phyla compared with the global mean from our null hypothesis in Fig. 2. *E. grandis* and *E. robusta* had the highest number of bacterial taxa with significant shifts under sufficient P, and most of the phyla exhibited negative shifts (Fig. 2A and Supplementary Table 5). Under low P, *E. propinqua* and *E. resinifera* had the highest number of bacterial taxa with significant shifts, with mostly positive shifts (Fig. 2B and Supplementary Table 6).

The heatmap in Supplementary Fig. 1 shows the complete bacterial community composition in the rhizosphere of each of the 24 eucalypt species under each P condition alongside a dendrogram showing the hierarchical clustering of the eucalypt species. A total of 184 bacterial taxa (Supplementary Table 1) exhibited changes in relative abundance in the eucalypt rhizosphere under sufficient and low soil P (Supplementary Fig. 1).

Fig. 3 (and Supplementary Fig. 1) shows the hierarchical clustering of the eucalypt species according to similarities in rhizosphere bacterial community composition. Under sufficient P, the bacterial taxa in the rhizosphere grouped the eucalypt species into two main clusters: cluster I comprising *E. deanei*, *E. microcorys*, *E. urophylla* and *E. acmenoides* and cluster II comprising the remaining 20 eucalypt species. Cluster II had two subclusters: sub-cluster IIa comprising *E. propinqua*, *E. grandis*, *E. robusta*, *E. urograndis* and *E. exserta* and sub-cluster IIb comprising *E. botryoides*, *E. amplifolia*, *E. resinifera*, *E. camaldulensis*, *E. brassiana*, *E. tereticornis*, *E. pellita*, *E. dunnii*, *C. maculata*, *E. saligna*, *E. cladocalyx*, *E. globulus*, *E. crebra*, *C. citriodora*, and *C. henryi* (Fig. 3A and Supplementary Fig. 1A). In the rhizosphere of the species in cluster I, most taxa decreased in relative abundance, whereas in the rhizosphere of the species in cluster IIa,

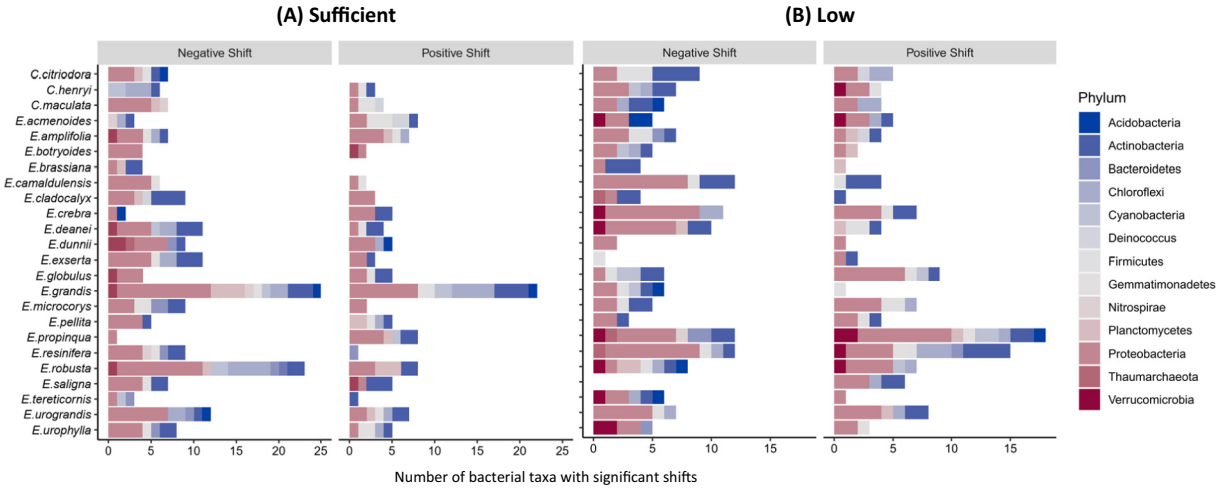

Fig. 2. Bacterial taxa with significant negative and positive shifts (relative to the global mean from the null hypothesis) at the phylum level in the rhizosphere of the 24 eucalypt species under low (A) and sufficient (B) P availability.

most taxa increased in relative abundance (Fig. 3A and Supplementary Fig. 1A).

The arrangement of the relative abundance of bacterial taxa under low P differed from that under sufficient P (Fig. 3B and Supplementary Fig. 1B). Under low P, the bacterial taxa grouped the eucalypt species into two main clusters: cluster I comprising *E. camaldulensis*, *E. resinifera*, *E. deanei*, *E. robusta*, *E. propinqua* and *E. urograndis* and cluster II, which comprised the remaining species and could be subdivided into subclusters IIa and IIb. Most of the bacterial taxa in the rhizosphere of eucalypts in cluster I increased in relative abundance, whereas those in the rhizosphere of eucalypts in cluster IIa (*C. maculata*, *C. citriodora*, *E. crebra*, *E. microcorys*, *E. amplifolia* and *E. pellita*) decreased in relative abundance (Fig. 3B and Supplementary Fig. 1B). Bacterial taxa with opposing changes in relative abundance in the rhizosphere between clusters I and IIa belonged to the genera *Lawsonella* and *Turicella* (phylum *Actinobacteria*), *Staphylococcus* (*Firmicutes*), *Methylobacterium* and *Escherichia/Shigella*, *Leptothrix* and *Massilia* (*Proteobacteria*) and the families *Roseiflexaceae* (phylum *Chloroflexi*), *Isosphaeraceae* (*Planctomycetes*), *Burkholderiaceae* and *Nitrosomonadaceae* (*Proteobacteria*) (Supplementary Table 6).

Among the eucalypt species with similar rhizosphere bacterial communities under sufficient and low P, only *E. brassiana* and *E. tereticornis* had similar bacterial communities (Fig. 3).

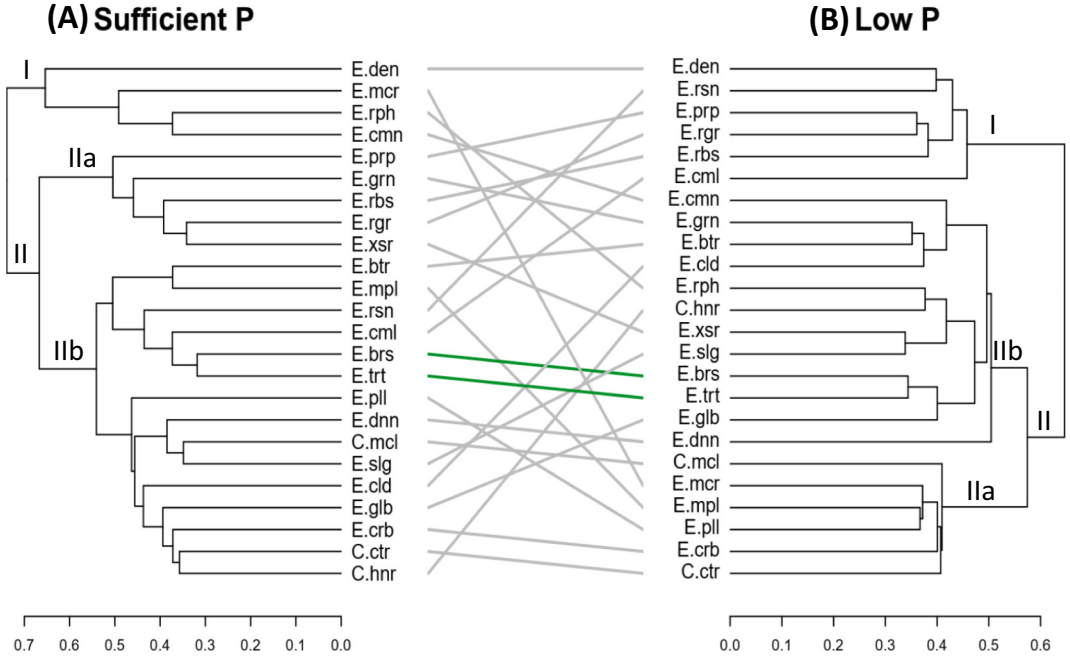

Fig. 3. Dendrogram based on the regression coefficients of the bacterial community in the rhizospheres of 24 eucalypt species under sufficient (A) and low (B) P availability. Similarity scale according to Ward's algorithm: values closer to zero indicate rhizosphere bacterial communities with greatest similarity. The species are linked with grey lines when differently clustered under low and sufficient P availability, while they are linked with green lines when present within the same cluster in both P availability. *Eucalyptus acmenoides* (E.cmn), *E. amplifolia* (E.mpl), *E. brassiana* (E.brs), *E. camaldulensis* (E.cml), *Corymbia citriodora* (C.ctr), *E. deanei* (E.den), *E. cladocalyx* (E.cld), *E. dunnii* (E.dnn), *E. exserta* (E.xsr), *E. globulus* (E.glb), *E. grandis* (E.grn), *C. maculata* (C.mcl), *E. microcorys* (E.mcr), *E. pellita* (E.pll), *C. henryi* (C.hnr), *E. propinqua* (E.prp), *E. resinifera* (E.rsn), *E. robusta* (E. rbs), *E. saligna* (E.slg), *E. tereticornis* (E.trt), *E. urograndis* (E.rgr), *E. urophylla* (E.rph), *E. crebra* (E.crb) and *E. botryoides* (E.btr).

3.4. Fungal community assembly in the rhizosphere of eucalypt species

The numbers of fungal taxa with significant positive or negative shifts in the rhizosphere at the class level for each eucalypt species are shown in Fig. 4. Under sufficient P, *E. exserta* and *E. robusta* had the highest numbers of fungal taxa with significant positive shifts (Fig. 4A and Supplementary Table 7). Under low P, the highest numbers of significant shifts occurred in the rhizosphere of *E. crebra* and *C. henryi*, with mostly positive shifts (Fig. 4B and Supplementary Table 8). For several eucalypt species, no significant shifts in the numbers of fungal taxa were observed, including *E. microcorys* and *E. cladocalyx* under low P and *E. acmenoides*, *E. amplifolia*, *E. dunnii* and *E. tereticornis* under sufficient P.

Similar to the rhizosphere bacterial community, rhizosphere fungal community structure differed among the eucalypt species independently of soil P levels. In addition, the clustering of eucalypt species according to rhizosphere fungal community composition differed between low P and sufficient P (Supplementary Fig. 2). The hierarchical clustering of eucalypt species according to their fungal community composition is shown in Fig. 5. Within the rhizosphere fungal community, the relative abundances of 25 fungal taxa changed significantly in the rhizosphere of all 24 eucalypt species under both soil P conditions (Supplementary Fig. 2 and Supplementary Table 2).

Under sufficient P, Ward's hierarchical clustering of eucalypt species according to rhizosphere fungal community composition formed 2 main groups (Fig. 5A): cluster I, which comprised *E. exserta*, *C. henryi*, *C. citriodora*, and *E. urophylla* and was characterised by increases in the relative abundances of most fungal taxa, and cluster II, which comprised *C. maculata*, *E. brassiana*, *E. amplifolia*, *E. pellita*, *E. dunnii* and *E. tereticornis* and featured decreases in the relative abundances of most fungal taxa (Fig. 5A).

Under low P, the relative abundances of the 25 identified fungal taxa differed depending on the eucalypt species (Fig. 5 and Supplementary Fig. 2) and compared with the abundances observed under sufficient P (Fig. 5). The eucalypt species were clustered into two main groups under low P: cluster I, which was characterised by increases in the relative abundances of most fungal taxa, particularly in the rhizosphere of *E. crebra* and *C. henryi*, and cluster II, which included *E. camaldulensis*, *E. robusta*, *E. urophylla*, *E. pellita*, *E. botryoides*, *E. propinqua* and *E. exserta* and was marked by decreases in the relative abundances of most fungal taxa (Fig. 5B and Supplementary Fig. 2). Among the eucalypt species, *E. exserta* and *E. crebra* exhibited the greatest shifts in their rhizosphere fungal assemblages in response to P. Under low P, the relative abundances of taxa of the genus *Rhizophagus* (*Mucoromycota*), of the family *Herpotrichiellaceae* (*Ascomycota*) and of the orders *Hypocreales* and *Pleosporales* (*Ascomycota*) increased in the rhizosphere of *E. crebra* and *C. henryi*. Interestingly, the relative abundances of members of the genus *Arthrinium*, family *Apiosporaceae*, and family *Sporocadaceae* all from class *Sordariomycetes*, phylum *Ascomycota*, increased in the rhizosphere of *E. dunni*, *C. henryi*, *C. maculata*, *E. acmenoides* and *E. grandis* in response to low P (Fig. 4B and Supplementary Table 8).

3.5. Co-dependency relationships between rhizosphere bacterial and fungal communities and plant variables

Co-inertia analysis was performed to observe global associations of the structure of rhizosphere microbial (bacterial and fungal) communities imposed by P soil availability with plant traits such as nutritional status and plant growth. Fig. 6 shows the percentage of co-variance between these two datasets and whether the co-dependence was significant. Significant co-dependence was observed between the bacterial and fungal rhizosphere communities and between the microbial communities and plant factors under both soil P conditions. When we focused on the microbial taxa that underwent significant changes in the rhizosphere depending on the eucalypt species (BactSig and FungiSig), we observed an increase in the co-inertia strength (lower *p*-values). Under sufficient P, the composition of the rhizosphere fungal communities with significant changes depending on eucalypt species. Under sufficient P, the composition of the rhizosphere fungal communities with significant changes in different eucalypt species (FungiSig) showed 61% covariance with the composition of the rhizosphere bacterial community that changed significantly in different eucalypt species (BactSig) (Fig. 6A). BactSig also showed 48% covariance with the non-significantly affected bacterial community (BactNS) (Fig. 6A). Under low P, the covariance of FungiSig and BactSig, and the covariance of BactSig and BactNS reached 54% (Fig. 6B).

Compared with sufficient P, low P intensified the co-dependence between bacterial community composition and plant biomass (Fig. 6B). Under low P, there were near-significant relationships between BactSig and plant biomass (Biomass) and between BactNS and Biomass, with covariances reaching 12% for low P (Fig. 6B) and 9% for sufficient P (Fig. 6A).

Under sufficient P, significant relationships were observed between BactSig and the plant micronutrient content (ContMicro) with 20% covariance, and between BactNS and ContMicro, with 16% covariance. FungiSig and BactSig had covariances of 10% and 17% respectively, with the plant content of macronutrients (ContMacro) (Fig. 6A). Under low P, the

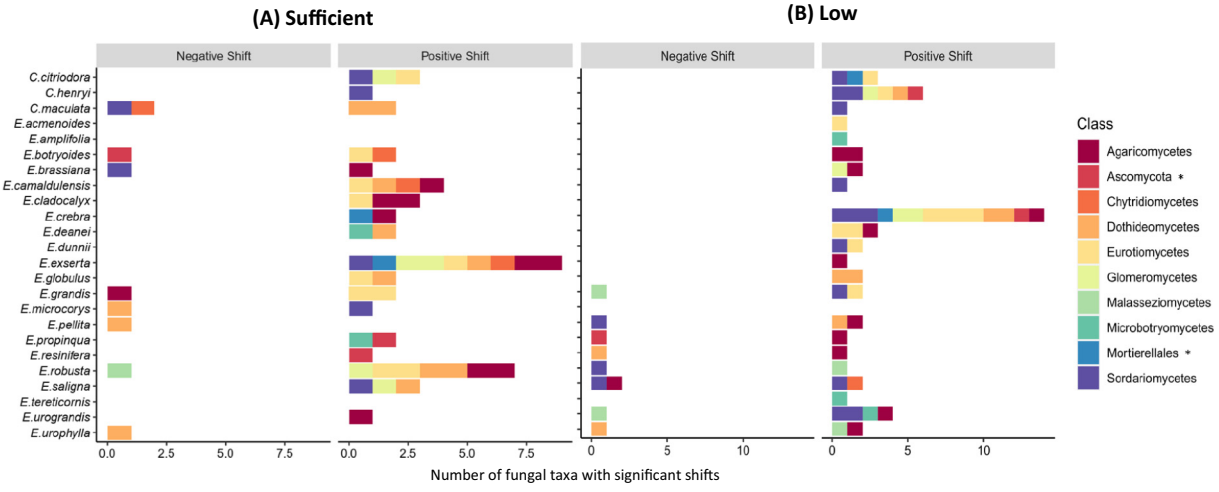

Fig. 4. Fungal taxa with significant negative and positive shifts (relative to the global mean from the null hypothesis) at the class level in the rhizospheres of the 24 eucalypt species under low (A) and sufficient (B) P availability. **Ascomycota*, class entitled "NA", so phylum is used instead of class; *Mortierellales*, class entitled "incertae sedis", so order is used instead of class.

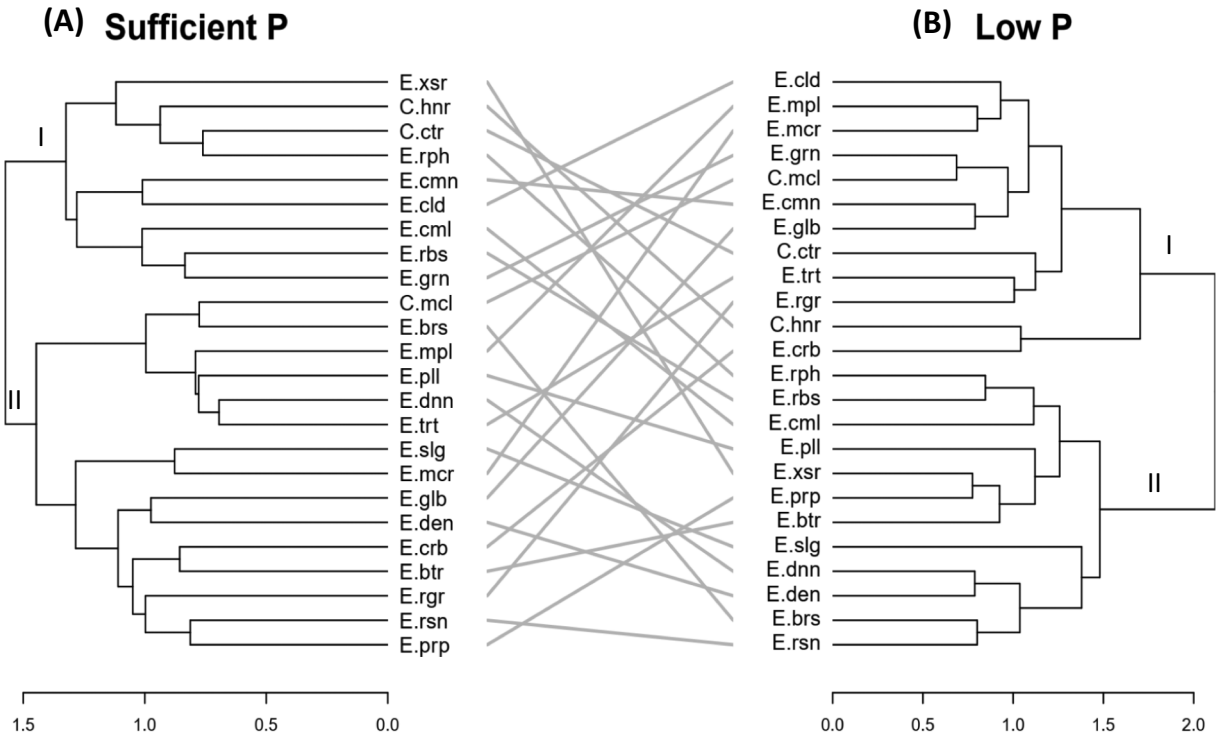

Fig. 5. Dendrogram based on the regression coefficients of the fungal community in the rhizospheres of plants of 24 eucalypt species under sufficient (A) and low (B) P availability. Similarity scale according to Ward's algorithm: values closer to zero indicate rhizosphere bacterial communities with greatest similarity. The species are linked with grey lines when differently clustered under low and sufficient P availability. *Eucalyptus acmenoides* (E.cmn), *E. amplifolia* (E.mpl), *E. brassiana* (E.brs), *E. camaldulensis* (E.cml), *Corymbia citriodora* (C.ctr), *E. deanei* (E.den), *E. cladocalyx* (E.cld), *E. dunnii* (E.dnn), *E. exserta* (E.xsr), *E. globulus* (E.glb), *E. grandis* (E.grn), *C. maculata* (C.mcl), *E. microcorys* (E.mcr), *E. pellita* (E.pll), *C. henryi* (C.hnr), *E. propinqua* (E.prp), *E. resinifera* (E.rsn), *E. robusta* (E.rbs), *E. saligna* (E.slg), *E. tereticornis* (E. trt), *E. urograndis* (E.rgr), *E. urophylla* (E.rph), *E. crebra* (E.crb) and *E. botryoides* (E.btr).

covariation of FungSig and plant micronutrient content (ContMicro) and covariation of BactSig and plant micronutrient content (ContMicro) were the same as those for sufficient P (Fig. 6A and B); however, the covariation of FungSig and plant macronutrient content (ContMacro) and covariation of BactSig and plant macronutrient content (ContMacro) were lower (Fig. 6B) than those for sufficient P (Fig. 6A).

4. Discussion

Our results show that species identity played a dominant role in shaping the rhizosphere microbial community of eucalypts, while soil P availability exerted a secondary influence, modulating the microbial composition of the rhizosphere in a species dependent manner.

4.1. Eucalypt species identity shapes the rhizosphere community

Independent of soil P availability, the bacterial and fungal composition of the rhizosphere varied among the eucalypt species. The strong effect of plant species in shaping the rhizosphere microbial community may be related to differences in root physiological and biochemical activities (Senior et al., 2016). Eucalypt species are all native to the Australasian region but have undergone a wide array of adaptations to varied edaphoclimatic conditions and evolved strategies to grow in resource-poor soils (Steane et al., 2011). Eucalypts as hyper-diverse group of trees also show diverse metabolic capacities, including the synthesis of root primary and secondary compounds such as organic acids, phenolics, terpenoids and tannins (Senior et al., 2016). Phenolics exuded by roots cause striking shifts in the composition of rhizosphere microbial communities (Stassen et al., 2021; Zwetsloot et al., 2020), which in turn may affect plant nutrient acquisition (Steane et al., 2011; Stassen et al., 2021). While several studies with maize, soybean and switchgrass report the influence of plant species or genotype on rhizosphere microbiome (Gomes et al., 2018; Sawyer et al., 2019; Liu et al., 2019), for eucalypts, the relationship between root exudates composition and rhizosphere microbiome was not yet investigated. Eucalypt species differ in their nutrient requirements (Bulgarelli et al., 2019) and might also differ in root exudate composition (Broeckling et al., 2008). Thus, considering that plant-microbes-soil interactions form a complex feedback system, eucalypt species are likely to have specific rhizosphere microbial compositions.

4.2. Changes in eucalypt rhizosphere microbial communities in response to low soil P

The bacterial and fungal communities in the rhizosphere were strongly shaped not only by the eucalypt species but also by soil P availability, although the effects of soil P availability varied depending on the species. Strategies to improve P acquisition in P-poor soils include associations with soil microorganisms that enhance soil P availability and plant P uptake (Richardson and Simpson, 2011). Symbioses with arbuscular mycorrhizal fungi (AMF) contribute to enhanced plant P absorption, as the extraradical hyphae explore the soil beyond the root P-depletion zone (Wen et al., 2019). Bacterial and fungal PSM adopt different strategies to solubilise unavailable forms of soil P to allow phosphate uptake by plants (Raymond et al., 2021). Soil P mobilisation can also be enhanced by changes in plant root physiology, such as alterations of the quality and amounts of exudates or the secretion of phosphatases (Wang and Lambers, 2020). Hence, young plants of eucalypt species in this study may have adjusted root exudation in response to P availability, thereby influencing interactions with microbes in the rhizosphere (Sasse et al., 2018).

Under low P availability, not all eucalypt species exhibited enrichment of microorganisms with P-mobilising capacities in the rhizosphere. We previously demonstrated that the studied eucalypt species differ significantly in their capacity to grow under low P and may also differ in their prevalent P-acquisition strategies (Bulgarelli et al., 2019). Interestingly, *E. urograndis*

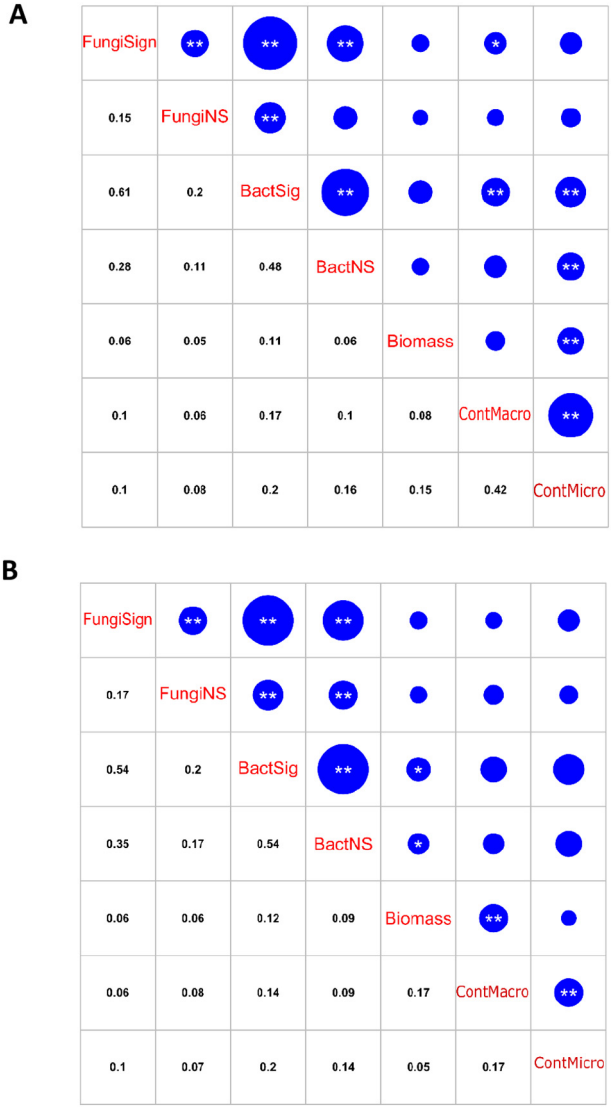

Fig. 6. Co-inertia analysis of the plant-related datasets and rhizosphere bacterial and fungal community composition under sufficient (A) and low (B) P availability. The fungal and bacterial communities were subset into those that were significantly affected by the treatments (FungiSig and BactSig) and those that did not show significant changes (FungiNS and BactNS). Leaf, stem and root biomass production (Biomass); macronutrient contents: phosphorus, nitrogen and magnesium contents in shoots (ContMacro); micronutrient contents: copper, iron, zinc, and manganese contents in shoots (ContMicro). * shows near-significant relationship with p-value < 0.10; ** shows significant relationship with p-value < 0.05.

and *E. cladocalyx* exhibited the smallest shifts in bacterial community composition but marked shifts in fungal community composition. Conversely, *E. microcorys* and *E. globulus* exhibited the smallest shifts in rhizosphere fungal community composition but relatively large shifts in bacterial community composition. Curiously, low P does not affect the root growth of *E. urograndis* and *E. cladocalyx* but reduces the root growth of *E. microcorys* and *E. globulus* (Bulgarelli et al., *2019*).

E. deanei, *E. acmenoides*, *E. microcorys* and *E. camaldulensis* exhibited significant shifts in rhizosphere bacterial community composition under low P, but not in fungal community. We previously classified these species as non-efficient in the use of P and responsive to P supply, with poor growth under low P (Bulgarelli et al., 2019). By contrast, *E. robusta*, *E. exserta* and *E. urophylla*, previously classified as efficient under low P, exhibited significant shifts in the rhizosphere fungal community due to P availability (Bulgarelli et al., 2019). Thus, it seems that eucalypt species with higher P use efficiency rely more on changes in the fungal community than in the bacterial community in the rhizosphere. For example, a large number of shifts in fungal composition were observed in the rhizosphere of *E. exserta*, including in AMF taxa, which may increase P acquisition (Smith and Read, 2008). On the contrary, responsiveness to P addition seems to be associated with shifts in the bacterial community in the rhizosphere.

4.3. Notable bacterial taxa in the eucalypt rhizosphere

Under low P, plant growth-promoting microorganisms (PGPM) and PSM were recruited by some eucalypt species. Several bacteria taxa from the *Actinobacteria* were identified, being that members from this phylum have been described as PGPM and PSM (Yadav et al., 2018; Anwar et al., 2016). PSM genera such as *Kocuria*, *Micrococcus*, *Pseudarthrobacter* and *Streptomyces* were significantly selected under low P by eucalypt species such as *E. microcorys*, *C. citriodora*, *E. brassiana*, *E. globulus*, *E. propinqua*, *E. tereticornis* and *E. urograndis*.

Members of the phyla *Acidobacteria*, *Firmicutes* and *Proteobacteria*, which increased in abundance under low P, were previously found in deep soil layers of *E. grandis* plantations (Pereira et al., 2017). The presence of members of *Proteobacteria* has also been reported in acid soils and with low total P (Eldridge et al., 2018). In fact, *Proteobacteria* was the most prevalent phylum in our study, with 88 genera, and showed the greatest number of positive shifts under low P. Conversely, *Acidobacteria* and *Firmicutes* phyla were underrepresented, with six and eight genera, respectively. *Burkholderia*, *Bacillus* and *Rhizobium*, which have been reported to possess phosphate-solubilising capacities (Castagno et al., 2021), were found in the rhizosphere of eucalypts under both soil P availability.

4.4. Mycorrhizal, pathogenic and dark-septate fungi were present in the eucalypt rhizosphere

Eucalypts can establish associations with AMF in the early stages of growth and with ectomycorrhizal (ECM) fungi as adult trees (Lapeyrie and Chilvers, 1985). In our study, most of the fungal taxa in the rhizosphere belonged to the phyla *Ascomycota* (15 taxa) and *Basidiomycota* (6 taxa), including members that can form ECM symbiosis. We also found increases in two AMF taxa belonging to *Glomeromycetes* in the rhizospheres of *C. henryi*, *E. brassiana* and *E. crebra* under low P. Under sufficient P, these AMF taxa increased in abundance in the rhizospheres of *E. robusta*, *C. citriodora*, *E. exserta*, and *E. saligna*. Therefore, we cannot establish a direct relationship between the presence of AMF taxa and the growth efficiency of eucalypt species under low P or their response to P addition, as colonization rates were not assessed. The greatest increases in fungal taxa abundance in the rhizosphere assemblage were observed for *E. exserta* and *E. robusta* under sufficient P and *E. crebra* and *E. henryi* under low P. In response to low P, the relative abundance of the genus *Arthrinium* (class *Sordariomycetes* in *Ascomycota*) significantly increased. This class of fungi is an ecologically and functionally diverse group comprising generalist endophytes, pathogens and saprobic species (Zhang et al., 2006; Vujanovic et al., 2012; Bonthond et al., 2018). Soil P may influence the plant microbiome directly or indirectly via changes in plant physiology that shape microbial niches in the rhizosphere. In addition, P starvation responses can negatively regulate plant immunity and lead to changes in the rhizosphere microbiome (Chan et al., 2021).

Under low P, the abundance in members of the order *Pleosporales* increased for *C. henry*, *E. crebra*, and *E. pellita* and *Chaetothyriales* for *E. crebra*, *E. deanei*, *E. dunnii*, *C. citriodora*, and *E. acmenoides*. By contrast, under sufficient P, only the abundance of *Venturiales* increased, in the rhizosphere of *C. maculata*. These fungal taxa has members of the so-called dark-septate endophytes (DSE), that have been found in eucalypt roots (Uma et al., 2012) as well as in the rhizosphere, where they may mineralise organic compounds (Carvajal Janke and Coe, 2021). DSE have been found in the roots and rhizosphere of crop species, Velloziaceae in *campos*

rupestres in Brazil, and trees of subarctic and boreal forests (Fors et al., 2020; Ruotsalainen, 2018; Abrahão et al., 2020). Although the function of DSE in plants remains elusive; they are frequently present in nutrient-limited environments, which suggests they are important for plants under adverse soil conditions (Abrahão et al., 2020; Knapp et al., 2018).

The relative abundance of *Mortierella* increased in the rhizospheres of *C. citriodora* and *E. crebra* under low P and *E. crebra* and *E. exserta* under sufficient P. *Mortierella* is a diverse genus described as saprobic that may have the ability of desorb P from soil particles (Ning et al., 2020; Osorio and Habte, 2009).

4.5. Low P intensifies the co-dependence between microbial communities and plant factors

Co-inertia analysis revealed a strong relationship between bacterial and fungal community structure under low P, suggesting close interactions between these rhizosphere components. This relationship may also indicate that P limitation strengthens the interaction between the bacterial and fungal communities (Marschner et al., 2001). For example, the relative abundance of members of the AMF genus *Rhizophagus* increased in the rhizospheres of some eucalypt species under low P. AMF are known to influence bacterial community composition in soil and the rhizosphere (Magallon-Servín et al., 2020), as the symbiosis alters root exudates and the AMF hyphae themselves exude compounds that attract bacteria (Toljander et al., 2007).

The co-inertia analysis also allowed us to observe the global associations between the microbial community structure shaped by soil P availability and plant-related factors. Studies in sorghum and willows have also shown that the rhizosphere bacterial and fungal communities affect each other's composition (Schlemper et al., 2018; Bell et al., 2014). The co-inertia analysis revealed significant relationships of bacterial community composition with the macro and micronutrient contents in eucalypt shoots. Fungal community composition showed a near-significant relationship with plant macronutrient contents, suggesting that the microbial community selected by the plant was responsive to the increase in soil P availability and plant nutrition status (Ai et al., 2018).

A relationship between microbiota assembly and plant growth was observed only under low soil P availability. The bacterial communities significantly affected by plant identity (BactSig) were significantly related to plant growth, while the bacterial communities that were not significantly affected by plant identity (BactNS) had a near-significant relationship with plant growth. The eucalypt trees used in this experiment were previously reported to have reduced growth under low P compared with sufficient P (Bulgarelli et al., 2019). The stronger co-dependence between BactSig and plant growth suggests that plants act to shape rhizosphere microbial composition once P limitation begins to impact plant growth. As noted above, many of the identified bacterial taxa were plant growth-promoting bacteria (PGPB), which promote growth either directly by facilitating resource acquisition or modulating phytohormone signalling processes or indirectly by decreasing the inhibitory effects of pathogenic agents on plant growth and development (Glick, 2012). The identification of potential PGPB in the rhizosphere of the 24 eucalypt species suggests an important association of these bacteria with this forestry group.

5. Conclusion

The response of microbial community composition in the rhizosphere of tree species to P limitation has received little attention. Here, we showed that eucalypt species was a greater determinant of rhizosphere bacterial and fungal community composition than soil P availability. Interestingly, soil P availability had no significant influence on the rhizosphere microbiome structure of some eucalypt species but shifted the composition of the bacterial and fungal communities of others. The relative abundances of bacterial and fungal taxa in the rhizosphere were related to each other at both P availability levels, and co-dependence was observed between the rhizosphere microbiome and plant traits such as shoot nutrient contents and biomass production. Further studies will contribute to deciphering the role of P in modulating the interaction between root exudation and microbial functions in the rhizospheres of different eucalypt species.

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Availability of data and material

The raw sequences were submitted to the European Nucleotide Archive (ENA) under study accession number PRJEB48727.

CRediT authorship contribution statement

P. M. and S.A.L.A designed the research; R.G.B. conducted the experiment and DNA extraction; M.F.A.L.and M.H. performed the statistical and bioinformatic analyses; R.G.B., P.M., S.A.L.A. and E.E.K. wrote the manuscript. P.M., S.A.L.A. and E.E.K. critically reviewed the manuscript. All authors reviewed the manuscript.

Ethics approval and consent to participate

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155667.

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