Root morphological changes in response to low phosphorus concentration in eucalypt species

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Abstract

Key message **Eucalypt seedlings differently modulate root morphology in response to phosphorus availability, with changes in the length or density of root hairs being more common that changes in root length.**

Abstract Phosphorus (P) is an essential nutrient for plant growth and development and thus can restrict biomass accumulation when it is at low levels in the soil. Eucalypts cover large areas of planted forests in the world, including regions with naturally low P availability. This study was conducted to evaluate the morphological changes in the roots of seedlings of five eucalypt species: *Eucalyptus acmenoides*, *E. globulus*, *E. grandis*, *E. tereticornis* and *Corymbia maculata* in response to low P concentration. Seedlings were grown in pots with vermiculite and received a nutrient solution of low (25 μM), and sufficient concentration (500 μM) of P. Root hair length and density were evaluated in secondary root segments, and the production of plant biomass and P concentration in the shoots were determined. The species modulated root morphology differently in response to P limitation. *E. tereticornis* showed the lowest plasticity of these morphological traits in response to P concentration. The total root length increased in some species, but changes in the length and/or density of root hairs were the commonest response to low P concentration. P concentrations in the shoots and biomass production were not related to increase of root length or root hair density and length.

Keywords Root hairs · Root plasticity · Eucalyptus · Corymbia · Phosphorus limitation

Introduction

Phosphorus (P) is an essential macronutrient for plant development. Besides being a component of nucleic acids and phospholipids, it is a critical component in physiological and molecular functions of plants, such as photosynthesis, energy transfer and carbohydrate metabolism (Vance et al. 2003). P is absorbed by plants in the form of inorganic P, predominantly as orthophosphate, $H_2PO_4^-$. Its availability for plants is usually limited because $H_2PO_4^-$ can form

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stable and/or insoluble complexes with Fe and Al oxides and hydroxides or with Ca in the soil (Holford 1997). The mineralogical characteristics of the soil and the quantity and quality of soil organic matter are also intrinsically related to P availability for plants (Binkley and Vitousek 2000).

The concentrations of inorganic P in the soil solution are usually very low, ranging from 2 to 10 μM (Hinsinger and Gilkes 1996). This low level is particularly true in the acidic and weathered soils of tropical regions, which have high P retention ability, leading to plant deficiency (Friesen et al. 1997). Besides, P diffusion to plant roots is a slow process (Huang et al. 2017), which makes P one of the least available nutrients for plants (Marschner 2012). Therefore, understanding the response of plant roots to P restriction is essential for the development of plants for efficient P uptake (Lambers and Plaxton 2018).

Planted forests represent around 7% of global forests (Valadares et al. 2020). In Brazil, these forests cover around 7.83 million hectares, and although this area corresponds to less than 1% of the Brazilian territory, they supply approximately 91% of the country's timber market. Eucalypt plantations

occupy 72.7% of the total area of planted forests in Brazil, and many of these areas have soils of low natural fertility (Valadares et al. 2020), showing the eucalypts ability to adapt to the edaphoclimatic conditions in Brazil (Grattapaglia and Kirst 2008; Pérez-Cruzado et al. 2011).

Australia and nearby islands are the centres of eucalypt diversification, in regions where P concentration in the soil is naturally very low (Flores et al. 2016; Rossel and Bui 2016). As a result, eucalypts may have evolved mechanisms to increase the efficiency of P uptake and use (Del-Saz et al. 2018).

Response to P availability varies according to the species and developmental stage of eucalypt plants (Thomas et al. 2006; Bulgarelli et al. 2019). Recently, a comparison of 24 eucalypt species showed contrasting responses to P availability in the soil, with varied efficiency and responsiveness (Bulgarelli et al. 2019). Species such as *E. acmenoides* and *E. crebra* were responsive to the addition of P and inefficient in P use. In contrast, other species, such as *E. tereticornis* and *E. globulus*, were efficient in biomass production under low P availability but had no response to the addition of P. Bulgarelli et al. (2019) did not find a correlation between P concentration in the leaves and biomass accumulation. They suggested that foliar P concentration is not a good indicator of the response of eucalypts to P, which is probably related to the varied P use efficiencies and mechanisms related to P acquisition.

Adaptive plant strategies to increase P uptake in conditions of low availability include: (i) mining strategies with an increase in the desorption of P adsorbed into soil colloids through root exudation of organic molecules, such as organic acids (Miguel et al. 2015); (ii) increased expression of genes encoding high-affinity phosphate transporter proteins located in the membranes (Tang et al. 2020); (iii) foraging strategies to increase soil exploration by promoting root growth, changes in root architecture or changes in root hair emergence and growth (Trindade and Araujo 2014); (iv) development of symbiotic associations with mycorrhizal fungi (Miguel et al. 2015). Therefore, plants can adapt to low P conditions, increasing the ability of soil exploration and uptake and optimizing the use of absorbed P (Tang et al. 2020).

Changes in root morphology and physiology are important in plant adaptation to P limitation (Tang et al. 2020). P exploration capacity is closely linked with the morphological and architectural plasticity of roots (Cao et al. 2013; Tang et al. 2020), considering plasticity as the ability of a given genotype to express different phenotypes under different environmental conditions (Palmer et al. 2012). In situations of P limitation, plants can adapt the morphological traits of roots to increase the volume of soil explored for the acquisition of P (Xia et al. 2020; Miguel et al. 2015). A study that analyzed root plasticity in eight commercial

eucalypt hybrids under P limitation showed root morphology alteration in response to the varied supply of P in the soil (Zhou et al. 2017). Roots of some hybrids proliferated in places where P was more abundant, while the opposite was observed with other genotypes, with broader root proliferation in places where P availability was lower (Zhou et al. 2017). Plants of *Cunninghamia lanceolata* (Lamb.) Hook., *Pinus massoniana* Lamb., and *Phoebe zhennan* S. Lee changed root growth patterns to adapt to lower P availability in the soil, showing an increase in the root-shoot ratio (Yan et al. 2019).

Root hairs increase the surface area of roots, consequently increasing the contact between root and soil (Rongsawat et al. 2020; Ruiz et al. 2020; Bates and Lynch 2001). The emergence of root hair can be driven by low P availability, representing a strategy of lower metabolic cost for increasing the P uptake (Klamer et al. 2019; Foehse and Jungk, 1983). For several plant species, these two characteristics increase during P deficiency, as observed in *Poncirus trifoliata* Raf. orange (Cao et al. 2013) and *Triticum aestivum* L. wheat (Yuan et al. 2016).

However, studies have demonstrated that only longer hair length may not be a guarantee of higher P uptake by plants and that other morphological and physiological characteristics of roots significantly contribute to an efficient acquisition of this nutrient (Brown et al. 2013). Transgenic mutants of *Brachypodium distachyon* L. with shorter root hairs than wild-type plants showed lower growth and P uptake in conditions of low P availability than mutants of longer root hairs (Zhang et al. 2018).

Here, we analyzed the morphological changes in roots and root hair of seedlings of five eucalypt species in response to the low P concentration. We aimed to have a better understanding of the relationship among phenotypic plasticity of the root, P availability, and accumulation of biomass and P in eucalypts. We hypothesized that eucalypt genotypes have different root morphological traits in response to low P concentration and that such responses are related to the ability of P uptake and accumulation by plants.

Material and methods

Experimental design and biological material

The eucalypt species studied were: *Eucalyptus acmenoides* Schauer, *E. globulus* Labill., *E. grandis* W. Hill ex Maiden, *E. tereticornis* Sm., and *Corymbia maculata* (Hook.). These species were used in this study because previous research conducted by our group showed they differ regarding P uptake (Bulgarelli et al. 2019). Among 24 species, *E. acmenoides* was classified as non-efficient under low P availability in soil and responsive to added P, *C. maculata* was

non-efficient and non-responsive, *E. globulus* and *E. teretircornis* were efficient and non-responsive, while *E. grandis* presented an intermediate response.

The seeds of the species used here were obtained from clonal garden plants (Caiçara Sementes, Brejo Alegre, São Paulo, Brazil; Instituto de Pesquisas e Estudos Florestais—IPEF, Piracicaba, São Paulo, Brazil). Seeds were germinated in trays with vermiculite. Since germination, seedlings received modified Hoagland solution, ½ strength (Hoagland and Arnon 1938), with low level (25 µM) and sufficient level (500 µM) of P (Bahar et al. 2018). When the seedlings had between four and five leaves, they were transplanted into 3 L pots containing vermiculite, with four seedlings per pot, and four pots per treatment. The seedlings continued to receive the same nutrient solutions according to the treatments (200 mL per pot and three times a week). Thus, the statistical experimental design was a 5×2 factorial scheme, with five eucalypt species and two P concentrations in the nutrient solution; with four replicates.

Plant harvest

After 21 days of transplantation, the seedlings were carefully removed from the pots, washed in running tap water, and had the shoots and roots separated. The shoots were dried at 60 °C, weighed, and ground. The roots were stored in 50% ethanol for the analysis of morphological traits.

Morphological traits of roots

The roots were scanned at a resolution of 600 dpi (Epson Expression 12000XL, Epson America), and the images were analyzed with WinRHIZO (Regent Instruments Inc., Canada). Data obtained were: average diameter (mm), total length (cm), volume (cm^3), and surface area (cm^2). After scanning, segments of root tips were reserved for root hair analysis. Data for length and surface area were used to calculate specific root length (SRL) and specific root area (SRA), dividing them by the root dry matter mass. Root tissue density (RTD) was calculated as the ratio between dry mass and root volume.

Root hair length and density

We used five 0.5 cm segments from each of all four treatment replicates to analyze root hair length and density. These segments were obtained from the apex of second-order roots. They were cut with a scalpel under a stereomicroscope (Leica LED5000SLI) and then stained with 2% trypan blue for 10 min and then used to obtain images on a microscope equipped with image system (Leica DM2700M). We obtained root hair length and abundance using the images processed in the ImageJ software (https://imagej.net/Fiji/

Downloads). In each segment, the length of ten root hairs was measured. An area between 0.018 and 0.088 mm^2 was delimited for determining the abundance of root hairs per area.

Determination of aerial and root masses

After the morphological analysis of the roots, roots and aerial parts of the plants were dried at 60 °C and weighed to obtain the dry masses.

Determination of the concentration of P and other nutrients

The dried shoots and roots were ground and submitted to nitric-perchloric acid digestion (HNO_3–$HClO_4$) to determine the contents of nutrients (P, K, Mg, Zn, Cu, B, Cu and Mn) by plasma emission spectrophotometry (ICP-OES) (JobinYvon, JY50P Longjumeau, France).

Statistical analysis

Data were analyzed by two-way ANOVA, Scott-Knott test for comparison of means at 5% significance using the SISVAR software (Ferreira 2011). Data related to counts and percentages were transformed into log ($x + 1$) and arcsine $\sqrt{(x/100)}$], respectively, before statistical analysis. Pearson's correlation was used to check the correlation between the main variables, and the concentration of P. Principal component analysis (PCA) was conducted to identify the variables that best explained the highest proportion of data set variance using Minitab software (Minitab Statistical version 17).

Results

Root morphology

In general, species and P concentration significantly influenced root morphological parameters. *E. globulus* was the species with higher values of root length and surface area, while *E. acmenoides* and *E. tereticornis* had the lowest values (Fig. 1). Regarding the response to P concentration, *E. globulus* and *C. maculata* showed higher values of root length and surface area in sufficient P concentration (Fig. 1a, b). *E. grandis* seedlings showed higher values for these parameters in low P concentration (Fig. 1a, b).

For specific root length (SRL), the species showed a similar response and only *C. maculata* had significantly lower values when compared to the other species (Fig. 1c). Regarding the response of SRL to P, no significant difference was observed among plants at low and sufficient P concentration (Fig. 1c). For specific root area (SRA), *E. globulus*,
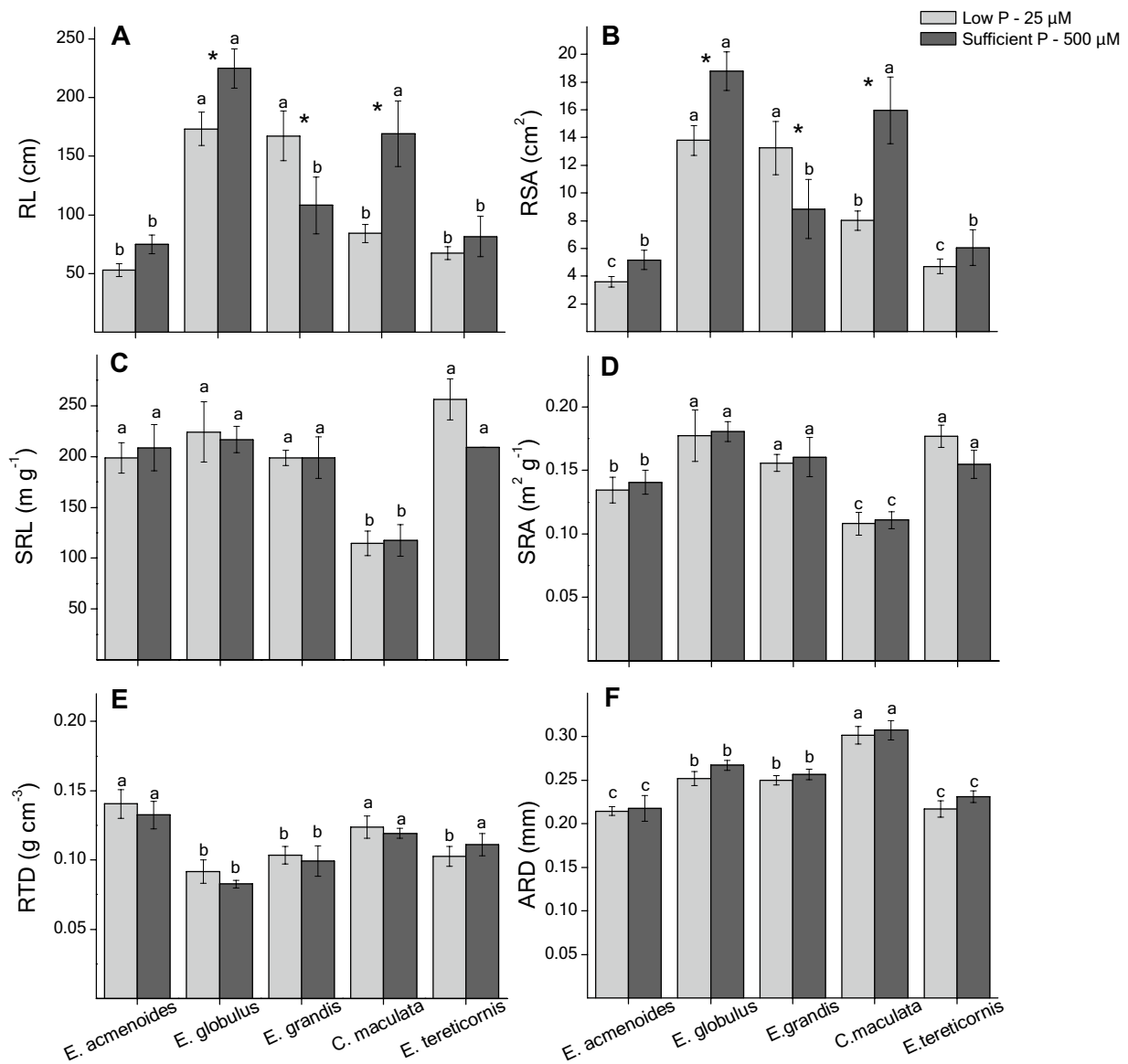
Fig. 1 Morphological traits of the seedling root system of five eucalypt species grown with a nutrient solution of low (25 µM) and sufficient (500 µM) P concentration. **a** Total length (RL) of the root system, **b** surface area (RSA), **c** specific root length (SRL), **d** specific surface area (SRA), **e** root tissue density (RTD) and **f** average root diameter (ARD). Different letters indicate a significant difference among species for each P concentration, whereas the asterisk indicates a significant difference between P concentrations for each species, by the Scott-Knott test at 5% probability. Bars indicate standard error ($n = 16$)

E. grandis, and *E. tereticornis* had the highest values, followed by *E. acmenoides* and *C. maculata* (Fig. 1d). For this root trait, no significant difference was observed in response to the P concentration (Fig. 1d).

Regardless of the P concentration, *E. globulus* and *E. grandis* had the lowest root tissue density (RTD), and *E. acmenoides* and *C. maculata* showed the highest RTD. RTD did not respond significantly to the increase in P concentration in any of the studied species (Fig. 1e). Despite the average root diameter (ARD) did not respond to P concentration it was significantly different among eucalypt species

(Fig. 1f). *C. maculata* showed the greatest ARD and *E tereticornis* and *E. acmenoides* the smallest ARD (Fig. 1f).

Root hair length and density

In general, root hair length varied significantly among species and with P concentration (Fig. 2a). *E. globulus* showed the longest root hairs, around three times those of *E. acmenoides*, which had the shortest root hairs (Fig. 2a). The other studied species, *E. grandis*, *E. tereticornis* and *C. maculata*, had intermediate root hair length (Fig. 2a). In response to P
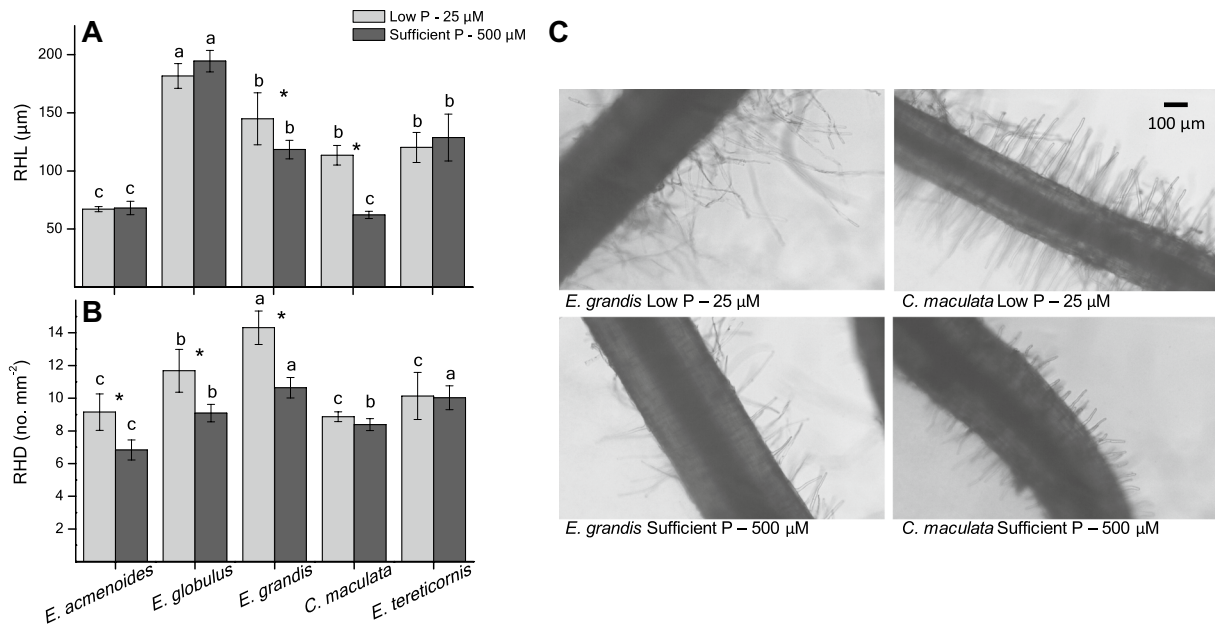
Fig. 2 Root hair length (**a**) and root hair density (**b**) on secondary roots of eucalypt species grown with a nutrient solution of low (25 μM) and sufficient (500 μM) P concentration. Different letters indicate a significant difference among species for each P concentration, whereas the asterisk indicates a significant difference between P concentrations for each species, by the Scott-Knott test at 5% probability. Bars indicate standard error (*n* = 20). **c** Photographs showing contrasting responses in root hair length and density at low and sufficient P concentration (scale bar: 100 μm)

concentration, *E. grandis* and *C. maculata* had longer root hair length at low P concentration, and *C. maculata* seedlings developed root hairs up to twice the length of seedlings developed at sufficient P concentration (Fig. 2a, c).

Root hair density varied in response to P level and species (Fig. 2b). In general, *E. grandis* roots had the highest root hair density among all species that grew in low P conditions, followed, in decreasing order, by *E. globulus, E. tereticornis, E. acmenoides* and *C. maculata*. When the seedlings were grown in sufficient P concentration, *E. grandis* and *E. tereticornis* had a higher hair density than *E. globulus* and *C. maculata*, which, in turn, had a higher density when compared to *E. acmenoides*, which was the species with the lowest value (Fig. 2b). When comparing root hair density as a function of P concentration, seedlings of *E. grandis*, *E. globulus* and *E. acmenoides* had between 33 and 40% higher hair density at low P than those grown at sufficient P concentration (Fig. 2b). *C. maculata* and *E. tereticornis* did not show significant differences in root hair density in response to P concentration (Fig. 2b).

Biomass production

In general, shoot biomass accumulation varied among species in response to P concentrations (Fig. 3a). In particular,

at sufficient P, *E. globulus* and *C. maculata* presented higher shoot biomass than the other species (Fig. 3a) and produced a significantly smaller amount of shoot biomass when grown at low P concentration, with around 40% less biomass accumulation than plants grown with sufficient P. The same growth pattern was found in *E. tereticornis* that produced around 50% less biomass at low P. In contrast, *E. grandis* accumulated 46% more shoot biomass when grown at low P compared to sufficient P (Fig. 3a).

Regarding the root biomass, at sufficient P *C. maculata* stood out among all species, producing around three times more root biomass than *E. acmenoides*; the latter species, along with *E. grandis* and *E. tereticornis*, showed the lowest root biomass production. At low P, *E. globulus*, *E. grandis* and *C. maculata* had higher root biomass than *E. acmenoides* and *E. tereticornis* (Fig. 3b). When comparing the response to P, *E. grandis* had higher root biomass at low P, and *C. maculata* had higher root biomass at sufficient P (Fig. 3b).

The root-shoot (R:S) ratio varied between species (Fig. 3c). At sufficient P concentration, *E. grandis* and *C. maculata* had the highest R:S ratio, while at low P, *E. acmenoides* had the lowest ratio. *E. tereticornis* was the only species with a significant increase of R:S ratio at low P (Fig. 3c).
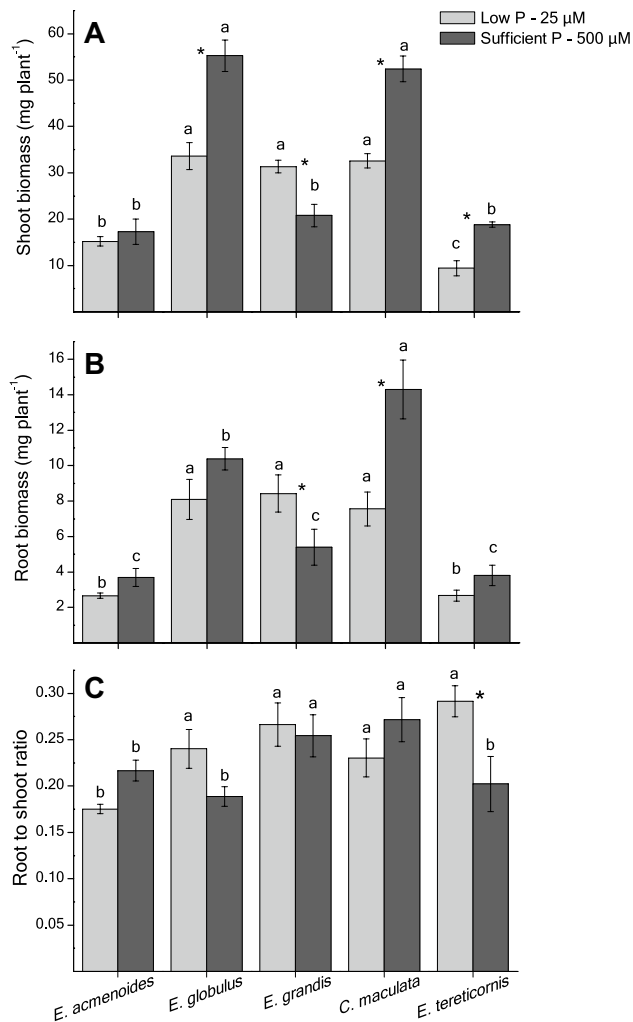
Fig. 3 Biomass production of the shoot (**a**), root (**b**) and root to shoot ratio (**c**) of eucalypt species grown at low and sufficient P concentration. Different letters indicate a significant difference among species for each P concentration, whereas the asterisk indicates a significant difference between P concentrations for each species, by the Scott-Knott test at 5% probability. Bars indicate standard error ($n=4$)

P concentration and accumulation in the shoots

The concentration of P in the shoots varied significantly depending on the concentration of P in the nutrient solution. Species that grew at sufficient P showed higher shoot P concentrations than species grown at low P (Fig. 4a). At sufficient P, *E. tereticornis* presented higher P concentration in the shoots when compared to all other species, being 155% higher than in *E. acmenoides* plants, which presented the lowest concentration of P (Fig. 4a). At low P, the five species showed similar shoot P concentrations (Fig. 4a).

As observed for P concentration in the shoots, P content (P concentration multiplied by shoot biomass) was highest in plants that grew at sufficient P (Fig. 4b). At sufficient
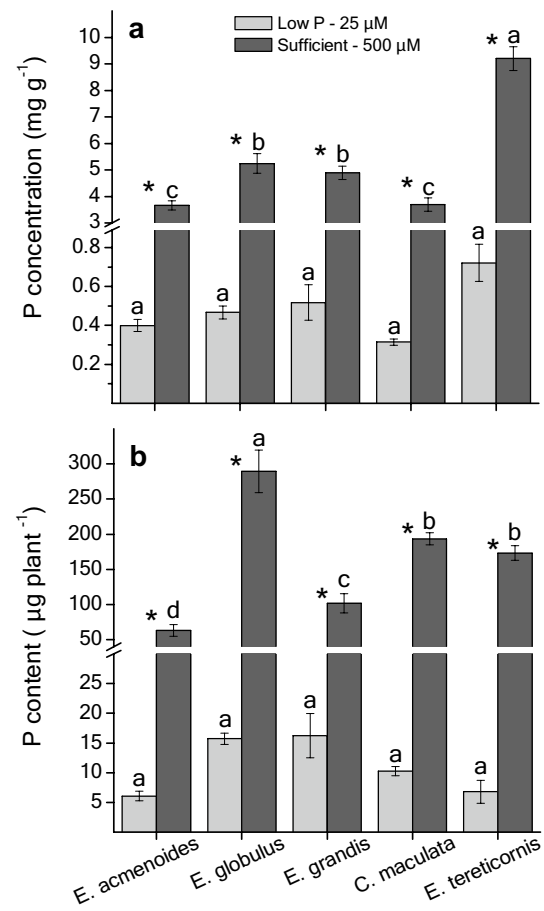

Fig. 4 Phosphorus concentration (**a**) and content (**b**) in the shoot of eucalypt species grown at low and sufficient P concentration. Different letters indicate a significant difference among species for each P concentration, whereas the asterisk indicates a significant difference between P concentrations for each species, by the Scott-Knott test at 5% probability. Bars indicate standard error ($n=4$)

P conditions, *E. globulus* accumulated 2.8- and 4.6-times higher P contents than *E. grandis* and *E. acmenoides*, respectively; the later species presented the lowest P content (Fig. 4b). However, at low P, no significant differences were observed among the species in P accumulation in the shoots (Fig. 4b).

Regarding other nutrients, Online resource Table 1 shows the concentrations of potassium (K), magnesium (Mg), boron (B), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), and zinc (Zn) in the shoots. In general, seedlings grown at sufficient P had a higher concentration of the evaluated nutrients, except for Zn and B, which had higher concentrations in seedlings grown at low P. For B and Mg, *E. acmenoides* and *E. tereticornis* presented significantly higher concentrations than the other species (Online resource Table 1).

Principal component analysis and correlations

Principal component analysis (PCA) was performed separately for P levels (Fig. 5). At low P, the first (PC1) and the second (PC2) components explained 59.2% and 26.8% of the total variation, respectively. For P sufficiency, PC1 and PC2 explained 59.8% and 22.8% of the total variation, respectively. At low P, the root morphological and root hairs traits contributed to the separation of *E. grandis* and *E. globulus* (Fig. 5a, c). These variables were grouped with shoot P content, indicating that for these species, P accumulation and biomass had a strong correlation with the characteristics of roots and root hairs. On the other hand, *E. tereticornis*, at both low and sufficient P, segregated for the loading plot quadrants without the presence of root characteristics, indicating that these characteristics were not so determinant for the responses to P (Fig. 5a). *E. acmenoides* was separated mainly due to the high variance of root tissue density, and oppositely, due to the morphological traits of root hairs

(Fig. 5). B and Mg concentrations strongly contributed to the separation of *E. acmenoides* and *E. tereticornis* in the PC2 axis; as these species showed the higher concentrations of these nutrients, while in PC1 axis they were in opposite quadrants influenced mainly by P concentrations, that were low in *E. acmenoides* and high in *E. tereticornis* (Fig. 5).

Discussion

The adverse effects of P limitation are significant in the early stages of eucalypts development (Graciano et al. 2005). Here, we observed that the negative impact of low P on shoot growth was not observed for *E. grandis*, which produced the larger biomass, and for *E. acmenoides*, which had no significant changes. These results support our previous findings on the response variability of eucalypt species to P availability (Bulgarelli et al. 2019). These responses may be related to changes in biomass allocation, with increased investment in
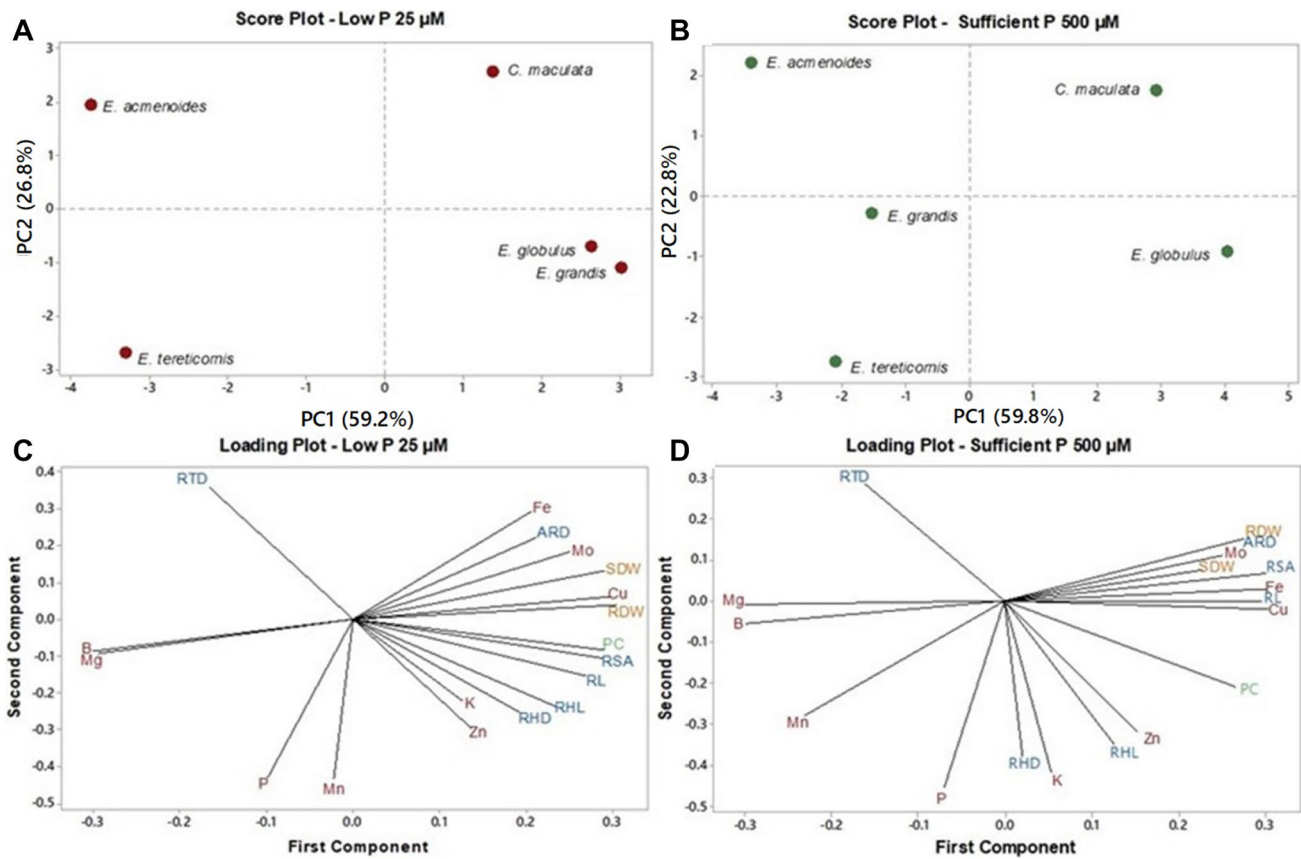

Fig. 5 Principal component analysis (PCA) from five eucalypt species grown at low (**a** and **c**) and sufficient (**b** and **d**) P concentration, score plots (**a** and **b**) and loading plots (**c** and **d**) of the analyzed variables dispersed in the first (PC1) and second (PC2) components. Numbers in parentheses indicate the variation percentage explained by PC1 and PC2. Text colours indicate the cluster to which parameters have been assigned: blue, root morphology; red, nutrient con-

centrations; green, P content; orange, biomass. shoot biomass (SDW), root biomass (RDW), root length (RL), average root diameter (ARD), root surface area (RSA), root hairs length (RHL), root hairs density (RHD), phosphorus content (PC), root tissue density (RTD) and phosphorus (P), potassium (K), magnesium (Mg), boron (B), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), and zinc (Zn) concentrations in the shoots

root growth, changes in root architecture and morphology, the emergence of more prolonged or more abundant root hairs, among others (Kramer-Walter and Laughlin 2017; Poorter et al. 2012). Physiological adaptations of the root system can also contribute to more efficient phenotypes in environments of low P availability (Wang et al. 2020). So, the characteristics that determine how plants will cope with low P concentration represent different strategies of plant phenotype (White and Hammond 2008).

In our study, changes were observed in root morphology of seedlings of all five eucalypt species in response to P supply, but the response varied depending on the species. We observed that biomass was preferentially distributed in the shoots than in the roots, a fact that has already been reported by other authors (Wu et al. 2014). However, changes in characteristics that could contribute to improving the uptake of P were not associated with higher shoot P concentration, although in P deficiency, root biomass production, length, and root surface area correlated positively and significantly with accumulated P in the shoots (Online resource Fig. 1).

Among the morphological traits evaluated, only average root diameter was not altered by P deficiency in any species. Some species showed changes in root length and/or surface area in response to low P concentration, but differently, depending on the species. Compared with sufficient P, while *E. globulus* and *C. maculata* had longer roots and larger root surface area at sufficient P, *E. grandis* had these characteristics increased at low P. The association between an increase of the root length with low P availability have been observed in *Zea mays* (Wang et al. 2020), *Rosa multiflora Thunb. ex Murr.* (Ma et al. 2020), *Fagus sylvatica* (Loew et al. 2020), and *Eucalyptus dunii* (Dias et al. 2017).

E. acmenoides and *E. tereticornis* did not present significant changes in the main root morphology traits evaluated, although SRL and SRA tended to increase at low P in *E. tereticornis*. Longer SRL has been related to improved efficiency in P uptake in tree species (Lugli et al. 2019; Li et al. 2017) and several herbaceous species (Haling et al. 2018; Sandral et al. 2018).

E. acmenoides and *C. maculata* had the highest RTD at low P and *E. globulus* and *E. grandis* the lowest, with RTD presenting negative correlation with root length and root hair length (Online resource Fig. 1). Under limited P, low root tissue density may reduce the metabolic costs of producing new roots (Eissenstat et al. 2000), mainly in those species with short root length. These results might be related to lower dependence on root proliferation and the existence of other physiological strategies for P uptake, which may be metabolically more economical than investing in the development of new tissues (Xia et al. 2020; Funayama-Noguchi et al. 2015). However, RTD can also be a plant genotype characteristic, as reported for different coffee genotypes (Silva et al. 2019).

Improved contact between roots and soil particles can be achieved with longer or more numerous root hairs (Haling et al. 2013), considering it promotes a larger specific surface of the roots (Raghothama and Karthikeyan 2005). Changes in root hairs characteristics have been associated with a strong ability to absorb nutrients of low diffusivity in the soil, as seen with P (Ma et al. 2001; Miguel et al. 2015). Eucalypt species showed different responses to P availability in terms of morphological plasticity of root hairs, which in general were longer and more abundant at low P. *E. grandis* was the only species with an increase in both length and root hairs density at low P. Higher root hairs abundance was the commonest characteristic in the studied species. However, although some studies relate an increase in the number and length of root hairs under low P availability (Ruiz et al. 2020), our study did not find a correlation between longer and/or most abundant root hairs with higher P concentration or accumulation in the shoots of plants (Online resource Fig. 1).

Because our study was carried with plants growing in vermiculite and receiving nutrient solution, one may argue that it does not reflect the potential benefit of more abundant or longer root hairs in P uptake. Although root hairs can be more effective in P uptake when plants grow in the soil, where P-depletion areas can form around roots (Ruiz et al. 2020) our results show that eucalyptus genotypes responded solely to the low concentration of P in the nutrient solution.

E. tereticornis was the species with the lowest plasticity of root morphological traits. However, it was the only species that increased the root-shoot ratio at low P. Such behaviour indicates that biomass allocation was a response to low P in this species. On the contrary, an increase in biomass allocation to the roots has been widely reported in young tree plants growing with limited P (Santiago et al. 2012; Yan et al. 2019). On the other hand, *E. grandis* was the species with the highest root plasticity at low P, since it had an increase in root hair density and length, as well as in root length and surface area. *E. grandis* was also the only species that accumulated more biomass in the shoots at low P than at sufficient P. Such results suggest that for this species changes in the root traits may be related to higher biomass production, even not leading to higher P accumulation in the shoots.

Root morphological response to P limitation may be dependent on plant genotype (Zhou et al. 2017). In a study with eucalypt hybrids, the responses to P limitation varied depending on the genotype/hybrid, with an overall increase in root length and volume, and an increase in root hairs length and density at low P (Zhou et al. 2017). For *C. lanceolata* plants grown at P low concentrations, an increase in P uptake was strongly related to root plasticity, and plants with longer roots were more efficient in P absorption (Yan et al. 2019). This behaviour suggests the pattern of root growth may change so that plants can adapt to a lower P

availability in the soil, increasing the root to shoot ratio to improve P uptake.

Increases in root hairs length and density can act synergistically in response to a low P availability (Brown et al. 2013), favouring P influx in plants and indicating an intrinsic relationship between root hairs and P uptake (Bates and Lynch 2001). This type of response to low P availability in the soil has been reported for *Eucalyptus spp.* (Zhou et al. 2017), *Oryza sativa* (Kakade et al. 2017), *Phaseolus vulgaris* (Miguel et al. 2015), and *Zea mays* (Zhu et al. 2010). However, this relationship between root hairs length and density and increased P uptake was not observed in different genotypes of *Zea mays L.* (Tang et al. 2020; Yuan and Chen 2015). Mutants of *Brachypodium distachyon* grass also did not show this relationship, involving other traits to improve P uptake (Zhang et al. 2018). Therefore, root hairs plasticity does not seem to be a universal trait, but specific to some species and/or genotypes to increase P uptake from soil (Vandamme et al. 2013). However, for species presenting such response to low P, this strategy may be beneficial for P uptake, offering a relatively low metabolic cost when compared to exudation of organic acids and protons, association with soil microorganisms, or development of new roots (Lynch and Ho 2005, Van de Wiel et al. 2016). Some studies showed that arbuscular mycorrhizal symbiosis affect the length and density of root hairs by inducing changes in the metabolism of cell wall and phytohormones, such as auxins (Liu et al. 2020; Ma et al. 2021). In this context, further studies under natural soil conditions are needed to elucidate the effects of the interaction between mycorrhizae and P availability on root morphology and root hairs characteristics.

In conclusion, our results showed that seedlings of eucalypt species present different abilities to adapt their root morphological traits in response to P limitation. However, this response did not reflect positively on P uptake and accumulation under our experimental conditions. Root morphological parameters, such as length and surface area, differed between species, showing root plasticity in eucalyptus regarding P availability. Plasticity, in response to P availability, reveals the extent to which growth processes and interactions can be altered and how environmental factors can be perceived and translated into morphological changes by plants (Gruber et al. 2013). Thus, our results strongly support that root morphological traits, such as long and dense root hairs, are important for plant growth, especially in agroforestry systems under limited P conditions.

Author contribution statement PM and SALA conceptualized and designed the study. SB conducted the experiment, supervised by SALA, and wrote the first manuscript draft. PM and SALA edited draft manuscripts and PM obtained the funding to conduct the research.

Availability of data and materials Data are available on reasonable request to the corresponding author.

Declarations

Conflict of interests The authors declare that there are no conflicts of interest/competing interests in this study.

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