



RESEARCH ARTICLE

Low soil phosphorus availability has limited effects on wood traits in young plants of five eucalypt species

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(Received 23 January 2024; revised 21 April 2024; accepted 22 April 2024)

Summary

Plant-derived products rely heavily on the availability of phosphorus (P) in the soil. With reserves of P-rocks being limited, there is a growing demand to enhance the efficiency of P utilization by crops. Eucalypts, an important economic crop in many countries, is a source of timber, coal, essence oils, and cellulose. After identifying low P tolerant and susceptible species in a previous study, we explored the various physiological and biochemical responses of these same species to low P availability. The aim was to expand our understanding of how different P-nutrition responses might impact eucalypt wood production and traits related to its quality. Our results indicate that low soil P minimally affects physiological wood parameters in the young trees of *Eucalyptus acmenoides*, *Corymbia maculata*, *E. grandis*, *E. globulus*, and *E. tereticornis*. Decreases in cellulose contents and increases in lignin content and syringyl and guaiacyl (S/G) ratios were observed under low P and only in *E. acmenoides* plants. Wood density remained unaffected in all species. Additionally, bark, stem, and root P concentrations increased under sufficient P conditions in *E. globulus*, *E. grandis*, and *E. tereticornis*. These findings suggest that these plant parts may act as reserve pools of this nutrient.

Keywords: cellulose; eucalypt; lignin; plant nutrition; phosphorus use efficiency; wood quality

Introduction

Phosphorus (P) is an essential nutrient for all life forms on Earth. In plants, it plays a fundamental role in several physiological processes, including energy metabolism, cell division and the synthesis of crucial molecules such as nucleic acids, phospholipids, and ATP (White and Hammond, 2008; Lambers and Plaxton, 2015; Xu *et al.*, 2019).

P entry into the food chain occurs through plants, with fertilizers frequently serving as the primary source for cultivated plants due to the low P availability of most agricultural soils and the relatively high plant demands. The inorganic P used as a fertilizer is derived from the extraction of phosphate rock reserves, with the largest reserves located in Morocco, China, South Africa, and the USA. Projections based on current global phosphate rock usage estimate a depletion of these reserves in approximately 90 years (Vaccari, 2009; Veneklaas *et al.*, 2012). These raise significant concerns about the sustainability of P availability for agriculture, given that P reserves are non-renewable. Consequently, identifying mechanisms to enhance P-use efficiency is essential and ranks high in the actions of agricultural science (Vaccari, 2009; van de Wiel *et al.*, 2016). Although alternative approaches are conceivable, such as recovering P from human-generated waste, they still encounter substantial technical and cost challenges (Vaccari, 2009; Pavinato *et al.*, 2020).

Because of the high adsorption of P in soil oxides, predominantly found in tropical soils, it is estimated that only 20% of the P present in applied fertilizers is absorbed by crops (McLaughlin *et al.*, 1988; Zhang *et al.*, 2008). The primary chemical form absorbed by plants is the orthophosphate ion (PO_4^{2-}), and in weathered soils, it strongly binds with Fe and Al oxides/hydroxides, rendering it unavailable. Additionally, part of the soil P can be immobilized in organic forms. In both scenarios, P retained by oxides and in organic compounds can be released into the soil solution through the action of organic acids and enzymes exuded by plants and microorganisms, making it available to plants (Lambers, 2023).

Throughout evolution, plants have developed various mechanisms to obtain P from the soil or to increase its use efficiency by recycling and remobilizing it within cellular metabolism (White and Hammond, 2008; Bulgarelli *et al.*, 2019; de Oliveira Silva *et al.*, 2022). These mechanisms collectively contribute to the P-use efficiency, defined as the amount of biomass produced per unit of absorbed P. (Baker *et al.*, 2015)

The availability of P in tropical forests is a crucial determinant for wood production and the subsequent increase in productivity, responding to total soil P contents (Jucker *et al.*, 2016; de Andrade *et al.*, 2024). In these highly weathered soils, characterized by low P availability, the growth and productivity of eucalypt plantations are significantly restricted (de Moraes Gonçalves *et al.*, 2004). Studies have demonstrated notable effects of P fertilization on eucalypt plantations. For example, the application of superphosphate fertilizer in *E. urophylla* plantations resulted in a higher percentage of stem wood and a lower relative allocation of biomass to the roots compared to unfertilized treatments (Xu *et al.*, 2002). Similarly, in native stands of *E. tereticornis*, P fertilization led to an increase in stem basal area growth (Crous *et al.* 2015). The impact of low P availability extends to wood formation by disrupting processes such as photosynthesis and leading to a shift in carbon allocation towards root growth (Jucker *et al.*, 2016). Nevertheless, the addition of nitrogen and P fertilizers may adversely affect certain wood traits, such as density and fibre length, depending on the soil and climatic conditions. This phenomenon has been observed in *E. globulus* plantations (Raymond and Muneri, 2000). A correlation between wood density and P soil supply was identified in *E. grandis* seedlings. Low P supplies were associated with increased wood density, which was linked to the inhibition of stem cambial activity (Thomas *et al.*, 2005).

Eucalyptus, the world's most extensively cultivated hardwood tree (Carnus *et al.*, 2006; Valadares *et al.*, 2020), encompasses over 700 species, providing a large genetic variability in response to P availability. In previous work, we categorized 25 eucalypt species into four groups based on their responsiveness and tolerance to low soil P availability (Bulgarelli *et al.*, 2019). This information provides an opportunity to explore P allocation strategies in response to different soil P conditions, as these species exhibit variations in their ability to acquire, allocate, and utilize P. Ultimately, these factors influence their overall performance and survival in P-limited environments.

Here, we examined five eucalyptus species – *Eucalyptus acmenoides*, *Corymbia maculata*, *E. grandis*, *E. globulus*, and *E. tereticornis* – selected based on their differing abilities to use P (Bulgarelli *et al.*, 2019) Our investigation aimed to explore the following hypothesis: (i) the availability of P in the soil affects wood compositional traits and density; (ii) species differing in P-use efficiency adjust their photosynthetic rates to optimize P utilization; (iii) stem and bark serve as potential sites for long-term P storage.

Materials and Methods

Plant material and experimental design

The experiment utilized outdoor cultivation in 50-L plastic pots equipped with a dipping water supply system. Seeds of the species *E. acmenoides*, *Corymbia maculata*, *E. grandis*, *E. globulus*, and *E. tereticornis* were obtained from a clonal garden (Caiçara Sementes – <https://sementescaicara.com/>) and germinated in seedbeds containing a mixture of commercial substrate and soil. When

the seedlings reached two months of age, they were transferred into the 50 L pots, each featuring two levels of available soil P: low P (4 mg kg^{-1}) and sufficient P (25 mg kg^{-1}). The transplantation took place in September 2020, and the plants were collected 18 months later. The experimental setup followed a 5×2 factorial design, comprising five eucalyptus species and two P levels, with four biological replicates.

Soil preparation

The substrate for plant growth was prepared by mixing soil and coarse sand in a 1:1 (v:v) ratio. The soil employed was a ferrosol (IUSS Working Group WRB, 2015); its chemical composition can be found in Table S1A (Supplementary material). Potassium phosphate (KH_2PO_4) was incorporated into the substrate to achieve the desired P level in the treatment with sufficient P. No additional P was added for the low P treatment. Additionally, in both P treatments we adjusted the concentrations of copper (Cu), magnesium (Mg), zinc (Zn), manganese (Mn), and boron (B) to levels suitable for forest species at a medium fertility level in the state of São Paulo, Brazil (van Raij *et al.*, 1996). Specific amounts of each nutrient (B, Cu, Mn, Zn, and Mg) added to the soil in milligrams per kilogram (mg kg^{-1}) were as follows: B (H_3BO_3) – 2.25, Cu (CuCl_2) – 2.7, Mn (MnSO_4) – 15.6, Zn (ZnCl_2) – 52.7, and Mg (MgSO_4) – 418.3. Nutrient salts were solubilized in water and slowly added to the soil-sand mixture using a concrete mixer. Following the preparation, the soil-sand substrate was stored for 20 days, after which a sample was taken for soil chemical analysis (Table S1B, supplementary material). Additionally, N, in the form of NH_4NO_3 , was added to the soil at 125 mg kg^{-1} . This N was applied in three separate fertilizations during the experiment, at seedlings transplantation, and after one and two months.

Dry mass accumulation and nutrient analysis

At the end of the experiment, leaves and stems (including branches) were separately collected and air-dried at 60°C until a constant dry mass was achieved. The roots were washed under tap water and also dried at 60°C . Following dry mass determination, the dried material was used for nutrient analysis. A section of the main stem was used for nutritional analysis, and bark was separated from the stem in this section using a blade. Concentrations of P and other macronutrients were analysed in samples of the leaves, stems, bark, and roots using inductively coupled plasma optical emission spectrometry (ICP-OES; Varian Vista MPX, Palo Alto, CA, USA), following nitro-perchloric digestion of the samples.

Determination of gas exchange parameters

Physiological parameters related to leaf gas exchange were assessed in 18-month-old plants using an infrared gas analyser IRGA (Li 6400R, Li-Cor, Lincoln, USA). Measurements of photosynthetic rate (A), stomatal conductance (gs), and intracellular CO_2 concentration (Ci) were conducted on young fully expanded leaves between 9 h and 14 h. The measurements were taken under ambient CO_2 concentration (approximately 400 ppm) and a light intensity of $1,500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with 10% blue light supplied by the equipment.

Wood parameters

Cellulose content was determined using the protocol of Chen *et al.* (2002). Lignin content was determined using the acetyl bromide method (Fukushima *et al.*, 2015). The lignin syringyl/guaiacyl (S/G) ratio was determined according to Mokochinski *et al.* (2015). Wood density was determined using Archimedes' principle (Hacke *et al.*, 2000).

		N	K	Ca	Mg	S
BARK	<i>C. maculata</i>		↓			
	<i>E. acmenoides</i>			↓		
	<i>E. globulus</i>			↓	↓	
	<i>E. grandis</i>			↑	↑	
	<i>E. tereticornis</i>	↑				
ROOT	<i>C. maculata</i>					
	<i>E. acmenoides</i>					
	<i>E. globulus</i>					
	<i>E. grandis</i>					
	<i>E. tereticornis</i>		↓			
STEM	<i>C. maculata</i>					
	<i>E. acmenoides</i>	↑				
	<i>E. globulus</i>	↑				
	<i>E. grandis</i>					
	<i>E. tereticornis</i>		↓			
LEAVES	<i>C. maculata</i>					
	<i>E. acmenoides</i>		↑			
	<i>E. globulus</i>				↓	
	<i>E. grandis</i>		↓			
	<i>E. tereticornis</i>					

Figure 1. Schematic presentation of significant changes in nutrient concentrations in tissues of five Eucalyptus species. The blue arrow up indicates a significant increase of the nutrient in the low P treatment in relation to the sufficient P treatment, and the red down arrows indicate a significant decrease.

Statistical analysis

The influence of soil P availability and eucalypt species on growth variables, leaf and stem nutrient concentration, gas exchange parameters, and biochemical traits was evaluated using a two-factor analysis of variance (two-way ANOVA). Mean values were compared using the Tukey’s test at a significance level of $p \leq 0.05$, employing the Rbio software (Bhering, 2017).

Results

Macronutrient concentrations

The concentrations of nutrients other than P (N, Ca, K, Mg, and S) were determined in roots, stems, bark, and leaves, and values can be found in Table S2 (supplementary material). A concise overview of the significant differences between low and sufficient P treatments in each species is shown in Figure 1. Minimal alterations were observed, and no distinct pattern emerged regarding species or organs/tissues. Most of the significant changes were identified in the bark, where, when significantly altered, N concentrations consistently increased while K decreased (Figure 1). In the stems, N concentrations increased in *E. acmenoides* and *E. globulus* plants when compared to plants under sufficient P conditions (Figure 1). However, in the leaves, only minor alterations in nutrient concentrations were observed in some species (Figure 1).

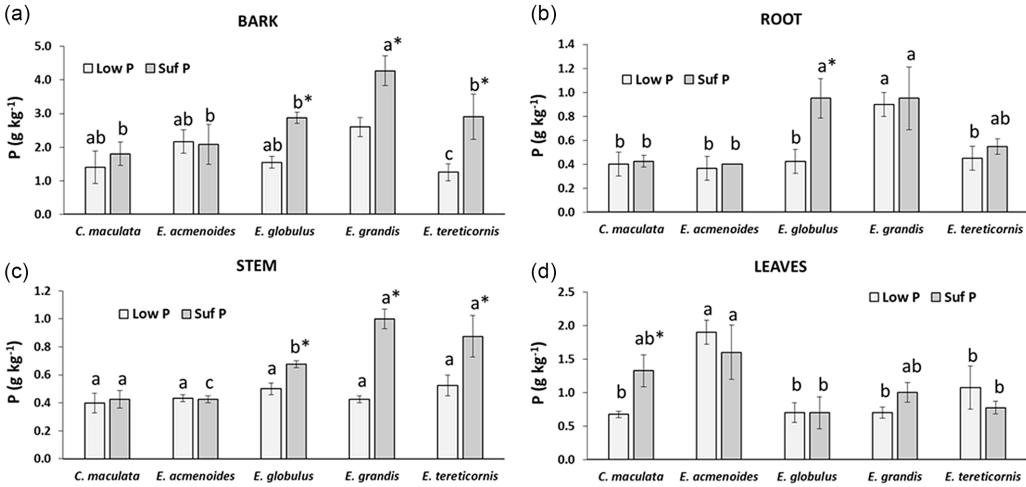


Figure 2. Phosphorus concentrations in different tissues of five eucalyptus species subjected to low and sufficient P. Lowercase letters indicate statistical differences ($p < 0.05$, Tukey's test) among species for the same P treatment, and asterisks indicate statistical differences between P treatments within the same species.

Figure 2 shows the P concentrations in each tissue/organ. *E. grandis* roots exhibited higher P concentrations in both low and sufficient P treatments than the other species. Notably, *E. globulus* was the sole species showing a significant difference between plants subjected to low P and sufficient P conditions. Compared to low P conditions, plants of *E. globulus*, *E. grandis*, and *E. tereticornis* receiving sufficient P showed higher P concentrations in the bark and stem. Conversely, in the leaves, *E. acmenoides* showed the highest P concentrations among the studied eucalypt species (Figure 2), while only in *C. maculata* were P concentrations significantly higher under sufficient compared to low P conditions (Figure 2).

Plant growth

Plants of *E. globulus*, *E. grandis*, and *E. tereticornis* grown under low P and sufficient P exhibited comparable dry mass accumulation in all organs (Figure 3). However, plants of *C. maculata* and *E. acmenoides* showed better growth under sufficient P compared to low P. Specifically, under low P, *E. tereticornis* was the species accumulating the highest dry mass in all plant organs. Under low P conditions, root biomass production in *C. maculata* and *E. acmenoides* was higher compared to plants under sufficient P. For *E. globulus* and *E. tereticornis*, P did not significantly influence root biomass production, whereas for *E. tereticornis*, root biomass production was higher under low P conditions compared to sufficient P conditions (Figure 3a).

Photosynthetic traits

Except for *E. globulus*, a clear pattern of reduced CO₂ assimilation rates was evident in plants under low P conditions (Figure 4a); however, the only significant difference was observed in *E. grandis* plants. There were also minor alterations in stomatal conductance (Figure 4b) and internal leaf CO₂ concentration (Figure 4c).

Wood density, cellulose, lignin, and S/G ratio

Minimal alterations were observed in terms of wood traits due to low P availability and among eucalypt species (Figure 5). Under low P conditions, an interesting trade-off was identified in

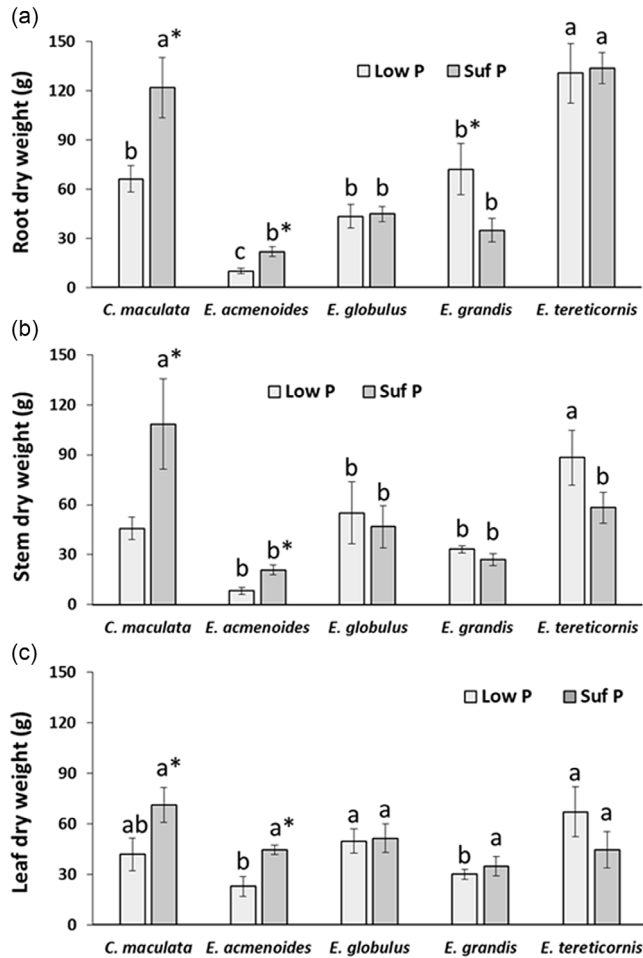


Figure 3. Dry mass of different tissues of five eucalyptus species subjected to low and sufficient P. Lowercase letters indicate statistical differences ($p < 0.05$, Tukey's test) among species for the same P treatment, and an asterisk indicates statistical differences between P treatments within the same species.

E. acmenoides plants, exhibiting reduced cellulose contents (Figure 5b) but increased lignin contents (Figure 5c). The S/G ratio also increased in plants of this species under low P compared to plants under sufficient P conditions (Figure 5d). Under sufficient P conditions, *E. tereticornis* exhibited the lowest lignin contents among the studied species, with levels up to four times lower than those observed in *E. grandis* plants (Figure 5c).

Discussion

Phosphorus and plant growth

The importance of P in plants is highlighted by its essential role as a major component of vital molecules, such as ATP and sugar-phosphates. It participates in enzyme phosphorylation and contributes to the formation of cellular structures, such as the case of phospholipids in membranes. Deficiency in P strongly impacts plant growth and productivity (Hawkesford *et al.*, 2012).

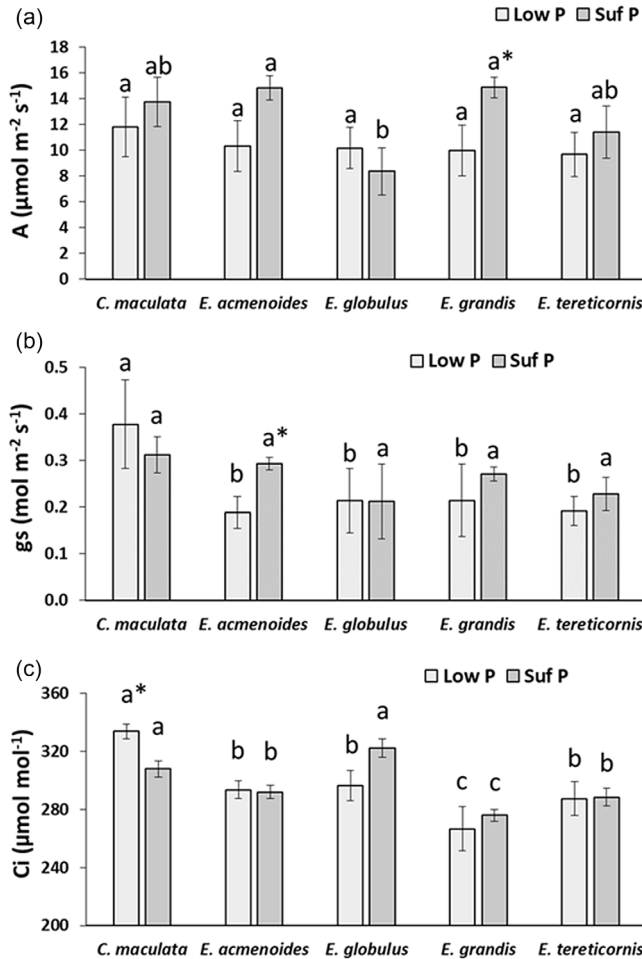


Figure 4. Photosynthesis (a), stomatal conductance (b), and leaf internal CO₂ concentration of different tissues of five eucalyptus species. Lowercase letters indicate statistical differences ($p < 0.05$, Tukey's test) among species for the same P treatment, and asterisk indicates statistical differences between P treatments in the same species.

Our investigation revealed that *E. globulus*, *E. grandis*, and *E. tereticornis* plants subjected to low P conditions had lower P concentrations in the stem and bark than plants under sufficient P (Figure 2). Surprisingly, we did not find a positive correlation between P concentrations and dry matter accumulation in these species. Conversely, *C. maculata* and *E. acmenoides*, despite not showing an increase in P concentration (Figure 2), were the species that accumulated more biomass (dry mass-basis) (Figure 3). Furthermore, plants of *C. maculata* and *E. acmenoides* under sufficient P did not show a significant increase in photosynthetic rates, although a noticeable trend was observed.

Previous studies with eucalypts, specifically *E. tereticornis*, showed that P enhances plant height and stem biomass over time with a stable photosynthetic capacity (Crous *et al.*, 2015). Similarly, P was found to lead to a 30 % increase in wood production in *E. pauciflora*, which was related to a reduction in photoassimilate allocation to roots rather than an improvement in canopy biomass (Keith *et al.*, 1997). In our study, only *E. maculata* and *E. acmenoides* exhibited increased root dry mass accumulation with P, although all studied species had the same or higher root mass than stem dry mass (Figure 3).

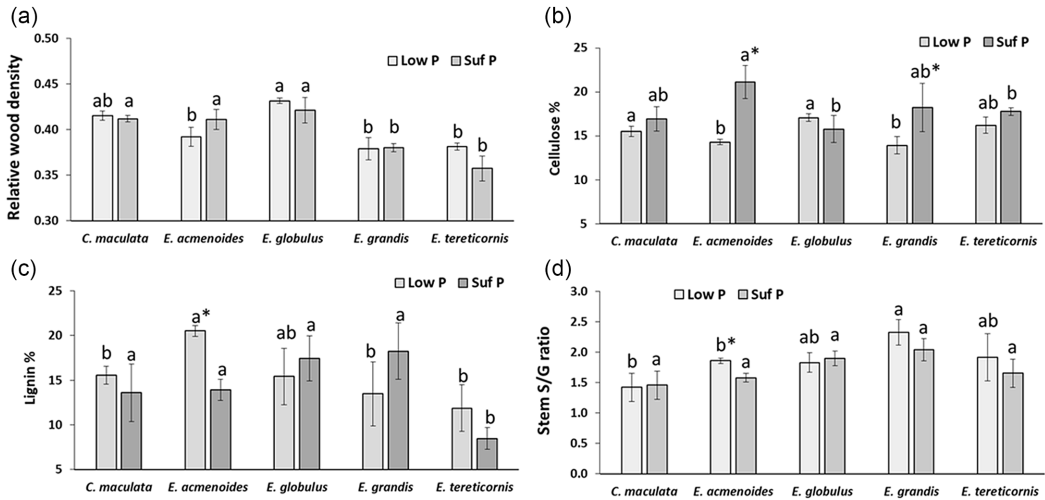


Figure 5. Wood density (a), cellulose (b), lignin (c) concentrations, and S/G ratios in the wood of five eucalyptus species subjected to low and sufficient P. Small letters indicate statistical differences ($p < 0.05$, Tukey's test) among species for the same P treatment, and asterisk indicates statistical differences between P treatments within the same species.

A previous study on beech trees, in which the impact of added P on forest soil was evaluated, revealed that new P was preferentially allocated aboveground. This resulted in increased bound P in xylem tissue and enhanced soluble P in bark, indicating increased storage and transport in sufficient P conditions (Keith *et al.*, 1997). Similarly, a study by Netzer *et al.* (2018) identified several processes and metabolites contributing to adequate phosphate supply in low P soil, including the accumulation of phospholipids and phosphates in the bark and storage of phosphate in the wood. Therefore, the higher P accumulation in the bark observed in our study for *E. globulus*, *E. grandis*, and *E. tereticornis* may suggest a strategy employed by eucalyptus species to store P for subsequent remobilization and use under low P conditions.

It is widely acknowledged that low P availability can disrupt the photosynthetic machinery and electron transport chain, redirecting assimilated carbon for root growth, contrary to the observations made by the species in this study (Lambers *et al.*, 2006; Carstensen *et al.*, 2018). However, the responses of several eucalypt species may vary considerably regarding their response to low P (Bulgarelli *et al.*, 2019). Using responsiveness and use efficiency for P as traits to categorize species into four possible groups (responsive and tolerant to low P, responsive and non-tolerant, non-responsive and tolerant, and non-responsive and non-tolerant), most species were grouped in responsive and non-tolerant to low P and non-responsive and tolerant categories. *C. maculata* was distinctly positioned in the non-responsive and non-tolerant group, while *E. acmenoides* was typically positioned as responsive and non-tolerant. The distinctions among these two species, as well as the other three included in this study, may indicate different metabolic mechanisms in response to low P, as previously suggested (de Oliveira Silva *et al.*, 2022), including the buffering of P deficiency in one organ by P accumulated in other organs, such as roots, as observed in other eucalyptus species (Mulligan, 1988).

The dose of P supplied to the plants is also noteworthy for discussion. In this study, a high P dose of 25 mg P kg⁻¹ soil was used, requiring the addition of 21 mg of P to reach this level compared to the low P treatment of 4 mg P kg⁻¹ soil. In 50-L pots filled with substrate up to about 90% of their volume, which had approximately a 0.4 m² diameter, the amount of P added was calculated considering a soil density of 1.3 g cm⁻³. This resulted in the addition of about 3 g of P per m². For comparison, in a field with *E. tereticornis* plantation, (Crous *et al.*, 2015) applied

50 kg ha⁻¹, equivalent to 5 g P per m². Therefore, depending on the amount of P used, the nutrient accumulation may differ because of the plant species and the nutrient availability or supply.

Phosphorus and wood quality

Wood quality is a critical attribute in various industries, including timber production, construction, pulp, and paper manufacturing, and contributes to the economic value of tree stems (Lourenço *et al.*, 2013).

In *E. grandis*, a lack of P fertilization over 20 months resulted in a notable 50% decrease in wood volume (Rocha *et al.*, 2019). An earlier study on the effects of P limitation on *E. grandis* seedlings revealed that, although plant height, stem diameter, and total biomass increased with increasing P supply, stem wood density sharply decreased with increasing P supply until a threshold was reached, beyond which further P supply did not affect wood density (Thomas *et al.*, 2005). The increase in wood density was primarily attributed to the increased thickening of secondary walls in stem fibre cells under low soil P supply, altering biomass partitioning within the stem in favour of secondary wall thickening (Thomas *et al.*, 2005).

Changes in the metabolome of leaves, roots, and stems of seedlings from the same five eucalyptus species examined in this study (de Oliveira Silva *et al.*, 2022) found substantial variation among species in concentrations of sugars, organic acids, amino acids, and lipids under low P supply. Some species exhibited a reduction in P-sugars and increased sulphur-lipids, indicative of enhanced P-use efficiency. In agreement with our study, irrespective of species and P treatment, stems accumulated more P than leaves and roots.

The physical and chemical properties of wood directly influence its utilization in various applications. When considering wood constituents, cellulose emerges as a primary component due to its unique characteristics. As the main component of the cell wall and wood, cellulose is a complex polysaccharide composed of repeating units of glucose molecules linked together by β -1,4-glycosidic bonds, forming long chains characterized by their high degree of polymerization and linear arrangement, which contributes to the strength and integrity of wood fibres (Mahood and Cable, 1922; Horikawa, 2022). These cellulose fibres are organized into crystalline structures known as microfibrils, which are assembled into oriented sheets stacked on top of each other, forming the cell wall. The density and orientation angle of these microfibrils influence wood stiffness and utility (Tabet and Aziz, 2013; Horikawa, 2022).

Another crucial component significantly influencing wood quality is lignin, the second most abundant polymer in vascular plants, providing rigidity and strength to plant cell walls. Its composition and structure can vary among different plant species and even within different cell types within the same species (Vanholme *et al.*, 2010; Liu *et al.*, 2018).

Limited information is available about the effects of low P availability on cell wall components. A recent review showed that P deficiency may affect cellulose and lignin synthesis in the root cells, as roots alter growth patterns to increase P absorption from the soil (Ogden *et al.*, 2018). At the molecular level, PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT1) may be affected by P nutrition. PAP1 may act as a positive or negative regulator of the lignin biosynthesis pathway. The transcript levels of PAP1, and its close homolog PAP2/MYB90, are strongly induced nitrogen and/or P limitation, promptly reversed by nitrate fertilization (Ogden *et al.*, 2018).

A leaf metabolome analysis of young *E. globulus* plants grown at different P concentrations showed significant changes in metabolite profiles (Warren, 2011). P-sugars like glucose-6-P and fructose-6-phosphate decreased with low P, indicating a reallocation of P pools. Minimal changes were observed regarding carbohydrates, organic acids of the tricarboxylic acid (TCA) cycle, and amino acids. However, low P led to a decrease in phenolic compounds, such as coumaroylquinic acid and catechin, suggesting a relation to increased lignin synthesis. P deficiency has been reported to increase lignification in proteoid roots of lupin (Uhde-Stone *et al.*, 2003) and leaves of young cotton plants (Luo *et al.*, 2021).

In our study, low P availability did not affect the wood density of all species. However, it increased lignin content in *E. acmenoides* and decreased cellulose contents. This trade-off might explain why the relative wood density was not altered in this species. Additionally, in *E. acmenoides*, the ratio between syringyl (S) and guaiacyl (G) units increased under low P. A higher S/G ratio is particularly advantageous for pulp and paper manufacturing due to the easier S-lignin degradation, resulting in lower alkali consumption and higher pulp yield (González-Vila *et al.*, 1999). Furthermore, the S/G ratio is an important factor in biomass recalcitrance to bioethanol production, with a higher S/G ratio leading to higher recalcitrance (Cesarino *et al.*, 2012; Vicentini *et al.*, 2015). For timber production and construction, however, a low S/G ratio is more desirable as it contributes to a denser and more compact wood structure, ideal for the furniture and civil construction market (Yoo *et al.*, 2018; Börcsök and Pásztor, 2021).

Conclusion

This study explored biochemical and physiological responses associated with low P availability to determine if a low P supply might affect wood formation and quality in five eucalypt species. The observed effects were minimal and non-related to P availability or the ability of the species to respond or accumulate P. However, an interesting finding was that three species, *E. globulus*, *E. grandis* and *E. tereticornis*, accumulated more P in the stem and bark than in leaves, suggesting that these tissues may act as reserve pools of this nutrient.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0014479724000115>

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments. FMOS and AAB thank the São Paulo Research Foundation (FAPESP) for student fellowships (grants 2018/09624-0 and 2019/10614-2, respectively), and PM thank the Brazilian National Council for Scientific and Technological Development (CNPq) for research fellowship. This work was supported by FAPESP (grant 2016/25498-0). The funders had no role in the study design, data collection, analysis, interpretation, manuscript writing, or decision to publish the results.

Authors contributions. FMOS: investigation, formal analysis, data curation, and writing – Original Draft; HAG: Data curation and writing – original draft; AAB: investigation and data curation; SALA: conceptualization, writing – original draft and reviewing, and supervision; PM: conceptualization, writing – original draft and reviewing, and supervision.

Competing interests. The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Cite this article: Silva FMO, Gioppato HA, Borghi AA, Andrade SAL, and Mazzafera P. Low soil phosphorus availability has limited effects on wood traits in young plants of five eucalypt species. *Experimental Agriculture*. <https://doi.org/10.1017/S0014479724000115>