

## Article

# Macro- and Micronutrient Contents and Their Relationship with Growth in Six Eucalyptus Species

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**Abstract:** Knowing nutrient allocation dynamics in the tissues and the characteristics related to growth in different forest species is crucial to fertilization management and selecting better species for specific environments, ensuring greater fertilization efficiency and consequent sustainability in the forestry sector through the rational use of fertilizers. The objectives of this study were (i) to evaluate the content of macro- and micronutrients in different tissues of eucalyptus species and (ii) to relate them with their growth. The treatments were composed of six eucalyptus species (*Eucalyptus camaldulensis* Dehnh., *Corymbia citriodora* Hook., *E. saligna* Sm., *E. grandis* W.Hill ex Maiden, *E. urograndis*, and *E. urophylla* S. T. Blake). Macro- (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) and micronutrient (boron, copper, iron, manganese, and zinc) contents were determined in the leaves, bark, and sapwood. To study the functional patterns in macro- and micronutrient contents, Canonical Variable Analysis (CVA) was performed. The first two canonical variables in nutrient content of leaves, bark, and sapwood and the growth variables of eucalyptus species accumulated values greater than 80% of variance. The species *E. grandis* and *E. urograndis* showed the highest means for volume and total height but showed no differences regarding the concentration of major elements in the tissues, except the iron content in the bark, which was higher compared to other species. CVA proved to be an excellent tool for understanding, identifying, and classifying the strategies of *Eucalyptus* sp. regarding the content of nutrients in the shoot biomass tissues and may support genetic improvement programs aiming at identifying potential species. Future research involving the use of remotely piloted aircraft and remote sensors could be a strategy to monitor nutrient contents in different parts of trees throughout the cycle of different eucalyptus species.

**Keywords:** nutritional diagnosis; functional traits; forestry improvement; canonical variables



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## 1. Introduction

Using higher nutrient-efficient species will play a key role in increasing or maintaining forest productivities in the future. Additionally, with the advent of the bioeconomy, there is a growing demand for ecologically sustainable production of food and biomass [1]. For example, the nitrogen (N) and phosphorus (P) use efficiency have been proposed as indicators of progress toward a goal of promoting sustainable agriculture [2]. In the case of N, for example, excessive fertilization leads to environmental pollution due to the escape of reactive N from agricultural soils, which can lead to groundwater contamination, emissions of nitrogen oxides and ammonia gas, as well as the accumulation of nitrous

oxide, a harmful greenhouse gas that reduces stratospheric ozone [1–3]. Consequently, these environmental impacts pose serious risks to the climate and human health, making the adoption of management and techniques that promote the rational use of fertilizers indispensable in modern agriculture. However, several studies point to crop-specific needs for different nutrients in different environments.

For assessing the yield potential of forest crops, only climate and altitude information are considered, as the soil can be managed through fertilization and other techniques. However, functional perspectives on the level of plant characteristics related to nutrient allocation in the tissues and the characteristics related to growth [3] are especially relevant, as they can indicate both limiting and promoting factors of forest production. In addition to the analysis of specific characteristics, synthetic measures, such as functional indices and functional composition, are useful for indicating overall patterns of forest functioning [4].

A functional perspective of forests is useful for understanding forest dynamics and proposing management of planted forests, since it identifies and improves the understanding of the patterns and behavior of different species, but its use is not common for this purpose, being mostly restricted to ecological studies. Functional traits are important for understanding the relationship between species and the environmental factors that influence their growth and dynamics [5].

Functional characteristics of a forest community can help explain species behavior in space and time. The functional attributes of plants related to resource uptake and allocation (e.g., nutrients) indicate the ecophysiological advantages of different plant strategies for different environmental conditions [3,4]. Thus, information on the functional composition of forest species can improve our ability to understand and predict the dynamics of plantations, besides helping to select better species or genotypes for specific environments, ensuring a more adequate forest management, particularly regarding fertilization, which is costly in silviculture. Additionally, the identification of nutrient-use efficient species is essential for promoting the rational use of fertilizers and guaranteeing sustainable production systems.

Eucalyptus cultivation is among the most productive forest crops in the world, having considerable importance in the global scenario by providing raw material for several uses [6]. Widely planted in tropical and subtropical regions, this forest genus has a wide environmental adaptation due to its phenotypic plasticity, besides showing high growth rates and satisfactory wood density [7]. *Eucalyptus grandis* is the most widely cultivated species in Brazil; among the reasons are its adaptation to the climate and soil and the moderate basic density that allows various uses, especially for the production of cellulose and charcoal. The species *E. saligna*, *E. urophylla* and the hybrid *E. urograndis* also show great prominence due to their environmental plasticity [8].

Eucalyptus species often have high nutrient uptake efficiency, and under favorable fertilization conditions, plantations can uptake large amounts of nutrients, increasing macro- and micronutrient levels in their biomass [9], with direct effects on volumetric and height growth. Several studies have already reported that nutrient concentration in eucalyptus is variable according to the different tissues of the plant [10–18], mainly regarding N, P, and K, and that the nutrient contents occur in a descending gradient in the following order: leaves > bark > branches > belowground > stemwood [19,20].

In this sense, knowing the patterns of macro- and micronutrient contents in the different tissues of trees, such as leaf, bark, and sapwood, and identifying which eucalyptus species have the highest macro- and micronutrient levels in each of these tissues, considering the same fertilization conditions, is of great importance for the forestry sector from both production and sustainability perspectives. Plants with higher leaf nutrient content, for example, tend to have better photosynthetic efficiency, which promotes greater tree growth [19,20]. Likewise, species with a higher nutrient content in the leaves and stem are

indicative of better efficiency in uptake and accumulating nutrients for their vital processes, which is an advantage in nutritional terms. Thus, the use of such species can contribute to a more rational use of fertilizers, since they show greater nutritional efficiency when compared to others under the same soil fertilization conditions.

Given the abovementioned, the objectives of the study were (i) to evaluate the content of macro- and micronutrients in different tissues of eucalyptus species and (ii) to relate them with the growth of trees to identify production patterns that contribute to more sustainable agriculture.

## 2. Materials and Methods

### 2.1. Conducting the Experiment

The experiment was installed in January 2014 at the experimental area of the Federal University of Mato Grosso do Sul, Chapadão do Sul campus, State of Mato Grosso do Sul, Brazil. The altitude is 820 m, and the soil is classified as medium-textured red oxisol. According to the Köppen classification system, the climate is tropical humid (Aw), with a rainy season from October to April and a dry season from May to September [10]. Average rainfall ranges from 750 to 1800 mm year<sup>-1</sup> and the average annual temperature ranges from 20 to 25 °C.

All fertilization requirements were determined from chemical soil analyses, whose results are as follows: pH (CaCl<sub>2</sub>): 4.9; organic matter: 31.5 g dm<sup>-3</sup>; phosphorus: 13.6 mg dm<sup>-3</sup>; hydrogen + aluminum (H + Al): 5.4; potassium: 0.29 cmol<sub>c</sub> dm<sup>-3</sup>; calcium: 2.8 cmol<sub>c</sub> dm<sup>-3</sup>; magnesium: 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity (CEC): 9.0 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation: 39.9%. Limestone was applied three months before the implementation of the experiment to increase base saturation to 60%. The proportions of clay, sand, and silt were 46%, 46%, and 8%, respectively. Crowning, weeding, ant control, and herbicide application (glyphosate) were performed when necessary. No irrigations were carried out during the experiment.

### 2.2. Experimental Design

The experimental design adopted was randomized block with three replications, with 20 plants within each experimental plot. Treatments were composed of six species (*Eucalyptus camaldulensis* Dehnh., *Corymbia citriodora* Hook., *E. saligna* Sm., *E. grandis* W. Hill ex Maiden, *E. urograndis*, and *E. urophylla* S. T. Blake). All species were implanted via seeds, with the exception of *E. urograndis*, which used the GG100 clone. The seedlings were transplanted to the field six months after emergence. The spacing used was 3.0 m between rows and 3.5 m between plants.

### 2.3. Variables Assessed

Measurements and plant collections were performed in December 2019. Growth variables were assessed in two trees from each plot: diameter at breast height at 1.30 m (DAP, cm), total height of tree (HT, m) and wood volume (VOL, m<sup>3</sup>). A tape measure was used to determine the circumference at breast height, which was later converted to DAP. The Ht (m) was measured using a Haglof<sup>®</sup> hypsometer model ECII. For obtaining VOL, the trees were cubed using the Smalian method [21]. A disc with a thickness of 3–5 cm was removed from each of the trees cubed, at a position of 1.3 m from ground level. The sapwood was then separated from the bark using a penknife, allowing samples of sapwood and bark to be obtained for the analysis of macro- and micronutrient levels. Leaves were also collected from these same trees. To sample the leaves, around 300 g of leaves were collected from each tree, taken from the middle third of the canopy, according to the recommendations for assessing the nutritional status of plants proposed by Malavolta et al. [22]. All the samples (leaves, bark, and sapwood) were dried in an oven at 65 °C until a constant dry mass was obtained.

The content of macronutrients nitrogen (N) ( $\text{g kg}^{-1}$ ), phosphorus (P) ( $\text{g kg}^{-1}$ ), potassium (K) ( $\text{g kg}^{-1}$ ), calcium (Ca) ( $\text{g kg}^{-1}$ ), magnesium (Mg) ( $\text{g kg}^{-1}$ ), and sulfur (S) ( $\text{g kg}^{-1}$ ), and micronutrients boron (B) ( $\text{mg kg}^{-1}$ ), copper (Cu) ( $\text{mg kg}^{-1}$ ), iron (Fe) ( $\text{mg kg}^{-1}$ ), manganese (Mn) ( $\text{mg kg}^{-1}$ ), and zinc (Zn) ( $\text{mg kg}^{-1}$ ) were determined in the leaves, bark, and sapwood using wet digestion with  $\text{HNO}_3 + \text{HClO}_4$  (3:1). Acid digestion consists of completely oxidizing the organic matter in the plant's tissues using acids and high temperature, with the aid of a digester block [23]. Once the extract had been digested, the elements were determined using spectrophotometry (P and S) and atomic absorption spectrophotometry (Ca, Cu, Fe, K, Mn, and Zn).

P contents were determined using the vanadate yellow spectrophotometry method, where  $\text{H}_2\text{PO}_4^-$  reacts with  $\text{MoO}_4^{2-}$  and  $\text{VO}_3^{2-}$  to form a yellow-colored complex with light absorption in the 420 nm region. S contents were determined using the principle that  $\text{SO}_4^{2-}$  forms a white precipitate with  $\text{Ba}^{2+}$  [23]. A UV-VIS spectrophotometer was used for both analyses and analytical curves were constructed to estimate the concentration of P and S in the digested extract. The contents of Ca, Cu, Fe, K, Mg, Mn, and Zn in the extracts were determined using atomic absorption spectrophotometry. For this purpose, an atomic absorption device with an air-acetylene flame and hollow cathode lamps, specific for each element analyzed, was used [23].

Unlike the elements mentioned above, in order to determine the N content, the dry matter was weighed (0.1 g) using the Kjeldahl digestion, distillation, and titration technique. The material was digested using  $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$  in a digester block (1); distilled using a semi-micro Kjeldahl distiller (2); and titrated using an automatic titrator (3). According to Miyazawa et al. [23],  $\text{NH}_4^+$  obtained via digestion with  $\text{H}_2\text{SO}_4$  is distilled in an alkaline medium, so that the condensed  $\text{NH}_4^+$  is collected in the  $\text{H}_3\text{BO}_3$  solution and titrated with the HCl solution. Finally, in order to determine the B content, 0.2 g of the material was weighed and dry digested in a muffle furnace at  $600^\circ\text{C}$  for four hours, with the B content determined using spectrophotometry with azomethine-H [23].

#### 2.4. Statistical Analysis

Initially, an analysis of variance (F test) was carried out and the Scott–Knott test was applied to group the eucalyptus species according to the variables evaluated. A significance level of 5% probability was adopted. A canonical variable analysis (CVA) was performed to study functional patterns in macro- and micronutrient contents. The multivariate analysis based on canonical variables is a process for assessing the degree of similarity between accessions that takes into account both the residual covariance matrix and covariance between phenotypic means of the evaluated traits [24]. This technique was applied for the following groups of variables: macronutrients and micronutrients in different tissues of the shoot biomass (leaf, bark, and sapwood). In each analysis, the variables DAP, HT, and VOL were entered to verify the influence of nutrients on the growth variables in the eucalyptus species. Statistical analysis was performed using the *candisc* package of the R software 3.8.1 version [25].

### 3. Results

When we analyzed the DAP (Tables 1 and S4), the species *E. grandis*, *E. urograndis*, *E. urophylla*, and *E. saligna* had the highest means, differing statistically from the others. The species *E. grandis* and *E. urograndis* had the highest HT. Consequently, these species presented the highest VOL averages in relation to the other species evaluated.

All species used in this study showed no statistical differences regarding the content of N, P, Mg, and S in the leaves (Tables 2 and S1). However, when analyzing the potassium concentration in this organ, it was observed that the species *C. citriodora*, *E. urophylla*, and *E. camaldulensis* differed from the others, showing higher levels. *E. camaldulensis* showed high levels of calcium, significantly different from the other species.

An analysis of the leaf's micronutrient content showed that the species had no statistical difference for B, Cu, and Mn (Table 2). As for Fe, only the *E. grandis* and *E. saligna* species differed from the others, with higher concentration values. Zn content in the leaves was higher in *C. citriodora*, statistically different from the other species, followed by *E. camaldulensis*, and the latter from the other species.

**Table 1.** Mean values for wood volume (VOL, m<sup>3</sup>), diameter at breast height (DAT, cm), and total height (HT, m) in different eucalyptus species.

Species	DAP (cm)	HT (m)	VOL (m <sup>3</sup> )
CA	14.93 b	14.56 b	0.1272 b
CI	14.17 b	17.12 b	0.1419 b
SA	18.67 a	18.37 b	0.2273 b
GR	21.43 a	22.14 a	0.3467 a
UG	20.50 a	23.56 a	0.3575 a
UR	19.20 a	19.49 b	0.2488 b

Means followed by the same letters in the column do not differ by the Scott–Knott test at 5% probability level. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR).

**Table 2.** Mean values for macro- and micronutrients in leaves in different eucalyptus species.

Species	Macronutrients					Micronutrients					
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg <sup>-1</sup>					mg kg <sup>-1</sup>					
CA	12.77 a	0.67 a	12.80 a	19.03 a	2.20 a	1.07 a	41.67 a	29.67 a	352.33 b	150.33 a	26.33 b
CI	14.97 a	0.83 a	14.87 a	9.23 b	2.37 a	0.97 a	51.67 a	57.33 a	385.00 b	196.33 a	42.33 a
SA	14.33 a	1.00 a	9.10 b	10.70 b	3.07 a	0.97 a	57.33 a	49.00 a	587.67 a	151.00 a	14.33 c
GR	14.00 a	0.93 a	7.73 b	11.17 b	2.07 a	1.03 a	46.33 a	43.67 a	502.33 a	153.67 a	11.00 c
UG	13.73 a	0.90 a	9.77 b	9.40 b	2.10 a	1.13 a	58.67 a	37.33 a	303.33 b	109.67 a	8.00 c
UR	12.43 a	1.03 a	11.80 a	12.60 b	2.73 a	1.03 a	51.67 a	47.00 a	420.67 b	107.00 a	17.67 c

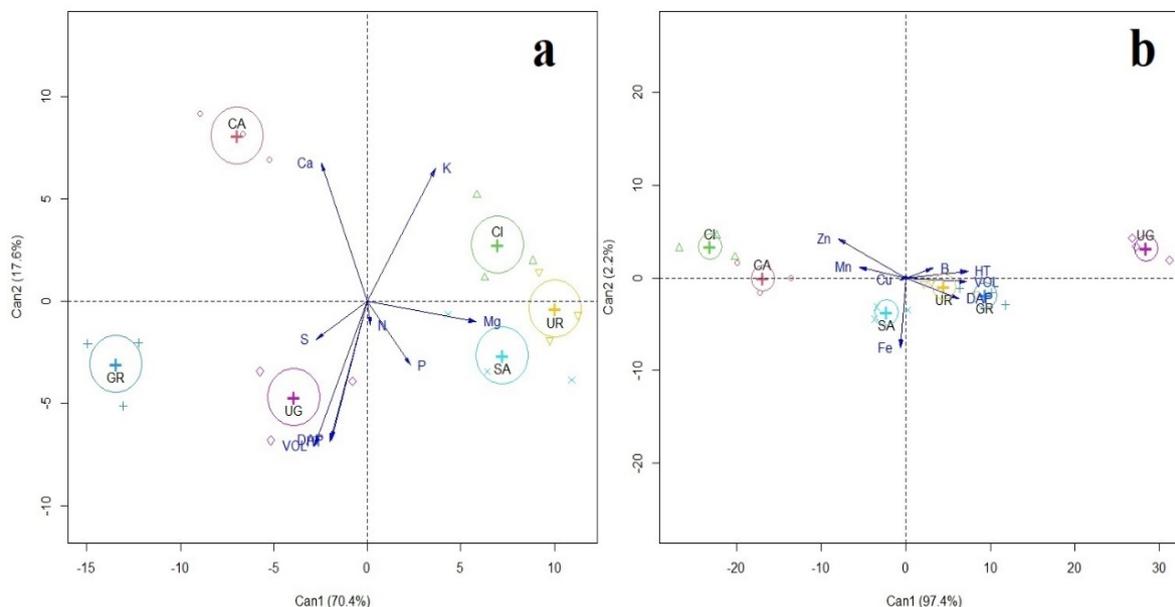
Means followed by the same letters in the column do not differ by the Scott–Knott test at 5% probability level. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Canonical variable analysis proved to be satisfactory. In all analyses, the first two canonical variables (Can1 and Can2) used as the axis of the Cartesian system involved a considerable fraction of the total variation, higher than 80%. Canonical variables of macronutrients in the leaves of eucalyptus species accumulated an eigenvalue of 88.0%. The first and second canonical variables resulted from the linear combination of the nine studied variables, which explained 70.4% and 17.6% of the variance, respectively (Figure 1a).

The species *E. saligna*, *E. urophylla*, and *C. citriodora* were similar based on the magnesium content (Figure 1a). The species *E. grandis* and *E. urograndis* showed similarity based on growth parameters (volume, diameter at breast height, and total height) and S content, while *E. camaldulensis* was dissimilar to the others (Figure 1a).

Regarding the leaf micronutrients, the canonical analysis considered the first two components, accumulating an eigenvalue of 99.6%, Can1 (97.4%), and Can2 (2.2%) (Figure 1b). *C. citriodora* and *E. camaldulensis* had similar contents for almost all micronutrients and were grouped based on Zn and Mg contents. *E. grandis*, *E. urograndis*, and *E. urophylla* showed similarity based on higher B content in leaf tissue and growth variables. *E. saligna* showed patterns different from the others, separated based on higher Fe accumulation. Micro- and macronutrients N, P, Mg, and S contents in the sapwood showed the same patterns as those

observed in the leaves for all species (Tables 3 and S2). K content in sapwood was higher for *E. camaldulensis* species, differing from the others. Meanwhile, Ca was higher in the *C. citriodora* and *E. camaldulensis* species.



**Figure 1.** Analysis of canonical variables and grouping of eucalyptus species based on the macro- (a) and micronutrient (b) contents in leaves. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). DAP: diameter at breast height; Ht: total height; VOL: wood volume.

**Table 3.** Mean values for macro- and micronutrients in sapwood in different eucalyptus species.

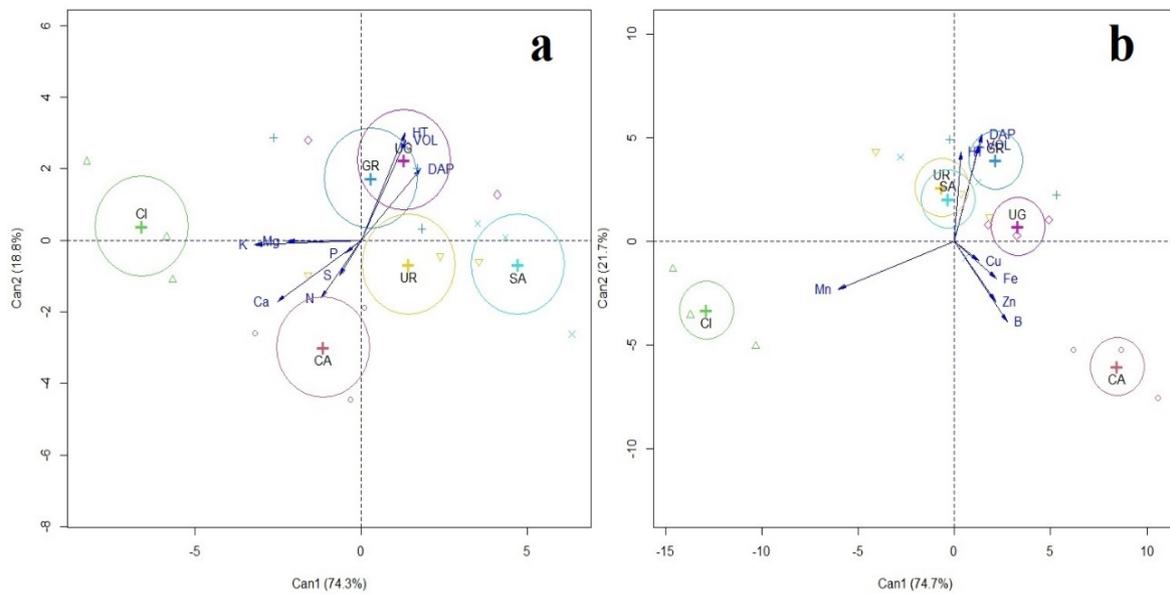
Species	Macronutrients						Micronutrients				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg <sup>-1</sup>						mg kg <sup>-1</sup>				
CA	1.03 a	0.27 a	1.93 a	1.80 a	0.37 a	0.47 a	1.67 a	9.67 a	36.33 b	16.33 a	4.67 b
CI	1.40 a	0.33 a	1.10 b	1.93 a	0.27 a	0.57 a	4.67 a	9.67 a	111.00 b	7.33 a	25.67 a
SA	0.83 a	0.30 a	0.93 b	0.93 b	0.33 a	0.40 a	1.33 a	10.00 a	24.33 a	7.00 a	4.33 c
GR	0.73 a	0.30 a	0.70 b	1.27 b	0.17 a	0.40 a	1.67 a	10.33 a	78.67 a	8.67 a	2.67 c
UG	0.83 a	0.10 a	0.53 b	0.83 b	0.23 a	0.57 a	1.67 a	9.67 a	66.67 b	8.33 a	7.33 c
UR	1.00 a	0.17 a	1.17 b	1.10 b	0.23 a	0.40 a	1.33 a	9.33 a	56.00 b	8.33 a	7.33 c

Means followed by the same letters in the column do not differ by the Scott–Knott test at 5% probability level. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Canonical analysis of the macronutrient contents in sapwood revealed an accumulated eigenvalue of 93.1%, in which the first canonical variable accumulated 74.3% of the total variance and the second canonical variable 18.8% (Figure 2a). *E. grandis*, *E. urophylla*, and *E. urograndis* had a similar pattern, as they did not show association with any macronutrient, only with the growth variables. The other species were not grouped by presenting different patterns based on tissue macronutrient.

Canonical analysis of sapwood micronutrients had an accumulated eigenvalue of 96.4%, in which Can1 and Can2 explained 74.7% and 21.7% of the total variance, respectively

(Figure 2b). *E. saligna*, *E. grandis*, *E. urograndis*, and *E. urophylla* were grouped for having similar contents of all micronutrients in the sapwood, not being associated with any micronutrient, only with the growth variables. On the other hand, *E. camaldulensis* was not associated with any growth variable but with all micronutrients except Mn. Whereas, *C. citriodora* was associated with a single nutrient (Mn).



**Figure 2.** Analysis of canonical variables and grouping of eucalyptus species based on the macro- (a) and micronutrients (b) contents in sapwood. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). DAP: diameter at breast height; Ht: total height; VOL: wood volume.

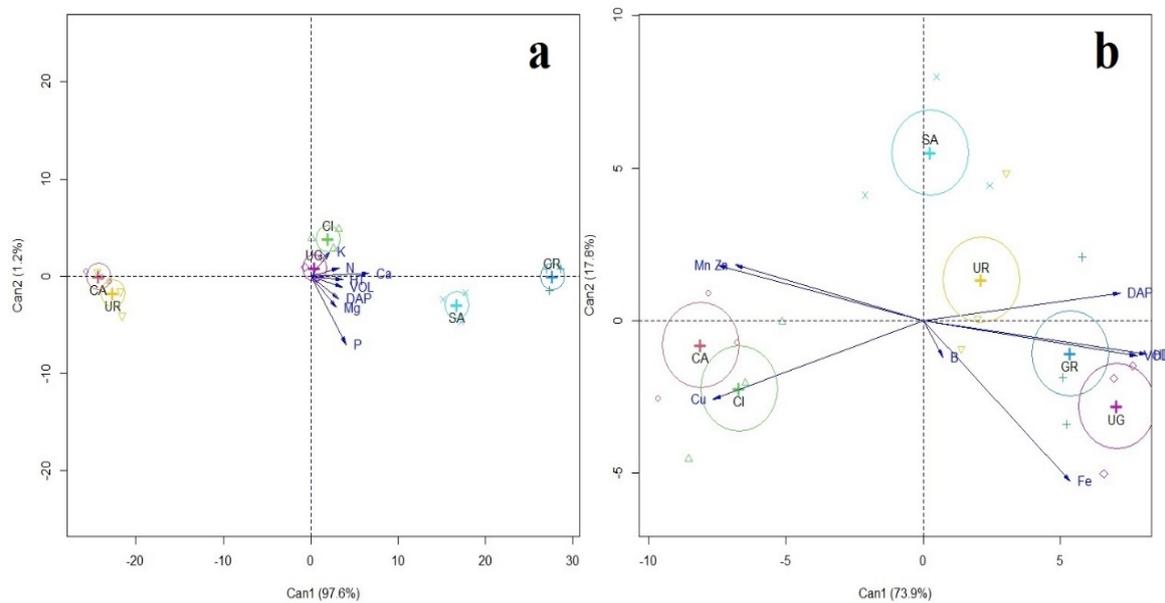
No difference was observed for N, P, K, and S contents when analyzing the bark (Tables 4 and S3). However, when considering Ca, the species *E. grandis* and *E. saligna* showed high contents of this element. Magnesium was higher in *E. saligna* species, statistically different from the others, followed by *E. grandis* and *E. camaldulensis*, which differed from the others.

**Table 4.** Mean values for macro- and micronutrients in bark in different eucalyptus species.

Species	Macronutrients						Micronutrients				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg <sup>-1</sup>						mg kg <sup>-1</sup>				
CA	2.33 a	0.20 d	3.83 a	9.37 b	1.90 b	0.43 a	8.33 b	10.33 a	42.33 d	77.33 a	21.00 a
CI	3.23 a	0.23 d	5.83 a	12.90 b	1.60 c	0.53 a	13.33 a	10.33 a	99.00 c	90.67 a	22.67 a
SA	3.07 a	1.37 a	4.97 a	19.53 a	2.63 a	0.50 a	9.33 b	8.33 b	25.33 d	72.67 a	19.00 a
GR	3.07 a	0.87 b	4.63 a	25.53 a	2.07 b	0.60 a	17.33 a	8.00 b	320.33 a	43.00 b	3.67 b
UG	3.03 a	0.47 c	3.97 a	13.90 b	1.37 c	0.53 a	7.67 b	8.33 b	187.33 b	23.67 b	4.67 b
UR	3.13 a	0.80 b	4.43 a	8.70 b	1.23 c	0.57 a	9.00 b	8.33 b	135.67 c	21.67 b	5.33 b

Means followed by the same letters in the column do not differ by the Scott–Knott test at 5% probability level. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Can1 (97.6%) and Can2 (1.2%) for bark macronutrient content showed a total accumulated variance of 98.8% (Figure 3a). Regarding the macronutrient contents in the bark, only a single group was formed, and the other species were not similar. This group is composed by *E. camaldulensis*, *E. grandis*, *E. saligna*, and *E. urophylla*, as they were not based on any macronutrient and growth variable.



**Figure 3.** Analysis of canonical variables and grouping of eucalyptus species based on the macro- (a) and micronutrients (b) contents in bark. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). DAP: diameter at breast height; Ht: total height; VOL: wood volume.

Micronutrient contents in the bark showed the highest variations compared to the other tissues. B content was higher in *E. grandis* and *C. citriodora* (Table 4). *C. citriodora* and *E. camaldulensis* had higher Cu contents in this tissue. Fe contents were higher in *E. grandis*, significantly different from the other species, followed by *E. urograndis*, *E. urophylla*, and *C. citriodora*. Mn contents were higher in *C. citriodora*, *E. camaldulensis*, and *E. saligna* species. Likewise, Zn contents were higher in *C. citriodora*, *E. camaldulensis*, and *E. saligna* species.

Canonical analysis of the micronutrient contents in the bark accumulated 91.7% of the total variance in Can1 and Can2, forming three groups. The first group was composed of *E. camaldulensis* and *C. citriodora* based on higher contents of Cu, Mn, and Zn, while the second group was composed of *E. grandis*, *E. urograndis*, and *E. urophylla* by the higher contents of Fe and B, and growth variables. Finally, a third group was formed by only *E. saligna*, showing a low association with all variables.

#### 4. Discussion

*Eucalyptus* genus shows a certain stability as to the nutrient content in the plant from the sixth planting year onwards [10]. However, we verified here that the species *E. grandis* and *E. urograndis* showed higher volume and total height. Among the variables evaluated in this research, the Fe content in the bark of these two species had higher values. Simultaneously, it is known that remobilizing nutrients from the bark significantly contributes to the biochemical cycle in eucalyptus trees [21].

Fe is an important element for plant growth, mainly by its role in photosynthesis as a chlorophyll synthesis catalyst [12], in nucleic acid metabolism [13], and in activity of

several enzymes, such as Fe-SOD, involved in oxidative stress metabolism in plants [14]. Despite its low mobility in the plant [15], red latosols (oxisols) are rich in iron [16], which would guarantee an excellent nutrient supply in plants grown in this class of soils. These facts associated with the genetics of these two species possibly ensured *E. grandis* and *E. urograndis* a higher volumetric growth and height compared to the other species.

In this sense, the need for studies to identify the different patterns of nutrient content, especially Fe, in different tissues of these two species is evident. This information would be helpful in genetic improvement programs, seeking a superior performance of these species in different growing locations considering their soil fertility conditions, minimizing the need for fertilization, and thus making forestry more sustainable.

The variation of response trait attributes across gradients needs further investigation. For example, according to Grassein et al. [17], species strategy is defined by trait values and plasticity. These authors reported that conservative species are characterized by constant leaf dry matter content across a gradient of resource availability (e.g., nutrients), while the explorer species exhibit significant variability in this trait. Conversely, the exploitative species can be characterized by their ability to express high and constant values of N content in the leaves [17].

As eucalyptus trees perform their vital functions, they move and store the different nutrients in their organs [10–18]. Both the amount stored and concentration of these elements vary in the different parts of the tree [11,16]. For example, the leaves are rich in N, the bark in Ca, and the sapwood in K. However, there is variation in some species, which leads to higher levels of specific nutrients in the tissues [19,20].

In fact, the high metal contents in the tissues protect the plant against herbivory (e.g., insects) and microorganisms, providing the plants a defense against pests and diseases [26–28]. This is evidenced by *E. saligna*, which has a relationship based on Cu, Mn, and Zn contents in bark and sapwood. The resistance of this species to xylophagous agents, such as the wood wasp, has been reported in the literature [26–28].

It is worth noting that environmental factors can be considered filters that restrict which individuals with specific “response trait” attributes can succeed in forest communities [29], including commercial forestry. Different sets of response traits to environmental factors have been recognized in plants [30–32].

A strategy for storing nutrients in specific tissues was observed in *E. urograndis*, which did not show high levels of macronutrients in the sapwood and bark, but exhibited high contents of macronutrients in the leaves, indicating a greater targeting of nutrients to the leaf. In practice, this ensures a stoichiometric balance more suitable for photosynthesis, possibly promoting higher diameter at breast height and wood volume.

The planted forest’s productivity and the forest ecosystem’s perpetuity are closely related to our understanding of the nutrient balance in the different tissues of the tree [33–36]. Investigating the trees in the location where the reforestation will be carried out and the species or clones that will be planted is a basic requirement. Furthermore, it is essential to develop genetic improvement programs to obtain trees with higher productivity and nutrient uptake and use efficiencies. This will make possible a more sustainable production of the forest, since the identification of more nutrient-use efficient plants, i.e., which show higher uptake and accumulation of nutrients in their different tissues at a minimum fertilization rate, contributes to a more rational use of fertilizers.

## 5. Conclusions

*E. grandis* and *E. urograndis* showed higher values for total height and volume but showed no differences regarding the macro- and micronutrient contents in the tissues, except for the Fe content in the bark, which was higher than the other species. Eucalyptus species presented different strategies regarding the macro- and micronutrient contents in the different tissues of the shoot biomass.

Canonical variables proved to be an excellent tool for understanding, identifying, and classifying the strategies of *Eucalyptus* sp. in terms of the nutrient content of the shoot biomass tissues aiming at a better understanding of the functioning of the forest and may subsidize genetic improvement programs to identify potential species.

Future research involving the use of remotely piloted aircraft and remote sensors could be a strategy to monitor nutrient contents in different parts of trees throughout the cycle of different eucalyptus species.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su152215771/s1>, Table S1: Summary of the analysis of variance for macro- and micronutrients in leaves of different eucalyptus species. Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn); Table S2: Summary of the analysis of variance for macro- and micronutrients in the sapwood of different eucalyptus species. Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn); Table S3: Summary of analysis of variance for macro- and micronutrients in the bark of different eucalyptus species. Species: *E. camaldulensis* (CA), *C. citriodora* (CI), *E. saligna* (SA), *E. grandis* (GR), *E. urograndis* (UG), and *E. urophylla* (UR). Nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn); Table S4: Summary of the analysis of variance for volume (VOL), diameter at breast height (DAP), and total height (HT) of different eucalyptus species.

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## References

1. Alongi, D.M. Impact of global change on nutrient dynamics in mangrove forests. *Forests* **2018**, *9*, 596. [CrossRef]
2. Zhang, X.; Davidson, E.A.; Mauzerall, D.L.; Searchinger, T.D.; Dumas, P.; Shen, Y. Managing nitrogen for sustainable development. *Nature* **2015**, *528*, 51–59. [CrossRef] [PubMed]
3. Schmidt, M.; Veldkamp, E.; Corre, M.D. Tree species diversity effects on productivity, soil nutrient availability and nutrient response efficiency in a temperate deciduous forest. *For. Ecol. Manag.* **2015**, *338*, 114–123. [CrossRef]
4. Chiang, J.M.; Spasojevic, M.J.; Muller-Landau, H.C.; Sun, I.F.; Lin, Y.; Su, S.H.; Chen, Z.S.; Chen, C.T.; Swenson, N.G.; McEwan, R.W. Functional composition drives ecosystem function through multiple mechanisms in a broadleaved subtropical forest. *Oecologia* **2016**, *182*, 829–840. [CrossRef] [PubMed]
5. Zhang, T.; Niinemets, Ü.; Sheffield, J.; Lichstein, J.W. Shifts in tree functional composition amplify the response of forest biomass to climate. *Nature* **2018**, *556*, 99–102. [CrossRef] [PubMed]

6. Bayle, G.K. Ecological and social impacts of eucalyptus tree plantation on the environment. *J. Biodivers. Conserv. Bioresour. Manag.* **2019**, *5*, 93–104. [[CrossRef](#)]
7. Gonçalves, J.L.; Alvares, C.A.; Rocha, J.H.; Brandani, C.B.; Hakamada, R. Eucalypt plantation management in regions with water stress. *South. For. J. For. Sci.* **2017**, *79*, 169–183. [[CrossRef](#)]
8. Rocha, J.H.T.; de Moraes Gonçalves, J.L.; Gava, J.L.; de Oliveira Godinho, T.; Melo, E.A.; Bazani, J.H.; Hubner, A.; Junior, J.C.A.; Wichert, M.P. Forest residue maintenance increased the wood productivity of a Eucalyptus plantation over two short rotations. *For. Ecol. Manag.* **2016**, *379*, 1–10. [[CrossRef](#)]
9. Kotttek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [[CrossRef](#)]
10. Leite, F.P.; Silva, I.R.; Novais, R.F.; Barros, N.F.D.; Neves, J.C.L.; Villani, E.M.D.A. Nutrient relations during an eucalyptus cycle at different population densities. *Rev. Bras. Cien. Solo* **2011**, *35*, 949–959. [[CrossRef](#)]
11. Grove, T.S.; Thomson, B.D.; Malajczuk, N. Nutritional physiology of eucalypts: Uptake, distribution and utilization. In *Nutrition of Eucalypts*; Attiwill, P.M., Adams, M.A., Eds.; CSIRO Publishing: Collingwood, Australia, 1996; pp. 77–108.
12. Akmakjian, G.Z.; Riaz, N.; Guerinot, M.L. Photoprotection during iron deficiency is mediated by the bHLH transcription factors PYE and ILR3. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2024918118. [[CrossRef](#)] [[PubMed](#)]
13. Khodour, Y.; Kaguni, L.S.; Stiban, J. Iron–sulfur clusters in nucleic acid metabolism: Varying roles of ancient cofactors. *Enzymes* **2019**, *45*, 225–256. [[PubMed](#)]
14. Alscher, R.G.; Erturk, N.; Heath, L.S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [[CrossRef](#)] [[PubMed](#)]
15. Schmidt, W.; Thomine, S.; Buckhout, T.J. Iron nutrition and interactions in plants. *Front. Plant Sci.* **2020**, *10*, 1670. [[CrossRef](#)] [[PubMed](#)]
16. Moreira, R.S.; Mincato, R.L.; Santos, B.R. Heavy metals availability and soil fertility after land application of sewage sludge on dystroferic red latosol. *Cienc. Agrotec.* **2013**, *37*, 512–520. [[CrossRef](#)]
17. Grassein, F.; Till-Bottraud, I.; Lavorel, S. Plant resource-use strategies: The importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. *Ann. Bot.* **2010**, *106*, 637–645. [[CrossRef](#)] [[PubMed](#)]
18. Turner, J.; Lambert, M.J. Nutrient cycling in age sequences of two Eucalyptus plantation species. *For. Ecol. Manag.* **2008**, *255*, 1701–1712. [[CrossRef](#)]
19. De Souza Kulmann, M.S.; de Jesus Eufraide-Junior, H.; Dick, G.; Schumacher, M.V.; de Azevedo, G.B.; Azevedo, G.T.D.O.S.; Guerra, S.P.S. Belowground biomass harvest influences biomass production, stock, export and nutrient use efficiency of second rotation Eucalyptus plantations. *Biomass Bioenergy* **2022**, *161*, 106476.
20. Taiz, L.; Zeiger, E.; Møller, I.M.; Murphy, A. *Plant Physiology and Development*, 6th ed.; Sinauer Associates Incorporated: Sunderland, MA, USA, 2015; 761p.
21. Campos, J.C.C.; Leite, H.G. *Mensuração Florestal: Perguntas e Respostas*; UFV: Viçosa, Brazil, 2013; 605p.
22. Malavolta, E.; Vitti, G.C.; Oliveira, S.A.D. *Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações*, 2nd ed.; Potafos: Piracicaba, Brazil, 1997; 319p.
23. Miyazawa, M.; Pavan, M.A.; Muraoka, T.; do Carmo, C.A.F.S.; de Melo, W.J. Análise química de tecido vegetal. In *Manual de Análises Químicas de Solos, Planta e Fertilizantes*, 2nd ed.; Silva, F.C., Ed.; Embrapa Informação Tecnológica: Brasília, Brazil, 2009; 627p.
24. Cruz, C.D.; Carneiro, P.C.S.; Regazzi, A.J. *Modelos Biométricos Aplicados AO Melhoramento Genético*, 4th ed.; UFV: Viçosa, Brazil, 2013; Volume 1, 514p.
25. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021. Available online: <https://www.R-project.org/> (accessed on 1 April 2023).
26. Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmael, Q.; El Hamss, H.; Belabess, Z.; Barka, E.A. Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms* **2022**, *10*, 596. [[CrossRef](#)]
27. Cabot, C.; Martos, S.; Llugany, M.; Gallego, B.; Tolrà, R.; Poschenrieder, C. A role for zinc in plant defense against pathogens and herbivores. *Front. Plant Sci.* **2019**, *10*, 1171. [[CrossRef](#)]
28. Hörger, A.C.; Fones, H.N.; Preston, G. The current status of the elemental defense hypothesis in relation to pathogens. *Front. Plant Sci.* **2013**, *4*, 395. [[CrossRef](#)] [[PubMed](#)]
29. Keddy, P.A. Assembly and response rules: Two goals for predictive community ecology. *J. Veg. Sci.* **1992**, *3*, 157–164. [[CrossRef](#)]
30. Ackerly, D. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecol. Monogr.* **2004**, *74*, 25–44. [[CrossRef](#)]
31. Kraft, N.J.; Valencia, R.; Ackerly, D.D. Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* **2008**, *322*, 580–582. [[CrossRef](#)] [[PubMed](#)]
32. Poorter, H.; Niinemets, Ü.; Poorter, L.; Wright, I.J.; Villar, R. Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytol.* **2009**, *182*, 565–588. [[CrossRef](#)] [[PubMed](#)]
33. Du, B.; Ji, H.; Liu, S.; Kang, H.; Yin, S.; Liu, C. Nutrient resorption strategies of three oak tree species in response to interannual climate variability. *For. Ecosyst.* **2021**, *8*, 70. [[CrossRef](#)]
34. Raulino, W.N.C.; Freire, F.J.; Assunção, E.A.D.A.; Ataíde, K.M.P.D.; Silva, H.V.D.; Silva, A.C.F.D. Nutrition of tree species in tropical dry forest and rainforest environments. *Rev. Ceres* **2020**, *67*, 70–80. [[CrossRef](#)]

35. Giweta, M. Role of litter production and its decomposition, and factors affecting the processes in a tropical forest ecosystem: A review. *J. Ecol. Environ.* **2020**, *44*, 11. [[CrossRef](#)]
36. Tang, Z.; Xu, W.; Zhou, G.; Bai, Y.; Li, J.; Tang, X.; Chen, D.; Liu, Q.; Ma, W.; Xiong, G.; et al. Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4033–4038. [[CrossRef](#)]

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