Original Research Article

BIOMASS AND STOCK OF NUTRIENTS IN DIFFERENT GENOTYPES OF *Eucalyptus* IN SOUTHERN BRAZIL

Abstract: The objective of this study was to estimate the biomass, nutrient stocks, and nutrient utilization efficiency of six genotypes of ~~*Eucalyptus*~~eucalypts. The experiment was conducted in Eldorado do Sul (Cfa - Climatic conditions???; soil???), Rio Grande do Sul, Brazil. The selected trees were fractionated into leaves, branches, stembark and stemwood. The amount of total biomass ranged from 68.40 to 117.52 Mg ha^{-1}, with the highest production being *E. uroglobulus*, and *E. dunnii* the lowest. The canopy (leaves and branches) accumulated between 17% and 52% of the total macronutrients in *E. benthamii* (P1) and *E. uroglobulus* and from 24% to 34% of the total micronutrients in *E. dunnii* and *E. uroglobulus*. While the stem (wood and bark) accumulated between 48 to 83% and 66 to 76% of the total macro and micronutrients, respectively. For the stemwood, it was observed that *E. benthamii* (P2) presented the highest values of nutritional efficiency for N, Ca, Cu and Fe, and *E. uroglobulus* for P, Mg and B. The different eucalypts genotypes~~ of *Eucalyptus*~~, under the same edaphoclimatic conditions, presented different biomass production. Variations in concentration, in the allocation of the amount of nutrients in the different genotypes, and in the different components of the same genotypes were observed.

Keywords: *Eucalyptus* productivity, Forestry nutrition, Silviculture, Sustainability.

1. INTRODUCTION

Comment [A1]: Suggest – eucalypts – popular name

Comment [A2]: How old? four years?

Formatted: Font: Not Italic

Comment [A3]: Hybrid - Eucalyptus urophylla x E. globulus (E. *uroglobulus*)

Comment [A4]: ???

Comment [A5]: confuse

Eucalyptus silviculture has expanded worldwide, mainly because of the increasing demand for wood and the high potential ~~of the genus~~ for biomass production [23]. In Brazil, the expansion of forestry was boosted by a government policy that subsidized reforestation programs from 1967 to 1989, with the aim of developing an internationally competitive logging industry [7].

The possibility of using eucalypt~~us~~ wood for various purposes led both large and small companies to establish eucalyptus plantations for multiple uses [7]. Currently, eucalyptus plantations occupy 5.6 million hectares of the country's forest plantation area, with an annual growth of 2.8% [11]. This rate of increase has been constant for more than 40 years [7], with growth rates strongly dependent on the genetic of clones, forestry practices, and climate [4]. Thus, improving the use efficiency of natural resources through the creation of genotypes and using appropriate practices of site management is a fundamental challenge of maintaining or increasing productivity in a sustainable manner [7].

Biomass production varies according to the availability of resources at different sites, mainly through influences in the processes of photosynthesis, respiration, compartmentalization of carbon, underground flow, and leaf production, among others [15]. The quantification of forest biomass allows the determination of the production potential, or adequacy, of certain species for specific purposes, and the prediction of crop yields, thus helping to assess the loss or accumulation of biomass over time [12].

To define management practices in forest plantations, it is important to choose species that achieve maximum biomass production for a given location by maximizing the uptake of nutrients [9]. For this, the prolongation of the harvest cycle is necessary. In order

Formatted: Highlight

Formatted: Strikethrough

Formatted: Highlight

Formatted: Strikethrough, Highlight

Formatted: Highlight

to achieve maximum efficiency during nutrient cycling, it is important to reduce the unnecessary export of nutrients [17]. In this context, the objectives of future studies on forest biomass should reconsider traditional practices and seek new alternatives to maintain an efficiently balanced crop [5].

Studies on the biomass production and the nutrient stocks of different ~~species/provenances~~clones, planted under the same edaphoclimatic conditions, are key to select genotypes which are able to achieve high productivity in a sustainable way. Therefore, the objective of the present studywas to estimate the biomass, nutrient stocks, and nutrient use efficiency in six different genotypes of *Eucalyptus* established in Eldorado do Sul, Rio Grande do Sul (RS), Brazil.

Formatted: Highlight

2. METHODS

2.1 Characterization of the site

The experiment was conducted in Eldorado do Sul, Rio Grande do Sul, southern of Brazil, in the Horto Florestal Terra Dura, owned by Celulose Riograndense – CMPC (30º 11’30.3”S and 51º 37’47.7”W). The approximate altitude of the place is 158 m.

The climate of the region is characterized as subtropical humid (Cfa), according to the climatic classification of Köppen presenting an average temperature of 19 °C. The average annual precipitation reaches 1,400 mm [2].   In the period from 2012 to 2016, the average rainfall was 1283.6 mm per year. The annual mean temperature was approximately 17.6 ° C (Figure 1). The soil in the experimental area is of the type Red-Yellow Argissol. Table 1 presents the clay and chemical atributes of the soil a depths from 0 to 130 cm.

Comment [A6]: And frost???

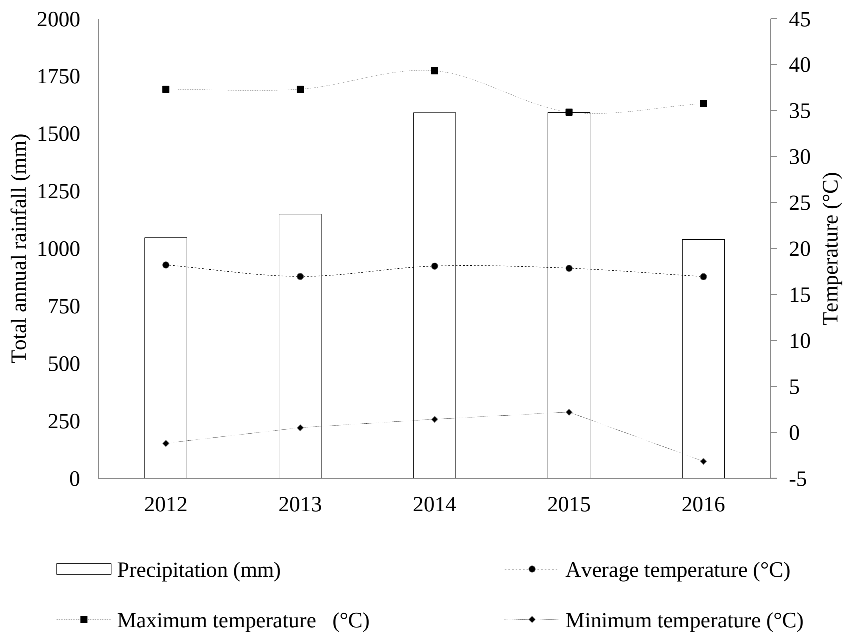

Figure 1 - Climatic diagram of the municipality of Eldorado do Sul, RS, Brazil, during the study period (2012 to 2016).

Table 1 – Physical and chemical attributes of the soil of the area implanted with different genotypes of *Eucalyptus* at 49-months-old in Eldorado do Sul, RS, Brazil

Prof.	**pH**	**Argila**	**C.O**	**V**	**m**	**Al**	**T**	**N**	**P**
	H_2O	**-----------------%-----------------**				**--cmol$_c$ dm^{-3}--**		**%**	**mg g^{-1}**
0-30	5,0	17	0,88	35	34	0,9	10,3	0,10	2,0
30-60	4,3	9	0,77	11	71	3,7	14,0	0,09	1,6

60-90	4,4	25	0,66	15	69	4,8	15,3	0,08	1,0
90-100	4,6	4	0,42	17	64	3,6	12,0	0,06	0,7
100-130	4,7	6	0,22	20	61	3,1	10,0	0,04	0,6

Prof.	**K**	**Ca**	**Mg**	**S**	**B**	**Zn**	**Mn**	**Cu**	**Fe**
	------cmol$_c$ dm^{-3}------			**------------------------mg dm^{-3}------------------------**					**g dm^3**
0-30	0,14	3,3	0,9	19,4	0,4	0,5	13	0,8	0,1
30-60	0,14	0,9	0,5	32,5	0,7	0,5	13	1,2	0,1
60-90	0,15	1,0	0,8	61,7	0,5	0,3	7	1,2	0,1
90-100	0,14	1,0	0,9	60,9	0,3	0,3	5	1,0	0,1
100-130	0,12	0,9	0,9	59,0	0,3	0,3	5	0,7	0,1

O.C: organic carbom; V = saturation by base; m = saturation by aluminum; T = total cation exchange capacity.

2.2 Planting of the experimental area

The genotypes were planted in April 2012, with spacing each plant in a plot of 3 m x 3 m. Subsoiling was performed at a depth of 60 cm, using a subsoiler with three stems, and a liming treatment was applied consisting of 2 Mg ha^{-1} of limestone, and 200 kg ha^{-1} of single superphosphate. Three different fertilizers were applied under different methods: fertilization during planting, coverage fertilization, and maintenance fertilization. The fertilizer used during planting consisted of, 110 g plant^{-1} of N-P_2O_5-K_2O (06:30:06) + 0.3% Zn and 0.2% Cu. For coverage fertilization 200 kg ha^{-1} of N-P_2O_5-K_2O (12:00:20) + 0.7% of B were applied, and for the maintenance fertilization, 300 kg ha^{-1} of N-P_2O_5-K_2O (24:00:26) + 0,5% B were applied.

Before planting, a chemical weeding with 2.5 kg ha^{-1} of glyphosate was carried out. After planting chemical weeding was carried out at 120 and 300 days, with 1.7 kg ha^{-1} of Scout (glyphosate) at the interow. Also, it was carried out to combat leaf-cutting ants.

Formatted: Highlight

The following *Eucalyptus* clones were planted: *E. benthamii* (P1), *E. benthamii* (P2), *E. saligna*, *E. dunnii*, hybrid of *E. urophylla* × *E. globulus* (*E. uroglobulus*), and hybrid of *E. urophylla* × *E. grandis* (*E. urograndis*). *E. benthamii* (P1) is a provenance originating from Guarapuava, Paraná, Brazil and *E. benthamii* (P2) is from Telêmaco Borba, Paraná, Brazil. At the time of data collection, the stands were 49 months old.

Comment [A7]: Not provenance – seedlot or clone, insert some information about the clones (provenance, level of improvement, etc)

Formatted: Highlight

For each genotype of eucalyptus, a plot of 720 m^2 was demarcated, where the DBH (diameter at breast height, measured at 1.30 m above ground level) of all individuals was measured with diametrical tape. The heights of 20% of the plants were measured using a Vertex hypsometer; thus, the heights that were not measured in the field were estimated through hypsometric models. According to Table 2, the mean volume varied from 73.96 to 114.99 m 3 ha (*E. dunnii* and *E. benthamii* (P2). The highest mortality of trees occurred in the settlement of *E. dunnii* (21%). In contrast, the hybrid *E. urograndis* had a 100% survival.

Comment [A8]: Wrong place - results

Table 2 - Dendrometric characterization of different genotypes of *Eucalyptus* at 49-month-old in Eldorado do Sul, RS, Brazil

Genotypes of *Eucalyptus*	**N° of individuals per ha**	**Basal area (m^2 ha^{-1})**	**Volume (m^3 ha^{-1})**

E. benthamii (P1)	986	24,4ab	105,19a
	(192)[**]	(8,8)	(51,1)
E. benthamii (P2)	1.000	22,7b	114,99a
	(216)	(6,3)	(48,0)
E. saligna	972	23,7ab	103,63a
	(206)	(3,5)	(29,8)
E. dunnii	875	16,7c	73,96b
	(195)	(6,9)	(40,62)
E. uroglobulus	903	22,2b	100,27a
	(183)	(7,3)	(43,56)
E. urograndis	1.111	26,4a	111,93a
	(229)	(4,9)	(43,24)

Mean of each variable in different treatments (genotypes of *Eucalyptus*) followed by equal letters, do not differ significantly by the Tukey test at the 5% level of error. [*]Values in parentheses are the standard deviation of the mean.

2.3 Biomass and nutrient stocks

According to the data obtained in the plot inventory, three trees with a mean diameter were sampled for each genotype of eucalyptus. The selected trees were felled and separated in the following components: leaves, branches, stembark and stemwood.

Formatted: Highlight

A sampling of the wood and bark of the stem was done by dividing the trunk into three sections of equal parts, with the sampling performed on three points in the median position of each section. All biomass samples were weighed in the field with a precision scale to determine the moisture content. Subsequently they were sent to the laboratory and dried in an oven at 70 °C with circulation and air exchange until weight stabilization. Based on the dry biomass of each component and the number of trees per hectare of each genetic material, the total biomass per hectare was estimated.

For nutrient determination, the samples were milled with Wiley-type blades, with 30 mesh sieves and submitted to chemical analysis to determine N content by the Kjeldahl method; Ca, Mg, Cu, Fe, Mn and Zn content by atomic absorption spectrometry; P and B content by spectrophotometry; K content by flame photometry, and S content by turbidimetry. The nutrients were analyzed according to the methodology of [24,13]. The estimates of the nutrient stock for each component was obtained by multiplying the dried biomass by the concentration of nutrients. The estimate per hectare was performed by extrapolating the stock per individual based on the number of individuals present in each sampling unit.

2.4 Nutrient Use Efficiency (NUE)

The values of nutrient use efficiency (NUE) were obtained by dividing the amount of biomass of each component and the amount of nutrient from each biomass component, according to the equation:

$$\text{NUE} = \frac{(\text{Amount of biomass})}{(\text{Amount of nutrient})}$$

2.5 Statistical procedures

Statistical analyses were performed at a 5% error probability level with the statistical software Assistat 7.7 [21]. The biomass and nutrient concentration data were subjected to analysis of variance and Tukey's test for comparison of means between treatments (genotypes of eucalypt~~u~~s).

3. RESULTS AND DISCUSSION

3.1 Aboveground biomass

Formatted: Highlight

The highest total biomass production was observed in *E. uroglobulus* and the lowest in *E dunnii*, with 117.52 and 68.40 Mg ha^{-1}, respectively (Table 3). Similar values to the genotype of the present study were reported by [19] while evaluating *E. globulus* in a four-year-old plantation in Butiá, RS, Brazil (83.2 Mg ha^{-1}). Lower values were reported by [20] while evaluating *Eucalyptus* spp. in plantations of two and four years of age in Vera Cruz (RS), Brazil (26.70 and 44.55 Mg ha^{-1}); and by [16], studying *E. saligna* at 1.1 years of age in Telêmaco Borba, Paraná (PR), Brazil (37.35 Mg ha^{-1}). In a study conducted by [28], in the Pearl River Delta region of southern China, when grouping species of eucalyptus into three age classes: < 6 years, 6–15 years, and 16 years of age, the authors found a marked increase in the accumulation of biomass with the increase of age with values of 54.63, 136.94, and 186.43 Mg ha^{-1}, respectively. This suggests that the production of biomass is influenced by plant age, species specific characteristics and planting location.

Comment [A9]: So, why work with dunnii? Insert something in the discussion

In relation to the stemwood biomass, the *E. uroglobulus* hybrid produced 25 and 44% more than the *E. saligna* and *E. dunnii* clones, respectively. Genetic factors (improvement and provenance), edaphoclimatic conditions, and management practices are directly related to the production capacity of the species [8].

Comment [A10]: Where are the information?

Table 3 - Production and partition of biomass for the different components of genotypes *Eucalyptus* at 49-month-old established in Eldorado do Sul, RS, Brazil

Genotypes of *Eucalyptus*	**Leaves**	**Branches**	**Stembark**	**Stemwood**	**Biomass**
			Mg ha^{-1}		
E. benthamii (P1)	4,36b*	7,04a	8,17ab	73,04b	92,19b
	(4,73)**	(7,64)	(8,86)	(79,23)	(100,00)
E. benthamii (P2)	3,92bc	5,08b	8,60a	84,44ab	102,04ab
	(3,84)	(4,98)	(8,43)	(82,75)	(100,00)
E. saligna	3,22c	5,60b	7,92ab	72,50b	89,25bc
	(3,61)	(6,28)	(8,87)	(81,24)	(100,00)
E. dunnii	3,09c	4,59b	6,05c	54,68c	68,40c
	(4,51)	(6,70)	(8,84)	(79,94)	(100,00)
E. uroglobulus	6,52a	7,47a	6,69bc	96,84a	117,52a
	(5,55)	(6,36)	(5,69)	(82,40)	(100,00)
E. urograndis	3,05a	7,41a	7,76ab	83,58ab	101,80ab
	(2,99)	(7,28)	(7,62)	(82,10)	(100,00)

*Averages of each fraction of biomass in different treatments (genotypes of *Eucalyptus*) followed by equal letters, do not differ significantly by the Tukey test at the 5% level of error. **Values in parentheses refer to the percentage of each component in relation to the total biomass of each genotype.

Comment [A11]: %???

The greatest contribution to total biomass was from the stemwood, followed by the stembark, branches, and leaves, except in the clone *E. uroglobulus*, from which the greatest contribution to total biomass was from the stembark. The relative distribution of biomass, considering the same components, was the same as that found by: [25] while studying *E. urophylla* × *E. globulus* at 10 years of age, in Eldorado do Sul, RS, Brazil; by [16] while evaluating *E. saligna* at 6.7 years of age in Telemaco Borba, PR, Brazil; and by [6] while studying the biomass of eucalyptus plantations of different ages in the Central-Eastern Region of the State of Minas Gerais, Brazil. In plantations of *E. nitens* in northern Spain, the distribution trend in terms of total biomass was wood > bark > thick twigs > dried twigs > leaves > fine twigs > twigs [14].

Formatted: Highlight

Formatted: Highlight

By adding the value of the bark to that of the wood, the biomass of the stem represents from 88 to 91% of total aboveground biomass, whose the lowest value was found in *E. benthamii* (P1) and the highest in *E. benthamii* (P2), while the canopy (leaves and branches) represents 9 to 12% of the total aboveground biomass. Some previous studies have reported contrasting results: [8] while evaluating *E. dunnii* at 4 years of age, reported that 81% of the aerial biomass was found in the wood and bark components; and [19], estimating the biomass of *E. globulus*, also at 4 years of age, reported that 77% of the biomass was found in the same components.

In an experiment with *E. saligna* and *E. urophylla* × *E. grandis* at 18 months of age, the authors observed that even at an early age the contribution of the wood component to biomass was the largest relative to total aerial biomass, while the contribution from the bark component was the lowest. The average proportions were 41.5 and 37.4% for the wood and 7.5 and 7.1% for the bark, for *E. saligna* and *E. urophylla* x *E. grandis*, respectively [27].

Before the closure of the canopy, there is a period of intense growth in which most of the photoassimilates synthesized by the plant are channeled into the canopy and root systems. In this phase, the roots partially exploit soil volume and trees do not compete with each other for growth factors (e.g., light, water, and nutrients). After the crowning of the tree canopy, the accumulation of nutrients in the trunks occurs with more intensity, as the formation of the canopy reaches a phase of relative stability, due to auto-shading that imposes a maximum leaf area limit [20].

Formatted: Highlight

3.2 Concentration of nutrients

Nutrient concentrations varied between genotypes and between different components within ~~the same~~ genotype (Table 4). In general, the leaves had the highest concentrations of nutrients and the wood the lowest concentrations, while the branches and bark exhibited intermediate values (Insert new references and discussion – there are many references available - eucalypt in brazil). The tendency for most nutrients to accumulate in the leaves is because leaves have a higher metabolic activity than other components of the plant [25].

Table 4 - Nutrient concentrations in the different biomass components of genotypes of *Eucalyptus* at 49-month-old established in Eldorado do Sul, RS, Brazil

Genotypes of *Eucalyptus*	Fractions	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		g kg^{-1}						mg kg^{-1}				
E. benthamii (P1)	Leaves	22,83a	1,38a	8,24ab	6,07a	2,85ab	1,35a	19,68bc	4,90a	133,63a	460,59a	16,10^a
	Branches	1,91a	0,34a	3,34a	5,58a	1,83^a	0,31a	6,20ab	3,72b	51,44a	307,19a	10,77^a

	Stembark	5,38a	0,58a	5,92a	15,94a	3,69ab	0,32a	13,97a	3,18a	32,33ab	508,08a	15,82[a]
	Stemwood	0,89a	0,17a	1,97a	0,57ab	0,28bc	0,18a	2,90[a]	1,08ab	69,03a	20,51a	4,94[a]
E. benthamii (P2)	Leaves	23,52a	1,27ab	6,63bc	5,19a	2,72ab	1,28a	24,48ab	5,74a	124,37ab	358,99a	13,13ab
	Branches	1,57a	0,26ab	2,90a	2,90a	1,31ab	0,29a	5,20b	2,84b	45,03a	162,36ab	9,42[a]
	Stembark	5,32a	0,69a	4,91a	8,08b	3,68ab	0,35a	13,14a	2,56a	27,70b	285,22ab	13,29[a]
	Stemwood	0,45a	0,11b	1,52bc	0,37b	0,20c	0,18a	1,86ab	0,73b	20,26a	14,66a	4,58ab
E. saligna	Leaves	20,61a	1,26ab	8,31a	4,63a	3,11ab	1,26a	28,72a	5,45a	77,23b	179,76a	11,96ab
	Branches	1,57a	0,29ab	3,14a	5,56a	1,93a	0,34a	7,13[a]	6,55a	39,74a	107,19b	8,32[a]
	Stembark	2,16bc	0,53a	4,19a	9,10b	3,72a	0,33a	11,25a	3,72a	32,32ab	238,13b	7,26[a]
	Stemwood	0,74a	0,08b	1,32c	0,47b	0,36b	0,21a	1,81ab	1,10ab	27,69a	6,49a	4,25ab
E. dunnii	Leaves	21,62a	1,23ab	6,08c	6,49a	3,55a	1,26a	19,52bc	6,38a	100,98ab	300,37a	15,17[a]
	Branches	1,89a	0,25ab	2,44a	4,21a	1,79a	0,31a	6,40ab	4,54ab	47,51a	165,63ab	7,71[a]
	Stembark	4,08ab	0,42a	5,84a	9,17b	3,23ab	0,25a	12,83a	3,06a	32,14ab	288,47ab	9,61[a]
	Stemwood	0,80a	0,09b	1,37c	0,70a	0,55a	0,19a	2,75[a]	0,92ab	23,34a	18,94a	2,84c
E. uroglobulus	Leaves	18,46a	0,99b	6,27bc	4,18a	2,13b	1,01b	15,20c	4,27a	83,19ab	222,94a	10,32b
	Branches	1,54a	0,19b	3,34a	3,76a	0,99b	0,26a	6,20ab	3,15b	46,76a	95,45b	7,38[a]
	Stembark	2,70bc	0,42a	6,45a	7,84b	2,92b	0,35a	14,21a	2,72a	29,16ab	231,58b	8,75[a]
	Stemwood	0,91a	0,08b	1,60bc	0,44b	0,18c	0,23a	1,30b	1,06ab	65,58a	10,37a	3,41bc
E. urograndis	Leaves	21,10a	1,21ab	7,67abc	5,35a	3,98a	1,29a	30,72a	6,49a	74,51b	196,63a	12,81ab
	Branches	0,81a	0,22ab	3,14a	5,08a	1,86a	0,26a	4,87b	4,58ab	48,48a	172,69ab	8,55[a]
	Stembark	1,90c	0,59a	4,42a	9,33b	3,36ab	0,34a	8,49[a]	3,22a	56,56a	284,29ab	6,88[a]
	Stemwood	0,87a	0,09b	1,71ab	0,55ab	0,27bc	0,18a	3,10[a]	1,47a	30,55a	10,20a	4,74[a]

Averages of each fraction of biomass in different treatments (genotype of *Eucalyptus*) followed by equal letters, do not differ significantly by the Tukey test at the 5% level of error.

This same trend, i.e., the highest concentration of nutrients in the leaves and the lowest in the wood, was also reported in populations of *E. urograndis* at 18 months of age in Piratini, RS, Brazil [25], in an *E. dunnii* stand at four years of age in Alegrete, RS, Brazil [8], and in *E. globulus* in Chile [1].

In relation to the analyzed macronutrients, N, P, K, and S were more concentrated in the leaves in most genotypes, except for *E. uroglobulus*, where K was more concentrated in the bark. Ca and Mg were found in higher concentrations in the bark in most genotypes, except for *E. dunnii* and *E. urograndis*, in which the highest Mg content occurred in the leaves. In an *E. dunnii* stand at 9 years of age in Algorta, Uruguay, the highest concentrations of N, P, and K were found in the leaves, but the bark also accumulated high concentrations of nutrients, mainly Ca [10].

For micronutrients, the highest concentrations occurred in the leaves, except for Cu in *E. saligna*, which the highest concentration was observed in the branches; and for Mn in *E. benthamii* (P1), *E. saligna*, *E. uroglobulus*, and *E. urograndis*, and Zn in *E. benthamii* (P2), which the highest concentrations were observed in the bark. This same trend, with higher content of micronutrients in leaves, was also found by [26] in *Eucalyptus urograndis* stands at 18 months of age in Piratini-RS municipality.

3.3 Amount of nutrients

Nitrogen occurred in greater quantities in the leaves of most genotypes, with the exception of *E. urograndis* in which higher concentrations of N were found in the wood. P, K, and S had greater representation in the wood, and Ca and Mg in the bark in most genotypes, except in *E. dunnii*, in which the highest amount of Mg was observed in the wood (Table 5). Micronutrients were stored more in the wood, with the exception of Mn which accumulated in higher concentrations in the bark.

Table 5 - Amount of nutrients in the biomass components of different genotypes of *Eucalyptus* at 49-month-old established in Eldorado do Sul, RS, Brazil

Genotypes of *Eucalyptus*	Fractions	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		---kg ha^{-1}---						---g ha^{-1}---				

E. benthamii (P1)	Leaves	99,70	6,00	36,00	26,50	12,40	5,90	86,00	21,40	586,30	2.033,50	70,40
	Branches	13,40	2,40	23,50	39,30	12,80	2,20	43,60	26,30	362,90	2.173,40	76,20
	Stembark	44,10	4,80	48,60	129,80	30,20	2,70	115,30	26,10	265,20	4.164,70	130,70
	Stemwood	65,50	12,30	143,50	41,90	20,10	13,20	210,30	78,30	4.956,10	1.515,90	360,90
	Total	222,70	25,60	251,60	237,60	75,50	24,00	455,20	152,20	6.170,60	9.887,50	638,20
E. benthamii (P2)	Leaves	92,50	5,00	26,00	20,20	10,70	5,00	95,60	22,40	490,40	1.400,70	51,30
	Branches	7,70	1,30	14,60	14,90	6,70	1,50	26,40	14,20	228,60	835,00	47,00
	Stembark	45,60	6,00	42,30	70,40	31,80	3,00	113,20	21,90	240,50	2.452,30	113,30
	Stemwood	37,90	9,60	127,60	31,40	16,70	15,50	155,20	61,40	1.688,90	1.230,00	383,60
	Total	183,70	21,80	210,50	136,90	65,80	25,00	390,40	119,70	2.648,50	5.918,00	595,20
E. saligna	Leaves	66,50	4,00	26,70	14,90	10,00	4,00	92,50	17,60	248,40	574,90	38,60
	Branches	8,70	1,60	17,60	31,30	10,80	1,90	39,80	36,60	221,60	594,70	46,40
	Stembark	17,00	4,20	33,10	72,10	29,50	2,60	89,20	29,50	255,20	1.882,40	57,50
	Stemwood	53,60	5,90	95,70	34,50	25,90	15,10	131,20	80,00	1.993,90	466,70	309,80
	Total	145,70	15,70	173,10	152,80	76,10	23,70	352,80	163,80	2.719,10	3.518,70	452,30
E. dunnii	Leaves	66,00	3,80	18,50	19,90	10,80	3,90	59,50	19,50	309,90	941,40	46,70
	Branches	8,20	1,10	10,90	19,30	8,10	1,40	29,30	20,40	217,20	791,90	34,80
	Stembark	24,50	2,50	34,70	55,30	19,40	1,50	77,50	18,30	193,50	1.818,30	57,00
	Stemwood	41,50	4,80	74,10	38,20	30,30	10,40	150,40	51,00	1.304,20	1.070,80	155,20
	Total	140,30	12,20	138,20	132,70	68,60	17,10	316,70	109,20	2.024,80	4.622,30	293,70
E. uroglobulus	Leaves	120,00	6,40	40,80	27,30	14,00	6,60	99,40	27,60	548,80	1.457,60	67,80
	Branches	11,40	1,40	25,40	28,20	7,40	1,90	46,80	23,50	349,40	729,80	55,40
	Stembark	17,90	2,80	43,50	51,90	19,70	2,40	95,40	18,40	198,00	1.556,70	59,20
	Stemwood	88,10	7,70	154,80	42,80	17,50	22,10	125,80	103,20	6.782,90	1.018,30	328,50
	Total	237,40	18,40	264,50	150,20	58,70	33,00	367,40	172,70	7.879,00	4.762,30	511,00
E. urograndis	Leaves	64,30	3,70	23,40	16,30	12,10	3,90	93,60	19,80	227,10	599,40	39,10
	Branches	6,00	1,70	23,20	37,70	13,80	1,90	36,10	33,90	359,20	1.279,60	63,30
	Stembark	14,80	4,60	34,30	72,40	26,10	2,70	65,90	25,00	438,90	2.206,10	53,40
	Stemwood	73,00	7,30	143,00	46,10	22,90	14,80	259,50	122,50	2.553,30	852,30	396,40
	Total	158,00	17,20	223,90	172,50	74,90	23,40	455,00	201,10	3.578,50	4.937,40	552,10

The concentrations of macronutrients in the total biomass followed the order: K > Ca > N > Mg > S > P in most genotypes. In *E. benthamii* (P1), however, P content was higher than S content. In *E. benthamii* (P2) and *E. uroglobulus*, N content was higher than Ca content. For *E. dunnii*, the concentrations of macronutrients in the total biomass followed the order: N > K > Ca > Mg > S > P. In stands of *E. urograndis* at 30 and 60 months of age in Seropédica, Rio de Janeiro, Brazil, the following order was observed: K > N > Ca > Mg > P [18].

For micronutrients, the order of concentrations in most genotypes was: Mn > Fe > Zn > B > Cu, except for *E. dunnii*, whose the amount of B was greater than that of Zn; and for *E. uroglobulus*, whose the amount of Fe was higher than that of Mn.

The highest amount of P, Ca, B, Mn, and Zn was found in *E. benthamii* (P1); of N, K, S, and Fe in *E. uroglobulus*; of Mg in *E. saligna*; and of Cu in *E. urograndis*. In *E. uroglobulus* was observed with 39 and 41% more than N and 35 and 48% more of K than the *E. saligna* and *E. dunnii* clones, respectively. In *E. benthamii* (P1) P concentrations were found to be 33, 39, and 52% higher compared to *E. urograndis*, *E. saligna*, and *E. dunnii*, respectively.

The canopy (leaves and branches) accumulated between 17 and 52% of the total macronutrients in *E. benthamii* (P1) and *E. uroglobulus*, and from 24 to 34% of total micronutrients in *E. dunnii* and *E. uroglobulus*. The stem (wood and bark) accumulated between 48 to 83% and 66 to 76% of the total macro and micronutrients, respectively.

The distribution and total content of nutrients in the canopy are affected mainly by changes in the amount of biomass and by differences that occur owing to age, both of the tree and the leaves, in their different physiological stages [3].

Insert new references and discussion – there are many references available

3.4 Nutrient use efficiency

Genotypes and their different components showed variations in nutrient use efficiency (NUE) (Table 6). With the exception of Fe in *E. benthamii* (P1) and *E.*

uroglobulus, in which NUE was larger in the stembark, and of N in *E. urograndis*, where the branches had the highest concentrations, the stemwood presented the highest values of NUE, which is very relevant to forest companies, because this is the main product taken from forest plantations.

Table 6 – Nutrient use efficiency in the biomass components of different genotypes of *Eucalyptus* at 49-month-old established in Eldorado do Sul, RS, Brazil

Genotypes of *Eucalyptus*	Fractions	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
E. benthamii (P1)	Leaves	44	723	121	164	351	740	50.706	203.368	7.437	2.144	61.975
	Branches	526	2.907	300	179	549	3.176	161.441	267.639	19.407	3.241	92.390
	Stembark	185	1.699	168	63	271	3.070	70.875	312.609	30.799	1.961	62.494
	Stemwood	1.116	5.939	509	1.742	3.634	5.514	347.279	933.091	14.737	48.182	202.399
E. benthamii (P2)	Leaves	42	791	151	194	368	784	40.987	175.244	7.992	2.798	76.460
	Branches	660	3.878	347	341	757	3.473	192.308	358.293	22.212	6.080	108.033
	Stembark	189	1.441	203	122	271	2.907	75.968	393.553	35.754	3.507	75.873
	Stemwood	2.228	8.795	662	2.692	5.071	5.437	544.211	1.376.084	49.995	68.651	220.141
E. saligna	Leaves	49	798	121	216	322	797	34.868	182.763	12.979	5.608	83.607
	Branches	645	3.482	319	179	520	2.981	140.612	152.949	25.284	9.421	120.734
	Stembark	466	1.891	239	110	269	2.989	88.700	268.164	31.023	4.206	137.632
	Stemwood	1.353	12.391	758	2.103	2.803	4.807	552.606	905.966	36.364	155.370	234.061
E. dunnii	Leaves	47	817	167	155	285	798	51.850	158.141	9.962	3.279	66.107
	Branches	556	4.047	419	237	564	3.307	156.571	224.899	21.106	5.790	131.794
	Stembark	247	2.379	174	109	312	4.018	78.002	330.696	31.254	3.326	106.134
	Stemwood	1.317	11.439	738	1.431	1.807	5.279	363.615	1.071.303	41.925	51.065	352.245
E. uroglobulus	Leaves	54	1.014	160	239	465	988	65.593	236.128	11.878	4.472	96.073
	Branches	657	5.269	294	265	1.009	3.892	159.548	317.854	21.379	10.236	134.777
	Stembark	374	2.356	154	129	339	2.823	70.113	363.003	33.799	4.299	112.953
	Stemwood	1.100	12.617	626	2.265	5.519	4.376	770.081	938.682	14.277	95.103	294.774
E. urograndis	Leaves	47	825	130	187	252	776	32.552	154.162	13.421	5.086	78.057
	Branches	1.241	4.467	319	197	538	3.803	205.480	218.341	20.629	5.791	117.012
	Stembark	525	1.684	227	107	297	2.914	117.801	310.776	17.681	3.518	145.423
	Stemwood	1.145	11.475	584	1.811	3.645	5.634	322.119	682.490	32.734	98.058	210.872

In general, the highest values of NUE were found in micronutrients, where Cu stood out in all biomass components but presented greater values in the stemwood. However, Mn had a lower NUE in most components, with the exception of the stemwood, in which the lowest NUE was found for Fe. For this component, nutrient use efficiency decreased in the following order: Cu > B > Zn > Mn > Fe.

In relation to macronutrients, P stood out as the most utilized element in the stemwood. In contrast, N presented the least efficiency in the leaves. The NUE of the stemwood for macronutrients decreased in the following order in most genotypes: P > S > Mg > Ca > N > K, with the exception of *E. uroglobulus* in which Mg was higher than S. Similar results, although with inversion in the distribution of some nutrients, were reported by [18] while studying *E. urograndis* at the age of five in Seropédica, RS, Brazil (P > Mg > Ca > N > K); by [17] while evaluating the provenance of *E. grandis* and *E. saligna* in forest sites of São Paulo, Brazil (P > Mg > K > N > Ca); and by [5] while studying *E. urograndis* at two years of age in Botucatu, São Paulo, Brazil (P > Mg > S > N > K > Ca). The variation in nutrient use efficiency can occur due to several factors, such as: the intrinsic characteristics of the genotype, the failure to obtain optimal or critical nutritional balance between the soil and the plant and water conditions [17].

In general, the lowest NUE values were found in the leaves, with the exception of some elements, in which the lowest coefficients were observed in the stembark, as was the case for Ca, Mg, and Mn in the clones *E. benthamii* (P1) and *E. saligna*; for K, Ca, Mg, and Mn in *E. uroglobulus*; for Ca and Mg in *E. benthamii* (P2); for Ca and Mn in *E. urograndis*; and for Ca in *E. dunnii*. In this context, the harvesting of the leaves will result in the greatest export of nutrients, especially N and K. In contrast, considering only the harvesting of the stemwood with bark, Ca and Mg are the limiting nutrients in terms of the productivity of the next cycle, but this limitation may be reduced if only the wood is harvested. In relation to the other biomass components, P presented the highest NUE for the leaves and branches in most genotypes, except for *E. benthamii* (P1) where S had the highest value. As for the bark of the shaft, the largest NUE was found for S.

Taking into account the greater commercial interest in stemwood, it was observed that the highest biomass yields were accompanied with the highest values of nutritional efficiency for some elements, that is, the highest efficiency values for *E. uroglobulus* (P, Mg, and B) and *E. benthamii* (P2) (N, Ca, Cu, and Fe). Regarding the other genotypes, *E. saligna* showed higher efficiency for K and Mn, *E. urograndis* for S, and *E. dunnii* for Zn. The high efficiency presented by a species in the use of nutrients implies that it has a lower nutritional requirement, therefore, a parameter of great utility in the selection of species to be used in reforestation, especially in nutrient poor soils [22].

4. CONCLUSIONS

The different genotypes of *Eucalyptus*, under the same edaphoclimatic conditions, present different biomass production.

Comment [A12]: Ok, try answer why in your discussion.

There are a great variation in the concentration and allocation of the amount of nutrients in the different genotypes of *Eucalyptus* and in the different components of the same genotypes.

The highest biomass yields were accompanied with the highest values of nutritional efficiency for some elements, that is, the highest efficiency values for *E. uroglobulus* (P, Mg, and B) and *E. benthamii* (P2) (N, Ca, Cu, and Fe).

5. REFERENCES

1. Albaugh TJ, Rubilar RA, Maier CA, Acuña EA, Cook RL. Biomass and nutrient mass of *Acacia dealbata* and *Eucalyptus globulus* bioenergy plantations. Biomass and Bioenergy. 2017; 97: 162-171.

2. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift. 2013; 22 (6): 1-18.

3. Bellote AFJ, Silva HD. Sampling techniques and nutritional evaluations in eucalypt plantations, In: Gonçalves JLM, Benedetti V. Forest nutrition and fertilization. Piracicaba: IPEF; 2004.

4. Binkley D, Campoe OC, Alvarez C, Carneiro RL, Cegatta I, Stape JL. The interactions of climate, spacing and genetics on clonal *Eucalyptus* plantations across Brazil and Uruguay. Forest Ecology and Management. 2017; 405: 271-283.

5. Eufrade Junior HJ, Melo RX, Sartori MMP, Guerra SPS, Ballarin AW. Sustainable use of eucalypt biomass grown on short rotation coppice for bioenergy. Biomass and Bioenergy. 2016; 90: 15-21.

6. Gatto A, Barros NF, Novais RF, Silva IR, Leite HC, Villani EMA. Estoque de carbono na biomassa de plantações de eucalipto na região centro-leste do estado de Minas Gerais. Revista Árvore. 2011; 35 (4): 895-905.

7. Gonçalves JLM, Alvares CA, Higa AR, Silva LD, Alfenas AC, Stahl J et al. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. Forest Ecology and Management. 2013; 301: 6-27.

8. Guimarães CC, Schumacher MV, Witschoreck R, Souza HP, Santos JC. Biomassa e nutrientes em povoamento de *Eucalyptus dunnii* Maiden no Pampa Gaúcho. Revista Árvore. 2015; 39 (5): 873-882.

9. Guo LB, Sims REH, Horne DJ. Biomass production and nutrient cycling in *Eucalyptus* short rotation energy forests in New Zealand.: I: biomass and nutrient accumulation. Bioresource Technology. 2002; 85 (3): 273-283.

10. Hernández J, Pino A, Salvo L, Arrarte S. Nutrient export and harvest residue decomposition patterns of a *Eucalyptus dunnii* Maiden plantation in temperate climate of Uruguay. Forest Ecology and Management. 2009; 258 (2): 92-99.

11. IBÁ, Indústria Brasileira de árvores: ano base 2016/IBÁ. Brasília/DF, 2016.

12. Kuyah S, Dietz J, Muthuri C, Noordwijk M, Neufeldt H. Allometry and partitioning of above- and Bellow-ground biomass in farmed eucalyptus species dominant in Western Kenyan agricultural landscapes. Biomass and Bioenergy. 2013; 55 (1): 276-284.

13. Miyazawa M, Pavan MA, Muraoka T. Análises químicas de tecido vegetal. In: Silva, F.C. Manual de análises químicas de solos, plantas e fertilizantes. Brasília: Embrapa Comunicação para Transferência de Tecnologia, p. 171-224; 1999.

14. Pérez-cruzado C, Rodríguez-Soalleiro R. Improvement in accuracy of aboveground biomass estimation in *Eucalyptus nitens* plantations: Effect of bole sampling intensity and explanatory variables. Forest Ecology and Management. 2011; 261(1): 2016-2028.

15. Ryan MG, Stape JL, Binkley D, Fonseca S, Loos RA, Takahashi EM et al. Factors controlling *Eucalyptus* productivity: How water availability and stand structure alter production and carbon allocation. Forest Ecology and Management. 2010; 259 (9): 1695-1703.

16. Salvador SM, Schumacher MV, Viera M, Stahl J, Consensa CB. Biomassa e estoque de nutrientes em plantios clonais de *Eucalyptus saligna* Smith. em diferentes idades. Scientia Forestalis. 2016; 44 (110): 311-321.

17. Santana RC, Barros NF, Neves JCL. Eficiência de utilização de nutrientes e sustentabilidade da produção em procedências de *Eucalyptus grandis* e *Eucalyptus saligna* em sítios florestais do estado de São Paulo. Revista Árvore. 2002; 26 (4): 447-457.

18. Santos FM, Chaer GM, Diniz AR, Balieiro FC. Nutrient cycling over five years of mixed-species plantations of *Eucalyptus* and *Acacia* on a sandy tropical soil. Forest Ecology and Management. 2017; 384: 110-121.

19. Schumacher MV, Caldeira MVW. Estimativa da biomassa e do conteúdo de nutrientes de um povoamento de *Eucalyptus globulus* (Labillardière) sub-espécie Maidenii. Ciência Florestal. 2001; 11(1): 45-53.

20. Schumacher MV, Witschoreck R, Calil FN. Biomassa em povoamentos de *Eucalyptus* spp. de pequenas propriedades rurais em Vera Cruz, RS. Ciência Florestal. 2011; 21 (1), 17-22.

21. Silva FAZ, Azevedo CAV. Principal components analysis in the software assistat statistical attendance. In: Word Congress on Computers in Agriculture 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers; 2009.

22. Silva HD, Poggiani F, Coelho LC. Eficiência de utilização de nutrientes em cinco espécies de *Eucalyptus*. Boletim de Pesquisa Florestal. 1983; (6/7): 1-8.

23. Silva PHM, Poggiani F, Libaldi PL, Gonçalves AW. Fertilizer management of eucalypt plantations on sandy soil in Brazil: Initial growth and nutrient cycling. Forest Ecology and Management. 2013; 301: 67-78.

24. Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. Análise de solo, plantas e outros materiais. (2.ed.). Porto Alegre, RS: Departamento de Solos, UFRGS; 1995.

25. Viera M, Schumacher MV, Trüby P, Araújo EF. Biomassa e nutrientes em um povoamento de *Eucalyptus urophylla* x *Eucalyptus globulus*, em Eldorado do Sul-RS. Revista Ecologia e Nutrição Florestal. 2013; 1 (1) :1-13.

26. Viera M, Bonacina DM, Schumacher MV, Calil FN, Caldeira MVW, Watzlawick LF. Biomassa e nutrientes em povoamento de *Eucalyptus urograndis* na Serra do Sudeste-RS. Semina: Ciências Agrárias. 2012;33 (1): 2481-2490.

27. Viera M, Schumacher MV, Bonacina DM, Ramos LOO, Rodríguez-Soalleiro R. Biomass and nutrient allocation to aboveground components in fertilized *Eucalyptus saligna* and *E. urograndis* plantations. New Forests. 2017; 47: 1-18, 2017.

28. Zhang H, Guan D, Song M. Biomass and carbon storage of *Eucalyptus* and *Acacia* plantations in the Pearl River Delta, South China. Forest Ecology and Management. 2012; 277: 90-97.