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Toposequence effects on leaf and wood traits of *Eucalyptus* clones under water and light gradients

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TECHNOLOGY OF FOREST PRODUCTS

ABSTRACT

Background: In Brazil, the production of wood derivatives is mainly based on planted eucalyptus forests, often established in areas marginal to agriculture, such as steep terrain. However, intra-site variability in these plantations is still poorly explored, especially in operational silviculture contexts. Topographic gradients can modify the availability of water and light, influencing plant performance and wood quality. In this context, this study aimed to evaluate the effect of topographic variation along a toposequence on the morphological and anatomical characteristics of leaves, as well as on the anatomical and chemical properties, basic density, and higher heating value of the wood, in a clonal plantation of Eucalyptus spp.

Results: Position in the toposequence significantly influenced several characteristics ($p < 0.10$). Trees located at the base of the slope exhibited, on average, higher leaf water content (1.37 g. g^{-1}), larger leaf area (47.95 cm^2) and lower total extractive content in the wood (3.47%). The leaves of trees in the middle of the slope had the lowest water content (1.16 $g.g^{-1}$). At the top of the slope, where the trees with the lowest height growth were found (15.26 m), the leaves had a higher proportion of palisade and spongy parenchyma (0.62), one of the smallest leaf areas (28.9 cm^2) and higher total extractive content (7.20%).

Conclusions: Topographic variation promotes integrated adjustments in leaf and wood characteristics, reflecting adaptive strategies to local resource availability conditions. These results reinforce the importance of considering intra-site variability in forest management and in the selection of genetic materials better adapted to heterogeneous environments.

Keywords: *Intra-site variability; leaf anatomy; topographic gradient; wood chemistry.*

HIGHLIGHTS

Topography widely affected leaf area and water content in sampled forest trees.
Wood extractives ranged from 3.47% (base) to 7.20% (top of slope).
PCA explained up to 71% of total variance, discriminating slope positions.
PCA suggested similarity between middle- and top-slope trees.

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INTRODUCTION

The growth and development of trees, along with wood quality, are shaped by a complex interaction of genetic and environmental factors. Although the form of plant organs is largely genetically determined, plants can modify the morphology and physiology of these organs to optimize the use of environmental resources (Wright *et al*., 2017; Lin *et al*., 2021), which, in turn, affects the overall growth and development of the plant. Leaves, for example, can undergo physiological and structural changes to optimize light absorption. Under low light conditions, leaves can expand their surface area to capture morelight and increase chlorophyll content to improve light absorption. Conversely, under high light conditions, leaves generally reduce their size to minimize light exposure and may produce more protective pigments, such as carotenoids, to prevent damage (Tang *et al*., 2022; Zhang *et al*., 2022).

In Brazil, the main raw material for wood-derived products comes from eucalyptus plantations (IBÁ, 2025). Brazil's vast territorial extension encompasses significant climatic, edaphic, and topographical diversity, resulting in a wide range of cultivation locations. Considering that these plantations are frequently established in areas considered unsuitable for traditional agriculture, such as steep terrain (Gonçalves *et al*., 2013), it is relevant to assess to what extent the variability of light and water along the toposequence would lead to significant changes in plant characteristics. While the availability of light and water is expected to be contrasting when comparing upper (sunnier and drier) and lower (shadier and wetter) slopes, what can be expected from mid-slope conditions? And, more importantly, how will the anatomical, physical, and chemical characteristics of the wood and leaves respond to such conditions?

Many studies have examined the performance of Eucalyptus clones in different locations in Brazil (Silva *et al*., 2016; Barbosa *et al*., 2019; Araujo *et al*., 2019; Rocha *et al*., 2020; Almeida *et al*., 2026). However, these studies have mainly focused on broad environmental gradients or isolated characteristics, with little exploration of intra-site variability associated with topography, especially through an integrated approach combining leaf and wood traits. In this context, investigating xylem-related variables from both leaves and wood, which are highly responsive to factors such as water availability and solar radiation, represents a useful approach for understanding how topographic variation influences tree growth and wood quality under operational forestry conditions.

Therefore, the objective of this study is to investigate the effect of topographic variation on leaf morphology and anatomy, anatomical and chemical properties, higher heating value, and basic wood density in a toposequence (base, middle, and top of the slope) of an area cultivated with clonal planting of *Eucalyptus* spp. We hypothesis is that topographic variation along the slope would lead to detectable anatomical, physical, and chemical adjustments in both leaves and wood, reflecting adaptive responses to gradients in water and light availability.

MATERIAL AND METHODS

The study was conducted using leaves and wood from *Eucalyptus* spp. trees, originating from a clonal plantation along a toposequence in Viçosa, MG (20° 45′ 0.097″ S; 42° 49′ 7″ W), southeast Brazil. According to the Köppen classification, the climate is of type Cwa, with hot, rainy summers and cold, dry winters (Martins *et al*., 2018), with temperature and precipitation average annual of 21.9°C and 1289 mm, respectively (Fialho and Santos, 2022). The natural vegetation of the area, situated in the Atlantic Forest biome, consists of Semi-deciduous Seasonal Forest, and the predominant soils in the region are dystrophic red-yellow latosols.

Biological material

The plantation, five years old and established with a spacing of 3 × 3 meters, was located on undulating terrain with an average slope of 17° relative to the base of the stream. Trees were situated at different altitudes (672, 707, and 724 meters), corresponding to the experimental treatments: base (T1), mid-slope (T2), and hilltop (T3). A stratified random sampling was performed, in which the population was divided into strata according to topographic position, and within each stratum, three trees were randomly selected as experimental units, totaling three replicates per treatment (Figure 1). The reduced number of replicates reflects operational limitations associated with field sampling and the detailed anatomical and chemical analyses performed. Therefore, the results should be interpreted considering the exploratory nature of the study.

Saturated water content of leaves (SWC)

Leaf saturated water content was determined according to (Turner, 1981), with modifications. Five fully expanded leaves from the fourth or fifth pair of leaves at the apex of each individual's branches were collected. The leaves were stored in zip-lock plastic bags, placed in a refrigerated, hermetically sealed container, and taken to the laboratory. One leaf from each plant was used for the SWC analysis. The petioles were immersed in water for 12 hours. Then, the petioles were dried, and using an analytical balance, the mass of the saturated leaves was measured. After 4 days in an oven at 70ºC, the dry mass was measured, with SWC calculated by equation 1.

$$\mathrm{SWC} = \frac{\text{saturated mass} - \text{dry mass}}{\text{dry mass}}. \quad (1)$$

Leaf area

The remaining four leaves were scanned and, from the digitized images obtained (Lucena *et al*., 2011), the leaf area was determined using the Image Pro-Plus software, version 4.5.

Leaf anatomy

The collected leaves were fixed in FAA50 for 48 h and then stored in 70% (v/v) aqueous ethanol (Johansen,

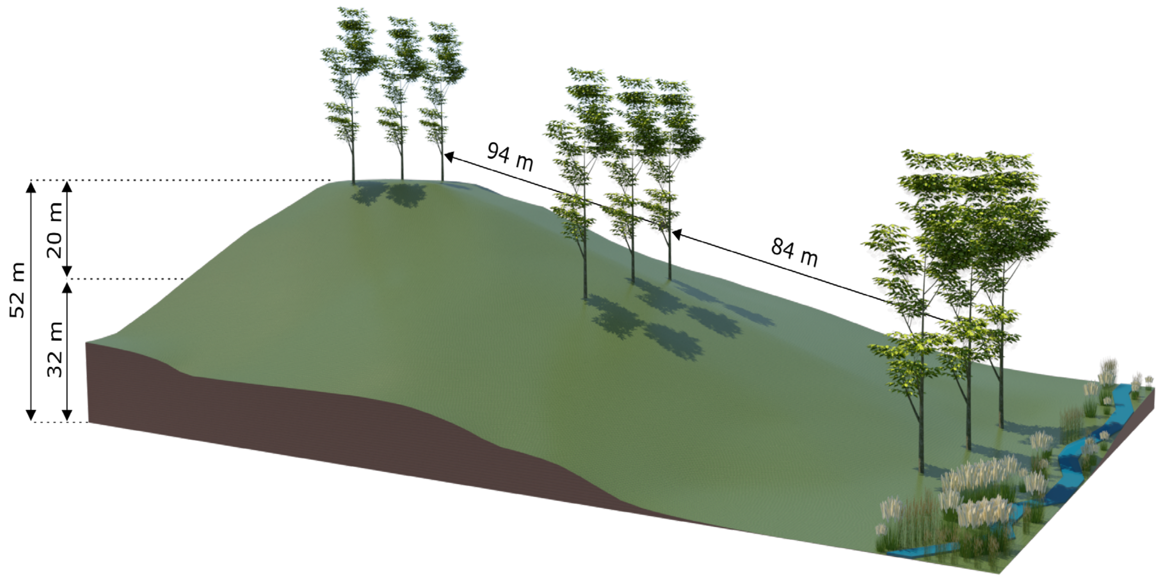

Figure 1: 3D model with the real topology of the territorial portion from which the studied material was obtained. The slope of the top in relation to the base of the stream is, on average, 17º.

1940). Subsamples from the middle region of each leaf blade were embedded in methacrylate (Historesin-Leica Microsystems Nussloch, Heidelberg, Germany), following the manufacturer's instructions. Cross sections (5 µm thick) were obtained using an automatically advancing rotary microtome (Leica RM2155, Leica Microsystems Inc., Deerfield, USA), stained with toluidine blue (O'Brien *et al*., 1964) and mounted in synthetic resin (Permount®). For obtaining stomatal density (DE) and venation density (Dv), the leaves were cleared as described by (Strittmatter, 1973) and modified for the species, following these steps: boiling for 5 minutes in absolute ethyl alcohol; boiling for 5 minutes in a solution of absolute ethyl alcohol + 5% sodium hydroxide (1:1); washing in running water for 20 seconds; rinsing in distilled water twice for 3 minutes; clearing with an aqueous solution of sodium hypochlorite for 10 to 15 minutes, followed by five washes in distilled water for 3 minutes each; dehydration in 70% ethanol for 10 minutes; staining in 1% hydroalcoholic safranin solution for 30 minutes; immersion in 80%, 70%, and 50% ethanol for 5 minutes each; immersion in distilled water and mounting the slide in glycerin gelatin.

The slides were photographed using a light microscope (AX70 TRF, Olympus Optical, Tokyo, Japan) coupled to a digital camera (Zeis AxioCan HRc, Göttinger, Germany) and a microcomputer with the Axio Vision image capture program. The following anatomical data were quantified using the previously described image analysis software: (i) thickness of the palisade (PP) and spongy (PL) parenchyma, leaf thickness (EF); (ii) stomatal (DE) and venation (DV) density. Xylem conduits were measured (quantity and internal diameter) to determine theoretical axial hydraulic conductance (K_t) and treated as ellipses to calculate K_t by equation 2.

$$K_t = \sum \frac{\pi a^3 b}{64\eta(a^2 b^2)}, \tag{2}$$

where *a* and *b* are the major and minor internal diameters of the conduits, and *η* is the viscosity of water at 25ºC, normalized by length and leaf area. The summation symbol refers to the sum of the conductivity of all measured conduits.

Wood sample preparation

Discs approximately 5 cm thick were collected from the base, at 25%, and at 75% of the commercial height of each tree trunk. From each disc, two opposite wedges passing through the pith were obtained, identified, and used to determine the basic wood density. The remaining material from each disc was used to prepare samples for anatomical analyses of fibers and pores, while another portion was processed into sawdust for chemical composition analyses and determination of the higher heating value, both performed with three repetitions per treatment in duplicate (two repetitions per experimental unit).

Basic density of wood

To determine the basic density of the wood, the analysis followed the procedures described in NBR 11941 (ABNT, 2003), using the water immersion method with the aid of a hydrostatic balance. The density values were calculated from the arithmetic mean of the densities of the respective wedges.

Heartwood/Sapwood ratio (H/S)

Initially, the peripheral heartwood region and the boundary between heartwood and sapwood were identified in each disc using a 10x magnifying glass, observing the color variation and the occurrence of tyloses in the heartwood.

Two perpendicular lines were drawn from one end to the other of each disc, passing through the center of the pith. Using a ruler with 0.1 cm precision, the total diameter and the heartwood diameter were measured. From the obtained measurements, the average cross-sectional area of each disc, the area occupied by heartwood, the area occupied by sapwood, and the percentage of heartwood and sapwood were calculated. Based on the calculation results, the heartwood/sapwood ratio was obtained according to the methodology described by (Castro *et al.*, 2016).

Morphological and anatomical analysis of xylem fibers and vessels

In the section corresponding to the transition region between heartwood and sapwood, rectangular test specimens with dimensions of 2 x 2 x 3 cm (tangential x radial x longitudinal, respectively) were taken. For fiber analysis, the wood was macerated according to the method recommended by (Dadswell, 1972), and 30 fibers were measured to determine length, width, and lumen diameter. Subsequently, the test specimens were softened in hot water for a week, and then thin sections of wood in the transverse plane were obtained using a microtome. Semi-permanent slides were mounted and stained with 1% aqueous safranin solution. Thirty lumen diameter measurements of vessels were taken for each tree, and frequency was measured in 5 micrographs per disc, per tree. For the measurements, an optical microscope with an image acquisition system and Axion Vision Rel. 4.8 software were used. From the fiber dimension measurements, the wall thickness (EP) and wall fraction (FP) were calculated, by equations 3 and 4, respectively. with EP = [(fiber width – lumen diameter) / 2] and FP = [(2 x wall thickness / fiber width) x 100].

Table 1: Statistical analysis for comparison of means among treatments. Values expressed as mean ± standard deviation (SD), (n = 3); Coefficiente of variation (CV). Means followed the same letter, on the same line, do not differ from each other by Tukey test at 10% probabily.

Material	Features	Position on the slope					
		Base		Middle		Top	
		Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Leaf	Saturated Leaf Water Content ($g{\cdot}g^{-1}$)	1.376a ± 0.18	8.584	1.161b ± 0.046	3.923	1.224ab ± 0.031	2.512
	Leaf Area (cm^2)	47.9a ± 3.8	8.1	30.7b ± 3.2	10.5	28.9b ± 2.4	8.2
	Leaf Thickness (µm)	294.558a ± 19.547	6.636	286.932a ± 25.749	8.974	309.144a ± 34.098	11.03
	Palisade/lacunate parenchyma	0.464b ± 0.085	18.265	0.588ab ± 0.022	3.797	0.629a ± 0.060	9.578
	Stomatal Density (n/mm^2)	324.778b ± 40.103	12.348	411.869a ± 33.759	8.197	372.649ab ± 38.656	10.373
	Venation Density (mm/mm^2)	10.355b ± 0.380	3.667	11.609a ± 0.739	6.365	10.894ab ± 0.359	3.295
	Average hydraulic diameter (µm)	7.627a ± 1.608	21.07	6.632a ± 0.369	5.565	6.506a ± 0.461	7.091
	Number of xylem vessels	911a ± 467.479	51.296	792a ± 292.475	36.898	1082a ± 74.907	6.923
	Xylem vessel area (μm^2)	55.252a ± 18.998	34.388	40.265a ± 15.803	39.249	534.122a ± 8.886	16.638
	Hydraulic conductance ($\mu mol\ m\ s^{-1}\ MPa^{-1}$)	0.414a ± 0.270	65.317	0.417a ± 0.163	39.107	0.587a ± 0.181	30.812
Wood	Tree height (m)	21.05a ± 4.17	19.82	20.50a ± 0.50	2.44	15.26b ± 1.07	6.99
	Heartwood/Sapwood Ratio (H/S)	0.42a ± 0.04	11.45	0.05b ± 0.01	2.76	0.35ab ± 0.03	9.30
	Basic density (g/cm^3)	0.411a ± 0.051	12.401	0.468a ± 0.005	1.125	0.466a ± 0.006	1.185
	Wall fraction (%)	52.829b ± 1.697	3.211	57.358a ± 1.538	2.681	59.049a ± 1.164	1.971
	Vessel fire diameter (µm)	104.60a ± 6.45	6.16	92.89b ± 5.08	5.47	96.99ab ± 5.32	5.49
	Vessel frequency (n/mm^2)	7.93c ± 0.64	8.09	9.69b ± 0.47	4.94	10.82a ± 0.22	2.09
	Total Lignin (%)	30.1a ± 0.3	1.1	29.4b ± 0.3	1.0	30.4a ± 0.1	0.1
	Syringyl/Guaiacyl Lignin Ratio (S/G)	2.72a ± 0.6	6.07	2.36b ± 0.10	4.22	2.35b ± 0.08	3.62
	Total extractives (%)	3.47b ± 0.37	10.88	4.61b ± 1.05	22.9	7.20a ± 0.68	9.5
	Higher Calorific Value (%)	19.13b ± 0.11	0.6	19.40a ± 0.17	0.89	19.63a ± 0.05	0.29
	Ash Content (%)	0.53a ± 0.09	17.27	0.42a ± 0.08	20.2	0.46a ± 0.04	9.65
	Carbon (%)	48.90a ± 0.30	0.61	48.87a ± 0.05	0.11	49.37b ± 0.15	0.3
	Nitrogen (%)	0.11a ± 0.01	15.73	0.07b ± 0.02	35.43	0.11ab ± 0.01	14.65
	Hydrogen (%)	5.38a ± 0.1	2.19	5.25ab ± 0.13	2.56	5.08b ± 0.03	0.71
	Hemicellulose (%)	20.25c ± 0.18	0.89	21.03b ± 0.20	0.96	21.58a ± 0.28	1.31
	Cellulose (%)	42.87a ± 1.48	3.48	43.2a ± 0.15	0.34	43.62a ± 0.20	0.46

$$WT = \frac{\text{fiber width} - \text{lumen diameter}}{2}. \quad (3)$$

$$WF = \frac{2 \times \text{wall thickness}}{\text{fiber width}} \times 100. \quad (4)$$

Elemental chemical composition

The elemental analysis of the wood was conducted according to the methodology described by (Paula *et al*., 2011), using a CHNS-O analyzer, model LECO. The gases required for operation were helium, as the carrier gas, and oxygen, as the combustion gas. The chemical elements (carbon, nitrogen, and hydrogen) were determined using the TruSpec CHNS Micro module and identified through a thermal conductivity detector, where each element interacts with and produces a specific peak.

Total extractives

The total extractives content of the wood was determined according to TAPPI 204 om-88 (TAPPI, 2001), using the total extractives determination method, replacing ethanol/benzene with ethanol/toluene.

Structural Chemical Composition

The insoluble lignin content was determined using the Klason method, modified according to the procedure proposed by (Gomide and Demuner, 1986). Soluble lignin was determined by spectrometry, as per Goldschmid (1971), from the dilution of the filtrate obtained from the insoluble lignin procedure. The total lignin content was obtained by summing the values of soluble and insoluble lignin. Using the same filtrate diluted to 250 ml obtained for insoluble lignin analysis, carbohydrate analysis was conducted. The cellulose content was determined by considering the glucose percentage, subtracting a percentage equivalent to the mannan content, as glucoses monomers are components of glucomannans, in a 1:1 ratio (Rowell et al., 2005). The hemicellulose content was estimated by assessing acetyl groups (2.9%) and uronic acids (4.1%) based on Gomide et al. (2005).

Syringyl/Guaiacyl lignin ratio (S/G)

Oxidation of extractive-free sawdust (0.20 g dry weight) with nitrobenzene was carried out according to the procedures of Goldschmid (1971) and Lin and Dence (1992). The oxidized sawdust was analyzed by high-performance liquid chromatography (HPLC) using a Shimadzu CTO-10A apparatus, equipped with a UV-VIS SPD-10AV vp detector, operating at a wavelength of 280 nm, an LC-10AD vp pump, and an SCL-10A vp injector. An LC analytical column, SHIM-PACK CLC ODS 4.6 mm x 25 cm, was used. The solvent system consisted of a mixture of acetonitrile (1:6 v/v), maintained at pH 2.6, adjusted with trifluoroacetic acid solution. The column temperature was 40°C, and the mobile phase flow rate was 0.4 ml/min. Vanillin and syringaldehyde standards (Aldrich, Milwaukee, WI, USA) were used for quantifying the guaiacyl and syringyl unit derivatives, respectively. Solutions were prepared in a mixture of acetonitrile/water (1:1 v/v) at pH 2.6.

Ash content

The ash content of the wood was determined following the ABCP M11/77 standard (ABCP, 1974) at 575 °C (±25 °C), with samples kept in a muffle furnace until constant weight on an analytical balance.

Higher calorific value

The higher heating value of the wood was determined according to the methodology described by ABNT NBR 8633 (ABNT, 1984), using a Parr 6300 adiabatic calorimeter.

Statistical analysis

Th results were subjected to analysis of variance (ANOVA) to evaluate the effects of the treatments, followed by Tukey's test with a significance level of 10%, using OriginPro 8.5 software. Data are presented as mean ± standard deviation. The assumptions of normality and homogeneity of residual variances were evaluated using the Kolmogorov-Smirnov, Lilliefors or Shapiro-Wilk tests and the Levene or Bartlett tests, respectively. Additionally, a principal component analysis (PCA) was performed separately for the properties of the leaves and wood, using STATISTICA 12 software, to evaluate the occurrence of discrimination between the treatments adopted. Due to the small sample size (n = 3), the statistical power of the analyses is limited. In addition, potential pseudoreplication effects cannot be completely excluded, therefore, the observed patterns should be interpreted as indicative trends associated with topographic position and should be validated in future studies using larger independent sample sets.

RESULTS

Analysis of variance (ANOVA)

According to the results of the analysis of variance and Tukey's test (Table 1), many of the wood and leaf properties differed among the treatments ($p < 0.10$).

Leaf traits that did not differ among treatments ($p > 0.10$) included leaf thickness, mean hydraulic diameter, number and area of xylem vessels, and xylem hydraulic conductivity (Table 1). Among the variables that differed across slope positions ($p < 0.10$), leaf saturated water content was higher in trees located at the bottom (1.376 ± 0.118 g g^{-1}) compared to those at the mid-slope position (1.161 ± 0.046). Leaf area was also greater at the bottom (47.9 ± 3.8 cm^2), differing from the mid-slope (30.7 ± 3.2 cm^2) and top positions (28.9 ± 2.4 cm^2). The ratio between palisade and spongy parenchyma was higher in leaves from trees at the top (0.629 ± 0.060) and lower at the bottom of the slope (0.464 ± 0.085). Finally, stomatal and venation densities were higher at the mid-slope and top positions compared to the bottom (see Table 1).

For wood, the characteristics that did not differ among treatments ($p > 0.10$) were basic density, ash content, and cellulose content (Table 1). Among the variables that differed along the slope ($p < 0.10$), tree height was higher at the bottom (21.05 ± 4.17 m) and mid-slope (20.50 ± 0.50 m) compared to the top (15.26 ± 1.07 m). Fiber wall fraction and higher heating value were also greater in trees located at the top and mid-slope positions, whereas carbon content showed the opposite pattern, with higher values observed at the top of the slope (see Table 1). The heartwood/sapwood ratio, vessel lumen diameter, and nitrogen content were higher in trees located at the bottom of the slope compared to the mid-slope position (Table 1). Total lignin content was lower in trees at the mid-slope position (29.4 ± 0.3 %) than at the bottom (30.1 ± 0.3 %) and top (30.4 ± 0.1 %), while the S/G ratio was higher at the bottom (2.72 ± 0.16 %), differing from the other positions. Total extractives were higher at the top (7.20 ± 0.68 %), followed by the mid-slope (4.61 ± 1.05 %) and bottom (3.47 ± 0.37 %). Finally, hemicellulose content and xylem vessel frequency were highest at the top of the slope, followed by the mid-slope and bottom positions (see Table 1).

Principal component analysis (PCA)

Figure 2 and 3 present the graphical results of the Principal Component Analysis for leaf and wood properties, respectively. Figure 2a and Figure 3a show the ordination diagrams of the original variables and the scores of the first two components for leaf and wood data, respectively. In the case of leaves, the first two components explain 69% of the total variation, while for wood, this explanation is 71%. Figure 2b and Figure 3b display the grouping of individuals based on their variances, where the variables of the first principal component suggested separation among treatments, grouping trees according to their position on the slope for both leaf and wood traits.

Regarding leaf anatomy, the variables closest to the unit circle were leaf area, followed by the leaf saturated water content. The variables associated with trees located at the top and middle of the slope included the ratio of palisade to spongy parenchyma, stomatal and venation densities, and the number of xylem vessels. In contrast, the leaves of trees at the bottom were more associated with leaf water content, leaf area, and the mean hydraulic diameter of xylem vessels.

Overall, among the variables contributing more strongly to the PCA ordination of wood properties were total extractive content, followed by tree height and vessel frequency. The variables most related to trees at the top and middle of the slope included carbon content, total extractives, hemicellulose content, vessel frequency, fiber wall fraction, higher heating value, basic density, and total cellulose and lignin content. In contrast, trees at the bottom were more associated with height, the S/G lignin ratio, ash content, hydrogen and nitrogen content, heartwood-sapwood ratio, and the lumen diameter of xylem vessels.

DISCUSSION

Leaves of trees located in the slope base show higher water content and exhibit typical shade leaf characteristics

Leaf shape and structure are fundamental to photosynthetic efficiency, and many species adjust these characteristics in response to light availability (Tang *et al*., 2022; Zhang *et al*., 2022). In this study, trees located at the base of the slope showed higher water content, larger leaf area, lower stomatal density, and lower vein density compared to trees in the middle of the slope, characteristics frequently associated with leaves adapted to more shaded environments (Dörken and Lepetit, 2018; Poorter *et al*.,

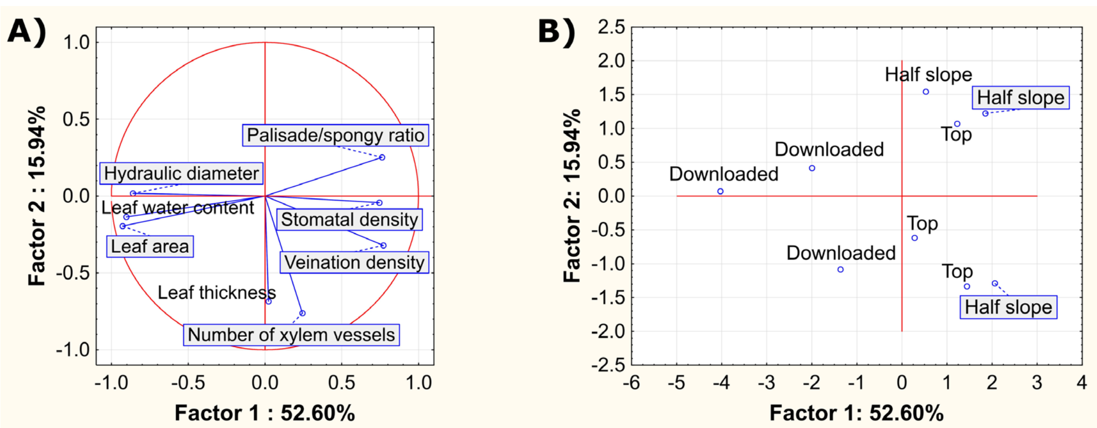

Figure 2: Principal component analysis (PCA) of leaves properties of eucalipto trees along the toposequence. (A) Correlation circle of the original variables associated with the first two principal components. Variables closer to the unit circle contributed more strongly to treatment discrimination. (B) Score plot showing the grouping of trees according to slope position.

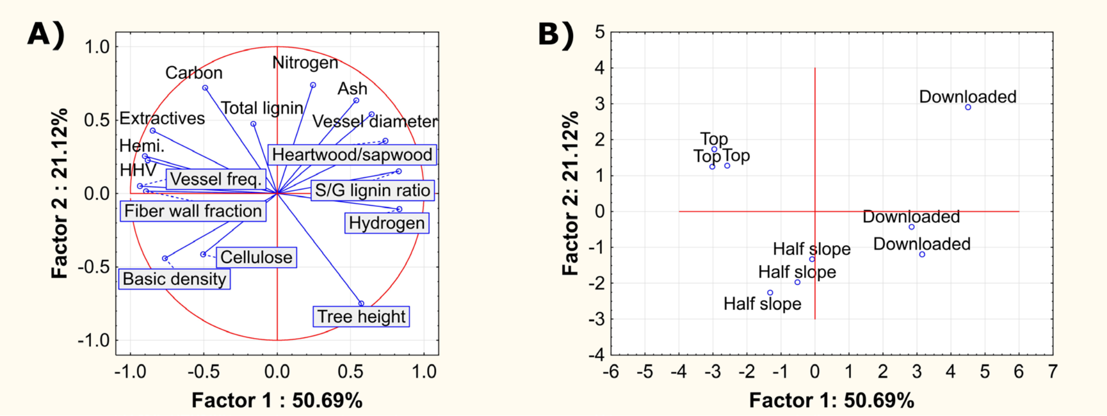

Figure 3: Principal component analysis (PCA) of wood properties of eucalipto trees along the toposequence. (A) Correlation circle of the original variables associated with the first two principal components. Variables closer to the unit circle contributed more strongly to treatment discrimination. Hemi.: hemicellulose; HHV.: higher heating value; Vessel freq.: vessel frequency. (B) Score plot showing the grouping of trees according to slope position.

2019). The lower density of veins and stomata in larger leaves may be related to the "dilution" effect resulting from leaf expansion, as proposed by (Murphy *et al*., 2012). Furthermore, leaves from trees located at the base of the slope showed a lower proportion of palisade parenchyma relative to spongy parenchyma when compared to trees at the top. Palisade parenchyma plays an important role in photosynthesis and tends to develop more intensely under higher light incidence, often being associated with sun leaves (Han *et al*., 2016; Wyka *et al*., 2022). In contrast, the leaves of trees located in the middle of the slope showed the lowest water content, while the trees at the top exhibited a higher proportion of palisade parenchyma compared to spongy parenchyma, characteristics that may be associated with variations in water availability and solar radiation along the slope. In a boreal forest of *Larix gmelinii*, (Li *et al*., 2021) also observed variations in leaf traits and tree growth along a toposequence, associating these responses with differences in soil moisture and fertility between slope positions. Similarly, (Méndez-Toribio *et al*., 2017) verified a strong influence of topographic position on the functional traits of tree communities in dry tropical forests, reinforcing the role of environmental heterogeneity in modulating the functional characteristics of plants.

Trees located at the slope top exhibit shorter height and wood with higher total extractive content

Among the wood characteristics analyzed in this study, basic density, ash content, and cellulose content did not vary between the different treatments, suggesting relative uniformity of these characteristics along the toposequence, considering the slope and altitude range studied. Elissetche *et al*. (2024), when evaluating *Eucalyptus* genotypes under water limitation, found distinct responses in basic density among genetic materials cultivated in different environmental conditions, indicating that the influence of the environment on this characteristic may vary according to the genotype and the intensity of environmental restrictions. Trees at the top of the slope showed lower height, higher frequency of xylem vessels, and higher total extractives content compared to those at the base and middle of the slope. Vessel elements, essential for water transport in plants, are particularly sensitive to environmental variations. Studies on wood anatomy under different environmental conditions have shown that plants subjected to water deficit tend to develop smaller and more numerous vessel elements, which has been associated with adjustments that can optimize water conduction and minimize the risk of hydraulic conductivity loss under these conditions (Pfautsch *et al*., 2016; Zhang *et al*., 2023; Santini *et al*., 2024; Keret *et al*., 2024). In juvenile *Eucalyptus grandis*, Costa *et al*. (2023) also observed an increase in the frequency and a reduction in the size of vessels under water restriction, highlighting the high anatomical plasticity of the xylem in response to water availability.

Under water deficit conditions, plants also can increase osmotically active solutes, promoting osmotic adjustment and maintaining cell turgor under water restriction (Tschaplinski *et al*., 2019). These mechanisms can stimulate the production of secondary metabolites, such as extractives. Furthermore, a relative increase in cell wall lignin may occur, a strategy that increases mechanical strength and contributes to protection against abiotic stresses (Cesarino, 2019; Bezerra *et al*., 2020; Kumar *et al*., 2021). Both lignin and extractives are carbon-rich and oxygen-poor compounds, thus increasing the percentage of elemental carbon in wood (Demirbaş, 2001). In the present study, the variation in elemental carbon content appears to be associated with the production of total extractives, which in turn were associated with the variations observed in gross calorific value. This effect is evidenced by the fact that, although the trees at the base had a total lignin content similar to that of the trees at the top and a lower hemicellulose content, their low extractives content resulted in the lowest net calorific value observed. The more pronounced differences observed in the trees located at the top of the slope, compared to those in other positions, may reflect anatomical and chemical responses associated with environmental heterogeneity along the topographic gradient.

Trees located at the middle of the slope resemble those at the slope top

In general, the trees located in the middle and at the top of the slope presented leaf and woody characteristics that were more similar to each other, as suggested by PCA. This trend suggests functional responses associated with environmental heterogeneity along the slope, indicating potentially less favorable conditions for tree growth in these positions.

CONCLUSION

Topographic variation along the slope promoted significant changes in leaf and wood traits of eucalyptus clones, particularly in leaf water content, leaf area, stomatal and venation density, tree height, and total extractives content. The integration of leaf and wood characteristics allowed a broader understanding of plant responses to environmental heterogeneity within the same stand, highlighting the importance of intra-site variability in forest plantations. From a practical perspective, variations in wood chemical composition, especially in extractives and lignin, may directly affect industrial processing and wood utilization. Therefore, considering topographic heterogeneity may contribute to more efficient forest management and to the selection of genotypes better adapted to contrasting environmental conditions within the same plantation area.

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Data availability:

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

ABNT, ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. NBR 8633: carvão vegetal: determinação do poder calorífico. Rio de Janeiro, 1984. 13 p.

ABNT, ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. NBR 11941: madeira: determinação da densidade básica. Rio de Janeiro, 2003. 6 p.

ABCP, ASSOCIAÇÃO BRASILEIRA TÉCNICA DE CELULOSE E PAPEL. ABCP M11/77. São Paulo: ABTCP, 1974.

ALMEIDA, M. N. F.; LOUZADA, J. L.; PEZZOPANE, J. E. M.; et al. Influence of clone-site interaction on wood basic density of Eucalyptus clones in Brazilian tropical zones. CERNE, v. 32, n. 1, 2026. DOI: 10.1590/01047760202632013566.

ARAUJO, M. J.; PAULA, R. C.; CAMPOE, O. C.; et al. Adaptability and stability of eucalypt clones at different ages across environmental gradients in Brazil. Forest Ecology and Management, v. 454, 2019. DOI: 10.1016/j.foreco.2019.117631.

BARBOSA, T. L.; OLIVEIRA, J. T. S.; ROCHA, S. M. G.; et al. Influence of site in the wood quality of Eucalyptus in plantations in Brazil. Southern Forests, v. 81, p. 247–253, 2019. DOI: 10.2989/20702620.2019.1570453.

BEZERRA, J. D. C.; FRANÇA, S. A.; JÚNIOR, J. R. S. N.; et al. Biossíntese de lignina em plantas submetidas ao déficit hídrico. Pubvet, v. 14, n. 9, p. 1–14, 2020. DOI: 10.31533/pubvet.v14n9a653.1-14.

CASTRO, A. F. N. M.; CASTRO, R. V. O.; CARNEIRO, A. C. O.; et al. Correlations between age, wood quality and charcoal quality of Eucalyptus clones. Revista Árvore, v. 40, n. 3, p. 551–560, 2016. DOI: 10.1590/0100-67622016000300019.

CESARINO, I. Structural features and regulation of lignin deposited upon biotic and abiotic stresses. Current Opinion in Biotechnology, v. 56, p. 209–214, 2019. DOI: 10.1016/j.copbio.2018.12.012.

COSTA, L. S.; VURALHAN-ECKERT, J.; FROMM, J. Effect of elevated CO2 and drought on biomass, gas exchange and wood structure of Eucalyptus grandis. Plants, v. 12, n. 1, 2023. DOI: 10.3390/plants12010148.

DADSWELL, H. E. The anatomy of Eucalypt woods. Melbourne: Commonwealth Scientific and Industrial Research Organization, 1972. 28 p.

DEMIRBAŞ, A. Relationships between lignin contents and heating values of biomass. Energy Conversion and Management, v. 42, p. 183–188, 2001. DOI: 10.1016/S0196-8904(00)00050-9.

DÖRKEN, V. M.; LEPETIT, B. Morpho-anatomical and physiological differences between sun and shade leaves in Abies alba Mill. (Pinaceae, Coniferales): a combined approach. Plant, Cell & Environment, v. 41, p. 1683–1697, 2018. DOI: 10.1111/pce.13213.

ELISSETCHE, J. P.; ALZAMORA, R. M.; ESPINOZA, Y.; et al. Wood basic density assessment of Eucalyptus genotypes growing under contrasting water availability conditions. Forests, v. 15, n. 1, 2024. DOI: 10.3390/f15010185.

FIALHO, E. S.; SANTOS, L. G. F. Unidades mesoclimáticas de Viçosa-MG, na Zona da Mata Mineira. Revista Brasileira de Climatologia, v. 31, p. 230–258, 2022. DOI: 10.55761/abclima.v31i18.15841.

GOMIDE, J. L.; COLODETTE, J. L.; OLIVEIRA, R. C.; et al. Caracterização tecnológica, para produção de celulose, da nova geração de clones de Eucalyptus no Brasil. Revista Árvore, v. 29, n. 1, p. 129–137, 2005.

GOMIDE, J. L.; DEMUNER, B. J. Determinação do teor de lignina em material lenhoso: método Klason modificado. O Papel, v. 47, p. 36–38, 1986.

GONÇALVES, J. L. M.; ALVARES, C. A.; HIGA, A. R.; et al. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. Forest Ecology and Management, v. 301, p. 6–27, 2013. DOI: 10.1016/j.foreco.2012.12.030.

GOLDSCHMID, O. Ultraviolet spectra. In: SARKANEN, K. V.; LUDWIG, C. H. (ed.). Lignins. New York: Wiley Interscience, 1971. p. 241–266.

HAN, S.; CHEN, S. M.; SONG, A. P.; et al. Photosynthetic responses of Chrysanthemum morifolium to growth irradiance: morphology, anatomy and chloroplast ultrastructure. Photosynthetica, v. 55, p. 184–192, 2016. DOI: 10.1007/s11099-016-0219-5.

IBÁ, INDÚSTRIA BRASILEIRA DE ÁRVORES. Relatório anual 2025. São Paulo: IBÁ, 2025. 100 p. Available at: https://iba.org/wp-content/uploads/2025/10/IBA_relatorio_digital_POR.pdf.

JOHANSEN, D. A. Plant microtechnique. New York: McGraw-Hill, 1940. 523 p.

KERET, R.; DREW, D. M.; HILLS, P. N. Xylem cell size regulation is a key adaptive response to water deficit in Eucalyptus grandis. Tree Physiology, v. 44, 2024. DOI: 10.1093/treephys/tpae068.

KUMAR, M.; PATEL, M. K.; KUMAR, N.; et al. Metabolomics and molecular approaches reveal drought stress tolerance in plants. International Journal of Molecular Sciences, v. 22, 2021. DOI: 10.3390/ijms22179108.

LI, Z.; WANG, C.; LUO, D.; et al. Toposequence variability in tree growth associated with leaf traits for Larix gmelinii. Forest Ecology and Management, v. 479, 2021. DOI: 10.1016/j.foreco.2020.118611.

LIN, S. Y.; DENCE, C. W. Methods in lignin chemistry. Berlin: Springer-Verlag, 1992. 578 p.

LIN, Y.; KUANG, L.; TANG, S.; et al. Leaf traits from stomata to morphology are associated with climatic and edaphic variables for dominant tropical forest evergreen oaks. Journal of Plant Ecology, v. 14, p. 1115–1127, 2021. DOI: 10.1093/jpe/rtab060.

LUCENA, R. R. M.; BATISTA, T. M. V.; DOMBROSKI, J. L. D.; et al. Medição de área foliar de aceroleira. Revista Caatinga, v. 24, n. 2, p. 40–45, 2011.

MARTINS, F. B.; GONZAGA, G.; SANTOS, D.; et al. Classificação climática de Köppen e de Thornthwaite para Minas Gerais: cenário atual e projeções futuras. Revista Brasileira de Climatologia, p. 129–156, 2018. DOI: 10.5380/abclima.v1i0.60896.

MÉNDEZ-TORIBIO, M.; IBARRA-MANRÍQUEZ, G.; NAVARRETE-SEGUEDA, A.; et al. Topographic position, but not slope aspect, drives the dominance of functional strategies of tropical dry forest trees. Environmental Research Letters, v. 12, 2017. DOI: 10.1088/1748-9326/AA717B.

MURPHY, M. R. C.; JORDAN, G. J.; BRODRIBB, T. J. Differential leaf expansion can enable hydraulic acclimation to sun and shade. Plant, Cell & Environment, v. 35, p. 1407–1418, 2012. DOI: 10.1111/j.1365-3040.2012.02498.x.

O'BRIEN, T. P.; FEDER, N.; MCCULLY, M. E. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma, v. 59, p. 368–373, 1964. DOI: 10.1007/BF01248568.

PAULA, L. E. R.; TRUGILHO, P. F.; NAPOLI, A.; et al. Characterization of residues from plant biomass for use in energy generation. CERNE, v. 17, n. 2, p. 237–246, 2011. DOI: 10.1590/S0104-77602011000200012.

PFAUTSCH, S.; HARBUSCH, M.; WESOLOWSKI, A.; et al. Climate determines vascular traits in the ecologically diverse genus Eucalyptus. Ecology Letters, v. 19, n. 3, p. 240–248, 2016. DOI: 10.1111/ele.12559.

POORTER, H.; NIINEMETS, Ü.; NTAGKAS, N.; et al. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. New Phytologist, v. 223, p. 1073–1105, 2019. DOI: 10.1111/nph.15754.

ROCHA, S. M. G.; VIADAURRE, G. B.; PEZZOPANE, J. E. M.; et al. Influence of climatic variations on production, biomass and density of wood in Eucalyptus clones of different species. Forest Ecology and Management, v. 473, p. 1–10, 2020. DOI: 10.1016/j.foreco.2020.118290.

ROWELL, R. M.; PETTERSEN, R.; HAN, J. S.; et al. Cell wall chemistry. In: ROWELL, R. M. (ed.). Handbook of wood chemistry and wood composites. Boca Raton: CRC Press, 2005. p. 121–138.

SANTINI, L.; CRAVEN, D.; RODRIGUEZ, D. R. O.; et al. Extreme drought triggers parallel shifts in wood anatomical and physiological traits in upper treeline of the Mediterranean Andes. Ecological Processes, v. 13, 2024. DOI: 10.1186/s13717-024-00486-9.

SILVA, R. M. L.; HAKAMADA, R. E.; BAZANI, J. H.; et al. Fertilization response, light use, and growth efficiency in Eucalyptus plantations across soil and climate gradients in Brazil. Forests, v. 7, p. 1–12, 2016. DOI: 10.3390/f7060117.

STRITTMATTER, C. G. C. Nueva técnica de diafanizacion. Boletín de la Sociedad Argentina de Botánica, v. 15, n. 1, p. 126–129, 1973.

TANG, W.; GUO, H.; BASKIN, C. C.; et al. Effect of light intensity on morphology, photosynthesis and carbon metabolism of Alfalfa (Medicago sativa) seedlings. Plants, v. 11, n. 13, p. 1–18, 2022. DOI: 10.3390/plants11131688.

TAPPI, TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY. TAPPI test methods T 204 om-88: solvent extractives of wood and pulp. In: TAPPI Standard Method. Atlanta, 2001.

TSCHAPLINSKI, T. J.; ABRAHAM, P. E.; JAWDY, S. S.; et al. The nature of the progression of drought stress drives differential metabolomic responses in Populus deltoides. Annals of Botany, v. 124, p. 617–626, 2019. DOI: 10.1093/aob/mcz002.

TURNER, N. C. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil, v. 58, p. 339–366, 1981. DOI: 10.1007/BF02180062.

WRIGHT, I. J.; DONG, N.; MAIRE, V.; et al. Global climatic drivers of leaf size. Science, v. 357, n. 6354, p. 917–921, 2017. DOI: 10.1126/science.aal4760.

WYCA, T. P.; ROBAKOWSKI, P.; ŻYTKOWIAK, R.; et al. Anatomical acclimation of mature leaves to increased irradiance in sycamore maple (Acer pseudoplatanus L.). Photosynthesis Research, v. 154, p. 41–55, 2022. DOI: 10.1007/s11120-022-00953-4.

ZHANG, K. Y.; YANG, D.; ZHANG, Y. B.; et al. Vessel dimorphism and wood traits in lianas and trees among three contrasting environments. American Journal of Botany, v. 110, 2023. DOI: 10.1002/ajb2.16154.

ZHANG, Y.; CHEN, C.; JIN, Z.; *et al*. Leaf anatomy, photosynthesis, and chloroplast ultrastructure of Heptacodium miconioides seedlings reveal adaptation to light environment. Environmental and Experimental Botany, v. 195, p. 1–11, 2022. DOI: 10.1016/j.envexpbot.2022.104780.