

Article

Soil Effects on Stem Growth and Wood Anatomy of Tamboril Are Mediated by Tree Age

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Abstract: Soil influences the growth of trees and the characteristics of the wood; but could this influence change during the ontogeny of trees? To answer this question, we analyzed the wood anatomy of 9-year-old trees and 2-year-old saplings of *Enterolobium contortisiliquum*, known as “tamboril”, growing in eutrophic and oligotrophic soil in the Brazilian Cerrado, and assessed the effect of age on plant–soil relationship. Sapwood samples were collected from the main stem, anatomical sections were prepared in the lab, and 12 anatomical wood traits were measured. Individuals in eutrophic soil had greater stem diameter and height than those in oligotrophic soil. Trees in eutrophic soil had vessel-associated parenchyma cells with abundant storage compounds. Fibers walls were 47% thicker and intervessel pits diameter were 14% larger in trees of eutrophic soil. A greater proportion of solitary vessels (74%) was observed in trees rather than in saplings (50%). The secondary xylem of trees was mainly formed by fibers (63%) whereas in saplings it was mainly formed by storage tissue (64%). Our study provides evidence that the influence of soil conditions on tree growth reflects variations in wood anatomical features. No significant response to soil type was observed in saplings, thus demonstrating that the relationship between soil type and wood growth is mediated by tree age. These findings help to develop reliable reforestation strategies in tropical ecosystems characterized by different levels of soil fertility.

Keywords: *Enterolobium contortisiliquum*; eutrophic; oligotrophic; ontogeny; plant–soil relationships



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1. Introduction

Environmental conditions influence the biological processes involved in plant development, thus affecting the final growth. Variations in the secondary growth of woody plants can be an answer to gradients in resource availability. Identifying the influence of environmental conditions in wood structure and function of trees growing under different conditions is of particular interest because the environment directly affects xylem cell differentiation, i.e., enlargement and secondary wall thickening, and, consequently, the efficiency of water and nutrient transport from roots to leaves [1–3].

Soil offers structural support and nutrition, participates in physiological processes, and provides the elements for building plant tissues [4]. Phosphorus and magnesium are essential components of molecules involved in photosynthesis [5]. Moreover, according to the same authors, calcium and potassium are involved in transpiration, and boron content influences calcium absorption and utilization, which is, in turn, a component of the cell walls. The lack of the essential soil mineral elements might trigger nutritional deficiencies and modify plant structure and function [6].

Commonly, the relationship of plant–soil has been studied by evaluating the effects of one or two mineral elements on growth rates, biomass production, or leaf nutrient concentration either in nursery [7–9] or nature [10–12]. Soil fertility has induced xylem tissue modifications [6,10], including the size and arrangement of xylem cells. Such modifications have consequences for the efficiency and security of hydraulic transport in woody plants [13–16].

Studies of plant–soil relationships can gain more insights when individuals of different ages are compared, since structure and function of the conducting system in plants change during ontogeny. The nutrient acquisition depends on the age and size of the individuals [4,17]. Thus, investigations of anatomical traits of the xylem could explain why trees of different ages develop different strategies for nutrient acquisition [18,19].

Enterolobium contortisiliquum (Vell.) Morong (Fabaceae), known as “tamboril”, is a tree species spreading in various regions of Brazil [20,21], such as Caatinga, Cerrado, and Atlantic Forest, and exhibiting a good adaptation to different conditions. Because of its fast growth, this species can be used to facilitate the natural regeneration of woody shrubs in reforestations and for phytoremediation of oil-contaminated soils [22,23]. Previous observations have detected that “tamboril” trees growing in eutrophic and clay soil reached a larger stem diameter than those in oligotrophic and sandy soil (personal communication V. L. Engel). Due to the importance of this species for the recovery of degraded areas and abandoned agricultural lands, it is necessary to understand its biological strategies in response to soil restrictions. Survival and growth performance under specific soil conditions are key factors that ensure the success of artificial plantations.

In this study, we compared stem growth and wood anatomy in 9-year-old trees and 2-year-old saplings of “tamboril” growing in eutrophic and oligotrophic soils. We expected that the influence of soil on tree growth also reflects changes in wood anatomical features. Accordingly, we tested the hypotheses that (1) soil type affects wood production and secondary xylem anatomy, and that (2) the responses to soil type are mediated by tree age. Our analysis assesses the relationships between growth and soil during the ontogeny of trees.

2. Materials and Methods

2.1. Study Sites

This study was carried out in two areas of semi-deciduous seasonal forest, nearby Botucatu (22°52′32″ S and 48°26′46″ W), São Paulo State, Brazil, that belongs to a geographic region of the Cerrado Domain [24]. This Domain is categorized into main floristic groups, based on tree species composition (savannas, cerradão, seasonal dry tropical forest, evergreen forest, semideciduous seasonal forest) [25]. The climate of the region is subtropical, wet, and hot [26]. The mean annual temperature is 20.5 °C, with the coldest and warmest months being July and February, respectively. Total precipitation is 1494 mm, with a rainy season lasting from October to March.

The two sites have diverging soil types and fertilities, characterized by either eutrophic or oligotrophic soil [27]. The soil chemistry was obtained from Nogueira Jr. [28] and the chemical attributes of the mineral nutrients of the soils were based on the SiBCS [29] and IAC classification [30]. The eutrophic soil has base-cations saturation > 50%, a suitable proportion of exchangeable cation nutrients and is clayey, moderately acid, with a medium content of organic matter (Table 1). The oligotrophic soil has a base saturation < 50% and is sandy, highly acid, and poor in organic matter, calcium, magnesium, and potassium.

Table 1. Chemical attributes of the studied soils. Adapted from [28].

Chemical Attributes	Eutrophic Soil	Oligotrophic Soil
pH CaCl_2	5.6 (moderately acid)	4.3 (highly acid)
Organic matter (g dm^{-3})	26 (medium)	3.1 (low)
P_{resin} (mg dm^{-3})	13 (medium)	2.6 (low)
S-SO_4^{-2} (mg dm^{-3})	22 (high)	5.5 (medium)
Base-cation saturation (%)	72 (eutrophic)	36 (oligotrophic)
K ($\text{mmol}_c \text{ dm}^{-3}$)	1.8 (medium)	1.2 (low)
Ca ($\text{mmol}_c \text{ dm}^{-3}$)	64 (high)	6.6 (low)
Mg ($\text{mmol}_c \text{ dm}^{-3}$)	15 (high)	1.8 (low)
Al ($\text{mmol}_c \text{ dm}^{-3}$)	0.4 (low)	2.5 (high)
B (g dm^{-3})	0.24 (medium)	0.42 (medium)
Cu (g dm^{-3})	13.2 (high)	0.56 (medium)
Fe (g dm^{-3})	13.8 (high)	22.45 (high)
Mn (g dm^{-3})	176 (high)	34 (high)
Zn (g dm^{-3})	2.06 (high)	0.44 (low)

2.2. Plant Material

We have analyzed ten adult tamboril trees (9-year-old), five growing in eutrophic soil and five in oligotrophic soil. These trees were planted as part of a larger experimental study on restoration ecology [22].

Ten 2-year-old saplings were also selected, i.e., five growing in eutrophic soil and five growing in oligotrophic soil. For the saplings, we collected seeds from adult trees growing in the same areas where the study was carried out. We broke the dormancy by mechanical scarification with sandpaper and sowed three seeds inside each plastic pot (volume of 5 l). Each pot was filled with soils collected in the study sites at 20 to 40 cm depth, resulting in five pots with eutrophic soil and five pots with oligotrophic soil. After germination, we maintained the saplings at the nursery for two years.

2.3. Sampling

We collected wood samples (with 3 cm in width, 5 cm in height, and 5 cm in depth) from the main stems of the trees at 1.30 m from the ground using hacksaw and chisel and kept the sapwood for wood-anatomical analyses, which were stored in alcohol 70%. All trees were sampled on the same day.

Saplings were sampled when they were 2-years-old. They were collected from the stem 10 cm above the soil level in the pot to avoid the shoot-root transition area. The samples were fixed in FAA₇₀ (formaldehyde, acetic acid, ethanol 70%, (1:1:9)) according to Johansen [31] for 36 h and stored in alcohol 70%. Saplings in oligotrophic soil were collected one week before the ones in eutrophic soil.

2.4. Sample Preparation

All samples were reduced to cubes with 1 cm^3 , and were cut along the three sections, i.e., cross (CS), tangential (TLS), and radial (RLS) at a thickness of 15–20 μm with a sliding microtome. The sections were clarified with sodium hypochlorite 50%, washed with water and acetic water 1%, double-stained with aqueous safranin 1% and Astra blue 1% [32], dehydrated at increasing alcohol concentrations from 30 to 100%. The stain was fixed with butyl acetate. We prepared permanent histological sections in synthetic resin (Entellan). For maceration, we followed the Franklin [33] method modified by Kraus and Arduin [34]. The cells were dissociated in acetic acid and hydrogen peroxide (1:1), stained with safranin 1% in alcohol 50% according to Berlyn and Miksche [35] and mounted in semi-permanent slides with water and glycerin (50%).

For scanning electron microscopy (SEM), wood samples were cut along the three sections with a sliding microtome at 12–18 μm in thickness. These sections were dehydrated in a ketonic series before being attached to carbon tape on aluminum stubs at critical point

dried (Leica EM CPD030, Leica microsystems, Wetzlar, Alemanha). Then, they were coated with 20 nm gold in the sputter (Emitech K550X, Quorum Technologies Ltd, Ashford, Kent, UK) before being submitted to SEM.

2.5. Data Collection and Statistics

We measured the adult trees stem diameter at breast height (DBH) of the trees, i.e., at 1.3 m from the ground. Tree height was estimated using a meter stick as a reference. The diameter and height of saplings were measured with a digital pachymeter and a measuring tape, respectively.

We used the recommendations of the IAWA Committee [36] as a guide for qualitative and quantitative wood anatomical analysis. Eleven wood anatomical features were measured as follow: vessel element length (μm), vessel diameter (μm), vessel density ($\text{n}^\circ/\text{mm}^2$), vessel grouping index, intervessel pit diameter (μm), vessel-ray pit diameter (μm), fiber length (μm), fiber diameter (μm), fiber wall thickness (μm), rays height (μm), and rays density ($\text{n}^\circ/\text{linear mm}$). We analyzed the cells parameters under a light microscope (Axioscop 40 Zeiss, Jena, Germany) equipped with an AxioCAM MRC and Axiovision software. The fractions of xylem cell types (vessels, fibers, axial parenchyma, and rays) were obtained by measuring five areas of 1 mm^2 in cross-section images. The area of each group of cell types was manually painted using Adobe Photoshop CS6, and the area was calculated using Color Counter in ImageJ [37]. The vessel grouping index was calculated as a ratio between the number of vessels and the number of groups [38]. Wood sections were observed with a scanning electron microscope Zeiss EVO 040 (Zeiss, Jena, Germany).

We tested the effect of age (saplings and trees) and soil (eutrophic and oligotrophic) on the size of individuals and anatomical characteristics of wood using two-way ANOVA and assessed the relationships among variables with principal component analysis (PCA). We used multivariate analysis of variance (MANOVA) to test for global differences. Since different units of measurement were present, the variables were standardized according to their standard deviations. All statistics were carried out in JMP 15.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Plant Size

Trees growing in eutrophic soil had larger stem diameters and heights (25.5–43.0 cm and 10–16 m, respectively) compared to those growing in oligotrophic soil (14.6–22.0 cm and 3.7–7.5 m, respectively) (Table 2; Figure 1). Moreover, saplings had stem diameters ranging from 2.46 to 2.76 cm and heights ranging from 0.65 to 0.90 m in eutrophic soil compared to those growing in oligotrophic soil (from 1.47 to 3.30 cm and from 0.41 to 0.99 m, respectively) (Table 2; Figure 1).

Table 2. Studied trees and saplings of *Enterolobium contortisiliquum*. DBH = diameter at breast height; H = height; CD = collar diameter.

Soil	9-Year-Old Trees			2-Year-Old Saplings	
	Individual	DBH (cm)	H (m)	CD (cm)	H (cm)
Eutrophic	1	29.6	11	2.73	75.5
	2	25.5	10	2.59	82.7
	3	28.7	16	2.76	90
	4	29	13	2.46	65
	5	43	15	2.71	74
Oligotrophic	1	16.9	3.75	3.30	99.7
	2	22	5.75	1.96	50
	3	14.9	4.5	2.55	55.9
	4	14.6	7.5	2.53	65.6
	5	19.7	7	1.47	41



Figure 1. Studied 9-year-old trees (A,B) and 2-year-old saplings (C,D) of *Enterolobium contortisiliquum*. (A–C) Plants in eutrophic soil. (B–D) Plants in oligotrophic soil. Note the saplings are taller in eutrophic soil (C) than in oligotrophic soil (D). Blue vertical bar = 35 cm in (C).

3.2. Wood Anatomy

From a qualitative point of view, wood anatomy was similar in eutrophic and oligotrophic soil, except for storage compound contents (Figure 2). Abundant storage compounds were observed inside vessel-associated parenchyma cells in the wood of five trees growing in eutrophic soil, which was not observed in the wood of the trees growing in oligotrophic soil. Differences were observed in quantitative wood anatomy (Table 3). Trees in eutrophic soil had longer and wider vessels, higher density of vessels, larger intervessel pits diameter, longer and wider fibers, thicker fibers wall, also longer rays when compared with those in oligotrophic soil. Saplings growing in eutrophic soil had larger intervessel pits diameter, larger vessel-ray pits diameter, longer fibers, thicker fibers wall, and larger density of rays.

In saplings of both soils, we observed a large amount of gelatinous fibers (Figure 3A), and parenchyma-like fiber bands alternating with ordinary fibers (Figure 3A). Rays were 1 to 3 cells wide (Figure 3B) sometimes 4 in trees, and predominantly uniseriate in saplings (Figure 3C). Large amount of starch grains were observed in axial parenchyma cells and in parenchyma-like fiber bands (Figure 3D,E) in saplings. Prismatic crystals are present in chambered axial parenchyma cells in both saplings (Figure 3F) and trees. A complete description of the wood anatomy of trees and saplings is reported in Supplementary Material (Figure S1).

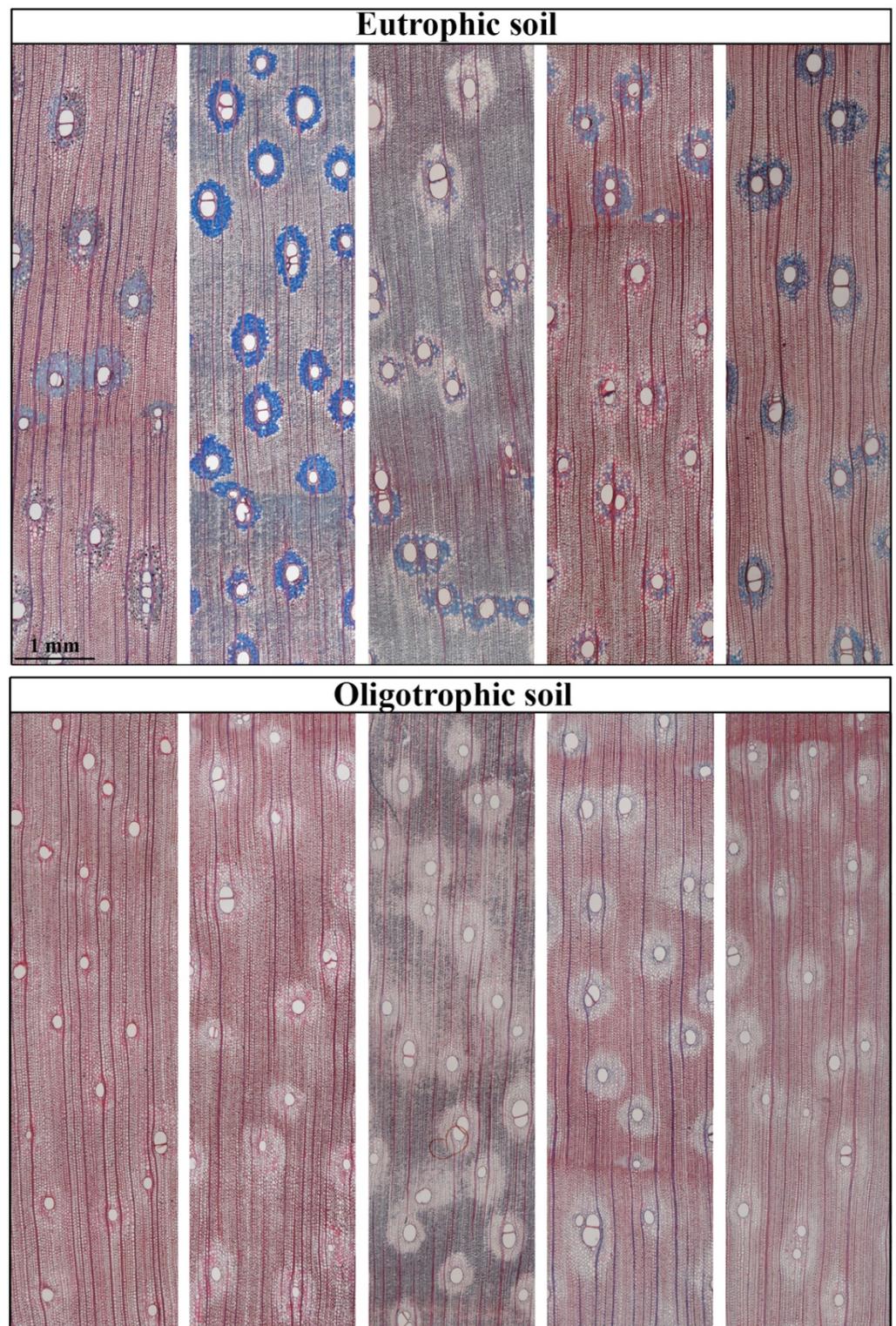
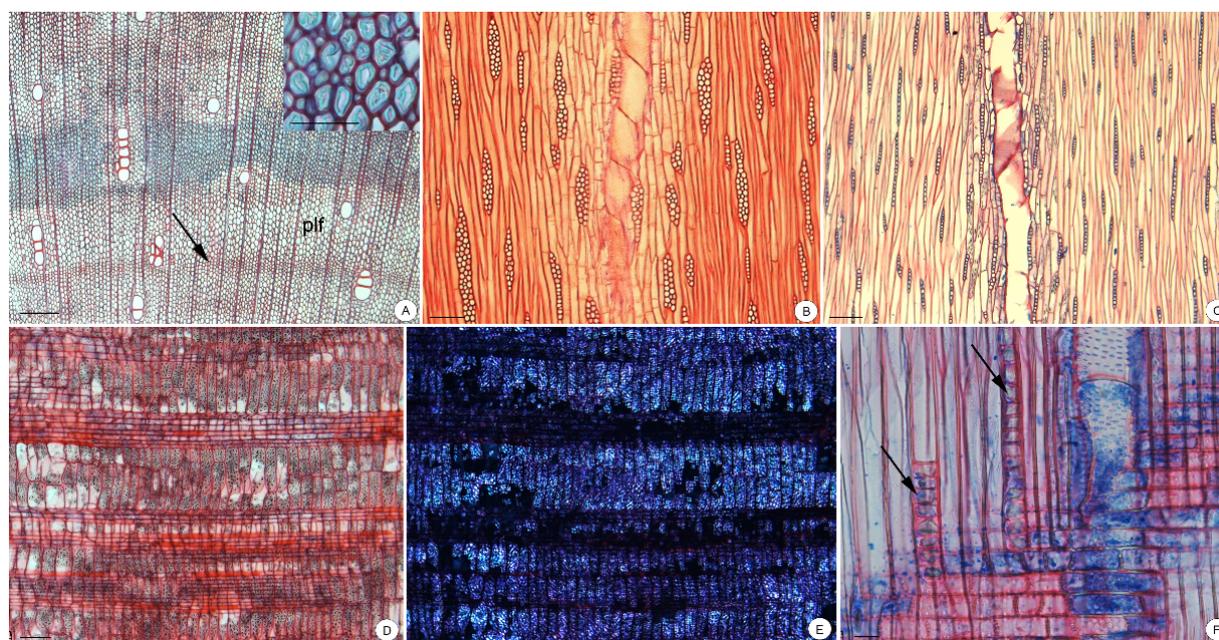


Figure 2. Cross-sections of *Enterolobium contortisiliquum* 9-year-old trees wood in eutrophic soil and oligotrophic soil. Note abundant storage compounds inside vessel-associated parenchyma cells in the wood of trees grown in eutrophic soil.

Table 3. Wood anatomy of *Enterolobium contortisiliquum* 9-year-old trees and 2-year-old saplings growing under different soil conditions. Values are reported as mean \pm standard deviation.

Features/Soil	Trees		Saplings	
	Eutrophic	Oligotrophic	Eutrophic	Oligotrophic
Vessel density (n mm ²)	3.24 \pm 0.27	3.07 \pm 0.56	14.25 \pm 3.83	15.95 \pm 7.18
Vessel grouping	2.17 \pm 0.13	2.21 \pm 0.20	4.18 \pm 0.63	4.79 \pm 1.56
Vessel element diameter (μ m)	143.25 \pm 13.07	122.72 \pm 20.27	57.00 \pm 7.42	58.24 \pm 8.81
Vessel element length (μ m)	315.08 \pm 33.75	291.88 \pm 28.07	229.38 \pm 19.40	229.82 \pm 37.91
Intervessel pit diameter (μ m)	8.57 \pm 0.40	7.51 \pm 0.75	6.51 \pm 0.53	6.20 \pm 0.63
Vessel-ray pit diameter (μ m)	8.18 \pm 0.52	8.38 \pm 0.73	5.76 \pm 0.51	5.34 \pm 0.96
Fiber length (μ m)	874.39 \pm 47.68	849.14 \pm 100.42	439.92 \pm 6.36	407.15 \pm 45.18
Fiber diameter (μ m)	31.10 \pm 4.38	27.62 \pm 4.59	22.01 \pm 1.50	22.36 \pm 3.62
Fiber wall thickness (μ m)	6.55 \pm 1.12	4.46 \pm 0.12	2.91 \pm 0.29	2.67 \pm 0.40
Rays height (μ m)	148.58 \pm 22.73	144.26 \pm 7.10	112.51 \pm 5.39	123.25 \pm 19.02
Rays density (n mm ²)	5.24 \pm 0.36	5.41 \pm 0.23	7.27 \pm 0.58	6.70 \pm 0.54
Vessels fraction (mm ²)	30,227.05 \pm 5739.13	24,082.29 \pm 7480.81	27,501.15 \pm 6212.37	49,836.43 \pm 25,224.47
Fibers fraction (mm ²)	665,599.27 \pm 62,255.31	609,342.54 \pm 66,568.52	312,430.42 \pm 71,249.96	426,151.15 \pm 102,494.11
Axial parenchyma fraction (mm ²)	202,790.99 \pm 50,710.13	275,121.46 \pm 69,229.71	574,358.50 \pm 70,979.53	452,113.57 \pm 101,125.87
Rays fraction (mm ²)	101,382.70 \pm 33,542.00	91,453.71 \pm 16,913.73	85,709.94 \pm 10,271.79	71,898.85 \pm 14,087.33

**Figure 3.** Wood of *Enterolobium contortisiliquum*. (A). Larger amount of gelatinous fibers in the stem of a 2-year-old sapling. Inset: Higher magnification of gelatinous fibers. Note also the growth rings delimited by flattened and thick-walled latewood fibers (arrow), wood diffuse-porous, paratracheal axial parenchyma, and parenchyma-like fibers (plf). (B,C). Tangential sections. Rays 1 to 3 cells wide (B) in the stem of 9-year-old tree, and uniseriate (C) in the stem of a 2-year-old sapling. Note also 2 to 4 cells per axial parenchyma strand (B,C). (D,E). Starch grains in axial parenchyma cells and parenchyma-like fibers seen under light microscopy (D) and shine under polarized light (E) in 2-year-old saplings. F. Prismatic crystals (arrows) in chambered axial parenchyma cells in xylem of a 2-year-old sapling. Bars = 200 μ m in A; 50 μ m inset; 100 μ m in (B–E); 20 μ m in (F).

The vessel grouping index (1.87–2.37 in trees and 3.04–6.78 in saplings), vessel density (2.47–3.82/mm² in trees and 8.33–26.47/mm² in saplings), and ray density (4.67–5.67/mm² in trees and 6.10–7.60/mm² in saplings) were higher in saplings than in trees. The secondary xylem of trees was mainly formed of fibers (63%), whereas the xylem of saplings exhibited a large amount of axial parenchyma (64%) (Figure 4).

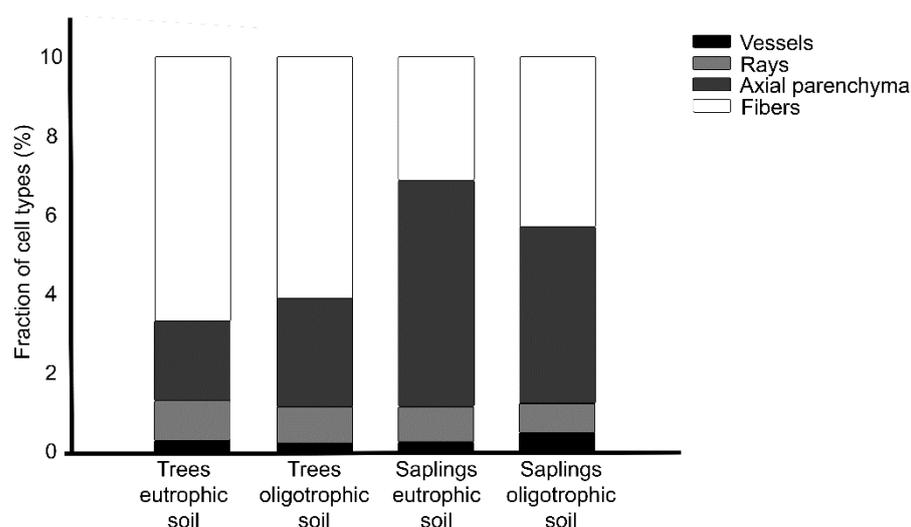


Figure 4. Fraction of cell types (%) of *Enterolobium contortisiliquum* 9-year-old trees and 2-year-old saplings in eutrophic and oligotrophic soil. Bars: black = vessels fraction; white = fibers fraction; dark grey = parenchyma fraction; light grey = rays fraction.

3.3. Statistical Analysis

Statistical analysis demonstrated that the soil type produced significant differences in growth ($p < 0.001$) (Table 4). Age influenced the stem's diameter and height, resulting in $p < 0.001$. The interaction age \times soil was significant ($p < 0.01$ for diameter and $p < 0.001$ for height), indicating that plants at different ages responded differently to soil type.

Table 4. Two-way ANOVA testing for the effect of age and soil on size and wood anatomy of *Enterolobium contortisiliquum* trees and saplings.

Feature	Age		Soil		Age \times Soil	
	F-Value	p	F-Value	p	F-Value	p
Diameter of stem	168.1	<0.0001	16.78	0.0008	15.41	0.0012
Height	164.4	<0.0001	30.48	<0.0001	28.08	<0.0001
Vessel density	42.95	<0.0001	0.1789	0.6779	0.2677	0.612
Vessel grouping	36.32	<0.0001	0.7258	0.4068	0.5581	0.4658
Vessel element diameter	158.9	<0.0001	2.638	0.1238	3.284	0.0888
Vessel element length	28.95	<0.0001	0.804	0.3832	0.6581	0.4291
Intervessel pit diameter	43.05	<0.0001	7.074	0.01713	1.988	0.1777
Vessel-ray pit diameter	76.32	<0.0001	0.1881	0.6703	1.172	0.295
Fiber length	266	<0.0001	1.166	0.2963	0.0196	0.8905
Fiber diameter	18.52	0.0005	0.8778	0.3627	1.329	0.2659
Fiber wall thickness	97.95	<0.0001	17.89	0.0006	11.3	0.0039
Rays height	13.59	0.002	0.1753	0.6811	0.9354	0.3479
Rays density	47.14	<0.0001	0.1752	0.6826	1.482	0.2412
Vessels fraction	3.472	0.0809	1.716	0.2087	5.31	0.0349
Fibers fraction	3.618	0.0753	1.643	0.2182	0.0439	0.8366
Parenchyma fraction	66.49	<0.0001	0.5505	0.4689	8.365	0.0106
Rays fraction	60.21	<0.0001	0.6911	0.418	6.047	0.0257

Statistics revealed that fiber wall thickness (4.38–8.04 μm in trees and 2.00–3.22 μm in saplings, minimum and maximum, respectively) and intervessel pit diameter (6.85–9.03 μm in trees and 5.03–6.80 μm in saplings, minimum and maximum, respectively) varied significantly between soil types ($p < 0.05$ for intervessel pit diameter and $p < 0.001$ for fiber wall thickness) (Table 4). All measurements varied in function of age, except for the fraction of vessels and fibers. The interaction age \times soil influenced fiber wall thickness ($p < 0.01$), axial parenchyma fraction ($p < 0.05$), and ray fraction ($p < 0.05$).

PCA demonstrated that Main Component 1 clearly separated trees and saplings, explaining 66.1% of the variance (Figure 5). The correlation coefficient was 0.31 for vessel diameter and 0.3 for fiber length, which are the variables that meaningfully influenced Component 1 (Table S1). On the other hand, the fraction of vessels and fibers influenced more Component 2 (correlation coefficient = 0.59 and 0.32, respectively). Vessel diameter was positively correlated to fiber wall thickness in Main Component 1 and inversely correlated to vessel density. Rays fraction was inversely correlated to axial parenchyma fraction.

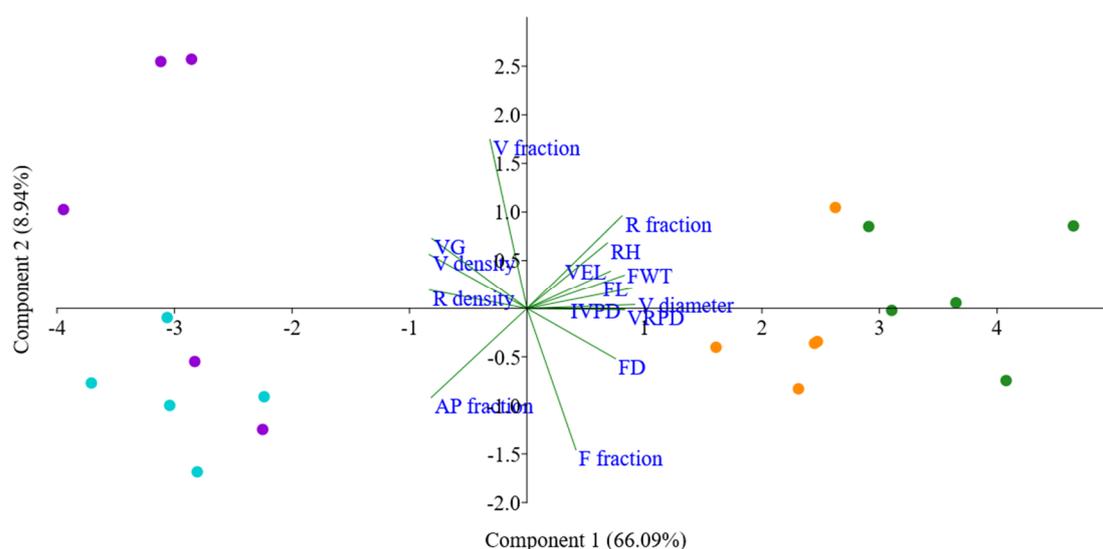


Figure 5. Main component analysis of *Enterolobium contortisiliquum* 9-year-old trees and 2-year-old saplings. Legends: ● TE = trees in eutrophic soil; ● TO = trees in oligotrophic soil; ● SE = saplings in eutrophic soil; ● SO = saplings in oligotrophic soil; Vdensity = vessel density (n/mm^2); VG = vessels grouping; VD = vessel diameter (μm); VEL = vessel element length (μm); IVPD = intervessel pit diameter (μm); VRPD = vessel-ray pit diameter (μm); FL = fiber length (μm); FD = fiber diameter (μm); FWT = fiber wall thickness (μm); RH = rays height (μm); Rmm² = rays density (n/mm^2); Vfraction = fraction of vessels; Ffraction = fraction of fibers; APfraction = fraction of axial parenchyma; Rfraction = fraction of rays.

Main Component 2 explained 8.94% of the variance. The density of vessels and rays (number of cells/ mm^2) and vessel grouping were positively correlated. The fraction of vessels was inversely correlated to the fraction of fibers.

MANOVA confirmed the effect of age (Wilk's $\lambda = 0.0007$, $p < 0.01$), and soil type (Wilk's $\lambda < 0.0001$, $p < 0.0001$) on wood anatomical traits. We detected a significant interaction age \times soil (Wilk's $\lambda = 0.0020$, $p < 0.01$).

4. Discussion

4.1. Allometric Features

Individuals growing in eutrophic soil were taller and larger than those growing in oligotrophic soil. Normally, plants in fertile soils have a higher growth performance than the ones subjected to nutrient limitations [7,11], and our results corroborate this statement. We expected that the differences in soil that have influenced tree growth would reflect changes in wood anatomy. We found plasticity in fiber wall thickening, intervessel pit diameter, and storage material inside vessel-associated parenchyma cells of trees. We discuss these findings in the next sections.

4.2. Wood Anatomy in Response to Soil Type

Fibers with thicker walls (47%) were detected in eutrophic soil trees compared with oligotrophic soil. According to De Melo, Amorim, and Soffiatti [15], fertile soils affect the carbon allocation and, consequently, influence wood structure. The same authors also observed fibers with thicker walls in the xylem of *Ficus cestrifolia* growing in more fertile

soil. Thicker walls could contribute to the main trunk stiffness [39] of taller “tamboril” trees in eutrophic soil. Even so, the wood fibers are considered thin or thin to thick-walled (according to IAWA 1989 parameters), regardless of soil types. Combined to the incidence of gelatinous fibers, the thickness of the fibers wall can ensure flexibility and flexural strength in tall trees in high winds [40].

A functional advantage of thicker-wall fibers in the wood of taller trees of “tamboril” grown in eutrophic soil is to reinforce weakness areas, such as those close to the vessels [41]. Our findings demonstrated that fiber wall thickness was positively correlated to vessel diameter, suggesting that the thickening of the fiber walls would form in secondary xylem a matrix of fibers protecting the vessels of possible drought-induced negative pressure [42].

Besides fiber wall thickening, the diameter of intervessel pits also varied with the differences of soil types where plants of “tamboril” have grown. This result is in agreement with Cary et al. [43], showing that the pit aperture size and depth might be influenced by soil nutrient restrictions. Soil nutrients may have affected the carbon allocation and partitioning at cellular levels [44]. The way that variations of the size of intervessel pits could influence the water transport and solutes between adjacent vessels of studied plants requires further analysis, considering that vessels in groups were observed both in trees and saplings.

Vessel diameter and density were negatively correlated, as also observed in the literature [41,45,46]. Both features, thus, contribute to the trade-off between efficiency and safety of the conducting system of trees, considering that there should be a sufficient number of vessels to compensate for vessel diameter to ensure water transport.

The relationship between vessel traits and water transport in the studied plants should consider that both soils occur naturally in close sites, which are evidently submitted to the same or similar water regimes. However, the two soil types have different fertility and capacity of water retention [18,28]. The variations observed in vessel features were probably a response to nutrient content and water availability, but the potential implications for the water transport require further investigations.

4.3. Storage Compounds inside the Parenchyma

“Tamboril” 9-year-old trees growing in eutrophic soil had storage compounds in the reserve cells that were not observed in the oligotrophic soil. In trees, storage compounds were mostly observed in the parenchyma cells associated with the vessels. In saplings, the storage compounds were spread throughout the axial parenchyma cells.

According to Morris et al. [47], vessel-associated cells are generally not expected to have the capacity for storage because they lack cell storage compartments, i.e., the vacuole. Although starch and non-identified compounds could be observed in the wood of our studied species, the storage could be temporary. De Lima, Oliveira, and Rodrigues [48] also observed starch grains in the wood of the same species. Morris et al. [47,49] have discussed the role of the starch grains, i.e., storage or contribution to the osmoregulation. Therefore, the starch grains possibly contributed to the maintenance of the water conductivity in vessels during the drier season.

4.4. Age Effect on Wood Anatomy

A greater proportion (74%) of solitary vessels was observed in trees compared to saplings (50%), which had higher amounts of multiple vessels (up to 7 vessels and clusters). Moreover, the higher values of vessel grouping index were observed in saplings of oligotrophic soil. Although saplings growing in controlled conditions received the same water amount at the nursery, multiple vessels might have been produced to ensure hydraulic safety in oligotrophic soil, which is characterized by lower water retention [18,28]. In addition, the larger proportion of multiple vessels in saplings is very likely related to juvenile wood and this gain has a controversial advantage [50]. On the one hand, there are vessels left to alternatively take over waterway transport, if any of the group’s vessels

is incapacitated by embolism. On the other hand, vessels in a group might also spread air-seeding through vessel-adjacent pit aperture.

Parenchyma-like fiber bands alternating with ordinary fibers might be a functional advantage for saplings because they were filled with starch grains, as shown in Figure 3D,E. Usually, these fibers are septate and function as a storage cell, besides the support function (see Carlquist [51]). Parenchyma-like fibers are not easily distinguished in transverse sections but could be confirmed in longitudinal sections of “tamboril” saplings.

The difference found in the width of the rays, that is, seedlings presenting predominantly uniseriate rays and trees with uniseriate and multiseriate rays, was expected. Carlquist [38] explains that most dicotyledons initially produce uniseriate rays, and the ray initials divide them to multiseriate throughout time, so much that the ray width (number of cells) proportionally raises to the stem circumference during the secondary growth. Therefore, multiseriate rays are a functional advantage for the adult plants of “tamboril”, contributing to the storage and radial mobilization of water, starch, sugar, and other compounds throughout the stem [52].

As for the fraction of cell types produced in the xylem of “tamboril” plants, we have found that trees have produced more resistant tissue (63% are fibers), regardless of the soil type. Saplings have prioritized the formation of storage tissue (64% axial parenchyma and ray cells), specifically in eutrophic soil. This difference among trees and saplings is due to the cambium age, considering that young plants may change considerably in their xylem structural and functional features as a tree grows and matures [19].

Given the difference in age between saplings and trees, different responses to soil conditions were expected [53]. Saplings might have different nutrient demands than trees. Therefore, it is necessary to consider their ability to allocate mineral nutrients that are essential for physiological events that lead to wood formation and differentiation.

5. Conclusions

Our study provides evidence that the influence of soil conditions on tree growth reflects variations in wood anatomical features. We detected changes in fiber wall thickening, intervessel pit diameter, and storage tissues in the secondary xylem of trees. In opposition, no significant response to soil type was observed in saplings, thus demonstrating that the relationships between soil type and wood growth is mediated by tree age. Compared to the trees, saplings had more multiple vessels, more parenchymatous cells, and predominantly uniseriate rays. The difference of age between them could explain the different responses to the soil conditions. However, the soil nutrient involved in these variations remained undetected.

In addition to water, soil nutrients have important effects on plant development, and the allocation of essential elements plays an important role in plant function and structure, with potential implications for water transport and the survival of trees [4,13,43]. Therefore, assessing reliable soil-plant relationships is necessary to explain the anatomical strategies of tropical species in response to the edaphic conditions. These strategies would be crucial for plant survival under degraded conditions where soil restrictions may occur in the future.

Considering the growth performance of “tamboril”, our model species, in eutrophic and oligotrophic soil, we conclude that this species is able to develop wood anatomical strategies to improve water use and nutrient absorption. This finding contributes to the current knowledge about the establishment of forest species in reforestation of areas characterized by different levels of soil fertility. Furthermore, we suggest investigating the limiting factors under low fertility that impede the successful growth of “tamboril” in a context of forest restoration and artificial regeneration.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12081058/s1>. The Supplementary Material reports the description of the wood anatomy of trees and saplings of *Enterolobium contortisiliquum*. Figure S1: Wood of *Enterolobium contortisiliquum*. Table S1: Loadings of the principal component analysis for the measured wood anatomical features.

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