

Do root secondary xylem functional traits differ between growth forms in Fabaceae species in a seasonally dry Neotropical environment?

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- **Background and Aims** Whole-plant performance in water-stressed and disturbance-prone environments depends on a suitable supply of water from the roots to the leaves, storage of reserves during periods of shortage, and a morphological arrangement that guarantees the maintenance of the plants anchored to the soil. All these functions are performed by the secondary xylem of roots. Here, we investigate whether different growth forms of Fabaceae species from the seasonally dry Neotropical environment have distinct strategies for water transport, mechanical support and non-structural carbon and water storage in the root secondary xylem.
- **Methods** We evaluated cross-sections of root secondary xylem from species of trees, shrubs and subshrubs. We applied linear models to verify the variability in secondary xylem anatomical traits among growth forms.
- **Key Results** Secondary xylem with larger vessels and lower vessel density was observed in tree species. Vessel wall thickness, vessel grouping index, potential hydraulic conductivity and cell fractions (vessels, fibres, rays and axial parenchyma) were not statistically different between growth forms, owing to the high interspecific variation within the groups studied.
- **Conclusion** Our results showed that the variability in anatomical traits of the secondary xylem of the root is species specific. In summary, the cellular complexity of the secondary xylem ensures multiple functional strategies in species with distinct growth forms, a key trait for resource use in an environment with strong water seasonality.

Key words: Chaco vegetation, Leguminosae, shrub, subshrub, tree, wood anatomy.

INTRODUCTION

Woody plants of different growth forms coexist in seasonally dry tropical forests, where water availability is the major limiting resource for the composition and structure of these plant communities (Chaturvedi *et al.*, 2011). The coexistence of plants with different growth forms is associated with a complex set of functional strategies that enable partitioning in the use of limited resources in environments with strong water seasonality (Rossatto and Franco, 2017). The different xylem cell types (water-conducting cells, fibres, and axial parenchyma and rays) provide numerous cellular arrangements that can result in the development of different ecological strategies to ensure simultaneous longitudinal water transport, mechanical support and storage of carbohydrates and water throughout the plant body (Baas *et al.*, 2004). Water transport from roots to leaves and the storage of water and non-structural carbohydrates are primary functions performed by the secondary xylem of roots in woody species (Carlquist, 2001). In addition, the secondary xylem provides mechanical support for the aerial organs and anchors the plants to the soil (Meijer, 2021). However, despite the numerous functions performed by the secondary xylem of roots,

little is known about their functional strategies in woody species of different growth forms in environments with strong water seasonality.

Tree and shrub species, in general, develop an extensive root system, with vertically oriented taproots in the soil reaching many metres in length and with lateral roots distributed on the soil surface (Villagra *et al.*, 2009). Despite the morphological similarity in the root system, trees and shrubs can differ in the functional traits of the secondary xylem (Martínez-Cabrera *et al.*, 2011). Generally, trees have wider and less dense vessels than shrubs (Carlquist and Hoekman, 1985; Martínez-Cabrera *et al.*, 2011). Given that the potential hydraulic conductivity of a vessel increases to the fourth power of its diameter (Hagen–Poiseuille laminar flow equation; Tyree and Zimmermann, 2002), wide vessels in trees have the potential to conduct a larger volume of water per unit of time and offer low resistance to water flow (Hacke *et al.*, 2006). However, wide vessels in the secondary xylem decrease its mechanical resistance, and the formation of a larger area of thick-walled fibres would be an important strategy to increase the mechanical support to the vessels and, consequently, to the organ as a whole (Hacke and Sperry, 2001; Jacobsen *et al.*, 2007). In contrast, narrower and more numerous vessels in shrubs have

less water-conducting potential but are considered to be important for water security in environments with strong water seasonality (Dória *et al.*, 2016). The greater number of vessels per millimetre cubed increases the possibility of pathways for water flow in the event of embolisms (Hacke and Sperry, 2001), while at the same time increasing the water-conducting area (Tyree *et al.*, 1994). As the number of vessels increases, so does the likelihood of an organ reaching maximum water transport safety, which is an essential requirement for the survival of plants from water-limited environments (Ewers *et al.*, 2007, 2023).

Subshrub species, in contrast, have shallow, thickened or non-thickened taproots vertically oriented in the ground, with slender lateral roots near the soil surface (Schenk and Jackson, 2002; Silva *et al.*, 2020). Overgrazing, trampling by cattle, severe droughts and frosts are frequent causes of above-ground biomass loss in subshrub species distributed in seasonally dry environments (Silva and Rossatto, 2019; Silva *et al.*, 2020). After such disturbances, resprouting from buds in roots or root crowns is an important functional trait to increase above-ground branching (Appezato-da-Glória, 2015). This strategy requires secondary xylem with a large area of parenchyma cells, with storage of non-structural carbohydrates and water to support the formation of new buds and growth of new stems (Chapin *et al.*, 1990). Furthermore, water and carbohydrates stored in underground organs are a key resource to buffer the effects of low water availability during drought (Borchert, 1994).

Here, we examined the secondary xylem traits in tree, shrub and subshrub Fabaceae species from seasonally dry vegetation to test whether the secondary xylem of roots in plants of different growth forms is associated with distinct strategies for water transport, mechanical support, and carbon and water storage. We hypothesized that tree species would have larger vessels that contribute more efficient water transport than shrub and subshrub species. Also, we expected that tree species would have secondary xylem with a larger fraction of fibre to provide more mechanical support. In contrast, because of their ability to resprout (Silva *et al.*, 2020), we hypothesized that subshrub species would have a higher percentage of parenchyma cells as a key ecological strategy to store carbohydrates and water.

In order to test our hypothesis, we collected roots of Fabaceae species from seasonally dry environments of the Neotropics, specifically in the South American Chaco. Chaco vegetation covers areas of woodlands and xeromorphic forests in central South America (Pennington *et al.*, 2000) and supports a rich assemblage of Fabaceae species that experience regular annual droughts and frosts (Lima *et al.*, 2015; Baptista *et al.*, 2020). Fabaceae species are particularly important in dry environments owing to their capacity to fix nitrogen through nodulation, increasing soil fertility (Baptista *et al.*, 2020). Moreover, native Fabaceae species are important food sources for wild and domestic animals and economic resources for the local people in the Chaco area (Felker *et al.*, 2003). The coexistence of several species of Fabaceae in Chaco vegetation makes this a good opportunity to test whether different growth forms submitted to similar climatic and edaphic conditions show distinct strategies in the root secondary xylem.

MATERIALS AND METHODS

Study area

We performed this study in a wet Chaco remnant located in the municipality of Porto Murtinho (21°41'0.50"S, 57°46'42.90"W), State of Mato Grosso do Sul, Brazil. Brazilian Chaco remnants are restricted to the far southern region of the Pantanal wetland. The vegetation of the study area is classified as a 'thorn forest' (UNESCO, 1973) characterized by shrubby-arboreal strata, interspersed with herbaceous plants and mainly composed of microphyll, deciduous and thorny species.

The Brazilian Chaco remnant experiences marked seasonality, with a dry season occurring from May to September and a rainy season from November to March (Fig. 1). The climate is hot and dry, with an average annual rainfall of 970 mm and a mean temperature of 25.1 °C. During the rainy season, temperatures can exceed 40 °C, and temporary floods occur owing to the poor drainage of the compacted soil. In the dry season, frosts are frequent during the colder months (from June to August), when temperatures may drop below 5 °C. Meteorological data were provided by the CEMTEC-Monitoring Centre for Weather, Climate and Water Resources of Mato Grosso do Sul State, Brazil. The soil in the area is classified as natric planosol with a clay texture, dystrophic, saline and compacted, with slow drainage (Couto and Oliveira, 2014; Assunção *et al.*, 2021).

Studied species and sampling

We selected 14 co-occurring woody Fabaceae species considered more abundant in areas of Brazilian Chaco remnant. The species selection was based on a previous phytosociological and floristic survey by Baptista *et al.* (2020), Carvalho and Sartori (2015), Alves and Sartori (2009) and Noguchi *et al.* (2009). The species studied were divided into three functional groups based on their growth form: trees, shrubs and subshrubs. We consider as trees those woody species that have only one main stem supporting the branches; as shrubs, plants with numerous stems that are entirely woody and branched from the stem base; and as subshrubs those plants with multiple, ascending, woody stems, except in the apical portion of the branches (Pérez-Harguindeguy *et al.*, 2016). The species studied are described in Table 1.

To determine the variation in secondary xylem traits among the different plant functional groups (trees, shrubs and subshrubs), we excavated trenches ≥ 50 cm deep and collected taproots from three individuals of each species studied. For botanical identification, we collected and herborized the branches with flowers and/or fruits, in addition to those with vegetative parts. The exsiccates were deposited at the CGMS Herbarium, Federal University of Mato Grosso do Sul, Campo Grande, Brazil.

Wood anatomical study

We fixed the root samples in FAA₅₀ (10 % formaldehyde, 5 % glacial acetic acid and 50 % ethanol) for 48 h, then stored them in 70 % ethanol (Johansen, 1940). We cut transverse and longitudinal (tangential and radial) histological sections of the secondary xylem at a thickness of 14–18 μm using a

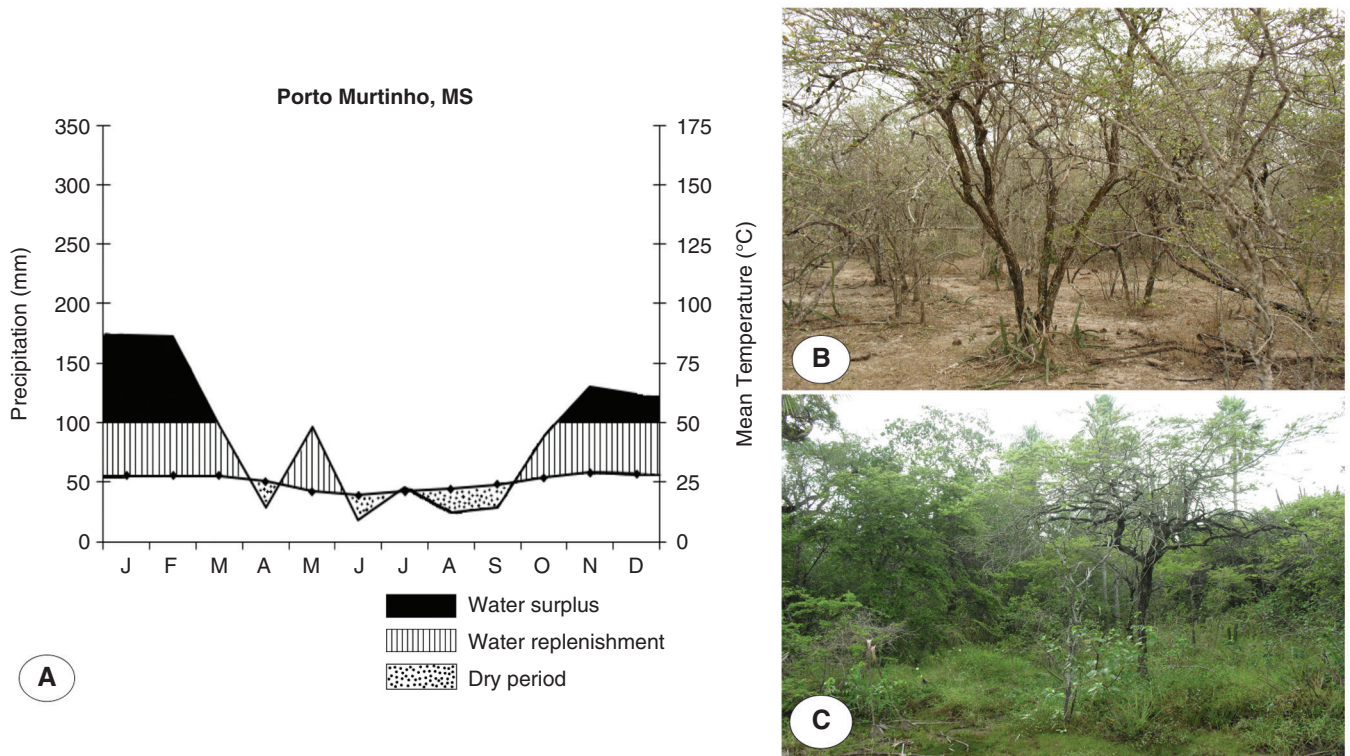


FIG. 1. (A) Climatic diagrams with precipitation and mean temperature. (B, C) Brazilian Chaco remnants in the dry season (B) and the rainy season (C).

TABLE 1. Root length and diameter and plant height of Fabaceae species sampled. The values are shown as the mean ± s.d.

Species	Root length (cm)	Root diameter (cm)	Plant height (m)
Tree			
<i>Mimosa glutinosa</i> Malme	26.3 ± 1.5	1.5 ± 0.1	1.1 ± 0.05
<i>Mimosa hexandra</i> Micheli	33.6 ± 2.1	2.0 ± 0.2	1.2 ± 0.05
<i>Parkinsonia praecox</i> (Ruiz & Pavon ex. Hook.) J. Hawkins	30.0 ± 0.3	1.7 ± 0.1	0.6 ± 0.2
<i>Prosopis rubriflora</i> Hassl.	36.0 ± 4.0	2.0 ± 0.1	1.0 ± 0.2
<i>Prosopis ruscifolia</i> Griseb	33.5 ± 1.5	1.6 ± 0.1	1.1 ± 0.1
Shrub			
<i>Bauhinia hagenbeckii</i> Harms	24.0 ± 3.6	4.0 ± 2.0	0.90 ± 0.07
<i>Mimosa polycarpa</i> Kunth.	14.0 ± 1.0	0.6 ± 0.1	0.40 ± 0.01
<i>Mimosa sensibilis</i> var. <i>urucumensis</i> Barneby	20.3 ± 1.5	1.6 ± 0.1	0.85 ± 0.05
Subshrub			
<i>Arachis lignosa</i> (Chodat & Hassl.) Krapov. & W.C. Gregory	15.0 ± 1.3	1.2 ± 0.3	0.27 ± 0.03
<i>Galactia paraguariensis</i> Chodat & Hassl.	17.2 ± 0.2	0.7 ± 0.17	0.40 ± 0.01
<i>Neptunia pubescens</i> Benth.	22.3 ± 2.5	1.1 ± 0.15	0.32 ± 0.04
<i>Senna pilifera</i> (Vogel) H.S. Irwin & Barneby	14.6 ± 1.5	0.7 ± 0.12	0.29 ± 0.01
<i>Stylosanthes hamata</i> (L.) Taub.	23.0 ± 3.0	0.9 ± 0.15	0.37 ± 0.01
<i>Tephrosia chaquenha</i> R. T. Queiroz & A. M. G. Azevedo	20.0 ± 2.6	0.8 ± 0.1	0.40 ± 0.02

Leica SM2000R sliding microtome. The histological sections were stained with aqueous 1 % Astra Blue (Roeser, 1972) and aqueous 1 % Safranin (Bukatsch, 1972) and mounted in

glycerin and water solution (1:1 v:v). We also made histological sections of root samples, embedded them in plastic resin (Bennett et al., 1976) and sectioned them using a Leica

RM2145 rotary microtome at 5–10 μm thickness. The histological sections were stained in 0.1 % Toluidine Blue at pH 4.7 (O'Brien *et al.*, 1964). To dissociate the cellular elements of the xylem, we placed small fragments of secondary xylem in a solution of glacial acetic acid and hydrogen peroxide (1:1 v:v) at 60 °C for 24 h, rinsed them in water, stained them with aqueous 1 % Safranin, and mounted semi-permanent slides in a glycerin and water solution (1:1 v:v) (Franklin, 1945 modified by Kraus and Arduin, 1997). We used the dissociated cells of the secondary xylem for description of the following qualitative features: perforation plate type, presence or absence of vasicentric tracheids and fibre morphology.

We analysed the root secondary xylem slides under a light microscope equipped with a Moticam Pro 252B digital camera. We followed the recommendations of the International Association of Wood Anatomists (IAWA) Committee (1989) for anatomical description of the secondary xylem. Based on the IAWA list (1989), we wrote an anatomical description of the following secondary xylem features in transverse sections: growth rings; porosity, arrangement and grouping of vessels; fibre wall thickness; presence of gelatinous fibres; and arrangement of axial parenchyma. We used longitudinal radial sections to describe the cellular composition of rays. Longitudinal tangential sections were used to describe the arrangement of intervessel pits and vessel-ray pitting, strand length of the axial parenchyma, and the width and height of rays (number of cells).

We performed scanning electron microscopy to observe the features of intervessel pits. Secondary xylem samples 3 mm in length fixed in FAA₅₀ were split in a tangential plane, dehydrated in an ethanol series from 50 to 100 %, then air dried for 24 h. The split wood samples were fixed to stubs using an electron-conductive carbon sticker and gold coated in a Baltec SCD 050 sputter coater for 3 min. We observed the intervessel pits using a Jeol JSM-6380LV scanning electron microscope at an accelerating voltage of 15 kV. We used scanning electron microscopy to confirm the presence of vestured pits in the vessel walls.

Wood data collection

We evaluated the following wood features: vessel diameter (in micrometres); vessel wall thickness (in micrometres); vessel density (number per millimetre squared); vessel grouping index; fractions of vessel (as a percentage), fibre (as a percentage), ray (as a percentage) and axial parenchyma (as a percentage) in transverse section; and potential hydraulic conductivity (in kilograms per metre per megapascal per second). We performed 30 measurements of vessel lumen area and vessel wall thickness per sampled individual of each species studied. We used the vessel lumen area to calculate the vessel diameter (D) based on the following equation:

$$D = \sqrt{\frac{4A}{\pi}}$$

where A is the vessel lumen area in micrometres (Scholz *et al.*, 2013).

To quantify the vessel density (number of vessels/area), we counted the number of vessels in three areas of 1 mm² per sampled individual in each species studied. We decided to count

the vessels in three areas per individual to avoid overlapping of vessel counting areas in those species with roots of narrow diameter. We estimated the fractions of vessels, fibres, rays and axial parenchyma in three areas of 1 mm² in wood transverse sections of each sampled individual. We performed all wood measurements using ImageJ 1.6.0 (<https://imagej.nih.gov/ij/download.html>). Primary xylem vessels (protoxylem and metaxylem) and newly produced vessels were excluded from the measurements. We considered protoxylem vessels to be those with smaller diameters and thinner cell walls and the metaxylem vessels to be those with larger diameters and thicker walls (Evert, 2006). New vessels formed are those nearest the vascular cambium (Evert, 2006).

We calculated the vessel grouping index as the total number of vessels divided by the total number of vessel groupings (Carlquist, 1984; Scholz *et al.*, 2013). For each sampled individual, we selected 25 vessel groups and counted the total number of vessels in these groups, then divided the total number of vessel groups by 25. We followed recommendations made by Carlquist (1984) and considered both solitary vessels and grouped vessels as vessel groups.

To determine the potential hydraulic conductivity, we first calculated the hydraulically weighed vessel diameter (D_h) for each sampled individual:

$$D_h = \left(\frac{\sum D^4}{n} \right)^{\frac{1}{4}},$$

where D is the vessel diameter (in micrometres) and n is the number of vessels measured. Then we used the result of hydraulically weighed vessel diameter (D_h) and vessel density (VD) to calculate the potential hydraulic conductivity based on the Hagen–Poiseuille law (Poorter *et al.*, 2010):

$$K_p = \left(\frac{\pi \rho_w}{128 \eta} \right) \times VD \times D_h^4,$$

where K_p is the potential hydraulic conductivity (in kilograms per metre per megapascal per second), ρ_w is the density of water at 20 °C (998.2 kg m⁻³), η is the viscosity of water at 20 °C (1.002 $\times 10^{-3}$ Pa s), VD is the vessel density (number of vessels per millimetre squared) and D_h is the hydraulically weighed vessel diameter (in metres).

Data analyses

To test whether growth form differs in the anatomical traits of root secondary xylem, we performed mixed linear models, using groups (trees, shrub and subshrub) as a fixed factor and with species and individuals nested in species as a random factor for the following wood features: vessel diameter, density and wall thickness, and the fraction of vessels, fibres, rays and axial parenchyma. This design avoided inflating the variation of the fixed factors, because the variation of individuals is already nested within species by default (Schielzeth and Nakagawa, 2013). For vessel grouping index and potential hydraulic conductivity, we performed mixed linear models using groups (trees, shrubs and subshrubs) as a fixed factor and species as a random factor. To determine whether there were differences in wood traits between functional groups (trees,

shrubs and subshrubs), we used one-way ANOVA. Tukey’s test was used as a post-hoc analysis to explore differences in the response variables among functional groups. To avoid multiple comparisons, we adjusted the *P*-value using the Bonferroni–Holm method, and we considered a significant level of 5 % (*P* < 0.05).

All the analyses were conducted by the R program (R Core Team, 2020), with the additional packages ‘lme4’ (Bates et al., 2015), ‘car’ (Fox and Weisberg, 2019) and ‘multcomp’ (Therneau et al., 2015).

RESULTS

The mixed model analysis revealed that the vessel diameter and density differed between tree, shrub and subshrub species. Tree species generally had larger vessels than shrub and subshrub species (Fig. 2; Tables 2 and 3). The average vessel diameter was 40 ± 15.6 µm in tree species, 25.7 ± 10.15 µm in shrub species and 32.1 ± 14.8 µm in subshrub species. The vessel diameter in shrub and subshrub species was statistically similar. Concerning vessel density, tree species had lower vessel density than shrub species. The vessel density was not statistically different between trees and subshrub species and between shrub and subshrub species. The vessel density in tree species ranged from 34.9 ± 5.2 vessels mm⁻² in *Parkinsonia praecox* to 75.5 ± 36.4

vessels mm⁻² in *Prosopis ruscifolia*, and the vessel density in shrub species ranged from 96 ± 15.6 vessels mm⁻² in *Bauhinia hagenbeckii* to 302 ± 26 vessels mm⁻² in *Mimosa polycarpa*.

Vessel wall thickness, vessel grouping index, potential hydraulic conductivity and fractions of secondary xylem cells (vessel, fibre, ray and axial parenchyma) were not statistically different between functional groups (Fig. 2; Tables 2 and 3). However, these wood traits varied substantially across tree, shrub and subshrub species. The largest variations in wood traits were observed across subshrub species: potential hydraulic conductivity varied 13.9-fold (from 1 kg m⁻¹ MPa⁻¹ s⁻¹ in *Arachis lignosa* to 13.9 kg m⁻¹ MPa⁻¹ s⁻¹ in *Stylosanthes hamata*), vessel fraction varied 4.2-fold (from 7.7 % in *Senna pilifera* to 32.4 % in *Stylosanthes hamata*), fibre fraction varied 33.1-fold (from 0.6 % in *Senna pilifera* to 19.9 % in *Stylosanthes hamata*), ray fraction varied 3.6-fold (from 6.6 % in *Stylosanthes hamata* to 23.5 % in *Tephrosia chaquenha*), and axial parenchyma fraction varied 2-fold (from 41.1 % in *Stylosanthes hamata* to 82.2 % in *Senna pilifera*). Vessel grouping index and axial parenchyma fraction also varied substantially across shrub species: vessel grouping index varied 4.6-fold (from 1.8 vessels per group in *Bauhinia hagenbeckii* to 8.4 vessels per group in *Mimosa polycarpa*) and axial parenchyma fraction varied 28-fold (from 1.9 % in *Mimosa polycarpa* to 52.4 % in *Mimosa sensibilis* var. *urucumensis*). Among tree species, potential hydraulic conductivity varied

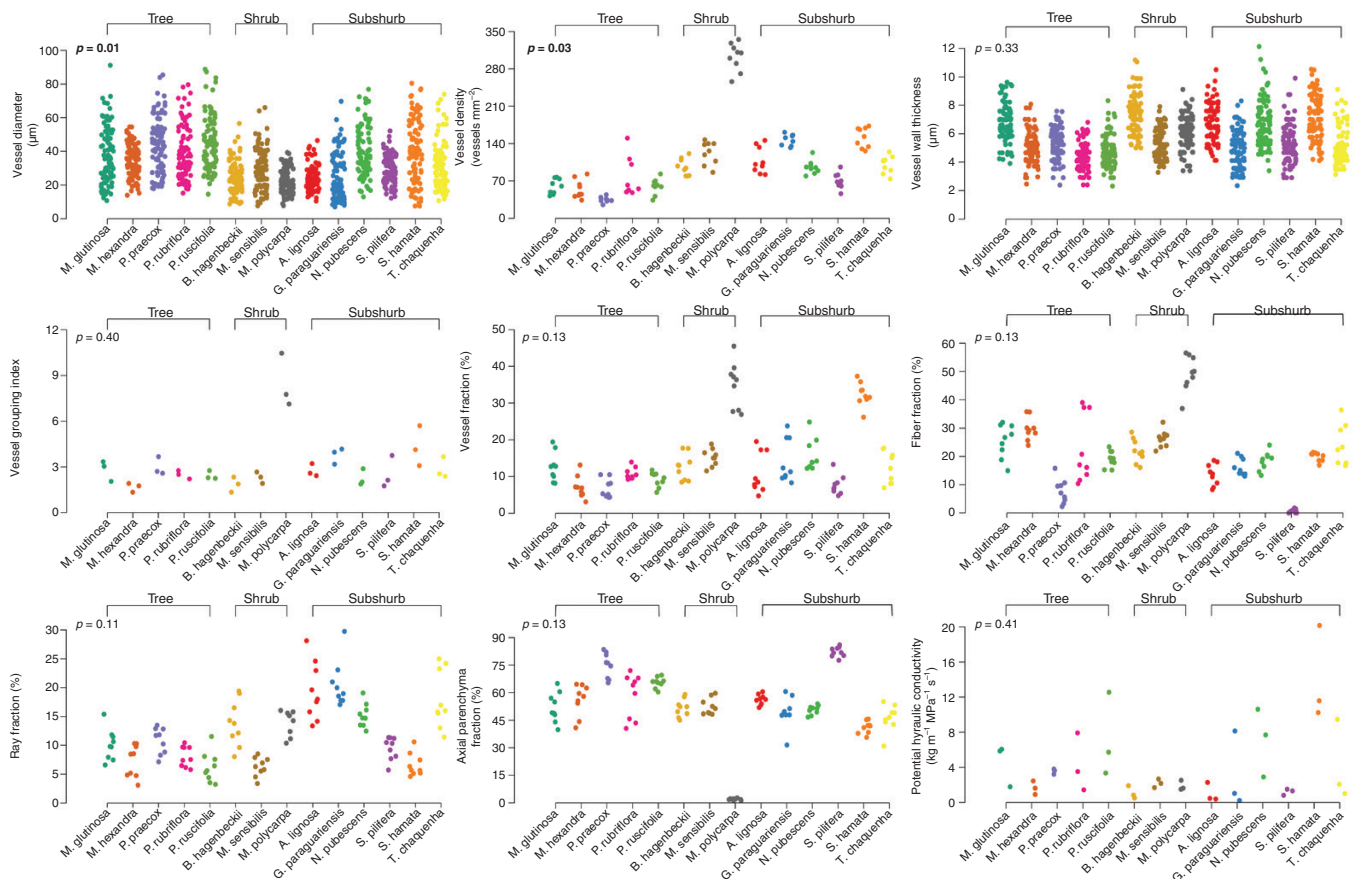


FIG. 2. Stripchart showing wood features of woody Fabaceae species from a Brazilian Chaco remnant. A *P*-value in bold indicates that the secondary xylem trait differs statistically between growth forms (tree, shrub and subshrub).

TABLE 2. Secondary xylem features of Fabaceae woody species from Brazilian Chaco remnants. The values are shown as the mean \pm s.d.

Species	Vessel diameter (μm)	Vessel density (number mm^{-2})	Vessel wall thickness (μm)	Vessel grouping index	Potential hydraulic conductivity ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$)	Vessel fraction (%)	Fibre fraction (%)	Ray fraction (%)	Axial parenchyma fraction (%)
Tree species									
<i>Mimosa glutinosa</i>	38.3 \pm 16.9	59.4 \pm 14.2	6.8 \pm 1.3	2.8 \pm 0.7	4.6 \pm 2.4	12.6 \pm 3.9	25.4 \pm 5.9	10 \pm 2.7	51.9 \pm 8.1
<i>Mimosa hexandra</i>	34.4 \pm 9.5	54.5 \pm 17.2	5.1 \pm 1.1	1.7 \pm 0.3	1.7 \pm 0.8	7.1 \pm 3	29.7 \pm 4	7.2 \pm 2.7	56 \pm 8.5
<i>Parkinsonia praecox</i>	43.8 \pm 16.1	34.9 \pm 5.2	5.3 \pm 1	2.9 \pm 0.6	3.6 \pm 0.3	6.8 \pm 2.5	7.6 \pm 4.2	10.8 \pm 2.3	74.8 \pm 6.7
<i>Prosopis rubriflora</i>	38.6 \pm 15.8	75.5 \pm 36.4	4.4 \pm 0.9	2.5 \pm 0.3	4.3 \pm 3.3	10.7 \pm 1.6	22.5 \pm 11.8	8.1 \pm 1.8	58.6 \pm 12
<i>Prosopis ruscifolia</i>	45.6 \pm 15.9	59.7 \pm 14.7	4.7 \pm 1.1	2.4 \pm 0.3	7.2 \pm 4.8	9.2 \pm 2	19.1 \pm 2.8	6.2 \pm 2.6	65.5 \pm 2.9
Shrub species									
<i>Bauhinia hagenbeckii</i>	24.9 \pm 9.1	96 \pm 15.6	7.5 \pm 1.3	1.8 \pm 0.5	1.1 \pm 0.7	12.6 \pm 3.5	22 \pm 4.1	13.8 \pm 3.9	51.6 \pm 4.7
<i>Mimosa polycarpa</i>	22.4 \pm 7.2	302.2 \pm 26	6.1 \pm 1.1	8.4 \pm 1.8	1.9 \pm 0.5	34.9 \pm 6.2	49.2 \pm 6.2	14 \pm 2.1	1.9 \pm 0.4
<i>Mimosa sensibilis</i> var. <i>urucumensis</i>	29.5 \pm 12.3	122.3 \pm 21.3	5.3 \pm 1	2.3 \pm 0.4	2.2 \pm 0.5	15.2 \pm 2.4	26.1 \pm 3	6.3 \pm 1.6	52.4 \pm 4.4
Subshrub species									
<i>Arachis lignosa</i>	24.4 \pm 7.4	108.7 \pm 24.6	6.6 \pm 1.3	2.7 \pm 0.41	1 \pm 1.1	10.9 \pm 5.5	13.6 \pm 3.8	19.4 \pm 4.9	56.1 \pm 2.8
<i>Galactia paraguayariensis</i>	25.5 \pm 13.6	147.2 \pm 10.5	4.8 \pm 1.3	3.7 \pm 0.5	3.1 \pm 4.3	14.1 \pm 5.8	16.2 \pm 3	20.4 \pm 3.9	49.3 \pm 8.2
<i>Neptunia pubescens</i>	39.8 \pm 15.1	95.7 \pm 12.6	6.9 \pm 1.6	2.2 \pm 0.5	7.1 \pm 3.9	15.8 \pm 4.3	18.4 \pm 3.2	15.2 \pm 2	50.6 \pm 2.5
<i>Senna pilifera</i>	29.7 \pm 8.5	69.4 \pm 14.6	5.4 \pm 1.3	2.5 \pm 1.1	1.2 \pm 0.3	7.7 \pm 2.6	0.6 \pm 0.6	9.5 \pm 1.9	82.2 \pm 2.7
<i>Stylosanthes hamata</i>	40.9 \pm 18.7	151.1 \pm 18.6	7.1 \pm 1.5	4.3 \pm 1.3	13.9 \pm 5.3	32.4 \pm 3	19.9 \pm 1.5	6.6 \pm 1.9	41.1 \pm 3.3
<i>Tephrosia chaquenha</i>	32.1 \pm 14.1	100.5 \pm 16.1	5.1 \pm 1.2	2.8 \pm 0.7	4.1 \pm 4.6	12.3 \pm 4.3	17.9 \pm 4.9	23.5 \pm 7.1	46.3 \pm 7

TABLE 3. Results of the mixed linear models used to test the difference between functional groups in woody Fabaceae species from Brazilian Chaco remnants. The values are shown as the mean \pm s.d. *Significant difference ($P < 0.05$). Abbreviations: S, shrub species; Sb, subshrub species; T, tree species.

Wood features	MLM results: random effects		One-way ANOVA		Tukey's test		
	Species	Species:individual	F-value	P-value	Sb vs. S	T vs. S	T vs. Sb
Vessel diameter	18.98 \pm 4.3	34.22 \pm 5.8	6.52	0.01*	0.10	0.001*	0.03*
Vessel density	2722.1 \pm 52.1	232 \pm 15	4.60	0.03*	0.17	0.007*	0.17
Vessel wall thickness	0.87 \pm 0.9	0.26 \pm 0.51	1.22	0.33	0.65	0.45	0.45
Vessel grouping index	2.63 \pm 1.62	–	0.98	0.40	0.69	0.48	0.69
Vessel fraction	0.009 \pm 0.09	0.002 \pm 0.05	2.38	0.13	0.32	0.10	0.31
Fibre fraction	0.01 \pm 0.14	0.003 \pm 0.05	2.40	0.13	0.08	0.37	0.37
Ray fraction	0.004 \pm 0.006	0.001 \pm 0.03	2.67	0.11	0.60	0.60	0.06
Axial parenchyma fraction	0.04 \pm 0.2	0.03 \pm 0.05	2.41	0.13	0.16	0.09	0.56
Potential hydraulic conductivity	9.32 \pm 3.05	–	0.94	0.41	0.51	0.64	0.69

4.2-fold (from 1.7 kg m⁻¹ MPa⁻¹ s⁻¹ in *Mimosa hexandra* to 7.2 kg m⁻¹ MPa⁻¹ s⁻¹ in *Prosopis ruscifolia*), and fibre fraction varied 3.9-fold (from 7.6 % in *Parkinsonia praecox* to 29.7 % in *Mimosa hexandra*).

The root wood anatomical features were similar among functional groups (tree, shrub and subshrub species), except for vestured pits, axial parenchyma arrangement and fibre type. Poorly defined growth rings were observed in all species studied (Fig. 3A, B). Vessels were diffuse-porous (Fig. 3A–I), solitary (Fig. 3A–D), and with two (Fig. 3C, D) to eight (Fig. 3F) vessels clustered; simple perforation plates (Fig. 4A), alternate intervessel pits (Fig. 4B) and vessel-ray pits with distinct borders, similar to intervessel pits in size and shape throughout the ray cell. Vessels with vestured pits were observed in all species (Fig. 4C), except the shrub species *Bauhinia hagenbeckii* (Fig. 4D) and subshrub species *Senna pilifera*. We observed gelatinous fibres in all species studied (Fig. 3A, D–I), except for the tree species *Parkinsonia praecox* and *Prosopis rubriflora*. In these species, we observed thin-walled libriform fibres in *Parkinsonia praecox* (Fig. 3B) and very thick-walled libriform fibres in *Prosopis rubriflora* (Fig. 3C). In all studied species, except the shrub species *Mimosa polycarpa*, the axial parenchyma was paratracheal in bands more than three cells wide (Fig. 3A–E, G–I). In the shrub species *Mimosa polycarpa*, the axial parenchyma was vasicentric (Fig. 3F). Non-lignified paratracheal parenchyma was observed in the shrub species *Bauhinia hagenbeckii* (Fig. 3D) and subshrub species *Arachis lignosa*, *Galactia paraguariensis*, *Stylosanthes hamata* (Fig. 3H) and *Tephrosia chaquenha* (Fig. 3I). We observed rays one to three cells wide (Fig. 4E, F) and the body with procumbent cells in all species (Fig. 4G). Detailed anatomical descriptions of the root secondary xylem of the studied species are provided in the Supplementary Data S1.

DISCUSSION

In this study, we investigated whether the secondary xylem traits of roots differ between growth forms (tree, shrub and subshrub) of Fabaceae species that co-occur in the seasonally dry

Neotropical environment. The results showed that only vessel diameter and density varied among growth forms, with larger vessels being observed in tree species. Other vessel features (wall thickness, grouping index and potential hydraulic conductivity) and xylem cell fractions (vessels, fibres, rays and axial parenchyma) were not statistically different among the growth forms, indicating the wide range of variation in root secondary xylem traits within the functional groups studied.

As expected, tree species were found to have larger vessels than shrub and subshrub species. In general, our results confirm the findings of previous studies based on anatomical studies of the secondary xylem of roots (Carlquist and Hoekman, 1985; Martínez-Cabrera *et al.*, 2011) and indicate that the vessel diameter varies as a function of plant size (Olson and Rosell, 2013) and probably also as a function of root size. Tree species also showed lower vessel density, highlighting the inversely proportional ratio of vessel diameter and density in wood (Baas *et al.*, 2004). Despite the larger vessels in the tree species, potential hydraulic conductivity was not statistically different between growth forms. This is likely to be explained by the broad range of variation in vessel density within the studied functional groups.

Larger vessels in tree species enhance water transport efficiency, but they can also increase the vulnerability to drought-induced cavitation (Hacke and Sperry, 2001; Hacke *et al.*, 2006). Taking into account that species occurring in the Brazilian Chaco are exposed to the stress conditions of drought in winter and flood in summer (Pott *et al.*, 2011), both conductivity efficiency and safety are key traits to guarantee water transport. The species and groups presented high intra- and interspecific variation of wood traits. This could help to maintain the species in the environment, given that narrow vessels, with a minor tangential area, promote more tension to maintain the integrity of the water column under higher vapour pressure, which might cause cavitation and/or embolism events during the dry season (Hacke and Sperry, 2001; Bucci *et al.*, 2004). This trait is common and expected in species inhabiting dry environments (Alves and Angyalossy-Alfonso, 2000; Sonsin *et al.*, 2012). In contrast, wide vessels ensure efficiency during the hot and flooded season, when it is necessary to move a



FIG. 3. Root secondary xylem cross-sections of Fabaceae species. (A–C) Cross-sections of the tree species *Mimosa hexandra* (A), *Parkinsonia praecox* (B) and *Prosopis rubriflora* (C). (D–F) Cross-sections of shrub species *Bauhinia hagenbeckii* (D), *Mimosa sensibilis* var. *urucumensis* (E) and *Mimosa polycarpa* (F). Note the non-lignified paratracheal parenchyma in *Bauhinia hagenbeckii* (D) and gelatinous fibres in *Bauhinia hagenbeckii* (D) and *Mimosa sensibilis* var. *urucumensis* (E). (G–I) Cross-sections of the subshrub species *Senna pilifera* (G), *Stylosanthes hamata* (H) and *Tephrosia chaquenha* (I). Note gelatinous fibres (*) and non-lignified paratracheal parenchyma (arrowheads) in *Stylosanthes hamata* (H) and *Tephrosia chaquenha* (I). Scale bars: 100 μ m in A–I.

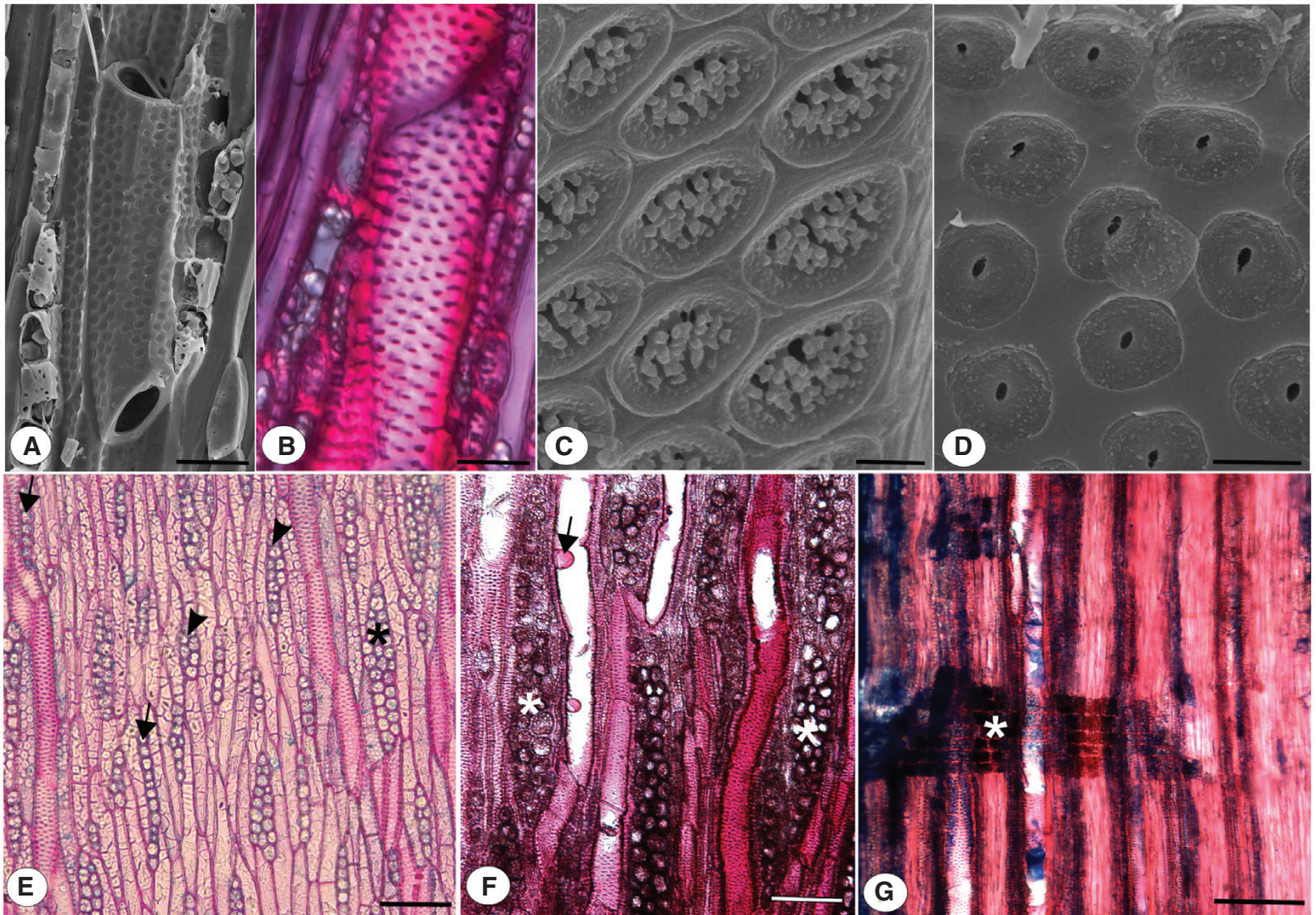


FIG. 4. Details of the root secondary xylem of Fabaceae species. (A) Simple perforation plate in vessel of *Bauhinia hagenbeckii*, scanning electron micrograph. (B) Alternate intervessel pits *Mimosa sensibilis* var. *urucumensis*, tangential longitudinal section. (C) Vestured pits in scanning electron micrograph of the tree species *Mimosa glutinosa*. (D) Non-vestured pits in *Bauhinia hagenbeckii*. (E) Rays with a width of one (arrows), two (arrowheads) and three cells (*) in *Parkinsonia praecox*, tangential longitudinal section. (F) Rays with a width of three cells (*) and tyloses (arrow) in *Mimosa polycarpa*, tangential longitudinal section. (G) Procumbent ray cells (*) in *Mimosa polycarpa*, radial longitudinal section. Scale bars: 20 μm in A, B; 1 μm in C; 2 μm in D; 50 μm in E–G.

larger volume of water to maintain transport and transpiration. All of these vessels communicate and create a net, combining different vessel sizes and densities to ensure safety and efficiency in different environmental conditions (Zanne *et al.*, 2010). In our study, most Fabaceae species, regardless of growth habit, showed vessels with different diameter classes (see Fig. 2; Table 2). The occurrence of different vessel diameter classes within the secondary xylem, known as diameter polymorphism, could be a valuable feature to provide both efficient and safe transport in a strongly seasonal environment (Jacobsen and Pratt, 2023).

In angiosperms, the intervessel pit features are important anatomical traits that can influence vessel function and minimize air-seeding dispersion (Lens *et al.*, 2011). There are two hypotheses that suggest the function of vestured pits in the air-seeding mechanism: first, vestured pits could trap air bubbles during air seeding; and second, vestured pits could prevent the rupture of the pit membrane during its stretching in air seeding (Carlquist, 2001; Jansen *et al.*, 2003). Despite limited knowledge of how vestured pits function to minimize air bubble dispersal during air seeding, such structures are common in

species that evolved in warm and dry environments, such as most Fabaceae species.

We hypothesized that tree species would have a larger fibre cross-sectional area than shrub and subshrub species. Contrary to our expectation, the amount of fibre was not statistically different among the growth forms studied. This result can be explained, in part, by the wide variation in fibre cross-sectional area within the groups studied. Fibre fraction varied 3.9-fold in tree species (from 7.6 % in *Parkinsonia praecox* to 29.7 % in *Mimosa hexandra*), 2.2-fold in shrub species (from 22 % in *Bauhinia hagenbeckii* to 49.2 % in *Mimosa polycarpa*) and 33.1-fold in subshrub species (from 0.6 % in *Senna pilifera* to 19.9 % in *Stylosanthes hamata*). Fibres are specialized cells that provide mechanical strength to support the plant body against gravity and provide mechanical stability (Ennos, 1993). In the root, the surrounding soil matrix and soil physical properties also contribute to mechanical support and maintenance of plants anchored to the soil (Pratt *et al.*, 2007; Fortunel *et al.*, 2014). For example, soil moisture content influences plant stability, because the decrease in the surrounding soil moisture caused by root water uptake leads to an increase in matrix

suction and soil mechanical strength (Easson *et al.*, 1995; Schwarz *et al.*, 2010). Clayey soils also provide greater resilience to plant movement than other soil types (Ennos, 2000). Thus, in environments with clay soils and prolonged periods of drought, such as the Brazilian Chaco, low soil moisture and the soil physical properties would act as major drivers for plant anchorage.

We observed gelatinous fibres in the root secondary xylem of the studied species, except in the tree species *Parkinsonia praecox* and *Prosopis rubriflora*. Gelatinous fibres are characterized by the presence of an inner cell wall with concentric cellulose layers and other polysaccharides, such as hemicelluloses, pectins or arabinogalactan-proteins (Evert, 2006; Bowling and Vaughn, 2008). Gelatinous fibres are related to organ movement, providing a strong tensile force that enables plant organs to bend, straighten or remain upright (Chery *et al.*, 2022). The unique cell wall of gelatinous fibres could increase the mechanical resistance of roots against bending stress during external events, such as wind, and mitigate rotational effects when the plant is pulled from the soil by large mammals in grazing. In addition, the cellulose-rich inner layer of gelatinous fibres has a high water-absorption capacity (Carlquist, 2001), which could act as an important source of water storage in the roots of the studied species. However, experimental studies are necessary to understand the function of gelatinous fibres in water storage in plant organs.

It was expected that there would be a larger parenchyma cell fraction (rays and axial parenchyma) in subshrub species, because these species have resprouting potential from the root crown and roots. However, we did not observe a statistically different amount of rays and axial parenchyma among the functional groups studied. Paratracheal parenchyma occupying a large cross-sectional area of the secondary xylem is a common feature in Fabaceae species (Dória *et al.*, 2022) and, except for the subshrub species *Mimosa polycarpa*, the species studied had >40 % of the area of secondary xylem of the roots occupied by parenchyma tissue, highlighting the storage function of this organ. Parenchymal cells act in the storage and transport of non-structural carbohydrates (Morris and Jansen 2016; Plavcová *et al.*, 2016) that support the growth of new stems after overgrazing, trampling by cattle, droughts and frosts, which are recurring events in Chaco vegetation. Furthermore, it is essential to emphasize that in Brazil, the remaining Chaco vegetation is located in the Pantanal wetlands (Pott *et al.*, 2011), which are exposed to a high frequency of fire in the dry season, especially in recent years (Kumar *et al.*, 2022), and a high proportion of parenchymal cells could be a key trait for post-fire plant persistence (Clarke *et al.*, 2013).

A high fraction of parenchyma in roots could provide an extra area to store carbohydrates synthesized in green stems, a common feature in the studied tree species. Chloroplasts were observed in branches of all the tree species, and in *Parkinsonia praecox* chloroplasts were also found in the bark and wood tissues of the main stem and roots when exposed to light. Photosynthesis in bark and wood tissue is considered an additional strategy for whole-plant carbon gain and water economy in species with microphyllous or absent leaves during dry conditions and high temperatures in a seasonally

dry environment (Aschan and Pfanz, 2003). Furthermore, photosynthesis in bark and wood tissue plays a role in repair of xylem embolism, contributing to the maintenance of hydraulic function in periods of reduced water availability (Bloemen *et al.*, 2016).

In general, distinct growth forms occupy different niches within and across the environments, hence they exhibit different ecological strategies for the use and conservation of the major resources (water, light and soil nutrients; Martínez-Cabrera *et al.*, 2011; Rossatto and Franco, 2017). Our findings, however, showed a high interspecific variation of root secondary xylem traits, regardless of growth form. Interspecific variability provides diversification of functional strategies in plants to cope with limited resources in seasonal environments, thus enabling their coexistence in the environment and reducing competitive interaction between species (Silva and Batalha, 2011).

Secondary xylem is a vascular tissue that exhibits high plasticity in the dimensions and number of its cells, features that have been used to differentiate growth forms in ecological anatomy studies. In our study, the similarity of secondary xylem trait estimates among growth forms might have been limited by our sample size. The inclusion of further specimens (or individuals), even if it does not change the estimates found, could enhance our understanding of these traits in the diverse spectrum of growth forms. Furthermore, we highlight that the estimates presented here could also be used as support for a broader study.

In this study, we measured root secondary xylem anatomical traits in 14 Fabaceae species from the seasonally dry Neotropical environment to assess whether the growth forms (tree, shrub and subshrub) show distinct strategies for water transport, mechanical support and non-structural carbon and water storage. Larger vessels in tree species suggest an effect of organ size on vessel diameter. Our results also highlighted high interspecific variability in potential hydraulic conductivity and secondary xylem cell fractions (vessels, fibres, rays and axial parenchyma) within growth forms. Altogether, our results suggest that the high variability in secondary xylem traits at the species level allows multiple strategies for water transport, mechanical support and non-structural carbon storage by species of different growth forms in environments with strong seasonal variation in water availability.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Data S1: anatomical descriptions of secondary xylem.

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The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

J.R.S., T.S.Y., and E.S.-D. conceived and designed the study. J.R.S. and T.S.Y. collected and prepared the samples and conducted the anatomical analyses. J.R.S., and A.C.A.R.

analysed the data. J.R.S., T.S.Y., A.C.A.R. and E.S.-D. wrote the manuscript.

DATA AVAILABILITY

The authors declare that all the data is available in the tables in the manuscript and as supplementary material.

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