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Incidence of controlled water restriction on density and chemical profiles in tree rings of *Araucaria araucana* seedlings

Sofía Papú^{1*} , Daigard Ricardo Ortega-Rodriguez², Fidel Alejandro Roig¹ and Florencia Navas³

Abstract

Background Dehydration of plant tissues caused by water stress affects the dynamics of the lateral cambium, the rate of cell division and differentiation in cell lumen size, wall thickness and wall chemical properties. Based on evidence that ongoing climate change projects longer and more intense water stress conditions, forest dynamics and decline are predicted to be affected by increases in drought intensity and frequency. To verify the interaction of these phenomena, we described the modifications in the anatomy and mineral contents of annual growth rings of *Araucaria araucana* seedlings subjected to water stress treatments, using X-ray densitometry and X-ray fluorescence techniques.

Results Severe water stress conditions during the growing period produced narrower tree rings, with reduced cell lumen size (5–7 μm) and higher tracheid reinforcement factor values, but with lower wood density. Plants subjected to moderate water stress generated intra-annual density fluctuations coinciding with periods of decreased soil moisture. Under the precept that the essential nutrients play a relevant role in the functioning of trees, we found evidence of element allocation and concentration in response to drought. Calcium and phosphorus concentration increased significantly as stress becomes more severe, but with small differences between early- and latewood. On the contrary, potassium and sulfur presented lower values in the most stressed plants, and manganese had the lowest values only for moderate water stress. Finally, S/Ca and K/Ca molar ratios decreased, while Ca/Mn increased as stress becomes more severe.

Conclusions Our findings suggested that *A. araucana* seedlings invested resources aimed at increasing structural components of the cell wall to prevent cavitation. This would maintain metabolism and cell growth even in unfavorable environmental conditions. Furthermore, the imbalance of manganese and calcium and their consequent ratio (Ca/Mn) could be linked to early adaptive signals to avoid dieback.

Keywords Wood density, Wood anatomy, Wood chemistry, Water stress, Intra-annual density fluctuation, Climate change

Background

Drought is one of the most important environmental stresses causing changes in physiological, morphological, biochemical, and anatomical traits in plants (Salehi-Lisar and Bakhshayeshan-Agdam 2016). In the context of global climate change, a combination of natural and man-made processes increases frequency, duration and/or severity of drought and thermal stress events, altering forest ecosystem dynamics (Crausbay et al. 2017). In

*Correspondence:

Sofía Papú
spapu@mendoza-conicet.gov.ar

¹ Instituto Argentino de Nivología Glaciología y Ciencias Ambientales (IANIGLA, CONICET), Mendoza, Argentina

² Universidade de Sao Paulo, Escola Superior de Agricultura Luiz de Queiroz, Departamento de Ciências Florestais, Piracicaba, Brazil

³ Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina



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Northern Patagonia, an increase in the number of hot and dry periods during the summer months has been recorded during the twentieth century, characterizing an anomalous climatic situation for this South America region, causing impacts on forest ecosystems (Mundo et al. 2012). One of these ecosystems is represented by the forests of *Araucaria araucana* (Molina) K. Koch, a conifer currently considered endangered by the International Union for Conservation of Nature (IUCN) due to natural and anthropogenic disturbances. The species shows a certain degree of sensitivity to water availability (Mundo et al. 2012; Hadad et al. 2014), so decreases in precipitation have caused alterations in its growth (Arco-Molina et al. 2019). Furthermore, in some cases extreme manifestations of adult tree mortality have been observed during the last decade (Amoroso et al. 2012; Rodríguez-Catón et al. 2016). The lack of information regarding the anatomical responses and the critical conservation state of the *A. araucana* old-growth forests, make relevant to expand the studies about the ecological responses of this tree species to environmental changes.

Considering that dehydration phenomena may broadly affect reproductive tissues and vegetative meristems (Buttó et al. 2021), it is expected that water stress induces modifications in the cambial division rate, radial and tangential xylem cell diameter and wall growth (Larson 1995; Abe and Nakai 1999). These structural variations are linked to the seasonal wood formation cycle, linked also to climatic conditions during the active cell division period. Therefore, the frequency and size of the conductive cells that represent the hydraulic structure of the stems, may explain the effects of climatic stress on the annual cycles of growth ring formation (Islam et al. 2019). One of these effects is the production of higher density wood or intra-annual density fluctuations (IADFs) (Battipaglia et al. 2014; De Micco et al. 2015; 2016). In this sense, conifer trees respond by adjusting their hydraulic system to reduce the risk of xylem cavitation, which is accomplished via tracheid lumen reduction and cell wall thick increase, resulting in IADFs (Campelo et al. 2007; Martinez-Meier et al. 2008). Therefore, more pronounced periods of drought may have a severe impact on IADF occurrence and other characteristics related to hydraulic safety (De Micco et al. 2015). For the study of this phenomenon, X-ray densitometry has been used as a classical methodology to analyze the wood density (WD) pattern related to seasonal growth, which in turn describes the climate seasonal sensitivity of tree-ring formation (Hervé et al. 2014; Jacquin et al. 2017).

The WD represent a proxy to understand the volume of wood produced in each growth-ring, whereas wood chemical elements are proxies resulted from combined nutritional and physiological processes

(Ortega-Rodriguez et al. 2022, 2023). Hietz et al. (2015) and Santini-Junior et al. (2019) have verified a relationship between wood density and wood chemical fluctuations along tree rings. This bond can be useful to identify a deterioration occurrence, a mobilization of defense substances, or an alteration in the nutritional supply in plants. In addition, both parameters are essential to analyze key mechanisms related to tree responses to changing environmental conditions affecting the inter- and intra-annual tree-ring variability (Hevia et al. 2019; Ortega-Rodriguez et al. 2022).

Recently, the content and distribution of elements in tree rings has allowed to determine chemical markers related to the cambial activity and storage characteristics of xylem cells (Barrelet et al. 2006; Poussart et al. 2006; Cherubini et al. 2013). Hence, the monitoring of xylem minerals, has proven to be a novel source of environmental and climate archives at worldwide forests (e.g., Martins et al. 1999; Poussart et al. 2006; Smith et al. 2008; Balouet et al. 2009; Locosselli et al. 2018; Muñoz et al. 2019; Hevia et al. 2019; Alterio et al. 2020; Durand et al. 2020; Binda et al. 2020). Some of these minerals implies essential nutrients in tree tissue (Ca, K, P, Mn, Fe), and its imbalances may increase a long-term sensitivity to drought (Horsley et al. 2000; Helama et al. 2009), so trees may modify their use and allocation in response (Hevia et al. 2019). Some nutrients are mobile in wood, even in a low concentration, due to its translocation to growing tissues (K, P); others are immobile, remaining in higher concentrations in wood (Ca, Fe, S).

Although there is evidence on the impact that climate change and extreme droughts are exerting on growth, physiology and biochemistry of *A. araucana* trees (Arco-Molina et al. 2019; Papú et al. 2021), little is known about the incidence of drought on the chemical composition of tree rings. We hypothesize that lower availability of water in soils triggers changes in the anatomical structure and mineral contents of wood. This implies changes in wood nutrient concentrations, increases in WD, a reduction of lumen size and ultimately a decrease in tree growth visualized by a narrower ring width, to maintain tracheid's functions which enables vital functions during stress and plant survival. Then the main objective of this paper was to identify wood anatomical responses and chemical profiles in tree rings of *A. araucana* seedlings subjected to contrasting water availability under controlled experimental conditions. This study (i) quantifies soil and leaf relative water content to find out the stress level and the plant water status; (ii) evaluates anatomical responses in wood density, ring width, lumen and cell wall thickness, and tracks intra-annual wood density fluctuations in tree rings; (iii) determines the chemical profile in the tree rings during the corresponding year of experimentation.

This approach would provide new information on the response of the radial cambium to the incidence of droughts.

Materials and methods

Plant material and experimental design

Araucaria araucana (Molina) K. Koch (Araucariaceae) is a north Patagonian endemic tree distributed between 37° 20' and 40° 20' S on both sides of the Andes in Argentina and Chile, South America (Roig 1998; Hadad et al. 2014; Roig et al. 2014). Commonly known as araucaria or by its Mapuche name *pehuén*, this long-lived dioecious gymnosperm can reach more than 1000 years old, 40 m in height, and 2 m in trunk diameter (Aguilera-Betti et al. 2017). The growth of this species is extremely low, with a mean raw tree-ring width of 0.43 mm in adult trees (>276 years) and 1.04 mm in young trees (<120 years) (Hadad et al. 2014). This growth occurs under the influence of a Mediterranean-type climate, with a precipitation gradient between 2000 and 600 mm yr⁻¹ from the Andes Cordillera to the Patagonian steppe (Paruelo et al. 1998; Bianchi et al. 2016).

Five-year-old *A. araucana* seedlings were germinated and cultured in 2.5 l plastic pots filled with the following substrate mixture: 40% *Nothofagus pumilio* and *N. antarctica* leaf litter, 40% *A. araucana* leaf litter, 10% sand, and 10% pumice. This combination simulates the natural mulch of the predominant soil where araucaria grows. Seedlings were allowed to grow with constant water, refilled every two days to field capacity, until they reached a height of 35 cm, which occurred within 5 years of germination. The experiment lasted 120 days from November 2016 to March 2017, which approximates the average length of the growing season in the temperate Southern Hemisphere. Pots were placed on a suspended platform and covered with a transparent polyethylene cover (1 m above plants and laterally extended to prevent rain input) that had a 90% transmittance of solar photosynthetically active radiation (PAR; 400–700 nm), as measured with an MQ-200 quantum meter (Apogee Instruments Inc., Utah, USA). Plants were covered with a 50% shade cloth, which PAR values were 360–437 μmol m⁻² s⁻¹ at 9 am and 445–475 μmol m⁻² s⁻¹ at 12 am. Water regimes were set using a completely randomized block design with one plant as the experimental unit and 27 replicates. There were three watering regimes at field capacity: control (C, watered every 2 days); moderate water stress (MWS, watered every 3 weeks) and severe water stress (SWS, only watered at the beginning and the end of the treatment). The amount of water in C and MWS was equivalent to 2000 mm yr⁻¹ and 400 mm yr⁻¹, respectively, simulating the extremes of total annual rainfall within the species' natural distribution (Bianchi et al. 2016; Hadad

et al. 2020), while SWS simulated extreme water shortages less than 400 mm yr⁻¹.

Soil and plant moisture measurements

To determine the water status of plants, relative water content (RWC, %) was measured when the experiment was over but before the final recovery watering. Samples of 10 apical, 10 basal and 10 middle leaves from 6 plants per treatment were used. In evergreen forests, the tree canopy consists of leaves from several age cohorts, and each year's cohort could have different physiological properties (Wang et al. 2020). Since *A. araucana* leaves stay alive for a long time (~24 years, Lusk 2001), physiological responses could vary between leaves of different ages or in different ontogenetic stages (Arco-Molina, personal communication).

Leaf relative water content (RWC %)

From 5 individuals per treatment, 3 basal (BL), 3 middle (ML), and 3 apical leaves (AL) per plant were collected. The leaves were immediately wrapped in nylon bags and stored in a portable refrigerator, to later be weighed in the laboratory with a precision balance of 0.1 mg resolution, obtaining the fresh weight of the leaves (LFW). Subsequently, the leaves were hydrated with distilled water for 24 h under dark conditions at 24 °C, in order to obtain the full leaf turgor weight (LTW). Then, the samples were dried in an oven at 40 °C for 24 h, obtaining the leaf dry weight (LDW) (Barr and Weatherley 1962). The following equation was used to calculate the relative water content:

$$\text{RWC}(\%) = \frac{\text{LFW} - \text{LDW}}{\text{LTW} - \text{LDW}} \times 100$$

The soil moisture content is related to its matrix potential values, that is, the tension with which water is retained in the soil. Matrix suction (kPa) was measured every 3 days in 18 plants, 6 per treatment (2 per block), using Watermark[®] Soil Moisture Meter sensors placed 10 cm deep.

Sample preparation

Six seedlings per watering regime, 2 per block were randomly selected ($n=6$) and 4 cm long stem portions were cut from the base of each stem and dried at 40 °C to constant weight. A stem fraction of 1.5 cm long was used for density and chemical analysis, while similar portion of wood was used for anatomical analysis.

Wood anatomical measurements

Wood sections were boiled during 2 h to rehydrate and stored in 3% sodium hypochlorite solution for one day. Transverse histological sections of 0.10 to 0.20 μm thick were cut using a sliding microtome (Leica SM 2000 R,

Leica Biosystems, Nussloch, Germany). Then they were stained with safranin and fast green solution at 1%. The samples were fixed between slide and cover object with Canada balsam and digital photographed with a camera (Olympus Evolt E-330 SLR) attached to an optical microscope (Leica®). Five digital photographs were taken at a magnification of 40× of the cross-section, covering the entire cut area of the treatment year. Measurements of the cell lumen, both in radial and tangential directions, and the wall thickness were taken on 20 cells per image, using the ImageJ® program (National Institutes of Health, free program). The protocols of the International Association of Wood Anatomists (IAWA 1989) were adopted for the anatomical description.

To estimate the lumen mean diameter (LD) in early and latewood tracheids we used the following equation:

$$LD = \frac{2 \times y}{(x + y)} (\mu\text{m})$$

where x and y are the radial and tangential diameters, respectively (Lewis and Boose 1995).

The tracheid reinforcement factor, in both early and latewood, as a safety measure against cell implosion due to stress, was estimated as:

$$\text{Tracheid reinforcement factor} = \left(2 \frac{tw}{dl}\right)^2$$

where tw is the cell wall thickness and dl the lumen diameter (Hacke and Sperry 2001).

The early and latewood percentage, lumen diameter and mean cell wall thickness was measured according to Fernández and Gyenge (2010).

To achieve a better understanding of the reaction of xylem development to water availability, a final test was performed with plants subjected to SWS followed by a period of irrigation to recover the level of field capacity.

X-ray densitometry analysis

For the analysis of WD properties, samples were glued on a wood support and cut transversely (1 mm thick) with a precision sectioning circular saw ISOMET 4000 (Buehler, IL, USA). Transverse wood slices were scanned and kept at 20 °C and 60% relative humidity until reaching a stable moisture content of 12% (~24 h). Then, the samples were scanned with a cellulose acetate calibration scale using an X-ray densitometry chamber (Faxitron MX20-DC12, Faxitron X-Ray, IL, USA; Quintilhan et al. 2021). Digital images (TIFF, 513 dpi) were analyzed with Windendro® program to obtain tree-ring density profiles with 0.017 mm step intervals, mean ring wood density (WD), and early-(WD_E), and latewood density (WD_L). The earlywood-latewood boundary was defined by the equation:

$ELb = WD_{max} + (X/100) \times (WD_{min} - WD_{max})$, using ring-specific minimum (WD_{min}) and maximum (WD_{max}) WD profile values to find the position of the ELb as a floating threshold for each tree ring (WINDENDRO Density 2017a® software, Regent Instruments Inc.). IADFs were defined based on the comparison of density profiles between earlywood and latewood with respect to the control plants.

X-ray fluorescence analysis

The samples were microtome-polished (Gärtner and Nievergelt 2010) in their transverse surface and analyzed following a line scan area of 30 μm polycapillary X-ray optic, 128 points, and steps of 0.03 mm. The microanalysis was performed in an X-ray fluorescence spectrometer (μXRF), Orbis PC model with EDAX (Ametek, New Jersey, USA), Rh tube, silicon drift chamber—SDD detector (30 kV, 600 μA), vacuum condition and 10 s/measurement point (dead time of 12%).

The line scan was built with the X-ray transmission intensity values (counts per second, cps). The selected elements were: P (K-alpha emission line of element [Kα] 2.01), a structural component of plant compounds (Peterson and Anderson 1990) and with a critical role in wood formation (Stark and Spitzner 1985); S (Kα 2.31), essential to define the cell-architecture (Fairchild et al. 2009); K (Kα 3.31), involved in the cambial activity related to cell expansion (Fromm 2010); Ca (Kα 3.69), involved in the cambial activity related to cell differentiation (Lautner and Fromm 2010); Mn (Kα 5.90), important growth-limiting factor in acid soils (Kogelmann and Sharpe 2006) and which could replace Ca in the xylem cation exchange sites (McClenahan et al. 1989; Peterson and Anderson 1990); and Fe (Kα 6.40) essential for plant growth (meristematic cell division), although highly reactive and toxic under acid ambient conditions (Stark and Spitzner 1985; Morrissey and Guerinot 2009). Furthermore, the molar ratio of K/Ca related to the wood formation (cell expansion/cell differentiation; Fromm 2010), Ca/Mn ratio, as early-warning signal of dieback (Hevia et al. 2019). S/Ca ratio, as an indicator of the interaction between the neutralization of cellular stress caused by free radicals (S effect) (Fairchild et al. 2009; Yadav 2010) and the promotion of cambial activity (Ca effect) (Lautner and Fromm 2010).

Statistical analysis

For soil moisture data, we compared different treatments with one-way ANOVA and Fisher's LSD tests ($p < 0.05$), previously checking data normality and homocedasticity. The leaf RWC was used to evaluate the plant water state and the differences between treatments was defined by a non-parametric Kruskal–Wallis test. Then, a pairwise

comparisons pos-hoc analysis was performed to evaluate differences between groups, as proposed by Lee and Lee (2018) for non-parametric analysis, since these variables did not meet assumptions of normality and homocedasticity.

The effects of water stress on WD were analyzed with a two-way ANOVA and means were compared by Fisher's test ($p \leq 0.05$). The normality assumption was checked using the Shapiro–Wilk test (H_0 =residuals normal) and the homogeneity of variance was performed by using Bartlett's test (H_0 =variances equal).

The following parameters were analyzed: ring width (RW, in mm), latewood proportion (LW, %), whole tree ring wood density (WD, $g\ cm^{-3}$), earlywood density (WD_{E_r} , $g\ cm^{-3}$), latewood density (WD_{L_r} , $g\ cm^{-3}$) and the average counts of P, S, Ca, K, Mn and Fe of each ring (cps). Furthermore, Spearman's rank correlation analysis was used to assess the agreement shared between tree-ring parameters. To integrate wood anatomical

parameters and wood chemicals measurements, a correlation was estimated using Pearson coefficient.

Results

Soil and plant moisture measurements

Soil water content can be visualized through matric potential (Ψ_m) and its deviations through the experiment days (Fig. 1 a). Differences were found between treatments, with SWS soils being the ones with the highest retention potential. Then, leaf RWC decreased significantly, as water stress increased: 80.95% in C, 45.25% in MWS, and 31.32% in SWS (Fig. 1 b). There were no significant differences due to leaf age for RWC.

Wood anatomical measurements

In C seedlings, a normal variation of WD between earlywood and latewood was observed (Additional file 1: Fig. S1a), with lower values in earlywood increasing towards the latewood. On the contrary, fluctuations of WD were

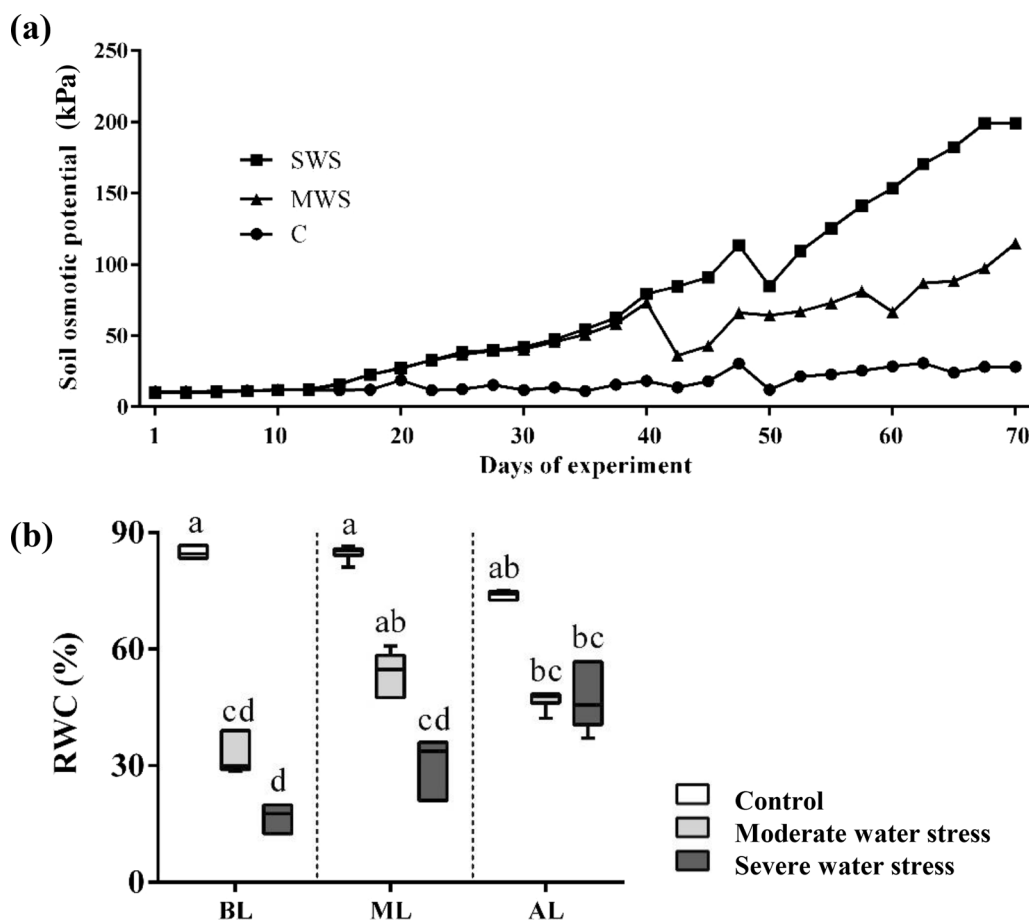


Fig. 1 Measurement of soil water content measured as osmotic potential (Ψ_o) in centibars for the three treatments, values obtained by Watermark® meter (a). Relative water content in leaves (RWC) for control (C; white bars), moderate (MWS; light grey bars), and severe (SWS; dark grey bars) water stress regimes ($n = 10$), for basal (BL), middle (ML), and apical leaf (AL) (b). Letters above individual boxplots indicate significant differences ($p < 0.05$), which were determined by post-hoc pairwise comparisons analysis of Kruskal–Wallis test results. Vertical broken lines separate the leaf type

found in MWS in the fifth tree ring of life, corresponding to the year's stress treatment. A greater increase in WD was observed at the beginning of latewood, reaching, later, a plateau with two lower peaks caused by irrigation events that allowed to reach field capacity level (Additional file 1: Fig. S1b). Under severe water stress conditions, two responses were observed: a stagnation in tree-ring growth and a sudden increase in WD (Additional file 1: Fig. S1c).

Plants under C treatment had wider tree rings than both stress treatments (38% lower in MWS and 53% in SWS), which was consistent for both early- and latewood. Similarly, plants under MWS produced WD similar to C plants, while those under SWS produced a 15% lower average of WD, a consistent response to earlywood (Table 1).

Plants under MWS treatment had two WD peaks, 16% and 37%, higher than the average WD_E , and differences were also evident between those peaks and its next immediate period of rehydration, with a reduction of 1% for the first peak, and 13% for the second (Fig. 2). Significant differences were observed in the lumen size and between the different treatments and wood type (earlywood and latewood) correlated to WD (Figs. 3, 4a and b). For earlywood lumen was 8% lower in MWS and 60% in SWS, and for latewood, lumen was 18% and 63% lower, respectively; however, cell wall thickness did not reach significant differences between treatments (Fig. 4c). The contribution to hydraulic conductivity on these cells with a reduced lumen diameter (5–7 μm), is considered negligible. In addition, a great difference was observed in the tracheid reinforcement factor, where the SWS treatment cells presented the highest values, being 40% higher than C and 28% than MWS (Fig. 4d).

Wood chemical measurements

The chemical element variability related to water stress treatment responded in a similar way both EW and LW (Additional file 1: Fig. S2, Figs. 5 and 6). According to

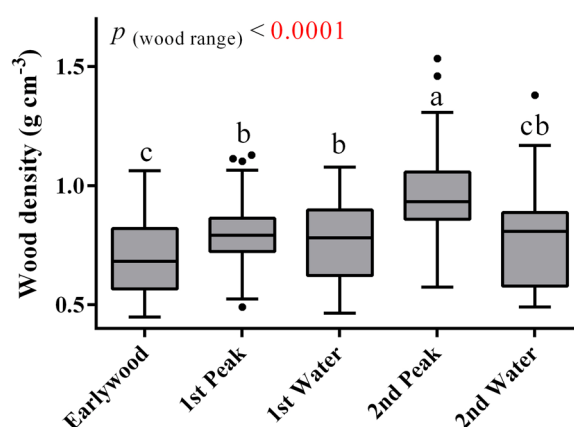


Fig. 2 Boxplot and Tukey whiskers representing the differences found in wood density (WD) only in moderate water stress (MWS) plants treatment. Difference between earlywood zone, first water-stress related wood-density peak, first water event, second water-stress related wood density peak, and the second water event zone. P : wood rank effect on WD ($p < 0.05$). Different letters indicate significant differences

Kruskal–Wallis, the explained variability for chemical elements was strongly variable depending on the considered element. Ca and P increased by 63% and 81% respectively, as water stress increases, with small differences between EW and LW (Fig. 5). The Ca concentration increased in both wood types as the stress level intensified, by 53% in MWS and 63% in SWS (Fig. 5a). In contrast, the P trend did not change significantly in EW between treatments, but did in LW, being MWS the lowest values of cps relative increase of P concentration (2.17, regarding the 3.2 value presented by SWS) (Fig. 5b).

On the other hand, K and S presented a negative trend for both wood types (Fig. 5c and d), excepted for S in LW where the lowest values of cps relative increase (30% lower than C) were in MWS treatments trees. Conversely, Mn followed the same pattern, with the lowest values in MWS (0.09 cps relative increase), but in this case the same for both early- and latewood, respectively

Table 1 Anatomical measurements of the 3 water stress treatments in *A. araucana* tree rings

	Control (C)	Moderate water stress (MWS)	Severe water stress (SWS)	$P_{(\text{treat})}$
Ring width (mm)	0.45 ± 0.03 a	0.28 ± 0.05 b	0.21 ± 0.03 b	< 0.0001
Earlywood width (mm)	0.33 ± 0.03 a	0.21 ± 0.04 b	0.14 ± 0.03 b	0.0001
Latewood width (mm)	0.13 ± 0.01 a	0.07 ± 0.02 b	0.07 ± 0.01 b	0.0149
Earlywood (%)	69.55 ± 4.32 a	78.71 ± 6.11 a	64.62 ± 4.32 a	0.1786
Wood density (g cm^{-3})	0.85 ± 0.03 a	0.85 ± 0.04 a	0.72 ± 0.03 b	0.0081
Earlywood density (g cm^{-3})	0.79 ± 0.03 a	0.80 ± 0.04 a	0.64 ± 0.03 b	0.0003
Latewood density (g cm^{-3})	0.96 ± 0.09 a	0.70 ± 0.12 a	0.73 ± 0.09 a	0.0996

The data presented correspond to mean values ($n=6$). $P_{(\text{treat})}$: effect of water stress, bold numbers indicates significant differences between regimes. The different letters indicate significant differences between treatments ($p \leq 0.05$)

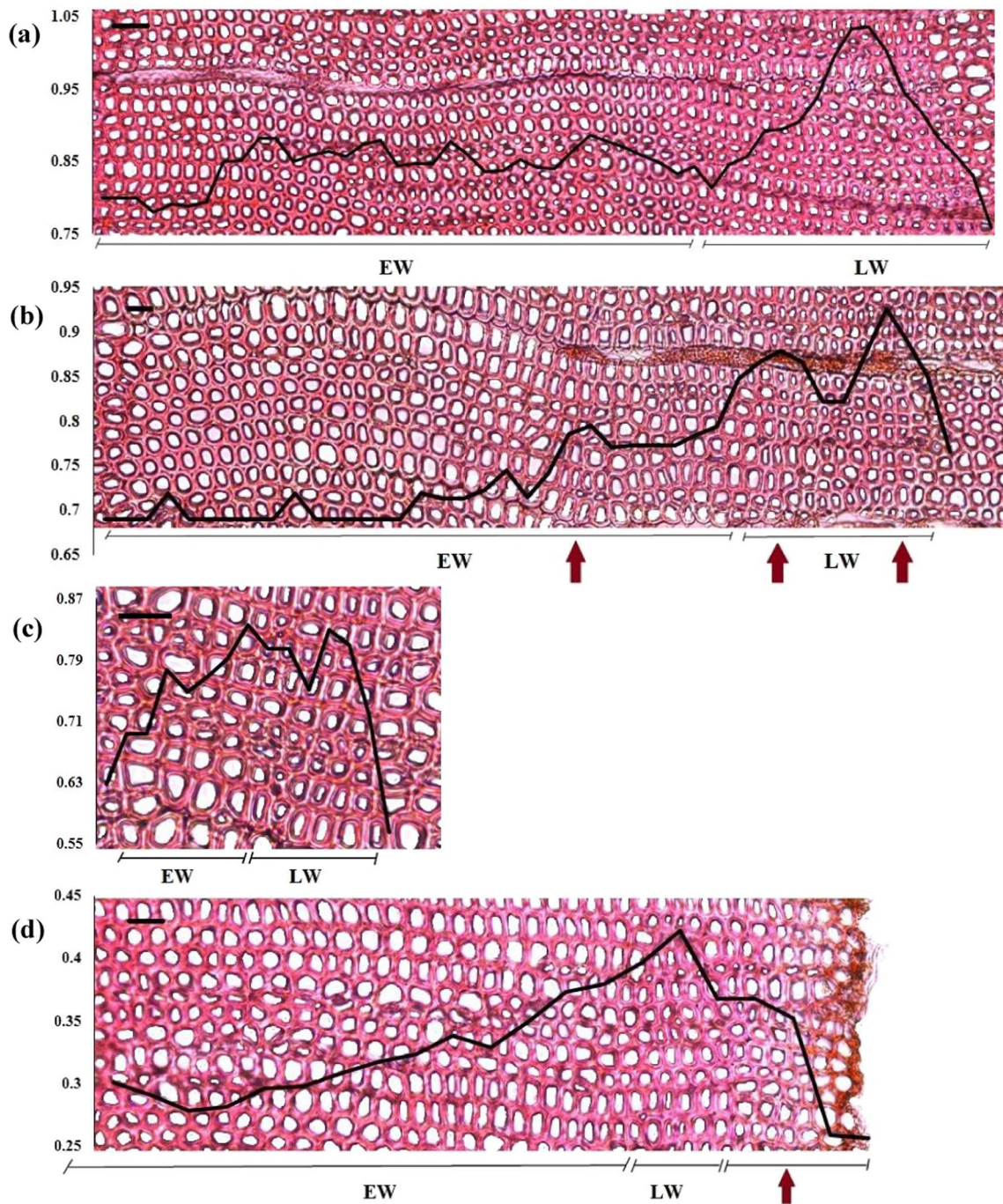


Fig. 3 Light microscope photographs showing transversal xylem views of safranin-stained histological wood sections of *A. araucana* seedlings corresponding to the ring formed during the year of the experiment, overlaid with the X-ray wood density (g cm^{-3}) graph. Tree ring development under control (a); moderate water stress, the arrows indicate the moments of stress (b); severe water stress (c); and severe water stress followed by an irrigation recuperation pulse, the arrow shows the xylem recovery (d). EW (earlywood); LW (latewood). The upper left line shows the scale (20 μm)

(Fig. 5e). Finally, Fe remained almost the same in its content between treatments, with no significant differences for earlywood and a lightly increasing trend in

MWS (Fig. 5f). The Kruskal–Wallis showed that most molar ratios of cps relative increases related to growth decreased while the stress raises (44% for S/Ca and 71%

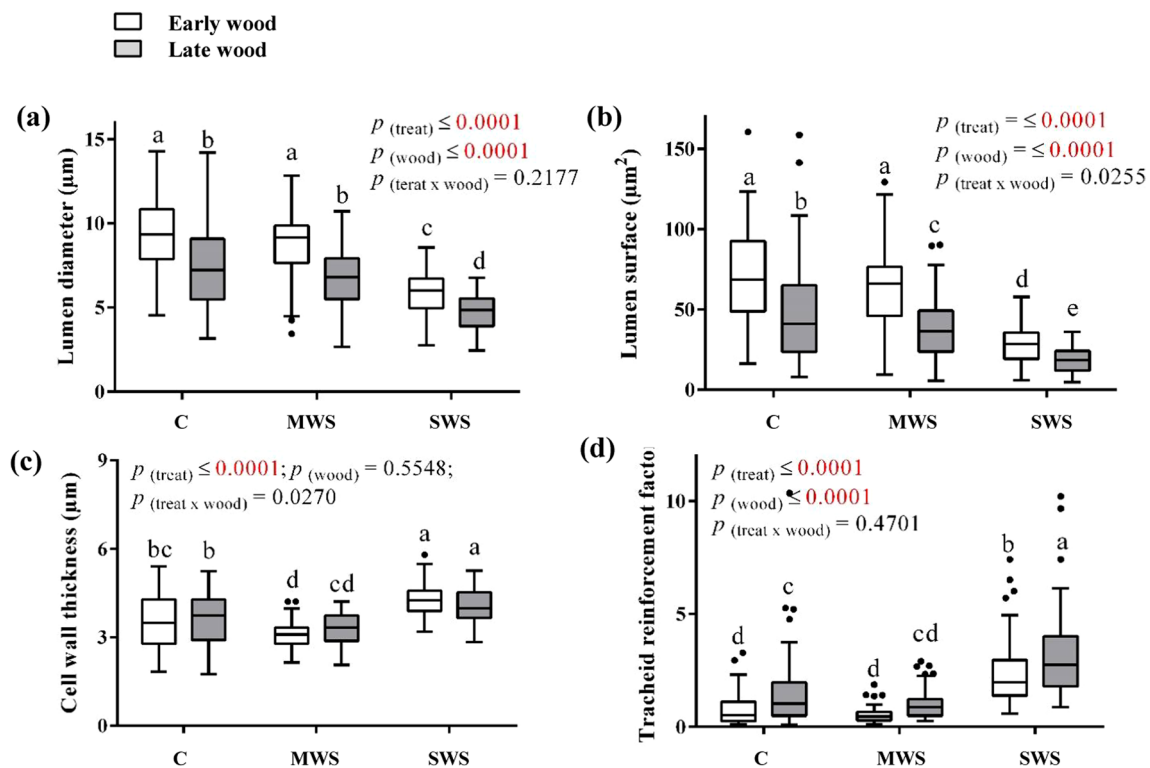


Fig. 4 Boxplot and Tukey whiskers explaining differences in xylem anatomical parameters of *A. araucana* seedlings: lumen diameter (a), lumen surface (b), cell wall thickness (c) and tracheid reinforcement factor (d). Those parameters related to different water stress treatments: control (C), moderate (MWS), and severe (SWS) water stress regimen ($n=10$); and wood: earlywood (white bars) and latewood (light grey bars). Letters above individual boxplots indicate significant differences ($p < 0.05$), determined by post-hoc pairwise comparison analysis of Kruskal–Wallis test results

for K/Ca; Fig. 6a and b); on the contrary, Ca/Mn ratio increased by 41% (Fig. 6c). Finally, no significant correlation was found between anatomical and chemical measurements (Table 2).

Discussion

Tree rings are an important source of retrospective information about tree growth, nutritional status, and environmental changes (De Micco et al. 2016; Mendivelso et al. 2016; Hevia et al. 2019; Ortega-Rodríguez et al. 2023). Our findings provide new information on the role of nutrient imbalances and variations in wood anatomy that underlie physiological responses to the incidence of water stress in *A. araucana* seedlings.

Wood anatomy and density responses to drought

A. araucana presented variations during xylogenesis depending on the water stress treatment (Additional file 1: Fig. S1, Fig. 4). Control plants showed a normal increase in WD between earlywood and latewood, while MWS plants exhibited temporal fluctuations in WD (IADFs). Increases in WD due to irrigation suppression periods were preceded by lower density wood segments

in response to two irrigation pulses carried out at field capacity (Additional file 1: Fig. S1, Fig. 3b). Then, the density values reached in both drought events were similar to latewood values and significantly different to WD_E (Fig. 2). Similar reactions have been identified in *Tectona grandis* earlywood, where dry conditions in spring followed by wet summers caused the formation of IADFs (Priya and Bhat 1998), establishing a positive relationship between the IADFs occurrence and the abundant water supply at the end of the growing season. Likewise, Masioskas and Villalba (2004) related abnormally hot and dry springs followed by hot and humid summers with IADFs in *Nothofagus pumilio* trees growing in temperate-cold climate regions of southern Patagonia.

It is known that tracheids with thin walls and large lumens, typical of earlywood portions of the tree ring, are more efficient for water conduction, while tracheids with a thicker wall and smaller lumen, present in latewood, are less efficient for transporting liquids (Campelo et al. 2007; Copenheaver et al. 2006; Battipaglia et al. 2014). Smaller lumen size is associated with physiological responses that avoid exposing the transport tissues to cavitation processes. When the conductive tissue experiences extreme

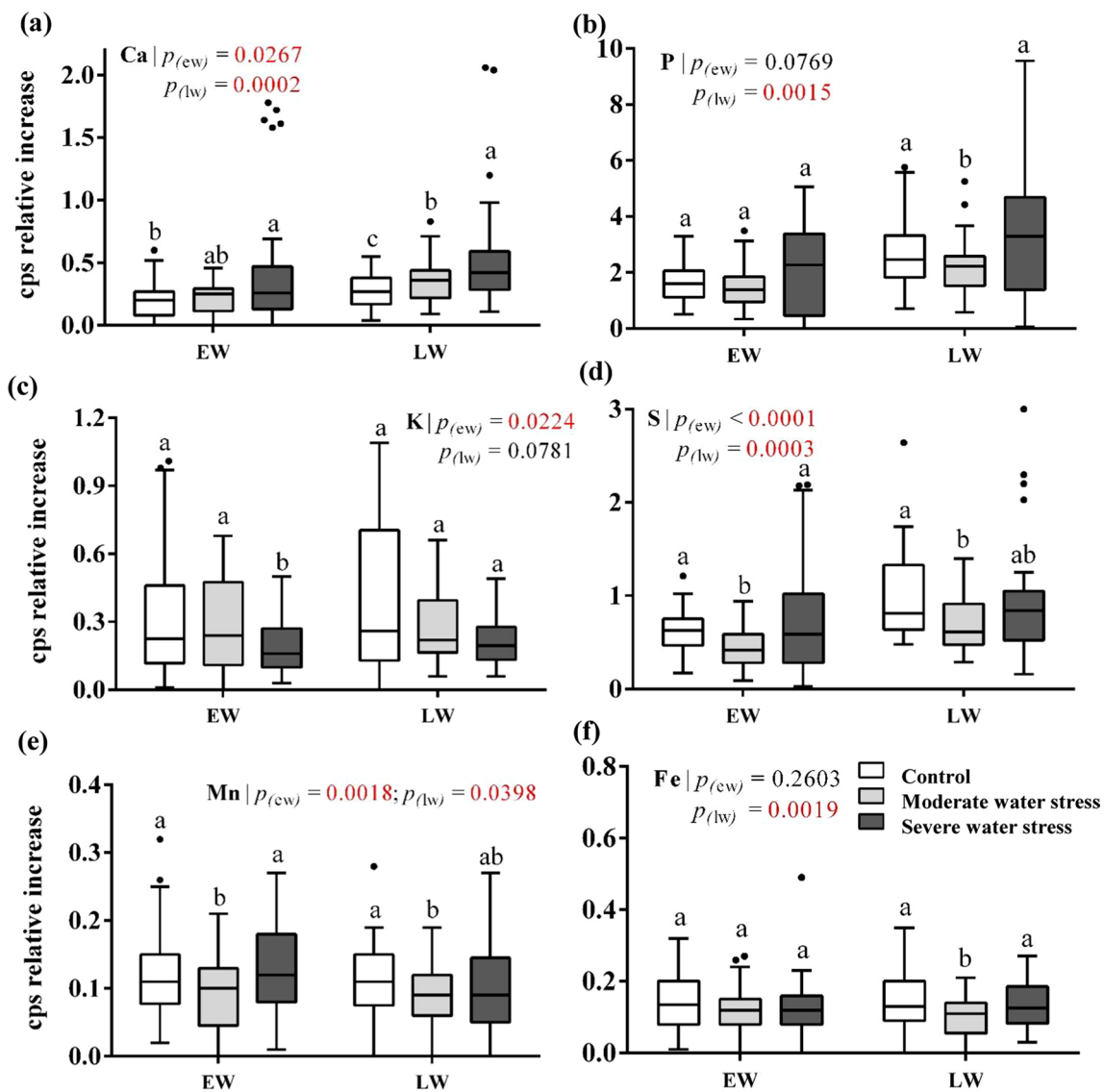


Fig. 5 Boxplot and Tukey whiskers explaining differences in wood chemistry relative increases of counts per second measurements of *A. araucana* seedlings xylem: calcium (Ca; **a**), phosphorus (P; **b**), potassium (K; **c**), sulfur (S; **d**), manganese (Mn; **e**) and iron (Fe; **f**). Those parameters associated to different water stress treatments: control (C, white bars), moderate (MWS, light grey bars), and severe (SWS, dark grey bars) water stress regimes ($n = 10$) and type wood, earlywood (EW) and latewood (LW). Letters above individual boxplots indicate significant differences ($p < 0.05$), determined by post-hoc pairwise comparisons analysis of Kruskal–Wallis test results

negative pressure conditions, caused for example by water stress, tracheids may be at risk of cavitation. This causes not only a percentage reduction of the conductive xylem, but also a risk of the hydraulic system collapse, with the consequent premature death of roots, trunk and branches (Domec and Gartner 2002). In this sense, *A. araucana* expresses this behavior through the development of latewood tracheids with a thick wall and reduced lumen (Fig. 3a), reaching a greater tolerance to extreme decreases in water potential and maintaining a minimum level of hydraulic conductivity (Bouriaud et al. 2005).

Therefore, it is possible to observe thick-walled tracheid bands in earlywood, conforming IADFs (Campelo et al. 2007; Copenheaver et al. 2006; Battipaglia et al. 2014). In this sense, our study evidenced anatomical responses to water stress, likewise other trees such as *Picea abies* (L.) Karst. (Bouriaud et al. 2005), *Quercus ilex* L. (Zhang and Romane 1991), and *Pinus taeda* L. (Cregg et al. 1986).

According to Table 1, the WD was significantly lower in individuals with SWS treatment than in C ones. In turn, the tree rings formed during the year of water stress treatment had a lower WD than the previous ones without

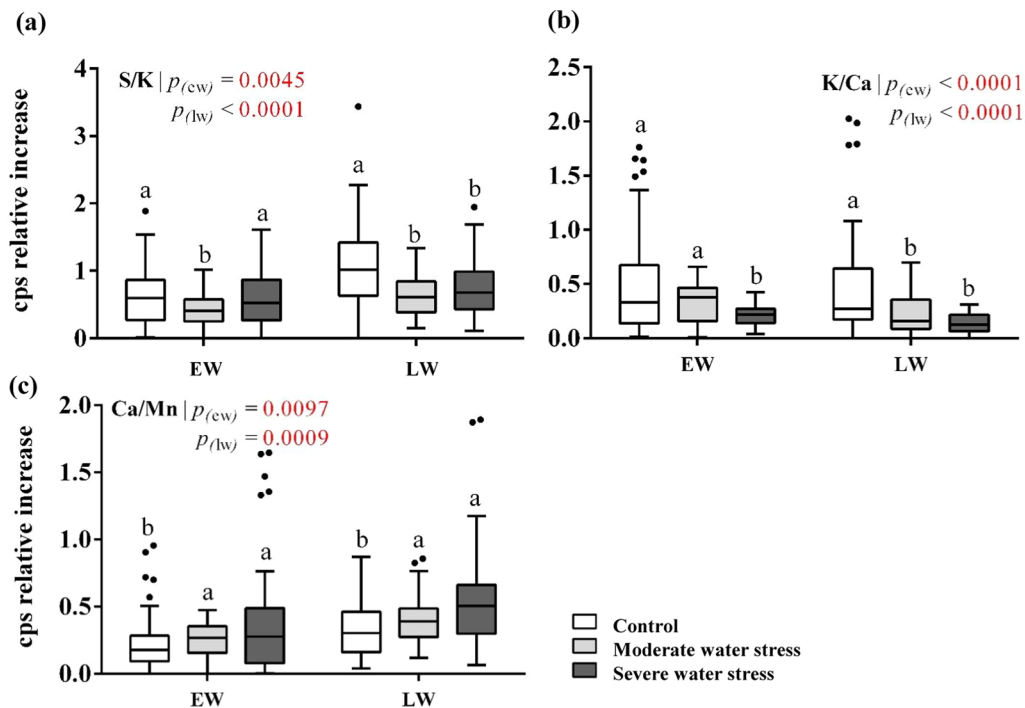


Fig. 6 Boxplot and Tukey whiskers explaining differences in wood chemistry relative increases of cps ratios of *A. araucana* seedlings xylem: sulfur potassium ratio (S/K; **a**), potassium calcium ratio (K/Ca; **b**) and calcium manganese ratio (Ca/Mn; **c**). Those parameters associated to different water stress treatments: control (C, white bars), moderate (MWS, light grey bars), and severe (SWS, dark grey bars) water stress regimes ($n = 10$) and type of wood, earlywood (EW) and latewood (LW), respectively. Letters above individual boxplots indicate significant differences ($p < 0.05$), determined by post-hoc pairwise comparisons analysis of Kruskal–Wallis test results

Table 2 Pearson correlation between anatomical and chemical measurements in *A. araucana* tree rings

	Wood density	Tracheid reinforcement factor	Cell wall thickness	Lumen diameter	Lumen surface
P (cps relative increase)	0.19**	0.06	0.00	-0.06	-0.05
S (cps relative increase)	0.21**	0.04	0.02	-0.04	-0.04
K (cps relative increase)	0.37**	-0.19**	-0.26**	0.29**	0.32**
Ca (cps relative increase)	0.06	0.18**	0.10	-0.20**	-0.19**
Mn (cps relative increase)	-0.17**	0.03	0.06	-0.03	-0.03
Fe (cps relative increase)	-0.19**	-0.05	-0.02	0.04	0.04

The data presented correspond Pearson coefficient (r) ($n = 332$) **. Significant at 1 percent probability level

stress. These results are contradictory to the findings in most tree species, where the WD is higher as water stress increases (Trifilò et al. 2015). An explanation could lie in the fact that the growth of most SWS seedlings stopped abruptly due to a persistent stomatal closure caused by water deficiency. This could have caused a decreased carbohydrate accumulation due to a decrease in the CO_2 flow to the cellulose biosynthesis centers. Therefore, a lower amount of carbohydrates focused on the formation of the tracheid cell wall and meristem dehydration,

resulted in a stoppage in wood production, and in consequence a poor devolvement of latewood with its corresponding high WD. Consequently, the whole tree ring WD falls regarding the previous years and the C treatment (Eilmann et al. 2009; Fig. 3c: 6 cells rows). If this process remains over time, the seedling would reduce its ability to conduct water to the photosynthetic reaction centers, weakening their physiological performance and, ultimately, increasing the risk of death. However, after a recovery irrigation applied, and with temperatures

still appropriate to stimulate cell reproduction, the plant rehydration allowed the resumption of earlywood tracheid formation (Fig. 3d). Consequently, plants subjected to SWS followed by a period with recovered field capacity, produced a zone of higher WD at the middle of the tree ring, commonly recognized as IADFs.

Tree ring width was also affected, indicating reduction accumulated biomass of tree growth. Eilmann et al. (2009) revealed a decrease in total radial increments of *Pinus sylvestris* under water stress conditions. The same result was found in several studies (Bigler et al. 2006; Weber et al. 2007) and also in *A. araucana*, plants under stress resulted in a less developed growth ring, or showed a strong reduction of the latewood portion (Table 1). With these results it is possible to assume that *A. araucana* experiences rapid responses to environmental stimuli (Papú et al. 2021), reducing significantly tree ring width ($p < 0.0001$), caused principally by a lower growth and a smaller lumen size. Then, wood cell anatomy analysis identified significant differences in lumen diameter and surface, being lower in SWS treatment plants for both earlywood and latewood zones (Fig. 4). The reduction in lumen diameter could indicate, newly, an adaptation to avoid cavitation under dry conditions, as observed in several studies (Jenkins 1974; Nicholls and Waring 1977; Sheriff and Whitehead 1984; Sterck et al. 2008). However, the results are not as expected for cell wall thickness, being lower in SWS treatment plants. Nevertheless, the tracheid reinforcement factor was significantly higher, evidencing a mechanism to actively reinforce the tracheids to prevent embolism (Hacke and Sperry 2001).

Changes in wood nutrients related to water stress

Relatively few dendrochemistry studies report historical nutrient uptake changes associated to climatic variables (Watmough 1997; Poussart et al. 2006; Ortega Rodriguez et al. 2018; Hevia et al. 2019). As found in some tree species, like *Pinus taeda* and *P. sylvestris*, an inter-annual synchronization of several chemical elements indicates a common link to environmental fluctuations (Ortega Rodriguez et al. 2018), a fact that was also identified in *A. araucana* (Additional file 1: Fig. S2, Figs. 5 and 6). Low precipitation may decrease the bio-availability of nutrients (St. Clair et al. 2008; Hevia et al. 2019) and the uptake by the plant-roots (Robson and Pitman 1983; Ortega Rodriguez et al. 2023). A long-term effect of water stress triggers a reduction in hydraulic conductivity, which could alter the plant nutritional status, given the importance of water flow along the soil-plant continuum for nutrient uptake. Moreover, it has been argued that the tree hydraulic properties are modulated

by changes in xylem sap cation concentrations (Hevia et al. 2019).

In most studies, high concentration of Ca in tree-rings, as a structural component of the cell wall, has been associated with rainfall increases (Poussart et al. 2006; Ortega-Rodriguez et al. 2018; Hevia et al. 2019). According to our results, Ca increased significantly as water stress increases, with small differences between EW and LW (Fig. 5a). We have accomplished two possible explanations for this discrepancy. On the one hand, Ca is involved in lignin polymerization within the cell wall, affecting the division and differentiation of cambial cells during xylem development (Fromm 2010; Lautner and Fromm 2010; Ortega-Rodriguez et al. 2022). Then, a thicker cell wall implies more ionic Ca binding sites, so a higher concentration is expected at the end of latewood (Fromm 2010). The same occurs with WD peaks observed in the IADFs formed in MWS treatments (Fig. 2) and on the last portions of latewood in SWS treatments. This lignification increases WD magnified in thicker cell wall, which in turn is associated with a high concentration of Ca (Ortega-Rodriguez et al. 2022). But, these results contradicted decrease in WD of SWS tree rings. This could be indicating a stoppage in wood production, and in consequence a poor devolvement of latewood with its corresponding low WD, even though the cells have thick walls with higher concentrations of Ca (Eilmann et al. 2009; Fig. 3c).

Mn in stems is mainly distributed in the xylem cell wall, being a residual Mn reservoir during the transport process intended to leaf formation (Ortega-Rodriguez et al. 2022). Therefore, a higher concentration is expected in well-watered plants, due to a sizeable leaf formation, as occurs in control plants, contrary to the reduction observed in MWS (Fig. 5e). In the same sense, Mn is essential for cell wall elongation (Hevia et al. 2019), so the decrease in MWS could cause reduced expressions of earlywood and latewood widths in *A. araucana* seedlings (Table 1).

Most studies indicated an opposite intra-annual Ca and Mn concentration (e.g. Pearson et al. 2009; Hevia et al. 2019), possible due to its lower susceptibility to leaching and in consequence interfering in Ca uptake sites in root (Ortega-Rodriguez et al. 2022), as observed in *A. araucana* under MWS treatment (Table 1 and Fig. 5a, e). Ca concentration increased significantly respect to C treatment, whereas Mn decreases. Then, drought affects the nutrient balance in the wood as a response to hydraulic failures, in which Mn and Ca imbalance and their ratios (Ca/Mn) can indicate early signs of forest dieback. The increase of Ca/Mn ratio in *A. araucana* tree-rings as the water stress increases (Fig. 6c) can be a response signal to avoid imminent tree death. Hevia et al. (2019)

observed that *Abies alba* and *Pinus sylvestris* trees in growth decline due to extreme droughts presented lower Ca/Mn values, associated with lower earlywood density values. Furthermore, according to Kogelmann and Sharpe (2006), higher concentrations of Mn in the sapwood and leaves have been considered predisposing or contributing factors to forest dieback. This may explain why we found higher Mn values in the EW of seedlings with increasing water stress (Fig. 5e).

In *A. araucana* tree-rings, cps relative increase of K and S declined significantly as water stress increases (Fig. 5c, d). This is consistent with other studies where tree ring concentrations of K (essential for cambium activity) and S (essential to define the cell-architecture) are associated with rainfall increases (Poussart et al. 2006; Ortega-Rodriguez et al. 2018; Hevia et al. 2019). Wind et al. (2004) observed that K content in *P. tremula* cambium tissue was higher where wood formation was more active. K plays an important osmotic role during cambial activity affecting cell expansion, cambial zone width, and vessel size during the development of xylem (Ortega-Rodriguez et al. 2022). Therefore, its decrease during stress events, as occurs in MWS and SWS treatments, could influence the decline of earlywood and latewood tree-ring widths (Fig. 5c and Table 1). Then, the K/Ca ratio decreased under the EW and LW stress conditions (Fig. 6b), which could be linked to a reduction in cambium activity. The relation between cambium activity and high K/Ca has been found in *Sequoia sempervirens* (Boulay 2012) and *Pinus taeda* (Ortega-Rodriguez et al. 2022).

The same pattern is followed by S, an element stored mostly in primary cell walls where proteins define the cell architecture (Ortega-Rodriguez et al. 2022). In consequence, its decrease could decline the early and latewood tree-ring widths in *A. araucana* (Fig. 5d). In addition, S is the constituent of several compounds that directly or indirectly take part in ameliorating the adverse effects of different types of biotic and abiotic stresses. Therefore, the reduction in S shown in *A. araucana* seedling under waters stress, could compromise plant resistance to waters stress. Then, a reduction in S/Ca ratio (Fig. 6a) could indicate that *A. araucana* inverts resources on cell wall structural components to avoid cavitation by sulfur-containing compounds.

Finally, the water deficit could decrease the basipetal leaf-xylem S mobility and in consequence the xylem storage observed in LW tree rings of *A. araucana* (Fig. 5d; Ihsan et al. 2019; Ortega-Rodriguez et al. 2023). These cellular spaces left by S could be occupied by Mn, increasing its values (Fig. 5e), when acropetal mobility occurs and could generate toxicity due to the decrease in water-soluble nonprotein thiols (-SH, functional group), which become defense mechanisms when cellular Mn

concentrations increase (Neves et al. 2017; Ortega-Rodriguez et al. 2023).

P did not yield conclusive results, since no significant differences were found on EW, but a decrease was found on latewood MWS plant treatments with respect to C and SWS (Fig. 5b). A reduction in soil water content induced a mobility decrease, since P moves primarily by diffusion through pores filled with water. By decreasing the water flow and P, the meristematic activity decreases, interrupting the formation of apical and cambial growth. Low P availability can also alter its re-translocation from the meristematic regions to the phloem and xylem parenchyma cells during the senescence period (Gutiérrez-Boem and Thomas 1998; Ortega-Rodriguez et al. 2023), a process that could be taking place in plants under MWS.

Despite the lack of water, plants maintain a good P proportion for growth maintenance in its early stages in all treatments for EW, allowing *A. araucana* to prioritize wood formation (Stark and Spitzner 1985). However, the regulation of P transport will depend on the species adaptations of vascular structures (Ortega-Rodriguez et al. 2023).

Conclusions

Signals from anatomical-densitometric wood traits and cell wall chemical compounds in response to differential water stress conditions could be used as early warning signals of impending tree mortality. Moderate and severe water stress treatments triggered responses in *A. araucana* seedlings that promoted resilience and adaptation under drought-like conditions. *A. araucana* develop fluctuations in wood density (IADFs) and reduced lumen of tracheids in responses to water stress. However, in cases of more severe stress, tracheid production could be reduced or even stop wood development, reducing its ability to conduct water. Therefore, it is expected that drought affects wood nutrient balance as a response to hydraulic failures, in which Mn (low) and Ca (high) and Ca/Mn ratio (high) may be early adaptative signals of *A. araucana* to avoid dieback as water stress increases. Higher Ca values were related to thicker cell walls, while lower deposition of Mn (essential for cell elongation) in xylem during the transport process was associated to a narrow tree ring. The decreases of K, S and the K/Ca ratio result in reductions of the tree-ring widths, since K has an osmotic role during cambial activity and S-proteins define cell architecture. Moreover, a reduction in S/Ca ratio suggests that *A. araucana* inverts resources on cell wall structural components to avoid cavitation. WD represents a proxy to understand the volume of wood produced in each growth ring, whereas wood chemical elements are proxies of combined nutritional and physiological processes, affecting the inter- and intra-annual

tree-ring variability. In summary, the variables investigated are useful to suspect causes of mortality and particular functional responses during drought events, as global climate models project to be more frequent and intense in the twenty-first century. Extreme and persistent droughts could push *A. araucana* to the edge of their functional hydraulic limits, causing accelerated reductions in growth and wide-spreading forest dieback, as seen in forests around the world.

Abbreviations

C	Control
Ca	Calcium
cps	Counts per second
dl	Lumen diameter
EW	Earlywood
WD _E	Earlywood density
Fe	Iron
IADFs	Intra-annual density fluctuations
K	Potassium
LD	Lumen mean
LDW	Leaf dry weight
LFW	Leaf fresh weight
LTW	Leaf turgor weight
LW	Latewood
WD _L	Latewood density
WD _{max}	Maximum wood density
WD _{min}	Minimum wood density
Mn	Manganese
MWS	Moderate water stress
N	Nitrogen
P	Phosphorus
RW	Ring width
RWC	Relative water content
SWS	Severe water stress
tw	Cell wall thickness
WD	Wood density

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-024-00519-3>.

Additional file 1: Fig. S1. X-ray images of trunk cross-section of 5-year-old *A. araucana* seedlings subjected to different water stress treatments, control (a), moderate water stress (b) and severe water stress (c). The images are under the same magnification. Wood density is expressed in g cm^{-3} . The red line represents the density reading line. Black triangles specify the edge of the tree rings, and red triangles indicate the irrigation pulses as proposed in the protocol. The experiment was carried out for 120 days, during the 5th year of the seedling's life. Scale: 1 mm. **Fig. S2.** RX images of *A. araucana* wood cross-section showing the chemical profiles (in counts per second, cps) of calcium (Ca, yellow), potassium (K, green), manganese (Mn, light blue), sulfur (S, pink), phosphorus (P, light green) and iron (Fe, orange). Scale bars in microscopic images correspond to 0.7 mm. The line scan area using 30 μm wide X-ray optic is represented by a red line.

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Author contributions

PS and RFA conceived and designed the experiments. The experiment execution and samples collection was performed by PS. Wood chemistry and wood density laboratory analysis were carried out by O-RRD, wood anatomy analysis by PS, RFA and NF. Statistical analysis was performed by PS, O-RDR and RFA. The first draft of the manuscript was written by PS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no financial or non-financial conflict of interest.

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