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No carbon shortage in declining trees of the isohydric species *Araucaria araucana* (Molina) K. Koch under drought

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Abstract

Key message: At the sixth and seventh years of a drought event in south of Chile, non-structural carbohydrate (NSC) concentrations were similar between healthy and unhealthy trees of *Araucaria araucana* (Molina) K. Koch, and growth did not decrease, suggesting that leaf loss prevented C shortage in unhealthy trees.

Context: Tree drought resistance and resilience may be impaired by decreasing growth and non-structural carbohydrates (NSC). During a 7-year drought, the isohydric species *Araucaria araucana* (Araucariaceae) evidenced decline (foliage loss and browning).

Aims: To determine whether tree decline was related to an impaired carbon status and reduced growth.

Methods: In two sites of southern Chile, we selected healthy- and unhealthy-looking trees to study drought effects on NSC and growth. We measured the basal area increment (BAI) and NSC concentrations of needles and roots after 6 years of drought (2016) and following one less severe year in terms of drought (2017).

Results: At both years, healthy and unhealthy trees had similar NSC and sugar concentrations in needle and roots, and furthermore, they maintained their growth rates. In 2017, NSC, starch, and sugar concentrations of needles (but not roots) increased in both healthy and unhealthy trees at one of the study sites, while growth did not vary.

Conclusion: Unhealthy trees likely prevented C shortage through an acclimation mechanism such as foliage loss. The remarkable similar NSC concentrations found between healthy and unhealthy trees indicates the absence of C starvation in trees that lost a substantial fraction of their foliage under drought.

Keywords: Climate change, Isohydric, Monkey puzzle tree, Soluble sugars, Starch

1 Introduction

Global changes including increasing temperature and decreasing precipitation are altering the performance of tree species, causing reduced tree growth, forest decline, and massive events of tree mortality worldwide (e.g., Anderegg et al. 2012; Camarero et al. 2015; Fajardo et al. 2019a; Camarero et al. 2020; Miranda et al. 2020). Drought-induced decreased tree performance and potential mortality are particularly dramatic for long-lived species that have a marginal distribution, as they generally have low genetic variation and long generational times which hamper adaptation to rapid climatic changes (Savolainen et al. 2007). For such species, it is critical to know whether and how drought events impact their performance.

The level of carbon (C) reserves at drought, largely represented by non-structural carbohydrates (NSC) (Chapin et al.

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1990), may be important for drought resistance and subsequent recovery in trees (Sala et al. 2012; Martínez-Vilalta et al. 2016). The pool of NSC has the potential to mitigate drought stress since it is a source of osmotic compounds, which increase the cell water potential and thus prevent reductions in the cell turgor (Arndt et al. 2001; Körner 2015; Ravi et al. 2021). Thus, trees under drought conditions commonly exhibit higher sugar concentrations than their counterparts under more humid conditions (Piper et al. 2017; Schönbeck et al. 2018; Fajardo and Piper 2021). Furthermore, seedlings experimentally enriched with NSC are more drought resistant, have greater sugar concentrations, and show better hydraulic functioning under drought than non-enriched counterparts (O'Brien et al. 2014; Ravi et al. 2021; Sapes et al. 2021). Likewise, trees that reduce their vigor during drought generally exhibit lower sugar concentrations than their healthier counterparts (Klein et al. 2014; Camarero et al. 2015). At the interspecific level, more drought-tolerant tropical tree species are able to increase leaf sugar concentrations in response to drought, while drought-sensitive species are not (Signori-Müller et al. 2021). Also, tree species inhabiting biomes recurrently affected by drought (e.g., Mediterranean) show high sugar concentrations in the dry period (Martínez-Vilalta et al. 2016). Additionally, NSC concentrations at the time of drought influence the tree recovery once drought is over. For example, re-foliation and the reversal of xylem embolism require carbohydrates as source of energy, structures, and osmotic compounds (Galiano et al. 2011; Tomasella et al. 2019; Tomasella et al. 2021).

One general consequence of drought is the reduction of growth (Klein et al. 2014; Piper et al. 2017). The magnitude of this reduction is used as an indicator of drought resistance, i.e., the ability of individuals to maintain their functioning during drought (Lloret et al. 2011; Gazol et al. 2018; Bose et al. 2020; Gessler et al. 2020; Bottero et al. 2021), and in some cases, it can predict the survival/mortality response of trees (Cailleret et al. 2017; Rodríguez-Catón et al. 2019). Drought causes growth decline because the process of tissue formation (i.e., mitosis, cell differentiation, cell elongation) requires sufficient turgor (Muller et al. 2011; Körner 2015). Because photosynthesis is less drought-sensitive than growth, tree C reserves can increase under moderate dry conditions whereas growth has already stopped (Körner 2003; Muller et al. 2011; Zweifel et al. 2021). Nonetheless, if drought prolongs, C reserves will also decrease as a result of their continued use to cover metabolic demands, potentially leading to C depletion and subsequent death (McDowell et al. 2008; Galiano et al. 2011). Trees typically also exhibit defoliation (foliage loss or browning) after prolonged drought (Galiano et al. 2011; Klein et al. 2014; Lloret et al. 2018; Schönbeck et al. 2018; Vélez et al. 2020). This response might represent an acclimation mechanism to cope with drought by which trees improve their C balance, e.g., if dead leaves had already a negative C balance or reduced photosynthetic capacity before their death. Drought, either acting alone or along with pathogens, may reduce the tree photosynthetic capacity by limiting stomatal conductance and leaf turgor, and causing photodamage (Damour et al. 2009). Foliage loss may negatively feedback on the tree C status by limiting the C gain at periods of drought release, thus aggravating and prolonging the NSC depletion and limiting future growth (Galiano et al. 2011; Schönbeck et al. 2018). However, defoliation may also represent an acclimation response to drought that improves the water balance by reducing the transpiratory leaf area (Martínez-Vilalta et al. 2009; Papú et al. 2021). As a result, the remaining leaf area is expected to experience less stomatal limitations and higher photoassimilation. This mechanism could explain the similar NSC concentrations between defoliated and undefoliated trees reported in some studies (Anderegg et al. 2012; Camarero et al. 2016; Colangelo et al. 2017). Alternatively, similar NSC concentrations between defoliated and undefoliated trees could result if defoliated trees favor the formation of C reserves over growth (Piper et al. 2015).

Besides stomatal closure and defoliation, other factors may influence the level of C reserves at drought. High growth rates before drought have been found to reduce the resistance and the physiological resilience to droughts in terms of survival and cambial growth (Martínez-Vilalta et al. 2012; Bose et al. 2020). Although the mechanisms behind this pattern remain unclear, it is possible that fast growth implies a greater reliance on C remobilization (Piper 2021), restricting the capacity of trees to face drought with adequate NSC levels. In addition, species that avoid severe variations in plant water potential through acute stomatal regulation (i.e., isohydric species) are expected to be particularly prone to experience reduced levels of C reserves by drought (McDowell et al. 2008). Gymnosperm species show higher hydraulic safety margins than angiosperms (Choat et al. 2012) and are more vulnerable to deplete their NSCs under drought (Adams et al. 2017). However, gymnosperm species themselves may greatly differ in their hydraulic strategies, with some taxa being relatively more anisohydric (e.g., *Juniperus*) than others (e.g., Pinaceae and Araucariaceae species) (McDowell et al. 2008; Brodribb et al. 2014). Two families, Araucariaceae and Pinaceae, stand out at the extreme of fast stomatal closure due to an ABA-mediated highly efficient regulation, which means that species of these families are potentially the most prone to C depletion (Brodribb et al. 2014). While mechanisms of tree decline have extensively been investigated in Pinaceae (Galiano et al. 2011; Klein et al. 2014; Aguade et al. 2015), they remain much less explored in species of Araucariaceae (Vélez et al. 2020).

Araucaria araucana (Molina) K. Koch, also known as monkey puzzle tree, is a long-lived and slow-growing tree species endemic to Chile and Argentina with a very restricted distribution, between 37° and 40° S in the southern Andes and the Coastal mountain range (Veblen et al. 1995). Extensive logging and human-set fires have reduced the species distribution (i.e., it is currently considered as an endangered species by the IUCN Red List, Premoli et al. 2013), and it is placed in the list of the 10 conifer species with the highest risk of extinction worldwide (Forest et al. 2018). *Araucaria araucana* populations are genetically isolated (Ruiz et al. 2007; Sanguinetti 2014), which may contribute to a reduced capacity to adapt to novel climatic conditions (Premoli et al. 2013). For the 2010-2018 period, an unprecedented megadrought affected southern Chile and Argentina (Garreaud 2018; Garreaud et al. 2020), causing tree decline and mortality in some *A. araucana* populations (Saavedra and Willhite 2017; Medina et al. 2018; Puchi et al. 2021). It has been hypothesized that pathogens have contributed to the decline (Pérez et al. 2018; Vélez et al. 2020), although the direct effects of drought on *A. araucana* have not been investigated. In addition, it is unknown what the consequences of decline are in terms of C reserves and growth. Here, we examined the performance of *Araucaria araucana* affected by drought through the study of growth and C reserves in drought-induced declining and non-declining trees. We aimed at determining (i) whether decline was associated to C shortage and low growth, i.e., unhealthy trees have lower NSC concentrations and growth than healthy ones, (ii) whether NSC concentrations, growth, or both change with time in response to a long drought, and (iii) whether pre-drought growth had an influence on the tree C status several years into the drought period. To accomplish all these, we measured the needle and root NSC concentrations after 6 and 7 years of drought and the basal area growth for the drought and pre-drought period in healthy and unhealthy trees of *A. araucana*.

2 Material and methods

2.1 Species sites

The sampling was conducted in Conguillío National Park in Southern Chile (38° 40′ S, 71° 39′ W), an area where *A. araucana* is the dominant tree species. Occasionally, species of the *Nothofagus* genus, particularly *N. pumilio*, appear as co-dominants. The mean annual temperature in the area is 9.5 °C, and the mean annual precipitation for the period 1990–2009 is 2202 mm (Fig. 1). However, from 2010 to 2018, a continued and severe precipitation deficit along with high temperatures was recorded in central Chile and the influence of this anomaly, called megadrought, reached the south of the country (Garreaud et al. 2017; Garreaud et al. 2020). As a result, the annual rain deficit in the study area reached − 25% on average during the 2010–2017's period, relative to the 1990–2009's historical period (Tricauco weather station, 38° 50′ 38″ S, 71° 33′ 05″ W, 520 m.a.s.l.; Dirección General de Aguas). Climatic water balance was, accordingly, 1648 and 1023 mm for the 1990-2009's and for the 2010-2017's periods, respectively. Likewise, the average precipitation from September to March for the period 2010-2017 was − 21% of that between 1990 and 2009, while temperature was 3 °C higher and evapotranspiration was 62 mm higher (Fig. 1). As a result, the growing season's climatic water balance was 318 mm and 90 mm for the 1990–2010's and for the 2010-2017's periods, respectively. However, the September 2016 to March 2017's growing season was relatively less dry and warm than the previous season; precipitation and mean temperature from September 2016 to March 2017 were 609 mm and 13.6 °C, respectively, while from September 2015 to March 2016, they were 506 mm and 15.1 °C, respectively (Fig. 1). Accordingly, the mean monthly evapotranspiration and climatic water balance were, respectively, 54 and 97 mm for the period September 2015–March 2016 and 51 and 145 mm for September 2016–March 2017.

Given that a significant variation in the tree response to drought has been found to depend on site features (Bose et al. 2020; Camarero et al. 2020), particularly for *Araucaria araucana* (Puchi et al. 2021), we worked with two locations. The first site was located on the west slope of Llaima volcano (site Los Paraguas, 38° 40′ 48″ S, 71° 51′ 01″ W, 1059 m.a.s.l.), whereas the second location was in the Conguillío lake area (site Lake Area, 38° 38′ 42″ S, 71° 37′ 47″ W, 1141 m.a.s.l.). The soil in both sites is volcanic and well drained. Given the short distance (15 km) that separates the two sites, we assume there are no major climatic differences between the two locations except for approximately 0.5 °C lower temperatures at the 100 m higher site.

2.2 Sampling and sample processing

In 2016, we identified 14 trees at the Lake Area site and 11 trees at Los Paraguas site, which were subsequently categorized as healthy and unhealthy (Table 2 in the Appendix). Unhealthy trees were defined as those with 50–70% damaged crown (i.e., brown and/or defoliated across leaf cohorts), whereas healthy trees where those trees without any evidence of crown damage. All selected trees were sun-exposed, and they were 2–10 m tall (Table 2 in the Appendix). In addition, selected trees were separated by a minimum of 10 m from one another. Tissue sampling for NSC concentrations in trees was carried out in November 2016 (i.e., mid-spring), after 6 years of drought, and also in November 2017, following one less severe year in terms of drought (Fig. 1). The sampling date was selected to represent the peak of growth and carbohydrate demand in evergreen

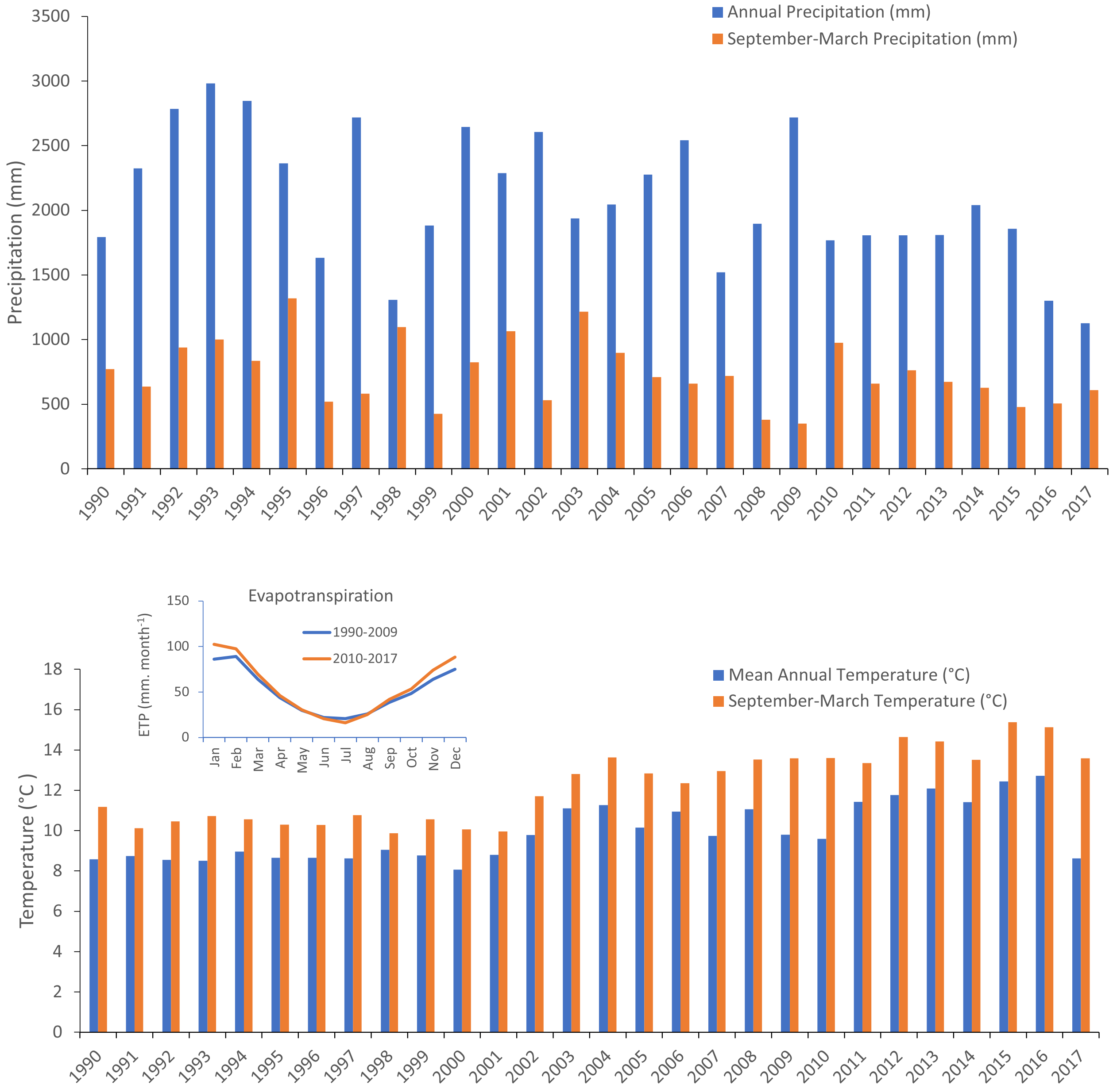

Fig. 1 Precipitation and temperature for the study area, Conguillío National Park area (Tricauco weather station 38° 50′ 38″ S, 71° 33′ 05″ W, 520 m.a.s.l.; Dirección General de Aguas, 1990–2017). The precipitation is quoted as the sum of mean monthly values for the annual and growing season (01 September–01 March) periods. The temperature is quoted as the average of mean monthly values for the annual and growing season (01 September–01 March) periods. The inset shows the corrected potential evapotranspiration (ETP) (Thornthwaite 1948)

species (Löiez and Piper 2021), which is when healthy and unhealthy trees could show differences in the C status and growth. During the second sampling date, one to-the-pith long stem core at breast height (1.35 m) was sampled for tree growth determination using a 5.15-mm increment bore (Haglöf, Långsele, Sweden). In order to have a more precise representation of the growth trends and the C status, we increased the number of sampled trees in 2017 by including new individuals of each category at both study sites, in addition to those sampled in 2016 (Table 2 in the Appendix). Coarse roots and needles were sampled between 10:00 and 16:00 h at a single point in time. For each individual tree and sampling date, one terminal, *c.* 1 m long, fully expanded sun-exposed branch was identified and cut using (when necessary) a 5.6-m telescopic pole (ARS Corporation, Sakai, Japan). From this branch, leaves of all cohorts were sampled for chemical analyses, except for unhealthy trees, where we sampled green leaves only. To sample root tissue, the root of the target tree was dug and identified and then cut a *c.* 1 cm-diameter piece; its bark was then removed in the field with a knife. Leaves and roots were all bagged, labeled, and stored in a cooler for transportation, while increment cores were placed in labeled straws for future tree growth determination. Samples for NSCs determination were heated in a microwave oven at 600 W for 90 s to denaturalize enzyme. In the laboratory, all samples for chemical analyses were placed to dry in a forced-air stove (Memmert GmbH, Schwabach, Germany) at 65 °C for 72 h, and then ground into a fine powder and stored under cool, dry, and dark conditions, until chemical analyses were conducted. In addition, we measured tree height and diameter at breast height (DBH, 1.35 m) of each individual using a clinometer and a diameter tape, respectively.

2.3 Chemical analyses

The assessment of C reserves was based on the determination of NSC, as the sum of the three most abundant low molecular-weight soluble sugars (glucose, fructose and sucrose; *hereafter* sugars) and starch. NSC concentrations were analyzed in all samples following the procedure of Hoch et al. (2002). About 13 mg of dried powder were extracted with 1.6 ml of distilled water at 100 °C for 60 min. An aliquot of the extract was used to determine low molecular carbohydrates after enzymatic conversion (invertase and phosphoglucose isomerase from *Saccharomyces cerevisiae*, Sigma Aldrich I4504 and P5381, respectively, St. Louis, MO, USA) of sucrose and fructose to glucose. The concentration of free glucose was determined photometrically after the enzymatic conversion of glucose to gluconat-6-phosphate (Glucose Assay Reagent, G3293 Sigma Aldrich, St. Louis, MO, USA) on a 96-well multiplate reader. Following the degradation of starch to glucose using a purified fungal amylase (“amiloglucosydase” from *Aspergillus niger*, Sigma Aldrich 10115, St. Louis, MO, USA) at 45 °C overnight, NSC was determined in a separate analysis. The starch concentration was calculated as NSCs minus the sum of free sugars. Total sugar, starch, and NSC concentrations are presented on a percent of dry matter basis.

2.4 Tree growth determination

Cores were prepared following standard dendrochronological techniques (Stokes and Smiley 1996). In brief, cores were dried, mounted, glued firmly on grooved wooden sticks, and sanded with successively finer grades of sandpaper until optimal surface resolution allowed the annual rings to be distinguished under magnification (10-fold). Inside-bark bole radius and annual radial increments from 2001 were measured to the nearest 0.01 mm using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system (Bloomfield, NY, USA). Cross-dating accuracy was checked visually since rings of *A. araucana* are easily identified. We calculated annual basal area increment (BAI), which represents a relative measure of gains in growth. BAI was estimated as:

$BAI = \pi (R^2_t - R^2_{t-1})$,

where R_t is the radius of the stem at year t, and R_{t-1} is the radius of the stem of year t − 1. The radius of the stem, R_t, was computed as the distance between the pith and the t year’s ring in the core. Each BAI value is referred to the year of tree formation. Thus, BAI for 2016 represents the BAI of the relatively less dry year, as for convention for the southern hemisphere the tree ring starts its formation in the spring of the previous years (Schulman 1956), in this case, spring of 2016.

We quantified the predrought BAI as the mean annual BAI for the rings formed between 2001 and 2009, and the BAI at drought time as the mean BAI for the rings formed between 2010 and 2016 (the latest corresponding to spring 2016–autumn 2017). Resistance was calculated for all trees of both sites as in Lloret et al. (2011). The resistance index quantifies the ratio between BAI at drought and predrought BAI, representing thus the capacity of the trees to buffer the stress and maintain growth during drought.

2.5 Data analysis

We used linear models to test for the influence of years, health status, and the interaction between them, on the concentrations of NSC, starch, and sugars. Analyses were performed for each site and tissue (needles and roots) separately. We also used linear models to test for the influence of health status on pre-drought BAI, BAI at drought, and resistance, and for the influences of year, health status, and the interaction between both, on the annual basal area increment for the whole period (2001-2016). Again, these models were run separately for each site. All linear models included tree height as a covariate. Values are presented as averages ± SE. All analyses were run with JMP 14.0 (SAS Institute, Cary, NC, USA).

3 Results

3.1 Carbohydrate concentrations

3.1.1 Needles

NSC and starch concentrations in needles were similar between healthy and unhealthy trees at both study sites in 2016 and 2017. The concentrations of NSC, starch, and sugar in needles at Los Paraguas site were significantly higher in 2017 (moderate drought) than in 2016 (severe drought) (Fig. 2). At the Lake Area, NSC and starch concentrations were similar between years, while sugar concentrations were higher in 2017. For both sites, sugar concentrations were similar between healthy and unhealthy trees in 2016 and higher for healthy than for unhealthy trees in 2017, although, only at Lake Area, this difference turned out to be statistically significant (i.e., significant year * health status interaction, Table 1, Fig.2). Also, at the Lake Area site, tree height related negatively and significantly to the concentrations of NSC and sugars in needles (Table 1).

3.1.2 Roots

NSC and sugar concentrations in roots were similar between healthy and unhealthy trees in both sites and for both years (Fig. 2, Table 1). The concentration of starch in roots across years was also similar between health statuses at Los Paraguas site but significantly higher in healthy than in unhealthy trees at the Lake Area site. At this site, soluble sugar concentrations in roots decreased in 2017 in unhealthy trees, but this decrease was marginally significant (P = 0.056 for the year * health status interaction, Table 1).

3.1.3 BAI and tree-drought resistance

At Lake Area, BAI was higher in healthy than unhealthy trees for the predrought period ($F_{1,\ 143}$ = 45.17, $P < 0.0001$) and afterwards ($F_{1,\ 111}$ = 46.6, $P < 0.001$) (Figs. 3 and 4). Both health statuses exhibited a similar temporal dynamic with the highest BAI in 2010. This BAI value, however, was only significantly different to the BAI of 2001 ($P < 0.0001$, Tukey test). As a result, healthy and unhealthy trees had similar drought resistance ($F_{1,\ 15}$ = 1.11, P = 0.310) (Fig. 4). At Los Paraguas, mean pre-drought BAI was higher in unhealthy than healthy trees ($F_{1,\ 179}$ = 6.55, P = 0.011) (Figs. 3 and 4); however, healthy trees had significantly higher BAI during drought ($F_{1,\ 139}$ = 3.80, P = 0.053) and higher drought resistance than unhealthy trees ($F_{1,\ 19}$ = 5.13, P = 0.036) (Fig. 4). This result was mostly driven by the

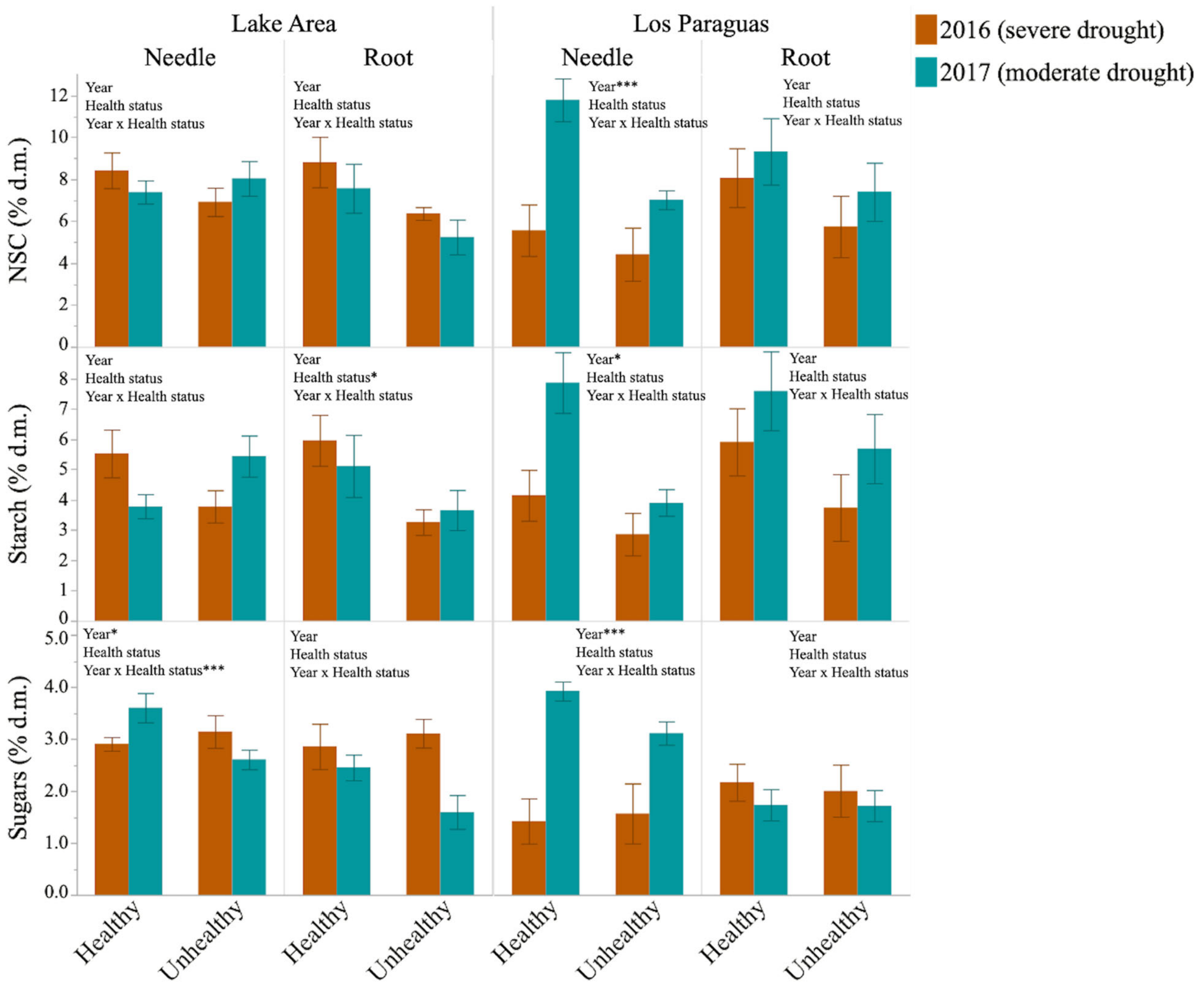

Fig. 2 Total non-structural carbohydrates (NSC), starch, and soluble sugars (SS) concentrations in needle and root of healthy and unhealthy *Araucaria araucana* trees during the spring of a year with severe drought (2016) and a year of moderate drought (2017). Insets show the statistical significance of year, health status, and the interaction between them on the concentrations. The lack of asterisk stands for non-significant effect of the factor, while *, **, and *** stands for significant effects at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

Table 1 Results of linear models examining the effects of the year, tree health status, the interaction of them, and tree height on leaf and root concentrations of non-structural carbohydrates (NSC), starch, and sugars in *Araucaria araucana* during a dry (2016) and a relatively less dry year (2017). *F* stands for *F*-ratios, subindices stand for the degree of freedom of the numerator and the denominator, respectively, and number in parentheses show *P*-values

	NSC_{needle}	$starch_{needle}$	$Sugar_{needle}$	NSC_{root}	$starch_{root}$	$Sugar_{root}$
Lake Area						
Year	$F_{1, 33}$ = 2.19 (0.149)	$F_{1, 33}$ = 0.85 (0.365)	$F_{1, 33}$ = 4.82 (**0.036**)	$F_{1, 33}$ = 0.65 (0.427)	$F_{1,33}$ = 0.26 (0.611)	$F_{1, 33}$ = 1.59 (0.218)
Health status	$F_{1, 33}$ = 0.25 (0.619)	$F_{1, 33}$ = 0.39 (0.538)	$F_{1, 33}$ = 0.01 (0.927)	$F_{1,33}$ = 3.62 (0.068)	$F_{1, 33}$ = 5.05 **(0.033)**	$F_{1, 33}$ = 0.05 (0.821)
Year * status	$F_{1,33}$ = 0.033 (0.857)	$F_{1, 33}$ = 2.67 (0.113)	$F_{1, 33}$ = 14.60 **(< 0.001)**	$F_{1, 33}$ = 0.05 (0.944)	$F_{1, 33}$ = 0.70 (0.411)	$F_{1, 33}$ = 3.99 (0.056)
Height	$F_{1, 33}$ = 4.35 **(0.046)**	$F_{1, 33}$ = 1.96 (0.172)	$F_{1, 33}$ = 7.88 (**0.009**)	$F_{1, 33}$ = 0.00 (0.969)	$F_{1, 33}$ = 0.24 (0.627)	$F_{1, 33}$ = 1.40 (0.246)
Los Paraguas						
Year	$F_{1, 30}$ = 15.00 **(0.007)**	$F_{1, 30}$ = 6.92 **(0.014)**	$F_{1, 30}$ = 25.25 **(< 0.001)**	$F_{1, 30}$ = 1.66 (0.209)	$F_{1,30}$ = 2.75 (0.109)	$F_{1, 30}$ = 0.08 (0.783)
Health status	$F_{1, 30}$ = 3.11 (0.089)	$F_{1, 30}$ = 2.91 (0.100)	$F_{1, 30}$ = 1.02 (0.321)	$F_{1,30}$ = 0.00 (0.981)	$F_{1, 30}$ = 0.10 (0.759)	$F_{1, 30}$ = 1.02 (0.321)
Year * status	$F_{1, 30}$ = 2.60 (0.119)	$F_{1, 30}$ = 1.94 (0.176)	$F_{1, 30}$ = 1.80 (0.192)	$F_{1, 30}$ = 0.05 (0.819)	$F_{1, 30}$ = 0.36 (0.851)	$F_{1, 30}$ = 0.11 (0.740)
Height	$F_{1, 30}$ = 0.13 (0.721)	$F_{1, 30}$ = 0.39 (0.536)	$F_{1, 30}$ = 0.246 (0.624)	$F_{1, 30}$ = 1.95 (0.174)	$F_{1, 30}$ = 1.47 (0.235)	$F_{1, 30}$ = 3.22 (0.085)

increasing growth rates in healthy trees during drought, along with the rather constant growth rates in unhealthy trees (Figs. 3 and 4).

4 Discussion

4.1 Is tree decline associated with C shortage and lower growth?

Overall, healthy and unhealthy trees had similar needle and root NSC concentrations at both years, 2016 and 2017. Although in one of the study sites, Los Paraguas, unhealthy trees had significantly lower root starch concentration than healthy trees, the levels were far from a complete depletion. In addition, growth was not reduced during drought in any health category. Thus, our study suggests that defoliation (foliage loss and browning), a main characteristic defining drought-induced unhealthy trees, was generally not associated with C shortage. This is an unexpected result, given that members of the family Araucariaceae are among the most isohydric species on Earth (Brodribb et al. 2014), and are thus expected to respond to drought through a tight stomatal regulation and a concomitant use of C reserves to meet metabolic demands (McDowell et al. 2008). Accordingly, other studies have found that declining trees of Pinaceae species, which are also relatively isohydric species, do have lower NSC concentrations than healthier counterparts in the same organs analyzed by our study (Klein et al. 2014; Aguade et al. 2015). However, similar C status between trees of differing health status or defoliation degree has been found in other species as well, suggesting that causes other than insufficient C availability provoked defoliation. For example, healthy and unhealthy ramets of trembling aspen in an area severely affected by drought had similar starch concentrations across organs, and the health status was rather explained by hydraulic failure, phloem impairment, or inaccessibility to C stores (Anderegg et al. 2012). Any of these mechanisms could explain our results. Also, defoliation itself could be an acclimation response to drought that may prevent C shortage by reducing the leaf area and hence transpiration, improving the water balance and preventing stomatal closure (Martínez-Vilalta et al. 2009). In fact, seedlings of *A. araucana* acclimated to restricted water regimes through a reduction in leaf area and mass (Papú et al. 2021), supporting the idea that defoliation was indeed an acclimation response to drought in the adult trees examined by our study. Defoliation might have also prevented C shortage by reducing the proportion of leaves with low photosynthetic capacity due to photodamage or stomatal limitations. Those leaves represent a higher metabolic cost, and the tree C balance consequently improves when they are removed (Oikawa et al. 2008; Reich et al. 2009). By improving the water and C balance during drought, defoliation likely permitted the maintenance of growth and metabolic demands without the need of remobilization of stored C. Finally, unhealthy trees had lower growth than healthy trees during the dry years, suggesting that the maintenance of NSC concentrations at levels similar to those of healthy trees could be explained by limitations to invest photoassimilates (C sink limitation) in unhealthy trees (Zweifel et al. 2016).

The fact that growth was not reduced during drought is unexpected to say the least, given the high sensitivity of growth to drought (Muller et al. 2011). Moreover, there was even a trend of increasing

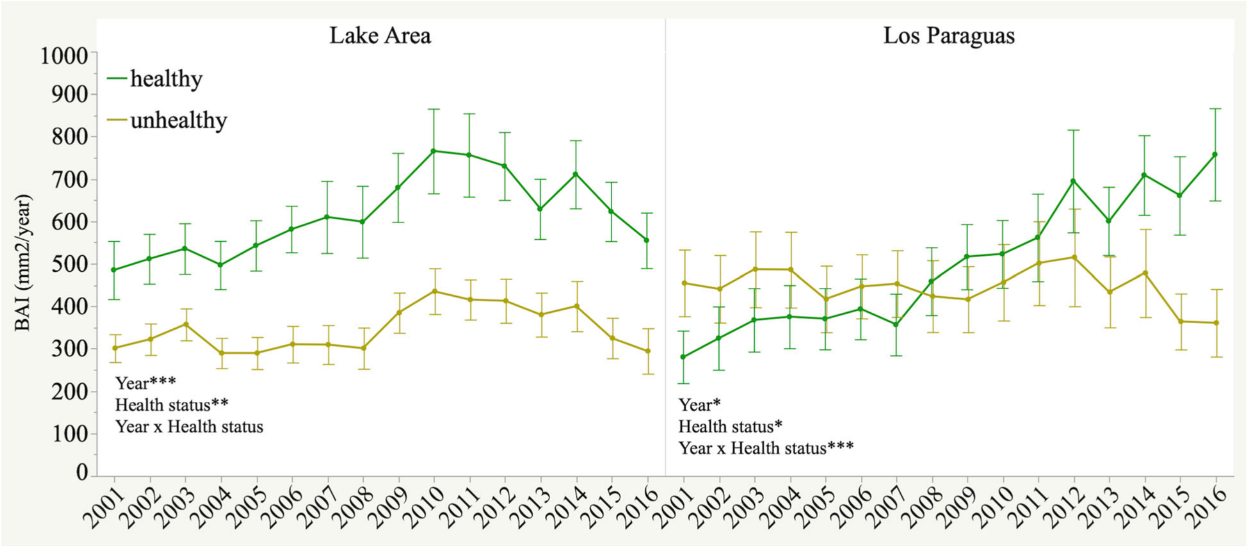

Fig. 3 Annual basal area increment (BAI) during the period 2001–2016 of healthy and unhealthy *Araucaria araucana* trees sampled in November 2017 in Conguillío National Park, Chile. Asterisks stand for statistically significant differences in BAI between healthy and unhealthy trees, for the specified years

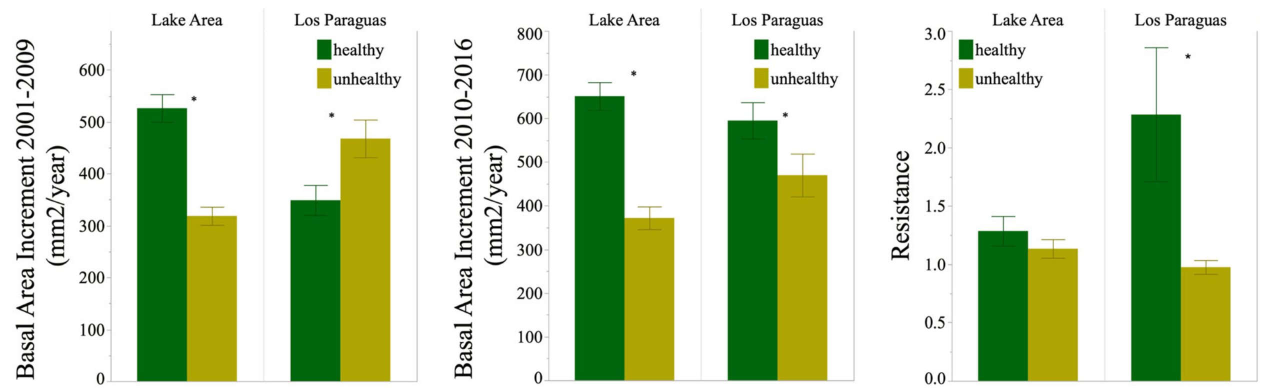

Fig. 4 Mean basal area increment for the periods 2001–2009 (pre-drought) and 2010–2016 (drought), and drought resistance calculated as Lloret et al. (2011) and Bose et al. (2020), of healthy and unhealthy *Araucaria araucana* trees sampled in November 2017 in Conguillío National Park, Chile

growth rates in healthy trees during drought (Fig. 4). This result is in strong contrast with studies in other regions, which have found systematic significant decreases in radial growth in response to moderate or severe drought (Gazol et al. 2018; Camarero et al. 2020; Bottero et al. 2021). However, the maintenance of the radial growth under the precipitation deficit that affected southern Chile between 2010 and 2017 seems to be more the rule than the exception. Fajardo and Piper (2021) found that across a precipitation gradient in Patagonia, neither wide- nor narrow-niche breath angiosperm tree species decreased its BAI as a consequence of the most drastic drought event in the last 70 years. In the same line, Urrutia-Jalabert et al. (2020) found that the decrease in radial growth of *Fitzroya cupressoides* (Cupressaceae) under the same megadrought occurred in only one of two study sites, and it was a rather small decrease. As suggested by these two previous studies, it is possible that the water demands for growth in *A. araucana* are low, given the inherent slow growth of this species. Thus, the precipitation reduction could have been not large enough to ultimately restrict growth. Indeed, it is important to note that the term megadrought was defined for central Chile, where mean precipitation deficits were of 70%. However, for southern Chile, where our study was performed, deficits were less severe (c. 10–30%). Still, in line with our study, a recent study in *Araucaria araucana* reports defoliation associated with precipitation reductions and temperature increases of similar magnitude to those occurred in the area of our study (Puchi et al. 2021). More importantly, and also in line with our results, Puchi et al. (2021) did not find a significant reduction in radial growth during the megadrought, neither for healthy nor for unhealthy trees. Together, these findings support the idea of defoliation as an acclimation response of drought-avoidance, which warrants the maintenance of the water balance through a reduction of the transpiratory area. On the other hand, the health decline observed during the drought could reflect the effect of factors other than drought, but indirectly promoted by drought (McDowell et al. 2008). Indeed, defoliation and leaf desiccation have been suggested to result from phloem impairment driven by pathogen infection (Pérez et al. 2018; Vélez et al. 2020). Lower starch root concentrations in unhealthy than in healthy trees at Lake Area are compatible with phloem impairment. Regardless the cause of defoliation, our study shows that defoliation and leaf browning do not cause reductions in NSC concentrations at spring. However, it remains possible that NSC reductions had been more evident at other times of the year (Klein et al. 2016), and in other tissues (Piper and Paula 2020).

4.2 Were NSC concentrations and growth lower during a year of severe drought than during a year of less severe, moderate drought?

Radial growth and NSC concentrations were generally similar between 2016 and 2017. Specifically, concentrations of soluble sugars in needles of healthy trees at one of the sites (Lake Area) and concentrations of NSC, starch, and sugar in needles of both healthy and unhealthy trees at the other site (Los Paraguas) were higher in 2017 than in 2016. However, NSC, starch, and soluble sugar concentrations in roots were statistically similar between years at Los Paraguas. Also, NSC, starch, and soluble sugar concentrations in both tissues at the Lake Area site showed no significant changes from 2016 to 2017. Overall, these results suggest a lack of C depletion after 6 years of drought in comparison to 2017, when drought was moderate. Most probably, the climatic difference between 2016 and 2017 was insufficient to drive consistent physiological differences between sites in *Araucaria*. Although we did not compare tree responses between sites, it is remarkable that in 2016 the concentrations of the different carbohydrate pools were higher at the Lake Area site than at Los Paraguas site. We thus suggest that increased NSC, starch, and sugar concentrations at Los Paraguas site reflected C replenishment under the relatively more mesic climatic conditions of 2017. By contrast, at the Lake Area site, the C status of trees might have been less affected or transitorily affected. It has been found that some species tend to achieve homeostatic NSC levels after a long period of drought such as the one examined in this study (Schönbeck et al. 2018). The lack of change in the NSC concentrations from 2016 to 2017 at the Lake Area site could be explained by homeostasis in NSC concentrations. Alternatively, the local soil and topographic conditions of this site could have determined less severe drought effects than at Los Paraguas site. Increasing needle soluble sugar concentrations in 2017 relative to 2016, as observed in Los Paraguas, support a role of soluble sugars in the avoidance of tissue desiccation and damage through osmoregulation and osmoprotection during drought (Arndt et al. 2001). Similarly, soluble sugar concentrations in needles of *Pinus sylvestris* were significantly higher for trees deprived of irrigation than for their irrigated counterparts (Schönbeck et al. 2018). Also, several angiosperm tree species increased their soluble sugar concentrations in twigs following a punctual severe drought event in Patagonia (Fajardo and Piper 2021).

Although a reduced leaf area may limit water stress during and after droughts and may favor resilience if it leads to improved stomatal conductance and higher photosynthesis in the remaining leaves, it may also hamper carbohydrate reserves buildup and may lead to C depletion and starvation (McDowell et al. 2008). Other studies have found that reduced leaf area limit the C gain, and in turn the growth recovery, during rainy periods (Galiano et al. 2011; Schönbeck et al. 2018). In one of our study sites, Los Paraguas, reduced leaf area presumably allowed unhealthy trees to recover their C reserves similarly as healthy trees. Here, it seems that the higher leaf area and vigor of healthy trees allowed them to fulfill C demands of both storage and growth. In the same study site, unhealthy trees were less resistant to drought. Most probably, reduced leaf area was again a factor contributing to this result. Although C reserves remobilization fuel growth at early season, a greater proportion of seasonal radial growth might have been supported from current photoassimilation.

4.3 Did pre-drought growth influence the C status?

Our results showed that higher pre-drought BAI was not associated with an impaired C status (e.g., reduced NSC or starch concentrations). For instance, we found that unhealthy trees had higher pre-drought BAI than healthy ones at Los Paraguas, but NSCs concentrations were similar between health statuses at this site. Nonetheless, unhealthy trees had significantly lower resistance at this site. These results suggest that high growth rates before drought did not restrict the capacity of trees to face drought with adequate levels of C reserves but may constrain growth at drought, as found by other studies (Martínez-Vilalta et al. 2012; Bose et al. 2020). On the other hand, we found that the concentrations of NSC and soluble sugars in needles were negatively correlated with tree height at one of our study sites (Lake Area). This result may imply that tree size increases the vulnerability to drought by reducing the pool of C reserves that can be potentially remobilized at drought. In fact, unhealthy trees were taller than healthy trees, which supports the idea that taller trees were more drought-affected. The NSC pool, however, results from NSC concentrations scaled by biomass, thus even though NSC concentrations were lower in taller trees, their pool was probably still higher. However, even slight decreases in the NSC pool may have still an impact in the tree performance. This is likely because taller trees have higher maintenance costs associated with hydraulic integrity (Sala et al. 2012) and are more prone to suffer hydraulic embolism than shorter trees because of the higher occurrence of wider hydraulic conduits (Olson et al. 2018; Fajardo et al. 2019b).

5 Conclusion

In long-lived species, as *Araucaria araucana*, drought may reduce fitness at a faster pace than the species' ability to respond to it (Quintero and Wiens 2013). It might be that *A. araucana* cannot follow the pace of climate change. Our study shows that a long and continued drought period in southern Chile did not severely decrease the C status or growth in *A. araucana*. More importantly, our results strongly point to defoliation and leaf browning as an acclimation mechanism, rather than as a consequence of impaired C status.

Tree decline can be also caused by pathogens acting alone or co-occurring with drought, which may exacerbate C shortage (McDowell et al. 2008; Aguade et al. 2015), or to cause themselves C shortage (Li et al. 2019). Indeed, tree decline and defoliation in *A. araucana* under the influence of the recent drought have been suggested to result from pathogen infection (Saavedra and Willhite 2017; Medina et al. 2018). While the pathogen infection was not examined by our study, we can assert that regardless of this possibility decline was not related to impaired C status.

6 Appendix

Table 2 Tree height (in m, as mean ± standard error) for the sites included in this study. Last column shows statistical results for the comparison of tree height between healthy and unhealthy trees within each site and year. Subindices stand for degree of freedom for the numerator and denominator, respectively

Site	*n*	year	Health status	Height (mean ± standard error)	*F*-ratio (*P*-value)
Lake Area	7	2016	Healthy	3.00 ± 0.31	
Lake Area	7	2016	Unhealthy	5.86 ± 0.46	$F_{1,25} = 50.17$ ($P < 0.0001$)
Lake Area	10	2017	Healthy	6.80 ± 0.47	
Lake Area	10	2017	Unhealthy	6.65 ± 0.41	$F_{1,39} = 0.73$ ($P = 0.7290$)
Los Paraguas	4	2016	Healthy	2.80 ± 0.32	
Los Paraguas	7	2016	Unhealthy	4.80 ± 0.53	$F_{1,21} = 15.22$ ($P = 0.0009$)
Los Paraguas	11	2017	Healthy	3.50 ± 0.38	
Los Paraguas	9	2017	Unhealthy	5.90 ± 0.40	$F_{1,39} = 38.29$ ($P < 0.0001$)

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Authors' contributions

Conceptualization: Mylthon Jiménez-Castillo and Frida I. Piper; methodology: all authors; formal analysis and investigation: all authors; writing—original draft preparation: Mylthon Jiménez-Castillo; writing—review and editing: Frida I. Piper; funding acquisition: Mylthon Jiménez-Castillo; resources: Mylthon Jiménez-Castillo, Alex Fajardo, Frida I. Piper. The authors read and approved the final manuscirpt.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The authors declare that they obtained the approval of Corporación Nacional Forestal (CONAF) ethics committee for conducting the study on *Araucaria araucana* (Molina) K. Koch in Conguillío National Park.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

The authors declare that they have no competing interests.

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