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RESEARCH ARTICLE

Planting density: Key strategy for optimizing soil health and fruit antioxidant activity in a calafate orchard

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ABSTRACT

Limited information exists regarding the impact of planting density on soil fertility, plant productivity, and the phenolic content of calafate (*Berberis microphylla* G. Forst.) fruits. This study aimed to assess the effects of planting density on the soil, plants, and fruits in a calafate orchard. Four treatments were employed: High-density (HD; 6667 plants ha^{-1}), traditional density (TD; 3333 plants ha^{-1}), medium density (MD; 2222 plants ha^{-1}), and low density (LD; 1667 plants ha^{-1}). In the soil, TD exhibited a 28% increase in basal respiration compared with MD, and a 29% increase in urease compared with HD. Additionally, TD enhanced soil N availability by 57%, ammonium availability by 58%, and Mn availability by 33% compared to LD. Photosystem II experienced an increase with MD and LD ($p > 0.05$), surpassing TD and HD by 3%. The LD significantly outperformed the other treatments in terms of yield per plant, reaching up to 873 g. Conversely, despite its lower yield per plant, HD produced larger and heavier fruits, albeit at the expense of phenolic content. However, HD and MD, averaging 2.1 t ha^{-1}, generated more fruit per unit area than LD and TD did. Notably, planting density did not affect the fruit antioxidant capacity. These findings suggest that TD planting density in calafate preserves the biological and chemical functions of the soil, while maintaining the antioxidant capacity of calafate fruit.

Key words: Basal respiration, *Berberis microphylla*, berberis, berries, polyphenols, stomatal conductance.

INTRODUCTION

The calafate (*Berberis microphylla* G. Forst.) is a shrub belonging to the genus *Berberis* and the family Berberidaceae, flourishing in the wild landscapes of Chile and Argentina. Purplish-black berries, utilized since ancient times, play a crucial role in the crafting of diverse food products. In particular, they are distinguished by their sweetness and phenols with antioxidant properties that exceed up to four times those of blueberries (Rodoni et al., 2014). Recent studies have highlighted the pharmacological and nutritional potential of calafate berries in vivo, with substantial improvements in blood glucose tolerance (Soto-Covasich et al., 2020).

In recent years, the scarcity of calafate has spurred the nascent cultivation in southern Chile, encompassing an area of 0.24 ha (CIREN-ODEPA, 2022). Additionally, an organic orchard dedicated to research and focused on the domestication of calafate was established in south-central Chile, covering an area of 0.15 ha (Pinto-Morales et al., 2022). These emerging orchards, managed by both conventional and organic practices, have embraced conventional planting densities. This density, similar to that used for berries, such as blueberries, implies a 3 m row spacing and a plant spacing of approximately 1 m. However, the optimum planting density that improves biological and chemical soil conditions, encourages plant development, and amplifies the distinctive phenolic content of the fruit remains unknown.

Establishing an optimal density in orchards is crucial for fostering soil health, encompassing both chemical and biological properties, such as inorganic nutrients and microbial communities that enhance agricultural systems (Wang et al., 2019). This is achieved by optimizing resource distribution and minimizing interplant competition, thereby amplifying the performance of each individual plant. However, the appropriateness of density requires tailored evaluation for each fruit species, as exemplified in olive orchards, where, higher densities (401-1500 plants ha^{-1}) have demonstrated an increase in soil biodiversity and an enhancement of N balance compared to lower densities (201-400 plants ha^{-1}) (Sobreiro et al., 2023). Furthermore, a high-density of 1850 plants ha^{-1} resulted in a remarkable 29% increase in soil organic C (SOC) and root biomass compared to a lower density of 300 plants ha^{-1} (Gómez et al., 2022). Similarly, in blueberries, high densities result in greater efficiency in the use of water and soil nutrients (Fang et al., 2020).

In recent decades, there has been a trend to establish high-density orchards to optimize early-stage performance and achieve favorable profitability. However, the implications of high planting density require thorough evaluation given its potential adverse impact on plant development. This effect is evident in specific leaf area requirements, for example, in a coffee (*Coffea arabica* L.) orchard planted at a density of 10 000 plants ha^{-1}, the leaf area index (LAI) per plant decreased by 9% compared to a density of 6000 plants ha^{-1}, with no increase in yield, which remained at 5.7 t ha^{-1} (Rakocevic et al., 2021). Furthermore, because yield is closely related to the concentration of soluble solids and phenolic compounds, planting density can affect fruit quality, as happened in olive trees, where yield reduction attributable to increased density increased relative oil content from 16.8% to 18% when the density increased from 179 to 286 plants ha^{-1} (Lavee et al., 2012).

In cultivated calafate, soil disturbance alters plant growth and physiology, influencing the productivity and phenolic content of the fruit (Betancur et al., 2023). Consequently, this study was devoted to investigating the impact of different planting densities on the soil, plant, and fruit parameters of calafate grown in south-central Chile. The main objective was to establish a baseline for identifying the most appropriate planting density to ensure the soil health and phenolic content of the calafate. The knowledge acquired in this study can serve as a reference for the strategic establishment of new calafate orchards under sustainable productivity.

MATERIALS AND METHODS

Edaphoclimatic characteristics and agronomic management of orchards

The calafate orchard is located in the Chillán (36°31' S; 71°54' W), Ñuble Region, Chile. The soil of the study site was classified as Melanoxerands and was chemically characterized at the Soil Laboratory of the Instituto de Investigaciones Agropecuarias (INIA): pH 6.4, 9.7% organic matter (OM), 19 mg kg^{-1} available N, 15.3 mg kg^{-1} available P; 496 mg kg^{-1} available K; 8.7 cmol kg^{-1}exchangeable Ca; 1.6 cmol kg^{-1} exchangeable Mg, 1.3 cmol kg^{-1} exchangeable K; 0.01 cmol kg^{-1} exchangeable Na and 0.02 cmol kg^{-1} exchangeable Al. The climate is temperate Mediterranean, with mean temperatures and cumulative annual precipitation of 13.5 °C and 649 mm by 2021 and, 13.2 °C, and 920 mm by 2022. The orchard was established in 2017 using 2-yr-old plants obtained from seeds from Valdivia, Los Rios Region, Chile. Weed control was performed over the row using a 100 µm thick black polypropylene geotextile mulch (Protekta, Santiago, Chile). Irrigation was carried out annually from August to March, replenishing 100% of the daily potential evapotranspiration (ET_0) of the crop, according to Betancur et al. (2022).

Experimental trial setup

In the orchard, which had a total area of 1.5 ha, four planting density treatments were established: High density (HD) with 0.5 m plant spacing and 6667 plants ha^{-1}; traditional density (TD) with 1 m plant spacing and 3333 plants ha^{-1}; medium density (MD) with 1.5 m plant spacing and 2222 plants ha^{-1}; and low density (LD) with 2 m plant spacing and 1667 plants ha^{-1}. An inter-row distance of 3 m was maintained for all the treatments. The statistical design used was a randomized complete block with four treatments and four replicates (n = 16). Each replicate consisted of four plants, with the two central plants that were evaluated

independently as the experimental unit. The results obtained from both central plants were averaged for each treatment and replicate.

Chemical and microbiological soil analysis

Soil samples for microbiological and chemical analyses were collected at the end of the study on 10 October 2023, at a depth of 0-30 cm. Soil chemical analysis was performed at the Laboratory of Chemical Analysis of Soils and Plants of the University of Concepción, Chillán, Chile, as described by Sadzawka et al. (2006). For microbiological analyses, the soil adhering to the roots was collected. Subsequently, the samples were homogenized and sieved at 2 mm before being conditioned to 60% of their field capacity.

Soil microbial activity was estimated by fluorescein diacetate (FDAse) activity, as described by Alef and Nannipieri (1995), and basal respiration, as described by Joergensen (1995), expressed as μg FDA g^{-1} and μg CO_2 g^{-1} h^{-1}, respectively. The following enzymatic activities were determined: Urease, acid phosphatase (Nannipieri et al., 1980), and dehydrogenase (Garcia et al., 1997). Urease and phosphatase were expressed as ammonium-N and *p*-nitrophenol (PNP) units, respectively, per gram of soil (dry weight) and hour (μmol NH_4^+ g^{-1} h^{-1} and PNP g^{-1} h^{-1}), whereas dehydrogenase was expressed as micrograms of iodonitrotetrazolium formazan (INTF) per gram of soil (dry weight) (μg INTF g^{-1}).

Foliar and physiological plant measurements

Plant foliar and physiological measurements were made on leaves exposed to the sun and from the second third of the shoot during the season. On each plant, four subsamples were taken from each of the cardinal points at an average plant height of 1.2 m. Measurements were performed after harvest in December 2021 and 2022.

Leaf temperature (°C) was measured using a portable infrared thermometer CTR1000 (Instrumentos WIKA S.A.U., Barcelona, Spain) four times of the day (09:00, 12:00, 15:00, and 18:00 h). The leaf area index (LAI; m^2 m^{-2}) was determined at 12:00 h using a portable ceptometer (AccuPAR LP-80, Decagon Devices, Washington D.C., USA), which averages the readings of 80 quantum sensors to determine direct and residual photosynthetically active radiation from below the plant canopy (Sonnentag et al., 2007). The soil plant analysis development (SPAD) chlorophyll index was measured at 12:00 h using the portable chlorophyll meter kit MC-100 (Apogee Instruments, Logan, Utah, USA) (Cunha et al., 2015).

The maximum photochemical efficiency of photosystem II (F_v/F_m) was calculated using the following relationship: $F_v/F_m = (F_m - F_0)/F_m$, where, the maximum fluorescence intensity (F_m) as well as the minimum chlorophyll fluorescence intensity (F_0) was measured with a portable fluorimeter model OS-5p (Opti-Sciences, Hudson, New Hampshire, USA). Stomatic conductance (mmol m^{-2} s^{-1}), was measured using portable porometer equipment (SC-1 Decagon Devices). Stomatal conductance and F_v/F_m measurements were performed four times a day (09:00, 12:00, 15:00, and 18:00 h) (Retamal-Salgado et al., 2017).

Physical-chemical measurements of the fruit

The physicochemical parameters of the fruit were measured in December, immediately after manual harvest, 130 d after full bloom (Pinto-Morales et al., 2022). Physical and solid parameters were determined in the 2021 and 2022 seasons, and chemical parameters were determined only in the 2022 season.

Fruit productivity (g $plant^{-1}$) was determined by weighing the total fruit of each plant using an analytical balance (Series 360 ES; Precisa Gravimetrics AG, Dietikon, Switzerland). For the fresh weight of 10 fruits, 10 fruits were randomly selected from the total production per plant (n = 4) on the same analytical balance. The polar and equatorial diameters (mm) of each fruit (n = 10) were measured using a digital meter foot model E5001002 ± 0.003 mm (Veto y Cia Ltda., Santiago, Chile). The concentration of soluble solids (°Brix) was measured in the sample juice using a digital refractometer (model HI96801 ± 0.2%; Hanna Instruments S.R.L., Woonsocket, Rhode Island, USA) from a random sample of fresh fruit (n = 4) from each total yield per plant.

Total polyphenol concentration was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as mg gallic acid in fresh fruit (mg gallic acid 100 g^{-1} FW). Antioxidant capacity was determined using 2,2-diphenyl-picryl-hydrazyl (DPPH) and oxygen radical absorbance antioxidant capacity (ORAC) (Romero-Román et al., 2021), which were expressed as µmol Trolox equivalents in fresh fruit (µmol TE 100 g^{-1} FW). Individual anthocyanins were identified using high-performance liquid chromatography (HPLC) (Romero-Román et al., 2021) and expressed as mg of fresh fruit (mg 100 g^{-1} FW).

Statistical analysis

The data obtained were analyzed (ANOVA) and compared using Fisher's least significant difference (LSD) test at a significance level of 0.05. The relationship between soil, plant, and fruit variables was analyzed by principal component analysis (PCA) and correlation analysis with R software (Allaire, 2011) using the FactoMineR and ggplot2 packages, focusing on the mean based on eigenvalues.

RESULTS

Chemical and microbiological soil parameters

The activity of FDAse showed nonsignificant response to planting density, with an average of 42.33 µg FDA g^{-1}. The same lack of response was evidenced in dehydrogenase and acid phosphatase enzymes, which presented an average activity of 85.36 µg INTF g^{-1} and 92.10 µmol PNP g^{-1} h^{-1} respectively. In contrast, basal respiration was 28% higher ($p < 0.05$) in TD and LD than in MD. The HD with a respiration of 0.59 µg CO_2 g^{-1} h^{-1} did not present significant differences between treatments. Urease responded to planting density, being favored ($p < 0.05$) by 29% in TD compared to HD (Table 1).

Chemical analysis of the soil at the end of the experiment indicated that planting density significantly influenced soil fertility (Table 2). Specifically, there was a significant increase of 10% in OM with HD compared to LD. Similarly, HD significantly improved Zn by more than 21% with respect to MD and LD. In contrast, TD significantly improved the availability of N (57%), ammonium (58%), and Mn (33%) with respect to LD, and even with this treatment, ammonium was higher than MD (43%) and HD (50%). The other parameters were not influenced ($p > 0.05$) by planting density.

Table 1. Soil microbiological properties and enzyme activity in response to planting density at the end of the study (2023). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 4$). FDAse: Fluorescein diacetate; INTF: iodonitrotetrazolium formazan; PNP: *p*-nitrophenol; HD: high density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}).

Treatments	FDAse activity	Soil basal respiration	Urease activity	Dehydrogenase activity	Acid phosphatase activity
	µg FDA g^{-1}	µg CO_2 g^{-1} h^{-1}	µmol NH_4^+ g^{-1} h^{-1}	µg INTF g^{-1}	µmol PNP g^{-1} h^{-1}
HD	40.68 ± 4.63^{a}	0.59 ± 0.03^{ab}	0.47 ± 0.03^{b}	95.59 ± 4.61^{a}	88.73 ± 9.72^{a}
TD	46.24 ± 3.54^{a}	0.68 ± 0.02^{a}	0.66 ± 0.04^{a}	83.09 ± 11.72^{a}	97.07 ± 12.39^{a}
MD	40.19 ± 1.33^{a}	0.53 ± 0.03^{b}	0.55 ± 0.03^{ab}	74.30 ± 11.90^{a}	90.29 ± 1.76^{a}
LD	42.20 ± 3.99^{a}	0.67 ± 0.06^{a}	0.55 ± 0.06^{ab}	88.46 ± 6.18^{a}	92.32 ± 6.42^{a}
ANOVA p values	0.6400	0.0357	0.0359	0.4462	0.9088

Table 2. Chemical analysis of the soil in response to planting density at the end of the study (2023). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error (n = 4). HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). CEC: Cation exchange capacity.

	Treatments				
Analysis	HD	TD	MD	LD	P-value
Organic matter, %	11.64 ± 0.37^{a}	11.04 ± 0.07^{ab}	11.10 ± 0.19^{ab}	10.46 ± 0.29^{b}	0.0367
pH (water)	6.52 ± 0.05^{a}	6.47 ± 0.03^{a}	6.43 ± 0.07^{a}	6.45 ± 0.04^{a}	0.6066
Nitrate ($N\text{-}NO_3^-$), mg kg^{-1}	5.77 ± 2.15^{a}	9.97 ± 2.94^{a}	5.17 ± 1.16^{a}	4.47 ± 1.96^{a}	0.3282
Ammonium ($N\text{-}NH_4^+$), mg kg^{-1}	5.00 ± 0.17^{b}	9.97 ± 2.53^{a}	4.33 ± 0.29^{b}	4.17 ± 0.22^{b}	0.0366
N availability, mg kg^{-1}	10.77 ± 2.32^{ab}	19.90 ± 5.31^{a}	9.53 ± 0.99^{b}	8.63 ± 1.73^{b}	0.0281
Olsen P, mg kg^{-1}	26.07 ± 9.25^{a}	17.73 ± 4.31^{a}	26.80 ± 13.76^{a}	12.27 ± 2.66^{a}	0.6062
K availability, mg kg^{-1}	357.73 ± 48.88^{a}	411.33 ± 75.63^{a}	375.10 ± 57.10^{a}	285.90 ± 46.91^{a}	0.5189
S availability, mg kg^{-1}	12.87 ± 2.27^{a}	14.83 ± 1.34^{a}	13.13 ± 1.43^{a}	14.27 ± 0.56^{a}	0.7758
Exchangeable Ca, $cmol_+$ kg^{-1}	9.06 ± 0.98^{a}	8.03 ± 0.36^{a}	8.12 ± 0.61^{a}	7.44 ± 0.35^{a}	0.3926
Exchangeable Mg, $cmol_+$ kg^{-1}	2.50 ± 0.24^{a}	2.30 ± 0.21^{a}	1.99 ± 0.30^{a}	2.16 ± 0.43^{a}	0.6952
Exchangeable K, $cmol_+$ kg^{-1}	0.92 ± 0.12^{a}	1.05 ± 0.19^{a}	0.96 ± 0.15^{a}	0.73 ± 0.12^{a}	0.5179
Exchangeable Na, $cmol_+$ kg^{-1}	0.15 ± 0.02^{a}	0.11 ± 0.02^{a}	0.10 ± 0.03^{a}	0.11 ± 0.02^{a}	0.3935
Sum of bases, $cmol_+$ kg^{-1}	12.63 ± 1.15^{a}	11.50 ± 0.54^{a}	11.16 ± 0.86^{a}	10.44 ± 0.82^{a}	0.4037
CEC, $cmol_+$ kg^{-1}	12.65 ± 1.15^{a}	11.51 ± 0.54^{a}	11.18 ± 0.86^{a}	10.46 ± 0.82^{a}	0.4053
Al saturation, %	0.09 ± 0.04^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}	0.3984
K saturation, %	7.51 ± 1.65^{a}	9.18 ± 1.60^{a}	8.73 ± 1.51^{a}	7.03 ± 1.02^{a}	0.7095
Ca saturation, %	71.45 ± 1.47^{a}	69.76 ± 0.27^{a}	72.63 ± 0.58^{a}	71.57 ± 2.48^{a}	0.6069
Mg saturation, %	19.72 ± 0.16^{a}	19.94 ± 1.26^{a}	17.65 ± 1.85^{a}	20.26 ± 2.82^{a}	0.7367
B, mg kg^{-1}	0.47 ± 0.09^{a}	0.50 ± 0.06^{a}	0.37 ± 0.03^{a}	0.40 ± 0.01^{a}	0.3662
Cu, mg kg^{-1}	1.83 ± 0.15^{a}	1.90 ± 0.15^{a}	1.70 ± 0.10^{a}	1.57 ± 0.09^{a}	0.3106
Zn, mg kg^{-1}	0.80 ± 0.01^{a}	0.73 ± 0.03^{ab}	0.63 ± 0.07^{bc}	0.57 ± 0.07^{c}	0.0440
Fe, mg kg^{-1}	37.13 ± 3.38^{a}	40.40 ± 7.41^{a}	32.87 ± 2.34^{a}	32.07 ± 2.89^{a}	0.5498
Mn, mg kg^{-1}	2.67 ± 0.32^{ab}	2.80 ± 0.31^{a}	2.20 ± 0.23^{ab}	1.87 ± 0.22^{b}	0.0334

Foliar and physiological plant parameters

Leaf temperatures in 2021 (Figure 1a) and 2022 (Figure 1b) did not show significant differences between treatments ($p > 0.05$). However, in both seasons, an increasing trend was observed as the measurement period progressed during the day, increasing by 26.8% during the 2021 season and by 29% during the 2022 season, from 09:00 to 18:00 h.

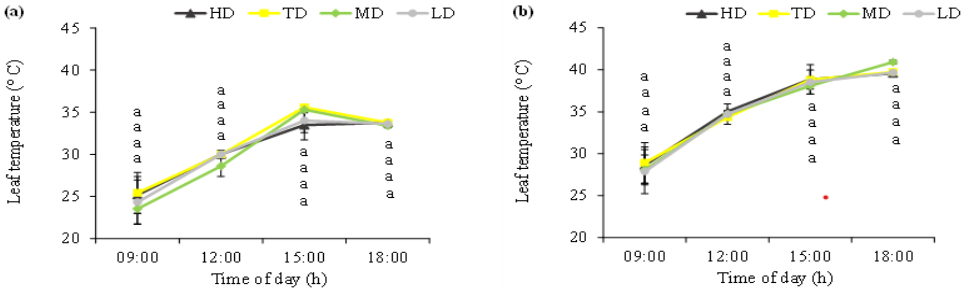

Figure 1. Leaf temperature in calafate plants in 2021 (a) and 2022 season (b), at different times of the day: 09:00, 12:00, 15:00 and 18:00 h. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Mean ± standard error (n = 4). The bars correspond to the experimental error for each treatment.

The LAI in 2021 (Figure 2a) was significantly higher ($p < 0.05$) with LD than with MD (27%), TD (42%), and HD (71%). In the same period, MD and TD, without differences between them, were significantly higher than HD (56%). In the 2022 season, nonsignificant variations ($p > 0.05$) were observed in the LAI, with an average of 2.8 m^2 m^{-2}.

The SPAD index in 2021 (Figure 2b) was not influenced by the planting density, with an average SPAD value of 9. However, in 2022, an increase of 31% was observed in the MD and LD treatments with respect to TD and HD ($p > 0.05$).

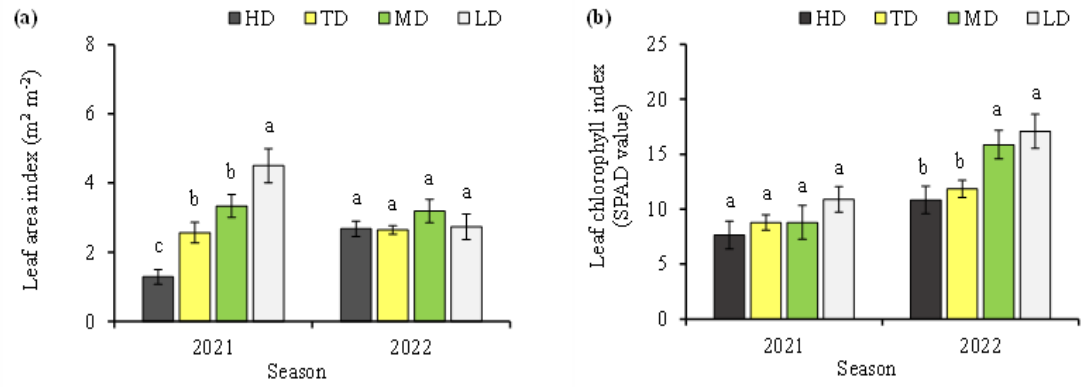

Figure 2. Leaf area index (LAI) (a) and leaf chlorophyll index (b) in calafate plants, evaluated in the 2021 and 2022 seasons. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error (n = 4). The bars correspond to the experimental error for each treatment.

The maximum quantum yield of photosystem II in 2021 (Figure 3a) showed nonsignificant differences between treatments. However, a slight decreasing trend was observed during the last hour of the measurement. In 2022 (Figure 3b), the MD and LD treatments were significantly higher by 3% than TD and HD at 15:00 h and 4% at 18:00 h. In general, all treatments showed a decreasing trend with advancing measurement time, with a decrease of up to 14% from 09:00 to 18:00 h.

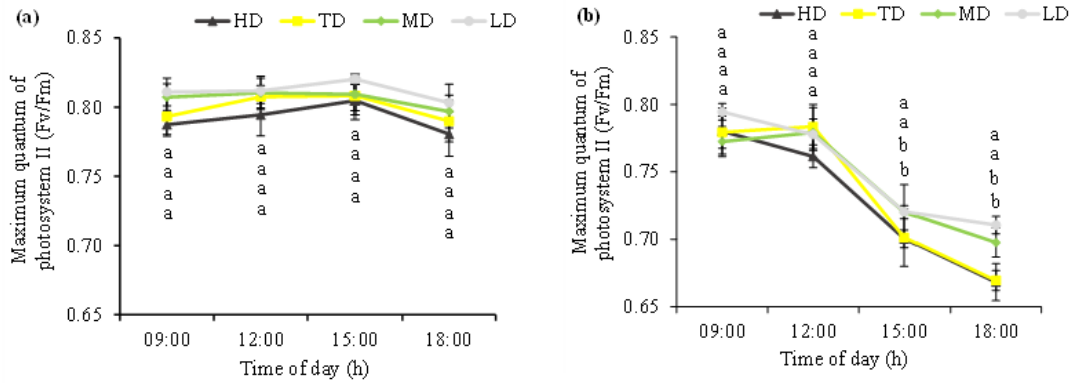

Figure 3. Maximum quantum yield of photosystem II (F_v/F_m) in calafate plants season 2021 (a) and 2022(b); evaluated at different times of the day: 09:00, 12:00, 15:00 and 18:00 h. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error (n = 4). The bars correspond to the experimental error for each treatment.

The stomatal conductance in 2021 (Figure 4a) significantly increased with LD to 25.7% and 30.5% at 12:00 and 18:00 h, respectively, compared to HD. In 2022 (Figure 4b), stomatal conductance was significantly higher at 09:00 h with LD than with TD (27%) and HD (40%). In both seasons, a decreasing trend in stomatal conductance was observed, with a 17% decrease in 2021 and 23.5% decrease in 2022 from 09:00 to 18:00 h.

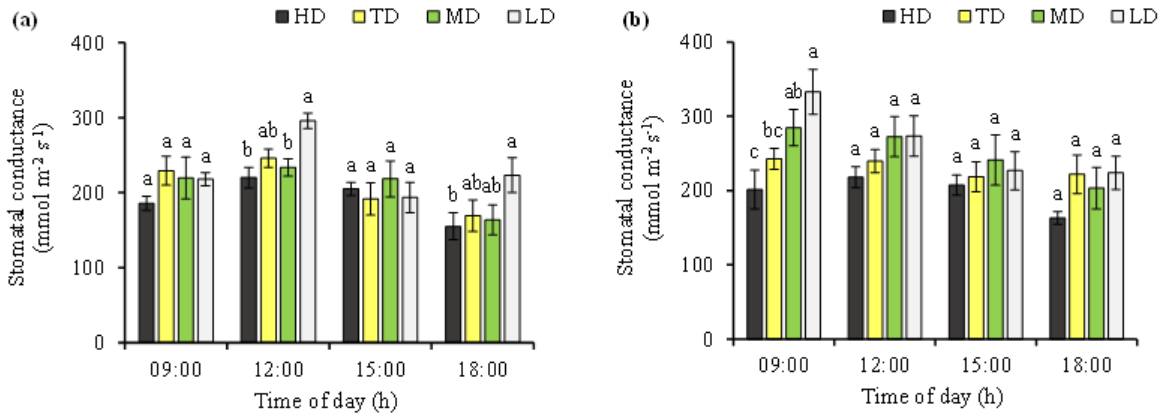

Figure 4. Recorded values of stomatal conductance in calafate plants season 2021 (a) and season 2022 (b); evaluated at different times of the day: 09:00, 12:00, 15:00 and 18:00 h. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 4$). The bars correspond to the experimental error for each treatment.

Physicochemical fruit parameters

Fruit yield in 2021 (Figure 5a) increased significantly with LD, reaching 377 g $plant^{-1}$, compared with the other treatments, with an average of 151 g $plant^{-1}$ in all other treatments. In 2022, yield (g $plant^{-1}$) significantly increased in the LD and MD treatments, with an average of 873 g $plant^{-1}$, compared to the HD and TD treatments, with an average of 277 g $plant^{-1}$. In terms of fruit yield per hectare (t ha^{-1}), in 2021, HD presented 0.9 t ha^{-1} being significantly superior to the TD and MD treatments, which averaged 0.4 t ha^{-1}. In 2022, HD and MD treatments, with an average yield of 2.1 t ha^{-1} were significantly superior to LD and TD. During that period, LD, with a yield of 1.3 t ha^{-1}, was superior to TD, which had 0.8 t ha^{-1}.

Regarding the 10-fruit weight (Figure 5b), in 2021, HD was 32% higher than the other treatments. However, in 2022, the 10-fruit weight did not change significantly with planting density. The equatorial diameter of fruits (Figure 5c) in 2021 was significantly greater with HD than with LD (6%). In the same period, the TD and MD treatments averaged 6.9 mm. In the 2022 season, nonsignificant differences were found in equatorial diameter among the treatments. As for the polar diameter (Figure 5d), similar patterns were observed with respect to the equatorial diameter in both measurement seasons, but with slightly lower values.

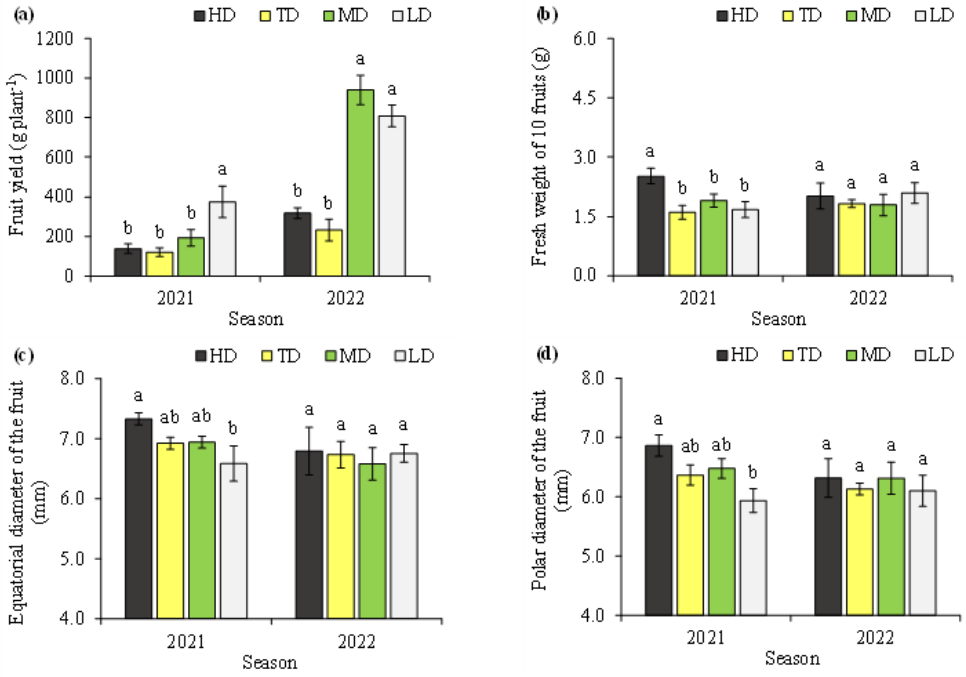

Figure 5. Fruit yield (a), weight of 10 fruits(b), fruit equatorial diameter (c), and fruit polar diameter (d) for the 2021 and 2022 seasons. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 4$). The bars correspond to the experimental error for each treatment.

Fruit soluble solids (Figure 6a) were not influenced by planting density, with an average of 40 °Brix. The same response was evident in the antioxidant capacities DPPH (Figure 6c) and ORAC (Figure 6d), with average values of 6066 and 12998 µmol TE 100 g^{-1} FW, respectively, which were not significantly different from each other. However, there was a 36% higher total phenolic content in fruits under TD and MD than under HD. The phenolic content of fruits in LD with an average of 407 mg gallic acid 100 g^{-1} FW did not show significant differences with respect to the other treatments.

Parameter interaction soil-plant-fruit

Principal component analysis (PCA) of the variables (Figure 7a) and individuals (Figure 7b) was performed for 31 soil, plant, and fruit parameters. The principal components PC1 and PC2 retained a low percentage of variance, corresponding to 23.9% and 20.6%, respectively. In the biplot, each parameter is represented as a vector, and the vector length shows how well the variables are represented in the plot. The treatments in the PCA of individuals (Figure 7b) were represented by the numbers 1-3 for HD, 4-6 for TD, 7-9 for MD and 10-12 for LD, in the 2022 season.

The PCA results confirmed what was indicated in the correlation matrix described in Figure 8 and were analyzed using Pearson's correlation coefficient (r). Moderate correlations were observed between soil indicators, such as N (N, NH_4^+, and NO_3^-), FDAse, basal soil respiration, and urease. Likewise, there were higher correlations between soil S and K availability and FDAse ($r = 0.78$) and acid phosphatase ($r = 0.74$), respectively. Moderate correlations were observed between the soil and plant indicators. The OM and soil Fe, Mn, Zn and Cu availability were moderately correlated ($r > -0.51$ and $r < -0.72$) with chlorophyll index, F_v/F_m and plant stomatal conductance. However, there were high correlations between fruit parameters. Polyphenols were positively correlated with the antioxidant activities of DPPH ($r = 0.81$) and ORAC ($r = 0.72$).

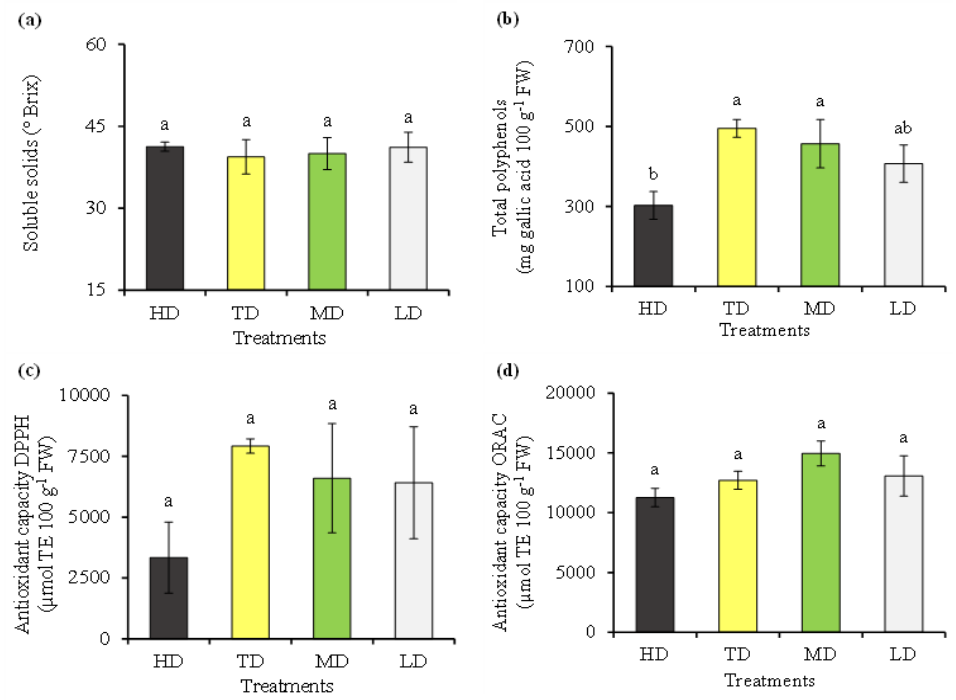

Figure 6. Soluble solids (a), total polyphenols (b), DPPH antioxidant capacity (c), and ORAC antioxidant capacity (d) of calafate fruit in 2022. HD: High density (6667 plants ha^{-1}); TD: traditional density (3333 plants ha^{-1}); MD: medium density (2222 plants ha^{-1}); LD: low density (1667 plants ha^{-1}). Different letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error (n = 4). The bars correspond to the experimental error for each treatment.

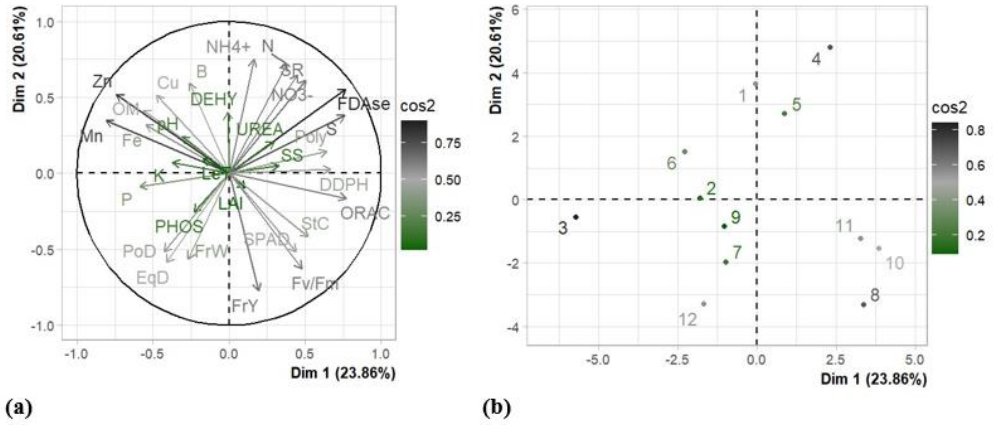

Figure 7. Principal component analysis (PCA) for soil, plant and fruit variables in 2022; PCA of variables (a) and PCA of individuals (b). Soil variables: pH: pH; OM: organic matter; NO3-: nitrate; NH4+: ammonium; N: available N; P: available P; K: available K; S: available S; FDAse: microbial activity; SR: basal soil respiration; UREA: urease; PHOS: acid phosphatase; DEHY: dehydrogenase. Plant variables: LAI: leaf area index; SPAD: chlorophyll index; F_v/F_m: photosystem II maximum quantum yield; StC: stomatal conductance; LeT: leaf temperature. Fruit variables: SS: soluble solids; Poly: total polyphenols; DPPH: DDPH antioxidant activity; ORAC: ORAC antioxidant activity; FrY: yield; EqD: equatorial diameter per fruit; PoD: polar diameter per fruit; FrW: 10 fruit weight.

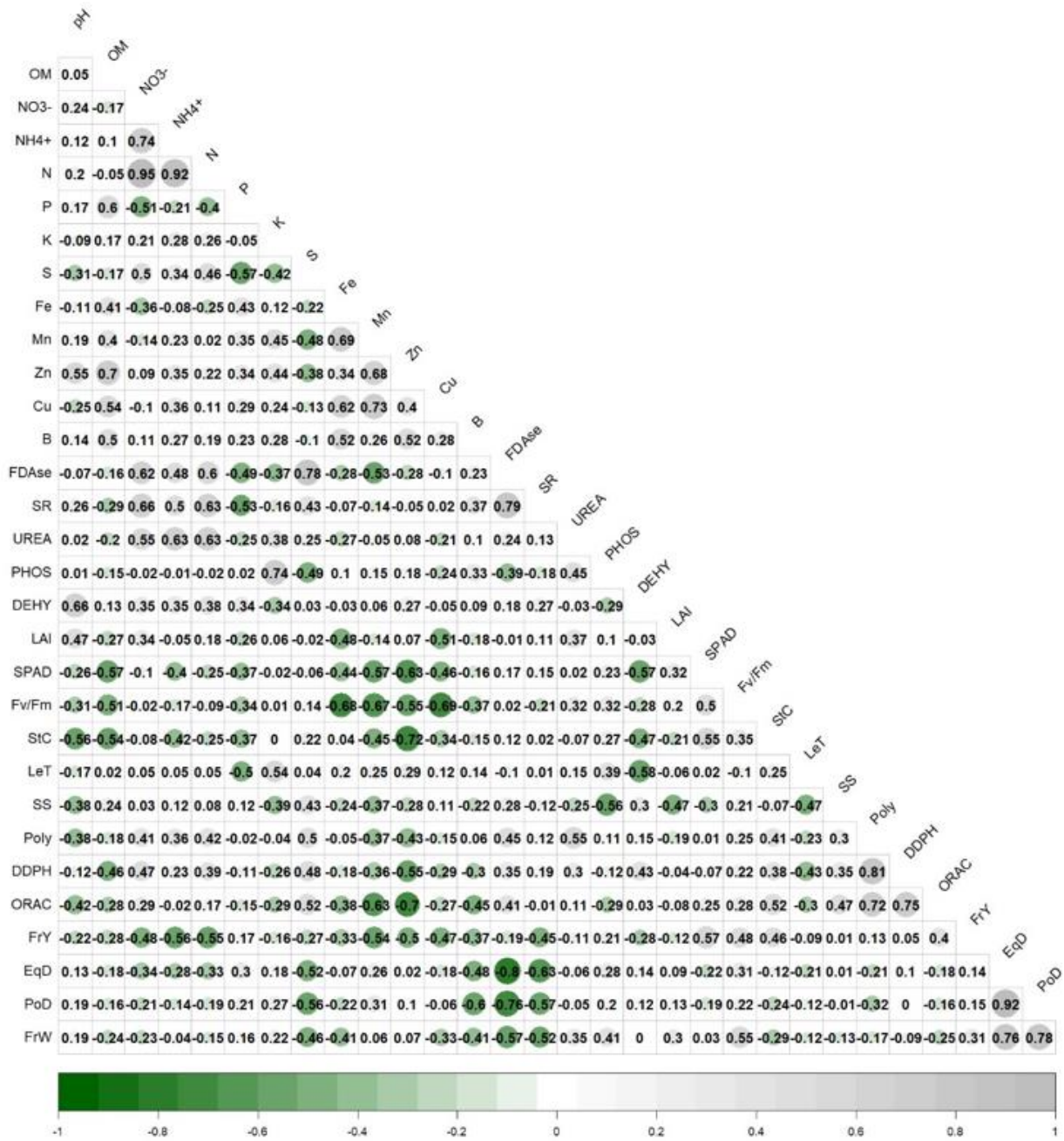

Figure 8. Correlation matrix for soil, plant and fruit variables in 2022. Soil variables: pH: pH; OM: organic matter; NO3-: nitrate; NH4+: ammonium; N: available N; P: available P; K: available K; S: available S; FDAse: microbial activity; SR: basal soil respiration; UREA: urease; PHOS: acid phosphatase; DEHY: dehydrogenase. Plant variables: LAI: leaf area index; SPAD: chlorophyll index; F_v/F_m: photosystem II maximum quantum yield; StC: stomatal conductance; LeT: leaf temperature. Fruit variables: SS: soluble solids; Poly: total polyphenols; DPPH: DDPH antioxidant activity; ORAC: ORAC antioxidant activity; FrY: yield; EqD: equatorial diameter per fruit; PoD: polar diameter per fruit; FrW: 10 fruit weight.

DISCUSSION

Chemical and microbiological soil responses

The different planting densities did not affect FDAse, considered as an indicator of microbial activity, dehydrogenase involved in the C cycle through the oxidation of soil organic C, or phosphatase that catalyzes the mineralization of organic P. It is likely that the Andisol soil type, due to its high OM content, as well as the agronomic practices, irrigation and mulching, used throughout the study attenuated this response by modulating the stabilization of the soil microclimate, in particular, soil moisture, which is crucial for the activity of FDA and these enzymes. The FDAse increases its sensitivity to changes in soil moisture in the short term (Guntiñas et al., 2013). Although lower planting densities may result in lower evaporative soil moisture, it is likely that constant water replenishment and summer ground cover mitigated these losses in the short term (Betancur et al., 2022; 2023). However, the lack of response in acid phosphatase between planting density treatments can be attributed to the high OM content of the soil in all treatments, with high buffer capacity and alkaline mineral content, such as Ca, Mg, and K, which maintained a neutral pH (Yang et al., 2022), with a lower response than in acidic conditions, where this enzyme improves its action. The lack of dehydrogenase response could also be explained by the high OM content in the soil at the beginning of the study. Although there was a higher OM content in HD, previous studies in the same area indicated that OM levels higher than 9% reached a threshold where there was no change in the activity of this enzyme (Betancur et al., 2022; 2023). However, it is important to note that the response of these enzymes is more pronounced with fertilization treatments in soils with low fertility because it promotes the formation of organo enzyme complexes that improve their stability (Nannipieri et al., 2012).

Despite this, TD led to higher basal soil respiration, which is another indicator of soil microbial community activity with respect to MD. This could be due to the fact that TD probably generated a greater aerial biomass and a greater number of roots per surface area, which contributed more OM to the soil, thus favoring microbial activity (Gómez et al., 2022; Hou et al., 2023). It is important to note that HD did not show differences in soil respiration with respect to the other treatments, despite having a high OM content. A similar situation was demonstrated by Rui et al. (2016), where soils, despite having a high OM content, presented a higher stable proportion of OM that generated slow decomposition and activity by microorganisms. Similarly, in our study, TD generated a regulated extraction of soil nutrients, such as N, leaving a stock available as a substrate source for microbial and enzymatic growth and activity (Selvalakshmi et al., 2022). However, HD increased the soil N uptake and decreased the available N at the end of the study. It should be noted that in this study, the disparity in the response of basal respiration and soil FDAse could be explained because the former measures the overall release of CO_2 from all microbial processes in the long term, which in our study corresponded to 7 yr, while FDAse would indicate an effect in the short term.

As expected, there was a change in soil chemical properties under the different planting densities, HD improved the availability of Zn, an element that is mobilized in the soil by root exudates, and was probably higher in this treatment (Hamzah Saleem et al., 2022). The TD significantly improved the availability of N, ammonium and Mn with respect to HD, MD and LD. Therefore, we highlight that TD treatment, to some extent, improved the biological activity of the soil and maintained greater chemical fertility towards the end of the study. However, it is important to note that more research is needed on the cycling and movement of nutrients in the soil, as well as on their uptake by calafate plants.

Foliar and physiological plant responses

In terms of foliar indices, LD led to a greater leaf area and chlorophyll, which is due to less competition between plants, and even stress, as there is more availability of fundamental resources for vegetative development, such as nutrients and water (Huang et al., 2021). Similar results were reported by Casanova-Gascón et al. (2019), who demonstrated lower SPAD values in less vigorous trees.

Also, in the research it is noteworthy that there was lower LAI in HD with respect to TD and MD, probably due to the shading which affects photosynthetic activity that depends on the light energy disappointed by chlorophylls, so that a lower amount limits photosynthesis and growth (Yan et al., 2019).

Likewise, it has been indicated that a decrease in the availability of photosynthetically active radiation (PAR) induces an altered phytochrome response, which alters shoot elongation and leaf area (Retamal-Salgado et al., 2017).

The HD and TD treatments resulted in a lower maximum quantum yield of photosystem II, and HD led to lower stomatal conductance. Similar cases have been documented in species such as apples, where a reduction in tree planting distance to four times (from 1 to 0.25 m) produced more stress, decreasing photosynthetic rate by 39% and up to 2.5 times stomatal conductance (Laužikė et al., 2020). Therefore, HD, being a plant that is more repressed in size and leaf pigments, is more affected by abiotic stress, which would explain the decrease in photosynthesis and gas exchange. It is important to mention that there was a greater effect on the chlorophyll index, maximum quantum yield of photosystem II, and stomatal conductance in the second evaluation season than in the first, which could be explained by climatic conditions such as temperature and more intense precipitation during the evaluation season. Likewise, the lack of change in LAI in the second season with respect to the first could be due to the higher environmental temperature of that season, which could have influenced the development of the crop, as was the case for blueberries (Retamal-Salgado et al., 2017). However, the need for long-term monitoring to verify the continuity of these effects on the physiological parameters of calafate has been highlighted.

Physicochemical fruit response

In the present study, HD and TD led to lower fruit production per plant. These fruit yield results agree with the moderate correlations found in our study, where this productive parameter was positively correlated with physiological parameters, such as SPAD or maximum quantum yield of photosystem II. Therefore, it was confirmed that plants in a better physiological state were more productive, but not HD or TD. Moreover, these results agree with those reported in species such as *Citrus aurantifolia* Swingle, where the yield per plant was always lower at a high-density of 1600 plants ha^{-1} compared to 800 and 400 plants ha^{-1}, respectively (Ladaniya et al., 2020).

However, it should be noted that in terms of cumulative production (t ha^{-1}) HD always produced higher productivity, which would be due to the greater number of trees per surface area compared to TD (2 times more), MD (3 times more) and LD (4 times more), which has also been reported in species such as apple (Lordan et al., 2018) and olive (Díez et al., 2016), which is also indicated as an advantage in productive terms by conferring precocity of young orchards and reducing cultural costs associated with harvesting and pruning (Lavee et al., 2012). Despite these results, we highlight that fruit production per tree and area with MD was high in the second season, reaching the cumulative production of HD.

In addition, in this study, it was demonstrated that higher fruit production per plant was associated with a greater number of fruits and not necessarily with larger fruits, as we found that the plants with lower individual productivity (HD) were those with higher fruit weight and size. These results are in agreement with those reported for blueberries, where plants with lower fruit production showed better quality attributes such as fruit size and weight (Hirzel et al., 2023). This could be attributed to the fact that a lower amount of fruit per plant reduces competition for resources such as sunlight, water, and nutrients, which allows individual fruits to develop more fully (Lordan et al., 2018), which was also corroborated by the moderate correlations in this study between soil nutrients such as N, Mn, and Zn, and fruit yield. On the other hand, in coffee plants, it has been shown that yield decreases at high density because of decreased light and branching frequency, which has a strong photomorphogenetic effect on sprouting (Rakocevic et al., 2021). Therefore, self-shading is a fundamental factor affecting berry quantity and quality (Cheng et al., 2020).

The HD led to a decrease in the total polyphenol content, but not enough to affect the antioxidant capacity measured using different methodologies. In addition, the higher correlation found in this study corroborates that the antioxidant capacity of calafate is conferred by phenolic compounds, among which anthocyanins (Romero-Román et al., 2021) would stand out. Also, in this study, it was possible to demonstrate that planting density did not generate differences in the soluble solid content of the fruit, which is fundamental because it can be used as an indicator to plan the ripening period of the fruit as the soluble solid determinants of the sugar content in the pulp and its state of maturity (Arena et al., 2003). In addition, despite the lower phenolic content of HD, it produced a higher antioxidant activity than other berries such as blueberry and

strawberry, which is beneficial for scavenging reactive oxygen radicals and counteracting oxidative stress (Rodoni et al., 2014). This is interesting for the natural pigment industry because these phenolic compounds provide sensory attributes such as berry pigmentation (Romero Román et al., 2020) and their characteristic antioxidant capacity. However, it is noteworthy that any density generates a significantly higher antioxidant capacity than wild calafate (Romero-Román et al., 2021), probably because of the edaphoclimatic conditions of our study, such as high summer temperatures capable of generating abiotic stress in the plant, inducing changes in secondary metabolism and its accumulation in the fruit (Mariangel et al., 2013).

The results of this study indicate that HD improves calafate productivity per unit area, but at the expense of phenolic content. In contrast, a moderate density, such as MD, which in turn reduces the cost of plants per area, can produce high individual and area productivity, as well as fruits with adequate phenolic content and antioxidant capacity.

This study signifies a notable progression in the assessment of planting density in calafate orchards, as it illustrates the role of planting density in enhancing soil microbial and enzymatic activity, thereby influencing the antioxidant activity of calafate fruit (Betancur et al., 2022; 2023). Nevertheless, due to their moderate interrelationships, a comprehensive analysis of these factors is warranted.

CONCLUSIONS

The impact of planting density on soil health and phenolic compounds in calafate fruits was investigated in this study. The results revealed that traditional density (TD, 3333 plants ha^{-1}) was the most effective in enhancing basal respiration, urease activity, and the availability of essential nutrients such as soil N, Zn, and Mn compared to the other planting density treatments. Moreover, medium density (MD, 2222 plants ha^{-1}) and low density (LD, 1667 plants ha^{-1}) demonstrated a significant increase in leaf area index, chlorophyll content, photosystem II maximum quantum yield, and stomatal conductance relative to the other treatments. Additionally, MD and LD resulted in higher fruit yield per plant, while MD and high density (HD, 6667 plants ha^{-1}) exhibited the highest fruit yield per hectare. The study also found that planting density influenced the phenolic content, with HD showing lower phenolic content compared to the other treatments. Based on the findings, it is recommended that TD be considered due to its positive impact on microbial activity, soil nutrient availability, and the production of fruits with high phenolic content and antioxidant activity. However, in terms of productivity per surface area, MD was observed to generate a high fruit content per plant and surface area with high antioxidant capacity. The study also suggests that future research on calafate should incorporate soil biological parameters to gain a more comprehensive understanding of soil health.

Author contribution

Conceptualization: M.S. J.R.-S., M.D.L. Methodology: M.S., J.R.-S., M.B., R.V-R., M.D.L. Software: M.B., M.D.L. Validation: M.S., J.R.-S., M.D.L. Formal analysis: M.S., J.R.-S., M.B. Investigation: M.B., M.S., J.R.-S., R.V-R., M.D.L. Resources: M.S., M.D.L., J.R.-S., R.V-R., M.B. Data curation: M.B., M.S., J.R.-S., M.D.L. Writing-original draft preparation: M.B., M.S., J.R.-S., M.D.L. Writing-review & editing: M.B., J.R.-S., M.S., M.D.L. Visualization: M.B., M.S., J.R.-S., M.D.L. Supervision: M.S., J.R.-S., R.V-R., M.D.L. Project administration: M.S., J.R.-S. Funding acquisition: M.S., J.R.-S., R.V-R., M.D.L. All co-authors reviewed the final version and approved the manuscript before submission.

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