

Organic mulch as a strategy to improve the health and productivity of calafate (*Berberis microphylla* G. Forst) in south-central Chile

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ABSTRACT

Objective: Calafate (*Berberis microphylla* G. Forst) is a shrub with berries of high functional value that is cultivated in early stages in Chile. Fundamental agronomic management practices, such as physical weed control, have been scarcely evaluated. This study analyzed, over two seasons (2022 and 2024), the effect of different types of mulch.

Design/methodology/approach: The treatments of no mulch (CK), hazelnut shell (HAZ), oat straw (OAT), and geotextile (GEO) were evaluated on plant health indicators (photosystem II quantum yield, stomatal conductance, leaf area index, and foliar pigments) and productivity in an orchard in south-central Chile.

Results: In 2022, OAT and GEO significantly increased leaf development and chlorophyll content, registering increases of up to 115% and 108%, respectively, compared to the control, without significant increases in fruit production. In 2024, the mulches did not significantly alter leaf pigments (chlorophyll, anthocyanins, flavonoids); however, OAT and GEO significantly increased yield and fruit weight, reaching increases of up to 85% and 75%, respectively, compared to the control. The number of seeds per fruit did not vary between treatments ($P > 0.05$).

Findings/conclusions: OAT and GEO improve physiological performance and plant health in the short term and, cumulatively, promote productivity in subsequent seasons.

Keywords: Pigments; antioxidants; yield; berries; chlorophyll.

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INTRODUCTION

The Calafate (*Berberis microphylla* G. Forst), is a species of the genus *Berberis*, which can grow up to 1.5 meters and has small, spiny and evergreen leaves, yellow flowers grouped in clusters and blue fruits with five to seven seeds (Silva *et al.*, 2020). This wild species grows in cool climates and moist soils, and is commonly found associated with temperate forests in the Patagonian region of Chile and Argentina (Silva *et al.*, 2020). Calafate has been shown to have high edaphoclimatic adaptability, being able to grow and fruit in extreme and warm climates (Radice *et al.*, 2018). For example, in Chile, Calafate has been identified in the Maule Region, where maximum temperatures can reach up to 32 °C in summer (Agrometeorología, 2025).

In the last 20 years, research in Calafate has had a notable increase due to the nutritional content and phenolic profile of its fruits beneficial to human health, which



can have up to four times greater antioxidant capacity than the blueberry (Rodoni *et al.*, 2014). For this reason, public and private initiatives have developed its nascent cultivation for small-scale commercial purposes, currently reporting an area of 0.24 ha (Natural Resources Information Center, 2022). However, information on plant management to assess its physiological state and fruit production is still scarce, requiring experimentation with key practices used in fruit orchards, such as weed control.

Soil cover, or mulch, is a practice that is being implemented more frequently in fruit orchards, due to its benefits as a physical suppressor of weeds, allowing the replacement of herbicides (Paušič *et al.*, 2021), and for favoring biodiversity and the activity of microbial communities, which contribute to the nutrition and productivity of plants (Rodoni *et al.*, 2014). However, it has been shown that the effects on plant physiology and yield are strongly determined by the type of mulch used. For example, in an apple orchard, it was shown that highly degradable organic materials such as cereal straw, when more easily incorporated into the soil, can significantly improve fertility through changes in pH, organic matter and nutrients such as P and N, generating improvements in the plant (Selvalakshmi *et al.*, 2022). On the other hand, in some cases, black synthetic mulches that absorb solar radiation can lead to a decrease in biological activity and soil fertility with repercussions for the plant (Meyer *et al.*, 2021). In cultivated Calafate, mulches were previously evaluated by Betancur *et al.* (2023), who demonstrated that mulch suppressed weeds and significantly improved soil moisture retention, increasing soil biological activity by up to 38% compared to plants without mulch. Despite this evidence, a significant knowledge gap remains regarding the effects of different mulch types on plant health components such as physiology, morphology, leaf pigments, and fruit quality.

This study aimed to determine the effect of different soil mulch types (no mulch, hazelnut shell, oat straw, and geotextile) on plant health components of Calafate (stomatal conductance, photosystem II yield, chlorophyll concentration, anthocyanins, and flavonoids) and productivity (total fruit yield, weight, and size). The hypothesis suggests that oat straw mulch treatment, being easier to degrade, will significantly improve the plant's physiological response and concentrate more pigments such as chlorophyll, which will have a favorable impact on fruit production. This effect is attributed to the ability of cereal straw to improve soil fertility by providing organic matter and promoting microbial activity (Selvalakshmi *et al.*, 2022). On the other hand, we hypothesize that hazelnut shell and geotextile treatments, being less degradable, do not significantly influence the plant's pigment content, but will improve fruit production due to greater soil moisture retention (Betancur *et al.*, 2023). The results of this study allow us to determine the types of soil mulch that offer the greatest vegetative and productive benefits to calafate, a species that produces fruit with functional properties for human health.

MATERIALS AND METHODS

Location, soil, and climate conditions of the study site

The study was conducted in an experimental orchard in Calafate, located at the Adventist University of Chile, Ñuble Region, Chile (36° 31' S; 71° 54' W). The soil corresponded to the Andisol order, which was characterized by a chemical analysis.

The climate was temperate Mediterranean, characterized by cold, wet winters with temperatures and precipitation of 21.1 °C and 528.4 mm, respectively, between April and July; summers, meanwhile, had temperatures of 40 °C and low precipitation of 24.4 mm (INIA, 2024).

Planting and management of the Calafate crop

Plants originating from southern Chile and propagated from seed in 2015 were established in 2017 in a 352-plant orchard with a spacing of 3 m between rows and 1 m within rows. At establishment, a base fertilization was applied for pest control. This consisted of 150 g of urea (45% N), 200 g of triple superphosphate (46% P₂O₅), and 200 g of potassium sulfate (50% K₂O). Water replenishment was standardized for all treatments, and the daily potential evapotranspiration of the crop (ET_c) was estimated using the methodology suggested by Romero *et al.* (2010).

Establishment of the trial and experimental design

In 2017, weeding was carried out around the Calafate plants, and subsequently, soil mulches were applied with a minimum thickness of 5 cm and a 1 m diameter around each plant. The organic mulches consisted of dry oat straw and European hazelnut shells, while the geotextile used was composed of black polyester and polypropylene. The oat straw mulch was renewed annually due to its rapid decomposition, while the hazelnut shell mulch was renewed every four years. The experimental design of the trial corresponded to a randomized complete block design, where the experimental unit consisted of the average of two Calafate plants.

Evaluation of physiological variables and foliar pigments

Stomatal conductance and photosystem II peak quantum yield were measured during the 2022 growing season (December) at four times of day (09:00 h, 12:00 h, 15:00 h, and 18:00 h). Stomatal conductance was measured using a portable SC-1 porometer (Decagon Devices, Washington, DC, USA), as described by Pinto-Morales *et al.* (2022). Photosystem II peak quantum yield was measured with a portable OS-5p fluorometer (Opti-Sciences, Hudson, NH, USA), as indicated by Pinto-Morales *et al.* (2022). Leaf area index was measured at 12:00 h with an AccuPAR LP-80 ceptometer (Decagon Devices Inc., Washington, DC, USA), also following the recommendations of Pinto-Morales *et al.* (2022). In addition, the surface temperature of the leaf and mulch (°C) was measured using a CTR1000 portable infrared thermometer (WIKA Instruments S.A.U., Barcelona, Spain).

Between September and December 2024, once a week on clear days at 12:00 PM, the foliar pigment content of Calafate leaves was evaluated. The concentration of chlorophyll, flavonoids, anthocyanins, and the nitrogen-flavonol index were measured by the fluorescence ratio at wavelengths F660 nm/F325 nm and F660 nm/F525 nm, respectively. A portable multipigment analyzer, model MPM-100 (Opti-Sciences, Hudson, NH, USA), was used for this purpose, following the same criteria used for the physiological variables.

Productivity and fruit yield assessment

In 2022 and 2024, fruit yield (kg plant^{-1}) was measured by manually harvesting the total production per plant, 130 days after full bloom, during December. The harvested fruits were then weighed on a Gravimetrics 360 ES Series analytical balance (Precisa Gravimetrics AG, Dietikon, Switzerland). Each fruit was also characterized individually by measuring the weight of ten selected fruits and determining their polar diameter with calipers, as described by Betancur *et al.* (2023). In 2024, seed weight was also included. Ten fruits were randomly selected, each fruit was manually opened, and the seeds were extracted, weighed individually, and the average weight was calculated.

Statistical analysis

An analysis of variance (ANOVA) was performed, and Fisher's LSD test was applied to compare treatments, considering a significance level of 0.05. Statistical analysis was performed using InfoStat software (Di Rienzo *et al.*, 2020). To evaluate the relationships between vegetative and productive variables of the Calafate plant affected by the types of mulch, a principal component analysis (PCA) was performed, applying a multivariate analysis of variance (MANOVA) with a significance level of $p=0.05$ using RStudio software, version R 4.5.1 (R Core Team, 2024).

RESULTS AND DISCUSSION

Leaf and mulch temperature, 2022 season

Leaf and mulch temperature measurements taken during 2022 indicated significant differences between treatments. Leaf temperature (Figure 1a) increased progressively throughout the day, reaching its peak at 3:00 PM, coinciding with the period of greatest solar radiation. The mulch treatments (HAZ, GEO, and OAT) exhibited the lowest leaf temperatures at the time of highest temperature (3:00 PM) compared to the control (CK), being 12%, 16%, and 19% lower, respectively. Mulch temperature (Figure 2b) showed a similar pattern, increasing from the early morning hours until the end of the evaluation

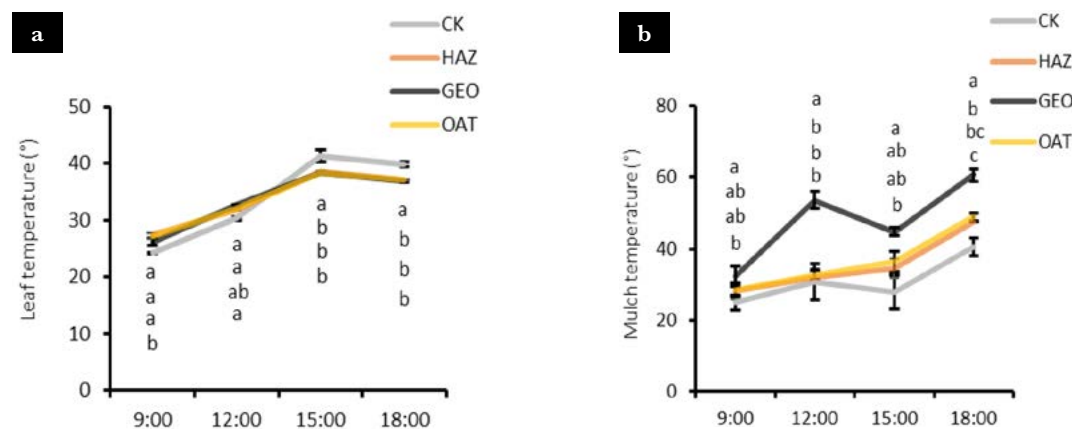


Figure 1. Leaf temperature (a) and mulch (b) 2024 season. CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ($p<0.05$). Mean \pm standard error ($n=4$). Bars represent the experimental error for each treatment.

period. The GEO treatment had the highest mulch temperature at the end of the day (6:00 PM) compared to the other treatments, being 18% higher than HAZ, 30% higher than OAT, and 44% higher than CK.

Plant physiological parameters, 2022 season

Regarding the plant's physiological variables evaluated during 2022, the results indicated that the GEO and OAT treatments favored some variables compared to the other treatments (CK and HAZ). The leaf area index (LAI) (Figure 2a) was significantly higher with OAT and GEO than with HAZ (115%) and CK (85%). For the chlorophyll content index (SPAD) (Figure 2b), GEO showed the highest average value, being approximately 108% higher than CK and 85% higher than HAZ. The photosystem II quantum yield (F_v/F_m) (Figure 2c) decreased during the hours of highest radiation (3:00 PM), followed by a recovery towards the end of the day, but no significant differences ($p > 0.05$) were observed between treatments. Stomatal conductance (Figure 2d) showed an increase around midday (12:00 PM), although without significant differences between treatments. Average stomatal conductance values ranged between 180 and 350 $\text{mmol m}^{-2} \text{s}^{-1}$.

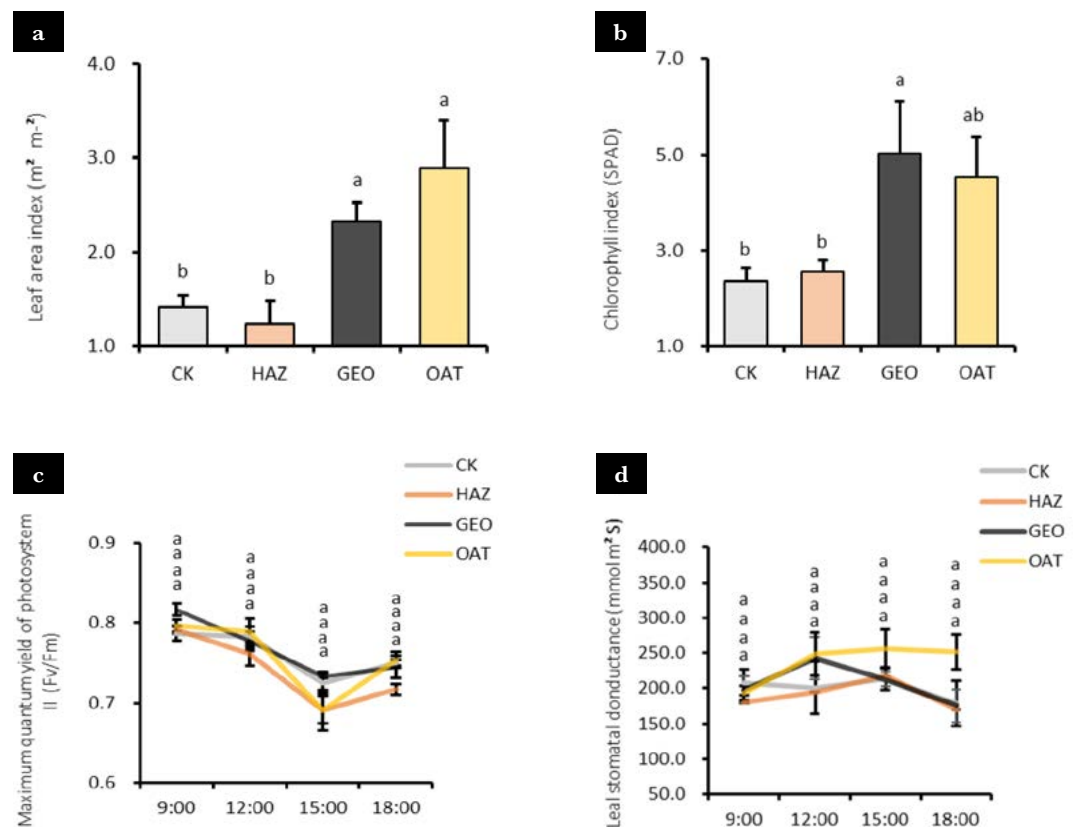


Figure 2. Plant physiological parameters measured in the 2022 season: a) leaf area index, b) chlorophyll index, c) photosystem II quantum yield, and d) stomatal conductance. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean \pm standard error ($n = 4$). Bars represent the experimental error for each treatment.

Fruit performance parameters 2022

The fruit parameters analyzed in 2022 showed no statistically significant differences between treatments. Regarding fruit yield (Figure 3a), the GEO treatment showed the highest average value, reaching approximately 1.9 kg plant⁻¹, followed by OAT, while the control (CK) registered the lowest yield, at approximately 0.8 kg plant⁻¹. Despite this trend, there were no statistically significant differences ($p > 0.05$) between treatments. Regarding the average weight of 10 fruits (Figure 3b), the values ranged from 2.5 g to 2.9 g, with no significant differences between treatments. As for the morphological dimensions of the fruit, both the equatorial diameter (Figure 3c) and the polar diameter (Figure 3d) showed average values between 6.5 and 7.5 mm, respectively.

Analysis of plant and fruit variables 2022

Principal component analysis (PCA; Figure 4) showed that the first two components explained 57.5% of the total variation. Some trends were observed with the mulch treatments; for example, the GEO treatment was slightly associated with higher yield and fruit weight, while OAT was linked to physiological variables such as leaf area index and chlorophyll content. In contrast, CK and HAZ clustered more closely, reflecting similar physiological responses. However, PERMANOVA analysis showed no significant differences between treatments ($F = 2.099$; $p = 0.133$), with high overlap observed between groups.

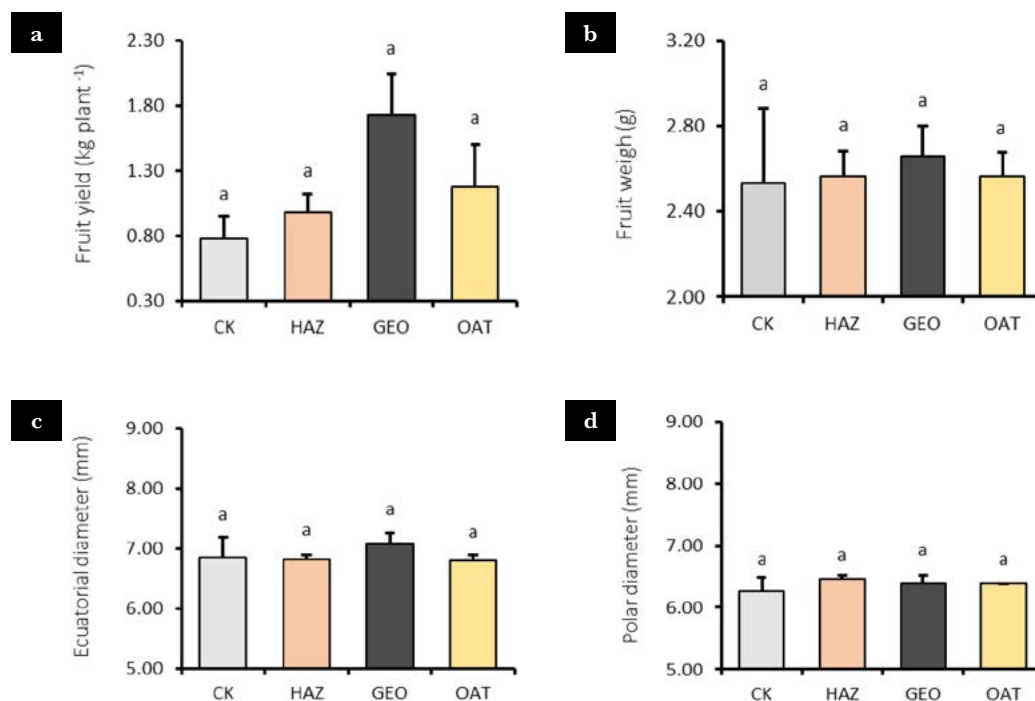


Figure 3. Fruit parameters measured in the 2022 season: a) fruit yield, b) average weight of 10 fruits, c) equatorial diameter, and d) polar diameter. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean \pm standard error ($n = 4$). Bars represent the experimental error for each treatment.

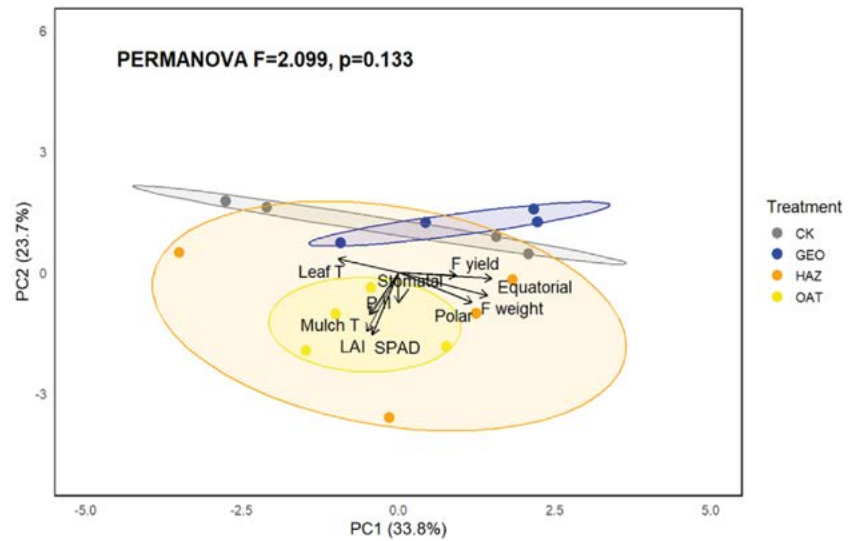


Figure 4. Fruit parameters measured in the 2022 season. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Leaf T=leaf temperature; Mulch T=mulch temperature; LAI=leaf area index; SPAD=chlorophyll index; Stomatal=stomatal conductance; PII=photosystem II maximum quantum yield; Polar=polar diameter; Equatorial=equatorial diameter; F yield=fruit yield per plant.

Leaf pigment parameter, 2024 season

The averaged foliar pigments evaluated, chlorophyll (ChlM; Figure 5), flavonoids (FIVM; Figure 5b), anthocyanins (AnthM; Figure 5c) and nitrogen-flavonol index (NFI; Figure 5d), did not have significant differences between treatments.

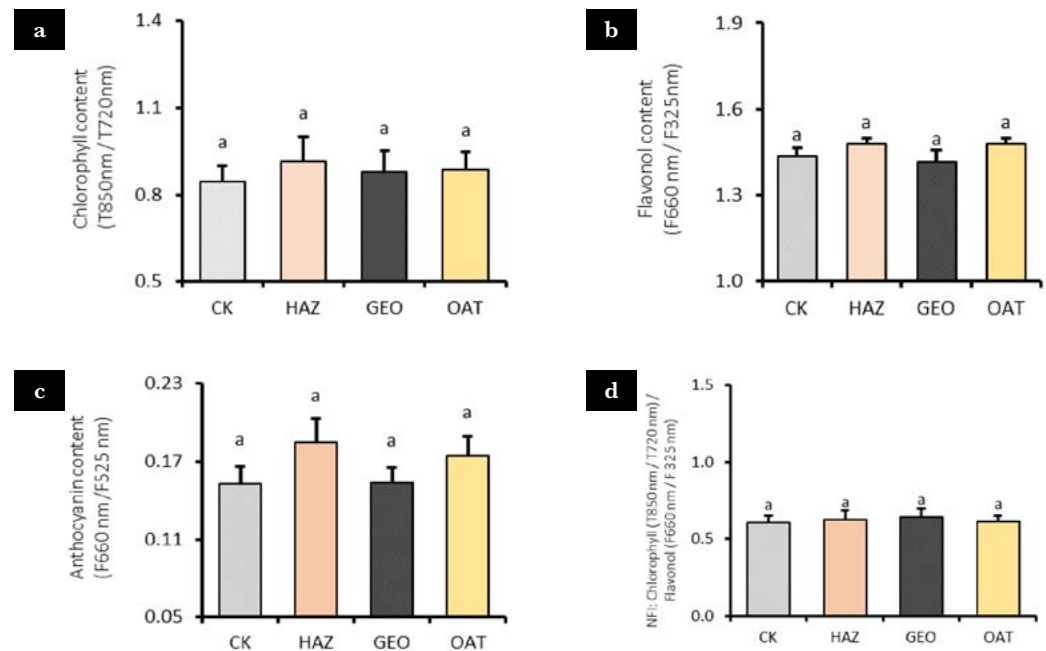


Figure 5. Leaf pigment variables: a) chlorophyll measurement, b) flavonoid measurement, c) anthocyanin measurement, d) flavonol nitrogen index. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher’s LSD test ($p < 0.05$). Mean \pm standard error ($n=4$). Bars represent the experimental error for each treatment.

Fruit parameters for the 2024 season

Regarding the behavior of fruit variables in 2024, fruit yield (Figure 6) was statistically higher with HAZ, GEO, and OAT compared to the control, with increases of 75%, 85%, and 60%, respectively. Fruit weight (Figure 6b) was statistically higher with GEO and OAT compared to CK, being up to 41% and 31% higher, respectively. As for fruit dimensions, the equatorial diameter (Figure 6c) and polar diameter (Figure 6d) did not change significantly due to the treatments, with averages close to 5.5 mm for both equatorial and polar diameters.

Seed parameter season 2024.

The fruit seed variables (Figures 7 and 7b) showed no statistically significant differences ($p > 0.05$) between treatments. The average seed weight ranged from 0.47 g to 0.59 g, while the average number of seeds per fruit ranged from 6.5 to 7.3.

Analysis of plant and fruit variables 2024

In the PCA (Figure 8), the first two principal components explained 50.8% of the total variation. GEO and OAT cover types maintained their association with higher yields and fruit weight, as in 2022, while physiological variables (such as leaf pigments) showed a more diffuse distribution without defined clustering. However, no significant differences were detected between treatments ($F = 0.49$; $p = 0.87$), with high overlap between groups.

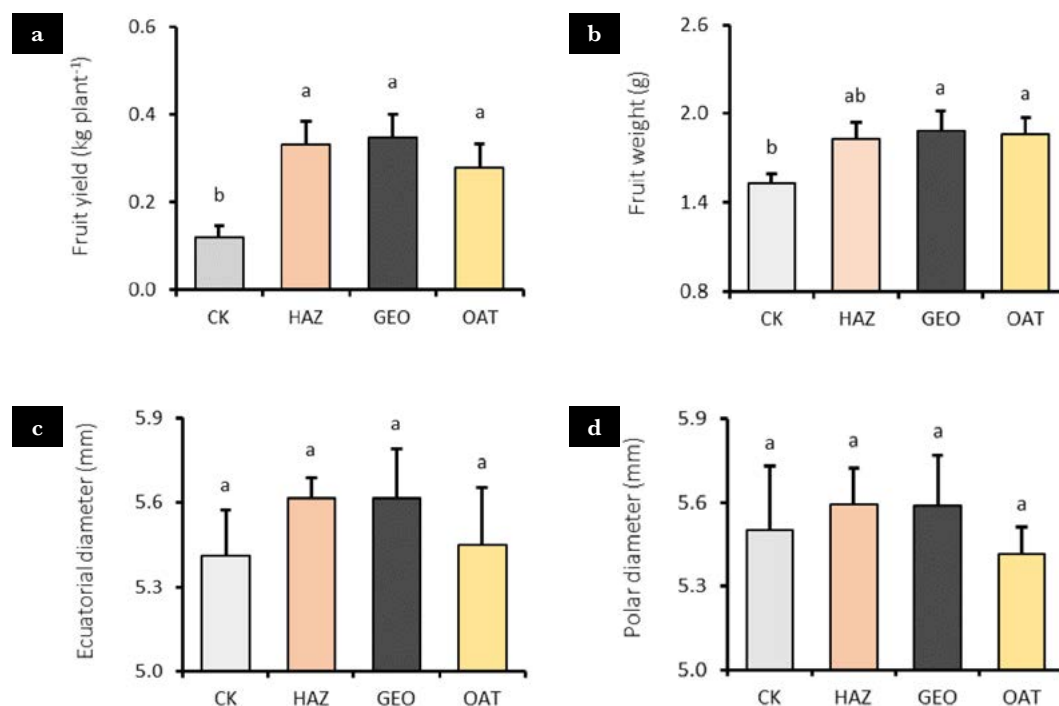


Figure 6. Fruit parameters for the 2024 season: a) yield, b) fruit weight, c) equatorial diameter, d) polar diameter. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean \pm standard error ($n = 4$). Bars represent the experimental error for each treatment.

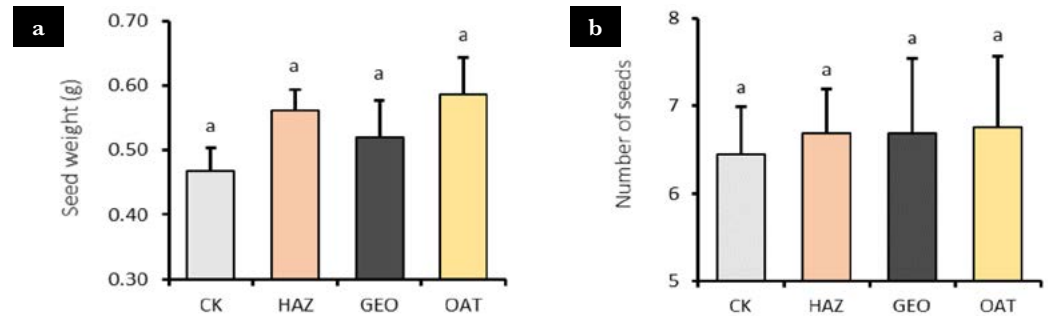


Figure 7. Seed measurements, 2024 season: a) seed weight, b) number of seeds. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean \pm standard error ($n=4$). Bars represent the experimental error for each treatment.

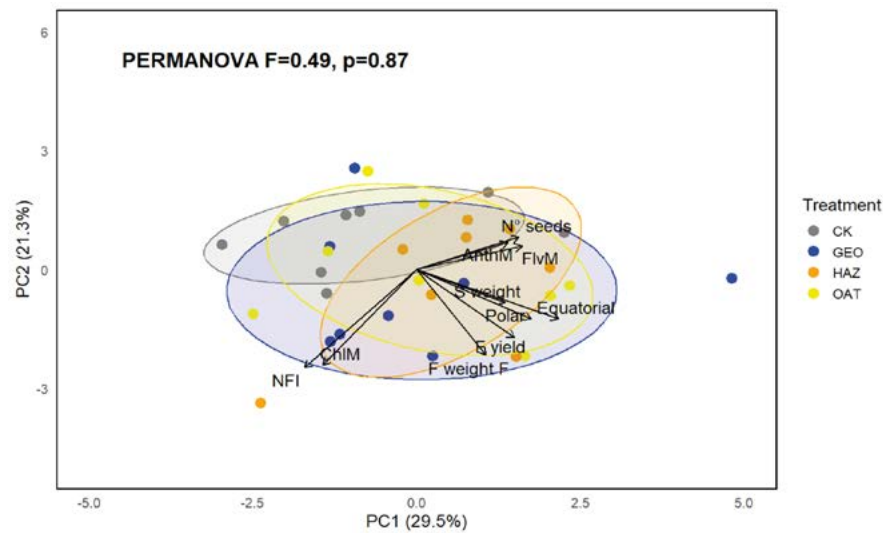


Figure 8. Seed measurements, 2024 season: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. N° seeds=number of seeds; AnthM=anthocyanins; FlvM=flavonoids; Polar=polar diameter; Equatorial=equatorial diameter; F yield=fruit yield per plant; F weight F=fruit weight; ChlM=chlorophyll; NFI=flavonol nitrogen index.

This study evaluated the effect of different types of mulch on physiological variables and fruit production of Calafate (*Berberis microphylla*) over two seasons (2022 and 2024). The results showed that OAT and GEO mulches affected the plant's physiological functioning in the first season (2022), but no significant differences were observed in the 2024 season. In terms of production, OAT and GEO resulted in higher fruit yields in the second season (2024). These results are consistent with those reported for other fruit crops, where different mulches can initially modulate the plant's photosynthetic activity, and in subsequent seasons, this translates into improvements in yield and/or fruit quality (Zhang *et al.*, 2021; Betancur *et al.*, 2023). These findings are relevant because they show that agronomic management through the use of mulch has effects both at the plant level and on the characteristics of the fruit.

Effect of mulches on plant and fruit 2022

In the 2022 growing season, chlorophyll content and leaf area index were sensitive to mulch use, with the highest responses observed primarily with GEO and OAT. Surface mulches have been shown to modify the soil microclimate by increasing soil temperature, conserving moisture and improving nutrient availability, favoring the development of foliar biomass and the accumulation of photosynthetically active area (Chauhan *et al.*, 2023). The improved physiological performance observed under GEO is consistent with reports indicating that synthetic or semi-synthetic mulches can generate a more stable root environment, promoting water and nutrient efficiency, and sustaining higher levels of photosynthetic pigments (Li *et al.*, 2022). Likewise, organic mulches such as OAT maintain high levels of organic matter in the soil and suppress weeds, which also improves the availability of resources to promote vegetative growth (El-Beltagi *et al.*, 2022; Betancur *et al.*, 2023). In this study, the principal component analysis (PCA) of the 2022 season, although it did not detect significant differences, showed patterns of association between GEO and fruit weight and yield variables, while OAT was mainly linked to physiological variables such as leaf area index and chlorophyll index.

In the 2022 season, other physiological parameters such as photosystem II quantum efficiency and stomatal conductance did not vary significantly between treatments. However, under conditions where water availability is not critically limiting, mulch may not generate marked differences in photosystem II quantum efficiency or conductance, even though it does improve the root microenvironment (Zhang *et al.*, 2014; Guo *et al.*, 2021; Betancur *et al.*, 2023). This is consistent with what has been observed in blueberries and other berries managed with different mulch systems and supplemental irrigation, where the plant maintains a relatively stable physiology at the level of photoprotection even under contrasting treatments (Retamal-Salgado *et al.*, 2023; Guo *et al.*, 2021). Even so, a diurnal variation was observed in photosystem II quantum efficiency and conductance where they decreased around the point of maximum radiation (close to 3:00 pm) and recovered towards the end of the afternoon. This pattern is typical of transient photochemical stress due to high radiation and leaf temperature, followed by partial recovery when conditions moderate, and has been described in Calafate as well as in other fruit trees in areas with high summer radiation (Arena *et al.*, 2020; Betancur *et al.*, 2023).

Regarding fruit yield in 2022, there were no significant differences between mulch treatments. In other fruit trees, it has been described that the productive response to mulch can take several seasons to become apparent, especially in young orchards (Zhang *et al.*, 2021). Despite this, the absence of yield differences could also be affected by other factors such as the genetics of the plant material, pruning management, and nutrition, which can mask the direct effect of mulch on the number of fruits per plant (Betancur *et al.*, 2023). On the other hand, the lack of effect of mulch on fruit yield is more consistent in soils of low fertility, unlike in our study (Romero-Román *et al.*, 2021; Pinto-Morales *et al.*, 2022).

Effect of mulches on plant and fruit 2024

In the 2024 growing season, foliar pigments such as chlorophyll, anthocyanin, and flavonoid content were not affected by the different mulches. Since pigments like

anthocyanins typically increase under stress conditions, the lack of differences between mulches indicates that none of the conditions generated severe physiological stress in the plant (Arena *et al.*, 2020; Guo *et al.*, 2021; Retamal-Salgado *et al.*, 2023). Multivariate analysis using PCA reinforced this interpretation, although without significant differences. In terms of yield, in the 2024 growing season, plant weight and individual fruit weight increased significantly with OAT and GEO. Mulches have been shown to significantly improve yield through better soil moisture conservation and nutrient availability (El-Beltagi *et al.*, 2022; Betancur *et al.*, 2023). Specifically, OAT provided more highly degradable organic material that can improve soil organic matter and phosphorus content, which has been associated with more efficient fruit filling in berries (Sun *et al.*, 2017; Duan *et al.*, 2022). Meanwhile, GEO, with its black color, is able to stabilize and increase soil temperature, limit weed competition, and improve water use by the plant—effects that translate into increased fruit yields (Chauhan *et al.*, 2023; Betancur *et al.*, 2023). This study also measured the number of seeds per fruit and showed no significant effect from the use of mulch. This result is consistent with what has been observed in other perennial fruit trees, where soil management through mulch tends to affect fruit filling and quality more than seed formation, which is a genetic factor (Zhang *et al.*, 2014; Retamal-Salgado *et al.*, 2023).

Our results demonstrate that OAT is more beneficial for both the plant and the fruit. From a sustainability perspective, this is relevant because the use of plant residues such as cereal straw has been proposed as a management practice that simultaneously controls weeds, reduces soil water loss, and improves microbial activity, thus decreasing the need for external inputs (El-Beltagi *et al.*, 2022). This type of organic mulch has also been linked to increases in soil microbial biomass, basal respiration, and key enzymatic activities associated with the nitrogen and carbon cycles (Li *et al.*, 2022).

Future perspectives of the study

The results of this study demonstrated that the effects of mulch can be cumulative across seasons, beginning with physiological improvements that are subsequently reflected in increased fruit weight and yield per plant. This has also been described in other perennial fruit trees such as grapevines, apples, and peaches, where mulching gradually improves soil properties, reduces weed competition, and, over time, enhances fruit productivity and quality (Giese *et al.*, 2014; Sharma *et al.*, 2023; Mairata *et al.*, 2023). However, future studies should evaluate the effects of mulch over consecutive seasons to fully capture the physiological and productive dynamics under mulch. In this study, the orchard evaluated is still young, so yield per plant may increase as the orchard progresses toward physiological and reproductive maturity, which could amplify the differences between mulch treatments in future seasons (Pinto-Morales *et al.*, 2022; Radice *et al.*, 2018). Given that these results demonstrate benefits from the use of reusable organic mulches such as OAT and HAZ, future research should also evaluate other types of mulch made from organic products and residues on cultivated and native plants such as Calafate.

CONCLUSIONS

This study demonstrated that mulches, especially OAT and GEO, significantly improved the plant's physiological processes in the first instance (2022 season), increasing the leaf area index and chlorophyll content by more than 100% compared to the control. Subsequently (2024 season), Calafate fruit production increased significantly by over 70% with these treatments (OAT and GEO) compared to the control. Although HAZ had a less pronounced effect compared to OAT and GEO, it showed moderately superior productivity compared to the control. Given that these results demonstrate the benefits to the plant from the use of reusable organic mulches, particularly OAT, future research should evaluate other types of mulch made from organic products on native plants cultivated as Calafate. This study demonstrates that mulches are useful for enhancing Calafate productivity and represent an environmentally responsible alternative.

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