



## Article

# Novel Approach to Organic Mulching from Natural-Based Solutions to Enhance Soil Health and Functional Value of Calafate Fruit

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**Abstract:** Mulching suppresses weeds, improves soil biology, and increases physical or bioactive fruit yield in fruit orchards. However, there is no information on its impact on calafate (*Berberis microphylla* G. Forst.) orchards, which produce berries with high antioxidant content. To address this gap, in 2021, an experiment was conducted to evaluate the effect of 5 years of mulching on soil, plants, and calafate fruit. Four mulching treatments were established: no mulch (control), geotextile, oat straw, and hazelnut shell. All mulches suppressed weeds (43%) and maintained more soil moisture (5%) than the control. Soil microbial activity increased only with hazelnut shell compared with the control, up to 46%. Only oat straw and hazelnut shell increased basal respiration and urease up to 31% and 15% more than the control. Oat straw produced the highest fruit yield with 0.44 t ha<sup>-1</sup>, while the lowest yield was produced by the control and hazelnut shell with 0.1 and 0.15 t ha<sup>-1</sup>, respectively. The geotextile with 0.35 t ha<sup>-1</sup> of fruit produced no differences between treatments. The ORAC antioxidant capacity was only higher in the control and hazelnut shell, with a mean of 3272 μmol TE 100 g<sup>-1</sup>. Hazelnut shell mulch is recommended to improve the biological functions of the soil and the antioxidant capacity of the calafate fruit.

**Keywords:** *Berberis*; soil respiration; polyphenols; antioxidants; urease

## 1. Introduction

A widely accepted practice in fruit orchards is the use of ground covers for their efficient physical suppression of weeds and inhibition of seed germination [1] and for their ability to replace herbicides [2]. In soil, the use of certain types of mulch favors biodiversity and the activity of microbial communities, which contribute to plant nutrition and productivity [3]. However, the composition of the mulch must be carefully evaluated. Plastic mulch significantly enhances the enzymatic activities of alkaline phosphatase, invertase, catalase, and urease, as well as the carbon (C) and nitrogen (N) contents of soil microbial biomass [4]. Synthetic mulches of polyethylene and other black synthetic polymers can absorb solar radiation by emitting energy in the form of heat into the soil [5], which also leads to a decrease in soil biological activity [6].

Mulching, implemented in berry species such as grapevine, blueberries, or strawberries, modifies the soil environment, influencing plant growth, development, and aspects of berry quality, such as concentrations of sugars, anthocyanins, and total polyphenols [7,8]. Taparauskienė and Miseckaitė [9] showed that black plastic mulch improved fruit yield by 60% compared with soil without mulch and by 56% compared with soil with wheat straw mulch in a strawberry orchard due to an increase in soil temperature. In contrast, when a combination of rice straw mulching with an irrigation strategy was applied in a grapevine orchard, there was an increase in berry diameter, fruit yield, water use efficiency, and berry sugar concentration by 2.8 mm, 271.5 g tree<sup>-1</sup>, 33% and 15%, respectively, compared with treatment without mulching and irrigation [10]. In another case, compost mulching increased soil C and N content by 50%, and straw mulching suppressed weeds by 90% and increased soil moisture by 5% compared with soil without mulching but induced smaller changes in fruit yield in an apple orchard [11].

Chen et al. [12] demonstrated that mulching with corn stover significantly increased soil bacterial communities because of the contribution of organic matter (OM) and improvements in soil physicochemical conditions caused by this treatment in an apple orchard. Similarly, mulching with sawdust, corn straw, and wild grass improved the richness and diversity of soil bacterial and fungal communities, as well as the stability of the edaphic ecosystem, in response to changes in soil pH, phosphorus (P), C, and C:N ratios [13]. For this reason, organic mulches are often preferred, innovating with materials high in lignin and of greater durability, coming from industrial wastes, such as the timber industry [14]. In this context, the nut industry in Chile, such as hazelnut production for export, is increasing and currently has 36,000 ha planted [15], which produces high amounts of hazelnut shells as waste, with potential use as mulch due to its woody composition.

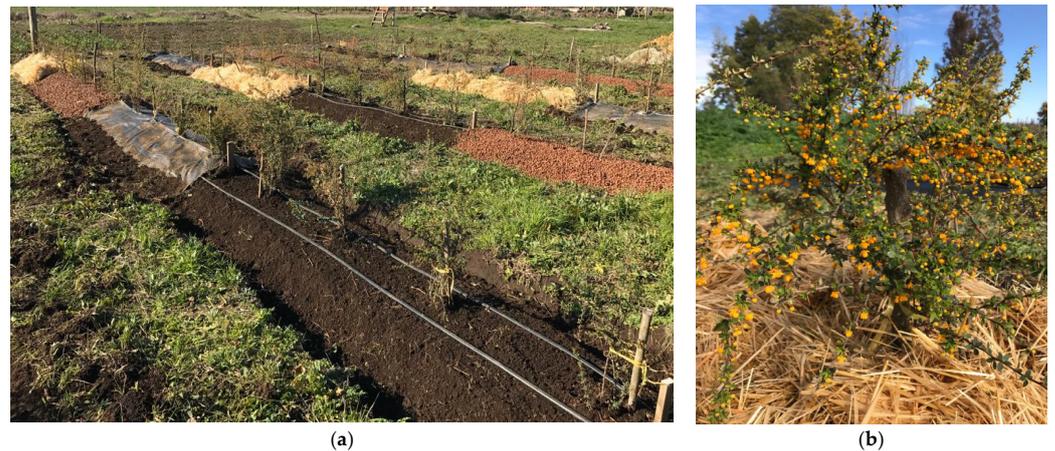
In the last decade, it has been determined that the calafate (*Berberis microphylla* G. Forst.) berry has a high concentration of phenols, higher than fruits such as oranges, blueberries, and strawberries [16,17] and that it substantially reduces the presence of degenerative, cardiovascular and carcinogenic diseases [18]. In addition, it has a higher sugar content (14.6%) compared with other fruits, such as cherry (9.9%), strawberry (5.5%), blueberry (6%), and raspberry (4.8%) [19]. The production of calafate fruit in Chile is mainly wild, having a cultivated area in Southern Chile of only 0.24 ha [15]. However, in the Ñuble Region, Central Southern Chile, an orchard was established in 2017 with the purpose of evaluating its response to different agronomic managements, such as organic fertilization and hydric replenishment. Pinto-Morales et al. [20] demonstrated that fertilization with compost at a dose of 10 t ha<sup>-1</sup> significantly improved the yield and DPPH antioxidant capacity of the fruit by 100% and 20%, respectively, compared with a plant without fertilization. On the other hand, Betancur et al. [21] demonstrated that it is possible to reduce water replenishment from 100% to 50% of the reference evapotranspiration (ET<sub>0</sub>), improving soil microbial activity by 38% and fruit production by 86%. Despite these initial advances, other aspects of agronomic management—such as sustainable weed control, which reduces environmental pollution and contributes to better soil health—require further research in calafate orchards.

Despite this background, there is no evidence of the effects of mulching on the soil, plant, and fruit of the calafate orchard. Therefore, the purpose of this study is to investigate the impacts on soil microbiological parameters, physiological aspects of the plant, and physicochemical aspects of the calafate fruit using different types of mulches, highlighting the geotextile, widely used in agriculture, as well as organic mulches from organic residues, such as hazelnut shells and oat straw. This research contributes to the development of sustainable management practices by using agricultural residues, with the purpose of establishing future organic calafate orchards.

## 2. Materials and Methods

### 2.1. Agronomic Management of the Orchard and Soil and Climatic Conditions

The establishment of the orchard was carried out in 2017 at the experimental station of the Adventist University of Chile located in the Ñuble Region, Chile (36°31' S; 71°54' W). Agronomic management was equally standardized for all treatments, according to Pinto-Morales et al. [20]. Water replenishment was carried out annually from October to April according to the criteria proposed by Betancur et al. [21]. The orchard (Figure 1a) has 352 plants (Figure 1b) that are established 1 m above the row and 3 m between rows.



**Figure 1.** (a) Establishment of different mulch treatments in the calafate orchard, 2017; (b) flowering calafate plant with oat straw mulch, 2019.

The soil of the calafate orchard was Andisol order (Melanoxerand) [22] and was characterized by chemical soil analysis at the time of establishment. The results of soil chemical analysis (0–20 cm) are shown in Supplementary S1.

The climate of the Ñuble Region is temperate Mediterranean. For the growing seasons 2020–2021 and 2021–2022, the mean annual temperatures were 13.5 and 13.2 °C, respectively, and accumulated precipitation was 920 mm and 650 mm, respectively (accumulated monthly temperatures and precipitation for 2021 and 2022 in the Ñuble Region can be found in Supplementary S2).

### 2.2. Experimental Setup

In 2017, four treatments were established: a control treatment without mulch (CK), geotextile mulch (GEO), oat straw mulch (OAT), and hazelnut shell mulch (HAZ). The mulches of organic origin, oat straw and hazelnut shell (chemical analysis of oat straw and hazelnut shell mulches, shown in Supplementary S3), were applied to the soil with a thickness of 10 cm and a coverage area of 1 m radius around each plant. HAZ was applied only once at initiation, GEO was installed only once at initiation, and OAT was renewed on an annual basis.

The ground cover model geotextile mulch (Polytex, Santiago, Chile) was composed of black polypropylene with 98% absence of light and 100 µm thickness. The statistical design used was a randomized complete block design with four treatments and four replicates ( $n = 16$ ). In turn, each replicate consisted of the average of two plants that were evaluated independently. The results obtained from the two plants were averaged to obtain each replicate for each treatment.

### 2.3. Weeds, Soil Moisture, Temperature of Mulch, and Leaves

The dry weight of weeds was determined by weighing those growing at a 1 m radius from each plant, and the weeds were subsequently dried in a BOV-VF Series forced air oven (BIOBASE, Jinan, China) at 105 °C for 72 h.

Volumetric soil water content (%) was determined by averaging three measurements taken 1 d after each irrigation during December 2021, at a depth of 0.2 m with a Diviner 2000 portable soil moisture probe (Sentek, Stepney, Australia).

Leaf and mulch surface temperature (°C) was determined with a portable infrared thermometer CTR1000 (Instrumentos WIKA S.A.U., Barcelona, Spain) at four times of the day (09:00, 12:00, 15:00, and 18:00 h) in December 2021.

#### 2.4. Microbiological and Chemical Soil Analysis

Soil samples for microbiological and chemical analyses were collected on 21 October at a depth of 0–20 cm [21]. Samples were stored at  $-20^{\circ}\text{C}$  for analysis and were subsequently sieved at 2 mm and conditioned to 60% field capacity. For soil microbiological analyses, four technical replicates per treatment and replicate were used.

Fluorescein diacetate (FDA) activity [23] and basal soil respiration [24] were estimated, expressed as  $\mu\text{g FDA g}^{-1}$  and  $\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ , respectively. Urease [25], dehydrogenase [26], and acid phosphatase [27] enzyme activities were estimated. Urease and acid phosphatase were expressed as  $\mu\text{mol ammonium (NH}_4^+)$  and *p*-nitrophenol (PNP) per gram of soil (dry weight) per hour (h), respectively. In contrast, dehydrogenase was expressed as  $\mu\text{g iodionitrotetrazoliumformazan (INTF)}$  per gram of soil (dry weight).

#### 2.5. Plant Physiological Measurements

Physiological measurements were made on 12 January 2021, using as criteria plants and leaves exposed to the sun and from the second third of the season's shoot.

Leaf area index (LAI;  $\text{m}^2 \text{ m}^{-2}$ ) was measured at 12:00 h, with an AccuPAR LP-80 ceptometer (Decagon Devices Inc., Washington, DC, USA).

The maximum quantum yield of photosystem II ( $F_v/F_m$ ) and stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{ s}^{-1}$ ) were measured at four times of the day (09:00, 12:00, 15:00, and 18:00 h), with a portable fluorometer OS-5p (Opti-Sciences, Hudson, NH, USA), adapting the leaves to darkness for 30 min and with a portable porometer equipment model SC-1 (Decagon Devices, Washington, DC, USA), respectively [28,29].

#### 2.6. Yield and Physical Fruit Measurements

Yield and physical parameters of fruit were measured immediately after harvest on 30 December 2021, by weighing total fruit per plant and individually ( $n = 10$ ) with a Precisa Gravimetrics 360 ES Series analytical balance (Precisa Gravimetrics AG, Dietikon, Switzerland) and measuring polar and equatorial diameter (mm) ( $n = 10$ ) with a digital meter foot model E5001002  $\pm 0.003$  mm (Veto Y Cia Ltd., Santiago, Chile).

#### 2.7. Soluble Solids and Antioxidant Properties of the Fruit

The concentration of soluble solids (°Brix) and antioxidant properties of the fruit were evaluated after harvest [21]. For the chemical analysis of the fruit, four technical replicates per treatment and repetition were used.

Soluble solids concentration (°Brix) was measured by extracting a random sample of fresh fruit ( $n = 4$ ) from each total yield per plant, which was squeezed to obtain two drops of liquid. The liquid was measured in a digital refractometer model HI96801  $\pm 0.2\%$  (Hanna Instruments S.R.L., Woonsocket, RI, USA). Total polyphenol content was measured using the Folin–Ciocalteu reagent, as described by [30]. The absorbance was measured at 760 nm and the results were expressed as mg gallic acid 100  $\text{g}^{-1}$  FW [31]. The antioxidant capacity of DPPH was performed as suggested by Romero-Román et al. [32]. The absorbance readings were performed at 515 nm. ORAC antioxidant capacity was determined as suggested by Romero-Román et al. [33] with excitation and emission wavelengths of 485 and 520 nm. The results of fruit antioxidant capacity were expressed as  $\mu\text{mol Trolox equivalent (TE)}$  per 100 g fresh fruit (FW) [33,34]. Individual anthocyanins were quantified with high-performance liquid chromatography (HPLC-DAD), as suggested by Romero-Román et al. [33], and the results were expressed as mg 100  $\text{g}^{-1}$  FW [34].

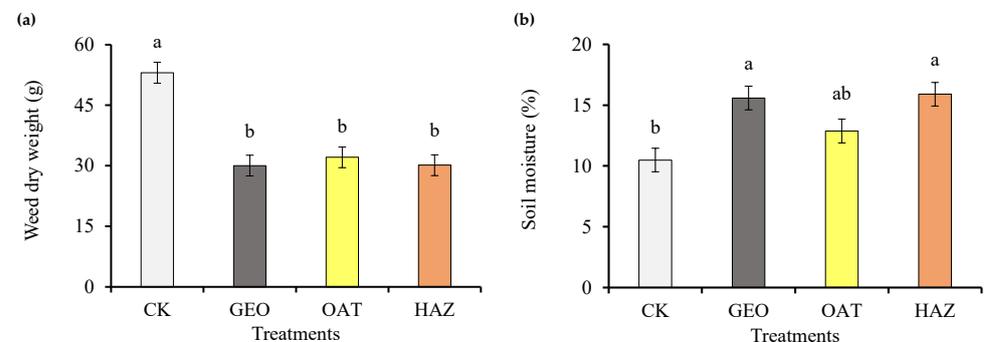
## 2.8. Statistical Analysis

The results were subjected to analysis of variance (ANOVA), where the comparison of means was performed with Fisher's least significant difference (LSD) test with a significance of 0.05. Principal component analysis (PCA) and analysis of correlations between soil–plant–fruit variables were performed, and the data were processed with R Studio software 4.2.1 [35] using the packages FactoMineR and ggplotOAT [36], focusing on the mean based on eigenvalues.

## 3. Results

### 3.1. Weeds, Soil Moisture and Temperature, and Leaf Temperature

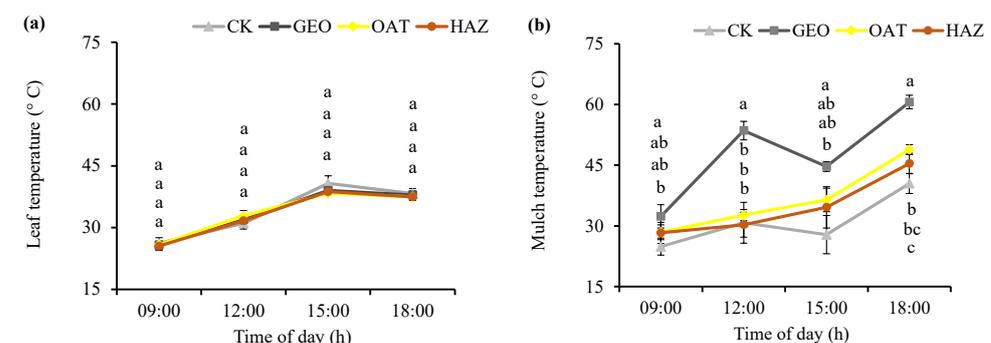
Weed weight (Figure 2a) was 43% higher ( $p < 0.05$ ) in the control treatment (CK) with respect to the other treatments, with no differences between them ( $p > 0.05$ ). The main weeds were monocotyledonous weeds of the *Poaceae* family, such as *Paspalum vaginatum* Sw. and *Avena fatua* L., and, to a lesser extent, dicotyledonous weeds of the *Asteraceae* and *Boraginaceae* families, such as *Centaurea solstitialis* L. and *Echium vulgare* L., respectively.



**Figure 2.** (a) Measurement of weed dry weight (g) harvested from different mulch treatments. (b) Measurement of soil moisture (%) from different mulch treatments. Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). Bars correspond to experimental error for each treatment.

Soil moisture (Figure 2b) had a significant effect ( $p < 0.05$ ) with GEO and HAZ treatments at 15.8%, compared with CK at 10.5%. However, no significant difference was observed in OAT treatments.

Leaf temperature (Figure 3a) did not show significant differences among treatments ( $p > 0.05$ ) at different times of the day but increased to 40 °C at 15:00 h and decreased slightly to 38 °C at 18:00 h. Mulch temperature (Figure 3b) increased significantly ( $p < 0.05$ ) with GEO treatment compared with the other treatments, reaching 54 and 61 °C at 12:00 and 18:00 h, respectively. On the other hand, the CK treatment generally presented the lowest temperature at different measurement hours with a minimum value of 26 °C at 09:00 h.



**Figure 3.** Measurement of calafate leaf temperature (°C) at four times of the day: 09:00, 12:00, 15:00, and 18:00 h. (a) Measurement of mulch surface temperature (°C) at four times of the day: 09:00,

12:00, 15:00, and 18:00 h. (b) Measurement of mulch surface temperature ( $^{\circ}\text{C}$ ) at four times of the day: 09:00, 12:00, 15:00, and 18:00 h. Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). The bars correspond to the experimental error for each treatment.

### 3.2. Soil Microbiological and Chemical Parameters

Soil microbial activity (Table 1) increased significantly ( $p < 0.05$ ) with HAZ treatment, and was 40% higher than that of CK. Likewise, basal soil respiration (Table 1) increased significantly with HAZ with respect to the other treatments, reaching  $1.9 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ . GEO presented the lowest respiration with  $1.0 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ .

**Table 1.** Soil microbiological properties and enzyme activity in response to mulch.

Treatments	Microbial Activity	Soil Basal Respiration	Urease Activity	Dehydrogenase Activity	Acid Phosphatase Activity
	( $\mu\text{g FDA g}^{-1}$ )	( $\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ )	( $\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ h}^{-1}$ )	( $\mu\text{g INTF g}^{-1}$ )	( $\mu\text{mol PNP g}^{-1} \text{ h}^{-1}$ )
CK	$33.38 \pm 3.20 \text{ b}$	$1.32 \pm 0.10 \text{ bc}$	$1.25 \pm 0.04 \text{ c}$	$36.36 \pm 6.10 \text{ a}$	$27.29 \pm 2.36 \text{ a}$
GEO	$41.42 \pm 7.31 \text{ ab}$	$0.98 \pm 0.16 \text{ c}$	$1.34 \pm 0.01 \text{ bc}$	$38.96 \pm 3.95 \text{ a}$	$17.57 \pm 2.03 \text{ b}$
OAT	$49.75 \pm 3.71 \text{ ab}$	$1.46 \pm 0.15 \text{ b}$	$1.44 \pm 0.04 \text{ ab}$	$32.36 \pm 2.60 \text{ a}$	$17.28 \pm 1.89 \text{ b}$
HAZ	$55.75 \pm 7.99 \text{ a}$	$1.90 \pm 0.15 \text{ a}$	$1.48 \pm 0.05 \text{ a}$	$29.16 \pm 1.39 \text{ a}$	$19.66 \pm 2.10 \text{ b}$
Anova <i>p</i> values	0.0095	0.0053	0.0063	0.3462	0.0181

Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). FDA: Fluorescein diacetate activity, INFT: Iodonitrotetrazoliumformazan, PNP: *p*-nitrophenol.

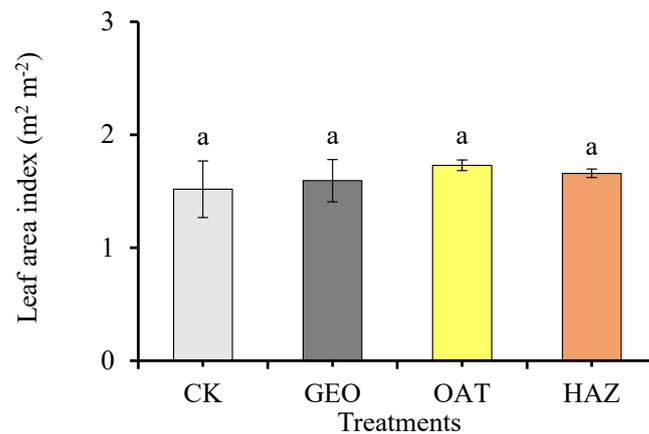
Soil enzyme activity showed different responses to mulching treatments. In this sense, urease activity increased significantly in HAZ treatment by 20% and 13% compared with CK and GEO, respectively. Dehydrogenase activity with a mean of  $34.21 \mu\text{g INTF g}^{-1}$  showed no differences among treatments. Acid phosphatase activity was significantly higher in the CK treatment and was 33% higher than that of the mulch treatments.

Chemical analysis of the soils at the end of the experiment compared with the beginning of the study indicated that MO increased by 2.8% with the OAT treatment. Chemical analysis of the soil (0–20 cm) affected by the compost treatments at the end of the study is shown in Supplementary S4. The pH with mulch treatments (GEO, OAT, and HAZ) increased by an average of 2.3% compared with CK. There was 46% higher N availability in the CK treatment compared with the GEO and HAZ treatments. The availability of S and exchangeable Ca reached the highest value in GEO with  $34.5 \text{ mg kg}^{-1}$  and  $9.19 \text{ cmol}_+ \text{ kg}^{-1}$ , respectively. The K availability was 31.9% higher in OAT than in CK. In all treatments, the availability of micronutrients such as Zn, Fe, and Mn tended to decrease toward the end of the study compared with the beginning of the study. There was, on average, 31.5% and 28.5% higher availability of Cu and Mn, respectively, with the mulched treatments (GEO, OAT, and HAZ) compared with the control.

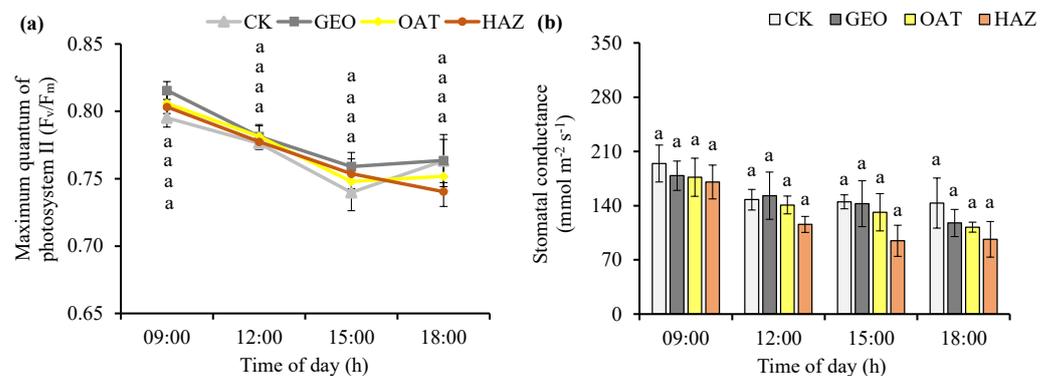
### 3.3. Plant Physiological Parameters

The leaf area index (LAI) (Figure 4) with a mean value of  $1.6 \text{ m}^2 \text{ m}^{-2}$  did not show significant differences between treatments ( $p > 0.05$ ).

The maximum quantum yield of photosystem II ( $F_v/F_m$ ) (Figure 5a) did not show significant differences ( $p > 0.05$ ) among treatments, with a decreasing trend as the day progressed until an average value of 0.75 at 18:00 h. Stomatal conductance (Figure 5b) did not show significant differences between treatments. The average values of stomatal conductance were 180, 139, 128, and  $117 \text{ mmol m}^{-2} \text{ s}^{-1}$  at 09:00, 12:00, 15:00, and 18:00 h, respectively.



**Figure 4.** Leaf area index in calafate plants. Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fisch-er's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). The bars correspond to the experimental error for each treatment.



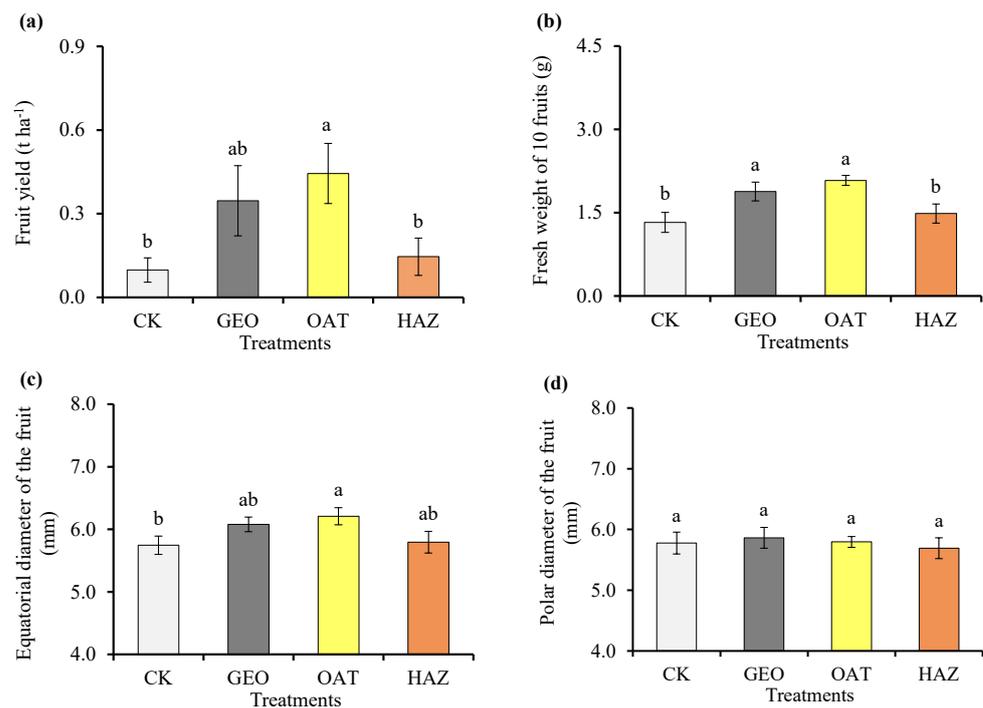
**Figure 5.** Variation of maximum quantum yield of photosystem II ( $F_v/F_m$ ) (a) and values recorded for stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) in calafate plants (b); evaluated at different times of the day: 09:00, 12:00, 15:00, and 18:00 h. Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fisch-er's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). The bars correspond to the experimental error for each treatment.

### 3.4. Yield and Physical Parameters of the Fruit

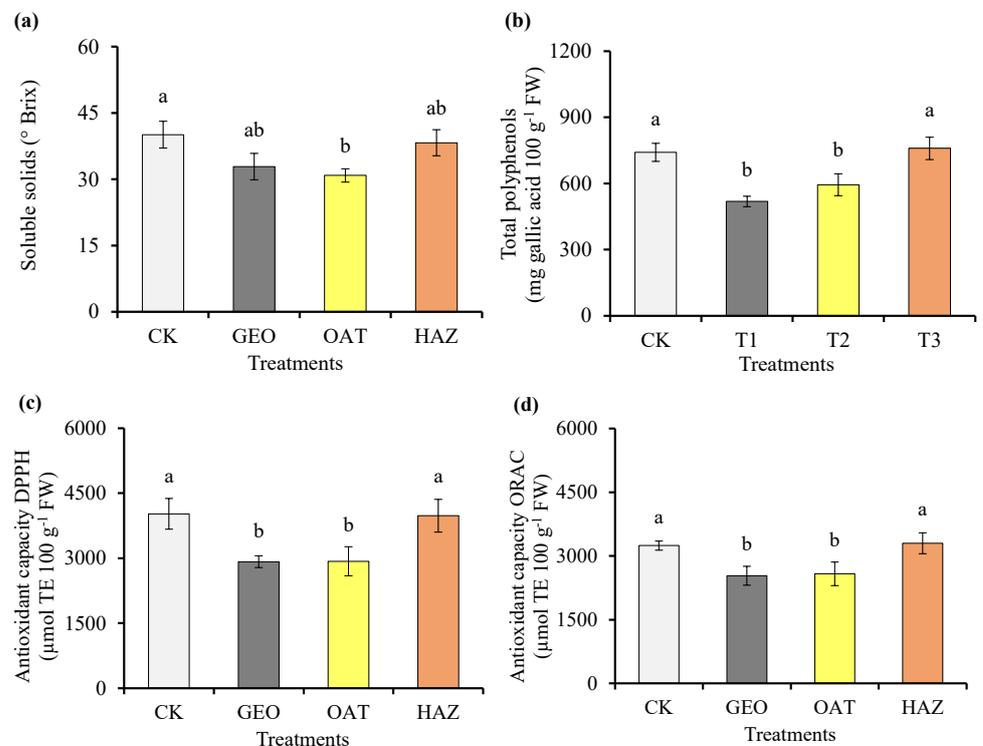
Fruit yield (Figure 6a) increased significantly ( $p < 0.05$ ) with the OAT treatment, which presented  $0.4 \text{ t ha}^{-1}$  compared with the CK and HAZ treatments, which presented  $0.1$  and  $0.2 \text{ t ha}^{-1}$ , respectively. Likewise, OAT presented a higher fruit weight (Figure 6b) of  $1.89 \text{ g}$  and an equatorial diameter (Figure 6c) of  $6.2 \text{ mm}$  compared with the control, but there were no significant changes ( $p > 0.05$ ) in the polar diameter of the fruit (Figure 6d).

### 3.5. Soluble Solids and Antioxidant Parameters of the Fruit

Soluble solids (Figure 7a) increased significantly ( $p < 0.05$ ) with the CK treatment, which presented  $40^\circ \text{Brix}$ , compared with  $31^\circ \text{Brix}$  in OAT. Total polyphenols (Figure 7b) were significantly higher in the CK and HAZ treatments with values  $741$  and  $760 \text{ mg gallic acid } 100 \text{ g}^{-1} \text{ FW}$ , respectively. Likewise, the antioxidant activity DPPH (Figure 7c) and ORAC (Figure 7d) were significantly higher ( $p < 0.05$ ) in the CK and HAZ treatments, with values of  $4021$  and  $3981 \text{ } \mu\text{mol TE } 100 \text{ g}^{-1} \text{ FW}$ , respectively, for DPPH, and with values of  $3245$  and  $3299 \text{ } \mu\text{mol TE } 100 \text{ g}^{-1}$ , respectively, for ORAC.



**Figure 6.** Average fresh fruit yield of calafate (a); average weight of 10 fresh fruits (b); average equatorial diameter (c); average polar diameter of fresh fruit (d). Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). The bars correspond to the experimental error for each treatment.



**Figure 7.** Soluble solids (a); total polyphenols (b); DPPH antioxidant capacity (c); and ORAC antioxidant capacity (d) determined from fresh fruit of calafate plants. Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ). The bars correspond to the experimental error for each treatment.

The total anthocyanin concentration (Table 2) was significantly higher ( $p < 0.05$ ) for the CK treatment with  $461.2 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$  compared with the OAT treatment that presented  $319.1 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$ . Total anthocyanins were 86%, 82%, 83%, and 85% in the CK, GEO, OAT, and HAZ treatments, respectively, represented by the anthocyanins delphinidin, petunidin, and malvidin 3-glucoside.

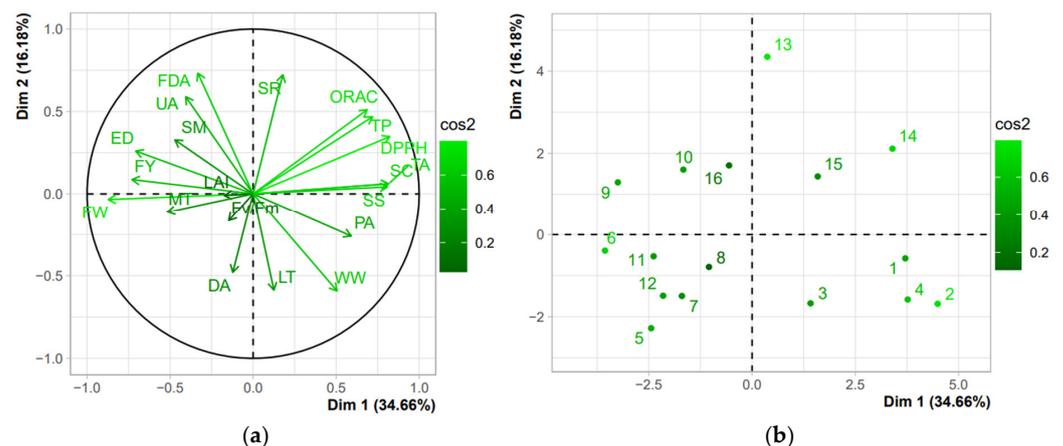
**Table 2.** Anthocyanins ( $\text{mg } 100 \text{ g}^{-1}$ ) of fresh calafate fruit by HPLC.

Anthocyanins	Treatments				Anova $p$ Values
	CK	GEO	OAT	HAZ	
Delphinidin 3,3-dihexoside	$2.4 \pm 0.6 \text{ a}$	$1.8 \pm 0.4 \text{ a}$	$2.2 \pm 0.2 \text{ a}$	$2.3 \pm 0.4 \text{ a}$	0.8147
Petunidin 3,5-dihexoside	$9.4 \pm 0.4 \text{ a}$	$9.8 \pm 2.1 \text{ a}$	$10.4 \pm 3.0 \text{ a}$	$8.6 \pm 1.5 \text{ a}$	0.9253
Malvidin 3,5-dihexoside	$5.8 \pm 0.2 \text{ b}$	$8.7 \pm 1.1 \text{ ab}$	$8.2 \pm 2.3 \text{ ab}$	$11.5 \pm 0.8 \text{ a}$	0.0446
Delphinidin 3-glucoside	$190.6 \pm 28.8 \text{ a}$	$109.3 \pm 14.9 \text{ b}$	$105.6 \pm 20.5 \text{ b}$	$137.3 \pm 11.9 \text{ ab}$	0.0395
Delphinidin 3-rutinoside	$1.7 \pm 0.3 \text{ a}$	$1.5 \pm 0.3 \text{ ab}$	$1.0 \pm 0.2 \text{ b}$	$1.7 \pm 0.3 \text{ ab}$	0.0371
Cyanidin 3-glucoside	$29.7 \pm 1.3 \text{ a}$	$20.5 \pm 2.7 \text{ a}$	$20.5 \pm 3.3 \text{ a}$	$22.4 \pm 4.6 \text{ a}$	0.1939
Petunidin 3-glucoside	$133.9 \pm 12.6 \text{ a}$	$99.3 \pm 8.2 \text{ bc}$	$92.0 \pm 12.0 \text{ c}$	$124.6 \pm 6.9 \text{ ab}$	0.0380
Petunidin 3-rutinoside	$3.1 \pm 0.2 \text{ a}$	$2.1 \pm 0.6 \text{ ab}$	$1.1 \pm 0.1 \text{ b}$	$2.1 \pm 0.4 \text{ ab}$	0.0239
Peonidin 3-glucoside	$11.9 \pm 0.7 \text{ a}$	$20.9 \pm 3.7 \text{ a}$	$10.2 \pm 1.2 \text{ a}$	$13.2 \pm 1.7 \text{ a}$	0.2730
Malvidin 3-glucoside	$72.7 \pm 3.8 \text{ a}$	$79.4 \pm 18.8 \text{ a}$	$67.8 \pm 1.7 \text{ a}$	$98.5 \pm 9.9 \text{ a}$	0.2553
Total anthocyanins	$461.2 \pm 46.0 \text{ a}$	$353.3 \pm 27.8 \text{ ab}$	$319.1 \pm 39.0 \text{ b}$	$422.2 \pm 28.3 \text{ ab}$	0.0439

Treatments: CK: no mulch, GEO: geotextile mulch, OAT: oat straw mulch, and HAZ: hazelnut shell mulch. Different lowercase letters indicate significant differences between treatments according to Fischer’s LSD test ( $p < 0.05$ ). Mean  $\pm$  standard error ( $n = 4$ ).

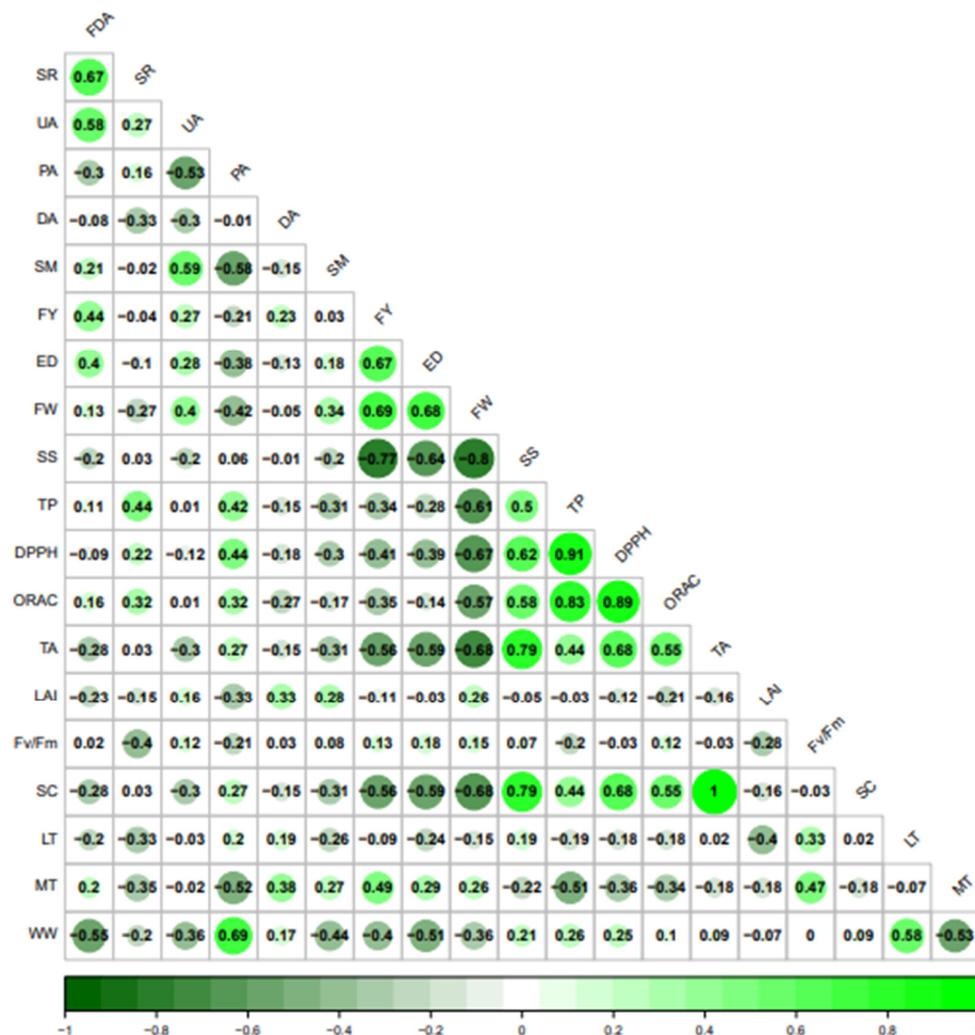
### 3.6. Soil–Plant–Fruit Parameter Interaction

Of the 20 parameters evaluated (Figure 8), the principal components PC1 and PC2 retained 34.66% and 16.18% of the variance, respectively. In the biplot, each parameter is represented as a vector whose length indicates its importance in the analysis (Figure 8a). Treatments are represented by numbers in the PCA (Figure 8b): 1–4 for CK, 5–8 for GEO, 9–12 for OAT, and 13–16 for HAZ.



**Figure 8.** Principal component analysis (PCA) of variables (a) and individuals (b) of soil microbiological, plant physiological, and physicochemical variables of fruit. Soil microbiological activity (FDA); soil respiration (SR); urease activity (UA), dehydrogenase activity (DA); acid phosphatase activity (PA); leaf temperature (LT); leaf area index (LAI); stomatal conductance (SC); maximum photosystem II efficiency (Fv/Fm); fruit productivity (FY); fruit equatorial diameter (ED); fruit fresh weight (FW); soluble solids (SS); total polyphenols (TP); DPPH antioxidant activity (DPPH); ORAC antioxidant activity (ORAC); total anthocyanins (TA); soil moisture (SM); mulch temperature (MT); weed dry weight (WW).

Correlations among soil, plant, and fruit (Figure 9) were analyzed using Pearson’s correlation coefficients ( $r$ ). Moderate correlations ( $r > 0.59$  or  $r < -0.59$ ) were observed among soil biological indicators. There were moderate relationships between environmental and soil biological variables ( $r > 0.5$  or  $r < -0.5$ ). Plant physiological variables interacted moderately with plant yield ( $r < -0.5$ ), but there was a higher relationship with soluble solids ( $r > 0.7$ ) and DPPH antioxidant activity ( $r > 0.6$ ). Fruit physical parameters had a high relationship with chemical attributes ( $r > -0.8$ ) and also between the latter ( $r > 0.5$  or  $r < 0.8$ ). In addition, the correlation matrix corroborated the closeness between variables demonstrated in the PCAs (Figure 8a,b).



**Figure 9.** Correlation matrix for soil variables, plant physiological parameters, yield parameters, and fruit bioactive compounds. Soil microbiological activity (FDA); soil respiration (SR); urease activity (UA), dehydrogenase activity (DA); acid phosphatase activity (PA); leaf temperature (LT); leaf area index (LAI); stomatal conductance (SC); maximum photosystem II efficiency (Fv/Fm); fruit productivity (FY); fruit equatorial diameter (ED); fruit fresh weight (FW); soluble solids (SS); total polyphenols (TP); DPPH antioxidant activity (DPPH); ORAC antioxidant activity (ORAC); total anthocyanins (TA); soil moisture (SM); mulch temperature (MT); weed dry weight (WW).

#### 4. Discussion

##### 4.1. Weeds, Soil Moisture and Temperature, and Leaf Temperature

In our study, different types of mulches showed a significant effect on weed suppression compared with the control. This reaffirms the ability of mulches, regardless of the material used, to exert a physical effect on weed suppression and weed seed germina-

tion [37]. It is important to note that both GEO and HAZ mulches showed greater efficacy in soil moisture retention. This retention capacity is directly related to the less permeable composition of these materials, which act as an effective barrier to soil water vapor losses [38]. Also, it is likely that HAZ mulching led to increased soil aggregation, reduced bulk density, and the appearance of larger pore spaces, increasing water retention [39]. In contrast, the CK and OAT treatments, which did not show significant differences between them, showed a lower capacity to retain moisture. This may be attributed to the higher permeability and degradability of OAT [11], as well as the relatively lower capacity of weeds to retain moisture in the case of CK [40].

As for leaf temperature, the results indicate that the different mulches did not have a significant effect. This suggests that the ambient temperature in the study area, with maximums of up to 30 °C at the time of the measurements, may not have been a determining factor in this parameter [41]. However, when analyzing the soil surface temperature, it was observed that the GEO mulch showed a higher temperature compared with the control, while it presented similarity in this aspect with the other mulching treatments. These findings support the inherent ability of mulches to absorb a wide range of visible and infrared wavelengths of solar radiation, leading to an increase in soil surface temperature due to heat emission [8]. Importantly, despite the similarities in solar radiation absorption capacity among the different mulches, the GEO mulch showed a tendency to record higher temperatures than the OAT and HAZ mulches, especially during midday hours and at the end of the evaluation period. This pattern suggests that the black color of the GEO mulch plays a key role in absorbing solar radiation and, thus, heat emission [42,43].

Our results demonstrate that HAZ mulch emerges as an effective option for weed control, maintaining greater moisture reserves and moderate soil temperatures compared with soil without mulch. Furthermore, these comparative benefits highlight it in relation to the other mulches evaluated in this study. We emphasize the need to evaluate other types of low-degradability agricultural residue mulches that contribute to maintaining adequate soil temperature and moisture levels in calafate orchards.

#### 4.2. Soil Microbiological and Chemical Parameters

The results of our investigation show that there was higher fluorescein diacetate (FDA) activity in HAZ. However, the other mulches did not generate significant changes in FDA activity. It is important to mention that the FDA is considered an indicator of hydrolysis carried out by living microorganisms in the soil [24]. It is plausible that after a period of 5 years since the implementation of HAZ, the convergence of environmental factors such as soil moisture and temperature may have contributed to the increase in live microbial biomass and, therefore, higher microbial activity observed. Similar findings were presented by Huang et al. [14] who evidenced an increase in soil microbial activity due to higher soil aeration, temperature, and moisture, after the application of woody mulch from Acacia trees. Likewise, it was indicated in a peach orchard (*Prunus persica* L. Batsch) that organic pine needle mulch, which regulated soil moisture and temperature regimes, significantly improved microbial activity and microbial biomass carbon [44]. It is likely that the use of organic mulch increased soil water content by reducing soil evaporation and, in turn, by increasing water storage and infiltration capacity [45]. Given the increase in FDA activity observed in the HAZ treatment after a 5-year period, it would be beneficial to conduct longer-term studies to understand how the effects of different mulch types on soil microbial activity evolve over time.

On the other hand, in our study, OAT, despite having induced an adequate soil temperature, caused greater soil moisture loss. It is likely that this reduction in moisture in turn decreased the abundance, distribution, and activity of soil microorganisms [46]. Meanwhile, GEO, despite increasing soil moisture, generated increased temperatures above the ambient temperature, which negatively affects microbial growth [6]. This condition could also explain the lower basal soil respiration rate observed in this treatment. In contrast, higher basal respiration was observed in HAZ, which is congruent with the

previously mentioned results. Basal respiration in OAT was significantly higher compared with GEO. This suggests that mulches derived from readily decomposable materials, such as oat straw, may promote a higher basal respiration rate. This could be attributed to the contribution of new microbial communities from OAT that enrich native soil bacterial diversity [13], as well as soil structural improvements in bulk density and proportion of larger soil aggregates that favor soil microclimate and microbial action [47]. However, we emphasize the need to investigate alterations in microbial diversity and composition, which would provide more background on the mechanisms involved in the response to the types of mulches applied.

Urease activity increased significantly with HAZ and OAT compared with CK. This increase could be explained by the strong correlation of this enzyme with soil microbial biomass content [48]. It is important to highlight that the activity of this enzyme may also be related to the activity of extracellular enzyme-organic complexes, which are influenced by the organic amendments applied [49]. On the other hand, the lower urease activity in CK is consistent with the higher nitrate levels present toward the end of the study, which may inhibit the action of this enzyme through repression of microbial synthesis [50]. It should be noted that urease is involved in urea hydrolysis and would not explain nitrogen mineralization [49], suggesting that its understanding requires further investigation. The mulches used in our study showed a nonsignificant impact on dehydrogenase activity, which is responsible for the oxidation of organic carbon in the soil [51]. According to the literature, the incorporation of organic compounds into the soil generates a high response due to the formation of organo-enzyme complexes, which improve their stability [49]. However, this response could be more pronounced in soils with low fertility, as previously observed [52]. Therefore, by presenting considerable levels of organic matter from the orchard establishment to the end of the study (9.7% at the time of orchard establishment and an average between treatments of 9.2% at the end of the study), our results suggest that the treatments with mulch and without mulch have reached a threshold in which the action of this enzyme is not significantly affected. On the other hand, acid phosphatase activity increased significantly with CK. This finding may be explained by the decrease in pH treatment, which may stimulate a higher activity of this enzyme [27]. It is likely that the soil under CK, lacking a physical barrier limiting exposure to precipitation in winter and constant irrigations in summer, experienced a reduction in pH due to a decrease in bases and an increase in anions such as nitrate [53].

In relation to the chemical composition of the soil, it is relevant to highlight that the HAZ treatment resulted in a decrease in OM after a period of 5 years from its incorporation, without it having been replenished during this period. This decrease could be related to the environmental conditions favored by this treatment, which favored an increase in active microbial biomass. As a consequence, it is possible that this increased biomass consumed more efficiently the C readily available in the soil, using this component as an energy substrate [54]. In contrast, the OAT treatment, due to its high degradability, experienced a more frequent release to the soil, requiring replenishment annually over the same 5-year period. This trend could explain the observed increase in MO in the OAT treatment. These findings are consistent with the observations of Von Lützow and Kögel-Knabner [55] who emphasize that the impact of temperature on the degradation rate of organic compounds may vary according to the type of material applied. It is worth mentioning that the OAT treatment also showed a positive effect on soil fertility, particularly on the availability of elements such as S, Ca, sum of bases, and CEC. A similar situation was demonstrated in a vineyard with straw mulch, in which the OM content and available P, K, Na, Mg, and S increased after 4 years [45]. It is important to note that the control treatment showed a significant accumulation of available nutrients, such as N and P, during the same evaluation period. This accumulation can be attributed to the annual recycling of the most abundant weeds in this treatment, which can contribute significantly to the improvement of soil fertility [5]. Our results demonstrate that the favorable soil microclimate provided by organic mulch covers, especially HAZ, dampens elevated temperatures and improves soil

moisture, which slows the decline of the bacterial population in the soil. In addition, our results are consistent with Micallef et al. [56] who indicated that organic mulch covers have the ability to modify the carbon and nitrogen fractions in the soil, which increases soil respiration and leads to changes in fungal and bacterial diversity.

Furthermore, our results support the benefits of the application of organic mulches such as oat straw despite its high degradability and lower efficacy in improving the evaluated soil biological properties, proved to be more beneficial than no mulching and geotextile mulching in terms of these properties. However, we highlight the need for longer-term research to better understand the sustainability of organic mulches on the stability and persistence of soil organic matter and nutrients.

#### 4.3. Plant Physiological Parameters

Mulches did not affect the leaf area. These results contrast with previous studies in pear and apple trees where polypropylene geotextile mulches for 4 years in pear trees and organic mulches of straw and pine bark for 3 years in apple trees positively impacted photosynthetic rate, stomatal conductance, and stimulated plant growth, due to improvements in environmental conditions, such as soil moisture and soil nutrition [57,58]. However, it has been shown that the leaf area index of locally cultivated calafate plants is favored by applications of organic, highly decomposable, and N-rich components, such as compost, at doses higher than  $10 \text{ t ha}^{-1}$  [20]. A similar situation occurred in an olive orchard where the easily decomposable animal manure mulch, high in N, had the greatest influence on plant morphophysiological traits [59]. It has also been shown that leaf area index can be negatively affected by extreme soil moisture deficiency (0%  $ET_0$  replenishment) [21], which did not occur in our study where irrigations were constant with 100%  $ET_0$  replenishment.

Regarding the maximum photosystem II yield and stomatal conductance, there were no differences with mulch applications. This suggests that initial soil fertility is adequate for the physiological functions of this species, which exhibits a high level of hardiness [60]. However, the general tendency to decrease as the day progressed indicates that the plant, when subjected to a higher intensity of environmental factors such as light or temperature, presents resistance strategies such as decreasing the maximum photosystem yield [28] or reducing the flow of gas exchange through the stomata [61].

These results indicate that the different types of mulches did not have a significant impact on the leaf area index and physiological parameters in the calafate plant. However, it is essential to emphasize the importance of conducting research over several years to achieve a more comprehensive understanding of their effects.

#### 4.4. Physicochemical Parameters of Fruits

The OAT treatment had a higher fruit yield compared with the HAZ and CK treatments. These results are in agreement with the higher fertility levels observed in the OAT treatment and the lower levels in HAZ. Soil fertility has been documented to be closely related to crop productivity, with K, which exhibited a higher presence in OAT, being one of the main components of calafate fruit [62]. Soil-available K that can be extracted by the plant improves fruit firmness, caliber, flavor, and aroma [62]. Our results are in agreement with previous literature, with cereal straw mulch being more effective in contributing to soil fertility and producing increases in yield, fruit size, and weight [63,64].

On the other hand, the HAZ treatment, despite showing higher soil biological activity, did not show a significant increase in fruit production. This could be related to the lower fertility and availability of nutrients in HAZ, which are essential factors for the production of wild and cultivated calafate [19,20]. In fact, this treatment reached a production level similar to the control (CK). On the other hand, fruit weight in the GEO treatment was higher than the control despite the suppression of soil biological activity caused by high temperature. However, it is relevant to mention that in the case of calafate, temperature accumulation has been pointed out as a requirement for the adequate growth of its roots [62]. It is known that roots play a crucial role in the absorption of nutrients and translocation of

organic compounds for fruit formation. This increase in yield due to the use of synthetic black mulch covers has been documented in other berries, such as grape, strawberry, raspberry, and blueberry [8].

In all treatments, a close correlation was observed between yield, fruit number, and equatorial diameter, which may be inherent to calafate since the same relationship was found in wild calafate from Central Southern Chile [65]. However, it is important to consider that this study covered only 1 year, so longer-term research is needed to obtain a deeper understanding of the effects of different covers on the yield of calafate.

#### 4.5. Soluble Solids and Antioxidant Parameters of the Fruit

The study revealed an inverse relationship between the soluble solid concentration and yield. Smaller fruit treatments, HAZ and CK, exhibited higher soluble solids or sugar concentrations due to reduced water content. In contrast, the larger fruit treatments, OAT and GEO, with higher yields showed lower sugar concentrations, similar to those reported in strawberries [66]. This trend was also reflected in the phenolic compounds of the fruit and their antioxidant capacity as has been previously reported in calafate berries [64]. Although OAT and GEO had lower phenolic values and antioxidant activity compared with HAZ and CK, they proved superior to berries such as blueberries and strawberries with a high potential to counteract oxidative stress [17].

Anthocyanin content was significantly lower with OAT compared with CK, but no significant differences were observed among other mulching treatments. This suggests that calafate plants, both with HAZ and GEO and with CK, produce fruit with high levels of anthocyanins, which contribute to intense tones in the fruit [67], relevant to the natural pigment industry [32]. All treatments showed three predominant anthocyanins: delphinidin, petunidin, and malvidin, which is in agreement with previous studies in cultivated [21] and wild [33] calafate. The prevalence of delphinidin, which represented 40% of total anthocyanins, was similar to that reported in blueberries [68], gaining relevance for its potent antioxidant properties attributed to a higher number of hydroxyl groups on the B-ring [69].

Our study provides evidence that the use of HAZ mulching significantly increases bioactive compounds, especially anthocyanins such as delphinidin (Table 2), and these higher levels of delphinidin generate darker fruits, which better counteracts cellular oxidative stress. Therefore, these findings are valuable for their benefits to health and the natural pigment industry. It is important to take into consideration that more years of study are required to address in depth the physical and chemical productivity of calafate as an excessive increase in yield may compromise its phenolic composition and antioxidant capacity for which it is renowned.

#### 4.6. Soil–Plant–Fruit Parameter Interaction

The moderate retentions in the principal components PC1 with 34.66% and PC2 with 16.18% contribute to improving the understanding of the interaction of soil, plant, and fruit variables. The moderate correlations between environmental and soil microbiological parameters demonstrate that the use of mulch promotes soil microbial and enzymatic activity through improvements in environmental conditions, which is consistent with previously reported [4,12]. Moderate correlations between plant parameters with fruit yield demonstrate that mulching contributes to fruit yield increases without affecting plant morphophysiology as has been demonstrated in other berry species, such as grapevine [10]. However, the highest correlations between physical parameters and fruit bioactive compounds demonstrate that increases in fruit yield due to mulching lead to a decrease in phenolic and antioxidant content of the fruit, a situation that has been corroborated in berries such as grapes and strawberries [7,9]. This study represents a significant advance in the evaluation of mulch used in calafate orchards, analyzing in an integrative manner a total of 20 variables, which is higher than the 13 in previous research in cultivated calafate [21]; however, due to their moderate interrelationship, they should be analyzed in depth.

## 5. Conclusions

Our research highlights the benefits of mulch for the soil, plant, and fruit of cultivated calafate. The results show that mulch, regardless of the material, has high effectiveness in the control of weeds, up to 43%, compared with the soil without mulch. In particular, hazelnut shell (HAZ) mulch emerges as a low degradable soil microbiological enhancer, significantly increasing soil microbial activity, basal respiration, and urease enzyme activity by 40%, 31%, and 20%, respectively, compared with the control (CK). However, we see the need to investigate alterations in microbial diversity and composition. On the other hand, the highly degradable oat straw (OAT) mulch generates increases of 8% in OM and 31% in P available compared with CK. Long-term research would help to better understand the sustainability of mulches in changing soil OM and nutrient levels. Despite these results, the physiology and morphology of the calafate plant were not affected. The caulk without mulch, in itself, is a plant with a high phenolic content, but with the use of OAT and geotextile (GEO) mulches, this decreases significantly. However, HAZ mulch is capable of maintaining adequate levels of total polyphenols, anthocyanins, and antioxidant capacity with values of 760 mg gallic acid 100 g<sup>-1</sup> FW, 422 mg 100 g<sup>-1</sup> FW, and 3299 μmol TE 100 g<sup>-1</sup>, respectively. We highlight the need to evaluate these parameters in a comprehensive way for a longer time since the changes in yield caused by this type of management can compromise the bioactive composition of the calafate fruit. On the other hand, we recommend the use of HAZ mulch, as an innovative strategy to enhance the bioactive compounds of the calafate fruit through efficient, environmentally conscious soil management.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae9111202/s1>, S1: Soil chemical analysis (0–40 cm) of the study site in the Ñuble Region of Chile; S2: Monthly accumulated temperatures and precipitation during the evaluation seasons (2021 and 2022) in the Ñuble Region, Chile; S3: Chemical analysis of oat straw and hazelnut shell mulches; S4: Soil chemical analysis at end of study.

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**Data Availability Statement:** The data presented in this study are available in the article.

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