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Physiological and Biochemical Responses of 'Burlat' Sweet Cherry to Pre-Harvest Foliar Application of Calcium and Seaweed Extracts

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Abstract: Sweet cherry (*Prunus avium* L.) is a highly valued fruit, and optimal nutrient management is crucial for enhancing yield and fruit quality. However, the over-application of chemical fertilizers in cherry cultivation leads to environmental issues such as soil degradation and nutrient runoff. To address this, foliar application, a more targeted and eco-friendly fertilization method, presents a promising alternative. This study evaluates the effects of pre-harvest foliar application of calcium (Ca) (150 and 300 g hL^{-1}) and seaweed extracts (75 and 150 mL hL^{-1}), both individually and in combination, on the physiological and biochemical responses of 'Burlat' sweet cherry trees. Key physiological parameters, including plant water status, photosynthetic performance, and leaf metabolites, were analyzed. Results show that trees treated with seaweed extracts or with combined Ca and seaweed application had improved water status, higher sugar, starch, and protein content, as well as enhanced antioxidant activity and phenolic content compared to those treated solely with calcium. However, the combined treatment did not significantly enhance overall tree performance compared to individual applications. This study highlights the potential of seaweed-based biostimulants in sustainable cherry production.

Keywords: antioxidant activity; foliar analysis; leaf metabolites; photosynthetic performance; water status

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1. Introduction

Sweet cherry (*Prunus avium* L.) is a highly valued non-climacteric fruit known for its taste, attractive appearance, and nutritional quality, making it increasingly popular worldwide [1]. Growing consumer demand and high market value have pushed producers to intensify cultivation practices to maximize yield and fruit quality. However, despite advances in nutrient management knowledge, many cherry growers continue to over-apply chemical fertilizers. These excessive inputs often surpass the trees' actual needs, causing nutrient imbalances and inefficiencies that do not meaningfully enhance crop performance [2]. The environmental consequences of over-fertilization include nutrient leaching, soil degradation, and contamination of nearby water bodies, contributing to broader issues like eutrophication and biodiversity loss [2].

To address these challenges, foliar fertilization has emerged as a promising alternative to traditional soil-based approaches, allowing nutrients to be absorbed directly by the leaves and enhancing uptake efficiency while reducing nutrient waste and environmental impact [3]. Foliar applications are also increasingly employed to deliver

biostimulants—substances that improve plant growth, nutrient absorption, and stress resilience, further decreasing reliance on chemical fertilizers [4].

Among biostimulants, calcium (Ca) and seaweed extracts are particularly effective in improving physiological and biochemical responses in fruit crops. Ca plays an essential role in plant cell structure and tissue strength [5], with foliar Ca sprays widely used in fruit crops to enhance firmness, color, and reduce physiological disorders such as fruit cracking, especially in cherries and apples [6,7]. In cherries, Ca application is effective in reducing fruit splitting and enhancing resistance to handling and transport [8,9].

Seaweed extracts are increasingly recognized as powerful biostimulants due to their high concentration of biologically active compounds, including plant hormones, amino acids, polysaccharides, vitamins, and phenolic compounds [10–13]. Applied as a foliar spray, seaweed extracts have been shown to stimulate plant growth, increase nutrient uptake, and enhance fruit quality by bolstering plant resilience to abiotic stresses such as drought, heat, and salinity [14]. In cherry production, seaweed-based biostimulants have been associated with reduced fruit cracking, improved skin quality, and greater overall plant vigor [15–18].

Optimizing leaf physiology is crucial for plant productivity, as leaves are the primary site of photosynthesis, transpiration, and nutrient uptake [19]. In cherries, where yield and quality are key, enhancing leaf function supports overall plant health and resilience [20]. Photosynthetic efficiency is closely linked to carbohydrate production essential for growth and fruit development [20], while leaf water status and stomatal regulation play a vital role in balancing water conservation and gas exchange, influencing both stress tolerance and photosynthetic performance [21].

In recent years, biostimulants have become an effective strategy to improve leaf physiological performance in cherry trees. Natural biostimulants like seaweed extracts contain bioactive compounds that influence leaf physiology by enhancing processes such as nutrient absorption, photosynthetic pigment synthesis, and stress resilience [16]. Studies in cherry trees indicate that biostimulant applications can increase antioxidant activity and reduce oxidative stress, supporting growth and fruit quality [17,18]. Seaweed products, which are rich in plant hormones, vitamins, and amino acids, have been shown to regulate stomatal conductance and improve water use efficiency, which is especially beneficial under drought stress [16,22].

Moreover, foliar applications of biostimulants can enhance leaf biochemical properties, including the accumulation of sugars, starch, and proteins critical for cellular health under stress. Research on cherry trees suggests that biostimulants can increase phenolic content and antioxidant defenses in leaves, potentially improving resilience to environmental challenges [18]. These compounds not only protect the photosynthetic apparatus from damage but may also enhance post-harvest fruit quality [20]. Through direct effects on leaf physiology, biostimulants such as Ca and seaweed extracts provide a sustainable approach to improving cherry orchard productivity and fruit quality while supporting environmental sustainability [23]. In fact, understanding plant nutrient requirements allows for more efficient and well-timed fertilization, leading to improved application efficiency and, consequently, enhanced fruit yield and quality [24].

This study aims to assess the physiological and biochemical responses of cherry leaves to pre-harvest foliar applications of calcium (150 and 300 g hL^{-1}) and seaweed extracts (75 and 150 mL hL^{-1}), both individually and in combination. By evaluating markers such as water status, photosynthetic activity, and metabolite composition, we seek insights into sustainable cultivation practices that optimize cherry production and minimize environmental impact. Additionally, while the combination of calcium and seaweed extracts shows promise for enhancing plant physiology and fruit quality, limited research exists on their synergistic effects in cherry trees. Therefore, we also examine whether combining these treatments offers additive benefits in improving physiological and biochemical responses in sweet cherry leaves (cv. Burlat).

2. Materials and Methods

2.1. Experimental Design

This experiment was conducted in a 10-year-old sweet cherry orchard established in Resende (latitude 41°6′52.08″ N, longitude 7°56′12.50″ W, altitude 235 m), north of Portugal, in 2019. The soil in the experimental area is classified as Arenosol (WRB, 2014), with a water pH of 6.2 and low contents of organic matter (18 g kg^{-1}) and total nitrogen (0.4 g kg^{-1}). Extractable phosphorus (P_2O_5) and potassium (K_2O) are 48.9 and 149.1 mg kg^{-1}, respectively.

Weather data (Table 1) were recorded by a weather station located near the experimental site.

Table 1. Weather Data Recorded By A Weather Station Located Near The Experimental Site.

	Minimum Temperature (°C)	Maximum Temperature (°C)	Total Rainfall (mm)
March	3.7 to 12.2	11.9 to 22.6	4.1
April	2.0 to 14.3	10.9 to 26.0	8.1
May	6.4 to 17.4	16.2 to 32.9	0.3

In this study, 25 *Burlat* sweet cherry trees grafted onto *Prunus avium* rootstock were used, spaced 2.5 m apart both between and within rows. These trees were divided into five experimental groups, with each group consisting of five trees. Leaf compound applications were performed twice (at the flowering stage and in the fruit maturation stage) and two different products were applied: seaweed extracts (FORALG) (75 mL hL^{-1} and 150 mL hL^{-1}) and calcium (KIPLANT Ca) (150 g hL^{-1} and 300 g hL^{-1}) (Asfertglobal, Espanha). A control treatment with 150 mL hL^{-1} of FORALG + 300 g hL^{-1} of KIPLANT Ca was also used. The control treatment served as a positive control, providing a known and expected response that helps validate the experimental setup, assess sensitivity, ensure quality assurance, standardize comparisons, identify variability, and enhance scientific rigor (Table 2). The applied concentrations were defined considering the maximum dose recommended by the manufacturer. With the exception of the respective treatment, all the trees received uniform management practices, including the application of 100 g hL^{-1} of ENERMAX (P 8.7%; K 28.3%; Fe 0.6%; B 0.04%; Mn 0.2%; Mo 0.025%) and 250 g hL^{-1} of KIPLANT Mg.

Table 2. Commercial fertilizers and respective treatment doses applied in the experiment.

Treatment	FORALG (mL hL^{-1})	KITPLANT Ca (g hL^{-1})	KALENGOR K (g hL^{-1})	KITPLANT Mg (g hL^{-1})
Ca_150	0	150	150	250
Ca_ 300	0	300	150	250
Seaweed_75	75	0	150	250
Seaweed_150	150	0	150	250
Control	150	300	150	250

In this study, given the known nutritional demands of sweet cherry trees and the commercial context of the orchard, the decision to exclude a negative control was made to prioritize the overall health and productivity of the orchard. The positive control treatments were carefully chosen and applied to enhance the nutritional status of the trees in accordance with established agricultural practices. While the absence of a negative control limits direct comparison, the focus remains on evaluating the impact of specific treatments relevant to the practical needs of sweet cherry cultivation in a real-world orchard environment. The fruit harvest took place on the 9th and 10th of May 2019.

2.2. Gas Exchange Measurements

Leaf gas exchange measurements were performed on the five trees of each treatment by using a portable LCpro + Infrared Gas Analyser System (IRGA) (ADC BioScientific, Ltd., Hoddesdon, UK), with a 2.5 cm^2 leaf chamber, operating in the open mode, on well-exposed

leaves during the morning (09:30–11:00 h) of the first day of harvesting. The measurements of the gas exchanges at midday were not performed because of the rain (1.1 mm).

Net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), and intercellular CO_2 concentration (*Ci*) were estimated from gas exchange measurements, using the equations developed by Von Caemmerer and Farquhar [25]. Intrinsic water-use efficiency was calculated as the ratio of *A* to *gs* (*A*/*gs*) [26].

2.3. Relative Water Content

After the gas exchange measurements, the same leaves (5 per treatment) were detached and immediately placed into air-tight tubes to determine the relative water content (RWC). In the laboratory, leaves were weighed to obtain fresh mass weight (FMW). Then, the leaf petioles were immersed in cold ultra-pure water and kept at 4 °C, during 24 h. Afterwards, leaves were weighed again to obtain the turgid mass weight (TMW). Finally, leaves were placed at 70 °C, until reached constant weight, obtaining the dry mass weight (DMW). RWC (%) was calculated as follows: RWC = [(FMW-DMW)/(TMW-DMW)] × 100 [27].

2.4. Electrolyte Leakage

One foliar disc (Ø = 8 mm), from a previously washed and blotted dry leave for each tree (5 per treatment), was used to determine the electrolyte leakage. The disc was placed in a Falcon tube with 10 mL of deionized water. Tubes were shaken (85 rpm) in a water bath at 25 °C for 24 hours and the initial conductivity was measured (84 μS cm^{-1}). Then, samples were heated to 121 °C for 15 min in an autoclave, and after the samples had cooled, total conductivity was measured again. Electrolyte leakage was quantified as the ratio between initial conductivity and final conductivity multiplied by 100 [28].

2.5. Metabolite Composition Determination

2.5.1. Photosynthetic Pigments

One fully expanded leaf per tree (from 5 trees per treatment) was collected and immediately frozen in liquid nitrogen for the determination of the photosynthetic pigments. Leaf discs (1 per leaf) with an area of 1.57 cm^2 each were used for the extraction of chlorophylls *a* (Chl *a*) and *b* (Chl *b*) and carotenoids (carot). Chl *a* and Chl *b* were extracted in 80% acetone and quantified spectrophotometrically [29]. Total carotenoids were extracted with chlorophylls and determined via the equations of Lichtenthaler [30].

2.5.2. Total Soluble Sugars and Starch

Total soluble sugars (SSs) were quantified following the method of Irigoyen et al. [31]. Foliar discs (one per leaf, from one leaf per tree, across five trees per treatment) were heated in 5 mL of 80% ethanol for 1 h at 80 °C. After reacting the alcoholic extract with fresh anthrone in a boiling water bath (100 °C) for 10 min, SS levels were measured by recording absorbance at 625 nm. Starch (St) was extracted from the remaining solid fraction by heating the leaf discs in 30% perchloric acid at 60 °C for 1 h, based on the method by Osaki et al. [32]. The St concentration was then determined using the same anthrone method as for SS quantification. Glucose served as the standard for both SS and St measurements.

2.5.3. Soluble Proteins

Total soluble proteins (SPs) were determined following the Bradford method [33], utilizing a phosphate buffer (pH 7.5) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 100 mM phenylmethylsulfonyl fluoride (PMSF), and 20 g L^{-1} polyvinylpyrrolidone (PVP). Absorbance was measured at 595 nm, with bovine serum albumin (BSA) used as the standard.

2.6. Lipid Peroxidation

Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by

Heath and Packer [34] and Costa et al. [35]. One foliar disc (Ø = 8 mm) of each leaf (1 leaf from each of five trees per treatment) was homogenized in liquid nitrogen, with 3 mL of 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 4000 rpm for 20 min. To 1 mL of the aliquot of the supernatant, 1 mL of 20% TCA containing 0.5% TBA and 100 mL of 4% butylated hydroxytoluene in ethanol (BHT) were added. The mixture was vortexed and heated at 95 °C for 30 min and then quickly cooled on ice. The contents were centrifuged at 3000 rpm for 20 min and the absorbance was measured at 532 nm.

2.7. Total Antioxidant Activity (ABTS)

Total antioxidant activity of sweet cherry leaves, after maceration, was measured through the 2,2′-azinobis (3-ethlybenzthiazoline)-6-sulfonic acid (ABTS) method, according to Re et al. [36] and Sratil et al. [37]. The results were calculated based on the calibration curve and expressed as micromoles of Trolox equivalent per gram of sample.

2.8. Total Polyphenol Content

Total phenolic content was determined following the method of Singleton and Rossi [38]. Forty milligrams of freeze-dried leaf samples (n = 5) were dissolved in 1 mL of 70% aqueous methanol, then vortexed and heated to 70 °C for 30 min. The extracts were subsequently centrifuged at 6000 rpm for 15 min, and the supernatants were filtered through a 0.2 µm filter and stored at −80 °C. For analysis, 20 µL of each extract was combined with 100 µL of Folin–Ciocalteu reagent (diluted 1:10 in double-distilled water) and 80 µL of 7.5% sodium carbonate (Na_2CO_3). After a 15 min incubation at 45 °C, absorbance was measured at 765 nm using a 96-well microplate reader, with a methanol blank. Gallic acid served as the standard.

2.9. Thiol Content

Total thiol content was evaluated taking in account the method described by Leão et al. [39]. A total of 250 µL of the previously prepared extract (total polyphenol content) was used for thiol content determination. Amounts of 1 mL of potassium phosphate buffer (0.2 M pH 8.2), 50 µL of Ellman reagent (5.5′-dithiobis-(2-nitrobenzoic acid), 0.01 M in methanol), and 1.5 mL of 100% methanol were added to the extract. The mixture was heated to 37 °C for 15 min. After the incubation time, the absorbance values were recorded at 412 nm against a methanol blank using a 96-well microplate reader.

2.10. Statistical Analysis

The statistical analysis was conducted using SPSS software (Version 25, SPSS-IBM, Orchard Road, Armonk, NY, USA). Differences among treatments (with five samples per treatment group) were assessed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons at a significance level of $p < 0.05$. Additionally, Pearson's rank correlation was applied to evaluate relationships between parameters. Principal component analysis (PCA) was also utilized, primarily as a tool for exploratory data analysis and predictive modeling. PCA was conducted by performing eigenvalue decomposition on the data correlation matrix (Corr-PCA) after normalizing the dataset for each parameter.

3. Results

3.1. Gas Exchange Measurements

The results of the physiological parameters measured in this work are presented in Figure 1.

There were significant differences in all analyzed leaf gas exchange parameters between treatments. In relation to transpiration rate (E), this parameter was significantly higher (p = 0.000) in sweet cherry trees treated with the higher dose of Ca, with both doses of seaweed extract, and with the combined application of both compounds, when compared with trees treated with the lower dose of Ca. The photosynthetic rate (A) was significantly

higher (p = 0.005) in the leaves of trees with calcium application (both doses) and with the lower dose of seaweed extract, comparing with trees with the combined application of both compounds. An opposite profile was observed in the stomatal conductance (*gs*), since the significantly higher values (p = 0.002) were observed in trees with application of both doses of seaweed extract and with the combined application of both compounds (Control), compared with trees treated with the lower dose of calcium. On the other hand, in relation to the intrinsic water use efficiency (*A/gs*), trees treated with the lower dose of calcium presented higher values (p = 0.000) than all the other treatments. Regarding intercellular CO_2 concentration (*Ci*), the trees treated with the higher dose of calcium, with both doses of seaweed extract, and with both compounds (Control) presented significantly higher (p = 0.000) intercellular CO_2 concentrations than sweet cherry trees treated with an application of the lower dose of calcium. Furthermore, plants treated with both compounds also presented significantly higher intercellular CO_2 concentration than trees treated with the higher dose of calcium.

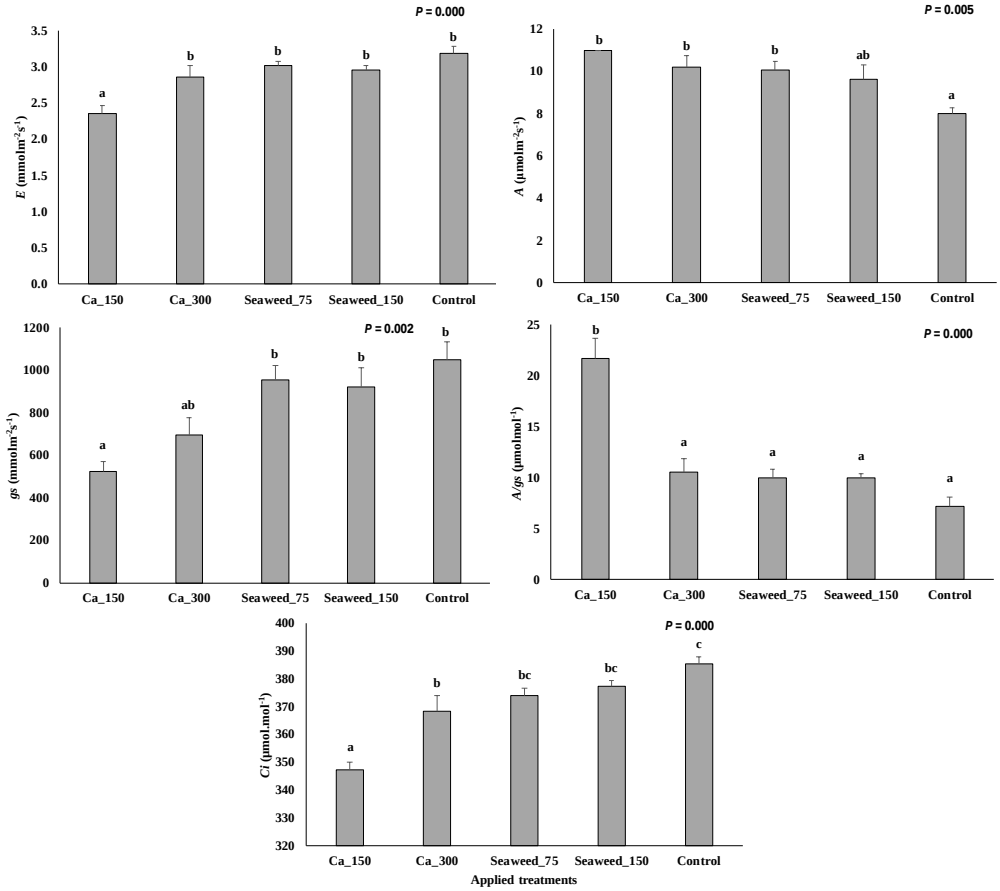

Figure 1. Transpiration rate (*E*), net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), intrinsic water use efficiency (*A/gs*), and intercellular CO_2 concentration (*Ci*) of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.2. Relative Water Content

The relative water content (%) of leaves from sweet cherry trees of different treatments is presented in Figure 2. A significantly higher RWC (p = 0.003) was observed in trees with

application of the higher dose of both compounds, separately and in combination (control), compared with trees treated with the lower dose of calcium.

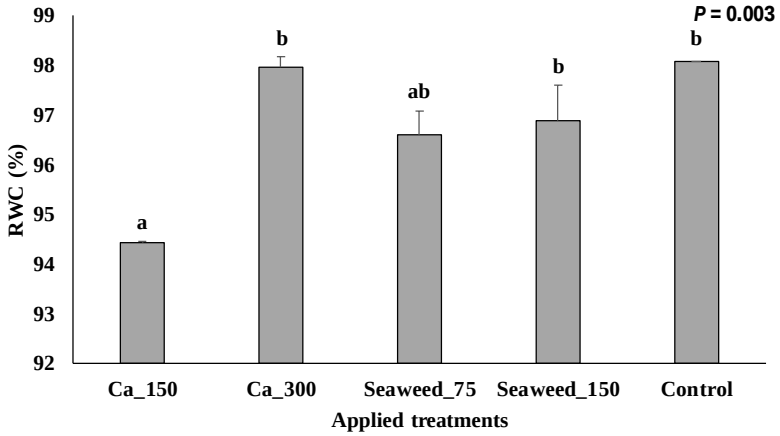

Figure 2. Relative water content (RWC), in %, of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.3. Electrolyte Leakage

The electrolyte leakage (%) of leaves from sweet cherry trees of different treatments is presented in Figure 3. No significant differences (p = 0.270) were observed in electrolyte leakage between the applied treatments.

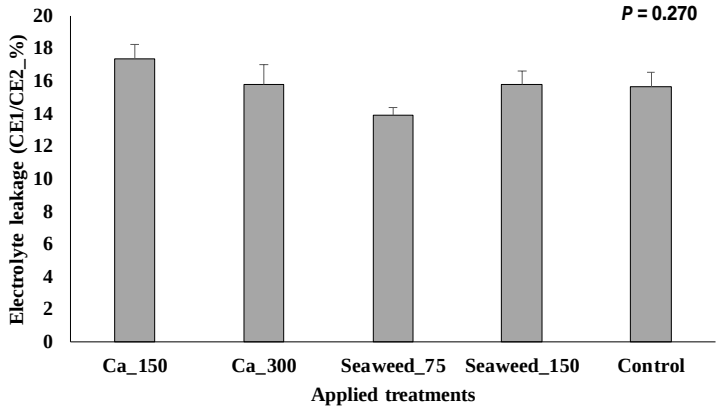

Figure 3. Electrolyte leakage, in %, of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.4. Metabolite Composition Determination

3.4.1. Photosynthetic Pigments

The photosynthetic pigments (chl *a*, *b*, and total and carotenoids) quantified in sweet cherry leaves from different treatments are presented in Table 3. Although no significant differences were observed between treatments in chl *a* (p = 0.916), chl *b* (p = 0.355), and chl total (p = 0.475), the higher values were observed in trees treated with the higher

concentration of seaweed extract, which simultaneously had lower carotenoid contents ($p = 0.585$).

Table 3. Photosynthetic pigments (chlorophyll *a*, *b*, and total and carotenoids) (mg g^{-1}) of sweet cherry trees subjected to foliar applications of calcium and seaweed ($n = 5$).

	Chl a	*Chl b*	*Chl Total*	*Carot*
Ca_150	4.50 ± 0.76 a	3.65 ± 0.59 a	8.15 ± 1.06 a	0.76 ± 0.08 a
Ca_300	3.92 ± 0.68 a	2.71 ± 0.38 a	6.63 ± 1.00 a	0.63 ± 0.07 a
Seaweed_75	4.10 ± 0.08 a	3.62 ± 0.50 a	7.72 ± 0.76 a	0.73 ± 0.06 a
Seaweed_150	4.80 ± 0.26 a	3.99 ± 0.38 a	8.79 ± 0.76 a	0.59 ± 0.10 a
Control	4.68 ± 1.05 a	2.75 ± 0.35 a	7.43 ± 1.48 a	0.76 ± 0.06 a

Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.4.2. Total Soluble Sugars and Starch

The total soluble sugars (SSs) and total starch (St) of leaves from sweet cherry trees with application of different compounds and doses are presented in Figure 4. The total SS were significantly higher ($p = 0.006$) in sweet cherry trees treated simultaneously with both compounds (control), when compared with trees with calcium application (both doses). On the other hand, St content was significantly higher ($p = 0.000$) in sweet cherry trees with application of the higher dose of seaweed extract, when compared with trees with calcium application (both doses) and with control trees. Additionally, sweet cherry trees treated with the lower dose of seaweed extract presented significantly higher St content than trees treated with the lower dose of calcium.

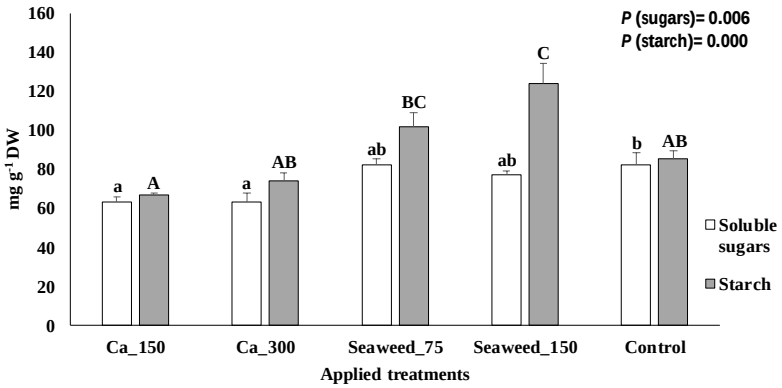

Figure 4. Total soluble sugars (mg g^{-1} DW) and starch (mg g^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed ($n = 5$). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.4.3. Soluble Proteins

The content of soluble proteins presented in the leaves of trees with different applications can be observed in Figure 5. The soluble protein content was significantly higher ($p = 0.003$) in sweet cherry trees with application of the higher dose of seaweed extract, when compared with trees treated with both doses of calcium. Furthermore, trees treated with both compounds (Control) also presented a significantly higher protein content than trees with application of the higher dose of calcium.

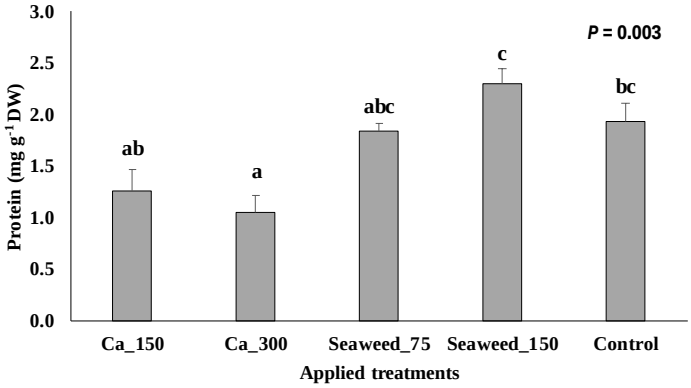

Figure 5. Soluble protein (mg g^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.5. Lipid Peroxidation (TBARS)

The content of TBARS in sweet cherry trees with application of different compounds and doses is presented in Figure 6. No significant differences (p = 0.444) were observed in this parameter between trees from different treatments.

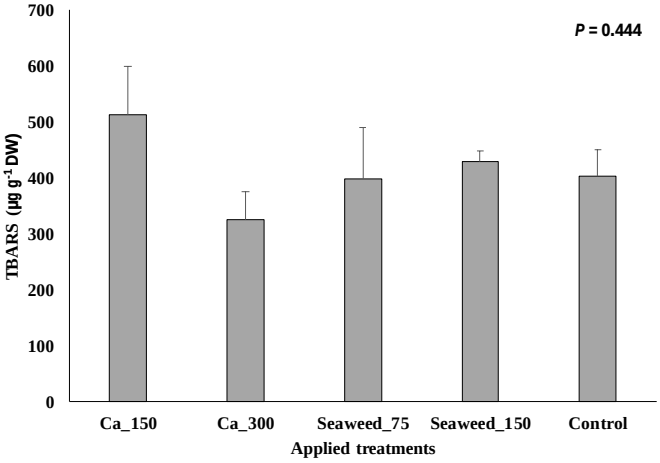

Figure 6. Lipid peroxidation quantified by the TBARS method (µg g^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.6. Antioxidant Activity (ABTS)

The antioxidant activity, quantified by ABTS method, of sweet cherry leaves subjected to the application of different compounds/doses can be observed in Figure 7. A significantly higher antioxidant activity (p = 0.000) was observed in sweet cherry leaves with seaweed extract application (both doses), when compared with trees treated with both doses of calcium. Trees treated with both compounds also presented significantly higher antioxidant activity than trees treated with a lower dose of calcium. Additionally, the application of the higher dose of seaweed extract led to a significantly higher antioxidant activity than in all other applications.

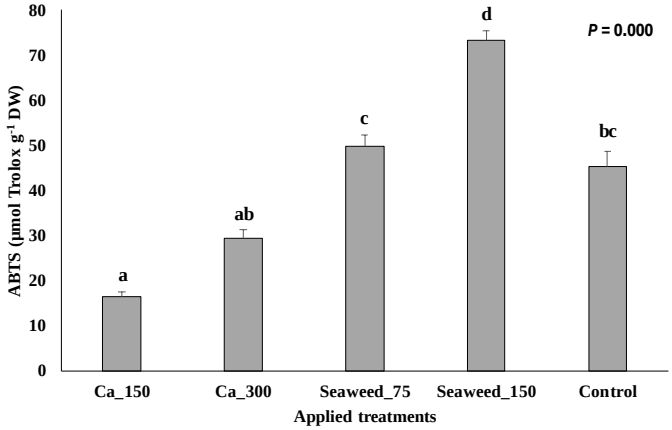

Figure 7. Antioxidant activity quantified by the ABTS method (µmol Trolox g^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed ($n = 5$). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.7. Polyphenolic Compounds

The content of polyphenolic compounds in trees from different treatments is presented in Figure 8. In this parameter, only the sweet cherry trees with application of the lower dose of seaweed extract presented significantly higher values ($p = 0.029$) than trees treated with the lower dose of calcium, despite higher values also being observed in trees treated with the higher dose of seaweed extract and in trees treated with both compounds (control).

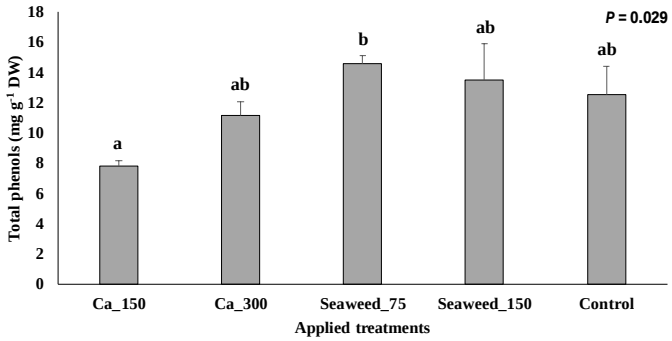

Figure 8. Total phenols (mg g^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed ($n = 5$). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.8. Thiol Content

The results of thiol content in trees with different applications are presented in Figure 9. Similarly to phenolic compounds, the highest thiol content was also observed in trees treated with the lower dose of seaweed extract. In fact, this treatment presented significantly higher ($p = 0.000$) thiol content than all the other treatments. Furthermore, plants treated with both compounds also presented significantly higher thiol content than trees treated with the lower dose of calcium.

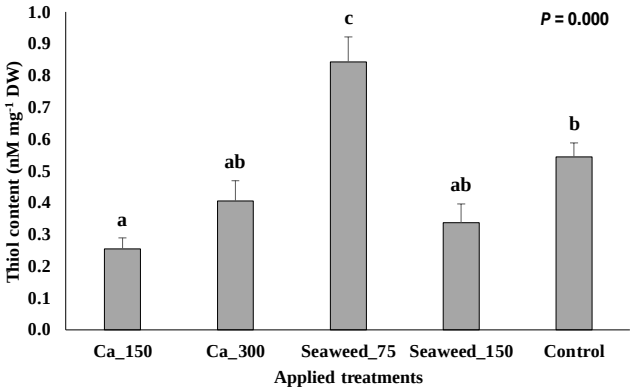

Figure 9. Thiol content (nM mg^{-1} DW) of sweet cherry trees subjected to foliar applications of calcium and seaweed (n = 5). Both compounds were applied in two different doses, namely low and high—150 and 300 g hL^{-1} for calcium and 75 and 150 mL hL^{-1} for seaweed. A positive control was also utilized, consisting of higher doses for each compound. Data are expressed as means ± standard error. Different letters indicate significant differences ($p < 0.05$), and the absence of letters indicates no statistically significant differences ($p > 0.05$) between treatments, according to Tukey's test.

3.9. Principal Component Analysis (PCA)

To gain deeper insights into the correlations among all evaluated parameters, a chemometric analysis was conducted by integrating the complete dataset (Figure 10). Principal component analysis (PCA), based on the correlation matrix, was used to standardize the data. In this PCA, the first two principal components (PC1 and PC2) account for 51.82% of the total variance, with PC1 contributing the largest proportion at 37.55%. The parameters RWC, chl *a*, chl *b*, chl total, and starch were together in the right PCA quadrant, as well as sugars, protein, antioxidant activity, phenols, thiols, and the gas exchange measurements *E*, *gs*, and *Ci*, although these were located further away from the remaining parameters. On the other hand, the electrolyte leakage, lipid peroxidation, carotenoids, *A*, and *A/gs* were spatially separated and placed in the left PCA quadrant, corroborating the negative correlations observed between parameters.

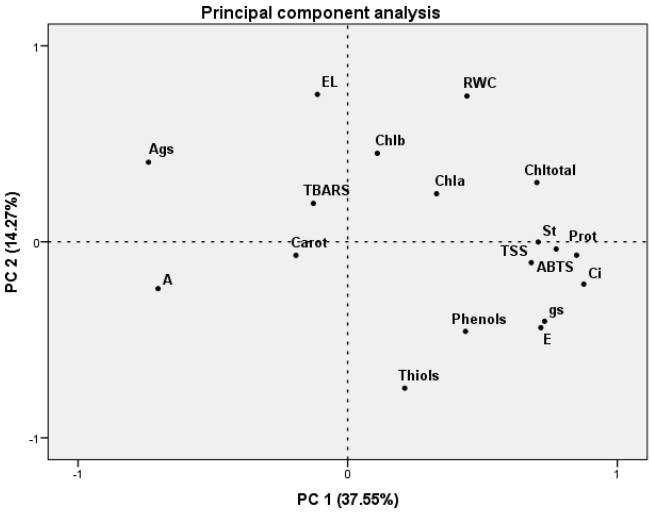

Figure 10. Principal component analysis using the whole dataset of all applied treatments (n = 5). Analyzed parameters: *E*—transpiration rate; *A*—net CO_2 assimilation rate; *gs*—stomatal conductance; *Ci*—intercellular CO_2 concentration; *A/gs*—intrinsic water use efficiency; Chl*a*—chlorophyll *a*; Chl*b*—chlorophyll *b*; Chltotal—chlorophyll total; Carot—carotenoids; RWC—relative water content; EL—electrolyte leakage; TSS—total soluble sugars; St—starch; Prot—protein content; TBARS—lipid peroxidation; ABTS—antioxidant activity; Phenols—total phenols; and Thiols—thiol content.

4. Discussion

The results of this study highlight the role of foliar applications of Ca and seaweed extracts on the physiological and biochemical responses of sweet cherry trees (cv. Burlat). Similar to findings in other crops, seaweed extracts, either alone or in combination with Ca, influenced several key parameters related to plant health and stress resilience. These results align with previous research, which has demonstrated that seaweed extracts can improve crop performance, increase resistance to both biotic and abiotic stresses, and extend post-harvest shelf life in perishable fruits such as cherries [40–43].

In terms of gas exchange, the low stomatal conductance (*gs*) observed in trees treated with Ca suggests a possible reduction in stomatal opening, likely a mechanism to prevent excessive water loss under certain conditions [44]. Interestingly, this reduced *gs* did not appear to limit the photosynthetic rate (*A*) in Ca-treated trees, which remained higher compared to those receiving combined treatments. This suggests that stomatal closure is not the only factor governing photosynthetic efficiency in sweet cherry trees, as biochemical or non-stomatal factors may play a role [45]. This observation is consistent with the assertion that using photosynthetic rate as a yield indicator requires the careful consideration of multiple variables, including environmental conditions and plant phenology Mebrahtu and Hanover [46]. Indeed, gas exchange measurements are of extremely importance; however, it is necessary to take into account the momentary nature of these measurements [47]. Transpiration rate (*E*) was higher in trees treated with seaweed extracts and in those receiving the combined treatments, showing a positive correlation with *gs* ($r = 0.839$, $p = 0.000$) (Supplementary Figure S1). These two parameters followed the same trend. In fact, the co-application of Ca and seaweed extract or seaweed extract alone may improve water regulation by facilitating greater stomatal opening, thereby enhancing water fluxes and gas exchange [48]. Positive correlations between *E* and intercellular CO_2 concentration (*Ci*) ($r = 0.847$, $p = 0.000$) (Supplementary Figure S1) further support the idea that seaweed extracts help maintain more favorable water relations under varying environmental conditions [49–51].

Relative water content (RWC), a crucial parameter for assessing plant water status, was significantly higher in trees treated with the higher doses of both compounds. These findings are in line with previous studies reporting that biostimulants, particularly seaweed extracts, improve RWC, likely due to their ability to enhance water uptake and retention under stress conditions [16,22,52,53]. RWC values below 65% indicate water stress conditions [54]. In this study, RWC values remained consistently high (95–98%), indicating that irrigation levels were adequate, and the trees were not under water stress. The improvement in RWC across treatments reflects the beneficial role of seaweed extracts in promoting better water-use efficiency.

The biochemical analysis revealed interesting trends in the metabolite composition of the leaves. Despite no significant differences in chlorophyll levels between treatments, trees treated with Ca and the combined treatments exhibited lower chlorophyll content, potentially indicating damage to the photosynthetic machinery. This observation is consistent with studies suggesting that Ca may negatively affect chloroplast integrity under certain conditions [55]. The increase in carotenoid content observed in these trees may be a compensatory response to lower chlorophyll levels, as carotenoids protect plants from oxidative damage and help optimize light absorption in lower radiation environments [56].

Starch is the major carbohydrate storage in plants. In the leaves, starch is synthesized during the day and mobilized during the following night to guarantee a steady supply of carbon and energy [57]. In the present work, starch accumulation in leaves was significantly higher in trees treated with seaweed extract, which, coupled with lower photosynthetic rates, suggests that these trees relied more on stored carbohydrates during periods when photosynthesis was limited [57]. This could be a reflection of the plants' metabolic adjustments to maintain energy homeostasis under varying light and water availability. In the guard cells which border the stomatal pores that control water and CO_2 exchange with the environment, starch is degraded very rapidly upon light exposure, helping to

generate organic acids and sugars to increase guard cell turgor and promote stomatal opening [58]. In this study, higher sugar content in trees receiving the combined treatments further supports the role of seaweed extracts in promoting carbohydrate accumulation, particularly glucose, fructose, and sucrose, which are critical for fruit development in cherries [59–61]. The observed sugar accumulation in the combined seaweed + Ca treatment may result from synergistic interactions between calcium and seaweed compounds, which could influence carbohydrate synthesis pathways differently than treatments with seaweed alone. A positive correlation was found between total sugars content and starch (r = 0.424, p = 0.035) (Supplementary Figure S1).

Higher protein content in trees treated with seaweed extract (higher dose) is another notable finding. Proteins are essential for plant growth, enzyme activity, and stress defense. In this work, protein content was positively correlated with total soluble sugars (r = 0.564, p = 0.003), with starch (r = 0.682, p = 0.000), with antioxidant activity (r = 0.812, p = 0.000), and with phenol content (r = 0.440, p = 0.028) (Supplementary Figure S1), suggesting that seaweed extracts contribute to overall metabolic enhancement, allowing trees to maintain better biochemical status. These findings are in agreement with previous studies, where biostimulants like seaweed extracts have been shown to enhance protein synthesis and metabolic activity under stress [18,62,63].

Oxidative stress indicators, such as TBARS, which measure lipid peroxidation, did not show significant differences between treatments, although trees treated with the lower dose of Ca exhibited slightly higher TBARS levels. This suggests that Ca may not be as effective in mitigating oxidative stress as seaweed extracts, which contain natural antioxidants and bioactive compounds that enhance plant defenses against oxidative damage [64,65].

The ABTS method used in the present work gives a measure of the antioxidant activity of the range of carotenoids, phenolics, and some plasma antioxidants, determined by the decolorization of the ABTS•+, through measuring the reduction in the radical cation as the percentage inhibition of absorbance at 734 nm [36]. In this work, an increase in antioxidant activity was observed in trees treated with seaweed extracts and the combined treatments, which can be attributed to the higher phenolic and thiol content in these trees. In fact, a positive correlation was observed between antioxidant activity and phenol content (r = 0.554, p = 0.004) (Supplementary Figure S1). The phenol content was also positively correlated with thiol content (r = 0.560, p = 0.004) (Supplementary Figure S1). Phenolics and thiols play critical roles in scavenging free radicals and protecting plant cells from oxidative stress. The strong positive correlations between antioxidant activity, phenolic content, and thiol levels further underscore the role of seaweed-based biostimulants in enhancing the antioxidant defense system of sweet cherry trees [18]. The effect of Ca on phenolic content appears to be cultivar-dependent, as some studies reported an increase in phenolics with calcium application [66], while others showed a decrease, particularly in sweet cherry cultivars cv. Germersdorfi 3 [67]. This suggests that the response to Ca treatments may vary based on genetic factors and environmental conditions, emphasizing the need for further research on the interactions between calcium and phenolic metabolism in different cherry cultivars.

In general, sweet cherry trees treated with seaweed extracts presented better performance than the other trees, which could be due to the presence of active compounds, micro-and macronutrients in the extract of seaweeds (macroalgaes), which can stimulate plant growth and development [68,69].

5. Conclusions

This study demonstrates that pre-harvest foliar applications of calcium and seaweed extracts, both individually and in combination, effectively enhance the physiological and biochemical responses of 'Burlat' sweet cherry trees. Although a negative control was not included, the positive control with higher concentrations of calcium and seaweed provided a benchmark to assess the relative efficacy of each treatment. Results indicate that while calcium improved certain photosynthetic metrics, seaweed extracts contributed

notably to water retention, elevated sugar and protein levels, and boosted antioxidant activity, reflecting increased resilience and overall plant health. Interestingly, combining calcium and seaweed did not consistently produce synergistic effects, suggesting that each treatment may optimize plant responses through distinct mechanisms.

The findings underscore the potential of seaweed extracts as a sustainable biostimulant in cherry production, promoting plant vigor and quality outcomes without excessive reliance on chemical inputs. Future research should consider long-term effects on fruit yield and quality, along with the ecological benefits of integrating seaweed-based biostimulants into routine orchard management practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10111173/s1, Figure S1: Pearson's correlation matrix of all the analysed parameters represented by a heat map with a colour scale from red (+1) to blue (−1).

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