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Enhanced growth and photosynthetic efficiency in wild rocket (*Diplotaxis tenuifolia* L.) following multi-species microalgal biostimulant application

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ABSTRACT

Foliar spraying is a simple and efficient technique that enables targeted delivery of biostimulants directly onto plant surfaces, minimizing losses and environmental dispersion. Among biostimulant categories, microalgae-based formulations represent an eco-friendly solution to improve crop productivity, thanks to their richness in bioactive compounds and rapid growth in low-input systems. In this study, the crude extract of three green microalgae with high commercial value and broad biotechnological potential—*Auxenochlorella protothecoides*, *Tetradesmus obliquus* and *Chlamydomonas reinhardtii*—along with their consortium, was tested as foliar biostimulants for the first time on wild rocket (*Diplotaxis tenuifolia* L.) at an early vegetative stage. Plants received three sequential treatments, and physiological and biochemical responses were evaluated at 24, 48, and 72 h after the final application. Biostimulation led to up to 32 % increases in fresh biomass and significantly enhanced photosynthetic efficiency (F_v/F_m, PI), particularly in consortium-treated plants. The multi-step application also triggered a late and transient rise in antioxidant compounds (carotenoids and phenolics), potentially improving post-harvest quality. Remarkably, these effects were observed even in the absence of abiotic stress, highlighting the intrinsic biostimulant potential of the treatments. Notably, when the consortium crude extract was applied, higher chlorophyll content, nitrate accumulation, and enhanced nitrogen assimilation (indicated by lower $\delta^{15}N$ values) were also observed. These results suggest a compositional and functional uniqueness of the consortium, likely due to interspecies interactions. Overall, early-stage, multi-step foliar biostimulation with selected microalgal species or consortia represents a promising and sustainable strategy to improve crop performance and modulate quality traits in leafy vegetables.

1. Introduction

As global food demands rise, the agricultural sector faces urgent pressure to adopt more sustainable practices that balance productivity with environmental responsibility (Velten et al., 2015; Searchinger et al., 2018; Khan et al., 2021). Biostimulants are emerging as promising tools in this context, enabling farmers to enhance plant growth, optimize nutrient use, and improve resilience to stress (Bulgari et al., 2015; du Jardin, 2015). According to the European Biostimulants Industry Council (EBIC, https://biostimulants.eu/), biostimulants differ from fertilizers and pesticides in that they do not provide nutrients directly; instead, they stimulate natural plant processes that support growth and stress resistance, complementing traditional inputs (Calvo et al., 2014; Ricci et al., 2019).

Among the various sources of biostimulants, algae have gained increasing attention due to their distinct advantages. Historically, algae-based products have been utilized in agriculture as soil amendments, particularly in coastal agricultural communities (Borowitzka, 1998). However, the application of algae as biostimulants is a recent development. The most commonly used algae in biostimulant formulations include brown algae species, such as *Ascophyllum nodosum*, *Ecklonia maxima*, and *Laminaria* spp., which are known for their high content of beneficial compounds such as polysaccharides and growth hormones (Ali et al., 2021). Additionally, red and green algae, such as *Chondrus*

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crispus and *Ulva* spp., are used, but to a lesser extent. Although the use of microalgae is less common and still under development (Colla and Rouphael, 2020; González-Pérez et al., 2022), it presents several advantages, bolstered by advances in biotechnology and an increasing understanding of the bioactive compounds within microalgae. These microscopic, photosynthetic organisms grow quickly, adapt well to various conditions, and can be cultivated in non-arable land or even wastewater. Utilizing wastewater can provide a cost-effective source of nutrients while simultaneously aiding in its remediation (Kaloudas et al., 2021). The biomass produced can then be processed into biostimulants, creating a closed-loop system that reduces resource waste and minimizes the environmental footprint of agricultural production (Stiles et al., 2018; Kholssi et al., 2019; Olabi et al., 2023).

Bioactive compounds found in microalgae, including amino acids, phytohormones, polysaccharides, and antioxidants, trigger positive physiological responses in plants (Michalak et al., 2016). These compounds help increase photosynthetic efficiency, enhance tolerance to abiotic stresses such as drought or salinity, and boost overall vigour (Khan et al., 2009). Additionally, the nutritional value of plants treated with biostimulants is often elevated due to the stimulation of secondary metabolite pathways (Brito-Lopez et al., 2025), resulting in the accumulation of beneficial compounds like carotenoids, polyphenols, anthocyanins, and glucosinolates (Gatti et al., 2025).

Research has demonstrated the effectiveness of microalgal biostimulants derived from species such as *Tetradesmus obliquus*, *Chlamydomonas reinhardtii*, and *Auxenochlorella protothecoides* as well as various cyanobacteria, in improving seed germination rates and root and shoot growth (Calvo et al., 2014; Qu et al., 2022; Braun and Colla, 2023). Microalgae biostimulants have proven to be effective across a range of crops, including tomatoes, cucumber, wheat, and barley. For example, *A. protothecoides* increased the final germination percentage of tomato seeds up to 100 % when an algal extract was applied during seed priming (Mollo and Norici, 2025). In a similar experiment, the germination index of wheat treated with 0.2 g L^{-1} of *A. protothecoides* and *T. obliquus* increased by 177 % and 82 %, respectively, compared to the control condition. A study involving *C. reinhardtii* as an algal species reported strain-specific biostimulant effects when applied using the soil drench method in *Medicago truncatula* (Gitau et al., 2022). The mechanisms by which microalgae function as biostimulants remain inadequately understood; however, existing evidence suggests that microalgae can significantly enhance plant growth, improve photosynthetic efficiency, and enrich biochemical composition (Chabili et al., 2024).

Until now, none of the three mentioned microalgal species, or any other microalgae, have been evaluated on wild rocket, the experimental subject of this study. Nevertheless, biostimulants derived from seaweed extracts, particularly brown algae, have demonstrated efficacy in enhancing biomass production, improving water use efficiency, and increasing tolerance to abiotic stresses (Giordano et al., 2020; Candido et al., 2023; El-Nakhel et al., 2023; Ciriello et al., 2024). In the case of wild rocket, biostimulation has resulted in improved water use efficiency and a substantial increase in fresh biomass, reaching up to 30 %, under both optimal and stressful growth conditions. However, variations in mineral composition, especially the levels of nitrogen in the form of nitrates, have been reported inconsistently across the literature, suggesting that the effects may be contingent upon the specific biostimulant employed.

Unlike fertilizers that require large doses to deliver measurable effects, microalgal biostimulants have been shown to be effective even in low concentrations, sometimes as low as 0.1 mg mL^{-1} (Carillo et al., 2020; Kapoore et al., 2021; Braun and Colla, 2023). This low-dose efficacy underscores the potency of the bioactive compounds in microalgal extracts and supports their classification as biostimulants rather than nutrient supplements. Biostimulant application concentration range is generally within 0.1 mg mL^{-1} and 1 mg mL^{-1}, with lower values rarely found on literature.

A key application method for microalgae-based biostimulants is foliar spraying, which involves applying the biostimulant solution directly onto the plant's leaves (Khan et al. 2009; Preininger et al., 2018; Jindo et al., 2022). This method offers precise control over dosage, minimizing both waste and environmental runoff, and allows for rapid absorption of active compounds through leaf tissues and delivers these compounds precisely where they are most needed. Studies indicate that microalgae-derived biostimulants applied via foliar spraying enhance nutrient uptake, stimulate chlorophyll production, and boost overall photosynthetic capacity. Foliar spraying is particularly effective in mitigating abiotic stresses, as it enables plants to quickly mobilize stress-response pathways (Refaay et al., 2021; Hariharan et al., 2024). Furthermore, the advantages of this application method have been documented even in non-stressed conditions. Studies involving walnut (Pascoalino et al., 2025), mandarin (ValizadehKaji and Mohammaei, 2025), lettuce (Álvarez-González et al., 2025), and rapeseed (Mohseni et al., 2025) demonstrate that biostimulated plants, despite the absence of stress, commonly exhibit increased fresh weight, improved nutritional quality, and enhanced fruit characteristics. These findings underscore the potential role of biostimulants in enhancing overall crop performance. Consequently, foliar spraying emerges as a particularly effective technique for maximizing the resilience and productivity of microalgae-based biostimulants.

The objective of the present study was to investigate the potential of three green microalgae species (*Tetradesmus obliquus*, *Chlamydomonas reinhardtii*, and *Auxenochlorella protothecoides*) as biostimulants, as well as their consortium. The same bio-stimulants were previously tested by the same authors in an experiment involving tomato seeds and various concentrations of algal extracts (Mollo and Norici, 2025). Differences were observed across all treatments, but the most significant variations were attributed to the concentration and the usage of the consortium. In fact, the consortium generally demonstrated a lower effect compared to the respective monocultures, indicating that co-cultivation may significantly alter the biochemical composition of the algal biomass. The species were grown in a synthetic standard medium (BG11), and once they reached the stationary phase, the biomass was collected and disrupted to obtain a cellular extract. The algal extracts were then diluted to a concentration of 20 mg L^{-1} and used as a foliar spray on wild rocket plants. *Diplotaxis tenuifolia* is valued both for its distinctive peppery flavour and its rich nutritional profile (Caruso et al., 2020) and was chosen as experimental species for its rapid growth cycle and its common use in ready-made salads (Ciriello et al., 2024). Differences in fresh weight, photosynthetic efficiency, and biochemical composition were assessed in comparison with a control group of plants treated with water, at 24, 48, and 72 h after treatment. This study also aimed to investigate whether the effects of biostimulation persist over time. Moreover, this research focuses on the unique effects of the algal consortium, with the intent to elucidate the impact of co-cultivation on the biostimulant properties of the algal biomass, as it is known that the algal consortia exhibit distinctive qualities compared to the individual cultures of its constituent species.

2. Materials and methods

2.1. Microalgal biostimulants preparation

Three microalgae were tested as biostimulants: *Auxenochlorella protothecoides* (CCAP 211/8D[2]), *Tetradesmus obliquus* (CCAP 276/3 A[2]), and *Chlamydomonas reinhardtii* (RCC125[3]). A stable consortium of these three species was also evaluated (Chieti et al., 2024; Mollo et al., 2024b, 2024a). Biostimulants were prepared from algal cultures grown in BG11 standard growth medium (Allen and Stanier, 1968). Algae were grown

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[2] https://www.ccap.ac.uk/

[3] https://roscoff-culture-collection.org/

until reaching the stationary phase at controlled conditions: 20°C, illuminated with white fluorescent lamps at 100 mmol m^{-2} s^{-1} and 24 h photoperiod light. The algal biomass obtained from the centrifugation of cultures was washed twice with deionised water. Thus, it was resuspended in deionised water to achieve a final concentration of 20 g L^{-1}. Therefore, the algal biomass was treated using a cell bomb (Parr Instrument Company) to break cell walls and membranes, release its inner content and obtain a crude extract. The *cell bomb* uses high N_2 pressure and operates at room temperature, avoiding damage and denaturation of temperature-sensitive molecules.

The crude extracts, already tested as biostimulants on tomato seeds (Mollo and Norici, 2025), were then characterised as reported by Mollo and Norici (2025) to quantify pigments, proteins, C and N content, and microelements and to semi-quantify carbohydrates and lipids. Biomass composition is reported in Table 1. Algal extracts were stored at −20°C before use. Before use, the four microalgal extracts were diluted at the concentration of 20 mg L^{-1} (by diluting 1 mL of extract in 1 L of water).

2.2. *Cultivation*

Trials were carried out from April to May in a greenhouse at the Faculty of Agricultural and Food Science of Milan. Wild rocket plants (*Diplotaxis tenuifolia* L., ISI Sementi S.p.A., Parma, Italy) were grown in plastic pots filled with a peat-based substrate. For each pot, 0.5 g of wild rocket seeds were sown, and the experiment was carried out in 5 replicates per condition. Pots were regularly irrigated to avoid the drying of the growing substrate.

The trial aimed to evaluate the effect of the 4 microalgal extracts at the concentration of 20 mg L^{-1}. Treatments were reported as TO, CR, AP and CS depending on the type of biostimulant (see Table 1). Distilled water was applied on control plants (CTR). After 20 days, once plants reached a full-expanded leaves stage, three foliar applications of biostimulants were performed within a week of each other. Plants were sprayed with the algal extracts until they ran off. Physiological and biochemical analysis samples were collected 24, 48 and 72 h after the last biostimulant application. All samples were stored at −20°C before analysis. At 72 h after the last treatment, plants were harvested to measure fresh and dry weight.

2.3. *Total fresh biomass and dry matter*

Plants were harvested by cutting them at the collar, and biomass for each pot was measured and weighed by the aerial parts (fresh weight, FW). The sample's dry weight (DW) was determined after oven drying at 105 °C for at least 24 h.

2.4. *Chlorophyll fluorescence*

Chlorophyll *a* fluorescence-relate parameters were measured *in vivo* using a hand-portable fluorimeter (HandyPEA, Hansatech Instruments, King's Lynn, UK). At least ten measurements per pot were carried out on darkness-adapted leaves, previously kept in the dark for 30 min, using leaf clips of 4 mm diameter. Leaf fluorescence analysis was performed by exposure of leaves to saturating light for 1 s. The maximum quantum efficiency of photosystem II (F_v/F_m) and the performance index (PI) obtained by automatic calculation performed by the device were considered for further analysis. F_v/F_m represents a robust indicator of the maximum quantum yield of PSII photochemistry by Misra et al. (2012), while the PI measures the overall vitality and functionality of the photosynthetic apparatus, reflecting the efficiency of energy capture, transfer, and utilization within Photosystem II (Tsimilli-Michael, 2020).

2.5. *Total chlorophylls and carotenoids*

Leaf pigments (chlorophyll *a*, *b* and carotenoids) were quantified by extraction of 30 mg leaf disc samples incubated in 5 mL of 99.9 % v/v methanol and kept in the dark for 24 h at 4°C (Lichtenthaler, 1987; Misra et al., 2012; Tsimilli-Michael, 2020). Quantification was performed spectrophotometrically (Thermo Scientific Evolution 300 UV-Vis Spectrophotometer) by assessing the absorbance at 665.2, 652.4 and 470 nm. Pigments were expressed on a fresh weight basis.

2.6. *Phenolic index and total anthocyanins*

Phenols and anthocyanins concentration were quantified using the spectrophotometric protocol reported by Klein and Hagen (1961). Leaf disc samples of 5 mm diameter of about 30 mg weight were incubated with 3 mL of hydrochloric acid acidified methanol and kept in the dark for 24 h at 4°C. Absorbance was read at 320 nm for total phenols concentration and at 535 nm for total anthocyanins (Thermo Scientific Evolution 300 UV-Vis Spectrophotometer). Phenols were expressed as Phenolic Index ($ABS_{320\ nm}$ g^{-1} FW), while anthocyanins were expressed as cyanidin-3-glucoside equivalents using a molar extinction coefficient ε of 29,600 L M^{-1} cm^{-1}.

Table 1
biochemical characterisation of the algal extracts considering a DW of the algal extract of 20 g L^{-1}.

Parameter	**Algal extract as biostimulant**			
	***T. obliquus* (TO)**	***C. reinhardtii* (CR)**	***A. protothecoides* (AP)**	**Consortium (CS)**
Protein (g L^{-1})	9.30 ± 0.45	5.13 ± 0.02	4.55 ± 0.77	4.15 ± 0.22
Carbohydrate (UA)	12.41 ± 0.04	5.99 ± 0.00	9.51 ± 1.00	14.86 ± 3.01
Lipids (UA)	0.20 ± 0.00	0.06 ± 0.00	0.79 ± 0.12	0.28 ± 0.06
Chlorophyll A (mg L^{-1})	9.44 ± 0.38	44.78 ± 0.33	11.97 ± 0.55	156.25 ± 1.16
Chlorophyll B (mg L^{-1})	2.51 ± 0.45	56.47 ± 2.92	2.58 ± 0.94	197.00 ± 10.19
Carotenoids (mg L^{-1})	1.49 ± 0.04	7.52 ± 0.62	5.16 ± 0.46	26.25 ± 2.17
C (%)	52.0 % ± 2.0 %	51.8 % ± 3.3 %	51.9 % ± 0.8 %	52.1 % ± 2.2 %
N (%)	9.9 % ± 0.4 %	11.7 % ± 0.2 %	9.6 % ± 0.4 %	10.5 % ± 0.4 %
P (mg L^{-1})	54.69 ± 1.12	253.17 ± 16.08	221.72 ± 11.55	43.96 ± 2.77
S (mg L^{-1})	15.59 ± 0.50	64.01 ± 3.66	75.80 ± 11.29	13.41 ± 1.14
Cl (mg L^{-1})	6.73 ± 0.96	6.29 ± 0.37	13.83 ± 5.78	0.88 ± 0.11
K (mg L^{-1})	6.54 ± 0.26	17.12 ± 1.23	68.65 ± 0.14	8.06 ± 0.40
Ca (mg L^{-1})	58.08 ± 2.44	105.82 ± 5.78	30.04 ± 0.72	6.36 ± 0.42
Mn (mg L^{-1})	5.59 ± 0.29	13.52 ± 0.70	2.76 ± 0.03	0.55 ± 0.06
Fe (mg L^{-1})	18.94 ± 1.71	64.05 ± 2.90	9.31 ± 0.51	11.06 ± 2.15
Ni (mg L^{-1})	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
Cu (mg L^{-1})	0.28 ± 0.01	0.97 ± 0.05	0.64 ± 0.02	0.16 ± 0.01
Zn (mg L^{-1})	1.05 ± 0.07	2.36 ± 0.13	1.62 ± 0.01	0.30 ± 0.02

2.7. Nitrate concentration

The nitrate concentration of fresh leaf tissue was determined spectrophotometrically according to the method of Cataldo et al. (1975). Quantification was done by homogenising 1 g of fresh leaf tissue in 4 mL of deionised water in a mortar. The obtained extract was then centrifuged at 4000 rpm for 15 min, and the recovered supernatant was used for the analysis. 20 μL of the extract was added to 80 mL of 5 % (w/v) sulfuric-salicylic acid, prepared using concentrated H_2SO_4 and salicylic acid. After that, 3 mL of 1.5 NaOH was added, and absorbance at 410 nm was measured once samples were cooled at room temperature (Thermo Scientific Evolution 300 UV-Vis Spectrophotometer). Nitrate concentration was calculated referring to a KNO_3 standard calibration curve. Results were reported as mg of NO_3 per kg of fresh weight.

2.8. Sucrose, reducing and total sugars

The water-homogenised extract used for nitrates quantification was also used to determine sucrose and sugars (total and reducing) concentration by colorimetric method (Thermo Scientific Evolution 300 UV-Vis Spectrophotometer). Sucrose quantification was carried out as reported in Rorem et al. (1960): 20 μL of the extract were added to 200 μL of 2 N NaOH, incubated in a water bath at 100°C for 10 min, and then added to 1.5 mL of a resorcinol buffer (30 % hydrochloric acid, 1.2 mM resorcinol, and 4.1 mM thiourea 1.5 M acetic acid). Samples were then kept in a water bath at 80°C for 10 min, and once samples cooled at room temperature, absorbance was read at 500 nm. Quantification was carried out using a standard sucrose calibration curve.

Reducing sugar concentration was quantified using the Miller protocol (Miller, 1959). 200 μL of aqueous leaf extract was added to 200 μL of dinitrosalicylic acid and incubated at 99°C for 5 min. After that, 1.5 mL of water was added, and the sample was quantified spectrophotometrically at 530 nm, referring to a standard glucose calibration curve.

The total sugar concentration was quantified according to a modified anthrone method (Yemm and Willis, 1954). 2.5 mL of anthrone 10.3 mM H_2SO_4 95 % solution was added to 0.5 mL of aqueous leaf extract. After 5 min of incubation in ice, the samples were heated at 95°C for 10 min and then vortexed vigorously. Using a standard glucose calibration curve as a reference, the quantification was done by reading absorbance at 620 nm.

2.9. Protein content

Protein content per mg of dry weight (μg mg^{-1}) was quantified in 0.5 mg of dried leaf samples collected 24/48/72 h after the last treatment. Proteins were quantified spectrophotometrically (UV-1900i spectrophotometer, SHIMADZU CORP.) using the Lowry colorimetric method, as reported by Peterson (1977).

2.10. Elemental analysis and isotopic fractionation

Leaf samples were dried at 80°C until their weight stabilised. Approximately 1 mg and 10 mg of the dried leaf were respectively analysed using a ECS 4010 (Costech, Pioltello, Italy) for the quantification of C and N as reported by (Trotti et al., 2024), and a XRF Olympus Vanta Core for the quantification of all the elements with a molecular mass greater than 24 Da (Mg, Si, P, S, Cl, Ca, Fe, Cu, Zn, Mo, I). Stable isotopes composition of C and N (δ^{13}C and δ^{15}N) were determined by connecting the ECS 4010 to an ID Micro EA isotope ratio mass spectrometer (Compact Science Systems, Newcastle-Under-Lyme, UK). Urea isotopic standard was used to calibrate the mass spectrometer.

2.11. Statistical analysis

Each biochemical and physiological analysis was performed on at least 3 samples collected from each pot. Data are reported as mean ± standard deviation. GraphPad Prism 9.5.0 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. One-way ANOVA was used to analyse fresh weight, dry weight and fresh weight/dry weight ratio as a function of the treatment (source of algal extract). Two-way ANOVA followed by Tukey's *post-hoc* test was used to analyse the parameters as a function of the time after last treatment and the source of algal extracts. Tukey's test allowed the comparison between CTR and each treatment. Principal Component Analysis (PCA) was carried out using Quasar 1.9.1 (Toplak et al., 2021) on wild rocket plants' analysed parameters (dependent variable) between different experimental conditions (independent variables). All statistical analyses were performed with a significance level of $\alpha = 0.05$. Asterisk (*) were used in figures to distinguish significantly different groups ($p < 0.05$).

3. Results

3.1. Growth comparison and photosynthetic parameters

The fresh weight of wild rocket plants increased due to microalgal treatment compared to with that of CTR treated with water (Fig. 1a). Except for plants treated with TO, fresh weight of all other plants significantly increased ($p = 0.0143$), and biostimulated plants weighted 27–32 % more than CTR plants. When biostimulants were applied, particularly CR and CS, the plants appeared healthier and carried larger leaves than the CTR (Fig. 1b-f).

The maximum quantum efficiency of photosystem II (F_v/F_m) in treated plants was always higher than in CTR one, as shown in Fig. 2a. Among the treated plants, the highest value of F_v/F_m was achieved with CR extract, while the lowest value was registered for TO treatment. The effect of biostimulation was also confirmed by analysing the PI parameter, which was significantly higher in treated plants ($1.5 < PI < 2.5$) than in CTR plants (0.90 ± 0.36) (Fig. 2b, $p < 0.0001$). The effect on the PI was also time-dependent ($p = 0.0140$) since the parameter increased during the first 24 and 48 h but decreased to CTR values at 72 h after the algal treatment. AP was the only treatment that showed a significant difference at 72 h.

The higher biomass content and PSII efficiency of the treated plants were unrelated to the higher pigment content (Table 2). Of all the treatments, only the consortium extract significantly increased the chlorophyll *a* and total carotenoids in the plants (Tab. S.1). Nonetheless, these values decreased over time, as did the difference with CTR.

3.2. Antioxidant content

The microalgal extracts did not significantly affect the concentration of antioxidant compounds (reported as phenolic index and total anthocyanins) (Table 2). As for chlorophyll *a* and carotenoids, the only treatment which led to a significant increase compared with CTR was CS. Nonetheless, significant differences were observed only during 48 of treatment, while at 72 h, the effects were no longer visible. It should be noted that cyanidin and phenol content decreased during time both in CTR and treated plants with average values which decreased from 24.2 to 19.4 mg 100 g^{-1} FW for total anthocyanins and from 26.0 to 21.6 $ABS_{320\ nm}$ g^{-1} for phenolic index.

3.3. Nitrate content

The nitrate concentration at 24 h and 48 h from treatment in biostimulated plants was statistically similar to that in CTR plants, with values ranging from 16.3 to 7.1 mg g^{-1} (Table 2). On average, the nitrate concentration in the CTR was slightly higher than that in the other conditions. Nonetheless, an increase in the content of treated plants was observed at 72 h after algal treatment; indeed, while CTR nitrate concentration decreased over time (from 14.3 to 9.6 mg g^{-1}), due to treatments, the content increased up to 20.1 mg g^{-1} as in the case of

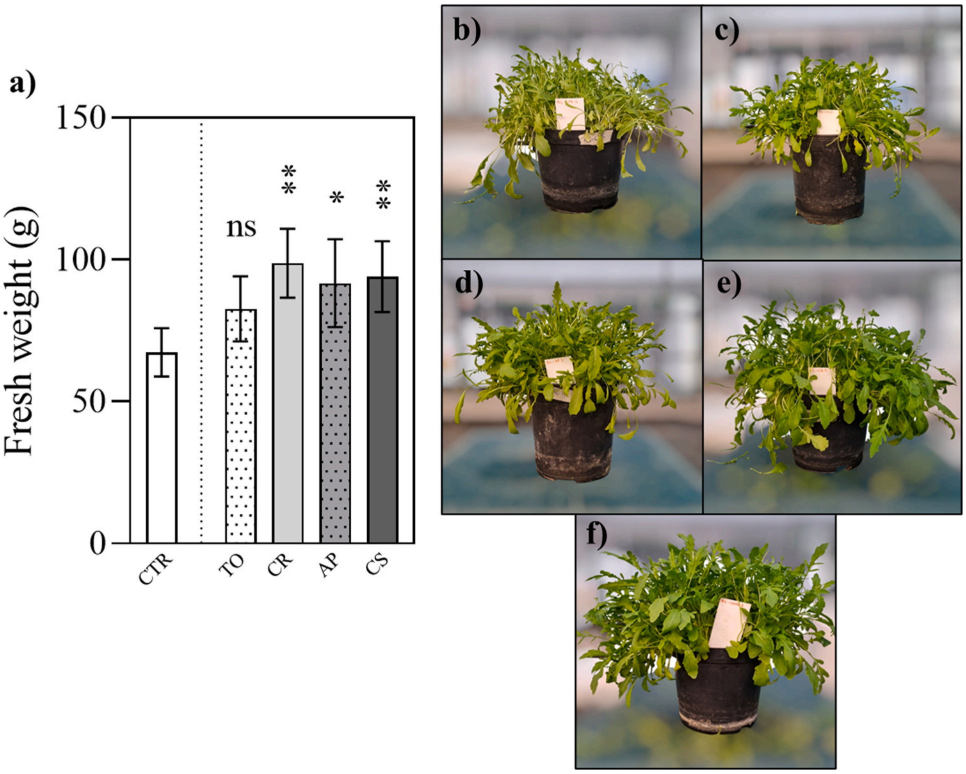

Fig. 1. a) fresh weight of rocket plants. Data are reported as mean ± standard deviation. Asterisks (*) represent the degree of significative difference between the CTR and treatment conditions (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). b-f) pictures of plants are reported in the following order: CTR (b), TO (c), CR (d), AP (e), CS (f).

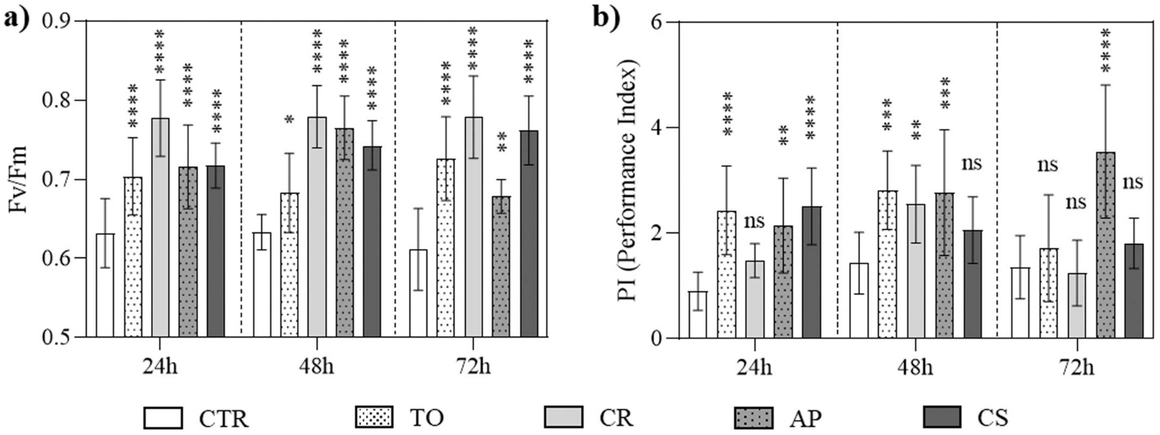

Fig. 2. a) Maximum quantum efficiency of photosystem II (F_v/F_m) and b) performance index (PI) of rocket plants. Data are reported as mean ± standard deviation. Asterisks (*) represent the degree of significative difference between the CTR and treatment conditions (ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

A. protothecoides treatment.

3.4. *Sugar and protein content*

The sugar content and composition remained mostly unaffected by treatment with the algal extracts. Significant differences were observed only for the sucrose concentration at 48 h after biostimulation, where treated plants, except when CS treated, had lower content compared to the content of CTR plants (Table 2). The observed variability was only explained by the type of algal extract ($p = 0.0004$), and not by the time of sampling after the algal application (not significant, $p = 0.1740$). Differences in reducing sugars and total sugar content were not significant as also reported by two-way ANOVA ($p > 0.05$).

The protein content was slightly affected by different biostimulant applications ($p = 0.0257$), with the highest concentrations recorded in the CTR and TO plants (Table 2). Nonetheless, the content did not change over time and was stable over the 72 h of sample collection.

3.5. *Leaves mineral composition*

C and N content in CTR and treated plants did not change over time, with only exception of the CS treatment, where C and N content decreased from 619 to 506 μg mg^{-1} DW and from 98 to 76 μg mg^{-1} DW respectively (Table 3, Tab. S.2). Overall, the biostimulation with

Table 2
biomass content, photosynthesis and biochemical parameters of wild rocket plants treated with water (CTR) or algal extracts. Where data is missing, “n.a.” is reported. All data are reported as mean ± standard deviation. Pigments, phenols, anthocyanins, nitrate and sugars are expressed on a fresh weight basis. Proteins are expressed on a dry-weight basis. The p values from the one- and two-way ANOVA for the various parameters, considering the factors Treatment, Time, and their interaction, are reported in the final column of the table. Significant p values are shown in bold.

Parameter	Time	Treatment					*p* value		
		CTR	TO	CR	AP	CS	Treatment	Time	Interaction
Fresh weight (g)	72 h	67.30 ± 8.45	82.64 ± 11.41	98.68 ± 12.11	91.68 ± 15.51	93.99 ± 12.44	**0.0143**	/	/
Dry weight (g)	72 h	4.03 ± 0.47	5.08 ± 1.03	5.81 ± 0.63	5.49 ± 1.02	6.07 ± 0.82	**0.0160**	/	/
PI	24 h	0.90 ± 0.36	2.43 ± 0.84	1.48 ± 0.32	2.14 ± 0.90	2.51 ± 0.73	**< 0.0001**	**0.0140**	**0.0004**
	48 h	1.43 ± 0.59	2.81 ± 0.75	2.55 ± 0.74	2.76 ± 1.19	2.06 ± 0.63			
	72 h	1.35 ± 0.60	1.71 ± 1.01	1.24 ± 0.62	3.55 ± 1.26	1.80 ± 0.48			
F_v/F_m	24 h	0.63 ± 0.04	0.70 ± 0.05	0.78 ± 0.05	0.72 ± 0.05	0.72 ± 0.03	**< 0.0001**	0.3889	**0.0022**
	48 h	0.63 ± 0.02	0.68 ± 0.05	0.78 ± 0.04	0.77 ± 0.04	0.74 ± 0.03			
	72 h	0.61 ± 0.05	0.73 ± 0.05	0.78 ± 0.05	0.68 ± 0.02	0.76 ± 0.04			
Chlorophyll *a* (μg mg^{-1})	24 h	0.93 ± 0.11	1.00 ± 0.14	1.24 ± 0.35	0.98 ± 0.09	1.52 ± 0.64	**< 0.0001**	**< 0.0001**	0.8572
	48 h	0.87 ± 0.12	1.03 ± 0.16	1.02 ± 0.16	0.86 ± 0.12	1.29 ± 0.21			
	72 h	0.60 ± 0.08	0.72 ± 0.09	0.94 ± 0.09	0.75 ± 0.14	1.10 ± 0.13			
Chlorophyll *b* (μg mg^{-1})	24 h	0.43 ± 0.13	0.45 ± 0.21	0.54 ± 0.19	0.35 ± 0.10	0.55 ± 0.21	**0.0200**	**< 0.0001**	0.3763
	48 h	0.32 ± 0.13	0.44 ± 0.18	0.28 ± 0.05	0.24 ± 0.03	0.38 ± 0.05			
	72 h	0.21 ± 0.03	0.23 ± 0.03	0.29 ± 0.03	0.25 ± 0.04	0.35 ± 0.04			
Carotenoids (μg mg^{-1})	24 h	0.23 ± 0.05	0.23 ± 0.05	0.30 ± 0.16	0.27 ± 0.04	0.41 ± 0.18	**< 0.0001**	**0.0081**	0.9874
	48 h	0.23 ± 0.04	0.23 ± 0.05	0.28 ± 0.05	0.24 ± 0.03	0.40 ± 0.06			
	72 h	0.17 ± 0.03	0.19 ± 0.02	0.25 ± 0.03	0.21 ± 0.04	0.31 ± 0.03			
Phenolic index (Abs_{300} g^{-1})	24 h	23.90 ± 1.46	24.00 ± 3.61	25.20 ± 7.38	25.58 ± 5.05	31.42 ± 6.24	**< 0.0001**	**0.0011**	0.5389
	48 h	23.78 ± 2.06	20.47 ± 3.96	24.58 ± 3.29	23.06 ± 3.53	33.04 ± 3.99			
	72 h	21.01 ± 2.00	20.22 ± 1.79	23.19 ± 1.24	18.96 ± 3.04	24.83 ± 6.81			
Cyanidin equivalent (mg 100 g^{-1})	24 h	22.15 ± 3.03	21.75 ± 3.23	28.15 ± 9.86	22.32 ± 4.61	26.73 ± 4.24	**< 0.0001**	**0.0005**	0.9476
	48 h	20.37 ± 1.61	20.26 ± 3.65	24.18 ± 3.62	19.84 ± 2.49	27.91 ± 2.47			
	72 h	16.80 ± 1.77	18.49 ± 2.25	21.90 ± 0.87	17.36 ± 2.55	22.55 ± 6.40			
Nitrate (mg kg^{-1})	24 h	14330 ± 3260	10850 ± 2160	8290 ± 2210	9770 ± 990	7450 ± 750	**0.0290**	**0.0118**	**0.0039**
	48 h	11860 ± 2660	16330 ± 8010	10120 ± 1930	9810 ± 2390	7190 ± 1200			
	72 h	9560 ± 5080	13330 ± 8940	12770 ± 6350	20100 ± 2680	12770 ± 3470			
Reducing sugars (mg kg^{-1})	24 h	2276 ± 350	2218 ± 225	2248 ± 438	2637 ± 190	2484 ± 136	**< 0.0001**	**< 0.0001**	0.1538
	48 h	2557 ± 147	1940 ± 180	2260 ± 380	2575 ± 445	2837 ± 332			
	72 h	2127 ± 374	1221 ± 270	1824 ± 341	1929 ± 518	2160 ± 227			
Sucrose (mg kg^{-1})	24 h	997 ± 65	868 ± 51	625 ± 278	772 ± 89	742 ± 132	**0.0004**	0.1740	0.1444
	48 h	995 ± 106	671 ± 40	669 ± 88	623 ± 118	743 ± 215			
	72 h	769 ± 232	671 ± 138	553 ± 169	859 ± 188	698 ± 341			
Total sugars (mg kg^{-1})	24 h	2579 ± 423	2172 ± 338	2834 ± 942	2764 ± 294	2737 ± 593	**0.0153**	**< 0.0001**	0.2251
	48 h	3323 ± 384	2607 ± 430	2386 ± 433	3623 ± 487	3074 ± 770			
	72 h	2437 ± 1037	1525 ± 479	2262 ± 772	1998 ± 712	2183 ± 388			
Proteins (mg g^{-1})	24 h	232 ± 14	231 ± 29	219 ± 59	220 ± 50	194 ± 25	**0.0257**	0.7035	0.9104
	48 h	258 ± 42	286 ± 91	155 ± 34	206 ± 67	194 ± 47			
	72 h	236 ± 72	271 ± 91	n.a.	210 ± 79	185 ± 46			

Table 3

elemental composition of wild rocket plants treated with water (CTR) or algal extracts. Where data is missing, “n.a.” is reported. All data are reported as mean ± standard deviation. Values are expressed as ppm (part per million) on a dry weight basis. The p values from the two-way ANOVA for the different elements, considering the factors Treatment, Time, and their interaction, are reported in the final column of the table. Significant p values are shown in bold.

Parameter	Time	Treatment					*p* value		
		CTR	TO	CR	AP	CS	Treatment	Time	Interaction
C	24 h	504953 ± 41051	531033 ± 57959	536513 ± 62939	535826 ± 58509	608386 ± 91278	0.4472	0.3084	0.7948
	48 h	504460 ± 88565	495933 ± 101080	596700 ± 163336	501113 ± 52098	539086 ± 64004			
	72 h	524628 ± 109435	446891 ± 49066	n.a.	469402 ± 80057	504866 ± 63635			
N	24 h	86660 ± 11305	93640 ± 12315	95053 ± 6316	88140 ± 15359	98446 ± 16653	0.9128	0.2094	0.9503
	48 h	91940 ± 14277	95160 ± 25752	94780 ± 20669	88740 ± 12750	86940 ± 17194			
	72 h	90763 ± 9649	82511 ± 6103	n.a.	78485 ± 11449	76858 ± 19352			
Ca	24 h	158063 ± 28289	100965 ± 19934	112666 ± 49018	67054 ± 41295	74352 ± 24628	0.1954	0.0099	0.1591
	48 h	168351 ± 9891	152371 ± 57883	97952 ± 83153	155243 ± 7881	158401 ± 5424			
	72 h	134214 ± 27486	150089 ± 76942	n.a.	152269 ± 7350	95310 ± 22657			
Cl	24 h	65887 ± 13997	56584 ± 14864	76224 ± 26341	52806 ± 24056	92642 ± 25551	**< 0.0001**	**< 0.0001**	**0.0175**
	48 h	75334 ± 11350	95184 ± 27915	65006 ± 36830	124267 ± 8392	146881 ± 2357			
	72 h	63588 ± 9236	85119 ± 29789	n.a.	104768 ± 693	90394 ± 5326			
Cu	24 h	20 ± 2	12 ± 2	42 ± 2	38 ± 1	21 ± 1	**0.0005**	**0.0046**	**< 0.0001**
	48 h	28 ± 9	28 ± 12	39 ± 4	28 ± 1	23 ± 2			
	72 h	24 ± 2	28 ± 11	n.a.	20 ± 2	16 ± 2			
Fe	24 h	463 ± 42	307 ± 149	274 ± 128	195 ± 191	237 ± 135	0.2605	0.1596	0.1471
	48 h	422 ± 27	377 ± 59	228 ± 121	419 ± 96	429 ± 26			
	72 h	305 ± 52	401 ± 272	n.a.	372 ± 31	269 ± 40			
I	24 h	230 ± 74	131 ± 46	165 ± 86	116 ± 18	89 ± 47	0.3699	0.0513	0.1089
	48 h	228 ± 33	187 ± 77	139 ± 64	201 ± 14	215 ± 12			
	72 h	155 ± 62	185 ± 90	n.a.	185 ± 29	120 ± 39			
Mg	24 h	28698 ± 704	25638 ± 816	16586 ± 1208	22571 ± 966	17740 ± 951	0.1334	0.0834	**< 0.0001**
	48 h	29031 ± 2084	30061 ± 8029	17268 ± 483	25532 ± 830	17831 ± 5943			
	72 h	29478 ± 1001	34581 ± 4348	n.a.	23097 ± 163	20818 ± 4043			
Mo	24 h	10 ± 1	9 ± 3	11 ± 2	12 ± 4	9 ± 2	0.0811	0.1158	0.1789
	48 h	12 ± 3	11 ± 1	14 ± 2	9 ± 1	13 ± 2			
	72 h	14 ± 4	10 ± 1	n.a.	13 ± 1	8 ± 0			
P	24 h	11408 ± 444	10214 ± 2116	7470 ± 1446	5789 ± 2342	6830 ± 1292	**0.0003**	**0.0004**	**0.0447**
	48 h	12985 ± 1225	11916 ± 981	7785 ± 3042	10936 ± 792	9948 ± 515			
	72 h	13085 ± 2715	10649 ± 2694	n.a.	12962 ± 1858	8642 ± 1932			
S	24 h	64661 ± 4917	51103 ± 6811	51791 ± 13860	39586 ± 15812	45254 ± 13172	0.2194	**0.0039**	0.1828
	48 h	65956 ± 9570	66978 ± 13280	49295 ± 25807	68094 ± 1427	72058 ± 3945			
	72 h	62170 ± 8778	60774 ± 11114	n.a.	63652 ± 2916	51482 ± 12030			
Si	24 h	1185 ± 249	1113 ± 118	1302 ± 534	1309 ± 388	1520 ± 274	0.0569	**0.0106**	**< 0.0001**
	48 h	1035 ± 29	1501 ± 237	2283 ± 1082	1445 ± 162	1321 ± 36			
	72 h	3699 ± 1014	1845 ± 656	n.a.	1108 ± 138	1289 ± 165			
Zn	24 h	198 ± 24	104 ± 59	107 ± 51	111 ± 132	125 ± 80	0.3381	0.8589	0.2458
	48 h	158 ± 24	132 ± 41	64 ± 27	181 ± 67	161 ± 18			
	72 h	100 ± 58	118 ± 62	n.a.	143 ± 20	124 ± 33			

microalgae did not affect the two elemental pools. The analysis of stable isotopes showed an interesting effect on the fractionation of N isotopes ($\delta^{15}N$). Generally, compared to CTR, treated plants had a lower $\delta^{15}N$ (Fig. 3), indicating an increased fractionation in favour of the heavier isotope and the fractionation significantly increased over time (Fig. 3, Tab. S.2). Notable evidence of the higher fractionation was found in the consortium treated plants where the $\delta^{15}N$ value decreased from 3.00 ± 0.67 to −0.02 ± 2.11. In contrast, differences in C fractions between CTR and treated plants were not observed (Tab. S.2): $\delta^{13}C$ values ranged from −32 to −35 regardless of the growing condition and values did not change during time.

Excluding carbon and nitrogen, the elemental composition of the treated wild rocket plants was similar to that of the control (CTR) plants (Tab. S.3). However, as indicated in Table 3, calcium (Ca) and chlorine (Cl) were the only elements that significantly differed from the CTR conditions. Specifically, the Ca concentration was significantly lower in the treated plants 24 h after biostimulant application, while the difference became less pronounced at 48 and 72 h (Table 3). Conversely, the Cl concentration was higher in the treated plants at 24 h post-treatment, but the Cl content matched that of the CTR plants at 48 and 72 h (Tab. S.3). Notably, plants treated with the CS biostimulant consistently showed higher Cl levels than those of the CTR plants throughout the measurement periods.

3.6. Overall comparison between tested biostimulants

The Principal Component Analysis (PCA) carried out on the elemental composition could not distinguish the treatments from the control condition (Fig. S.4a), further confirming the results reported in Table 2 where most of the elements within the plants did not change following the treatment with the algal extract.

The PCA carried out on the biomass content and, physiological and biochemical parameters (Fig. S.4b) allowed to distinguish a certain pattern and to separate the different treatment groups from the control group. Both PC1 and PC2 allowed the separation of the samples based on the treatment condition and sampling time: from right to left and from the bottom to the top, within the same group the samples were divided based on the sampling time. The treatment groups were divided from left to right and from bottom to top, with the CTR group being in the top left of the graph and the CS treatment group on the bottom right of the graph. Based on the loading values (Tab. S.4), parameters related to the photosynthesis (PI, F_v/F_m, carotenoids, chlorophyll *a*, and chlorophyll *b*) positively correlated with each other and the CS treated plants were characterized by higher values than CTR plants. Although a certain pattern was discernible, PCA components explained only 30.9 % of the variability (Fig. S.4b).

4. Discussion

4.1. Enhanced growth of biostimulated plants

Biostimulant products are known for their ability to increase nutrient uptake, tolerate abiotic stress, and enhance photosynthetic performance (Bulgari et al., 2015). Overall, these changes can result in more remarkable growth than in untreated plants, as it was the case in our study. Similar findings have been reported by Aloui et al. (2023) and Supraja et al. (2020) in lettuce and tomato plants, respectively, following treatment with algal biostimulants. Supraja et al. (2020) observed that an increase in tomato fresh and dry weight was closely linked to the concentration of microalgal extract, with the highest values reaching 60 %. However, as reported by Aloui et al. (2023) and other authors (Afonso et al., 2021; Melo et al., 2024), it appears that more significant effects of biostimulation can be achieved when plants experience abiotic stresses, such as water stress or heat stress (Toscano et al., 2023). In addition to abiotic stress, plant species also play a significant role: indeed, as reported by Toscano et al. (2023), the application of three different biostimulants (in the absence of abiotic stress) produced similar effects within the same plant species (turnip greens and radish microgreens) but varied between the two. The treatment of wild rocket plants with microalgal extracts produced similar results, with effects mostly independent of the algal source (except for *T. obliquus* treatment). The increase in biomass production could be related to the protein and aminoacidic content of the algal extracts, which are well known for their biostimulant properties (Khan et al., 2019; Almadi et al., 2020; Al-Karaki and Othman, 2023) stimulating the primary metabolism (Navarro-Morillo et al., 2023; Rosa et al., 2023).

In addition to the increase in biomass content, algal application enhanced the vigour and health of treated plants (Fig. 1), while control plants appeared floppier. Studies on lettuce reported remarkably similar results, where the aerial part of plants treated with 1 mg L^{-1} of *Chlorella vulgaris* extracts were healthier and much larger than the control plants, while the roots underwent fewer changes in terms of length and weight (La Bella et al., 2021). Stimulation of the aerial parts could be related to the carbohydrate content of the algal extracts, as also suggested by Rachidi et al. (2020) in tomato plants. As for the protein content, carbohydrates are one of the main components of our algae (Table 1), and together, these two macromolecular pools could have contributed to the enhanced growth of the treated plants. Overall, the enhanced growth of the aerial/edible part as evidenced by the increase in fresh weight is significant from a commercial perspective.

However, in other cases, the ratio changed depending on the growth condition, especially favouring fresh weight (Plaza et al., 2018; Supraja and Behera, 2020). Our study found that the fresh weight (FW)/dry weight (DW) ratio remained consistent across all experimental conditions (Fig. S.1) despite the biostimulant treatment increasing the plant weight. This indicated that our treatment did not increase the water content but rather an overall improvement in plant growth and biomass

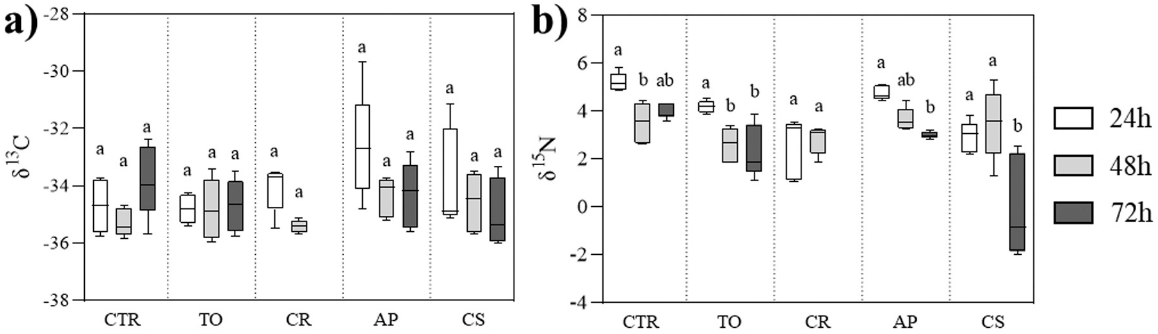

Fig. 3. Carbon (a) and Nitrogen (b) isotopic fractionation of control and treated plants. Data are reported as mean ± standard deviation. Letters represent significant differences between different sampling times within each experimental condition.

content. The observed positive effect may be attributed to the hormone-like compounds found in the microalgal extracts (González-Pérez et al., 2022). Young olive plants treated with a protein hydrolysate, rich in amino acids with a hormone-like activity, exhibited enhanced growth compared to control plants (Almadi et al., 2020). Researchers have suggested that the increase in plant biomass may be linked to heightened photosynthetic activity, as noted in other studies (Chovanček et al., 2023; Aloui et al., 2023). As shown in Fig. 2, the treated plants exhibited a general increase in F_v/F_m and PI, indicating high photosynthetic performance for at least 72 h after biostimulant application. Although the literature suggests that the content of chlorophylls and carotenoids in treated plants increase (Croft et al., 2017; Bulgari et al., 2019; Cristiano and De Lucia, 2021; Raza et al., 2022; Arif et al., 2023) our study found that the content remained comparable to that of the control conditions (Table 2). Therefore, enhanced growth is not linked to greater light capture but rather to improved light and nutrient utilization (Wobbe et al., 2016; Vitale et al., 2021).

4.2. *Variation in the biochemical and mineral profile*

Sugars are directly related to the photosynthetic performance of plants, and changes in the performance indicators PI and/or F_v/F_m are usually associated with changes in sugar (Jolayemi et al., 2023). However, on average, the sugar content in the treated plants was similar to that in the control plants. Despite expectations of increased sugar content due to enhanced photosynthetic performance (Fig. 2), similar results have been reported in the literature, such as in the case of strawberry plants treated with microalgae-based biostimulants (Žunić et al., 2024). Conversely, studies on common beans treated with different microalgae extracts (Refaay et al., 2021) indicated an increase in soluble sugar, polysaccharides, and total carbohydrates following each treatment. Our results, together with numerous reports in the literature, indicate that biostimulant efficacy depends not only on the algal extract composition but also critically on the treated plant species. Each plant species differs in the repertoire of peptide and hormone receptors, cuticle structure and stomatal absorption efficiency, as well as in the specific enzymatic and transcriptional networks that mediate biochemical signaling (Bulgari et al., 2015). For instance, foliar application of protein hydrolysates in soilless lettuce increased phenolic content by up to 30 %, with effects persisting for 72 h, whereas other species showed no such enhancement without abiotic stress (Al-Karaki and Othman, 2023). These variations are likely due to differences in amino acid transporter expression, endogenous phytohormone levels, and activation of signaling cascades such as MAP-kinases and calcium-dependent protein kinases. The divergent responses observed in wild rocket compared to tomato, wheat, or lettuce genotypes in our study underscore the need to tailor biostimulant formulations and dosages to the specific crop species.

In addition, it's worth noting that the leaf sucrose levels in treated plants were transiently lower during the first 48 h, returning to control values only by 72 h. We propose that this decrease reflects a shift in carbon allocation: microalgal biostimulants likely enhanced the mobilisation of photoassimilates from source leaves to growing sink tissues (e.g., stems and roots), thereby driving biomass accumulation. Comparable source–sink rebalancing has been reported after application of protein hydrolysates, which modulate carbon metabolism and upregulate sugar transporter activity to promote phloem loading (Colla et al., 2014; Malécange et al., 2023). This suggests that the treatment stimulates more efficient use of foliar sugars to fuel growth rather than their retention in leaves.

Antioxidant compounds are produced in plants as protective agents against various abiotic stresses to reduce the harmful effects of reactive oxygen species (Wang et al., 2019; Laddomada et al., 2021; Liu et al., 2021). Their accumulation is also beneficial in the post-harvest phase, where it helps preserve product quality (Spinardi et al., 2018; Meitha et al., 2020). Indeed, during storage, oxidative stress leads to the accumulation of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, which are neutralised by antioxidants previously synthesized. Accumulation occurs even in non-stressed plants, as in our trial, after biostimulant application. For example, an increase in anthocyanins and flavanols has been observed in strawberries treated with microalgae (Žunić et al., 2024). In our trial, only CS application temporarily increased the antioxidant content (at 24 and 48 h after treatment), but no changes were observed after single species-based treatments. This finding holds potential significance in the post-harvest phase and, given that wild rocket is typically marketed as a fresh-cut commody, implementing a pre-harvest treatment with a microalgal consortium extract to enhance antioxidant levels could be used to extend the shelf life and mitigate product degradation (Spinardi et al., 2018; Meitha et al., 2020).

Data on elemental composition indicated an increased uptake of nutrients in absolute terms, but not in concentration within plant tissues (Table 3, Fig. S4a). Notably, this increased nutrient uptake was correlated with higher biomass content (Fig. 1), suggesting that overall growth was enhanced. As a result, the plants had a greater need for the uptake of, and utilization of elements from the soil.

The C and N content, and fractionation proved what was just reported. As expected, C fractionation was comparable in each experimental condition because the ratio of C isotopes depends mainly on plant species, C source, and environmental conditions (the same between the control and the treated conditions). Even photosynthetic metabolisms (C4, CAM), carbon concentrating mechanisms (CCMs) and anaplerotic pathways are known to affect the C signature (Treves et al., 2021), but *Diplotaxis tenuifolia* L. lacks any of them, explaining the absence of variation.

Although C content per mg of dry biomass did not change following algae application, when expressed as total C per pot (multiplied by the plant biomass value), the content was much higher than that of the control plants, indicating higher photosynthetic performance (see paragraph 4.1) and carbon fixation. This trend was also observed for N content, with the concentration ($mg\ g^{-1}$) being the same in treated and control conditions, but the total N within the treated plants' biomass was higher. The same observation applies to all other elements analysed and reported in Table 3. The concentrations in the treated plants were similar to those in the control plants. This finding aligns with what has been reported in the literature, indicating that the use of biostimulants leads to increased nutrient concentrations in treated plants when they are subjected to abiotic stress (Baltazar et al., 2021). However, in the absence of such stress, the nutrient content remained unchanged.

The nitrate content and $\delta^{15}N$ of treated plants indirectly confirmed the higher uptake and metabolization of N. Indeed, as reported by other authors, N fractionation is mostly dependent on the transamination process where NH_2 is transferred from an amino acid to an α-keto acid (Macko et al., 1986, 1987; Yoneyama et al., 1991). A higher fractionation favouring lighter isotopes (more negative $\delta^{15}N$ values) corresponded to a higher incorporation of N into C skeletons (i.e. proteins).

However, the application of microalgal biostimulants significantly increased nitrate concentration in the leaves of *D. tenuifolia* at 72 h post-treatment, reaching levels as high as 20,000 $mg\ kg^{-1}$. This sharp increase may result from enhanced nitrate transporter activity and accelerated nitrogen uptake induced by biostimulant application, as reported in prior studies (Engel et al., 2023). However, the hyperaccumulation is also likely tied to the species' intrinsic ability to store nitrate in vacuoles as a metabolic reserve, particularly during rapid growth phases stimulated by biostimulants (Bulgari et al., 2020).

Indeed, *D. tenuifolia* is recognized as a nitrate hyperaccumulator among the Brassicaceae family. This trait is influenced by the species' genetic characteristics and environmental conditions, such as nitrogen availability in the soil and light intensity (Santamaria, 2006; Bian et al., 2020). While nitrates are essential for plant growth, excessive accumulation can pose risks to human health, as they can be converted into nitrites during digestion and contribute to the formation of potentially carcinogenic nitrosamines (Bian et al., 2020; Bulgari et al., 2020; Wuijts et al., 2022).

An important consideration is the balance between the agronomic benefits of biostimulants, and the potential risks associated with excessive nitrate accumulation. Strategies to manage this balance might include optimizing the timing and dosage of biostimulant applications or integrating them with other approaches that regulate nitrogen metabolism, such as light-level adjustments or the use of nitrate inhibitors (Zhang et al., 2018).

4.3. *Overall effect of biostimulation*

Overall composition of consortium-treated samples (CS) diverged from those of both control (CTR) and monospecific treated samples at 72 h post-application (Table 2, Fig. S4b).

Moreover, in CS-treated plants, the Performance Index (PI) and maximum quantum efficiency of PSII (F_v/F_m) were higher than in CTR, with peak gains of approximately 20–25 %. Total chlorophylls and carotenoids similarly exhibited consistent, albeit moderate, increases of 10–15 % above control levels, which persisted through 48 h before gradually declining. Such enhancements were absent in all monospecific treatments, providing evidence that an algal consortium extract may have applications beyond those achieved by single-species extracts since the interaction among individual species affect functional quality of the obtained biomass (Mollo et al., 2024b). From a sustainability and circularity perspective, the exploitation of such microalgal biomass becomes crucial since the use of microalgal consortia is becoming increasingly common in biotechnological applications, such as waste and wastewater treatment (Stiles et al., 2018; Rashid et al., 2019; Mugnai et al., 2023). The very low concentration proved as effective for the first time in the present study is also a promising fact for the feasible scale up of the biotechnology.

5. Conclusion

In conclusion, this study demonstrated the effectiveness of microalgal biostimulants, both monospecific and algal consortium extracts, in enhancing the growth and photosynthetic efficiency of *Diplotaxis tenuifolia* L. under controlled conditions. This suggests that the positive effects on growth resulted from increased photosynthetic efficiency rather than activation of stress-related pathways, which would typically lead to broader changes in the internal biochemical pool.

However, such biochemical changes, specifically in antioxidant content and pigment levels, were observed only in the consortium-treated plants, and not in those treated with monospecific extracts. This indicates that the compositional and functional uniqueness of the consortium, likely arising from interspecies interactions, plays a crucial role in modulating plant biochemical responses. These emergent effects support the idea that co-cultivation can result in a biostimulant with enhanced or novel bioactivity.

The effects, noted shortly after application, highlight the potential of microalgae-based foliar sprays as a sustainable strategy for improving crop quality and productivity. The findings indicate that microalgal biostimulants, in particular when applied as a consortium, can serve as valuable tools to enhance both the nutritional and commercial quality of crops such as wild rocket, contributing to improved vigour and extended post-harvest freshness. Additionally, it was observed that the effects of the algal consortium significantly differed from those produced by monoculture extracts, demonstrating that co-cultivation resulted in a completely new product.

Because of their environmental benefits and effectiveness at low doses, microalgal biostimulants could play a key role in sustainable agriculture, aligning with circular economy principles by utilizing readily cultivable and renewable algae sources. Future research should explore the long-term impacts, diverse environmental conditions, and various crop species to maximize the potential applications of these promising biostimulants.

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CRediT authorship contribution statement

Giacomo Cocetta: Writing – review & editing, Supervision, Methodology, Conceptualization. **Lorenzo Mollo:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Alice Petrini:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Norici Alessandra:** Writing – review & editing, Supervision, Funding acquisition. **Antonio Ferrante:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2025.112643.

Data availability

Data will be made available on request.

References

S. Afonso, M. Arrobas, M.Â. Rodrigues, Response of hops to algae-based and nutrient-rich foliar sprays, Agriculture 2021 11 (11) (2021) 798.

O. Ali, A. Ramsubhag, J. Jayaraman, Biostimulant properties of seaweed extracts in plants: implications towards sustainable crop production, Plants 2021 10 (10) (2021) 531.

G.N. Al-Karaki, Y. Othman, Effect of foliar application of amino acid biostimulants on growth, macronutrient, total phenol contents and antioxidant activity of soilless grown lettuce cultivars, South Afr. J. Bot. 154 (2023) 225–231.

M.M. Allen, R.Y. Stanier, Growth and division of some unicellular blue-green algae, J. Gen. Microbiol 51 (1968) 199–202.

L. Almadi, A. Paoletti, N. Cinosi, E. Daher, A. Rosati, C. Di Vaio, F. Famiani, A biostimulant based on protein hydrolysates promotes the growth of young olive trees, Agriculture 10 (2020) 618.

D. Aloui, F. Kalleli, G. Abid, C. Karmous, M. Manaa, M. M'Hamdi, Marine Algal extract as a biostimulant to improve tolerance to salinity in lettuce plants, Emir. J. Food Agric. 35 118 (2023).

A. Álvarez-González, L. Serrano, G. Gorchs, E. Uggetti, Exploring the biostimulant potential of Scenedesmus sp. grown in wastewater: impacts on plant growth and photosynthetic activity of lettuce, Chemosphere 382 (2025) 144494.

Y. Arif, A. Bajguz, S. Hayat, Moringa oleifera Extract as a Natural Plant Biostimulant, J. Plant Growth Regul. 42 (2023) 1291–1306.

M. Baltazar, S. Correia, K.J. Guinan, N. Sujeeth, R. Bragança, B. Gonçalves, Recent advances in the molecular effects of biostimulants in plants: an overview, Biomolecules 11 (2021) 1096.

Z. Bian, Y. Wang, X. Zhang, T. Li, S. Grundy, Q. Yang, R. Cheng, A review of environment effects on nitrate accumulation in leafy vegetables grown in controlled environments, Foods 9 (2020).

M.A. Borowitzka, Algae as food, Microbiol. Ferment. Foods (1998) 585–602.

J.C.A. Braun, L.M. Colla, Use of microalgae for the development of biofertilizers and biostimulants, Bioenergy Res 16 (2023) 289–310.

C. Brito-Lopez, N. van der Wielen, M. Barbosa, R. Karlova, Plant growth–promoting microbes and microalgae-based biostimulants: sustainable strategy for agriculture and abiotic stress resilience, Philos. Trans. R. Soc. B Biol. Sci. 380 (2025).

R. Bulgari, G. Cocetta, A. Trivellini, A. Ferrante, Borage extracts affect wild rocket quality and influence nitrate and carbon metabolism, Physiol. Mol. Biol. Plants 26 (2020) 649–660.

R. Bulgari, G. Cocetta, A. Trivellini, P. Vernieri, A. Ferrante, Biostimulants and crop responses: a review, Biol. Agric. Hortic. 31 (2015) 1–17.

R. Bulgari, A. Trivellini, A. Ferrante, Effects of two doses of organic extract-based biostimulant on greenhouse lettuce grown under increasing NaCl concentrations, Front Plant Sci. 9 (2019) 403468.

P. Calvo, L. Nelson, J.W. Kloepper, Agricultural uses of plant biostimulants, Plant Soil 383 (2014) 3–41.

V. Candido, V. Cantore, D. Castronuovo, M. Denora, M.I. Schiattone, L. Sergio, M. Todorovic, F. Boari, Effect of water regime, nitrogen level, and biostimulant application on the water and nitrogen use efficiency of wild rocket [Diplotaxis tenuifolia (L.) DC, Agronomy 13 (2023) 507.

P. Carillo, L.F. Ciarmiello, P. Woodrow, G. Corrado, P. Chiaiese, Y. Rouphael, Enhancing sustainability by improving plant salt tolerance through macro- and micro-algal biostimulants, Biology (Basel) 9 (2020) 253.

G. Caruso, C. El-Nakhel, Y. Rouphael, E. Comite, N. Lombardi, A. Cuciniello, S.L. Woo, Diplotaxis tenuifolia (L.) DC. yield and quality as influenced by cropping season, protein hydrolysates, and trichoderma applications, Plants 9 (2020) 697.

D.A. Cataldo, M. Maroon, L.E. Schrader, V.L. Youngs, Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid, Commun. Soil Sci. Plant Anal. 6 (1975) 71–80.

A. Chabili, F. Minaoui, Z. Hakkoum, M. Douma, A. Meddich, M. Loudiki, A comprehensive review of microalgae and cyanobacteria-based biostimulants for agriculture uses, Plants 13 (2024) 159.

M.G. Chieti, A. Petrucciani, L. Mollo, C. Gerotto, A.L. Eusebi, F. Fatone, A. Norici, J. González-Camejo, Acclimated green microalgae consortium to treat sewage in an alternative urban WWTP in a coastal area of Central Italy, Sci. Total Environ. 945 (2024) 174056.

E. Chovanček, J. Salazar, S. Şirin, Y. Allahverdiyeva, Microalgae from Nordic collections demonstrate biostimulant effect by enhancing plant growth and photosynthetic performance, Physiol. Plant 175 (2023).

M. Ciriello, E. Campana, G. Colla, Y. Rouphael, An Appraisal of Nonmicrobial Biostimulants' Impact on the Productivity and Mineral Content of Wild Rocket (Diplotaxis tenuifolia (L.) DC.) Cultivated under Organic Conditions, Plants 13 (2024) 1326.

G. Colla, Y. Rouphael, Microalgae: new source of plant biostimulants, Agronomy 10 (2020).

G. Colla, Y. Rouphael, R. Canaguier, E. Svecova, M. Cardarelli, Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis, Front Plant Sci. 5 (2014).

G. Cristiano, B. De Lucia, Petunia performance under application of animal-based protein hydrolysates: effects on visual quality, biomass, nutrient content, root morphology, and gas exchange, Front Plant Sci. 12 (2021).

H. Croft, J.M. Chen, X. Luo, P. Bartlett, B. Chen, R.M. Staebler, Leaf chlorophyll content as a proxy for leaf photosynthetic capacity, Glob. Chang Biol. 23 (2017) 3513–3524.

C. El-Nakhel, S. Petropoulos, I. Di Mola, L. Ottaiano, E. Cozzolino, Y. Rouphael, M. Mori, Biostimulants of different origins increase mineral content and yield of wild rocket while reducing nitrate content through successive harvests, Horticulturae 9 (2023) 580.

D.C.H. Engel, D. Feltrim, M. Rodrigues, J.L.C. Baptistella, P. Mazzafera, Algae extract increases seed production of soybean plants and alters nitrogen metabolism, Agriculture 13 (2023) 1296.

N. Gatti, M. Maghrebi, G. Serio, C. Gentile, V.V. Bunea, I. Vigliante, C. Boitte, C. Garabello, V. Contartese, C.M. Bertea, G. Mannino, Seaweed and yeast extracts as sustainable phytostimulant to boost secondary metabolism of apricot fruits, Front Plant Sci. 15 (2025).

M. Giordano, C. El-Nakhel, G. Caruso, E. Cozzolino, S. De Pascale, M.C. Kyriacou, G. Colla, Y. Rouphael, Stand-alone and combinatorial effects of plant-based biostimulants on the production and leaf quality of perennial wall rocket, Plants 9 (2020) 922.

M.M. Gitau, A. Farkas, V. Ördög, G. Maróti, Evaluation of the biostimulant effects of two Chlorophyta microalgae on tomato (Solanum lycopersicum), J. Clean. Prod. 364 (2022).

B.K. González-Pérez, A.M. Rivas-Castillo, A. Valdez-Calderón, M.A. Gayosso-Morales, Microalgae as biostimulants: a new approach in agriculture, World J. Microbiol Biotechnol. 38 (2022) 1–12.

G. Hariharan, N. Vathshalyan, H. Galahitigama, U. Wimalasiri, G. Don Kapila Kumara, Potential of foliar application of seaweed extracts as a biostimulant for abiotic stress alleviation on crop production, Rev. Agric. Sci. 12 (2024) 295–312.

P. du Jardin, Plant biostimulants: definition, concept, main categories and regulation, Sci. Hortic. 196 (2015) 3–14.

K. Jindo, T.L. Goron, P. Pizarro-Tobías, M.Á. Sánchez-Monedero, Y. Audette, A.O. Deolu-Ajayi, A. van der Werf, M. Goitom Teklu, M. Shenker, C. Pombo Sudré, J.G. Busato, R. Ochoa-Hueso, M. Nocentini, J. Rippen, R. Aroca, S. Mesa, M.J. Delgado, G. Tortosa, Application of biostimulant products and biological control agents in sustainable viticulture: A review, Front Plant Sci. 13 (2022).

O.L. Jolayemi, A.H. Malik, R.R. Vetukuri, G.V. Saripella, P.B. Kalyandurg, T. Ekblad, J.W. H. Yong, M.E. Olsson, E. Johansson, Metabolic processes and biological macromolecules defined the positive effects of protein-rich biostimulants on sugar beet plant development, Int J. Mol. Sci. 24 (2023) 9720.

D. Kaloudas, N. Pavlova, R. Penchovsky, Phycoremediation of wastewater by microalgae: a review, Environ. Chem. Lett. 19 (2021) 2905–2920.

R.V. Kapoore, E.E. Wood, C.A. Llewellyn, Algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices, Biotechnol. Adv. 49 (2021).

N. Khan, R.L. Ray, G.R. Sargani, M. Ihtisham, M. Khayyam, S. Ismail, Current progress and future prospects of agriculture technology: Gateway to sustainable agriculture, Sustainability (Switzerland) 13 (2021).

S. Khan, H. Yu, Q. Li, Y. Gao, B.N. Sallam, H. Wang, P. Liu, W. Jiang, Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability, Agronomy 9 (2019) 266.

W. Khan, U.P. Rayirath, S. Subramanian, M.N. Jithesh, P. Rayorath, D.M. Hodges, A. T. Critchley, J.S. Craigie, J. Norrie, B. Prithiviraj, Seaweed extracts as biostimulants of plant growth and development, J. Plant Growth Regul. 28 (2009) 386–399.

R. Kholssi, E.A.N. Marks, J. Miñón, O. Montero, A. Debdoubi, C. Rad, Biofertilizing effect of chlorella sorokiniana suspensions on wheat growth, J. Plant Growth Regul. 38 (2019) 644–649.

A.O. Klein, C.W. Hagen, Anthocyanin production in detached petals of *Impatiens balsamina* L, Plant Physiol. 36 (1961) 1–9.

La Bella, Emanuele, Andrea Baglieri, Ermes Ivan Rovetto, Piergiorgio Stevanato, and Ivana Puglisi. 2021. "Foliar Spray Application of *Chlorella vulgaris* Extract: Effect on the Growth of Lettuce Seedlings" Agronomy 11, no. 2: 308. https://doi.org/10.3390/agronomy11020308.

B. Laddomada, A. Blanco, G. Mita, L. D`amico, R.P. Singh, K. Ammar, J. Crossa, C. Guzmán, Drought and heat stress impacts on phenolic acids accumulation in durum wheat cultivars, Foods 10 (2021) 2142.

Lichtenthaler H.K. (1987) [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. pp. 350–382.

B. Liu, L. Zhang, L. Rusalepp, E. Kaurilind, H.Y. Sulaiman, T. Püssa, Ü. Niinemets, Heat priming improved heat tolerance of photosynthesis, enhanced terpenoid and benzenoid emission and phenolics accumulation in Achillea millefolium, Plant Cell Environ. 44 (2021) 2365–2385.

S.A. Macko, M.L.F. Estep, M.H. Engel, P.E. Hare, Kinetic fractionation of stable nitrogen isotopes during amino acid transamination, Geochim Cosmochim. Acta 50 (1986) 2143–2146.

S.A. Macko, M.L. Fogel, P.E. Hare, T.C. Hoering, Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms, Chem. Geol. (Isot. Geosci. Sect.) 65 (1987) 79–92.

M. Malécange, R. Sergheraert, B. Teulat, E. Mounier, J. Lothier, S. Sakr, Biostimulant properties of protein hydrolysates: recent advances and future challenges, Int J. Mol. Sci. 24 (2023) 9714.

K. Meitha, Y. Pramesti, S. Suhandono, Reactive oxygen species and antioxidants in postharvest vegetables and fruits, Int. J. Food Sci. 2020 (2020) 1–11.

G.B. Melo, A.G. da Silva, A.C. da Costa, A. Alves da Silva, M. Rosa, L.A. Bessa, C. R. Rodrigues, G. Castoldi, L.C. Vitorino, Foliar application of biostimulant mitigates water stress effects on soybean, Agronomy 2024 14 (14) (2024) 414.

I. Michalak, K. Chojnacka, A. Dmytryk, R. Wilk, M. Gramza, E. Rój, Evaluation of supercritical extracts of algae as biostimulants of plant growth in field trials, Front Plant Sci. 7 (2016).

G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.

A.N. Misra, M. Misra, R. Singh, A.N. Misra, M. Misra, R. Singh, Chlorophyll fluorescence in plant biology, Biophys. (Oxf.) (2012), https://doi.org/10.5772/35111.

H. Mohseni, K. Sorkheh, D.N. Ahmadi, Foliar application Ascophyllum nodosum extract biostimulants regulated the stem and root apical meristem by orchestrating miRNA-targets gene transcription and regulatory network in rapeseed (Brassica napus), J. Appl. Phycol. 37 (2025) 1373–1388.

L. Mollo, A. Norici, Biostimulant potential of three chlorophyta and their consortium: application on tomato seeds, Discov. Plants (2025), https://doi.org/10.21203/rs.3.rs-5394178/v1.

L. Mollo, A. Petrucciani, A. Norici, Selection of microalgae in artificial digestate: Strategies towards an effective phycoremediation, Plant Physiol. Biochem. 210 (2024a) 108588.

L. Mollo, A. Petrucciani, A. Norici, Monocultures vs. polyculture of microalgae: unveiling physiological changes to facilitate growth in ammonium rich-medium, Physiol. Plant (2024b), https://doi.org/10.1111/ppl.14574.

S. Mugnai, N. Derossi, Y. Hendlin, Algae communication, conspecific and interspecific: the concepts of phycosphere and algal-bacteria consortia in a photobioreactor (PBR), Plant Signal Behav. 18 (2023).

I. Navarro-Morillo, V. Navarro-Perez, E. Perez-Millan, E. Navarro-León, B. Blasco, J. M. Cámara-Zapata, F. Garcia-Sanchez, Effects of root and foliar application of corn steep liquor on pepper plants: a physiological, nutritional, and morphological study, Horticulturae 9 (2023) 221.

A.G. Olabi, N. Shehata, E.T. Sayed, C. Rodriguez, R.C. Anyanwu, C. Russell, M. A. Abdelkareem, Role of microalgae in achieving sustainable development goals and circular economy, Sci. Total Environ. 854 (2023).

L.A. Pascoalino, T.C. Pires, J. Pinela, M.Â. Rodrigues, I.C. Ferreira, L. Barros, J. C. Barreira, F.S. Reis, Foliar application of biostimulants improves nutritional and bioactive quality of walnuts, J. Sci. Food Agric. 105 (2025) 1138–1146.

G.L. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem 83 (1977) 346–356.

B.M. Plaza, C. Gómez-Serrano, F.G. Acién-Fernández, S. Jimenez-Becker, Effect of microalgae hydrolysate foliar application (Arthrospira platensis and Scenedesmus sp.) on Petunia x hybrida growth, J. Appl. Phycol. 30 (2018) 2359–2365.

C. Preininger, U. Sauer, A. Bejarano, T. Berninger, Concepts and applications of foliar spray for microbial inoculants, Appl. Microbiol Biotechnol. 102 (2018) 7265–7282.

Y. Qu, X. Chen, B. Ma, H. Zhu, X. Zheng, J. Yu, Q. Wu, R. Li, Z. Wang, Y. Xiao, Extracellular Metabolites of Heterotrophic AuxenoChlorella protothecoides: a New Source of Bio-Stimulants for Higher Plants, Mar. Drugs 20 (2022) 569.

F. Rachidi, R. Benhima, L. Sbabou, H. El Arroussi, Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution, Biotechnol. Rep. 25 (2020).

N. Rashid, A.J. Ryu, K.J. Jeong, B. Lee, Y.K. Chang, Co-cultivation of two freshwater microalgae species to improve biomass productivity and biodiesel production, Energy Convers. Manag 196 (2019) 640–648.

Q.-U.-A. Raza, M.A. Bashir, A. Rehim, R. Ejaz, H.M.A. Raza, U. Shahzad, F. Ahmed, Y. Geng, Biostimulants induce positive changes in the radish morpho-physiology and yield, Front Plant Sci. 13 (2022).

D.A. Refaay, E.M. El-Marzoki, M.I. Abdel-Hamid, S.A. Haroun, Effect of foliar application with Chlorella vulgaris, Tetradesmus dimorphus, and Arthrospira platensis as biostimulants for common bean, J. Appl. Phycol. 33 (2021) 3807–3815.

M. Ricci, L. Tilbury, B. Daridon, K. Sukalac, General principles to justify plant biostimulant claims, Front Plant Sci. 10 (2019).

E.S. Rorem, H.G. Walker, R.M. McCready, Biosynthesis of sucrose and sucrose-phosphate by sugar beet leaf extracts, Plant Physiol. 35 (1960) 269–272.

R. Rosa, L. Hajko, J. Franczuk, A. Zaniewicz-Bajkowska, A. Andrejiová, I. Mezeyová, Effect of L-tryptophan and L-glutamic acid on carrot yield and its quality, Agronomy 13 (2023) 562.

P. Santamaria, Nitrate in vegetables: toxicity, content, intake and EC regulation, J. Sci. Food Agric. 86 (2006) 10–17.

T. Searchinger, R. Waite, C. Hanson, J. Ranganathan, Creating a Sustainable Food Future, The World Resources Institute, 2018.

A. Spinardi, G. Cocetta, L. Martinetti, I. Mignani, A. Ferrante, Quality changes of lamb's lettuce during postharvest storage, Acta Hortic. (2018) 329–334.

W.A.V. Stiles, D. Styles, S.P. Chapman, S. Esteves, A. Bywater, L. Melville, A. Silkina, I. Lupatsch, C. Fuentes Grünewald, R. Lovitt, T. Chaloner, A. Bull, C. Morris, C. A. Llewellyn, Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities, Bioresour. Technol. 267 (2018) 732–742.

K.V. Supraja, B., P.B. Behera, Efficacy of microalgal extracts as biostimulants through seed treatment and foliar spray for tomato cultivation, Ind. Crops Prod. 151 (2020) 112453.

K.V. Supraja, B. Behera, P. Balasubramanian, Performance evaluation of hydroponic system for co-cultivation of microalgae and tomato plant, J. Clean Prod. 272 (2020) 122823.

M. Toplak, S.T. Read, C. Sandt, F. Borondics, L. Vaccari, H.J. Byrne, T.P. Wrobel, Quasar: easy machine learning for biospectroscopy, Cells 10 (10) (2021) 2300, 2021.

S. Toscano, M.J. Gómez-Bellot, D. Romano, M.J. Sánchez-Blanco, Physiological and biochemical changes in response to Moringa oleifera biostimulant in petunia plants under water deficit, Sci. Hortic. 319 (2023) 112187.

H. Treves, A. Küken, S. Arrivault, H. Ishihara, I. Hoppe, A. Erban, M. Höhne, T.A. Moraes, J. Kopka, J. Szymanski, Z. Nikoloski, M. Stitt, Carbon flux through photosynthesis and central carbon metabolism show distinct patterns between algae, C3 and C4 plants, Nat. Plants 8 (2021) 78–91.

J. Trotti, I. Trapani, F. Gulino, M. Aceto, M. Minio, C. Gerotto, E. Mica, G. Valè, R. Barbato, C. Pagliano, Physiological Responses to Salt Stress at the Seedling Stage in Wild (Oryza rufipogon Griff.) and Cultivated (Oryza sativa L.) Rice, Plants 13 (2024) 369.

M. Tsimilli-Michael, Revisiting JIP-test: an educative review on concepts, assumptions, approximations, definitions and terminology, Photosynthetica 58 (2020) 275–292.

B. ValizadehKaji, M. Mohammaei, Foliar application of biostimulants and potassium silicate enhances yield and fruit quality of Mandarin Cv. 'Page, Appl. Fruit. Sci. 67 (2025) 8.

S. Velten, J. Leventon, N. Jager, J. Newig, What is sustainable agriculture? A systematic review, Sustainability 2015 7 (7) (2015) 7833–7865. Pages 7833-7865.

E. Vitale, V. Velikova, T. Tsonev, I. Ferrandino, T. Capriello, C. Arena, The interplay between light quality and biostimulant application affects the antioxidant capacity and photosynthetic traits of soybean (Glycine max L. Merrill), Plants 10 (2021) 861.

J. Wang, B. Yuan, B. Huang, Differential heat-induced changes in phenolic acids associated with genotypic variations in heat tolerance for hard fescue, Crop Sci. 59 (2019) 667–674.

L. Wobbe, R. Bassi, O. Kruse, Multi-level light capture control in plants and green algae, Trends Plant Sci. 21 (2016) 55–68.

Wuijts S., Fraters D., Boekhold S., Van Duijnen R. (2022) Monitoring of nitrogen in water in the EU. Legal framework, effects of nitrate, design principles, effectiveness and future developments Directorate-General for Internal Policies PE.

E.W. Yemm, A.J. Willis, The estimation of carbohydrates in plant extracts by anthrone, Biochem. J. 57 (1954) 508–514.

T. Yoneyama, T. Omata, S. Nakata, J. Yazaki, Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia l by plants, Plant Cell Physiol. (1991) 32.

G.Bin Zhang, S. Meng, J.M. Gong, The expected and unexpected roles of nitrate transporters in plant abiotic stress resistance and their regulation, Int J. Mol. Sci. 19 (2018) 3535.

V. Žunić, T. Hajnal-Jafari, D. Stamenov, S. Djurić, J. Tomić, M. Pešaković, M.C. Grohar, F. Stampar, R. Veberic, M. Hudina, J. Jakopic, Application of microalgae-based biostimulants in sustainable strawberry production, J. Appl. Phycol. 36 (2024) 1219–1231.