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Nutrient and Nutraceutical Quality of Rocket as a Function of Greenhouse Cover Film, Nitrogen Dose and Biostimulant Application

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Abstract: The nutrient and nutraceutical quality of greenhouse wild rocket is strongly influenced by the light environment and nitrogen fertilization. We investigated the effects of two cover materials, a diffuse light film (Film1) and a traditional clear film (Film2), and three nitrogen regimes, no N supply (N0) and sub-optimal (N1) and optimal (N2) doses, also in combination with a biostimulant (Stimolo Mo), on the mineral composition, antioxidant properties and chlorophyll and carotenoid content of rocket plants grown in the autumn–spring cycle. The leaf concentration of most of the minerals was higher under Film1 compared to Film2. In general, K, Ca, Mg and Na were higher, and S was lower in the presence of N supply, and the addition of the biostimulant promoted the mineral uptake. Under Film1, the hydrophilic antioxidant activity (HAA) was higher in some harvests, and the ABTS antioxidant activity (ABTS AA) in the first one, while always lower afterward, than under Film2. Nitrogen fertilization did not affect the antioxidant activity, while it reduced the content of total phenols and ascorbic acid. The biostimulant application increased ABTS AA at the optimal N dose and reduced total phenols in unfertilized plants. Both the diffuse light and the N supply inhibited the synthesis of ascorbic acid, while N fertilization and the biostimulant promoted the synthesis of chlorophylls. The experimental treatments exerted variable effects over time and significant interactions with the harvest period were found for many of the investigated parameters.

Keywords: *Diplotaxis tenuifolia* L.; mineral composition; antioxidant capacity; ascorbic acid; chlorophyll; carotenoids

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1. Introduction

Diplotaxis tenuifolia L. (Family Brassicaceae), known in Italy as wild rocket, is a perennial herbaceous plant widely used across the world for fresh consumption and, recently, for baby leaf [1]. Rocket has a peculiar bitter taste and a good nutritional composition, being rich in A, B, C and K vitamins, iron, essential proteins and bioactive compounds [2]. However, wild rocket leaves are known to accumulate relevant concentrations of nitrate, which can be harmful to human health at high intake [3–5]. On the other hand, fresh-cut or minimally processed products and baby leaf vegetables are gaining importance worldwide, as they represent a good source of minerals, vitamins and phytochemicals with antioxidant properties beneficial for consumers [6].

Nitrate accumulation in plant tissues depends on many factors, including the dose and type of nitrogen (N) fertilizer, which modify the amount and the form of the available N [7], and the light intensity, which modulates the activity of nitrate reductase, regulating the N metabolism [8]. As a consequence, nitrate content in vegetables shows seasonal changes, with higher values in the autumn–winter season compared to spring–summer [9]. The N fertilization and the light environment influence not only the plant nitrogen metabolism but

also the uptake of other mineral elements. Indeed, the interaction of N with other nutrients (and among the other nutrients themselves) can determine antagonistic or synergistic effects [10]. As an example, it is well known that N availability promotes the uptake of magnesium [11]. In addition, the plant mineral nutrition is affected by both the intensity and spectral composition of light [12], and, as recently found, also the direction of light, as the angle of incidence of light rays on the plant canopy has been found to have an indirect impact on nutrient uptake as it alters the light interception and hence the rate of plant assimilation [13].

In this respect, the distribution of light inside the greenhouse assumes a relevant role in plant nutrition. Indeed, solar global radiation consists of direct and diffuse radiation. Direct light arrives directly from the sun, while diffuse light is reflected or scattered by molecules or larger particles in the atmosphere (e.g., clouds, water vapor, dust, pollutants); hence, it reaches a given surface from many directions simultaneously [14]. Diffuse radiation results in a more even vertical and horizontal light distribution in space, and it exhibits higher penetration capacity in the crop canopy, optimizing the contribution of lower and inner leaf layers to the whole plant assimilation [15]. In protected cultivation, light intensity is reduced compared to open fields, depending on the design of the facility and the optical and radiometric properties of the covering material (e.g., glass or plastics) [16], and also by changes in these properties due to ageing, damages and dust deposition [17]. Hence, in a protected environment, diffuse light represents an important fraction of the global radiation incident on the canopy, particularly in the winter period, when light can be a limiting factor because of the lower and more variable intensity. In fact, it has been demonstrated that plants use diffuse light more efficiently than direct light, and the increase in this fraction increases the light absorption, the rate of photosynthesis and the light use efficiency, while improving the crop growth uniformity and productivity [18].

In recent years, innovative smart materials have been developed with specific optical properties, able to modify both the light intensity and distribution, as well as the spectral composition of light entering the greenhouse [19]. Specifically, by means of the dispersion in the plastic material of interference pigments, gas microbubbles or hollow glass microspheres, diffuse light covers distribute light in a more uniform way, allowing better lighting of the plant profile [19]. This effect has been demonstrated to improve the overall plant growth and development compared to conventional materials [14]. Diffused light plastic films (e.g., low-density polyethylene, LDPE) or glass slabs applied in upright-growing species improved the light distribution along the plant vertical profile, with positive effects (particularly in winter) on the light interception of lower and inner leaves, normally shaded, and a reduction in stress related to light saturation and high temperatures (particularly in summer) in the upper leaves, exposed to direct solar light, compared to conventional clear covers [20]. In addition, exposure to more uniform light conditions can improve the product quality and their nutritional profile, as well as the synthesis of bioactive compounds, such as phenols, with beneficial effects on human health [12]. On this basis, Hemming et al. [18] recommended the use of cover materials with a transmittance of 90% and a minimum diffusivity of 50%.

Based on the above considerations, the modulation of the light environment and the functional management of the N supply in protected cultivation are both pivotal tools to improve crop productivity and the nutrient and nutraceutical properties of leafy vegetables. To promote plant growth while limiting nitrate accumulation and stimulating the synthesis of healthy compounds in rocket grown in unheated plastic tunnels, we studied an integrated strategy consisting of the use of diffuse light films, to increase the plant light use efficiency, and the optimization of nitrogen fertilization to prevent N losses in the soil and nitrate accumulation in leaves, to increase the N use efficiency. In addition, with the overall final objective of improving the sustainability of the horticultural industry, we tested the efficiency of a natural biostimulant to boost plant growth while reducing nitrate accumulation, as declared by the manufacturer. Plant biostimulants are formulations of natural substances or compounds, or microorganisms, able to activate several

physiological and molecular mechanisms, such as the enhancement of photosynthesis, increase of nutrient uptake and translocation and biosynthesis of secondary metabolites (including phytochemicals), resulting in the improvement of nutrition efficiency, abiotic stress tolerance, crop yield and product quality [21,22]. Biostimulants show broad use in horticulture, to integrate chemical fertilizers with non-microbial formulates based on seaweed and plant extracts, microalgae, protein hydrolysates and amino acids, accounting for approximately 75% of the total [23,24].

Although several studies addressed the effects of nitrogen fertilization and greenhouse covers on the yield and quality of vegetables, their interaction is still not clear. Within a series of experiments aiming at evaluating the potential application of the above-described strategy in leafy vegetables, we investigated the effects of two greenhouse cover films (diffuse light Film1 and clear Film2), and three N regimes, unfertilized (N0), sub-optimal (N1) and optimal (N2) doses, on rocket plants grown in the autumn–spring cycle, also in combination with the application of a natural biostimulant. We reported the results related to crop yield and nitrate accumulation in leaves in Di Mola et al. [25]. In the present paper, we present detailed data on quality in terms of mineral composition, antioxidant activity and pigment content, as a function of the light environment, nitrogen availability and biostimulant treatment.

2. Materials and Methods

2.1. Plant Material and Crop Management

The experiment was carried out from autumn 2020 to spring 2021, under two twin unheated tunnels at the Department of Agriculture of the University of Naples Federico II, in Portici (Naples, Italy; 40°48.870′ N; 14°20.821′ E; 70 m a.s.l.). Plants were grown in pots (0.38 m^2; Figure S1), filled with sandy soil (91% sand, 4.5% silt and 4.5% clay; USDA classification), with pH 7.4, containing 253 ppm P_2O_5, 490 ppm K_2O, 2.5% organic matter, 0.101% total nitrogen.

Wild rocket (*Diplotaxis tenuifolia* L.) cv. 'Reset' (Maraldi Sementi Srl, Cesena, Italy, https://www.maraldisementi.it/, accessed on 26 December 2022), with medium-sized lobe green leaves, was selected for its high productivity and good tolerance to pathogens (i.e., *Fusarium* spp.) and adaptability to different climatic conditions. Plantlets were transplanted on 8 October 2020, at the planting density of 18 plants per m^2 (7 groups of seedlings per pot), and harvested in 6 cuts: 27 November 2020, 3 February, 9 March, 8 April, 5 and 28 May 2021.

The air temperature in the growing environment was constantly measured by probes (Vantage Pro2, Davis Instruments, Hayward, CA, USA) located at 20 cm above the canopy and distributed randomly in the two tunnels.

Water management was based on the restitution of the plant water loss per evapotranspiration, estimated through the Hargreaves and Samani formula [26]. No pesticide treatments were performed.

2.2. Plastic Films Characteristics, Nitrogen Doses and Biostimulant Utilization

Polyethylene thermal films were used to cover two tunnels, 150 microns thick with distinctive optical properties. Film1 was a diffused light film (trade name Sunsaver Diff, produced by Ginegar Plastic Products and distributed by Polyeur Srl, Benevento, Italy). It had 58% diffusivity, 87% thermicity, a window of around 30% transmissivity in the UV-B waveband (280–315 nm) and a total transmittivity in photosynthetically active radiation (PAR) of 90% and an anti-drip effect. Film2 was a clear plastic film (commercial name Lirsalux, Lirsa Srl, Ottaviano, Naples, Italy) characterized by 75% thermicity, no transmission in the UV-B range, a total transmittivity of 85% in PAR and an anti-drip effect.

The nitrogen fertilization was performed at three rates, 0 (N0), 9 (N1) and 18 (N2) kg ha^{-1}, and N was applied as ammonium nitrate (34%), 18 days after the transplant and about 7 days after each harvest.

The two biostimulant treatments were untreated and treated with Stimolo Mo, hereafter indicated as Control and StMo, respectively. Stimolo Mo (Fertenia Srl, Bellizzi, Salerno, Italy) is an extract of alfalfa (*Medicago sativa* L.), seaweed (*Ascophyllum nodosum*) and molasses, rich in low-molecular-weight amino acids, with 5% organic nitrogen, 3% molybdenum (Mo) and 0.1% zinc (in weight), characterized by fast penetration into leaves and recommended for green leafy vegetables. According to the manufacturer, it improves plant growth and reduces nitrate accumulation by accelerating the conversion of nitric nitrogen in organic compounds (amino acids and proteins) and promoting the activity of nitrate reductase due to the fast supply of Mo. The biostimulant was sprayed on the leaves, at the concentration of 3 mL L^{-1}, 3 times per growing cycle, starting from the new leaf emission.

The timing of nitrogen supply and biostimulant applications and the dates of the harvests are reported in Table 1.

Table 1. Timing of the N fertilization, biostimulant application and harvests, in the different growing cycles, expressed as days after transplant (DAT, for the first cycle) and days after the previous harvest (DAPH, from the second cycle onwards) (further details in Di Mola et al. [25]).

Cycle	Nitrogen Fertilization	Biostimulant Application			Harvest Time (DAT/DAPH)
		1st	2nd	3rd	
I	18	26	33	40	50
II	7	14	21	30	68
III	6	14	21	27	34
IV	6	10	17	23	30
V	4	8	14	19	27
VI	3	5	11	17	23

2.3. Yield and Time Distribution of Production

At each of the 6 harvests, the whole aboveground parts of rocket plants were cut and the production was expressed as marketable yield in kg m^{-2}.

2.4. Product Quality

2.4.1. Mineral Composition

Samples of 250 mg of dried leaves were ground at 0.5 mm in a Wiley Mill, and then suspended in 50 mL of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) and shaken in a water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C for 10 min. The solution was centrifuged at 6000 rpm for 10 min (R-10 M, Remi Elektrotechnik Limited, India), and then filtered through a 0.45 µm nylon syringe filter (Phenomenex, Torrance, CA, USA) and analyzed by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector. An IonPac CG12A (4 × 250 mm, Dionex, Corporation) guard column and IonPac CS12A (4 × 250 mm, Dionex, Corporation) analytical column were used for the K, Ca, Na and Mg analysis, while, for nitrate and P determination, an IonPac AG11-HC guard (4 × 50 mm) column and IonPac AS11-HC analytical column (4 × 250 mm) were adopted, as detailed in Rouphael et al. [27]. P, K, Ca, Na and Mg were expressed as g kg^{-1} dw.

2.4.2. Antioxidant Activity and Total Phenol Content

The hydrophilic antioxidant activity (HAA) and ABTS antioxidant activity (ABTS AA) were assessed on an extract from freeze-dried rocket leaves (200 mg) added with methanol and distilled water, respectively. The ABTS antioxidant activity and hydrophilic activity were measured spectrophotometrically based on the methods of Re et al. [28] and Fogliano et al. [29], respectively. The absorbance of the solutions for ABTS and hydrophilic extracts was measured at 734 and 505 nm, respectively. ABTS and hydrophilic antioxidant activities were expressed as mmol of Trolox and mmol ascorbic acid 100 g^{-1} dw, respectively.

The total phenol content was assessed in methanolic extracts using the Folin–Ciocalteu method [30] with gallic acid as a standard. For this purpose, 100 mL of the supernatant was combined with 500 mL of Folin–Ciocalteau's reagent (Sigma-Aldrich Inc., Milano, Italy) and 400 mL of 7.5% sodium carbonate/water (*w*/*v*). The solution's absorbance was measured after 30 min at 765 nm by an ultraviolet–visible spectrophotometer, expressing the results as mg gallic acid (Sigma-Aldrich Inc.) 100 g^{-1} dw.

2.4.3. Total Ascorbic Acid, Chlorophyll *a* and *b* and Carotenoids

The total ascorbic acid (TAA), as the sum of ascorbic acid (ASA) and dehydroascorbate (DHA) acid, expressed as mg ascorbic acid on 100 g fw, was also assessed spectrophotometrically based on the protocol of Kampfenkel et al. [31]. The absorbance of the solution for total ascorbic acid was measured at 525 nm.

Chlorophylls and carotenoids were assessed on fresh samples of 1 g each, according to Lichtenhaler and Wellburn's [32] spectrophotometrical method.

2.5. Experimental Design and Statistical Analysis

A split-split plot design was adopted, comparing the two different greenhouse cover plastic films as the main factor, and the three nitrogen rates, the two biostimulant treatments and the harvest date as sub-factors. All treatments were replicated 3 times (3 pots per replicate, 36 pots in total).

A 4-way analysis of variance (ANOVA) was applied to the data using the SPSS software package (SPSS version 22, Chicago, IL, USA). Separation of the means was done using Tukey's multiple range test at $p = 0.05$.

3. Results

3.1. Air Temperatures Inside the Tunnels

Details about climate data are reported in Di Mola et al. [25], and in a table (Supplementary Table S1). The mean daily temperature in the tunnels ranged from the minimum value of 10.6 °C, recorded in the second ten days of January, to 28.4 °C in the middle of May. The mean temperature of the whole growing period was 16.3 and 16.1 °C for clear and diffuse light films, respectively (daily average values). The two cover films showed slight differences in thermal behavior throughout the seasons of the year. During autumn (October to December), under the diffusive Film1, the mean temperature was 15.6 °C vs. 15.4 °C under the clear Film2. Similarly, in winter months (December to March), the average temperature was 14.0 °C vs. 13.8 °C under Film1 and Film2, respectively. The trend was inverted in the spring (March till the end of May), when the mean temperature was 20.3 °C under the clear film and 19.3 °C under the diffuse light film.

3.2. Plant Productivity and Yield Distribution and Leaf Nitrate Content

Detailed results about yield and nitrate content, as a detrimental factor in rocket quality, are reported in Di Mola et al. [25]. Briefly, the plant productivity of rocket grown in unheated tunnels in the autumn–spring period was influenced by the cover material, the rate of nitrogen fertilization and the biostimulant application. Averaged on the other treatments, the diffusive Film1 determined a 36.5% higher yield than the clear Film2 (0.86 vs. 0.63 kg m^{-2}). Plant productivity also increased with the N dose (0.37, 0.82 and 0.99 kg m^{-2} for N0, N1 and N2 on average, respectively) and the application of Stimolo Mo (0.91 vs. 0.65 kg m^{-2} on average). It is worth noting that significant interactions for yield were observed between the plastic film and both the N dose and biostimulant, which determined the best plant performance under Film1, which improved productivity also in plants unfertilized and untreated with the biostimulant compared to Film2. Specifically, yield at the sub-optimal dose N1 under Film1 was even equal to those at the optimal dose N2 in Film2. The cover material also influenced the product distribution along the cultivation cycle, with increasing yields from the first to fourth harvest and stable values

from the fourth to the sixth under Film1, and lower and similar yields in the first two and higher yields in the remaining cuts under Film2.

Nitrate content in rocket leaves showed a typical seasonal trend, with lower values in the product harvested in spring, and it was boosted by the N supply (1096, 3696 and 4963 mg kg^{-1} fw, for N0, N1 and N2, respectively) and biostimulant (3924 vs. 2580 mg kg^{-1} fw in untreated control).

3.3. Statistical Results of Quality Parameters

Tables 2 and 3 show the effects of the experimental factors on the leaf mineral composition, antioxidant activity and pigment content in rocket leaves. The analysis of variance revealed that the experimental treatments significantly influenced the leaf content of all the considered mineral elements, except for K, which was unaffected by the plastic film, and P, which did not change under the two plastic covers and the different N doses (Table 2).

Table 2. Analysis of variance on mineral content of rocket as affected by the different experimental factors: significance of main factors and interactions.

	K	Ca	Mg	Na	S	P
Plastic film (P)	-	0.05	0.01	0.01	0.01	-
Fertilization (F)	0.01	0.01	0.01	0.01	0.01	-
Biostimulant (B)	0.01	0.01	0.01	0.01	0.01	0.01
Harvest (H)	0.01	0.01	0.01	0.01	0.01	0.01
P × F	0.05	-	-	-	0.05	
P × B	-	-	-	-	-	-
P × H	-	-	0.05	0.05	0.01	0.01
F × B	0.01	0.01	0.01	0.01	-	0.01
B × H	0.05	0.01	0.05	0.05	0.01	0.01
F × H	0.01	0.01	0.01	0.01	0.05	-
P × F × B	-	-	-	-	-	-
P × B × H	-	-	-	-	-	-
F × B × H	-	-	-	-	-	-
P × B × F × H	-	-	-	-	-	-

-: not significant.

Table 3. Analysis of variance of hydrophilic antioxidant activity (HAA), ABTS antioxidant activity (ABTS AA), content of total phenols, total ascorbic acid (TAA), chlorophyll *a* (Chl *a*) and *b* (Chl *b*), total chlorophyll and carotenoids, as affected by the different experimental factors: significance of main factors and interactions.

	HAA	ABTS AA	Total Phenols	TAA	Chl *a*	Chl *b*	Total Chl	Carotenoids
Plastic film (P)	0.01	0.01	0.01	0.05	-	-	-	-
Fertilization (F)	-	-	0.01	0.01	0.01	0.01	0.01	-
Biostimulant (B)	-	-	0.01	-	0.01	0.05	0.01	-
Harvest (H)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
P × F	-	-	-	0.05	-	-	-	-
P × B	-	-	-	-	-	-	-	-
P × H	0.05	0.01	0.01	-	0.01	-	0.05	0.01
F × B	-	0.01	0.01	-	-	-	-	-
B × H	-	-	-	-	0.01	0.05	0.05	0.05
F × H	-	0.05	0.01	-	0.01	-	0.01	0.01
P × F × B	-	-	-	-	-	-	-	-
P × B × H	-	-	-	-	-	-	-	-
F × B × H	-	-	-	-	-	-	-	-
P × F × B × H	-	-	-	-	-	-	-	-

-: not significant.

Significant second-degree interactions were found between the plastic film and nitrogen fertilization (P × F) in K and S content, plastic film and harvest (P × H) in all the elements except K and Ca, the biostimulant and N fertilization (F × B) in all the elements except S, and N fertilization and harvest (F × H) in all the elements except P. The interactions of the third and fourth degree were never significant.

The tunnel cover material affected significantly all the antioxidant parameters, while it did not influence the chlorophyll and carotenoid content (Table 3). The effect of N fertilization and biostimulant application was not relevant to the antioxidant activity and carotenoids, while it was stronger for the total phenols and chlorophylls, and fertilization also affected the total ascorbic acid (Table 3). The harvest affected all the considered features (Table 3).

A significant interaction between the plastic film and harvest (P × H) was found in most of the quality parameters, except TAA and Chl *b* (Table 3). The biostimulant treatment interacted with the N rate (F × B) on ABTS AA and total phenols, and with the harvest (B × H) in all chlorophylls and carotenoids. The interaction nitrogen fertilization × harvest (F × H) was significant in all the parameters, except HAA, TAA and Chl *b* (Table 3). The interactions of the third and fourth degree were never significant.

3.4. Mineral Composition as Affected by the Experimental Factors

Averaged on the other experimental treatments (N fertilization and biostimulant application), the two films influenced the leaf concentration of most of the considered minerals, except K and P (Table 2), with higher values recorded under Film1 (Table 4). However, the effects of film properties differed in the elements and changed over time, and significant interactions of plastic film × harvest (P × H) were found on most of the elements except K and Ca (Table 4). Specifically, compared to the clear Film2, under the diffusive Film1, Mg showed higher values in the first two harvests, S decreased in the second and the third harvests and P increased only in the first one while it decreased in the third and the sixth (Table 4).

Table 4. Interactions plastic film × harvest (P × H) and plastic film × nitrogen fertilization (P × F) on the average mineral content of rocket (g kg^{-1} dw). Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		K	Ca	Mg	Na	S	P
Film1	I	48.19 ± 1.37	22.23 ± 0.72	4.78 ± 0.17 de	3.74 ± 0.14 bc	10.12 ± 0.53 a	2.36 ± 0.11 c
	II	48.31 ± 1.78	23.30 ± 0.72	5.05 ± 0.19 cd	4.38 ± 0.20 a	4.12 ± 0.19 d	2.30 ± 0.07 cd
	III	52.57 ± 2.92	23.43 ± 0.79	5.65 ± 0.26 ab	4.37 ± 0.30 a	1.90 ± 0.24 e	2.12 ± 0.08 de
	IV	50.76 ± 2.72	20.45 ± 0.54	5.57 ± 0.21 ab	3.80 ± 0.28 bc	0.96 ± 0.12 ef	2.35 ± 0.16 cd
	V	49.44 ± 2.68	20.23 ± 0.74	5.63 ± 0.12 ab	3.17 ± 0.15 d	0.91 ± 0.13 ef	2.48 ± 0.11 c
	VI	48.38 ± 2.87	20.10 ± 1.21	5.59 ± 0.17 ab	3.10 ± 0.17 d	0.96 ± 0.14 ef	2.73 ± 0.13 b
Film2	I	45.39 ± 1.43	21.04 ± 0.66	4.05 ± 0.15 f	3.38 ± 0.15 cd	9.70 ± 0.39 a	2.01 ± 0.10 e
	II	45.79 ± 1.55	21.97 ± 0.66	4.42 ± 0.17 ef	3.62 ± 0.15 c	5.96 ± 0.18 b	2.30 ± 0.10 cd
	III	54.82 ± 2.55	22.82 ± 0.62	5.27 ± 0.21 bc	4.18 ± 0.31 ab	4.99 ± 0.52 c	2.39 ± 0.13 c
	IV	49.04 ± 2.45	21.22 ± 1.07	5.27 ± 0.20 bc	3.41 ± 0.29 cd	1.55 ± 0.25 f	2.25 ± 0.10 cd
	V	50.36 ± 2.27	20.18 ± 1.02	5.73 ± 0.20 a	3.00 ± 0.18 d	1.53 ± 0.31 f	2.44 ± 0.10 c
	VI	50.23 ± 2.05	18.53 ± 1.12	5.47 ± 0.19 ab	3.15 ± 0.17 d	1.55 ± 0.25 f	3.17 ± 0.21 a
Film1	N0	38.70 ± 1.30 e	19.41 ± 0.55	4.71 ± 0.13	3.31 ± 0.19	3.74 ± 0.63 bc	2.34 ± 0.09
	N1	53.87 ± 1.09 b	22.25 ± 0.58	5.86 ± 0.12	4.10 ± 0.15	2.84 ± 0.57 c	2.42 ± 0.08
	N2	56.26 ± 0.99 a	23.21 ± 0.51	5.56 ± 0.11	3.87 ± 0.15	2.85 ± 0.56 c	2.42 ± 0.09
Film2	N0	41.53 ± 1.35 d	18.62 ± 0.54	4.50 ± 0.17	3.04 ± 0.19	5.27 ± 0.54 a	2.40 ± 0.13
	N1	51.99 ± 1.36 c	20.84 ± 0.68	5.31 ± 0.16	3.70 ± 0.15	3.93 ± 0.56 b	2.36 ± 0.11
	N2	54.30 ± 0.98 b	23.41 ± 0.45	5.30 ± 0.12	3.63 ± 0.13	3.44 ± 0.54 bc	2.52 ± 0.08

A significant interaction of plastic film × fertilization (P × F) was found on S leaf content, which decreased at increasing N under Film2, while it was unaffected by the N dose under Film1 (Table 4).

Nitrogen fertilization and biostimulant application influenced the mineral composition of rocket leaves and both interacted with the harvest time (Tables 5 and 6), as shown by the analysis of variance (Table 2). The dose of N modified the content of all the elements investigated except P (Table 5). In general, K, Ca, Mg and Na were higher, and S was lower in the presence of the N supply, regardless of the N dose. However, the effect of N on mineral uptake changed in the growing periods, with greater increases from the first to the third harvests for all the cations, and lower increases (Na) or stable values (K, Ca and Mg) afterward, compared to the unfertilized control (Table 5).

Table 5. Interactions nitrogen fertilization × harvest (F × H) and nitrogen fertilization × biostimulant (F × B) on the average mineral content of rocket (g kg^{-1} dw). Different letters within each column indicate significant differences according at $p = 0.05$ (Tukey's test).

Treatments		K	Ca	Mg	Na	S	P
N0	I	46.77 ± 1.29 j	21.70 ± 0.72 ce	3.31 ± 0.18 fg	4.00 ± 0.23 g	10.94 ± 0.48 a	2.17 ± 0.10
	II	39.83 ± 1.75 m	21.29 ± 1.03 de	3.76 ± 0.24 de	4.16 ± 0.26 fg	5.16 ± 0.32 c	2.21 ± 0.11
	III	40.32 ± 2.84 l	20.41 ± 0.66 ef	3.09 ± 0.41 gi	4.47 ± 0.27 ef	4.88 ± 0.81 c	2.21 ± 0.20
	IV	37.39 ± 2.45 o	18.26 ± 0.46 gh	2.79 ± 0.45 i	4.66 ± 0.24 de	2.00 ± 0.33 e	2.31 ± 0.14
	V	37.07 ± 1.81 o	17.09 ± 0.61 hi	2.94 ± 0.29 hi	5.21 ± 0.16 c	2.03 ± 0.42 e	2.47 ± 0.16
	VI	39.31 ± 2.64 n	15.37 ± 0.58 i	3.17 ± 0.27 gh	5.14 ± 0.22 c	2.03 ± 0.32 e	2.85 ± 0.31
N1	I	45.66 ± 2.15 o	21.28 ± 0.63 ef	3.62 ± 0.21 ef	4.58 ± 0.19 de	9.63 ± 0.57 b	2.19 ± 0.16
	II	48.63 ± 1.10 h	22.88 ± 0.73 bd	4.07 ± 0.24 bd	4.91 ± 0.22 cd	4.76 ± 0.41 c	2.23 ± 0.11
	III	60.36 ± 1.27 a	24.22 ± 0.77 ab	5.04 ± 0.21 a	6.11 ± 0.18 a	3.20 ± 0.57 d	2.29 ± 0.12
	IV	54.91 ± 1.48 f	21.08 ± 1.14 ef	4.21 ± 0.24 b	6.02 ± 0.20 a	0.96 ± 0.09 f	2.24 ± 0.17
	V	55.62 ± 1.11 e	20.25 ± 0.98 ef	3.39 ± 0.11 fg	6.02 ± 0.14 a	0.89 ± 0.11 f	2.44 ± 0.12
	VI	52.37 ± 2.45 g	19.56 ± 1.68 fg	3.07 ± 0.19 gi	5.87 ± 0.18 ab	0.88 ± 0.11 f	2.95 ± 0.21
N2	I	47.93 ± 1.77 i	21.93 ± 1.16 ce	3.75 ± 0.16 de	4.66 ± 0.21 de	9.16 ± 0.56 b	2.21 ± 0.15
	II	52.68 ± 1.12 g	23.73 ± 0.67 ab	4.16 ± 0.25 bc	5.14 ± 0.13 c	5.19 ± 0.34 c	2.48 ± 0.07
	III	60.40 ± 1.06 a	24.75 ± 0.55 a	4.69 ± 0.15 a	5.80 ± 0.15 ab	2.25 ± 0.35 e	2.27 ± 0.08
	IV	57.40 ± 1.20 b	23.17 ± 0.87 ac	3.82 ± 0.16 ce	5.58 ± 0.11 b	0.81 ± 0.11 f	2.34 ± 0.18
	V	57.02 ± 1.25 c	23.28 ± 0.78 ac	2.94 ± 0.14 hi	5.80 ± 0.20 ab	0.61 ± 0.05 f	2.47 ± 0.10
	VI	56.24 ± 1.35 d	23.02 ± 0.79 ad	3.13 ± 0.18 gi	5.58 ± 0.21 b	0.84 ± 0.13 f	3.06 ± 0.14
N0	StMo	44.55 ± 1.12 d	20.03 ± 0.56 b	3.94 ± 0.13 ac	5.14 ± 0.13 b	4.37 ± 0.57	2.82 ± 0.10 a
	Control	35.68 ± 1.12 e	18.01 ± 0.49 b	2.41 ± 0.14 d	4.07 ± 0.12 c	4.64 ± 0.63	1.92 ± 0.05 c
N1	StMo	54.58 ± 1.03 b	23.78 ± 0.51 a	4.31 ± 0.14 a	5.78 ± 0.10 a	3.00 ± 0.49	2.78 ± 0.08 a
	Control	51.27 ± 0.95 c	19.31 ± 0.42 b	3.49 ± 0.13 bc	5.39 ± 0.13 ab	3.77 ± 0.60	2.00 ± 0.07 bc
N2	StMo	54.42 ± 1.14 b	24.12 ± 0.43 a	4.03 ± 0.14 ab	5.33 ± 0.13 ab	2.76 ± 0.52	2.71 ± 0.08 a
	Control	56.14 ± 1.27 a	22.51 ± 0.60 a	3.47 ± 0.14 c	5.52 ± 0.15 ab	3.52 ± 0.61	2.23 ± 0.06 b

Table 6. Interaction biostimulant × harvest (B × H) on the average mineral content of rocket (g kg^{-1} dw). Different letters within each column indicate significant differences according at $p = 0.05$ (Tukey's test).

Treatments		K	Ca	Mg	Na	S	P
StMo	I	46.35 ± 1.44 de	21.62 ± 0.77 b	3.85 ± 0.13 c	4.65 ± 0.17 c	8.93 ± 0.39 b	2.31 ± 0.12 ef
	II	49.30 ± 1.34 bd	24.28 ± 0.72 a	4.53 ± 0.18 ab	5.13 ± 0.17 b	5.02 ± 0.33 c	2.56 ± 0.05 cd
	III	56.04 ± 2.03 a	24.45 ± 0.57 a	4.89 ± 0.16 a	5.74 ± 0.17 a	2.96 ± 0.60 e	2.60 ± 0.08 cd
	IV	51.72 ± 1.70 bc	22.67 ± 0.80 ab	4.31 ± 0.16 b	5.66 ± 0.13 a	1.21 ± 0.19 f	2.78 ± 0.06 bc
	V	51.44 ± 2.05 bc	21.67 ± 0.64 b	3.47 ± 0.14 cd	5.73 ± 0.13 a	1.07 ± 0.19 f	2.84 ± 0.05 b
	VI	52.25 ± 2.12 ab	21.18 ± 1.12 b	3.52 ± 0.14 cd	5.60 ± 0.17 a	1.08 ± 0.23 f	3.53 ± 0.14 a
Control	I	47.23 ± 1.43 de	21.66 ± 0.64 b	3.27 ± 0.14 de	4.18 ± 0.18 d	10.88 ± 0.42 a	2.07 ± 0.10 g
	II	44.80 ± 1.84 e	20.98 ± 0.42 b	3.47 ± 0.12 cd	4.35 ± 0.17 cd	5.05 ± 0.25 c	2.05 ± 0.07 gh
	III	51.35 ± 3.23 bc	21.80 ± 0.70 b	3.66 ± 0.34 cd	5.17 ± 0.28 b	3.93 ± 0.48 d	1.91 ± 0.07 gh
	IV	48.08 ± 3.20 ce	19.01 ± 0.64 c	2.90 ± 0.29 ef	5.18 ± 0.25 b	1.30 ± 0.23 f	1.82 ± 0.0.7 h
	V	48.37 ± 2.81 ce	18.75 ± 0.96 c	2.71 ± 0.14 f	5.63 ± 0.19 a	1.28 ± 0.30 f	2.08 ± 0.05 fg
	VI	46.36 ± 2.65 de	17.45 ± 1.05 c	2.72 ± 0.15 f	5.46 ± 0.19 ab	1.42 ± 0.19 f	2.37 ± 0.10 de

The addition of the biostimulant generally improved the mineral uptake in rocket plants; however, significant interactions emerged between StMo and N fertilization for all the elements except S ($B \times F$; Table 2), with generally stronger effects in unfertilized plants (Table 5), and between StMo and harvest ($B \times H$; Table 2, Table 6).

In general, K, Ca, Mg and Na were higher while S was lower in plants treated with StMo; nevertheless, the extent of these effects changed among the minerals and varied over time (Table 6). Specifically, K increased in the second, the third and the last harvests, Ca in all the harvests except the first one and Mg in the first four compared to the untreated control (Table 6).

3.5. Antioxidant Properties as Affected by the Experimental Factors

The experimental treatments and the harvest period influenced in a different way the antioxidant properties of rocket, with the plastic film and harvest exhibiting the stronger impact (Table 3), and variable effects of the film over time (significant interaction P × H; Table 3). Specifically, under the diffusive Film1, the hydrophilic antioxidant activity (HAA) was higher at harvests II, III and VI, and the ABTS antioxidant activity (ABTS AA) was higher at harvest I and always lower afterward than under the clear Film2 (Table 7). The ABTS AA reached the highest value in the first-cut product and drastically decreased in the subsequent ones under diffusive Film1, while it showed much slighter variations over time under clear Film2 (Table 7).

Table 7. Interaction plastic film × harvest (P × H) on the average hydrophilic antioxidant activity (HAA), ABTS antioxidant activity (ABTS AA) and content of total phenols in rocket. Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		HAA *mmol Ascorbic Acid equ. 100 g^{-1} dw*	ABTS AA *mmol Trolox equ. 100 g^{-1} dw*	Total Phenols *mg Gallic Acid g^{-1} dw*
Film 1	I	7.56 ± 0.74 cd	10.94 ± 0.44 a	1.55 ± 0.06 f
	II	9.22 ± 0.15 ab	3.35 ± 0.34 g	1.55 ± 0.05 f
	III	9.09 ± 0.29 ab	3.37 ± 0.54 g	2.41 ± 0.12 c
	IV	8.31 ± 0.27 bc	5.02 ± 0.29 ef	2.12 ± 0.11 d
	V	9.28 ± 0.22 ab	5.87 ± 1.02 de	3.58 ± 0.20 b
	VI	8.79 ± 0.17 ab	4.22 ± 0.20 fg	1.91 ± 0.05 e
Film 2	I	7.60 ± 0.32 cd	8.04 ± 0.28 bc	1.54 ± 0.05 f
	II	6.57 ± 0.14 e	9.12 ± 0.31 b	1.61 ± 0.05 f
	III	7.65 ± 0.19 cd	8.04 ± 0.65 bc	2.58 ± 0.10 c
	IV	8.74 ± 0.29 ab	5.11 ± 0.23 ef	1.96 ± 0.07 de
	V	9.49 ± 0.18 a	9.18 ± 0.65 b	3.98 ± 0.19 a
	VI	7.00 ± 0.18 de	6.77 ± 0.20 cd	1.94 ± 0.05 e

Total phenols were unaffected by the cover material for most of the growing period, while it was reduced by the diffuse light in the first cut of May (harvest V), compared to the clear film (Table 7). Under Film1, phenols increased from the first to the fifth harvest, and then strongly decreased; under Film2, they showed a similar trend, but significantly higher values were recorded at harvest V compared to Film1 (Table 7).

Averaged on the plastic films, nitrogen fertilization did not affect the antioxidant properties of rocket, while it influenced the content of total phenols and TAA (Table 3), which were both reduced by the N supply (Table 8 and Figure 1).

Table 8. Interactions nitrogen fertilization × harvest (F × H) on the average hydrophilic antioxidant activity (HAA), ABTS antioxidant activity (ABTS AA) and content of total phenols in rocket. Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		HAA *mmol Ascorbic Acid equ. 100 g^{-1} dw*	ABTS AA *mmol Trolox equ. 100 g^{-1} dw*	Total Phenols *mg Gallic Acid g^{-1} dw*
N0	I	7.54 ± 0.74	9.10 ± 0.64 a	1.62 ± 0.05 h
	II	7.88 ± 0.41	6.13 ± 0.93 dg	1.60 ± 0.05 h
	III	8.97 ± 0.35	7.16 ± 0.97 cd	2.98 ± 0.13 d
	IV	8.42 ± 0.38	5.23 ± 0.31 fi	2.31 ± 0.14 e
	V	9.68 ± 0.28	7.60 ± 0.90 bc	4.81 ± 0.14 a
	VI	7.77 ± 0.31	5.41 ± 0.46 fi	1.98 ± 0.07 f
N1	I	7.51 ± 0.83	9.71 ± 0.57 a	1.57 ± 0.06 hi
	II	7.95 ± 0.47	5.94 ± 0.91 eh	1.54 ± 0.04 hi
	III	8.52 ± 0.33	5.56 ± 1.07 eh	2.26 ± 0.09 e
	IV	8.66 ± 0.32	4.92 ± 0.32 hi	1.83 ± 0.05 g
	V	9.33 ± 0.25	6.37 ± 1.01 df	3.39 ± 0.10 b
	VI	7.91 ± 0.35	5.64 ± 0.40 eh	1.86 ± 0.05 fg
N2	I	7.69 ± 0.51	9.65 ± 0.68 a	1.45 ± 0.08 i
	II	7.86 ± 0.44	6.64 ± 1.02 ce	1.61 ± 0.09 h
	III	7.63 ± 0.33	4.39 ± 0.85 i	2.25 ± 0.07 e
	IV	8.49 ± 0.35	5.05 ± 0.34 gi	1.98 ± 0.08 f
	V	9.15 ± 0.20	8.61 ± 1.44 ab	3.14 ± 0.08 c
	VI	8.01 ± 0.37	5.45 ± 0.50 fi	1.94 ± 0.06 fg
N0	StMo	8.38 ± 0.33	6.20 ± 0.47 ab	2.35 ± 0.18 b
	Control	8.38 ± 0.21	7.34 ± 0.46 a	2.75 ± 0.21 a
N1	StMo	8.47 ± 0.24	6.50 ± 0.65 ab	2.01 ± 0.10 c
	Control	8.16 ± 0.22	6.21 ± 0.50 ab	2.14 ± 0.10 c
N2	StMo	8.39 ± 0.33	7.26 ± 0.52 a	1.98 ± 0.11 c
	Control	7.88 ± 0.22	6.00 ± 0.49 b	2.14 ± 0.12 c

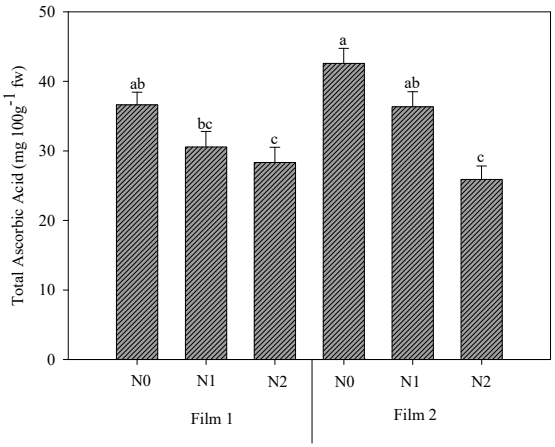

Figure 1. Effect of plastic films and nitrogen fertilization strategies on total ascorbic acid (TAA). Different letters indicate significant differences at $p = 0.05$ according to Tukey's multiple range test.

A significant interaction was found between the N dose and the date of harvest on ABTS AA and total phenols (Table 8). In general, ABTS AA reached the greatest value at the first and phenols at the fifth harvest, under all the N regimes (Table 8).

The N dose interacted also with the biostimulant (B × F) in influencing ABTS AA and total phenol content (Table 3). Indeed, ABTS AA was unaffected by StMo in unfertilized plants and at the sub-optimal dose N1, while it increased with the biostimulant application at the optimal supply N2 (Table 8). Total phenols were reduced by StMo in unfertilized

plants, while they did not change because of the biostimulant in plants fertilized with N (Table 8).

The plastic film and N fertilization both influenced the synthesis of TAA in rocket leaves, which was inhibited by both the diffuse light and the nitrogen supply (Figure 1). Specifically, under Film1, the TAA content showed a lower average value than under Film2, with similar values between the two N doses, differently to Film2, in which a further significant decrease was observed at the higher compared to the lower N dose (Figure 1).

The total ascorbic acid content of rocket changed in the harvest periods, with the highest values recorded in November and February and similar lower values from March to May (Figure 2).

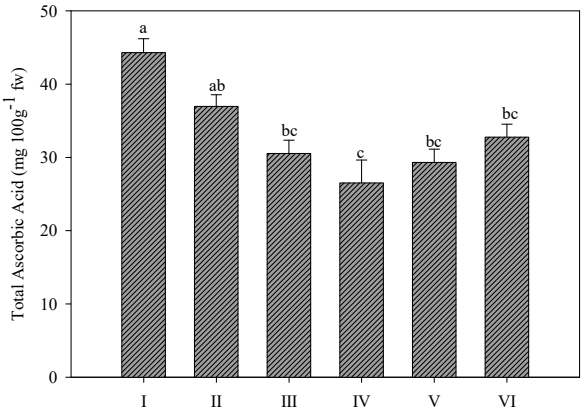

Figure 2. Effect of harvest on total ascorbic acid (TAA) (average of the experimental treatments). Different letters within each column indicate significant differences at $p = 0.05$ according to Tukey's multiple range test.

3.6. Chlorophyll and Carotenoid Content as Affected by the Experimental Factors

The leaf content of chlorophylls and carotenoids did not change in plants grown under the two plastic films; however, a significant interaction was found between the light environment and the growing period (P × H; Table 3). Specifically, the cover material did not influence the total chlorophyll content in most of the harvests, while Chl *a* decreased significantly under Film1 compared to Film2 at harvest V (Table 9). Carotenoids differed in plants under the two films only at harvests I and III. Indeed, under diffusive Film1, the average values were lower at harvest I and higher at harvest III compared to the clear Film2 (Table 9).

Table 9. Interactions plastic film × harvest (P × H) on the average content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll and carotenoids in rocket. Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		Chl *a* *mg g^{-1} fw*	Chl *b* *mg g^{-1} fw*	Total Chl *mg g^{-1} fw*	Carotenoids *mg g^{-1} fw*
Film1	I	1.04 ± 0.05 ab	0.60 ± 0.08 a	1.64 ± 0.11 a	0.327 ± 0.008 f
	II	1.04 ± 0.02 ab	0.49 ± 0.02 bc	1.54 ± 0.04 ac	0.359 ± 0.004 cd
	III	0.98 ± 0.02 bc	0.42 ± 0.02 cd	1.40 ± 0.04 ce	0.370 ± 0.005 bc
	IV	0.75 ± 0.02 e	0.37 ± 0.02 de	1.12 ± 0.04 f	0.404 ± 0.003 a
	V	0.85 ± 0.02 d	0.32 ± 0.01 e	1.16 ± 0.03 f	0.377 ± 0.0038 b
	VI	0.98 ± 0.03 bc	0.41 ± 0.03 ce	1.39 ± 0.05 de	0.361 ± 0.004 cd
Film2	I	1.10 ± 0.02 a	0.52 ± 0.02 ab	1.61 ± 0.04 ab	0.342 ± 0.005 e
	II	1.01 ± 0.02 bc	0.48 ± 0.02 bc	1.49 ± 0.03 bd	0.356 ± 0.004 cd
	III	1.00 ± 0.02 bc	0.47 ± 0.02 bd	1.46 ± 0.03 ce	0.354 ± 0.004 de
	IV	0.70 ± 0.02 e	0.37 ± 0.02 de	1.08 ± 0.03 f	0.408 ± 0.005 a
	V	0.94 ± 0.03 c	0.40 ± 0.02 ce	1.34 ± 0.05 e	0.375 ± 0.004 b
	VI	0.99 ± 0.03 bc	0.42 ± 0.03 bd	1.41 ± 0.06 ce	0.348 ± 0.005 de

The chlorophyll *a* and *b* in rocket leaves were influenced by both the N fertilization and the biostimulant and changed over time (Table 3), with generally higher values under treated plants. In addition, chlorophyll *a* and carotenoids responded to the N dose differently in the harvest periods (Table 10). Briefly, N fertilization increased the Chl *a* content compared to the unfertilized control only at the first and the last two harvests, with similar effects at the two N doses. Fertilization increased the carotenoids only at the first two harvests already at the sub-optimal N dose and without further effects at the optimal N dose (Table 10).

Table 10. Interactions nitrogen fertilization × harvest (F × H) on the average content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll and carotenoids in rocket. Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		Chl *a* *mg g^{-1} fw*	Chl *b* *mg g^{-1} fw*	Total Chl *mg g^{-1} fw*	Carotenoids *mg g^{-1} fw*
N0	I	0.99 ± 0.07 df	0.48 ± 0.03	1.47 ± 0.09 ce	0.324 ± 0.012 f
	II	1.02 ± 0.03 cd	0.49 ± 0.03	1.51 ± 0.06 ce	0.349 ± 0.006 e
	III	0.99 ± 0.02 df	0.44 ± 0.02	1.43 ± 0.04 df	0.379 ± 0.006 b
	IV	0.68 ± 0.02 l	0.34 ± 0.02	1.02 ± 0.04 i	0.412 ± 0.003 a
	V	0.82 ± 0.03 ij	0.30 ± 0.02	1.12 ± 0.05 hi	0.371 ± 0.006 bc
	VI	0.86 ± 0.03 hi	0.30 ± 0.02	1.17 ± 0.05 h	0.361 ± 0.008 cd
N1	I	1.12 ± 0.02 a	0.53 ± 0.03	1.65 ± 0.04 ab	0.348 ± 0.004 e
	II	1.02 ± 0.02 cd	0.48 ± 0.02	1.50 ± 0.04 ce	0.364 ± 0.003 cd
	III	0.96 ± 0.02 eg	0.44 ± 0.02	1.40 ± 0.03 eg	0.353 ± 0.005 de
	IV	0.78 ± 0.03 jk	0.40 ± 0.02	1.18 ± 0.04 h	0.405 ± 0.005 a
	V	0.93 ± 0.03 fg	0.39 ± 0.02	1.32 ± 0.05 fg	0.378 ± 0.004 b
	VI	1.03 ± 0.02 cd	0.45 ± 0.02	1.49 ± 0.04 ce	0.353 ± 0.004 de
N2	I	1.10 ± 0.02 ab	0.65 ± 0.12	1.75 ± 0.12 a	0.332 ± 0.007 f
	II	1.03 ± 0.02 cd	0.49 ± 0.02	1.52 ± 0.04 cd	0.359 ± 0.004 de
	III	1.01 ± 0.03 ce	0.44 ± 0.03	1.45 ± 0.05 ce	0.353 ± 0.003 de
	IV	0.72 ± 0.02 kl	0.37 ± 0.02	1.10 ± 0.04 hi	0.401 ± 0.006 a
	V	0.92 ± 0.03 gh	0.38 ± 0.02	1.30 ± 0.04 g	0.378 ± 0.003 b
	VI	1.05 ± 0.02 bc	0.49 ± 0.03	1.55 ± 0.04 bc	0.349 ± 0.004 e

In general, the application of the biostimulant promoted the synthesis of chlorophylls, while it did not affect the average concentration of carotenoids in rocket leaves; however, as observed per N fertilization, the effect of StMo changed in harvests (interaction $B \times H$; Table 3). Indeed, the biostimulant determined higher values of Chl *a* in the IV and V harvests, and a lower value of carotenoids in III compared to the non-treated control (Table 11).

Table 11. Interaction biostimulant × harvest (B × H) on the average content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll and carotenoids average in rocket. Different letters within each column indicate significant differences at $p = 0.05$ (Tukey's test).

Treatments		Chl *a* *mg g^{-1} fw*	Chl *b* *mg g^{-1} fw*	Total Chl *mg g^{-1} fw*	Carotenoids *mg g^{-1} fw*
StMo	I	1.05 ± 0.05 ab	0.51 ± 0.02	1.56 ± 0.06 ab	0.332 ± 0.008 d
	II	1.05 ± 0.01 ab	0.51 ± 0.02	1.56 ± 0.03 ac	0.352 ± 0.004 c
	III	1.00 ± 0.02 bc	0.49 ± 0.02	1.49 ± 0.03 bd	0.354 ± 0.004 c
	IV	0.77 ± 0.02 f	0.41 ± 0.02	1.18 ± 0.03 f	0.405 ± 0.004 a
	V	0.93 ± 0.02 d	0.38 ± 0.02	1.31 ± 0.04 ef	0.380 ± 0.003 b
	VI	1.00 ± 0.03 bd	0.42 ± 0.02	1.42 ± 0.05 ce	0.354 ± 0.005 c
Control	I	1.09 ± 0.02 a	0.60 ± 0.08	1.69 ± 0.09 a	0.338 ± 0.007 d
	II	1.00 ± 0.02 bc	0.47 ± 0.02	1.47 ± 0.04 bd	0.363 ± 0.004 bc
	III	0.97 ± 0.02 cd	0.40 ± 0.02	1.37 ± 0.03 de	0.370 ± 0.005 b
	IV	0.68 ± 0.02 g	0.34 ± 0.01	1.02 ± 0.03 g	0.406 ± 0.004 a
	V	0.86 ± 0.03 e	0.34 ± 0.02	1.19 ± 0.05 f	0.372 ± 0.004 b
	VI	0.97 ± 0.03 cd	0.41 ± 0.03	1.38 ± 0.06 de	0.355 ± 0.004 c

4. Discussion

Our research aimed at evaluating the influence of environmental parameters (i.e., light environment in terms of intensity and uniformity of distribution) and cultural factors (i.e., N availability and biostimulant application), as well as their interactions, on the yield and quality of wild rocket, grown in an unheated greenhouse under Mediterranean conditions, in the autumn–spring cycle. Moreover, we evaluated the interactions of the imposed experimental treatments with the changing climatic conditions during six harvests from November to May. The use of a diffusive film allowed us to obtain higher rocket yields already at half the dose of N compared to clear films and improved the productivity of both control plants and plants treated with Stimolo Mo, but the biostimulant did not confirm the capacity to contain nitrate, at least for this crop and in our cultivation system [25]. In this paper, we discuss the influence of the treatments on nutrient and nutraceutical features.

Effects of experimental factors on mineral composition

The leaf mineral content of rocket grown in a greenhouse under conventional plastic films in the autumn–spring period was found to be similar to that reported for the crop in a similar growing environment [1]. Averaged on the other treatments, the diffusive film determined a higher leaf concentration of Mg, Ca and Na, compared to the clear one, without affecting the nitrate content of rocket [25]. To the best of our knowledge, data on the influence of diffuse light and UV radiation on mineral uptake are scarce. In lettuce grown all year round, the foliar content of the main macronutrients increased under diffuse light but also the nitrate content rose [33]. However, in our experiment, the effects of film properties differed among the elements and changed over time. For instance, the diffusive film promoted the uptake in the first (P and Na) and the first two (Mg) harvests (November and February). This could be related to the lower solar radiation experienced by the crop in winter, which allows a better spatial distribution of light in the canopy due to the diffusive cover being more effective in promoting the plant activity, and hence in driving the mineral nutrition, in this period [34–36]. Consistently, the difference in leaf composition between the two light environments became irrelevant in the spring months, in the presence of non-limiting sunlight.

Nitrogen fertilization fostered the absorption of K, Ca, Mg and Na in rocket plants compared to the unfertilized control, with greater effects when increasing the N dose for K, and no further increase at the higher N dose for Ca and Mg. It is known that the availability of N in the growing media enhances the absorption of N itself, and this, in turn, has a synergistic effect on the absorption of some other macroelements (i.e., magnesium and, indirectly, phosphorus) [11]. In our case, the uptake of P was unaffected by the N supply, probably because it was already relatively high due to the good availability of P in the volcanic soil used in the experiment. The promoting effect of N on mineral uptake was stronger from the first to the third harvests (November to March) for all the cations. This is presumably related to the changing nutrient requirements during the plant developmental stages, which are greater in young plants at the full growth rate, as confirmed by the increasing yield in the same period [25], while they decline later because of both the plant ageing and the high temperature in the tunnel, which, in May, reached over 25 °C, representing the upper thermal limit for wild rocket [1].

The natural biostimulant Stimolo Mo applied as a foliar spray improved the uptake of K, Ca, Mg and Na, with a stronger effect in the absence of N fertilization. According to the label claims, it stimulates plant growth and limits nitrate accumulation, by accelerating the conversion of nitric N in amino acids and proteins and promoting nitrate reductase synthesis and activity by providing molybdenum. The beneficial effects on the plant performance of seaweed extracts and protein hydrolysates (PHs) have been attributed to several mechanisms, including biochemical changes (increasing the nutrient availability in the substrate), physiological effects (delaying of the senescence process), the functional control of stomatal conductance and the root-to-shoot ratio (enhancing the water use efficiency) and the stimulation of the rhizosphere microbiome (causing positive changes in the compo-

sition and activity of rhizobacteria and mycorrhizae) [23]. Moreover, among the multiple stimulating actions described for PHs, they increase the absorption and the use efficiency of nutrients, improve carbon and nitrogen metabolism, drive changes in hormonal activity and alleviate the effects of abiotic stress [37–39]. In our experiment, potential additive or synergistic effects among the different components of the mix probably contributed to eliciting the better mineral composition and overall rocket plant performance [23].

Effects of experimental factors on antioxidant capacity, total phenols and ascorbic acid

The antioxidant properties and the phenols and ascorbic acid content measured in our experiment are close to the reference values for rocket leaves reported in the literature [1]. The modified light environment exerted contrasting effects on the different components of the antioxidant capacity of rocket leaves. Indeed, compared to the clear film, the diffusive film determined higher hydrophilic antioxidant activity in February, March and the end of May, and comparable values in the other harvests, while it reduced the ABTS antioxidant activity in most of the production periods. This latter result partially agrees with our previous evidence in lamb's lettuce grown in spring under the same cover materials, in which both HAA and ABTS AA decreased under diffuse films [40]. Conversely, we found no significant effect of diffuse vs. clear films on ABTS AA in spinach in the winter cycle [41]. These conflicting results can be related to species-specific responses as well as to the diverse light environments (and, more generally, the climatic conditions) experienced by the crops in the different experiments. It can be hypothesized that rocket plants strengthen the synthesis of ABTS AA-related compounds under sub-optimal lighting (perceived as abiotic stress), which presumably occurred under the clear film because of the reduced light intensity in winter and excessive radiation in May. This hypothesis is consistent with the reduction in the antioxidant activity observed in several baby leaf vegetables (including rocket) harvested at high compared to low light [42], and suggests that the choice of the cover material needs to take into account the cultivation period, as diffuse light can be detrimental to this property, which is an important quality parameter of fresh leafy vegetables [43].

Total phenols in rocket leaves were not affected by the greenhouse cover film, in accordance with the previous results obtained in lamb's lettuce [40] and spinach [41], but contrary to what was expected based on the permeability to UV-B radiation of diffusive films. Phenolic compounds are plant secondary metabolites exerting a relevant antioxidant capacity in both plant and human cells [44]. Playing an active role in the photosynthetic process, their synthesis is strongly dependent on light exposure, and, specifically, it is boosted by high-intensity and UV radiation (perceived as environmental stressors), as a protective measure against light-induced damage (photoinhibition by reactive oxygen species). On this basis, the lack of difference in phenol content in rocket under the UV-B permeable diffusive film compared to the UV-B blocking clear film is surprising, although it could be partially explained by considering the naturally lower ultraviolet proportion in solar radiation during the winter season. Consistently, the increase that occurred in the harvest of May is in accordance with the stimulatory effect on their biosynthesis of high light intensity and UV in spring in leafy vegetables [45,46].

The synthesis of total ascorbic acid in rocket leaves was promoted by diffuse light and inhibited by N fertilization, as we already observed in spring lamb's lettuce [40]. It is well known that ascorbate in leaves depends on the light environment and, in general, plants synthesize more vitamin C under high rather than low light intensity, as it has several photoprotective functions [47]. Conversely, data on the effect of diffusive covers on vitamin C are scarce and somewhat contrasting, suggesting a species-specific response, and confirming the influence on this parameter of several environmental factors. Indeed, several studies report a reduction in lettuce compared to clear covers ([45] and references therein), and we also observed a drastic reduction under diffuse light, as well as a decrease under N fertilization in spinach in the winter cycle [41]. It is worth noting that our results on both rocket and lamb's lettuce confirm the stimulating effect on the synthesis of ascorbic acid as a protective agent for plants against UV-B radiation [47].

Averaged on the other experimental treatments, nitrogen fertilization did not affect the antioxidant properties of rocket, similarly to what was observed in lamb's lettuce and spinach, while it reduced the content of total phenols and total ascorbic acid, as in lamb's lettuce, for both the last two parameters [40], and in spinach for phenols [41]. Referring to the effect on phenols and ascorbic acid, it has been hypothesized that the low availability of N (creating nutritional stress) may trigger the synthesis of secondary metabolites in plants. However, the results of research addressing the effect of N fertilization (dose and chemical form) on vitamin C are often contrasting, as the increasing supply of N fertilizers may result in an decrease or increase, or simply no effect, highlighting that the plant response to N availability for this parameter strongly depends on the interaction among climatic and cultural conditions. For instance, in our experiment, N fertilization interacted with the biostimulant Stimolo Mo, which was effective in boosting the antioxidant activity (i.e., ABTS AA) only at the optimal N supply, and reduced the total phenols in unfertilized plants.

Effects of experimental factors on chlorophylls and carotenoids

The cover material did not influence the content of chlorophyll *a* and *b* and carotenoids in rocket leaves, while N fertilization and biostimulant application increased the total chlorophyll, although both types of pigment changed over time and responded to the N dose and biostimulant differently in the harvest periods. Specifically, N supply promoted the synthesis of Chl *a* at higher temperatures and solar radiation (April to May), as these conditions boost the photosynthetic process, requiring the strengthening of the light harvesting apparatus [12]. The application of the biostimulant also promoted the synthesis of chlorophylls in rocket leaves; however, as observed per N fertilization, also the effect of StMo changed in harvests, with higher values in the IV and V harvests. Chlorophylls are responsible for leaf greenness, which contributes to the esthetical value of green leafy vegetables. In addition, chlorophyll has been recently found to exert a preventive action against some forms of cancer [48]. Besides the effects on quality, in plants fertilized with N and treated with the biostimulant, the improvement in plant growth was associated with the stimulation of N uptake and chlorophyll biosynthesis, which may have enhanced the photosynthetic activity and increased the translocation of photosynthates to the sinks.

5. Conclusions

It is well known that both the amount of light and the nitrogen availability boost the biomass accumulation in several vegetable species. In the view of the environmental and economical sustainability of the greenhouse industry, exploiting solar radiation has to be preferred over artificial lighting; hence, optimizing the quantity and quality of natural light through the proper choice of cover materials is the first option. In the last 40 years, the global use of mineral N fertilizers has increased dramatically to support the increasing food demand, and the misuse of fertilizers determines serious environmental issues and plant food-related risks for human health. Hence, improving the N use efficiency of crops and cropping systems is imperative.

Our results demonstrate that both diffuse light and the proper N dose, also combined with a natural biostimulant, can be useful tools to improve the yield and nutritional quality of greenhouse rocket, and also to modulate the content of phytochemicals. However, the interaction among treatments, as well as the changing climatic conditions during the cultivation cycle, need to be considered every time, as they can become detrimental to quality and properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030638/s1, Figure S1: Pots used for the growth of Rocket plants under two different greenhouse plastic films; Table S1: Trend of average temperature during the whole growing period under the two greenhouse plastic films (Film1: diffuse-light film; Film2: clear film).

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Abbreviations

Chl a, chlorophyll a; Chl b, chlorophyll b; Chl, chlorophyll; dw, dry weight; fw, fresh weight; DAPH, days after the previous harvest; DAT, days after transplant; fw, fresh weight; HAA, hydrophilic antioxidant activity; ABTS AA, ABTS antioxidant activity; TAA, total ascorbic acid; UV, ultraviolet radiation.

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