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Combination of nitrogen reduction and algae-based biostimulants: a sustainable strategy for processing tomato

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Processing tomato (*Solanum lycopersicum* L.) is a key crop in the Mediterranean, where farmers are increasingly required to balance high yields with reduced inputs. In response to the EU Farm to Fork strategy, which targets a 20% reduction in fertilizer use by 2030, the aim of this study was to evaluate the potential of algae-based biostimulants to sustain crop performance under reduced nitrogen (N) supply. In this context, we carried out a two-year field trial (2023–2024) in Acerra, Southern Italy testing three N levels (100%, 90% and 80% of the optimal dose in 2023; 100%, 80% and 70% in 2024) and two commercial formulations (BIO1: Auximar + Procalcium; BIO2: Enerleaf + Pentacalcium), applied as foliar sprays. Reducing N dose led to a proportional decline in yield compared to the optimal dose (–16% in 2023, mean value of N80% and N90% and –30% in 2024 with N70%). In both years, all biostimulants increased marketable yield (+21% and +36%), mainly by raising fruit number. The N80% treatment combined with biostimulants maintained yields comparable to the full nitrogen dose, pointing to a more efficient use of fertilizer. Biostimulant treatments enhanced fruit firmness (+19% in 2023) and soluble solids total (+14% and +10% in 2023 and 2024, respectively), and boosted nutritional parameters such as ascorbic acid (12% in 2023 and 15% in 2024) and carotenoids. Lycopene was not influenced by biostimulants, but showed instead a strong dependence on seasonal conditions and N dose, with higher values recorded in 2024. Although main effects of nitrogen and biostimulants were largely independent, significant year × nitrogen × biostimulant interactions were detected for fruit nitrogen and nutraceutical compounds. The results indicate that algae-based products with a moderate ($\geq$20%) N reduction appears to be a practical way to cut fertilizer inputs while safeguarding both yield and fruit quality in processing tomato.

KEYWORDS
algae-based biostimulants, fruit quality, nitrogen reduction, *Solanum lycopersicum* L., sustainable agriculture

1 Introduction

Processing tomato (*Solanum lycopersicum* L.) represents one of the most important horticultural crops globally, both in terms of land area and economic relevance. In 2024, worldwide production of processing tomato is estimated at around approximately 45.8 million tons, showing a 3% rise over the previous year and about 13% more than the 2021–2023 average (WPTC, 2024). Italy, one of the leading European producers, recorded an 11% increase in cultivated area, although average yields fell by 2.4% due to adverse weather patterns, including drought episodes in the South and heavy rainfall in the North (ISMEA, 2025). Together with the United States and China, these countries account for the largest share of the world's total output. Even with this steady expansion, the sector is facing growing pressure to lower its environmental footprint and mitigate the effects of fertilizers use. In this context, European agricultural policies, especially the Green Deal and the Farm-to-Fork Strategy, are pushing towards more sustainable management by setting targets to reduce fertilizer use by 20% and nutrient losses by 50% by 2030 (European Parliament, 2020; European Commission, 2019). Achieving these goals requires a deep transformation of production systems, emphasizing low-impact agronomic practices such as biostimulants application and sustainable fertilization management. Nitrogen management plays a key role, being an essential nutrient for tomato growth. However, its inefficient use can cause severe environmental issues, including groundwater contamination and eutrophication of surface water bodies (Ronga et al., 2020). Optimizing nitrogen use efficiency (NUE) becomes a key strategy to reduce nitrogen (N) inputs, lower environmental damage, and reduce production costs associated with synthetic fertilizers (Rossini et al., 2018). NUE expresses how effectively crops convert available nitrogen into yield. Classical agronomic definitions include agronomic efficiency (yield increase per unit of applied N compared to only N available in soil), physiological efficiency (yield increase per unit of N uptake compared to only N available in soil), and partial factor productivity (yield per unit of N applied) (Dobermann, 2005). NUE is governed by two main physiological components: the ability of plants to acquire N from the soil (nitrogen uptake efficiency, NUpE) and their capacity to transform absorbed N into biomass or yield (nitrogen utilization efficiency, NUtE) (Ali et al., 2022). Improving NUE is widely recognized as essential for sustainable agriculture, both to safeguard yields and to reduce the leakage of reactive nitrogen into the environment. Excessive N inputs combined with low NUE accelerate soil acidification, nitrate leaching, gaseous losses (NH_3, N_2O), and negative effects on soil microbial functioning (Hirel et al., 2011; Tamagno et al., 2024). Recent studies emphasize that NUE must be interpreted through both classical agronomic indices and newer environmental indicators, such as N balance (Nb) and the ratio of N outputs to inputs (NUEb), which are increasingly used to assess risks of N surplus at field and policy scale (Congreves et al., 2021). In processing tomato, a crop with comparatively high nitrogen demand, fertilizer requirements vary according to soil fertility, climatic conditions and expected yield. Most studies conducted in Mediterranean systems report seasonal N inputs ranging between 180 and 300 kg N ha-1, typically supplied through split applications or fertigation to match crop uptake patterns (Farneselli et al., 2025; Tei et al., 2025). Despite these high inputs, nitrogen recovery is often limited, and 30-50% of applied N can be lost through volatilization or denitrification, underscoring the need to optimize fertilizer management and improve NUE. Among low-input strategies, biostimulants are increasingly considered promising tools to integrate or partially replace high N rates, enhancing root growth, nutrient uptake, and plant metabolic efficiency even under reduced N availability (Tei et al., 2025). Recent studies have validated the agronomic potential of these products, especially in improving nutrient utilization. For example, in a two-year organic tomato trial, Quintarelli et al. (2024) observed that the combined use of microbial biofertilizers and algae-based biostimulants improved plant vigor, and improved crop performance in terms of both yield and quality. As reported by Quille et al. (2025), biostimulants operate through complex physiological and molecular mechanisms that regulate nutrient uptake and assimilation, including nitrate absorption, nitrogen conversion, and amino-acid metabolism, offering a viable strategy to optimize nitrogen use while maintaining crop productivity. Biostimulants, according to Regulation (EU) 2019/1009 of the European Parliament and Council of 5 June 2019, comprise seaweed extracts, protein hydrolysates and natural organic compounds that improve nutrient uptake and utilization, although they do not directly supply nutrients (European Union, 2019). These formulations can stimulate root growth, photosynthetic activity, and abiotic stress tolerance, thereby improving both yield and product quality (Colla et al., 2017a; Di Mola et al., 2020). In addition, some biostimulants influence both primary and secondary metabolism, thereby improving tolerance to abiotic stress (Calvo et al., 2014; Rouphael et al., 2017). Amino-acid-rich extracts in particular enhance nitrogen assimilation and resistance to thermal and saline stress (Colla et al., 2014; Nardi et al., 2016; Lucini et al., 2015; Di Mola et al., 2021). Other mechanisms include hormone-like effects (auxin- and gibberellin-like), enhanced activity of key enzymes such as nitrate reductase (NR) and glutamine synthetase (GS), and the upregulation of genes involved in nitrogen assimilation (Carillo et al., 2019). The improvement of photosynthetic efficiency under low-N availability is especially important and it is linked to higher chlorophyll concentrations and increased synthesis of key amino acids like glutamate (Yaronskaya et al., 2006). In this context, biostimulants prove effective even in low-input systems and poor soils, where their action is especially beneficial (Monda et al., 2021). In extensive crops, such as durum wheat, Rossini et al. (2025) found that foliar application of biostimulants seaweed-based and plant growth-promoting bacteria (PGPB), under reduced nitrogen fertilization, increased root development, chlorophyll content, and NUE, by achieving potential nitrogen savings of up to 33% without yield losses. Seaweed-based formulations are one of the most commonly used groups within the broad category of plant biostimulants, thanks to their rich composition of bioactive molecules and consistent effects across diverse crops. Seaweed-based foliar sprays, particularly those derived from brown macroalgae such as Ascophyllum nodosum and *Ecklonia maxima*, contain a variety of bioactive compounds that contribute to their biostimulant properties (Battacharyya et al., 2015). These include polysaccharides such as alginate, laminarin and fucoidan, which can enhance water retention, stimulate root growth and modulate plant defence pathways; sugar alcohols, such as mannitol, which improve osmotic balance under stress; and betaines, polyphenols, vitamins and mineral micronutrients, which support metabolic activity and tolerance to oxidative stress (Deolu-Ajayi et al., 2022; Kumar et al., 2024). In addition, seaweed extracts often exhibit hormone-like activity, with auxin- and cytokinin-like effects that promote, leaf expansion and root system development. This combination of bioactive molecules can enhance nutrient uptake, improve photosynthetic efficiency and strengthen plant resilience. This explains why crops respond beneficially even when nitrogen availability is reduced (Kumar et al., 2024). Similarly, Cozzolino et al. (2021) observed that in processing tomato, foliar application of a seaweed extract (*Ecklonia maxima*) and a legume-derived protein hydrolysate markedly improved nitrogen efficiency and productivity even under low-input regimes. They reported an increase in marketable yield of 18.3% on average and a reduction in unmarketable production of 41.3%, compared with the untreated control. These treatments also enhanced nitrogen-related indices, raising nitrogen-use efficiency by 18.4% and nitrogen-uptake efficiency by 59.3%. There was also a 21.3% increase in fruit nitrogen content. Golin et al. (2024) conducted a literature review of 48 case studies on various horticultural crops (e.g., tomato, lettuce, strawberry), noting a positive impact on agronomic performance in 79% of cases. Among these, foliar application of *Ecklonia maxima* led to a 19% increase in processing tomato (cv. Coronel F1, Italy) yield compared to the control, along with improved fruit quality in terms of soluble solids, lycopene, and vitamin C content. Moreover, a multi-site trial on eight commercial farms (Mendes et al., 2023) showed that microbial biostimulants promoted vegetative growth, yield, and key soil parameters in open-field crops such as corn, soybean, cotton, and sugarcane, including physical structure, enzymatic activity (β-glucosidase, arylsulfatase), and the availability of exchangeable nutrients, resulting in improved chemical fertility (cation exchange capacity and exchangeable bases). Although biostimulants may increase production costs by 10-20% in some cases, Colla et al. (2017b) showed that the resulting yield increases lead to higher gross and net returns compared to untreated crops, largely offsetting the initial investment. In addition, potential indirect environmental benefits should also be considered, such as the reduction of synthetic inputs and their associated negative impacts. Based on these considerations, the present study aimed to evaluate the effect of the application of seaweed-based biostimulants on the productivity and quality of processing tomatoes. To this end, we tested three nitrogen doses per each year. In particular, in the first year we compared the optimal N dose to two sub-optimal doses (90% and 80%), this last one was chosen according to the indication of Farm to Fork Strategy that requires a 20% reduction in fertilizers use and we individuated an intermediate N level based on the high nutritional tomato requirements. Based on the first year results (no difference between 90 and 80% reduction), in the second year we chose to test again the 80% N reduction to respect the Farm to Fork Strategy indication and a further reduction of N level in order to verify the adaptability of the processing tomato to lower dose. In addition, in both years we combined the biostimulants application to nitrogen fertilizers in order to verify whether biostimulants could compensate, at least in part, for this reduction in inputs. The overall objective was to identify a sustainable agronomic strategy for reducing environmental impact without compromising yields, thus contributing to the ecological transition of agriculture and the development of more efficient and resilient cropping practices.

2 Materials and methods

2.1 Experimental setting, growing conditions, and tomato cultivar

The experiment was carried out at the private farm “Arca 2010” located in Acerra (Naples, Italy; 40°56′ N; 14°22′ E; 30 m a.s.l.) for two years (2023-2024) during the spring–summer period. The trial was conducted on a sandy loam soil that exhibited consistent physical and chemical characteristics across the two growing seasons. The average particle size distribution consisted of 60.5% sand, 24.9% silt, and 14.6% clay. Soil chemical analysis indicated a slightly alkaline pH (7.40–7.50) and low electrical conductivity (0.019–0.021 dS m-1). Organic matter content ranged from 2.65 to 2.69%, while total nitrogen remained constant at 0.18%. Available phosphorus (Olsen) and exchangeable potassium ranged between 203–216 ppm and 1555–1652 ppm, respectively.

Meteorological data for the two growing seasons are reported in Figure 1. During the first crop cycle (April–August 2023), the total rainfall was 103.0 mm, but its distribution was irregular, alternating between peaks during the second ten-day periods of May and June (exceeding 15 mm) and prolonged dry spells (July and most of August) (Figure 1A), Figure B, referring to the 2024 season (March–July), shows an overall drier pattern; indeed, the total amount of rainfall was 70.8 mm, but the distribution was more even across the growing season. As expected, in both years, a progressive rise in both maximum and minimum temperatures was observed throughout the growing period. In 2023, the highest values were generally recorded in the final part of the season, with a progressive increase from April to August and a maximum of 36.5 °C in the second ten-day period of July; minimum temperatures remained above 19 °C from mid-July onwards. In 2024, the trend was more gradual and consistent, with a peak of 35.4 °C in the second ten-day period of July, while minimum temperatures exceeded 20 °C only during July.

The plant tested was the processing tomato (*Solanum lycopersicum* L.) cultivar “Orion SWR F1 (BL 169)” (Blumen Group S.p.A., MI, Italy), a medium-early cycle hybrid with prismatic fruits good field performance and °Brix.

The tomato plants were transplanted on May 5, 2023, and on April 9, 2024, in the first and the second year, respectively. The seedlings were transplanted at a density of 33000 plants per hectare (60 cm row-to-row spacing within the paired row; spacing between the row pairs of 180 cm; plant-to-plant space on the single row of 33 cm). The harvest was made in early August in the first year and the last week of July in the second year.

All agricultural practices on the farm were carried out in accordance with ordinary local methods.

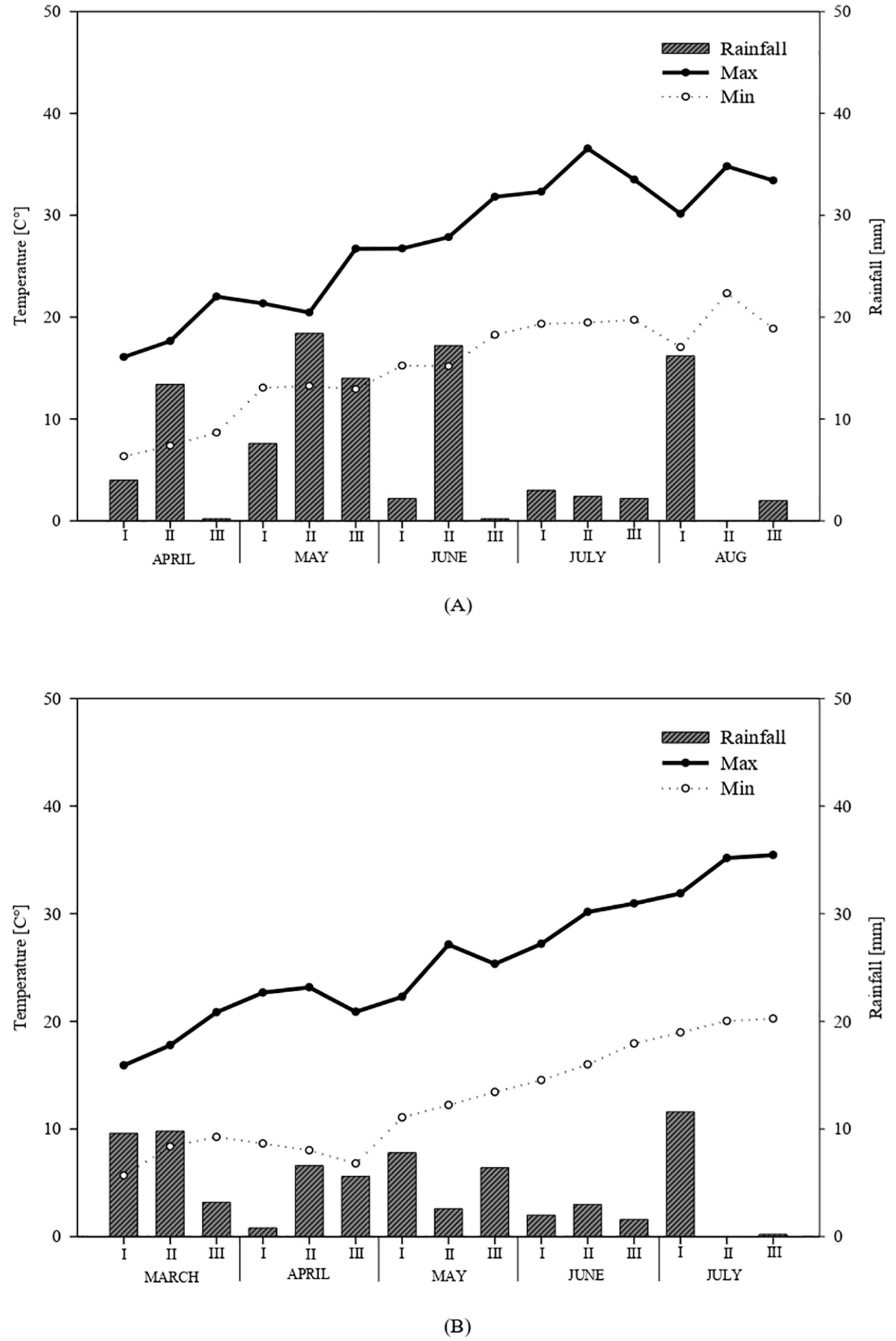

FIGURE 1
Trends in temperature and rainfall recorded during the two cultivation seasons. Figure above **(A)** shows data from the first year of the trial (2023), while figure below **(B)** refers to the second year (2024).

2.2 Experimental design and biostimulant application

The experimental design was a combination of three nitrogen fertilization levels and two biostimulant treatments, arranged in a split-plot design with three replicates per treatment. In the first year, the nitrogen levels were: I) the optimal nitrogen dose (N100%), II) a 10% reduced N dose (N90%); III) a 20% reduced N dose (N80%). Based on the first-year results, which indicated promising productive tomato response even with the lowest nitrogen input, we decided to further reduce the nitrogen rates in the second year. Specifically, in addition to N100% and N80% treatments, we introduced a new reduced N level that is a 30% reduction compared to the optimal dose (N70%).

The 3 biostimulants strategies were: I) an untreated control (NB); II) a combination of a seaweed-based biostimulant and calcium-based foliar treatment (produced by Menfin srl) -BIO1; III) a different combination of a seaweed-based biostimulant and calcium-based foliar treatment (produced by Diachem srl) -BIO2.

The optimal N dose was calculated with the Fertilization Plan of Campania Region, and it was 160 kg ha^{-1} in both years; nitrogen was added as ammonium nitrate (26%) twice: on June 9 and July 4, and on May 7 and June 2, respectively for the first and the second year.

2.2.1 Biostimulant composition and application protocol

The biostimulants are two different commercial algae-based, able to improve plant resistance to abiotic stresses, enhancing photosynthesis and promoting root development (S1).

The first biostimulant application (BIO1) included the use of Auximar^{TNF} and Procalcio (Menfin srl); Auximar, is a liquid seaweed extract derived from *Ascophyllum nodosum*, has a high content of natural phytohormones, polysaccharides (including 0.21% alginic acid), and the sugar alcohol mannitol (0.20%), together with small amounts of organic nitrogen (4.15% total N) and micronutrients such as Mn and Zn (0.44% each); while, Procalcio is a liquid fertilizer containing calcium (12.5% CaO) and low molecular weight organic acids that prevents apical rots and tomato blotching.

The second biostimulant application (BIO2) included the use of Enerleaf and Pentacalcium (Diachem srl); Enerleaf is rich in bioactive compounds such as mannitol (0.4%) and organic carbon (2.5%) derived from the seaweed *Ascophyllum nodosum*, Pentacalcium is a photosynthesis accelerator containing calcium (7%), magnesium (3.5%) and 5-aminolevulinic acid (>0.05% ALA), which stimulates chlorophyll production. The biostimulants were applied as a foliar spray at the rate of 3 mL L^{-1}. The two algae-based biostimulants were applied twice: on June 14 and 29 in the first year, and on May 22 and June 5 in the second year. The calcium-based products were applied once, on July 13 in the first year and on June 20 in the second year. Biostimulants were applied simultaneously under the same environmental conditions on the same day, during the early morning to ensure optimal and uniform absorption.

2.3 Yield and its components: classification and dry matter determination

The tomato was harvested on a 1.5 m^2 sample area per plot; then, the fruits were separated into marketable, not marketable (rotten), and green and were counted and weighed to determine the mean fruit weight. Finally, dry matter content was determined on a sub-sample of marketable fruits was oven-dried at 70 °C until reaching a constant weight to determine the dry matter percentage.

2.4 Nitrogen-use efficiency indicators

The efficiency of plants in using nitrogen can be determined through different indicators, which take different aspects into consideration. In this research, nitrogen use efficiency was estimated using partial factor productivity (PFP), which expresses the yield per unit of applied nitrogen (fertilizers), and nitrogen use efficiency (NUE), which also considers the nitrogen present in the soil at the beginning of the cycle.

PFP_N quantifies the overall productivity of applied N and expresses the harvested yield per unit of N fertilizer (often simply called nitrogen use efficiency):

$$PFP_N(kg\ product\ kg^{-1}N) = Y_N/F_N$$

Y_N is the crop yield obtained at a specific N dose applied (kg ha^{-1}), and F_N is the amount of N fertilizer applied (kg ha^{-1}), (Dobermann, 2005).

NUE was considered as an extended PFP index, and accounts for initial soil N availability. Following the conceptual distinction introduced by Moll et al. (1982) between N fertilizer and N soil-derived contributing to crop N supply, we calculated:

$$NUE\ (t\ product\ kg^{-1}N) = Y_N/(F_N + N_{soil})$$

Where F_N is amount of N fertilizer applied (kg ha^{-1}), and N_{soil} is the amount of N measured at transplanting (kg ha^{-1}).

This index is not equivalent to the physiological NUE described by Moll et al. (1982), which refers to the total N available to the crop during the total growing cycle. For this, we offer a practical way to account for differences in initial soil N when comparing treatments.

2.5 Colorimetric analysis of processing tomato fruits

At the harvest, the color space parameters (L*: brightness, ranging between 0 (black, no reflection) and 100 (white); a*, chroma parameter ranging between −60 (green) and +60 (red); b*, chroma parameter ranging between −60 (blue) and +60 (yellow)) were determined by a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). Colour measurements were performed on ten marketable fruits per plot, with two readings taken on opposite sides of each fruit. Analyses were carried out shortly after harvest under laboratory conditions at room temperature.

2.6 Chemical and physical quality parameters: nitrogen, total soluble solids, and firmness

Nitrate content was assessed on an oven-dried sample of marketable fruits (composite samples of marketable fruits collected from each replicate) according to the protocol of Sah, 1994; the solution absorbance was measured at 550 nm, and the results were expressed as mg kg^{-1} (dry weight; dw).

The nitrogen content of fruits was determined on dried samples (composite samples of marketable fruits collected from each replicate) by the Kjeldahl method (Bremner, 1965), and the results were expressed as g kg^{-1}.

On fresh fruit juice of five marketable fruits per replicate, total soluble solid (TSS) content was measured by a digital refractometer (Synergic Solutions, DBR35, Pescara, Italy), and it was expressed as °Brix.

Firmness was measured by a digital penetrometer (T.R. Turoni srl, Forlì, Italy) equipped with an 8 mm diameter probe; the measurements were performed on the two opposite sides of five fruits per replicate. Results were expressed in kg cm^{-2}.

2.7 Chemical quality parameters: antioxidant activities and bioactive compounds measurements

Finally, another sample of marketable fruits per each treatment and replicate was frozen at −80 °C and then lyophilized with a lyophilizer Crist, Alpha 1–4 (Osterode, Germany) for the determination of some qualitative characteristics, including hydrophilic and lipophilic antioxidant activity (HAA and LAA, respectively), ascorbic acid (AA), phenolic content, lycopene content, carotenoid content, nitrate concentration, total soluble solids content and firmness determination.

For freeze-dried tomato fruit samples, hydrophilic and lipophilic antioxidant activity (HAA and LAA, respectively), total phenols, and ascorbic acid content (AA) were assessed. HAA and LAA were determined using the DMPD (Fogliano et al., 1999) and ABTS (Re et al., 1999) methods, respectively, in order to account for the diverse chemical nature of tomato bioactive compounds. Indeed, HAA reflects the contribution of water-soluble antioxidants, while LAA represents the activity of fat-soluble fractions. The reduction in absorbance of the solutions obtained with the two methods was measured by UV–Vis spectrophotometry with an ONDA V-10 Plus (Giorgio Bormac s.r.l, Carpi, Italy), at 505 and 734 nm, respectively, using ascorbate and Trolox external standard calibration curves for HAA and LAA, respectively. Total phenols were also measured by a spectrophotometer, and the absorbance solution was detected at 765 nm, according to the Singleton et al. (Singleton et al., 1999) method. Total ascorbic acid was determined spectrophotometrically according to the protocol of Kampfenkel et al. (Kampfenkel et al., 1995) and the solution absorbance was measured at 525 nm.

The lycopene content was determined according to the method of Sadler et al. (Sadler et al., 1990). The samples of tomato (2.5 g) were mixed with 50 mL of a mixture of n-hexane:acetone:ethanol (2:1:1) at 0.5% BHT (2,6-di-tert-butyl-4metyl-phenol). The absorbance was read at 472 nm using spectrophotometer and the total lycopene content was expressed as mg lycopene 100 g^{-1}fw.

Carotenoid content was spectrophotometrically assessed on 1 g of fresh fruit after the extraction with ammoniacal acetone according to the method stated by Wellburn (Lichtenthaler and Wellburn, 1983), absorbance was measured at 450 nm, and it was expressed as mg g^{-1} fresh weight (fw).

2.8 Statistical analysis

The effects of nitrogen and biostimulants were evaluated using a split-plot experimental design, with the three N rates as the main experimental factor, and the biostimulant treatments as sub-factors. All treatments were replicated thrice for a total of 27 treatments (3 N rates × 3 Biostimulant treatments × 3 replicates). Each response variable was tested for normality using the Shapiro–Wilk test at the 0.05 significance level. Data from 2023 and 2024 were combined after confirming the homogeneity of error variances using Bartlett's chi-square test Gomez (Gomez and Gomez, 1984). The year was treated as a random effect in the analysis.

Statistical analyses were conducted using the GLM procedure in SAS/STAT (SAS® University Edition). Appropriate error terms were applied for each main effect and interaction. Mean comparisons were performed using the least significant difference (LSD) test, with p-values adjusted via the Bonferroni correction to account for multiple comparisons at the 0.05 significance level.

For the treatments common to the two years (N80 and N100% with or without biostimulants), all data were subjected to a three-way analysis of variance (ANOVA).

3 Results

Per each year we reported only the results for which the effects of single factors (nitrogen dose and biostimulants application) or their interaction was significant. In addition, as written in the previous paragraph, we carried out a statistical analysis on the treatments common to the two years, considering also these last ones as an experimental factor. Also in this case, we reported only the significant results (main or combined effects of experimental factors).

3.1 First year

3.1.1 Marketable and not marketable yield and their components

In the first year, the interaction between nitrogen fertilization and biostimulant application (N×B) was not significant formarketable yield. Therefore, only the main effect of the two factors was considered. Marketable yield decreased by approximately 16% under reduced nitrogen supply, irrespective of N reduction (N90% and N80%) compared to the optimal dose (N100%); indeed, no significant differences were detected between the two suboptimal nitrogen levels (Figure 2). Furthermore, the application of both biostimulants significantly increased marketable yield by about 21% compared to the untreated control (NB) (Figure 2).

This yield increase was mainly due to the number of fruits per square meter, which showed a 29% rise under biostimulant treatments with respect to the untreated control (Table 1), instead they did not affect the average fruit weight, which remained consistent across the treatments. In contrast, a statistically significant effect was observed with different nitrogen levels: the N80% fruits had a higher average weight, but a lower number of fruits per square meter, although not significant different from the other two treatments (Table 1). As regards green fruits, only BIO2 elicited an increase in both yield and fruit number per square meter: 42%, and 61%, respectively (Table 1). No significant differences were observed for the other green yield components and for non-marketable yield.

3.1.2 Physical and chemical parameters of processing tomato berries

The interaction nitrogen dose x biostimulant application as well as the single effect of N dose never affected the quality physical parameters, contrary to the biostimulants application that elicited a

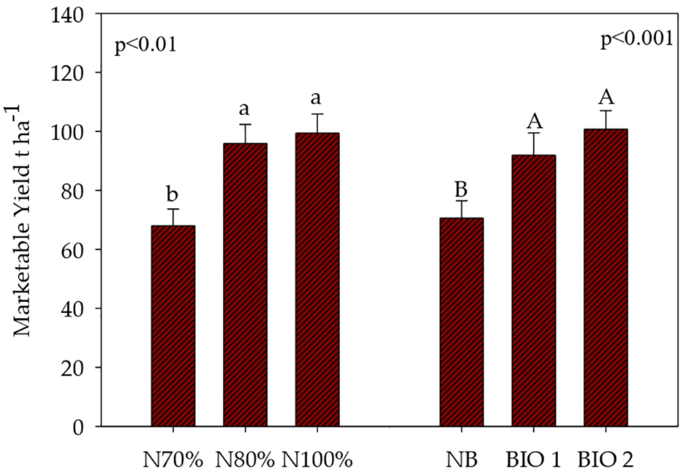

FIGURE 2
Tomato marketable yield as affected by nitrogen fertilization (N100%, full optimal dose; N90%, 10% N reduction; N80%, 20% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control). Each column indicates the mean value of 3 replicates. Vertical bars indicate standard error; different letters indicate significant differences according to the LSD test at the 0.05 significance level.

significant improvement in firmness and total soluble solids (TSS). Specifically, fruit firmness increased by 19% and TSS by approximately 14% compared to the untreated control (Table 2).

In the first year, among the chemical qualitative measurements, only the lipophilic antioxidant activity (LAA), and lycopene were significant affected by the interaction between nitrogen dose and biostimulant application (Figures 3 and 4, respectively).

N90% treatment showed the statistically higher values of LAA antioxidant activity, regardless of biostimulant application; while N80% treatment, that showed intermediate values, was not different from N100% with biostimulant application; finally, N100%-NB showed the lowest values (Figure 3).

Also for the lycopene content of the processing tomato fruits, the N90% treatments showed the highest value (+7%, and +20% compared to the mean value of N100% and N80%, respectively), regardless of the biostimulant application; the lowest values were recorded in N80% treatments with biostimulants application that were no different between them (Figure 4).

3.2 Second year

3.2.1 Marketable and not marketable yield and their components

In the second year of the experiment, as for the first one, no significant interaction was found between biostimulants and nitrogen levels, but only the main effects were significant. Specifically, the N70% marketable yield had a 30% reduction compared to the average of N100% and N80% treatments, which were not different between them (Figure 5). As for the effect of biostimulants, both ones elicited a 36% increase in yield compared to the untreated control (Figure 5).

TABLE 1 **Yield components (number of fruits per square meter, mean fruit weight, and yield for marketable, green, and non-marketable fraction) of processing tomato as affected by nitrogen fertilization nitrogen fertilization (N100%, full optimal dose; N90%, 10% N reduction; N80%, 20% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control).**

Treatments	Marketable yield			Green yield		Non-marketable yield		
	n° fruits m^{-2}	*g fruit^{-1}*	*t ha^{-1}*	*n° fruits m^{-2}*	*g fruit^{-1}*	*t ha^{-1}*	*n° fruits m^{-2}*	*g fruit^{-1}*
N80%	1517 ± 113	65.9 ± 1.6 a	26.2 ± 2.9	57.5 ± 6.6	46.6 ± 2.5	3.7 ± 0.6	8.4 ± 1.3	43.3 ± 2.5
N90%	1562 ± 59	60.5 ± 1.0 b	22.5 ± 2.1	59.6 ± 10.4	40.4 ± 2.7	7.2 ± 1.3	14.5 ± 2.5	49.0 ± 4.4
N100%	1791 ± 118	61.3 ± 1.7 b	29.5 ± 2.9	75.8 ± 7.6	39.4 ± 2.3	6.3 ± 1.0	13.5 ± 1.7	46.7 ± 6.1
NB	1358 ± 72 b	63.0 ± 2.0	22.7 ± 1.6 b	55.9 ± 4.5 b	41.6 ± 2.6	5.6 ± 1.5	13.2 ± 3.0	42.2 ± 53.1
BIO1	1749 ± 77 a	62.6 ± 1.5	23.0 ± 3.4 b	51.0 ± 7.1 b	45.2 ± 2.2	6.0 ± 0.9	12.5 ± 1.5	47.8 ± 3.8
BIO2	1762 ± 106 a	62.0 ± 1.5	32.5 ± 1.7 a	86.0 ± 8.7 a	39.7 ± 2.9	5.5 ± 1.0	10.8 ± 1.2	49.0 ± 6.1
Significance								
Fertilization (N)	ns	0.05	ns	ns	ns	ns	ns	ns
Biostimulant (B)	0.001	ns	0.05	0.05	ns	ns	ns	ns
NxB	ns	ns	ns	ns	ns	ns	ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns = not significant.

TABLE 2 **Technological quality traits (firmness, pH, dry matter -DM, total soluble solids -TSS) of processing tomato fruits as affected by nitrogen fertilization (N100%, full optimal dose; N90%, 10% N reduction; N80%, 20% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control).**

Treatments	Firmness *kg cm^{-2}*	pH	DM *%*	TSS *°Brix*
N80%	1.14 ± 0.03	4.30 ± 0.03	5.13 ± 0.10	4.59 ± 0.09
N90%	1.17 ± 0.03	4.32 ± 0.04	5.30 ± 0.06	4.70 ± 0.12
N100%	1.17 ± 0.05	4.32 ± 0.05	5.24 ± 0.15	4.65 ± 0.11
NB	1.03 ± 0.02 b	4.29 ± 0.04	5.13 ± 0.13	4.26 ± 0.03 b
BIO1	1.23 ± 0.02 a	4.33 ± 0.02	5.19 ± 0.11	4.80 ± 0.06 a
BIO2	1.23 ± 0.02 a	4.32 ± 0.05	5.35 ± 0.09	4.88 ± 0.05 a
Significance				
Fertilization (N)	ns	ns	ns	ns
Biostimulant (B)	0.0001	ns	ns	0.0001
NxB	ns	ns	ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns = not significant.

The yield increase was due to a higher number of fruits per square meter in the N100% and N80% treatments, without statistical difference in mean weight of fruits; similarly, biostimulant-treated plots showed a 24.7% increase in fruit number compared to the control (Table 3).

Regarding the other yield parameters (green and non-marketable), only the green (unripe) fruits were statistically affected by nitrogen fertilization; in particular, the N80% treatment resulted in a significant increase in yield (2.1 vs 0.3 t ha^{-1}), number (12.3 vs 4.3 fruits m^{-2}), and mean weight (16.5 vs 4.7 g fruit^{-1}) compared to N70% and N100% (Table 3).

3.2.2 Physical and chemical parameters of processing tomato berries

Regarding physical quality parameters, also in the second year, neither main effect nor interaction resulted significant, except for TSS that was statistically affected by biostimulants application, with a 10% increase compared to the untreated control (Table 4).

Among the nutraceutical quality traits, only the carotenoids content was significantly affected by the interaction nitrogen dose x biostimulant application (N x B), while the lipophilic antioxidant activity (LAA) and ascorbic acid content were influenced by both experimental factors, and total phenolic content only by biostimulants application (Table 5; Figure 6).

Specifically, for LAA antioxidant activity, among the nitrogen levels, the N80% dose showed a 27% increase compared to the mean value of N70% and N100%, as well as the BIO1 treatment elicited an 11% increase over the mean value of BIO2 and NB (Table 5). The ascorbic acid content was improved by N80% and N100% treatment with a 24.2% mean increase compared to N70%, as well as by biostimulants application that elicited a 15.6% increase over the NB (Table 5). Finally, for total phenolic content, BIO1 showed the highest value with a 12% increase compared to the mean value of the other two treatments (Table 5).

Irrespective of biostimulant application, the carotenoid content reached the highest values in N80% treatments; in particular, in both treatments with the biostimulants (+26% compared to the corresponding NB treatment) that were not different also from N70%-NB (Figure 6). The lowest values were recorded in N70%

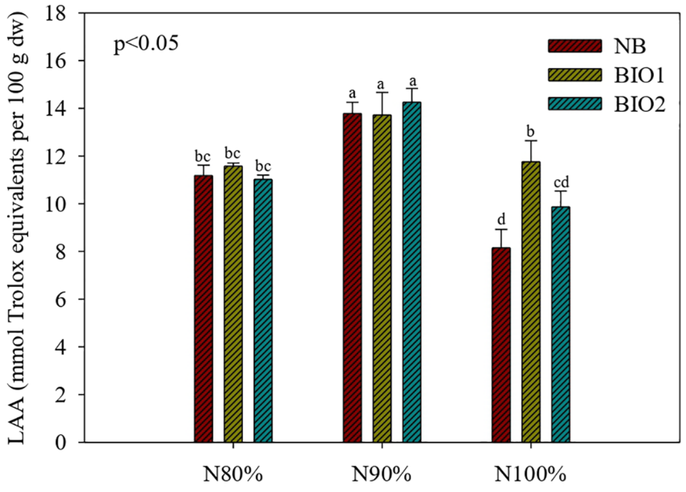

FIGURE 3
Interaction Nitrogen fertilization (N100%, full optimal dose; N90%, 10% N reduction; N80%, 20% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on lipophilic antioxidant activity (LAA assay, expressed as mmol Trolox equivalents per 100 g dw) in processing tomato berries. Values represent the mean ± standard error (n = 3). Different letters within columns indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

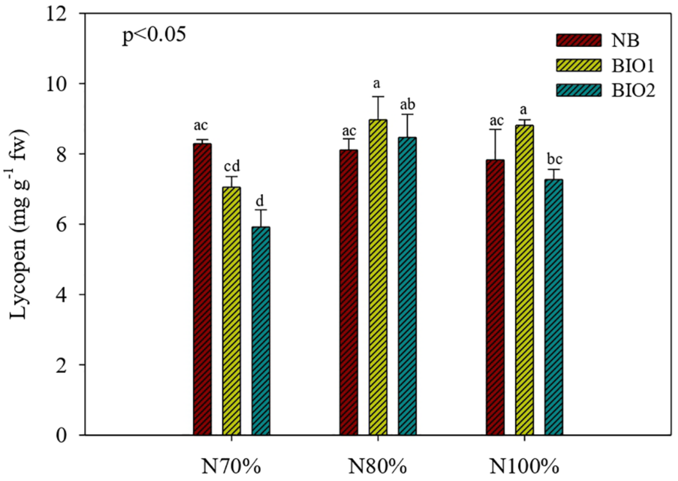

FIGURE 4
Interaction Nitrogen (N100%, full optimal dose; N90%, 10% N reduction; N80%, 20% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on lycopene content (expressed as mg g^{-1} fw) in processing tomato berries. Values represent the mean ± standard error (n = 3). Different letters within columns indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

with both biostimulants which were not different from all N100% treatments and N80%-NB (Figure 6).

3.3 Comparison between the two years

3.3.1 Effect of optimal (N100%) and suboptimal (N80%) N dose with or without biostimulants on processing tomato yield

The results of the treatments common to the two years were analyzed to determine the possible significant effect of the interaction between year, nitrogen dose and biostimulant application. In particular, statistical analysis showed a significant effect of the interaction year x nitrogen dose on marketable, green and non-marketable yield (Figure 7).

In the first year, the optimal N dose elicited a significant 17.8% increase compared to the suboptimal, while in the second year no statistical differences were recorded between the two treatments. Interestingly, the N80% treatment maintained a stable yield across both years, with a mean of 96.1 t ha^{-1} (Figure 7). A marked reduction in green (immature) berries between the two years was also observed. In particular, N100% showed an substantial decrease from 29.49 to 0.32 t ha^{-1} (98%); and N80% recorded a reduction from 26.26 to 2.12 t ha^{-1} (91%). Notably, in the first year there were no significant differences between N80% and N100%, while the suboptimal N dose showed significant higher value of green yield in the second year than N100% (Figure 7).

Finally, as for unmarketable yield, for each year the N treatments were not different between them; the only differences were between the two suboptimal doses in the two years.

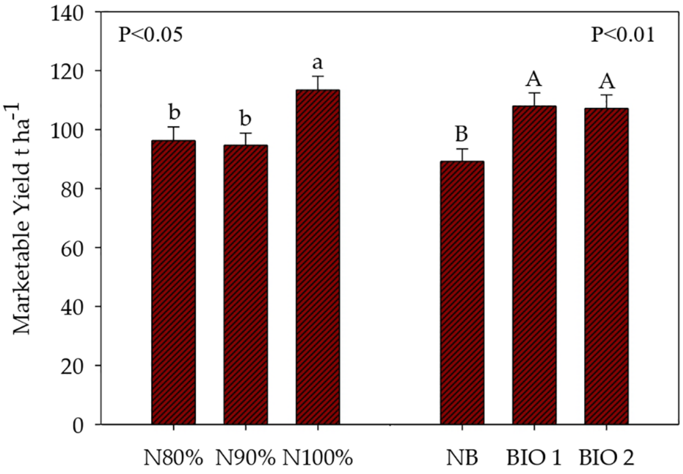

FIGURE 5
Tomato marketable yield as affected by nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction; N70%, 30% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control). Each column indicates the mean value of 3 replicates. Vertical bars indicate standard error; different letters denote significant differences according to the LSD test at the 0.05 significance level.

TABLE 3 **Yield components (marketable, green, and non-marketable number of fruits, mean weight, and yield) of processing tomato as affected by nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction; N70%, 30% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control).**

Treatments	Marketable yield		Green yield			Non-marketable yield	
	n° fruits m^{-2}	*g $fruit^{-1}$*	*t ha^{-1}*	*n° fruits m^{-2}*	*g $fruit^{-1}$*	*t ha^{-1}*	*n° fruits m^{-2}*	*g $fruit^{-1}$*
N70%	136 ± 14.7 b	47.3 ± 0.9	0.3 ± 0.1 b	4.2 ± 1.7 b	4.5 ± 1.7 b	7.6 ± 1.8	20.8 ± 4.7	37.0 ± 1.3
N80%	203 ± 11.5 a	47.4 ± 2.2	2.1 ± 0.5 a	12.3 ± 3.0 a	16.5 ± 3.1 a	11.7 ± 2.5	29.6 ± 5.9	38.3 ± 1.9
N100%	199 ± 10.4 a	49.6 ± 1.4	0.3 ± 0.1 b	4.4 ± 1.3 b	5.0 ± 1.4 b	4.94 ± 0.7	17.0 ± 2.6	33.2 ± 3.9
NB	154 ± 15.0 b	46.27 ± 1.6	0.5 ± 0.3	4.9 ± 1.8	5.9 ± 2.2	9.7 ± 1.7	24.3 ± 3.6	39.2 ± 1.3
BIO1	179 ± 17.0 ab	48.8 ± 2.0	1.0 ± 0.5	8.7 ± 2.9	9.5 ± 2.8	9.32 ± 2.7	28.5 ± 6.5	33.2 ± 3.7
BIO2	205 ± 12.0 a	49.1 ± 0.9	1.2 ± 0.5	7.3 ± 2.6	10.7 ± 3.4	5.27 ± 0.9	14.5 ± 2.6	36.0 ± 2.0
Significance								
Fertilization (N)	0.05	ns	0.01	0.05	0.01	ns	ns	ns
Biostimulant (B)	0.05	ns	ns	ns	ns	ns	ns	ns
NxB	ns	ns	ns	ns	ns	ns	ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns = not significant.

3.3.2 Response of nitrogen-use efficiency to optimal (N100) and suboptimal (N80%) N dose

Although both parameters (PFP_N and NUE) were not statistically affected by experimental factors, their trend is pivotal for interpreting the plants agronomic behavior. In both years, nitrogen-use efficiency indicators showed higher values under the N80% treatment compared to N100%: the increase was 12.8% for PFP_N, as mean value of the two years; less evident for NUE in 2023 with similar values, and about 18% in 2024 (Table 6).

TABLE 4 **Technological quality traits (firmness, pH, dry matter -DM, and total soluble solids -TSS) of processing tomato fruits as affected by nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction; N70%, 30% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control).**

Treatments	Firmness *kg cm^{-2}*	pH	DM *%*	TSS *°Brix*
N70%	0.97 ± 0.03	4.03 ± 0.02	5.33 ± 0.18	4.59 ± 0.09
N80%	1.00 ± 0.02	4.08 ± 0.03	6.13 ± 0.92	4.70 ± 0.12
N100%	0.99 ± 0.04	4.06 ± 0.03	5.72 ± 0.32	4.65 ± 0.11
NB	0.96 ± 0.03	4.04 ± 0.02	5.36 ± 0.37	4.29 ± 0.08 b
BIO1	1.01 ± 0.03	4.04 ± 0.03	5.76 ± 0.93	4.79 ± 0.06 a
BIO2	1.00 ± 0.03	4.08 ± 0.03	6.07 ± 0.12	4.64 ± 0.09 a
Significance				
Fertilization (N)	ns	ns	ns	ns
Biostimulant (B)	ns	ns	ns	0.001
NxB	ns	ns	ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns = not significant.

3.3.3 Effect of optimal (N100%) and suboptimal (N80%) N dose with or without biostimulants on nitrogen content and antioxidant activity and compounds in processing tomato

The nitrogen content ($N{-}NO_3$, and N total; Figure 8) in processing tomato fruits was significantly influenced by the third-degree interaction between N fertilization levels, biostimulant application and year.

The highest nitrate values were observed in the N100%-BIO1 in 2023 (32.22 mg kg^{-1}), which was significantly greater than all other treatments except N80%-BIO1 (Figure 8A). Conversely, N100%-NB had the lowest values of nitrate content in both years. Notably, all treatments with the BIO2 biostimulant, regardless of the nitrogen level applied, maintained relatively stable nitrate content, averaging about 24% lower compared to corresponding BIO1 treatments.

About the total nitrogen content (N-Kjeldahl) in fruits, the highest value was recoded in N100%-NB in the first year and it significantly different from all other treatments (Figure 8B). In contrast, in the second year, the same treatment (N100%-NB) recorded the lowest value statistically similar to the treatments N100%-BIO2 and N80%-BIO2.

As for the antioxidant activities and compounds, the lycopene and ascorbic acid content, and hydrophilic antioxidant activity (HAA) in tomato fruits were significantly affected by the third-degree interaction (Figure 9).

Specifically, the highest lycopene value was recorded in N80%-BIO1 treatment during the second year of experimentation, significantly greater than all other treatments (Figure 9A). On average, in 2024, the lycopene content was higher than in 2023, irrespective of N dose and biostimulant application with overall mean values of 12.65 and 7.53 mg g^{-1} fw, respectively. In the first year, the differences between the treatments were less marked, although the treatments with BIO2 always showed lower values, also if not different from all other 2023 treatments (Figure 9A).

TABLE 5 **Antioxidant activity (HAA: hydrophilic; LAA: lypophilic), total phenolic content and lycopene of processing tomato berries as affected by nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction; N70%, 30% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control).**

Treatments	HAA *mMol Ascorbic acid equ. 100 g^{-1} dw*	LAA *mMol Trolox equ. 100 g^{-1} dw*	Total Phenols *mg gallic acid g^{-1} dw*	Lycopene *mg 100 g^{-1} fw*	Ascorbic acid *mg g^{-1} fw*
N70%	9.27 ± 0.17	12.86 ± 0.86 b	2.26 ± 0.06	12.00 ± 0.83	31.43 ± 2.35 b
N80%	9.52 ± 0.15	16.38 ± 0.51 a	2.38 ± 0.09	13.79 ± 0.75	39.54 ± 3.06 a
N100%	9.82 ± 0.20	12.81 ± 0.70 b	2.3 ± 0.08	11.51 ± 0.41	38.55 ± 2.27 a
NB	9.59 ± 0.15	13.70 ± 0.73 b	2.29 ± 0.04 ab	12.36 ± 0.36	33.06 ± 3.60 b
BIO1	9.49 ± 0.19	15.01 ± 0.59 a	2.48 ± 0.09 a	12.40 ± 1.20	38.00 ± 1.48 a
BIO2	9.53 ± 0.22	13.33 ± 0.67 b	2.18 ± 0.05 b	12.54 ± 0.43	38.46 ± 2.75 a
Significance					
Fertilization (N)	ns	0.01	ns	ns	0.05
Biostimulant (B)	ns	0.01	0.05	ns	0.05
NxB	ns	ns	ns	ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns = not significant.

Like that observed for lycopene, also for ascorbic acid content, there was a marked seasonal influence, indicating a strong year-to-year effect, especially evident in the second experimental year, in which the highest values were recorded (39.05 vs 21.38 mg g^{-1} fw of 2023; Figure 9B).

In particular, the N80%-BIO2 treatment in the second year exhibited the highest value, significantly different from all other treatments except for N100%-NB in 2024. Interestingly, under the N optimal dose, the application of biostimulants reduced the ascorbic acid content in both years compared to NB treatments, while the trend was almost always opposite under suboptimal dose (Figure 9B).

Interestingly, treatments with a moderate (20%) reduction in nitrogen fertilization showed a slight increase in ascorbic acid content compared to the full nitrogen dose. In 2023, the mean values were 21.68 and 21.07 mg g^{-1} fw for N80% and N100%, respectively (+2.9%), while in 2024 the difference was similarly limited (39.54 vs 38.56 mg g^{-1} fw; +2.5%).

Like previous analysis parameters, also for HAA a clear seasonal effect emerged, with values generally higher in the second experimental year compared to the first (9.67 vs. 8.17 mMol Ascorbic acid equ. 100 g^{-1} dw, respectively; Figure 9C). Specifically, in the second year, the only difference was recorded between N80% and N100% treated with BIO2, and this last one showing the highest value. Conversely, during the first year, the lowest values were observed in the two N100% treatments treated with biostimulants (Figure 9C).

Finally, a significant interaction between year and nitrogen dose was observed for lipophilic antioxidant activity (LAA) and carotenoids (Figure 10), while, for phenolic compounds, the interaction between year and biostimulant application strategy was significant (Figure 11).

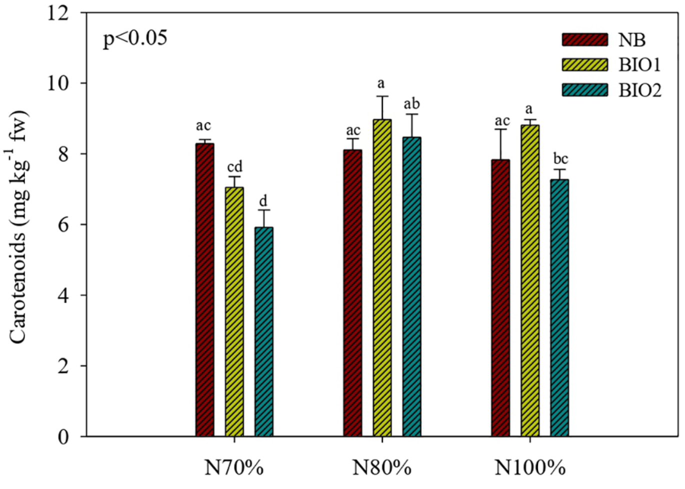

FIGURE 6
Interaction Nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction; N70%, 30% N reduction), and biostimulant application (BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on carotenoids content (expressed as mg kg^{-1} fw) in processing tomato fruits. Values represent the mean ± standard error (n = 3). Different letters within columns indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

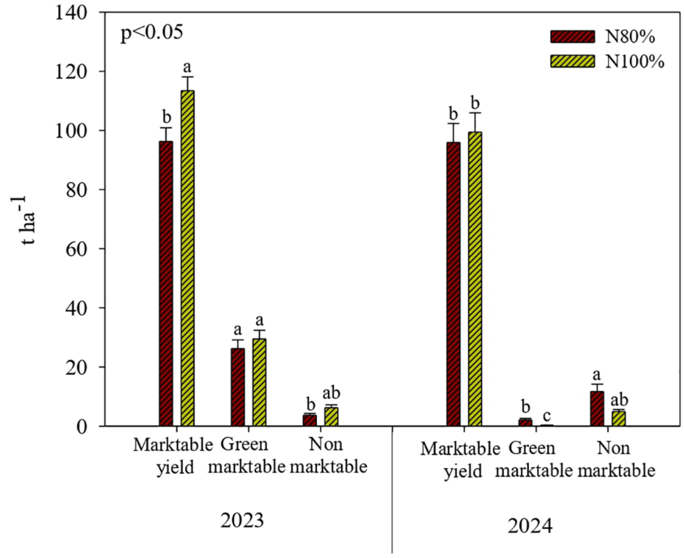

FIGURE 7
Effect of the interaction Year (2023, and 2024) × Nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction) on marketable yield, green, and non-marketable yield of processing tomato. Each bar represents the mean ± standard error of three replicates (n = 3). Per each parameter, different letters indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

For LAA a clear seasonal effect is observed, with overall higher values recorded in 2024 compared to 2023 (14.60 vs 10.60 mMol Trolox equ. 100 g^{-1} dw) (Figure 10A). The highest value was recorded in 2024 under the N80% treatment, while the lowest was in 2023 under the N100% treatment (16.38 and 9.93 mMol Trolox equ. 100 g^{-1} dw, respectively).

Carotenoids content showed a similar trend with higher values in 2024 than 2023 (0.20 vs 0.13 mg g^{-1} fw). The highest content was recorded in 2024 with the N80% treatment (0.22 mg g^{-1} fw), which is significantly higher than all other combinations. There were no significant differences between the two nitrogen levels in 2023, both with lower values (about 0.13 mg g^{-1} fw) (Figure 10B).

The total phenolic content in tomato processing fruits as influenced by the interaction between year and biostimulant application (Figure 11). In 2023, a different trend compared to other antioxidant compounds was observed, with the control treatment without biostimulants (NB) showing the highest phenolic content (2.9 mg gallic acid g^{-1} dw), significantly greater than both biostimulant treatments (BIO1 and BIO2), which had similar mean values of approximately 2.6 mg g^{-1} dw. In 2024, all treatments exhibited lower average values (2.27 mg gallic acid g^{-1} dw), with no significant differences among treatments, regardless of biostimulant application (Figure 11).

TABLE 6 **Indicators of nitrogen-use efficiency: partial factor productivity (PFP) and the supplementary nitrogen use efficiency that includes soil mineral N reported for the two common treatments (N100%, the optimal dose, and N80%, the reduction of 20%).**

Years	Treatments	PFP$_N$ *kg tomato kg^{-1}N*	NUE *t kg^{-1}*
2023	N80%	752.08 ± 51.9	0.65 ± 0.04
	N100%	708.92 ± 44.2	0.63 ± 0.04
2024	N80%	749.47 ± 49.0	0.65 ± 0.04
	N100%	621.42 ± 65.6	0.55 ± 0.06
Significance			
Years		ns	ns
Nitrogen		ns	ns
Y x N		ns	ns

Data are reported as mean ± standard error (n=3). Different letters within columns indicate significant differences according to the LSD test at the 0.05 significance level. ns, not significant.

4 Discussion

In line with EU agricultural policies to promote more sustainable agriculture by setting a 20% reduction in fertilizer use by 2030 (Farm to Fork, F2F:; European Commission, 2019), the current research was aimed at assessing whether seaweed-based biostimulants were able to mitigate the potential negative effects of non-optimal nitrogen doses (N90%, N80%, and N70%) on the productivity and quality of processing tomatoes. Our results indicate that reducing nitrogen supply, particularly to the N80% level, decreased marketable yield compared with the optimal N100% level. However, the application of algae-based biostimulants partially mitigated this reduction, suggesting that these products could help sustain productivity under reduced nitrogen inputs.

The yield response observed in our two-year field trial is very promising, although no significant interaction between nitrogen level and biostimulant application was found for yield or related agronomic parameters, in either year of the trial; this suggests largely independent main effects, within the range tested. In both years, regardless of the level of nitrogen reduction applied, yield decreased compared to the optimal N treatment (–16% in 2023 and –30% in 2024). This more pronounced yield penalty in 2024 could be partially attributed to the overall drier conditions experienced

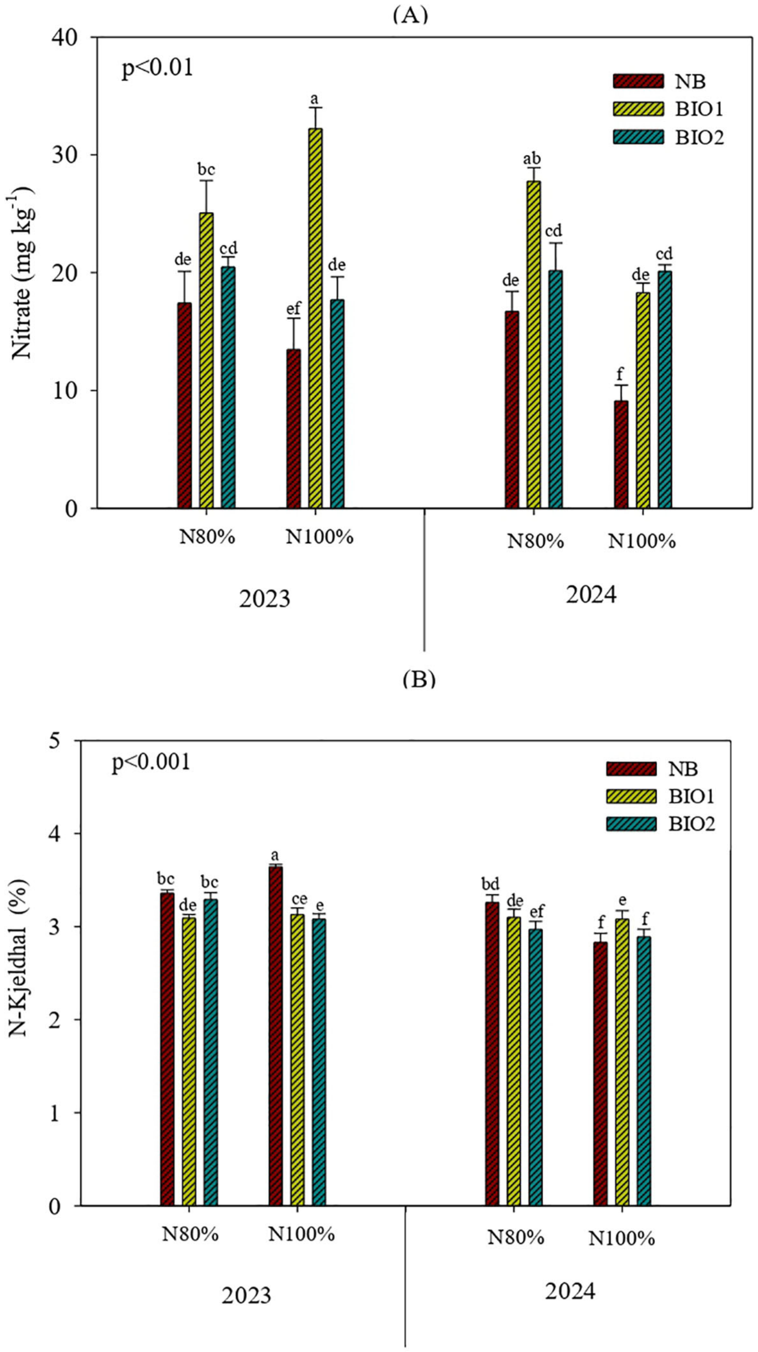

FIGURE 8
Effect of the third-degree interaction Year (2023, and 2024) x Nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction) x biostimulant application BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on nitrate ($N{-}NO_3$) **(A)** and N-Kjeldahl content **(B)** in processing tomato fruits. Each bar represents the mean ± standard error (n = 3). Different letters indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

during the second growing season (70.8 mm of total rainfall compared to 103.0 mm in 2023, Figure 1). This likely may have limited soil nitrogen mineralization and availability, particularly under reduced nitrogen supply, thereby exacerbating the yield decline observed at lower N rates.

These findings suggest that climatic variability, likely interacted with nitrogen availability to influence crop productivity across years. However, the application of biostimulants led to a substantial increase in marketable yield in both years (+21% and +36%, respectively), confirming their ability to support crop productivity even under sub-optimal fertilization.

Our results are consistent with previous studies showing that biostimulants can enhance yield performance even when nitrogen input is reduced. Ganugi et al. (2023) reported beneficial effects of microbial biostimulants (arbuscular mycorrhizal fungi and *Trichoderma*) enhanced fruit quality and metabolic responses in processing tomatoes grown under both conventional and reduced nitrogen fertilization regimes in Northern Italy.

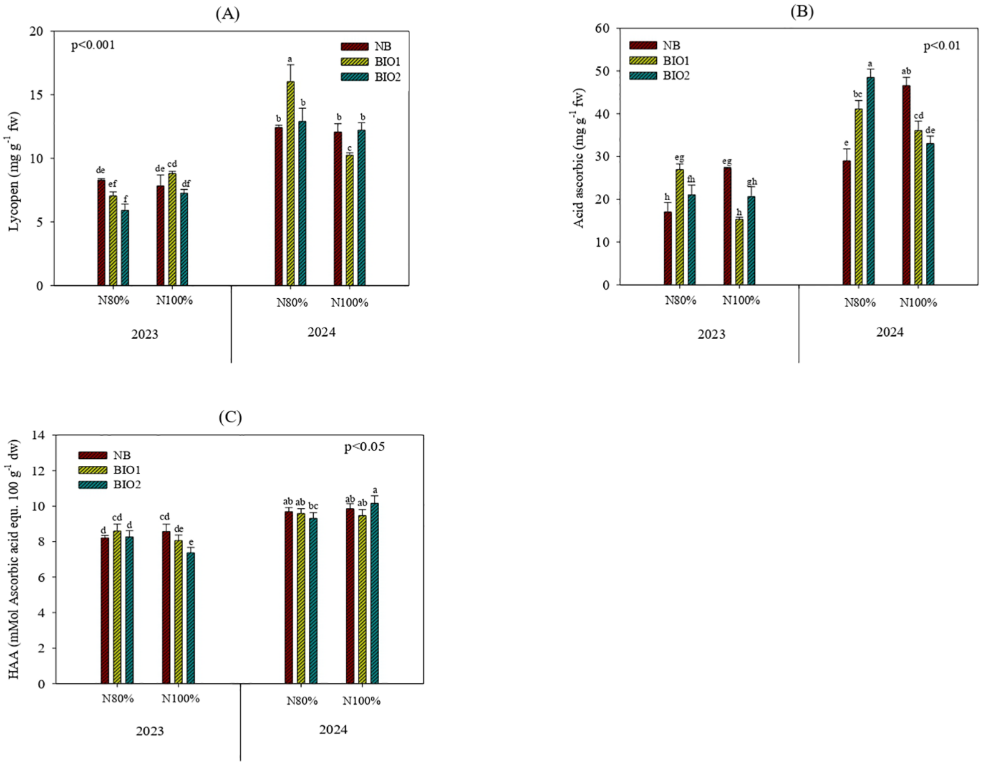

FIGURE 9
Effect of the third-degree interaction Year (2023, and 2024) × Nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction) x biostimulant application BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on lycopene **(A)**, acid ascorbic **(B)**, and hydrophilic antioxidant activity **(C)** content in processing tomato berries. Each bar represents the mean ± standard error (n = 3). Different letters indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

Similarly, Ntanasi et al. (2024) reported that seaweed extract and a microbial biostimulant improved yield and stress tolerance in two Greek tomato landraces under moderate salinity, although the response was genotype-dependent. Overall, these studies observe that biostimulants can enhance tomato performance under different conditions, but their effectiveness depends on the specific agronomic and environmental context.

In our trial, yield gains were mainly associated with a higher number of fruits per square meter, in line with findings by Ntanasi et al. (2024), who reported that biostimulant application enhanced fruit set under suboptimal conditions without affecting fruit weight. A similar response was described by Saraswathi and Praneetha (2013), using *Panchakavya*, a livestock-derived biostimulant, prepared from cow dung, urine, milk, curd, and ghee. This formulation contains macro- and micronutrients and bioactive compounds (including phytohormones, phenylacetic and benzoic acids), which significantly increased fruit number in tomato without altering fruit size. Similarly, Carmody et al. (2020) showed that *A. nodosum*-based biostimulants enhanced fruit set under heat stress without altering fruit weight. In the same direction, Boutahiri et al. (2024) concluded that biostimulants generally act by supporting fruit set and stress resilience rather than promoting fruit enlargement. Altogether, these results support the hypothesis that biostimulants primarily promote flowering and fruit set rather than fruit enlargement, regardless of their origin.

For both physical and chemical quality parameters, no significant interaction was observed between nitrogen level and biostimulant application, confirming that the effects of the two factors were also independent in terms of fruit quality. In addition, the application of biostimulants has significantly improved several technological and visual quality traits of processing tomato fruits. In particular, firmness (*kg* cm^{-2}) and total soluble solids (°*Brix*) were consistently increased by both BIO1 and BIO2. Specifically, TSS increased by 14% in the first year and by 10% in the second year, regardless of the biostimulant used. This improve in TSS and firmness suggests a synergistic effect of the biostimulants on fruits physiology. Moreover, the difference recorded in the two year may be linked to the stronger environmental pressure in the first year: when irregular rainfall distribution and higher temperature (36.5 °C vs. 35.4 °C in 2024) peaks likely increased evaporative demand despite the higher total rainfall recorded in 2023 (103.0 mm vs. 70.8 mm in 2024). According to Beckles (2012), temperatures exceeding 30 °C during fruit ripening increased fruit transpiration, which directly may promote higher TSS accumulation.

These results align with the findings of Ganugi et al. (2023), who reported that seaweed- and microbial-based biostimulants improved sugar accumulation in tomato fruits. Similarly, Sidhu (2017) suggested that biostimulants can activate primary metabolism and sugar partitioning, stimulating carbon assimilation and translocation. Fruit firmness was significantly improved in biostimulant-treated, but only in the first year, a result consistent with the hypothesis that seasonal variability strongly modulates the effectiveness of biostimulants. As reported in Figure 1, the two growing seasons were characterized by distinct climatic patterns. In

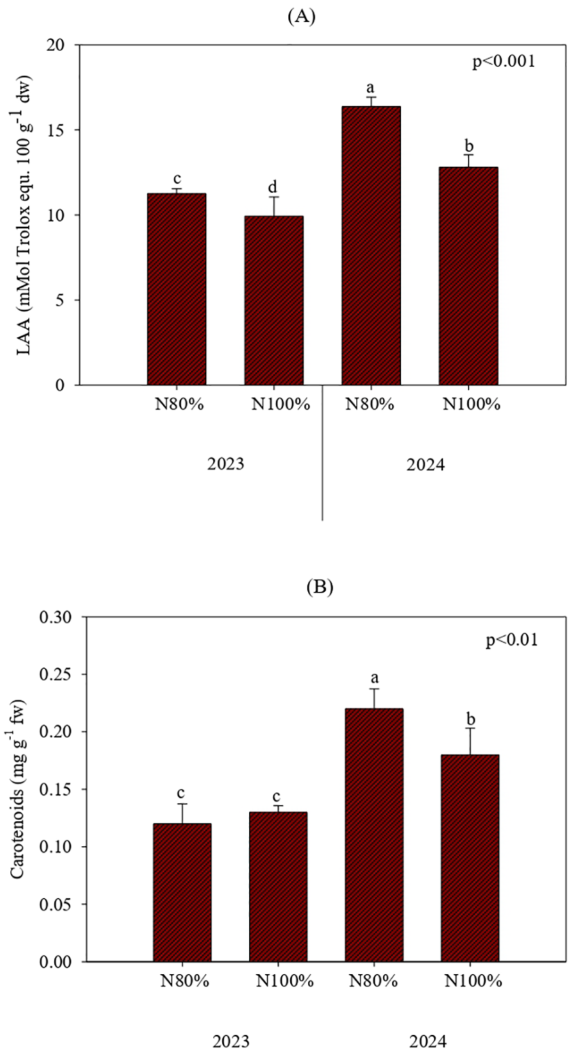

FIGURE 10
Interaction between Year (2023, and 2024) × Nitrogen fertilization (N100%, full optimal dose; N80%, 20% N reduction) on lipophilic antioxidant activity **(A)** and carotenoids **(B)** content in processing tomato berries. Each bar represents the mean ± standard error (n = 3). Different letters indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

2023 experienced irregular rainfall with prolonged dry spells and higher peak temperatures (up to 36.5 °C) during the ripening stage (July-August), while 2024 showed an overall drier but more evenly distributed rainfall pattern with a more gradual temperature trend. Fluctuations in water availability during the ripening stage probably affected the physical quality of the fruit, potentially enhancing its firmness under moderate stress conditions. However, the more gradual and consistent temperature pattern observed in 2024 may have reduced the intensity of these stress-related responses, thereby reducing the firmness-enhancing effect of biostimulants. In fact, Fusco et al. (2025) observed that the efficacy of plant-based biostimulants on yield and quality was largely overshadowed by interannual differences in climatic conditions, with the annual variation exerting a stronger effect than the treatments themselves. Leyva et al. (2013) reached similar findings, and they associated the improved fruit firmness with enhanced fruit structure and water-use efficiency in screenhouse-grown tomatoes under moderate environmental control. Additionally, Gu et al. (2023) linked firmness enhancement to physiological responses to nutrient availability and abiotic stress mitigation.

The pH of tomato juice was only slightly affected by treatments, with overall average values of 4.31 in 2023 and 4.05 in 2024, indicating a general trend toward higher acidity in the second year. These values fall within the recommended range for safe processing, as pH values below 4.5 are considered desirable for inhibiting microbial growth in tomato-based products (Tigist et al., 2013).

The application of biostimulants significantly influenced the nutritional quality of tomato fruits, particularly with respect to health-promoting compounds such as lycopene (*mg 100* g^{-1} *fw*) and ascorbic acid (*mg* g^{-1} *fw*). For lycopene, the most effective treatment in the first year was the combination of N90% with biostimulant application (both BIO1 and BIO2), while in the second year, the highest accumulation was recorded under the N80% regime always combined with biostimulant application.

For ascorbic acid, in 2023, the ascorbic acid was not affected by the experimental factors, instead in 2024, under both the nitrogen doses (N80% and N100%) combined with biostimulant treatment, the highest values were recorded. Additionally, BIO1 was particularly effective in enhancing total phenolic content in the second year. Overall, these findings suggest that the response of lycopene to nitrogen availability is strongly influenced by seasonal conditions. Lycopene accumulation in tomato fruits can be strongly influenced by environmental conditions and microclimatic factors. Excessive solar radiation and high temperatures have been shown to inhibit lycopene biosynthesis or promote its conversion into β-carotene (Leyva et al., 2013), whereas moderate environmental stress may stimulate carotenoid accumulation, while severe stress tends to reduce it (Paradiso et al., 2024). Our meteorological data strongly support this dynamic: the prolonged dry spells and higher maximum temperatures (36.5 °C) recorded in the late season of 2023 likely acted as a severe stress, impairing lycopene biosynthesis. Conversely, the more stable temperature trend and rainfall of 2024 provided the moderate stress conditions known to stimulate carotenoid accumulation. Our findings are in line with those of Hernández et al. (2020), who showed that moderate reductions in nitrogen supply can stimulate carotenoid biosynthesis, whereas more severe limitations tend to suppress it. Likewise, Benard et al. (2009) emphasized that the effect of nitrogen availability on lycopene accumulation is relatively minor compared with the strong influence of seasonal conditions. This was also observed by Fusco et al. (2025), who reported a marked year-to-year variation in lycopene content, independent of biostimulant application, underscoring the predominant role of environmental factors in regulating carotenoid synthesis. Our findings are consistent with those reported by Quintarelli et al. (2024), who also observed a clear increase in lycopene and β-carotene content in the second year of their field trial. In their case, the combined application of microbial biofertilizers and algae-based biostimulants was associated with enhanced fruit quality, especially through enhanced carotenoid accumulation.

Sidhu (2017) reported that darker-colored tomato fruits exhibited higher levels of lycopene and β–carotene, suggesting that fruit

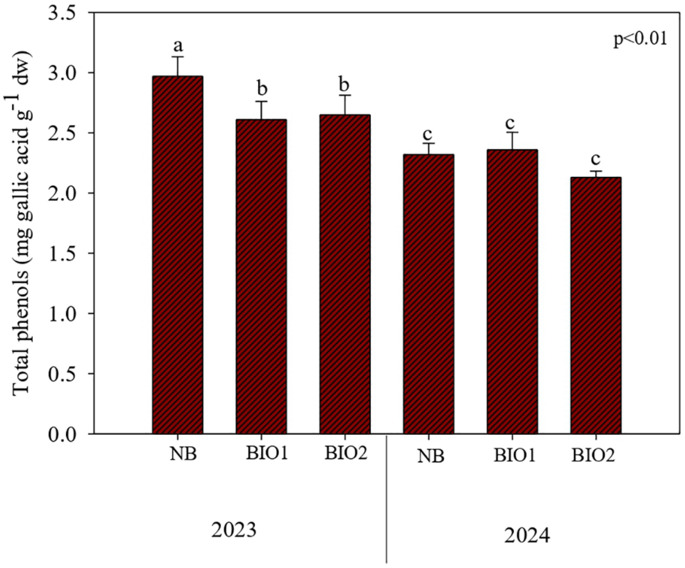

FIGURE 11
Interaction between Year (2023, and 2024) × Biostimulant application BIO1, Auximar + Pro Calcio; BIO2, Enerleaf + Pentacalcium; NB, untreated control) on total phenols content in processing tomato berries. Each bar represents the mean ± standard error (n = 3). Different letters indicate statistically significant differences among treatments according to the LSD test at the 0.05 significance level.

pigmentation may be a stronger determinant of carotenoid content. This is consistent with our findings, as fruits harvested in the second year showed significantly higher a* values, indicating more intense red pigmentation, regardless of biostimulant treatment, and also recorded a markedly higher lycopene content in the second year compared to the first year (12.4 vs 7.80 mg 100 g^{-1} fw).

Second year, both biostimulant applications resulted in higher ascorbic acid content. Conversely, hydrophilic antioxidant activity (HAA) remained stable across all treatments and years. This different response between HAA and LAA may be related to the distinct nature of the compounds involved. Lipophilic antioxidants, such as carotenoids, are directly associated with membrane protection and respond more to environmental stress conditions (Dumas et al., 2003), whereas hydrophilic antioxidants, including ascorbic acid and phenolic compounds, are often more tightly regulated and less sensitive to moderate variations in growing conditions (Dumas et al., 2003).

This effect is consistent with results obtained by Paradiso et al. (2024), who observed a significant increase in ascorbic acid levels in cherry tomato following the application of biostimulants in combination with nitrogen reduction. Although lycopene and ascorbic acid are biologically active compounds that contribute significantly to the nutritional value of tomato, their contribution to the measured total antioxidant activity is limited in analysis.

LAA was significantly influenced by nitrogen level and, to a lesser extent, by biostimulant application, with the highest values recorded under N90% (I year) and under N80% and BIO1 treatment (II year). These results may reflect a moderate stress-induced stimulation of antioxidant pathways, potentially involving lipophilic compounds such as carotenoids, as also suggested by Cozzolino et al. (2021), who reported increased LAA following foliar application of plant-based biostimulants. Conversely, hydrophilic antioxidant activity (HAA) remained stable across all treatments and years.

Our findings are partially in contrast with those reported in the literature. Paradiso et al. (2024) observed a different trend, with significant increases in hydrophilic antioxidant activity (HAA) following biostimulant application, while lipophilic antioxidant activity (LAA) remained stable across trial. Similarly, Ntanasi et al. (2024) found that the impact of biostimulants such as seaweed extracts and biofertilizer inputs on nutraceutical quality, particularly on HAA, was highly variable and strongly dependent on genotype and environmental stress levels. These findings support the hypothesis that hydrophilic and lipophilic antioxidant systems may require a threshold level of physiological stress or specific cultivar sensitivity to be effectively activated.

Our results about total phenolic content and carotenoids partially reflect what has been reported in previous studies. In our trial, a slight increase in total phenolics was observed under the BIO1 treatment, but only in the second year, while no significant differences emerged in the first year. Similarly, carotenoid content increased mainly at the N80% nitrogen level, especially when combined with biostimulant application, again only in the second year.

These outcomes are consistent with findings by Cozzolino et al. (2021), who reported no significant effect of plant-based biostimulants on total phenolic content, and by Sidhu (2017), who observed a cultivar-dependent increase in carotenoid levels, particularly lycopene and β-carotene, following the application of a seaweed-derived biostimulant. Leyva et al. (2013) further highlighted the strong influence of genotype and environmental conditions, showing variable results across seasons. They demonstrated that radiation and temperature can either stimulate or inhibit the biosynthesis of phenolic compounds and carotenoids, depending on their intensity. The consistent enhancement of these antioxidant compounds observed primarily in 2024 suggests that the steady, moderate water deficit of the second season (70.8 mm) created the optimal physiological baseline for biostimulants to trigger secondary metabolism. Altogether, these findings support the notion that the effectiveness of biostimulants in modulating antioxidant secondary metabolites is highly context-dependent and may require specific thresholds of abiotic stress or cultivar responsiveness to be fully expressed. Moreover, our results highlight the key role of environmental factors, such as temperature, solar radiation, and soil water availability, in shaping the biosynthetic potential of bioactive compounds in tomato, independently of agronomic treatments.

The trend of efficiency indicators (PFP_N, and the NUE), always showing higher values under N80% compared to N100%, suggesting a more efficient conversion of nitrogen inputs into marketable yield. Probably, the slight nitrogen deficiency stimulates plants to use it better, for example reducing the production of plant biomass and increasing fruit production. This behavior has also been observed in other studies on processing tomatoes and field crops under Mediterranean conditions.

Jalpa et al. (2020) reported that nitrogen-use efficiency declines as fertilizer rates increase, and that whole-plant N accumulation tends to plateau once moderate N levels are reached.

A similar trend emerges in Zhao et al. (2025), who reported a sharp decline in PFP_N at higher N rates. The authors quantified this decrease as an efficiency loss of roughly 30%, showing that increasing nitrogen beyond crop demand does not improve yield and instead lowers nitrogen-use efficiency. In their study, across two growing seasons, PFP_N values dropped from 450–630 kg kg^{-1} at 120 kg N ha^{-1} to 240–300 kg kg^{-1} at 240 kg N ha^{-1}, and further to 150–200 kg kg^{-1} at 360 kg N ha^{-1}.

From an environmental perspective, increasing nitrogen-use efficiency by reducing fertilizer inputs is particularly relevant, as excessive nitrogen supply is closely associated with greater risks of nitrate leaching and nitrous oxide (N_2O) emissions.

During the two-year trial, nitrates and total nitrogen showed different trends, which were also modulated by the application of biostimulants. In particular, BIO1 influenced nitrate accumulation in processing tomato fruits: in the first year in combination with the optimal nitrogen dose, and in the second year with the 20% reduced dose (32.22 and 27.74 mg kg^{-1} dw, respectively). Similar findings were reported by Cozzolino et al., which found all tested biostimulants increased fruit total nitrogen compared to the control, although variation in nitrogen doses was not considered. In our case, a more complex picture emerges due to a third-order interaction among year, nitrogen dose, and biostimulant, which plays a decisive role. Indeed, in the first year the trends of nitrates and total nitrogen were very similar, with the highest contents recorded at the optimal nitrogen dose (N100%). In the second year, however, the 20% reduced nitrogen dose(N80%), particularly when combined with biostimulant application, irrespective of product type, was associated with the highest values. Paradiso et al. likewise reported an interactive effect between biostimulants and nitrogen dose on fruit total nitrogen, confirming that the response to biostimulants can vary with nitrogen availability.

5 Conclusion

Our two-year field study indicates that algae-based biostimulants can be a solid tool to pursue the EU Farm to Fork (F2F) strategy's goal of reducing fertilizer inputs while safeguarding the performance of processing tomato. Although nitrogen reduction consistently decreased yield compared with the treatment with the optimal nitrogen dose, the application of biostimulants increased marketable yield in both years (+21% and +36%), mainly due to a higher number of fruits rather than greater fruit weight, underlining the contribution of biostimulants in sustaining fruit set. The treatments also enhanced several quality traits: fruits from biostimulant-treated plots were firmer, had higher soluble solids, and contained more health-promoting compounds such as lycopene and ascorbic acid. These benefits were obtained without affecting juice acidity, as pH values remained well within the safety range required for tomato processing.

The absence of interaction between nitrogen and biostimulant for yield and for most physical and qualitative characteristics of the fruits suggests that the main effects are independent. However, significant third-order interaction (year × nitrogen dose × biostimulant) were detected not only for fruit nitrogen but also for nutraceutical compounds (lycopene, ascorbic acid, carotenoids, and antioxidant activity) underscore a strong context-dependency. These results suggest that the effectiveness of biostimulants under reduced nitrogen conditions is deeply modulated by seasonal climatic variability. Therefore, when applying these products, it is crucial to monitor environmental factors (temperature and rainfall distribution).In practice, combining biostimulants with a 20% nitrogen reduction emerges as a balanced option to reduce nitrogen inputs. Stronger reductions (≥30%) are associated with higher marketable yield loss. This strategy is supported by the nitrogen-use efficiency indicators, which show that such a reduction enhances the crop's ability to convert nitrogen inputs into yield. Overall, our findings reinforce the idea that algae-based biostimulants act mainly as metabolic modulators rather than simple yield boosters. At the same time, their impact is far from uniform, as environmental variability strongly determines the outcome, as already highlighted in previous studies. This suggests that combining a moderate reduction in nitrogen with algae-based products offers a way to sustain yield while enriching nutritional quality, but its success will ultimately depend on how well environmental variability is considered in the design of sustainable agronomic practices.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

MP: Data curation, Formal analysis, Resources, Writing – original draft, Investigation, Writing – review & editing. ID: Methodology, Validation, Writing – review & editing, Conceptualization. LO: Methodology, Software, Data curation, Writing – review & editing. EC: Methodology, Conceptualization, Visualization, Writing – original draft. MS: Data curation, Software, Writing – review & editing. MM: Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

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