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Combining seaweed extract from *Ascophyllum nodosum* with nutrients enhances stalk yield when applied in the dry season during sugarcane development

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ABSTRACT

Sugarcane (*Saccharum officinarum*) is an important crop grown for the production of sugar, ethanol, and other by-products. The cultivation cycle from planting until harvesting usually takes at least a year, during which the plant is exposed to various environmental stress conditions. To improve tolerance to such stressful conditions, sugarcane plants can be treated with biostimulants, such as extracts from the brown seaweed *Ascophyllum nodosum*. Whether the application of such a biostimulant, alone or in combination with nutrients, improves stress resistance in sugarcane is unknown. In this study, we conducted two field trials: one in a cane-plant field and one in a ratoon field, with treatments applied to leaves during the fall (before the dry season) or spring (positive water balance in the soil). The treatments consisted of foliar application of nitrogen, potassium, boron, and zinc, provided alone (nutrient treatment) or together with *A. nodosum* extract (biostimulant treatment) and a control treatment (without foliar application). Overall, foliar application of biostimulant increased sugarcane stalk yield by 24 Mg ha^{-1} and sucrose yield by 4 Mg Pol ha^{-1} compared to nutrient treatment. In addition, agronomic efficiency and nutrient accumulation were higher in plants under biostimulant treatment compared to nutrient and control treatments. Therefore, the foliar application of *A. nodosum* extracts to sugarcane leaves is a promising strategy for improving drought tolerance, yield, and the nutritional status of the stalk.

1. Introduction

Climate change negatively affects agricultural production by decreasing plant yield due to more frequent, extreme weather events as well as severe dry seasons (Mubarik et al., 2021). As a consequence, the price of food has risen, particularly in developing nations (Mphande et al., 2020). To move away from fossil fuels, it is important to look for alternative, cleaner sources of energy. For example, ethanol and cogenerated energy can be produced from residues generated by the sugarcane (*Saccharum officinarum*) industry. A single sugarcane plant can serve as a source of both heat and electricity (Canabarro et al., 2023). However, sugar, ethanol, and energy production are directly influenced by long dry seasons, which decrease sugarcane development and yield (Nguyen et al., 2019; Anggraeni et al., 2022). Adverse weather conditions during the growing season result in an average yield loss of greater than 50 %, with drought stress being the major limiting factor for agriculture, food safety, and the global economy (Goñi et al., 2018; Elansary et al., 2019; Shukla et al., 2019).

Drought stress negatively affects numerous biochemical reactions and physiological responses in plants involved in nutrient homeostasis, photosynthesis, redox balance, energy production, photorespiration, water balance, and lipid metabolism (Nguyen et al., 2019; Kapoor et al.,

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2020; Rahman et al., 2022). To limit the water lost by transpiration, plants close their stomata under drought stress, concomitantly limiting their CO_2 uptake and fixation potential. Closed stomata directly affect photosynthesis and increase the production of reactive oxygen species (ROS) (Ali et al., 2020; Gupta, 2020), which leads to the oxidation of the lipid bilayers in the cell, therefore contributing to senescence and cell death (Tavanti et al., 2021).

The application of a biostimulant to leaves is one strategy employed to minimize the physiological problems arising from environmental stress, thereby increasing yield and enhancing final product quality (Du Jardin, 2012; Elansary et al., 2019). Biostimulants are sustainable, environmentally friendly, cost-effective products used to improve agricultural production (Calvo et al., 2014; Kawalekar, 2013; Shukla et al., 2019; Van Oosten et al., 2017; Yakhin et al., 2017). The concept of biostimulants has been around since 1933 but has received more attention in recent years as a possible solution to help mitigate the effects of the changing climate on agriculture (Yakhin et al., 2017; Shukla et al., 2019). According to the European Biostimulants Industry Council (EBIC), plant biostimulants contain substances and/or microorganisms that, when applied to plants or the rhizosphere, stimulate natural processes to benefit nutrient uptake, nutrient efficiency, abiotic stress tolerance, and crop quality (Shukla et al., 2019). Many substances act as biostimulants, such as seaweed extracts, humic substances, inorganic elements, biopolymers, and amino acids (Du Jardin, 2012).

Seaweeds are multicellular, macroscopic organisms found in marine ecosystems that are a plentiful source of polysaccharides, polyunsaturated fatty acids, enzymes, and bioactive peptides (Ahmadi et al., 2015; Courtois, 2009; Shukla et al., 2016, 2019). The most widely used plant biostimulant is the brown seaweed *Ascophyllum nodosum* (Shukla et al., 2019; Ugarte and Sharp, 2012). *A. nodosum* produces compounds not generated by terrestrial organisms that are thought to be vital for its survival in extremely stressful environments (Shukla et al., 2019, 2016). *A. nodosum* extract is a natural product that stimulates cytokinin production in plants (Wally et al., 2013) and may also help maintain turgor pressure in cells and a negative water potential, improve photosynthesis and the contents of photosynthetic pigments, and promote plant growth and defense responses (Mubarik et al., 2021).

The application of *A. nodosum* extract has been used to improve stress tolerance under controlled conditions in Arabidopsis (*Arabidopsis thaliana*), soybean (*Glycine* max), bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), spinach (*Spinacia oleracea*), sweet orange (*Citrus sinensis*), *Spiraea nipponica*, and lemonwood (*Pittosporum eugenioides*) (Carvalho et al., 2018; Elansary et al., 2016; Goñi et al., 2018; Santaniello et al., 2017). However, few studies have evaluated how sugarcane responds to the application of *A. nodosum* extract at different times under field conditions (Jacomassi et al., 2022). Such an investigation is important because sugarcane is a semi-perennial crop with a long growing season of approximately 18 months in cane-plant fields (using sugarcane cuttings) and ~12 months in ratoon fields (plants grown from underground stalks known as ratoons); during this long growth period, plants are exposed to extreme weather events.

In addition to their protective effects against stress, biostimulants are also a source of plant nutrients (Garcia-Gonzalez and Sommerfeld, 2016). Biostimulant treatment has been associated with greater nutrient uptake, higher biomass accumulation, and increased crop yields (Shaaban, 2001; Faheed and Abd-el Fattah, 2008). Nutrients play important roles in plant physiology, such as by increasing photosynthesis under a low supply of nitrogen (N) (Lyu et al., 2022) or potassium (K) (Wasaya et al., 2021). Likewise, the application of boron (B) and zinc (Zn) strengthens the cell wall and activates antioxidant enzymes to minimize the deleterious effects of ROS (Tavanti et al., 2021).

In this study, we investigated the following hypotheses: (1) Foliar application of a formulation containing extract from the seaweed *A. nodosum* combined with nutrients will enhance stalk yield and agronomic efficiency regardless of the method used for growth (cane-plant or sugarcane ratoon field). (2) The timing (in the fall or spring) of foliar application of *A. nodosum* extract will determine its effect on sugarcane yield. Accordingly, we performed field experiments in a cane-plant field and a sugarcane ratoon field using foliar application of nutrients alone or in combination with *A. nodosum* extract in the fall or spring season, before or after the dry season, respectively, in Southeast Brazil. Our findings indicate that the foliar application of *A. nodosum* extract as a biostimulant enhances stress tolerance in sugarcane, thereby improving the yield and technological quality of raw materials in a sustainable, environmentally friendly manner.

2. Materials and methods

2.1. Study sites

Two field trials were conducted in commercial sugarcane (*Saccharum officinarum*) fields in Sales Oliveira, São Paulo State, Brazil. The first trial was conducted in a cane-plant field located at 20°51′12’’S 47°56′36’’W; the second trial was conducted in a sugarcane ratoon field (1st ratoon) located at 20°51′06’’S 47°56′39’’W. The sugarcane variety in both fields was RB85 5156, a variety commonly grown in this area of Brazil that is responsive to agricultural management, e.g., N fertilization (Castro et al., 2019). Before initiating the experiments, the soil was sampled up to a depth of 0.6 m for chemical and physical analysis; soil fertility parameters and nutrient availability were analyzed as described by Raij et al. (2001). At both sites, the soils exhibited eutrophic characteristics and a clayey texture (Table 1), classified as Oxisol (Staff, 2014).

2.2. Experimental design and agronomic management

The experimental design used in both fields was a randomized block in a two-way factorial scheme with four replications. The first factor consisted of one of three foliar application treatments: (1) control (no foliar application of biostimulant or nutrients); (2) foliar application of nutrients; and (3) foliar application of biostimulant and nutrients. Nutrient application in treatments 2 and 3 consisted of 2.0 L ha^{-1} of a formulation containing 5.6 % N, 1.9 % K, 1.1 % B, and 1.1 % Zn (all w/w). The biostimulant used in treatment 3 was an extract of *Ascophyllum nodosum* (117 g of organic carbon [w/w]) known as YaraAmplix BIOTRAC™ (Yara International). The rate of biostimulant application was recommended by the manufacturer. The second factor was the time of application: fall or spring (Fig. 1). These application periods were chosen based on the soil water balance calculated for the experimental fields. The application in the fall took place after the soil in the field trial had transitioned from water excess to water deficit. Conversely, the application in the spring occurred at the end of the water deficit period and the return to water excess (Fig. 1). In both fields, the plots (each experimental unit) consisted of six sugarcane rows, spaced 1.5 m apart, with a length of 10 m.

In the cane-plant field, soybean (*Glycine* max) was grown prior to sugarcane planting in a no-tillage system. After the soybean harvest, sugarcane was mechanically planted in March 2020 by planting 24 buds per meter. The no-tillage system was also employed for sugarcane planting, with the billets placed in the bottom of the furrow (0.45 m depth). The fertilization rate was 45 kg N ha^{-1} (applied as ammonium nitrate [33 % N]), 65.5 kg P ha^{-1} (applied as triple super phosphate [45 % P_2O_5]), 66.4 kg K ha^{-1} (using potassium chloride [60 % K_2O]), 1.0 kg B ha^{-1} (boric acid), 1.75 kg Zn ha^{-1} (zinc sulfate), and 0.075 kg Mo ha^{-1} (ammonium molybdate), which was applied to the soil beneath the furrow. Three months after planting, an additional 62 kg K ha^{-1} (75 kg K_2O ha^{-1} applied as potassium chloride [60 % K_2O]) was applied along a band on both sides of each sugarcane row within each plot.

In the sugarcane ratoon field, sugarcane was mechanically harvested in March 2020. Fertilizer was incorporated into the soil at a depth of 0.08 m along a band on both sides of each row at 30 days after harvest. The fertilization rate was 105 kg N ha^{-1}, 22 kg P ha^{-1} (50 kg P_2O_5 ha^{-1}), and 100 kg K ha^{-1} (120 kg K_2O ha^{-1}) using the same fertilizer sources as

Table 1
Chemical and physical attributes of soil in the cane plant and sugarcane ratoon fields before the trials.

Soil Depth (m)	pH $CaCl_2$	SOM (g dm^{-3})	S (mg dm^{-3})	P	K ($mmol_c$ dm^{-3})	Ca	Mg	H+Al	Al	CEC	V (%)	Sand (g kg^{-1})	Silt	Clay	Texture
Cane-plant field															
0–0.2	5.0	34	8	9	0.7	37	11	50	0	98.7	49	140	340	520	Clayey
0.2–0.4	5.4	30	16	8	0.3	38	10	27	0	75.6	64	120	300	570	Clayey
0.4–0.6	5.5	20	9	8	0.2	33	8	28	0	70.0	59	120	230	650	Clayey
Sugarcane ratoon field															
0–0.2	5.1	29	8	11	1.4	38	16	38	0	93.3	59	140	310	550	Clayey
0.2–0.4	5.3	26	10	8	0.6	41	14	39	0	93.7	59	140	290	580	Clayey
0.4–0.6	5.6	19	8	10	0.2	34	9	25	0	68.1	63	160	270	570	Clayey

pH was measured in $CaCl_2$ (0.01 M); soil organic matter (SOM) was measured based on dichromate oxidation; S was extracted using calcium phosphate; P, K, Ca, and Mg were extracted using resin ($NaHCO_3$, 1 M, pH = 8.5); H+Al was extracted in SMP buffer; and Al was extracted using KCl (1 M). Sand, silt, and clay content were determined using the pipette method. CEC, cation exchange capacity; V, base saturation.

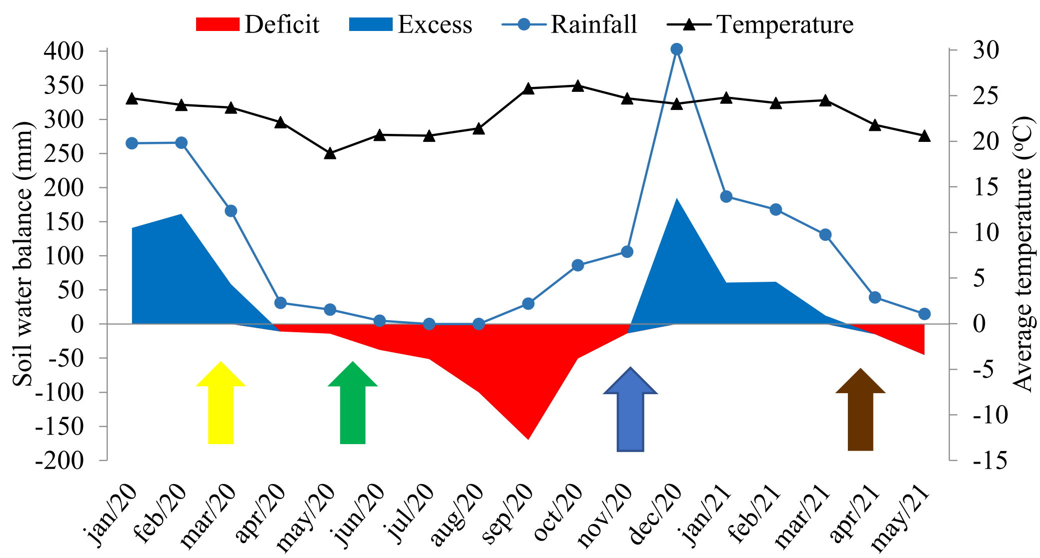

Fig. 1. Water balance throughout the experimental period.
Water balance was calculated according to Thornthwaite and Mather (1955). Yellow arrow represents the time of planting (cane-plant field) and harvesting (sugarcane ratoon field). Green and blue arrows represent foliar application in the fall and spring, respectively. Brown arrow represents harvesting at the end of the experiments.

for the cane-plant field. The micronutrients (0.15 kg B ha^{-1}, 0.75 kg Zn ha^{-1}, and 0.06 kg Mo ha^{-1}) were also applied to the soil in each sugarcane row after harvest.

2.3. Treatment conditions

Treatments were performed at 60 (May 25, 2020) and 240 (November 25, 2020) days after planting in the cane plant field for the fall and spring foliar applications, respectively. The fall application was conducted between 07:40 and 09:00 a.m. at an average temperature of 14 °C and relative humidity of 90 % in the absence of wind (0 km h^{-1}). The spring application was conducted between 05:20 and 08:00 a.m. at an average temperature of 19 °C, a relative humidity of 85 %, and a wind speed of 1.6 km h^{-1}. For the ratoon field trials, fall and spring applications were performed at 75 (May 27, 2020) and 255 (November 26, 2020) days after harvesting, respectively. The fall application was conducted between 09:00 and 11:20 a.m., with an average temperature of 16 °C, a relative humidity of 64 %, and a wind speed of 3.2 km h^{-1}. The spring application was conducted between 05:50 and 09:10 a.m., with an average temperature of 18 °C, a relative humidity of 61 %, and a wind speed of 2.8 km h^{-1}. Foliar applications were performed using a pressurized CO_2 spray pump coupled to a boom of 1.5 m with four nozzles (AXI 110 02) spaced 0.5 m apart, with a flow rate of 150 L ha^{-1}; no adjuvant was added.

2.4. Evaluation of plant growth, nutritional status, and yield

Biometric evaluations were performed at the time of sugarcane harvest (April 2021). Plants located in the center of each plot were collected, covering 2 m in the central position of the three rows for each plot. The stalk population size (stalks m^{-1}) and the aboveground biomass of three plant tissues (stalks, tops, and dry leaves) were determined and used to calculate the stalk yield per hectare (Mg stalk ha^{-1}). Additionally, 10 sugarcane stalks were randomly chosen from each plot to determine the technological quality of the raw material, that is, the contents of sucrose (Pol [%]), soluble solids (Brix [%]), fiber (%), and total recoverable sugars (TRSs; kg TRS Mg^{-1} of stalk), as described by Fernandes (2003). The sugar yield was calculated using sugarcane stalk yield and TRS values, expressed in tons of Pol per hectare (TPH; Mg Pol ha^{-1}). All plant tissues were milled in a forage grinder for homogenization, and a sub-sample was taken to assess moisture level and quantify macronutrient and micronutrient contents.

The sub-samples were oven-dried at 65 °C until reaching constant weight and ground again in a Wiley mill with a 0.5-mm mesh sieve before being assessed for macronutrient and micronutrient contents (N, P, K, Ca, Mg, S, B, Zn, Cu, and Mn) according to Bataglia et al. (1983). Dried and ground sub-samples were digested with sulfuric acid to measure N content or with nitric-perchloric acid to measure the contents of the other nutrients. Nutrients were quantified by spectrophotometry

(P and S), turbidimetry (S), micro-Kjeldahl (N), atomic absorption spectrometry (K, Ca, Mg, Zn, Cu, and Mn), and spectrophotometry after incineration (B). Plant tissue moisture was determined, and the biomass yield was corrected to dry matter to calculate nutrient accumulation (kg ha^{-1}).

Using the data obtained at harvest time, the agronomic efficiency of biostimulant and/or nutrient treatment in the fall or spring was calculated as described by Yang et al. (2017):

$$AE(p) = (Y1 \; - - \; YC)/R,$$

where AE(p) is the agronomic efficiency (Mg stalk L^{-1} of product) obtained over a given period (p; fall or spring), Y1 is the stalk yield (Mg stalk ha^{-1}) of the treatment group (e.g., biostimulant or nutrients), YC is the stalk yield (Mg stalk ha^{-1}) of the control group, and R is the rate of product applied (L ha^{-1}).

2.5. Weather conditions in the experimental areas

During the experimental period (March 2020 to May 2021), the weather parameters were monitored by an automatic meteorological station close to the fields to calculate water balance (Thornthwaite and Mather, 1955). Overall, the accumulated rainfall throughout the experimental period was 1388 mm, 403 mm (29 %) of which occurred in December 2020. The dry season was severe in 2020; the water deficit in the soil reached −170 mm in October 2020 (Fig. 1). Moreover, the accumulated rainfall from January to May 2021 was 540 mm, or 209 mm less than the rainfall of the previous year over the same period, according to the automatic weather station installed close to the sites. Therefore, the sugarcane growth season in which the experiments were conducted had extremely low soil moisture (adverse edaphoclimatic conditions).

2.6. Statistical analysis

A canonical discriminant analysis (CDA) was conducted using the variables of interest, including plant biomass (yield and stalk population size), technological quality of raw material (sucrose content [Pol], fiber content, and sugar yield [TPH]), and accumulation of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Mn, and Zn) across sites, treatments, and application times. CDAs were also performed for each site separately considering the same variables of interest mentioned above. All variables evaluated in this study were analyzed in a randomized block design in a two-factorial scheme (treatments and application time) with four biological replicates within each site. The data for each variable were submitted to analysis of variance (two-way ANOVA, $p < 0.05$). When the *p*-value was significant, the means of the variable were compared by Tukey's HSD post-hoc test ($p < 0.05$). Analyses were carried out in R version 4.1.0 (R Core Team R, 2021) using the candisc package for CDA (Friendly and Fox, 2022) and ExpDes for means comparison (Ferreira et al., 2014).

3. Results

To assess the effects of biostimulant (*A. nodosum* extract) application time (fall or spring), treatment, and field (cane-plant or ratoon field) on sugarcane biomass production, we compared the means for the variables reflecting plant biomass and the technological quality of the raw material. None of these variables were influenced by application time in the cane-plant or ratoon field (Table 2). By contrast, stalk yield and sugar yield differed among treatments in both fields. We observed a significant interaction between treatments and application time for nutrient accumulation in aboveground plant parts (Table 2) for most variables in the cane-plant field, except for P and S. In the ratoon field, the nutrient contents significantly differed only between treatments, except for P, K, Mn, and Zn, which showed no significant association with treatment

Table 2
Summary of statistical analysis (*p*-values) for the physical and chemical characteristics of sugarcane in the two field trials.

	Pop.	Yield	Brix	Fiber	Pol	TRS	TPH	N	P	K	Ca	Mg	S	B	Cu	Mn	Zn
Cane-plant field																	
Treatment (Tr)	0.89	0.001*	0.07	0.30	0.08	0.08	0.002*	0.408	0.64	0.948	0.711	0.667	0.598	0.098	0.084	0.115	0.067
Application time (AT)	0.87	0.774	0.50	0.18	0.78	0.77	0.990	0.434	0.68	0.108	0.862	0.786	0.617	0.777	0.983	0.646	0.348
Tr × AT	0.41	0.047*	0.10	0.99	0.12	0.12	0.242	0.030*	0.08	0.005*	0.015*	0.010*	0.386	0.008*	0.019*	0.008*	0.004*
Sugarcane ratoon field																	
Treatment (Tr)	0.18	0.000*	0.56	0.96	0.59	0.60	0.001*	0.034*	0.05*	0.153	0.026*	0.005*	0.010*	0.034*	0.048*	0.087	0.186
Application time (AT)	0.96	0.906	0.30	0.99	0.24	0.26	0.366	0.745	0.36	0.105	0.087	0.371	0.836	0.162	0.969	0.219	0.303
Tr × AT	0.49	0.646	0.96	0.67	0.84	0.86	0.863	0.352	0.29	0.884	0.554	0.687	0.588	0.181	0.231	0.289	0.352

p-values for the stalk population size (Pop.; stalks m^{-1}), yield (Mg ha^{-1}), technological quality of raw material (Brix [%], fiber [%], and Pol [%]), total recoverable sugar (TRS; kg TRS Mg $stalk^{-1}$), sugar yield (TPH; Mg Pol ha^{-1}), and total accumulation of macronutrients (N, P, K, Ca, Mg, and S; kg ha^{-1}) and micronutrients (B, Cu, Mn, and Zn; g ha^{-1}) in aboveground biomass in sugarcane plants grown in the cane-plant field and the sugarcane ratoon field at harvest under each treatment (Tr; biostimulant, nutrients, and control) and for each application time (AT; fall or spring) and the interactions between them (Tr × AT). The *p*-values were obtained by two-way ANOVA. The asterisk indicates a significant difference ($p < 0.05$).

type.

3.1. Biostimulant treatment increases sugarcane yield regardless of application time

In the cane-plant field, we observed a significant interaction between treatment type and application time and a significant effect of treatment on sugarcane stalk yield (Table 2). There was also a significant effect of treatment on sugar yield. In the ratoon field, both the sugar yield and stalk yield were significantly influenced by the treatment type (Table 2).

In the cane-plant field, foliar application of biostimulant in the fall or spring increased sugarcane stalk yield by 17.5 % and 24.1 %, respectively, compared to the control (Fig. 2A). The application of nutrients in the fall also enhanced stalk yield, reaching the same increase obtained with biostimulant; the application of nutrients in the spring did not affect stalk yield. In the ratoon field, regardless of the application time, biostimulant treatment increased stalk yield by 23.8 % relative to the average values obtained for the control and nutrient treatments (Fig. 2B).

In terms of the technological quality parameters of the raw material, biostimulant treatment improved sugar yield (TPH) in both fields compared to the control and nutrient treatments. Furthermore, the application of biostimulant increased the sugar yield by an average of 19.2 % and 22 % in the cane plant and ratoon field, respectively, compared to the other two treatments (Table 3). The other parameters were not influenced by treatment type or application time (Supplementary Tables 1 and 2).

We also investigated all plant biometric features by multivariate analysis in each field trial (Fig. 3). A CDA explained 93 % of the variance in both fields. Sugarcane stalk and sugar yield were the main variables between the combination of factors (treatment and application time). The canonical scores of biostimulant treatment were in the same direction as these two variables regardless of application time within the second main component of CDA (Can2, explaining 25.9 % of the total variance) in the cane-plant field and within the main component of CDA (Can1, explaining 81.5 % of the total variance) in the ratoon field. In the cane-plant field, the application of biostimulant in the spring was in the same quadrant as these vectors, separated from fall application by Can1 (explaining 67 % of the variance). Therefore, the application time of biostimulant onto sugarcane leaves does not appear to have any influence in ratoon fields, whereas its application in cane-plant fields after the dry season (in the spring) promotes an increase in stalk and sugar yield.

3.2. Nutrient accumulation in aboveground tissues

To calculate nutrient accumulation in aboveground sugarcane tissues, we measured the contents of multiple nutrients in stalks, dry leaves, and tops. The nutrient content of plant tissues was influenced by treatment type and application time only in the cane-plant field (Supplementary Tables 3A–3C). In this field trial, the contents of N, P, K, S, Cu, and Zn in the stalk differed among treatments for each application time (Supplementary Table 3A). Control and biostimulant treatments resulted in the greatest levels of these nutrients when plants were treated in the fall and spring, respectively. We detected no interaction between treatment type and application time for the nutrient contents of dry leaves or tops. The highest contents of all macronutrients and micronutrients were detected in samples collected from plants exposed to nutrient treatment. Different application times only produced a significant difference in the nutrient contents of the tops, in which fall treatment promoted the highest accumulation of N, Cu, and Zn. In the ratoon field trial, there were no significant differences in nutrient contents in any tissue (Supplementary Tables 4A–4C).

In the cane-plant field, the interaction between treatment and application time resulted in a significant difference in nutrient accumulation in aboveground tissues in the spring, but not in the fall (Table 4). When applied in the spring, the biostimulant promoted the greatest macronutrient and micronutrient accumulation in aboveground sugarcane tissues, except for P and S, whose contents remained constant regardless of treatment and application time. In the ratoon field, only treatment variable influenced nutrient accumulation in aboveground tissues (Table 2). Biostimulant treatment resulted in the greatest accumulation of N, Ca, Mg, S, B, and Cu in aboveground tissues, although these increases were similar to that from treatment with nutrients alone (Table 5).

We also investigated the accumulation of nutrients in the aboveground tissues of sugarcane plants by multivariate analysis for each field (Fig. 4). The CDA explained more than 75 % of the standing variation at both sites. Nutrient accumulation vectors mainly pointed in the direction of biostimulant application in the spring in the cane-plant field and

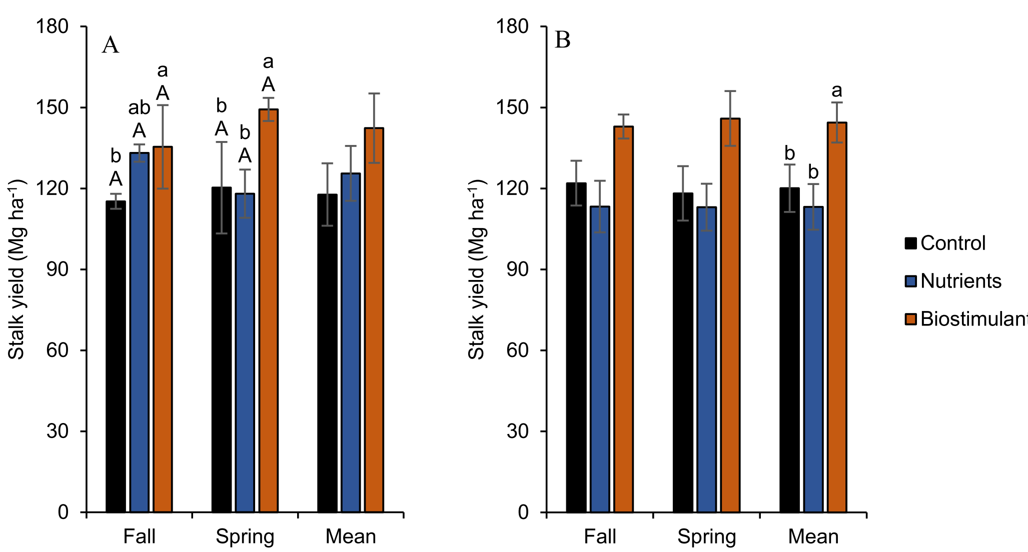

Fig. 2. Sugarcane stalk yield at harvest following each treatment in the fall or spring. Sugarcane stalk yield in the cane-plant field **(A)** and the ratoon field **(B)** following treatment with nutrients alone or together with biostimulant (*A. nodosum* extract) in the fall or spring. Control plants received neither nutrients nor biostimulant. The values are means ± standard error from four replicates. In (A), different lowercase letters indicate significant differences among treatments within each application time; different uppercase letters indicate significant differences between times within each treatment. In (B), different lowercase letters indicate significant differences among treatments for the mean of the two application times. All comparisons were performed using Tukey's HSD post-hoc test ($p < 0.05$).

Table 3
Sugar yields for all treatment groups. Sugar yield, reported as TPH (Mg Pol ha^{-1}), in plants grown in cane-plant and ratoon fields at harvest under each treatment (biostimulant, nutrients, and control) and application time (fall and spring).

	Cane-plant Field						Sugarcane Ratoon Field					
Application Time/Treatment	Fall		Spring		Mean		Fall		Spring		Mean	
Control	17.4		18.7		18.0	b	16.3		15.1		15.7	b
Nutrients	21.2		19.1		20.1	ab	14.4		14.2		14.3	b
Biostimulant	22.3		23.1		22.7	a	18.6		18.0		18.3	a
Mean	20.3	A	20.3	A			16.4	A	15.8	A		

Different lowercase letters indicate significant differences among treatments for the means of the two application times; different uppercase letters indicate significant differences between application times for the means of the three treatments according to Tukey's HSD post-hoc test ($p < 0.05$).

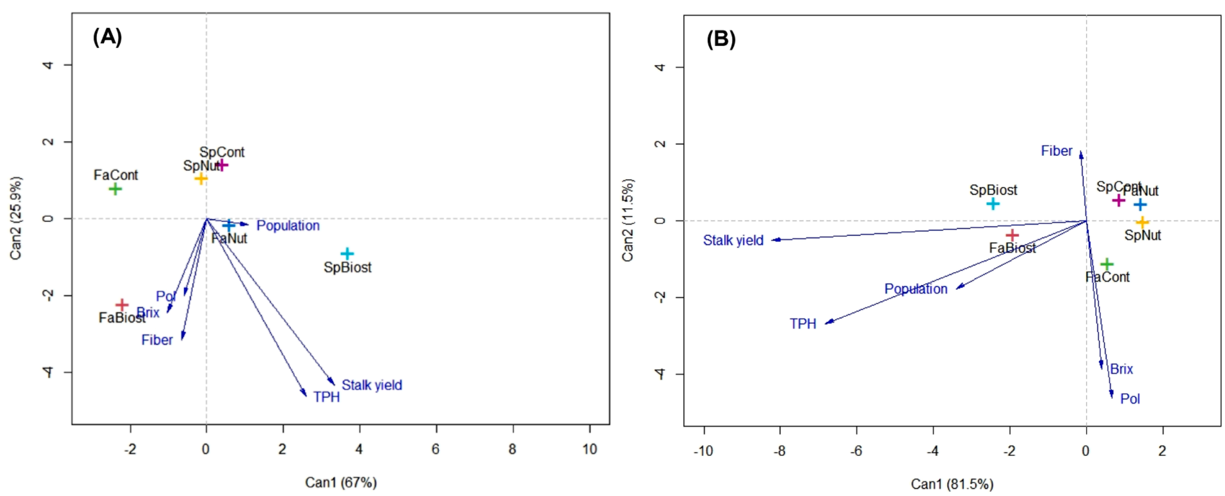

Fig. 3. Canonical discriminant analysis of plant biometric features from cane-plant and sugarcane ratoon field trials.
Canonical discriminant analysis (CDA) was conducted on stalk yield and stalk population size (population), sucrose content (Pol [%]), soluble solid content (Brix [%]), fiber content (%), and sugar yield (TPH; Mg Pol ha^{-1}) in the cane-plant **(A)** and ratoon **(B)** fields. The components Can1 and Can2 represent the first and second major components of the CDA, respectively. Fa, fall application; Sp, spring application; Cont, control treatment; Nut, nutrient treatment; Biost, biostimulant treatment.

biostimulant application regardless of application time in the ratoon field. We conclude that the application of biostimulant onto the leaves of sugarcane plants in the spring results in greater accumulation of nutrients in aboveground tissues in the cane plant field, whereas application time does not influence nutrient accumulation in the ratoon field.

3.3. Agronomic efficiency

The agronomic efficiency was influenced by the application time of biostimulant or nutrients in both field trials (Fig. 5). In the cane-plant field, the highest agronomic efficiency was obtained following treatment with biostimulant in the spring (14.5 Mg stalk^{-1} L product^{-1}), followed by biostimulant treatment in the fall (10.1 Mg stalk^{-1} L product^{-1}). By contrast, treatment with nutrients in the spring did not significantly improve agronomic efficiency in the cane-plant field. In the ratoon field, regardless of application time (fall or spring), the agronomic efficiency did not increase in response to nutrient treatment. Conversely, biostimulant treatment in either the spring or fall improved agronomic efficiency, with an average gain of 12 Mg stalk^{-1} L product^{-1}.

3.5. Effects of experimental factors on variables of interest

Multivariate analysis (CDA) using variables of interest as input explained 86 % of the standing variation (Fig. 6A). Field trials were positioned on opposite sides along the main component of CDA (Can1), which explained 73.4 % of the standing variation. To visualize the relationship between variables, that is, the combination of application time and treatment, we performed a CDA for each field trial separately. This second round of CDA explained 85 % of the variation in the cane-plant field (Fig. 6B) and 90.4 % in the ratoon field (Fig. 6C). In both field trials, the scores for biostimulant treatment were in the same direction as the stalk yield vector along Can1. This effect was greater in the ratoon field, where all variables except Pol were on the same side as biostimulant treatment along Can1 regardless of application time. We also conducted a univariate analysis using data specific to each field trial. In the cane plant field (Fig. 6B), foliar application of biostimulant during the spring had a more positive effect on stalk and sugar yield and nutrient accumulation in aboveground tissue. A similar response was observed at the ratoon site, but for both the fall and spring seasons (Fig. 6C).

4. Discussion

The application of biostimulant has emerged as a promising strategy to maximize sugarcane yields and promote crop longevity, specifically by increasing the number of ratoon harvests. Biostimulants can be applied to the soil or leaves, depending on their composition and the desired outcomes (Kunicki et al., 2010; Bulgari et al., 2015). In the current study, we treated the leaves of sugarcane plants grown in cane-plant and ratoon fields with *A. nodosum* extract before or after the dry season in Southeast Brazil (in the fall and spring, respectively). Treatment in the fall, when plants begin to experience water deficit, might enhance the plants tolerance to better survive during the upcoming drought period in winter (Jacomassi et al., 2022). Applying biostimulant and nutrients in the spring is thought to support plant

Table 4

Nutrient accumulation in the aboveground tissues of sugarcane from the cane-plant field at the time of harvest. Stalks, dry leaves, and tops were collected from plants exposed to each treatment (biostimulant, nutrients, and control) and for each application time (fall and spring). Nutrient accumulation is the sum of nutrient accumulation in each aboveground plant tissue.

Application Time/	Fall		Spring		Mean		Fall		Spring		Mean	
Treatment	**N (kg ha^{-1})**						**P (kg ha^{-1})**					
Control	217.3	aA	195.3	abA	206.3		24.6		19.1		21.8	a
Nutrients	230.4	aA	163.1	bB	196.7		23.7		19.2		21.5	a
Biostimulant	198.7	aA	248.9	aA	223.8		20.4		27.5		24.0	a
Mean	215.5		202.4				22.9	A	21.9	A		
	K (kg ha^{-1})						**Ca (kg ha^{-1})**					
Control	319.0	aA	256.5	abA	287.7		114.6	aA	109.4	abA	112.0	
Nutrients	387.0	aA	198.0	bB	292.5		133.4	aA	98.5	bA	116.0	
Biostimulant	250.9	aA	348.5	aA	299.7		99.4	aB	144.7	aA	122.1	
Mean	319.0		267.7				115.8		117.6			
	Mg (kg ha^{-1})						**S (kg ha^{-1})**					
Control	60.9	aA	57.9	bA	59.4		38.4		34.1		36.2	a
Nutrients	69.4	aA	55.4	bA	62.4		43.7		37.5		40.6	a
Biostimulant	53.6	aB	73.8	aA	63.7		35.8		40.9		38.3	a
Mean	61.3		62.4				39.3	A	37.5	A		
	B (g ha^{-1})						**Cu (g ha^{-1})**					
Control	331.9	aA	359.6	aA	345.8		229.6	aA	214.9	bA	222.2	
Nutrients	345.8	aA	197.4	bB	271.6		246.4	aA	186.0	bA	216.2	
Biostimulant	285.7	aB	435.3	aA	360.5		226.1	aB	302.3	aA	264.2	
Mean	321.1		330.8				234.0		234.4			
	Mn (g ha^{-1})						**Zn (g ha^{-1})**					
Control	5045.1	aA	4324.4	bA	4684.8		600.0	aA	534.8	bA	567.4	
Nutrients	5187.5	aA	4054.9	bA	4621.2		741.4	aA	457.5	bB	599.4	
Biostimulant	4453.5	aB	6921.5	aA	5687.5		614.0	aB	817.7	aA	715.8	
Mean	4895.4		5100.3				651.8		603.3			

Different lowercase letters indicate significant differences among treatments for the means of the two application times or within each application time; different uppercase letters indicate significant differences between application times for the means of the three treatments or within each treatment, according to Tukey's HSD post-hoc test ($p < 0.05$).

Table 5

Nutrient accumulation in the aboveground tissues of plants from the ratoon field at the time of harvest. Stalks, dry leaves, and tops were collected from plants exposed to each treatment (biostimulant, nutrients, and control) and at each application time (fall and spring). Nutrient accumulation is the sum of nutrient accumulation in each aboveground plant tissue.

Application Time/	Fall		Spring		Mean		Fall		Spring		Mean	
Treatment	**N (kg ha^{-1})**						**P (kg ha^{-1})**					
Control	180.7		191.8		186.2	b	24.1		29.0		26.5	a
Nutrients	215.0		223.9		219.5	ab	29.0		33.1		31.1	a
Biostimulant	250.8		217.3		234.0	a	35.1		32.1		33.6	a
Mean	215.5	A	211.0	A			29.4	A	31.4	A		
	K (kg ha^{-1})						**Ca (kg ha^{-1})**					
Control	282.9		247.4		265.1	a	111.7		94.1		102.9	b
Nutrients	318.5		280.0		299.2	a	123.7		120.2		121.9	ab
Biostimulant	365.9		300.5		333.2	a	149.9		122.4		136.1	a
Mean	322.4	A	276.0	A			128.5	A	112.2	A		
	Mg (kg ha^{-1})						**S (kg ha^{-1})**					
Control	61.2		58.0		59.6	b	47.7		46.2		47.0	b
Nutrients	64.7		65.3		65.0	b	54.7		59.1		56.9	ab
Biostimulant	78.3		71.8		75.1	a	66.0		60.7		63.4	a
Mean	68.1	A	65.0	A			56.2	A	55.4	A		
	B (g ha^{-1})						**Cu (g ha^{-1})**					
Control	184.5		179.5		182.0	b	251.3		250.1		250.7	b
Nutrients	191.7		194.5		193.1	ab	259.2		308.2		283.7	ab
Biostimulant	243.8		197.3		220.5	a	351.7		301.1		326.4	a
Mean	206.7	A	190.4	A			287.4	A	286.5	A		
	Mn (g ha^{-1})						**Zn (g ha^{-1})**					
Control	3416.1		3378.3		3397.2	a	664.2		647.4		655.8	a
Nutrients	3989.1		3935.8		3962.4	a	694.4		709.1		701.7	a
Biostimulant	5182.4		3826.0		4504.2	a	940.7		708.3		824.5	a
Mean	4195.9	A	3713.4	A			766.4	A	688.3	A		

Different lowercase letters indicate significant differences among treatments for the means of the two application times or within each application time; different uppercase letters indicate significant differences between application times for the means of the three treatments or within each treatment, according to Tukey's HSD post-hoc test ($p < 0.05$). .

growth under favorable conditions instead of during the dry season, which is characterized by extended water deficit, low temperature, and reduced solar irradiation (Jacomassi et al., 2024). Therefore, our study marks an initial step in evaluating the effects of biostimulants in conjunction with various weather conditions, including unfavorable (such as drought) and favorable conditions (such as spring, with higher temperatures, soil moisture, and solar irradiation).

We found that the foliar application of biostimulant during the

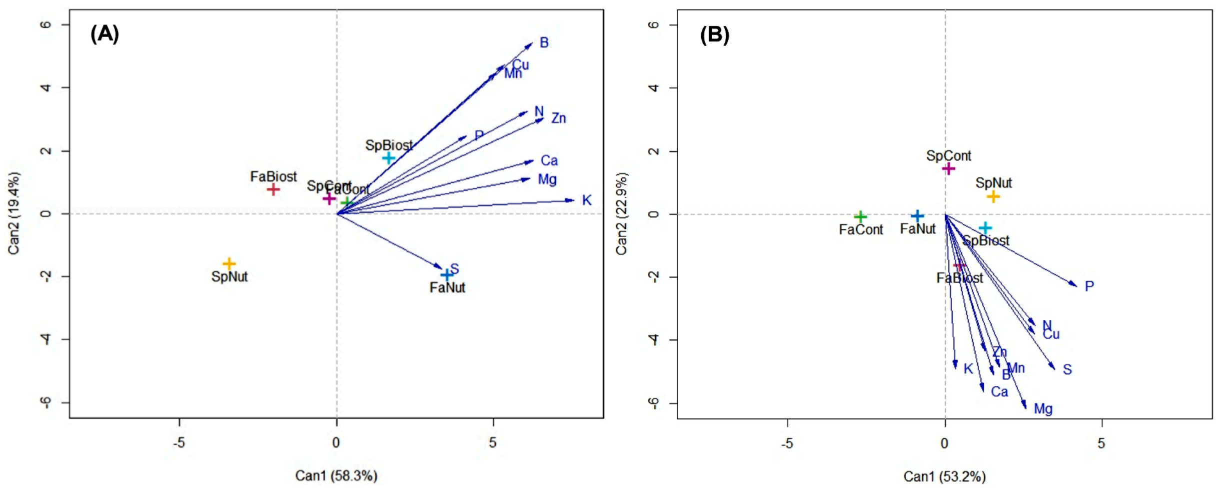

Fig. 4. Canonical discriminant analysis of plant nutrient accumulation in aboveground tissues.
Canonical discriminant analysis (CDA) was conducted on the accumulation of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) in kg ha^{-1} and boron (B), copper (Cu), manganese (Mn), and zinc (Zn) in g ha^{-1} in aboveground tissues from the cane-plant **(A)** and ratoon **(B)** fields. The components Can1 and Can2 represent the first and second major components of the CDA, respectively. Fa, fall application; Sp, spring application; Cont, control treatment; Nut, nutrient treatment; Biost, biostimulant treatment.

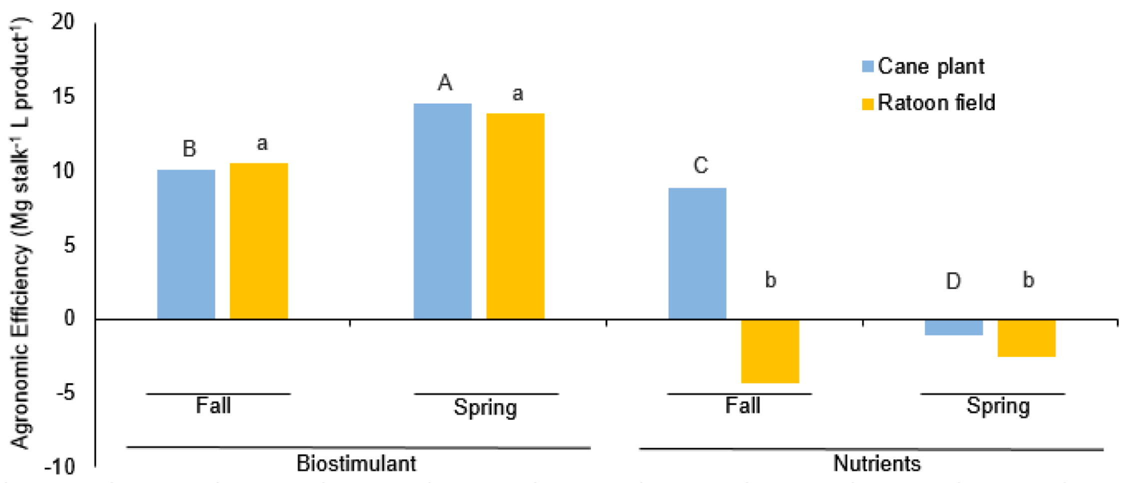

Fig. 5. Agronomic efficiency at the time of harvest.
Agronomic efficiency was calculated as Mg stalk^{-1} L product^{-1} at harvest in the cane-plant and ratoon fields following each treatment (biostimulant, nutrients, and control) and at each application time (fall or spring). Different uppercase letters indicate significant differences among the means for plants in the cane-plant field; different lowercase letters indicate significant differences among the means for plants in the ratoon field.

sugarcane crop cycle minimized the adverse effects of weather on plants, resulting in considerable development and high stalk yields, with an increase of 20 % over the nontreated controls (Fig. 3). Yield increases were also recently reported in sugarcane following biostimulant treatment (Gomathi et al., 2017; Jacomassi et al., 2022). In both studies, foliar application of biostimulant led to superior outcomes, with yield gains of 22 % (Gomathi et al., 2017) and between 11 % and 18 % (Jacomassi et al., 2022) compared to control treatment. This phenomenon might be attributed to the activation of various physiological responses in crops by the biostimulant, which enhances nutrient utilization efficiency, stimulates plant development, and enhances nutrient uptake from fertilized soil (Kunicki et al., 2010; Anggraeni et al., 2022).

Besides yield increases, the foliar application of biostimulant resulted in gains in sugar yield (TPH, Mg of Pol per hectare) of 7.3 Mg Pol ha^{-1}. Such an increase could lead to an economic return in sugarcane cultivation of approximately US$1700 ha^{-1} due to the low biostimulant application rate and product costs. However, in the present study, the application of nutrients alone or together with the biostimulant did not influence the levels of total recoverable sugars (TRSs). Among all seaweed-based biostimulants, those derived from *A. nodosum* extracts have received substantial attention due to their growth-stimulating activities when applied repeatedly and at low rates (Sharma et al., 2014; Van Oosten et al., 2017). Treatment with two commercial extracts of *A. nodosum* increased macronutrient (N, P, K, Ca, and S) and micronutrient (Mg, Zn, Mn, and Fe) contents in tomato (Di Stasio et al., 2018). Similarly, olive (*Olea europaea*) plants treated with *A. nodosum* extracts showed higher K and Cu uptake than untreated plants (Chouliaras et al., 2009).

A. nodosum extracts are known for their rich contents of bioactive phenolic compounds such as phlorotannins and unique polysaccharides (Holdt and Kraan, 2011; Yuan and Macquarrie, 2015; Moreira et al., 2017). When applied to plants experiencing stress, such as before the dry season (fall) in the current study, the combination of *A. nodosum* extract and nutrients mitigated the negative effects of the adverse environmental conditions, as reflected in the greater stalk yield (Fig. 2) and

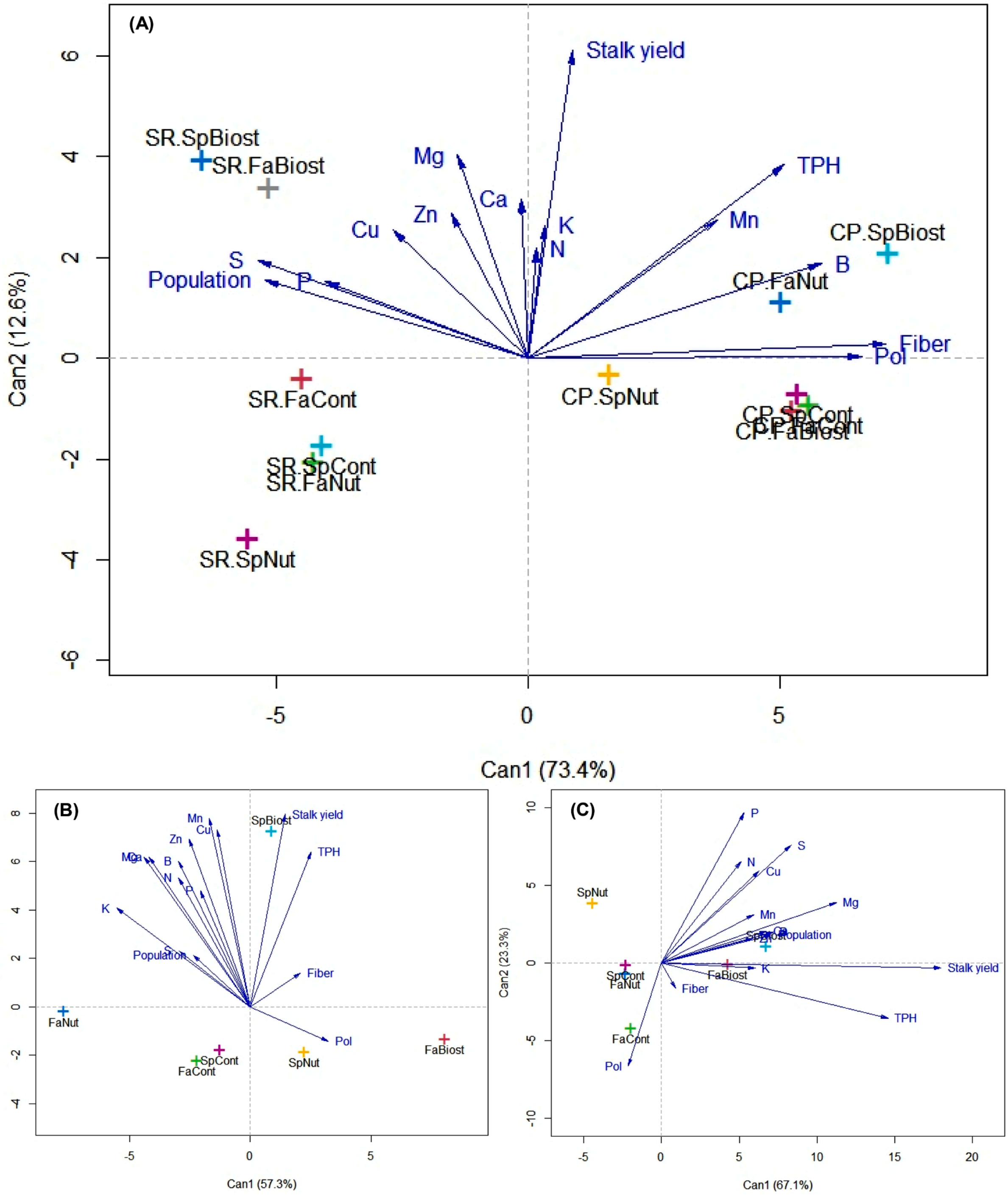

Fig. 6. Canonical discriminant analysis on variables of interest.
Canonical discriminant analysis (CDA) was conducted on the following variables of interest: stalk yield and stalk population size (population), sucrose content (Pol [%]), fiber content (%), sugar yield (TPH; Mg Pol ha^{-1}), and accumulation of macronutrients (N, P, K, Ca, Mg, and S; kg ha^{-1}) and micronutrients (B, Cu, Mn, and Zn; g ha^{-1}) for different combinations of application times and treatments **(A)** for the cane-plant field **(B)** and ratoon field **(C)** trials. The components Can1 and Can2 represent the first and second main components of the CDA, respectively. CP, cane-plant field; SR, ratoon field; Fa, fall application; Sp, spring application; Cont, control treatment; Nut, nutrient treatment; Biost, biostimulant treatment.

sugar yield (Table 3). The foliar application of nutrients alone or with the biostimulant did not affect the technological quality of sugarcane (Supplementary Tables 1 and 2), which is in agreement with previous findings (Castro et al., 2018; Rhein et al., 2016). Perhaps the high stalk yield resulted in a dilution effect on sucrose content due to increased plant water content. Several reports indicate that the application of a biostimulant containing (in g L^{-1}) organic carbon = 78.0, $N = 13.0$, $S =$ 40.3, $B = 1.17$, Co = 0.78, Fe = 16.9, Cu = 13.0, Mn = 14.3, Mo = 0.52, and Zn = 29.9 affected the technological quality of sugarcane raw material, such as sucrose content, reducing sugars, fiber, purity, and TRS (Anggraeni et al., 2022; Jacomassi et al., 2022).

Under drought conditions, plants often suffer from nutrient shortages due to diminished nutrient uptake by the roots and limited mineral mobilization caused by water scarcity (Hu and Schmidhalter, 2005;

Hosseini et al., 2021). Nutrients are vital to plant biology and are crucial for sustaining growth and development, particularly under abiotic stress conditions (Ahanger et al., 2019). Biostimulants were previously shown to positively affect plant nutrient uptake, as also observed in the current study (Fig. 5) in both the cane-plant (Table 4) and sugarcane ratoon fields (Table 5). The relationship between nutrient uptake and biostimulant application has been explored in various crops, including vegetables (e.g., cucumber [*Cucumis sativus*], lettuce [*Lactuca sativa*], and carrot [*Daucus carota*]), fruits (e.g., strawberry [*Fragaria* × *ananassa*], tomato, grapevine [*Vitis vinifera*], and apple [*Malus domestica*]), and grains (e.g., maize [*Zea mays*], soybean, and wheat [*Triticum aestivum*]) (Shukla et al., 2019). In general, biostimulant treatment enhanced nutrient uptake (Turan and Köse, 2004); maintained nutrient balance within the plant, mitigating the loss of biomass due to stress (Hosseini et al., 2021); and increased nutrient content in aboveground plant tissues, especially under drought conditions (Rathore et al., 2009). This study is among the first to investigate macronutrient and micronutrient levels in sugarcane in both cane-plant (Table 4) and ratoon fields (Table 5). We observed that biostimulant application increased the nutritional resilience of sugarcane plants, especially during unfavorable weather periods (dry season; e.g., winter in Southeast Brazil), with lower nutrient content compared to plants treated with nutrients alone or the control treatment. Conversely, when biostimulant was applied during favorable conditions (spring), nutrient uptake increased compared to other treatments. Therefore, the application of biostimulant to sugarcane during drought periods might help improve plant nutritional status, facilitating consistent growth and development, as observed in other crops (Rathore et al., 2009; Shukla et al., 2019).

To assess the true contribution of *A. nodosum* extract as a biostimulant in sugarcane, we included another treatment consisting of a nutritional mixture with similar nutrient composition and contents to the nutrients present in the commercially available biostimulant used in this study. This analysis, the first of its kind for sugarcane cultivation trials, confirmed the potential of *A. nodosum* extract as a biostimulant. Indeed, we observed an increase in agronomic efficiency of 12 Mg stalk L product^{-1} in both the cane-plant and ratoon fields in plants treated with biostimulant (Fig. 6). Furthermore, during the drought period (fall application), the amount of nutrients extracted by sugarcane plants was lower than that provided by the nutritional mixture applied to the leaves (Tables 4 and 5), yet this treatment yielded more sugarcane stalks and higher sugar content (Fig. 3). Managing biostimulant treatment throughout the cane-plant and ratoon cycles should be considered as an option to maximize the conversion efficiency of macronutrients into biomass (Goñi et al., 2018; Jacomassi et al., 2022; Shukla et al., 2019). Therefore, the use of biostimulant treatment in sugarcane cultivation provides nutritional balance, as biomass production is closely related to the potential extraction of nutrients by plants.

The use of *A. nodosum* extract as a biostimulant in agriculture is promising and presents numerous research opportunities. Several questions remain, such as determining the optimal application rate of *A. nodosum* extract and the best application method (e.g., drenching or spraying). Additionally, it will be important to establish the ideal timing of application and whether re-application is necessary during the growing season and at what intervals. The answers to these questions may vary among crops and climatic conditions (Shukla et al., 2019). Our findings increase our understanding of sugarcane cultivation by demonstrating the potential of biostimulant to increase stalk yield and the raw material quality of sugarcane in both the cane-plant and ratoon fields. Due to the long sugarcane crop cycle (15 months for the cane-plant and 12 months for the ratoon field), we recommend applying biostimulant before and/or after the dry season for cane-plant fields and at least once during the favorable development period, e.g., spring in Brazil, for ratoon fields. Finally, biostimulant application promotes better agronomic efficiency than nutrient mixture alone, enhancing plant nutritional resilience during unfavorable periods, such as during the dry season (Ahanger et al., 2019; Jacomassi et al., 2022).

Modern agricultural practices have become more sustainable and environmentally friendly (Bulgari et al., 2015). This agronomic approach strives to limit inputs without compromising crop yield and quality over a short timeframe and at a lower cost (Bulgari et al., 2015). The foliar application of a biostimulant is a compelling option for improving the yield of sugarcane and the technological quality of raw materials in a sustainable, environmentally friendly manner (Figs. 3 and 4, and Table 3).

5. Conclusions

The foliar application of seaweed extract from *A. nodosum* combined with nutrients enhanced the resilience of sugarcane plants to adverse environmental conditions, improved nutrient accumulation, and increased stalk yield. Applying biostimulant onto the leaves of sugarcane plants before or after the dry season (in the fall and spring in Brazil, respectively) resulted in a significant increase in stalk production (up to 24 Mg ha^{-1}) compared to the untreated controls and even to foliar application of nutrients in both cane-plant and ratoon fields. The foliar application of biostimulant, especially during the dry season (fall), resulted in the improved nutritional resilience of sugarcane plants. Furthermore, biostimulant treatment maximized the agronomic efficiency when used in both cane-plant and ratoon fields (more than 10 Mg stalk^{-1} L^{-1} product), regardless of application time (fall or spring) compared to the application of nutrients alone.

CRediT authorship contribution statement

Sérgio Gustavo Quassi de Castro: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Saulo Augusto Quassi de Castro:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Rosilaine Araldi de Castro:** Writing – review & editing, Writing – original draft, Visualization, Software, Conceptualization. **Renata Rebellato Linhares de Castro:** Writing – original draft, Visualization, Software, Methodology. **Luis Torres Dorante:** Writing – original draft, Visualization, Resources, Methodology. **Rejane Silva Souza:** Resources, Project administration, Funding acquisition. **Franz Walter Rieger Hippler:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100535.

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