







Article

# Exploring the Impact of Humic Biostimulants on Cassava Yield and Nutrition in Northeast Brazil

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**Abstract:** Cassava is a staple food mainly produced with low management inputs, causing soil depletion and low yields. The use of organic inputs, such as humic substances (HSs), represents a sustainable alternative to increase cassava growth and production, mainly in semi-arid regions such as the Brazilian Northeast. Thus, the objective was to evaluate the foliar application of a biostimulant based on humic substances on the morphophysiology, production, and mineral nutrient contents of cassava. The biofortified cultivar BRS Dourada was grown under field conditions and foliar application of a biostimulant based on humic substances (BHSs, treated plants) or water (untreated, control). The experiment was conducted in a randomized block design with four repetitions. At 225 days after planting, the growth, productivity, and mineral nutrient contents of soil, roots, and leaves were determined. No differences between treated and untreated plants were found for growth and productivity (average 15.2 t ha<sup>-1</sup>). On the other hand, BHS treatment reduced net carbon assimilation, water use efficiency, and carboxylation efficiency by 34%, 24%, and 47%, respectively. Moreover, BHS treatment reduced nutrient uptake from soil, and Na and K contents in roots and leaves, respectively. A foliar BHS application is not recommended for cassava production in the conditions evaluated here.

**Keywords:** agroecological management; biofortified cassava; fulvic acid; humic acid; *Manihot esculenta* Crantz



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## 1. Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the Euphorbiaceae family and is a root crop important for food security in many parts of the world, especially in semi-arid regions such as the Brazilian Northeast [1–3]. The world's production of cassava in 2022 was >330 million tons, with Nigeria showing the highest production (>60 million tons), followed by the Democratic Republic of the Congo, Thailand, Ghana, Cambodia, and Brazil [4]. In Brazil, the increases in the production of cassava and other crops have been mainly related to the incorporation of new land, instead of the use of technologies to improve productivity, which comes at the cost of the degradation of native biomes [5,6]. Moreover, due to the increasing demand for commodities, areas in which cassava is traditionally

produced have been replaced by other crops, such as soybean, corn, and sugar cane [5]. As cassava is mainly produced by small farmers as a form of subsistence and to meet domestic demands, the lack of incentive in its production also impacts socioeconomical and cultural aspects [7,8]. This, in turn, causes the erosion of the genetic variation in cassava varieties, also called ethnovarieties, which are maintained by these small farmers [7]. In this context, the valorization of this crop and the development of sustainable technologies to improve its productivity are essential, not only for food security but also for the preservation of natural biomes, local culture, and genetic basis, as well as the generation of income to small farmers.

Despite its high nutritional value, some cassava varieties have high levels of cyanide and thus are suitable only for industrial purposes, such as the production of flour and cassava starch [9]. On the other hand, the table varieties have low levels of cyanide (<100 ppm) and therefore are proper for fresh consumption and extensively consumed in natura [2,10]. In addition to the high levels of carbohydrates, fibers, minerals, and vitamins of cassava, efforts have been made to develop biofortified yellow varieties with increased levels of  $\beta$ -carotene, an important antioxidant compound and a precursor of vitamin A [1,9–11]. In addition to the roots, cassava leaves and stems, which are generally discarded, are also rich in nutrients, proteins, and vitamins, and, together with other by-products of flour production, can be used for biofuel [11,12]. These characteristics confer cassava a high sustainable potential that remains poorly explored so far.

Cassava is a very resilient crop, showing tolerance to unfavorable environmental conditions such as high temperatures, drought, and nutrient deficiency, and therefore has great potential for production in semi-arid regions [8,13]. In these regions, cassava is traditionally produced with low input investments, resulting in low yields (<15 t ha<sup>-1</sup>) and long-term soil depletion [4,14,15]. In this context, the use of chemical fertilizers on cassava is increasing, as it can highly increase the crop yield [16,17]. Nonetheless, these practices can strongly impact the ambient conditions, which raises the need for more sustainable practices that can also increase cassava yield, such as the use of humic substances (HSs) [6,18,19].

HSs originate from the decomposition of organic matter in the soil and are mainly composed of soluble (humic acids and fulvic acids—HAs and FAs) and insoluble (humin) fractions [6]. The soluble HAs and FAs are largely known for their effects on soil conditioning, as their structure enhances the chemical, physical, and biological qualities of the soil [20]. Additionally, HSs have biostimulant effects, promoting plant growth and productivity mainly by their hormone-like properties [6,21–23]. The biostimulant effects may vary according to the HS structure, dose, and application mode, as well as in response to the plant species, tissue, and phenological stage [22]. Depending on its source, HSs may vary in their polarity, molecular size, and functional groups present in their structure [20]. The polarity of HSs may affect their interaction with soil components and plant tissues, with hydrophilic HSs being more available to plants, while the functional groups determine their biological activity [20,23]. Moreover, depending on their molecular size, they can directly enter the cell and trigger responses (small molecular size), such as the activation of pathways involved in hormonal signaling, nitrogen assimilation, and cell division, or they can induce responses through the interaction with membrane receptors (high molecular size), such as nutrient transporters and plasma membrane H<sup>+</sup>-ATPases [23]. The dose–response curve of HSs generally shows a bell shape distribution as the dosage increases, with shoots requiring lower dosages compared to roots [23]. These differences may be related, for instance, to the effect of soil application of HSs in inducing higher nutrient uptake through the activation of H<sup>+</sup>-ATPases and transporters in roots, while it does not occur in foliar application [21]. Despite these differences, the foliar application of HSs has shown promising results in promoting photosynthesis, plant growth, defenses against pathogens and abiotic stresses, and higher post-harvest quality [24]. Moreover, the effectiveness of foliar HS application depends on leaf characteristics such as wax layers and stomatal aperture as well [24]. As a consequence of the well-known effects of HSs as

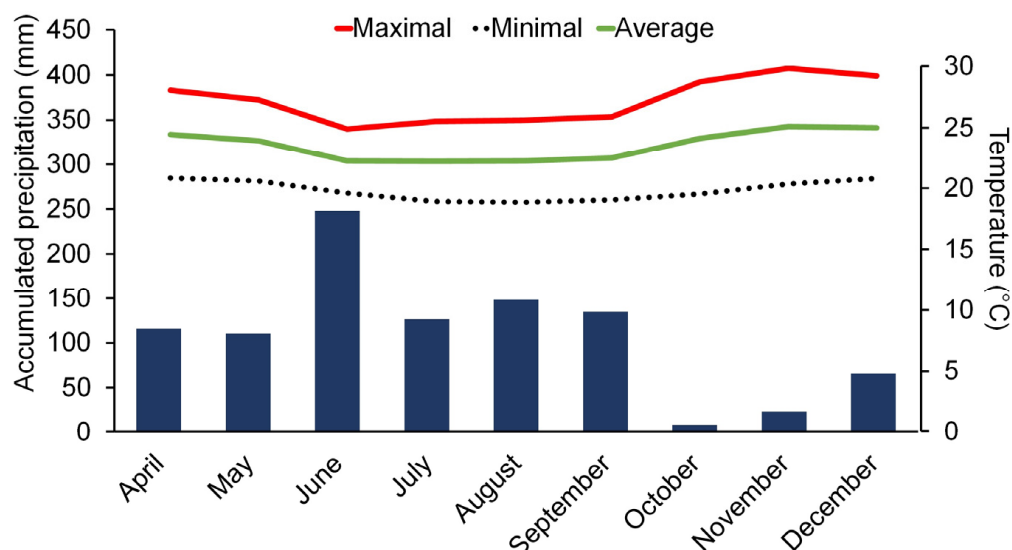
soil conditioners and biostimulants, most of the studies have focused on their application via roots.

Considering the importance of cassava as a staple food, as well as the need for sustainable practices that increase its yield, it is important to investigate the biostimulant potential of HSs in this crop. In this context, the objective was to evaluate the effects of the foliar application of a biostimulant based on humic substances on the morphophysiology, production, and mineral nutrient contents of a yellow cassava variety.

## 2. Materials and Methods

### 2.1. Experimental Site

The experiment was conducted from April to December 2023 in an experimental area at the Agriculture Sector of the Center for Human, Social and Agricultural Sciences of the Federal University of Paraíba (CCHSA/UFPB), located in the municipality of Bananeiras, Paraíba State, Brazil (6°45'25" S, 35°39'00" W, 624 m of altitude). The experimental site has been conducted under agroecological practices. The climate is classified as hot and humid [25]. The rainy season occurs from April to August and the dry season from September to March. The rainfall and temperature data during the experimental conduction are presented in Figure 1.



**Figure 1.** Accumulated precipitation (blue bars) and temperature (green, gray, and red lines) in the municipality of Bananeiras (PB) during the experimental period. Source: Aesa [26].

The soil in this area was classified as Oxisol, according to the criteria of the Brazilian Soil Classification System [27]. Table 1 shows the chemical characterization of the soil in the experimental area. The soil was analyzed in the Laboratory of Soils before the experiment (CCHSA/UFPB).

**Table 1.** Chemical characterization of the soil in the experimental area located in Bananeiras, Paraíba, Brazil.

pH <sub>(water)</sub>	P	K <sup>+</sup>	Na <sup>+</sup>	H+Al <sup>3+</sup>	Al <sup>3+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SB	CEC	OM
	mg dm <sup>3</sup>					cmol <sub>c</sub> dm <sup>3</sup>				g kg <sup>−1</sup>
6.36	34.09	30.52	0.02	2.64	0.05	2.30	3.20	5.60	8.24	27.69

P and K: Mehlich<sup>−1</sup> extractor; SB: sum of bases; CEC: cation exchange capacity; OM: organic matter.

## 2.2. Plant Material and Treatments

Stems of the biofortified yellow cassava cultivar BRS Dourada [28], donated by the Secretariat of Economic and Agrarian Development of the municipality of Mari-PB, were cut into cuttings with 2.5–3.0 cm length and at least 5 buds planted 10 cm deep. No additional irrigation was provided during the experiment. The experimental design was randomized blocks, with 4 blocks. Each block corresponded to two plots, each one containing 5 rows with 10 plants per line, totaling 400 plants. The spacing used was 1.00 m between rows and 0.60 m between plants, totaling 24 m<sup>2</sup> per plot. To avoid border effects, only the 3 central rows and the 6 central plants per row were considered, totaling 18 useful plants per plot (144 useful plants in total) distributed in 6 m<sup>2</sup>.

The treatments with the biostimulant based on humic substances (BHSs, treated) or water (untreated, control) started 60 days after planting (DAP) and lasted until the end of the experiment (225 DAP). The BHS was composed of 95% humic acids + fulvic acids + macro and micronutrients (1% N, 14% K<sub>2</sub>O, 1% Ca, 0.15% Mg, <0.001% Cu, 0.002% Zn, 0.50% Fe, and 0.02% B), with 50% of organic matter and 36% of total carbon. The solutions with BHSs or water were applied via foliar using a knapsack sprayer every 15 days, totaling 10 applications. The dosage used was 3 kg ha<sup>-1</sup> diluted in 500 L, according to the manufacturer's instructions. Briefly, 2.5 mL of mineral oil and 0.5 mL of a neutral detergent were added to the BHS and water (control) solutions to reduce surface tension.

## 2.3. Gas Exchanges and Chlorophyll Index

Gas exchanges were measured 225 DAP, in three fully expanded leaves of three plants per block. Measurements were performed from 8 h to 10 h in the morning using an infrared gas analyzer (model LCpro-SD Portable Photosynthesis System, ADC BioScientific, Hoddesdon, UK). The light source was an artificial light of 1000 µmol m<sup>-2</sup> s<sup>-1</sup>, while the reference CO<sub>2</sub> concentration, temperature, and humidity were kept ambient. The net CO<sub>2</sub> assimilation rate (*A*, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (*g<sub>s</sub>*, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (*E*, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), internal carbon concentration (*C<sub>i</sub>*), water use efficiency (WUE, *A/E*), and instantaneous carboxylation efficiency (*iCE*, *A/C<sub>i</sub>*) were determined.

Chlorophyll a and b indices were determined using a digital chlorophyll meter (ClorofiLOG<sup>®</sup> 1030, Falker, Porto Alegre, RS, Brazil). Measurements were taken in the same leaves as those used for measuring gas exchange parameters.

## 2.4. Growth and Productivity

Growth and productivity were determined 225 DAP in at least ten plants per block. The plant height and main stem diameter were determined with a tape measure and caliper. The leaf area and number of leaves were determined after separating, counting, and photographing the leaves, and analyzing the images using the ImageJ software version 1.53k (U.S. National Institute of Health, Bethesda, MD, USA). After that, the number of roots per plant was counted, and the shoot fresh mass (g), root fresh mass (g), and total fresh mass (g) were determined by weighing the different organs of cassava plants. Average root mass (g) was determined by dividing the total root mass per plant by the number of roots per plant, and the shoot/root ratio was determined by dividing shoot mass by root mass. The productivity (ton hectare<sup>-1</sup>) was calculated by extrapolating the root mass per plant per useful plot (18 plants, 6 m<sup>2</sup>) to the hectare area.

## 2.5. Soil Analysis and Mineral Nutrient Contents in Soil, Roots, and Leaves

Composite soil samples were collected before and after the experiment at a depth of 0–20 cm. The samples collected before the experiment were analyzed for soil fertility in the Soil Laboratory of the Center for Human, Social, and Agricultural Sciences of the Federal University of Paraíba (CCHSA/UFPB) according to Teixeira et al. [29] (Table 1). The soil samples collected after the experiment, as well as leaf and root samples, were analyzed for mineral nutrient contents at the National Semi-Arid Institute (INSA). For this, approximately 0.1 g of each sample was weighed directly into digestion tubes, and

then 5 mL of nitric acid was added. The tubes remained open for 15 min for pre-digestion. Then, the samples were subjected to the digestion process using a microwave digester with a closed system model MARS 6 (CEM, Matthews, NC, USA) with a 20 min ramp and 15 min of residence at 210 °C. After the digestion process, the sample extracts were dissolved in MilliQ water in 50 mL volumetric flasks. For the quantification of the analytes, a Microwave Plasma Atomic Emission Spectrometer (MP-AES), model 4200 Agilent (Agilent Technologies, Santa Clara, CA, USA), was used.

### 2.6. Statistical Analysis

The data were subjected to normality (Shapiro–Wilk) and homogeneity (Bartlett) tests, and, once the assumptions were met, an analysis of variance was performed (F-test,  $p \leq 0.05$ ). A principal component analysis was performed to assess the interrelation between variables and treatments. Also, a Pearson's correlation analysis was performed with 5% probability, using the Student's *t*-test. Statistical analyses were performed using the statistical software Genes version 2015.5.0 [30].

## 3. Results

None of the growth parameters evaluated here were affected by foliar application of BHSs (Table 2). Similarly, the BHSs did not affect root production (root mass per plant) and productivity (Table 2 and Figure 2).

**Table 2.** Growth, production, and physiological variables of 225-day-old BRS Dourada cassava plants treated with biostimulant based on humic substances (treated) or water (untreated).

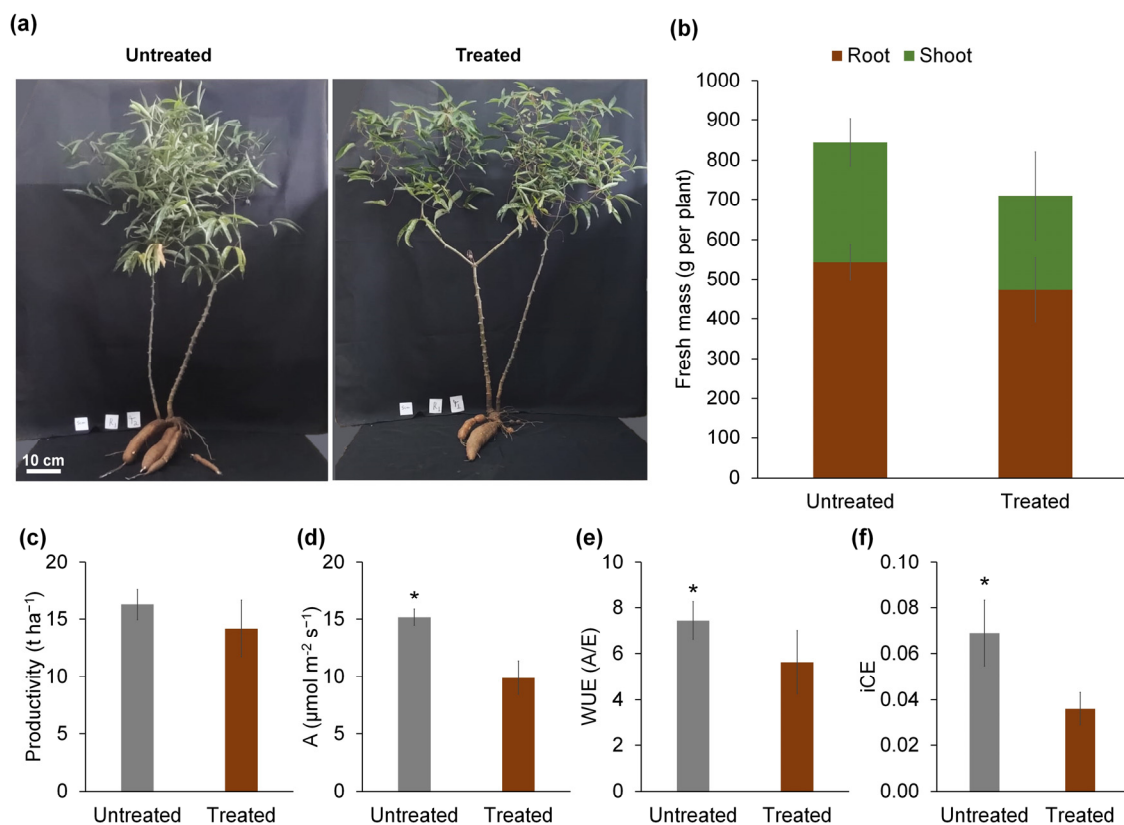
Variables	Untreated	Treated	<i>p</i> -Value
Plant height (cm)	65.03 ± 7.87	57.14 ± 2.66	0.13 <sup>ns</sup>
Main stem diameter (cm)	1.21 ± 0.20	1.14 ± 0.11	0.49 <sup>ns</sup>
Number of roots per plant	4.68 ± 0.67	4.13 ± 1.06	0.36 <sup>ns</sup>
Number of leaves per plant	73.64 ± 15.64	52.67 ± 11.60	0.17 <sup>ns</sup>
Root mass (g per plant)	542.87 ± 44.64	472.44 ± 82.20	0.19 <sup>ns</sup>
Shoot mass (g per plant)	300.96 ± 59.36	237.24 ± 110.84	0.50 <sup>ns</sup>
Total plant mass (g per plant)	843.83 ± 87.59	709.67 ± 171.33	0.31 <sup>ns</sup>
Average root mass (g per root)	133.43 ± 31.75	128.63 ± 29.22	0.85 <sup>ns</sup>
Shoot/root ratio	0.55 ± 0.10	0.49 ± 0.19	0.70 <sup>ns</sup>
Leaf area per plant (m <sup>2</sup> )	6598.61 ± 2395.78	11,664.05 ± 9326.61	0.26 <sup>ns</sup>
E (mmol H <sub>2</sub> O m <sup>−2</sup> s <sup>−1</sup> )	2.05 ± 0.16	1.81 ± 0.32	0.10 <sup>ns</sup>
gs (mol H <sub>2</sub> O m <sup>−2</sup> s <sup>−1</sup> )	0.21 ± 0.04	0.18 ± 0.02	0.18 <sup>ns</sup>
Chl a index	34.74 ± 3.25	37.68 ± 1.60	0.07 <sup>ns</sup>
Chl b index	10.63 ± 2.17	11.81 ± 1.05	0.27 <sup>ns</sup>
Total Chl index	45.36 ± 5.37	49.49 ± 2.59	0.11 <sup>ns</sup>

Values represent the mean ± standard deviation. <sup>ns</sup>: not significant by F-test ( $p \geq 0.05$ ).

No significant differences between treatments were found for fresh mass of shoots and roots and, consequently, for productivity (Figure 2a–c). Regarding physiological parameters, BHS application did not affect transpiration (E), stomatal conductance (gs), chlorophyll a, chlorophyll b, and total chlorophyll indices (Table 2). On the other hand, foliar BHS resulted in a lower net CO<sub>2</sub> assimilation rate (A), water use efficiency (WUE), and instantaneous carboxylation efficiency (iCE) compared to untreated plants (Figure 2d–f).

The foliar application of BHSs affected the content of Na, K, Zn, Fe, and Mn in the soil, while it only affected Na content in roots and K content in leaves (Table 3). The BHS application increased soil Na, K, Zn, and Mn contents but reduced Fe content in soil, compared to untreated samples. By contrast, BHSs reduced the contents of Na in roots and K in leaves (Table 3).





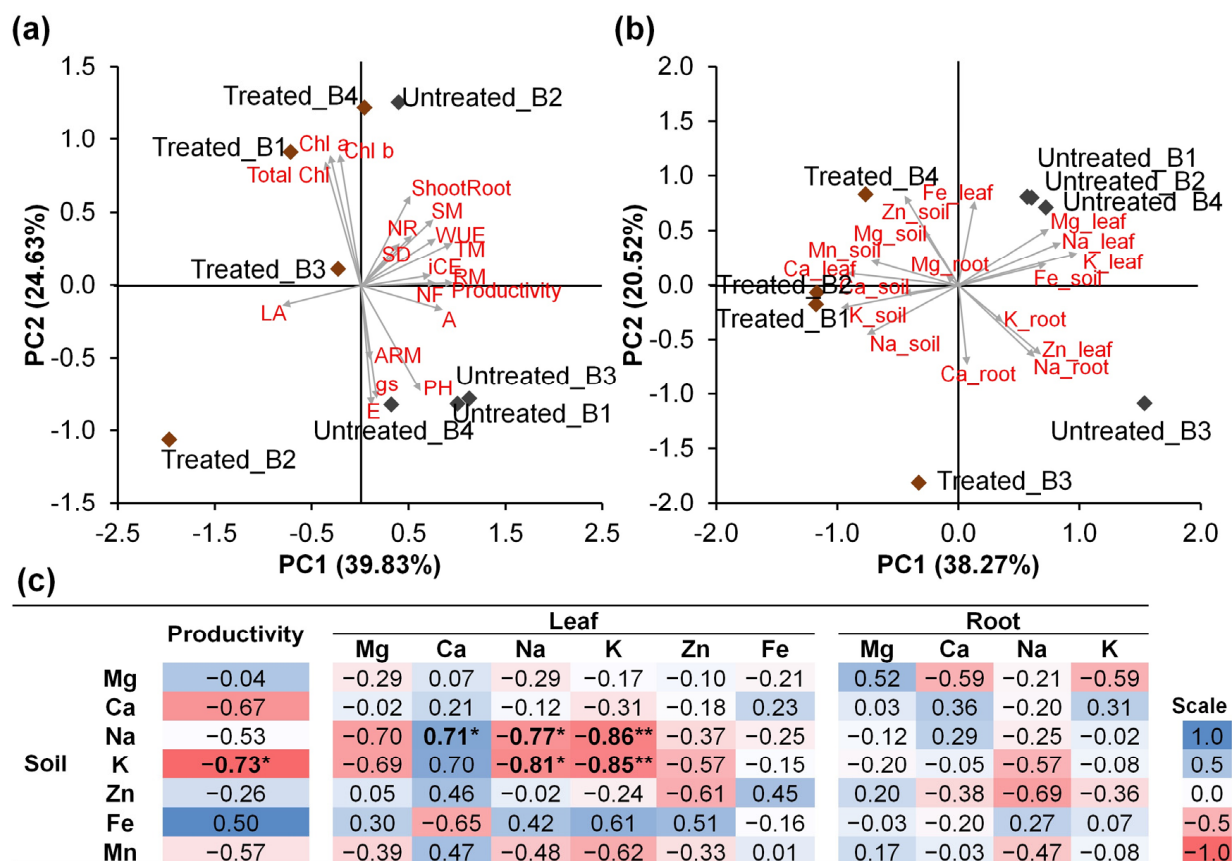
**Figure 2.** Images of representative plants, root and shoot biomass, productivity, and gas exchanges of 225-day-old cassava plants treated with biostimulant based on humic substances or water (untreated). Bars represent mean  $\pm$  SD. Asterisks indicate differences by F-test ( $p \leq 0.05$ ). (a) plant phenotype; (b) fresh mass of shoots and roots; (c) productivity; (d) net CO<sub>2</sub> assimilation rate—A; (e) water use efficiency—WUE; (f) instantaneous carboxylation efficiency—iCE.

**Table 3.** Mineral nutrient contents found in the soil after the experiment and in the roots and leaves of 225-day-old BRS Dourada cassava plants treated with biostimulants based on humic substances (treated) or water (untreated).

	Mineral	Untreated	Treated	p-Value
Soil	Mg (g/kg)	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.18 <sup>ns</sup>
	Ca (g/kg)	0.94 $\pm$ 0.07	1.04 $\pm$ 0.21	0.07 <sup>ns</sup>
	Na (g/kg)	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00	0.00 *
	K (g/kg)	0.10 $\pm$ 0.01	0.15 $\pm$ 0.03	0.00 *
	Zn (mg/kg)	6.77 $\pm$ 1.61	7.45 $\pm$ 1.98	0.01 *
	Fe (mg/kg)	100.90 $\pm$ 12.85	84.45 $\pm$ 14.86	0.00 *
	Mn (mg/kg)	11.75 $\pm$ 1.06	14.07 $\pm$ 1.99	0.00 *
Root	Mg (g/kg)	0.80 $\pm$ 0.20	0.81 $\pm$ 0.07	0.84 <sup>ns</sup>
	Ca (g/kg)	0.79 $\pm$ 0.08	0.85 $\pm$ 0.33	0.25 <sup>ns</sup>
	Na (g/kg)	0.50 $\pm$ 0.16	0.38 $\pm$ 0.21	0.00 *
	K (g/kg)	8.38 $\pm$ 2.67	7.51 $\pm$ 2.13	0.28 <sup>ns</sup>
Leaf	Mg (g/kg)	3.59 $\pm$ 0.03	3.51 $\pm$ 0.03	0.10 <sup>ns</sup>
	Ca (g/kg)	10.63 $\pm$ 0.08	10.73 $\pm$ 0.02	0.19 <sup>ns</sup>
	Na (g/kg)	0.12 $\pm$ 0.01	0.10 $\pm$ 0.01	0.07 <sup>ns</sup>
	K (g/kg)	10.55 $\pm$ 0.13	9.44 $\pm$ 0.06	0.00 *
	Zn (mg/kg)	46.59 $\pm$ 1.06	46.14 $\pm$ 0.75	0.13 <sup>ns</sup>
	Fe (mg/kg)	208.84 $\pm$ 1.42	208.03 $\pm$ 0.84	0.42 <sup>ns</sup>

Values represent the mean  $\pm$  standard deviation. <sup>ns</sup>: not significant by F-test ( $p \geq 0.05$ ). Asterisks indicate differences by F-test ( $p \leq 0.05$ ).

The principal component analysis based on morphophysiological characteristics and mineral nutrients allowed for the spatial separation of treatments, where the BHS treatment (treated) was mainly located in the third and fourth quarters, together with chlorophyll indices, leaf area (LA), and soil nutrients (Na, K, Ca, Mn, Zn, and Mg) (Figure 3a,b). On the other hand, the control treatment (untreated) was mostly located in the first and second quarters and was more related to growth, production, gas exchanges, and mineral nutrients in leaves (Fe, Mg, Na, K, and Zn) and roots (Ca, Na, K, and Mg) (Figure 3a,b).



**Figure 3.** Spatial dispersion of treatments and variables according to the first two principal components based on morphophysiological (a) and mineral nutrient (b) characteristics and Pearson's correlation analysis (c) between soil nutrient contents with productivity, leaf, and root mineral nutrient contents. Treated and untreated points on the graph represent the four blocks (B1, B2, B3, and B4). Asterisks indicate significant correlations by Student's *t*-test ( $p \leq 0.05$  or  $\leq 0.01$ ). A: net carbon assimilation; ARM: average root mass; Chl: chlorophyll; E: transpiration rate; gs: stomatal conductance; iCE: instantaneous carboxylation efficiency; LA: leaf area; NE: number of leaves; NR: number of roots; PH: plant height; RM: root mass; SD: stem diameter; SM: shoot mass; TM: total plant mass; WUE: water use efficiency. The arrows represent the contribution of original variables, and the diamonds represent the distribution of treatments. Blue represent positive correlations, white represent no correlations, and red represent negative correlations. \* and \*\* in bold values indicate significant correlations at 5 and 1% of probability, respectively (*t*-Student test).

Correlation analysis showed negative correlations between soil K with productivity (Figure 3c). Moreover, the contents of Na and K in soil were significantly correlated with nutrient contents in leaves but not in roots. For instance, soil Na was positively correlated with leaf Ca and negatively correlated with leaf Na and K. Soil K, in turn, was negatively correlated with leaf Na and K (Figure 3c).

#### 4. Discussion

The positive effects of HS Application on plant growth have been extensively demonstrated [6,21–24]. However, these effects depend on the source, structure, and dose of HSs, and the plant species, organ, and age [22]. Here, the spatial separation of treatments (treated and untreated) in the multivariate analysis based on morphophysiological variables pointed to a reduction in the growth and productivity of cassava upon foliar application of BHSs, as these variables were clustered in the same direction as the untreated samples (Figure 3a). Nonetheless, no significant differences were found between the growth and production of treated and untreated plants (Figure 2 and Table 2), indicating no effect of BHSs. In contrast, gas exchange parameters were grouped together and were closely distributed in the same quarters as untreated samples, which also coincided with univariate results that showed higher gas exchanges in untreated plants (Figure 2).

Stomatal closure, with subsequent impaired photosynthesis and growth inhibition, is an avoidance response of cassava against abiotic stresses, which is regulated by hormones, such as jasmonate (JA) and abscisic acid (ABA) [31]. HSs are reported to have stress-priming effects, inducing defense responses either through a weak acid effect or by triggering the biosynthesis of hormones such as auxins, ethylene, and (JA) [21,32,33]. Thus, the lower carbon assimilation upon HSB treatment observed here may be a result of the stress-priming effect of HSs. However, BHSs did not affect  $g_s$  and even reduced WUE, which do not correspond to defense responses, suggesting that, somehow, the BHS treatment impaired the photosynthetic capacity of cassava plants. This is further supported by the lower iCE, which indicates biochemical restraints during carboxylation. The reduction in carboxylation efficiency observed here may be related to the stress-induction effect of HSs [21]. Interestingly, leaf area and chlorophyll indices showed a dispersion pattern similar to that of the treated treatment, pointing to a possible effect of BHSs on increasing these characteristics; however, no statistical differences were found for these variables in univariate analysis.

BHSs are often provided to plants via roots/rhizosphere, where they improve the physical quality and fertility of soils while also inducing root-triggered physiological responses; however, foliar HS application has biostimulant effects, modulating physiological mechanisms and increasing growth without affecting soil properties [21]. Due to their effect on soil fertility, HSs are good options for areas where soils have low fertility. Under such conditions, a foliar application of BHSs has been reported to increase cassava production [6]. Nonetheless, this was not the case here, as the soil on the experimental site had good fertility attributes (Table 1), which may help to explain the lack of effects of BHS treatment on cassava growth and productivity.

The root application of HSs increases the activity of root  $H^+$ -ATPase and the shoot mineral nutrient contents, which does not occur upon foliar HS application [21]. As the activity of root  $H^+$ -ATPase affects the nutrient uptake, this may explain the results observed here, as mineral nutrient contents (Na, K, Zn, and Mn) were higher in the soil upon foliar BHS application, suggesting a lower nutrient uptake. In turn, root Na and shoot K were lower under BHS treatment, suggesting lower nutrient accumulation in shoots. These results are further confirmed by multivariate spatial dispersion, where leaf and root nutrients were clustered in similar directions as untreated samples, while soil nutrients were grouped in the opposite direction, together with treated samples (Figure 3). Moreover, soil K was negatively correlated with productivity, suggesting that higher soil K, found in BHS-treated plants, was a result of lower K uptake, probably affecting productivity. The negative correlations between soil K and Na with leaf Na and K also point to reduced K and Na uptake, which, in turn, reduces leaf Na and K contents.

Taken together, our results show that foliar application of BHS reduced the photosynthetic capacity and nutrient uptake and transport but did not affect cassava growth and productivity. The lack of effect of BHSs on cassava may be related to foliar application, as the efficiency of this application mode depends on leaf characteristics such as wax layers and stomatal aperture, as well as on adequate environmental conditions such



as temperature, winds, and precipitation [24,34]. Moreover, the evidence showing that foliar BHSs can induce stress responses [21,22,32] may help to explain the reduction in the photosynthetic capacity of cassava. Another aspect involved in the negative effects of BHS treatment observed here may be related to the dose, application mode, and frequency used, as high HS doses and application rates are reported to cause leaf burns, mainly under high-temperature conditions [22,24]. Thus, further studies are required to evaluate other doses, sources, and application modes.

## 5. Conclusions

Cassava is a resilient crop with great sustainable potential and is essential for food security in developing countries. The valorization of this crop and the development of sustainable agricultural management techniques, such as the use of HSs, are essential not only for food and income production but also for the preservation of biomes, ethnovarieties, and culture in traditional communities. Here, a foliar application of a biostimulant based on humic substances (BHSs) reduced the photosynthetic capacity and nutrient uptake but did not affect the growth and productivity of cassava plants. Thus, a foliar application of 3 kg ha<sup>−1</sup> BHS is not recommended for cassava under the application mode and frequency, climate, and soil characteristics evaluated here. More studies are necessary to compare other BHS doses and application modes, aiming at increasing cassava production.

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