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Fertilizer Performance of a Digestate from Swine Wastewater as Synthetic Nitrogen Substitute in Maize Cultivation: Physiological Growth and Yield Responses

Eduardo L. Buligon [1], Luiz A. M. Costa [1], Jorge de Lucas, Jr. [2], Francielly T. Santos [1], Piebiep Goufo [3,*] and Monica S. S. M. Costa [1,*]

[1] Agricultural Engineering Graduate Program, Universidade Estadual do Oeste do Paraná, Street University 2069, Cep, Cascavel 85819-110, Brazil
[2] Departamento de Engenharia Rural, Universidade Estadual Paulista "Júlio de Mesquita Filho", Via de Acesso Prof. Paulo Donato Castellane s/n—Jaboticabal/SP—Cep, São Paulo 14884-900, Brazil
[3] CITAB—Centro de Investigação e Tecnologias Agroambientais e Biológicas, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000–801 Vila Real, Portugal
* Correspondence: pgoufo@utad.pt (P.G.); monicasarollisilva@gmail.com (M.S.S.M.C.)

Abstract: Nitrogen (N) is the primary nutrient required for plant growth. During the last few decades, there has been extensive use of synthetic N-containing fertilizers in agriculture, resulting in increased environmental pollution. In this study, the feasibility of replacing synthetic N with biofertilizer in maize cultivation was investigated. A liquid biofertilizer (digestate obtained from the anaerobic digestion of swine wastewater) was obtained and applied to large plots as a total (100%) or partial (50%) substitute for synthetic N fertilizer. Moreover, the most efficient fertilization mode, i.e., basal versus foliar application, was studied. Physiological growth indices, leaf nutritional status, and grain yield were assessed for each biofertilization treatment and compared with the conventional treatment with synthetic minerals. Compared with the conventional treatment, the total substitution of synthetic N by the biofertilizer (basal application) did not affect the growth parameters and grain yield of maize; the other treatments usually resulted in lower growth rates and yields, although not statistically significant ($p \geq 0.05$). No difference was observed among the treatments for the contents of N, P, K, or Mg in the leaves. Generally, the highest means for Fe, Ca, Cu, Zn, and Mn contents in leaves were observed after in-row broadcast of synthetic fertilizers or basal application of the digestate as a total substitute for synthetic N, with a significant effect for Fe ($p < 0.05$). The mode of the biofertilizer application did not have any significant effect on either growth parameters or leaf nutrients. The data show that under the specific conditions of the study, the total substitution of mineral N with basal application of biofertilizer is the best strategy for minimizing the use of synthetic chemicals in maize cultivation without yield penalties.

Keywords: waste valorization; methane-rich biogas; bio-based fertilizers; soil amendments; organic fertilizers; foliar application; anaerobic digestion; corn growth dynamic; nutrient availability; fertilizer application rate/dose; drought stress mitigation

Citation: Buligon, E.L.; Costa, L.A.M.; de Lucas, J., Jr.; Santos, F.T.; Goufo, P.; Costa, M.S.S.M. Fertilizer Performance of a Digestate from Swine Wastewater as Synthetic Nitrogen Substitute in Maize Cultivation: Physiological Growth and Yield Responses. *Agriculture* **2023**, *13*, 565. https://doi.org/10.3390/agriculture13030565

Academic Editor: Maria Roulia

Received: 8 December 2022
Revised: 20 February 2023
Accepted: 22 February 2023
Published: 26 February 2023

1. Introduction

Brazil is nowadays a major player in international agricultural production and export [1,2]. However, Brazil is heavily dependent on the import of NPK (nitrogen, phosphorus, and potassium) fertilizers. It is estimated that 85% of the fertilizers used in Brazil are imported from the global market [1]. These data have prompted research on alternative local nutrient sources in agriculture, such as stabilized organic wastes, e.g., compost, vermicomposto, and biofertilizer. In 2020, manure production in Brazil was estimated at 61.8 million tons, of which 14.9 million tons was from poultry litter and 46.9 million tons from swine and cattle manure [3].

Before application to agricultural soils as a source of nutrients, organic waste needs to be stabilized, i.e., active biological decomposition processes need to be stopped to minimize disruption of the soil-plant-root interface as much as possible [4–7]. Stabilization can be achieved through several processes such as composting, vermicomposting, mechanical-biological treatments, or anaerobic digestion to produce bio-based products called "biofertilizers" [6,8–11]. Anaerobic digestion involves the degradation of organic matter in an oxygen-free environment to release a gas known as biogas and an organic effluent + residue called digestate [12,13].

According to Koszel and Lorencowicz [14], the main factors determining the use of digestates as biofertilizers are the physicochemical characteristics of the effluent and residue, the edapho-climatic conditions, and local regulations. Locoli et al. [15] found that digestates obtained from the anaerobic digestion of cattle manure, chicken litter, swine manure, and onion wastes had chemical and spectroscopic characteristics (e.g., C/N ratio, ammonium (NH_4^+) to nitrogen ratio (NH_4^+/N), and proportion of short-chain organic acids) similar to those of untreated wastes. Soils amended with the digestates in the study by Locoli et al. [15] emitted less CO_2 than soils amended with manure, and the fertilizing effect of the digestates on lettuce growth was related to the content of NH_4^+ [15]. However, recent review papers have found that feedstock, processing technology, and process operating conditions strongly influence the characteristics of digestates, and that without comprehensive management strategies, digestates can contribute to nutrient pollution [6,13]. Thus, each locally produced digestate should be assessed on its own merit based on optimal parameters suitable for adequate microbial activity such as the C/N ratio (15–30), the psychrophilic temperature (<20 °C), the mesophilic temperature (35–37 °C), and the thermophilic temperature (55 °C) [13].

Using indicators such as N uptake, P recovery rate, soil microbial stimulation, or yield, the biofertilizing effect of various digestates on different plants in different climates and soils has been confirmed. Results from a trial in Belgium indicated that the liquid fraction of a digestate obtained from swine manure could substitute synthetic N fertilizers without maize yield losses [16]. Tsachidou et al. [17] reported that the partial substitution of chemical fertilizers with a raw digestate from bovine manure as the sole source of N reduced the concentration of NO^{3-} in the soil without impacting biomass yield and N content in a pasture system. Zilio et al. [18] concluded that the maize grain yield obtained from plants grown in a soil that received a sewage sludge-based digestate is equivalent to the yield obtained from plants grown using urea. The abovementioned three examples show that a stable digestate can be used as a bio-based fertilizer to replace mineral N fertilizers without yield loss or without increasing the risk of environmental pollution. However, only a few large-scale studies have been conducted on the effects of digestates on crop growth. This highlights the need for more studies that contribute different variables to decision making on the use of biofertilizers in agriculture. Further studies in different locations are essential to gather the necessary information to formulate standardized international protocols for researchers and operators, to develop best management practices for farmers, and to promote digestate product commercialization as part of the organic waste circular economy paradigm.

The study reported in this paper aimed to contribute to the topic by testing different biofertilizer doses and application techniques. Specifically, the effects of a digestate from swine wastewater were evaluated on maize physiology, considering four application scenarios: (i) soil application with a total (100%) replacement of the amount of recommended mineral N; (ii) foliar application with a total (100%) replacement of the amount of recommended mineral N; (iii) soil application with a partial (50%) replacement of the amount of recommended mineral N; and (iv) foliar application with a partial (50%) replacement of the amount of recommended mineral N. The impact of the digestate on maize production was compared to that of the conventional fertilization practice using synthetic fertilizers. The applied treatments were evaluated over six months, with a focus on leaf morphology, physiological indices, dry matter (DM) content in maize, and grain yield.

2. Materials and Methods

2.1. Study Area

The field experiment was conducted at the Experimental Station of the Agricultural Engineering Department of the Western Paraná State University (UNIOESTE) in Cascavel municipality, PR, Brazil. Cascavel is located geographically between 24°57′21″ S and 53°27′19″ W. According to the Köppen–Geiger classification system, the predominant climate in Cascavel is Cfa. The Cfa climate is characterized by infrequent frosts, hot summers, and a trend of rainfall concentration in the summer [19]. The average annual temperature, atmospheric pressure, and rainfall at the experimental site were 20 °C, 936.34 hPa, and 1841 mm, respectively. Soil samples at the experimental site were collected from 0–20 cm depths. The samples were bulked, air dried, gently crushed, and sieved through a 2 mm sieve before analyses by Solanalise Central De Analises Ltd., an accredited laboratory in Cascavel. The clay-textured soil at the site was classified as Dystroferric Red Latosol (Oxisol), and its chemical characteristics are shown in Table 1.

Table 1. Main chemical characteristics of the soil in the experimental area before fertilization. Contents were classified as low, medium, or high based on the ranges specified in the Paraná State Handbook for Fertilization and Liming [20].

Nutrient	Unit	Content	Classification
Ca^{2+}	$cmol_c\ dm^{-3}$	4.59	High
Mg^{2+}	$cmol_c\ dm^{-3}$	1.61	High
PO_4^{3-}	$mg\ dm^{-3}$	6.38	Medium
K^+	$cmol_c\ dm^{-3}$	0.36	High
Al^{+3}	$cmol_c\ dm^{-3}$	0.31	Low
H + Al	$cmol_c\ dm^{-3}$	9.01	High
Sum of bases	$cmol_c\ dm^{-3}$	6.56	High
Cation exchange capacity at pH 7.0	$cmol_c\ dm^{-3}$	15.57	High
Cation exchange capacity efective	$cmol_c\ dm^{-3}$	6.87	High
Carbon	$g\ dm^{-3}$	25.75	High
Organic matter	$g\ dm^{-3}$	44.29	High
Aluminum saturation	%	4.51	Low
Base saturation	%	42.13	Low
B	$mg\ dm^{-3}$	0.24	Low
S	$mg\ dm^{-3}$	4.59	Low
Fe^{+2}	$mg\ dm^{-3}$	27.40	Medium
Mn^{+2}	$mg\ dm^{-3}$	43.40	High
Cu^{+2}	$mg\ dm^{-3}$	5.20	High
Zn^{+2}	$mg\ dm^{-3}$	1.80	Medium
pH ($CaCl_2$)	NA	4.60	NA

NA = not available or not applicable.

2.2. Materials

2.2.1. Test Crop

The maize cultivar used in this study was the hybrid P3380HR. The preceding crop was soybean. Following soybean harvesting, atrazine 500 SC (Nortox, Arapongas, Minas Gerais, Brazil) was applied at 1.0 kg ha^{-1} to control the remaining crops and weeds before maize planting. Maize was mechanically broadcast-seeded in March 2021 at a density of 2.8 seeds per m^2. After seedling emergence 5 d after sowing (DAS), plants were thinned to one plant per hole to homogenize the planting and avoid any influence of spacing and shading on nutrient absorption by individual plants. Harvesting occurred manually in August 2021. Insect control focused on the maize leafhopper, *Dalbulus maidis*, with three applications of the insecticide acephate 750 (Ameribrás, Cotia, São Paulo, Brazil) at 1.0 kg ha^{-1} and Galil 300 SC (Adama, Londrina, Paraná, Brazil) at 250 mL ha^{-1}. No other standard agricultural practices, e.g., irrigation, were implemented.

2.2.2. Mineral Fertilizers

The mineral fertilizers used in this study were urea (46% N), potassium chloride (60% K_2O), and single superphosphate (18% P_2O_5, 17.3% Ca, 3.3% Mg, and 5% S). Mineral fertilizers were obtained from a local vendor. The nutritional recommendations for maize were calculated based on the soil characteristics in Table 1, following the methodology proposed by the Paraná State Handbook for Fertilization and Liming [20]: 120 kg ha^{-1} of N, 110 kg ha^{-1} of P_2O_5, and 70 kg ha^{-1} of K_2O.

2.2.3. Biofertilizer

The biofertilizer was produced by the Laboratory of Agroindustrial Waste Analysis of UNIOESTE using swine wastewater (1.78% of total solids). Anaerobic digestion of the swine wastewater was performed in a horizontal tubular benchtop reactor tank operated in a semi-continuous flow with a volume of 60 L, a hydraulic retention time of 30 d, and a mesophilic-controlled temperature of 35 ± 1 °C. The obtained liquid digestate was used as biofertilizer without any post-processing treatment. The biofertilizer was stored in closed plastic barrels at room temperature until further use.

The biofertilizer was sampled and analyzed to determine its physicochemical characteristics and calculate the amount to be applied to the field. All chemicals used for the analyses were obtained from Química Moderna (Barueri, São Paulo, Brazil). Total Kjeldahl N was determined by digesting the samples with sulfuric acid, followed by distillation and titration using 0.0025 mol of H_2SO_4 [21]. The concentrations of P and K were determined by digesting the samples in a nitric-perchloric acid solution (3:1) with an external heat source, followed by dilution and filtration. Phosphorus was detected by measuring the absorbance at a wavelength of 725 nm in a 700 Plus UV/Vis spectrophotometer (Femto Indústria e Comércio de Instrumentos, São Paulo, São Paulo, Brazil) using the ascorbic acid method. Potassium was quantified using a DM-62 flame photometer (Digimed, Campo Grande, Mato Grosso do Sul, Brazil) as described by Malavolta et al. [21]. The levels of micronutrients (Fe, Zn, Cu, and Mn) and secondary macronutrients (Mg and Ca) were determined by atomic absorption spectroscopy (Shimadzu AA6300, Tokyo, Japan) prior to the digestion of samples with a nitric-perchloric solution (3:1) [22]. The results of the analysis are presented in Table 2.

Table 2. Main chemical characteristics of the liquid biofertilizer (digestate of swine wastewater) tested in the study.

Nutrient Unit	N	P_2O_5 g L^{-1}	K_2O	Ca	Mg	Cu	Fe mg L^{-1}	Mn	Zn
Content	2.50 ± 0.11	0.45 ± 0.15	0.15 ± 0.0	77.42 ± 5.8	0.08 ± 0.08	3.92 ± 0.02	7.45 ± 0.11	1.36 ± 0.08	2.08 ± 0.01

2.3. Experimental Design and Treatments

The experiment was conducted in a completely randomized block design with five treatments and four replications, totaling 20 plots spread across the field. Each of these plots was 3.2 m wide and 10 m long. The total area occupied by the experiment was 520 m^2, 13 m wide and 40 m long. The treatments consisted of two doses of biofertilizer (100 and 50%) established based on the recommended rate of N for maize cultivation in Paraná State [20], two forms of application (basal application on the soil surface and foliar application on the whole plant), and one control treatment (synthetic urea, potassium chloride, and single superphosphate as mineral fertilizers). A detailed description of these treatments is provided in Table 3.

Based on the chemical characterization of the digestate and the composition of synthetic chemicals, a combination of biofertilizers and mineral fertilizers was prepared. All combinations were made ensuring no limiting nutrients and the same N content for all treatments but with 100 and 50% reduced synthetic N amounts for T_1 and T_2 as well as T_3 and T_4, respectively, as shown in Table 4.

Table 3. Description of the five fertilization treatments used in the experiment.

Treatments	Application Mode	Description
T_1	Basal	Dose of biofertilizer corresponding to 100% of the amount of recommended mineral N + P and K supplementation with synthetic fertilizers
T_2	Foliar	Dose of biofertilizer corresponding to 100% of the amount of recommended mineral N + P and K supplementation with synthetic fertilizers
T_3	Basal	Dose of biofertilizer corresponding to 50% of the amount of recommended mineral N + N, P, and K supplementation with synthetic fertilizers
T_4	Foliar	Dosage of biofertilizer corresponding to 50% of the amount of recommended mineral N + N, P, and K supplementation with synthetic fertilizers
T_5	In-row broadcasting	Mineral fertilization as recommended for the maize crop in the Paraná State of Brazil

Table 4. Nutrient composition and application rates (L $Plot^{-1}$) for the different fertilization treatments.

Treatments	Biofertilizer (L)	Nutrient Composition of the Biofertilizer (g)			Synthetic Nutrients Applied Directly to Soil (g)		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O
T_1	122.88	307.20	56.30	19.00	0	225.30	160.20
T_2	122.88	307.20	56.30	19.00	0	225.30	160.20
T_3	61.44	153.60	28.10	9.50	153.60	253.50	169.70
T_4	61.44	153.60	28.10	9.50	153.60	253.50	169.70
T_5	0	0	0	0	307.20	281.60	179.20

The precise volume of biofertilizer for treatments T_1–T_4 was dispersed to the soil around the plants or on the leaves and all over the plants using watering cans. To satisfy crop nutrient requirements for treatments T_1–T_4, an adequate amount of mineral N-urea, P_2O_5, and K_2O powder was mixed and manually broadcast evenly over the soil surface. In the case of treatments T_3–T_4, mineral fertilizers were watered into the soil with 61.44 L $Plot^{-1}$ of tap water. As a reference treatment (T_5), mineral fertilizers were mixed, manually broadcast evenly over the soil surface, and watered into the soil with 122.88 L $Plot^{-1}$ of tap water, a volume equivalent to the volume of the digestate in treatments T_1 and T_2. The treatments were applied at two distinct phases of growth: at the beginning of the growing period to boost the development of plants, and during the period of high demand for nutrients and water for plants to enter reproductive growth. Specifically, 30% of fertilizer was applied at vegetative stage V1 (7 DAS), when plants had one visible leaf collar, and 70% at vegetative stage V10 (45 DAS), when plants began steady and rapid periods of growth and DM accumulation.

2.4. Parameter Measurements

2.4.1. Morphological and Physiological Parameters

Data on growth traits were collected at 20, 40, 60, 80, and 100 days after seedling emergence (DAE) at 20-day intervals to cover the main vegetative and reproductive stages of maize. During each sampling period, two plants were selected randomly, cut off at the base with a knife, and separated into leaves, stems, and ears, when present. Then, the number of leaves was counted. The width and length of all leaves were measured in centimeters using a ruler, averaged, and the leaf area was calculated and expressed using the formula proposed by Guimarães et al. [23] (Table 5). The leaves, stems, and ears were oven-dried separately at 105 °C until they reached a constant weight, recorded as DM. Total

DM and the ear DM: Total DM ratios were calculated. Based on leaf area and DM data, physiological indices were estimated using the mathematical equations (Table 5) proposed by Benincasa [24].

Table 5. Morphological and physiological parameters assessed in this study pertaining to maize growth and the respective equations used for their determination as described in Guimarães et al. [23] and in Benicasa [24].

Physiological Indices	Equation	Description of Abbreviations
Total dry matter (W)	$W = W_L + W_S + W_E$	W_L = leaf dry matter (g) W_S = stem dry matter (g) W_E = ear dry matter (g)
Total leaf area (TLA)	$TLA = 0.7458 \times L_W \times L_L$	L_W = leaf width (cm) L_L = leaf length (cm)
Leaf area index (LAI)	$LAI = TLA/S$	TLA = total leaf area (cm^2) S = soil surface area (cm^2)
Absolute growth rate (AGR)	$AGR = (W_2 - W_1)/(t_2 - t_1)$	W = total dry matter (g) t = time (d) 1,2 = two successive sampling periods Ln = Naperian logarithm
Relative growth rate (RGR)	$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$	W = total dry matter (g) t = time (d) 1,2 = two successive sampling periods Ln = Naperian logarithm
Leaf area relative growth rate (RGR_{LA})	$RGR_{LA} = (\ln TLA_2 - \ln TLA_1)/(t_2 - t_1)$	TLA = total leaf area W = total dry matter (g) t = time (d) 1,2 = two successive sampling periods
Net assimilation rate (NAR)	$NAR = [(W_2 - W_1)/(t_2 - t_1)] \times [(\ln TLA_2 - \ln TLA_1)/(TLA_2 - TLA_1)]$	TLA = total leaf area W = total dry matter (g) t = time (d) 1,2 = two successive sampling periods
Leaf area ratio (LAR)	$LAR = (TLA/W)$	TLA = total leaf area W = total dry matter (g)
Specific leaf area (SLA)	$SLA = (TLA/W_E)$	TLA = total leaf area W = total dry matter (g)

2.4.2. Leaf Nutritional Status

Maize leaves were collected at silking stage R1 when female inflorescences were visible on 50–75% of the plants (80 DAE). The R1 stage is characterized by complete K uptake and rapid N and P uptake, making R1 the most critical stage in determining the yield potential [25,26]. The third leaf, counted from the base and below the first (upper) ear, was cut with a knife, and the midrib was removed and discarded. The remaining leaf portion was washed with distilled water and oven-dried at 50 °C until a constant weight was achieved. The nutrient composition of leaves was determined using the method proposed by Martinez et al. [27]. Briefly, leaf samples were powdered in a home mixer and sieved. N in 1 g of powdered sample was estimated using the micro-Kjeldahl method, whereas P and K were analyzed after digestion of the samples in a nitric-perchloric acid solution using a UV/Vis spectrophotometer and a flame photometer, respectively (as described in Section 2.2.3). Ca, Mg, Cu, Fe, Mn, and Zn were analyzed in the nitric-perchloric extract using an atomic absorption spectrophotometer. The nutrient content of the leaves was expressed on a dry-weight basis. The nutritional values obtained were compared with the reference values in mg kg^{-1} considered suitable for maize in the State of Paraná in Brazil [20], that is, N (27,000–35,000), P (1900–4000), K (17,000–35,000), Ca (2300–8000), Mg (1500–5000), Cu (6–20), Fe (30–250), Mn (20–200), and Zn (15–100).

2.4.3. Maize Grain Yield

All ears were harvested at the full-grain maturity stage (120 DAS), at approximately 25% moisture content; the ears were collected manually from the plants in each plot, excluding border rows. The ears were mechanically dehusked and threshed to collect grains using a maize sheller coupled to a tractor. The grains were oven-dried at 105 °C until a moisture content of 13% was attained, which is a safe level for storage and commercialization. The mean weight of the samples was recorded using a scale and was expressed in kg ha^{-1}.

2.5. Statistical Analyses

All data were subjected to an analysis of variance using the statistical program Sisvar 5.6-Build 86 [28]. All variables satisfied the requirements of normal distribution and homoscedastic assumption of variance after examination using the Shapiro–Wilk test and Levene's test, respectively. Therefore, the mean value (n = 4) was calculated without data transformation. Differences between means were evaluated using Tukey's honest significant difference (LSD) test at the 5% probability level. The coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean to show the extent of variability concerning the mean for all treatments.

3. Results and Discussion

3.1. Effects of the Biofertilizer on Maize Grain Yield

In comparison with T_5 (mineral fertilizers), maize grain yield was not affected ($p < 0.05$) by the use of biofertilizers, although T_3 and T_4 (partial substitution of chemical fertilizers by the digestate) tended to lead to lower yields (Figure 1). The average grain yield of all treatments was 1274.52 kg ha^{-1}, which was well below the 5370 kg ha^{-1} average in Brazil in the experimental year [29]. The low maize yield in the testing field could be explained by the drought event in 2021, specifically acute in June, which coincided with the flowering and pollination stages of maize [25].

The data in Figure 1 expressly indicated the beneficial fertilizing properties of the biofertilizer. A major downside limiting the widespread adoption of biofertilizers by farmers is the long time it takes for organic matter to be oxidized into easily available nutrients [6,13]. However, bio-based fertilizers are known for their high variability in nutrients, and the starting material and processing conditions strongly influence the characteristics of the final product [4,12]. The yield data obtained in the present study could be explained by (i) a high level of NH_4^+ in the swine wastewater-based digestate and/or (ii) a fast and efficient conversion of organic N in the digestate into NH_4^+, an inorganic form of N easily absorbed by the plant root system. Although NH_4^+ levels were not determined in the present study, a reasonable correlation was established between vegetal growth and the level of NH_4^+ in a digestate obtained from cattle manure, poultry litter, and pig slurry [15]. Moreover, Costa et al. [4] observed that more than 60% of the total N in biofertilizers produced with beef cattle manure was NH_4^+. Results from trials in Argentina, Italy, and Belgium have also indicated that digestates applied at adequate dosages to the soil may substitute synthetic N fertilizers without crop yield losses [15–18]. In the former example, digestate application to soil produced a fast and short microbial stimulation [15]. In the latter examples, the digestates contributed to a short-term renewal of soil organic matter [16–18]. The results of the study presented in this paper further indicate that the N mineralization rate of some biofertilizers can be very fast, resulting in noticeable effects within months.

3.2. Effects of the Biofertilizer on the nutritional Value of Maize Leaves

There was a significant difference ($p < 0.05$) in the Fe content of maize leaves grown with biofertilizers and mineral fertilizers (Table 6). The lowest Fe content was observed in leaves from T_3 at 217.66 mg kg^{-1}, statistically different from the content in leaves from T_1. Overall, treatments T_1 and T_5 resulted in the highest Fe contents of 540.68 and 326.55 mg kg^{-1}, respectively. Although not significant ($p > 0.05$), higher Ca, Cu, Zn, and Mn contents were observed in leaves from T_1 and T_5 than in leaves from the other treatments. A lack of treatment effect on the contents of N, P, and K in maize leaves (Table 6) reinforces the grain yield results (Figure 1) and underlines the suitability of biofertilizers as partial or total substitutes for chemical fertilizers in the cultivation of maize. However, data from Figure 1 and Table 6 indicate better results with the total replacement of mineral fertilizers than with partial replacement. In a previous study [30], foliar application of a biofertilizer obtained from sewage sludge increased the contents of macro- and micronutrients in maize leaves; when the biofertilizer was applied directly to the soil, however, the contents of nutrients analyzed in both soil and leaves were not affected.

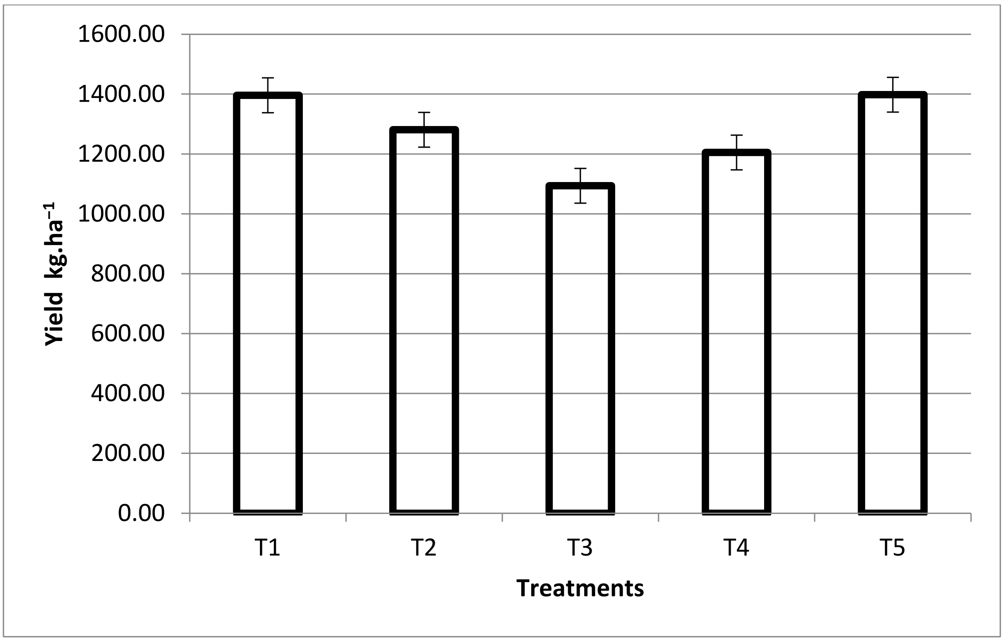

Figure 1. Effect of fertilization treatments on maize grain yield. T_1 = basal application of the digestate from swine wastewater as total substitute (100%) for synthetic N; T_2 = foliar application of the digestate as total substitute (100%) for synthetic N; T_3 = basal application of the digestate as partial substitute (50%) for synthetic N; T_4 = foliar application of the digestate as partial substitute (50%) for synthetic N; T_5 = in-row broadcast of mineral fertilizers. The treatments are fully described in Table 3. Mean bars with no letter or same letters are not statistically different (Tukey's HSD, $p < 0.05$, $n = 4$). Error bars indicate standard deviations.

Leaf nutrient analysis is an effective tool for diagnosing nutrient deficiency or excess in plants and for visualizing the capacity of plants to absorb nutrients from different fertilizers [21,27]. The Ca, Zn, and Mn contents in the leaves were within the reference range for maize in Paraná State; the N, Cu, and Fe contents in the leaves were above the reference values, whereas the P, K, and Mg contents were much lower than the minimum values reported in the literature [20] (Table 6). Cu [31], Fe ([32], Mn [33], and Zn [34] are among the micronutrients that directly affect photosynthesis. The biofertilizer contained a significant amount of Cu and Fe (Table 2), whereas mineral fertilizers were devoid of these two nutrients. However, the soil Cu content was considered high (Table 1), and the Dystroferric Red Latosol used in the experiment was characterized by the presence of high levels of Fe and aluminum oxide [35]; these two observations probably explain, respectively, the high contents of Cu and Fe in leaves following plant uptake. The P content was approximately 20 times less than the reference value, indicating a deficiency [26]. The low P level was however inconsistent with that in the soil (Table 1) or the digestate (Table 2), and the possible reason for this is that more Cu and Fe ions in the leaves affected P absorption and utilization [36,37]. Moreover, the low soil moisture resulting from the drought that occurred during maize growth could have damaged the root structure and reduced water and nutrient absorption, including P [37]. The same explanation holds for the K and Mg contents.

Table 6. Effect of fertilization treatments on the contents of nutrients in maize leaves at the R1 silking stage. T_1 = basal application of the digestate from swine wastewater as total substitute (100%) for synthetic N; T_2 = foliar application of the digestate as total substitute (100%) for synthetic N; T_3 = basal application of the digestate as partial substitute (50%) for synthetic N; T_4 = foliar application of the digestate as partial substitute (50%) for synthetic N; T_5 = in-row broadcast of mineral fertilizer. The treatments are fully described in Table 3.

Treatments	T_1	T_2	T_3	T_4	T_5	CV (%)	Reference
N (g kg^{-1})	40.60	38.60	42.00	40.50	37.80	12.40	27–35
P (g kg^{-1})	0.11	0.08	0.09	0.08	0.09	32.83	1.9–4.0
K (g kg^{-1})	11.40	12.10	8.40	10.30	9.80	18.88	17–35
Ca (g kg^{-1})	6.80	5.80	8.50	6.30	8.50	50.53	2.3–8.0
Mg (g kg^{-1})	0.035	0.029	0.041	0.034	0.037	30.11	1.5–5.0
Cu (mg kg^{-1})	140.90	68.61	69.34	85.27	172.00	66.19	6–20
Zn (mg kg^{-1})	67.54	54.43	54.73	50.25	71.24	30.99	15–100
Fe (mg kg^{-1})	540.68 ^{A}	313.03 ^{AB}	217.66 ^{B}	300.97 ^{AB}	326.55 ^{AB}	41.23	30–250
Mn (mg kg^{-1})	36.94	31.38	26.44	29.31	37.61	56.07	20–200

Mean within a row followed with no letter or same letters are not statistically different (Tukey's HSD, $p < 0.05$, $n = 4$). CV = coefficient of variation.

3.3. Effects of the Biofertilizer on the Growth Parameters of Maize

Data from samples collected at 100 DAE showed that the fertilization treatments influenced ($p < 0.05$) both leaf and stem productivity, as shown in Table 7. Overall, the highest values of total leaf area (TLA), leaf DM, stem DM, and total DM were observed in T_1 and T_5. The treatments had no effect on the number of leaves (average of 14 leaves), ear DM (average of 29.24 g), or ear DM: total DM ratio (average of 0.20). The key benefits of biofertilizers are their ability to increase the soil concentration of mineralized or partially available macronutrients and micronutrients, make the soil biologically alive with the presence of a wide diversity of beneficial microorganisms, build soil organic matter, and boost the amounts of humic substances [12,18,38,39]. These attributes allow biofertilizers to improve soil health and restore normal fertility through positive effects on the physical, chemical, and biological qualities of the soil system, thereby stimulating plant growth [14–18,30]. The data in Figure 1 and Tables 6 and 7 show that applying synthetic N to soil together with biofertilizers adversely affected the attributes of biofertilizers, as seen by the low performance of treatments T_3 and T_4, in which synthetic N was only partially replaced.

Table 7. Effect of fertilization treatments on the leaf morphology and dry matter of maize at the R5 dent stage. T_1 = basal application of the digestate from swine wastewater as total substitute (100%) for synthetic N; T_2 = foliar application of the digestate as total substitute (100%) for synthetic N; T_3 = basal application of the digestate as partial substitute (50%) for synthetic N; T_4 = foliar application of the digestate as partial substitute (50%) for synthetic N; T_5 = in-row broadcast of mineral fertilizer. The treatments are fully described in Table 3.

Treatments	Total Leaf Area (cm^2)	N° of Leaves	Leaf DM (g)	Stem DM (g)	Ear DM (g)	Total DM (g)	Ear DM: Total DM
T_1	5874 ^{AB}	14	42.54 ^{AB}	97.27 ^{A}	35.47	175.28 ^{A}	0.21
T_2	5709 ^{AB}	14	39.11 ^{AB}	76.22 ^{AB}	29.08	144.41 ^{AB}	0.20
T_3	5006 ^{B}	14	34.69 ^{AB}	72.71 ^{AB}	23.00	130.40 ^{AB}	0.18
T_4	5150 ^{B}	14	34.09 ^{B}	65.94 ^{B}	22.39	122.42 ^{B}	0.18
T_5	6424 ^{A}	14	44.15 ^{A}	86.63 ^{AB}	35.25	166.03 ^{AB}	0.22
CV (%)	13.43	7.22	16.05	22.7	36.68	21.61	NA

Mean within a column followed with no letter or same letters are not statistically different (Tukey's HSD, $p < 0.05$, $n = 4$). CV = coefficient of variation. DM = dry matter. NA = not applicable.

The fertilization treatments did not show statistical differences ($p > 0.05$) concerning phytometric parameters, except for a considerable decrease in relative growth rate (RGR) and absolute growth rate (AGR) 100 DAE in plants exposed to treatments T_2, T_3, and T_4 compared to T_5 (Table 8). The RGR represents the increase in DM of a plant or its organs relative to the existing DM when the observation period begins [24]. RGR is dependent on the leaf area ratio (LAR) and the net assimilation rate (NAR, the gross photosynthetic rate discounting respiration), and can also be expressed by the equation RGR = NAR × LAR [40,41]. RGR values decreased with sampling time (Table 8) because of an increase in the plant DM. The AGR represents the variation in DM with time, that is, the average growth rate over the observation period [24]. Maize growth behavior as a function of biomass accumulation was similar among the treatments up to 60 DAE, as shown by the variation in total DM (Figure 2a). At 100 DAE when the crop was close to physiological maturity, a treatment effect was observed, and the highest AGR values ($p < 0.05$) were calculated for T_5 and T_1, 4.53 and 4.05 g day^{-1}, respectively (Table 8).

Table 8. Effect of fertilization treatments on the phytometric parameters of maize. T_1 = basal application of the digestate from swine wastewater as total substitute (100%) for synthetic N; T_2 = foliar application of the digestate as total substitute (100%) for synthetic N; T_3 = basal application of the digestate as partial substitute (50%) for synthetic N; T_4 = foliar application of the digestate as partial substitute (50%) for synthetic N; T_5 = in-row broadcast of mineral fertilizer. The treatments are fully described in Table 3. CV = coefficient of variation. DAE = days after emergency; AGR = absolute growth rate; RGR = relative growth rate; RGRLA = leaf area relative growth rate; NAR = net assimilation rate; LAR = leaf area ratio; SLA = specific leaf area.

Treatments	DAE [1]	AGR	RGR	RGR_{LA}	NAR	LAR	SLA
Unit	(d)	(g d^{-1})	(g g^{-1}d^{-1})	(dm^{2}dm^{-2} d^{-1})	(g m^{-2} d^{-1})	(m^2 g^{-1})	(m^2 g^{-1})
T_1	40	1.25	0.10	0.08	8.75	1.60	0.02
	60	1.40	0.03	0.02	4.07	1.01	0.01
	80	1.88	0.03	0.00	3.87	0.86	0.01
	100	4.05 ^{AB}	0.03 ^{AB}	0.01	7.37	0.56	0.01
T_2	40	1.05	0.09	0.07	7.37	1.68	0.02
	60	2.11	0.05	0.04	5.48	1.05	0.01
	80	1.84	0.02	0.00	3.27	0.83	0.01
	100	2.02 ^{B}	0.02 ^{B}	0.00	3.64	0.53	0.01
T_3	40	0.90	0.09	0.07	7.59	1.58	0.02
	60	1.92	0.05	0.04	5.61	1.07	0.01
	80	1.03	0.01	0.00	2.20	0.82	0.01
	100	2.50 ^{B}	0.02 ^{B}	0.01	5.32	0.56	0.01
T_4	40	1.30	0.10	0.07	8.05	1.63	0.02
	60	1.88	0.04	0.03	4.70	1.01	0.01
	80	0.72	0.01	0.00	1.30	0.81	0.01
	100	2.00 ^{B}	0.02 ^{B}	0.00	4.16	0.63	0.01
T_5	40	1.26	0.10	0.08	8.54	1.64	0.02
	60	2.07	0.04	0.03	4.92	1.02	0.01
	80	0.26	0.00	0.00	0.59	0.80	0.01
	100	4.53 ^{A}	0.04 ^{A}	0.01	8.05	0.67	0.01

[1] Differences between data values of two successive sampling periods were used for the calculations. Since no data was collected at 0 DAE, data for 20 DAE are not applicable to the study. Mean within a column followed with no letter or same letters are not statistically different (Tukey's HSD, $p < 0.05$, $n = 4$).

Variations in leaf area index (LAI) according to the fertilization treatments (Figure 2b) were very similar to variations in AGR and RGR, with a statistical effect ($p < 0.05$) observed at 80 DAE and the highest values calculated for treatments T_5 and T_1 at 100 DAE. The LAI was calculated as the ratio of leaf area per plant to the soil area occupied by the plant. Remarkably reasonable correlations were observed between LAI and total DM at all sampling stages with the following equations: $y = -0.0030x^2 + 0.8231x + 5.3175$ ($R^2 = 0.9704$ for T_1), $y = -0.0044x^2 + 0.9918x + 3.8876$ ($R^2 = 0.9715$ for T_2), $y = -0.0047x^2 + 0.9609x + 3.4542$ ($R^2 = 0.9644$ for T_3), $y = -0.0056x^2 + 1.0759x + 2.8235$ ($R^2 = 0.9808$ for T_4), and $y = -0.0035x^2 + 0.943x + 3.6523$ ($R^2 = 0.9849$ for T_5).

RGR, AGR (Table 8), total DM (Figure 2a), and LAI (Figure 2b) results demonstrate that treatments T_1 and T_5 are highly comparable. The expectation was that, because of their high solubility, synthetic fertilizers in T_5 will promote higher growth rates and yields than biofertilizers in T_1, T_2, T_3, and T_4, especially under water deficit conditions, i.e., at 100 DAE. Rainfall levels recorded during the sampling period were 45, 67, 95, 270, and 0 mm at 20, 40, 60, 80, and 100 DAE, respectively, for a total of 477 mm of accumulated precipitation (data not shown). Soil application of biofertilizers with a total replacement of the amount of recommended synthetic N (treatment T_1) might have been beneficial to plants in coping with drought stress between 80 and 100 DAE when there was no precipitation. The beneficial microorganisms in the biofertilizer competitively colonizing the roots might have produced a more robust root system, which allowed the plant to seek water and nutrients in deeper soil layers [42].

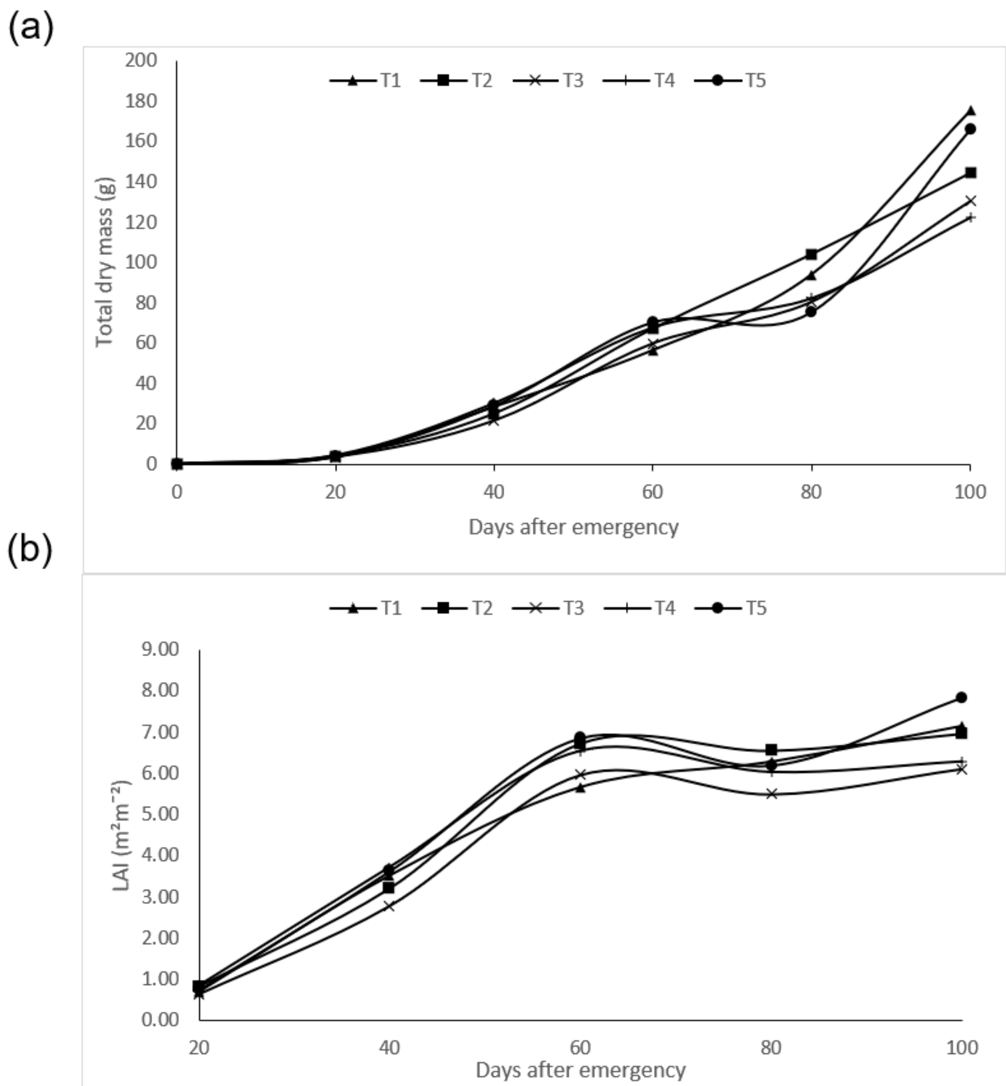

Figure 2. Variations in total dry matter DM (**a**) and leaf area index LAI (**b**) with maize growth development under different fertilization treatments. T_1 = basal application of the digestate from swine wastewater as total substitute (100%) for synthetic N; T_2 = foliar application of the digestate as total substitute (100%) for synthetic N; T_3 = basal application of the digestate as partial substitute (50%) for synthetic N; T_4 = foliar application of the digestate as partial substitute (50%) for synthetic N; T_5 = in-row broadcast of mineral fertilizer. The treatments are fully described in Table 3. Statistically, differences are not shown.

4. Conclusions

In this study, physiological growth and leaf nutrient parameters were measured at five different periods during the vegetative and reproductive stages of maize plants fertilized with biofertilizers (digestate from swine wastewater) or synthetic fertilizers. The experiment was conducted during the off-season maize crop, which is characterized by intermittent drought events. This is novel because previous studies have been conducted during the main growing season under favorable environmental conditions. Moreover, there are few previous studies on the effect of biofertilizers on phytometric parameters of crops. The results indicate that the biofertilizer applied at adequate dosages to the soil around the plants may totally (100%) substitute synthetic N fertilizers without crop yield losses. Partial replacement (50%) of synthetic N with the biofertilizer tended to yield data inferior to those obtained with synthetic fertilizers. The mode of application of the biofertilizer (basal versus foliar) did not have any significant effect on either growth parameters or leaf nutrients. In future studies, more doses of biofertilizers, maize varieties, and timing of applications should be evaluated before reaching a final conclusion. Organic fertilizers often act as a long-term carbon sink and a slow-release pool for nutrients. Thus, multi-year experiments are needed in order to gain a deeper understanding of the action of the swine wastewater-based digestate used in the present study.

Author Contributions: Conceptualization, J.d.L.J. and M.S.S.M.C.; methodology, E.L.B., L.A.M.C., J.d.L.J., F.T.S., P.G. and M.S.S.M.C.; software, M.S.S.M.C.; validation, L.A.M.C., P.G. and M.S.S.M.C.; formal analysis, E.L.B. and F.T.S.; investigation, E.L.B., J.d.L.J. and F.T.S.; resources, L.A.M.C. and M.S.S.M.C.; data curation, P.G.; writing—original draft preparation, E.L.B.; writing—review and editing, E.L.B., L.A.M.C., J.d.L.J., F.T.S., P.G. and M.S.S.M.C.; supervision, M.S.S.M.C. and P.G.; project administration, M.S.S.M.C.; funding acquisition, M.S.S.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES) who provided the scholarship to the first author, and the the Fundação para a Ciência e a Tecnologia (FCT grant number UIDB/04033/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available within the article.

Acknowledgments: The authors are grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES) and to the National Council for Scientific and Technological Development (CNPq).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Aragão, A.; Contini, E.O. *Agriculture in Brazil and in the World: A Synthesis of the 2000–2020 Period*, 1st ed.; SIRE/EMBRAPA: Brasília, Brazil, 2021; pp. 1–68. Available online: https://www.embrapa.br/documents/10180/62618376/O+AGRO+NO+BRASIL+E+NO+MUNDO.pdf/41e20155-5cd9-f4ad-7119-945e147396cb (accessed on 8 December 2022).
2. Asencios, Y.J.O. The Importance of hydrogen for Brazil: A source of clean energy and a path to the production of nitrogen fertilizers. *SciELO Prepr.* **2022**, 4408. [CrossRef]
3. Fioroni, B. *Deposits and Bioinputs Minimize the Lack of Foreign Fertilizer*; FAEP: Curitiba, Brazil, 2022; Volume 1558, pp. 18–27. Available online: https://www.sistemafaep.org.br/wp-content/uploads/2022/03/BI_1558_web.pdf (accessed on 8 December 2022).
4. Costa, M.S.S.M.; Lorin, H.E.F.; Costa, L.A.M.; Cestanaro, T.; Pereira, D.C.; Bernardi, F.H. Performance of four stabilization bioprocesses of beef cattle feedlot manure. *J. Environ. Manag.* **2016**, *181*, 443–448. [CrossRef] [PubMed]
5. Santos, F.T.; Costa, M.S.S.M.; Costa, L.A.M.; Trindade, H.; Tonial, L.M.S.; Lorin, H.E.F.; Goufo, P. Spectroscopic and physicochemical characterization of poultry waste-based composts and charcoal–compost mixtures for the prediction of dry matter yield of giant of Italy parsley. *Agronomy* **2021**, *12*, 256. [CrossRef]
6. Lamolinara, B.; Pérez- Martínez, A.; Guardado-Yordi, E.; Fiallos, C.G.; Diéguez- Santana, K.; Ruiz-Mercado, G.J. Anaerobic digestate management, environmental impacts, and techno-economic challenges. *Waste Manag.* **2022**, *140*, 14–30. [CrossRef] [PubMed]
7. Santos, C.; Goufo, P.; Fonseca, J.; Pereira, J.; Ferreira, L.; Coutinho, J.; Trindade, H. Effect of lignocellulosic and phenolic compounds on ammonia, nitric oxide and greenhouse gas emissions during composting. *J. Clean. Prod.* **2018**, *171*, 548–556. [CrossRef]
8. Santos, F.T.; Goufo, P.; Santos, C.; Botelho, D.; Fonseca, J.; Queirós, A.; Costa, M.S.S.M.; Trindade, H. Comparison of five agro-industrial waste–based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C. *Food Chem.* **2016**, *209*, 293–301. [CrossRef]

9. Novotny, E.H.; Balieiro, F.C.; Auccaise, R.; Benites, V.M.; Coutinho, H.L.C. Spectroscopic investigation on the effects of biochar and soluble phosphorus on grass clipping vermicomposting. *Agriculture* **2022**, *12*, 1011. [CrossRef]
10. Santos, C.; Monte, J.; Vilaça, N.; Fonseca, J.; Trindade, H.; Cortez, I.; Goufo, P. Evaluation of the potential of agro-industrial waste-based composts to control Botrytis gray mold and soilborne fungal diseases in lettuce. *Processes* **2021**, *9*, 2231. [CrossRef]
11. Santos, F.T.; Trindade, H.; Costa, M.S.S.M.; Costa, L.A.M.; Goufo, P. Effects of composts made from broiler chicken residues and blended with biochar on the minerals and phenolic compounds in parsley (*Petroselinum crispum* Mill.). *Agriculture* **2021**, *11*, 1168. [CrossRef]
12. Tambone, F.; Scaglia, B.; D'imporzano, G.; Schievano, A.; Orzi, V.; Salati, S.; Adani, F. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere* **2010**, *81*, 577–583. [CrossRef]
13. Samoraj, M.; Mironiuk, M.; Izydorczyk, G.; Witek-Krowiak, A.; Szopa, D.; Moustakas, K.; Chojnacka, K. The challenges and perspectives for anaerobic digestion of animal waste and fertilizer application of the digestate. *Chemosphere* **2022**, *295*, 133799. [CrossRef]
14. Koszel, M.; Lorencowicz, E. Agricultural use of biogas digestate as a replacement fertilizer. *Agric. Sci. Proc.* **2015**, *7*, 119–124. [CrossRef]
15. Locoli, G.A.; Zabaloy, M.C.; Pasdevicelli, G.; Gómez, M.A. Use of biogas digestates obtained by anaerobic digestion and co-digestion as fertilizers: Characterization, soil biological activity and growth dynamic of *Lactuca sativa* L. *Sci. Total Environ.* **2019**, *647*, 11–19. [CrossRef]
16. Sigurnjak, I.; Vaneeckhaute, H.; Michels, E.; Ryckaert, B.; Ghkiere, G.; Tack, F.M.G.; Meers, E. Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. *Sci. Total Environ.* **2017**, *599–600*, 1885–1894. [CrossRef] [PubMed]
17. Tsachidou, B.; Scheuren, M.; Gennen, J.; Debbaut, V.; Toussaint, B.; Hissler, C.; George, I.; Delfosse, P. Biogas residues in substitution for chemical fertilizers: A comparative study on a grassland in the Walloon Region. *Sci. Total Environ.* **2019**, *166*, 212–225. [CrossRef]
18. Zilio, M.; Pigoli, A.; Rizzi, B.; Herrera, A.; Tambone, F.; Geromel, G.; Meers, E.; Schoumans, O.; Giordano, A.; Adani, F. Using highly stabilized digestate and digestate-derived ammonium sulphate to replace synthetic fertilizers: The effects on soil, environment, and crop production. *Sci. Total Environ.* **2022**, *815*, 152919. [CrossRef] [PubMed]
19. Nitsche, P.R.; Caramori, P.H.; Ricce, W.S.; Pinto, L.F.D. *Climate Atlas of the State of Paraná*, 1st ed.; IAPAR: Londrina, Brazil, 2019; pp. 1–210. Available online: https://www.idrparana.pr.gov.br/system/files/publico/agrometeorologia/atlas-climatico/atlas-climatico-do-parana-2019.pdf (accessed on 8 December 2022).
20. Pavinato, P.S.; Pauletti, V.; Motta, A.C.V.; Adônis, M. *Fertilization and Liming Manual for the State of Paraná in Brazil*, 2nd ed.; SBCS/NEPAR: Curitiba, Brazil, 2017; pp. 1–482. Available online: https://issuu.com/editoracubo/docs/issuu-nepar-calagem (accessed on 8 December 2022).
21. Malavolta, E.; Vitti, G.C.; Oliveira, S.A. *Evaluation of the Nutritional Status of Plants: Principles and Applications*, 2nd ed.; POTAFOS: Piracicaba, Brazil, 1997; pp. 1–319. Available online: https://repositorio.usp.br/item/001070906 (accessed on 8 December 2022).
22. Silva, F.C. *Manual of Chemical Analysis of Soils, Plants and Fertilizers*, 2nd ed.; EMBRAPA/IT: Brasília, Brazil, 2009; pp. 1–319. Available online: https://www.infoteca.cnptia.embrapa.br/handle/doc/330496 (accessed on 8 December 2022).
23. Guimarães, D.P.; Sans, L.M.A.; Moraes, A.V.C. Estimativa da Área Foliar de Cultivares de Milho. In Proceedings of the XXIV Congresso Nacional de Milho e Sorgo, Florianópolis, Brazil, 1–5 September 2002; Available online: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/34888/1/Estimativa-area.pdf (accessed on 8 December 2022).
24. Benincasa, M.M.P. *Plant Growth Analysis, Basic Notions*, 1st ed.; FUNEP: Jaboticabal, Brazil, 1988; pp. 1–42. Available online: https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=418424&biblioteca=vazio&busca=autoria:"M"&qFacets=autoria:"M"&sort=&paginacao=t&paginaAtual=3 (accessed on 8 December 2022).
25. Weismann, M. Fases de desenvolvimento da cultura do milho. In *Technology and Production: Off-season Corn and Winter Crops*, 2008th ed.; Roscoe, R., Lourenção, A.L.F., Grigolli, J.F.J., Melotto, A.M., Pitol, C., Miranda, R.A.S., Eds.; Fundação MS: Maracaju, Brazil, 2008; pp. 4–7. Available online: https://www.fundacaoms.org.br/tipo_da_publicacao/conteudo-safrinha/ (accessed on 8 December 2022).
26. Bull, L.T.; Cantarella, H. *Maize Cultivation: Factors Affecting Productivity*, 1st ed.; POTAFOS: Piracicaba, Brazil, 1993; pp. 63–145. Available online: https://megaleitores.com.br/livro/estante/11993301-cultura-do-milho-fatores-que-afetam-a-produtividade-leonardo-theodoro-bull-e-heitor-cantarella/59797/4433/50300 (accessed on 8 December 2022).
27. Martinez, H.E.P.; Carvalho, J.G.; Souza, R.B. Diagnose foliar. In *Recommendations for the Use of Correctives and Fertilizers in Minas Gerais*, 5th ed.; Ribeiro, A.C., Guimarães, P.T.G., Alvarez, V.V.H., Eds.; CFSEMG/UFV: Viçosa, Brazil, 1999; pp. 143–168. Available online: https://www.sbcs.org.br/loja/index.php?route=product/product&product_id=133 (accessed on 8 December 2022).
28. Ferreira, D.F. SISVAR: A computer analysis system to fixed effects split plot type designs. *Rev. Bras. De Biom.* **2019**, *37*, 529–535. [CrossRef]
29. CONAB. Boletin da Safa de Grãos 2018/19, 9° Levantamento Junho 2019. In *Observatório Agrícola Acompanhamento da Safra Brasileira Grãos*, 1st ed.; Santos, C.M.R., Ruas, J.F., Salvador, M.A., Amazonas, L., Guth, T.L.F., Nogueira, B.P., Júnior, S.R.G.S., Soares, F.M.S., Campos, P.M., Eds.; CONAB: Brasília, Brazil, 2019; Volume 6, pp. 1–113. Available online: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos?start=4 (accessed on 8 December 2022).

30. Tejada, M.; Rodríguez-Morgado, B.; Gómez, I.; Franco-Andreua, L.; Benítezc, C.; Parrado, J. Use of biofertilizers obtained from sewage sludges on maize yield. *Eur. J. Agron.* **2016**, *78*, 3–19. [CrossRef]
31. Schulten, A.; Krämer, U. Interactions between copper homeostasis and metabolism in plants. *Prog. Bot.* **2017**, *79*, 111–146. [CrossRef]
32. Gao, F.; Robe, K.; Gaymard, F.; Izquierdo, E.; Dubos, C. The transcriptional control of iron homeostasis in plants: A tale of bHLH transcription factors? *Front. Plant Sci.* **2019**, *10*, 6. [CrossRef]
33. Fischer, W.W.; Hemp, J.; Johnson, J.E. Manganese and the evolution of Photosynthesis. *Orig. Life Evol. Biosph.* **2015**, *45*, 351–357. [CrossRef] [PubMed]
34. Hafeez, B.; Khanif, Y.M.; Saleem, M. Role of zinc in plant nutrition—A review. *Am. J. Exp. Agric.* **2013**, *3*, 374–391. [CrossRef]
35. Santos, H.G.; Jacomine, P.K.T.; Anjos, L.H.C.; Oliveira, V.A.; Lumbreras, J.F.; Coelho, M.R.; Almeida, J.A.; Araujo Filho, J.C.; Oliveira, J.B.; Cunha, T.J.F. *Brazilian System of Soil Classification*, 5th ed.; EMBRAPA: Brasília, Brazil, 2018; pp. 1–356. Available online: https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1094003/sistema-brasileiro-de-classificacao-de-solos (accessed on 8 December 2022).
36. Ambrosini, V.G.; Rosa, D.J.; Basso, A.; Borghezan, M.; Pescador, R.; Miotto, A.; Melo, G.W.B.; Soares, C.R.F.S.; Comin, J.J.; Brunetto, G. Liming as an ameliorator of copper toxicity in black oat (Avena strigosa Schreb.). *J. Plant Nutr.* **2017**, *40*, 404–416. [CrossRef]
37. Casarin, V. Dinâmica de nutrientes no sistema solo-planta visando BPUFs. In Proceedings of the IX Regional Symposium of the International Plant Nutrition Institute, Paragominas, Brazil, 30–31 August 2016. Available online: https://www.npct.com.br/npctweb/npct.nsf/article/BRS-3424/$File/Palestra%20Valter%20Casarin.pdf (accessed on 8 December 2022).
38. Tejada, M.; Rodríguez-Morgado, B.; Gómez, I.; Parrado, J. Degradation of chlorpyrifos using different biostimulants/biofertilizers: Effects on soil biochemical properties and microbial community. *Appl. Soil Ecol.* **2014**, *84*, 158–165. [CrossRef]
39. Nascimento, R.C. O uso do biofertilizante em solos agrícolas do cerrado da região do Alto Paranaíba (MG). *Bol. Goiano Geogr.* **2010**, *30*, 55–56. [CrossRef]
40. Lima, J.F.; Peixoto, C.P.; Ledo, C.A.S. Physiological indexes and initial growth of papaya plants (*Carica papaya* L.) under greenhouse conditions. *Ciênc. Agrotec.* **2007**, *31*, 1358–1363. [CrossRef]
41. Urchei, M.A.; Rodrigues, J.D.; Stone, L.F. Growth analysis of two bean cultivars under irrigation in no tillage and the conventional tillage. *Pesqui. Agropecuária Bras.* **2000**, *35*, 497–506. [CrossRef]
42. Najafi, S.; Nazari Nasi, H.; Tuncturk, R.; Tuncturk, M.; Sayyed, R.Z.; Amirnia, R. Biofertilizer application enhances drought stress tolerance and alters the antioxidant enzymes in medicinal pumpkin (*Cucurbita pepo* convar. pepo var. *Styriaca*). *Horticulturae* **2021**, *7*, 588. [CrossRef]