agronomy

Article

Deciphering the Effectiveness of Humic Substances and Biochar Modified Digestates on Soil Quality and Plant Biomass Accumulation

Jiri Holatko [1,2], Tereza Hammerschmiedt [1], Oldrich Latal [1,2], Antonin Kintl [1,3], Adnan Mustafa [1,4,5], Tivadar Baltazar [1], Ondrej Malicek [1] and Martin Brtnicky [1,5,*]

1 Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic; jiri.holatko@mendelu.cz (J.H.); tereza.hammerschmiedt@mendelu.cz (T.H.); oldrich.latal@mendelu.cz (O.L.); kintl@vupt.cz (A.K.); adnanmustafa780@gmail.com (A.M.); tivadar.baltazar@mendelu.cz (T.B.); ondrej.malicek@mendelu.cz (O.M.)
2 Agrovyzkum Rapotin, Ltd., Vyzkumniku 267, 788 13 Rapotin, Czech Republic
3 Agricultural Research, Ltd., Zahradni 400/1, 664 41 Troubsko, Czech Republic
4 Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Benatska 2, 128 00 Praha, Czech Republic
5 Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic
* Correspondence: martin.brtnicky@seznam.cz

Abstract: The effective use of digestate as exogenous organic matter to enhance soil carbon sequestration depends on the balance between labile and recalcitrant organic carbon, which is influenced by the type of feedstock, the fermentation process, and the fraction (liquid, solid) of the fermented product used. In this work, in order to change the ratio of labile to stable carbon in the resulting fertiliser, the digestate was mixed with organic carbon-rich supplements: biochar and Humac (a humic acid-rich substance). The pot experiment was carried out under controlled conditions with maize (*Zea mays* L.) in soil amended with the digestate (D), which was incubated with Humac (H), biochar (B), or a combination of both (D + B + H) before the application. Digestate enriched with Humac showed improved short-term nutrient (carbon, phosphorus, nitrogen) transformation, as indicated by soil enzyme activity and the highest maize biomass production of. Total carbon content, C:N ratio, short-term respiration activity, and nitrification were most enhanced by digestate enriched with either biochar or combined biochar + Humac). Long-term nitrogen mineralization was mostly enhanced by digestate + Humac, as indicated by amino-acid-induced respiration and urease activity. Short-term positive effects of digestate + biochar (eventually + Humac) on catabolism were proven, whereas their long-term effects on nutrient mineralization were negative (i.e., biochar-mediated immobilization, sequestration), which should be the focus of further research in future.

Keywords: digestate; soil health; respiration; sustainable agriculture; soil nutrients

check for updates

Citation: Holatko, J.; Hammerschmiedt, T.; Latal, O.; Kintl, A.; Mustafa, A.; Baltazar, T.; Malicek, O.; Brtnicky, M. Deciphering the Effectiveness of Humic Substances and Biochar Modified Digestates on Soil Quality and Plant Biomass Accumulation. *Agronomy* **2022**, *12*, 1587. https://doi.org/10.3390/agronomy12071587

Academic Editors: Maria Roulia and Domenico Ronga

Received: 30 May 2022
Accepted: 28 June 2022
Published: 30 June 2022

1. Introduction

To maintain ecosystem services for agricultural purposes, soil quality and fertility should be maintained at a level high enough to sustain intensive land use without soil organic matter (SOM) loss [1]. Therefore, there is a growing global demand for agricultural inputs that can increase the quantity and quality of SOM over a reasonably long period of time, both to ensure fertilization efficiency and to minimize negative environmental impacts, such as greenhouse gas (GHG) emissions. [2]. Low (neutral)-carbon agriculture (LCA) [3] strategies and environment-friendly agricultural practices [4] are examples of those approaches that focus, among other things, on balancing labile and stable soil organic carbon (SOC) in the soil [5]. Regarding organic-based fertilizers with conventional production technologies, there is still potential for further study and adoption of new types and combinations of organic sources in agricultural soils [6,7].

Anaerobic digestion (AD) is an important way of processing plant and waste biomass before its agronomic use. Since AD was developed for energy production, the potential use of digestate as exogenous organic matter (EOM) to enhance soil carbon sequestration is still being evaluated. However, the importance of the role of carbon mitigation in current and future EU policies on agricultural systems increases the significance of digestate as a potential contributor to SOC formation (and carbon dynamics) [8,9]. Several studies have concluded that digested EOM shows higher stability than undigested EOM in SOM [10–12]. Digestate-based fertilizers can be effective in microbial proliferation, carbon sequestration, and CO_2 emissions reduction in grasslands (cambisol, fluvisol) or tropical ultisol [13–15]. Other studies reported mostly long-term effects of digestate application: labile forms of C (hot-water extractable carbon) in soils were positively affected by digestate application [16], and increases in both labile organic carbon (LOC) in the topsoil and recalcitrant organic carbon (ROC) at greater depths (20–60 cm) were observed [17]. The balance between LOC and ROC determines soil fertility properties [18], such as plant biomass yield, C mineralization rate, soil respiration, carbon half-life [19], and nitrogen mineralization [20]. LOC:ROC ratio depends on the prevailing type of carbon fraction in applied organic amendment [19] and the interaction interval [21].

Agriculture plant waste-derived digestate is particularly recalcitrant due to its high lignin content, which is not degraded during AD [22], and other compounds, such as cellulose, hemicellulose, and raw protein, which are only partially degraded compared to compounds from livestock wastes [23]. Moreover, initial degradation of labile carbon components during AD is followed by their transformation into complex compounds (aliphatics, aromatics, and phenols) representing recalcitrant carbon [24]. However, we used a digestate produced from a mixed feedstock (including food waste). The aromatic chemical structures of food waste digestate are likely of a more proteinaceous nature [25], and their carbon parts may express different levels of recalcitrancy. Furthermore, separated applications of liquid and solid fractions of digestate has been reported as common. These fractions also differ in their contents of LOC and ROC [22,26]. The use of liquid digestate fraction, generally richer in LOC, was strongly encouraged by some authors [22,27]. On the other hand, its contribution to SOC stock is still weak compared to solid fraction digestate, due to low carbon input [9,22]. Because digestate based on food waste has properties close to those of the liquid fraction of digestate from agricultural waste, e.g., raw material with lower C:N ratio and low dry biomass of the final product, we had to assume lower recalcitrance of the digestate fertilizer. Blending of such digestate with other sources of stabilized organic carbon (e.g., biochar or humic acids) would likely alter the ratio of labile and stable carbon in the obtained blended fertilizer. Humic substances derived from low-rank coals (lignite, leonardite, etc.) could be used as soil conditioners due to their high humic acid content (25–85%) [28]. The addition of humic substances to the soil enhances biological (e.g., soil enzyme activity and microbial respiration), physical, and chemical soil properties and plant growth [29,30]. Biochar, the second tested material, is known to greatly increase the stable fraction of SOC in amended soils [31–33], as well as the abundance and activity of soil microbiota and crop yields [34,35]. Previous studies have mostly focused on the sole or combined use of digestate, biochar, or digestate as potential soil amendments to enhance soil fertility and crop yields. Few reports are available on how the aging of digestate can improve its beneficial properties as a soil amendment applied alone or in combination with other organic amendments. Therefore, in the present study, digestate prepared from biogas waste was aged with biochar and commercially available humic substances (Humac) and tested as a soil amendment.

The specific objectives of the present study were thus to (i) evaluate how the mixing of digestate with biochar or humic acids (or a combination of both) may affect the nutrient dynamics and microbial abundance in the aged digestate amendments, and (ii) evaluate the effects of applied digestate in combination with biochar and Humac on soil-nutrient mineralizing enzymes and microbial respirations. We hypothesized that:

i. Digestate enriched with Humac would improve microbial activities leading to enhanced mineralization of nutrients (C, N, P).
ii. Digestate enriched with biochar (or biochar + Humac) would increase total carbon, C:N ratio, and microbial respiration.
iii. Positive effects of digestate + biochar (eventually + Humac) on catabolism would be only short-term (until utilization of labile carbon in DG + amendment blend), with priming negative effects on microbial abundance.
iv. Long-term effects of digestate + biochar (+Humac) on nutrient mineralization would be negative, due to biochar-mediated immobilization and sequestration of nutrients.

2. Materials and Methods

2.1. Procurement and Preparation of Soil Amendments

Digestate obtained from mesophilic (≈40 °C) biogas-plant processing gastro-waste was mixed with the amendments (as shown in Table 1) in tightly closed sealable barrels with a volume of 50 L, and the mixture was incubated at room temperature for six weeks. Biochar pyrolyzed at approx. 650 °C from agricultural grain waste (Sonnenerde GmbH, Riedlingsdorf, Austria) and humic-acid-based product Humac AGRO prepared from leonardite-oxihumolite (Envi Produkt s.r.o., Prague, Czech Republic) were used as amendments.

Table 1. Experimental variants of digestate.

Variant	Abbrev.	Digestate per Barrel [L]	Biochar per Barrel [kg]	Humac per Barrel [kg]
Digestate	D	10	-	-
Digestate + Humac	D + H	10	-	0.1
Digestate + biochar	D + B	10	4	-
Digestate + biochar + Humac	D + B + H	10	4	0.1

The digestates thus prepared had the properties shown in (Table 2). Dry matter (DM) was measured gravimetrically [36], total nitrogen (N_{tot}) was determined by Kjeldahl method [37], available phosphorus (P) was determined according to [38], total sulphur (S) was determined according to [39], and potassium was determined according to [40]; gene copy numbers were determined as nitrifying = ammonium-oxidizing bacteria (AOB) according to [41], denitrifying microorganisms (nirS) according to [42], and bacteria in digestate according to [43].

Table 2. Properties of digestate experimental variants.

Variants	DM	N_{tot}	P	S	K	amoA	nirS	16S rDNA
	%	%	g/kg	g/kg	g/kg	cps/g	cps/g	cps/g
D	5.96	0.65	0.76	0.85	1.35	3.81×10^5	3.15×10^6	5.66×10^{10}
D + H	6.77	0.50	0.95	1.10	1.80	2.56×10^5	1.67×10^6	5.03×10^{10}
D + B	15.00	0.68	2.09	2.27	3.63	1.21×10^6	3.05×10^7	4.40×10^{11}
D + B + H	29.38	0.76	2.32	5.14	2.00	5.06×10^5	1.70×10^7	2.21×10^{11}

N_{tot} = total mitrogen, P = phosphorous, S = sulphur, K = potassiums; amoA = soil gene copy number, indicator of nitrifying microorganisms; nirS = soil gene copy number, indicator of denitrifying microorganisms; 16S (rDNA) = gene copy number, indicator of bacteria in digestate.

2.2. Experimental Design and Treatments

The prepared variants used a soil amendment in a pot experiment with maize (*Zea mays* L.). Pots with a volume of 2 L were filled with 1.7 kg of a soil–sand mixture prepared from a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) sieved through a 2 mm sieve and mixed in a 1:1 ratio with fine quartz sand (0.1–1.0 mm; ≥95% SiO_2). Eighty-five mL (equal to 50 $m^3 \cdot ha^{-1}$) of each type of digestate was applied to the surface and then covered with another 300 g of soil-sand mixture. Each pot was sown with 6 seeds of maize and placed in a greenhouse. Controlled conditions were set as follows: 12 h photoperiod, light intensity 370 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature (day/night) 20/12 °C, soil moisture maintained at 65% of water-holding capacity throughout the experiment. After germination, the seedlings were reduced to two in each pot. Maize was grown for 12 weeks. At the end of experiment, the aboveground biomass (AGB) was harvested at soil surface level, dried to constant weight at 60 °C and weighted on laboratory scales. A soil sample (100 g) was taken from each pot with a probe from for further analyses. Following the harvest of the crop after 12 weeks and the taking of samples, the same pots were used to grow maize for another 12 weeks under the conditions as described above, except that all variants were fertilized with unamended digestate in dose 70 mL (equal to 40 $m^3 \cdot ha^{-1}$).

2.3. Soil Analysis

The soil samples were homogenized by sieving through a 2 mm mesh. Air-dried samples were used for determination of soil pH in $CaCl_2$ [44], total soil carbon (TC), and nitrogen (TN) content [45,46]. The samples stored at 4 °C were used for determination of soil basal respiration (BR) and substrate-induced respirations: D-glucose (Glc-SIR), L-lysine (Lys-SIR), and L-arginine (Arg-SIR) [47]. The freeze-dried samples were prepared for the enzyme activity assays: β-glucosidase (GLU), phosphatase (Phos), N-acetyl-β-D-glucosaminidase (NAG), and urease (Ure) [48].

2.4. Statistical Analyses

Data obtained from the performed measurements were statistically analyzed using the multivariate analysis of variance (MANOVA), principal component analysis (PCA), one-way analysis of variance (ANOVA), Tukey HSD post-hoc test (at significance level $p = 0.05$), and Pearson correlation analysis (Program R, version 3.6.1—R CORE TEAM 2020) [49]. The results of Pearson's correlation analysis were mentioned when the value of the correlation coefficient r was: $0.5 < r < 0.7$ (moderate correlation) or $0.7 < r < 0.9$ (high correlation) [50]. The Rohlf's PCA was used to evaluate the mutual dependence among the properties and their values in individual compared variants of amended soil.

3. Results

As compared to the control digestate, the maize dry aboveground biomass (AGB_dry) was significantly increased in both variants enriched with Humac (D + H, D + B + H) in the first part of the experiment (Figure 1A). On the other hand, the second part of the experiment showed no significant long-term effect of soil amendments (Figure 1A).

In contrast to plant AGB_dry values, the total carbon (TC) and the carbon:nitrogen ratio (C:N) showed a clear trend during the whole experiment. There were significantly increased TC and C:N in variants D + B and DG + B + H as compared to the control and the D + H variant (Figure 1B,C). Further, variant D + B + H had the highest soil TC at the end of experiment, which documented the long-term synergic effects of digestate, biochar, and Humac on soil carbon sequestration. The absolute C:N values of both biochar-enriched variants decreased in the second part of the experiment, whereas the C:N of variant D + H increased. In line with the increased values of TC and C:N, pH increased in the D + B and D + B + H variants as compared to the control digestate (Figure 1D). Moreover, D + H soil also showed significantly increased pH compared to the control in the second part of experiment. However, it was significantly lower than the highest pH value of D + B + H variant.

Determination of basal (BR)-, L-lysine (Lys-)-, and L-arginine (Arg-)-induced respirations (SIR) showed a primary positive effect of D + B on the carbon oxidative mineralization, because all three properties were significantly increased as compared to other enriched digestate variants in the first part of experiment (Figure 2A,C,D). Aside from Lys-SIR, the control digestate showed significantly lower respiration values than D + B in the first half of trial., whereas the end of cultivation revealed an increase in the BR, Lys-SIR, and Arg-SIR of variants D + H and D, which were significantly higher (except of Lys-SIR of control digestate) than the values of variants D + B and D + B + H. These results indicated that labile carbon of biochar was more easily utilizable in the short term than humic carbon, while with an extending cultivation time, recalcitrant carbon putatively prevailed in the SOM of biochar-amended variants, and derived a long-term negative effect on carbon mineralization rate. Particularly, D + B + H increased carbon immobilization, which resulted in significantly decreased BR, Lys-SIR, Arg-SIR (the first part of experiment), and Lys-SIR (the second part of experiment) in variant D + B + H compared to D + B (Figure 2A,B). The stimulatory effect of D + H on microbial abundance was significant due to increased Glc-SIR compared to control digestate in the second part of the experiment; nevertheless, the highest value of Glc-SIR was displayed by variant D + B + H (Figure 2B).

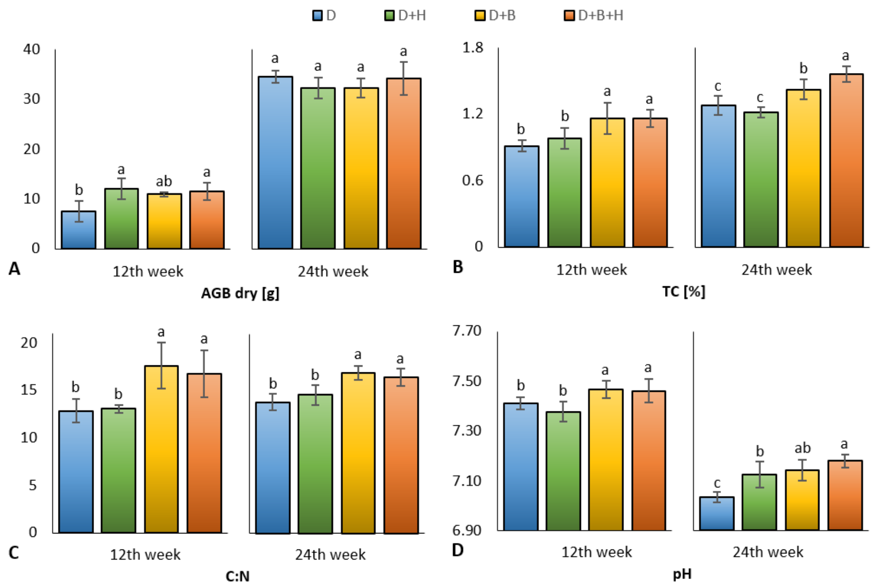

Figure 1. Dry aboveground biomass (**A**), total carbon (**B**), C:N ratio (**C**), and soil pH (**D**) under experimental variants in the first and the second part of pot experiment. Different letters indicate statistically significant differences of Tukey HSD post-hoc test at $p \leq 0.05$.

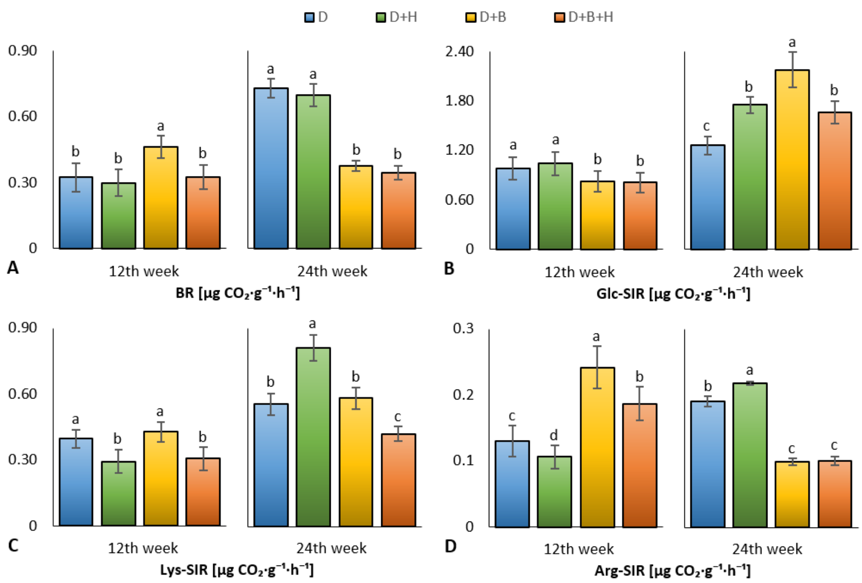

Figure 2. Basal respiration (**A**), D-glucose-induced respiration (**B**), L-lysine-induced respiration (**C**), and L-arginine-induced respiration (**D**) in the first and the second part of pot experiment. Different letters indicate statistically significant differences of Tukey HSD post-hoc test at $p \leq 0.05$.

β-glucosidase activity (GLU) also reflected the availability of soil organic carbon (SOC) in the second part of the experiment. Putative biochar recalcitrancy likely led to the significantly decreased GLU compared to the control (D), whereas Humac-enriched variants D + H and D + B + H were significantly increased (Figure 3A). In the first part of this experiment, only variant D + H showed a significant increase in GLU value compared to the control digestate, as well as it exerted a significant increase in the second, carbon transformation-related enzyme, N-acetyl-β-D-glucosaminidase (NAG, Figure 3C). We inferred from these results that activities related to carbon transformation and fungal biomass degradation were most enhanced by D + H. NAG activity was mitigated most by D + B in the first part of experiment compared to the control digestate, whereas the end of experiment revealed significant enhancement of NAG with all enriched digestates. The highest value was found in the D + B variant (Figure 3C).

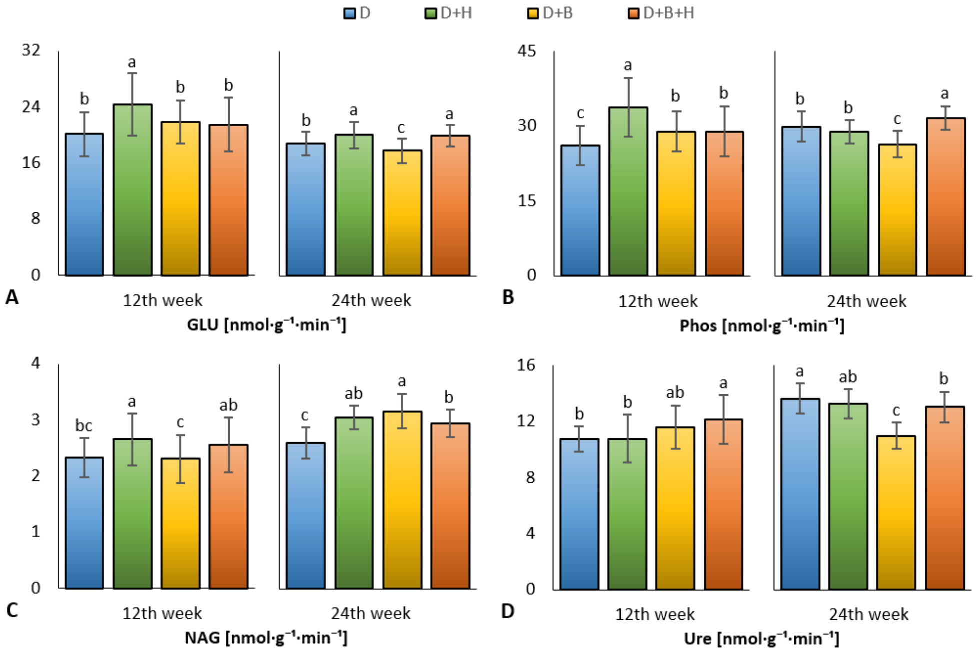

Figure 3. β-glucosidase (**A**), phosphatase (**B**), N-acetyl-β-D-glucosaminidase (**C**), and urease (**D**) under experimental variants in the first and second part of pot experiment. Different letters indicate statistically significant differences of Tukey HSD post-hoc test at $p \leq 0.05$.

All variants with added enriched digestate showed significantly increased activity of phosphatase (Phos) in comparison to the control (D), with the highest value observed in variant D + H. At the end of the experiment, the D + B variant showed the lowest Phos value, while variant D + B + H reached the highest Phos value (Figure 3B). This implied a biochar-mediated effect of digestate on the immobilization of nutrients, namely phosphorus, as well as nitrogen, because variant D + B exerted the lowest activity of urease (Ure) in the second part of experiment (Figure 3D). On the contrary, digestate amended with combined Humac + biochar determined significantly increased Ure activity in the first part of experiment. Thus, the nitrogen available in the biochar of this variant was presumably accessed by the effect of Humac, but in the long-term, the nitrification effect was mitigated.

4. Discussion

We hypothesized that the long-term effect of digestate + biochar on nutrient mineralization would be negative, because of the tendency for biochar-mediated immobilization and sequestration. Both humic acids and biochar represent amendments that are rich sources of carbon and also act as nutrient storehouses [51–54]. However, biochar contains both labile (easily available) and recalcitrant carbon, leading to higher heterogeneity of organic matter in the digestate. We observed a weaker short-term fertilizing effect of D + B compared to D + H on the aboveground biomass yield, despite significantly higher carbon mineralization associated with general SOM degradation, which was expressed as higher BR and Arg-SIR detected for D + B (as compared to D + H and control D) in the first part of the experiment. Therefore, biochar addition to digestate led to only insignificant positive effects on the fertility of amended soil, and did not increase AGB_dry in the D + B variant in either part of the experiment. Contrary to the biochar, Humac added to digestate in D + H variant mediated both higher microbial abundance (indicated by Glc-SIR) and increased enzyme activity (GLU, NAG, Phos) compared to the D + B variant, which could account for the observed increase in plant AGB_dry value in the first part of experiment. A previous study carried out with lettuce showed similar significant increases in microbial biomass content and enzyme activity values (ARS, GLU, Phos) for soil fertilized with digestate + Humac compared to soil fertilized with digestate + biochar [55]. This positive effect of Humac in digestate on AGB_dry was also revealed for the D + B + H variant (compared to the control D) in the 1st part of the trial. This might be due to the enhanced availability of soil nutrients under this amendment. Other studies found that the beneficial effect of digestate fertilization on the abundance of microorganisms was accompanied by an increase and higher levels of humic acids [56]. Other authors reported that the soil application of humic acids increased the activity of various enzymes compared to unamended soil [30,57].

As expected, higher total carbon content in D + B (compared to D + H) was detected due the higher application dose of biochar compared to Humac and general fact that biochar is a more carbon-abundant (approx. 74% of dry matter) source for SOM in comparison to Humac (carbon content 38.4% of d.m.). Throughout the experiment, there was a significantly higher TC content in the D + B and D + B + H variants compared to the D and D + H variants. Nevertheless, the variant D + B + H had the highest soil TC at the end of experiment, probably due to the synergistic effect of biochar and Humac on soil carbon sequestration. These results were partially in line with the results of our previous study carried out with biochar and Humac-enriched digestate [54].

Moreover, the high carbon content of D + B and D + B + H variant resulted in the highest values of C:N ratio as well. These results are in line with the previously reported findings [55,58] that a higher C:N ratio predicts nitrogen immobilization. This nitrogen immobilization, accompanied with enhanced carbon mineralization, results in decreased C:N values [58]. We observed that absolute C:N values decreased over time throughout the experiment (Figure 1C). In contrast, the D + H variant initially showed a lower but increasing C:N ratio in the long term (24 weeks). This positive relation of carbon content and mineralization rate was corroborated by a significant ($p \leq 0.01$ and 0.001, respectively) positive correlation of TC with BR and Arg-SIR (r = 0.43 and 0.53, respectively) in the first part of the experiment. These results were partially in line with the results of our previous study carried out with Humac and biochar [59], in which we observed a positive effect of single-applied biochar on soil respiration, although co-application with Humac produced a substantial benefit to aboveground biomass yield. Other authors also reported a positive effect of increased soil humic acid content due to digestate application on the aboveground biomass yield [60,61].

The high C:N ratio of variant D + B determined the higher fungal biomass and its presumed long-term increased turnover, which was reflected by the significantly increased NAG activity at the end of the experiment. C:N and NAG correlated significantly positively ($p \leq 0.01$, r = 0.5), and showed synergy on PCA biplot (Figure S4), NAG correlated with Glc-SIR ($p \leq 0.001$, r = 0.57) as well. However, the high C:N value in D + B variant contributed only insignificantly to the activity of nutrient-mineralizing enzymes (GLU, Ure) in the first part of the experiment, with a long-term negative final effect on both enzymes at the end of experiment (Figure 3A,D). A significant negative correlation and antagonism (PCA biplot) of C:N and Ure ($p \leq 0.001$, r = −0.58) indicated this relation, as shown in Figures S3 and S4. Additionally, phosphorus solubilization was mainly stimulated in the D + B + H variant, as the Phos value increased compared to the control, while final Phos activity decreased significantly (Figure 3B).

In the second part of experiment, carbon sequestration was presumably coupled with decreased carbon mineralization, because TC showed antagonism (PCA biplot) and negatively correlated with BR, Lys-SIR, and Arg-SIR ($p \leq 0.001$; r = −0.77, −0.7, −0.82, respectively)—Figures S3 and S4. This antagonism was proven in the D + B + H variant, where we observed the highest TC value, while BR, Lys-SIR, and Arg-SIR were the lowest. This was consistent with the results of a previous study [55], where we found a significant overlap in TC values for digestate + biochar + Humac compared to digestate + Humac. Nevertheless, BR-, L-lysine (Lys)-, and L-arginine (Arg)-induced respirations were significantly more enhanced by the addition of Humac than by the combination of both amendments (Figure 2A,C,D). While biochar, even combined with humic acids, helped maintain long-term low levels of nutrient mineralization in the soil [52], the enzymes involved in carbon catabolism (e.g., invertase, β-glucosidase) were stimulated by the humic acid access [62,63]. Further, in the second part of the experiment, the negative correlation between C:N and Ure (Figure S3) was due to the fact that the increased final C:N ratio in the D + H variant (compared to the value from 1st half of experiment) was caused by increased enzymatic activity and subsequently accelerated nitrogen mineralization, as indicated by the highest Lys-SIR and Arg-SIR values (Figure 2C,D). These changes led to the proportionately higher nitrogen uptake and efflux from soil. In contrast, the long-term effect of carbon access from biochar stimulated carbon mineralization with the final consequence of a decreased C:N ratio. This can be seen from the synergy (PCA biplot) and positive correlation (the most apparent in D + H variant) between C:N and BR or Arg-SIR ($p \leq 0.01$ and 0.001, respectively; r values were 0.41 and 0.65, respectively) in the first half of experiment (Figure S1 and S2), followed by antagonism (PCA biplot) and significant ($p \leq 0.001$) negative correlation between C:N and BR (r = −0.79) or Arg-SIR (r = −0.74) in the second half of the experiment (Figure S3 and S4).

Finally, the tested amendments and their effects on soil microbial activity, nutrient transformation, and plant biomass yield also depended on soil pH. C:N showed a strong synergy (PCA biplot) with soil pH during the whole experiment—Figures S2 and S4. Moreover, C:N ratio, respiration (BR, Arg-SIR), and nitrification (Ure) significantly ($p \leq 0.001$) positively correlated with pH (r = 0.5, 0.53, 0.52, and 0.63, respectively). This was consistent with the positive relation between pH, CO_2 evolution, and N mineralization reported in previous studies [64–66]. The long-term interaction of carbon-enriched digestate, soil, and plant led to an even broader effect on pH, whose values increased significantly compared to the control (D) in all other variants in the second part of the experiment. The significant correlation of pH with TC ($p \leq 0.01$, r = 0.43) suggested a general association of stabilized carbonaceous organic sorbents (i.e., biochar or humic acids) with alkaline (soil) pH [67,68]. However, the synergy (PCA biplot—Figure S4) and correlation (Figure S3) of pH with the indicator of aerobic abundance, Glc-SIR ($p \leq 0.001$, r = 0.57), as well as with NAG ($p \leq 0.01$, r = 0.44), suggested that the fungal fraction of the microbial soil community may be involved in the in the long-term interaction with the biochar matrix, as already reported [69].

5. Conclusions

Based on the results obtained, we verified our hypothesis that Humac-enriched digestate improved short-term nutrient (carbon, phosphorus, nitrogen) transformation in amended soil as determined by β-glucosidase, phosphatase, and N-acetyl-β-D-glucosaminidase. Application of Humac-enriched digestate resulted in the least-reduced activity of aerobic microbes in the soil and led to the highest yield of dry aboveground maize biomass. We further confirmed the hypothesis that digestate enriched with biochar (or biochar + Humac) had a beneficial effect on total carbon content, C:N ratio, short-term respiration activity, and nitrification. However, long-term nitrogen mineralization was most enhanced by the addition of digestate + Humac, as shown by the amino acid-induced respiration and the values of urease activity. The hypothesis of a short-term positive effect of digestate + biochar (possibly + Humac) on catabolism was confirmed. After the occurrence of a negative priming effect on microbial abundance, utilization of (mostly) labile carbon increased and led to an increased biomass of soil aerobes, but also to the negative long-term effect of digestate + biochar (+Humac) on nutrient mineralization and to biochar-mediated immobilization and sequestration. The positive effects of applied amendments on soil physico-chemical and biological properties were anticipated; however, further studies are required to confirm the necessity of microbial parameters involved in soil health enhancement.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071587/s1. Figure S1: Pearson's Correlation Analysis of Variables in the first part of pot experiment. Figure S2: PCA Biplot Analysis of Variables in the first part of pot experiment. Figure S3: Pearson's Correlation Analysis of Variables in the second part of pot experiment. Figure S4: PCA Biplot Analysis of Variables in the first part of pot experiment.

Author Contributions: Conceptualization, T.H., J.H. and M.B.; methodology, T.H., J.H., O.M. and M.B.; software, T.B. and A.K.; validation, M.B., T.H. and A.M.; formal analysis, O.L. and T.B.; investigation, J.H., A.M. and M..B.; re-sources, O.L., A.K. and O.M.; data curation, T.H., T.B. and O.L.; writing—original draft preparation, T.H. and J.H.; writing—review and editing, T.H., J.H., A.M. and M.B.; visualization, T.H., T.B. and A.K.; supervision, A.M. and M.B. project administration, J.H., A.K. and M.B.; funding acquisition, J.H., A.K. and M.B. All authors have read and agreed to the published version of the manuscript.

Funding: The work was supported by the projects of Technology Agency of the Czech Republic TH04030132, by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO1218, MZE-RO1722, and by Ministry of Education, Youth and Sports of the Czech Republic, grant number FCH-S-22-8001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Glæsner, N.; Helming, K.; De Vries, W. Do current european policies prevent soil threats and support soil functions? *Sustainability* **2014**, *6*, 9538–9563. [CrossRef]
2. COWI. *Technical Guidance Handbook—Setting up and Implementing Result-Based Carbon Farming Mechanisms in the EU Report to the European Commission*; Contract No. CLIMA/C.3/ETU/2018/007; Ecologic Institute: Berlin, Germany; IEEP: Bruxelles, Belgium; DG Climate Action: Kongens Lyngby, Denmark, 2021.
3. Kilpeläinen, S.; Aalto, P.; Toivanen, P.; Lehtonen, P.; Holttinen, H. How to achieve a more resource-efficient and climate-neutral energy system by 2030? Views of nordic stakeholders. *Rev. Policy Res.* **2019**, *36*, 448–472. [CrossRef]
4. Luo, L.; Qin, L.; Wang, Y.; Wang, Q. Environmentally-friendly agricultural practices and their acceptance by smallholder farmers in china-a case study in xinxiang county, henan province. *Sci. Total Environ.* **2016**, *571*, 737–743. [CrossRef] [PubMed]
5. Figueiredo, C.C.D.; Farias, W.M.; Melo, B.A.D.; Chagas, J.K.M.; Vale, A.T.; Coser, T.R. Labile and stable pools of organic matter in soil amended with sewage sludge biochar. *Arch. Agron. Soil Sci.* **2018**, *65*, 770–781. [CrossRef]
6. Klinglmair, M.; Thomsen, M. Using food waste in organic fertilizer: Modelling biogenic carbon sequestration with associated nutrient and micropollutant loads. *Sustainability* **2020**, *12*, 7399. [CrossRef]
7. Shaji, H.; Chandran, V.; Mathew, L. Chapter 13—Organic Fertilizers as a Route to Controlled Release of Nutrients. In *Controlled Release Fertilizers for Sustainable Agriculture*; Lewu, F.B., Volova, T., Thomas, S., Rakhimol, K.R., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 231–245. [CrossRef]
8. Barłóg, P.; Hlisnikovský, L.; Kunzová, E. Effect of digestate on soil organic carbon and plant-available nutrient content compared to cattle slurry and mineral fertilization. *Agronomy* **2020**, *10*, 379. [CrossRef]
9. Slepetiene, A.; Kochiieru, M.; Jurgutis, L.; Mankeviciene, A.; Skersiene, A.; Belova, O. The effect of anaerobic digestate on the soil organic carbon and humified carbon fractions in different land-use systems in lithuania. *Land* **2022**, *11*, 133. [CrossRef]
10. Chen, R.; Blagodatskaya, E.; Senbayram, M.; Blagodatsky, S.; Myachina, O.; Dittert, K.; Kuzyakov, Y. Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities. *Biomass Bioenergy* **2012**, *45*, 221–229. [CrossRef]

11. Stumpe, B.; Werner, S.; Jung, R.; Heinze, S.; Jüschke, E.; Strippel, C.; Marschner, B. Organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. *Agric. Sci.* **2012**, *3*, 104–113. [CrossRef]
12. Thomsen, I.K.; Olesen, J.E.; Møller, H.B.; Sørensen, P.; Christensen, B.T. Carbon dynamics and retention in soil after anaerobic digestion of dairy cattle feed and faeces. *Soil Biol. Biochem.* **2013**, *58*, 82–87. [CrossRef]
13. Béghin-Tanneau, R.; Guérin, F.; Guiresse, M.; Kleiber, D.; Scheiner, J.D. Carbon sequestration in soil amended with anaerobic digested matter. *Soil Tillage Res.* **2019**, *192*, 87–94. [CrossRef]
14. Slepetiene, A.; Kadziuliene, Z.; Feiziene, D.; Liaudanskiene, I.; Amaleviciute-Volunge, K.; Slepetys, J.; Skersiene, A.; Velykis, A.; Armolaitis, K. The distribution of organic carbon, its forms and macroelements in agricultural soils. *Zemdirb. Agric.* **2020**, *107*, 291–300. [CrossRef]
15. Leno, N.; Sudharmaidevi, C.R.; Byju, G.; Thampatti, K.C.M.; Krishnaprasad, P.U.; Jacob, G.; Gopinath, P.P. Thermochemical digestate fertilizer from solid waste: Characterization, labile carbon dynamics, dehydrogenase activity, water holding capacity and biomass allocation in banana. *Waste Manag.* **2021**, *123*, 1–14. [CrossRef] [PubMed]
16. Voltr, V.; Menšík, L.; Hlisnikovský, L.; Hruška, M.; Pokorný, E.; Pospíšilová, L. The soil organic matter in connection with soil properties and soil inputs. *Agronomy* **2021**, *11*, 779. [CrossRef]
17. Tang, Y.; Luo, L.; Carswell, A.; Misselbrook, T.; Shen, J.; Han, J. Changes in soil organic carbon status and microbial community structure following biogas slurry application in a wheat-rice rotation. *Sci. Total Environ.* **2021**, *757*, 143786. [CrossRef]
18. Kolář, L.; Kužel, S.; Peterka, J.; Štindl, P.; Plát, V. Agrochemical value of organic matter of fermenter wastes in biogas production. *Plant Soil Environ.* **2008**, *54*, 321–328. [CrossRef]
19. Sarma, B.; Farooq, M.; Gogoi, N.; Borkotoki, B.; Kataki, R.; Garg, A. Soil organic carbon dynamics in wheat—Green gram crop rotation amended with vermicompost and biochar in combination with inorganic fertilizers: A comparative study. *J. Clean. Prod.* **2018**, *201*, 471–480. [CrossRef]
20. Ruhlmann, J. Calculation of net nitrogen mineralization from the decomposable soil organic matter pool. In Proceedings of the International Workshop on Ecological Aspects of Vegetable Fertilisation in Integrated Crop Production in the Field, Wellesbourne Hastings, Warwick, UK, 27–31 July 1998; International Society Horticultural Science: Leuven, Belgium, 1998.
21. Ludwig, B.; Geisseler, D.; Michel, K.; Joergensen, R.G.; Schulz, E.; Merbach, I.; Raupp, J.; Rauber, R.; Hu, K.; Niu, L.; et al. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. *Agron. Sustain. Dev.* **2010**, *31*, 361–372. [CrossRef]
22. Piccoli, I.; Francioso, O.; Camarotto, C.; Delle Vedove, G.; Lazzaro, B.; Giandon, P.; Morari, F. Assessment of the short-term impact of anaerobic digestate on soil C stock and CO_2 emissions in shallow water table conditions. *Agronomy* **2022**, *12*, 504. [CrossRef]
23. Molinuevo-Salces, B.; Gomez, X.; Moran, A.; Garcia-Gonzalez, M.C. Anaerobic co-digestion of livestock and vegetable processing wastes: Fibre degradation and digestate stability. *Waste Manag.* **2013**, *33*, 1332–1338. [CrossRef]
24. Tambone, F.; Adani, F.; Gigliotti, G.; Volpe, D.; Fabbri, C.; Provenzano, M.R. Organic matter characterization during the anaerobic digestion of different biomasses by means of cpmas 13c nmr spectroscopy. *Biomass Bioenergy* **2013**, *48*, 111–120. [CrossRef]
25. Shakeri Yekta, S.; Hedenstrom, M.; Svensson, B.H.; Sundgren, I.; Dario, M.; Enrich-Prast, A.; Hertkorn, N.; Bjorn, A. Molecular characterization of particulate organic matter in full scale anaerobic digesters: An nmr spectroscopy study. *Sci. Total Environ.* **2019**, *685*, 1107–1115. [CrossRef] [PubMed]
26. Tambone, F.; Orzi, V.; Zilio, M.; Adani, F. Measuring the organic amendment properties of the liquid fraction of digestate. *Waste Manag.* **2019**, *88*, 21–27. [CrossRef]
27. Greenberg, I.; Kaiser, M.; Gunina, A.; Ledesma, P.; Polifka, S.; Wiedner, K.; Mueller, C.W.; Glaser, B.; Ludwig, B. Substitution of mineral fertilizers with biogas digestate plus biochar increases physically stabilized soil carbon but not crop biomass in a field trial. *Sci. Total Environ.* **2019**, *680*, 181–189. [CrossRef] [PubMed]
28. Akimbekov, N.; Qiao, X.; Digel, I.; Abdieva, G.; Ualieva, P.; Zhubanova, A. The effect of leonardite-derived amendments on soil microbiome structure and potato yield. *Agriculture* **2020**, *10*, 147. [CrossRef]
29. Al-Maliki, S.; Al-Mammory, H.; Scullion, J. Interactions between humic substances and organic amendments affecting soil biological properties and growth ofzea maysl. In the arid land region. *Arid. Land Res. Manag.* **2018**, *32*, 455–470. [CrossRef]
30. Li, Y.; Fang, F.; Wei, J.; Wu, X.; Cui, R.; Li, G.; Zheng, F.; Tan, D. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. *Sci. Rep.* **2019**, *9*, 12014. [CrossRef]
31. Atkinson, C.J.; Fitzgerald, J.D.; Hipps, N.A. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant Soil* **2010**, *337*, 1–18. [CrossRef]
32. Wang, J.; Xiong, Z.; Kuzyakov, Y. Biochar stability in soil: Meta-analysis of decomposition and priming effects. *GCB Bioenergy* **2015**, *8*, 512–523. [CrossRef]
33. Mousavi, S.M.; Srivastava, A.K.; Cheraghi, M. Soil health and crop response of biochar: An updated analysis. *Arch. Agron. Soil Sci.* **2022**, *62*, 1–26. [CrossRef]
34. Lehmann, J.; Joseph, S. *Biochar for Environmental Management*; Routledge: London, UK, 2015. [CrossRef]
35. Liao, N.; Li, Q.; Zhang, W.; Zhou, G.; Ma, L.; Min, W.; Ye, J.; Hou, Z. Effects of biochar on soil microbial community composition and activity in drip-irrigated desert soil. *Eur. J. Soil Biol.* **2016**, *72*, 27–34. [CrossRef]
36. Hoskins, B.; Wolf, A.; Wolf, N. Dry Matter Analysis. In *Recommended Methods of Manure Analysis*; Peters, J., Ed.; University of Wisconsin Cooperative Extension Publishing: Madison, WI, USA, 2003.

37. *ISO 11261*; Soil Quality—Determination of Total Nitrogen—Modified Kjeldahl Method. International Organization for Standardization: Geneva, Switzerland, 1995.
38. Egnér, H.A.; Riehm, H.; Domingo, W.R. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor-und Kaliumbestimmung. *K. Lantbr. Ann.* **1960**, *26*, 199–215.
39. *EN_15749*; Fertilizers—Determination of Sulfates Content Using Three Different Methods. European Standardization Organization CEN-CENELEC: Brussels, Belgium, 2009.
40. *ISO 9964-3*; Water Quality—Determination of Sodium and Potassium—Part 3: Determination of Sodium and Potassium by Flame Emission Spectrometry. International Organization for Standardization: Geneva, Switzerland, 2001.
41. Rotthauwe, J.H.; Witzel, K.P.; Liesack, W. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **1997**, *63*, 4704–4712. [CrossRef] [PubMed]
42. Kandeler, E.; Deiglmayr, K.; Tscherko, D.; Bru, D.; Philippot, L. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl. Environ. Microbiol.* **2006**, *72*, 5957–5962. [CrossRef] [PubMed]
43. Amann, R.I.; Ludwig, W.; Schleifer, K.H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* **1995**, *59*, 143–169. [CrossRef] [PubMed]
44. *ISO 10390*; Soil Quality—Determination of pH. International Organization for Standardization: Geneva, Switzerland, 2005.
45. *ISO 10694*; Soil Quality—Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). International Organization for Standardization: Geneva, Switzerland, 1995.
46. *ISO 13878*; Soil Quality—Determination of Total Nitrogen Content by Dry Combustion (Elemental Analysis). International Organization for Standardization: Geneva, Switzerland, 1998.
47. Campbell, C.D.; Chapman, S.J.; Cameron, C.M.; Davidson, M.S.; Potts, J.M. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl. Environ. Microbiol* **2003**, *69*, 3593–3599. [CrossRef]
48. *ISO 20130*; Soil Quality—Measurement of Enzyme Activity Patterns in Soil Samples Using Colorimetric Substrates in Micro-Well Plates. International Organization for Standardization: Geneva, Switzerland, 2018.
49. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
50. Hinkle, D.E.; Wiersma, W.; Jurs, S.G. *Applied Statistics for the Behavioral Sciences*; Houghton Mifflin: Boston, MA, USA, 2003.
51. Rawat, J.; Saxena, J.; Sanwal, P. Biochar: A Sustainable Approach for Improving Plant Growth and Soil Properties. In *Biochar—An Imperative Amendment for Soil and the Environment*; Abrol, V., Sharma, P., Eds.; IntechOpen: London, UK, 2019; p. 124.
52. Ndoung, O.C.N.; Figueiredo, C.C.; Ramos, M.L.G. A scoping review on biochar-based fertilizers: Enrichment techniques and agro-environmental application. *Heliyon* **2021**, *7*, e08473. [CrossRef]
53. Xu, J.; Mohamed, E.; Li, Q.; Lu, T.; Yu, H.; Jiang, W. Effect of humic acid addition on buffering capacity and nutrient storage capacity of soilless substrates. *Front. Plant Sci.* **2021**, *12*, 644229. [CrossRef]
54. Ampong, K.; Thilakaranthna, M.S.; Gorim, L.Y. Understanding the role of humic acids on crop performance and soil health. *Front. Agron.* **2022**, *4*, 10. [CrossRef]
55. Holatko, J.; Hammerschmiedt, T.; Kintl, A.; Danish, S.; Skarpa, P.; Latal, O.; Baltazar, T.; Fahad, S.; Akca, H.; Taban, S.; et al. Effect of carbon-enriched digestate on the microbial soil activity. *PLoS ONE* **2021**, *16*, e0252262. [CrossRef]
56. Gryń, G.; Gryń, G.; Paluszak, Z.; Olszewska, H.; Keutgen, A.J. Chemical and microbiological properties of luvisol after addition of post-fermentation residue. *J. Elem.* **2020**, *25*, 701–716. [CrossRef]
57. Sun, Q.; Liu, J.; Huo, L.; CLi, Y.; Li, X.; Xia, L.; Zhou, Z.; Zhang, M.; Li, B. Humic acids derived from leonardite to improve enzymatic activities and bioavailability of nutrients in a calcareous soil. *Int. J. Agric. Biol. Eng.* **2020**, *13*, 200–205. [CrossRef]
58. Egene, C.E.; Sigurnjak, I.; Regelink, I.C.; Schoumans, O.F.; Adani, F.; Michels, E.; Sleutel, S.; Tack, F.M.G.; Meers, E. Solid fraction of separated digestate as soil improver: Implications for soil fertility and carbon sequestration. *J. Soils Sediments* **2020**, *21*, 678–688. [CrossRef]
59. Holatko, J.; Hammerschmiedt, T.; Datta, R.; Baltazar, T.; Kintl, A.; Latal, O.; Pecina, V.; Sarec, P.; Novak, P.; Balakova, L.; et al. Humic acid mitigates the negative effects of high rates of biochar application on microbial activity. *Sustainability* **2020**, *12*, 9524. [CrossRef]
60. Fascella, G.; Montoneri, E.; Rouphael, Y. Biowaste-derived humic-like substances improve growth and quality of orange jasmine (*Murraya paniculata* L. Jacq.) plants in soilless potted culture. *Resources* **2021**, *10*, 80. [CrossRef]
61. Tan, F.; Zhu, Q.; Guo, X.; He, L. Effects of digestate on biomass of a selected energy crop and soil properties. *J. Sci. Food Agric.* **2021**, *101*, 927–936. [CrossRef]
62. Allison, S.D. Soil minerals and humic acids alter enzyme stability: Implications for ecosystem processes. *Biogeochemistry* **2006**, *81*, 361–373. [CrossRef]
63. Zhou, L.; Xu, S.-T.; Monreal, C.M.; McLaughlin, N.B.; Zhao, B.-P.; Liu, J.-H.; Hao, G.-C. Bentonite-humic acid improves soil organic carbon, microbial biomass, enzyme activities and grain quality in a sandy soil cropped to maize (*Zea mays* L.) in a semi-arid region. *J. Integr. Agric.* **2022**, *21*, 208–221. [CrossRef]
64. Xu, N.; Tan, G.; Wang, H.; Gai, X. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil Biol.* **2016**, *74*, 1–8. [CrossRef]

65. Shah, T.; Khan, S.; Shah, Z. Soil respiration, ph and ec as influenced by biochar. *Soil Environ.* **2017**, *36*, 77–83. [CrossRef]
66. Hossain, M.Z.; Bahar, M.M.; Sarkar, B.; Donne, S.W.; Ok, Y.S.; Palansooriya, K.N.; Kirkham, M.B.; Chowdhury, S.; Bolan, N. Biochar and its importance on nutrient dynamics in soil and plant. *Biochar* **2020**, *2*, 379–420. [CrossRef]
67. Santos, L.L.d.; Lacerda, J.J.J.; Zinn, Y.L. Partição de substâncias húmicas em solos brasileiros. *Rev. Bras. Ciência Solo* **2013**, *37*, 955–968. [CrossRef]
68. Kaźmierczak, B.; Wystalska, K.; Malińska, K.; Włodarczyk, R.; Chajczyk, O.; Kutyłowska, M.; Piekarska, K.; Jadwiszczak, P. Effects of pyrolysis parameters on the yield and properties of biochar from pelletized sunflower husk. *E3S Web Conf.* **2018**, *44*, 197. [CrossRef]
69. Wiedner, K.; Glaser, B. Biochar-fungi interactions in soils. In *Biochar and Soil Biota*; CRC Press: Boca Raton, FL, USA, 2013; p. 10. [CrossRef]