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Biochar and Sulphur Enriched Digestate: Utilization of Agriculture Associated Waste Products for Improved Soil Carbon and Nitrogen Content, Microbial Activity, and Plant Growth

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Abstract: A number of agriculture residues may be used either directly or after suitable treatment as amendments to improve soil quality. Such materials include biochar made of agriculture residues, digestate or elemental sulphur obtained from biogas desulphurisation. The joint use of these materials via pre-incubation may be more advantageous than only mixing prior the application to soil. In this study, digestates were mixed with amendments and incubated for 6 weeks before application to soil in a short-term pot experiment with lettuce (Lactuca sativa). The following treatments were tested: control digestate, digestate + biochar, digestate + elemental sulphur, digestate + biochar + elemental sulphur. The biochar-enriched digestate significantly increased soil microbial biomass, soil C:N, fresh above ground biomass, fresh and dry root biomass. Elemental sulphur-enriched digestate caused highest arylsulfatase and phosphatase, increased urease, microbial biomass in soil and fresh root biomass. Amendment of digestate + biochar + sulphur led to the significantly highest total soil carbon, microbial biomass, β-glucosidase, urease, and increased C:N ratio, arylsulfatase in soil and root biomass. It mitigated the adverse effect of either biochar or elemental sulphur on soil respiration. Properties of digestates were apparently affected by pre-incubation. This approach in digestate fertilizer production may contribute to sustainable farming.

Keywords: plant biomass; microorganisms; respiration; enzymes; pot experiment; biosorbent

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1. Introduction

The increasing use of renewable energy facilities, including biogas plants, leads to the growing interest in digestate—a by-product of anaerobic digestion usually used as an organic fertilizer. The digestate is a suitable supplement to mineral fertilizers providing a readily available nitrogen (N), phosphorus (P) and potassium (K) source [1]. Thus, by providing macro- and micro-nutrients to the soil, it can effectively promote plant growth.

Besides the favorable effects, the organic part of digestate may enhance the soil organic matter (SOM) turnover and modify soil biological and physicochemical properties [2]. Digestate positively affects soil microorganisms [3] which play vital roles in the soil ecosystem functioning. They are responsible for nutrient cycling, mineralization of organic matter, synthesis of new compounds (immobilization), humus formation, maintenance and stability of soil substructure and influence soil fertility and crop yields. In other words, the soil microorganisms serve as critical indicators of proper soil functioning and health. Microorganisms are sensitive to soil nutrient availability as well, enabling their use as indicators of ecosystem changes [4]. The postulate is that the disrupted microbial enzymatic activity may indicate potential harmful impacts of anthropogenic stress [5].

One of the main issues of digestate use is the sustainability of nutrients in the amended soil [6] and their possible loss by leaching. For instance, N is especially prone to transformations in soil, including mineralization, nitrification/denitrification, immobilization, and losses by nitrate leaching and ammonia volatilization [7]. These N losses can damage the environment and reduce fertilizer efficiency. Although, the N loss is dependent on the use of cover crops [8], and their proper selection may mitigate it [7], it is still the main digestate drawback.

Several suggestions for digestate improvement can be found in the scientific literature, e.g. more advantageous use and favorable modifications. These include application of digestate to the lower soil layers (under-surface injection) [9–11], modifications of the anaerobic digestion process by using more stable feedstock [7,12–14] or co-application with other amendments, such as biochar [15–18].

Biochar, a product of thermal decomposition of biomass at 300–1000 °C in oxygen-limited conditions [19], is being suggested as a tool to improve soil fertility and soil properties while helping to mitigate climate change. Given the possible agricultural and environmental benefits, it has received full attention in the last decade [20]. Biochar is rich in carbon (C), especially when wood material is used as a feedstock, which seems to be recalcitrant [21]; it has a low bulk density and pH primarily higher than 7 [22]. It may even contain some valuable nutrients [23–25], although, their release over time is questioned [25–27].

Instead of enriching the soil with a nutrient supply like in the case of digestate, the positive effect of biochar on soil fertility is mostly ascribed to the reduced nutrient leaching and mobility, leading to an increased chance of utilization by plants. Therefore, by combining digestate with biochar, a novel fertilizer with advantageous properties may be obtained, exploiting a high load of available nutrients while mitigating their losses via leaching and volatilization.

The co-application of digestate and biochar was evaluated only a few times in the scientific literature with different results [15–18]. These studies demonstrated subsequent adsorption of ammonium on biochar particles (up to 60%), resulting in reduction of nitrate leaching [17], reduction of N_2O atmospheric emissions [15,16] and positive effects on yields [18].

Apart from activation by biochar, several other materials may enhance digestate properties. For example, the elemental sulphur used in combination with digestate could be solution for the lack of available sulphur, which has been reported to reduce yields and quality of crop plants worldwide [28]. Sulphur is generally absorbed by plants in the form of sulphate, while elemental sulphur is unavailable [29]. Its transformation in the soil—oxidation to sulphate (SO_4^{2-})—happens only in a limited quantity, and it is primarily a microbial process [30].

To the best of our knowledge no experiment has been reported with modification of digestate with biochar or elemental sulphur before distribution to the soil. Therefore, the goal of this study is to obtain an improved fertilizer by mixing and combined maturation (i.e., activation) of digestate with biochar or/and elemental sulphur. Basic soil nutrient content (C, N), plant and microbial biomass, soil respiration types and enzymatic activities

(indicating microbial soil function) were determined to identify modified digestate impacts on the soil environment and present the core of this current study.

Hypotheses 1. *The amendments (biochar, elemental sulphur) in modified digestate would increase nutrient content via both enrichment and stabilization (adsorption to the biochar) and enhance fertilizing effect observed on soil microbiota and plant.*

Hypotheses 2. *The interaction among digestate and amendments (biochar, elemental sulphur) would mitigate possible adverse effects on the chemical and biological processes in the organic material and soil.*

Hypotheses 3. *The amendment of elemental sulphur to digestate would not only elevate the S content but potentially could convert a fraction of sulphur to the more available form by the joint maturation.*

2. Materials and Methods

2.1. Soil Amendments Preparation

At the beginning of the activation process, 10 L of digestate were dosed in the barrel of volume 50 L. Digestate was amended by biochar and S, as it is shown in Table 1, the ratios were determined on the basis of required doses of components in field conditions: 50 $m^3 \cdot ha^{-1}$ of digestate, 20 $t \cdot ha^{-1}$ of biochar, 40 $kg \cdot ha^{-1}$ of sulphur. All treatments were prepared in triplicates. Used digestate was obtained from biogas plant (Rapotin, Czech Republic), which processes food waste under continuous mesophilic (about 40 °C) conditions. Used biochar was produced from agricultural waste (cereal bran and chaff, sunflower hulls, fruit peels and pulp) at 600 °C (Sonnenerde GmbH, Austria) and possess the basic properties as follows: C 86.6%, C:N 288.7, pH 8.5, BET specific surface 288.5 $m^2 \cdot g^{-1}$. Elemental sulphur was produced as a biogas desulphurization waste product in THIOPAQ scrubber (Paques, The Netherlands). The activation process was carried out for 42 days under laboratory temperature. At the end of the process, the mixed sample was taken from each barrel and analyzed (methods are indicated and referenced in Table 2).

Table 1. Activation doses.

Treatment	Digestate [L·Barrel^{-1}]	Biochar [kg·Barrel^{-1}]	Sulphur [g·Barrel^{-1}]
Control	10	0	0
Biochar (BC)	10	4	0
Sulphur (S)	10	0	8
Biochar + sulphur (BC + S)	10	4	8

Table 2. Determined digestate properties, methods used for measurement, relevant references.

Property	Abbrev.	Method	Unit	Reference
Dry matter	DM	Gravimetric, on a mass basis	%	[31]
Total nitrogen	N_{total}	Dry combustion using TruSpec analyzer (LECO, USA)	$g \cdot kg^{-1}$	[32]
Nitrate nitrogen	$N\text{-}NO_{3-}$	Reduction using Devarda's alloy	$g \cdot kg^{-1}$	[33]
Ammonium nitrogen	$N\text{-}NH_4^+$	Reduction using Devarda's alloy	$g \cdot kg^{-1}$	[34]
Mineral nitrogen	N_{min}	Sum of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$	$g \cdot kg^{-1}$	-
Sulphur in sulphates	$S\text{-}SO_4^{2-}$	Acid-soluble sulphates	$mg \cdot kg^{-1}$	[35]
Boron	B	Azomethine H colorimetric method	$mg \cdot kg^{-1}$	[36]
Nutrients	P, Ca, Na, K, Mg / Mn	Atomic absorption spectrometry (AAS) in Mehlich 3 extract	$g \cdot kg^{-1}$ / $mg \cdot kg^{-1}$	[37]

2.2. Pot Experiment Preparation

The activated digestates were used as a soil amendment in a pot experiment with lettuce (*Lactuca sativa* L. var. *capitata* L. cv. Briliant). All experimental pots (volume 1 L, top diameter 11 cm, bottom diameter 9 cm, height 13 cm) were filled up with 1 kg of soil substrate (fine quartz sand (0.1–1.0 mm) mixed with sieved (2.0 mm) topsoil from the rural area near the town Troubsko (Czech Republic—49°10′28″ N 16°29′32″ E) in ratio 1:1, *w*/*w*). The properties of used a silty clay loam (according to USDA Textural Triangle), Haplic Luvisol (according to WRB soil classification) are shown in Table 3.

Table 3. Properties of soil used for the pot substrate preparation.

pH [-]	TC [%]	TN [%]	N_{min} [mg·kg^{-1}]	N-NO_3 [mg·kg^{-1}]	N-NH_4 [mg·kg^{-1}]
7.29	1.40	0.16	62.84	56.80	6.04
C:N [-]	**S [%]**	**K [mg·kg^{-1}]**	**Ca [mg·kg^{-1}]**	**Mg [mg·kg^{-1}]**	**P [mg·kg^{-1}]**
8.77	0.01	231	3259	236	97

TC—total carbon, TN—total nitrogen, N_{min}—mineral nitrogen, N-NO_3—nitrogen in nitrate form, N-NH_3—ammonium nitrogen, C:N—ratio of total values, S—total sulphur, K—available potassium, Ca—available calcium, Mg—available magnesium, P—available phosphorus.

Activated digestates were added into six pots per treatment in the following amount: 40 mL (~40 g) of digestate, 16 g of biochar and 32 mg of sulphur per pot, depending on treatment. Three lettuce seeds were sown 2 mm under the soil surface into each pot. After that, all pots were placed randomly into grow chamber Climacell Evo (BMT Medical Technology Ltd., Czech Republic). After 10 days, the most robust seedling was left in each pot. Controlled conditions were set as follows: 12 h long photoperiod, light intensity 20,000 lx, temperature (day/night) 22/18 °C, relative air humidity 70%. Each pot was watered with distilled water to 65% WHC, which was maintained throughout the experiment. Moreover, pots were randomly rotated every other day to ensure homogeneity of conditions for the treatments.

2.3. Plant Biomass

The lettuce seedlings were grown for 42 days. Subsequently, the leaves were cut at the ground level, and roots were removed gently from the soil and washed under tap water. The weight of aboveground (AGB) and roots fresh biomass was determined gravimetrically using the analytical scales (OHAUS Europe Gmb, Switzerland). The weight of dry AGB and roots biomass was also determined gravimetrically by weighting both biomasses dried at 60 °C to constant weight.

2.4. Soil Sampling and Preparation

The soil samples were taken after the harvesting of lettuce (1 mixed sample per pot). The homogenization of samples was done by sieving through a 2 mm mesh under sterile conditions. The samples for the enzyme activity assay (β-glucosidase (GLU), arylsulfatase (ARS), phosphatase (PHOS) and urease (URE)) were freeze-dried. Microbial biomass carbon (MBC), soil basal (BR) and substrate-induced respiration (SIR)—trehalose (Tre-SIR), lysine (Lys-SIR), L-alanine (Ala-SIR), D-glucose (Glc-SIR), N-acetyl-β-D-glucosamine (NAG-SIR)—were determined in samples stored at 4 °C. Total soil carbon (TC) and nitrogen (TN) content were analyzed using air-dried samples.

2.5. Chemical, Biological, and Statistical Analysis

Soil properties were determined, and the obtained data statistically analyzed by the downlisted methods (Table 4.) and their specifications were identical to our previously published work [38].

Table 4. Determined soil properties, methods used for measurement and statistics, relevant references.

Property	Method	Unit	Reference
Total soil carbon	Dry combustion using, TruSpec analyzer (LECO, USA)	$mg \cdot g^{-1}$	[39]
Total soil nitrogen			[32]
Microbial biomass carbon	Fumigation extraction method	$mg \cdot g^{-1}$	[40]
Soil enzyme activities	Microplate incubation, UV-Vis spectrophotometry	$\mu mol\ PNP \cdot g^{-1} \cdot min^{-1}$, $\mu mol\ NH_3 \cdot g^{-1} \cdot min^{-1}$	[41]
Basal soil respiration	MicroResp® device (The James Hutton Institute, UK)	$\mu g\ CO_2 \cdot g^{-1} \cdot h^{-1}$	[42]
Substrate induced soil respiration	MicroResp® device + inducers (sugars, amino acids)		
Processing	**Tool**	**Method**	**Reference**
Statistical analysis	Program R version 3.6.1.	Multivariate analysis of variance (MANOVA), principal component analysis (PCA), one-way analysis of variance (ANOVA), Tukey's range test, Pearson correlation analysis	[38]

3. Results

3.1. Composition of Modified Digestates

The parameters of modified digestates are shown in Table 5. There could be seen, that biochar amendment led to increased dry matter and nutrient (Ca, P, Na, K, Mg, Mn, B) content in BC and BC + S treatments as compared to control and S treatment. These all-nutrient traits showed a significant agonisms in the PCA biplot (Figure A2) and also a positive correlation e.g., Ca correlated absolutely with P, K ($r = 1.0$ for both) and highly with Na, Mg, Mn, B (r = 0.99, 0.89, 0.91, 0.99, respectively). The blending ratios between digestate and biochar, digestate and elemental S, or among all three materials was the simple reason for different nutrient values, which were most affected by biochar with high content of P (5.6% *w*/*w*), K (5.0% *w*/*w*), Ca (4.9% *w*/*w*), Mg (3.1% *w*/*w*). On the other hand, sulphur amendment (treatments S and BC + S) caused the expected increase of sulphur in sulphate form ($S\text{-}SO_4^{2-}$) and also the decrease in the nitrate N ($N\text{-}NO_3^{-}$) content (compared to control and BC treatment). Moreover, sulphur addition to the BC + S digestate mitigated a decline in the ammonium N ($N\text{-}NH_4^{+}$) content, which was detected in the BC digestate (as compared to the control). Due to these findings, there was a clear antagonism between $S\text{-}SO_4^{2-}$ and NH_4^{+} content on one hand and the NO_{3-} content on the other hand, apparent in PCA biplot (Figure A2). Further, this antagonism was corroborated by the Pearson's correlation analysis, which revealed a high positive correlation of $S\text{-}SO_4^{2-}$ with $N\text{-}NH_4$ ($r = 0.78$) and high negative correlation of $S\text{-}SO_4^{2-}$ with $N\text{-}NO_3$ ($r = -0.76$)—Figure A1.

Table 5. Properties of activated digestates (in fresh matter): BC—biochar, S—sulphur, BC + S—biochar and sulphur.

Variable	Unit	Control	BC	S	BC + S
Ca	$[g \cdot kg^{-1}]$	$1.15^{c} \pm 0.08$	$3.17^{b} \pm 0.28$	$1.67^{c} \pm 0.10$	$4.87^{a} \pm 0.38$
P	$[g \cdot kg^{-1}]$	$0.76^{c} \pm 0.04$	$2.09^{b} \pm 0.16$	$1.01^{c} \pm 0.08$	$3.15^{a} \pm 0.26$
Na	$[g \cdot kg^{-1}]$	$0.36^{c} \pm 0.02$	$0.92^{b} \pm 0.06$	$0.35^{c} \pm 0.02$	$1.26^{a} \pm 0.10$
K	$[g \cdot kg^{-1}]$	$1.35^{c} \pm 0.11$	$3.63^{b} \pm 0.23$	$2.11^{c} \pm 0.16$	$5.65^{a} \pm 0.43$
Mg	$[g \cdot kg^{-1}]$	$0.09^{b} \pm 0.01$	$0.23^{a} \pm 0.02$	$0.12^{b} \pm 0.01$	$0.22^{a} \pm 0.02$
Mn	$[mg \cdot kg^{-1}]$	$3.99^{b} \pm 0.32$	$10.80^{a} \pm 0.75$	$3.01^{b} \pm 0.21$	$10.99^{a} \pm 0.88$
B	$[mg \cdot kg^{-1}]$	$2.62^{c} \pm 0.21$	$4.65^{b} \pm 0.38$	$2.54^{c} \pm 0.20$	$7.23^{a} \pm 0.57$
DM	[%]	$5.96^{b} \pm 0.11$	$15.00^{a} \pm 0.36$	$5.78^{b} \pm 0.13$	$15.05^{a} \pm 0.40$
N_{total}	$[g \cdot kg^{-1}]$	$43.10^{a} \pm 3.47$	$44.50^{a} \pm 3.59$	$40.60^{a} \pm 3.19$	$41.2^{a} \pm 3.20$

Table 5. *Cont.*

Variable	Unit	Control	BC	S	BC + S
$N\text{-}NO_{3-}$	$[g \cdot kg^{-1}]$	$2.20^{a} \pm 0.15$	$2.51^{a} \pm 2.20$	$1.64^{b} \pm 0.12$	$0.79^{c} \pm 0.05$
$N\text{-}NH_4^{+}$	$[g \cdot kg^{-1}]$	$5.17^{a,b} \pm 0{,}42$	$4.02^{b} \pm 0.32$	$5.45^{a} \pm 0.39$	$5.96^{a} \pm 0.45$
N_{min}	$[g \cdot kg^{-1}]$	$7.37^{a} \pm 0{,}55$	$6.53^{a} \pm 0.53$	$7.09^{a} \pm 0.57$	$6.75^{a} \pm 0.52$
$S\text{-}SO_4^{2-}$	$[mg \cdot kg^{-1}]$	$8.52^{b} \pm 0.69$	$9.00^{b} \pm 0.70$	$13.89^{a} \pm 1.12$	$14.38^{a} \pm 1.14$

Values of variables (digestate properties) are displayed as meanvealues ± standard deviation (SD); lowercase superscript letters express the results of ANOVA Tukey's HSD Posthoc Test—the statistical differences at significance level $p \leq 0.05$.

3.2. Soil Nutrients, Microbial Abundance, and Plant Biomass

The amendment of digestate enriched with elemental sulphur did not change TC (Figure 1a). The BC treatment showed higher soil TC as compared to the control only in average values (insignificant), whereas digestate enriched with both BC + S statistically increased TC in the soil (Figure 1a). TC showed a direct dependence on digestates DM content (Table 5) and a high positive correlation with C:N ratio ($r = 0.81$).

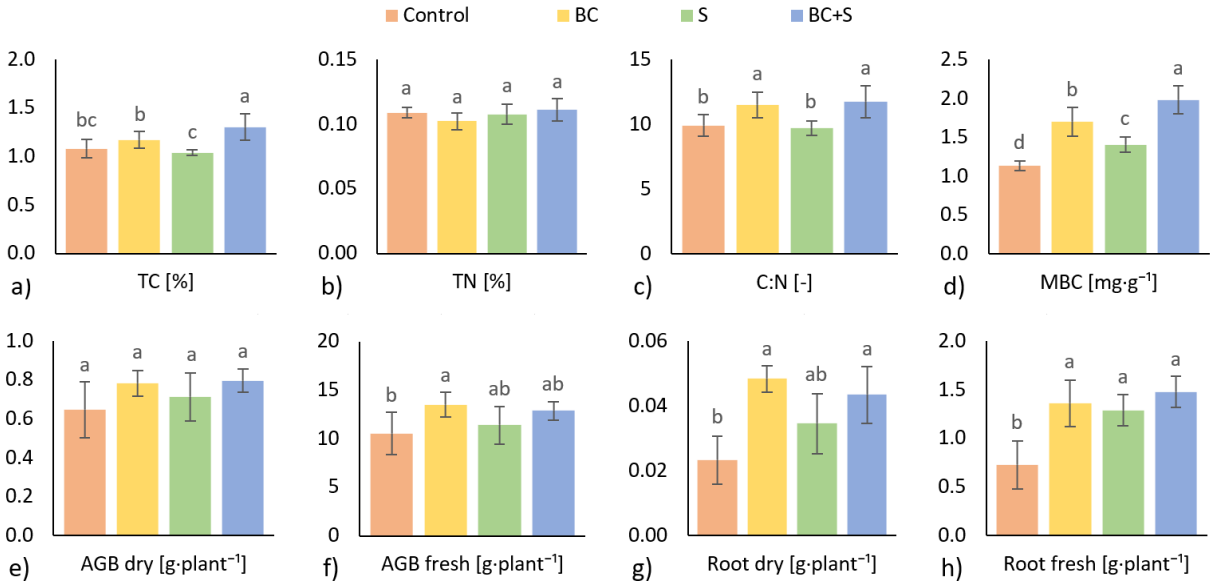

Figure 1. Total soil carbon (**a**) and nitrogen (**b**), C:N ratio (**c**), microbial biomass carbon (**d**); dry (**e**) and fresh (**f**) aboveground biomass (AGB), dry (**g**) and fresh (**h**) root biomass; tested treatments: BC—biochar, S—sulphur, BC + S—biochar and sulphur. Mean ± SD. The different letters express the results of ANOVA Tukey's HSD Posthoc Test—the statistical differences at significance level $p \leq 0.05$.

In contrast to TC, soil TN was not strongly affected by any of the enriched digestates as there was no significant difference in this parameter among the treatments (Figure 1b). The increase in TC content logically affected the C:N ratio values (Figure 1c), which were significantly higher in both biochar amended treatments (BC, BC + S) as compared to the control and S treatment. We also experienced a moderate positive correlation of C:N with MBC ($r = 0.54$) and dry root biomass ($r = 0.53$).

A significant increase in MBC compared to the control was detected in all experimental treatments (Figure 1d). The BC + S treatment reached the highest MBC value. MCB also showed a moderate positive correlation with TC, C:N, root fresh and dry biomass.

Although, the differences in the soil nutrient content and microbial abundance presumed an increase in soil fertility of all treatments amended with the modified digestate, the expected effect of the experimental digestate on the dry lettuce AGB was not confirmed (Figure 1e). Only fresh AGB in the BC treatment was significantly higher than control (Figure 1f). On the other hand, the changes in various soil properties led to the significant

increase in the root biomass (Figure 1g,h) in all modified digestate-amended treatments. The fresh AGB:root biomass ratio (of average values) was 14.5, 9.9, 8.8, and 8.7 in the control, BC, S, and BC + S, respectively. Dry root biomass was significantly increased in the BC and BC + S treatments (Figure 1h) and their dry AGB:root biomass ratio was 16.2 and 18.3 (compared to 27.8 in the control). Interestingly, the biomass increase was observed mainly after BC addition to the digestate. Compared to BC, elemental sulphur modification played a lesser role in plant growth. Biochar decomposition with simultaneous release of nutrients may have played a more significant role; thus, lettuce biomass properties were positively related to the mineralization indicators URE and ARS—as the PCA biplot (Figure A2) showed.

3.3. Soil Microbial Activity

The GLU was statistically higher only in the BC + S treatment (Figure 2a), resulting in the highest microbial biomass values and nutrient content. As ARS is the enzyme of sulphur transformation, it was expectedly significantly increased in both sulphur-amended treatments BC + S and S, compared to the control (Figure 2b). The highest ARS value was proven for the S treatment, which was amended with the digestate containing a high S-SO_4^{2-} concentration (Table 5). ARS moderately correlated to PHOS (r = 0.58) which was statistically the highest in the S treatment and lowest in the BC (Figure 2c). The last determined enzyme URE was in comparison to the control significantly increased in sulphur amended treatments BC + S and S (Figure 2d).

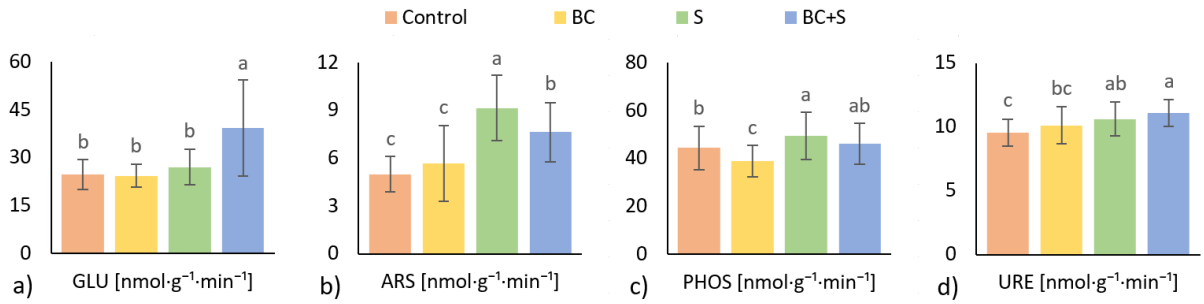

Figure 2. Soil activities of β- glucosidase—GLU (**a**), arylsulfatase—ARS (**b**), phosphatase—PHOS (**c**), and urease—URE (**d**); tested treatments: BC—biochar, S—sulphur, BC + S—biochar and sulphur. Mean ± SD. The different letters express the results of ANOVA Tukey's HSD Posthoc Test—the statistical differences at significance level $p \leq 0.05$.

The values of BR in the BC and S treatments were significantly lower compared to the control (Figure 3a), showing that aerobic decomposition is apparently negatively affected by the amendment of the respective enriched digestates. The co-enrichment of digestate with both the biochar and elemental sulphur mitigates the negative effect of each of the materials on the BR in the soil.

As all SIRs correlated highly or moderately positively with each other, the differences in the respiration properties were similar (Figure 3b–f). For example, the BC and S treatments' values were significantly lower than the control. In contrast, the BC + S digestate increased or did not change all SIRs and we assumed that the combined enrichment of digestate by biochar and sulphur mitigated the adverse effect of either BC or elemental Son soil aerobes. Moreover, the PCA biplot (Figure A2) showed a positive relationship among all types of soil respiration except for Glc-SIR.

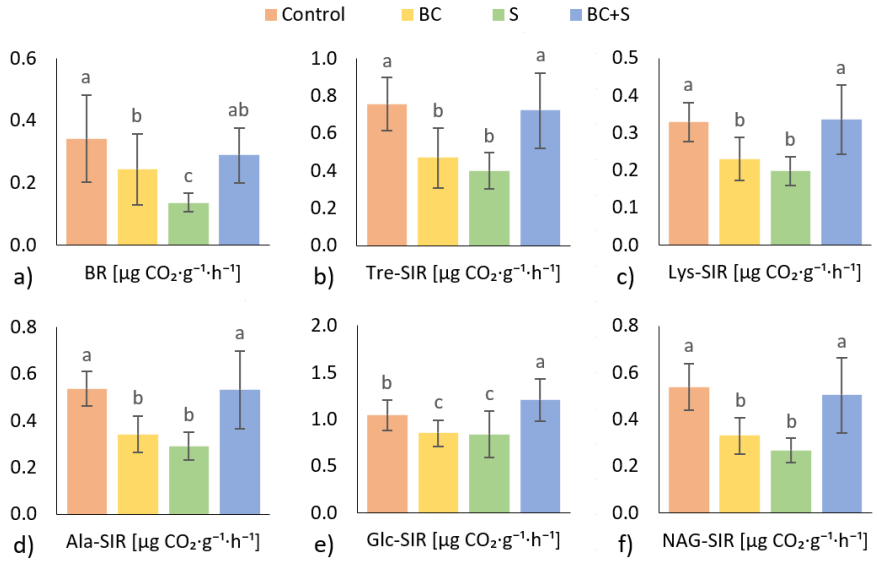

Figure 3. Basal respiration (**a**), trehalose SIR—Tre-SIR (**b**), L-lysine SIR—Lys-SIR (**c**), L-alanine SIR—Ala-SIR (**d**), D-glucose SIR—Glc-SIR (**e**) and N-acetyl-β-D-glucosamine SIR—NAG-SIR (**f**); tested treatments: BC—biochar, S—sulphur, BC + S—biochar and sulphur. Mean ± SD. The different letters express the results of ANOVA Tukey's HSD Posthoc Test—the statistical differences at significance level $p \leq 0.05$.

4. Discussion

4.1. Modified Digestates

There was a different relationship between the biochar or elemental sulphur amendment in the digestate and the content of N and sulphur chemical forms. The detected beneficial effect of elemental sulphur addition on the increase in S-SO_4^{2-} concentration in the digestate treatments S and BC + S was explainable by stimulated oxidation of elemental sulphur to the sulphates. In the previous study [43], an anaerobically digested sludge was matured in a similar way as our digestate incubation with amendment of elemental sulphur (4 g·L^{-1}), and there was reported a 70% sulphur oxidation efficiency to SO_4^{2-}. Concurrently with the expected increase of S-SO_4^{2-}, we experienced the decrease in the NO_{3-} content in the treatments S and BC + S (compared to control and BC treatment). The authors of research done in the aerobic wastewater reactor enviroment [44] referred to the completely inhibited ammonium oxidation under increased sulfide concentration in the liquid. If ammonia oxidation was retarded in the sulphur-amended digestate treatments (S and BC + S), then also the following steps in the nitrification (e.g., nitrites oxidation to nitrates) might be inhibited. This presumption could explain the shown significant low content of nitrates in the sulphur amended treatments. Nevertheless, after the sulphur oxidation proceeded to higher S-SO_4^{2-} content, ammonia oxidation presumable recovered again and might cause in the BC treatment decrease in the N-NH_4^+ and increase in the N-NO_3^- content. This N-NH_4^+ decrease in the BC treatment can be explained either by the highest ammonium-oxidation rate (no interference with an elemental sulphur oxidation) or due to the possible retention in organic N transformation [45]. However, this negative feature was mitigated by the addition of elemental sulphur to the biochar-amended treatment, which apparently caused a competition in complexing of ammonium with either SO_4^{2-} newly produced by elemental sulphur oxidation or with biochar surface. Then the ammonium ions were more bound to the formed sulphate (determined in the extract) and less adsorbed to BC (not determined in the extract) [46]. Nevertheless, it led to an insignificant increase in the N-NH_4^+ content among control and both sulphur-amended digestate treatments (S, BC + S).

4.2. Soil Nutrients, Microbial Abundance, and Plant Biomass

The BC + S treatment exerted significantly higher soil TC than the control, showing a clear, direct dependence on a dry matter of the BC + S digestate. Further we presume that the sulphur amendment to the digestate prior to maturation incubation increased the biochar fixed C which is the parameter closely related to stable C content [47]. It was reported that biochar amendment to digestate enhanced catabolic bacterial performance to degrade organic C during the fermentation [48], we anticipated the significantly stimulated microbial activity also in post-fermentation phase. On the other hand, the elemental sulphur was referred to inhibit microoorganisms [30,49], the bacterial destabilizing effect on the digestate organic C was apparently mitigated, at least in the early phase of digestate maturation. Subsequently retarded mineralization of labile organic matter in the BC + S digestate may be the reason for observed increased TC and C catabolism in thesoil treated with this digestate. These results verified our hypothesis of improved nutrient content via both enrichment and stabilization and enhance fertilizing effect of the maturation-modified digetates.

Contrarily, the joint amendment of digestate and elemental sulphur seemed to decrease C sequestration. The assumed reason for this feature of the S treatment is generally increased decomposition of SOM. At higher dose of elemental sulphur added to soil, organic matter acts as an energy source for sulphur-oxidizing microorganisms which increase the sulphur-mineralization rate in soil [50,51]. Thus, we detected significantly enhanced ARS activity, which indicated increased organic sulphur-mineralization rate in the soil of S treatment. We deduced from this an accelerated SOM degradation. This assumption corroborated also other enhanced enzyme activities (PHOS, URE) and higher MBC.

We observed a difference in C:N ratio among biochar amended treatments (BC and BC + S), the control and the sulphur-amended treatment (S). In the context of the reported close-to-optimum C:N ratio for better N uptake by plants, when <15 accelerates mineralization [52], the treatments BC and BC + S with C:N above 10 favoured the plant assimilation and growth more than the control and S, for which the C:N ratios were below 10, therefore favouring microbial decomposition [53], associated with higher values of ARS and PHOS in the S treatment.

MBC, i.e., C contained within living microorganisms (bacteria, fungi), is the next parameter explaining soil fertility [54]. MBC can serve as an early indicator of total organic C changes. The addition of all amended digestates caused a positive change in MBC compared to unmodified digestate. Moreover, the MBC value was almost doubled in the BC + S treatment as compared to the control. The possible explanation for the enhanced microbial abundance by all enriched digestates might be nutrient sequestration and stabilization via amendments (BC, S). Biochar was reported to adsorbed and stabilize various sources of macro- and microelements [55] which would be further utilized by microorganisms. The elemental sulphur was also referred to protect the ammonium N from volatilization [56], and co-stimulate N-uptake [57,58]. The biochar- and sulphur-mediated higher supply of these components increased the nutritional level of the soil environment, which was reflected by an increase in the populations of microorganisms as measured by the MBC value.

Despite the differences in the soil nutrient content, no significant differences in dry AGB were detectable among the treatments. On the other hand, the fresh and dry root biomass were increased by the amendment of modified digestates used here, most significant positive effect showed BC treatment, as already reported [59], and BC + S treatment exerting high TC, C:N, MBC values.

4.3. Soil Microbial Activity

The highest GLU value, detected in the BC + S, corresponded to the highest MBC and TC values. It can be concluded that GLU activities are ruled by both soil microbial demand for energy, C and nutrient availability [60]. This aspect is even more evident for the ARS. Its activity is enhanced in both treatments amended with the sulphur-modified digestates

(S and BC + S). Direct dependence of ARS values on the S-SO_4^{2-} content in the used digestates (Table 5) revealed the positive effect of sulphur enrichment within the BC + S digestate amendment, similar to the previously referred study [61]. Sulphate content in the S and BC + S digestate treatment, apparently increased as compared to the control, conferred the stimulation of elemental sulphur mineralization in the respective soil.

The application of sulphur-enriched digestate also increased the PHOS activity, which was significant only in the S treatment. A similar positive effect of fertilization with elemental sulphur on soil PHOS activity previously reported [62,63]. Nevertheless, similar effect of elemental sulphur enrichment in the treatment BC + S was not detected. It is known that PHOS activity is induced by low levels of available P thus its product of enzymatic reaction suppresses it [64]. Significantly lowered PHOS in the BC treatment compared to the control anticipated the increased phosphate supplement due to the P-richness of amended biochar. Considering these presumptions, low significance of the change in PHOS activity of the BC + S treatment may be explained.

The activity of URE is associated with deamination of organic N and indicates the microbial activities within of the range of soil biota. The URE was significantly increased in BC + S and S treatments. The results showed that the increase in URE activity is related to the higher content of N-NH_4^+ in sulphur-enriched digestates (Table 5). Thus, we presume that the protective effect of elemental sulphur against the ammonium volatilization is involved, similarly as previously reported [56,65], causing higher N availability for plant and microbial uptake and biomass gain via precursor supplied nitrification.

Another method to evaluate microbial soil activity in the experimental treatment was BR and SIR [66]. It represents the way to determinate the catabolic activity of the soil microflora, the intensity of utilization C and energy sources. As the biochar-enriched digestate had no (GLU) or negative (PHOS) effect on microbial enzyme activity in the soil, it was anticipated that BR was inhibited in the BC treatment. Similar negative effect of biochar addition to digestate on the CO_2 emission from fertilized soil has been already referred [67]. Furthermore, the elemental sulphur-amended digestate showed even more significantly negative effect on the BR. This contrasting feature of microbial activity in the S treatment is surprising, because the enzymatic activities (ARS, PHOS, URE) of this treatment were affected positively. Neither of the other authors referred to the significantly decreased soil respiration due to the elemental sulphur amendment [68]; on the contrary, the detrimental effect of elemental sulphur on the soil microbial diversity was reported [69]. From this and the results of SIR, which were all significantly decreased in the BC and S treatments, we presume negative effect of both types of single-enriched digestates on the abundance of aerobic microorganisms in soil. Nevertheless, we observed that the adverse effect of either biochar- or sulphur-enriched digestate on the BR and SIR was mitigated by the co-enrichment of the digestate with both materials together. This observation corroborated the multi-beneficial effect of the BC + S digestate on the soil properties and verified our hypothesis of the mitigation of possible adverse effects on the chemical and biological properties by the maturation-modified digestate.

5. Conclusions

Although no significant increase in the aboveground lettuce biomass was observed, a beneficial effect of modified digestates on root biomass and several soil quality properties in the presented pot experiment was proven as apparent. We were able to partially correlate the detected changes in the soil quality to the composition of the used digestates, which were prepared by novel common pre-incubation of digestates and tested amendments, which preceded the application. We evaluated that biochar may improve the fertilizing properties of digestate. The biochar-enriched digestate significantly increased microbial biomass, C:N ratio, fresh AGB, both fresh and dry root biomass. However, a negative effect on the soil respiration was observed. The sole sulphur enrichment of digestate maximized arylsulfatase and phosphatase activities. Sulphur-enriched digestate decreased soil respiration, but this adverse effect was mitigated and supressed by co-application of sulphur

and biochar. Elemental sulphur used in combination with biochar improved the effect of digestate on particular plant and soil properties—namely, showing significantly high fresh root biomass, TC, microbial biomass, β-glucosidase and urease activities. However, these are only preliminary results, leading to further testing and evaluation of this new approach in digestate fertilizer production, which may contribute to sustainable farming.

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Appendix A

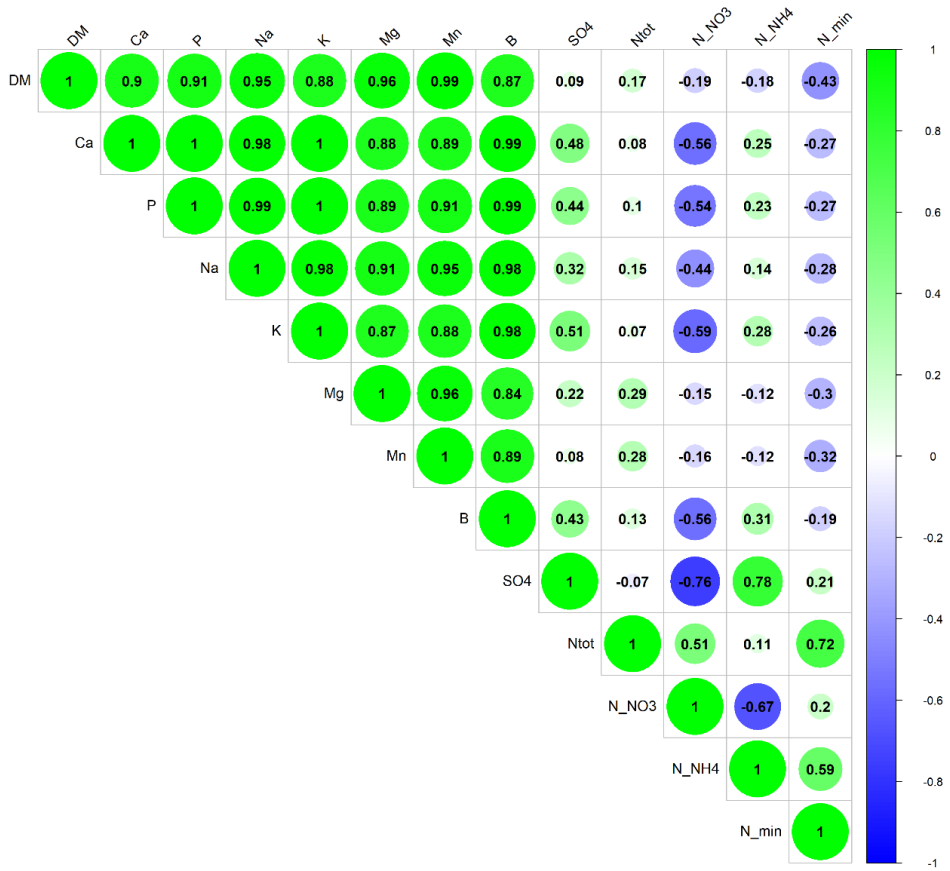

Figure A1. Pearson's Correlation matrix for properties of digestate treatments. Displayed values = correlation coefficient r.

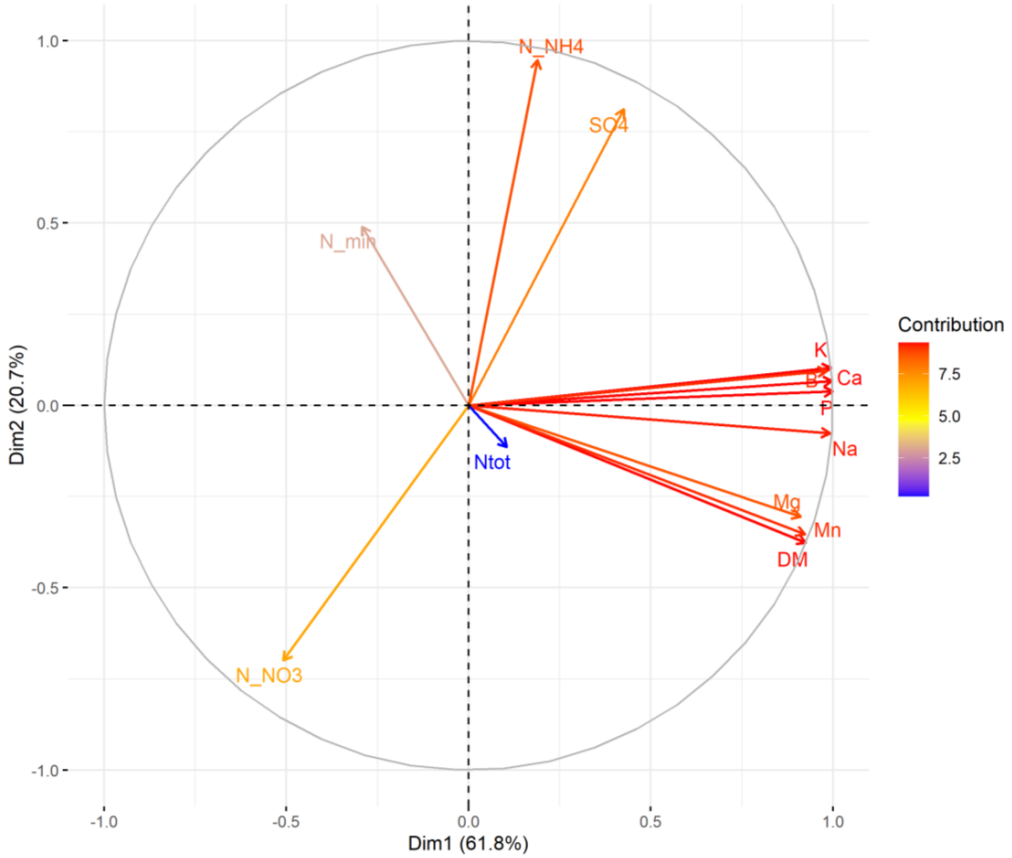

Figure A2. Rohlf's PCA biplot for properties of digestate treatments.

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