



Article Manure Maturation with Biochar: Effects on Plant Biomass, Manure Quality and Soil Microbiological Characteristics

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Abstract: Application of biochar and composts prepared from organic wastes as soil amendments has been recognized as a beneficial strategy to enhance soil fertility and crop production. However, the modification of manures with applied organic amendments such as biochar has not been well explained. Therefore, the preliminary study was designed to evaluate the impact of two doses of biochar (low 0.4 kg + 10 kg of manure and high 4 kg + 10 kg of manure) on the modification of resulting co-composted manure properties, and subsequently to evaluate the effect of matured manure amendment on the soil chemical and biological properties and plant yield in the pot experiment with barley (Hordeum vulgare L.). The following variants were tested: control, manure (M), manure + low biochar dose (M + LB), manure + high biochar dose (M + HB). Results revealed that, the M + HB significantly improved the co-composted manure properties as compared to control and M + LB, respectively. The most pronounced effects of M + HB treatment were observed on pH, NH₄-N and humic acid to fulvic acid ratio (used as an index for manure maturity) relative to other treatments. Similarly, significant variations were observed between AOB (ammonium oxidizing bacteria) and nirs genes under M + HB which lowered the AOB and increased the nirs abundance as compared to other treatments. Moreover, when applied to soil, M + HB increased the observed soil chemical parameters with the exception of TN contents as compared to M and M + LB treatments. Similarly, plant biomass was significantly enhanced under the applied M + HB treatment. However, statistically insignificant differences were observed regarding soil enzyme activities and soil respiration values under the applied amendments. Thus, it was concluded that the co-composted manure with high biochar dose can have the potential to enhance the manure properties, soil fertilization value and plant biomass. However, its effects on soil microbiological and enzyme activities were intended be explored under long-term field experiments.

Keywords: manure; crop production; soil enzymes; plant nutrients; respiration; microbial biomass

1. Introduction

Biochar is a heterogeneous material produced by the pyrolysis of various biomass such as wood, agriculture wastes, sewage sludge, etc., under controlled conditions in the absence



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). or limited access of oxygen. It has several specific properties such as stability, high carbon (C) content, large specific surface area, microporosity and sorption capacity [1]. Biochar is resistant to decomposition, which predetermines its potential year-long persistence in nature [2] and positive contribution to soil C sequestration [3]. Its surface and sorption properties enable reduction of greenhouse gas emissions [4], removal of diverse organomineral pollutants, e.g., agrochemicals, antibiotics, polycyclic aromatic hydrocarbons, polychlorinated biphenyls [5,6], heavy metals [7], ammonium [8], and hydrogen sulfide [9]. Yet, the foremost benefit of biochar was considered a 2000 year-long effect in the Amazonian "Terra Preta" soils, due to joint amendment with biochar and other organic materials (e.g., bones, plant tissues, animal feces) exerting higher pH, nutrient content and more abundant and diverse microbial populations than unamended Oxisols [10,11]. Taking these findings into account, biochar represents a carbonaceous material, which provides agronomic benefits in the improvement of soil fertility (carbon and other nutrients sequestration [12]) and quality, such as mitigation of soil acidity and salinity [13–15].

Application of biochar, manure or compost mixtures and co-composted biochar as soil amendment is the most advantageous agriculture usage of biochar [16,17]. Biochar as a supplement for composting and fermentation of different types of manure [18] showed a positive influence on mitigation of greenhouse gases [19,20] and ammonia [21] emissions, prevention of nutrient loss [22], process of manure maturation (thermodynamics, heat generation [23]), abundance and functional diversity of manure microflora [24] and microorganism-dependent biochemical processes of nutrient metabolism and mineralization [25]. The physicochemical properties of different biochar types, depending on the biochar feedstock and pyrolysis conditions [26,27], determine the variation in the process of composting and the final traits of blended product [28]. It is known that biochar prepared at low temperatures (\leq 400 °C) has a much higher ratio of potentially mineralizable C to stable C as well as a higher content of low-molecular-weight acids [29]. This feature enables greater incorporation of ammonium ions (NH₄⁺) into organic compounds during the microbial utilization of labile, soluble organic C and subsequent lower NH_3 emissions [29]. Therefore, maximum ammonia (NH₃) sorption occurs in biochar prepared by low-temperature pyrolysis at almost neutral pH (7.0–7.5).

The effect of application rate on the biochar behavior in either manure or solid waste has also been a focus of various scientists [21,30]. Depending on the increasing application rate in the composted material, biochar can exert either positive [31] or negative effects [32] on microbial diversity, composting efficiency and quality, and C mineralization in soil and amendment priming effect. There are many studies regarding biochar, manure or compost mixtures and composting process modification by biochar [33-35]. Yet, the knowledge of co-composted biochar-manure importance for soil fertility and quality improvement via affecting, e.g., biological traits and the microbe-controlled nutrient transformation, still calls for further improvement [36]. Therefore, the objectives of our pilot research were to evaluate the impact of different doses of biochar on the properties of resulting co-composted manure product. Subsequently, we investigated the effect of non-enriched and dose-dependent enriched manure amendment on the soil chemical and biological properties and plant biomass yield in the pot experiment using barley (Hordeum vulgare L.) as a test crop. We hypothesized that: (I) co-composted manure would positively affect the maturity of final product, mainly nutrient (C and plant-available nitrogen (N)) content, microbial abundance and fertilization value; (II) subsequently, the soil amended with the biochar-enriched manure would stimulate the microbial activity in soil, enhance the nutrient transformation and mineralization, increase their (C, N, S) content and C:N ratio, in addition to increased plant biomass.

2. Materials and Methods

2.1. Manure Modification

This pot experiment was intended as a preliminary study for further field trial. Experimental manures were prepared by mixing of unmatured manure with biochar, which was (according to manufacturer) pyrolyzed from agricultural waste (85% spelled husks and sunflower husks, 10% wood chips and 5% fruit pulp) at 600 °C for 30 min (extinguished with water). Properties of the commercial biochar (Sonnenerde GmbH, Riedlingsdorf, Austria) were the same as referred [37].

Unmatured manure (from cattle breeding without marketable milk production) and biochar were dosed into the 50-litre tightly closeable barrels (Table 1). Each manure variant was prepared in 3 replicates. In biochar-amended variants, the manure and biochar were thoroughly mixed.

Table 1. Manure variants and additives dosage.

Variant	Abbrev.	Manure (M) per Barrel	Biochar (B) per Barrel	Dry Matter Ratio M:B
Manure	М	10 kg	0	0
Manure + biochar low dose	M + LB	10 kg	0.4 kg	12.5:1
Manure + biochar high dose	M + HB	10 kg	4.0 kg	1.25:1

The barrels were tightly covered, and fermentation process was carried out for 8 weeks under laboratory temperature 22.2–25.2 °C and relative humidity 60–78%. The flow of air was not controlled but was limited due to the covering. At the end of process, the mixed sample of matured manure was taken from each variant and was analyzed. The determined properties, methods and relevant references are described in Table 2.

Abbrev.	Property, Method	Unit	Reference
pН	pH determined in CaCl ₂	-	[38]
DM	dry matter, gravimetry	%	[39]
Р	available phosphorus, extraction	$ m g\cdot kg^{-1}$	[40]
Ca	calcium, extractable (Mehlich III)	$g \cdot kg^{-1}$	[41]
TN	total Kjeldahl nitrogen	%	[42]
TC	total carbon, dry combustion	0/	[42]
TOC	total organic carbon, dry combustion	/0	[43]
HA:FA	humic acid:fulvic acid ratio	-	[44]
N-min	mineral nitrogen	%	
$N-NH_4$	ammonium nitrogen	$mg\cdot kg^{-1}$	[45]
N-NO ₃	nitrogen in nitrate form	$mg \cdot kg^{-1}$	[45]
AOB	ammonium-oxidizing bacteria, qPCR (gene amoA)	$copies \cdot g^{-1}$	[46]
nirS	denitrifying bacteria, qPCR (gene nirS)	$copies g^{-1}$	[47]

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

2.2. Pot Experiment

This pot experiment was intended as a preliminary study for further field trials. All three types of experimental manures were used as organic fertilizers in a pot experiment with barley (*Hordeum vulgare* L.). Each experimental pot of volume 5 dm³ was filled up with soil substrate prepared by mixing of fine quartz sand (0.1–1.0 mm) with topsoil (0–15 cm) from the rural area near the town Troubsko, Czech Republic (49°10′28″ N 16°29′32″ E) sieved through 2.0 mm in ratio 1:1 (w/w). The soil was a silty clay loam (USDA Textural Triangle), Haplic Luvisol (WRB soil classification), the soil properties were the same as previously reported [37].

The tested variants were made by thorough mixing of 5 kg of experimental soil with 200 g of particular manure type per pot (equal to field manure dose of 50 t \cdot ha⁻¹). Unamended control contained only 5 kg of experimental soil without any fertilizer. Each soil variant was prepared in 4 replications, in a minimal design sufficient for a preliminary study. The variants were marked equally to the used manure type: (1) (unamended) control, (2) manure (M), (3) manure + low dose of biochar (M + LB), (4) manure + high dose of biochar (M + HB). Each pot was sown with 16 barley seeds 2 cm under soil surface and was

watered with distilled water to achieve 65% water holding capacity (WHC). The moisture level was maintained at $65 \pm 5\%$ WHC throughout the experiment. All pots were placed randomly into the grow chamber (CLF Plant Climatics GmbH, Wertingen, Germany) and rotated every other day to ensure homogeneity of conditions for the treatments. Controlled conditions were set as follows: 12 h long photoperiod, temperature (day/night) 20/12 °C, relative air humidity (day/night) 45/70%. The number of plants was reduced to 12 in each pot after 14 days.

2.3. Plant Biomass

The barley seedlings were grown for 12 weeks. After that, they were cut at the ground level and dried at 60 °C to constant weight. The dry above ground biomass (AGB) was determined gravimetrically using the analytical scales.

2.4. Soil Sampling, Chemical and Biological Analyses

A mixed soil sample was taken from each pot after the harvesting of barley. The samples were homogenized by sieving through a 2 mm mesh. Air-dried samples were used for total soil carbon (TC), nitrogen (TN), and sulphur (S) content determination by analyzer TruSpec (LECO Corporation, St. Joseph, MO, USA). The freeze-dried samples were prepared for the enzyme activity assays according to (ISO 20130: 2018) [48], namely: β -glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), *N*-acetyl- β -D-glucosaminidase (NAG), and urease (Ure). The samples stored at 4 °C were used for determination of dehydrogenase activity (DHA) according to [49], and for determination of soil basal (BR) and substrate-induced respiration (expressed in $\mu g CO_2 \cdot g^{-1} \cdot h^{-1}$) via MicroResp method according to [50], namely: D-glucose (Glc-SIR), *N*-acetyl- β -D-glucosamine (NAG-SIR), L-lysine (Lys-SIR), L-arginine (Arg-SIR).

2.5. Statistical Analysis

Data obtained from the performed measurements were statistically analyzed using the methods of 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).

3. Results

3.1. Effect of Amendments on Physical and Chemical Properties of Manure after Maturation

The pH value of the un-amended manure (M) was significantly higher in comparison to the M + HB variant, but there was no difference compared to M + LB variant (Table 3). We found a very highly positive correlation of pH with TN (r = 0.96), AOB (r = 0.96) and N-NO₃ (r = 0.82), whereas the correlation with N-NH₄ (r = -0.85) was negative (Figure A1a).

Content of DM was affected by the added biochar and therefore the un-amended manure (M) showed a significantly lower DM compared to M + LB and M + HB. The significantly highest TC was detected in the M + HB variant. However, M + LB did not differ from the un-amended manure variants, which was unexpected (Table 3). Further, the very high negative correlation (Figure A1a) with TOC (r = -0.93) and mutual antagonism (PCA biplot—Figure A2a) was found. Contrarily, TOC content in M + HB was significantly decreased as compared to both M + LB and M, and TOC value of M + LB was lower in comparison to the unamended manure (Table 3). The TOC property positively highly correlated with AOB (r = 0.85), and N-NO₃ (r = 0.91).

TN value of M + HB was lowered significantly in comparison to both other variants. However, M + LB variant did not differ from the control manure M in the TN content (Table 3). The positive correlation of TN with AOB (r = 0.91), TOC (r = 0.87), and N-NO₃ (r = 0.77) was observed. The M variant showed a significantly higher N-min content in comparison to both biochar-amended variants (Table 3). N-min correlated highly positively with N-NO₃ (r = 0.92), P (r = 0.78), and TOC (r = 0.74). N-NH₄ was significantly the highest in the M + HB variant as compared to the other two variants, and the M variant was also surprisingly higher compared to M + LB (Table 3). The high negative correlation and antagonism (Figures A1a and A2a) of N-NH₄ with TN (r = -0.87) and AOB (r = -0.78) was found. The content of N-NO₃ was significantly the highest in the control manure M in comparison to both biochar-amended variants, and M + HB showed the significantly lowest value (Table 3). The correlation analysis (Figures 1 and A1) revealed high relation between N-NO₃ and P (r = 0.80), AOB (r = 0.82), *nirS* (r = -0.86).

Table 3. Properties of matured manures—pH, dry matter (DM), total carbon (TC), total organic carbon (TOC), total nitrogen (TN), mineral nitrogen (N-min), ammonium nitrogen (N-NH₄), nitrogen in nitrate form (N-NO₃), humic acid:fulvic acid ratio (HA:FA), available phosphorus (P), available calcium (Ca).

Property [Linit]	М	M + LB	M + HB
	Mean \pm SD *	Mean \pm SD *	Mean \pm SD *
pH [-]	$9.04\pm0.01~\mathrm{a}$	$9.05\pm0.01~\mathrm{a}$	$8.71\pm0.01~\mathrm{b}$
DM [%]	$30.01\pm0.02~\mathrm{c}$	$31.48\pm0.02b$	$36.01\pm0.02~\mathrm{a}$
TC [%]	$9.10\pm0.12~\mathrm{b}$	$9.13\pm0.18\mathrm{b}$	21.01 ± 0.34 a
TOC [%]	13.50 ± 0.24 a	$13.01\pm0.12\mathrm{b}$	$11.89\pm0.09~\mathrm{c}$
TN [%]	$2.48\pm0.05~\mathrm{a}$	$2.54\pm0.02~\mathrm{a}$	$1.99\pm0.07\mathrm{b}$
N-min [%]	$16.70\pm0.03~\mathrm{a}$	$14.58\pm0.09\mathrm{b}$	$14.33\pm0.55\mathrm{b}$
N-NH₄ [mg·kg ^{−1}]	$2.06\pm0.01~b$	$1.44\pm0.07~{ m c}$	$2.58\pm0.07~\mathrm{a}$
N-NO ₃ [mg·kg ^{-1}]	$14.64\pm0.02~\mathrm{a}$	$13.14\pm0.03~\mathrm{b}$	$11.75\pm0.50~\mathrm{c}$
HA:FA [-]	$0.79\pm0.01~\mathrm{b}$	$0.69\pm0.02~\mathrm{b}$	$2.04\pm0.18~\mathrm{a}$
P [g·kg ^{−1}]	$4.22\pm0.31~\mathrm{a}$	$3.45\pm0.55~\mathrm{ab}$	$3.04\pm0.30\mathrm{b}$
$Ca [g \cdot kg^{-1}]$	$20.93\pm0.76~\mathrm{c}$	$32.95\pm1.69b$	$159.34\pm8.03~\mathrm{a}$

Mean values calculated as average from independent replicates (n = 4) ± SD (standard deviation). * Different letters express the statistical differences at the significance level $p \le 0.05$.



Figure 1. Mean values of chemical properties of soil and above ground biomass production. (a) TC = total soil carbon, (b) TN = total soil nitrogen, (c) C:N = total soil carbon/nitrogen ratio, (d) S = total soil sulphur, (e) Dry AGB = dry plant aboveground biomass. Average values of independent replicates (n = 3), error bars = standard deviation; the different letters express the statistical differences at the significance level $p \le 0.05$.

The P content was significantly lowered in M + HB in comparison to the control manure (Table 3). A high positive correlation of P with N-NO₃ (r = 0.80) and N-min (r = 0.78) and synergism in PCA biplot (Figure A2a) was revealed. On the contrary, Ca was significantly higher in the M + HB variant as compared to the other variants (Table 3), and M + LB was higher compared to the control manure. The significantly highest ratio of HA:FA was observed in M + HB variant (Table 3). However, we observed decreased HA:FA ratio in the M + LB variant (in average) as compared to the un-amended manure (M).

3.2. Effect of Amendments on Microbial Properties of Manure after Maturation

A significantly decreased amount of ammonium oxidizing bacteria (AOB, indicated by gene marker *amoA*) was observed in M + HB, compared to the variants M and M + LB, which did not differ (Table 4). High correlation and synergism (Figures A1a and A2a) of AOB with TOC (r = 0.85), TN (r = 0.91) and pH (r = 0.96) was already mentioned.

М	M + LB	M + HB
Mean \pm SD *	Mean \pm SD *	Mean \pm SD *
$2.11 imes 10^8 \pm 2.48 imes 10^7$ a	$2.09 imes 10^8 \pm 3.43 imes 10^6$ a	$7.46 imes 10^7 \pm 5.26 imes 10^6 ext{ b}$
$1.07 \times 10^9 \pm 1.50 \times 10^8 \ \mathrm{c}$	$1.40 imes 10^9 \pm 1.59 imes 10^8 \ { m b}$	$1.85\times10^9\pm2.06\times10^8$ a
	$\begin{tabular}{ c c c c c } \hline M & \\ \hline Mean \pm SD * \\ \hline 2.11 \times 10^8 \pm 2.48 \times 10^7 \ a \\ 1.07 \times 10^9 \pm 1.50 \times 10^8 \ c \\ \hline \end{tabular}$	MM + LBMean \pm SD*Mean \pm SD* $2.11 \times 10^8 \pm 2.48 \times 10^7$ a $2.09 \times 10^8 \pm 3.43 \times 10^6$ a $1.07 \times 10^9 \pm 1.50 \times 10^8$ c $1.40 \times 10^9 \pm 1.59 \times 10^8$ b

Table 4. Microbial properties of matured manures—ammonium-oxidizing bacteria (AOB), denitrifying bacteria (*nirS*).

Mean values calculated as average from independent replicates (n = 4) \pm SD (standard deviation). * Different letters express the statistical differences at the significance level $p \le 0.05$.

M + HB was significantly more abundant in *nirS* (determinant of microorganisms reducing nitrates) in comparison to both other variants (Table 4). *nirS* of the M + LB variant was significantly increased compared to the control manure M.

3.3. Effect of Matured Manure Types on Soil Chemical Properties and Plant Biomass

The soil TC was significantly increased in the variants M + LB and M + HB, as compared to the variants M and control, and the un-amended control soil showed the significantly lowest TC value (Figure 1a). The moderate correlation (Figure A1b) with dry AGB (r = 0.67), DHA (r = 0.70), and Ure activity (r = 0.74) was shown by the Pearson's correlation and synergism was found in the PCA biplot (Figure A2b).

The soil TN was significantly increased as compared to the control in the variants M and M + LB (Figure 1b). The Pearson's analysis (Figure A1b) revealed moderate positive correlation with TC (r = 0.70), DHA (r = 0.60), Ure (r = 0.59). The significantly highest soil C:N ratio (Figure 1c) was received in the M + HB variant. However, neither strong negative correlation (Figure A1b) nor any antagonism (Figure A2b) was observed in the relation to the dry AGB. The soil S was significantly the highest in the M + HB variant as well (Figure 1d). Both other manure-amended soil variants M and M + LB showed also significantly higher S compared to the control.

The significantly highest dry AGB was found in the M + HB variant (Figure 1e). Both other manure-amended soil variants M and M + LB exerted also significantly higher dry AGB as compared to the control. Dry AGB correlated positively (Figure A1b) with BR (r = 0.71), various types of SIRs (0.46 < r < 0.82), Ure (r = 0.58).

3.4. Effect of Matured Manure Types on Soil Microbial Properties

The BR and SIR were used as the indicators of aerobic C and N mineralization rate in soil. BR was significantly the highest in M + HB (Figure 2a), and the control soil showed the significantly lowest BR. The Glc-SIR was significantly higher (compared to the control and M) in both M + LB and M + HB, BR and Glc-SIR correlated positively with dry AGB (r = 0.71 and 0.48, respectively), with NAG-SIR (r = 0.80 and 0.81, respectively), with Lys-SIR (r = 0.81 and 0.78, respectively) and each other (r = 0.67). The significance of highest and lowest values was identical for BR and NAG-SIR, Arg-SIR (Figure 2a,c,e), and similar significant differences among variants were found for Glc-SIR, NAG-SIR and Lys-SIR (Figure 2b–d).

All manure-amended variants showed the significant increase in the DHA (dehydrogenase activity) in comparison to the control (Figure 3a). The ARS (arylsulfatase) was significantly decreased in all manure-amended variants in comparison to the control (Figure 3b). The activity of *N*-acetyl-b-D-glucosaminidase (NAG) was significantly increased only in M + LB variant (Figure 3c) compared to the control. Significantly increased Ure was revealed in all manure-based variants as compared to the control (Figure 3d). The significantly highest Phos value (compared to the control) was observed in M + HB (Figure 3f).



Figure 2. Mean values of soil basal (BR) and substrate induced (SIR) respirations. (**a**) BR = basal respiration, (**b**) Glc-SIR = respiration induced by D-glucose, (**c**) NAG-SIR = respiration induced by *N*-acetyl- β -D-glucosamine, (**d**) Lys-SIR = respiration induced by L-lysine, (**e**) Arg-SIR = respiration induced by L-arginine. Average values of independent replicates (*n* = 3), error bars = standard deviation; the different letters express the statistical differences at significance level *p* \leq 0.05.



Figure 3. Mean values of soil enzyme activities. (a) DHA = dehydrogenase activity, (b) ARS = arylsulfatase activity, (c) NAG = *N*-acetyl- β -D-glucosaminidase activity, (d) Ure = urease activity, (e) GLU = β glucosidase activity, (f) Phos = phosphatase activity. Average values of independent replicates (*n* = 3), error bars = standard deviation; the different letters express the statistical differences at significance level *p* \leq 0.05.

4. Discussion

4.1. Effect of Amendments on Physical and Chemical Properties of Manure after Maturation

The M + HB variant showed significantly lower pH compared to un-amended manure (M) and M + LB variant (Table 3). We assume that, similarly to the previously observed results by Hammerschmiedt et al. [51], the high biochar dose caused a negative priming effect on the mineralization of N, which was coupled with increased content of NH_4^+ , which has a slight acidifying effect [52]. We ascribed this presumption from the PCA biplot (Figure A2a) and the calculated positive correlation with both AOB and N-NO₃ as well as negative correlation with N-NH₄ (Figure A1a).

The M + HB had significantly increased DM compared to M + LB, which was simply explainable by the higher dose of added biochar with lower moisture than the amended fresh manure had (Table 3).

TC content was closely related to the DM values as well and the significantly highest TC was detected in the M + HB variant, in comparison to the M and M + LB variants. TC of M + LB was insignificantly higher compared to the un-amended manure value (Table 3). We ascribed from the very high negative correlation (Figure A1a) of TC and TOC (and antagonism apparent in the PCA biplot—Figure A2a) that the high portion of biochar-derived C was recalcitrant to enter the metabolism of manure microorganisms. The study by Jien et al. [53] referred to biochar-mediated increase in C mineralization at the beginning of application after 70 days incubation. Although this feature was observed for compost-amended soil, we presumed this feature occurs in manure as well. Thus, we assume that primarily enhanced utilization of organic C led to significantly lower TOC content in M + HB as compared to both M + LB and M, and M + LB in comparison to the unamended manure (Table 3). The positive high correlation of TOC with AOB and N-NO₃ implied that the higher TOC access stimulated the nitrification process and N mineralization.

N content of pyrolyzed matter was 0.3% and the manure N content was assumed to be eight-fold higher. The amendments were mixed in the dry matter ratio 1.25:1 in the M + HB variant, therefore, the N concentration in the final mixture was lowered significantly in comparison to both other variants. Less clear was the result received for the M + LB variant, which did not differ from the control manure M (Table 3). We presumed that early fermentation sorption of volatile forms of N (NH₃, N₂O), mediated by low biochar dose, caused the difference between M + LB and M variant. There was a report that biochar reduced the losses of N in the matured composted product [18]. The positive correlation of TN with AOB and N-NO₃ corroborated the presumption that mainly higher access of mineralizable organic N and C in manure organic matter (OM) enhanced nitrification during the maturation.

Nevertheless, the mineralization of N seemed to be repressed by the biochar adsorbing and stabilizing impact of the organic N-compounds in the treated manure: M revealed a significantly higher N-min content in comparison to both biochar-amended variants (Table 3). The study by Lentz et al. [54] showed that use of wood biochar led to 33% less cumulative net mineral N compared to manure. N-min correlated highly positively with N-NO₃ (r = 0.92), P (r = 0.78), and TOC (r = 0.74), which corroborated the relation between nitrification and the availability of nutrients for manure microorganisms.

N-NH₄ was significantly the highest in the M + HB variant as compared to the other two variants, and the M variant was also surprisingly higher compared to M + LB (Table 3). We explain these results obtained for M + HB with the biochar adsorption of NH₄⁺ reported by Kizito et al. [55], whereas in case of M + LB, we presumed higher losses of N during nitrification due to denitrification indicated by higher *nirS* number, which is also proven by low N-min. The high negative correlation of N-NH₄ with TN and AOB implied that a putative retardation in early nitrification phase (organic N deamination) could be involved. The content of N-NO₃ was significantly the highest in the control manure M in comparison to both biochar-amended variants. On the other hand, M + HB showed the significantly lowest value (Table 3). Overall restriction from further N oxidation via adsorption, availability of P, and putative losses of NO₃⁻ due to the denitrification are the presumed causes of the observed differences. These presumptions were corroborated by the high positive correlation and synergism between N-NO₃ and P, AOB, *nirS* (Figures A1a and A2a).

Similarly to the amount of TN in the manure types, the other nutrients (P, Ca) were determined by their content in the un-manured manure and biochar. Therefore, the P content was significantly lowered in M + HB in comparison to the control manure (Table 3), as the biochar exerted significantly lower P content (2.45% in DM). A high positive correlation of P with N-NO₃ and N-min which indicated the positive effect of P access on the nitrification in manure could be seen in PCA (Figure A2a). This finding is in line with the study by [56] reporting that P addition to composted manure positively affects N fixation and restrict denitrification. On the contrary, Ca was significantly higher in the M + HB variant as compared to the other variants (Table 3), and M + LB was higher compared to the control manure. This remarkable difference was presumably caused by the high cation sorption capacity of biochar, which was reported by several studies [57,58].

The recalcitrant nature of biochar may affect the character of humic substances formed during the manure composting; it was referred that biochar increases the polycyclic aromatic C in humified matter of biochar [59]. Further, it was reported that humic acids were adsorbed and co-flocculated on the biochar surface [60], a feature which might have decreased the HA:FA ratio in the M + LB variant, together with weaker contribution of low dose of biochar to the formation of humic acids. On the other hand, the significantly highest ratio of HA:FA was observed in the M + HB variant (Table 3). The HA:FA value, determining the degree of maturity of composted organic matter [61], indicated that M + HB may represent the most qualitatively fermented manure variant.

4.2. Effect of Amendments on Microbial Properties of Manure after Maturation

The gene marker *amoA* [46] was used as a determinant of bacteria capable to oxidize ammonium nitrogen (AOB). The variants M and M + LB exerted significantly increased AOB compared to M + HB, although AOB values of both M and M + LB did not differ (Table 4). This finding corresponded to the availability of N-NH₄ substrate to oxidation and to the overall nutrient availability and soil conditions as well. It was proven by high correlation and synergism of AOB with TOC, TN, and pH (Figures A1a and A2a).

The gene marker *nirS* [47] was used as a determinant of microorganisms which mediated reduction of nitrate. We received inverted proportions of AOB and *nirS* and thus, M + HB was significantly more abundant in *nirS* in comparison to both other variants (Table 4). And *nirS* of the M + LB variant was significantly increased compared to the control manure M. Putatively, the decreased pH, the lowest abundance of counteracting AOB and lowest humification intensity determined by low HA:FA ratio resulted in the enhanced denitrification coupled with the lower N-NO₃ content in the final manure M + LB. Other studies referred to increased nitrate reduction to nitrite in low pH [62] and increase in denitrification which was counteracted by the release of ammonium from OM [63]. These features (lower pH and N-NO₃, higher N-NH₄) were observed in the M + HB variant and may explain a detected high *nirS* value which represented abundance of denitrifiers in this manure.

4.3. Effect of Matured Manure Types on Soil Chemical Properties and Plant Biomass

Significantly increased TC was detected in M + LB and M + HB compared to the variants M and control, with the significantly lowest value in the un-amended control soil (Figure 1a). The increased access of C from the high dose biochar-enriched manure may explain the result in M + HB, however surprisingly high TC in M + LB implied that a C-sequestration role of biochar in the soil may be involved. The less easily degradable biochar-derived C putatively lowered the mineralization rate in early phase of the experiment, which was described similarly in previous study [64], and this short priming effect preserved more C to be degraded at the end of experiment. We evidenced moderate correlation of TC with dry AGB, DHA, and Ure activity (Figure A1b) which proved higher TC-mediated decomposition activity in soil, joint increased N mineralization and concurrent plant biomass yield. The PCA biplot data supported these findings in the mutual relation of traits (Figure A2b).

The soil TN of the variants M and M + LB was significantly increased as compared to the control (Figure 1b). These results clearly correspond to the values of N which were determined in the respective manure types, which were added to the soil variants. The Pearson's analysis (Figure A1b) revealed moderate positive correlation of TN with TC, DHA, Ure—these relations presumed the positive effect of the biochar-enriched manure on the soil OM decomposition.

The M + HB variant exerted the significantly highest soil C:N ratio (Figure 1c). However, neither strong negative correlation (Figure A1b) nor any antagonism (Figure A2b) was observed in the relation to the dry AGB, which was in contrast to the previously observed relation between C:N and plant biomass values in soil treated with a biochar-based amendment [17].

The soil S was the significantly highest in M + HB variant as well (Figure 1d). Both other manure-amended soil variants M and M + LB showed also significantly higher S compared to the control. We explain the results by the reported positive effect of the biochar on sorption of S forms in manure and other waste OM during composting [65]. However, partial immobilization of S in some soil types was observed too [66], which corresponded with higher S content in the M variant.

Further, the M + HB variant showed the highest dry AGB (Figure 1e). Both other manure-amended soil variants M and M + LB exerted also significantly higher dry AGB as compared to the control, which clearly corresponded to the values of TC and S in soil, and to the content of Ca and NH_4^+ in the prepared manures, used for the fertilization. From

this reason we consider role of biochar in the soil manure amendment as crucial for crop yield, similar results were already observed [67]. The used manures impacted the microbial properties of soil which exerted a certain effect on the plant growth, probably due to the enhanced mineralization activities, monitored as respiration and enzyme activities. This was corroborated by the positive correlation (Figure A1b) of dry AGB with BR, various types of SIRs, and Ure.

4.4. Effect of Matured Manure Types on Soil Microbial Properties

The BR and SIR were used as the indicators of aerobic C and N mineralization rate in soil. BR was significantly the highest in M + HB (Figure 2a), and the control soil showed the significantly lowest BR. The findings for Glc-SIR, which was significantly higher (compared to the control and M) in both M + LB and M + HB, were similar (Figure 2b). These findings corresponded to the overall content of TC and S. Moreover, BR and Glc-SIR correlated positively with dry AGB, with NAG-SIR, with Lys-SIR, and each other. These relations indicate that the soil respiration was coupled also with nitrification and N mineralization, as the substrates NAG, Lys, and Arg are important organic N sources. The significance of highest and lowest values and their comparison was identical for BR and NAG-SIR, Arg-SIR (Figure 2), and similar statistical significance and differences among variants were found for Glc-SIR, Tre-SIR and Lys-SIR (Figure 2). The study by [68] referred to biocharmanure interaction for increased CO_2 emissions representing higher BR, but the concurrent sequestration of manure-derived C. We proved similar features of biochar-enriched manure amendment in relation to the soil respiration and observed that the respective soil variant significantly more increased both, BR and SIR, without any significant effect on the soil functional diversity; we ascribe this from the similarities among the various SIR types.

The DHA monitors overall decomposition rate of soil OM and it is considered as one of the best indicators of microbiological redox systems [69]. All manure-amended variants showed the significant increase in the DHA, which was in relation to the increased access of degradable OM (Figure A1a). The study by [70] reported that soil amended with biochar and animal manure increased DHA. The activities of enzymes involved in the nutrient mineralization corresponded to the particular nutrient contents in the soil variants. The ARS is the enzyme that catalyzes desulphurization of organosulphates [71], and its activity was significantly decreased in all manure-amended variants in comparison to the control (Figure 3b), probably because of the higher access of mineralized S and lower demand for the organic S mineralization. The respective manure-amended soil variants showed both significantly higher total S and dry AGB and lower ARS activity, thus we presume a higher S content in the utilizable form for both plants and microbes in these soil variants.

The NAG is an enzyme involved in the N mineralization, which hydrolyzes the intermediate of chitin degradation [72]. Its activity was significantly increased only in the M + LB variant (Figure 3c). The enhanced NAG corresponded with the highest soil TN, its activity may also indicate higher fungi content in the M + HB soil. The study by [35] referred to increased fungi abundance, but lowered NAG activity due to co-composted biochar-chicken manure addition to soil.

The Ure activity is the most ubiquitous enzyme among the soil microbiota, which catalyzes the deamination of urea [73]. Significantly increased Ure (as compared to the control) was revealed in all manure-based variants (Figure 3d). Consist with our findings, it was reported that biochar and animal manure increased Ure activity in soil [70]. Ure is one of the key enzymes in the early nitrification pathway and we explain the results of its activity with the chemical and biological properties of the added manure types. The non-enriched manure showed the highest AOB abundance and N-NO₃, whereas the manure M + HB exerted the lowest values of all traits. These data of manures anticipated significantly enhanced nitrification in the M variant as compared to M + HB.

The slightly different aspects were the results of GLU estimation. GLU is a part of cellulase complex and is involved in the C mineralization [74]. Hussain et al. referred to maximum conversion of feed OC to biochar recalcitrant OC at 500 °C [75]. The biochar

serves as a source of both recalcitrant and labile C, and despite relatively high pyrolysis temperature (600 °C), we consider significant enrichment of soil with available carbon. Therefore, the significantly increased GLU was detected in M + LB and M + HB variants in comparison to the control and M variants (Figure 3e). The last evaluated soil enzyme was Phos, which catalyzes dephosphorylation of organophosphates in P mineralization [76]. Minhas et al. reported that biochar significantly promoted the availability and acquisition of nitrogen and phosphorus nutrients, as 2 t-ha^{-1} of amended biochar with half-recommended dose of NP fertilizer improved the fertilization performance of full dose of NP fertilizer [77]. The significantly highest Phos value was observed in M + HB (Figure 3f). We assumed that phosphatase activity corresponded to the amount of P added with respective amendment to soil, thus we put into context the highest Phos value and the lowest P content in the M + HB manure. In other variants, the manure-derived P was high enough to mitigate the demand for P mineralization. Our findings agreed with reported enhancement of Phos activity in soil amended with biochar-blended compost [78].

5. Conclusions

In this preliminary study, it was concluded that the co-composted manure with high biochar dose can lower the pH of the resultant matured amendment and hence has strong effect on nutrient availability. This was further supported by the increased NH₄-N content which further suggests the acidifying effects of co-composted amendment. Moreover, co-composted manure with high biochar dose caused higher maturation of the final product as indicated by increased HA:FA ratio. Further, biochar in manure caused a retardation in nitrification, whereas denitrification was promoted as revealed by reduced AOB and higher *nirS* abundance. The plant biomass was also enhanced, which suggests the ability of co-composted manures to supply essential plant nutrients in higher amounts. However, this is only a preliminary study with a small sized experiment, so it is necessary to investigate this issue on a larger scale to draw clear conclusions regarding soil enzyme activity and microbial respiration.

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Appendix A

Figure A1. The Pearson's correlation matrix of (**a**) matured manure properties and (**b**) soil and plant properties; values indicate a correlation coefficient (r). Explanation: · Significant at 0.10 level; * Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level.



Figure A2. The Rohlf's PCA biplot of (**a**) matured manure properties and (**b**) soil and plant properties. (**a**) M = manure, M + LB = manure + biochar low dose, <math>M + HB = manure + biochar high dose; (**b**) control = unamended soil, <math>M = soil amended with manure, M + LB = soil amended with manure + biochar low dose, M + HB = soil amended with manure + biochar high dose; position of points/arrows is placed on the basis of their role in the first and second component (Dim1 and Dim2); arrow length equals the rate of property effect.

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