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Long-Term Effects of Biochar-Based Organic Amendments on Soil Microbial Parameters

Martin Brtnicky [1,2], Tereza Dokulilova [1], Jiri Holatko [1], Vaclav Pecina [1], Antonin Kintl [3], Oldrich Latal [4], Tomas Vyhnanek [5], Jitka Prichystalova [2] and Rahul Datta [1,*]

1 Department of geology and pedology, Faculty of Forestry and Wood technology, Mendel University in Brno, Brno 61300, Czech Republic; martin.brtnicky@seznam.cz (M.B.); tereza.dokulilova@mendelu.cz (T.D.); jiri.holatko@centrum.cz (J.H.); vaclav.pecina@mendelu.cz (V.P.)
2 Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Brno 61300, Czech Republic; jitka.prichystalova@mendelu.cz
3 Agriculture Research, Ltd., Troubsko 66441, Czech Republic; kintl@vupt.cz
4 Agrovyzkum Rapotin, Ltd., Rapotin 78813, Czech Republic; oldrich.latal@vuchs.cz
5 Department of Plant Biology, Faculty of AgriSciences, Mendel University in Brno, Brno 61300, Czech Republic; tomas.vyhnanek@mendelu.cz
* Correspondence: rahulmedcure@gmail.com; Tel.: +420-773990283

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Abstract: Biochar application to the soil has been recommended as a carbon (C) management approach to sequester C and improve soil quality. Three-year experiments were conducted to investigate the interactive effects of three types of amendments on microbial biomass carbon, soil dehydrogenase activity and soil microbial community abundance in luvisols of arable land in the Czech Republic. Four different treatments were studied, which were, only NPK as a control, NPK + cattle manure, NPK + biochar and NPK + combination of manure with biochar. The results demonstrate that all amendments were effective in increasing the fungal and bacterial biomass, as is evident from the increased values of bacterial and fungal phospholipid fatty acid analysis. The ammonia-oxidizing bacteria population increases with the application of biochar, and it reaches its maximum value when biochar is applied in combination with manure. The overall results suggest that co-application of biochar with manure changes soil properties in favor of increased microbial biomass. It was confirmed that the application of biochar might increase or decrease soil activity, but its addition, along with manure, always promotes microbial abundance and their activity. The obtained results can be used in the planning and execution of the biochar-based soil amendments.

Keywords: biomass; biochar; soil; BPLFA; FPLFA; DHA; ammonia-oxidizing bacteria

1. Introduction

Fertilizers used in agricultural management influence soil quality and health [1,2]. The recent rise in concerns about environmental problems caused by the excessive use of chemical fertilizers necessitates detailed studies on alternate strategies to address such hazards. Long-term use of organic amendments to the soil helps in improving several soil parameters like organic carbon, aggregate stability and crop yield, in contrast to the application of chemical fertilizers [3–5]. Organic amendments also increase soil carbon sequestration and play a decisive role in mitigating the adverse effect of climate change [3,6,7]. Independent of the type and nature of the applied organic amendment, different changes in soil properties and fertility have been observed in a broad time horizon under different pedoclimatic conditions [8]. The positive effect on total soil carbon, soil nitrogen, soil microbial biomass carbon (MBC) and dehydrogenase activity (DHA) has been observed in soils treated with manure [9,10].

Organic amendment to the soil a has long term impact on soil restoration. Miller and Miller (2000) showed that long term application of manure has greater impact on soil properties as compared to short term application [11]. Long-term application of manure causes enhanced soil physical, chemical and biological properties [12]. In another study Shindo et al. (2006) [13] reported that continuous long term application of manure to a field drastically increases fulvic, humic acids and total humus content in the soil. On the contrary, the absence of organic fertilizer input to soil results in non-complex light weight humus [14]. A literature survey on the long-term application of manure pointed out that the use of manure with a mineral fertilizer (NPK) enhances soil properties and crop yield as compared to organic amendment alone [15,16].

Biochar is a carbon-rich material produced by pyrolysis reaction under limited or no oxygen environments, often used for soil amendment and carbon sequestration [17]. Biochar amendment improves soil physicochemical and biochemical properties. It increases the soil pH and cation exchange capacity (CEC) [18], improves soil structure [19], alters soil microbial populations [20] and enhances nutrient retention [21–24]. Although biochar is recalcitrant in nature, its ability to interact with soil properties makes it a good investment in soil [25]. Long term application of biochar brings change in the physio chemical properties of the soil. That leads to an alternation in the soil bacterial community.

Many studies have reported a synergistic effect of biochar and organic fertilizer in (a) improving plant growth by nitrate-capture in co-composted biochar [23,26], (b) promoting carbon stabilization through the formation of organo-mineral complexes [27] and (c) affecting soil nutrient cycles [28]. Organic fertilizer may form a coating inside and outside the biochar particle and increase hydrophilicity, thus raising the nutrient retention [29]. However, the antagonistic or neutral effect of manure and biochar interaction has been reported in studies [30–32].

The positive effect of soil treatment with manure on total soil carbon, nitrogen, microbial biomass carbon and dehydrogenase activity in the surface soil (0–5 cm) has generally been observed [10]. However, the combined effect of manure and the other soil amendment (e.g., biochar) has not been widely studied. In a few studies in which manure-derived biochar was applied to soils, a mostly positive effect was seen [33,34], but also a converse [34–36] effect on plant growth and soil microbial diversity was observed.

This study aimed to determine and compare the long-term effect of the biochar-based organic amendments on selected soil properties (MBC, DHA and soil microbial community abundance) on the agricultural land of the temperate climate zone of Central Europe. Only a few studies have been done so far on the agricultural area of temperate soils [37–39]. Prior studies suggested that manure addition to biochar as a soil amendment might have a synergistic or antagonistic effect. We hypothesized that the supplement of manure with biochar will represent a valid strategy to further enhance the soil microbial biomass and related soil quality indicators.

2. Materials and Methods

2.1. Study Site and Rationale behind the Field Scale Experiment

Field-scale experiments were designed to evaluate the potential of biochar and its combination with manure in improving the studied soil properties in on-site conditions. Experiments were carried out at arable land (luvisols) during the cropping season from the year 2014 to 2017. Experimental plots were located in Rapotín locality, the Czech Republic, at an altitude of about 345 m a.s.l., and it comes under the temperate continental climate zone, with a mean annual temperature of 7 °C. The mean annual precipitation is about 705 mm in the area. The rainfall pattern is 400–450 mm in the vegetation season and 250–300 mm during the winter period. The experiment consisted of the application of four soil treatments that were only NPK (mineral fertilizer) as a control, NPK + cattle manure (50 t/ha), NPK + biochar (15 t/ha) and NPK + combination of manure (50 t/ha) with biochar (15 t/ha) (MB). Biochar was added at the start of the experiment, while manure was added every year. Dosage of cattle manure 50 t/ha is added as recommended by (Singh et al., 2011) [40].

Dosage of biochar 15 t/ha was chosen close to the maximum amount of biochar allowed to be amended to the arable soil on the field (Pereira et al., 2011) [41]. Variant 4 is a combination with the same dosage of both amendments i.e., cattle manure 50 t/ha and biochar 15 t/ha. The experimental area was divided as follows: three small-scale-plots (10 × 10 m) per each of four variants of soil amendment (12 small-scale-plots overall).

2.2. Soil Sampling and Preparation

At the end of third crop season, the samples for the final analyses of the application of amendments were collected in October 2017. Three spatially-independent mixed soil subsamples from each experimental variant were collected in the following way: A portion of topsoil from a depth 0–15 cm was taken by a soil drill at five spots of an experimental field and mixed in a plastic sampling bag. The samples (app. 500 g) were immediately cooled down and transported to the laboratory at 0–4 °C and homogenized by sieving the soil through a 2 mm mesh under sterile conditions [42]. Samples for the enzyme activity assays were stored at 4 °C until analyzed (within one week). Samples for qPCR and phospholipid fatty acids (PLFA) analysis were freeze-dried.

2.3. Dehydrogenase Activities

Triphenyl tetrazolium chloride-dehydrogenase activity (TTC-DHA) was used to determine microbial activity in the soil. The methodology was modified according to Tabatabi (1994) [43], based on (Casida et al., 1964) [44]: 3-gram soil sample was mixed with MgO and sealed with the standard solution (triphenyl tetrazolium chloride + distilled water). The samples were incubated in the thermostat at 37 °C for 24 h. Afterwards, triphenylformazan (TPF) was extracted from the samples using methyl alcohol, resulting in the color change of the solution. The spectrophotometer (DR 3900, Hach Lang, Duesseldorf Germany) was used to measure the color intensity at a wavelength of 485 nm. DHA was calculated according to the calibration curve and expressed in μg TPF·g^{-1}·h^{-1}.

2.4. Quantification of Microbial Biomass

The samples for PLFA analysis were extracted from the mixture of chloroform-methanol-phosphate buffer (1:2:0.8) [45]. Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 40, Merck, Bellefonte, PA, USA). The samples were then subjected to mild alkaline methanolysis and extracted to hexane as a final solvent [46]. The free methyl esters of phospholipid fatty acids were analyzed using gas chromatography-mass spectrometry (Agilent 7890A with FID detector, Agilent Technologies, USA). The gas chromatography instrument was equipped with a split/splitless injector, and a CP Sil 88 column was used for separation (100 m, 0.25 mm i.d., 0.2 μm film thickness). The temperature program started at 80 °C and was held for 1 min in splitless mode. Then the splitter was opened, and the oven was heated to 160 °C at a rate of 20 °C·min^{-1}. The second temperature ramp was up to 225 °C at a rate of 5 °C·min^{-1}; this temperature was maintained for 12 min.

Methylated fatty acids were identified according to their mass spectra and using a mixture of chemical standards obtained from Sigma Aldrich (Merck, USA)/Matreya LLC (USA). Fungal biomass was quantified based on the 18:2ω6,9 content (FPLFA), and bacterial biomass was quantified as a sum of i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0 and cy19:0 (BPLFA). The fatty acids found in both bacteria and fungi, 15:0, 16:0 and 18:1ω7, were excluded from the analysis. The relative content of individual PLFA molecules was also calculated. The total content of all PLFA molecules (PLFAT) was used as an indicator of total microbial biomass.

2.5. Microbial Biomass Carbon

Soil Microbial Biomass Carbon (MBC) was characterized and determined by the fumigation extraction method [47], based on the lysis of microbial cells upon contact with chloroform (24 h). Sample sets were duplicated, and only one set was subjected to fumigation, followed by the extraction of K_2SO_4 and comparison of fumigated and non-fumigated samples.

2.6. DNA Extraction and Real-Time qPCR

DNA was extracted from 0.5 g of lyophilized soil with the help of a DNeasy PowerSoil Kit (Qiagen, Valencia, CA, United States). Real-time PCR was performed to quantify partial bacterial (16S rDNA) and fungal (18S rDNA) rDNA gene in soil DNA extracts. Each sample was spiked with the DNA of plasmid vector derived from pUC18 serving as an internal standard for valuation of yield efficiency and contamination with PCR inhibitors. Isolated DNA was quantified using Picodrop. SYBR-green assays were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories). The primers used were 1108F (5′ATGGYTGTCGTCAGCTCGTG 3′) and 1132R (5′GGGTTGCGCTCGTTGC 3′) for bacteria and FF390 (5′AICCATTCAATCGGTAIT 3′) and FR1 (5′AICCATTCAATCGGTAIT 3′) for fungi [48]. Combination of primer was used for the quantification of ammonia-oxidizing bacteria (AOB) 16S rDNA, CTO189FA/B (5′GGAGRAAAGCAGGGGATCG 3′), CTO189FC (5′GGAGGAAAGTAGGGGATCG 3′), and RT1R (5′CGTCCTCTCAGACCARCTACTG 3′) primers at a 2:1:2 ratio [49]. DNA of pUC18-derivate (internal standard) was quantified by qPCR using SQP (5′GTTTTCCCAGTCACGAC 3′) and SQPR2 (5′CTCGTATGTTGTGTGGAA 3′) primers.

2.7. Statistics Analysis

Comparison of individual data sets was made by one-way analysis of variance (ANOVA) and comparison methods. Duncan's multiple range test was used to compare treatments means, and a ($p < 0.05$) was considered statistically significant. Two way (ANOVA) was used to find the interaction between manure and biochar on measured soil properties (MBC, DHA, microbial community abundance). All the data were analyzed by Statistica ver. 13.4.0.14 software package.

3. Results and Discussion

3.1. Dehydrogenase

Dehydrogenase enzyme catalyzes organic matter decomposition in soil by transferring H^+ from the organic substrate with the help of coenzyme such as $NAD^+/NADP^+$ [50]. It is located inside living soil bacteria (e.g., genus *Pseudomonas*) and acts as an extracellular enzyme. It also implies that the enzyme cannot be deposited extra-cellularly in the soil in its active form [51]. Therefore, a high DHA activity suggests a higher number of the bacterial community present in the soil [52].

There was a significant decrease in DHA activity for the soil samples treated with biochar as compared to the control (Figure 1A). Data concerning the effect of different types (sources and pyrolytic temperature) of biochar on the dehydrogenase activity are still limited and contradictory. Different research groups reported different effects of biochar amendments on BPLFA values i.e., positive [53,54], neutral [55], and negative [56] effects. At least one study reported that DHA activity (and C mineralization) was lower in the biochar amended soil, however-glucosidase activity and the extracted PLFA concentration was not affected by biochar treatment [57]. Mechanisms for these different responses remain unclear [58]. Biochar effect on dehydrogenase activity in soil depends on the extent of the interaction between the substrate, enzyme and biochar (e.g., sorption and desorption of substrates on the biochar surface) [59]. The substrate and enzyme are attracted toward functional groups present on the biochar surface. Sorption of the enzyme to biochar blocks the active site present on the enzyme; resulting in the reduction in dehydrogenase activity [60]. The application of biochar produced at high temperature (approx. 400 °C or more) decreases soil enzyme activity and affects the soil nutrition dynamics, as it nonselectively sorbs the enzyme as well as a substrate due to its high absorptivity [61]. This high absorptivity can be attributed to high surface area and porosity of biochar created due to high temperature.

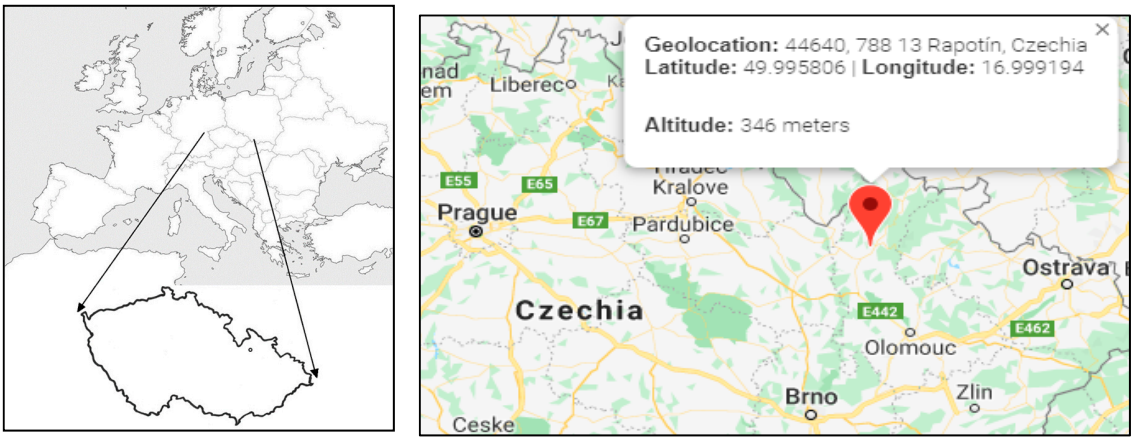

Figure 1. Geographical picture of study area.

MB amendment to biochar shows a significant increase in DHA activity as compared to biochar treatment (Figure 2A). The coapplication of manure caused a decrease in absorptive surface characteristics of biochar (sorptive sites occupancy) and hence neutralizing the negative effect of biochar. This agreed with the concept that net negative surface charge on biochar sorbs positive charge nutrients (NH_4^+, K^+, Ca^+, Fe^{++}, Cu^{++}) from manure [62]. Additionally, The sustainable and slow release of immobilized nutrient from biochar for an extended period is another reason for the increase in DHA activity [63–67]. The sorption of nutrient onto the biochar is reported to be a reversible phenomenon which favors the retention, and they are delay released into the soil [68–70].

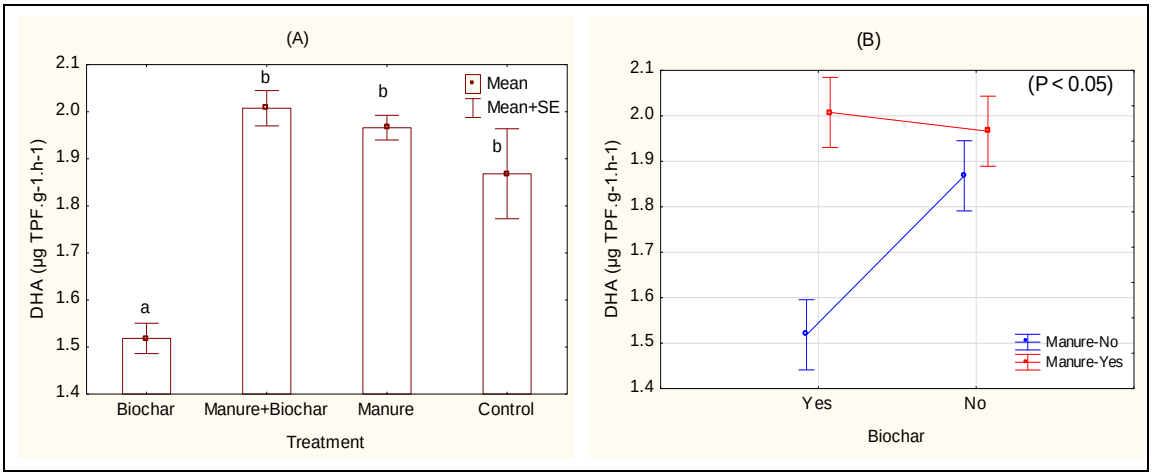

Figure 2. (**A**) DHA activity in soil amended with manure, biochar and MB. (**B**) Interaction graph of biochar and manure for DHA activity.

Two-factor ANOVA (with replication) was conducted to test the interaction effect of manure and biochar on DHA activity. The result shows that manure and biochar have a significant main effect on DHA activity (Figure 2B), whereas MB treatment shows a significant ordinal interaction on DHA activity (Figure 2B).

3.2. Soil Phospholipid Fatty Acid Analysis

PLFA, a rapid and sensitive method, was used to detect changes in the microbial community in soil. PLFAT, FPLFA and BPLFA can be viewed as indicators of total microbial biomass, fungal biomass, and bacterial biomass, respectively. Soil PLFAs analysis is a widely accepted method based on the rapid degradation of PLFAs after cell death [71].

Soil microbial community biomass represented by FPLFA was significantly higher in the sample treated with biochar, as compared to the control (Figure 3A). The highest value of FPLFA was recorded for soil treated with MB (Figure 3A). Generally, biochar is considered recalcitrant in nature [72], and microorganisms use it rarely, while mediating it in the rhizosphere as a source of nutrient for plants [73]. However, some studies suggest the existence of a labile-carbon fraction in it [31,41,74]. This labile fraction is available for the microorganism as a carbon source and supports microbial growth. This labile fraction may contain lipids, (up to 4.5%) with strong domination of glycolipids and phospholipids [75]. Additionally, biochar has been suggested to stimulate the dormant soil microorganism growth, thereby increasing the microbial biomass [76–79]. Saprotrophic fungi were shown to efficiently colonize biochar in association with decomposing fibrous organic matter [80], and the higher nutrient (namely P) content of the biochar increases its fungal colonization [81]. The unexpectedly higher FPLFA value in the sample treated with biochar (compared to manure treatment) can be explained due to the combined effect of initial biochar-derived phospholipids and the P-enhanced fungal colonization of biochar particles. This is also documented in agreement with high 18S rDNA content in the biochar-treated soil sample (Figure 5A). The synergy in co-application of manure and biochar on PLFA can be explained by their direct interaction and stimulation of microbial growth [82,83].

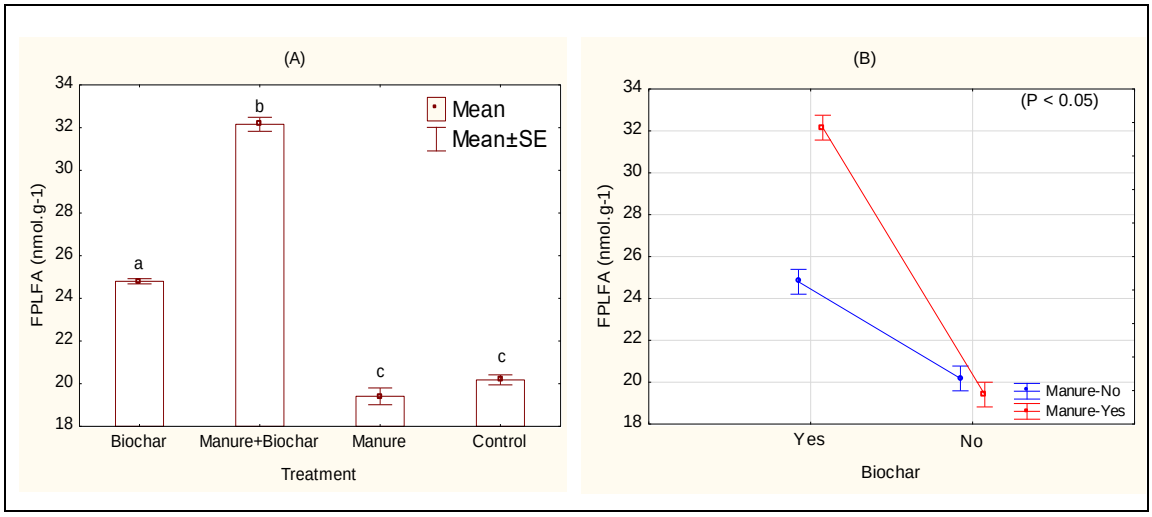

Figure 3. (**A**) Amount of FPLFA (nmol·g^{-1}) in soil amended with manure, biochar and MB. (**B**) Interaction graph of biochar and manure for FPLFA.

Two-factor ANOVA (with replication) was done to test the interaction effect of manure and biochar on the FPLFA value. The result shows that manure and biochar have a significant main effect on this FPLFA value (Figure 3B), whereas biochar and manure show significant ordinal interaction (Figure 3B).

BPLFA shows a similar response to biochar amendment (Figure 4) as FPLFA. There was a significant increase in BPLFA value following the addition of biochar. The noticeable increase in BPLFA concentration was observed when the mixture of manure with biochar is applied to the soil. BPLFA value for MB treatment was even higher than manure itself (Figure 4A).

Two-factor ANOVA (with replication) was conducted to test the interaction effect of manure and biochar on BPLFA values. The result shows that manure and biochar have a significant main effect on BPLFA values (Figure 4B), and a significant ordinal interaction was found between biochar and manure (Figure 4B).

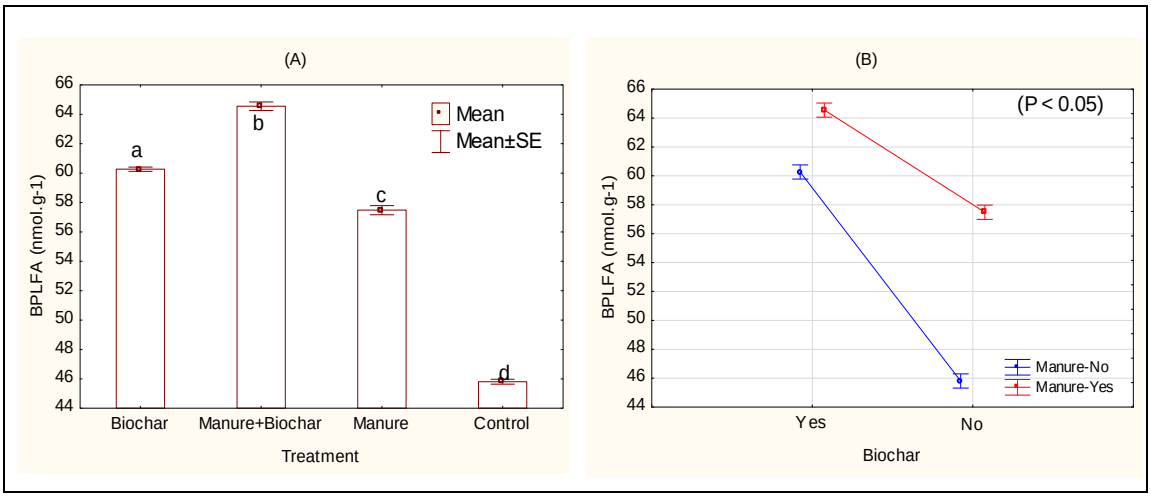

Figure 4. (**A**) Amount of BPLFA (nmol·g^{-1}) in soil amended with manure biochar and MB. (**B**) Interaction graph of biochar and manure for BPLFA.

3.3. Microbial Biomass Carbon

MBC is the main characteristic of soil organic carbon activity, so it is primarily used for the evaluation of soil quality [84]. It plays an essential role in biogeochemical cycles and is a major driver of ecosystem functioning [85,86]. Our experiment result shows a decline in the MBC value for the soil sample treated with biochar, and was lowest among all treatment (Figure 5A). Decrease in MBC upon biochar treatment has also been reported in the past [87,88]. We speculate that decline in the MBC value was either due to immobilization of carbon [89–91]. Or it could be due to the fact that biochar reduced the concentrations of nutrients through sorption and sequestration. Nutrients are protected from the microbes by their adsorption on the biochar surface. A decrease in the nutrient arability as a result reduces the microbial abundance. According to Dempster et al. (2012) [87] decrease in decomposition of SOM could be the main reason for the reduction in soil microbial biomass, upon biochar treatment. Our results appear to support this view. Several other studies show a positive effect of biochar on soil microbes [92,93]. Highest values for MBC were recorded when manure was applied in combination with biochar (Figure 5A). MBC values for biochar-only treatments are contradictory by PFLA values.

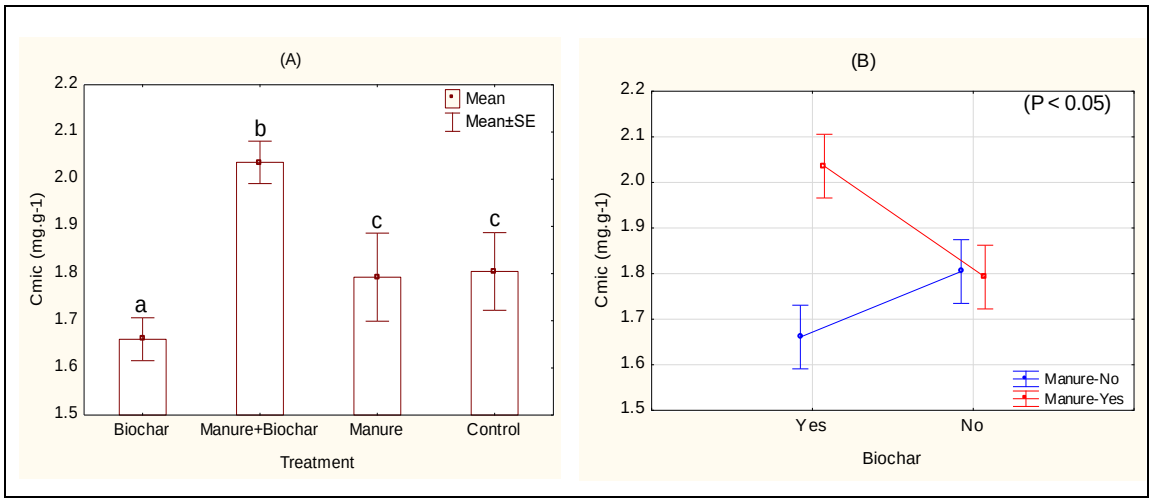

Figure 5. (**A**) MBC in soil amended with manure biochar and MB. (**B**) Interaction graph of biochar and manure for MBC.

These results indicate that chloroform fumigation used to determine microbial biomass and the decline in active cell numbers, does not always accompany with any decrease in microbial biomass. Hence, PLFAs, as a constant composition of living cell membranes, may also be unchanged when fumigation-sensitive microbial biomass decreases.

Two-factor ANOVA (with replication) was conducted to test the interaction effect of manure and biochar on MBC value. The result shows that biochar does not have any significant main effect on MBC (Figure 5B), whereas significant ordinal interaction was found between biochar and manure (Figure 5B).

3.4. DNA Extraction and Real-time qPCR

The effect of biochar, manure, and MB on the microbial community of treated soils is represented by the quantities of bacterial and fungal biomass, estimated as total 18S rDNA (fungal DNA) and 16S rDNA (bacterial DNA) content in the soil.

3.4.1. 18S rDNA

One-way analysis of variance shows a significant increase in log 18S rDNA copies relative to control on an application of biochar. The possible reason for an increase in 18S rDNA is that biochar can act as a habitat for many fungi [94–97]. Even though the extent of the hyphal colonization of biochar in soil is reported to be weak, extensive hyphal colonization of the surface of the biochar occurs, however it contrasts with a limited hyphal colonization of pores within the biochar [98]. Available nutrients like P, K and Ca on the biochar surface were possible reasons for fungal colonization [80,81], while the role of the labile-carbon fraction in the biochar was considered, similarly to the explanation of the observed FPLFA values (Topic 3.2). Small condo in biochar protects the microorganism from a natural soil predator such as mites, *Collembola* and larger (>16 µm in diameter) protozoans and nematodes [95,97,99–101]. The soil samples treated with MB showed a significant increase in log 18S rDNA copies, as compared to all treatments, including control (Figure 6). The increases in log 18S rDNA value followed the order: control < manure < biochar < MB (Figure 6A).

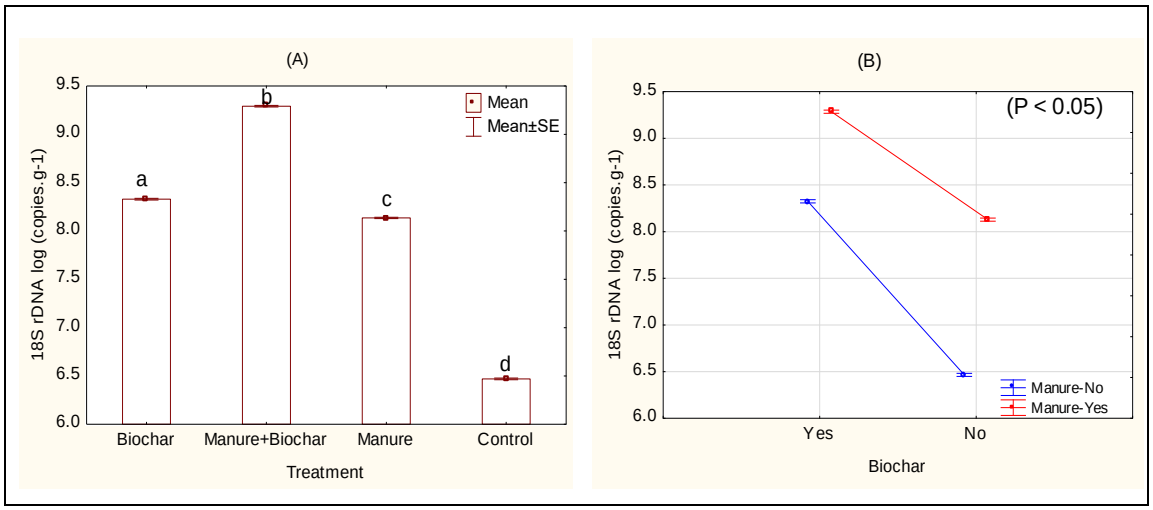

Figure 6. (**A**) 18S rDNA log (copies·g^{-1}) in soil amended with Biochar and MB. (**B**) Interaction graph of biochar and manure for 18S rDNA log (copies·g^{-1}).

The increase in log 18S rDNA copies upon MB treatment was putatively associated with a favorable environment for microbial proliferation [17]. Moreover, the functional group present on the biochar surface helps the sorption of dissolved organic carbon, decomposable organic compounds, and the chemisorption of the ammonium ion ($NH4^+$), and makes it a perfect microbial habitat [64]. Previous

research showed that manure supplies macro- and micronutrients to be sorbed on the biochar surface and provide a suitable environment for fungal and other microbial growth and proliferation [27,63,102].

Two-factor ANOVA (two-way ANOVA with replication) is conducted to test the interaction effect of manure and biochar on log 18S rDNA copies. The result shows that manure and biochar have a significant main effect on log 18S rDNA copies (Figure 6B). Also, manure and biochar show significant ordinal interaction (Figure 6B).

3.4.2. 16S rDNA

Similarly, observations of an increase in log 16S rDNA copies relative to control was observed for biochar treatment and the soil treated with biochar in combination with manure, which shows the highest value of log 16S rDNA copies, even higher than the only manure treatment (Figure 7). Some authors also reported a significant increase in bacterial 16S rDNA genes abundance in samples coupled with biochar poultry-manure [94,103].

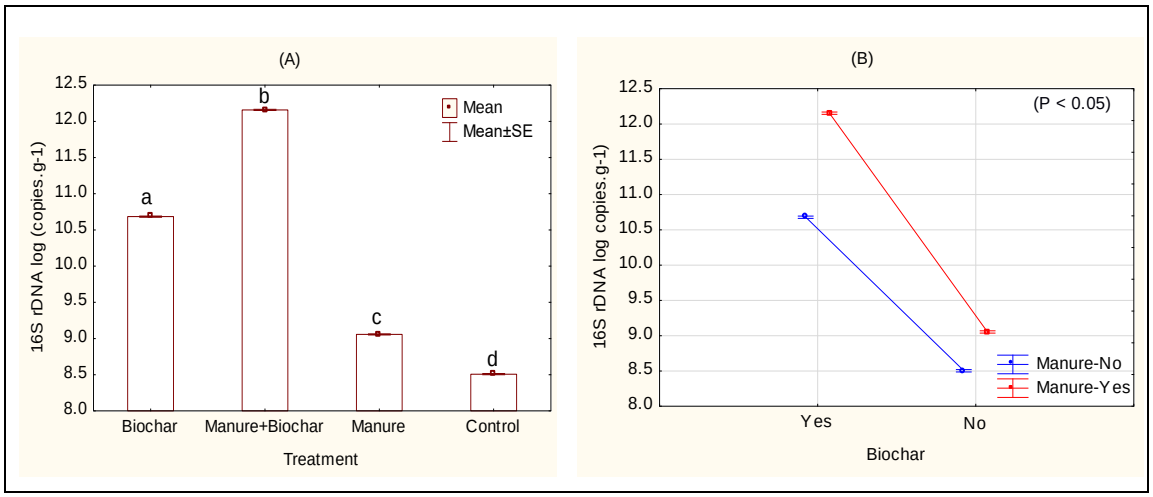

Figure 7. (**A**) 16S rDNA in soil amended with manure biochar and MB. (**B**) Interaction graph of biochar and manure for 16S, log (copies·g^{-1}).

Two-factor ANOVA (with replication) was conducted to test the interaction effect of manure with biochar on 16S rDNA gene copy. The result shows that manure and biochar have a significant main effect on the log-transformed 16S rDNA value (Figure 7B), whereas there was also a significant ordinal interaction found between biochar and manure (Figure 7B) with an increase in the 16S rDNA gene abundance, which was also observed in earlier studies [21].

The results obtained by estimation of 16S and 18S rDNA were supported by quantification of microbial biomass via phospholipidic fatty acids (PLFA). Two-factor ANOVA results of both bacterial BPLFA and fungal FPLFA show the statistical significance interactions between the amendments of biochar and manure.

3.4.3. 16S rDNA (AOB)

The effects of biochar and their combination with manure on 16S rDNA AOB copies are presented in Figure 8A. There was a significant increase in log 16S rDNA AOB copies for all the treatments compared to the control (Figure 8A). The 16S rDNA AOB value is an indicator of microbial activity in the process of nitrogen mineralization. It has been observed that the addition of organic fertilizers (e.g., compost) positively affects the microbial activity and utilization of nitrogen in contrast to the addition of the NPK fertilizer [104,105]. Among all the treatments, manure amendment with biochar shows the highest log 16S rDNA AOB copies.

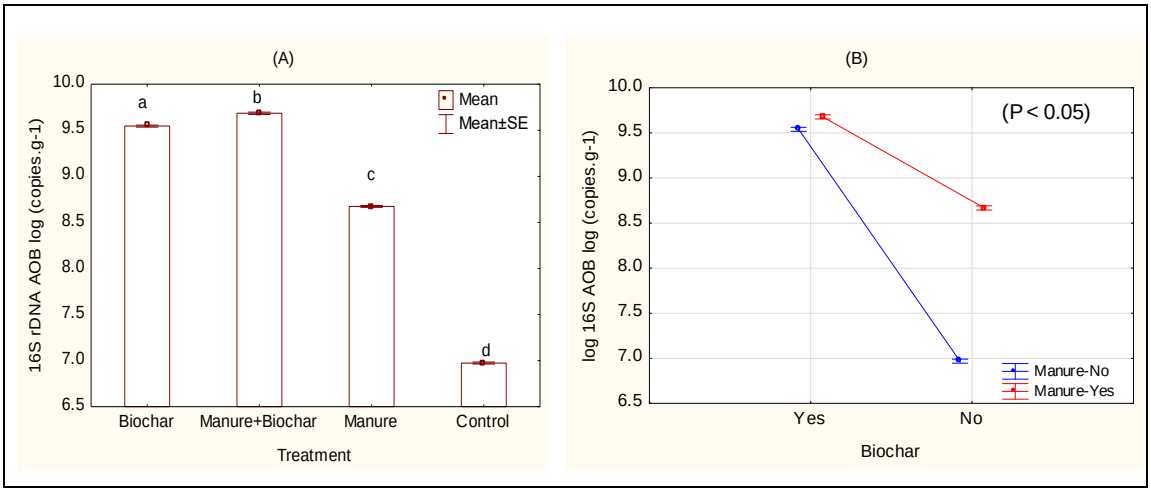

Figure 8. (**A**) 16S rDNA AOB rRNA in soil amended with biochar and combination with manure. (**B**) Interaction graph of biochar and manure for log 16S rDNA AOB copies.

Adding biochar into soils changes the soil structure [99,100] and alters soil microbial populations [79]. It is well known from the previous studies that adding biochar to soil, especially in combination with manure, can potentially alter the nitrification process in soil by affecting ammonia- and nitrite-oxidizing bacteria [106], decreasing N_2O emission [107,108] and increasing NH_4^+ storage [107].

Recently, several studies found that phenolic compounds (PHCs) and polycyclic aromatic hydrocarbons (PAHs) are retained in the biochar during the pyrolysis, and are really responsible for the inhibition of microbial activity, soil AOB and soil NO_3^- [107,109]. Previously published experimental outcomes proved that biochar addition to the soil retards the microbial nitrification mainly due to the toxicity of PHCs to AOB [110]. However, in our experiments, 16S rDNA AOB copies increase in response to the application of either biochar or its combination with manure. It was either due to lesser or no PHCs in the biochar, or increased availability of NH_4^+ sorbed on the biochar surface [62].

Two-factor ANOVA (with replication) was conducted to test the interaction effect of manure and biochar on log-transformed 16S rDNA AOB value (Figure 8B). Manure and biochar have a significant main effect on log 16S rRNA copies (Figure 8B), whereas significant ordinal interaction was found between biochar and manure (Figure 8B).

4. Conclusions

The results of this study demonstrate that the application of biochar with or without manure positively affect the fungal and bacterial biomass, as evident from the increased quantity of phospholipid fatty acid (BPLFA and FPLFA) and the DNA copy number (16S r DNA and 18S rDNA). Soil MBC and DHA activity decrease with the incorporation of biochar, but the maximum value is recorded for co-application with manure. These two properties were most affected by sorptive characteristics that might be directly dependent on the pyrolytic temperature used in biochar preparation. The results also revealed that the AOB population increased with the application of biochar and reached its maximum value when biochar is applied in combination with manure. However, further detailed studies are required to investigate the influence of biochars on nitrification and AOB community.

It can be concluded that the amendment of biochar in combination with manure changed the soil properties in three years of soil experiment in favor of increased microbial biomass. It was also confirmed that the application of biochar solely might increase or decrease soil activity, but their addition, along with manure, always promotes microbial abundance and their activity. However, quantity and sorption characteristics must be taken into account while planning the biochar-based soil amendments.

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