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Unveiling the Impacts of Biochar, Manure and Their Optimal Combinations on Microbiological Soil Health Indicators and Lettuce Biomass

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Abstract: Continuous use of chemical fertilizers has deteriorated soil health and crop productivity. Replenishing soil nutrients and microbial activity with eco-friendly soil amendments such as biochar and manure is therefore necessary to sustain soil health for crop production. However, studies are limited regarding the evaluation of biochar and poultry manure effects on soil health, attributed mainly to microbial extracellular enzymes and respiration. Therefore, the present study was designed to investigate the effects of poultry manure and biochar on soil physico-chemical and microbiological properties and lettuce biomass accumulation in a pot experiment. The pots were amended with poultry manure either alone and or in combination with low (10%) and high (20%) rates of biochar. The treatments included were; (i) control, (ii) manure alone (M), (iii) manure plus 10% biochar (M + B10, and (iv) manure plus 20% biochar (M + B20). Results revealed that soil extracellular enzymes related to C, N, and P mineralization, soil basal (BR), and substrate induced respirations (SIR) were significantly affected by applied manure and manure–biochar. However, there were large differences observed for applied amendments regarding various soil and crop parameters. Specifically, the manure combined with a high rate of biochar (M + B20) enhanced total carbon (TC) content, dehydrogenase activity (DHA), BR, and all SIRs except Arginine-IR. On the other hand, manure combined with a low rate of biochar (M + B10) resulted in enhanced lettuce aboveground dry biomass (AGB-dry). The manure treatment alone (M), however, proved to be the most influential treatment in improving soil enzymes (β-glucosidase, *N*-acetyl-β-D-glucosaminidase and phosphatase) involved in C, N, and P mineralization compared to the other treatments and control. Thus, it was concluded that the sole application of M and M + B20 improved both fertility and soil health, which therefore could be a promising direction for the future to enhance soil quality and crop productivity.

Keywords: poultry manure; plant nutrients; extracellular enzymes; manure maturation; soil health

1. Introduction

In recent years, the decline in soil fertility and its quality has become a global threat. In fact, Oldeman et al. [1] and Rashid et al. [2] estimated that nearly 30% of world's agricultural land will be converted to degraded soils in upcoming decades. This could be attributed to increased globalization and a growing human population on the one hand and to intensive agriculture and inappropriate management practices such as extensive tillage and indiscriminate chemical use, as well as low carbon inputs in the soil, on the other [3–5]. Furthermore, more agricultural intensification and frequent biotic and abiotic stresses induced by global climate change are known to impair soil fertility and crop production. These causative factors are among the chief contributors to soil degradation and contamination of groundwater quality. Soil, being the pedosphere, supports immense ecosystem services, and any change in its quality is interrelated with the deterioration of the whole environment. Therefore, there is a dire need to look for alternative strategies in agricultural soils that could ensure safe crop production and boost its fertility and health. Soil quality and fertility could be enhanced by increasing carbon inputs into the soil in the form of organic amendment, such as manure, biochar, compost, and other forms of carbon. By doing so, the soil nutrient-supplying capacity would be enhanced, soil health would be improved, and crop production would be increased [6,7].

Biochar is a carbonaceous material obtained through the destructive pyrolysis of organic materials (e.g., wood, manures, plant remains, food wastes), etc., in a low or zero oxygen environment [8]. It is distinguished from other similar materials and charcoal by virtue of its utilization as a soil amendment [9]. It is most often used to mitigate climate change via sequestration of soil organic carbon in the soil [10]. However, recently it has gained immense momentum for its use as a soil conditioner to improve soil fertility, reduce nutrient losses (mainly nitrate leaching), improve soil water retention, alleviate biotic and abiotic stresses in plants and enhance crop production [11,12]. In addition, a number of reports have acknowledged its stimulating effects on microbial populations and their activities in the soil [13,14]. Moreover, soil application of biochar has been considered as one of the best ways to recycle nutrients and convert biowastes into a useful product with agricultural benefits [8,9,13].

Biochar does so by altering the soil physico-chemical characteristics and by enhancing the availability of limiting nutrients [15]. Despite the fact that biochar has a high potential for improving soil properties, its application effects are further influenced by the type of feedstock, pyrolysis temperature, and rate of application in the soil. For instance, Zheng et al. [16] observed improved soil physico-chemical properties with the application of 0, 1, 2, 4, and 10% biochar. The authors reported an increasing trend of observed properties (soil pH, porosity, N, P, K, and amount of soil organic matter) with increasing biochar rate, suggesting a 10% biochar application rate as the best strategy for improving soil physical–chemical health. Another study by Du et al. [17] reported that biochar application at the rate of 1% enhanced microbial biomass and activity. However, Wang et al. [18] reported low positive effects (0.5%) and negative effects of biochar on microbial properties at (1%) biochar doses. Moreover, the pyrolysis of feedstock for biochar production have shown negative effects on the environment. A soil–water–atmosphere (gas) cycle possibly occurs during biochar production and transportation [19], which causes entry of biochar and its derivatives in unwanted media. Moreover, biochar sometimes contains minute amounts of primary environmental contaminants, such as heavy metals, poly aromatic hydrocarbons, and volatile organic compounds, which upon addition might accumulate in the soil [20–22]. These issues, together with soil textural constraints, affect the suitability and efficiency of biochar, which does not always result in positive effects. Therefore, the combination of biochar with other organic amendments, such as manure, has been recommended.

Manures are rich in plant nutrients and are a cheap source for improving soil fertility. Poultry manure production has been increased due to an increase in poultry production [23]. Poultry manure has been recognized as a rich source of nutrients especially N and C [24]. Its application in soil has been reported to enhance soil physico-chemical characteristics and plant biomass [25]. However, direct application of poultry manure on soil results in nitrate leaching and phosphorous runoff into freshwater bodies, which is one of the main environmental concerns of its direct application [26]. To date, many studies have been published on the sole utilization of biochar and/or poultry manure [25,27]. Little information is available on how the addition of poultry manure together with biochar impacts soil physico-chemical and microbiological properties. In particular, the role of manure in improving the efficiency of biochar under different rates has been the least explored, which in the present study constitutes the novelty. Thus, the responses of soil physico-chemical and microbiological health indicators through combined application of poultry manure and different rates of biochar should be further explored. Therefore, in the present study, we took advantage of a poultry rearing farm at Mendel University Brno, Czech Republic, to procure poultry manure and mix it with the biochar produced through the pyrolysis of organic wastes. Using such combinations could improve soil health and be ecologically promising. We thus hypothesized that (i) the sole application of poultry manure would enhance the soil physico-chemical properties, (ii) the combination of poultry manure and biochar at different rates would not only result in increased nutrients and plant biomass but also enhance the soil respirations; however, (iii) the differences in rates of biochar would influence the end point results. Therefore, the specific objectives of the present study were to (i) compare the effects of manure and manure–biochar amendments on soil chemical and health traits and crop biomass accumulation, and (ii) analyze soil basal and substrate-induced respirations and enzyme activities in order to track the changes in microbial functions caused by applied amendments. The results obtained from this work would essentially enhance our understanding of the amendment effects of poultry manure and biochar (at different rates) on main soil health indicators (soil respiration and extracellular enzyme activities), along with their potential for crop production.

2. Materials and Methods

2.1. Procurement of Manure and Preparation of Amendments

Poultry manure was produced at Mendel University in Brno. The biochar was introduced to the manure during the experimental rearing of broilers in the doses 0 $_{wt}$%, 10 $_{wt}$%, and 20 $_{wt}$% of used litter. Used biochar was produced at 650 °C from agricultural waste (cereal bran and chaff, sunflower hulls, fruit peels, and pulp) (Sonnenerde GmbH, Riedlingsdorf, Austria) and had the following basic properties: C 86.6%, N 0.3%, C:N 288.7, pH 8.5, and BET specific surface 288.5 $m^2 \cdot g^{-1}$.

2.2. Pot Experiment and Design

The experimental soil was a mixture (1:1, *w*:*w*) of a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) sampled (0–15 cm) near the town of Troubsko, Czech Republic (49°10′28″ N 16°29′32″ E) and a fine quartz sand (0.1–1.0 mm; ≥95% SiO_2). The following soil properties of Haplic Luvisol were determined before the start of the experiment: total carbon (TC) 7.0 $g \cdot kg^{-1}$, total nitrogen (TN) 0.80 $g \cdot kg^{-1}$, Phosphorous 0.049 $g \cdot kg^{-1}$, Sulphur (S) 0.073 $g \cdot kg^{-1}$, Ca (1.60 $g \cdot kg^{-1}$), Mg (0.118 $g \cdot kg^{-1}$), and K (0.115 $g \cdot kg^{-1}$); soil reaction, pH ($CaCl_2$) 7.3.

Fresh manure was matured in bags for 4 months at 14 °C ± 4 °C. Then, matured manure was mixed and applied into 5 kg of soil at a dose of 30 g, equivalent to 10 $t \cdot ha^{-1}$. The variants tested in the pot experiment and properties of matured manures are provided in (Table 1). The resulting soil–manure mixture was dosed into 3 replicates per variant (Table 1) and incubated in the greenhouse for 6 weeks before seeding. The incubation conditions were 22/18 °C, 45/60% air relative humidity, and a 12 h photoperiod, with soil moisture maintained at 60% of its water-holding capacity. After 6 weeks of incubation, the soil from all pots of each variant was mixed together and divided into 1 kg batches that were dosed into 1 L pots. All variants were prepared in 8 replications for the pot experiment. All pots were placed in the greenhouse under the same conditions as the incubation and sown with three sprouted lettuce seeds. The lettuce was grown for 7 weeks. At the end of the experiment, the plants were cut at ground level and aboveground biomass (AGB) was dried at 60 °C until a constant weight was obtained and then weighed gravimetrically using laboratory analytical scales to determine the weight of dry above-ground biomass (hereinafter referred to as AGB-dry).

Table 1. The tested variants in the pot experiment and manure properties (in fresh matter).

	Variant	Variant Code	pH [-]	C [%]	N [%]	C:N [-]
I.	Control without manure	Control	-	-	-	-
II.	Manure	M	6.5	20.1	1.1	18.2
III.	Manure + biochar 10%	M + B10	6.9	26.4	1.5	17.4
IV.	Manure + biochar 20%	M + B20	7.1	35.9	2.0	17.5

2.3. Soil Analyses

Soil samples were sieved through a sieve with a mesh size of 2 mm. Air-dried samples were used for pH ($CaCl_2$) analysis [28] and for total carbon (TC) and total nitrogen (TN) content determination using the Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Freeze-dried samples were assayed for enzymatic activities—β-glucosidase (GLU), phosphatase (Phos), and *N*-acetyl-β-D-glucosaminidase (NAG)—using methods based on spectrophotometric measurement of coloration evolved from *p*-nitrophenyl-derivates of respective enzyme substrates [29]. The samples stored at 4 °C were used for determination of dehydrogenase activity (DHA)—using a standard method based on triphenyltetrazolium chloride (TTC) [30]—and soil basal respiration (BR) and substrate induced respirations (IR)—with inductors D-glucose (Glc-IR), L-alanine (Ala-IR), L-arginine (Arg-IR) [31]—using a MicroResp® device (The James Hutton Institute, Aberdeen, UK).

2.4. Statistical Analyses

To evaluate the effects of the applied amendments, the obtained data were statistically analyzed using one-way analysis of variance (ANOVA). The Tukey HSD post hoc test was used to compare treatment means (at a significance level of $p = 0.05$). Principal component analysis (PCA) was performed to evaluate the mutual relationships between measured variables and applied amendments.

3. Results

3.1. Effects of Amendments on Soil Properties and Plant Biomass

There were no significant changes in soil pH observed after the application of amendments, namely, manure (M), manure + biochar 10% (M + B10), and manure + biochar 20% (M + B20). In all cases, the effect of applied amendments remained statistically non-significant among each other and with control (Figure 1a). The application of M + B20 resulted in the highest total carbon (TC), which was statistically significant compared to control (Figure 1b). This trend was followed by M + B10 and M alone treatments, but they remained statistically non-significant relative to control (Figure 1b). Conversely, there was no clear trend observed for TN contents under the applied amendments (Figure 1c). In the same way, there were no significant differences observed for C:N under the applied amendments (Figure 1d). The application of M + B10, however, yielded a slightly higher C:N ratio compared to other amendments and in the control (Figure 1d). The aboveground plant dry biomass (AGB-dry) was differently affected with the applied amendments. Seemingly, the application of M + B10, M + B20, and M alone resulted in similar increases in AGB-dry as compared to control soil (Figure 1e).

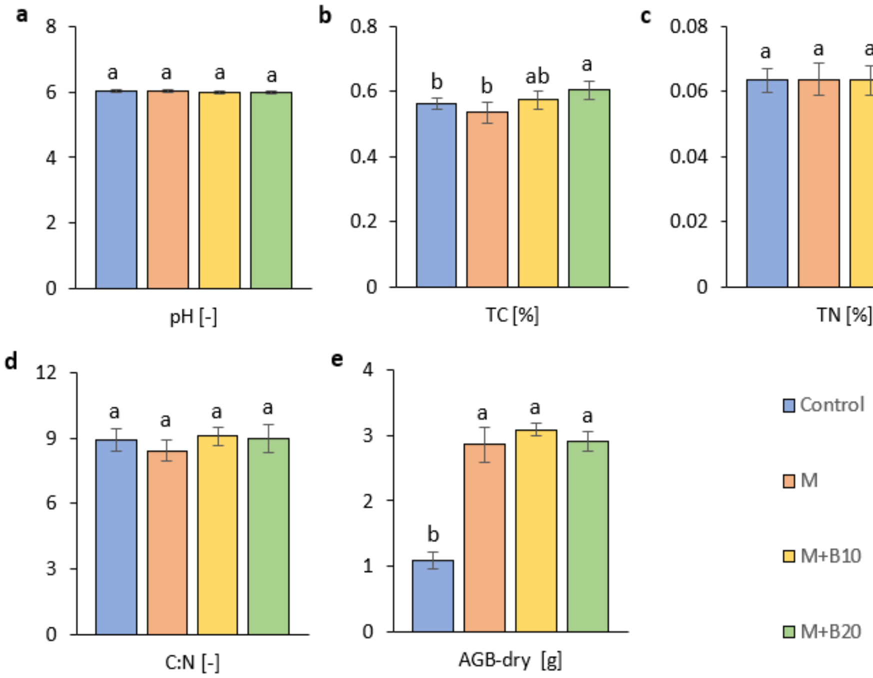

Figure 1. The effects of different amendments on: (**a**) soil pH; (**b**) total carbon; (**c**) total nitrogen; (**d**) carbon: nitrogen ratio; and (**e**) aboveground dry biomass of lettuce in a pot experiment. Different lowercase letters represent statistical difference at $p = 0.05$, ($n = 3$).

3.2. Effects of Amendments on Soil Microbial Properties

All three amendments yielded variable results regarding different soil enzyme activities. Dehydrogenase activity (DHA) was significantly higher in the soil amended with M + B20 compared to other amendments and the control, while the effects of other amendments remained statistically non-significant to the control (Figure 2a). The β-glucosidase (GLU) activity was significantly higher in the soil amended with M alone than in the soil amended with M + B10 and M + B20, respectively (Figure 2b). Likewise, the soil amended with M showed a significantly higher value for phosphatase (Phos) activity compared to control and other amendments. In the case of urease, the application of M and M + B20 treatments yielded similar values, both of which were significant compared to control and M + B10 as well (Figure 2c). The M treatment also enhanced the activity of *N*-acetyl-β-D-glucosaminidase (NAG), followed by M + B20 and M + B10 compared to control (Figure 2d).

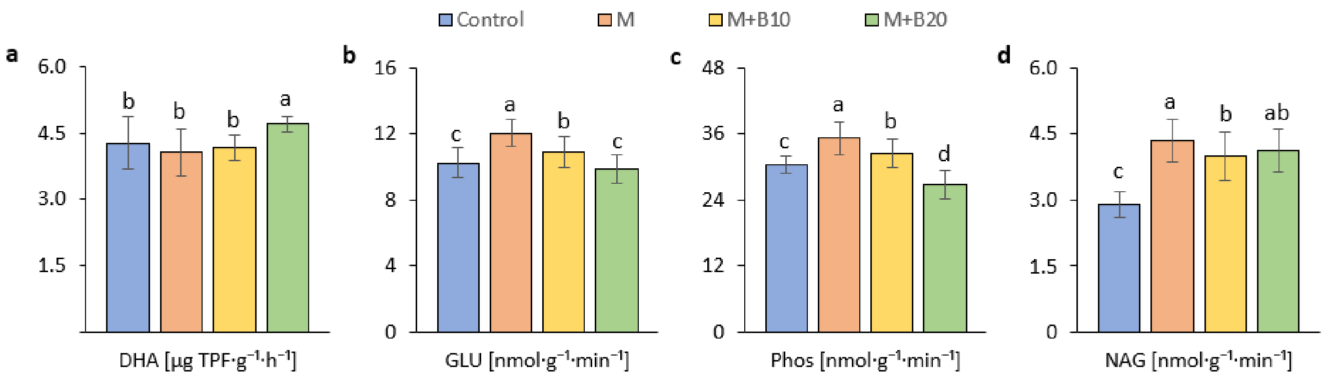

Figure 2. The effects of different amendments on: (**a**) dehydrogenase activity; (**b**) β-glucosidase activity; (**c**) phosphatase activity; and (**d**) *N*-acetyl-β-D-glucosaminidase. Different lowercase letters represent statistical difference at $p = 0.05$, ($n = 3$).

Surprisingly, the application of M + B20 resulted in the highest basal respiration (BR), which was significantly different than other treatments and control (Figure 3a). Considerable differences were observed for various substrate-induced respirations subjected to different amendments. Specifically, in line with the results of BR, the amendment of soil with M + B20 also enhanced the glucose-induced soil respiration (Glu-IR) and alanine-induced (Ala-IR) which were statistically significant compared with other amendments and control treatment (Figure 3b,c). This was followed by M and M + B10, but the effect of the M + B10 amendment remained non-significant compared to control (Figure 3b,c). Conversely, in the case of arginine-induced respiration (Arg-IR), the significantly highest values were recorded in soil amended with M compared to control. However, the amendment of soil with M + B10 and M + B20 yielded similar but statistically significant differences from control (Figure 3d). The association of Arg-IR in M treatment as shown by PCA further supports this result (Figure 4).

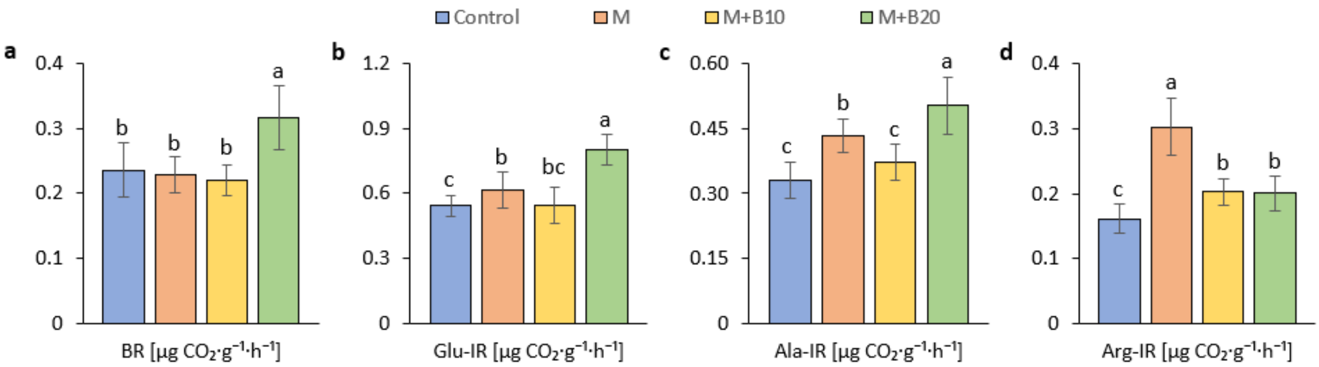

Figure 3. The effects of different amendments on: (**a**) basal respiration; (**b**) D-glucose-induced respiration; (**c**) L-alanin induced respiration; and (**d**) L-arginine-induced respiration. Different lowercase letters represent statistical difference at $p = 0.05$, ($n = 3$).

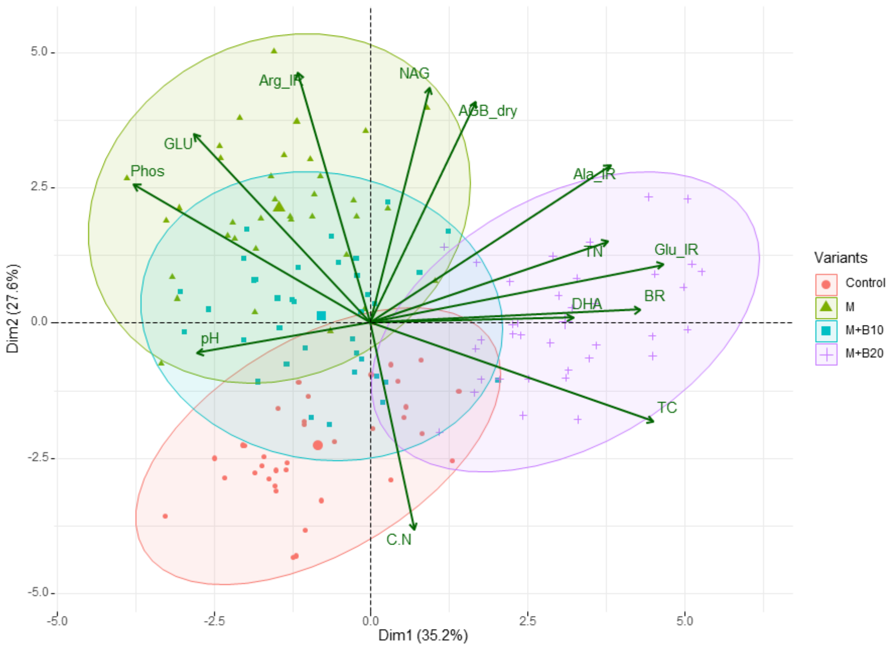

Figure 4. Principle component analysis (PCA) biplot of observed variants. Abbreviations are: AGB-dry, aboveground dry biomass; pH, soil reaction ($CaCl_2$); TN, total nitrogen; TC, total carbon; C:N, ratio of carbon to nitrogen; DHA, dehydrogenase activity; Glu, β-glucosidase activity; Phos, phosphatase activity; NAG, *N*-acetyl-β-D-glucosaminidase; BR, basal respiration; Glu-IR, D-glucose-induced respiration; Ala-IR, L-alanin-induced respiration, Arg-IR, L-arginine-induced respiration.

3.3. Results from Principal Component Analysis

The score and loading plots of observed parameters and variants are shown in (Figure 4). Both the extracted components PC1 (hereinafter referred to as Dim 1) and PC2 (hereinafter referred to as Dim 2) accounted for 62.8% variation in the dataset. The applied variants were markedly separated as shown by different colors (Figure 4), showing the positive effects of amendments on the observed attributes. The positive relationship was found among the parameters (NAG, AGB-dry, Ala-IR, TN, Glu-IR, BR, DHA, TC, and C:N) separated by principal component one. While, the principal component two separated mutually positively related parameters (Arg-IR, GLU, Phos, and pH). This clearly shows the displacement of observed parameters under the applied amendments.

4. Discussion

4.1. Effects of Amendments on Soil Physico-Chemical Properties and Plant Biomass

Biochar and manures have been recognized to enhance soil fertility, physical and chemical soil health, and crop production. The soil reaction (pH) is an important soil chemical property and is driven by changes in soil mineral composition, soil nutrient status, and other microbiological characteristics [32]. In the present study, however, the applied amendments, manure alone (M), and combined with various biochar levels as M + B10 and M + B20, did not show any significant changes in soil pH compared to control (Figure 1a). These results are in agreement with the previous results reported by Zahra et al. [33], who reported no significant effects of biochar and compost application on soil pH due to the higher pH of the soil than the pH of applied amendments. The role of biochar on changing soil pH has been inconclusive. Different authors have reported enhanced or reduced pH of the soil due to the application of manure and biochar. For instance, Rehman et al. [34] reported a decreased pH of the soil under the application of manure and composted biochar, while the pH was increased due to the addition of rice-straw- and cotton-stick-derived biochars. This shows that the pH of the soil further depends on the nature and feedstock of the applied biochar and/or manure. Organic amendments have been perceived as enhancing the soil carbon and total nitrogen concentration in the soils [14,35]. We observed enhanced TC content under the combined application of manure and biochar at both levels (M + B10 and M + B20), with the highest increase being observed at the higher biochar level (Figure 1b). The enhanced TC content in the present study might be the result of the direct addition of soil organic carbon (SOC) through applied biochar and manure amendments in the soil and due to the improved soil aggregation. Yang and Lu [36] found that adding biochar to soil increased TC content. The authors argued increased TC content is due to the improved soil aggregate stability and soil aggregation with the addition of biochar. Furthermore, the higher TC content under a higher dose of biochar combined with manure M + B20 can be attributed to the resistance of C in biochar, which resists microbial decomposition after application in soil and thus appears in the TC pool of observed soil [37]. Our results of enhanced TC are in line with the findings of [38], who reported increased C content under the applied biochar and compost amendments. This is consistent with previous research that has established the role of organic amendments (biochar, compost, and manure) in improving C buildup [39,40]. These results were further verified by the strong association of TC with the M + B20 variant as depicted by PCA (Figure 4). In contrast, in the present study, the positive role of amendments in improving TN content was not verified (Figure 1c), and therefore no significant differences were observed for C:N ratio (Figure 1d). We ascribe these changes to the relatively shorter duration of the experiment and to the highly recalcitrant nature of biochar, which in combined treatments M + B10 and M + B20 might have resulted in stabilized N forms and ultimately less mineralizable N in the soil [41].

The present study revealed that the applied amendments positively affected the plant biomass accumulation (Figure 1e). The highest average aboveground dry biomass (AGB-dry) was observed in the pots amended with M + B10, followed by M + B20 and M alone (Figure 1e). These observed effects suggest the positive role of combined application of chicken manure and biochar in improving plant production. Increased plant biomass improves soil nutrient availability and uptake in plants via the combined use of biochar and manure, as previously reported by Cao et al. [42] and Dubey et al. [43]. The enhanced plant growth might also be the outcome resulting from improved soil physical conditions such as water-holding capacity, soil structure, and soil aeration due to applied amendments [44]. This might be the reason for efficient plant biomass production under combined manure plus biochar amendments in the present study.

4.2. Effects of Amendments on Microbial Properties

Organic amendments such as biochar and manures can maintain high microbial biomass and result in higher microbial activities. We found considerable variations in the measured soil enzyme activities subjected to different amendments. The dehydrogenase activity (DHA) was highest in the treatment M + B20, which is suggestive of higher C mineralization potential under this particular treatment. The results of enhanced DHA activity in the present study (Figure 2a) are advocated by previous works of [14], who reported enhanced DHA activity under the addition of biochar and humic substances in the soil. We further ascribe this improved DHA activity to a manure plus biochar mediated enhancement in SOM-degrading microbial communities, which under M + B20 amendment might have proliferated and raised the levels of DHA. In the present study, all other enzymes showed the highest activities under the M amendment compared to control (Figure 2b–d). This shows that manure alone (M) provided easily accessible substrates for microbes, which resultantly enhanced enzyme activities in a pattern similarly observed by [13]. Our results of enhanced C, N, and P acquiring enzymes under manure and, in some cases, manure combined with biochar are in agreement with [45], who reported enhanced soil enzymatic activities under manure alone and/or combined with a low or high dose of biochar. Moreover, Irmak et al. [46] also confirm our results of enhanced microbial enzymes under the application of manure and biochar. The differential responses of all enzymes under manure combined with biochar treatments might be associated with the variable substrate–enzyme interactions in the presence of biochar and to the chemistry of OC derived from biochar and manure [47], which further depend upon biochar surface characteristics and the rate of biochar applied. This is in line with Cardenas-Aguiar et al. [48], who stated that the variations in the measured soil enzymes could be related to the changes in the decomposition rate of the applied substrate (manure and biochar) in the present study.

Soil basal and substrate-induced respirations (SIR) are measures of microbial activity and their potential to mineralize nutrients in soil. The exogenous supply of organic amendments has been recognized to stimulate microbial respiration. In the present study, manure and biochar combinations at different rates showed a stimulating effect on soil basal (BR) and different substrate-induced respirations (Figure 3a–d). In all the cases (except for Arg-IR), we observed the highest respiration values in the soils amended with manure and high rates of biochar (M + B20). These results are in accordance with the previous results by [49], who reported enhanced microbial respiration due to the interactive application of manure and biochar. In line with our findings, another study by Trupiano et al. [40] reported enhanced microbial respiration under the application of manure combined with higher biochar doses. We ascribe these results to the enhanced microbial activity and proliferation due to freshly available C sources in the simultaneous addition of manure [40] and the shielding effect of biochar as the biochar is utilized by microbes as a niche [50]. Additionally, the enhanced sorption of organic substances on the biochar surface might have increased the microbial population [51] and hence eventually resulted in enhanced respiration (Figure 3) in the present study.

5. Conclusions

Soil degradation and a decline in its fertility have been recognized as a serious issue in the face of global climate change. The role of biochar and manures in improving soil productivity in this regard has gained momentum in recent years. This study concluded that all applied amendments, either sole manure, or combined with low and high doses of biochar, differed in their potential to improve soil physico-chemical and biological health indicators but not significantly in the biomass of crop sown in a pot experiment. As predicted, M + B20 enhanced TC content, confirming the C sequestration potential of this particular treatment. The same treatment enhanced the basal- and substrate-induced respirations (except for Arg-IR), which was coupled with enhanced DHA activity. The application of M alone enhanced all other soil enzymes, which is suggestive of the higher nutrient-supplying capacity of M treatment. Moreover, the applied amendments enhanced the AGB-dry of the crop at the same potential. Taken together, these findings suggest that the choice of a suitable amendment is imperative for sustaining soil health and crop benefits. However, the evaluation of direct mechanisms related to manure and biochar surface chemistry and their effects on microbial activities, community composition, and soil nutrient dynamics needs to be further studied under long-term studies in agroecosystems.

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