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Comparison of the Responses of Soil Enzymes, Microbial Respiration and Plant Growth Characteristics under the Application of Agricultural and Food Waste-Derived Biochars

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Abstract: The conversion of bio-wastes to useful products such as biochar provide a suitable option not only to minimize the mass of wastes, but also to use the biochar as soil amendment. In the present study, food waste biochar (FWB) and agricultural waste derived biochar (AB), either alone or in combination (FWB + AB), were tested for their effects on soil microbiological characteristics (soil enzymes, soil basal as well as substrate-induced respiration), plant growth and photosynthetic parameters. Four treatments were tested: control, FWB, AB and FWB + AB. The results indicated that the application of AB significantly enhanced the plant above ground and below ground fresh and dry biomass as compared to other amendments and control (+41 to +205% compared to control). The application of FWB enhanced the quantum yield of photosystem II (QY-max, +4% compared to control) and normalized difference vegetation index (NDVI, +13% compared to control). Moreover, the FWB application improved the soil dehydrogenase (DHA) activity (+24% compared to control). Furthermore, the soil basal respiration was found to be increased under AB application (+46% compared to control) and the substrate-induced respirations were relatively decreased, depicting negative effects of applied biochars on substrate-induced respirations. Thus, we concluded that the differential responses of observed crop and soil attributes might be related to the biochar specific effects on soil properties.

Keywords: biochar; soil quality; sustainable management; nutrient cycling; soil respiration

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1. Introduction

The world's population is expected to reach >9 billion by 2050, which poses a great challenge to produce food from the available resources [1]. To meet this challenge, scientists are now focusing to increase the food production to meet the global food demand. However, with this rapid increase of population and increased food demand, the competition for land, water, energy and other natural resources has also exponentially increased [2]. Therefore, increased food requirements will essentially put pressure on soil, water and the overall environment. In the meantime, the higher food production is concomitantly linked with higher waste generation (food and agricultural), which is another of the main concerns in the face of climate change.

However, safe recycling and utilization of such wastes for sustainable soil management is a promising approach which may help in achieving the sustainable development goals [3]. Many different types of organic food as well as agricultural waste materials are being generated annually around the globe, which have the potential to contribute to enhancing soil fertility and mitigate climate change [4,5]. All these wastes can be bio converted to safer organic products with agricultural benefits for enhancing soil health and supporting crop production, and hence, limiting reliance on agro-chemicals [6–8]. Such products, when used as soil amendments, enhance the soil fertility and its quality for prolong periods of time, unlike chemical fertilizers, which negatively affect soil fertility in the long term and may deteriorate its biodiversity [9–11].

One of the major products obtained through biomass (agricultural and food waste in the present study) conversion is biochar. Biochar is a carbon rich porous organic material obtained through the complete pyrolysis of feedstock in the absence of oxygen [5,12]. Biochar is a promising soil amendment that is widely adapted and utilized for enhanced soil health and sustainable crop production [13–15]. Previously, a large number of studies have shown that the addition of biochar as soil amendment enhance soil physico-chemical properties [16,17], soil health [18,19] and crop performance [20,21]. Moreover, the combination of biochar with other organic and inorganic materials has also been acknowledged for their promoting effect on soil fertility and crop production [22,23]. However, it should be noted that the specific effects of biochar amendment on soil properties and or crop biomass depend on soil type, feedstock and the nature of the applied biochar [24–26].

Microbial activity in terms of respiration and or extracellular enzymes is an important indicator of soil quality. Maintaining an adequate level of microbial biomass and supporting their activities are important for nutrient cycling and ecosystem function. It has been reported that biochar application considerably alters microbial population and its activity in soils [27,28], and hence, aid in ecosystem services of soils. However, the information on biochar derived from different feedstocks and their effects on soil fertility and crop production is lacking. Previously, a number of studies have reported inconsistent results for soil properties, crop production and plant nutrients [29,30]. These variations in the obtained results could be attributed to soil properties, pyrolysis conditions and feedstock properties [31,32]. To the best of our knowledge, to date, few studies have compared the biochars obtained from food wastes and agricultural wastes in terms of their comparative efficacy. In particular, the responses of soil microbiological health indicators (soil respiration, extracellular enzymes) have been least explored. Additionally, the comparative effects of biochars derived from different feedstocks on plant growth and photosynthetic efficiency needs to be further studied, which the present study aims to do. Thus, we hypothesized that the food and agricultural waste-derived biochars would have differential effects on the measured soil properties and plant growth (lettuce in the present study). The specific objectives of the present study were to (i) compare the effectiveness of using food and agricultural waste biochars on soil health indicators (soil enzymes and microbial respiration) and (ii) evaluate the responses of plant growth and physiological attributes under the applied amendments.

2. Materials and Methods

2.1. Biochar Production

For the purposes of this pot experiment, biochar from mixed food waste (collected from the university canteen) was prepared in two steps. (i) A pre-treatment process, which consists of two consequent steps. Firstly, the dried food waste (dry matter approximately 90%) was mixed with 25% of spruce sawdust, and subsequently, the mixture was pelletized at a briquetting press for the production of pellets type JGE 260 with a matrix at a size of 6 mm of extrusion holes and a pellet length of 40 mm. (ii) Heat treatment of the samples took place in the second step, whereby thermal pyrolysis (TP) was performed in laboratory, small-scale conditions, in a small-scale TP unit working under 650 °C. This unit works discontinuously, and the maximum capacity was around 5 kg·batch^{-1} of feedstock (FS).

The glass condenser attached to the pyrolyzer was used for the separation of gaseous products and the pyrolysis oil. The input weight of FS samples was 3000 g·batch^{-1}. The FS was placed into the TP unit in a stainless-steel cylindrical reactor. During the experiment, the residence time was 340–410 min, and the temperature did not exceed 650 °C [33].

Moreover, commercial biochar from agricultural waste was purchased from the manufacturer (Sonnenerde GmbH, Riedlingsdorf, Austria). This biochar was produced with a high-technology production unit Pyreg500 from grain husks, sunflower pods and pulp mud. The process temperature was set up at 650 °C. The physico-chemical properties of the applied biochars are given in (Table 1) and has been mentioned in our previous study [33].

Table 1. Physico-chemical properties of used biochars in this study.

Biochar	TC [%]	ROC [%]	TIC [%]	TOC [%]	N [%]	H [%]	O [%]	C:N	H:C	O:C
AB	50.13 ± 0.02	0.45 ± 0.06	0.33 ± 0.00	49.80 ± 0.02	1.01 ± 0.06	1.60 ± 0.04	17.28 ± 0.21	49.67 ± 2.89	0.03 ± 0.00	0.34 ± 0.00
FWB	81.25 ± 0.03	0.28 ± 0.01	0.07 ± 0.00	81.18 ± 0.03	3.58 ± 0.05	3.04 ± 0.06	8.10 ± 0.25	22.71 ± 0.30	0.04 ± 0.00	0.10 ± 0.00

TC: total carbon, ROC: resistant organic carbon, TIC: total inorganic carbon, TOC: total organic carbon, N: nitrogen, H: hydrogen, O: oxygen.

2.2. Experimental Soil and Pot Experiment

The growth substrate used for the pot experiment was prepared by mixing one part fine quartz sand (0.1–1.0 mm; ≥95% SiO_2) with one part arable soil, a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification) sampled (0–15 cm) near the town Troubsko, Czech Republic (49°10′28″ N 16°29′32″ E), sieved through 2 mm. The soil properties were as follows (g·kg^{-1}): total C 14.0, total N 1.60, P 0.097, S 0.145, Ca 3.26, Mg 0.236, K 0.231; pH ($CaCl_2$) 7.3. One kilogram of growth substrate was mixed with 32 g (equivalent to 40 t ha^{-1}) of a food waste biochar (FWB), agricultural biochar (AB) or their mixture in weight ratio 1:1 (FWB + AB) and filled to experimental plastic pots (volume 1 L, top diameter 11 cm, bottom diameter 9 cm, height 13 cm). The treatments included (i) Control (no biochar), (ii) food waste biochar (FWB), (iii) agricultural waste biochar (AB) and (iv) combined food plus agricultural waste biochar (FWB + AB). Each treatment was carried out in three replicates (pots).

The pot experiment with lettuce (*Lactuca sativa* L. var. *capitata*) took place in growth chamber Climacel EVO (BMT, Czech Republic) under controlled conditions: light intensity 20,000 lux; photoperiod 12 h; temperature 18/22 °C (night/day); relative humidity 70%. A two-day sprouting of the lettuce seeds on wet filter paper preceded sowing to the depth of approximately 2 mm in each pot. After sowing, each pot was watered with 100 mL of distilled water. The 10-day-old seedlings were reduced to one plant per pot. Pot placement in the growth chamber was randomized. Soil humidity was controlled, and water content was maintained during the experiment at approximately 60% of water holding capacity. The pots were variably rotated once per week. The plants were harvested 8 weeks after sowing.

2.3. Plant Growth and Physiological Attributes Determination

At harvest time, determination of photochemical efficiency of photosystem II (PSII) of lettuce plants was carried out. The quantum yield of the PSII (ΦPSII) was determined (at light intensity 2400 µmol·m^{-2}·s^{-1}) by the fluorometer PAR-FluorPen FP 110-LM/S (Photon Systems Instruments, Drásov, Czech Republic) and the software FluorPen 1.1 (Photon Systems Instruments, Drásov, Czech Republic) was used for the analysis of the measured data. Determination of normalized difference vegetation index (NDVI) was carried out too with PlantPen NDVI 310 (Photon System Instruments, Drásov, Czech Republic). The spectral reflectance of chlorophyll pigments, expressed as NDVI, is a measure of chlorophyll content [34] and its integrity [35] and correlates with the photosynthetic rate [34]. The lettuce shoots were cut at ground level, and the roots were gently cleaned of soil and washed with water. Fresh aboveground (AGB) and root biomass were estimated gravimetrically by weighing on the analytical scales. The lettuce shoots and roots were dried at 60 °C to a

constant weight, and dry aboveground and root biomass were estimated gravimetrically by weighing on the analytical scales.

2.4. Soil Sampling and Preparation for Analyses

A mixed soil sample was taken from each pot after harvesting the lettuce. The collected soil samples were homogenized by sieving through a sieve with mesh size 2 mm, followed by air drying the samples, which were analyzed for soil pH measurement [36]. Freeze-dried samples were used for the analyses of enzymatic activities according to (ISO 20130: 2018) [37], including β-glucosidase (hereinafter referred to as GLU), phosphatase (hereinafter referred to as PHOS), urease (hereinafter referred to as URE) and N-acetyl-β-D-glucosaminidase (hereinafter referred to as NAG). The samples stored at 4 °C were used for determination of dehydrogenase activity (hereinafter referred to as DHA) using the standard method based on triphenyltetrazolium chloride (TTC) [38], soil basal respiration (hereinafter referred to as BR) and substrate-induced respirations (IR), such as D-glucose-induced respiration (hereinafter referred to as Glu-IR), L-alanine-induced respiration (hereinafter referred to as Ala-IR) and L-arginine-induced respiration (hereinafter referred to as Arg-IR) [39], using a MicroResp® device (The James Hutton Institute, Aberdeen, Scotland).

2.5. Statistical Analyses

The obtained data were statistically analyzed using the one-way analysis of variance (ANOVA), to evaluate the effects of applied amendments. Data normality and homogeneity of variance was performed using the Shapiro–Wilk and the Levene tests (at $p \leq 0.05$). Treatment means were compared using principal component analysis (PCA) and Tukey's HSD post-hoc test (at significance level $p = 0.05$) using Program R (version 3.6.1).

3. Results

3.1. Plant Growth and Chlorophyll Fluorescence

Considerable variations were recorded regarding the effects of the applied biochars (FWB and AB) on plant growth and physiological parameters related to chlorophyll fluorescence (Figures 1 and 2). The plant aboveground fresh biomass (AGB_fresh) was significantly ($p < 0.05$) enhanced under the applied agricultural waste biochar (AB) as compared to control (Figure 1A), followed by the combined application of FWB + AB. The same treatment enhanced the aboveground dry biomass (AGB_dry) as compared to control, and this was followed by FWB + AB (Figure 1B). Regarding root fresh weight (hereinafter root_fresh), the applied AB and food waste-derived biochar (FWB) resulted in the statistically ($p < 0.05$) highest significant increase as compared to control (Figure 1C), whereas root dry biomass (root_dry) was found to be highest under applied AB as compared to control, followed by FWB + AB (Figure 1D).

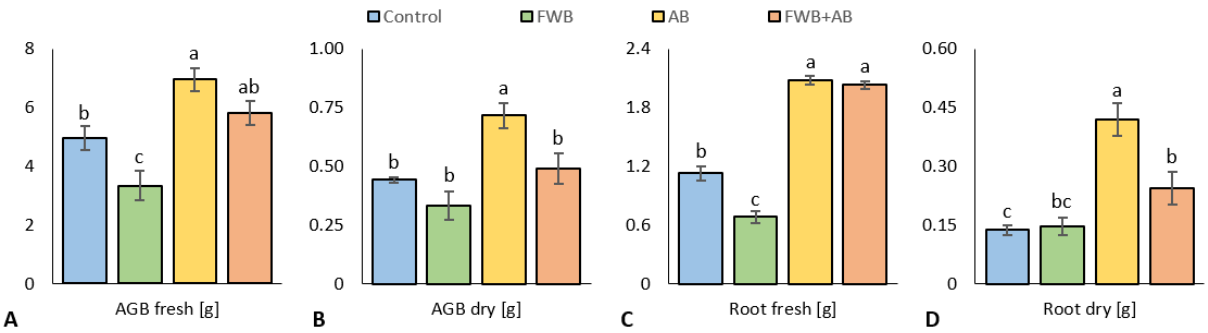

Figure 1. The responses of plant growth characteristics under the applied amendments on (**A**) above ground fresh biomass; (**B**) above ground dry biomass; (**C**) root fresh weight; and (**D**) root dry weight). Different lowercase letters indicate statistically significant differences within columns. $n = 3$.

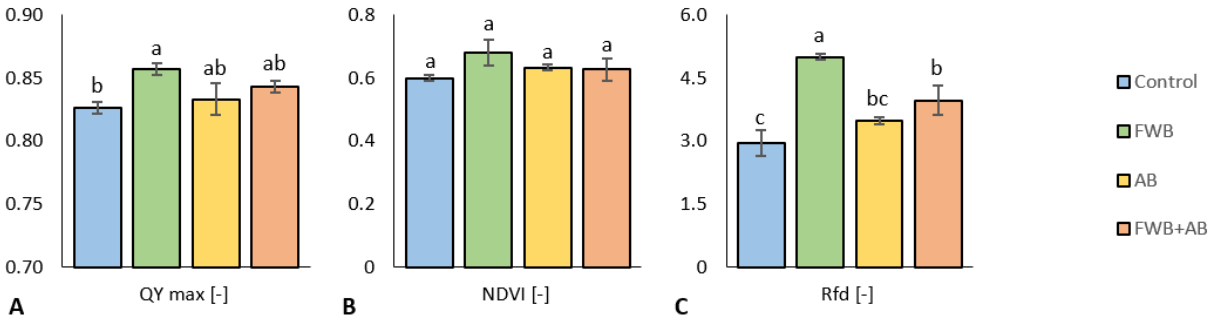

Figure 2. The responses of plant chlorophyll fluorescence characteristics under the applied amendments on (**A**) quantum yield of electron transport chain; (**B**) normalized difference vegetation index; and (**C**) chlorophyll fluorescence decrease ratio. Different lowercase letters indicate statistically significant differences within columns. $n = 3$.

The results regarding chlorophyll fluorescence parameters showed mild effects of applied amendments (Figure 2A–C). Specifically, the application of FWB found superior to other amendments, significantly ($p < 0.05$) enhancing the quantum yield of the electron transport of the PSII (QY-max) and Rfd, as compared to control, while remaining statistically non-significant in case of the spectral reflectance of chlorophyll pigments (NDVI) (Figure 2A–C). In all cases, this was followed by the combined FWB + AB application.

3.2. Soil pH and Extracellular Enzyme Activities

All the applied amendments reduced the soil pH. The maximum reduction was recorded for the combined application of FWB + AB (Figure 3A), which was also statistically significant as compared to control. This was followed by FWB. Dehydrogenase activity (DHA) was significantly ($p < 0.05$) enhanced by the application of FWB as compared to control and other amendments (Figure 3B). The combined use of FWB and AB, however, yielded highest β-glucosidase (GLU), which remained statistically non-significant as compared to control (Figure 3C). The sole application of AB and FWB, however, reduced the GLU activity as compared to control. Moreover, the same treatments resulted in reduced phosphatase (PHOS) and urease (URE) activities as compared to control; however, the combined application of AB + FWB resulted in similar values as control (Figure 3D,E). The N-acetyl-β-D-glucosaminidase (NAG) activity was significantly reduced under the applied amendments as compared to control (Figure 3F). The highest significant decrease in this case was recorded under the applied FWB.

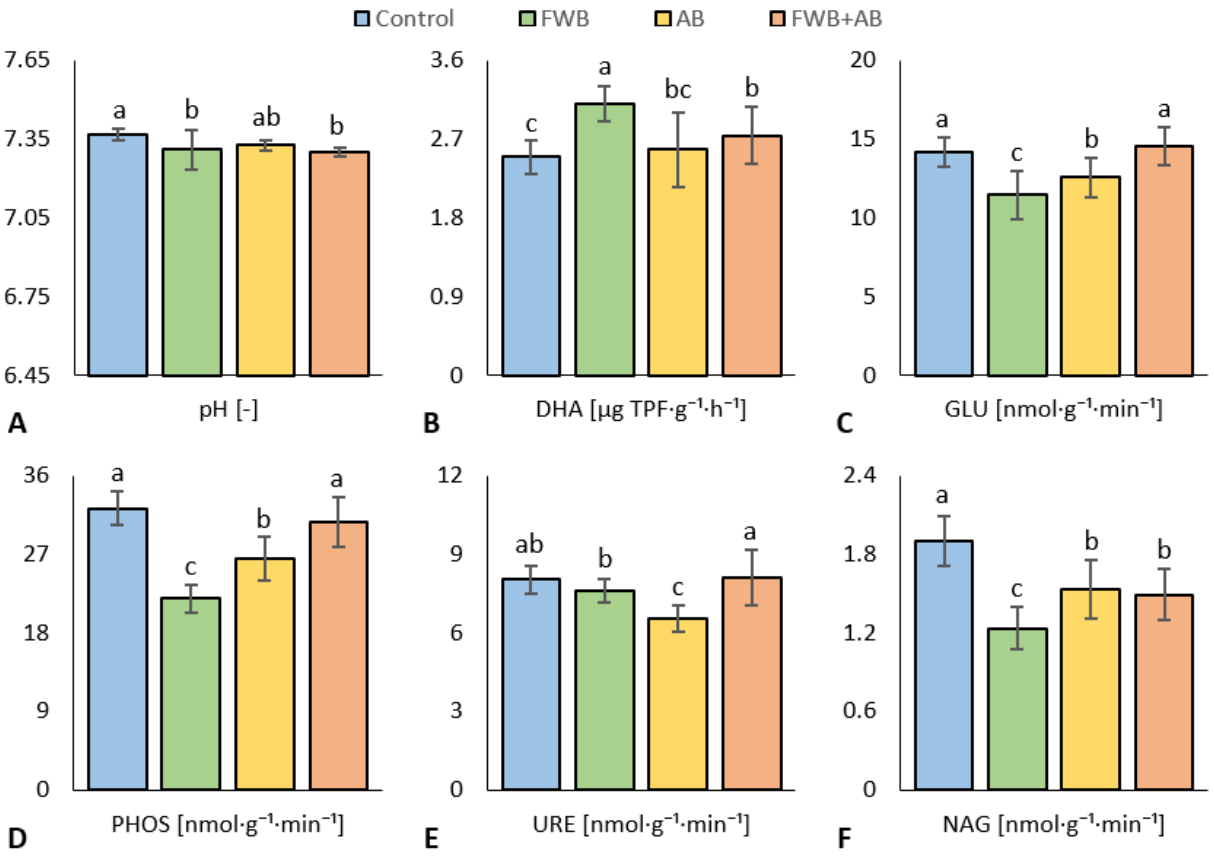

Figure 3. The responses of soil pH (**A**) and extracellular enzyme activities (**B**–**F**) under the applied amendments. Different lowercase letters indicate statistically significant differences within columns. $n = 3$.

3.3. Soil Basal and Substrate-Induced Respirations

The application of AB significantly improved the soil basal respiration (BR) as compared to control and other amendments (Figure 4A). The glucose-induced respiration (Glu-IR) was significantly reduced by AB and FWB, while the effect of the two combined (AB + FWB) remained statistically non-significant as compared to control (Figure 4B). Similarly, all the applied amendments reduced the Arg-IR as compared to control, whereas the application of AB enhanced the Ala-IR, but remained non-significant relative to control (Figure 4D).

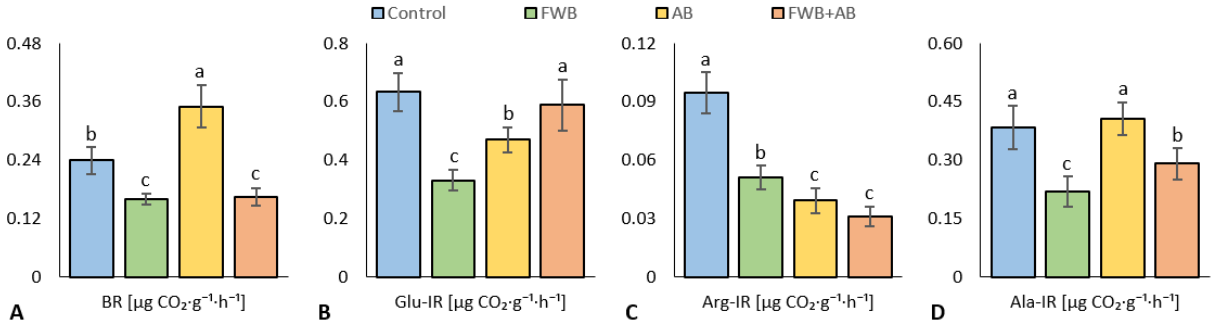

Figure 4. The responses of soil basal (**A**) and substrate-induced respiration (**B**–**D**) under the applied amendments. Different lowercase letters indicate statistically significant differences within columns. $n = 3$.

3.4. Results from Principal Component Analysis

The score and loading plots of observed parameters and applied amendments are been shown in Figure 5. Of the extracted components, the principal component 1 contributed 51.6% and principal component 2 contributed 26.4% of the total variations in the dataset. In case of PC1, the following properties have the large contributions: Rfd (10.33%), QY_max (9.89%), Ala-IR (9.53%), AGB-fresh (8.27%), NDVI (7.94%), Root-fresh (7.15%), NAG (6.29%), Glu-IR (6.10%), AGB-dry (6.08%) and BR (5.98%). In case of PC2, the following properties have the large contributions: Root-dry (16.27%), URE (15.46%), AGB-dry (9.58%), BR (8.04%), GLU (7.50%), Glu-IR (7.12%), Phos (6.62%) and Arg-IR (6.10%). Other soil properties do not have large contribution in accounting for the variability in a first two principal component. All the applied amendments successfully distributed under these two components. This distribution of amendments gave clear indication of the effects of treatments on studied parameters. The first component with which the parameters were positively correlated collected (in descending order): Ala-IR (r = 0.91, $p < 0.001$), AGB-fresh (r = 0.85, $p < 0.001$), Root-fresh (r = 0.79, $p < 0.001$), NAG (r = 0.74, $p < 0.001$), Glu-IR (r = 0.73, $p < 0.001$), AGB-dry (r = 0.73, $p < 0.001$), BR (r = 0.72, $p < 0.001$), PHOS (r = 0.69, $p < 0.001$), pH (r = 0.58, $p < 0.001$), GLU (r = 0.50, $p < 0.001$), Root-dry (r = 0.49, $p < 0.001$) and Arg-IR (r = 0.45, $p < 0.001$), while a significantly negative correlation of PC1 parameters was recorded (in ascending order) in case of DHA (r = −0.68, $p < 0.001$), NDVI (r = −0. 83, $p < 0.001$), QY-max (r = −0.93, $p < 0.001$) and Rfd (r= −0.95, $p < 0.001$). In case of the second component, positive correlation was observed (in descending order) with Root-dry (r = 0.86, $p < 0.001$), AGB-dry (r = 0.66, $p < 0.001$), BR (r = 0.60, $p < 0.001$), Root-fresh (r = 0.47, $p < 0.001$), AGB-fresh (r = 0.47, $p < 0.001$), NDVI (r = 0.43, $p < 0.001$) and Rfd (r = 0.24, $p < 0.001$). Negative correlations were observed in case of the following properties (in ascending order): DHA (r = −0.19, $p < 0.001$), pH (r = −0.22, $p < 0.001$), NAG (r = −0.44, $p < 0.001$), Arg-IR (r = −0.52, $p < 0.001$), PHOS (r = −0.55, $p < 0.001$), Glu-IR (r = −0.57, $p < 0.001$), GLU (r = −0.58, $p < 0.001$) and URE (r = −0.83, $p < 0.001$) (Figure 5).

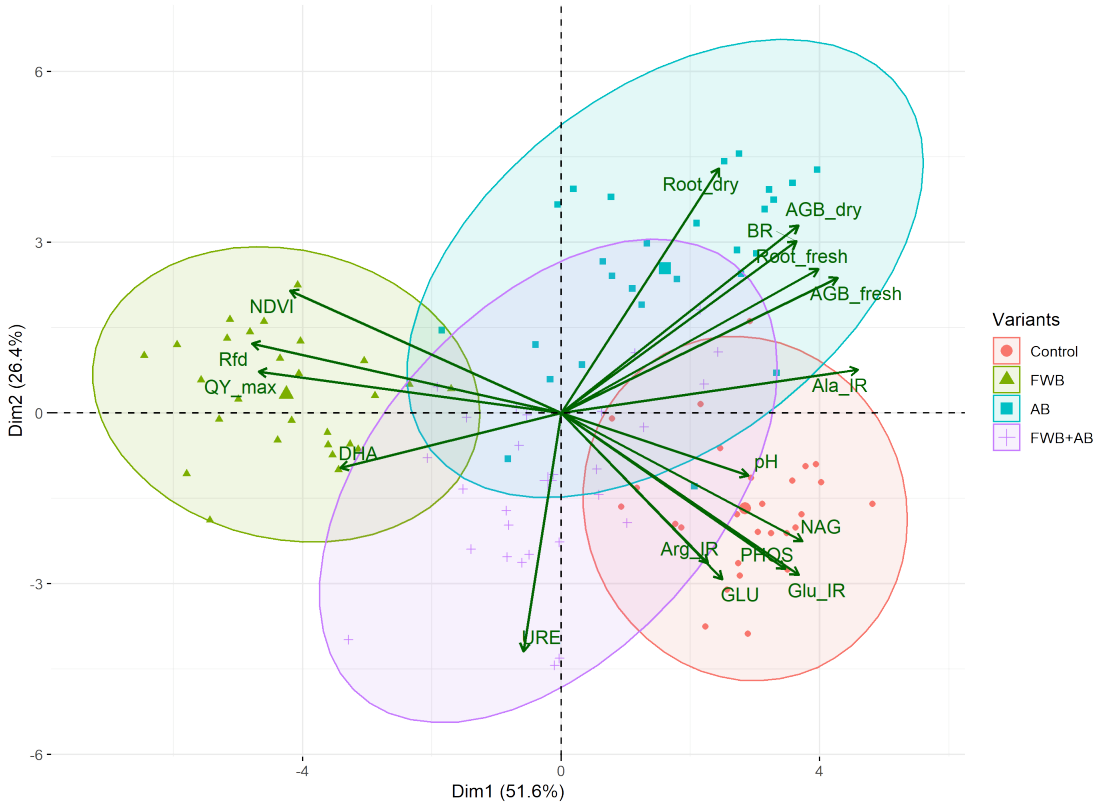

Figure 5. Principal component analysis of the observed parameters and applied amendments. Abbreviations

are: Root-dry, root dry biomass; Root-fresh, root fresh biomass; AGB-dry, above ground dry biomass; AGB-fresh, above ground fresh biomass; pH, soil reaction ($CaCl_2$); DHA, dehydrogenase activity; Glu, β—glucosidase activity; PHOS, phosphatase activity; NAG, N-acetyl-β-D-glucosaminidase; URE, urease activity; BR, basal respiration; Glu-IR, D-glucose-induced respiration; Ala-IR, L-alanin-induced respiration, Arg-IR, L-arginine-induced respiration; QY-max, quantum yield of photosystem II; NDVI, normalized vegetation index.

4. Discussion

Chemical fertilizers have been recognized to improve plant nutrition and crop production, but are often associated with negative environmental consequences. Therefore, alternative strategies are required to boost crop production in an environmentally safe way. In the meantime, the application of organic amendments obtained by the conversion of wastes to the soil have shown the ability to enhance soil quality, sustaining crop production and the overall environment [3,9]. In the present study, we utilized food and agricultural waste-derived biochars as potential amendments for enhancing soil fertility and crop growth. Biochar application in agricultural soils has been seen as a win-win strategy to improve crop growth and yield formation [5,40]. In the present study, the application of AB, either alone or combined with FWB, improved the observed plant growth characteristics, with the significantly highest effects being observed under AB application (Figure 1A–D). Our results are in line with Tian et al. [41], who observed enhanced plant biomass and leaf area under the application of green waste biochar. This enhancement in growth characteristics in the present study could be attributed to the higher nutrient retention and improvement in the soil properties after biochar application. Previously, a number of reports have shown that biochar application results in the improvement of soil physical properties, such as soil porosity, water holding capacity and aggregate stability [41–43], chemical properties including pH modification, soil nutrients availability and enhanced cation exchange capacity [44,45], microbial soil health indicators related to soil enzymes and microbial respiration [46,47]. Biochar application might have resulted in the modifications of these properties, which provided better growth conditions for plants and eventually enhanced the crop growth and development in the present study (Figure 1 A–D). This was further supported by positive correlations observed for plant growth characteristics and the nutrient mineralizing enzymes (Glu, PHOS, NAG (Figure 5)). However, it must be acknowledged that the biochar effects on plant growth enhancement are mostly indirect [48] and enhanced plant growth and development is the consequence of direct effects of biochar on improvement of soil physico-chemical properties and microbial communities [49]. The increased plant biomass could be attributed to enhanced plant available nutrients and soil moisture supply [48]. Moreover, the direct stimulation of microbial activity, as indicated by higher respiration activities (Figure 4) under biochar addition, might explain the higher nutrient mineralization and easy access to plants, which eventually results in higher plant biomass. This fact was further clarified by the principal component analysis that showed a positive relationship between plant biomass and microbial respiration (Figure 5). Furthermore, there were differences among the applied AB and FWB on their responses to plant biomass and physiological parameters (Figures 1 and 2). Such differences are directly related to the large variations in composition of applied biochars (Table 1), which after application might have differently modified the soil environment.

The activity of the photosystem determines the photochemical reactions, which are an important component influencing photosynthesis [50]. The reduction in photosynthetic pigments can inhibit the light captured by plants, and hence, reduced photosynthesis [51]. The intensity of light absorption and its utilization can be reflected by measuring chlorophyll fluorescence parameters [52]. Moreover, biochar application has shown to enhance physiological responses of crops [53]. We found a moderate effect of applied biochars on the observed photosynthetic machinery (Figure 2A–C). Specifically, we noted a relatively higher quantum yield of the electron transport of the PSII (QY-max), which shows the capacity of PSII for biochemical reactions and the spectral reflectance of chlorophyll pigments

(hereinafter NDVI), which represent an important indicator of chlorophyll formation. The enhancement of these photosynthesis indicators might be related to enhanced soil nutrients, mainly nitrogen under applied biochar [54].

Soil enzymes are an important indicator of microbial processes involved in nutrient cycling in agro-ecosystems [55]. We found marked differences in the responses of soil enzymes, mainly those related to C, N and P cycling under the applied amendments (Figure 3). Dehydrogenase activity (DHA) in the soil represent the C mineralization potential [10]. We found higher DHA under the applied FWB. This might be due to the presence of a higher proportion of easily mineralizable carbon [42,56]. However, there were differences observed for DHA between AB and FWB application. This could be explained by the fact that FWB showed the higher TC (81.25%), but lower amount of recalcitrant organic carbon (0.28%, Table 1), and hence, a major part of TC might have been mineralized, as depicted by the higher DHA under FWB application (Figure 3). Moreover, the strong loading of DHA in AB (Figure 5) further supported these findings. The enhancement of DHA under applied biochars is in reasonable agreement with findings of Irmak et al. [57], who reported enhanced DHA under the application of biochar and manures. Previously, a number of studies have shown that the inconsistency exists on the responses of soil enzymes under biochar application [58,59]. The activities of other soil enzymes (GLU, PHOS, URE) in the present study remained relatively similar under the applied FWB + AB as compared to control, with a slight decrease in the activity of NAG. This shows that the observed soil enzymes were limited by nutrient constraints. However, we found a decreased tendency of all the enzymes, except DHA under the sole application of either FWB or AB (Figure 3). Our results contrast with the findings of Bailey et al. [60] and Ameloot et al. [61], who found enhanced activities of soil enzymes under the application of biochar produced at lower temperatures (350 °C). The decreased activities of nutrient cycling enzymes in the present study might be due to the reason that the biochar used in our study was prepared at 650 °C, which might have cast negative effects on microbial enzyme synthesis in the test soil. These results are corroborated with the findings of Ameloot et al. [62], who found decreased activities of soil enzymes under the application of biochar produced at 700 °C, which is a similar pattern observed in the present study. However, some other researchers observed no effects of biochar application on the soil enzyme activities [63]. This suggests the variable effects of applied biochars, which further depend on type of the biochar, pyrolysis conditions and the modification effects of biochar on the soil after application. Moreover, the decreased microbial extracellular enzymes might also be associated with the release of toxic substances from biochars, such as polyaromatic hydrocarbons, which might have affected microbial biomass in the test soil resulting in reduced production of enzymes [64]. This demands for the future studies on the quantification of toxic substances present in biochar and their aftereffects on soil properties.

The soil respiration is an indication of the indigenous microbial activity in the soil and is influenced by the management practices. The applied biochar in the present study differently affected the soil basal (BR) as well as substrate-induced respiration (SIR) (Figure 4). In particular, we found significantly increased basal respiration under the applied AB, which was further supported by the strong loading by PCA (Figure 5). The increased respiration under biochar might be due to the fact that biochar might have resulted in enhanced oxidation of native soil organic matter and enhanced microbial population [65,66]. This can be explained by the fact that biochar application enhances the microbial reproduction rate and activity, which results in enhanced oxidation of soil organic matter [48], and hence, showing higher respiration rates (Figure 4). The substrate-induced respiration is an indicator of the total active microbial biomass in the soil [67,68]. All the substrate-induced respirations were generally decreased with all biochar types in the present study. The decrease observed in substrate-induced respiration might be associated with the fact that nearly 20% of soluble compounds in biochar can be respired by microbes, while the rest undergo precipitation as aromatic compounds, which are resistant to degradation, and hence, results in reduced microbial respiration [69]. Conversely, some other researchers

have advocated the positive effects of biochar on biological soil health indicators through providing microbial microsites, providing nutrients to microbes and providing physical barrier from outside predators [69–71]. Our results are in line with previously published work by Holatko et al. [72], reporting decreased substrate-induced respirations under the application of biochar either alone or combined with humic substances. The authors described this decrement to be related with the negative priming effect of biochar on microbial respiration. In addition, irrespective of its highly stable nature, biochar after application to soil undergoes physico-chemical and biological alterations in its chemical structure [73,74], which hinders the quantitative estimation of its effects on soil properties [75]. This necessitates the exploration of possible negative effects of biochar application in agricultural soils [76]. Moreover, biochar application has shown to sorb certain chemical and organic substances with inhibitory effects on soil enzymes or their substrates through the blocking of biochemical reaction sites [77,78]. This might be another reason of reduced microbial respiration under combined FWB + AB application. The findings on reduced BR under a combined application of FWB + AB are in line with the findings of [79] Dempster et al. (2012), who reported reduced basal soil respiration under corn stover biochar. Singh et al. (2019) [80] also reported that the application of biochar results in the clogging of soil micropores and limits the water holding capacity and nutrient deficiency. This would have ultimately negatively affected the microbial activity resulting in reduced BR and Arg-IR under combined FWB + AB treatment in the present study (Figure 4A,C). Furthermore, the differences observed in basal (BR) and substrate-induced respiration (Glu-IR, Arg-IR and Ala-IR) in the present study (Figure 4A–D) could be due to the differences in chemistry and type of organic carbon (OC) between the applied biochars [81], FWB and AB in the present case. In addition, there were large differences in the physico-chemical composition of the applied AB and FWB in the present study (Table 1), which could be the chief reason for the comparable responses observed for two biochars. The clear distribution of AB and FWB alone and in combination (AB + FWB), as indicated by PCA (Figure 5), further support the obtained differential responses on the observed parameters.

5. Conclusions

Bioconversion of organic wastes (such as agricultural and food wastes) to biochar is a viable option not only to mitigate the magnitude of wastes but also the utilization of biochar as a soil amendment. In the present study, food and agricultural waste-derived biochars were used as potential soil amendments to check their efficacy on soil quality attributes. We found differential responses of plant growth, physiological responses and soil health indicators, specifically soil extracellular enzymes and microbial respirations (both basal as well as different substrate induced). In particular, the findings of this study demonstrated that the applied agricultural waste-derived biochar (AB) improved growth parameters (above and below ground biomass) of lettuce, while the food waste-derived biochar (FWB) improved the chlorophyll fluorescence parameters of lettuce crop. Moreover, all the studied enzymes related to C, N and P cycling showed variable responses to the applied amendments. The dehydrogenase activity was most enhanced by FWB as compared to others, while PHOS activity was improved with the combined application of both biochars (FWB + AB). All other enzymes showed decreased activities under applied biochars, suggesting biochar-specific effects on the observed enzymes. We further noted that all the substrate-induced respirations were decreased under the applied biochars, showing negative priming effects of biochar on microbial activity. These findings suggest that the applied biochars differ in their mechanisms of action, probably due to their initial composition and C and N transformation rates. Therefore, for most of the parameters, there were preferential responses between the applied biochars, depending on the feedstock during the experimental duration. However, further studies are required to unveil the differences and compare the surface chemistry and functional group characterization to better understand the mechanisms by which biochar affects plant and soil characteristics.

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