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Response of β-glucosidase enzyme activity of soil to biochar applications in a crop rotation at Blacksea agroecosystem

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Abstract

The use of biochar has emerged a potentially effective approach to improve soil function and promote crop performance. However, the specific impact of biochar on β-glucosidase enzyme activity (BGA) within crop rotation systems in the Black Sea agroecosystem requires further investigation. This study was conducted to determine the effects of rice husk biochar (RHB) and poultry manure biochar (PMB) on BGA in soils. Six biochar doses (0-control, 10, 20, 30, 40 and 50 t ha^{-1}) were applied at the beginning of two wheat-cabbage red pepper rotation periods. The mean BGA at second rotation (73.71 μg pNP g^{-1}) was significantly lower compared to the BGA of the first period (93.39 μg pNP g^{-1}). The BGA value in control (94.51 μg pNP g^{-1}) decreased with increasing biochar application doses (76.05 μg pNP g^{-1}, 50 t ha^{-1}) treatment. The mean BGA value in PMB treatment was slightly higher than that of RHB, but it was not statistically different between two biochar types. However, the decrease in BGA value (25.0%) in the highest RHB dose compared to control was more than two-fold compared to the decrease in PMB application (12.1%). The difference in carbon/nitrogen ratio between RHB and PMB can be attributed to the variation in BGA values observed at the application of same biochar doses. The decrease in BGA over the course of the two rotation cycles implies that biochar may have a long-term influence on soil carbon cycling.

Keywords: Biochar, Biochar type, Poultry manure, Rice husk, Crop Rotation, β-glucosidase.

Introduction

Biochar, a carbonaceous substance produced through the thermal processing of biomass at high temperatures, has gained significant interest as a soil amendment for enhancing soil fertility and mitigating atmospheric carbon levels (Günal et al., 2019; Abhishek et al., 2022). An abundance of studies has been attempted to determine the impacts of biochar on the physical, chemical, and biological properties of soils. A recent comprehensive review analyzing 26 global meta-analyses (Schmidt et al., 2022; Long and Dung, 2023) provides evidence that biochar application has a positive overall impact on various agronomic parameters, including crop yield, root biomass, water use efficiency, microbial activity, soil organic carbon, and greenhouse gas emissions. The findings of this review provide strong evidence supporting the overall positive impact of biochar on the aforementioned parameters. However, the impact of incorporating biochar into soil enzyme depends on factors such as the type of feedstock and the pyrolysis temperature employed during biochar production, as well as the variability in soil texture (Feng et al., 2023). The meta-analysis revealed that the use of biochar in research examining soil organic carbon (SOC) has consistently resulted in a significant increase in SOC levels, irrespective of variations in feedstock, pyrolysis temperature, and soil types. In particular, the application of wood and herbs derived biochars led to the highest increase in SOC compared to the manure and agricultural residues. In general, biochars produced at high temperatures have a greater positive impact

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on SOC as compared to the biochars produced at lower and medium temperatures. Furthermore, the addition of biochar had a more pronounced effect on SOC in loamy and clay soils that the sandy soils (Gross et al., 2021).

The effect of biochar applications on soil enzymes has garnered significant attention, as enzymes play a vital role in nutrient cycling and organic matter decomposition in soil ecosystems (Wojewódzki et al., 2022; Rahmanian et al., 2023). The enzymes are proteins that catalyze chemical reactions and are essential for the decomposition and mineralization of organic materials into nutrients that can be used by plants and other organisms. Soil enzymes are produced by soil microorganisms, and they are influenced by a variety of factors, including the type of soil, the amount of organic matter, the moisture content, and the temperature (Tabatabai, 1994; Burns et al., 2013).

The Black Sea agroecosystem consists of dynamic agricultural landscapes characterized by a prevalent practice of crop rotation (Gülser et al., 2021). Consequently, it is crucial to investigate the impact of biochar applications on the activity of specific soil enzymes. The β-glucosidase enzyme is important in the breakdown of cellulose, a major component of plants. Therefore, the activity of β-glucosidase enzyme is commonly used an indicator of the soil quality and high β-glucosidase e activity indicates that the soil has a high quality of soil organic matter (Adetunji et al., 2020; Acir et al., 2022). Previous studies have clearly revealed that the biochar is a promising material for improving soil quality and crop production (Günal and Erdem, 2021; Murtaza et al., 2022; Wang et al., 2023). However, the specific effects of biochar on β-glucosidase enzyme activity in crop rotation systems in the Black Sea region are not well understood. This study investigated the effects of rice husk and poultry manure biochar applications on β-glucosidase enzyme activity in soils under a wheat-pepper-red cabbage rotation. An in-depth investigation of the complex relationship between biochar, soil enzymes, and crop rotation practices offers significant knowledge and understanding regarding the improvement of soil management techniques to achieve sustainable and productive agricultural solutions within this ecologically diverse region. The ramifications of this research extend beyond the Black Sea region and have relevance for agricultural operations in agroecosystems globally. This will contribute to our understanding of the potential utilization of biochar to improve soil quality and increase crop yields.

Material and Methods

Study area

Field experiments were conducted in Bafra experimental Station Research and Application fields of the Black Sea Agricultural Research Institute in Samsun province, Turkey. Bafra Plain is located between 400 26' and 410 45' north latitude and 350 30'' and 360 11' east longitude. The research area has warm and dry summers and mild and rainy in winters, displaying the characteristic climatic attributes commonly observed in the Black Sea Region. The precipitation in the study area occurs mainly throughout the fall and winter seasons, whilst the summer and spring months have low precipitation.

The initial crop rotation commenced with the cultivation of winter wheat from November 2014 to July 2015, succeeded by the planting of red cabbage from July 2015 to February 2016, and concluded with the production of paste peppers from May 2016 to September 2016. Similarly, the second cycle of crop rotation commenced with the cultivation of winter wheat from November 2016 to July 2017. This was followed by the planting of red cabbage from July 2017 to January 2018, and ended with the cultivation of paste pepper from May 2018 to September 2018. The experimental design comprised the application of rice husk and poultry manure biochar in the main plots, whereas the sub plots consisted of six different biochar doses ranging from 0 to 50 tons per hectare (0, 10, 20, 30, 40 and 50 t ha^{-1}). At the beginning of the experiment, biochars were applied to the soil surface and subsequently incorporated to a depth of 15 cm in the experimental field using a disc harrow. were in the sub plots. The layout of the experiment was split plots in randomized blocks with 3 replications. The experiments were continued for 4 years. The experiments were conducted over a period of four years. The dimensions of the plots were 3.6 meters by 5.6 meters.

Details of the Crop Rotation and Agricultural Practices

In the experiment, a crop rotation system consisting of winter wheat, red cabbage, and paste red pepper, which is commonly used in the Bafra plain, was applied. Bread wheat (Canik 2003), red cabbage (Caballero F1), and paste red pepper (Yalova 28) were used in the crop rotation. This study was carried out over two crop rotations. The Canik 2003 wheat variety is a moderately late-maturing type with white spikes, long awns, and red-hard seeds. The Caballero F1 red cabbage variety has a maturation period of 130-140 days. Its fruit head structure is oval cylindrical with a waxy layer. The Yalova 28 red pepper variety is a dwarf-looking plant with low branching, abundant foliage, and is moderately early maturing.

The soil analysis results of the experimental field indicated that P and K concentrations were at sufficient levels; therefore, only ammonium sulfate ($(NH_4)_2.SO_4$ - 21% N, 24% S) was used as the nitrogen source for all the crops grown in the rotation.

Characteristics of Biochars

Biochars were produced using poultry manure received from poultry farms in the region and processing waste from intensively planted paddy rice in the Central Black Sea Region. The rice husk was obtained from a rice processing facility and subjected to pyrolysis at a temperature of 400 ºC. The poultry manure biochar utilized in this study originated from a commercial power generation facility. The pyrolysis process of poultry waste was carried out at 400 ºC.

Some chemical properties of biochars used in the experiments and soil physical and chemical properties of experimental field are given in Table 1. The pH and electrical conductivity (EC) values of biochars were measured in a solution containing a mixture of biochar and water at a ratio of 1:5 using a pH-EC meter (Thomas, 1996). Total carbon and total nitrogen contents were determined using an Elemental Analyzer instrument (EA 3000 Eurovector SpA, Milan, Italy).

Table 1. Some properties of the biochars and soil used in the experiment (Birol, 2020)

Properties	Soil	Rice Husk Biochar	Poultry Manure Biochar
pH	7.74	10.20	11.57
EC[1] (dS m^{-1})	0.35	3.20	4.28
Organic Matter (%)	2.01	N/A[2]	N/A[2]
Sand (%)	17.7	N/A[2]	N/A[2]
Clay (%)	47.5	N/A[2]	N/A[2]
Silt (%)	34.8	N/A[2]	N/A[2]
Texture Class	Clay	N/A[2]	N/A[2]
Total N (%)	0.14	0.45	0.88
Total C (%)	N/A[2]	61.7	58.8
C:N	N/A[2]	137.1	66.8

[1]EC: Electrical Conductivity; [2]N/A = Not Applicable

Soil Sampling and Analysis

Soil samples were collected from a depth of 0-20 cm in each plot before to the application of biochar, as well as after the completion of the first and second rotation periods. Particle size distribution was determined using the hydrometer method in a sedimentation cylinder; with sodium hexametaphosphate as the dispersing agent (Gee and Bouder, 1986). The method employed to determine β-glucosidase enzyme activity (BGA) in soil samples was based on the protocol established by Eivazi and Tabatabai (1988). In the method, a 4 ml solution of hydroxyethyl aminomethane buffer at a pH of 12 and a 0.05 M 1 ml p-nitrophenyl β-Glucopyranoside solution were added to the 1 g sample. The samples were incubated at 37ºC for 60 minutes. The concentration of p-nitrophenol was quantified at 410 nm wavelength using a spectrophotometer and the BGA was reported as micrograms of p-nitrophenol per gram of dry sample. Soil reaction (pH) (Thomas, 1996) and electrical conductivity (EC) (Rhoades, 1996), were measured in 1:2 soil–water suspensions. Soil organic matter was analyzed by using the Walkley–Black dichromate oxidation procedure (Walkley and Black, 1934).

Data Analysis

The normality test of the data showed a normal distribution. The Levene's test was performed to confirm the equality of variances of BGA between replicated measurements. An analysis of variance (ANOVA) was conducted to assess the impact of rotation period, biochar type and biochar doses on BGA. The mean values of BGA in various treatments were grouped using the least significant difference test (LSD) at a significance threshold of 0.05. Correlation analysis was done for the determination of the relationships between some properties of soils and β-glucosidase enzyme activity. The statistical analyses were conducted using the SPSS (SPSS Inc., Chicago, IL, USA) statistical software (version 26.0, SPSS Inc., Chicago, IL, USA).

Results and Discussion

The biochars utilized in this study are derived from plant (rice husk) and animal (poultry manure) feedstocks that are readily accessible in the region and frequently used to examine the effects of biochar application on soil characteristics and plant growth. The chemical and physical properties of biochars indicated notable differences (Table 1). Poultry manure biochar (PMB) had higher pH and EC values, total nitrogen, available phosphorus, potassium, DTPA extractable iron, copper, zinc, and manganese, while total carbon content of rice husk biochar (RHB) is higher compared to the PMB. Rich nutrient content of PMB is consistent with the previous reports indicating that the plant-based biochars can be considered a good soil conditioner due the low content of extractable nutrients compared to the biochars produced from manure, which is rich in nutrients and may be used as a soil fertilizer and conditioner (Uchimiya et al., 2010; Clemente et al., 2018; Jaaf et al., 2022). The soil of the experimental field had a silty clay loam texture, slightly alkaline pH, low in salinity, calcareous, rich in available P, K and micronutrients. Mean sand, clay and silt contents of experimental soils were 17.7, 47.5 and 64.8%, respectively. Average soil pH was 7.74, EC was 0.353 dS m^{-1}; lime and organic matter contents were 7.45% and 2.01%. Average nitrogen, phosphorus and potassium contents of the experimental soils were 0.14%, 21.1 mg kg^{-1} and 267 mg kg^{-1}, respectively (Table 1).

The results for individual effects of rotation period, biochar type and biochar application rate on BGA, two- and three-way interactions among individual factors have been presented separately.

Effects of individual factors on β-glucosidase enzyme activity of soil

β-glucosidase enzyme activity (BGA) is an indicator of biological activity and is one of the most widely used indicators to determine the effect of land use changes or application of agricultural practices on organic matter content of soils (Acir et al., 2022). The effects of rotation period and biochar application dose on BGA were statistically significant (p<0.01), while biochar type regardless of biochar dose and rotation period had no effect on the activity of β-glucosidase (Table 2). Like our findings, Zhang et al. (2014) reported a notable impact of 2-year corn-soybean rotation on BGA, that was significantly higher (p<0.05) in reduced tillage soils under monoculture corn compared to the activity under corn-soybean rotation. The difference was attributed to the changes in the structure of microbial communities associated with plants used in the crop rotation system.

Table 2. The effects of individual factors and interactions on β-glucosidase enzyme activities of soils

Source	DF	Sum of Square	Mean Square	F	P
Rotation period (RP)	1	6971.640	6971.640	390.974	0.003**
Biochar type (BT)	1	21.813	21.813	0.292	ns
Biochar dose (BD)	5	2650.806	530.161	17.173	<0.001**
RP x BT	1	48.626	48.626	0.650	ns
RP x BD	5	177.606	35.521	1.151	ns
BT x BD	5	470.072	94.014	3.045	0.020*
RP*BT*BD	5	79.033	15.807	0.512	ns

**: significant at p<0.01, **: significant at p<0.01, ns: not significant; DF: Degrees of freedom

The variation in organic matter incorporated into the soil with different crops grown during crop rotation has resulted in a differentiation of the BGA in the soil (Figure 1). In the first rotation, organic matter content of soils consistently increased with the increased application doses of rice husk biochar. Although the increase was not statistically significant, the organic matter content in the control plots was 2.09%, rising to 2.34% at the highest biochar dose (Figure 1). A similar trend was observed with poultry manure applications. The organic matter content, which was 2.02% in the control plot, increased to 2.33% at the highest biochar dose. In the second rotation period, a similar pattern was observed with both types of biochar, where higher biochar doses corresponded to increased organic matter content in the soils, similar to the first period. The mean BGA at the end of the first rotation period (93.39 μg pNP g^{-1}) was significantly higher than the BGA value measured at the end of second rotation (73.71 μg pNP g^{-1}) (Figure 1). The decline in BGA value as biochar ages is a significant factor to consider when applying biochar to soils. This observation aligns with the findings reported in the studies conducted by Chen et al. (2016) and Yadav et al. (2019). Yadav et al. (2019) showed a decrease of 60% and 39% in the BGA in aged and fresh biochar soil mixture, respectively, compared to the control on the 90^{th} day. Additionally, Chen et al. (2016) observed a decrease of 20% in BGA after 18 months of biochar application in soil, followed by a subsequent increase of 56%. According to Yadav et al. (2019), the decline in BGA with aging of biochar can be related to the sorption of the enzyme onto the surfaces of the biochar particles. Although biochars can sometimes stabilize enzymes and enhance their activity, they more frequently reduce enzyme activity due to substrate sorption or direct interactions with biochar's hydrophobic and surface properties. The sorption process involves various interactions, such as electrostatic, pH-controlled, hydrophobic, and physical interactions, which can either preserve the enzyme's structure and function or alter the active site, reducing activity (Swaine et al., 2013; Foster et al., 2018).

The correlation analysis for rice husk biochar and poultry manure biochar treatments reveals significant insights into the interplay between some of soil properties and BGA (Table 3). The variation in organic matter incorporated into the soil through different crops during crop rotation and biochar has notably impacted the BGA, as illustrated in Figure 1. For rice husk biochar, the results show a positive correlation between BGA and soil pH (r=0.202), indicating that an increase in soil pH may enhance enzyme activity. However, there is a very weak negative correlation with electrical conductivity (EC) (r = -0.006), suggesting that EC has little to no impact on BGA in this context. The significant negative correlation between BGA and organic matter content (r=-0.443) suggests that higher organic matter levels might suppress enzyme activity (Table 3). The negative correlation between organic matter and BGA for both biochar types indicates that while biochar applications increase soil organic matter, they may simultaneously reduce enzyme activity. This suppression could be due to the sorption of enzymes onto the biochar surface or changes in soil microbial activity dynamics (Foster et al., 2018). Similarly, in soils treated with poultry manure biochar, BGA shows a positive correlation with EC (r =0.357) and pH (r=0.094). These positive correlations suggest that both higher EC and pH levels might promote enzyme activity. Nonetheless, BGA is negatively correlated with organic matter content (r =-0.273), indicating a potential inhibitory effect of increased organic matter on enzyme activity (Table 3). This inhibitory effect may result from the biochar's influence on organic matter dynamics or direct enzyme adsorption, reducing the bioavailability of the enzymes.

Table 3. The results of correlation analysis between β-glucosidase enzyme activity (BGA) and some of soil properties

	Rice Husk Biochar				Poultry Manure Biochar			
	pH	EC	OM	BGA[1]	pH	EC	OM	BGA[1]
pH	1				1			
EC	-0.018	1			0.163	1		
OM	-0.128	0.389*	1		0.300	-0.017	1	
BGA	0.202	-0.006	-0.443*	1	0.094	0.357*	-0.273	1

*P<0.05; [1] BGA: β-glucosidase enzyme activity. EC: Electrical Conductivity, OM: Organic Matter

Despite a C/N ratio of 66.8 in poultry manure and 137.1 in rice husk biochar (Table 1), the mean BGA in rice husk biochar added soil (84.10 μg pNP g^{-1}) was slightly higher compared to the BGA in poultry manure biochar added soil (83.00 μg pNP g^{-1}) (Figure 1). However, the difference in BGA between biochar types was not statistically significant (p=0.618) (Table 2). In contrast to our findings, Günal et al. (2018) reported a substantial variation (p<0.01) in the activity of the BGA between different types of biochar. The application of biochar derived from bean harvest residue resulted in the highest BGA (20.17 μg pNP g^{-1}), while the rice husk biochar application yielded the lowest BGA activity (18.54 μg pNP g^{-1}). The apparent similarity in enzyme activity among three distinct solid phases, namely pine biochar, grass biochar, and agricultural soil, has been attributed to the presence of negative surface charges on both the solid phases and the enzymes, despite the notable difference in their respective surface areas (Foster et al., 2018).

The highest mean BGA was recorded in control (94.51 μg pNP g^{-1}), while the BGA value decreased with increasing biochar application doses and the lowest average BGA value was recorded in BD5 (76.05 μg pNP g^{-1}) treatment (Figure 1). Inconsistent results have been reported regarding the effects of biochar application on BGA values of soils. Due to the complexity of the interactions between enzymes and solid surfaces, the effects of biochar applications on enzyme activities in soils cannot be sufficiently explained. The changes in soil structure and nutrient diffusion rates, sorption of substrate, or enzymes are the possible causes of decrease in enzyme activities with biochar application in soils (Foster et al., 2018). The application of higher biochar doses probably increased the C/N ratio in soils and lead to slow mineralization of soil organic matter. Previous studies reported that soil extracellular enzyme activities involved in carbon and sulphur cycling were higher in low biochar application doses and reduced with increasing biochar application doses (Wang et al., 2015). Demisie et al. (2014) reported an increase in microbial activity in low biochar application doses and indicated that high microbial activity contributed to higher carbon mineralization. Biochar applications caused 8.3, 10.0, 14.9, 16.6 and 16.4% decrease in BGA value compared to control with BD1, BD2, BD3, BD4 and BD5 application doses, respectively (Figure 1).

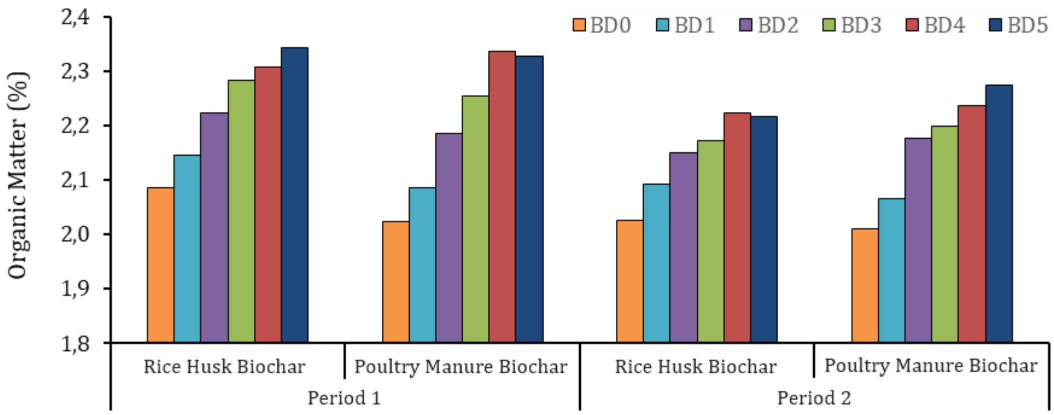

Figure 1. The effects of rotation period, biochar type and biochar dose interactions on organic matter content, % (RP1: First rotation period, RP2: Second rotation period) (Control; BD0: 0 t ha^{-1}, BD1: 10 t ha^{-1}, BD2: 20 t ha^{-1}, BD3: 30 t ha^{-1}, BD4: 40 t ha^{-1}, BD: 50 t ha^{-1})

The decrease in BGA can also be attributed to the presence of active functional groups on the surface and the pore space of biochar particles. The recorded decrease in BGA resulting from the application of biochar to soils has been attributed to the chemical modification or blocking of substrate binding sites by the applied biochar (Yoo and Kang, 2012; Chintala et al., 2014). The functional groups of biochar can selectively adsorb the enzyme substrate, potentially leading to a reduction in enzyme activity (Pokharel et al., 2018; Sial et al., 2022). Likewise, Foster et al. (2016) suggested that substantial sorption capacity of biochars could limit the accessibility of easily degradable carbon substrates for microorganisms, leading to reduced BGA in biochar applied soils. Consistent with our findings, Günal et al. (2018) showed that the application of 0.5, 1, 2 and 3% corn, bean and rice husk biochar to sandy loam and loamy soil resulted in a 3.7, 11.7, 16.4 and 16.4% decrease in mean BGA value compared to control, respectively. In their study, Moreno et al. (2022) compared the BGA activity of biochar derived from holm oak chips by slow pyrolysis at 600 °C with unamended soils in a crop rotation of barley-sunflower-wheat-barley-cameline under a Mediterranean agroecosystem. The researchers reported a notable increase in urease activity in biochar added soil, while the activities of BGA and protease were significantly reduced with the addition of biochar. The results revealed that biochar application to soil affected the activities of microbial community which is active in cellulose degradation and soil organic matter dynamics in soils. In contrast to our findings, Pérez-Guzmán et al. (2020) reported significantly higher ($P<0.05$) BGA values in soils incubated with corn and hardwood biochars produced under slow pyrolysis compared to the control group.

Effects of rotation period (RP), biochar type (BT) and biochar dose (BD) interactions on β-glucosidase enzyme activity

The statistical analysis revealed that the interaction between BT and BD had a significant influence on the BGA value ($p<0.05$), whereas the impact of other interactions on the BGA value was found to be insignificant (Table 2). The highest mean BGA value (94.77 µg pNP g^{-1}) in the RP x BT interaction was obtained in RP2 x rice husk biochar and the lowest average BGA value (73.44 µg pNP g^{-1}) was obtained in RP1 x rice husk treatment (Figure 2). The highest mean BGA value in RP x BD interaction was obtained in RP2x BD0 (104.70 µg pNP g^{-1}), while the lowest mean value was recorded in RP1 x BD5 (66.99 µg pNP g^{-1}) treatment (Figure. 2). The BGA value in BD1, BD2, BD3, BD4 and BD5 doses of rice husk biochar decreased by 12.1, 14.0, 21.7, 22.8, and 25.0%, respectively, compared to the control. The application of poultry manure biochar resulted in lower decreases of 4.0, 5.7, 7.4, 9.7 and 12.1% with respect to increasing doses of biochar. Overall, the difference in C/N ratio between rice husk biochar and poultry manure biochar is a major factor that contributes to the variation in BGA values observed at the application of the same biochar doses. The difference in C/N ratio has a significant impact on the way that biochar interacts with soil and microorganisms (Xu et al., 2023). Rice husk biochar is typically more resistant to decomposition and has a longer-term impact on soil carbon sequestration and BGA. Poultry manure biochar, on the other hand, is more easily decomposed and releases nutrients more quickly to plants, which may have a more immediate impact on BGA. The highest mean BGA value (100.07 µg pNP g^{-1}) in the BT x BD was obtained from rice husk x BD0 treatment, while the lowest average BGA value (75.04 µg pNP g^{-1}) was recorded in rice husk x BD5 treatment (Figure 2). The involvement of soil BGA, which is the rate-limiting enzyme, is crucial in the microbial metabolism, carbon (C) cycling, and sequestration of terrestrial ecosystems during the last phase of cellulose hydrolysis. In a study conducted by Raiesi and Khadem (2019), biochar derived from maize waste was incorporated into sandy loam and clayey soils at three different doses:

control (corn waste), 0.5% (corn waste biochar), and 1% (corn waste biochar), with the biochar being produced at a temperature of 600°C. Following a 90-day incubation period, the introduction of biochar resulted in a 81% increase in potential BG enzyme activity in sandy loam soil with only 1% biochar addition. Conversely, in clayey soil, the addition of 0.5% biochar led to a 10% reduction in potential BG enzyme activity, while addition of 1% biochar resulted in a 29% decrease.

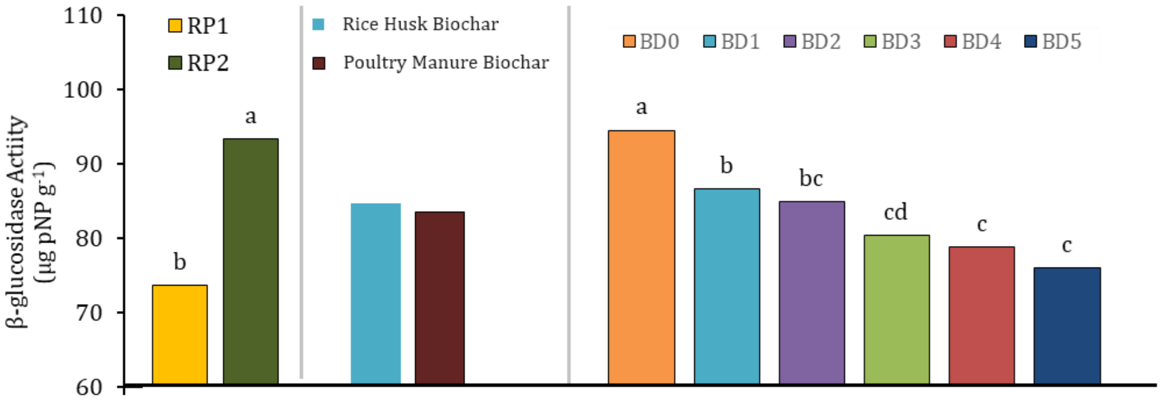

Figure 2. The effects of rotation period, biochar type and biochar doses on β-glucosidase enzyme activity (µg pNP g^{-1}) (RP1: First rotation period, RP2: Second rotation period) (Control; BD0: 0 t ha^{-1}, BD1: 10 t ha^{-1}, BD2: 20 t ha^{-1}, BD3: 30 t ha^{-1}, BD4: 40 t ha^{-1}, BD: 50 t ha^{-1}).

The highest mean BGA value in the RP x BT x BD interaction was obtained in RP2 x Rice Husk Biochar x BD0 (112.23 µg pNP g^{-1}) and the lowest BGA value was in RP1 x Rice Husk Biochar x BD5 (65.45 µg pNP g^{-1}) interactions (Figure 3). The findings suggest that suggest that the effects of biochar on BGA may be complex and depend on several factors, including the type of biochar, the biochar dose, and the rotation period. Rice husk biochar was found to have a more pronounced effect on reducing BGA than poultry manure biochar. The difference is likely due to the higher C/N ratio of rice husk biochar, which makes it more resistant to decomposition. The slower decomposition rate of rice husk biochar means that it persists in the soil for longer, allowing for a more prolonged interaction with soil microbes and enzymes. The decrease in BGA over the course of the two rotation cycles suggests that biochar may have a long-term impact on soil carbon cycling. This is a promising finding, as it suggests that biochar could be used as a sustainable approach to improving soil health in crop rotation systems.

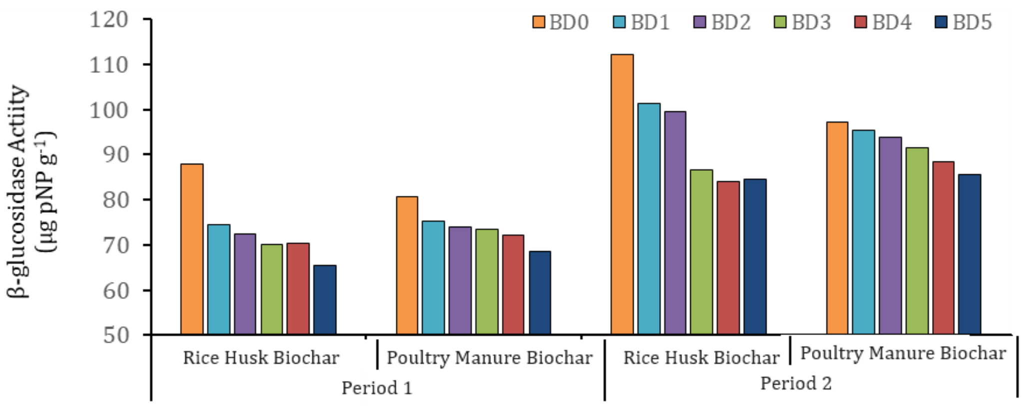

Figure 3. The effects of rotation period, biochar type and biochar dose interactions on β-glucosidase enzyme activity (BGA, µg pNP g^{-1}) (RP1: First rotation period, RP2: Second rotation period) (Control; BD0: 0 t ha^{-1}, BD1: 10 t ha^{-1}, BD2: 20 t ha^{-1}, BD3: 30 t ha^{-1}, BD4: 40 t ha^{-1}, BD: 50 t ha^{-1})

Conclusion

This study investigated the effects of rice husk and poultry manure biochars on β-glucosidase enzyme activity (BGA) within a wheat-cabbage-red pepper crop rotation system in the Black Sea agroecosystem. Biochar application significantly impacted BGA, with rice husk biochar exhibiting a more pronounced effect on reducing activity compared to poultry manure biochar. This suggests a potential long-term influence of biochar on soil carbon cycling through its effect on organic matter decomposition. The observed decrease in BGA was statistically significantly for both biochar types, but the reduction was more than twice as high with rice husk biochar compared to poultry manure biochar at each application rate. We attribute this disparity to the higher C/N ratio of rice husk biochar, which promotes slower decomposition and a more persistent influence on soil carbon sequestration and BGA. Conversely, the lower C/N ratio of poultry manure biochar likely leads to faster decomposition and p nutrient release, potentially exerting a more direct influence on BGA in the short term. These findings suggest that rice husk biochar may be a more effective strategy for long-term soil carbon sequestration within this specific crop rotation system. In addition, the negative correlation between organic matter content and BGA suggests that increased organic matter may hinder enzyme activity, possibly due to biochar-mediated enzyme adsorption or alterations in soil microbial dynamics. However, further research is necessary to validate these results under various field conditions and across diverse crop rotations to elucidate the broader applicability of different biochar types on BGA activity.

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