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Effect of Natural Aging of Biochar on Soil Enzymatic Activity and Physicochemical Properties in Long-Term Field Experiment

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Abstract: The effect of different rates of biochar on selected soil properties and enzymatic activity 48, 60, and 72 months after biochar application to soil was investigated. Soil enzymatic activity (dehydrogenase—ADh; phosphatase—Aph; urease—AU), the organic carbon content (TOC), the total nitrogen content (N_t), the mineral nitrogen content (NH_4^+, NO_3^-), and soil pH were determined. The study was conducted on Haplic Podzol originating from glaciofluvial fine-grained loamy sand. Biochar was applied to soil under winter rye (*Secale cereale* L.) at rates of 10 (BC_{10}), 20 (BC_{20}), and 30 t ha^{-1} (BC_{30}). Plots with biochar-unamended soil were the control treatment (BC_0). The pH, TOC, and N_t content in the biochar-amended soil were higher compared to the control soil. A broader C:N ratio was found in the BC_0 soil compared to BC_{10}, BC_{20}, and BC_{30}. With increasing biochar rate, the content of the ammonium nitrogen form (NH_4^+) decreased and was statistically lower than in the control soil (BC_0). The soil in the BC_{20} and BC_{30} treatments was characterized by the highest content of NO_3^-, whereas the lowest nitrate nitrogen content was found in the control soil (BC_0). Biochar application increased soil enzymatic activity. Dehydrogenase activity increased with increasing biochar rate. As far as phosphatase and urease activity is concerned, a similar relationship was not observed. In this case, the soil amended with biochar at a rate of 20 t ha^{-1} (BC_{20}) was characterized by the highest phosphatase and urease activity.

Keywords: biochar; enzymatic activity; nitrogen forms; *Secale cereale*; monoculture

1. Introduction

Biochar is a product of thermal decomposition of an organic substance in the process of pyrolysis during which, in the absence of oxygen and at a temperature of 300–1000 °C, products such as oil, synthetic gas, and biochar are formed. Biochar, depending on the substrate from which it was produced and on the conditions in which the pyrolysis process is carried out, has different properties [1–4]. Due to its high organic carbon content, well-developed porosity, and specific surface area, biochar can be used to improve properties of agriculturally used soils [5–8]. It increases soil water-holding capacity and pH, prevents nutrient leaching, and binds organic and inorganic contaminants [9]. Biochar can be an element that binds the active substances of plant protection products and heavy metals [10–13]. On account of its high ion exchange capacity, biochar is characterized by the ability to retain and exchange soil nutrients [14]. It also contains macro- and micronutrients that are a valuable source of minerals for soil microorganisms, which in consequence stimulates soil microbial activity [15–19]. Nevertheless, biochar can contain contaminants in the form of both heavy metals and polycyclic aromatic hydrocarbons [20].

Soil microorganisms determine the quality of soil environment and belong to its most active components. The activity of enzymes released into the soil environment is an indicator of their metabolic capacity [21,22]. Soil enzyme activity can effectively indicate soil biological activity and nutrient supply capacity, which can reflect soil ecosystem stability and soil health. Enzymes are involved in many biochemical processes in soils, such as organic matter decomposition, carbon (C) mineralization, and C, N, and P cycles [21–24]. Enzymatic processes manifest disturbances in matter cycling and the flow of energy through ecosystem elements. Research reveals that some enzymatic tests reliably reflect the impact of agronomic practices on soil ecological status [21,22,25–28].

In recent years, a lot of attention has been given to research on the use of biochar to improve soil chemical, physical, and biological properties [3,4,11,29–31]. However, there is sparse information on the effect of biochar on soil enzymatic activity and mineral nitrogen content in the arable layer of soil. Existing research has shown that biochar increases soil enzymatic activity. This is a beneficial phenomenon because it indicates proper soil microbiological processes [11]. Biochar application provides an additional source of carbon and nutrients, as well as enhanced sorption properties, probably stimulating the activity of the investigated enzymes [26]. Nonetheless, existing studies have focused on relatively short periods of time. The literature lacks information how biochar affects soil enzymatic activity, in particular in the long term (several years). It is known that, due to the aging process, biochar undergoes different changes that can have a significant impact not only on soil properties, but also on biological life. The research hypothesis assumes that biochar added to soil will change selected physicochemical and biological properties of soil. The aim of the present study was to determine the effect of different biochar rates on soil enzymatic activity, mineral nitrogen, TOC, and pH over a period of several years after biochar amendment.

2. Materials and Methods

2.1. Biochar Characteristics

Biochar applied to soil was provided by Mostostal Sp. z o.o. (Wrocław, Poland). Biochar was produced by process of pyrolysis from wheat straw at temperature 650 °C (maximum applied temperature) in an oxygen-poor atmosphere (<2% O_2). Biochar made from wheat straw was in the form of ash. The biochar was intentionally produced for the purposes of the experiment. To produce 1 t of biochar from wheat straw, 3 t of straw must be burned in the pyrolysis process [32]. The chemical properties of biochar were determined by standard methods. The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 10. The available potassium, phosphorus, and magnesium were determined according to procedures for soil analysis [29]. Total organic carbon (TOC) was determined by TOC-VCSH (Shimadzu) with Solid Sample Module SSM-5000. The total nitrogen (Nt) was determined by the Kjeldahl's method without the application of Dewarda's alloy (Cu–Al–Zn alloy reducer of nitrites and nitrates) [33].

The physicochemical characteristics of biochar were as follows: pH (in KCl) 9.9, available forms of phosphorous (P), 235.6 mg kg^{-1}; potassium (K), 2344.6 mg kg^{-1}; and magnesium (Mg), 163.2 mg kg^{-1}. Elemental composition was: 53.87% C, 0.91% N, and 1.76% H. The ratio of hydrogen to carbon was 0.033, and the ash content was 41.2%. The scanning electron microscope (SEM) pictures of biochar are presented in Figure 1. Pictures show typical features of biochar used in the study—large specific surface area and porosity.

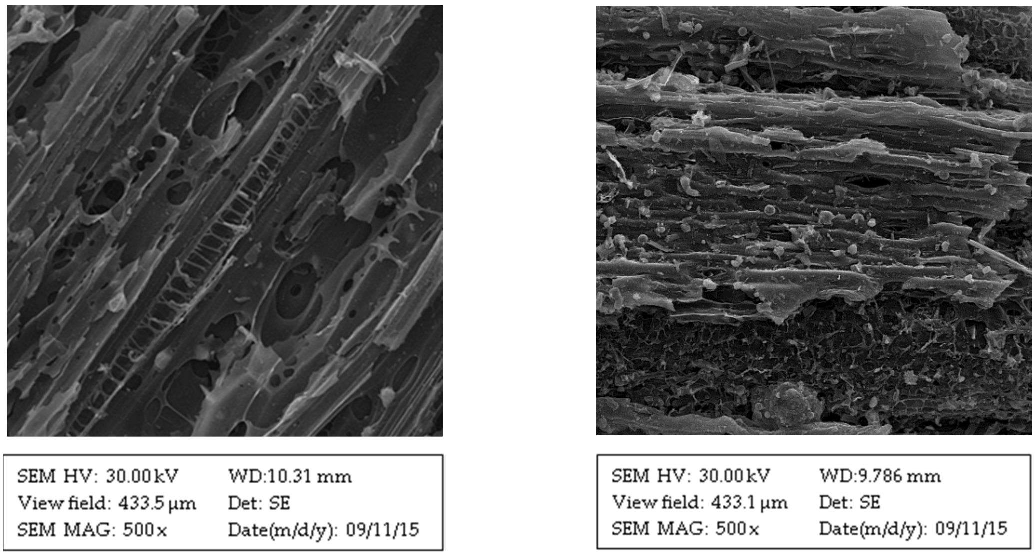

Figure 1. Scanning electron microscope (SEM) pictures of biochar used in the field experiment.

2.2. Weather Conditions

Weather conditions were described on the background of the multiyear period 1974–2010 based on the average monthly air temperature (°C) and total precipitation (mm). Additionally, hydrothermal Sielianinov coefficient (K) was calculated according to the formula: K = (P·10)/(T·L), where P is the total monthly precipitation, T is the average monthly temperature, and L is number of days in the month [34]. In the 2014–2015 season, the total rainfall was only 77% of the long-term average (Figure 2). The highest rainfall deficits occurred in the period from September to November and also from July to August. During these months, the total rainfall was lower than the long-term average for the relevant period by 30% and 65%, respectively. The Sielianinov hydrothermal coefficients confirmed that these months were very dry and extremely dry (Table 1). The 2015–2016 season was very humid, since during this season the total rainfall was higher than the long-term average by 31%. The months of September and July were most abundant in rainfall, and the total rainfall in these months was about 60% higher than to the long-term average. In the third year of the experiment, the total rainfall was similar to the long-term average; however, the distribution of precipitation in individual months varied. The highest amount of rainfall was recorded in October, in which the sum of precipitation was three times higher than the long-term average. At the same time, the highest rainfall deficit was found in the months of September, June, and August. During these months, the total rainfall was only 19%, 35%, and 56% of the long-term average, respectively. The average air temperature during the field experiment was above the long-term average for most of the months. In subsequent seasons the air temperature exceeded the long-term average by 2.0 °C, 2.2 °C, and 1.0 °C respectively.

Table 1. Sielianinov hydrothermal coefficients (K) during the growing seasons in the years of the experiment according to the Meteorological Station at Bezek.

Year	III	IV	V	VI	VII	VIII
2015	2.73	1.47	4.75	0.30	0.70	0.10
2016	4.49	2.40	1.23	1.23	2.20	0.94
2017	1.79	2.66	1.67	0.50	1.66	0.65

Extremely dry, K ≤ 0.4; very dry, 0.4 < K ≤ 0.7; dry 0.7, < K ≤ 1.0; quite dry, 1.0 < K ≤ 1.3; optimal, 1.3 < K ≤ 1.6; quite humid, 1.6 < K ≤ 2.0; humid, 2.0 < K ≤ 2.5; very humid, 2.5 < K ≤ 3.0; extremely humid, K > 3.0.

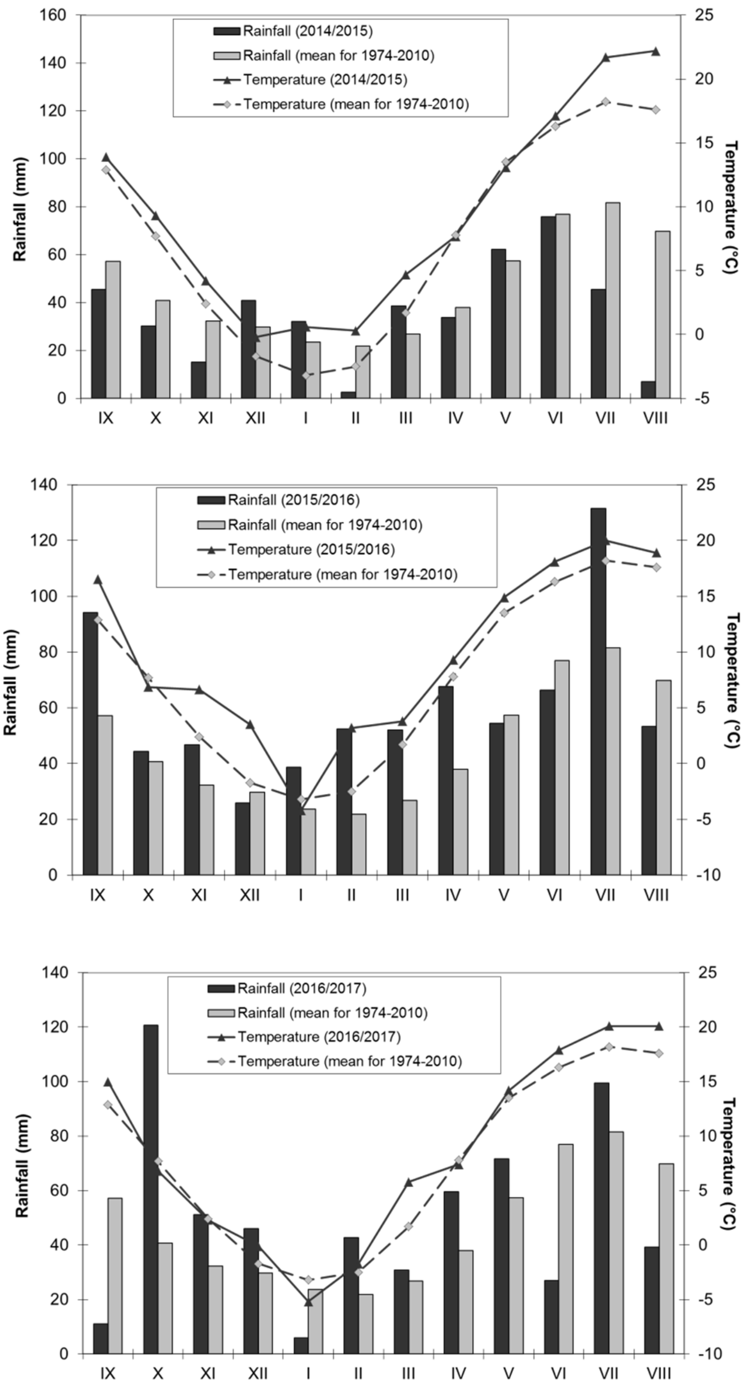

Figure 2. Rainfall and air temperature as compared to the long-term mean figures (1974–2010) according to the Meteorological Station at Bezek.

2.3. Experimental Site

The field study was established in 2011 at the Experimental Farm in Bezek (51°19′ N, 23°25′ E), Poland. The experiment was established on a Haplic Podzol (PZha) originating from glaciofluvial fine-grained loamy sand [35]. The particle size distribution of arable layer of this soil was as follows: 2.0–0.5 mm fraction—9%; 0.5–0.25 mm fraction—26%; <0.002 mm fraction—2% [31]. The average amount of P in the soil was 59.4 mg kg^{-1}; K—108.5 mg kg^{-1}; Mg—12.8 mg kg^{-1}; Cu—1.8 mg kg^{-1}; Zn—5.4 mg kg^{-1}; Mn—116.1 mg kg^{-1}; Fe—481.3 mg kg^{-1}; B—0.82 mg kg^{-1}; the pH in KCl was 5.32 [29]. The experiment, set up in a randomized block design in three replicates, compared the effect of three biochar rates in the cultivation of winter rye (*Secale cereale* L., 'Dańkowskie Diament') cultivated in a monoculture. The area of a single plot was 18 m^2. At the beginning of September 2011, biochar at rates of 10 (BC_{10}), 20 (BC_{20}), and 30 (BC_{30}) t per hectare was applied to the soil, and then the soil was ploughed. The plot without biochar was the control treatment (BC_0). The spacing between plots fertilized with the different rates of biochar was 2 m. Winter rye was sown every year in the fourth week of September.

The crop was sown at a rate of 5 million seeds per hectare at 12 cm inter-row spacing. Mineral fertilizers were applied every year of the experiment at the following rates: 70 kg ha^{-1} N (ammonium nitrate), 26 kg ha^{-1} P (triple superphosphate), and 66 kg ha^{-1} K (muriate of potash, KCl). Phosphorus and potassium fertilizers as well as 20 kg ha^{-1} N were applied before sowing. In the spring, the remaining portion of the nitrogen (N) rate was applied before plant growth began (30 kg ha^{-1}) and at the stem elongation stage (20 kg ha^{-1}). The winter rye crop protection program included the application of the herbicide Aminopielik Tercet 500 SL (2,4-D 300 g L^{-1}, Mecoprop 160 g L^{-1}, and Dicamba 40 g L^{-1}) at a rate of 2 L ha^{-1} (BBCH 23–29). Moreover, the fungicide Tango Star 334 SE (Fenpropimorph 250 g L^{-1} and Epoxiconazole 84 g L^{-1}) was applied at a rate of 1 L ha^{-1} (BBCH 51–56).

2.4. Sample Collection

Soil samples were collected from the 0–25 cm layer in August 2015, 2016, and 2017 (after 48, 60, and 72 months from the beginning of the experiment). The composite soil sample was an average from 15 subsamples taken from each treatment. Individual samples from specific plots were averaged and physicochemical and biochemical properties were determined in these samples in three simultaneous replicates. Tables 1 and 2 also give the values of evaluated parameters as means independent of biochar rates. These data were calculated as means for BC_0, BC_{10}, BC_{20}, and B_{30} after 48, 60, and 72 months after biochar incorporation to soil.

2.5. Enzymatic Activity and Physicochemical Properties

The enzymatic activities that play an important role in the transformation of organic carbon compounds (dehydrogenases), nitrogen compounds (ureases), and phosphorus compounds (phosphatases) were determined in the soil samples. Activity of dehydrogenases (ADh) was determined by Thalmann's method [36] using a 1% solution of TTC (triphenyl tetrazolium chloride) as a substrate, expressing their activity in mg 1,3,5-triphenyl formazan (TPF) kg^{-1} 24 h^{-1}. Determination of neutral phosphatase activity (APh) was performed according to Tabatabai and Bremner [37] using a 0.8% sodium p-nitrophenyl phosphate solution as a substrate in buffer pH 7.0. The APh were expressed in mmol p-nitrophenol (PNP) kg^{-1} h^{-1}. Urease activity (AU) was determined following Zantua and Bremner [38] using a 2.5% urea solution as a substrate, expressing the activity in mg N-NH_4^+ kg^{-1} h^{-1}. The content of organic carbon, total nitrogen, and ammonium and nitrate nitrogen were determined according to ISO 14235 [39], ISO 13878 [40], and ISO 14255 [41], respectively. The pH was measured potentiometrically in 1 M KCl [42].

2.6. Data Analysis

Statistica PL 13.3 (TIBCO Software Inc., Tulsa, OK, USA) was used to conduct ANOVA analysis, and Tukey's mean separation was used to determine statistical significance at $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Properties

The biochar-amended soil was characterized by higher pH compared to control soil. The pH increased with increasing of biochar rate (Table 2). The reason for the increase in soil pH due to biochar application could be the high surface area and porous nature of biochar, which increases the cation exchange capacity (CEC) of the soil by reducing the leaching of base cations in competition with H^+ ions [4,43].

The increase of soil pH after biochar application may be also related to ash content, which contains carbonates of alkali and alkaline earth elements, variable amounts of heavy metals, silica, sesquioxides, phosphates, and small amounts of organic and inorganic nitrogen [44,45]. These results confirmed earlier studies [4,11,29,46] where the increase of soil pH after biochar application was also observed. Furthermore, this study demonstrated that, over time, soil pH decreased in the biochar-treated plots, which is also a typical phenomenon in such cases related to the aging of biochar [4].

Table 2. Soil pH, total organic carbon (TOC), total nitrogen (Nt) and mineral form of nitrogen ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) contents, and C:N ratio in soil after 48, 60, and 72 months from biochar incorporation.

Assessment Date/Year	Biochar Rate	pH_{KCl}	TOC	N_t	C:N	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
			g kg^{-1}			mg kg^{-1}	
After 48 months	BC_0	5.38 a	5.95 a*	0.47 a	12.75 a	16.17 a	57.53 a
	BC_{10}	6.76 b	6.32 b	0.56 b	11.35 b	13.67 b	69.24 b
	BC_{20}	6.85 b	7.41 c	0.64 c	11.52 b	10.54 c	80.81 c
	BC_{30}	7.15 c	7.59 d	0.69 d	11.05 c	9.16 d	77.62 d
After 60 months	BC_0	5.37 a	5.94 a	0.46 a	12.91 a	11.68 a	74.81 a
	BC_{10}	6.38 b	6.48 b	0.63 b	10.32 b	9.30 b	90.44 b
	BC_{20}	6.65 b	7.42 c	0.68 c	10.99 c	4.59 c	101.32 c
	BC_{30}	6.73 b	7.79 d	0.71 d	10.90 c	3.01 d	92.85 d
After 72 months	BC_0	5.40 a	5.93 a	0.47 a	12.63 a	10.86 a	121.29 a
	BC_{10}	5.72 b	6.56 b	0.65 b	10.02 b	8.65 b	131.31 b
	BC_{20}	5.99 c	7.78 c	0.70 c	11.17 c	3.94 c	190.89 c
	BC_{30}	6.16 d	7.88 c	0.74 d	10.68 d	2.07 d	142.89 d
After 48 months	Average for biochar rates		6.82 a	0.59 a	11.58 a	12.38 a	71.30 a
After 60 months			6.91 a	0.62 b	11.28 a	7.14 b	89.86 b
After 72 months			7.04 a	0.64 c	11.00 a	6.38 b	146.60 c

* different letters indicate significant difference at $p \leq 0.05$; BC_0—plots without biochar (control treatment); BC_{10}—biochar rate of 10 t h^{-1}; BC_{20}—biochar rate of 20 t ha^{-1}; BC_{30}—biochar rate of 30 t ha^{-1}.

After 48, 60, and 72 months after biochar application to soil, the total organic carbon (TOC) and total nitrogen (Nt) in the biochar-amended soil were significantly higher than in the control (BC_0) (Table 1). The soil content of these nutrients was found to increase with increasing biochar rate. The highest values of TOC in the biochar-treated soils indicate the recalcitrance of organic C in biochar. High TOC and Nt in biochar-amended soil have been similarly reported by a number of authors [29,43,47]. In successive years of the study, TOC showed statistically insignificant fluctuations. In turn, the content of Nt behaved differently compared to TOC in biochar-amended soil. Nt increased over the experimental period, which could have been due to the fact that biochar is characterized by the ability to retain and exchange soil nutrients [14]. The values of the C:N ratio in the control soil were shown to be significantly wider than in the soil with biochar addition. In successive growing seasons, the C:N ratio did not change significantly ($p \leq 0.05$) (Table 2). The $N\text{-}NH_4^+$ content was the highest in the control soil and gradually decreased in the treatments with the increased biochar rate. At 48 months after biochar application, the $N\text{-}NH_4^+$ content was higher than after 60 and 78 months (Table 2). Biochar amendment caused a significant ($p \leq 0.05$) increase of the nitrate nitrogen ($N\text{-}NO_3^-$). The highest content of $N\text{-}NO_3^-$ over the entire study period was observed for the soil where biochar was applied at a rate of 20 t ha^{-1}. A significantly lower $N\text{-}NO_3^-$ content was determined in the experiment BC_{30}, while the lowest was determined in the experiment BC_{10} (Table 2). Biochar application to the soil modifies the nitrogen cycle in the environment. As a soil amendment, biochar exhibits the ability to store nitrogen by reducing the leaching of $N\text{-}NO_3^-$ ions and also by inducing the growth of nitrogen-fixing bacteria [48–50]. Lehman et al. [15] observed greater activity of microorganisms participating in soil nitrogen fixation already after application of 10 t ha^{-1} biochar.

3.2. Dehydrogenase Activity

In the biochar treatments, dehydrogenase activity (ADh) was higher than in the control soil. The ADh increased significantly with increasing biochar rate. Moreover, in successive years of observation, ADh decreased to a small extent, regardless of the biochar rate (Table 3). Błońska et al. [51] suggested that decrease of ADh with time may be related to the grown conditions (monoculture) and affects activity of the microorganisms. ADh is a very sensitive indicator of soil property changes and is related to living microbial cells [26,51,52]. Oleszczuk et al. [11] found an increase in ADh in the treatments where biochar was applied at 30 and 45 t ha^{-1}. Sopeña and Bending [53] also indicated the protective role of biochar with regard to ADh. Brzezińska and Włodarczyk [54], in turn, demonstrated a close relationship between ADh and organic matter content, soil fertility, soil microbe numbers, proteolytic activity, nitrification, denitrification, respiration (release of CO_2, absorption of O_2), and also the activity of other enzymes present in the soil environment. The above observations show that the effect of biochar on ADh varies and can take different directions, probably depending on the type of biochar (properties, origin), soil type, or environmental conditions.

Table 3. Enzymatic activity of soils after 48, 60, and 72 months from biochar incorporation.

Assessment Date/Year	Biochar Rate	ADh	APh	AU
After 48 months	BC_0	1.99 a*	81.27 a	2.86 a
	BC_{10}	3.66 b	92.63 b	3.43 b
	BC_{20}	4.12 c	97.42 c	4.42 c
	BC_{30}	7.30 d	95.38 d	4.32 c
After 60 months	BC_0	1.66 a	59.44 a	2.36 a
	BC_{10}	3.07 b	60.37 b	2.88 b
	BC_{20}	3.38 c	81.01 c	4.28 c
	BC_{30}	5.17 d	65.41 d	3.43 d
After 72 months	BC_0	1.25 a	43.03 a	2.05 a
	BC_{10}	2.80 b	49.21 b	2.32 b
	BC_{20}	3.29 c	59.32 c	2.98 c
	BC_{30}	5.04 d	53.81 d	2.51 d
After 48 months	Average for biochar rates	4.27 a	91.67 a	3.76 a
After 60 months		3.32 a	66.56 b	3.24 b
After 72 months		3.10 a	51.34 c	2.47 c

ADh—dehydrogenases in mg TPF kg^{-1} 24 h^{-1}; APh—phosphatase in mmol PNP kg^{-1} h^{-1}; AU—urease in mg N-NH_4^+ kg^{-1} h^{-1}; * different letters indicate significant difference at $p \leq 0.05$; BC_0—plots without biochar (control treatment); BC_{10}—biochar rate of 10 t ha^{-1}; BC_{20}—biochar rate of 20 t ha^{-1}; BC_{30}—biochar rate of 30 t ha^{-1}.

3.3. Phosphatase Activity

Phosphatases play an important role in soil, stimulating organic transformations of phosphorus compounds into inorganic phosphates (HPO_4^{-2} and $H_2PO_4^-$) which are directly available to plants and soil organisms [55]. Phosphatase activity (APh) in the soil environment reflects the activity of enzymes associated with soil colloids and humic substances, free phosphatases in the soil solution, and phosphatases associated with living and dead plant cells and microorganisms [56]. APh can be a good indicator of the organic phosphorus mineralization potential and soil biological activity [57]. The highest APh was found in the soil amended with biochar at 20 t ha^{-1}, which was significantly ($p \leq 0.05$) higher compared to 30 and 10 t ha^{-1}. On the other hand, the control soil was characterized by lowest phosphatase activity (Table 3). Gong et al. [58] found that biochar applied at low rates (< 1%) can increase the enzymatic activity of soil microbes. Over the experimental period, APh was found to decrease. According to Gregorich et al. [59], periodic changes in enzyme activity are associated with changes in soil moisture content and oxygenation, and they do not depend on small differences in soil C and N content. In the study period presented, the monthly rainfall totals were very variable, whereas the mean air temperatures exceeded the long-term mean, which could have influenced the observed changes in APh (Figure 2).

3.4. Urease Activity

Ureases catalyze the hydrolysis of urea in soil to carbon dioxide and ammonium. These enzymes are found in cells of many higher plants and microorganisms, in particular bacteria [60]. According to Vaheda et al. [61], soil urease activity (AU) correlates with TOC and Nt content. These authors also noted a significant relationship between urease activity and soil $N\text{-}NH_4^+$ content. Similarly, in the present study, both TOC and Nt content and AU were highest in the treatments where the highest biochar rates (BC_{20} and BC_{30}) were applied. The highest AU was found in the same treatments where the $N\text{-}NO_3^-$ content was the highest (Tables 2 and 3). In each successive growing season, AU decreased. This could have been attributable to the decline in soil properties due to monoculture cropping of rye and also to the effect of biochar aging. Attention is drawn to this phenomenon by Gul et al. [4], who found that the properties of biochar change due to its aging in soil, in particular on account of its oxidation and the accumulation of H^+ from the soil solution. In turn, Natywa et al. [62] found the respiratory activity of monoculture soils to decrease compared to those where crop rotation was used. Vahed et al. [61] report that for AU the optimum value of pH is between 6 and 7. Hence, this was probably the reason for the lower AU in the third year of the experiment, in which a slightly lower soil pH was found in all studied treatments compared to the earlier period (Tables 2 and 3).

4. Conclusions

Application of biochar to the soil resulted in a significant increase of TOC and Nt as well as $N\text{-}NO_3^-$. In comparison to the control soil, the C:N ratio and the $N\text{-}NH_4^+$ content decreased. The biochar-amended soil showed higher ADh, APh, and AU. However, when analyzing soil enzymatic activity, it was found that ADh, APh, and AU were increasingly lower in successive years of the study, regardless of the biochar rate, which was probably associated with the effect of biochar aging and monoculture cropping of rye. The present study clearly confirms that it is possible to use biochar to improve soil biological activity, in particular during the initial period after biochar application. Its positive impact on enzymatic activity is limited and weakens as a result of biochar aging. Soil enzymatic activity should therefore be monitored, and after several years biochar should be applied again, which in consequence will result in an increase in the soil's biological activity.

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