The Impact of Biochar Doses on Soil Quality and Microbial Functional Diversity

Anna Gałązka,* Krzysztof Jończyk, Karolina Gawryjołek, and Jarosław Ciepiel

Changes of soil biological activity and microbial diversity were assessed after application of biochar in different doses. The biochar doses were determined based on the initial carbon content in the soil, and they were increased to 2.5%, 5%, 10%, 20%, 50%, and 100% in the experimental conditions. The experiment design also included a control condition, which was not treated with biochar. The basic biological activities in the soil, *i.e.*, enzymatic activity and the content of carbon and nitrogen in the microbial biomass, were determined. Additionally, the functional biodiversity of soil microorganisms was assessed using the Biolog EcoPlates method. It was demonstrated that biochar added to the soil at the dose of 10 to 20% significantly increased soil biological activity and functional diversity. The biochar dose of 10 to 20% was the optimal dose for enhancement of soil biological activity. This dose induced a significant increase in the total carbon content in the microbial biomass, enzymatic activity, and the overall content of total and easily extractable glomalins. The highest values of the Shannon and AWCD indices were determined in soil supplemented with 5 to 20% of biochar.

Keywords: Biochar; Soil biological activities; Glomalin; Wheat; Community Level Physiological Profiles (CLPP)

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INTRODUCTION

Biochar production is a subject of recent research interest in Europe (Jones *et al.* 2012; Ahmed and Schoenau 2015). Biochar is a solid renewable fuel extracted from various types of biomass in the pyrolysis process, in which liquid and gas fuels are generated. The material used to produce biochar varies greatly and comprises energy plants, forest waste, rapeseed and sunflower biomass, corncobs, sewage sludge, and organic waste (Schulz and Glaser 2012; Xu *et al.* 2012). Biochar has been used mainly as fuel for energy production (Kalton *et al.* 2011). In recent years, however, biochar has been used frequently for other purposes such as environmental protection and agriculture. Biochar can serve as a natural fertiliser for soil applications (Novak *et al.* 2016; Cao *et al.* 2017). Previous studies of the agricultural applications of biochar indicate its multidirectional effects on the natural environment and a number of other benefits associated with its use. These benefits include improved nutrient retention in the soil, increased crop production, improved quality indices of soils (mainly in degraded and low-productivity soil), enhanced efficiency of fertiliser utilisation, a long-lasting increase in soil carbon content, and reduced CO_2 emission (Anderson *et al.* 2011; Artiola *et al.* 2012; Cui *et al.* 2013; Gałązka *et al.* 2018).

Biochar has a positive effect on soil quality and yields of plants (Solaiman *et al.* 2011; Jones *et al.* 2012; Shi *et al.* 2015; Meng *et al.* 2019). Biochar application can enhance the organic matter content in soil, which leads to increased soil fertility (Xu *et al*. 2012). Gebremedhin *et al*. (2015) demonstrated a beneficial effect of biochar on the yield and traits of the wheat yield structure. The application of biochar at a dose of 4 $t \cdot ha^{-1}$ resulted in an increase in grain and straw yields, compared with the control. Biomass introduced into the soil exerts a positive impact on the formation of a strong sorption complex, which is especially important in the case of light soils. However, the application of large quantities of biomass and decomposition thereof by microorganisms releases organic acids, leading to increased acidification of soils (Jones *et al.* 2012). The benefits of using biochar are: increased bioavailability of nutrients, increased soil pH, exchange of cations, and increased activity and number of microorganisms (Mierzwa-Hersztek *et al.* 2019).

The addition of biochar has a positive effect on soil microorganisms, which play a key role in soil structure and function. Microorganisms are good indicators of soil quality due to their participation in many biochemical processes, essential for the environment and ecological functions of the soil (Furtak *et al*. 2017). Biochar supports the activity of many soil microorganisms that are important for agriculture. The porous structure of biochar serves as a suitable habitat for many microorganisms, providing them with carbon, energy, and minerals (Anderson *et al.* 2011; Kolton *et al.* 2011; Jones *et al.* 2012; Fletcher *et al.* 2014).

Bacterial growth and abundance may be associated with the sorption surface of biochar, and bacteria can be attached to biochar particles in a variety of ways. With its sorption properties, biochar also protects microorganisms against drought. Seasonal periods of drought in soils that are not supplemented with biochar can lead to stress and, hence, reduce the activity of certain bacteria. Woolf (2008) demonstrated that biochar exerts a positive effect on the mycorrhiza and production of mycorrhizal glomalin, *i.e*., a glycoprotein stabilising soil structure.

Given the necessity to improve the greenhouse gas (GHG) balance, sustain the increase in the soil carbon content, and include agriculture as an important sector in the production of energy from renewable sources, the agricultural application of biochar is promising (Cui *et al.* 2013; Fletcher *et al.* 2014). Despite the benefits of using biochar in agriculture, the current knowledge in this field is fragmentary and insufficient. An additional problem in investigations of biochar and determination of doses that are best suited for production and environmental effects is the large variety of biochar forms that can be applied in practice (diversity of fractions, sources, and origins of biochar). There are no literature reports on the assessment of the effects of biochar doses on changes in the soil and, especially, in the structure and function of soil microorganisms. The choice of an appropriate dose of biochar has economic importance; additionally, it should focus on maintenance of soil biological activity and prevention of loss of biodiversity in the soil environment. Although some research has been conducted to assess the properties and possibilities of using biochar in agriculture, the problem of its impact on the biological activity of soil is unclear. This is evidenced by the limited number of reports on biochar doses and their impact on the comprehensive assessment of soil microbiology. The control of microbial processes occurring in soil supplemented with biochar is essential.

The aim of the study was to assess the effect of application of different biochar doses on soil biological activity and the functional biodiversity of soil microorganisms.

EXPERIMENTAL

Materials

Field experiment

The research was conducted on the only available Polish biochar manufactured on a large scale in the pyrolysis process and patented by FLUID S.A. (Sędziszów, Poland).

The biochar was originated from pyrolysis of wood (various types of wooden parts – deciduous and coniferous wood) and constituted a heterogeneous structure. The biochar composition was as follows: dry mass 96.6%; P_2O_5 83.9 mg/100 g; K_2O 356 mg/100 g; Mg 15.37 mg/100 g; Ca 21.77 g/kg; C 72.63%.

The investigations were carried out in a micro-plot experiment in the period 2016 to 2018. The 1 m^2 micro-plots were filled with luvisols with the particle size distribution of strong loamy sand with the preservation of soil genetic zones.

The experiments were conducted in seven plots supplemented with different doses of biochar. The doses were determined based on the initial carbon content in the soil (1.5% of humus, which corresponds to 0.87% of C in the soil) and increased in individual objects by 2.5%, 5%, 10%, 20%, 50%, and 100% (Table 1).

Table 1. Micro-plot Experiment: Applied Biochar Doses

Combination	Biochar doses [kg/m^2]
Control	0
2.5%	0.21
5%	0.42
10%	0.85
20%	1.70
50%	4.24
100%	8.48

The experiment design also included a control plot with no biochar addition. Biochar was mixed evenly in the 0 to 25 cm soil layer. In the analysed period, winter triticale and spring wheat were grown in 2017 and 2018, respectively. The same agrotechnical treatments of fertilisation and plant protection were applied in all plots. Soil samples for the microbial analyses were collected in June 2017 and 2018 in accordance with the ISO 10381-6 (2009). Soil samples for the chemical analyses were collected at the beginning of the experiment and every year after the harvest of plants. The soil samples were taken in three replicates from the 0 to 30 cm layer, sieved through a 2 mm sieve, and stored at 4 ºC until analysis. During the experiment, the meteorological conditions were controlled. Table 2 shows the monthly temperature and precipitation sums in 2017-2018.

Table 2. Meteorological Conditions during the Growing Season

Sum of precipitation [mm]						
	Months					
Years	IV	V	VI	VII	VIII	IX
2017	62	48	35	150	77	105
2018	30	59	38	122	28	48
Average air temperature [°C]						
2017	7.9	14.7	18.9	19.4	20.1	14.1
2018	13.9	17.8	19.6	21.1	21.0	15.5

Methods

Physico-chemical parameters

The organic carbon was determinate using the Turin method [PB 20.1 Wyd. I – 20.05.1999] and total nitrogen - flow spectrometry, wet sample digestion. The exchangeable cations Ca and Mg was determined according to atomic absorption spectrometry [PN – R –04020:1994]. The available P_2O_5 and K_2O was determined according Egner-Riehm colorimetric method [PN – R - 04023:1996].

Analysis of bacterial communities and activities

Enzymatic activities were determined spectrophotometrically based on the soil dehydrogenase activity using the TTC method (Casida *et al.* 1964) and phosphatase activity with the p-NPP method (Tabatabai 1982). Microbial biomass was determined with the chloroform-fumigation-extraction method (Ghani *et al.* 2003). The results of microbial biomass C and N were calculated according to the following formula: C_{mic}.=EC/kEC, where EC= soluble C in fumigated samples – soluble C in the control (un-fumigated) samples and kEC= 0.45 as well as N_{mic}.= EN/kEN, where EN= soluble N in fumigated samples – soluble N in the control (un-fumigated) samples and kEN=0.54 (Ghani *et al.* 2003).

The soil glomalin content

The glomalin content was determined as proposed by Wright *et al.* (1996). The concentrations of both total glomalins (TG) and easily extractable glomalins (EEG) were determined. The content of glomalin-related soil proteins (GRSP) was determined as well. The glomalin concentrations were determined spectrophotometrically. A detailed description of the methodology and the introduced modifications is provided in Gałązka *et al.* (2017b and 2018).

Assessment of the functional diversity of microorganisms using Biolog EcoPlate

The basic parameters of soil biodiversity and the metabolic profile were determined with the Biolog EcoPlate method (Biolog Inc., Harward, CA). The Biolog EcoPlate method is very popular and often used in assessing the functional diversity of soils. In particular, this method gives good results in assessing changes in functional biodiversity of soils under the influence of the addition of external factors to the soil, *e.g.* biochar. First, 1 g of freshly collected soil samples was suspended in a bottle containing 99 mL of sterile water, followed by shaking for 20 min at 20 °C (Garland and Mills 1991). For further analysis, readings at 144 h were chosen as the most suitable values representing optimum optical density. Average well colour development (AWCD) after 144 h of incubation was calculated for each plate as a mean of optical densities (OD_{590}) from 31 wells (corrected by subtracting the optical density of the control well without a carbon source). The methodology is described in detail in Gałązka *et al.* (2017a). Additionally, AWCD was calculated for each group of substrates, assuming OD_{590} = 0.25 as a threshold value, below which the substrate is considered as unmetabolized. Based on data obtained at 144 h, the Richness (*R*), Shannon-Weaver (*H*'), Evenness (*E*), and average well colour development (AWCD) indices were calculated. The AWCD was evaluated according to Garland and Mills (1999) in accordance with the formula AWCD = Σ (*C*-*R*)/95; where *C* was the absorbency in each well, and *R* was the absorbency in the control well. The Shannon–Wiener (*H*') index was evaluated in accordance with formula $H' = -\Sigma pi(\ln pi)$, pi was the ratio of the absorbance of each well to the absorbency of all wells (Gomez *et al.* 2004).

Statistical analysis

The main statistical analyses were performed using the package STATISTICA.PL (StatSoft. Inc., version 10.0, Tulsa, OK, USA). The collected data was subjected to an analysis of variance (ANOVA) for the comparison of means, and significant differences were calculated according to post-hoc Tukey's HSD test at a $P < 0.05$ significance level. The cluster analysis methods were applied to standardised data from the average absorbance values at 144 h (Biolog EcoPlate, Biolog Inc., Harward, CA, USA). The results were also subjected to principal component (PC) analysis to determine the common relations between the biochar doses and microbial activity and functional diversity.

RESULTS AND DISCUSSION

Assessment of the Microbial Diversity

The basic parameters of the biological activity in the soil were determined in relation to the biochar doses applied (Fig. 1). Soil enzymes are strongly associated with microorganisms and they play an important role in catalyzing reactions indispensable in life processes of soil microorganisms, decomposition of organic residues, circulation of nutrients, and forming organic matter and soil structure. Dehydrogenases are closely related to the microbiological activity of soils, as they occur not only within living cells, where they catalyze the oxidoreductive processes. The highest dehydrogenase activity was determined in the soil supplemented with 5 to 20% of biochar (Fig. 1a).

These results may indicate higher enzymatic activity after using biochar. Dehydrogenases belong to the enzymes displaying strong fluctuations in their activities caused by addition some substances such as biochar to soil, as they are in close relation to microbial activity dynamics. The highest acid phosphatase activity was found in the soil with the addition of 10 and 20% of biochar (Fig. 1b), and alkaline phosphatase exhibited the highest values in the soil treated with a biochar dose of 10% (Fig. 1c). Determining alkaline and acid phosphatase in the soil samples provides a large amount of information about biological characteristics of the soil. Phosphatase activity in soil reflects the activity of enzymes associated with soil colloids and humic substances and frees phosphatases in the soil solution and phosphatases associated with live and dead cells, plants, and microorganisms.

The relationship Increased enzymatic activity in the soil indicates enhanced activity of soil microorganisms, which is reflected in soil quality. Higher enzymatic activity in soil after application of biochar was also reported by Anderson *et al.* (2011). However, it is very important to choose an optimal biochar dose sustaining biological activity. It was found in the present study that the doses over 20% caused a decline in soil enzymatic activity.

The activity of these enzymes showed the highest correlation with microbial biomass and microbial community in soils. The addition of biochar to the soil can increase the organic carbon content, which promotes the growth of microflora, thereby enhancing its mineralisation activity (Anderson *et al.* 2011). An increase in the content of organic matter contributes to increased total soil porosity, which in turn leads to reduction in soil specific density (Vitkova *et al.* 2017).

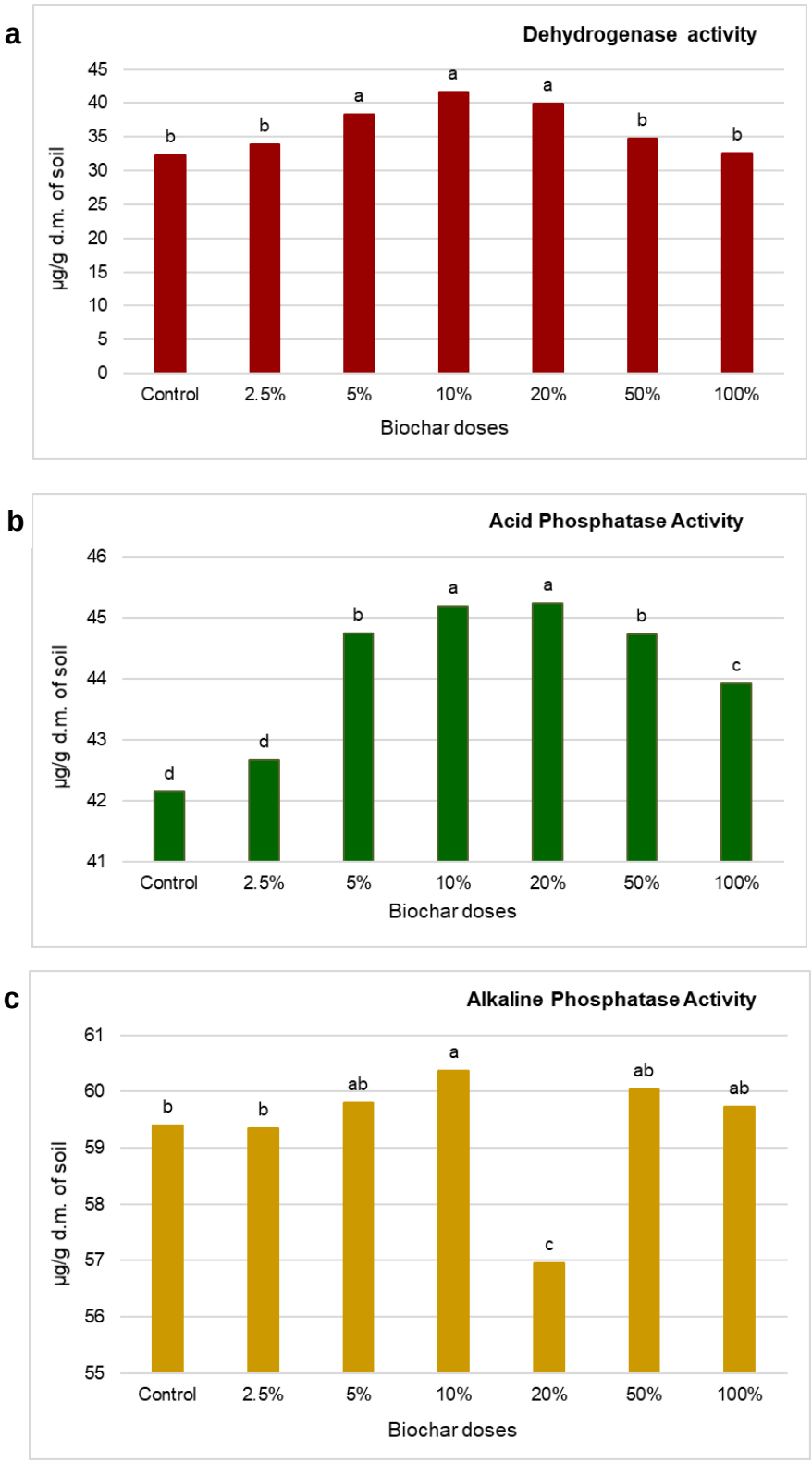

Fig. 1(a,b,c). Basic microbial parameters in soil depending on the biochar doses. Treatment means separated by different letters are significantly different (Tukey's mean separation test, $P < 0.05$), average from 2017-2018 (N=9).

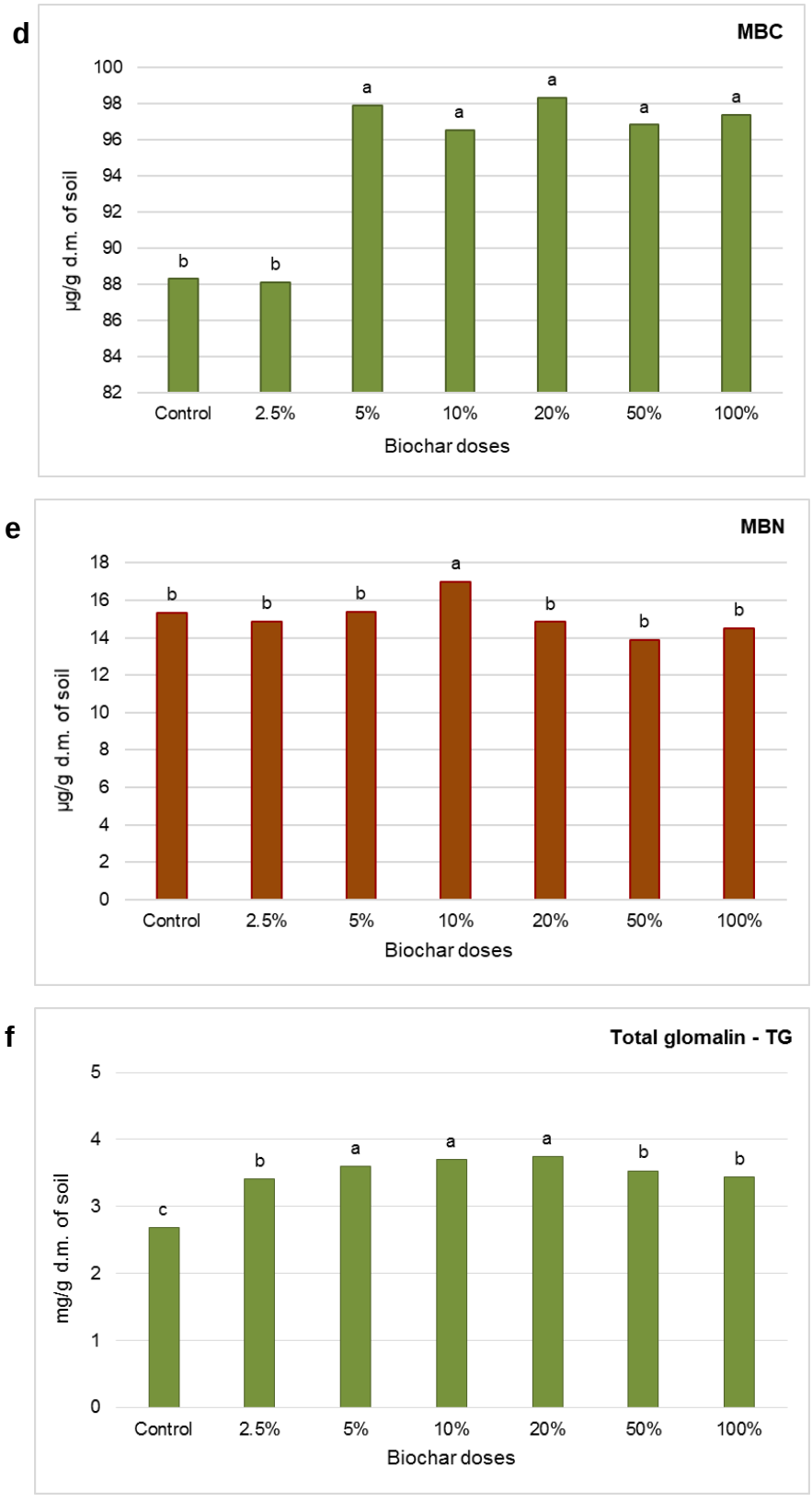

Fig. 1. (d,e,f). Basic microbial parameters in soil depending on the biochar doses. Treatment means separated by different letters are significantly different (Tukey's mean separation test, $P < 0.05$), average from 2017-2018 (N=9).

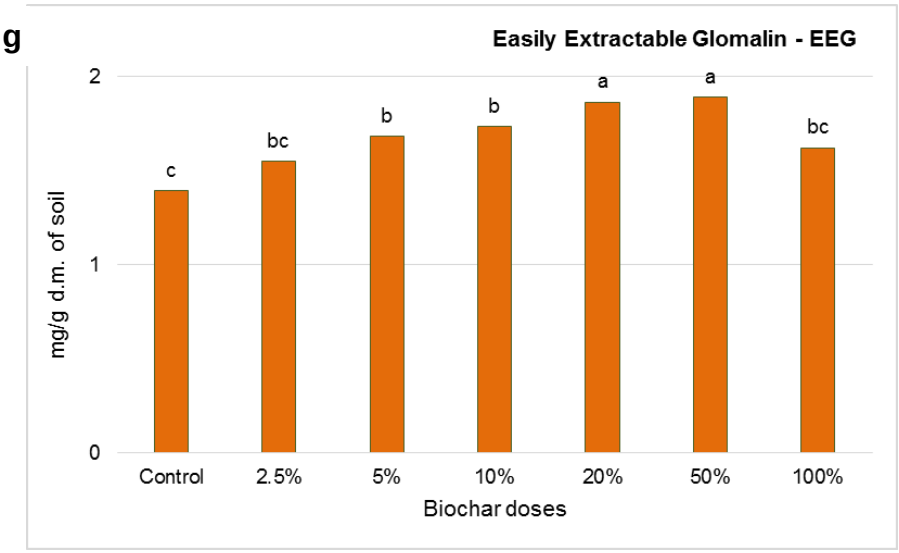

Fig. 1(g). Basic microbial parameters in soil depending on the biochar doses. Treatment means separated by different letters are significantly different (Tukey's mean separation test, $P < 0.05$), average from 2017-2018 (N=9).

There were no significant differences in the MBC content in the microbial biomass between the doses from 5 to 100%. The MBC content increased compared with the control, at 5% biochar addition (Fig. 1d). In turn, the highest nitrogen content in the microbial biomass was noted in the soil supplemented with 10% of biochar (Fig. 1e). The quality of the soil environment and therefore the improvement of the soil structure were greatly influenced by the content of glomalins in the soil (Gałązka and Gawryjołek 2015). Glomalins are soil glycoproteins produced by mycorrhizal fungi. Their content in the soil indicates increased abundance of mycorrhizal fungi and greater soil stability (Gałązka *et al.* 2015, 2017). The highest content of total glomalins (TG) was found in the soil with the 5 to 20% addition of biochar (Fig. 1f), whereas the highest levels of easily extractable glomalins were determined at the 20 to 50% biochar doses (Fig. 1g). The chemical properties of soils in relation to the applied biochar dose is presented in Table 2. The biocarbon doses induced no statistically significant changes in the soil chemical parameters, except for the C_{org} content in soils with the addition of biochar above 20%. The pH of the soil remained at the same level. In addition, an increase in the phosphorus and potassium contents between the samples tested in 2016 and 2018 was observed. The Mg content remained at the same level (Table 3).

Table 3. Chemical Properties of Soils in Relation to the Applied Biochar Dose

Combination	pH_{KCl}		C_{org} [%]		mg per 100 g of soil					
					P_{Egner}		K_{Egner}		Mg	
	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018
Control	6.9	6.5	0.858	0.823	12.5	13.1	3.8	6.2	5.3	5.2
2.5%	6.6	6.5	0.847	0.841	13.0	13.7	4.3	6.5	6.2	5.2
5%	6.8	6.6	0.899	0.870	12.8	14.7	4.0	6.6	5.8	5.3
10%	6.8	6.6	0.910	0.870	13.6	13.9	4.1	6.3	5.7	5.0
20%	6.7	6.6	0.963	0.978	13.9	13.9	4.3	6.2	6.0	5.3
50%	6.7	6.6	0.922	0.997	13.1	14.3	4.0	7.3	6.0	5.3
100%	6.6	6.6	0.846	1.200	12.8	13.9	3.7	7.7	5.7	4.9

The addition of biochar into the soil had a statistically significant impact on changes in the functional soil biodiversity and the utilisation of particular groups of carbon and nitrogen compounds. From the five main groups of compounds used as carbon sources, all groups were utilised most easily by the microorganisms (Fig. 2). Increased utilisation of amino acids at the 10% biochar dose (Fig. 2a) and amines and amides (Fig. 2b) at 5 and 10% of biochar was observed. In the case of carboxylic acids (Fig. 2c) and carbohydrates (Fig. 2d), there was no significant effect of the biochar dose on an increase in the utilisation of these compounds, compared with the control. In turn, there was a significant decrease in the activity of carboxylic acids at the biochar dose of 5 to 10% and carbohydrates at the biochar dose of 10 to 20%. Higher increased utilisation of polymers was observed at 5% and 20% of biochar (Fig. 2e). Increased utilisation of selected carbon groups at the 5, 10, and 20% doses of biochar may indicate higher microbial activity in the soil.

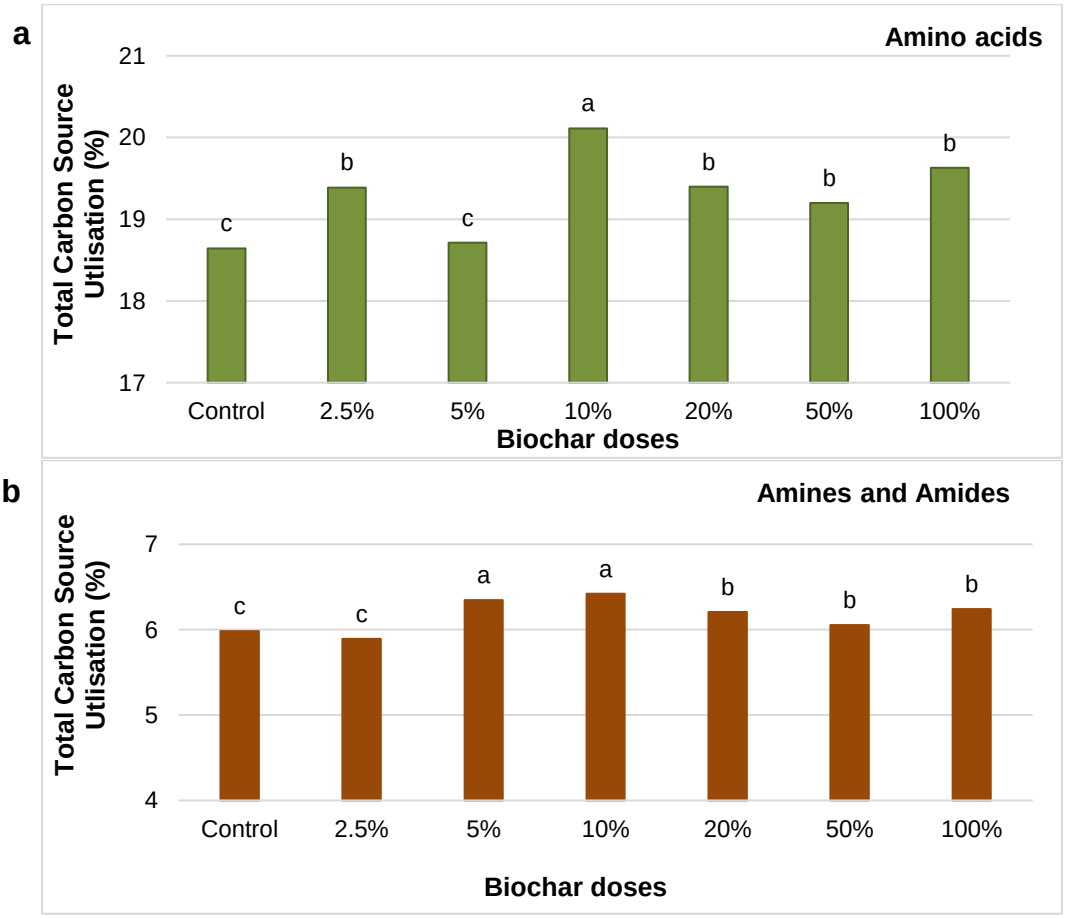

Fig. 2(a,b). Effect of different biochar doses on microbial community catabolic diversity evaluated by substrate utilisation in the Biolog EcoPlate incubated for 144 h. Treatment means separated by different letters are significantly different (Tukey's mean separation test, $P < 0.05$). a). percentage of utilisation of amino acids; b) percentage of utilisation of amines and amides; c) percentage of utilisation of carboxylic and acetic acids; d) percentage of utilisation of hydrocarbons; e) percentage of utilisation of polymers.

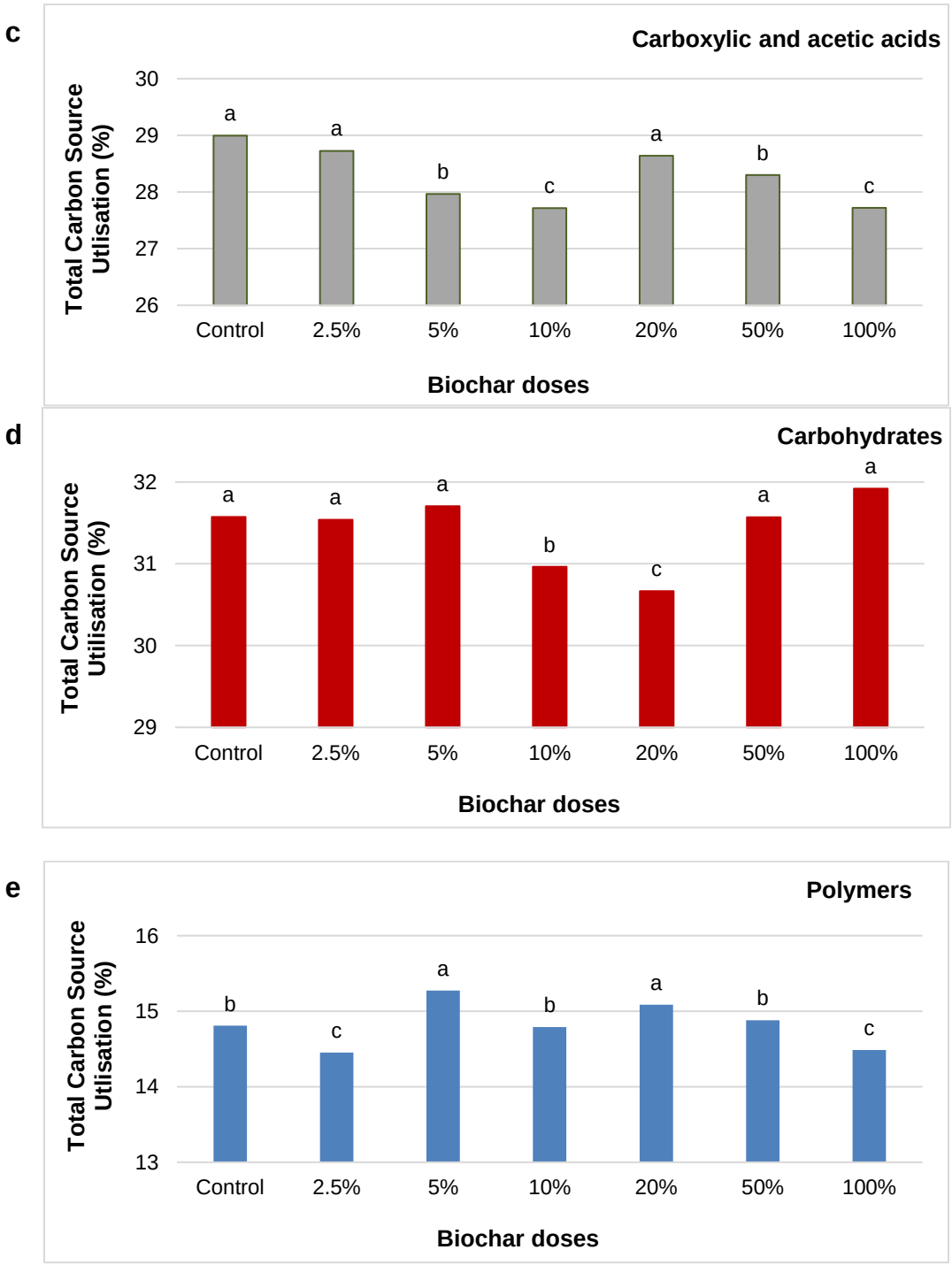

Fig. 2(c,d,e). Effect of different biochar doses on microbial community catabolic diversity evaluated by substrate utilisation in the Biolog EcoPlate incubated for 144 h. Treatment means separated by different letters are significantly different (Tukey's mean separation test, $P < 0.05$). a). percentage of utilisation of amino acids; b) percentage of utilisation of amines and amides; c) percentage of utilisation of carboxylic and acetic acids; d) percentage of utilisation of hydrocarbons; e) percentage of utilisation of polymers.

From the 31 different carbon sources, the highest rates of substrate utilisation was recorded for the soil supplemented with 5% and 10% of biochar, and D-Gluconic Acid gamma-Lactone, Glucose-1-Phosphate, Putrescine, L-Threonine, Glycyl-L-Glutamic Acid, Glycerol Phosphate, L-Serine, L-Asparagine, and D-Malic Acid were the most commonly utilised substrates (Fig. 3). Lower activity in the utlisation of carbon sources was observed using a biochar dose of 2.5%, 50%, and 100%.

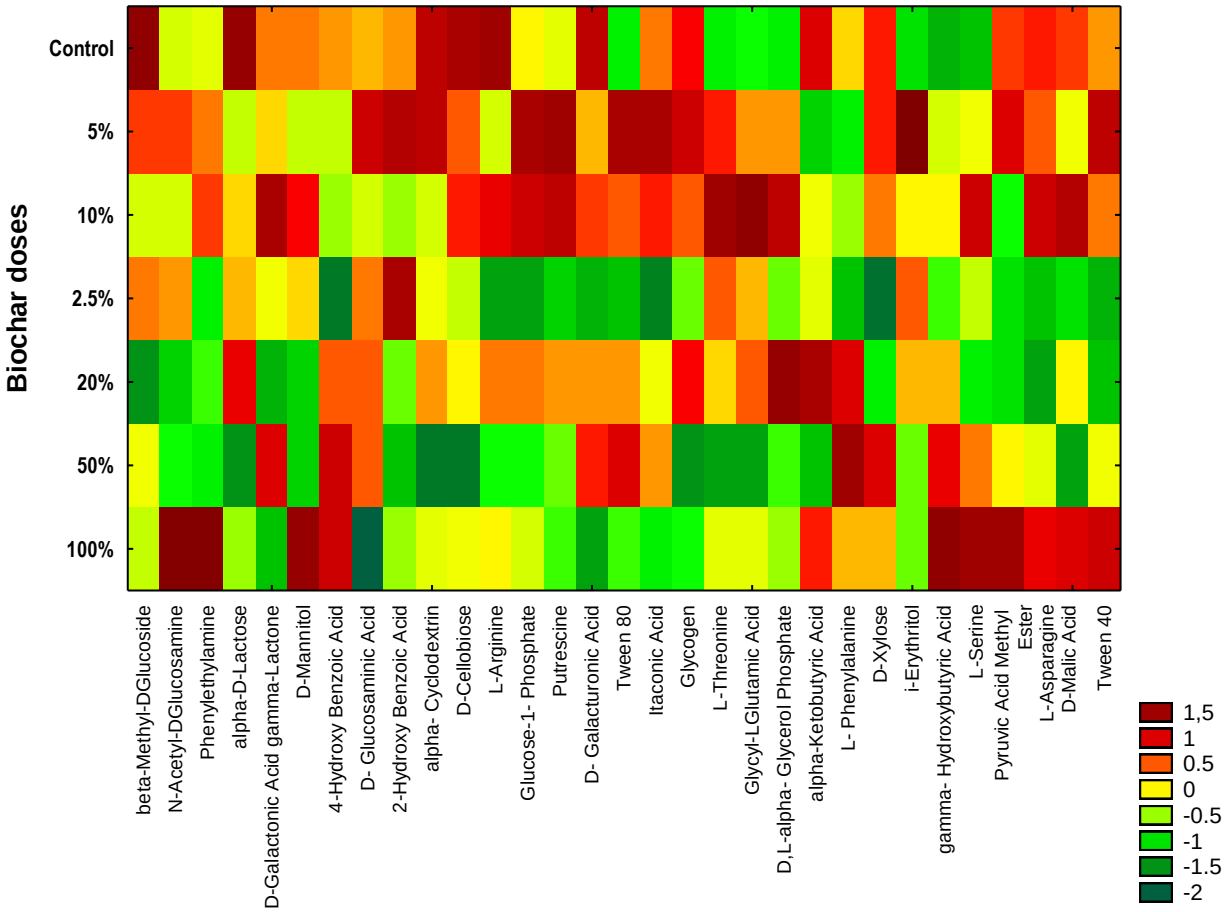

Fig. 3. The carbon utilisation patterns of the 31 substrates located only on the Biolog EcoPlates date incubated for 144 h.

Based on the diversity indices (Shannon-Weaver diversity index (*H*'), substrate richness (*R*), substrate evenness (*E*), and average well colour development ($AWCD_{590}$) in the Biolog EcoPlate incubated for 144 h, the highest soil biodiversity was in the biochar supplementation dose of 5 to 20% (Table 3).

The carbohydrates and amines/amides were characterised by the highest metabolic activity, while the lowest activity was determined for polymers. The samples supplemented with 10% and 20% of biochar were characterised by the highest AWCD values and biodiversity indices (Table 3). These samples were also characterised by the most intense metabolic activity.

Table 3. Effect of Biochar Doses on the Catabolic Diversity of the Microbial Community Evaluated by the Shannon-Weaver Diversity Index (*H'*), Substrate Richness (*R*), Substrate Evenness (*E*), and Average Well Colour Development ($AWCD_{590}$) in the Biolog EcoPlate Incubated for 144 h.

Combination	Shannon-Weaver	*Richness*	*Evenness*	$AWCD_{590}$
Control	3.36 ± 0.003	28.89 ± 0.192	0.990 ± 0.001	1.829 ± 0.077
2.5%	3.37 ± 0.013	28.78 ± 0.509	0.993 ± 0.001	1.758 ± 0.031
5%	3.57 ± 0.008	30.11 ± 0.385	0.991 ± 0.004	1.876 ± 0.057
10%	3.57 ± 0.004	30.78 ± 0.694	0.983 ± 0.005	1.943 ± 0.089
20%	3.66 ± 0.007	29.56 ± 0.385	0.983 ± 0.003	1.988 ± 0.121
50%	3.34 ± 0.015	26.89 ± 0.769	0.993 ± 0.011	1.744 ± 0.084
100%	3.36 ± 0.004	26.67 ± 0.000	0.990 ± 0.001	1.805 ± 0.021

Note: The values are means ± standard error (n = 9).

Cluster analysis, including grouping of the treatments and features, was performed on standardised data from the average absorbance values at 144 h (Biolog EcoPlate). The dendrogram was prepared with scaled bond distances on the axis (Ward's method) and boundary marked according to Sneath's criteria. Based on Ward's cluster analysis, two main groups and four subgroups were distinguished. The first group included soils with the addition of biochar at a dose of 5, 10, and 20%, while the second group included soils with biochar supplementation at a dose of 2.5, 50, and 100% as well as the control soil (Fig. 4).

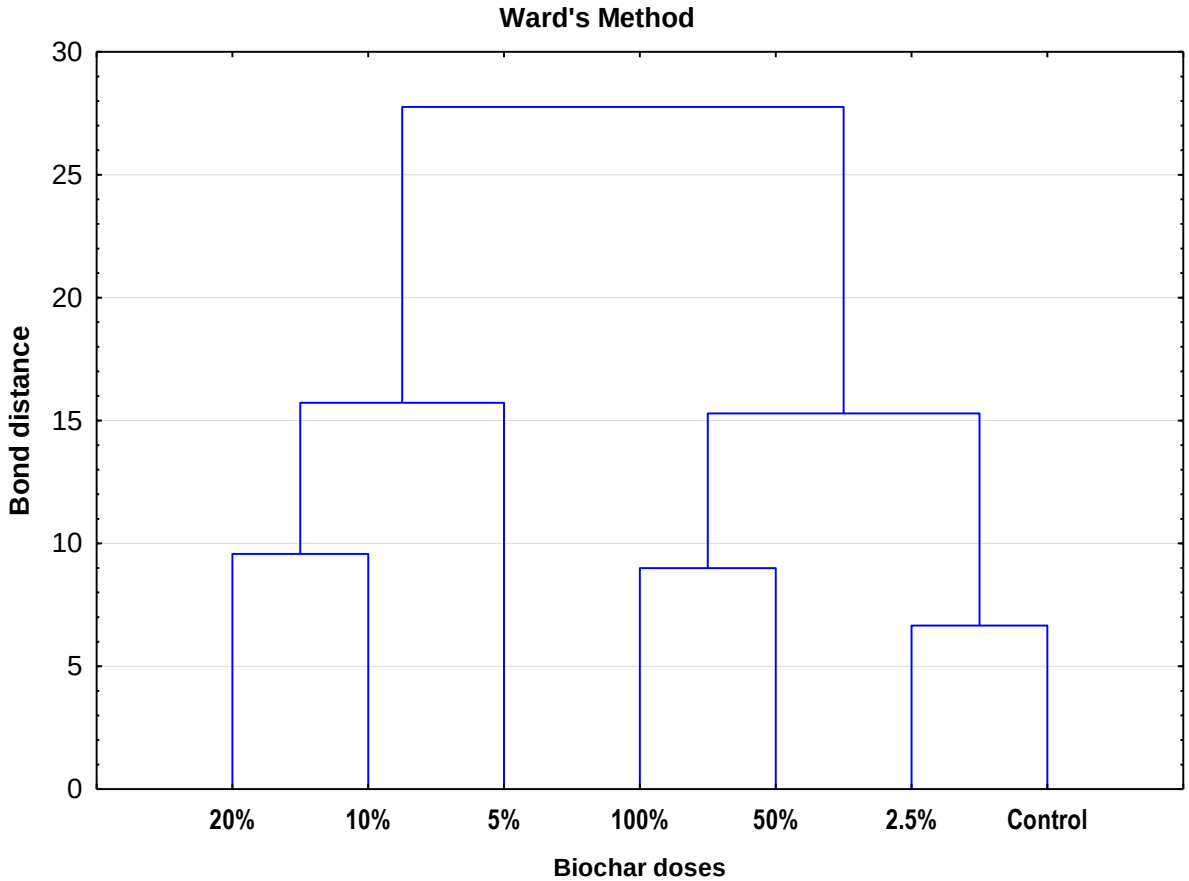

Fig. 4. Dendrogram of the bond distances between the carbon utilisation patterns of the substrates located on the Biolog EcoPlates and the biodiversity indicators.

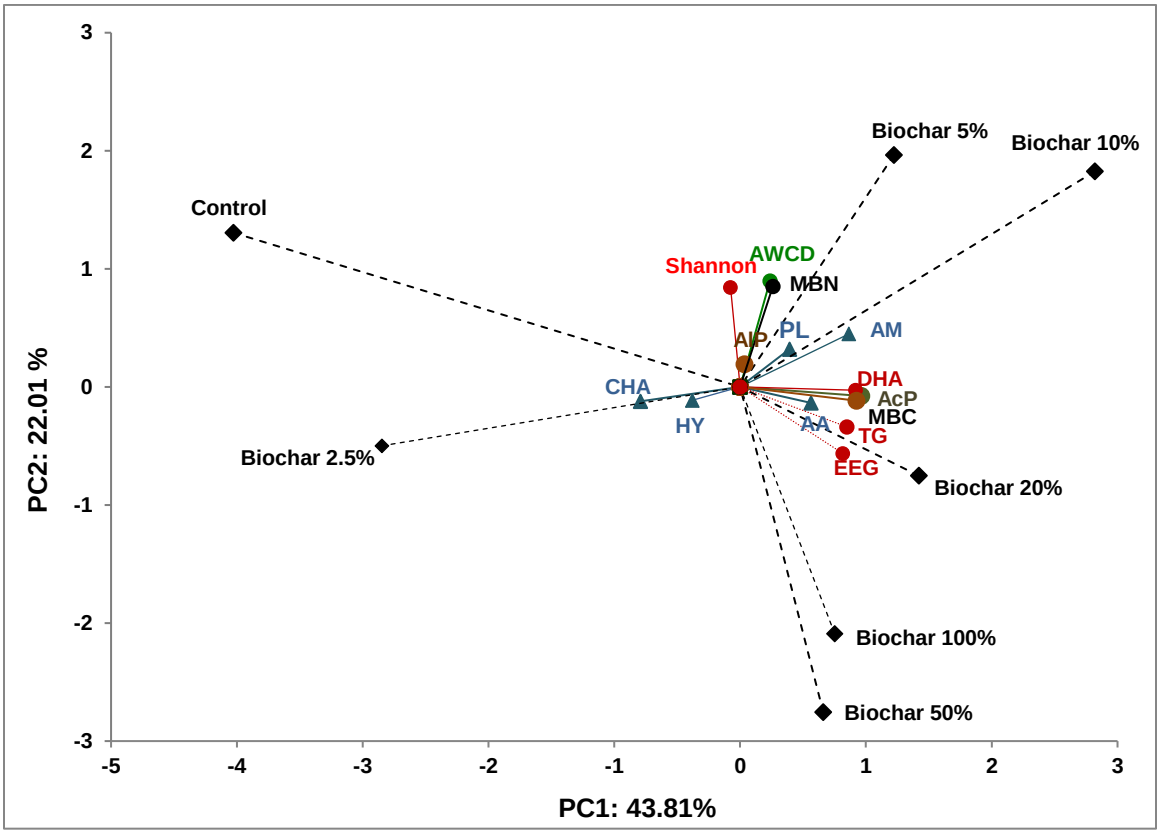

Fig. 5. Results of the PCA analysis taking into account of basic microbial parameters, biodiversity indices, and Biolog EcoPlates; average from 2017-2018. Note: Dehydrogenases (DHA) [ug formazan/g d.m. of soil/24 h]; alkaline phosphatase (AlP) [ug p-nitrophenol/g d.m. of soil/h]; acid phosphatase (AcP) [ug p-nitrophenol/g d.m. of soil/h]; microbial biomass carbon (MBC) [ug/g d.m. of soil]; microbial biomass nitrogen (MBN) [ug/g d.m. of soil]; total glomalin content (TG) [mg/g d.m. of soil]; easily extractable glomalin content (EEG) [mg/g d.m. of soil].

Three main groups were distinguished based on the principal component analysis PCA, with strong positive correlations between the biological activity parameters and the biochar doses of 5, 10, and 20% (Fig. 5). There were also strong positive correlations observed between such parameters of soil biological activity as enzymatic activity, carbon content in the microbial biomass, and glomalin content. A strong correlation between parameters of soil biological activity and biodiversity indicators was found in the case of biofuel addition to the soil in an amount from 5 to 20%. Based on the obtained results, it can be concluded that the addition of biochar to the soil in an amount of 5 to 20% may increase biological activity and biodiversity in the soil.

Cui *et al.* (2013) unequivocally demonstrated that addition of 20 and 40 tons/ha of biochar to cadmium- and lead-contaminated soil caused a significant increase in the enzymatic activity of soil microorganisms and thus improved the quality of the soil environment. The authors reported an increase in the activity of cellulases, urine enzymes, and phosphatases, as well as reduced cadmium and lead bioavailability and an increased total number of actinomyces and fungi in the soil. Changes in the biological activity of soils after biochar application were demonstrated by other authors as well. Shi *et al.* (2015) reported significant reduction in the activity of the nitrification inhibitor dicyandiamide in soil after the application of two types of biochar of plant origin (biochar produced from eucalyptus wood as well as coconut and rice straw). Their investigations were carried out

on two different soils: Cambisol (pH 7.14) and Latosol (pH 4.83). The inhibition of dicyandiamide resulted in significant changes in nitrogen transformations in the soil and increased the processes of soil nitrogen metabolism and bioavailability.

The results obtained in this work coincide with the results of other authors. Mend et al. (2019) confirmed the impacts of three levels of the wheat straw-derived biochar (1%, 2%, and 4% (w/w) on rhizosphere microbial communities of the wheat (*Triticum aestivum*) seedlings under the fomesafen stress using high-throughput sequencing. Compared with the three addition amounts, amendment with 2% of biochar exhibited the best effects of stimulation of microbial community structure in soil contaminated with fomesafen. Also it was shown that the level of biochar application influences the structure and diversity of soil microbiome and plant performance under abiotic stress conditions (Mend *et al*. 2019). The research of Mierzwa-Hersztek *et al.* (2019) also confirmed that the addition to the soil of wheat straw (WS) and wheat straw biochar (WSB) (300 °C) at 0.2%, 0.5%, 1%, and 2% doses can increase the dehydrogenase activity index, carbon and nitrogen fractions contents, and also changed the microbial community composition (bacteria, fungi, actinobacteria, *Azotobacter* spp., ammonifers, nitrifers, denitrifers, *C. pasteurianum*). Similar results were obtained by Quan *et al*. (2018) who confirmed that the addition of biochar in content 20 t bamboo biochar ha^{-1} significantly increased soil MBC and bacterial diversity, while biochar BC40 (40 t bamboo biochar ha^{-1}) significantly decreased soil MBC but increased bacterial diversity.

CONCLUSIONS

1. The highest microbiological activity determined with the Biolog EcoPlate analysis and the basic biological activity parameters was exhibited by the soil supplemented with 5 to 20% biochar.
2. The addition of 20% biochar contributed to a statistically significant increase in the total carbon content in the microbial biomass, dehydrogenase activity, acid phosphatase activity, and the overall content of total and easily extractable glomalins.
3. The highest values of the Shannon and AWCD indices were calculated for the soil supplemented with biochar at a dose from 5 to 20%.

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