INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2023, 37, 111-128
doi: 10.31545/intagr/158897

Chemical, biological and respirometry properties of soil under perennial crops fertilized with digestate**

Wioleta Radawiec[1]*, *Janusz Gołaszewski*[2], *Barbara Kalisz*[3], *and Sebastian Przemieniecki*[4]

[1]Department of Genetics, Plant Breeding and Bioresource Engineering, Faculty of Agriculture and Forestry,
[2]Center for Bioeconomy and Renewable Energies,
[3]Department of Soil Science and Microbiology, Faculty of Agriculture and Forestry,
[4]Department of Entomology, Phytopathology and Molecular Diagnostics, Faculty of Agriculture and Forestry,
University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10-719 Olsztyn, Poland

Received September 19, 2022; accepted January 3, 2023

Abstract. The aim of this study was to evaluate the effect of thea digestate which originated fromon a widely cultivated perennial crop (*Miscanthus giganteus*). Cchanges in the physicochemical properties of the soil, the abundance of soil microorganisms, and soil respiration were all assessed. Three types of digestate: fresh in the liquid form, processed – dried and pyrolysed, were tested and compared with mineral fertilization and an unfertilized control. Soil samples were taken in spring 2014, summer 2015 and autumn 2016. In total, 14 variables were analysed: total carbon, hot water extractable carbon, total nitrogen, C/N ratio, phosphorus, magnesium, potassium, pH, bacteria, fungi, O_2 intake, CO_2 emission, total carbon mineralized after 7- and 100-day-long respiration. Overall, regardless of the form of the digestate, the chemical parameters of the soil improved, although the extent of the improvement depended on the applied form of the digestate. The highest TC 12.79, N 1.29, K 257.95 and P 149.96 g kg^{-1} DM were determined in the plots fertilized with biochar. All digestate forms had a positive influence on the bacterial DNA abundance, and biochar also affected the abundance of the fungal DNA and the potential carbon sequestration in the soil. Pyrolysed digestate may have a particular value in the fertilization of perennial industrial crops.

*Corresponding authors e-mail: wioleta.radawiec@uwm.edu.pl
**This research is the result of a long-term study carried out at the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering, topic number 30.610.007-110.
Project financially supported by the Minister of Education and Science under the program entitled "Regional Initiative of Excellence" for the years 2019-2023, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN."

Keywords: digestate, soil chemical properties, soil respiration, *Miscanthus giganteus*

INTRODUCTION

The growth in agricultural biogas production has stimulated more intensive research into the rational management of digestate, including its use for fertilization purposes. The importance of digestate application to soil is being determined across the world (Nakamya *et al.,* 2020; Castro *et al.,* 2017; Roubík *et al.,* 2018; Ogwang *et al.,* 2021). The wide array of substrates that are processed in agricultural biogas plants produces a qualitative diversity of digestate. Depending on the substrate used, the physical and chemical parameters of the digestate are highly varied. Numerous studies have provided detailed information concerning the chemical composition of digestate and its impact on soil properties (Alburquerque *et al.,* 2012; Battista *et al.,* 2019; Bułkowska *et al.,* 2012; Goberna *et al.,* 2011; Jurgutis *et al.,* 2021; Kaparaju and Rintala, 2008; Karimi *et al.,* 2022; Odlare *et al.,* 2008; Panuccio *et al.,* 2021; Pranagal *et al.,* 2017; Siebielec *et al.,* 2018; Smith *et al.,* 2007; Tambone and Adani, 2017; Walsh *et al.,* 2012).

Digestate may be applied directly to the soil either in an unchanged form or having been separated into two phases, solid and liquid (Akhiar *et al.,* 2017; Battista *et al.,* 2019;

Tambone and Adani, 2017). Currently, the latter digestate application method is gaining in importance owing to its beneficial effects on agriculture and the environment as it reduces the loss of nutrients through runoff or oxidation, and is capable of generating positive economic outcomes by limiting the costs of both storage and transporting the digestate to plantations (Battista *et al.,* 2019; Nelissen *et al.,* 2014; Zheng *et al.,* 2013). However, despite increasingly effective separation methods, the solid mass obtained from digestate still contains quite a large fraction of water, which favours the metabolic activity of microbiota and therefore the undesirable processes of organic matter degradation. As a consequence, a longer storage period for the digestate may lead to a decreased fertilizing value and also create a possible threat to the environment (Baldé *et al.,* 2016; Bruun *et al.,* 2011).

A prospective solution in terms of the direct fertilization use of fresh or composted digestate could be the conversion of digestate solid mass to biochar using pyrolysis (Troy *et al.,* 2013). During pyrolysis, biomass undergoes numerous chemical reactions, which lead to a change in the chemical composition of the substance so that the biochar acquires several new characteristics (Radawiec *et al.,* 2014). The most significant changes in the context of the soil application of biochar are connected with an increase in the total carbon concentration, including the fraction of bound/stable carbon, which has a direct influence on the carbon stability in soil. The fraction of stable carbon has either an aromatic or a heterocyclic character, which makes it highly tolerant to decomposition (Bornemann *et al.,* 2008; Lehman *et al.,* 2009). In turn, the labile carbon fraction in biochar is composed for the most part of carbonates and organic compounds soluble in the soil solution. The proportion of the carbon fractions (stable and labile) conditions the degradability of the biochar and the rate of mineralization in the soil (Lehman *et al.,* 2009). According to Bai *et al.* (2014) and Spokas (2010) the content of bound carbon has a fundamental effect on the stability of carbon in the soil environment, and therefore on effective carbon sequestration in the soil.

Biochar can have a positive effect on the properties and functions of soil in the environment, as proven by numerous studies (Blackwell *et al.,* 2009; Burrell *et al.,* 2016; Hardie *et al.,* 2014; Lehmann *et al.,* 2006; Ojeda *et al.,* 2015; Razzaghi *et al.,* 2020; Tryon, 1948). The high temperature at which biochar is produced results in the formation of a large inner surface area and a large cation exchange capacity (Jindo *et al.,* 2014). Due to these qualities, biochar can play an important role in the remediation of soil pollutants, such as pesticides, heavy metals and polycyclic aromatic hydrocarbons, therefore it has a positive effect on the functioning of the soil and the entire environment (Beesley *et al*., 2011; Bornemann *et al.,* 2008; Bruun *et al.,* 2011; Cabrera *et al.,* 2011; Galvez *et al.,* 2012; Lehmann, 2007; Lehmann *et al.,* 2011; Smith *et al.,* 2007). Improving the physical properties of the soil and the remediation of harmful compounds in the soil are examples of the beneficial influence of biochar on the environment. Research has shown that biochar can either directly or indirectly improve the fertility of soil, thereby improving the growth, development and yields of crops. The adsorption of nutrients in the biochar structure contributes to the improvement in the availability of essential micro- and macroelements to plants (Galvez *et al.,* 2012; Lehmann *et al.,* 2006, 2011; Smith *et al.,* 2007). The application of biochar to the soil results in the improved phytoavailability of phosphorus, potassium as well as total nitrogen and, to a lesser extent, of calcium and copper (Lehmann *et al.,* 2011; Schulz and Glaser, 2012). It has also been demonstrated that the application of biochar to acid soils raises soil pH (Topoliantz *et al.,* 2002). The improved fertility of the soil may also be a consequence of the participation of biochar in the formation of humic substances, and the stabilization of humus and soil aggregates (Bai *et al.,* 2014; Bornemann *et al.,* 2008; Jining *et al.,* 2014). Moreover, the improved availability of nutrients to plants stems from the direct application of nutrients to plants together with the biochar added to the soil, the increased retention of nutrients in the soil and their slower release, and from positive changes in soil microbiological dynamics. The porous structure of the biochar ensures physical protection while the inner surface of the pores with their ability to adsorb soluble organic matter provides a suitable environment for colonization and the development of soil microorganisms: bacteria, fungi and actinomycetes (Lehmann *et al.,* 2011).

Soil microorganisms play a decisive role in the decomposition of soil organic matter carbon (SOC); they are also key regulators of the dynamics of SOC transformation in the soil. By constantly competing for nutrient substrates, fungi and bacteria have developed various mechanisms to adapt to the prevailing soil conditions. According to De Boer *et al.* (2005), fungi have adopted better to the decomposition of complex and hardly degradable lignocellulose materials (including lignin), while bacteria utilize easily degradable organic compounds (such as sugars). Tian *et al.* (2015) maintain that organic carbon fractions play a significant role in the shaping of the functional diversity of soil microorganisms. Likewise, the functional diversity of microorganisms in the soil, as measured by their catabolic potential, depends on the type of organic carbon available (Degens, 1998). The main consumer of organic matter can therefore influence the direction and intensity of decomposition processes in the soil, thereby affecting the carbon cycle. The soil microbial community largely regulates the relative proportions of the different types of carbon fractions (Six *et al.,* 2006). Changes in the structure and abundance of fungi in the soil induced by intensive soil use was found to determine the chemical composition of SOC on bamboo plantations (Li *et al.,* 2017). The study conducted by Zhang *et al.* (2015) showed that the presence

of different microbiological taxa in the soil determines the carbon fractions. Thus, changes in the composition and functioning of the soil microbial community in response to different agricultural treatments should play an important role in the determination of the stability of the carbon fraction in the soil (Zhang *et al.,* 2015).

The chemical properties of fresh digestate and its influence on soil properties have been the subject of numerous studies. The time limitations of soil application have made it necessary to improve the methods of the "fixation" of digestate, thereby allowing it to be stored for a longer period without the loss of nutrients and environmental pollution.

The aim of the study was to assess the effect of the addition of processed digestate (biodrying, pyrolysis) to the soil on its physicochemical and biological properties, as well as the rate of degradation in the soil. The research was used to verify the research hypothesis that the greatest effect with regard to improving the physicochemical and biological properties of the soil will be demonstrated for pyrolysed digestate. Moreover, the pyrolysed digestate will show a greater resistance to decomposition in the soil as compared to the application of fresh digestate and dried digestate.

MATERIALS AND METHODS

The test plant was the giant miscanthus (*Miscanthus giganteus*). It is a perennial crop used in industrialized agriculture, it is grown for combined biomass production and the ecological restoration of contaminated and marginal land (Wang *et al.,* 2020). Field experiments were carried out in 2013-2016, in the fields of the Teaching and Research Station of the University of Warmia and Mazury located in Łężany in Poland (53°967'N 21°133'E). The soil of the experimental field was classified as a Luvisol. The average air temperatures in the consecutive years of the experiment (January-December) and plant growing seasons (April-October) were similar, they ranged within 8.5-9.0°C and 13.4-14.2°C, respectively. Larger variations in rainfall were noted during the years of the study. While the average precipitation in 2014 and 2015 was similar for the whole year and also during the growing season: 550 and 572 mm in 2014 and 240 and 372 mm in 2015, respectively, the results were much higher in 2016, reaching 853 mm for the whole year and 642 mm for the plant growing period.

Five fertilization treatments were tested in the experiment (factor A); fresh digestate in a liquid form (fresh digestate, Fd), the separated and dried solid fraction of the digestate (dried digestate, Dd), the separated and dried solid fraction of the digestate submitted to pyrolysis (pyrolysed digestate, Pd), and for reference – mineral fertilization with an ammonium salt (NPK) and an unfertilized plot (control treatment, Ct). Fertilization was applied in 2013, 2014 and 2015, while the year 2016 was used as a source of reference data for testing the residual effects of fertilization. Soil samples were collected on three dates: spring 2014 (April 11, 2014) (2014Sp), summer 2015 (August 17, 2015) (2015Su) and autumn 2016 (October 30, 2016) (2016Au) (factor B). The characteristics of the digestates are presented in Table 1.

Digestate was obtained from a pilot agricultural biogas plant located at the Teaching and Research Station of the UWM in Bałdy. The digestate used in that biogas plant was bovine manure with added maize silage. Fresh digestate was applied directly to the field. Dried digestate was obtained by dewatering fresh digestate using a screw separator equipped with a slotted filter, after which the solid fraction was biodried. Pyrolysed digestate was produced by heating the solid fraction of digestate in a flow-through reactor at a temperature of 400°C. The doses of digestate were established with reference to a dose of nitrogen equal 85 kg N ha^{-1}. Mineral fertilization was applied in the following doses: N 68.0, P 22.7, K 62.8 kg ha^{-1}. Fertilization was performed in 2013, 2014 and 2015. The soil studies commenced in the spring of 2014 (in May, before fertilization) and they were continued in summer 2015 and autumn 2016.

The research was based on the results of laboratory analyses of soil samples with respect to the chemical, biological and respirometric properties of the soil. In total, 14 variables were analysed, including: total carbon (TC), hot water extractable carbon (HWC), total nitrogen (TN), the C/N ratio, phosphorus available to plants (P), magnesium available to plants (Mg), potassium available to plants (K), pH, bacteria load (BL), fungi load (FL), O_2 intake (O_2), CO_2 emission (CO_2), total carbon mineralized after 7 days of respiration (TC-7) and total carbon mineralized after 100 days of respiration (TC-100).

The content of total carbon was determined using a CHS 500 analyser (Eltra, Germany). The fraction of labile carbon – hot water extractable – was determined according to the method described by Sparling *et al.* (1998) and the extracts

Table 1. Characteristics of digestates

Fertilizer	Solids %	TN	$N\text{-}NH_3$	TC	C_{fixed}	Organic matter	C/N	P_2O_5	K_2O	MgO	pH
		g kg^{-1} DM									
Fresh digestate (Fd)	5.45	71.56	33.58	420.0	185.4	689.7	5.9	23.85	66.06	12.84	8.24
Dried digestate (Dd)	58.23	35.79	1.19	480.0	240.7	764.3	13.4	12.65	31.60	2.05	9.03
Pyrolysed digestate (Pd)	84.17	24.83	0.41	520.0	412.4	820.5	20.9	15.33	26.02	8.79	8.86

obtained were analysed using a Multi C/N 3100 analyser (Analityk Jena, Germany). The content of total nitrogen was determined with the Kjeldahl method using K435 mineralization and B324 distillation sets (BUCHI, Switzerland). The soil pH was determined according to ISO 10390:1997 with a potentiometric method using a CPC-505 pH-meter (Elmetron, Poland), coupled with an IJ44A electrode (Elmetron, Poland). The content of plant available phosphorus and potassium were determined using the Egner-Riehm method, in accordance with this method, an SQ 118 (Merck, US) photometer was used with a Jenway PFP7 flame photometer (VWR, Germany). The content of plant available magnesium was determined using the Schachtschabel method with a GBC 932 Plus atomic absorption spectrometer (GBC Scientific Equipment Ltd, UK).

In order to determine the abundance of microorganisms in the soil microbial community, an assay of the genetic material was conducted using Real-Time PCR on a RotorgeneQ (Qiagen, Germany) Real-Time PCR. The analysis was carried out with TaqMan technology using a molecular probe (for total Bacteria) and SybrGreen (for total Fungi). Prior to the isolation of the genetic material, 0.5 g of the soil was homogenized in a TissueLyserLT homogenizer (Qiagen, Germany), after which the extraction of the total DNA was carried out using a GeneMATRIX Soil DNA Purification Kit (EurX, Poland), according to the manufacturer's instructions. The determination of the total quantities of DNA was accomplished using a Qubit 2.0 fluorometer (Invitrogen Life Technologies, USA), and the quality of the products obtained was verified with a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). The RealTime PCR reaction of the fragments of the gene encoding 16S rRNA of bacteria was conducted using specific starters BAC338F and BAC805R and a BAC516F probe (Martin and Rygiewcz, 2005). Amplification was conducted using the reaction mixture Maxima Probe qPCR Master Mix 2x (Thermo Fisher Scientific, Waltham, MA). In order to assess the quantities of fungi, a Real-Time PCR reaction was run on a fragment of the ITSI region with the starters NSI1 and 58A2R (Martin and Rygiewcz, 2005) and a reaction mixture of SYBR Green qPCR Master Mix 2x (Thermo Fisher Scientific, Waltham, MA).

The RealTime PCR reaction for bacteria comprised the following steps: preliminary denaturation at 95°C for 10 min, then 45 cycles, followed by denaturation at 95°C for 15 s, attachment of starters and elongation (60°C, 1 min). In turn, the RealTime PCR reaction for fungi consisted of these steps: preliminary denaturation (95°C, 10 min), 40 cycles, denaturation (95°C, 15 s), attachment of starters (52°C, 30 s), elongation (72°C, 30 s) and collecting fluorescent data at 78°C for 15 s. The melting curve analysis comprised the steps of denaturation at 95°C for 15 s, elongation at 60°C for 1 min, and melting stepwise at 0.3°C intervals to 95°C for 15 s. A standard curve of amplification, which allows for the determination of the initial content of nucleic acids, was derived using the genomic DNA from pure cultures of *Bacillus subtilis* (Bacteria) and *Fusarium culmorum* (Fungi) respectively for the two domains. Determinations of the quantities of gene copies in the analysed samples were based on comparisons with the standard curves. The efficiency of the reactions was 0.99 and 0.98 for Total Bacteria and Total Fungi, respectively.

In order to estimate the intensity of the mineralization of fertilizers in the soil, respirometry analyses of the oxygen processes in the soil were conducted using a Micro-Oxymax respirometer (Columbus Instruments International, US). The tests consisted of a 100-day incubation period during which the amounts of absorbed O_2 and released CO_2 were recorded. The soil samples were placed in bioreactors with a capacity of 250 ml, and then secured against light with aluminium foil. The fresh mass of the soil in a pot was 200 g. The dry matter content determined in the soil was used to establish the water content using the weight method at a level of 20%. Two bioreactors were set up for each fertilization treatment. The soil samples prepared as described above were incubated at a room temperature of 21°C for 100 days (Diochon *et al.,* 2013). During that time, the cumulative amounts of CO_2 produced and O_2 consumed (μg g^{-1} DM of soil) were recorded. The results concerning CO_2 emissions were then converted to the C content according to the molar mass.

The assumptions of a normal distribution and the homogeneity of variance of the results from the fertilization plots were tested for each variable. Next, a two-factorial analysis of variance was conducted in order to evaluate the significance of the influence of fertilization (factor A), season (factor B) and the interactions between these factors on soil properties. The following steps in the statistical processing of post-hoc comparisons of means were performed using Tukey's test (HSD) at a significance level of $p<0.01$. Pearson's correlation coefficients were used to evaluate the mutual relationships between the variables, followed by a PCA (Principal Component Analysis). All statistical analyses were carried out using the STATISTICA (StatSoft, Inc., 2014, Poland) software.

RESULTS

The analysed soil variables along with the basic statistics as well as the *F*-statistics and the significance level for the fertilization x season interaction according to the ANOVAs of the chemical and biological soil parameters are given in the Supplement (Table S1 and S2). The soil tested in the experiment was characterized by a low total carbon content (9.5 g kg^{-1} DM) and a low total nitrogen content (1.12 g kg^{-1} DM). Conversely, it was found to be rich in macronutrients, especially phosphorus and potassium (89.9 and 36.9 g kg^{-1} DM, respectively). With respect to the

abundance of bacteria in the soil as well as its respiratory parameters, these characteristics were noted to be highly variable.

The fertilization x season interaction for all of the studied soil chemical and biological parameters was highly significant (Table S2) which indicates that the variation in parameters between seasons was specific to the fertilization applied.

The application of digestate, regardless of the form, affected the analysed chemical properties of the soil (except HWC), in addition to which it raised the pH and increased the soil content of nutrients. The effects which occurred in particular seasons depended on the type of digestate applied. Figure 1 shows the average values of the variables for the type of fertilizer x season interaction. The highest average concentration of carbon was determined for soil

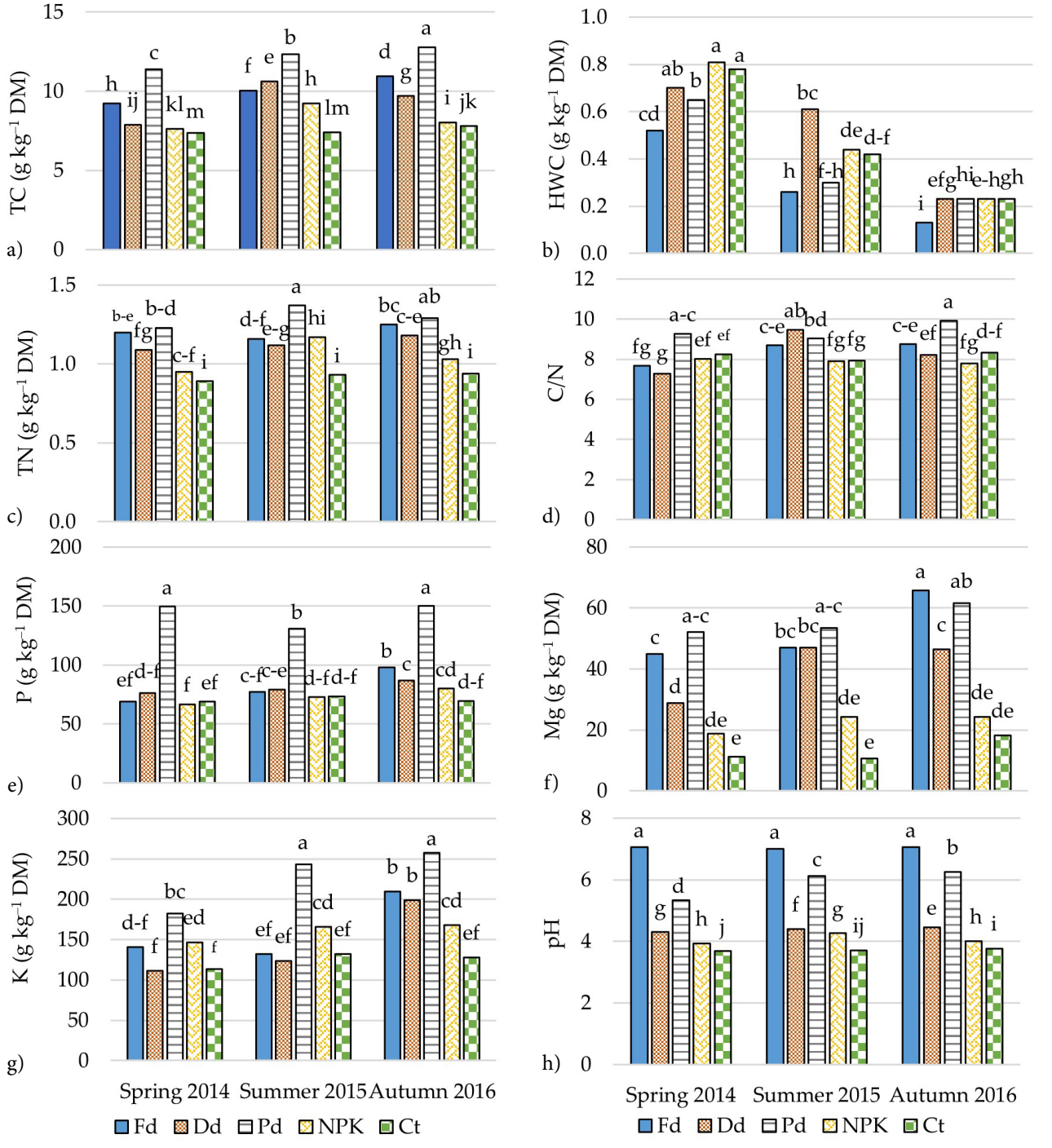

Fig. 1. Changes in the chemical properties of the soil in the seasons of spring 2014, summer 2015 and autumn 2016, depending on the type of fertilizer used (Fd – fresh digestate, Dd – dried digestate, Pd – pyrolysed digestate, NPK – mineral fertilizer, Ct – control treatment). The use of the same letter indicates insignificant differences between means at $p<0.01$.

amended with pyrolysed digestate (12.18 g kg^{-1} DM), and slightly lower ones were found for soil following the application of fresh digestate (10.08 g kg^{-1} DM) and dried digestate (9.41 g kg^{-1} DM). During the 3-year-long experiment, the highest total carbon concentration in the soil was found in the plots fertilized with dry digestate and mineral fertilizer and occurred in the summer of 2015, whereas the TC concentration in the other plots increased gradually and peaked in the autumn of 2016. The content of labile carbon (HWC) decreased in all fertilizer plots. The average HWC value was highest in the plots fertilized using dry digestate (0.57 g kg^{-1} DM), and lowest (lower than in the NPK and the control treatments) in the ones fertilized with pyrolysed digestate (0.39 g kg^{-1} DM) and also the fresh one (0.30 g kg^{-1} DM). However, the plots fertilized with pyrolysed and fresh digestate were found to have the highest concentration of total nitrogen, *i.e.* 1.30 and 1.20 g kg^{-1} DM, respectively. With regard to the total nitrogen content, an increase was observed in the plots fertilized with fresh and dry digestate, while the soil supplied with mineral fertilizer and pyrolysed digestate was found to have the highest total nitrogen content in summer 2015. Similarly, pyrolysed, fresh and dry digestate contributed to the enrichment of the soil with elements essential to crops, such as phosphorus, magnesium and potassium, and the values obtained in these experimental treatments were much higher than the ones determined for the mineral fertilization and control treatments. The highest average concentrations of phosphorus (143.43 g kg^{-1} DM), potassium (227.89 g kg^{-1} DM) and magnesium (55.74 g kg^{-1} DM) were achieved in the plots fertilized with pyrolysed digestate. Fertilization with digestate also resulted in an increase in the soil pH. A neutral soil reaction was noted after the application of fresh digestate, while a slightly acidic one occurred in the variants fertilized with pyrolysed and dried digestate.

All of the applied fertilization treatments produced a differentiated abundance of bacteria and fungi. The study demonstrated a significant increase in the abundance of fungal DNA in all fertilization treatments (Fig. 2a). The greatest increase relative to the soil samples taken for analysis in the spring of 2014 occurred after the application of liquid digestate (a 2.5-fold increase) and pyrolysed digestate (a 2.2-fold increase), and the highest abundance of fungi was noted in summer 2015 following the application of liquid digestate. In general, the abundance of fungi in the NPK fertilized and also in the control plots in spring and autumn was higher than in the plots fertilized with dried and fresh digestate. At the same time, the abundance of bacteria in autumn 2016 was lower than in spring 2014 (Fig. 2b). The greatest decrease in the content of bacteria should be linked to the intensive rise in the abundance of fungi, which, through competition for nutrients are an antagonistic group of microorganisms towards bacteria. This is confirmed by a strong, negative correlation between the abundance of fungi and bacteria (–0.768) (Table 2).

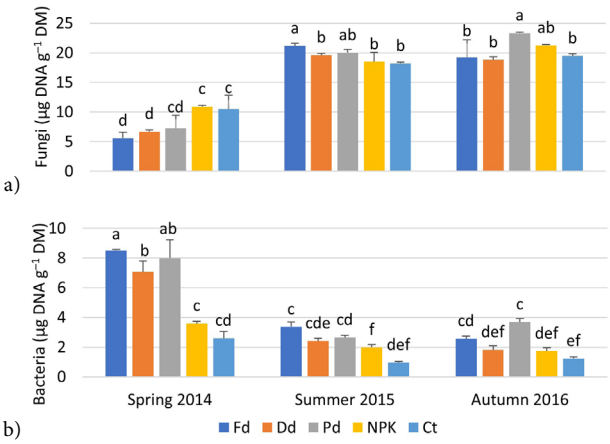

Fig. 2. Fungal load (a) and bacteria load (b) in the soil depending on the type of fertilizer and season (Fd – fresh digestate, Dd – dried digestate, Pd – pyrolysed digestate, NPK – mineral fertilizer, Ct – control).

Overall, it may be assumed that – regardless of the form that the digestate is applied in – the abundance of fungi increased while the abundance of bacteria decreased. As for the plots with mineral fertilization and the control one, the course of the changes was the same, but the scale was smaller than among the plots fertilized with digestate.

An analysis of the results concerning soil respiration indicates that changes in absorbed O_2 and released CO_2 followed a similar course during consecutive seasons: spring 2014, summer 2015 and autumn 2016 (Fig. 3). The highest degree of intensity of the ongoing soil respiration processes was noted successively in soil fertilized with liquid digestate, dried digestate, mineral fertilizer, and pyrolysed digestate. The amounts of CO_2 released from these soils were much higher than in the control, in which the soil remained unfertilized. Moreover, in all of the fertilized soils the intensity of the uptake of O_2 and the release of CO_2 increased in the subsequent years of the study. In the spring of 2014, the total amount of CO_2 released from soil fertilized with liquid digestate was 1 703.26 µg CO_2 kg^{-1} DM of soil, compared to 1 499.25 µg CO_2 kg^{-1} DM when dried digestate was applied, 1 505.92 µg CO_2 kg^{-1} DM of soil treated with mineral fertilizer, and 1 430.25 µg CO_2 kg^{-1} DM of soil when pyrolysed digestate was applied. In the third year of the study, in autumn 2016, these values were 5 540.99, 5 414.06, 5 281.10, 4 625.17 and 3 415.65 µg CO_2 kg^{-1} DM of soil, respectively.

Regardless of the form of fertilizer used, a high rate of O_2 uptake and CO_2 emission was noted in the first week of incubation, after which it declined in all of the analysed fertilization treatments. The amount of carbon mineralized in 7 days reflects the content of the most active fraction of easily available carbon (Table 3). The percentage of mineral carbon in the first 7 days was 1.64-2.59% TC in spring 2014, 5.90-9.46% TC in summer 2015, and 9.26-14.94% TC in autumn 2016 (Table 3). The highest values were

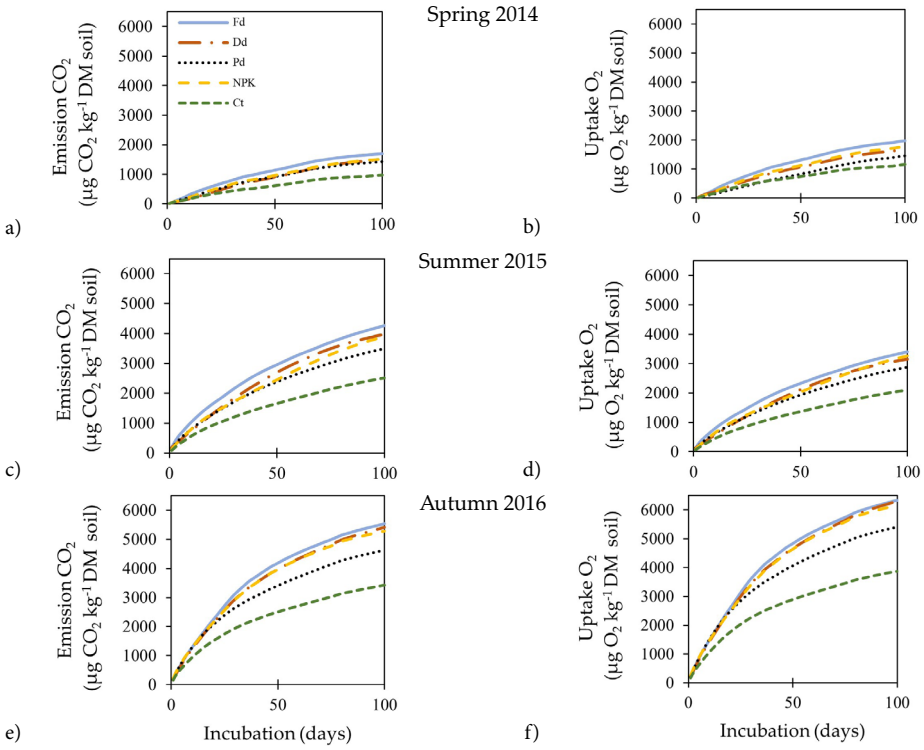

Fig. 3. The emission of CO_2 and the uptake of O_2 during the incubation of the soil, depending on the type of fertilizer used and the season (Fd – fresh digestate, Dd – dried digestate, Pd – pyrolysed digestate, NPK – mineral fertilizer, Ct – control) (a, b – spring 2014, d, e – summer 2015, e, f – autumn 2016).

Table 2. Pearson's correlation coefficients for the chemical, biological and respirometric properties of soil (n = 15)

Variable	TC	HWC	TN	C/N	pH	P	Mg	K	FL	BL	TC-7	TC-100
HWC	–0.458	1										
TN	0.910**	–0.469**	1									
C/N	0.818**	–0.299*	0.507	1								
pH	0.750**	–0.499**	0.877**	0.363*	1							
P	0.840**	–0.282	0.703**	0.743**	0.443**	1						
Mg	0.911**	–0.481**	0.901**	0.656**	0.869**	0.681**	1					
K	0.792**	–0.568**	0.761**	0.566**	0.458**	0.793**	0.685**	1				
FL	0.334*	–0.766**	0.187	0.431	0.096	0.152	0.212	0.440	1			
BL	0.172	0.409**	0.312*	–0.074	0.429**	0.254*	0.301	–0.083	–0.793**	1		
TC-7	0.109	–0.806**	0.091	0.105	0.113	–0.016	0.154	0.333	0.833**	–0.657**	1	
TC-100	0.053	–0.719**	0.086	0.002	0.114	–0.126	0.131	0.261	0.785**	–0.651**	0.970**	1
CO_2	0.404	–0.816**	0.399	0.297	0.375	0.150	0.462	0.519**	0.844**	–0.557**	0.910**	0.924**

Significant at: *p<0.05 and **p<0.01.

recorded for the plot fertilized with liquid digestate (spring 2014, summer 2015) and with mineral fertilizer (autumn 2016), while the lowest ones were determined in the plots treated with pyrolysed digestate, regardless of the year of the study. After 100 days, the amount of carbon which had undergone mineralization was 15.05-23.76% TC in spring 2014, 33.89% TC in summer 2015, and in 43.39-78.79% TC in autumn 2016. The highest values were determined in the soil with mineral fertilization (spring 2014, autumn 2016) and also in the soil fertilized with liquid digestate (summer 2015), while the lowest ones – as was the case after 7 days – were recorded in the soil fertilized with pyrolysed digestate.

An analysis of Pearson's correlation coefficient (Table 2) revealed a significant, positive linear relationship between the total carbon content and the total nitrogen content, the available forms of macronutrients (K, P, Mg) and soil pH. The HWC content correlated negatively with the

Table 3. Amount and percentage of mineralized carbon relative to the initial amount of total carbon in the bioreactor after 7 (TC-7) and 100 (TC-100) days of incubation

Season	Fertilizer	Carbon initial			Carbon mineralized	
		TC	HWC	HWC 100/TC	TC-7	TC-100
		g kg^{-1} DM soil		%	% TC	
Spring 2014	Fd	9.24	0.52	5.63	2.59	22.12
	Dd	7.89	0.70	8.87	1.73	22.77
	Pd	11.40	0.65	5.70	1.64	15.05
	NPK	7.61	0.81	10.64	2.30	23.76
	Ct	7.35	0.78	10.61	1.96	16.03
Summer 2015	Fd	10.04	0.26	2.59	9.46	51.02
	Dd	10.63	0.61	5.74	6.24	44.78
	Pd	12.35	0.30	2.43	5.90	33.89
	NPK	9.24	0.44	4.76	7.54	50.68
	Ct	7.39	0.42	5.68	6.87	40.62
Autumn 2016	Fd	10.95	0.13	1.19	10.83	60.73
	Dd	9.71	0.39	4.02	11.58	66.90
	Pd	12.79	0.23	1.80	9.26	43.39
	NPK	8.04	0.33	4.10	14.94	78.79
	Ct	7.80	0.29	3.72	11.19	52.56

Table 4. Summary of the four significant PCs

Principal component	R^2X	R^2X(cum.)	Eigen values	Q^2	Limit	Q^2(cum.)
PC1	0.376	0.376	7.904	0.180	0.116	0.180
PC2	0.258	0.634	5.412	0.273	0.123	0.403
PC3	0.109	0.743	2.285	–0.007	0.132	0.399
PC4	0.083	0.826	1.752	0.064	0.141	0.438

content of potassium, the abundance of fungal DNA and soil respirometry parameters. A negative correlation was shown between the abundance of the bacterial and fungal DNA, with a higher fungal DNA abundance correlating with a more intensive soil respiration process, while a higher bacterial DNA correlated with a decreased intensity in the soil respiration process.

The PCA analysis identified four significant components (Table 4). The cumulated R_2X(cum.) = 0.826 indicates that PCs summarize the total variance very well. The global goodness of fit and the predictive quality of the model measured by Q_2X were significantly improved by the inclusion of PC_2 and the next PCs did not increase the general utility of the model.

When analysing the loadings in Fig. 4, we can see that the first two PC coordinates classify variables into four distinctive groups, *i.e.* variables associated with seasons 2014Sp (I group), 2015Su (II group) and 2016Au (III group) as well as those associated with biochar application (IV group).

In the first group of variables that were highly correlated with PCs, the labile carbon (HWC) and bacteria abundance (B) were correlated with the first season of the study (2014Sp). This confirms the results presented above which found that in spring 2014Sp before fertilizer application but after fertilization in the previous year (2013) the soil was rich in labile carbon and contained a high abundance of bacteria. The second group of variables is composed of the intermediate season 2015Su and the associated fertilization treatments Ct, NPK, Dd and Fd. The variables are weakly associated with the first two PCs. The third group closely correlates the last season of the 2016Au study with a high abundance of soil fungi and the intensive processes of respiration and CO_2 emission. It should be noted that no fertilization was applied in 2016, thus it represents the follow-up effect of different types of mineralization and their associated emissions. In the fourth group, there was a set of chemical properties closely correlated with the application of pyrolysed digestate (Pd), which caused a higher pH value and content of total carbon, total nitrogen, phosphorus, potassium, magnesium and C/N ratio.

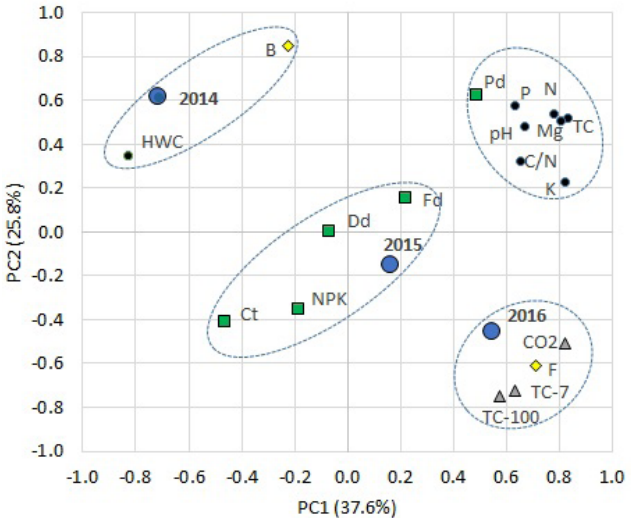

Fig. 4. The first two principal components in the classification of soil related variables (blue circle points – seasons: 2014 – spring, 2015 – summer, 2016 – autumn; black circle points – chemical variables; yellow diamond points – biological variables; gray triangle points – respirometry variables).

DISCUSSION

In comparison with mineral fertilization and the control, fertilization with digestate had a positive effect on most of the analysed soil chemical parameters (except for HWC). The effect of an increase in the total carbon content following soil enrichment with a large load of organic matter contained within the digestate has been well documented in the literature (Beni *et al.,* 2012; Fernández-Bayo *et al.,* 2017; Nabel *et al.,* 2014; Nabel *et al.,* 2016). Also, an increase in the soil content of nitrogen induced by digestate fertilization has been demonstrated by Alberquerque *et al.* (2012), Bachmann *et al.* (2011), Lošák *et al*. (2012), Nabel *et al*. (2014, 2016), Risberg *et al.* (2017). Moreover, the research results show that the application of fermented fertilizer may bring about better effects as opposed to the direct use of natural fertilizer. A rise in the mineral nitrogen concentration by 36% after three years of soil fertilization with cattle slurry, as compared to the application of swine manure, has been reported by Möller *et al*. (2008). Although some researchers (Barłóg *et al.,* 2020) have reported the lack of any significant improvement in the key soil fertility parameters and have only determined a positive tendency for changes after the application of digestate, they attribute these results to the insufficiently long duration of the experiment. Mineral fertilization can lead to the mineralization of soil organic matter, and consequently to some depletion of SOC in the soil (Menšík *et al.,* 2008). Our study also demonstrated a distinct increase in the total carbon content in summer 2015 and its decrease in the autumn of 2016 in NPK fertilized plots. As expected, the application of pyrolysed digestate, owing to the high carbon concentration in this fertilizer, produced the highest increase in the total carbon content in the soil. Numerous papers have confirmed that with its high carbon content, including stable carbon, biochar contributes to the improvement of the carbon balance in the soil (Galvez *et al.,* 2012; Ginebra *et al.,* 2022; Mohan *et al.,* 2014; Sigua *et al.,* 2014).

The mass of fresh digestate added and its various derivative forms can result in an increase in the soil content of phosphorus, potassium and magnesium. The positive effect of the phosphorus contained in the fertilizer on the soil content of phosphorus has been proven in experiments by Beni *et al.* (2012), Bachmann *et al.* (2011), Fernández-Bayo *et al*. (2017), Koszel and Lorencowicz (2015) and Nabel *et al*. (2016). The improved soil richness with regard to potassium following the application of digestate has been demonstrated by Fernández-Bayo *et al.* (2017) and by Koszel and Lorencowicz (2015). The impact of digestate application on the soil content of available magnesium was not unambiguous. An increase in the content of magnesium in the soil in response to the application of digestate has been shown by Koszel and Lorencowicz (2015), and by Barłóg *et al*. (2020), in contrast to the findings of Krzywy-Gawrońska (2013), who reported a decrease thereof. However, many scholars have emphasized that digestate may be an alternative solution to mineral or organic fertilization. Beni *et al.* (2012) showed that digestate can fulfil the demand of crops for potassium better than organic-mineral fertilizer, and may lead to a smaller loss of available magnesium in the soil than mineral or organic-mineral fertilization. Nabel *et al.* (2016) demonstrated that potassium concentrations in soil fertilized with digestate can be higher than in soil from the control plots or from plots treated with mineral fertilizer. Grigatti *et al.* (2019) proved that digestate can be used as an alternative to mineral fertilization in terms of soil enrichment with available phosphorus. Barłóg *et al.* (2020) analysed the results of a 4-year experiment and showed a significant increase in the concentrations of phosphorus and potassium in plots fertilized with digestate relative to the control, mineral fertilization and cattle slurry, in which the content of phosphorus was balanced at an identical level. As with those reported in the literature, the results presented in this article confirm that the application of digestate as a source of P, K and Mg is well-founded. Regardless of its form, digestate can be successfully used as a substitute for mineral fertilization in terms of supplying phosphorus, magnesium and potassium with the exception of dried digestate. Attention should be focused on the highest increase in the content of available phosphorus, potassium and magnesium after the application of pyrolysed digestate in comparison with the other forms of digestate.

The soil pH is a determinant of the soil microbiological activity and the bioavailability of mineral ingredients to plants. The regulation of soil pH through the use of organic fertilizers, which act as a buffer, is an important aspect of the research on fertilization. Digestate can also contribute to the lowering of soil acidity. A significant increase in soil pH after the application of fresh digestate or the solid fraction

of digestate has been demonstrated by Bachman *et al.* (2016), with the effect being more pronounced when fresh digestate is used. A slight increase in soil pH following the application of digestate has been demonstrated by Brod *et al.* (2015), Koszel and Lorencowicz (2015), and Nabel *et al.* (2016). In our study, all of the tested forms of digestate had an alkaline reaction, but a particularly high increase in soil pH was achieved when fresh digestate was added to the soil, this may be explained by the high dose of digestate used in that case, which resulted from balancing the nitrogen dose.

The abundance of bacteria and fungi in the soil was determined using the quantitative PCR method and is an important manifestation of the changes in the biological life of the soil induced by agronomic treatments, including fertilization. In considering the competition for nutrients and habitats between these two groups of microorganisms, and an evident quantitative prevalence of fungi over bacteria shown in this experiment, it may be concluded that fungi adapt better to soil conditions modified by the application of organic matter, and to the use of nutrients contained in digestate (de Boer *et al.,* 2005). The quantitative prevalence of fungi over other groups of microorganisms, such as bacteria, may also be a consequence of fungi being able to utilize both organic and mineral forms of nitrogen (Kucharski and Wałdowska, 2001). Moreover, as highlighted by Jia *et al.* (2020), nitrogen forms are an important factor influencing the response of soil microbes and soil microbial characteristics after nitrogen enrichment. NH_4^+ and mixed NH_4^+ with NO_3^- enrichment had positive effects on bacterial and fungal biomass, while urea enrichment increased the bacterial biomass (Jia *et al.,* 2020). In addition to the direct effect of increasing the availability of nitrogen in the soil, nitrogen fertilization indirectly affects the level of biodiversity and the composition of the bacterial and fungal communities by acidifying the soil and changing the plant communities (Guo *et al.,* 2019; Jia *et al*., 2020; Wang *et al.,*2016; Zeng *et al.,* 2016).

Despite the overall decreasing trend in the abundance of bacteria, the plot fertilized with pyrolysed digestate stood out among all of the fertilized plots by having the highest counts of bacteria as well as fungi. The considerable abundance of soil microorganisms in the plot fertilized with pyrolysed digestate seems to be linked to the high degree of porosity of the fertilizer and the high specific surface area of such materials, this is because they provide a suitable environment for colonization by soil microorganisms (Lehmann, 2007; Lehmann *et al.,* 2011). Relative to the control treatment and the one treated with mineral fertilizer, all digestates promoted the development of bacteria. The majority of papers dedicated to this research subject showed the positive influence of organic fertilization on the counts of soil bacteria (Ndubuisi-Nnaji *et al.,* 2011; Zhong *et al.,* 2010). The beneficial influence of organic fertilization on the growth in the overall abundance of soil microorganisms has been reported by Gong *et al.* (2009), Mandic *et al*. (2012), Sapp *et al.* (2015), Zhang *et al.* (2015) and Zhen *et al*. (2014), and the extent of this effect resulted from the content of organic matter in the fertilizer. Nevertheless, a study by Wolna-Maruwka *et al.* (2017) showed a lack of any significant increase in the total abundance of bacteria following the application of sewage sludge.

The pertinent literature delivers ambiguous research results concerning the influence of organic fertilizers, including digestate, on soil colonization by fungi. The absence of any significant effect of organic fertilization on the counts of fungi in the soil has been shown by Cwalina-Ambroziak and Bowszys (2009), Lazcano *et al*. (2013), Pratt (2008), Wolna-Maruwka *et al.* (2017). By contrast, a rise in the abundance of soil fungi due to the application of digestate to the soil was demonstrated by Ndubuisi-Nnaji *et al.* (2011). Likewise, a higher content of soil fungi after the application of fresh digestate relative to the control and only slightly higher than that in the mineral fertilization plot were shown by Walsh *et al.* (2012). Moreover, according to Cwalina-Ambroziak and Bowszys (2009) as well as Cwalina-Ambroziak and Wierzbowska (2011), organic fertilizers have a positive effect on the structure of the fungal community in the soil, this is manifested by a lower abundance of pathogenic fungi than in the plots with mineral fertilization or without fertilization.

The examples cited above indicate that a relationship exists between the development soil microorganisms and the type of organic matter added to the soil. They also point to a significant relationship with soil pH. Long-term mineral fertilization, especially with large doses of nitrogen, by producing a strong acidifying effect on the soil induces a more intensive development of fungi, which are more tolerant to unfavourable conditions. As is the case in our experiment, a rise in the content of fungi in plots treated with mineral fertilizers was demonstrated by Zhong *et al.* (2010). This result was also confirmed by a simultaneously lower abundance of fungi in plots fertilized with liquid digestate, where the soil pH was highest.

The discussion above concerning our research results reveals that some of reported scientific findings are contradictory. The results obtained from our experiment confirm that the development of soil microorganisms is closely dependent on the type of fertilizer applied and its properties.

The respiration processes in soil are connected with the activity of microorganisms and the quantities of nutrients available in the soil (Stenberg, 1999). Soil microorganisms play a key role in the biodegradation of organic matter by decomposing complex organic compounds to simpler ones. Measuring the amount of released CO_2 provides an important indicator of the intensity of the mineralization processes known as soil respiration. The soil samples analysed in our study showed a high rate of O_2 absorption and CO_2 release in the first weeks of incubation, after which the emission rate in all of the analysed treatments slowed down. The more intensive mineralization rate in the early incubation period was associated with the use of the labile carbon fraction from

organic matter. Likewise, an initially high mineralization rate was demonstrated by Diochan *et al.* (2013). Moreover, regardless of the form of fertilization, the volume of soil respiration (both CO_2 emission and O_2 absorption) increased gradually; it was much higher in autumn 2016 than in spring 2014 and occurred in parallel to the increasing abundance of soil microorganisms, especially of fungi.

Regardless of the date of soil sampling, the proportion of the CO_2 emissions in the experimental treatments was similar – from the highest value for the fresh digestate, followed by the dried digestate, NPK fertilization and pyrolysed digestate, to the lowest in the control treatment. The high counts of both bacteria and fungi in soil fertilized with pyrolysed digestate, with the simultaneously high total carbon content, were not reflected in the intensity of soil respiration, which was lower than in soil fertilized with liquid and dried digestate. The low CO_2 emission indicates the low level of mineralization of this digestate. It also suggests that the added organic matter, which was previously subjected to thermal conditioning (pyrolysis), shows significant resistance to decomposition in soil due to its higher content of stable carbon. Schmidt and Noack (2000) also demonstrated the lower decomposability of these types of materials. Lower CO_2 emission from soil under the influence of biochar application has also been demonstrated by Marchettti *et al*. (2012), Kuzyakov *et al*. (2014) and Konobluch *et al*. (2011). The presence of stable carbon which is resistant to decomposition may contribute to the long-term enlargement of the carbon pool by participating in what is commonly known as carbon sequestration.

In addition, many authors point to the inhibition of the mineralization of organic matter, including the native fraction in the soil, under the influence of biochar, which is referred to as a negative priming effect (Liu *et al.,* 2018, Zheng *et al.,* 2018). The application of biochar together with another source of organic matter in the form of unprocessed digestate resulted in lower emission in comparison with the application of digestate alone (Marchetti *et al.,* 2012). Studies carried out with the use of labelled carbon showed that the addition of biochar decreased the intensity of soil organic matter mineralization by 21±3% over a 21-day-long incubation period (Jones *et al.,* 2011). The inhibition of CO_2 release from the soil following the application of biochar has also been demonstrated by Liang *et al.* (2010). Moreover, research has proven that biochar can lower the emission of CO_2 as well as the release of nitrous oxide (N_2O) and methane (CH_4) from the soil (Ameloot *et al.,* 2015; Ginebra *et al.,* 2022; Nelissen *et al.,* 2014). In our study, however, no inhibition of CO_2 emission was observed. The emission of CO_2 from soil amended with pyrolysed digestate was higher than that from the control soil. A slightly higher soil emission of CO_2 from soil fertilized with pyrolysed digestate than from unfertilized soil has been shown by Jones *et al.* (2011), and Liu *et al.* (2018), the higher degree of CO_2 emission from soil fertilized with pyrolysed digestate was a consequence of the low content of labile carbon in the biochar. The size of the labile carbon fraction in biochar depends on the type of substrate used and the parameters set for the production of biochar, such as residence time and temperature (Jindo *et al.,* 2014). Jones *et al*. (2011) emphasized that the carbon loss was very minor relative to the total carbon content in the biochar itself (about 0.1%), and that the short-term release of carbon should not compromise the ability of biochar to be utilized for the long-term sequestration of carbon in the soil environment. These findings were confirmed by Keith *et al*. (2011), who proved that biochar affects the intensity of the decomposition of native organic matter, and that both the direction and the scale of the changes depend on the type of soil and biochar. However, there are other studies in the literature which indicate that biochar may increase the rate of mineralization, soil microbial abundance, and enhance the activity and structure of soil microorganisms, this is referred to as a priming effect (Hardy *et al.,* 2019; Yu *et al.,* 2018). The scale of the effect is conditioned by the type of substrate and the temperature of the biochar production process (Fang *et al.,* 2015; Yu *et al.,* 2018). Moreover, under specific conditions, the priming effect may be driven by a combination of several mechanisms and shown to be related to the properties of both biochar and soil (Luo *et al.,* 2011; Wang *et al.,* 2016; Yang *et al.,* 2022).

As for the remaining types of digestate, in a liquid and dried form, the organic matter they contained underwent more rapid mineralization, which was reflected by the highest intensity of CO_2 emission and, simultaneously, a lower concentration of total carbon (Fd, Dd) and labile carbon (Fd) in the soil relative to Pd. The labile form of organic carbon, due to its high rate of biodegradation, is an important source of CO_2 emission from the soil (Gregorich *et al.,* 2003; Kim *et al.,* 2012). The lowest average content of labile carbon (HWC) and the highest abundance of bacteria in soil fertilized with liquid digestate indicates that by using the easily available carbon as a source of energy, bacteria induce a high rate of soil respiration and the mineralization of organic matter within the soil. The effects of the application of organic matter in the form of digestate such as an increase in the emission of CO_2 from soil has been described by Alburquerque *et al.* (2012), and Marchetti *et al*. (2012). An increase in the intensity of the respiration processes in the soil after the application of organic fertilizer, including digestate, has been demonstrated by other researchers (Abubaker *et al.,* 2012; Abubaker *et al.,* 2015; Odlare *et al.,* 2008). The differences that were determined in the intensity of CO_2 emission from soil treated with different forms of digestate confirm the varied sensitivity of various materials to microbial decomposition. The higher CO_2 emission from soil with added organic fertilizer (with no thermal processing) than from soil with added biochar has been proven by Kulczycki *et al.* (2020), and Liu *et al.* (2014).

The intensity of respiration can also be expressed as a percentage of mineralized carbon relative to the initial, total carbon content, as measured after 7 and 100 days of incubation. In soil fertilized with biochar, it can range from 0.02 to 1.9 g C kg^{-1} of soil, and 1.5% of the total carbon content in the soil (Diochon *et al.,* 2013; Robertson *et al.,* 1999). A small fraction, which amounts to less than 1% of the content of the active fraction of the total carbon undergoing mineralization during a 7-day laboratory incubation has been reported by Schwendenmann *et al*. (2007). In our experiment, the percentage of carbon mineralized in the first seven days of incubation was 1.64-2.59% TC in 2014Sp, 9.26-14.94% TC in 2016Au, with Pd being the lowest by a significant margin, at 1.64% TC and 9.26% TC, respectively. The amount of carbon mineralized over a long period of time – in a 100 day period – reflects the pool of labile carbon, but with relatively slow turnover, and can range from 0.03 to 18.19 g C kg^{-1} of soil, and 15% of the total carbon in soil with added biochar (Diochon *et al.,* 2013). Another study, by Bruun *et al*. (2011), showed that 3-12% of the added biochar was emitted as CO_2 during 115 days of incubation, of which, 90% of CO_2 losses occurred in the first twenty days. The CO_2 emission achieved in the case of pyrolysed digestate which was higher than that reported in the literature may be explained by the applied temperature of pyrolysis and the type of substrate used. At the same time, there is a significant relationship between the aforementioned parameters and the properties of biochar, which has been proven in numerous studies (Zhao *et al.,* 2018; Tan *et al.,* 2017; Meng *et al.,* 2018; Lee *et al.,* 2013). The results obtained in this experiment, namely that the smallest share of mineralized carbon as a proportion of the total carbon content was determined in soil amended with pyrolysed digestate, this may suggest that the application of biochar, of all the tested forms of digestate, can play a significant role in the sequestration of carbon in the soil.

The PCA results justified the categorization of 4 groups of variables in a system where two first principal components explained 53.4% of the total variance. The first and third group of variables were connected with the soil samples that were taken in spring 2014 and autumn 2016 and attest to the relationship between the development of a chosen group of microorganisms with the type of available nutrients in fertilizers. In the spring of 2014, the soil was rich in labile carbon and therefore contained a large abundance of bacteria. As the source of easily available carbon was being depleted, the abundance of bacteria was gradually decreasing, while the abundance of fungi able to decompose and use more complex substrates increased. Moreover, the third group comprised variables correlated with the intensive processes of respiration and emission of CO_2 and with a high abundance of the DNA of soil fungi. As no fertilization was carried out in 2016, the PCA results confirm the differentiation in the intensity of mineralization depending on the sensitivity of the substrate to decomposition and the resultant emissions. The second group of variables encompassed different forms of the applied fertilizers, with a relatively weak correlation between the first two principal components. The fourth group was composed of variables closely correlated with the application of biochar, which is significant in the context of soil fertility improvement (pH, TC, P, K, Mg, C/N).

CONCLUSIONS

1. The application of digestate leads to an improvement in the chemical properties of the soil, such as the concentrations of total carbon, total nitrogen as well as in the macronutrients: P, K and Mg, and the extent of such changes depends on the applied form of the digestate. The effects obtained after the application of digestate are more pronounced than after mineral fertilization, which means that digestate can be both a valuable organic fertilizer and a substitute for mineral fertilizer.

2. The high content of nutrients in the digestate stimulated a rise in the abundance of soil microorganisms and their activity (soil respiration). All forms of digestate cause changes in the abundance of soil microorganisms.

3. The differences in the intensity of CO_2 emission between different forms of digestate confirm the varied vulnerabilities of digestates to decomposition, including microbial breakdown. The intensity of soil respiration depends on the type of digestate applied, and more particularly on the availability of nutrients, including solid carbon, than on the abundance of bacteria and fungi. Digestate in the form of biochar appears to be more resistant to decomposition in the soil.

4. When digestate from a biogas plant is applied to the soil, it is a significant factor which is capable of improving soil fertility by supplying plants with easily available nutrients and by stimulating the microbiological activity of the soil. The positive effects derived from the application of digestate to the soil suggest that this material can sustain the richness of soil, in addition to which biochar can contribute effectively to the sequestration of carbon.

Conflict of interest: The Authors declare they have no conflict of interest.

REFERENCES

Abubaker J., Risberg K., Jönsson E., Dahlin A.S., Cederlund H., and Pell M., 2015. Short-term effects of biogas digestates and pig slurry application on soil microbial activity. Appl. Environ. Soil Sci., 1-15, https://doi.org/10.1155/2015/658542

Abubaker J., Risberg K., and Pell M., 2012. Biogas residues as fertilisers – Effects on wheat growth and soil microbial activities. Applied Energy, 99, 126-134, https://doi.org/10.1016/j.apenergy.2012.04.050

Akhiar A., Battimelli A., Torrijos M., and Carrere H., 2017. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Management, 59, 118-128, https://doi.org/10.1016/j.wasman.2016.11.005

Alburquerque J.A., de la Fuente C., Ferrer-Costa A., Carrasco L., Cegarra J., Abad M., and Bernal M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy, 40, 181-189, https://doi.org/10.1016/j.biombioe.2012.02.018

Ameloot N., Sleutel S., Das K., Kanagaratnam J., and Neve S., 2015. Biochar amendment to soils with contrasting organic matter level: effects on N mineralization and biological soil properties. GCB Bioenergy, 7, 135-144, https://doi.org/10.1111/gcbb.12119

Bachmann S., Uptmoor R., and Eichler-Löbermann B., 2016. Phosphorus distribution and availability in untreated and mechanically separated. Sci. Agric., 73(1), 9-17, https://doi.org/10.1590/0103-9016-2015-0069

Bachmann S., Wentzel S., and Eichler-Löbermann B., 2011. Codigested dairy slurry as a phosphorus and nitrogen source for *Zea mays* L. and *Amaranthus cruentus* L. J. Plant Nutr. Soil Sci., 174, 908-915, https://doi.org/10.1002/jpln.201000383

Bai M., Wilske B., Buegger F., Bruun E.W., Bach M., Frede H.G., and Breuer L., 2014. Biodegradation measurements confirm the predictive value of the O:C-ratio for biochar recalcitrance. J. Plant Nutr. Soil Sci., 77(4), 633-637, https://doi.org/10.1002/jpln.201300412

Baldé H., Vander Zaag A.C., Burtt S.D., Wagner-Riddle C., Crolla A., Desjardins R.L., and MacDonald D.J., 2016. Methane emissions from digestate at an agricultural biogas plant. Bioresource Technol., 216, 914-922, https://doi.org/10.1016/j.biortech.2016.06.031

Barlóg P., Hlisnikovský L., and Kunzová E., 2020. Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. Agronomy, 10, 379, https://doi.org/10.3390/agronomy10030379

Battista F., Frison N., and Bolzonella D., 2019. Energy and nutrients' recovery in anaerobic digestion of agricultural biomass: An Italian perspective for future applications. Energies, 12(17), 3287, https://doi.org/10.3390/en12173287

Beesley L., Moreno-Jiménez E., Gomez-Eyles J., Harris E., Robinson B., and Sizmur T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut., 159(12), 3269-3282, https://doi.org/10.1016/j.envpol.2011.07.023

Beni C., Servadio P., Marconi S., Neri U., Aromolo R., and Diana G., 2012. Anaerobic digestate administration: effect on soil physical and mechanical behavior. Communications in Soil Science and Plant Analysis, 43(5), 821-834, https://doi.org/10.1080/00103624.2012.648359

Blackwell P., Riethnuller G., and Collins M., 2009. Biochar Application to Soil. In: Biochar for Environmental Management: Science Technology (Eds J. Lehman, S. Joseph). London, 207-226.

Bornemann L., Welp G., Brodowski S., Rodionov A., and Amelung W., 2008. Rapid assessment of black carbon in soil organic matter using mid-infrared spectroscopy. Org. Geochem., 39(11), 1537-1544, https://doi.org/10.1016/j.orggeochem.2008.07.012

Brod E., Øgaard A.F., Wragg D., Haraldsen T.K., and Krogstad T., 2015. Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH. Nutr. Cycl. Agroecosyst., 103, 167-185, https://doi.org/10.1007/s10705-015-9734-1

Bruun E.W., Hauggaard-Nielsen H., Ibrahim N., Egsgaard H., Ambus P., Jensen P.A., and Dam-Johansen K., 2011. Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. Biomass Bioenerg., 35(3), 1182-1189, https://doi.org/10.1016/j.biombioe.2010.12.008

Bułkowska K., Pokój T., Klimiuk E., and Gusiatin Z.M., 2012. Optimization of anaerobic digestion of a mixture of *Zea mays* and *Miscanthus sacchariflorus* silages with various pig manure dosages. Bioresour. Technol., 125, 208-216, https://doi.org/10.1016/j.biortech.2012.08.078

Burrell L.D., Zehetner F., Rampazzo N., Wimmer B., and Soja G., 2016. Long-term effects of biochar on soil physical properties. Geoderma, 282, 96-102, https://doi.org/10.1016/j.geoderma.2016.07.019

Cabrera A., Cox L., Spokas K., Celis R., Hermosin M.C., Cornejo J., and Koskinen W.C., 2011. Comparative sorption and leaching study of the herbicides fluometuron and 4-chloro-2-methylphenoxy acetic acid (MCPA) in a soil amended with biochars and other sorbents. J. Agric. Food Chem., 59, 12550-12560, https://doi.org/10.1021/jf202713q

Castro L., Escalante H., Jaimes-Estévez J., Díaz L.J., Vecino K., Rojas G., and Mantilla L., 2017. Low cost digester monitoring under realistic conditions: rural use of biogas and digestate quality. Bioresource Technology, 239, 311-317, https://doi.org/10.1016/j.biortech.2017.05.035

Cwalina-Ambroziak B. and Bowszys T., 2009. Changes in fungal communities in organically fertilized soil. Plant, Soil and Environment, 55, 25-32, https://doi.org/10.17221/327-PSE

Cwalina-Ambroziak B. and Wierzbowska J., 2011. Soil fungal communities shaped under the influence of organic fertilization. J. Elem., 16(3), 365-375, https://doi.org/10.5601/jelem.2011.16.3.02

de Boer W., Folman L.B., Summerbell R.C., and Boddy L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews, 29, 795-811, https://doi.org/10.1016/j.femsre.2004.11.005

Degens B.P., 1998. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. Soil Biol. Biochem., 30, 1981-1988, https://doi.org/10.1016/S0038-0717(98)00070-4

Diochon A., Gregorich E.G., and Tarnocai C., 2013. Evaluating the quantity and biodegradability of soil organic matter in some Canadian Turbic Cryosols. Geoderma, 202-203, 82-87, https://doi.org/10.1016/j.geoderma.2013.03.013

Fang Y., Singh B., and Singh B.P., 2015. Effect of temperature on biochar priming effects and its stability in soils. Soil Biol. Biochem., 80, 136-145, https://doi.org/10.1016/j.soilbio.2014.10.006

Fernández-Bayo J.D., Achmon Y., Harrold D.R., McCurry D.G., Hernandez K., Dahlquist-Willard R.D., Stapleton J.J., Vander Gheynst J.S., and Simmons Ch.W., 2017. Assessment of two solid anaerobic digestate soil amendments for effects on soil quality and biosolarization efficacy. J. Agric. Food Chem., 65(17), https://doi.org/10.1021/acs.jafc.6b04816

Galvez A., Sinicco T., Cayuela M.L., Mingorance M.D., Fornasier F., and Mondini C., 2012. Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. Agric. Ecosyst. Environ., 160, 3-14, https://doi.org/10.1016/j.agee.2011.06.015

Ginebra M., Muñoz C., Calvelo-Pereira R., Doussoulin M., and Zagal E., 2022. Biochar impacts on soil chemical properties, greenhouse gas emissions and forage productivity: A field experiment. Sci. Total Environ., 806, 2, 150465, https://doi.org/10.1016/j.scitotenv.2021.150465

Goberna M., Podmirseg S.M., Waldhuber S., Knapp B.A., García C., and Insam H., 2011. Pathogenic bacteria and mineral N in soil following the land spreading of biogas digestates and fresh manure. Appl. Soil Ecol., 49, 18-25, https://doi.org/10.1016/j.apsoil.2011.07.007

Gong W., Yan X., Wang J., Hu T., and Gong Y., 2009. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. Geoderma, 149, 318-324, https://doi.org/10.1016/j.geoderma.2008.12.010

Gregorich E.G., Beare M.H., Stoklas U., and St-Georges P., 2003. Biodegradability of soluble organic matter in maize cropped soils. Geoderma, 113, 237-252, https://doi.org/10.1016/S0016-7061(02)00363-4

Grigatti M., Boanini E., Bolzonella D., Sciubba L., Mancarella S., Ciavatta C., and Marzadori C., 2019. Organic wastes as alternative sources of phosphorus for plant nutrition in a calcareous soil. Waste Manag., 93, 34-46, https://doi.org/10.1016/j.wasman.2019.05.028

Guo O., Yan L., Korpelainen H., Niinemets Ü., and Li C., 2019. Plant-plant interactions and N fertilization shape soil bacterial and fungal communities. Soil Biol. Biochem., 128, 127-138, https://doi.org/10.1016/j.soilbio.2018.10.018

Hardie M., Clothier B., Bound S., Oliver G., and Close D., 2014. Does biochar influence soil physical properties and soil water availability? Plant Soil, 376, 347-361, https://doi.org/10.1007/s11104-013-1980-x

Hardy B., Sleutel S., Dufey J.E., and Cornelis J.T., 2019. The long-term effect of biochar on soil microbial abundance, activity and community structure is overwritten by land management. Front. Environ. Sci., 7, 110, https://doi.org/10.3389/fenvs.2019.00110

Jia X., Zhong Y., Liu J., Zhu G., Shangguana Z., and Yana W., 2020. Effects of nitrogen enrichment on soil microbial characteristics: From biomass to enzyme activities. Geoderma, 366, 114256, https://doi.org/10.1016/j.geoderma.2020.114256

Jindo K., Mizumoto H., Sawada Y., Sanchez-Monedero M.A., and Sonoki T., 2014. Physical and chemical characterization of biochars derived from different agricultural residues. Biogeosciences, 11, 6613-6621, https://doi.org/10.5194/bg-11-6613-2014

Jining Z., Fan L., Chenghao L., Liming S., and Pinjing H., 2014. Humification characterization of biochar and its potential as a composting amendment. Res. J. Environ. Sci., 26, 390-397, https://doi.org/10.1016/S1001-0742(13)60421-0

Jones D.L., Murphy D.V., Khalid M., Ahmad W., Jones G.J., and Deluca T.H., 2011. Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. Soil Biol. Biochem., 43, 1723-1731, https://doi.org/10.1016/j.soilbio.2011.04.018

Jurgutis L., Šlepetienė A., Amalevičiūtė-Volungė K., Volungevičius J., and Šlepetys J., 2021. The effect of digestate fertilisation on grass biogas yield and soil properties in field-biomass-biogas-field renewable energy production approach in Lithuania. Biomass Bioenerg., 153, 106211, https://doi.org/10.1016/j.biombioe.2021.106211

Kaparaju P.L.N. and Rintala J.A., 2008. Effects of solid-liquid separation on recovering residual methane and nitrogen from digested dairy cow manure. Biores. Technol., 9, 120-127, https://doi.org/10.1016/j.biortech.2006.11.046

Karimi B., Sadet-Bourgeteau S., Cannavacciuolo M., Chauvin C., Flamin C., Haumont A., Jean-Baptiste V., Reibel A., Vrignaud G., and Ranjard L., 2022. Impact of biogas digestates on soil microbiota in agriculture: a review. Environ. Chem. Lett., 20, 3265-3288, https://doi.org/10.1007/s10311-022-01451-8

Keith A., Singh B., and Singh B.P., 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. Environ. Sci. Technol., 45, 9611-9618, https://doi.org/10.1021/es202186j

Kim Y.S., Yi M.J., Lee Y.Y., Son Y., and Koike T., 2012. Characteristics of soil CO_2 efflux in even-aged alder compared to Korean pine plantations in Central Korea. J. Forest Sci., 28(4), 232-241, https://doi.org/10.7747/JFS.2012.28.4.232

Knoblauch C., Maarifat A.A., Pfeiffer E.M., and Haefele S.M., 2011. Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. Soil Biol. Biochem., 43, 1768-1778, https://doi.org/10.1016/j.soilbio.2010.07.012

Koszel M. and Lorencowicz E., 2015. Agricultural use of biogas digestate as a replacement fertilizers. Agric. Agric. Sci. Proc., 7, 119-124, https://doi.org/10.1016/j.aaspro.2015.12.004

Krzywy-Gawrońska E., 2013. Effect of combustion wastes and sewage sludge compost on the chemical properties of soil. Pol. J. Chem. Technol., 15(3), 48-54, https://doi.org/10.2478/pjct-2013-0043

Kucharski J. and Wałdowska E., 2001. Biological results of long-term fertilization with slurry. Zesz. Probl. Post. Nauk Roln., 476, 197-204.

Kulczycki G., Magnucka E.G., Oksińska M., Kucińska J., Kobyłecki R., Pawleska K., Zarzycki R., Kacprzak A., and Pietr S.P., 2020. The effect of various types of biochar mixed with mineral fertilization on the development and ionome of winter wheat (*Triticum aestivum* L.) seedlings and soil properties in a pot experiment. Agronomy, 10, 1903, https://doi.org/10.3390/agronomy10121903

Kuzyakov Y., Bogomolova I., and Glaser B., 2014. Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. Soil Biol. Biochem., 70, 229-236, https://doi.org/10.1016/j.soilbio.2013.12.021

Lazcano C., Gómez-Brandón M., Revilla P., and Domínguez J., 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. Biol. Fertil. Soils, 49, 723-733, https://doi.org/10.1007/s00374-012-0761-7

Lee Y., Park J., Ryu C., Gang K.S., Yang W., Park Y.K., Jung J., and Hyun S., 2013. Comparison of biochar properties from biomass residues produced by slow pyrolysis at 500°C. Biores. Technol., 148, 196-201, https://doi.org/10.1016/j.biortech.2013.08.135

Lehman J., Czimczik C., Laird D., and Sohi S., 2009. Stability of Biochar in Soil. In: Biochar for Environmental Management: Science Technology (Eds J. Lehman, S. Joseph). London, UK.

Lehmann J., 2007. Bioenergy in the black. Front. Ecol. Environ., 5, 381-387, https://doi.org/10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2

Lehmann J., Gaunt J., and Rondon M., 2006. BIO-CHAR sequestration in terrestrial ecosystems - a review. Mitigation and Adaptation Strategies for Global Change, 11, 403-427, https://doi.org/10.1007/s11027-005-9006-5

Lehmann J., Rillig M.C., Thies J., Masiello C.A, Hockaday W.C., and Crowley D., 2011. Biochar effects on soil biota. Soil Biol. Biochem., 43(9), https://doi.org/10.1016/j.soilbio.2011.04.022

Li Y.C., Li Y.F., Chang S.X., Liang X., Qin H., Chen J.H., and Xu Q., 2017. Linking soil fungal community structure and function to soil organic carbon chemical composition in intensively managed subtropical bamboo forests. Soil Biol. Biochem., 107, 19-31, https://doi.org/10.1016/j.soilbio.2016.12.024

Liang B., Lehmann J., Sohi S.P., Thies J.E., Neill B.O., Trujillo L., Gaunt J., Solomon D., Grossman J., Neves E.G., and Luizão F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Org. Geochem., 41, 206-213, https://doi.org/10.1016/j.orggeochem.2009.09.007

Liu C., Lu M., Cui J., Li B., and Fang C., 2014. Effects of straw carbon input on carbon dynamics in agricultural soils: A meta-analysis. Global Chang. Biol., 1366-1381, https://doi.org/10.1111/gcb.12517

Liu Y., Chen Y., Wang Y., Lu H., He L., and Yang S., 2018. Negative priming effect of three kinds of biochar on the mineralization of native soil organic carbon. Land Degrad. Dev., 29, 3985- 3994, https://doi.org/10.1002/ldr.3147

Lošák T., Čermák P., and Hlušek J., 2012. Changes in fertilisation and liming of soils of the Czech Republic for the past 20 years. Agroma Soil Sci., 58, https://doi.org/10.1080/03650340.2012.698732

Luo Y., Durenkamp M., De Nobili M., Lin Q., and Brookes P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol. Biochem., 43, 11, 2304-2314, https://doi.org/10.1016/j.soilbio.2011.07.020

Mandic L., Djukic D., and Pesakovic M., 2012. Microbial characteristics of vertisol under different fertilization systems. J. Cent. Eur. Agric., 13(1), 1-9, https://doi.org/10.5513/JCEA01/13.1.1005

Marchetti R., Castelli F., Orsi A., Sghedoni L., and Bochicchio D., 2012. Biochar from swine manure solids: influence on carbon sequestration and Olsen phosphorus and mineral nitrogen dynamics in soil with and without digestate in-corporation. Ital. J. Agron., 7, 189-195, https://doi.org/10.4081/ija.2012.e26

Martin K. and Rygiewcz P., 2005. Fungal specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol., 5, 28-39.

Meng J., Tao M., Wang L., Liu X., and Xu J., 2018. Changes in heavy metal bioavailability and speciation from a Pb-Zn mining soil amended with biochars from co-pyrolysis of rice straw and swine manure. Sci. Total Environ., 633, 300-307, https://doi.org/10.1016/j.scitotenv.2018.03.199

Menšík L., Hlisnikovský L., Pospíšilová L., and Kunzová E., 2008. The effect of application of organic manures and miner-al fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. J. Soils Sediments, 18, 2813-2822, https://doi.org/10.1007/s11368-018-1933-3

Mohan D., Sarswat A., Ok Y.S., and Pittman Jr. C.U., 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent. Biores. Technol., 160, 191-202, https://doi.org/10.1016/j.biortech.2014.01.120

Möller K., Stinner W., Deuker A., and Leithold G., 2008. Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic farming systems. Nutr. Cycl. Agroecosyst., 82, 209-232, https://doi.org/10.1007/s10705-008-9196-9

Nabel M., Bueno D., Barbosa P., Horsch D., and Jablonowski N.D., 2014. Energy crop (*Sida hermaphrodita*) fertilization using digestate under marginal soil conditions: a dose-response experiment, Energy Procedia, 16, https://doi.org/10.1016/j.egypro.2014.10.358

Nabel M., Temperton V.M., Poorter H., Lücke A., and Jablonowski N.D., 2016. Energizing marginal soils - The establishment of the energy crop *Sida hermaphrodita* as dependent on digestate fertilization, NPK, and legume intercropping. Biomass Bioenerg., 87, https://doi.org/10.1016/j.biombioe.2016.02.010

Nakamya J., Tumuhairwe J.B., Sabiiti E.N., Strachan N.J.C., Avery L.M., and Smith J., 2020. Influence of biogas digesters on faecal indicator organisms in digestate and around homesteads in Ethiopia. Biomass Bioenerg., 142, 105746, https://doi.org/10.1016/j.biombioe.2020.105746

Ndubuisi-Nnaji U.U., Adegoke A.A., Ogbu H.I., Ezenobi N.O., and Okoh A.I., 2011. Effect of long-term organic fertilizer application on soil microbial dynamics. Afr. J. Biotechnol., 10(4), 556-559.

Nelissen V., Saha B.K., Ruysschaert G., and Boeckx P., 2014. Effect of different biochar and fertilizer types on N_2O and NO emissions. Soil Biol. Biochem., 70, 244-255, https://doi.org/10.1016/j.soilbio.2013.12.026

Odlare M., Pell M., and Svensson K., 2008. Changes in soil chemical and microbiological properties during 4 years of application of various organic residues. Waste Manag., 28, 1246-1253, https://doi.org/10.1016/j.wasman.2007.06.005

Ogwang I., Kasedde H., Nabuuma B., Kirabira J.B., and Lwanyaga J.D., 2021. Characterization of biogas digestate for solid biofuel production in Uganda. Scientific African, 12, e00735, https://doi.org/10.1016/j.sciaf.2021.e00735

Ojeda G., Mattana S., Àvila A., Alcañiz J.M., Volkmann M., and Bachmann J., 2015. Are soil-water functions affected by biochar application? Geoderma, 249-250, 1-11, https://doi.org/10.1016/j.geoderma.2015.02.014

Panuccio M.R., Romeo F., Mallamaci C., and Muscolo M., 2021. Digestate application on two different soils: agricultural benefit and risk. Waste and Biomass Valorization, 12, 4341-4353, https://doi.org/10.1007/s12649-020-01318-5

Pranagal J., Oleszczuk P., Tomaszewska-Krojańska D., Kraska P., and Różyło K., 2017. Effect of biochar application on the physical properties of Haplic Podzol. Soil Till. Res., 174, 92-103, https://doi.org/10.1016/j.still.2017.06.007

Pratt R.G., 2008. Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations. Appl. Soil Ecol., 38, 223-229, https://doi.org/10.1016/j.apsoil.2007.10.013

Radawiec W., Dubicki M., Karwowska A., Żelazna K., and Gołaszewski J., 2014. Biochar from a digestate as an energy product and soil improver. Agric. Engin., 3(151), 149-156, https://doi.org/10.14654/ir.2014.151.067

Razzaghi F., Obourb P.B., and Arthur E., 2020. Does biochar improve soil water retention? A systematic review and meta-analysis. Geoderma, 361, 114-155, https://doi.org/10.1016/j.geoderma.2019.114055

Risberg K., Cederlund H., Pell M., Arthurson V., and Schnürer A., 2017. Comparative characterization of digestate versus pig slurry and cow manure - Chemical composition and effects on soil microbial activity. J. Waste Manag., 61, 529-538, https://doi.org/10.1016/j.wasman.2016.12.016

Robertson G.P., Coleman D.C., Bledsoe C.S., and Phillip S., 1999. Standard Soil Methods for Long Term Ecological Research. Oxford University Press. New York.

Roubík H., Mazancová J., Phung L.D., and Banout J., 2018. Current approach to manure management for small-scale Southeast Asian farmers - Using Vietnamese biogas and non-biogas farms as an example, Renew. Energy, 115, 362-370, https://doi.org/10.1016/j.renene.2017.08.068

Sapp M., Harrison M., Hany U., Charlton A., and Thwaites R., 2015. Comparing the effect of digestate and chemical fertiliser on soil bacteria. Appl. Soil Ecol., 86, 1-9, https://doi.org/10.1016/j.apsoil.2014.10.004

Schmidt M.W.I. and Noack A.G., 2000. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. Glob. Biogeochem. Cycles, 14, 777-793, https://doi.org/10.1029/1999GB001208

Schulz H. and Glaser B., 2012. Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. J. Plant Nutr. Soil Sci., 175, 410-422, https://doi.org/10.1002/jpln.201100143

Schwendenmann L., Pendall E., and Potvin C., 2007. Surface soil organic carbon pools, mineralization and CO(2) efflux rates under different land-use types in Central Panama. Stability of Tropical Rainforest Margins: Linking Ecological, Economic and Social Constraints of Land Use and Conservation, 109-131.

Siebielec G., Siebielec S., and Lipski D., 2018. Long-term impact of sewage sludge, digestate and mineral fertilizers on plant yield and soil biological activity. J. Clean. Prod., 187, 372-379, https://doi.org/10.1016/j.jclepro.2018.03.245

Sigua G.C, Novak J.M., Watts D.W., Cantrell K.B., Shumaker P.D., Szögi A.A., and Johnson M.G., 2014. Carbon mineralization in two ultisols amended with different sources and particle sizes of pyrolyzed biochar. Chemosphere, 103, 313-321, https://doi.org/10.1016/j.chemosphere.2013.12.024

Six J., Frey S.D., Thiet R.K., and Batten K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J., 70, 555-569, https://doi.org/10.2136/sssaj2004.0347

Smith K.A., Metcalfe P., Grylls J., Jeffrey W., and Sinclair A., 2007. Nutrient Value of Digestate from Farm-Based Biogas Plants in Scotland. Report for Scottish Executive Environment and Rural Affairs Department - ADA/009/06. http://www.scotland.gov.uk/Topics/Environment/Water/15561/biogas

Sparling G., Vojvodic-Vukovic M., and Schipper L.A., 1998. Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biol. Biochem., 30(10/11), 1469-1472, https://doi.org/10.1016/S0038-0717(98)00040-6

Spokas K.A., Baker J.M., and Reicosky D.C., 2010. Ethylene: potential key for biochar amendment impacts. Plant Soil, 333, 443-452, https://doi.org/10.1007/s11104-010-0359-5

Stenberg B., 1999. Monitoring soil quality of arable land: microbiological indicators. Acta Agric. Scand., Soil Plant Sci., 49, 1-24, https://doi.org/10.1080/09064719950135669

Tambone F. and Adani F., 2017. Nitrogen mineralization from digestate in comparison to sewage sludge, compost and urea in a laboratory incubated soil experiment. J. Plant Nutr. Soil Sci., 180, 355-365, https://doi.org/10.1002/jpln.201600241

Tan Z., Lin C.S.K., Ji X., and Rainey T.J., 2017. Returning biochar to fields: A review. Appl. Soil Ecol., 116, 1-11, https://doi.org/10.1016/j.apsoil.2017.03.017

Tian J., McCormack L., Wang J., Guo D., Wang Q., Zhang X., Yu G., Blagodatskaya E., and Kuzyakov Y., 2015. Linkages between the soil organic matter fractions and the microbial metabolic functional diversity within a broad-leaved Korean pine forest. Eur. J. Soil Biol., 66, 57-64, https://doi.org/10.1016/j.ejsobi.2014.12.001

Topoliantz S., Ponge J.P., Arrouays D., Ballof S., and Lavelle P., 2002. Effect of organic manure and the endogeic earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) on soil fertility and bean production. Biol. Fertil. Soils, 36, 313-319, https://doi.org/10.1007/s00374-002-0535-8

Troy S.M., Lawlor P.G., O'Flynn C.J., and Healy M.G., 2013. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol. Biochem., 60, 173-181, https://doi.org/10.1016/j.soilbio.2013.01.019

Tryon E.H., 1948. effect of charcoal on certain physical chemical, and biological properties of forest soils. Ecol. Monogr., 18, 81-115, https://doi.org/10.2307/1948629

Walsh J.J., Jones D.L., Edwards-Jones G., and Prysor Williams A.P., 2012. Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. J. Plant Nutr. Soil Sci., 175(6), 840-845, https://doi.org/10.1002/jpln.201200214

Wang C., Kong Y., Hu R., and Zhou G., 2020. Miscanthus: A fast-growing crop for environmental remediation and biofuel production. GCB Bioenergy, 13, 58-69, https://doi.org/10.1111/gcbb.12761

Wang J., Xiong Z., and Kuzyakov Y., 2016. Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenergy, 8, 512-523, https://doi.org/10.1111/gcbb.12266

Wolna-Maruwka A., Sawicka A., and Kayzer D., 2007. Size of selected groups of microorganisms and soil respiration activity fertilized by municipal sewage sludge. Pol. J. Environ. Stud., 16(1), 129-138.

Yang Y., Sun K., Han L., Chen Y., Liu J., and Xing B., 2022. Biochar stability and impact on soil organic carbon mineralization depend on biochar processing, aging and soil clay content. Soil Biol. Biochem., 169, 108657, https://doi.org/10.1016/j.soilbio.2022.108657

Yu Z., Chen L., Pan S., Li Y., Kuzyakov Y., Xu J., Brookes P.C., and Luo Y., 2018. Feedstock determines biochar-induced soil priming effects by stimulating the activity of specific microorganisms. Eur. J. Soil Sci., 69, 521-534, https://doi.org/10.1111/ejss.12542

Zeng J., Liu X., Song L., Lin X., Zhang H., Shen C., and Chu H., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biol. Biochem., 92, 41-49, https://doi.org/10.1016/j.soilbio.2015.09.018

Zhang Q., Zhou W., Liang G., Wang X., Sun J., He P., and Li L., 2015 Effects of different organic manures on the biochemical and microbial characteristics of albic paddy soil in a short-term experiment. PLOS One, 10(4), https://doi.org/10.1371/journal.pone.0124096

Zhao B., O'Connor D., Zhang J., Peng T., Shen Z., Tsang D.C.W., and Hou D., 2018. Effect of pyrolysis temperature, heating rate, and residence time on rapeseed stem derived biochar. J. Clean. Prod., 174, 977-987, https://doi.org/10.1016/j.jclepro.2017.11.013

Zhen Z., Liu H., Wang N., Guo L., Meng J., Ding N., Wu G., and Jiang G., 2014. Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. PLOS One, 9(10), https://doi.org/10.1371/journal.pone.0108555

Zheng H., Wang X., Luo X., Wang Z., and Xing B., 2018. Biochar-induced negative carbon mineralization priming effects in a coastal wetland soil: Roles of soil aggregation and microbial modulation. Sci. Total Environ., 610-611, 951-960, https://doi.org/10.1016/j.scitotenv.2017.08.166

Zheng H., Wanga Z., Denga X., Herbert S., and Xing B., 2013. Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. Geoderma, 206, 32-39, https://doi.org/10.1016/j.geoderma.2013.04.018

Zhong W., Gu T., Wang W., Zhang B., Lin X., Huang Q., and Shen W., 2010. The effect of mineral fertilizer and organic manure on soil microbial community and diversity. Plant and Soil, 326, 511-522, https://doi.org/10.1007/s11104-009-9988-y

SUPPLEMENT

Table S1. General statistics for chemical, biological and respirometric soil parameters

Variable	Symbol	Unit	Min	Max	Mean	Standard deviation
Total carbon	TC	g kg^{-1} DM	7.24	12.87	9.50	1.79
Hot water extractable carbon	HWC	g kg^{-1} DM	0.10	0.81	0.46	0.21
Total nitrogen	TN	g kg^{-1} DM	0.87	1.42	1.12	0.14
C/N ratio	C/N	–	7.19	10.19	8.44	0.74
Phosphorus available to plants	P	g kg^{-1} DM	61.46	155.03	89.9	29.1
Magnesium available to plants	Mg	g kg^{-1} DM	8.08	66.69	36.9	18.4
Potassium available to plants	K	g kg^{-1} DM	100.66	260.05	163.6	46.3
pH	pH	–	3.68	7.11	5.02	1.28
Bacteria load	BL	µg DNA g^{-1} DM	0.89	9.24	3.48	2.42
Fungi load	FL	µg DNA g^{-1} DM	4.55	23.49	16.04	6.02
O_2 uptake	O_2	µg O_2 kg^{-1} DM	1159.09	6335.8	3005.54	1733.29
CO_2 emission	CO_2	µg CO_2 kg^{-1} DM	981.09	5540.99	3301.8	1592
Carbon mineralized after 7 days	TC-7	%	1.64	14.94	6.94	4.25
Carbon mineralized after 100 days	TC-100	%	15.05	78.79	41.54	19.21

Table S2. The *F*-statistics and significance level for fertilization x season interaction according to ANOVAs of chemical and biological soil parameters

	Chemical and biological soil parameters									
	TC	HWC	TN	C/N	pH	P	Mg	K	BL	FL
F-value	180.82	6.75	10.17	18.44	245.08	14.12	3.39	15.88	22.69	8.11
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.007	<0.001	<0.001