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RESPONSE OF CHEMICAL AND MICROBIAL PROPERTIES TO SHORT-TERM BIOCHAR AMENDMENT IN DIFFERENT AGRICULTURAL SOILS

ABSTRACT: The objective of this study was to assess the effect of biochar soil amendment (BSA) on chemical and microbial properties in different agricultural soils in Vojvodina Province. Short-term pot experiment consisted of five biochar application doses (0, 0.5, 1, 2, and 3%) and five contrasting soil types (Mollic Gleysol, Eutric Cambisol, Calcaric Fluvisol, Gleyic Chernozem, and Haplic Chernozem), planted with sunflower (*Helianthus annuus* L.) and winter wheat (*Triticum aestivum* L.). The examined chemical and microbial properties were significantly influenced by soil type and interaction of experimental factors. Significant influence of biochar on the contents of calcium carbonate (CaCO₃), total nitrogen (N), total carbon (C), soil organic carbon (SOC), humus and potassium (K) of the tested soils was observed. Biochar also significantly affected the number of azotobacters (AZB), fungi (FNG), actinomycetes (ACT) and copiotrophic bacteria (CB). The effect of BSA varied depending on the applied dose, with higher values of the examined chemical and microbial parameters at higher doses of application. Further studies on using biochar in soils with low fertility will be necessary to establish its efficiency as an enhancer for agricultural production in Serbia.

KEYWORDS: biochar, carbon, humus, nitrogen, microbial number, soil type

INTRODUCTION

Biochar is a stable C-rich material produced by thermal degradation of plant-derived biomass under oxygen-free to oxygen-deficient conditions (Sohi et al., 2009). Application of biochar into agricultural soils can improve soil fertility, increase carbon sequestration and mitigate greenhouse effects (Brassard

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et al., 2016). Numerous studies confirmed that biochar soil amendment (BSA) changes physical and chemical soil properties (water holding capacity, soil aeration and pH, soil structure, nutrient retention and availability, release of soluble C and availability of micronutrients), decreases fertilizer requirements, and increases sorption of toxic compounds (Lehmann and Joseph, 2009). Positive priming effects of biochar on crop biomass and yield have also been reported (Castaldi et al., 2011).

Studies of BSA have mainly been conducted in regions with tropical and humid climate, on soils which are more degraded, while the possibility of biochar application on soils in regions with temperate climate remains unexplored (Jeffery et al., 2011; Liao et al., 2016). There is no experimental confirmation on using biochar and its effects on soil properties in the environmental conditions of Vojvodina Province (South Pannonian Plain), Serbia. The soils in Vojvodina are potentially fertile. However, a downward trend in the content of humus and soil organic carbon (SOC) has been observed over the past few decades as a result of tillage, insufficient fertilization, removal and burning of crop residues (Šeremešić et al., 2013).

For the long-term preservation of soil fertility and protection of agroecological systems, it is necessary to enable intensification of microbiological activity by applying appropriate measures, such as BSA. Soil microorganisms can be affected by BSA because of their role in mineralization of organic matter and involvement in nutrient cycles (Ferrell et al., 2013). Improved understanding of the dynamics and interrelationship between chemical and microbial properties in response to biochar amendment can provide valuable information on soil fertility potential and assessment of strategic biochar application to agricultural soils (Quilliam et al., 2013). Therefore, the objective of this study was to assess the effect of biochar amendment on chemical and microbial properties in different agricultural soils in Vojvodina Province (Serbia).

MATERIAL AND METHODS

Soil description

Soil types were selected based on the most common use by farmers in the study area (Vojvodina Province): Mollic Gleysol (clayic): GL-mo-ce (Novi Bečej, 45° 58' N; 20° 08' E; 70 m), Calcaric Fluvisol (arenic): FL-ca-ar (Šangaj, 45° 29' N; 19° 87' E; 75 m MASL), Eutric Cambisol (clayic): CM-eu-ce (Bukovac, 45° 20' N, 19° 90' E, 198 m MASL), Gleyic Chernozem (arenic): CH-gl-ar (Futog, 45° 24' N; 19° 70' E; 78 m MASL) and Haplic Chernozem (loamic): CH-ha-lo (Čenej, 45° 35' N; 19° 79' E; 79 m MASL). Soils were classified according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). Vojvodina Province extends in the Pannonian Plain, characterized by typically temperate, continental climate.

Biochar

Biochar was purchased from a local company in Serbia. Chemical properties of biochar were analyzed according to the methods applied in soil analysis. Ash content of biochar was < 4 %, with the density of 0.402 g cm⁻³, and moisture of 4–10%. Biochar contained 74.51% carbon (C), 95.03% organic carbon (C_{org}), and 0.547% nitrogen (N). Available phosphorus (P) content was 53.8 mg / 100 g, available potassium (K) content was 291 mg / 100 g, whereas pH of biochar was 7.54 (in KCl) and 8.24 (in H₂O). Before application into the soil, biochar was milled and passed through a brass sieve with a 2 mm aperture size and weighed in bags with the planned amount for each pot.

Pot experiment

The experiment was conducted in a greenhouse under semi-controlled conditions, characterized by the complete control of soil moisture, partial control of light and protection from adverse mechanical influences, without controlling the air temperature. Soils and biochar were simultaneously distributed in pots (v/w 10 l) in the amount of 5 kg per pot. The experiment consisted of five biochar application rates: 0 (0%), 25 (0.5%), 50 (1%), 100 (2%) and 150 (3%) g pot⁻¹ (w/w). Treatments were arranged in a randomized block design with four replications. The experimental crops were winter wheat (*Triticum aestivum* L.) and sunflower (*Helianthus annuus* L.). Sunflower was sown in early April and matured in late August. Winter wheat was sown in early October and harvested in early June of the following year.

Soil sampling

After harvesting winter wheat at the end of the experiment, the samples were collected from the experimental pots assembled for each soil type and biochar dose in order to examine the effect of biochar application. After removing approximately 3 cm of the soil surface, about 0.5–1 kg of soil was taken from each pot. The samples were placed into polyethylene bags and transported to the laboratory. The samples were sieved at < 2 mm and stored at room temperature for chemical analysis. An aliquot of each soil sample was stored in refrigerator at 4 °C before microbiological analysis. All chemical and microbial analysis were performed in four replicates.

Soil chemical analysis

The main chemical properties of examined soils were determined using standard methods. pH in soil suspension with water or 1M KCl was analyzed potentiometrically (Mettler Toledo SevenCompact pH/ion). The content of

calcium carbonate (CaCO_3) (%) was determined with a Scheibler calcimeter. Humus content (%) was determined by oxidation of organic matter by the method of Tyurin. Contents of total nitrogen (N) and total and organic carbon (C and C_{org}) (%) were analyzed on elemental CHNS analyzer (Vario EL III, Elementar). C_{org} (%) content was expressed in the SI unit as g C/kg soil (SOC g/kg). Available K_2O and P_2O_5 (mg/100 g) were analyzed by AL-method according to Egner-Riehm, by extraction with ammonium lactate. Potassium content (K) was determined by the flame photometer (Evans Electro Selenium Ltd.) and phosphorus (P) content was assessed using the blue method in a spectrophotometer (Agilent Cary 60, Agilent Tehnologies).

Soil microbial analysis

Total cultivable bacterial and fungal colony forming units (CFU) were measured by the dilution plate method on the appropriate nutritive media. The total number of microorganisms (TNM) was determined on a soil agar (5 days, at 28 °C), the number of *Azotobacter* sp. (AZB) and free N_2 -fixers (NFB) on nitrogen-free medium (Fyodorov's medium) (48 h and 5 days, respectively, at 28 °C). The number of ammonifiers (AMN) was determined on a meat peptone agar (3 days, at 28 °C), actinomycetes (ACT) on Krasilnikov's agar (7 days, at 28 °C), fungi (FNG) on Czapek-Dox agar (5 days, at 28 °C), copiotrophic (CB) and oligotrophic (OB) bacteria on high and low C content medium (7 and 14 days, at 28 °C). The average number of colony forming units (CFU) was calculated per 1.0 g of soil dry weight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using software STATISTICA 12.6 (Statsoft, Tulsa, Oklahoma, USA). Means were separated using Tukey's HSD (honest significant difference) test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Chemical properties of the examined agricultural soils varied significantly depending on the type of soil (Table 1). Investigated soil types had a slightly alkaline pH reaction, which is a favorable environment for the growth and development of most plants and microorganisms. Variability of the other tested chemical properties was very apparent because of differences between the studied soil types. The highest contents of total N, total C, SOC, and humus were observed in Haplic Chernozem, followed by Mollic Gleysol. The lowest values of these parameters were detected in Gleyic Chernozem, where the highest contents of available P and K were found. Lower contents of humus, N and SOC were also determined in Eutric Cambisol and Calcic Fluvisol (Table 1).

Biochar had significant influence on the content of CaCO₃, total N, total C, SOC, humus and K. Interaction of soil type and biochar doses significantly affected the pH, total N, total C, SOC humus and K. Except pH and CaCO₃, concentration of all the examined chemical properties showed an upward trend after applying an increased dose of biochar (Table 1). The highest increase of examined chemical properties was recorded at 2% and 3% biochar treatments. The results are in accordance with those presented by Ippolito et al. (2014), who reported positive effects of BSA on chemical properties in calcareous soils. A significant increase in humus content along with increased doses of BSA could be explained by chemical content of BSA and high content of total C (74.51%). The positive effects of BSA on C and humus content suggested that application of biochar may promote the formation of stable soil organic matter (SOM) (Muhammad et al., 2014; Prayogo et al., 2014).

Table 1. Chemical properties depending on examined soil types and biochar doses

Factor/Variable	pH	CaCO ₃ (%)	Total N (%)	Total C (%)	SOC (g kg ⁻¹)	Humus (%)	P (mg)*	K (mg)*	
Soil (S)	GL-mo	7.23 e	0.68 d	0.25 b	4.34 a	35.5 a	3.74 b	14.42 c	25.78 b
	CM-eu	7.42 d	0.67 d	0.15 d	2.01 b	14.2 c	1.82 d	9.16 d	16.13 d
	FL-ca	7.75 a	21.66 a	0.17 c	4.35 a	17.8 b	2.21 c	15.07 c	7.41 e
	CH-gl	7.61 b	5.40 c	0.14 e	1.79 b	13.2 c	1.71 d	40.95 a	39.67 a
	CH-ha	7.49 c	7.35 b	0.30 a	4.68 a	36.3 a	4.75 a	23.45 b	19.03 c
Bio- char (B)	0%	7.51 a	7.57 a	0.18 d	2.60 b	17.3 c	2.38 d	20.30 b	20.61 b
	0.5%	7.50 a	7.18 ab	0.19 c	2.96 b	19.3 c	2.56 c	20.48 ab	21.26 b
	1%	7.52 a	7.09 ab	0.20 b	3.13 b	22.8 b	2.81 b	20.57 ab	21.56 b
	2%	7.47 a	6.83 ab	0.22 a	4.12 a	28.4 a	3.16 a	20.62 ab	21.14 b
	3%	7.49 a	7.08 b	0.23 a	4.36 a	29.2 a	3.32 a	21.08 a	23.45 a
P	S	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.069	0.008	0.000	0.000	0.000	0.000	0.076	0.000
	S × B	0.024	0.085	0.000	0.012	0.000	0.000	0.115	0.007

Values are the means of 4 replicates. Values in a row with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. GL-mo: Mollic Gleysol, CM-eu: Eutric Cambisol, FL-ca: Calcaric Fluvisol, CH-gl: Gleyic Chernozem, CH-ha: Haplic Chernozem.
* In a 100 g sample of soil.

Soil properties have a strong impact on a range of processes influencing crop yield, including microbial diversity (Coleman, 2011). The abundance and activity of certain microbial groups are positively or negatively correlated with soil chemical properties (Falkowski et al., 2008; Liang and Balser, 2011). Soil microorganisms can be affected by BSA because of their role in maintaining crop productivity through mineralization of complex organic compounds in soil, as well as their sensitivity to environmental change (Ferrell et al., 2013; Muhammad et al., 2014). The analysis of variance revealed that the soil type significantly

influenced the number of all investigated microbial groups (Table 2). The TNM, NFB, AMN, CB and OB were significantly higher in Mollic Gleysol compared with other soil types. AZB and ACT were most abundant in Chernozem soils, while the number of FNG was the highest in Calcaric Fluvisol (Table 2).

BSA had significant influence on the number of AZB, FNG, ACT and CB. Interaction of experimental factors significantly affected the number of all the examined microbial groups (Table 2). The effect of BSA depended on microbial group and the applied dose. Better effects on the tested microbial parameters were obtained with higher doses of biochar (1–3%). The treatments negatively affected the number of AMN and FNG.

Table 2. Microbial properties depending on examined soil types and biochar doses (g^{-1} soil)

Factor/ Variable	AZB (CFU $\times 10^2$)	TNM (CFU $\times 10^6$)	AMN (CFU $\times 10^6$)	NFB (CFU $\times 10^5$)	FNG (CFU $\times 10^3$)	ACT (CFU $\times 10^3$)	OB (CFU $\times 10^6$)	CB (CFU $\times 10^6$)	
Soil (S)	GL-mo	46 ab	467 a	242 a	637 a	67 b	2 d	371 a	371 a
	CM-eu	12 c	255 b	111 b	402 bc	62 b	4 cd	294 b	224 c
	FL-ca	40 b	325 b	153 b	500 b	85 a	7 bc	389 a	283 bc
	CH-gl	60 a	149 c	90 b	327 c	29 c	11 b	156 c	302 ab
	CH-ha	54 ab	137 c	117 b	361 c	35 c	19 a	160 c	256 bc
Bio- char (B)	0%	38 b	270 ab	172 a	454 a	75 a	6 b	270 a	250 b
	0.5%	36 b	271 ab	162 a	430 a	45 b	12 a	255 a	247 b
	1%	42 ab	254 ab	125 a	428 a	50 b	12 a	295 a	373 a
	2%	39 ab	313 a	152 a	475 a	52 b	6 b	304 a	336 a
	3%	57 a	225 b	100 a	439 a	56 b	7 b	247 a	229 b
P	S	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.019	0.052	0.076	0.689	0.000	0.000	0.124	0.000
	S \times B	0.012	0.000	0.008	0.004	0.000	0.011	0.000	0.000

Values are the means of 4 replicates. Values in a row with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. GL-mo: Mollic Gleysol, CM-eu: Eutric Cambisol, FL-ca: Calcaric Fluvisol, CH-gl: Gleyic Chernozem, CH-ha: Haplic Chernozem. AZB: azotobacters, TNM: total microbial number, AMN: ammonifiers, NFB: N_2 -fixers, FNG: fungi, ACT: actinomycetes, OB: oligotrophs, CB: copiotrophs.

Our results are similar to those reported by Hu et al. (2014) who found 12%, 30%, 37% higher bacterial diversity, and 17%, 40%, 23% lower fungal diversity in biochar amended soil. Similarly, biochar application to a calcareous soil caused an increase in soil C content, soil respiration rates and bacterial populations (Ippolito et al., 2014). Prayogo et al. (2014) observed that the amount of bacterial biomass was increased by BSA, providing evidence of stimulated abundance of Gram-negative bacteria and actinobacteria. Anderson et al. (2011) discovered that BSA had a positive influence on the abundance of bacterial families involved in nitrate denitrification, while organisms involved in nitrification were less abundant.

AMN participate in the processes of decomposition and transformation of organic nitrogen compounds in the soil, while NFB have the ability to reduce atmospheric nitrogen, transform it into plant-available forms, and thus enrich the soil with this important element. CB and OB were selected to determine bacterial response to alteration in C availability. As active decomposers of organic matter, ACT and FNG are included in the cycle of C, N, P, and other nutrients. Positive effects on the analyzed chemical and microbial properties revealed that BSA could potentially affect soil C and N cycling in the examined agricultural soils. Although it is accepted that most of the biochar-C is largely unavailable to microbes (Thies and Rillig, 2009; Farrell et al., 2013), it is clear that BSA can have positive influence on microbial community structure and soil fertility (Sun et al., 2013; Gul et al., 2015), which was confirmed by this research.

CONCLUSION

The examined soil properties were significantly influenced by soil type and interaction of experimental factors. Biochar amendment significantly affected the contents of calcium carbonate, total nitrogen, total and soil organic carbon, humus, potassium, as well as the number of azotobacters, fungi, actinomycetes and copiotrophic bacteria of tested soils. This was the first experiment examining biochar implementation in Serbia, in the continental ecological conditions of Vojvodina Province, and presents the preliminary results of its effect on the chemical and microbial properties of agricultural soils. Further research will include testing the effect of biochar application in field conditions, on a range of crop species and soil types. In addition to soil properties, it will be necessary to establish the effect of biochar application on yield components and quality of agricultural products.

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ПРОМЕНЕ ХЕМИЈСКИХ И МИКРОБИОЛОШКИХ СВОЈСТАВА
НАКОН ПРИМЕНЕ БИОУГЉА НА РАЗЛИЧИТЕ ТИПОВЕ
ОБРАДИВОГ ЗЕМЉИШТА

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РЕЗИМЕ: Циљ ових истраживања био је да се процени утицај биоугља, као оплемењивача земљишта на хемијска и микробиолошка својства на различитим типовима пољопривредног земљишта у Војводини. Краткорочни експеримент у судовима састојао се од пет доза примене биоугља (0, 0.5, 1, 2 и 3%) и пет различитих типова земљишта (ритска црница, еутрични камбисол, алувијално земљиште, чернозем на алувијалном наносу и чернозем на лесној тераси), где су у две производне године гајени озима пшеница и сунцокрет. На испитивана хемијска и микробиолошка својства значајно су утицали тип земљишта и интеракција експерименталних фактора. Уочен је значајан утицај биоугља на садржај калцијум-карбоната (CaCO_3), укупног азота (N), укупног угљеника (C), органског угљеника (SOC), хумуса и калијума (K) у испитиваном земљишту. Биоугаљ је такође значајно утицао на број азотобактера (AZB), гљива (FNG), актиномицета (ACT) и копиотрофних бактерија (CB). Ефекат биоугља варирао је у зависности од примењене дозе, са вишим вредностима испитиваних хемијских и микробиолошких параметара при вишим дозама примене. Да би се утврдила његова ефикасност, као оплемењивача земљишта и унапређења пољопривредне производње у Србији, потребно је урадити још истраживања са коришћењем биоугља на земљиштима лошијих производних својстава.

КЉУЧНЕ РЕЧИ: азот, биоугаљ, бројност микроорганизама, тип земљишта, угљеник, хумус