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Original

Potential of biochar after the biological activation by native soil microflora

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ABSTRACT

Objective. Dehydrogenase activity after the biological activation of biochar by the native soil microorganisms was studied. The main aim was to improve biochar properties by activation and make it more friendly for the soil microflora. **Materials and methods.** The activation was reached by aerating with the soil solution for two weeks. No special inoculum of microorganisms was applied. The following treatments in four replicates were prepared: conventional raw biochar (BR), activated biochar (BA), mineral fertilizer DAM 390 (NF), and control (C). A statistical test for comparing treatments means (Fisher $p \leq 0.05$; program STATISTICA 12.0; StatSoft software Inc., Tulsa, Oklahoma, USA) was used. **Results.** Statistically significant differences in the dehydrogenase activity between the treatments BR, BA, and C were found. Application of mineral fertilizers had a negative effect and increasing of nitrogen leaching was observed. **Conclusions.** Activating of biochar is suitable metods for impove soil biota conition compared with convention biochar.

Keywords: Biochar; carbon; dehydrogenase activity; nitrogen; soil microorganisms (*Source: CAB*).

RESUMEN

Objetivo. Se estudió la actividad de la deshidrogenasa tras la activación biológica del biocarbón por los microorganismos nativos del suelo. El objetivo principal era mejorar las propiedades del biocarbón mediante su activación y hacerlo más amigable para la microflora del suelo. **Materiales y métodos.** La activación se logró mediante la aireación del suelo con la solución durante dos semanas. No se aplicó ningún inóculo especial de microorganismos. Se prepararon los siguientes tratamientos en cuatro réplicas: biocarbón crudo convencional (BR), biocarbón activado (BA), fertilizante mineral DAM 390 (NF) y control (C). Se utilizó una prueba estadística para comparar las medias de los tratamientos (Fisher $p \leq 0.05$; programa STATISTICA 12.0; StatSoft software Inc., Tulsa, Oklahoma, USA). **Resultados.** Se encontraron diferencias estadísticamente significativas en la actividad deshidrogenasa entre los tratamientos BR, BA y C. La aplicación de fertilizantes minerales tuvo un efecto negativo y se observó un aumento de la lixiviación de nitrógeno. **Conclusiones.** La activación del biocarbón es un método adecuado para mejorar la condición de la biota del suelo en comparación con el biocarbón convencional.

Palabras clave: Biochar; carbono; actividad deshidrogenasa; nitrógeno; microorganismos del suelo (*Fuente: CAB*).

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INTRODUCTION

Biochar, carbonized organic matter, is applied to the soil for environmental purposes. Besides, it is very stable, contains more than 60% of carbon, and can remain in the soil for 100–1000 years (1). It consists of the highly condensed aromatic particles, which are slowly decomposed, having a positive effect on carbon sequestration in soil. Biochar affects the soil physical and chemical properties (porosity and water holding capacity, soil pH, cation exchange capacity, and nutrient availability) as well 1.

Acording to Lehmann (1) and Zhang (2) biochar is not a very friendly place for soil biota, and has no effect or even the negative effect on plant growth (1,2). Quilliam (3) studied the settlement of microorganisms on the biochar surface and concluded that even after three years of the application the colonisation was negligible.

Only a few more observations showed how to make biochar more attractive for soil biota using the chemical and physical ageing (4,5,6).

The effect of biochar on to the microbial activity is evaluated by the dehydrogenase activity (1,3). Either stimulation or inhibition effect is registered. The dehydrogenase activity depends also on soil type, moisture, aeration, pH, temperature, organic matter content and quality, fertilizers, and pollution (7).

This research aims at the evaluation of the dehydrogenase activity after biochar ageing using native soil solution. Furthermore, we studied the nitrogen availability and leaching in pot experiments.

MATERIAL AND METHODS

Soil characterization. Studied locality Březová nad Svitavou (Czech Republic) is one of the water source protected area in the Czech Republic. Monitoring of soil and water quality is therefore very important there. The soil was sampled within the profile to the depth 1 m and classified as Gleyic Planosol (8). Soil samples for studying of microbiological properties were taken from the upper *Ap* humic horizon (0-25 cm). All of the selected samples were stabilized for one week at laboratory temperature before analysis. Soil samples for determination of other properties were air-dried at room temperature and sieved (2 mm mesh). Total organic carbon (TOC) was determined by oxidimetric titration method (9) The exchangeable soil reaction (pH/KCl) was measured in 1M KCl (10g of soil and 25 mL of 1M KCl) using digital pH meter (Metrohm, Switzerland). The texture was determined by the pipette method (10). Basic soil properties are shown in table 1.

Table 1. Basic soil properties of Gleyic Planosol.

TOC* (%)	pH/KCl	Nt** (%)	Texture (%)		
			Clay <0.002 mm	Sand 2.00-0.25 mm	Silt 0.05-0.002 mm
1.52	5.19	0.13	31.86	3.48	45.16
			Silty Loam		

* TOC = total organic carbon; **Nt = total nitrogen

Biochar preparation. Biochar was produced by the company PYREQ (Germany) using dry carbonization method (500-700°C). Waste plant biomass was cutting (> 30 mm), then crushing and degassing. Received biochar was certified according to the European Biochar Certificate (31.12.2017, No. 70401) (Table 2).

Table 2. Selected properties of biochar (BR) (PYREQ, Germany).

Titulo	Titulo
TOC* (%)	2.22
pH/KCl	7.84
Conductivity (mS/cm)	0.06
P (mg.kg^{-1})	260.20
K (mg.kg^{-1})	141.80
Ca (mg.kg^{-1})	1474.00
Mg (mg.kg^{-1})	51.60

* TOC = total organic carbon

Experimental design. Initially, the pot experiment was carried out in the phytotron (PlantMaster, Germany). Day temperature 24°C, relative moisture 75%, light intensity 380 μmol.m^{-1}.s^{-1}, night temperature 20°C and moisture 65%, which are recommended for our type of phytotron. Four variants in four replicates were set: conventional biochar (BR), activated biochar (BA), DAM 390 = 39 kg of N in 100 liters (NF), and control – bare soil (C). The experimental pots were filled with 1 kg of mineral soil and the appropriate dose of amendments (Table. 3). The samples were moistured to the field water capacity. Each pot was provided by a disc on the bottom of the pot to capture the leaching nitrogen (Figure 1). As an experiment plant lettuce (*Latuca sativa*) was chosen for its sensitivity and rapid growth. Tree seeds of this plant were given into each pot. After five days of the experiment, only the one seed was left (Figure 1).

Table 3. Design of pot experiment in phytotron PlantMaster, Germany.

Variants	Doses
BR[1]	50 $t.ha^{-1}$
NF[2]	140 $kg.N^{-1}.ha^{-1}$
BA[3]	50 $t.ha^{-1}$
C[4]	0

[1]BR – conventional biochar; [2]NF -Mineral fertilizer DAM 390; [3]BA – activated biochar; [4]C – control

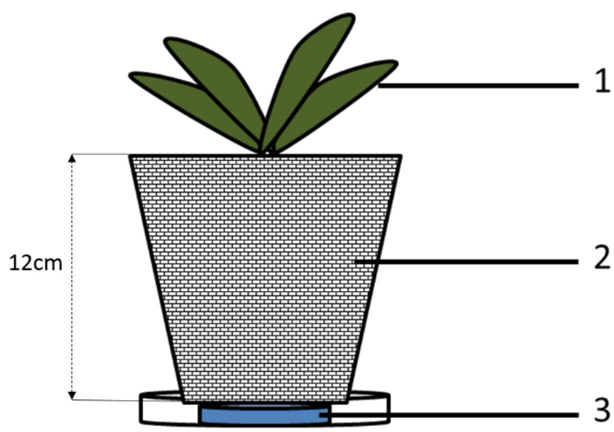

Figure 1. Schema of pot experiment (1- Plant, 2 – pot with mineral soil, 3 – ion exchange disc).

Biochar activation. Schema of biochar ageing and activation is shown in Figure 2.

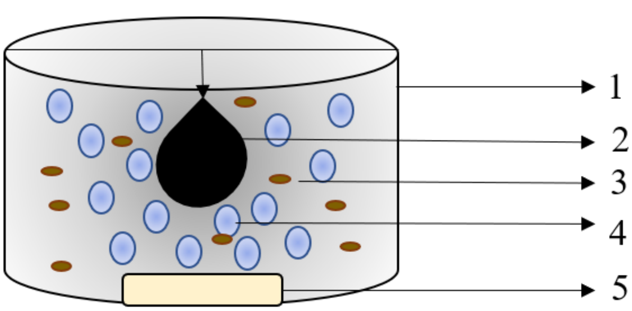

Figure 2. Scheme of biochar activation (1- plastic barrel (20 L) with distilled water; 2 – biochar in fabric bag; 3 – soil solution with native microflora; 4 – bubbles of the air; 5 – aerator)

The method of activation was as follows: plastic barrel (20 L) was poured with distilled water. A fabric with biochar (1 kg) and approximately 20 g of mineral soil was added. Water in the barrel was aerating for two weeks and then was biochar in the fibre dried at the room temperature for 24 h. Besides activated biochar also conventional biochar we used as a control.

Analytical methods

Polycarbonates. They were extracted by toluene using Soxtec as follows: 2 mg of activated or conventional biochar was extracted by toluene for 2 h at 180°C, then filtrated, evaporated in vacuum, and dried under nitrogen atmosphere. The rest of toluene was dissolved in 1 mL of isooctane, filtrated using nylon filter (0.45 μm) and analyzed by GC/MS-TOF, Pegasus IVD (11).

Plant biomass evaluation. The weight of above and underground biomass was registered and their separation was made. The above and underground biomass was washed and dried (105°C) and the constant weight was registered (12).

Dehydrogenase activity (DHA). DHA was determined according to Casida et al (13). The specific dyes such as the triphenyl-tetrazolium chloride (TTC) were used. By the reduction of a colourless, water-soluble substrate (TTC) by dehydrogenases present in the soil environment, an insoluble product with red colour (triphenyl-formazan-TPF) is formed. TPF can be easily quantified calorimetrically at the range of visible light (485 nm). This test, however, reflected positive answer only at a neutral range of pH and in the presence of calcium carbonate for buffering soil system. Briefly, if the red colours of soil samples prepared for spectrophotometer analyses are more intensive, the measured level of DHA is higher. Consequently, soil samples without colour (= no DHA activity) or those with are red (=high DHA activity) are distinguished.

Mineral nitrogen leaching. Nitrogen loss was measured continually during the whole experiment. The mixture 1: 1 of cations ion-exchange grains (CER, Purolite C100E) and anions ion-exchange grains (AER, Purolite A520E) was used. This mixture was placed into the permeable nylon discs UHELON 130T with diameter 42 μm (Silk & Progress; Czech Republic). The discs were installed under the outflows of the experimental pots. Total mineral nitrogen leaching was determined after the lettuce harvesting. Captured mineral nitrogen was determined by distillation – titration method (14). The distillation apparatus Behr S3 StreamDistillation Unit (BehrLabor Technik; Düsseldorf; Germany).

Index of nitrogen availability. Available nitrogen is supposed to be a part of total nitrogen, which is easily got by microorganism or plant roots within a short time (= relatively quickly). Available nitrogen was determined as follows: 20 g of the soil sample is poured with 50 mL of 2 M KCl and shaking for an hour, and filtrated. Similarly, 20 g of the soil sample is poured with 50 mL of distilled water, shaking for an hour, and filtrated (15). Content of total mineral nitrogen was determined by distillation – titration method (14). Parallelly, the same soil samples were prepared this way and placed into the thermostat at 40°C for seven-days incubation. The last caused spontaneous cells lysis followed by nitrogen mineralization of death microbial biomass. Then 50 mL of 4 M KCl is added, this suspension is shaking for an hour and filtrated. The content of NH_4^+-N was determined by distillation – titration method (14). The difference between the total nitrogen and NH_4^+-N nitrogen is supposed to be the easily available nitrogen.

The obtained data were assessed by one-way analysis of variance (ANOVA) and for comparing treatments means (Fisher p≤0,05) the program STATISTICA 12.0; StatSoft software Inc., Tulsa, Oklahoma, USA) was used.

RESULTS

Acording to FAO studied Gleyic Planosol is classified as a low-rank arable soil, silty loam textured, with acid soil reaction, low humus content and quality, and low total nitrogen content.

Comparison of the elemental composition of activated biochar is given in table 4.

Table 4. Elemental composition of studied types of biochar.

Variants	N (weight %)	C (weight %)
BR[1]	0.38	56.05
BA[2]	0.35	58.66

[1]BR – conventional biochar; [2]BA – activated biochar

As it is evident carbon and nitrogen content is higher to compare with conventional certified biochar. It is supposed that this is a result of nitrogen immobilization and a result of carbon increase due to microbial biomass presence. After lettuce harvesting total biomass was studied. The lowest total biomass was in control variant C (1.31 g) and the highest after mineral fertilizer application NF (3.88 g). Biomass after biochar ageing BA was higher (2.57g) to compare with conventional biochar BC (1.63 g).

Obtained results of pot experiment are documented in Figures 3 and 4. Statistically significantly higher is biomass under mineral fertilizer treatment (NF) followed by activated biochar (BA) and conventional biochar (BR). A positive effect of nitrogen fertilizing on biomass production was also confirmed by Pittelkow (16) Also according to Lehman et al. (1) application of mineral fertilizers and simplifying of food chains may lead to reducing of the diversity of soil biota. As a result, plants production is more and more depending on mineral fertilizing and pesticides. Pauli (17) showed that improving soil biological properties and conditions for soil biota has a direct effect on plant production. Chebotarew (18) stated that mineral fertilizers are important in the intensively used soils, where lack of nutrients is taken place due to their loss after harvesting.

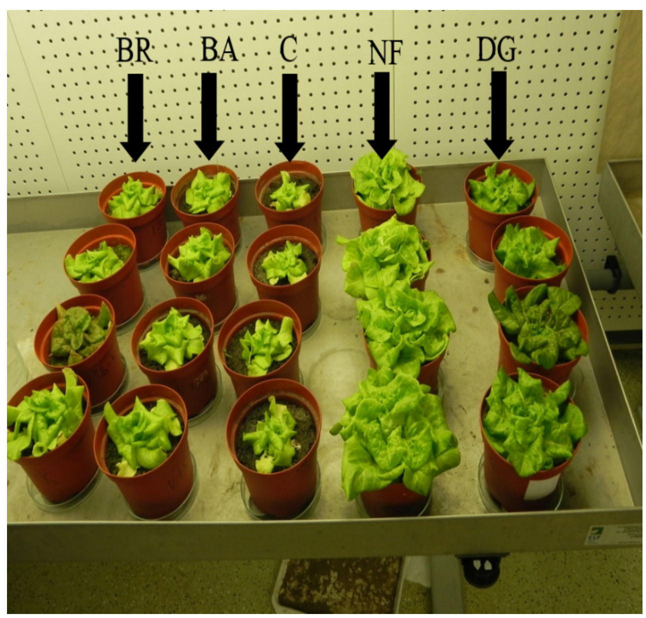

Figure 3. Pot experiments in phytotron PlantMaster, Germany (BR – conventional biochar, BA – activated biochar, C – control, NF – mineral fertilizer DAM 390).

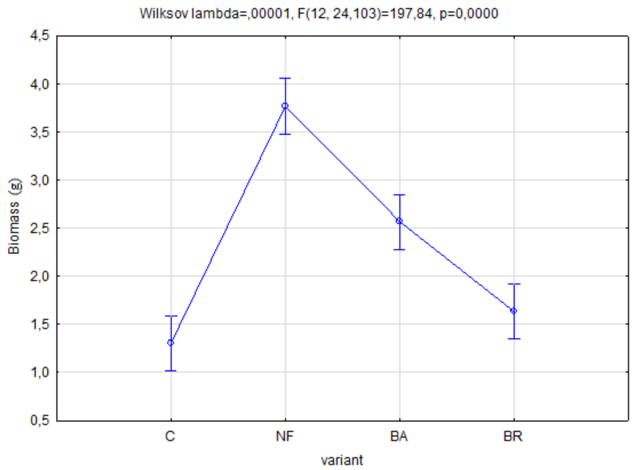

Figure 4. Statistically significant differences in biomass production (C – control, NF – mineral fertilizer DAM 390, BA – activated biochar, BR – conventional biochar).

Dehydrogenase activity (DHA) – evaluation of DHA is shown in Figure 5. DHA activity was decreasing in the order: BA (17,95 TPF/ g*h) > BR (11,51 TPF/ g*h) > NF (10,8 TPF/ g*h) > C (10,32 TPF/ g*h). Statistically significant differences between BA, BR and control (C)were determined. No differences between control (C) and mineral fertilizer NF were found.

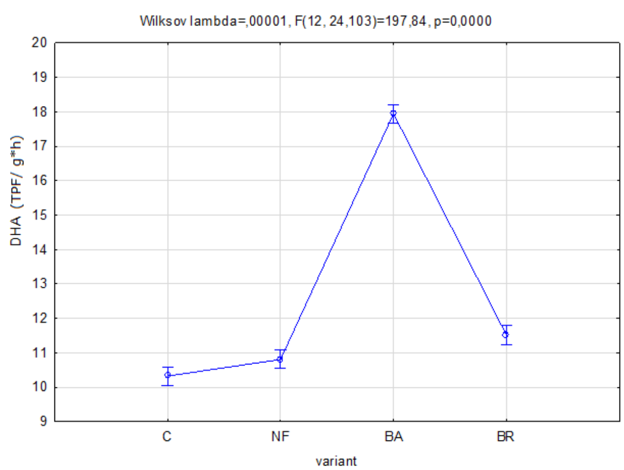

Figure 5. Statistically significant differences in DHA activity (C – control, NF – mineral fertilizer DAM 390, BA – activated biochar, BR – conventional biochar).

The amount of mineral nitrogen, which is leaching to the soil, is an important environmental parameter and the results of our study are shown in Figure 6. The highest nitrogen loss was in NF variant (2.06 mg.mm^{-2}). The increasing sequence is as follows: BA (0.14 mg. mm^{-2}) <BR (0.16 mg.mm^{-2}) <C (0.17 mg.mm^{-2}) <NF (2.06 mg. mm^{-2}). The lowest nitrogen loss was determined after biochar activation (BA; 0.14 mg.mm^{-2}). It was also found out that conventional biochar application did not affect nitrogen leaching much. Statistically significant results (ten times higher) were found only after mineral fertilizers application (NF).

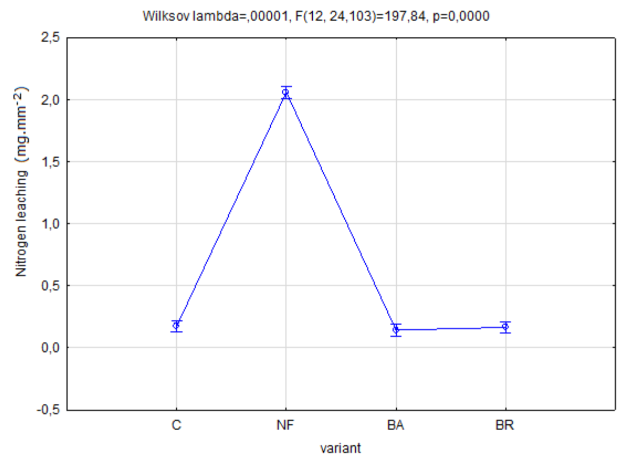

Figure 6. Statistically significant differences in nitrogen leaching (C – control, NF – mineral fertilizer DAM 390, BA – activated biochar, BR – conventional biochar).

Nitrogen availability index derives from the microbial mineralization of easily decomposed organic material and correlates with total nitrogen in microbial biomass. Figure 7 shows the obtained results in each studied variants.

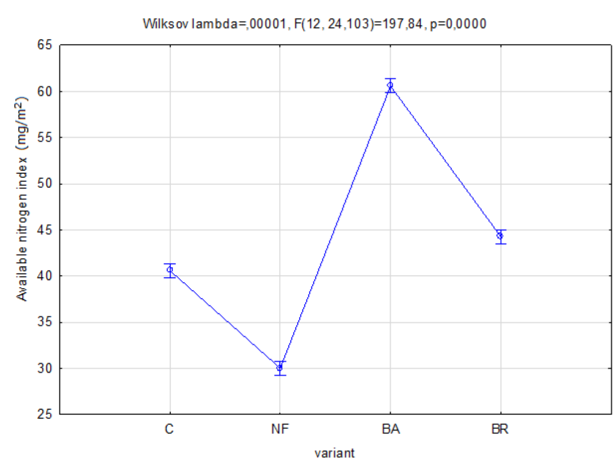

Figure 7. Statistically significant differences of available nitrogen index (C – control, NF – mineral fertilizer DAM 390, BA – activated biochar, BR – conventional biochar).

Statistically significant differences between variants were found. The amount of easily decomposed nitrogen was increasing as follows: NF (30.01 mg/m^2) <C (40.57 mg/m^2) <BR (44.25 mg/m^2) <BA (60.5 mg/m^2).

DISCUSSION

Pauli (17) stressed the importance of mineral fertilizers application together with exogenous organic materials. This may improve the conditions for soil biota (19). According to Lehmann (1) biochar application could have a positive or negative effect on soil biological properties. Presented results showed that the negative effect of biochar is possible to suppress by biochar activation and ageing. As a result, the special biofilm is formed on the surface and more friendly conditions are given for soil biota. Therefore, a complex community of soil biota can better communicate and cooperate. Biofilm is usually formed in those places, and the colonisation starts. Similarly, the stones in the water, are typical by this microbial colonisation (e.g. first bacterial biofilm and later colonies of algae and protozoa). This remains of the natural process, where the biochar pores are covered by a microbial film containing water and nutrients.

Kuzyakov (20) also quoted that biochar can absorb the signal substances responsible for nodulation and depending on duration in the soil. Authors also came to the conclusion, that biochar application is increasing the ability of soil biota for air nitrate fixation. This might be a reason for different nitrogen content in the different treatments (BR, BA, NF). The most important consequence was the decreasing of aromatic compounds. Typically, naphthalene and pyrene are determined in biochar. If their concentration is too high the negative effect on soil biota is recognizing. The biochar activation and aeration lead to the decreasing of naphthalene and pyrene due to the oxidation. The results showed that amending soil by raw biochar had a negative effect, the microbial mortality was high, and no DHA activity was registered. This was a consequence of the high concentration of aromatics. Pauli (17) and Quilliam (3) showed that the metabolic pathway system of soil biota is very rich and decomposition of all kind of anthropogenic substances (pesticides, insecticides, industrial poisons etc.) occurred.

Zhang (2) used determination of DHA as an indicator of enzymatic activity and they came to the same conclusion. In our case, DHA was mainly influenced by biochar activation and no effect of mineral fertilizing was recognized. Major (21) confirmed that activation of biochar can increase the comfort and activity of soil biota.
The negative consequences of mineral nitrogen leaching were studied by Xu (22). They showed that arable soil under conventional management is mainly threatened by this negative process. They suppose that it is given by losing interaction and ability of soil biota to communicate between themselves, with plats roots, and their loss of nutrients availability control. During the period when the arable soil is covered by plants mineral nitrogen is consumed by soil biota, but there is quite a long period of nitrogen leaching from the soil. Di and Cameron (23) stressed that the main problem is in high dozens of mineral fertilizers, especially nitrogen, which is nitrified by bacteria to the mobile form ($-NO^{3-}$).

The last caused nitrogen leaching and water contamination. In conventional management, it is recommended to supply the mineral fertilizer together with organic fertilizers, which can partly solve this problem. Nitrates accumulation in soil and water is one of the biggest environmental problems of today. Nitrates directly influence the quality of production and human health. Many authors showed that biochar ageing is the way how to prevent this negative process (1).

In the case of biomass production, activated biochar caused better results than conventional biochar. According to Lehmann (1), the state of the microbial community is directly reflected in crop production, which is well related to our biomass production result (Table 4).

Lehmann (1) proved that the biochar ageing had a positive effect on the microbial community and their activity. Similarly to our results, the negative effect of aromatics was diminished and soil pH increased. As confirmed also Geisseler (24) the application of mineral fertilizer leads to the decrease of available nitrogen in the soil. This was also proved by our research. It was also shown that the enormous increase of microbial biomass (NF variants) lead to the lack of available nitrogen in a short time (NF-30.01 mg/

m2). Kuzyakov (20) suggested that this could be explained by the changes of the nutrients cycle in time, by the induction of root exudates, and by the side effect of the whole spectrum of soil biota. Morriën (25) also described the same negative back stimulation effect after the high doses of mineral fertilizers.

In conclusions the activation of biochar by aerating with the native soil solution had a positive effect and biochar became more friendly for soil biota. The increase of dehydrogenase activity and dry biomass of lettuce in the pot experiment was observed after amending soil with activated biochar. A positive effect was also registered concerning the decrease of nitrogen leaching from the soil. Calculated index of nitrogen availability and the content of leaching nitrogen were determined as two important indicators of nitrogen transformation in the soil. The research also showed that the effect of biochar is influenced by the soil type and properties, biochar quality, soil microflora composition and activity. Further detailed studies are necessary to assess the effect of biochar on the soil environment.

Conflicts of interest

The authors declare having no conflicts of interest.

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REFERENCES

1. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. Biochar effects on soil biota–a review. Soil biology and biochemistry. 2013; 43(9):1812-1836. https://doi.org/10.1016/j.soilbio.2011.04.022

2. Zhang C, Nie S, Liang J, Zeng G, Wu H, Hua S. Effects of heavy metals and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure. Science of the Total Environment. 2016; 557:785-790. https://doi.org/10.1016/j.scitotenv.2016.01.170

3. Quilliam RS, Glanville HC, Wade SC, Jones DL. Life in the 'charosphere'–Does biochar in agricultural soil provide a significant habitat for microorganisms?. Soil Biol Biochem. 2013; 65:287-293. https://doi.org/10.1016/j.soilbio.2013.06.004

4. Sorrenti G, Masiello CA, Dugan B, Toselli M. Biochar physico-chemical properties as affected by environmental exposure. Science of the total Environment. 2016; 563:237-246. https://doi.org/10.1016/j.scitotenv.2016.03.245

5. Wang B, Jiang YS, Li FY, Yang DY. Preparation of biochar by simultaneous carbonization, magnetization and activation for norfloxacin removal in water. Bioresour Technol. 2017; 233:159-165. https://doi.org/10.1016/j.biortech.2017.02.103

6. Sajjadi B, Zubatiuk T, Leszczynska D, Leszczynski J, Chen WY. Chemical activation of biochar for energy and environmental applications: a comprehensive review. Rev Chem Eng. 2019; 35(7):777-815. https://doi.org/10.1515/revce-2018-0003

7. Wolińska A, Stępniewska Z, Bielecka A, Ciepielski J. Bioelectricity production from soil using microbial fuel cells. Appl Biochem Biotechnol. 2014; 173(8):2287-2296. https://doi.org/10.1007/s12010-014-1034-8

8. IUSS Working Group WRB. World reference base for soil resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps. Reports No. 106. World Soil Resources; 2015. http://www.fao.org/3/i3794en/I3794en.pdf

9. Sato JH, Figueiredo CCD, Marchão RL, Madari BE, Benedito LEC, Busato JG, Souza DMD. Methods of soil organic carbon determination in Brazilian savannah soils. Scientia Agricola. 2014; 71(4):302-308. http://dx.doi.org/10.1590/0103-9016-2013-0306

10. Pospíšilová H, Jiskrova E, Vojta P, Mrizova K, Kokáš F, Čudejková MM, Dzurova L. Transgenic barley overexpressing a cytokinin dehydrogenase gene shows greater tolerance to drought stress. N Biotechnol. 2016; 33(5):692-705. https://doi.org/10.1016/j.nbt.2015.12.005

11. Kučerík J, Tokarski D, Demyan MS, Merbach I, Siewert CC. Linking soil organic matter thermal stability with contents of clay, bound water, organic carbon and nitrogen. Geoderma. 2018; 316:38-46. https://doi.org/10.1016/j.geoderma.2017.12.001

12. José M, Paneque M, Miller AZ, Knicker H. Relating physical and chemical properties of four different biochars and their application rate to biomass production of Lolium perenne on a Calcic Cambisol during a pot experiment of 79 days. Sci Total Environ. 2014; 499:175-184. https://doi.org/10.1016/j.scitotenv.2014.08.025

13. Casida LE, Klein DA, Santoro T. Soil dehydrogenase activity. Soil Science. 1964; 98:371-376

14 Peoples MB, Faizah AW, Rerkasem B, Herridge DF. Methods for evaluating nitrogen fixation by nodulated legumes in the field (No. 435-2016-33692). 1989

15. Keeney DR, Nelson DW. Nitrogen—inorganic forms. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties. American Society of Agronomy. 1983. https://doi.org/10.2134/agronmonogr9.2.2ed.c33

16. Pittelkow CM, Linquist BA, Lundy ME, Liang X, van Groenigen KJ, Leevan Kessel C. When does no-till yield more? A global meta-analysis. Field Crops Research. 2015; 183:156-168. https://doi.org/10.1016/j.fcr.2015.07.020

17. Pauli N, Abbott LK, Negrete-Yankelevich S, Andrés P. Farmers' knowledge and use of soil fauna in agriculture: a worldwide review. Ecology and Society. 2016; 21(3):19. https://www.jstor.org/stable/26269977

18. Chebotarev NT, Yudin AA, Konkin PI, Oblizov AV. Efficiency of using organic and mineral fertilizers in fodder crop rotation on northern soddy podzols. Russian Agricultural Sciences. 2017; 43(2):162-166. https://doi.org/10.3103/S1068367417020045

19. Kučová L, Záhora J, Pokluda R. Effect of mycorrhizal inoculation of leek Allium porrum L. on mineral nitrogen leaching. Horticultural Science. 2016; 43(4):195-202. https://doi.org/10.17221/182/2015-HORTSCI

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

21. Major J, Rondon M, Molina D, Riha SJ, Lehmann J. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil. 2010; 333(1-2):117-128. https://doi.org/10.1007/s11104-010-0327-0

22. Xu N, Tan G, Wang H, Gai X. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur J Soil Biol. 2016; 74:1-8. https://doi.org/10.1016/j.ejsobi.2016.02.004

23. Di HJ, Cameron KC. Inhibition of nitrification to mitigate nitrate leaching and nitrous oxide emissions in grazed grassland: a review. J Soils Sediments. 2016; 16(5):1401-1420. https://doi.org/10.1007/s11368-016-1403-8

24. Geisseler D, Scow KM. Long-term effects of mineral fertilizers on soil microorganisms–A review. Soil Biol Biochem. 2014; 75:54-63. https://doi.org/10.1016/j.soilbio.2014.03.023

25. Morriën E. Understanding soil food web dynamics, how close do we get?. Soil Biol Biochem. 2016; 102:10-13. https://doi.org/10.1016/j.soilbio.2016.06.022