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The biochar effect on soil respiration and nitrification

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ABSTRACT

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Soil microorganisms play a main role in the nutrient cycle and they also play an important role in soil health. This article studies the influence of three rates of biochar (0.5, 1 and 3%) in comparison with control (0 biochar) in two different soils (Valečov and Čistá) on soil microbiota activities. The biochar was prepared from 80% of digestate from *Zea mays* L. and 20% of cellulose fibres by pyrolysis (470°C, 17 min). The biochar ability to influence microbial processes in soil was determined by respiration and nitrification tests. There were no significant differences between basal respiration of control samples and biochar-amended samples. Basal respiration in the Valečov soil reached average amounts from 1.32 to 1.52 mg CO_2/h/100 g. In the Čistá soil, basal respiration reached average amounts from 1.40 to 1.49 mg CO_2/h/100 g. No significant differences were proved also in nitrification tests of both soils. Nitrifying potential was the highest in 3% rate of biochar amendment. There were no negative changes in the measured soil parameters. CO_2 efflux was not higher in biochar-amended soil.

Keywords: microbial activity; mineralization; soil amendment; cambisol

Many scientists studied biochar because of its great potential. The biochar could be used as a soil amendment thanks to its influence on soil characteristics. According some authors (Lehmann et al. 2011), it can improve physicochemical properties of soils and helps to eliminate accessibility to hazardous elements and also has an impact to soil fertility. Biochar can influence soil nutrient cycle, especially carbon and nitrogen losses by leaching and volatilization. Microorganisms play an important role in these processes and adding different amendments could sometimes change their activity, amount and diversity.

Soil microbial mineralization is an important characteristics, which can show changes in soil microbial activity. Too high activity index could be undesirable for soil fertility, because mineralization of organic compounds prevails to humification and also rises soil CO_2 emission. However, really low activity index can show some soil diseases. Mineralization activity evaluated by the respiration tests was reported higher after the biochar amendment for the first few days or months (2–60 days); after this initial time, the respiration rates are similar to non-amended soil (Ameloot et al. 2013).

Biochar made from different types of biomass sources may react in soil differently. It is because of different size of particles and composition. It could be the reason why biochar in some studies

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increased soil respiration and decreased in others. Not only the material could change the biochar influence on mineralization, it could be also the pyrolysis temperature and time, C:N and C:O ratios in labile phase of the biochar.

In the European Union countries the amount of biogas plants increased because of the strategy to produce energy from renewable sources. The result of biogas production, digestate, is more stable than the material used for biogas production and it is poorer in nutrients, which mostly remains in the liquid phase – fugate. To transform this material to biochar is one of the possibilities how to make it more useful for improving soil properties. It could be also primarily used as a form of soil carbon sequestration if there were no negative effects on soil properties. Firstly, the aim of our experiment was to evaluate the influence of biochar made from the digestate from *Zea mays* L. to soil nutrient cycle. Secondly, the study is focused on the microbial response of three biochar rates compared with control.

MATERIAL AND METHODS

Experimental design. A long-term (12 months) pot experiment was carried out with soil collected from the top layer (0–20 cm) of haplic cambisol (according to WRB 2006) of arable field located in Čistá (50°01'31.1''N, 13°35'04.9''E), Czech Republic and from an arable field located in Valečov (49°38'53.5''N, 15°29'39.7''E), Czech Republic.

After collection, soil was homogenized and sieved at 2 mm; after that soil was stabilized for three weeks at 4°C. The samples from Valečov were air-dried for two days before homogenization because of their high humidity impeding the sieving. Subsequently, both samples of Valečov and Čistá soils were amended with biochar at three rates (0.5, 1 and 3%; control without biochar), placed into pots and moisturized.

The biochar was prepared from 80% of digestate from *Zea mays* L. and 20% of cellulose fiber; pyrolysis temperature was 470°C, heating time 17 min.

The pots were cultivated in the temperature room at 29°C, moisturized 2 times a week for 12 months. Samples were analysed at the beginning of April 2016 (microbial characteristic, physical and chemical properties) and in the following intervals: 0, 2, 4, 6, 10 and 12 months.

Physical and chemical properties. pH_{H2O} and pH_{KCl} were measured potentiometrically (pH meter inoLab pH Level 1 WTW, Weilheim, Germany). Soil salinity was measured conductometrically in the alcohol extract. Soil was mixed with 50% ethylalcohol, shaked for 2 h, filtrated and measured.

Nitrogen, carbon, sulphur was determined by the Elementary Analyzer Flash 2000 NCS (Thermo Scientific, Milano, Italy). Samples were homogenized and ground by mill (Vibratory Micro Mill Pulverisette 0, Fritsch, Idar-Oberstein, Germany). Vanadium pentoxide in the amount of 5 mg was added to each sample to complete conversion of inorganic sulphur. Organic carbon content (C_{org}) was measured by a modified Tyurin's oxidimetric titration (Pospíšil 1964).

Exchangeable Ca, Mg, K, Na, Al, Fe and Mn, risk elements and heavy metals were determined by means of the coupled plasma optical emission spectrometry (ICP-OES) in 0.1 mol/L $BaCl_2$ solution according to the UNECE (2006), effective cation exchange capacity (CEC) was calculated as a sum of the exchange cations and potentiometrically measured H^+.

According to UNECE (2006) the concentration of selected elements (Ca, Mg, K and Fe) in $BaCl_2$ solution and *aqua regia* extract were determined by the ICP-OES (iCAP 7000, Thermo Scientific, Bremen, Germany).

Carbonate content (C_{inorg}) was determined using the volumetric calcimeter method (Loeppert and Suaréz 1996).

Particle size distribution was determined by the hydrometer method (Gee and Bauder 1986).

Determination of microbial activities. The microbial metabolic activities were determined through the respiratory tests and nitrification tests. Respiration, the ability of microorganisms to degrade organic carbon into CO_2, was monitored using the static titration methods to evaluate current (basal) and potential respiration. Activity of the microorganisms in the N cycle was assessed with nitrification tests.

Respiration was tested according to the ISO 16072 (2002). Basal respiration (BR); potential respiration with $(NH_4)_2SO_4$ (N); glucose (G); $(NH_4)_2SO_4$ and glucose (NG) were established.

Actual content of NO_3^-, control nitrification and potential nitrification with $(NH_4)_2SO_4$ was measured by the ion-selective electrode (Löbl and Novák 1964).

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Table 1. Physical and chemical properties of biochar and both soils: Valečov and Čistá

	Biochar	Valečov	Čistá
pH_{H_2O}	9.67	4.63	6.04
pH_{KCl}	9.12	4.08	5.34
Electrical conductivity (μS/cm)	–	21.90	9.93
Carbonates (%)	–	not*	0.14
C_{ox} (%)	–	2.28	1.48
< 0.002 mm (%)	–	9.43	9.62
< 0.01 mm (%)	–	15.42	30.11
0.01–0.05 mm (%)	–	12.92	16.65
0.05–0.1 mm (%)	–	11.18	8.55
0.1–2 mm (%)	–	60.46	44.67

*not measured because of low pH; C_{ox} – oxidizable carbon

Microbial biomass carbon (C_{mic}) was determined by a modified rehydration method (Růžek et al. 2015).

Metabolic quotient qCO_2 $(BR/C_{mic}) \times 1000$ (modified according to Anderson and Domsch 1986) was calculated as the ratio of CO_2 captured in BR to C of microbial biomass.

Statistical analyses. Results were evaluated using the statistical programme Statistica 13 (TIBCO Software Inc., Palo Alto, USA). To assess the differences between the samples a one-way analysis of variance (ANOVA) and the Scheffe's test were used to evaluate the significance of differences of the means at the level of $P < 0.05$. ANOVA was also used to calculate the least significant differences (*LSD*).

RESULTS AND DISCUSSION

Physical and chemical properties. Properties of both chosen soils were different; for example, the soil from Valečov had lower pH (4.63) than the soil from Čistá (6.03). Biochar had the highest pH value (9.67), as was expected. Results describing the main physical and chemical properties are summarized in Table 1. Table 2 presents the content of elements of both soils and biochar.

Respiration by titration. The biochar amendment in both soils (Valečov and Čistá) at all three concentrations did not raise any statistically significant differences in respiration rates during the whole year compared to the control samples. Basal respiration in the Valečov soil reached the average amount 1.32 in the control and 1.56, 1.32, 1.52 in biochar amendment samples at the rates of 0.5, 1 and 3%, respectively (amounts are given in mg CO_2/h/100 g). In the Čistá soil, basal respira-

Table 2. Input values of the content of macro- and micronutrients and risk elements (mg/kg) in biochar and both soils: Valečov and Čistá

	Biochar*	Biochar**	Valečov*	Valečov**	Čistá*	Čistá**
P	846.33	3891.15	257.22	907.13	101.84	640.04
K	3500.00	14 396.68	287.40	2100.88	228.51	3270.61
Ca	9688.47	49 668.90	403.88	794.57	1535.86	1897.51
Mg	982.49	3930.11	49.60	2000.85	90.60	4418.04
Na	213.65	129.54	28.17	6.46	19.83	6.46
Zn	23.42	63.55	4.45	48.76	8.06	69.02
Fe	119.96	3156.78	229.39	12 456.37	175.83	24 345.50
Mn	39.28	134.96	39.27	327.57	91.79	845.55
As	0.34	1.68	0.79	4.80	0.34	11.69
Cd	0.03	0.17	0.12	0.63	0.11	1.13
Cr	0.27	12.57	0.20	10.05	0.14	24.55
Cu	14.31	37.49	5.20	25.08	7.25	31.57
Pb	2.29	6.64	3.95	14.01	4.74	23.43

*0.1 mol $BaCl_2$/L extract; ***aqua regia* extract

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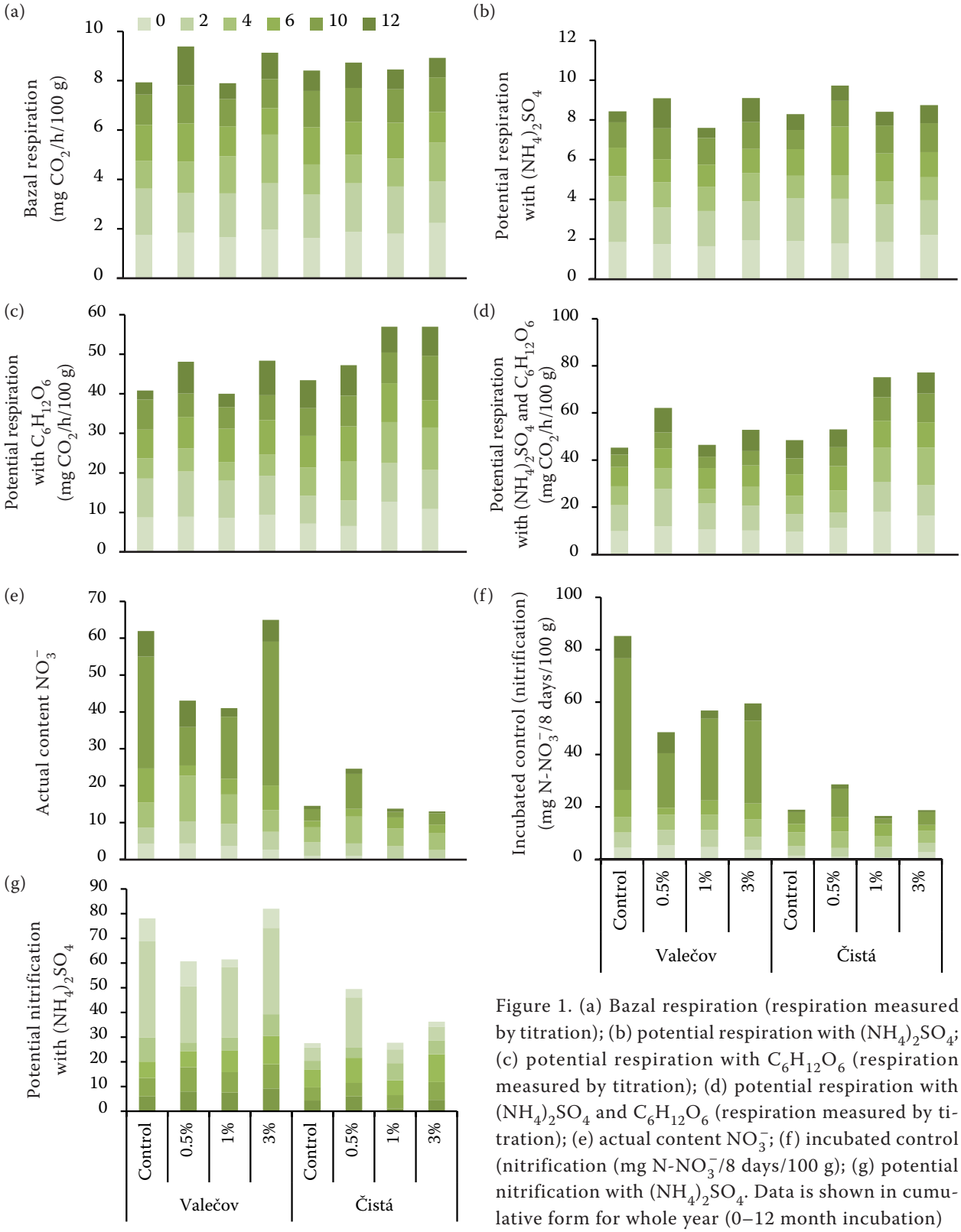

Figure 1. (a) Bazal respiration (respiration measured by titration); (b) potential respiration with $(NH_4)_2SO_4$; (c) potential respiration with $C_6H_{12}O_6$ (respiration measured by titration); (d) potential respiration with $(NH_4)_2SO_4$ and $C_6H_{12}O_6$ (respiration measured by titration); (e) actual content NO_3^-; (f) incubated control (nitrification (mg N-NO_3^-/8 days/100 g); (g) potential nitrification with $(NH_4)_2SO_4$. Data is shown in cumulative form for whole year (0–12 month incubation)

tion had average amounts 1.40 in the control and 1.46, 1.41, 1.49 in the biochar-amended samples at the rates of 0.5, 1 and 3%, respectively. Figure 1a shows changes in basal respiration during the year in both soils. Similarly, Figures 1b–d show the results of potential respiration.

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Mitchel et al. (2015) and Xu et al. (2016) observed significantly higher respiration rates after biochar treatment; these results were found out after 5 months and 6 months. Rutigliano et al. (2014) observed that the respiration rate increased in the first 3 months and was statistically significantly higher than control, but after 14 months there were no differences between samples. This information confirms the claim of Ameloot et al. (2013) and the results from their experiments, where samples with biochar and control had no statistically significant differences after 12 months. Biochar influence on soil C sequestration differs not only over time, biochar type and different pyrolysis conditions but also depends on the soil conditions especially soil texture (Zhou et al. 2017). Zhou et al. (2017) declared that the soil respiration after biochar addition increased in temperate forests while there were no statistically significant changes in subtropical forests during the 24-month experiment. In conclusion, the respiration rate in our study did not increase in comparison with control and thus there was not higher CO_2 efflux to the atmosphere.

Actual content of N-NO_3^- in incubated control and potential nitrification with $(NH_4)_2SO_4$. Actual contents of N-NO_3^- released from the Valečov soil were 10.33 in the control sample and 7.18, 6.84 and 10.83 in biochar-amended soil at the rates of 0.5, 1, and 3%, respectively; data are given in average amounts from all sampling periods in mg N-NO_3^-/100 g dry soil. Čistá reached much lower amounts than Valečov (that is slightly surprising because of Valečov soil lower pH); it is because nitrification in Valečov soil significantly increased after 10-month incubation. The average amounts of released N-NO_3^- in Čistá were 2.42 in control, 4.10, 2.29 and 2.17 in biochar-amended samples at the rates of 0.5, 1 and 3%, respectively. Actual N-NO_3^- content is shown in Figure 1e.

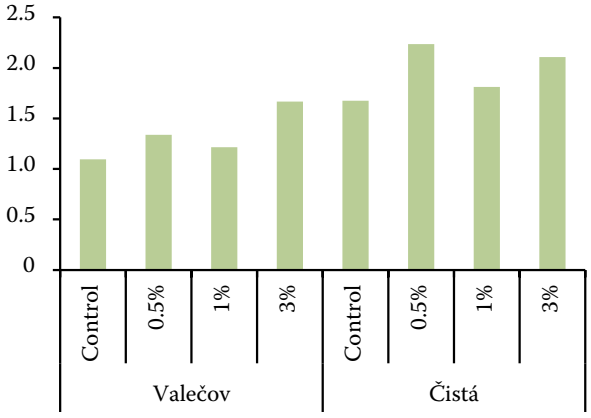

Figure 2. Nytrifying potential. Data is shown in average values (potential nitrification with $(NH_4)_2SO_4$/control nitrification)

Average N-NO_3^- amounts of incubated control (Figure 1f) were also higher in the Valečov soil samples than in the Čistá soil samples. Valečov reached average amounts between 6.84–10.83 mg N-NO_3^-/8 days/100 g dry soil and the Čistá reached average amounts between 2.17–4.10 mg N-NO_3^-/8 days/100 g dry soil. There were no significant differences between the control and biochar amendment samples during the whole experiment, but significant differences were observed between these two sites.

Potential nitrification of biochar variants (Figure 1g) had a similar value as the incubated control. The nitrifying potential (Figure 2; a ratio of potential nitrification and control nitrification) varied from 1.09 to 2.24 for both soil samples. Values of the nitrifying potential are statistically significantly higher in the Čistá soil samples than in the Valečov soil samples, but there is the same evident tendency in both soils. The nitrifying potential was the highest in the samples with the highest biochar rate (3%). Control and biochar amendment in the rate of 1% had a similar value of the nitrifying potential. The nitrifying potential of 0.5% biochar amendment had the higher level than control, the biochar amendment in the rate 1% was lower than 3% rate.

Wang et al. (2016) observed that biochar had no influence on soil N-NO_3^- similarly as in our study. However, Kelly et al. (2015) found out that the net nitrification in their experiment significantly decreased with increasing amounts of biochar addition. However, in both these cases the study was run over a short time period. In a 12-month experiment of Bai et al. (2015) it was demonstrated that the amount of N-NO_3^- increased significantly dependently to the increasing amount of biochar compared to the control, which is in contrast with our results and those of Wang et al. (2016).

Microbial biomass carbon (C_{mic}) and metabolic quotient qCO_2 (Table 3). There were no statistical differences in microbial biomass and metabolic quotient between the biochar-amended samples and the control. Similar observation was also published by Rutigliano et al. (2014).

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Table 3. Microbial biomass (C_{mic}) and metabolic quotient (qCO_2)

		Valečov				Čistá			
		C_{mic}*		qCO_2**		C_{mic}*		qCO_2**	
		0 month	2 months	0	2 months	0 month	2 months	0 month	2 months
Control		194.03	270.03	0.0247	0.0189	237.15	170.02	0.0186	0.0282
Biochar	0.5%	194.03	275.03	0.0257	0.0160	194.03	–	0.0264	–
	1%	186.85	195.02	0.0242	0.0248	186.85	135.01	0.0263	0.0386
	3%	201.22	230.03	0.0267	0.0223	179.66	145.02	0.0340	0.0317

*mg C/kg dry mater; **(mg C/CO_2/h/kg)/(mg C/kg) dry mater

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