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Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard

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ABSTRACT

Zemanová V., Břendová K., Pavlíková D., Kubátová P., Tlustoš P. (2017): Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard. Plant Soil Environ., 63: 322–327.

The objective of this study was to assess the effect of biochar on growth and metabolism of spinach (*Spinacia oleracea* L.) and mustard (*Sinapis alba* L.) planted in crop rotation: spinach (spring)-mustard-spinach (autumn). The impact of biochar soil application (5% per mass of soil) on the availability of Ca, Fe, K, Mg, Na and P to plants as well as the content of free proline and total amino acids contents were evaluated at degraded Chernozem soil. The results showed that biochar soil addition significantly increased spinach growth by 102% and 353% in spring and autumn, respectively. Biochar limited plant content of Ca, Mg and Na, however K content increased in all plants. Inconsistent effect was determined for Fe and P content in plants biomass. Total content of free amino acids was higher in plants harvested at amended treatments, except autumn spinach. Biochar increased proline content in all plants in comparison to control. The highest increase was obtained in mustard – by 186%. The results showed a more sensitive reaction of mustard to biochar application than spinach.

Keywords: carbonaceous amendment; macroelement; plant; stress metabolism

Biochar (BC) is a carbon-rich material produced by pyrolysis. In recent decade, it is widely described as a soil amendment improving soil quality. The main reason for the positive impact on soil properties, plant and microbial ecosystem is a direct BC influence on soil physical-chemical properties, nutrients available contents, and on its ability to sorb nutrients and release them slowly into soil solution (Atkinson et al. 2010). Nutrient composition of BC depends on the feedstock material and conditions of pyrolysis (Mukherjee and Zimmerman 2013). However, many studies described a positive effect of BC application to soil, whereas the responses on crop yields are not consistent (Novak et al. 2016). According to Břendová et al. (2015) BC ability to increase pH value is probably one of the crucial factors of reduction of element leachability.

As mentioned by Rizwan et al. (2016), there is a limited number of reports describing the effect of BC on the biochemical and physiological activities of plants. One of the important indicators of plant metabolism is composition and content of amino acids (AAs), which are affected by environmental conditions (Nikiforova et al. 2006). According to Singh (1999), amino acid metabolism may play an important role in plant stress resistance, by osmotic

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adjustment and the accumulation of compatible osmolyte, detoxification of active oxygen species and heavy metals, and intracellular pH regulation. Proline (Pro) is specific free AA which is involved in stress metabolism and tolerance of plants (Pavlíková et al. 2014).

Our study aimed to compare the ability of spinach and mustard accumulation of nutrients and amino acids as a response to biochar application to soil.

MATERIAL AND METHODS

Experimental design and soil characteristics. The experiment was conducted at greenhouse controlled conditions. Spinach (*Spinacia oleracea* L.) and mustard (*Sinapis alba* L.) were chosen as experimental plants, while the crop rotation was spinach-mustard-spinach. Firstly, spinach was sown in March and harvested after 64 days. Mustard was sown in May and harvested after 35 days. Late spinach was sown in August, harvested after 64 days. Biomass of both spinaches was sampled once during vegetation, after 42 days. Modal Chernozem soil (2.5 kg; Prague-Suchdol, Czech Republic; pH_{KCl} = 7.2, cation exchange capacity (CEC) = 258 $mmol_+$/kg, C_{org} = 1.83%, available content of elements: Ca = 6754 mg/kg; K = 233 mg/kg; Fe = 153 mg/kg, Mg = 191 mg/kg; P = 74 mg/kg) was uniquely fertilized with 0.1 g N, 0.16 g P and 0.4 g K per 1 kg of soil (applied in the form of NH_4NO_3 and K_2HPO_4), only at the beginning of the experiment. The experiment consists of 2 treatments: (i) control (without BC) and (ii) 5% of the applied BC from total mass of soil (BC 5%) thoroughly mixed with soil volume.

Biochar characteristics. Detailed biochar properties were described in Břendová et al. (2016). Biochar was characterized by: ash content = 13%; pH_{CaCl_2} = 6.9; CEC = 176 $mmol_+$/kg; specific surface area = 324 m^2/g and particle fraction = 5 × 6 × 0.5 mm. The total contents of elements in biochar were: K = 16.1 g/kg; Ca = 28 g/kg; Fe = 2.8 g/kg; C_{total} = 64% (w/w) and N = 1.1% (w/w). Total content of Mg was not determined.

Analysis of plant biomass. Dry-ashing decomposition was used for analyses of total element contents in plants (Street et al. 2006). The total contents of elements were determined by ICP-OES (Agilent 720, Agilent Technologies Inc., Torrance, USA) for Ca, Fe, Mg and P and by FAAS (Varian SpectrAA-280, Mulgrave, Australia) for K and Na. Aliquots of the certified reference material RM NCS DC 73350, poplar leaves (Analytika, Prague, Czech Republic), were determined under the same conditions to test quality assurance.

The contents of free AAs were determined after their derivatisation in extracts (1.0 g of fresh biomass, 15 mL of methanol + redistilled H_2O (7:3, v/v), 24 h) by EZ:faast set (Phenomenex, Santa Clara, USA). Samples were measured by GC-MS (Hewlett Packard 6890N/5975 MSD, Agilent Technologies, Torrance, USA) with a ZB-AAA 10 m × 0.25 mm AA analysis GC column (Zemanová et al. 2013).

Statistical analysis. All statistical analyses were performed using the Statistica 12.0 program (StatSoft, Tulsa, USA, www.statsoft.com). Collected data were performed using the non-parametric Kruskal-Wallis test.

RESULTS AND DISCUSSION

Biomass yield and proline content. Results presented in Table 1 showed fresh biomass yield of all treatments. Dry biomass of control and BC-amended spinaches were 9.6% and 9.7% on average, respectively. Application of BC increased dry biomass of both spinaches by 0.1% on average in comparison to control. The highest dry matter of biomass was found in mustard – 11% and was decreased by 2% at BC treatment. Application of BC significantly increased spinach biomass yields by 102% (spring) and 353% (autumn) on average, respectively in comparison to control. Same effect of BC was determined in mustard biomass yield (increase by 69%), but this effect was not statistically significant. According to Mukherjee and Lal (2014) application of BC may have positive, mixed or negative effect on yield of biomass. Jones et al. (2012) showed that BC application had no effect on maize growth, but did enhance the growth of the subsequent grass crop. Evangelou et al. (2014) using contaminated BC achieved higher yields of ryegrass in comparison to unamended treatment.

Amino acids are critically important for plant metabolism to make bridges between C and N metabolisms (Foyer et al. 2003). Plants can produce high quantities of AAs under limited stress conditions (Younis et al. 2015). In previous papers, there is a lack of information of the BC effect on composition and content of amino acids. Only

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Table 1. Biomass yield (BY), total content of amino acids (Σ AAs) and free proline content (Pro) of spinach and mustard

	42 days		64 days	
	control	BC 5%	control	BC 5%
Spinach – spring				
BY (g per pot FM)	23.5 ± 2.3^{aA}	46.4 ± 1.6^{bA}	28.5 ± 2.5^{aA}	58.7 ± 2.3^{bA}
Σ AAs (μmol/kg FM)	5386 ± 789^{aA}	5420 ± 279^{aA}	5908 ± 364^{aA}	6709 ± 144^{aB}
Pro (μmol/kg FM)	349 ± 34^{aB}	1052 ± 369^{bB}	147 ± 13^{aA}	167 ± 36^{aA}
Spinach – autumn				
BY (g per pot FM)	12.4 ± 1.3^{aA}	57.8 ± 2.9^{bA}	15.3 ± 2.4^{aA}	67.4 ± 3.5^{bA}
Σ AAs (μmol/kg FM)	22 890 ± 5483^{bA}	10 110 ± 2305^{aA}	17 397 ± 677^{bA}	9335 ± 802^{aA}
Pro (μmol/kg FM)	131 ± 10^{aA}	154 ± 20^{aA}	149 ± 32^{aA}	180 ± 60^{aA}

	35 days	
	control	BC 5%
Mustard		
BY (g per pot FM)	30.6 ± 1.4^{a}	51.7 ± 2.2^{a}
Σ AAs (μmol/kg FM)	8217 ± 451^{a}	13 484 ± 3190^{a}
Pro (μmol/kg FM)	2371 ± 395^{a}	6771 ± 2191^{a}

The values represent the means (± standard error) of data obtained in the experiment (*n* = 4). Different letters indicate significantly different values ($P < 0.05$) between treatments (a, b) and sampling period (A, B). FM – fresh matter

Zhang et al. (2014) and Younis et al. (2015) measured AAs content in plants grown in B-amended soil. The results presented in Table 1 showed total contents of free AAs in all tested plants. In spring spinach and mustard planted at BC treatment, total content of free AAs was increased by 7% on average and by 64%, respectively, however not significantly, in comparison to control. Similarly, Younis et al. (2015) obtained increasing production of AAs by BC application under toxic levels of Cd and Ni in spinach and fenugreek. According to these authors, high production of AAs and also proteins indicated the activation of defensive mechanism against oxidative damage. Opposite effect of BC – decrease of total content of free AAs (by 51% on average) was observed in spinach-autumn. However, this decrease can be partly influence due to the inhibition of photosynthesis by lower irradiance. Synthesis of AAs depends on photosynthetic activity, which, in turn, depends on other factors e.g. nutrient availability (Weckopp and Kopriva 2015).

Proline is a multifunctional AA accumulated in plant cells in response to various stresses (Szabados and Savouré 2010). In our study, Pro found in spring spinach, mustard and autumn spinach represented 8, 40 and 1% on average of total content of free AAs (Table 1). The highest content of Pro was accumulated in mustard, and the content was 15-fold higher on average and 31-fold higher on average than in spring spinach and autumn spinach, respectively. As presented by Jogaiah et al. (2013), Pro is known as an osmoprotectant and antioxidant, playing an important role in stress management in plants. Application of BC increased Pro content in all tested plants, but significantly only in spring spinach after 42 days of growth. Different results were obtained by Kammann et al. (2011) in the study with *Chenopodium quinoa*. In these plants under drought stress, application of BC decreased Pro content in leaves. Also Zhang et al. (2014) reported a decrease of Pro content in rice by BC soil application in comparison to unamended treatments.

Elements contents in plant tissues. The results, presented in Figure 1, showed diverse accumulation of selected elements in spinach and mustard after BC application. The contents of Ca, Mg and Na were reduced by BC application in all tested plants. The content of Ca was decreased in both spinach treatments – spring and autumn – by 45% and 30% on average, and in mustard – by 34%. The highest Ca content was measured in mustard both at control (3.5%) and at BC treatment (2.3%). The same effect of BC on the Ca content was found in corn ear-leaf and soybean plants (Brantley et al. 2016, Waqas et al. 2017). In comparison to

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control, BC application decreased Mg content in spring spinach (26% on average), mustard (27%) and autumn spinach (20%). Significant changes of Mg content in lettuce plants by BC soil application were confirmed by Woldetsadik et al. (2016). These authors observed a decrease of Mg content on sandy loam soil and an increase of Mg content on silty loam soil after BC application. Increase of Mg was found in dry beans (*Phaseolus vulgaris* L.) by Gao et al. (2016). Potassium concentration in plant tissues indicated high consumption in our experiment, thus the content of Mg could be depressed by antagonistic interaction mechanism of these two elements, which is seated in the translocation step from the root to the shoot (Ohno and Grunes 1985). The highest Mg content was measured in autumn spinach at both treatments – 1.4% at control and 0.9% at BC. The lowest Mg content was measured in mustard – 0.3% at control and 0.2% at BC. Like Mg, Na content could be reduced by high K consumption. In all plants, Na content decreased in spring spinach (88% on average), mustard (64%) and autumn spinach (79% on average) by BC application. Decrease of sodium content was found in maize (Kim et al. 2016) by BC treatment and in mixed biomass of grasses and forbs on temperate grassland (Schimmelpfennig et al. 2015).

The positive effect of BC application on plant accumulation of elements was observed only for K. Although this element was applied to the soil as a fertilizer at the beginning of the experiment, BC increased K availability and its accumulation in the tested plants during the whole experiment (Figure 1, Table 2). The same results – increase of K

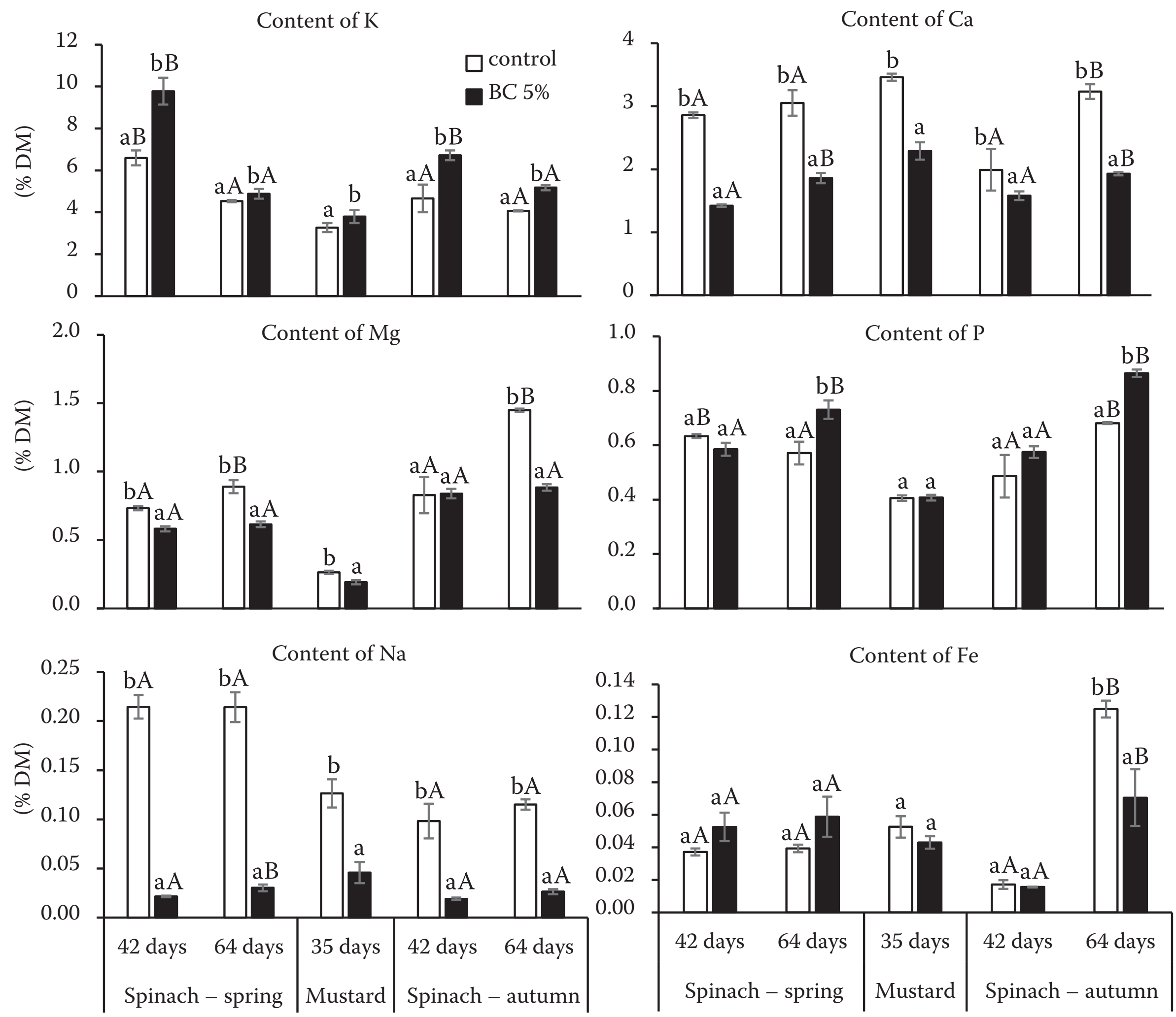

Figure 1. Content of nutrients (%) in spinach and mustard dry biomass. The values represent the means (± standard error) of data obtained in the experiment (n = 4). Different letters indicate significantly different values (P < 0.05) between treatments (a, b) and sampling period (A, B). DM – dry matter

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Table 2. Content of soil available nutrients (Mehlich III) and pH at the end of the experiment

	Ca	Fe	K	Mg	Na	P	pH
	(mg/kg)						
Control	11 784	0.26	279	225	30.3	75.1	6.51
BC 5%	13 704	0.47	480	350	34.3	199	6.61

BC – biochar

availability, was found by Kraska et al. (2016) after application of BC derived from wheat straw. The content of K was increased in spring spinach (72% on average), mustard (16%) and autumn spinach (36% on average) by BC. Significantly increased K content was found in stems, leaves and roots of green bean (*Vigna radiata* L.) by Prapagdee and Tawinteung (2017). Also Zhang et al. (2016b) observed an increase of K content by BC application. The highest K content was measured in autumn spinach in both treatments – 4.4% in control and 5.9% in BC. Evangelou et al. (2014) found that the BC application increased significantly K and Zn content in plant shoots, however no differences were found in P, Fe, Mg, Mn and Cu concentration comparing control and amended treatments.

The mixed effect of BC application on nutrients accumulation in plants was obtained for Fe and P content. The content of Fe was non-significantly affected by BC application, except autumn spinach at 64 days. The content of P was significantly affected by BC application only in spinaches (Figure 1). Similar results were found by Prapagdee and Tawinteung (2017), who reported no significant difference of P content in green bean parts at all BC treatments. Biochar can be a potential P source and some BC can also adsorb P efficiently from solutions (Peng et al. 2012, Yao et al. 2013). They suggested that BC could play a role in retaining P applied as fertilizer. However, information of the effect of BC on phosphate retention in soils is limited (Zhang et al. 2016a). In our experiment, P was applied as fertilizer – just at the beginning (before spring spinach sowing) and the positive effect of BC on the P plant content was shown in the end of spring and autumn spinach plants (increase by 27.5% on average). Gonzaga et al. (2017) observed same effect of BC derived from biosolids. Phosphorus contents in above-ground biomass of maize increased with increasing BC application rates. According to Chintala et al. (2014) and Zhang et al. (2016a), the ability of BC to increase P retention in soils is quite variable and it varies with concentration of P in the soil solution. Although the Mehlich III – available contents of Fe and P increased with BC application (Table 2), only Fe was not accumulated into plant tissues. Similar trend was observed for Fe in Evangelou et al. (2014). Sorrenti et al. (2016) showed a decrease of Fe content in nectarine leaf. Opposite effect – Fe increase by BC was found by Gao et al. (2016) in dry beans.

Biochar from contaminated biomass has a positive effect on plant growth. Positive effect of BC application on accumulation of K and P was shown especially after longer exposure. Biochar increased proline and reduced availability of some nutrients (Ca, Mg and Na), which means that BC amendment may cause stress for plants, especially at the beginning of the growth. Our results of nutrient and proline contents showed a different response of mustard and spinach. According to the lowest yield of biomass and higher proline content, mustard is more sensitive to BC soil application than spinach.

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