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Evaluation of the changes induced by gasification biochar in a peat-sand substrate**

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A b s t r a c t. Gasification biochar represents one of the biochar types tested for agricultural needs. The aim of this study was to clarify the physico-chemical and biological changes occurring in a peat-sand substrate amended with hardwood-derived gasification biochar in the rates of 2, 4 and 20 g l^{-1}. The pH(H_2O) of the substrate with 4 g l^{-1} and 20 g l^{-1} biochar was increased from 5.6 to 6.2 and 6.7, respectively. The testing of the substrate in the respirometry device showed that the increase in the biochar rate led to a decrease in the amount of CO_2 evolved at the maximum pressure drop. The continuous decrease in pressure observed in the respirometry bottles filled with pure biochar allows explaining this effect by biochar sorption activity. Addition of 2 and 4 g l^{-1} biochar to the peat-sand substrate stimulated the growth of cucumbers in an 18-day pot vegetation experiment. An increase in the number of root tips and root volume with a decreasing average root diameter was shown in the presence of biochar. Stimulation of plant growth on the background of low rates of biochar requires a further study with emphasis on the specific combination of biochar, soil type, plant species, and climatic conditions.

K e y w o r d s: gasification biochar, plant growth, CO_2 evolution, soil, buffering capacity

INTRODUCTION

The use of different types of biochar (BC) in agriculture for soil quality improvement attracts great attention of both, researchers and farmers. In spite of a huge number of reports regarding the effects of BC on soil physico-chemical properties, plant growth and microbial activity, the impact of BC under different conditions is still not understood. Life cycle assessments and economic calculations of BC application should be conducted on a site-specific basis (Stavi, 2013). Liu *et al*. (2013) summarized the recent publications on the topic and found that greater responses to BC addition were shown in pot experiments than in the field, in acid than in neutral soils, in sandy textured than in loam and silt soils.

Peat is one of the few materials available on the market, which possess entirely suitable aeration and water retention qualities, which is important for plant roots. Peat used for soil-less horticultural production systems can be amended with some products, especially to enhance the growing medium aeration and water holding capacity; besides, this contributes indirectly to reduction of the use of peat in horticulture (Johnson *et al*., 2012; Michel, 2010; Steiner and Harttung, 2014; Tian *et al*., 2012). As reported by many authors, the experiments with peat-containing substrates amended with BC have shown their potential in agriculture. In particular, moss peat amended with pellets containing equal proportions of BC and wood flour was shown to be a suitable substrate for cultivation of plants. A mixture of 75% peat and 25% BC pellets had enhanced hydraulic conductivity and greater water availability (Dumroese *et al*., 2011).

Another important issue in the discussion on peat substitution by biochar is peat as a source of greenhouse gases once the peat land is drained, extracted, aerated, limed, and fertilized (Cleary *et al*., 2005, Steiner and Harttung, 2014). Components of biochar are more recalcitrant than soil organic matter, and, therefore, biochar provides carbon input into soil to be increased greatly compared to the carbon output through soil microbial respiration (Lehmann *et al*., 2006; Verheijen *et al*., 2009).

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The liming potential of BC plays a positive role as an amendment to the acidic peat substrate. Different physico-chemical and biological changes in soil amended with BC are BC-specific and can depend on soil type, BC particle size, contact time *etc*. (Olszewski *et al*., 2013). Along with pH changes, the shift in soil physico-chemical properties is characterized by electrical conductivity (EC), cation exchange capacity (CEC), and exchangeable acidity (Chintala *et al*., 2013).

Biochars produced at higher temperature had a higher pH, whereas those produced at lower temperatures had more sites for holding nutrients for plant growth (Novak *et al*., 2009). An increase in pyrolysis temperature and BC activation decreased the availability of K, P, and S compared to non-activated biochar produced at 350°C. BC increased the content of dissolved organic carbon, total N and P, PO_4^{3-}, SO_4^{2-}, and K at a rather high application rate (40 g kg^{-1}) (Hass *et al*., 2012). In the leaching experiments, the presence of BC increased the pH of column leachate from 0.08 to 1.70 and significantly decreased the cumulative amount of mineral N leached from the soil (Angst *et al*., 2013). However, leaching of different elements is case-specific. For instance, K, P, and Mg have contrasting associations with hardwood biochar that govern the trajectory and ultimate extent of their release (Angst *et al*., 2013). Some BCs increased the soil water retention and pH, while other biochars increased soil sodium and phosphorus content (Novak *et al*., 2009).

Biological processes occurring in biochar-amended soil are also affected by pH changes. For instance, Cayuela *et al*. (2013) suggested a function of biochar as an 'electron shuttle' that facilitates the transfer of electrons to soil denitrifying microorganisms, which together with its liming effect would promote the reduction of N_2O to N_2.

Our previous results demonstrated a stimulating effect of the hard wood-derived gasification biochar (BC-G)on pea *Pisum sativum* L. in loamy sand soil and cucumber *Cucumis sativus* L. in a peat-sand substrate at a comparatively low application rate (Telysheva *et al*., 2013).

The aim of this study was to clarify the physico-chemical and biological changes occurring in a peat-sand substrate with addition of BC-G in the rate of 2, 4 and 20 g l^{-1} biochar. It was expected that a comparative study of the samples with different biochar concentrations in the respirometry device could give new data related to the effect of BC-G on physicochemical characteristics in the integral system containing peat, sand, BC-G, microorganisms, soluble nutrients *etc*.

MATERIALS AND METHODS

Biochar (BC-G) was obtained from hardwood by gasification under conditions of the original pilot plant 'Knavas granulas', Vilanu district, Latvia, with the total electroenergy capacity of about 500 MW. The original technology is licensed. The gasification hybrid technology GreenEngine was developed and commercialized in Latvia (www.entertecgreen.com). Gasification of granulated biomass proceeds in a two-stage reactor with separate zones of fast pyrolysis (zone of low temperature) and a zone of actual gasification (zone of high temperature). Gas temperature in the gasification reactor can reach 1150°C. The yield of the carbonized residue is up to 15 with 96% carbon content. The density of BC-G was 0.3 g cm^{-3}, and particle size 0.10-0.25 mm. The peat substrate (KKS-1, Laflora Ltd., Latvia) used had the following properties: particle size 0-5 mm; pH(KCl) 5.9±0.3; ES mS cm^{-1} 1.8 ±0.3.

Peat was mixed with sand in a ratio 3:1 by volume and then sieved through ∅ 2.5 mm. The peat-sand substrate obtained had 40% moisture content and one litre of it had a weight 500 g (or 300 g of dry mass). The substrate was divided into 4 parts to prepare BC-G-amended samples *ie* 0, 2, 4, and 20 g l^{-1} biochar. Testing of the peat-sand mix was performed in triplicate.

Dry weight was determined by drying the soil samples at 105°C until constant weight. The pH value was measured in distilled water, 1M KCl, and 1M CH_3COONa (10 g soil in 50 ml) with a pH-meter Hanna pH213.

The buffering capacity of the peat-sand substrate with 4 g l^{-1} biochar and without BC-G was assessed after the addition of increasing amounts of 0.1N H_2SO_4 or 0.1N NaOH to the 5 g substrate suspended in deionized water. The total volume of the suspensions was 13 ml. The pH value in the suspensions was measured after 24 h incubation at 22°C with periodical shaking.

Water holding capacity (WHC) of the peat-sand substrate was determined by immersing 50 cm^3 air-dried samples in water for 3 days until they reached constant weight. The tube was then placed in a vertical position for two hours to allow excess water to drain. Afterwards, the samples were dried at 105°C until constant weight. The maximum WHC was calculated as the amount of water retained by the soil against gravity, based on the oven-dry weight at 105°C.

The number of aerobic heterotrophic microorganisms and fungi was determined by cultivation on Tryptone Glucose Yeast Extract Agar and Sabouraud Chloramphenicol Agar (Sifin, Germany), respectively. Colony forming units (CFU) were counted after 96-h plate incubation at 28°C.

CO_2 evolution was measured using the OxiTop® OC 110 respirometric system (WTW, Germany). The OxiTop system represents an elegant, easy handled and sufficient alternative to the other, more expensive respirometric equipment (Černohlávková *et al*., 2009). The principle of the operation is a pressure drop in the closed system due to absorption of the released CO_2 in NaOH. 20 g substrate (dw 73÷76%) was placed in a bottle; afterwards 18 ml distilled water containing 1 g glucose was added. Fresh NaOH granules were added for each incubation set. Manometric measurement of the changes in the vacuum was performed automatically every 12 min. The incubation was performed at 20°C in dark without agitation. Data collected during the incubation were sent to the controller through infrared

interface and then to the computer using AchatOC software. The correlation between changes in the number of moles of substance and pressure drop was calculated using the following equation (Sadaka *et al*., 2006):

$$\Delta n = \frac{\Delta P V}{RT}$$

where: Δn is the changes in the number of moles of the substance (kmol), ΔP is the pressure drop (kPa), V is the gas volume (m^3), R is the general gas constant (8.134 kJ $kmol^{-1}$, $°K^{-1}$), T is the gas temperature (°K).

The vegetation test was carried out in triplicate in 10 l boxes (37x28x10 cm) in a warmed glasshouse. Boxes were filled with a peat-sand substrate amended with 2 and 4 g l^{-1} BC-G, moisturized till 70% of full water capacity. 20 cucumber plant (*Cucumis sativus* L., 'Grīvas') seeds were sown in each box. Seed germination was assessed on day 6 after sowing. Development of above and underground parts of seedlings was measured on day 12 and 18 after sowing using 10 plants from each box. The root system was evaluated using a calibrated rhizoscanner STD – 1600+ (Win Rhizo 2002 C). WinRHIZO is an image analysis system specifically designed for root measurement in different forms. It performs morphology (length, area, volume), topology, architecture, and colour analyses. It consists of a computer program and an image acquisition scanner with a working area 30x42 cm.

The experiment was performed in triplicate. Data presented in the figures are expressed as mean ± standard deviation at a 5% level of significance. Comparisons among the treatments were assessed by the Student t test and one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Judging by literature on the topic, the addition of BC to soil can lead to changes in the physico-chemical properties of soil, in particular, water-holding capacity, cation exchanging capacity, pH value, bulk density *etc*. (Barrow, 2012; Kookana *et al*., 2011; Lehmann and Joseph, 2009). This effect is likely to be biochar- and soil- specific, as well as dose-dependent. Sandy soils are known to be highly sensitive to the addition of BC. Otherwise, a peat substrate, which is rich in organic matter and has a rather high buffering capacity, is expected to exhibit another relationship with the pH level in the presence of BC. For example, as reported by Steiner and Harttung (2014), in experiments with wood-derived biochar, biochar peat blends containing up to 80% biochar did not show increased pH above 7.

In our study, the pH value of a peat-sand substrate amended with 2, 4 and 20 g g l^{-1} biochar was tested. The pH value of a non-amended substrate measured in water, KCl, and CH_3COONa was found to be 5.6, 6.0, and 7.0, respectively. The BC-G suspended in these three media showed a pH value of 11.0, 10.4, and 10.9, respectively. An increase in the pH level of the peat-sand substrate with the increasing biochar concentration was detected (Fig.1a). Statistical analysis showed that these changes were significant. In particular, the pH(H_2O) of the substrate with 4 and 20 g l^{-1}, compared with the non-amended substrate, showed changes with the level of significance of $p<0.04$ and $p<0.02$, respectively. Similar results were obtained for pH measurements in CH_3COONa. Conversely, in the analysis of the substrate amended with 4 g l^{-1} biochar in KCl solution, the increase in pH was not significant ($p=0.12$) in comparison with the non-amended substrate. In contrast, addition of 20 g l^{-1} biochar to the peat-sand substrate resulted in a significant ($p<0.04$) increase in pH(KCl).

Addition of acid and alkali led to changes in the pH values, which were detected in both the non-amended and the 4 g l^{-1} containing peat-sand substrate. The presence of 4 g l^{-1} BC-G in the substrate did not influence the relationship between the addition of alkali/acid and the pH value (Fig. 1b). Addition of 5 ml 0.1N NaOH to the substrate with the final volume of 13 ml resulted in raising pH by up to 3 units. In turn, 5 ml of 0.1N H_2SO_4 decreased the pH level

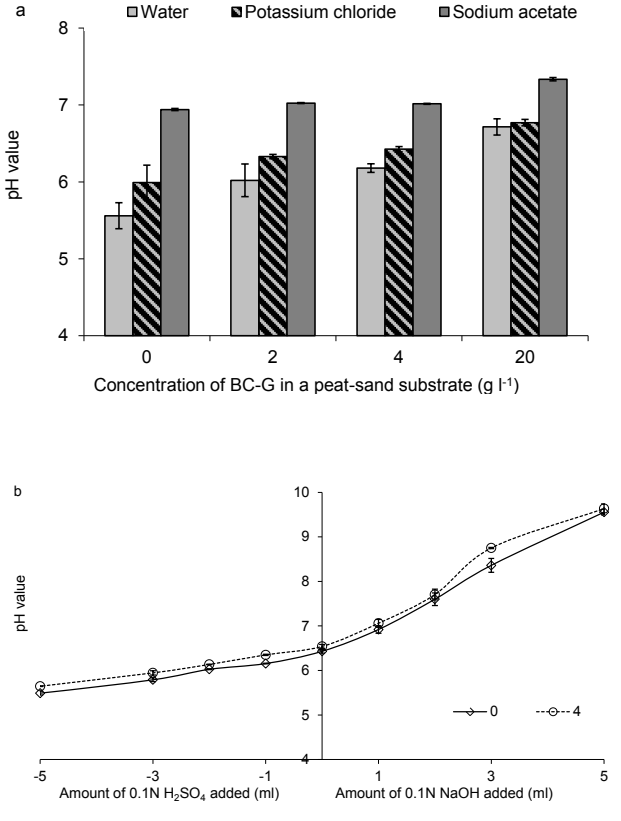

Fig. 1. pH values of the peat-sand substrate suspension amended with BC-G detected in different media: a – pH value of the peat-sand substrate amended with 0, 2, 4, and 20 g l^{-1} BC-G; 1N KCl and 1N CH_3COONa were used; b – effect of acid and alkali addition on the pH value of the suspension of the peat-sand substrate with 4 g l^{-1} BC-G (4) and without BC-G (0).

of the substrate suspension only by 1.2 units (Fig. 1b). The data obtained show that in the case of a comparatively high content of biochar in the substrate, the application of 1M KCl solution for pH measurement is not desirable due to the high cation-exchange capacity of biochar, because it could reduce the result, as found in the test with pure BC-G (data not shown).

Another important characteristic for agricultural soil/soil substrate quality is water holding capacity. In our experiments, the maximum moisture holding capacity of the peat-sand substrate amended with 2, 4, and 20 g l^{-1} biochar varied in the range from 173 to 186%. The average data for the non-amended substrate and pure BC-G were 190 and 269%, respectively (Table 1). However, statistical analysis of these results indicated that no significant difference in WHC between the pure substrate, the BC-G amended substrate, and the pure BC-G was found. In particular, the F-test for the pure substrate and pure BC-G showed p=0.216. As known from literature, introduction of biochar into soil can potentially increase its WHC; however, this effect depends on physico-chemical properties of biochar, in particular, its hydrophobicity (Sohi *et al*., 2010). Our results showed that WHC of BC-G is slightly higher than that for the substrate *ie* 269 and 190% (w/w), respectively. However, this difference is due to the considerably lower density of BC-G as compared to the substrate (Table 1).

Sohi *et al*. (2010) reported that the effect of biochar introduction in soil was soil-specific and did not depend on the application rate in the range of 5÷15 t ha^{-1}. An increase in WHC induced by biochar application was more effective in sandy textured soils (Dugan *et al*., 2010). Johnson *et al*. (2012) reported that biochar mixing ratios of 10% (v/v) and greater provided water holding capacity equivalent to peat-based potting mixes. In our experiments with the peat-sand substrate, the tested biochar did not significantly change the soil WHC in the studied BC-G application rates.

The mechanisms of the influence of biochar on soil microorganisms are studied by a wide spectrum of methodological approaches (Graber *et al*., 2010; Kolb *et al*., 2009; Steiner *et al*., 2008). Soil microbial respiration is one of the criteria for assessing microbial response to the presence of BC. However, sorption properties of biochars

T a b l e 1. Changes in water absorbing capacity of the peat-sand substrate in relation to the presence of BC-G

Parameter	BC-G rate in a peat-sand substrate (g l^{-1})				100% BC-G
	0	2	4	20	
Maximum moisture holding capacity % (w/w)	190.0	177.8	181.5	186.0	269.2
Water required to saturate 100 cm^{-3} of dw (g)	111.6	100.8	101.5	109.6	80.8

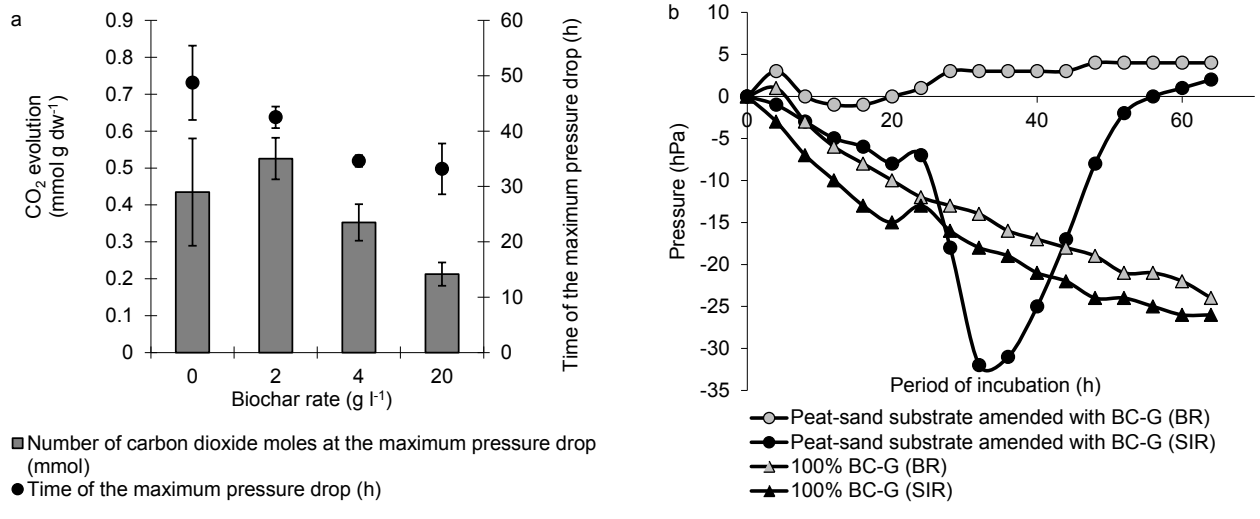

Fig. 2. Changes in CO_2 evolution dynamics for the peat-sand substrate amended with different amounts of BC-G. BR – basal respiration, SIR – substrate-induced respiration (1 g glucose per 20 g sample): a – number of CO_2 moles at the maximum pressure drop and time of the maximum pressure drop; b – typical profiles of the curves corresponding to the CO_2 evolution by pure BC-G and the peat-sand substrate containing 2 g l^{-1} BC-G.

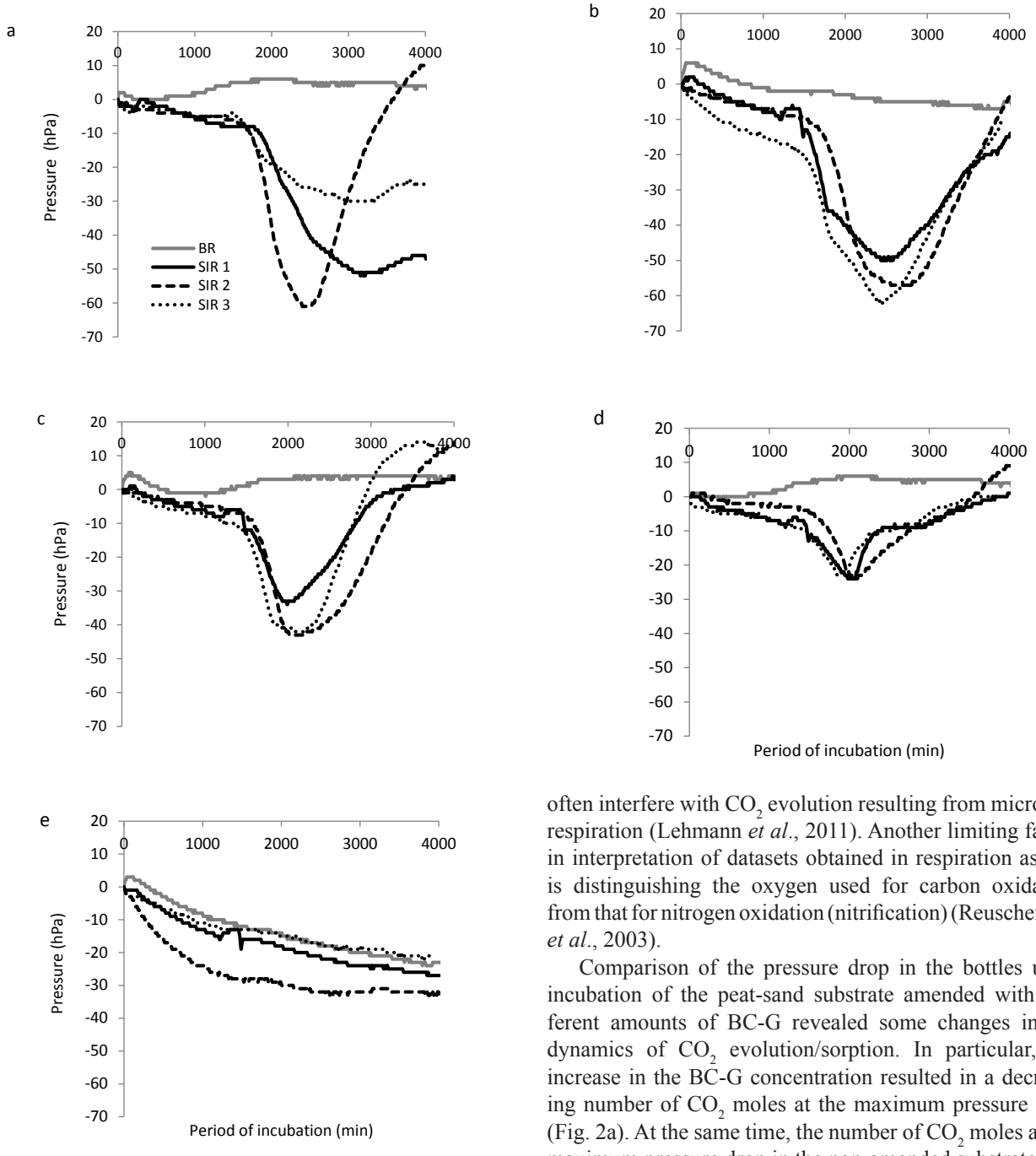

Fig. 3. Typical profiles of the pressure drop curves for the peat-sand substrate and BC-G performed with the OxiTop® OC 110 respirometric system: a – peat-sand substrate without BC-G; b – 2 g l^{-1} BC-G; c – 4 g l^{-1} BC-G; d – 20 g l^{-1} BC-G; e – BC-G without substrate. The incubation period was 67 h under conditions described in Section 2.4. BR – basal respiration, 20 g substrate amended with 18 ml water; SIR – substrate-induced respiration, 20 g substrate amended with 18 ml water and 1 g glucose. Testing of pure BC-G was performed under the same conditions as for the peat-sand substrate, except the mass of BC-G taken for testing was 10 g.

often interfere with CO_2 evolution resulting from microbial respiration (Lehmann *et al*., 2011). Another limiting factor in interpretation of datasets obtained in respiration assays is distinguishing the oxygen used for carbon oxidation from that for nitrogen oxidation (nitrification) (Reuschenbac *et al*., 2003).

Comparison of the pressure drop in the bottles upon incubation of the peat-sand substrate amended with different amounts of BC-G revealed some changes in the dynamics of CO_2 evolution/sorption. In particular, the increase in the BC-G concentration resulted in a decreasing number of CO_2 moles at the maximum pressure drop (Fig. 2a). At the same time, the number of CO_2 moles at the maximum pressure drop in the non-amended substrate was slightly lower as compared to the set with 2 g l^{-1} BC-G. The time to the maximum pressure drop ranged from 48 h (peat-sand substrate) to 33 h (substrate containing 20 g l^{-1} BC-G) and had a tendency to decrease with increasing BC-G concentrations in the substrate (Fig. 2a). Since the amount of CO_2 evolved daily from soil hectare is rather high (up to 50 kg for sandy soil and 7-12 time higher for organic matter-rich soil), the decrease in the amount of evolved CO_2 up to 1.2 and 2.0 times, respectively, for the BC-G rate of 4 and 20 g l^{-1} in comparison with the non-amended soil (Fig. 2a)

could be essential. Retention of some additional CO_2 quantity in the substrate may positively influence on the biodynamics of plant development. This was confirmed by the vegetation tests described below.

Typical profiles of the pressure drop curves during incubation of the peat-sand substrate amended with different concentrations of BC-G are presented in Fig. 3. The curve profiles differed in the amplitude of pressure drop in relation to the rate of BC-G in the substrate, while the pure BC-G without substrate demonstrated a completely different curve profile, which indicates CO_2 sorption (Fig. 3).

The sorption ability of BC-G is demonstrated in Fig.2. Incubation of BC-G caused a continuous decrease in pressure in the bottle regardless of the presence of glucose (Fig. 2b). Additional testing revealed the BC-G concentration-dependent character of the pressure drop under the studied conditions (results not shown). Otherwise, addition of glucose (substrate-induced respiration) to the peat-sand substrate amended with 2 g l^{-1} biochar resulted in a pressure drop, which had a typical profile for the soil containing microorganisms.

The number of culturable bacteria and fungi was determined in all the sets with the substrate and BC-G tested in these experiments. No considerable changes were found among the samples. The number of bacteria and fungi in all the tested samples was on average 2.03 x 10^6 CFU g dw^{-1} and 1.06 x 10^4 CFU g dw^{-1}, respectively.

The changes occurring during cucumber growth were evaluated using such criteria as seed germination and root development. After the first 6 days of the experiment, seed germination in the sets with 0, 2, 4 g l^{-1} biochar was found to be 100, 97, and 97%, respectively. Dry weight of the plant aboveground part and roots after 12 days in the presence of 2 g l^{-1} biochar was by 29 and 16% higher than in the control, respectively. Root volume of these plants was by 34% higher than in the control. In turn, after 18 days, the highest increase in the dry weight of the plant aboveground part was detected in the presence of 4 g BC-G *ie* by 36% as compared to control. Regarding the development of the root system of cucumber seedlings, considerable changes in the root structure of plants grown in the presence of BC-G were observed. In particular, a BC-G rate-dependent increase in the number of root tips was detected. At the same time, the average root diameter was decreased. Besides, addition of BC-G to the growth substrate resulted in an increase in the root volume, regardless of the biochar rate applied (Table 2, Fig. 4).

The positive impacts of low doses (1-5% by weight) biochar on the growth of plants were reported by Graber *et al.* (2010). Growth of pepper and tomato was stimulated by wood-derived biochar in a coconut fiber:tuff growing mix, but the changes observed were plant species-specific. The mechanisms of this effect were explained by the shift in the structure of soil microbial community towards beneficial rhizobacteria or fungi, as well as by the positive effect of low doses of biochar chemicals (hormesis) (Graber *et al*., 2010). However, even with the same application rate of biochar, a large variation in the effect size occurs, due to different biochar feedstocks used, different crops assessed, and differences in the soil type to which the biochar was added (Verheijen *et al*., 2009).

T a b l e 2. Effect of different rates of BC-G added to the peat-sand substrate on the development of seedlings of *Cucumis sativus* L. ('Grīvas') on day 12 and 18 after sowing

Rate of BC-G (g l^{-1})	Dry weight of plant aboveground part (mg)	Dry weight of plant roots (mg)	Root volume (cm^{-3})	Total root length (cm)	Average root diameter (mm)	Number of root tips (pcs)
12 days after sowing						
0	45.1±2.3	6.3±0.3	0.056±0.003	63.10±3.16	0.34±0.02	356±18
2	58.0±2.9	7.3±0.4	0.075±0.004	80.55±4.03	0.35±0.02	523±26
4	52.2±2.5	7.6±0.4	0.073±0.004	81.73±4.09	0.34±0.02	652±33
18 days after sowing						
0	100.2±5.0	6.4±0.3	0.065±0.003	95.69±4.78	0.30±0.02	500±25
2	110.1±5.5	7.5±0.4	0.077±0.004	99.66±4.98	0.31±0.02	585±29
4	136.0±6.8	7.6±0.4	0.078±0.004	100.48±5.02	0.31±0.02	670±34

Confidence intervals are calculated at a confidence level of 95%.

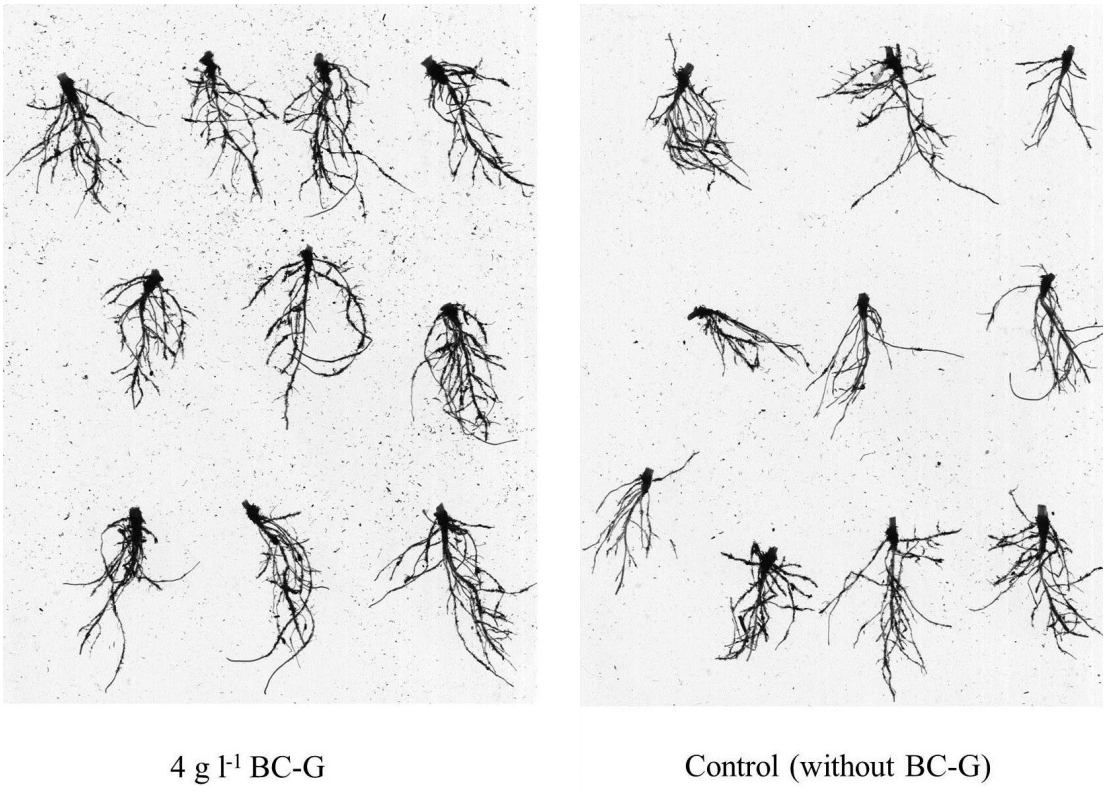

Fig. 4. Roots of *Cucumis sativus* L. ('Grīvas') grown during 18 days in the peat-sand substrate amended with 4 g l^{-1} BC-G and without BC-G. Images were made using an image analysis system Win Rhizo 2002 C.

CONCLUSIONS

1. Biochar obtained by gasification of hardwood fuel granules and used as amendment for a peat-sand (3 : 1) substrate with the biochar rate of 2, 4, and 20 g l^{-1} significantly ($p<0.05$) increased the pH level of the substrate. In particular, the pH(H_2O) of the substrate with 4 g l^{-1} and 20 g l^{-1} BC-G was increased from 5.6 to 6.2 and 6.7, respectively. Water holding capacity of the substrate was not considerably changed when amended with the tested concentrations of BC-G. Based on the results with pure BC-G, the use of 1M KCl for pH measurement of the substrate with a high BC-G rate is not recommended to due to its cation-exchange ability, which can be the cause of mistakes.

2. Introduction of BC-G into the substrate led to decreasing CO_2 evolved at the maximum pressure drop, which was detected by the respirometry device. Retention of some additional CO_2 quantity in the substrate may positively influence the biodynamics of plant development.

3. The 18-day vegetation test with cucumbers has shown a stimulation effect of 2 g l^{-1} and 4 g l^{-1} BC-G in the peat-sand substrate on the biometric indices of the aboveground part and roots. Considerable changes in the root structure of plants grown in the presence of BC-G were observed. In particular, an increase in the number of root tips and a decrease in the average root diameter with an increased root volume were shown.

4. Further investigations are required for optimization of BC-G dosages with emphasis on the specific BC combination, soil type, plant species, climatic condition, *etc*.

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