

EFFECTS OF BIOCHAR APPLICATION ON WINTER WHEAT (*TRITICUM AESTIVUM* L.) ROOTS UNDER LONG-TERM DROUGHT CONDITIONS

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Abstract

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The main objective of this paper was to evaluate the effect of applying biochar and activated carbon on winter wheat affected by drought in model laboratory conditions. Cultivation tests of the soil-microorganisms-plant (winter wheat) system were focused on understanding the interactions between microbial soil communities and experimental plants in response to specific cultivation measures, in combination with the modelled effect of drought. The containers were formed as a split-root rhizotron. In this container experiment, the root system of one and the same plant was divided into two separate compartments where into one half, biochar or activated carbon has been added. The other half without additives was a control. Plants favoured the formation of the root system in the treated part of the container under both drought and irrigation modes. In drought mode there was lower production of CO₂, lower overall length and surface of the roots of winter wheat compared to variants in irrigation mode. The application of biochar and activated carbon, therefore, supported the colonization of roots by mycorrhiza in general. The Scientific merit of this paper was to investigate the possibility of mitigating the effects of a long-term drought on winter wheat through the application of biochar or the application of activated carbon.

Keywords: soil, mycorrhiza, split-root rhizotron, biochar, CO₂, N₂O, roots

INTRODUCTION

In recent years, climate change has been increasingly influencing the temperature and precipitation conditions with an increased frequency of extreme situations, such as prolonged drought and increased temperatures (Liu and Allan, 2013; Rahmstorf and Coumou, 2011). These situations can adversely affect the yields of field crops including winter wheat (Trnka *et al.*, 2012; Hlavinka *et al.*, 2009). Many studies also draw attention to the likely continued increased frequency of droughts and high temperatures, which will also affect the cultivation of winter wheat (Semenov and Shewry, 2011; Gourdjji *et al.*, 2013; Trnka *et al.*, 2014). We can assume that the effect of the mentioned extreme weather conditions

will be influenced by the utilised systems of soil management. One of the ways to mitigate the negative impact of adverse weather conditions is the maintenance or improvement of soil fertility. This can be achieved by improving the biological and physico-chemical properties of soil.

An interesting option to achieve this is the application of biochar into the soil. Biochar is a fine-grained charcoal-like material produced by pyrolysis of biomass at temperatures between 300 °C and 600 °C, without access of air. During pyrolysis, carbonization of plant cells and chemical change produce structures that are resistant to microbial degradation. Thus, thermally converted material is approximately 1.5 to 2 orders of magnitude more stable in soil than the organic mass, which was not carbonized. Biochar in soil has a mean

lifetime of several hundreds to several thousands of years (Kuzuyakov *et al.*, 2009). Application of biochar to soil can also be used as a method of storage (sequestration) of carbon (Amonette *et al.*, 2007). Biochar can also be manufactured from waste materials such as green waste or manure. These waste materials can otherwise produce gases, such as CH₄ and N₂O, with a greater impact on the greenhouse effect than CO₂ (Lehmann and Stephen, 2009). After intensive fertilization (inappropriate application) a large surplus of ammonium or nitrate can be present (depending on the fertilizer) in the soil, which can be quickly removed simply via nitrification and through subsequent respiratory denitrification. And, also under a slight anaerobiosis, organic compounds from manure are only partially decomposed and form more methane. But the application of manure improves physical and chemical properties of soil and microbial conditions in soil, and organic fertilizer should always be preferable to mineral application. From the agricultural perspective, the application of biochar into the soil may have several positive effects. They include increase of soil fertility (Liang *et al.*, 2006, Novak *et al.*, 2016, Jeffery *et al.*, 2011), increased retention of agrochemicals (Spokas *et al.*, 2009), improvement of the physical properties of soil, such as increase in the pH level and cation exchange capacity as well as reduction in tensile strength (Chan *et al.*, 2007; Lehman *et al.*, 2011). The addition of biochar also significantly increases the content of available water in the soil by increasing the amount of water retained in the soil (field water capacity) and allowing plants to draw the soil water content and lower it before wilting (Koide *et al.*, 2015). This is caused mainly due to increasing capillary water capacity of the soil after application of biochar. This leads to increased productivity of plant cultivation, increased microbial activity in soil, and higher levels of availability of nutrients, particularly P and K (Biedermann and Harpole, 2013). Biochar has also shown the ability to change soil biological community composition and abundance (Lehmann, 2011). Atkinson (2010) summarises mechanisms that affect the application of biochar in soils of the temperate zone.

In order to test the hypothesis that the addition of biochar can improve the soil environment, it is necessary to obtain the direct roots' responses from the treated and untreated soil. For this purpose, the pot experiment was established where

the model plants were growing in two different soil compartments, the first one with, and the second without the addition of biochar. Based on the feedback of the plants the following hypotheses were tested: (i) application of biochar or charcoal will mitigate the effects of a prolonged drought on the winter wheat. (ii) the experimental plant will prioritize the formation of the root system in that half of the container with the addition of biochar or charcoal. To validate these hypotheses, the following parameters were monitored: The length and width of the root system in both halves of the container, the production of gaseous metabolites, and the percentage of roots colonized by mycorrhiza.

MATERIAL AND METHODS

Experimental design

In order to evaluate the influence of the addition of biochar to improve retention and storage capacity of the soil, and thus to increase the resistance of plants to drought, a container experiment was set up. The containers were formed as a split-root rhizotron.

The description of the sampling site and the experimental design is also given in Svoboda (2016). Soil sampling was conducted on the cadastral area of the Banín municipality (49°40'24.3"N 16°27'26.1"E), where the soil was degraded by a long absence of organic matter inputs to the soil. At the sampling site the main soil unit is cambisol. The sampling site is located in the climatic region MT2 with the average annual temperature of 7 °C to 8 °C and the total rainfall of 550 mm to 700 mm/year.

The containers for the experiment were set up so that the root system of one and the same pre-cultivated plant were divided by a plastic film into two compartments, one with and the other without the addition of biochar in order to study the root system responses during the induced drought stress. The root system of the two parts was separated from the experimental soil mixture by a 34 µm polyamide mesh, which would not allow root penetration into the soil. Two plants of winter wheat were placed into each test rhizotron container. The tested plants in vertical gaps between soil compartments and an impermeable plastic film developed two different root systems according to their own preferences. Contact with relevant

I: Summary of experimental variants

Variant	Variant Description
Ks	Drought Mode Control
Km	Irrigation Mode Control
As	Variant with application of activated carbon, 50 t/ha under drought mode
Am	Variant with application of activated carbon, 50 t/ha under irrigation mode
Bs	Variant with application of biochar, 50 t/ha under drought mode
Bm	Variant with application of biochar, 50 t/ha under irrigation mode

experimental variant of soil was facilitated primarily by microbial activities through the polyamide mesh. Retaining chambers were placed (4 cm³) into rhizotron at the depth of 15 cm for measuring emissions of gaseous microbial metabolites. A narrow tube was led out from the retaining chambers to help with the collection of gaseous metabolites. The experimental containers were set up according to variants described in Tab. I in four repetitions.

Both halves of the experimental containers of Ks and Km variants were filled only with experimental soil. In other variants, one half was always filled with experimental soil (control) while the second half included soil with an addition, according to the respective variants (A, B). In the text, letters A or B identify results from the given halves of the experimental containers where active carbon (A) or biochar (B) were applied. Letter C identifies the control halves of containers without additives. Ks – Control in drought mode, Km – Control in irrigation mode, As – Activated carbon 50 t/ha in drought mode, Am – Activated carbon 50 t/ha in irrigation mode, Bs – Application of biochar in an amount of 50 t/ha in drought mode, Bm – Application of biochar in an amount of 50 t/ha in irrigation mode. A, B represent those halves of the experimental containers where activated carbon or biochar was applied.

The activated carbon had a particle size from 2.36 mm to 4.75 mm, and was made from coconut shells, water content maximum 5%, ash content maximum 5%, bulk density of 500 ± 50 g/l, and pH 8 to 10. Application of active carbon was chosen to simulate biochar that had already stayed in the soil for a long period of time. It is assumed that after hundreds of years, only the physically chemical basis of biochar remains of pyrogenic material remained, in which there is nothing interesting for microorganisms. This material should approximate to the clean chemical product on the basis of active char, concerning its characteristics. This means we tested whether or not the positive changes are caused by the change of physical conditions of soil, or by the synergic interactions of microorganisms as well. The changes are caused, in part, by the positive ones, caused by the stimulation of MO pyrogenic co-products (for instance the liquid phase which can be 1–10% of pyrogenic admixtures), and also, in part, by the negative ones, as stressing of the soil microorganisms by shifting the soil reaction to an alkaline sphere, as the admixture of ashes is supposed in biochar, especially the basic cations of calcium, magnesium and potassium (approximately up to 5%). The used biochar was made from biomass waste. Composition: 1/3 Biochar, 1/3 cellulosic fiber and 1/3 sheep's manure, pyrolysed at 470 °C. To each half of the experiment container, we have placed mixed ion exchangers to catch mineral nitrogen escaping from the system. Ion exchange discs were buried under a layer of sand, which was

then covered with homogenized soil with pertinent additions depending on the variant.

Variants in both drought and irrigation modes were regularly irrigated so that the dry variant was kept at 30% of available water capacity (AWC). AWC is the maximum amount of water which a plant is able to utilise in the given soil profile; mathematically, it is a difference between the field water capacity and wilting point. Variants in irrigation mode were kept at 70% AWC. During the experiment, emissions of gaseous microbial metabolites were measured. The experiment was terminated after the tested plants reached maturity. After the termination of the experiment, the used containers were dismantled. The resulting two-dimensional system of plants was scanned and the area and length of the root system were evaluated. Simultaneously, microbial analysis of the root system was implemented and the degree of the root colonization by mycorrhizal fungi was assessed.

Assessment of mycorrhizal colonisation of roots

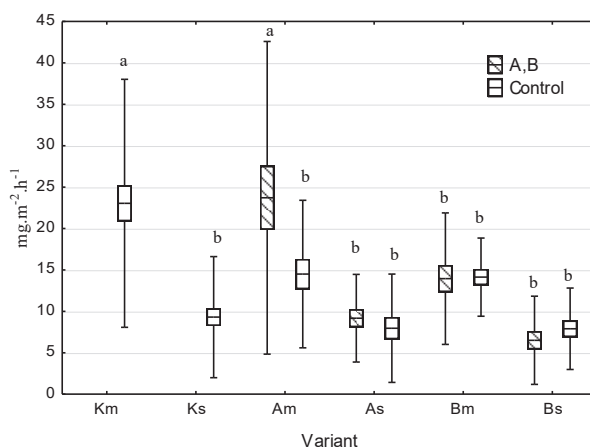
After the termination of the experiment, part of the fresh roots were collected to determine the mycorrhizal colonization. We determined the vesicular-arbuscular mycorrhiza (hereinafter referred to as the "mycorrhiza"). Prior to processing, the roots were stored in the FAA fixing solution – Formalin – Acetic Acid – Alcohol (Formaldehyde (38%) 5 ml + Acetic Acid 5 ml + Ethanol (50%) 90 ml). The evaluation of the mycorrhizal infection used the method of dyeing of roots using a 0.05% tropaeolum blue in lactoglycerol (Koske and Gemma, 1989). The percentage of roots colonized by mycorrhiza was determined by a microscopically modified preparation method (Giovannetti and Moss, 1980). The mycorrhizal colonization of roots was always assessed via two replicates from each root sample.

Determination of CO₂ and N₂O production

During the experiment, gas was removed three times from the above described chambers for collecting emission of microbial metabolites. The sampling was carried out once just before the end of the experiment, and twice with an interval of 20 days before that. A syringe was attached to the narrow tube leading out of the chamber. By using it, the gas was removed from each half of the test containers separately. In each syringe, we have assessed the content of gaseous microbial metabolites, CO₂ and N₂O, using Agilent 7890A gas chromatograph.

Determination of length and width of roots

After dismantling the experiment containers, the two halves of the resulting two-dimensional root systems were scanned by the Epson Perfection Photo



1: Graph of production CO₂; Midpoint represents average, box indicates standard error and error lines indicates standard deviation, treatment means with different letters are significantly different ($P < 0.05$).

V 700 device and evaluated by the WinRHIZO, Basic version (2009) program.

Statistical analysis

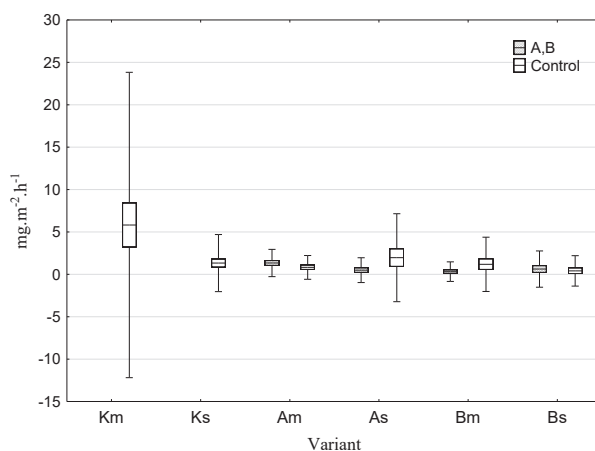
All results were subjected to one-way analysis of variance (ANOVA) in combination with post-hoc Tukey's test ($P < 0.05$). All analyses were done in Statistica 12 software.

RESULTS AND DISCUSSION

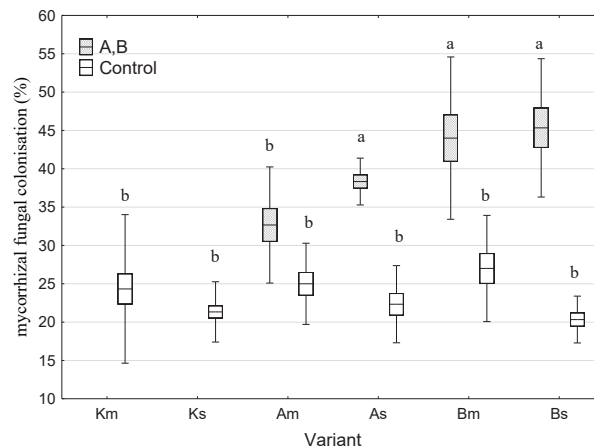
Fig. 1 shows a graph of CO₂ production in milligrams per cubic meter per hour. CO₂ production is proportional to the activity of microorganisms in the rhizosphere in the vicinity where chambers were placed to capture gases. Statistically detectable difference were found between the variant Km, and other variants except Am_A and also between variant Am_A and other variants, except Km and Bm_B. In the drought mode,

there was no statistically detectable difference in CO₂ production after the application of biochar and activated carbon. In absolute terms, the soil respiration between two parts of rhizosphere of one plant was only statistically different between significantly lower respirations in the compartment without the addition of activated carbon under the 70 per cent of AWC. In other cases, the respiration in both parts of root compartments was comparable. The application of biochar generally manifested itself by reducing respiration. Application of activated carbon increased the respiratory activity in both the 70 per cent of AWC, and in the drought mode, but not significantly. The same was not observed with application of biochar.

Fig. 2 shows a graph of the production of N₂O in milligrams per cubic meter per hour. Drought stress and application of biochar apparently decreased the variability in nitric oxide production. The reduction of N₂O soil emissions after



2: Graph of production N₂O; Midpoint represents average, box indicates standard error and error lines indicates standard deviation, treatment means with different letters are significantly different ($P < 0.05$).

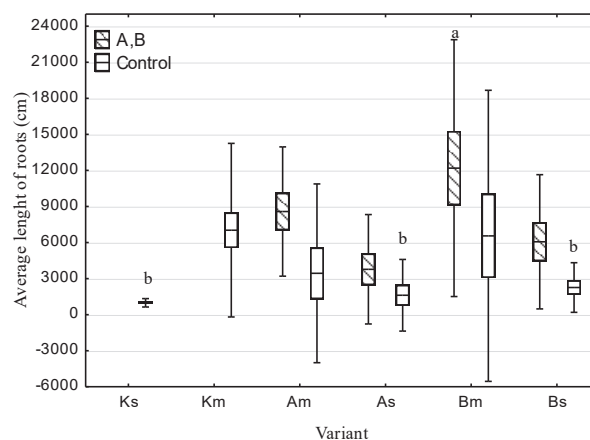


3: Graph of mycorrhizal fungal colonisation; Midpoint represents average, box indicates standard error and error lines indicates standard deviation, treatment means with different letters are significantly different ($P < 0.05$).

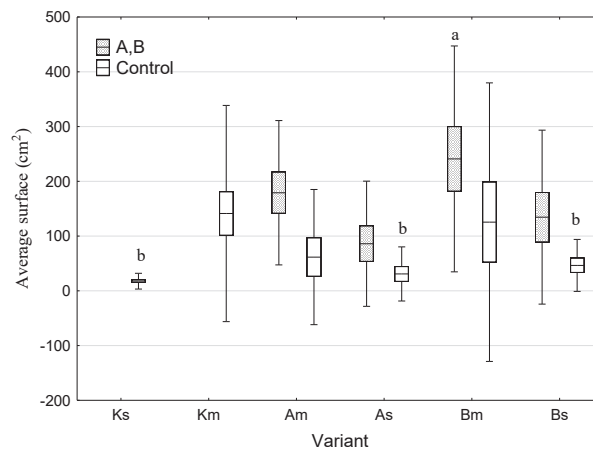
the application of biochar has been also observed by Lehmann (2011) and Atkinson (2010).

Fig. 3 shows a graph of mycorrhiza occurrence as percentages. Percentages represent parts of the roots, which were colonized by arbuscular mycorrhiza. Statistically detectable differences existed between the variants Bm_B and Bs_B and all other variants except As_A , and also between the As_A variant and control variants. It is notable that in the variants with the addition of biochar or activated carbon (A and B) there was always higher incidence of mycorrhiza in one and the same plant in comparison with the second control (C) half of the container, without the addition. In the Am_A variant, this difference was not statistically detectable when compared with Am_C . The application of biochar and activated carbon, therefore, supported the colonization of roots by mycorrhiza, in general. Lehmann (2011) states that the applications of carbonized substances increase the heterogeneity of the soil environment.

In the immediate vicinity of carbonized substances there are zones with a gradient of available cations. The following examples from the literature are assumed in this experiment. The increases of the cation exchange capacity, after biochar application to soil, are mentioned in many works (Glaser *et al.*, 2002, Liang, 2006 Atkinson, 2010). Warnock (2007) describes the increased availability of P and base cations. There is also an increase in water holding capacity of soil (Mickan *et al.*, 2016, Lehmann *et al.*, 2011) after the application of biochar. The experimental plants were induced to higher colonization by mycorrhiza. The importance of cooperation with filamentous microorganisms was apparently increased. Higher levels of mycorrhiza colonization were beneficial for wheat. The role of mycorrhizosphere for making available broader vicinity near the roots for fungal filaments to secure plant nutrition, and also for resistance to drought stress, is increased compared with the control half



4: Graph of the average length of the roots; Midpoint represents average, box indicates standard error and error lines indicates standard deviation, treatment means with different letters are significantly different ($P < 0.05$).



5: Graph of average surface of roots; Midpoint represents average, box indicates standard error and error lines indicates standard deviation, treatment means with different letters are significantly different ($P < 0.05$).

of the experiment container without the addition of carbonized substances. The application of biochar or activated carbon also affects other biological processes in the soil, as documented by Warnock *et al.* (2007), Lehmann *et al.* (2011) and Quilliam *et al.* (2012). Biochar also offers refuge for soil microorganisms and fungi (Warnock *et al.*, 2007) that are able to penetrate the structure of biochar under certain conditions (Hammer *et al.*, 2014), and significantly increase the intake of phosphorus by the host plant. Mickan *et al.* (2016) reported that biochar had little effect on the colonization of roots by mycorrhiza under the conditions of adequate irrigation, but increased colonization was recorded under drought conditions. This does not fully match our results as we recorded an increase, also under conditions of higher soil moisture (irrigation mode).

Fig. 4 shows the graph of the average length of roots and Fig. 5 shows the graph of the average root surface. The resulting average surface correlates with the resulting average length of roots. Statistically significant differences were found between the variant Bm_B and variants Ks_C and As_C in the root surface and between the variant Bm_B and variants Ks_C , As_C , and Bs_C . Although there are no statistically detectable differences ($P < 0.05$) among the other variants due to the high variation in the root length of respective experimental plants, we can observe a significant increase in the length of roots in variants Bs_B and As_B compared to the control variant Ks_C . In variants with biochar or activated carbon, plants always formed longer and wider root systems in the half of the container with the addition (Am_A , As_A , Bm_B , and Bs_B) compared with the other control half of the cultivation container. Moreover, this also corresponds to increased colonization by mycorrhiza in these variants. Other studies do not deal with measuring the length of the root system in connection with the application of biochar. Lehman *et al.* (2011) summarised the effects of biochar application on the formation

of underground biomass. Results of various studies vary considerably depending on the type of biochar, soil type, and tested plant. The reasons for the change in the growth of the root system after the application of biochar in the existing studies are rarely identified. They vary according to the properties of the biochar and different soil environments (Lehman *et al.* 2011).

The results of the measured lengths of the roots correspond to measured values of the dried underground biomass reported in Svoboda *et al.* (2016), who also lists the results of the production of aboveground biomass. In the Bs variant, the formation of total aboveground biomass was more than doubled compared with As and Ks variants. This effect can be explained by stimulating root colonization of experimental plants by mycorrhizal fungi after the addition of biochar (see Fig. 3). This is coupled with subsequent higher nutrient utilization efficiency, not only in the area of the rhizosphere, but also through extraradical fibres of mycorrhizal fungi from the area of the mycorrhizosphere. This interpretation is facilitated by the experimental design using dichotomous division of roots of tested plant in two separate soil compartments in the cultivation container, and their subsequent separate evaluations. This can thus support the hypothesis that the application of biochar and activated carbon increased the heterogeneity of the environment and thus the significance of the mycorrhizosphere towards the rhizosphere. The positive effects of the application of biochar on microbial communities under the drought mode in this experiment were published by Dvořáčková *et al.* (2016).

CONCLUSION

The application of biochar under drought conditions significantly increased the production of aboveground biomass. The addition of biochar or activated carbon (A and B) always induced higher incidence of mycorrhiza if compared with the second control pot compartment (C), without the addition of these additives. This situation occurred under both irrigation modes. In the drought mode, there was lower production of CO₂, lower overall length and surface of the roots of winter wheat compared to the variants in irrigation mode. The influence of biochar and activated carbon on the growth of the root system is not so clear. However, we can see the positive effect in the overall underground biomass and in length and surface of each root system after the application of biochar and activated carbon under simulated drought conditions. This supports the hypothesis that the plant roots preferred the pot compartment with the addition of biochar or activated carbon. It can be concluded that application of biochar or charcoal could mitigate the effects of a prolonged drought on the winter wheat. For further research, a more detailed study focused on the interactions between a tested plant, rhizospheric microorganisms, and a soil body is recommended, in order to better understand the fate of the key biogenic nutrients (especially nitrogen and phosphorus).

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