CARBON SEQUESTRATION VIA BIOCHAR APPLICATION INTO THE SOIL – IMPROVEMENT OF PLANT REACTION ON THE INDUCED DROUGHT STRESS

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Climate change impact is no longer just a global problem, but it could be observed locally, for example by the weather extremes. Still, the fact remains that even if we would be able to stop deforestation, to prevent the fossil fuels use or to contribute to the CO_2 reduction into the atmosphere in some other way; we have to take into account it's already manifested proportion. Therefore, an urgent question is how to reduce the amount of CO_2 in the atmosphere. One of the promising ways is CO_2 fixation in biochar´s carbon and its subsequent application into the soil. Biochar is a material which resembles charcoal with its formation and properties. The raw material might not be only wood material. Biochar can be produced from the waste or from the other by-products. In this work the effect of biochar and mineral fertilizers additions into the soil have been compared. Some of the experimental treatments have been exposed to simulated drought oscilations. Lettuce has been selected as an indicator plant; mainly due to its ability to respond immediately to different moisture regimes. The experiment has stated that soil with biochar is able to retain moisture better than other experimental treatments and that the planst growing in soil with biochar is able to survive better the simulated drought stress.

Keywords: Carbon, Biochar, Drought, Climate change

INTRODUCTION

Biochar is the material produced by the process of pyrolysis, which its properties resembles charcoal (Atkinson, 2010). Positive effect of biochar application on soil properties was observed already before and during the Middle Ages in the Amazon basin. It is still possible to find the patchy occurrence of this "Indians' black earth" ("Terra Preta de Indio" in Portuguese), and to observe its extraordinary fertility there. (Sombroek et al. 2003 Kernetal. 2003).

Biochar has shown a positive effect on the water holding capacity (Busch et al., 2012, Busscher et al., 2010, Kammann et al., 2012), on the water infiltration (Asai et al., 2009 and Ippolito et al., 2012), on the water retention in soil, on the nutrient retention and/or availability in soil (Clough et al., 2013 and Ventura et al., 2013), on the hydraulic conductivity (Buss et al., 2012), and on the soil aeration (Cayuela et al., 2013). Besides the improvement of soil physical properties after the biochar application, the biochar addition into the soil represents an exceptional way how to return carbon to its natural reservoir, into the earth, from where it was in recent decades actively pumping out (Woolf, et al, 2010). On the other hand it is necessary to mention the fact that Pietikäinen et al., (2000); Yin et al. (2000); Kim et al., (2006); O'Neill et al ., (2009); Liang et al., (2010) have observed negative characteristics of biochar on soil microorganisms. The biochar is a product of pyrolysis, it is very likely that during the mentioned process the biochar could be enriched by certain toxic hydrocarbons (Lehmann et al. 2011). These substances may or may not inhibit activity of the soil. According to (Mukherjee, 2011), microorganisms have the ability to withstand a certain amount of harmful hydrocarbons. It depends on the condition of soil biota and especially on the type and quantity of toxic substances. Biochar effects on microorganisms has not yet been described in detail (Lehmann 2011).

At the present stage of our knowledge, the drought period is an integral part of current agriculture, therefore is especially important to improve the soil water accessibility (Farooq, 2009).One way to achieve this is to inoculate plants plant root tissue with mycorhiza, and by this way to improve soil water availability (Augé, 2001).

Arbuscular mycorrhiza is endomycorrhizal type of symbiotic relationship between vascular plant and fungus, characterized by the ingrowing fungal hyphae into the cortical cells of the roots and by the formation of specific and unique structures vesicules and arbuscules (Parniske, 2008).

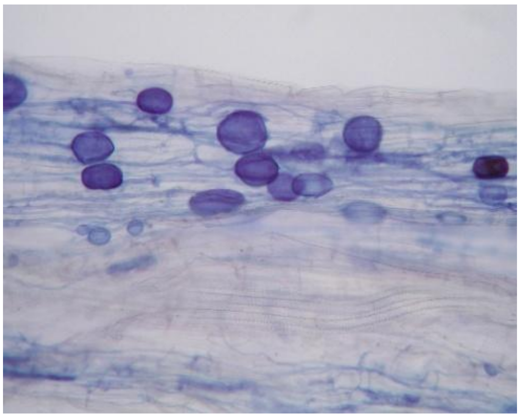

Fig. 1 Arbuscular mycorrhiza fungi (AMF)

If the plant´s roots are colonized by arbuscular mycorrhiza, their active area will increase, as well as the ability to obtain important compounds from the soil through extraradical fungal hyphae even from the extended distance (KOVÁŘOVÁ M. et al., 2011). Tobar et al., (1994), Davies et al., (1992) hypothesize that arbuscular mycorrhiza allows plant´s roots to have an access to the soil water which would be without this symbiosis inaccessible. The mechanism behind has not yet been described in detail, one of the theory states that the access of plants to water can improve glomalin (Wright, 1996). These substances affect the stability of aggregates and thereby indirectly the rainfall infiltration as well as the soil water retention Tobar et al., (1994).

This paper deals with the influence of biochar on soil organisms, namely arbuscular mycorrhiza (AM) fungi natively colonizing the host lettuce plants (*Lactuca sativa*) tested in the pot experiment under controlled conditions in growth chamber. Experimental pots were divided in two parts; one of them was exposed to water stress condition. Simultaneously, two different treatments were used; the first one with enrichment of the soil with biochar, and the second one with application of conventional nitrogen fertilizer. The aim of this work was to compare the effect of additives on the plant and AM fungi in the period when the soil is exposed to water stress.

MATERIALS AND METHODS

Design of the experiment

The experiment was arranged as the pot experiment with the lettuce plants (*Lactuca sativa*) growing at controlled growth box conditions for three months with two different water treatment in combination either with mineral nitrogen fertilizer and/or with biochar (see Table 1). Four replications were prepared for each treatment combination. The pots were laid out in a completely randomized block design with enough space between the pots to avoid the plants from shading each other.

Tab. 1 Experimental setup

Treatment	Irrigation regime	Application of
V1	40 % WHC	0.140 Mg $N.ha^{-1}$
V2	70 % WHC	0.140 Mg $N.ha^{-1}$
V3	40 % WHC	50 Mg $B_{ch}.ha^{-1}$
V4	70 % WHC	50 Mg $B_{ch}.ha^{-1}$
V5 (Control)	70 % WHC	---

Abreviations: WHC: water holding capacity; B_{ch}: biochar

The treatments V2, V4 and V5 were irrigated optimally, thus to reach approximately 70 % of WHC. The remaining treatments, V1 and V3 were cultivated at induced drought conditions; the WHC was reduced to 40 %. To compare plant reaction on the two water regimes after improving the soil nutrient status, the nitrogen fertilizer was applied. The same was made with biochar addition.

All the containers (total number of 20 pieces, the height of the pot was 12 cm and diameter 16 cm) were placed in a phytotron, where the conditions in the growth chamber were 24/20 °C (day/night) and 65 % relative humidity.

Nitrogen fertilizer

For the addition of nitrogen, DAM 390 fertilizer was selected at the recommended level of 0.140 Mg $N.ha^{-1}$ (this liquid fertilizer contains 30 % of nitrogen; the ratio of ammonium, nitrate and amidic nitrogen is 1:1:2).

Biochar

Biochar (Bch) has been prepared from beech wood chips mixture that have been exposed to the process of slow pyrolysis at temperatures from 350 °C to 500 °C. The amount of biochar addition was 50 Mg Bch. ha^{-1}.

Experimental plants

Lettuce (*Lactuca sativa* L., cv. Attraktion) seeds were soaked in destilled water overnight and then germinated in the dark on cheeseclotht. After 3 to 5 days, the seeds were transferred to a growth chambre. The plants were grown there for 1 to 2 weeks before transfered to polyethylene containers, described previously.

Fig. 2 Biochar

Assessment of AM fungi infection

The lettuce roots were stained in 0.05 % trypan blue in lactophenol according to the standard procedure (Phillipps and Hayman 1970). At the end of the experiment approximately 0.5 g of roots from each pot was sampled, cut to about 3 cm in length, washed thoroughly under water and placed in FAA solution (ethanol + acetic acid + formaldehyde). To improve stain penetration and clearing the whole mycorrhizal roots, samples were than rinsed with several changes of tap water to remove FAA and placed into a 10 % (w/v) KOH solution for 1 h at 90 °C. The next step is the acidification of the samples by transfering them to a beaker containing 10 ml 1 % HCl (enough to cover roots). Acidified roots were transfered directly from the 1 % HCl solution into a beaker containing 10 ml 1 % trypan blue in lactophenol solution (enough to cover roots), and left incubate for 1 h at 90 °C. For each plant at least 20 stained roots adjusted to 15 mm long fragments were randomly taken. Root segments were observed by light microscope Olympus CX-41st Observations conducted at a magnification of 100 and scored for percentage presence/absence of mycorrhizal fungi, mycorrhizal colonization (%).

The potential differences between AM fungi mycorrhizal root colonization have been analyzed by ANOVA analysis with post-hoc Fischer LSD test. All data were analyzed in Statistica 10 software. Graphic processing of measured data was performed in Microsoft Excel 2010.

RESULTS

Roots provide collaborative organisms including the AM fungi with carbonaceous substances, and they in turn obtain nutrients, because these microorganisms efficiently acquire nutrients from sources that are chemically or spatially unavailable to plants (Kuzyakov et Xu, 2013). In other words - plants dominate in soil environment by providing energy and carbon while soil microorganisms dominate by control of biochemical processes in soil (Záhora, 2015). Taking into account these basic facts we can assess our results.

Figure 3 shows, that the colonization of roots by AMF was significantly affected only by combination of soil moisture reduction and mineral fertilizer addition (V1). The highest production of plant biomass was found in V2, where mineral N was applied and soil moisture optimized, conversely the lowest in V3 treatment, where Bch was applied and experimental plants were exposed to water stress (Table 2).

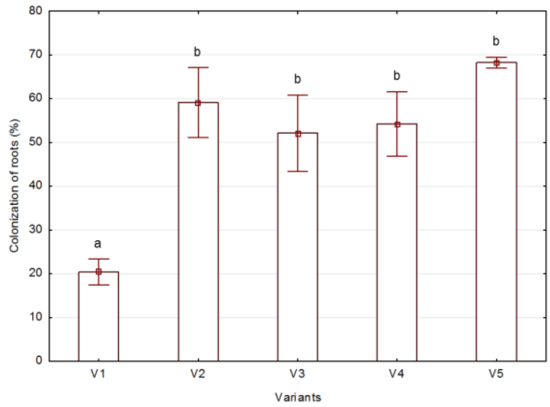

Fig. 3 colonization of root by AMF (different letters indicate a significant differences, ANOVA; Fischer LSD P<0.05)

Tab. 2 Effects of different soil moisture and various soil additives on lettuce biomass

Treatments	Mean [g of DM] ± Standard error
V1	2,404 ^{b} ± 0,165
V2	4,775 ^{c} ± 0,241
V3	1,410 ^{a} ± 0,080
V4	2,081 ^{b} ± 0,225
V5	2,417 ^{b} ± 0,360

Different letters above the mean values indicate significant ($p < 0.05$,Tukey's HSD) differences among treatments (modified according to Dvořáčková et al., 2015.

Considering that both, V1 and V3 treatments, were exposed to long period of drought we can observe how the higher nitrogen availability in soil can discriminate the supplies of plant assimilates which are supporting the AM fungi colonisation. In turn, the Bch addition stimulates the AM fungi at the cost of significantly lower plant production.

Tab. 3 Results of Tukey´s HSD post-hoc test for percentage root colonization by AMF

Tukey HSD test; a variable mycorrhiza Probability for a post-hoc test´s Error: intergroup = 163.10, sv = 15.000					
	V1	V2	V3	V4	V5
V1		0,005046	0,022319	0,014212	0,000842
V2	0,005046		0,933969	0,981620	0,846702
V3	0,022319	0,933969		0,999286	0,416625
V4	0,014212	0,981620	0,999286		0,548323
V5	0,000842	0,846702	0,416625	0,548323	

Tab. 4 Results of Fischer LSD post-hoc test for percentage root colonization by AMF

LSD test; a variable mycorrhiza Probability for a post-hoc test´s Error: intergroup = 163.10; sv = 15.000					
	V1	V2	V3	V4	V5
V1		0,00064	0,00312	0,00192	0,000089
V2	0,00064		0,45030	0,59723	0,328302
V3	0,00312	0,45030		0,81715	0,094394
V4	0,00192	0,59723	0,81715		0,141913
V5	0,00008	0,32830	0,09439	0,14191	

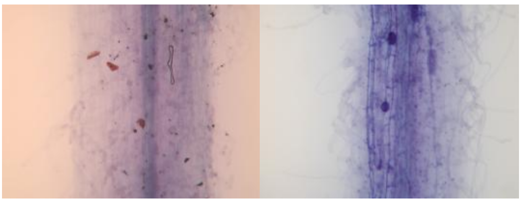

Fig. 4 Biochar in direct contact with the rhizoplane

CONCLUSION

It was demonstrated by this study, that the addition of biochar can improve the interactions between plant roots and arbuscular mycorrhizal fungi even at the prolonged drought stress. This phenomenon seems to be accompanied by the decrease of the plant biomass production. On the other hand, the addition of the nitrogen fertilizer to the experimental soil may pose a reason why to diminish the flux of plant assimilates into the root milieu, and why to increase the production of plant biomass.

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