BIOCHAR APPLICATION INTO THE SOIL AS A MEASURE OF CARBON SEQUESTRATION - NOT ALWAYS A PLANT GROWTH IMPROVER

IRINA MIKAJLO, JAKUB ELBL, HELENA DVOŘÁČKOVÁ, JAROSLAV ZÁHORA, JAROSLAV HYNŠT
Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

Biochar is the charred by-product of biomass pyrolysis, the heating of plant-derived material in the absence of oxygen that became of a particularly high attention in an investigation field. Many researchers state that the application of biochar into the soil can be a promising measure of reliable carbon sequestration. Furthermore, it has substantial potential for improvement of soil fertility due to its unique physical, chemical, and biological properties and their interactions with plant and soil communities. However, some interactions between soil, microorganisms and plants immediately after the application of freshly prepared biochar may cause deterioration of plant growth. The main aim of the presented work is to highlight an arguable effect of a biochar amendment along with the other soil conditioners on plant-soil system. Investigation has been conducted in a special growth box- phytotron with a test plant lettuce (Lactuca sativa) as a good indicator of soil state changes. Several variants with different concentrations of biochar and mineral fertilizer have been set up. In addition, we have applied two kinds of bacterial inoculums for our research. Changes in soil microbiocoenosis, soil respiration and above-ground biomass production have been analysed.

Keywords: biochar, soil, mineral fertilizer, carbon sequestration

INTRODUCTION

Climate change challenges along with flooding, desertification and extreme weather events resulted in the need to search for new methods and technologies to mitigate global warming with its increasing concentrations of greenhouse gases in the atmosphere (Lehmann, 2006; Laird, 2008; Singh, 2010; Sohi, 2010). Biochar production for use as a soil amendment is proposed as an up-to date strategy to mitigate climate change with potential to offset 1.8 to 9.5 Pg of carbon dioxide (CO_2)-C equivalent emissions annually (Lehmann, 2006). Some specific parameters of the biochar production such as temperature, residence time, rate of temperature increase, pre-and post-processing can also affect its resulting attributes and quality, furthermore it can impact the nutrient availability to crops and the amount of stable carbon sequestered. A lot of scientists have claimed about biochar as a solution to increase soil productivity by improving both chemical and physical soil properties (Czimczik, 2002; Downie 2009; Nguyen, 2010).

Biochar is believed to be more recalcitrant in soil than the original organic feedstock. Moreover, increasing number of studies report greater C-mineralization in soils amended with biochar than in unamended soils. In addition, soil organisms are playing almost a central role in this process and create the living component of soil organic matter (Sparling, 1992; Ameloot; 2013). Early studies have claimed on the fact that activity and size of the soil microbial communities are in most cases sensitive to the variation in soil physical and chemical properties (Aciego, 2009; Yang, 2010). Soil conditioning with special bacterial inoculums can improve its microbiological state with the soil organic matter increase. Expected benefits from mycorrhizal inoculation are likely to depend on both abundance (quantity) and on efficiency (quality) of all microorganismal populations (Onguenea, 2005). On the other hand changes in fertility, tilth and structure of the soil may take years after the biochar amendment to become evident. The main aim of our research is to study changes in production of plant biomass and in some basic soil microbiological characteristics while applying biochar with inoculums amendment to improve soil properties and increase soil organic matter amount.

MATERIALS AND METHODS

The experiment has been conducted in laboratory conditions in the special growth box with an indicator plant *Lactuca sativa* during the period from December 2015 till April 2015 with constant and precise ambient conditions: 24 °C daily temperature, 20 °C night temperature, 65 % humidity with a day length of 12 h and light intensity of 380 $\mu mol.m^{-1}.s^{-1}$. During our research twenty plastic experimental containers have been filled with 1 kg of topsoil from protection zone of underground water drinking source "Brezova nad Svitavou". Soil samplings have been made in accordance with ČSN ISO 10 381-6 (ČSN - Czech Technical Standard). All soil samples have been homogenized and have been sieved through a sieve with a grid size of 10 mm. We have also separated rhizosphere and non-rhizosphere root zones with special UHELON 130 T uni mesh bags.

In our research beech wood biochar have been applied into experimental containers with soil. This type of biochar have been made in low temperatures (350 °C or 500 °C) using slow pyrolysis. Five experimental variants have been prepared to demonstrate biochar amendment effect with four repetitions in each variant:

V1 – control soil with no biochar amendment;

V2 – with a biochar dose 50 $t.ha^{-1}$ and "Bactofil" inoculum *(Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus);*

V3 – with a biochar dose 50 $t.ha^{-1}$, "Bactofil" inoculum and mineral fertilizer (DAM 390) in a concentrartion of 140 kg N per ha;

V4 – with a biochar dose 50 $t.ha^{-1}$ and "NovaFerm" inoculum *(Azospirillum spp., Azotobacter spp., Bacillus megaterium, Bacillus subtilis);*

V5 – with a biochar dose 50 $t.ha^{-1}$, "NovaFerml" inoculum and mineral fertilizer (DAM 390) in a concentrartion of 140 kg N per ha (see Figure 1).

Fig. 1 Experimental design in growth box

Determination of basal respiration

Basal respiration (BR) has been determined by soil CO_2 production measuring from incubated soil samples in serum bottles for 24 h. 15 g of field moist soil samples have been weighed into each of three 120-ml serum bottles that were sealed with butyl rubber stoppers and incubated at 25 °C. After 3 and 24 h 0.5 ml sample of the internal atmosphere in each bottle has been analyzed by gas chromatography (Agilent Technologies 7890A GC System equipped with a thermal conductivity detector). Respiration has been calculated from the CO_2 increase during the 21 h incubation period (24–3 h). At the end of measurements the total headspace volume for each replicate bottle has been determined by measuring the volume of water required to fill the bottle. The measured CO_2 amounts have been corrected for the gas solved in the liquid phase. The results are expressed per gram of dry soil and hour (Šimek, 2011).

Determination of substrate induced respiration

Substrate induced respiration (SIR) has been determined by CO_2 production measuring from incubated in serum bottles soil samples for 4 h after the addition of glucose. Field-moist soil samoles have been added to three replicate serum bottles as described in the method of basal respiration determination in previous paragraph. In addition 2 ml of a glucose solution has been added to each bottle (4 mg $C.g^{-1}$ of dry soil). Bottles have been sealed with butyl rubber stoppers and soil samples have been incubated at 25 °C. After 2 and 4 h 0.5 ml of internal atmosphere samples have been analyzed by gas chromatography (see previous paragraph). SIR has been calculated from the CO_2 increase during the 4 h incubation period (4–2 h). The bottles have been further processed as described for BR measurement. The amount of glucose amendment necessary for maximal respiratory response and linearity of CO_2 evolution during first 4 h have been both checked in pilot experiments (Šimek, 2011).

Microbiological analysis

Colony forming unit method of different bacteria type counting (CFU; dilution plate method) has been used for microbial diversity determination in soil samples according to CSN EN ISO 6887-1 (Czech/International Technical Standard – "Part 1 – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). Total number of microorganisms indicative groups including spore-forming bacteria, fungi, actinomycetes and nitrogen-fixing bacteria have been analyzed. MPA nonselective medium has been used to estimate the total number of microorganisms and spore-forming microorganisms (the latter have been heated at 85 °C for 15 minutes before the seeding on MPA). Czapek Dox agar has been used to determine the number of fungi, starch and ammonia agar for actinomycetes and Ashby agar for nitrogen-fixing bacteria estimating (Figure 2). Final results have been expressed in CFU per g^{-1}.

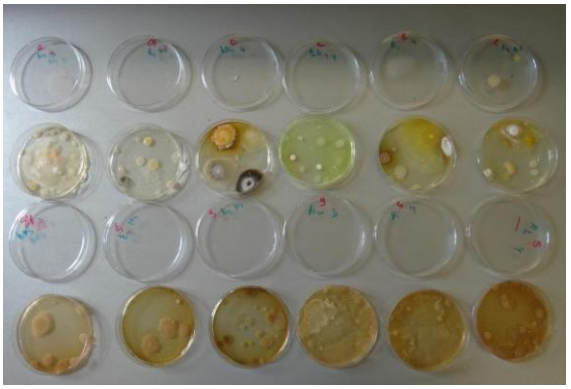

Fig. 2 Microbiological analysis on MPA nonselective medium and Czapek Dox agar

RESULTS

Plant biomass production

Obtained research results have shown that plant biomass production has increased with the application of biochar along wih DAM fertilizer in the variants V3 and V5 where the latter has the highest plant biomass production that reaches 2.328±0.61 (Figure 3).

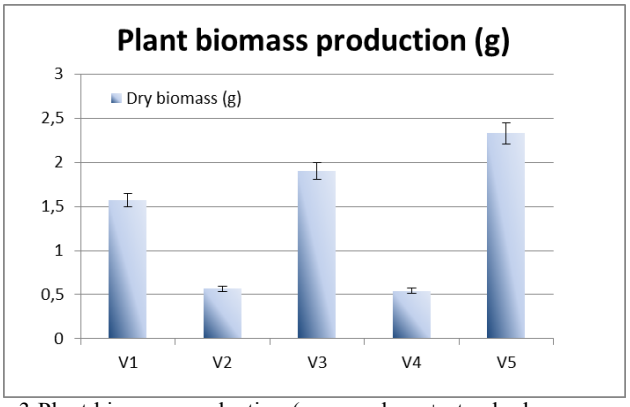

Fig. 3 Plant biomass production (mean values ± standard error; n = 4)

Inoculums amendment only have not influenced production of plant biomass positively so we may recognize a decrease in variants V2 and V4 compared to the control (1.571±0.21). We may state that soil properties improve in the case of the biochar and fertilizer application along with inoculum amendment.

Microbial activity

Soil respiration is considered to represent the overall microbial activity reflecting mineralisation of organic matter in soil (Vanhala, 2005). Referring to soil basal respiration we may claim that it is parameter of the soil biological activity which contributes mainly to aerobic respiration.

Experimental results have stated on the highest aerobic respiration with the variant V4 (biochar combined with "NovaFerm" inoculum) which have consisted 0.85 μg CO_2-$C.g^{-1}.h^{-1}$ (see Figure 4).

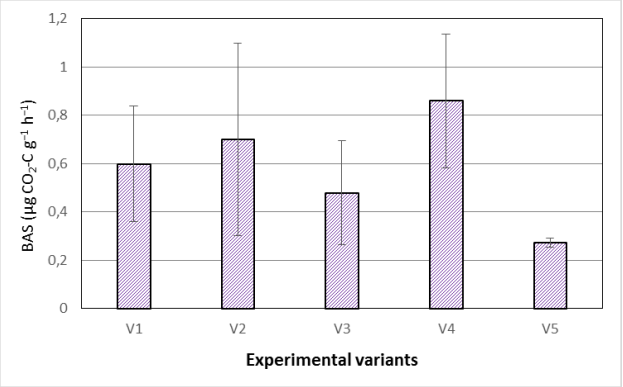

Fig. 4 Basal respiration (mean values ± standard error; n=4)

With the correlation analysis we have found that soil basal respiration data are highly correlated with quantitative characteristics of microorganisms in non-rhizosphere zone: actinomycetes up to 0.91; spore-forming bacteria index 0.84 and an average correlation with nitrogen-fixing bacteria in 0.69 (Table 1). Abundance of nitrogen-fixing bacteria in experimental variants V2 and V4 can be explained in the way that with N fertilizer additions plant may not need to rely on biological N_2 fixation as much as under N limitation (Lehmann, 2011). Great number of actinomycetes may indicate that in these variants soil has a high amount of active agents that are responsible for decomposition of organic matter.

Substrate induced respiration represents mainly microbe biomass measuring in soil. Final experiment data have stated on not so significant biochar amendment influence in microbiomass changes. Nevertheless we may observe an increse in the V5 variant combined with DAM application that is 4.25 µg CO_2-C.g^{-1}.h^{-1} (Figure 5).

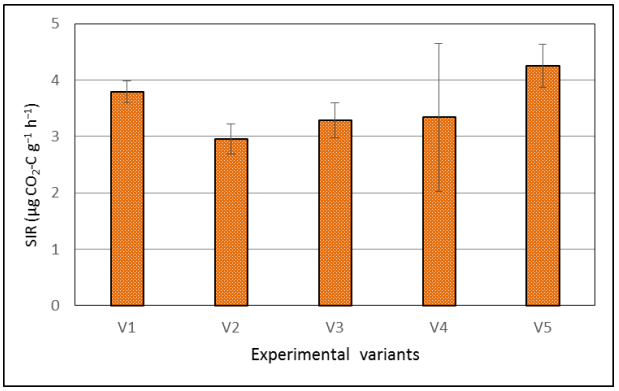

Fig. 5 Substrate induced respiration (mean values ± standard error; n=4)

Moreover correlation analysis between the quantity of total bacteria CFU and SIR and also between the quantity of fungi and SIR has been provided. Obtained results have argued on high correlation coefficients: 0.91 in the first case and up to 0.96 in the second case that indicate on strongly increased root colonization by bacteria and fungi.

Table 1 CFU quantitative changes in rhizosphere and non- rhizosphere zones (x from each variant; n = 3; ± σ)

Variants	**Colony forming units (CFU)**				
	Rhizosphere zone		**Non-rhizosphere zone**		
	Bacteria CFU (10^7) g^{-1} soil	**Fungi CFU** (10^4) g^{-1} soil	**Actinomycetes CFU** (10^5) g^{-1} soil	**Spore-forming bacteria** CFU (10^4) g^{-1} soil	**Nitrogen-fixing bacteria** CFU (10^4) g^{-1} soil
V1	19.5±1.1	13.6±1.5	17±1.6	12±0.5	68.5±4
V2	8.3±0.6	4±0.2	20±0.6	59±8	140±10
V3	14.6±0.8	5±0.2	13.5±1	18±1.2	53.5±1.2
V4	6±0.3	4.2±0.5	29±1	131±3	195±6
V5	38±1.8	17.4±1	13±0.5	10.5±1.7	101±3

CONCLUSION

The results presented in our experimental study indicate that the application of biochar can affect soil properties in positive way with other fertilizers or inoculums addition. In general we have not recognized significant soil improvement during our experiments on basal respiration, substrate induced respiration along with microorganisms colony forming units estimation. One of the possible reasons could be based in high rates of biochar amendment with too fast growing indicator plant planting within a small period of time left for the "adaptation of soil and rhizosphere organisms" in order to avoid strong impact on microorganism community. Future planned research will be aimed on the second generation of *Lactuca sativa* studying planted in the same soil but with already changed physical and chemical properties along with microbiological parameters in order to avoid extreme influence of biochar in high concentrations.

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