BIOCHAR APPLICATION INTO THE SOIL - SIMULATION OF THE LATE-PHASE EFFECT-MICROBIOLOGICAL ANALYSIS

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Abstract: Biochar is a fine-grained material produced by pyrolysis. During pyrolysis, plant cells carbonize and a chemical change occurs which increases the resistance to microbial decomposition. The application of biochar to soil brings many benefits. Among others, biochar can be used to "siphon" CO_2 from the air into stable forms in the soil, which could also contribute to carbon sequestration. In terms of agricultural management, the addition of biochar into soil increases its fertility, water and nutrient retention and accumulation of rainfall water. The improvement of the physical properties of the soil, in particular, increase in the capillary water capacity, leads to increased productivity of plant growing, higher microbial activity of the soil and greater availability of nutrients, particularly P and K. However, biochar loses its ability to directly stimulate microbial activity after remaining in the soil for a longer period of time, since the attractive substances in the biochar which accompany the pyrolysis process will have been used up by the microbes. In this later stage, biochar mainly improves the physical characteristics of soil and thus indirectly stimulates microorganisms and improves soil fertility. To simulate biochar depleted of nutrients, the experiment used activated carbon. To answer the question of how biochar which has remained in the soil for a long period of time influences the movement of nutrients and water in the soil after application and thus affects the fertility of the soil, we performed and evaluated a pot experiment. In the experiment, activated carbon was applied along with different doses of compost, and a relation was sought between the experimental variants. The study monitored mainly the activity of microorganisms, soil respiration, nitrogen availability index and colonization of roots by mycorrhizal fungi. The goal is to answer the question of the extent of stabilized biochar effectiveness on the soil-plant-microorganism system.

Key Words: activated carbon, compost, mycorrhiza, microbiological analysis, nitrogen

INTRODUCTION

Biochar is a fine-grained material similar to charcoal. It is produced by pyrolysis – heating of biomass to temperatures of 300°C to 600°C in the absence of air. During pyrolysis, a carbonization of plant cells occurs, which changes their chemical structure. The resulting material is then much more resistant to microbial decomposition. The advantage of biochar production is the freedom in the selection of starting material, which can come from a variety of sources of organic matter, including leftovers from forestry, agriculture (plant and animal remains), as well as biodegradable municipal waste. Material similar to biochar, resulting from forest fires, has always been an important component of the global carbon cycle in the soil. Since biochar is much more stable than other forms of soil carbon originating from biomass, it remains in the soil for much longer. Specifically, it is 1.5 to 2 orders of magnitue more stable than non-carbonized material and has a median lifespan of hundreds to thousands of years. The "level of saturation" of soil by carbon in the case of biochar application was significantly higher than when adding other forms of matter of organic origin. The addition of biochar can thus also be used for "siphoning" CO_2 from the air into stable forms in the soil, which could also contribute to the very welcome process of carbon sequestration (Amonette et al. 2007).

Biochar can be produced from waste materials, including those (such as green waste or dung) which can otherwise produce gases such as CH_4 or N_2O, which are even more effective greenhouse

gasses than CO_2 (Lehmann, Stephen 2009). In terms of agricultural management, the addition of biochar into the soil increases its fertility (Liang et al. 2006), retention and accumulation of rainfall water and the ability to retain agrochemicals. The improvement of the physical properties of the soil, in particular, increase in the capillary water capacity, leads to increased productivity of plant growing, higher microbial activity of the soil and greater availability of nutrients, particularly P and K. (Biedermann, Harpole 2013). Soil enriched with a significant amount of biochar has an order of magnitude greater ability to retain water in the land and eliminate the washing out of pollutants into watercourses. It is, therefore, the ideal material for biotechnical, anti-flood and anti-erosion measures.

There is, however, still the need to perform in-depth research of all the related aspects, as there are still many unknowns. For instance, there are the not quite answered questions in the area of interaction of soil microorganisms with biochar. Particularly lacking is holistic study of model situations and reactions of the entire soil-microbe-plant system to the application of biochar. Also missing are the reactions of soil biota to the application of biochar in alternative systems of agricultural management when compared to conventional agriculture. (Xu et al. 2014) state that the application of biochar accelerated the nitrification and denitrification processes and decreased the N_2O emissions, while the species diversity of communities within a single site increased dramatically. (Brennan et al. 2014) compared in their work the application of biochar with application of activated carbon. They conclude that these materials are comparable, which is why the present study uses activated carbon to simulate biochar which has been applied to the soil a long time ago.

MATERIALS AND METHODS

The delimitation of the sampling site and the reasoning for the choice is stated in (Svoboda, Záhora 2015). The article also included the yield of aboveground and underground biomass, the ratio of aboveground and underground biomass and the leakage of ammonium and nitrate nitrogen from the system.

Experiment design

A detailed description of the basis of the experiment is stated in (Svoboda, Záhora 2015). The test pots were assembled according to Table 1.

Table 1 Experiment setup

Treatment	Variant
A1	Default (soil sample)
A2	Default (soil sample + biochar)
A3	Default (soil + activated carbon)
B1	Soil + activated carbon + 50% of the recommended dose of compost
B2	Soil + activated carbon + 100% of the recommended dose of compost
B3	Soil + activated carbon + 200% of the recommended dose of compost
B4	Soil + activated carbon + 300% of the recommended dose of compost
C1	Agroperlite + 100% of the recommended dose of compost

The recommended dose for the application of compost, activated carbon and biochar used in the experiment was 50 $t.h^{-1}$. For agroperlite, a volume equivalent to the 50 $t.h^{-1}$ dose of activated carbon was used.

After the pots have been assembled, they were planted with *Lactuca sativa L.* Afterwards, the test pots were moved to a phytotron. The plants remained in the phytotron at a temperature of 20°C and humidity of 78% for 100 days with a circadian rhythm set to 16 hours of light and 8 hours of darkness. The experimental variants were irrigated throughout this period with equal amounts of water.

Mycorrhiza

After the end of the experiment, a portion of the roots was removed to determine the mycorrhiza in the individual test pots. The roots were cut into shorter segments and placed into closable glass

containers. Lactoglycerol was then poured into the containers to preserve the roots. The roots were then cleaned with water. The next step was clearing the colouration of the roots: a 10% solution of KOH was poured onto the roots, the containers were closed using aluminium foil to prevent evaporation, and the roots were left for one hour at a temperature of 90°C in a thermostat. This procedure was followed by washing on a fine sieve under running water and subsequent submersion of the root samples in 1% HCl for one hour. After draining the HCl from the samples, the roots were not washed and were dyed with 0.05% trypan blue in lactoglycerol. The samples submerged in trypan blue were again left for 1 hour in the thermostat at a temperature of 90°C. Afterwards, the roots were again washed on a sieve under running water. The individual 1.5 cm root segments were placed on a slide, poured over with gelatine and then covered with a cover slip. Each slide contained ten root segments. These sections were then studied under a microscope with a magnification of two hundred times in the following way: Each root segment was divided into ten visual fields. Each field was examined separately and the presence or absence of mycorrhiza was determined. The presence or absence was recorded. Therefore, for each sample, a hundred visual fields was studied. By adding up the occurrence of mycorrhiza, the percentage of roots colonized with mycorrhiza was determined.

Microbiological analysis

A microbiological analysis was performed for all samples, which focused on detecting the following groups of microorganisms: total amount of microorganisms (CAM) cultivated at 30°C (72 hours) on an MPA (meat peptone agar), nitrogen fixating bacteria cultivated on Ashby's agar (without nitrogen source) at 25°C (120 hours) and filamentous soil fungi cultivated on Czapek Dox Agar at 25°C (120 hours). The amounts were determined using a method of pouring a culture medium onto the inoculum. The preparation of the starting suspension and the tenfold dilution was performed according to ČSN EN ISO 6887-1. The culture medium was always poured onto a 1 ml sample at a corresponding dilution. After cultivation, the colonies on Petri dishes were added up and expressed as CFUs (colony-forming units).

Nitrogen availability index

The nitrogen availability index was determined after the completion of the experiment. A soil sample (20 g) was taken from each test pot. The soil samples were poured over with 50 ml of distilled water, creating anaerobic conditions. The samples were subsequently stored for 7 days in a thermostat at a temperature of 40°C. After 7 days, 50 ml of a 4 M solution of KCl were added to the samples. The ammonium ions were determined in the extraction solution via a distillation-titration method (Peoples et al., 1989). Distillation was performed on a Behr S3 device and titration using a Titronic 96 automatic burette.

Microbial respiration

Soil samples (10 g) taken immediately after the end of the experiment were humidified with distilled water for the determination of basal respiration. The content of CO_2 in the sample was determined as the cumulative increase in CO_2 accumulated due to microbial respiration during the incubation period. The amount of CO_2 in the container detected at the beginning of the measurement was subtracted from the value of CO_2 detected in individual samples. The respiration of soil microorganisms in individual experimental variants was thus determined. All samples were evaluated on a gas chromatograph 7890 A by Agilent Technologies USA using a TCD (thermal conductivity detector).

Statistical analysis

Potential differences in results were analysed by one-way analysis of variance (ANOVA) in combination with the post-hoc Tukey´s test. The analyses were performed using the Statistica 12 software. Microbiological analysis was performed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

The aim of the study was to determine the hypothetical compost dosage which would correspond with the exact portion of freshly applied biochar which serves as a source of carbon and energy for microorganisms and is responsible for an increase in the initial stimulation of microbial

activity in terms of nitrogen availability, microbial composition, root colonisation by micorrhiza fungi and soil respiration. In a study by (Svoboda, Záhora 2015), soil fertility, yields and nitrogen leakage from the system were the main factors monitored.

Figure 1 Graph of the mycorrhiza colonization

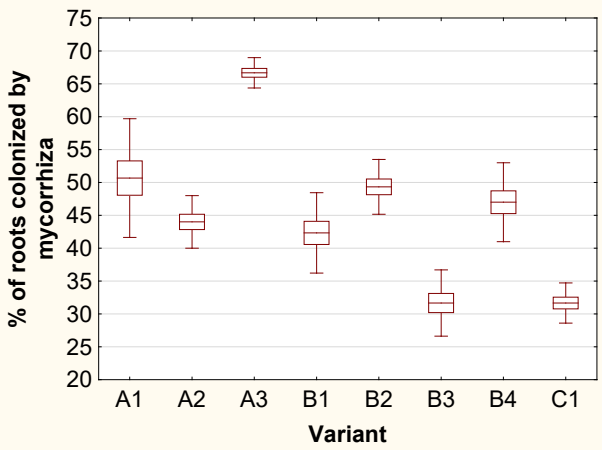


Figure 2 Graph of the nitrogen availability index

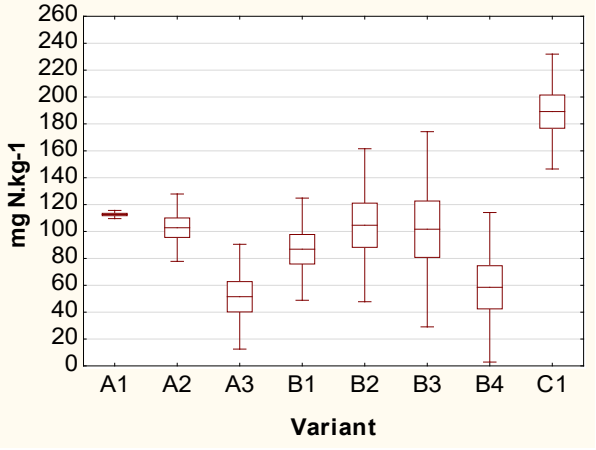


Figure 1 shows a statistically demonstrable difference especially in variant A3 when compared to the other variants. As reported for example by (Hammer 2014), arbuscular mycorrhizal (AM) fungi can use biochar as a physical growth matrix and nutrient source. However, variant A2 does not differ from the default variant in a statistically significant way. Colonization by mycorrhizal fungi in variant A3 with addition of activated carbon is statistically much higher, which can be explained by the mycorrhizal fungi using the activated carbon as a physical growth matrix, it is therefore an indirect effect. Why this was not the case in variant A2 should be the subject of further research. Variants B3 and C1 have been statistically demonstrably less colonized by mycorrhizal fungi.

The index of nitrogen availability is shown in Figure 2. The index of nitrogen availability was statistically demonstrably higher only in variant C1, which means C1 has higher potential mineralization. The biological nitrogen availability index (NAI) expresses the amount of N bound in less resistant organic bonds, which can transform into mineral structures available for plants within a few days to weeks.

Figure 3 Respiration graph

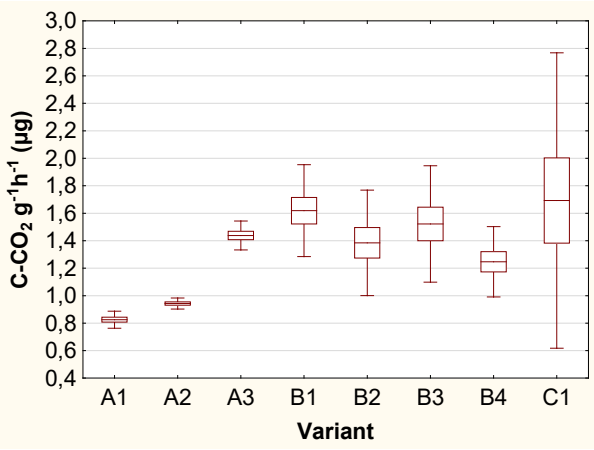
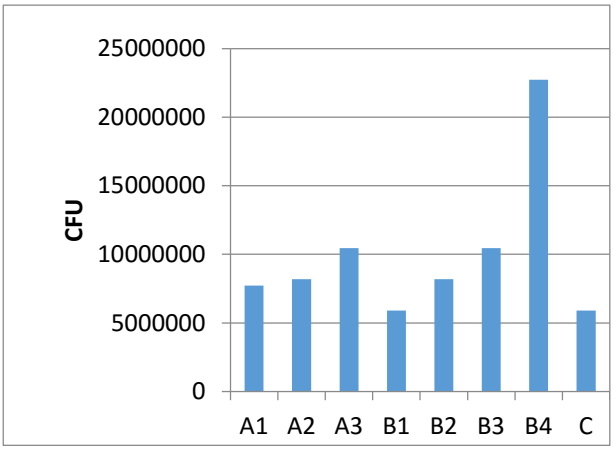


Figure 4 Total number of microorganisms



Statistically demonstrable differences were found between variants C1 and A1, A2. C1 reaches demonstrably higher values. Similar case is the comparison of B1 with A1 and A2. Other respiration values do not differ significantly from each other.

The microbiological analysis has shown a significant increase in the total number of microorganisms in variant B4 by more than 100% when compared to other variants, as shown in Figure 4. Variant B4 had more CFUs compared to other variants also in the determination of the amount of nitrogen fixating bacteria in Figure 6. Variants A2 and A3 have a significantly higher incidence of nitrogen fixating bacteria relative to other variants (with the exception of B4). Nitrogen

fixating bacteria are one of the significant microbial agents capable of using aerial nitrogen as a source for the production of organic compounds. The presence of such organisms is an important factor in soil fertility. This finding corresponds with the values measured by (Svoboda, Záhora 2015). The graph in Figure 5 shows that the greatest development of filamentous soil fungi appeared in variant A2 (after the addition of biochar). Filamentous soil fungi represent the most significant group of eukaryotic soil microorganisms, characteristic by their production of filamentous hyphas and cottony mycelia. A significant increase appears also in variant A3.

Figure 5 Filamentous soil fungi

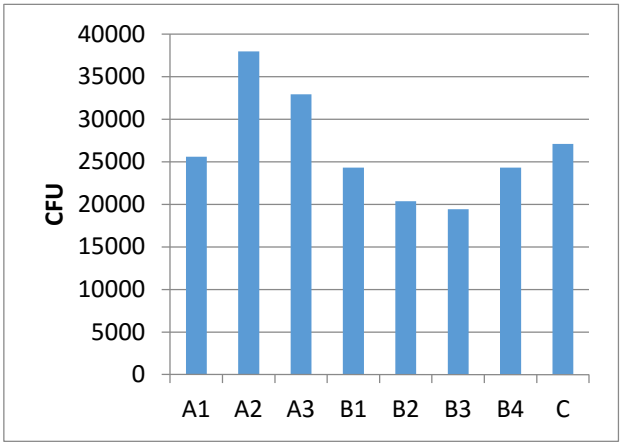
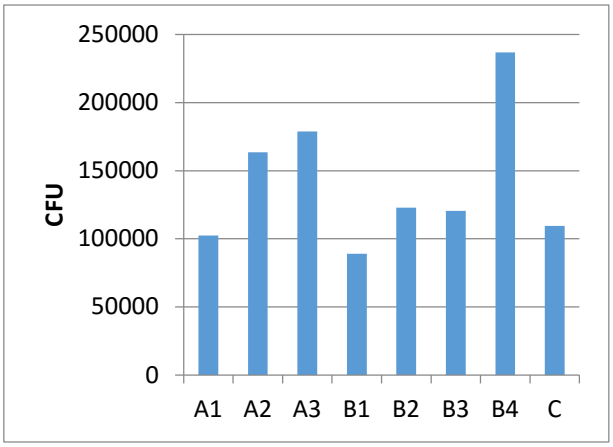


Figure 6 Nitrogen fixating bacteria



CONCLUSION

The addition of compost may not always lead to an increase in the development of microbial communities. Despite this, yield increased even after application of only a small amount of compost (Svoboda, Záhora 2015). The application of activated carbon on its own contributed to the colonization of roots by mycorrhizal fungi and the development of filamentous soil fungi. However, this was not the case when applying compost. On its own, biochar also increased the presence of filamentous soil fungi. High compost dosage increased the yield and significantly contributed to the development of microbial communities, while not increasing the leakage of mineral forms of nitrogen from the system.

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