INCREASING THE RESISTANCE OF MICROORGANISMS TO STRESS BY DROUGHT

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Abstract: Desertification is becoming a global problem and use of soil amendments is one of the options to decrease the danger. Many authors indicate that biochar can affect soil hydro-limits in a positive way. Unfortunately biochar also affects biological properties of soil. Predictions of the effect of biochar application are very difficult and must be based on aromatic substance concentration and on the dosage of biochar. In our experiment we prepared pots for establishing the effect on different substrates. Each container contained two separate parts with two different substrates. We measured the concentration of base groups of microorganisms on Lactuca sativa roots and the decomposition of cellulose. According to our results biochar can mitigate the impact of stress caused by drought on soil microorganisms. These amendments did not affect cellulose decomposition.

Key Words: biochar, winter wheat, activated carbon, CFU, desertification

INTRODUCTION

Climate changes cause loss of fertile soil all around the world. According to COM, more than 52 million ha in European Union are threatened by desertification and this number is growing. Hueso-Gonzalez et al. (2013) say using soil amendments can mitigate the impacts of climate changes that are soil erosion or desertification.

Biochar is a carbonized organic matter which can improve carbon sequestration, the stability of aggregates and soil retention as well (Besley et al. 2010). As Hardie et al. (2014) said the addition of biochar as fertilizer leads to increased water content at the permanent wilting point. On the other hand biochar is the product of pyrolysis. High temperature, high pressure and an atmosphere with low concentration of oxygen or oxygen–free are typical for this process. Unfortunately pyrolysis also used to produce toxic substances such as naphthalene or pyrene. According to Novák et al. (2009) studies these substances decrease biological activities during decades and centuries after biochar application, but eventually they decompose into non-toxic form as carboxyl acid.

The aim of our study was determinate effect of biochar or activated carbon on soil microbiota during dry season.

MATERIAL AND METHODS

Design of experiment

Experimental soil was collected in June 2015 from the area of Březová nad Svitavou, according to ČSN ISO 10 381-6 (ČSN – Czech Technical Standard). Soil samples were sieved through a 2 mm mesh. Experimental pots with two different chambers for substrates were used. These two chambers were separated by plastic iris. Between the substrate and the plastic iris was a polyamide mesh. As experimental plant Lactuca sativa was used. The plant was precultivated during a period of two weeks. After this period its roots were divided into two equal parts and put in the pot between the polyamide mesh and the plastic iris, according to figure 1. This means that in each container there was one plant, its root system divided into two equal parts and each part was placed in a different substrate. One part of the pot always included a control soil. Each container was filled with 4.2 kg of soil and two types of...
amendments were used (Table 1). Different water regime was maintained during the experiment. Thirty
percent available water capacity was maintained in containers with dry condition, and 70% available
water capacity was in containers with wet condition.

Figure 1 Sectional view of experimental pots

Legend: 1 – Experimental plant (Lactuca Sativa), 2 – Mash bag with cellulose, 3 – Control substrate (Bare soil), 4 –
Substrate with amendment, 5 – Plastic iris, 6 – IER discs

Table 1 Characteristic of variants

<table>
<thead>
<tr>
<th>Variants</th>
<th>Characteristics</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ks</td>
<td>Control – Dry condition</td>
<td>4</td>
</tr>
<tr>
<td>Km</td>
<td>Control – Wet conditions</td>
<td>4</td>
</tr>
<tr>
<td>Am</td>
<td>Active carbon (50t/ha) – Wet conditions</td>
<td>4</td>
</tr>
<tr>
<td>Am k</td>
<td>Active carbon – Wet conditions – Control</td>
<td>4</td>
</tr>
<tr>
<td>As</td>
<td>Active carbon (50t/ha) – Dry condition</td>
<td>4</td>
</tr>
<tr>
<td>As k</td>
<td>Active carbon – Dry condition – Control</td>
<td>4</td>
</tr>
<tr>
<td>Bm</td>
<td>Biochar (50t/ha) – Wet conditions</td>
<td>4</td>
</tr>
<tr>
<td>Bm k</td>
<td>Biochar – Wet conditions – Control</td>
<td>4</td>
</tr>
<tr>
<td>Bs</td>
<td>Biochar (50t/ha) – Dry condition</td>
<td>4</td>
</tr>
<tr>
<td>Bs k</td>
<td>Biochar – Dry condition – Control</td>
<td>4</td>
</tr>
</tbody>
</table>

Microbiological analysis

For estimation of the microbiological activity in roots the dilution plate method was used. Four
groups of microorganisms were analysed: the total amount of microorganisms, Actinomycetes,
Nitrogen-fixing bacteria and Fungi. The determination was carried out according to CSN EN ISO 6887–
1. The same methodology was used for choice and preparation of agars. One millilitre of the samples
was watered by agar and left in constant temperature to grow as seen in Table 2.

Table 2 Summarization of methodology for estimate basic soil microorganisms group

<table>
<thead>
<tr>
<th>Physiological groups of microorganisms</th>
<th>Agar</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of microorganisms</td>
<td>MPA nonselective medium</td>
<td>30 °C</td>
<td>72 h</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>Ammonia agar</td>
<td>30 °C</td>
<td>120 h</td>
</tr>
<tr>
<td>Nitrogen-fixing bacteria</td>
<td>Ashby agar</td>
<td>25 °C</td>
<td>120 h</td>
</tr>
<tr>
<td>Fungi</td>
<td>Czapek Dox</td>
<td>30 °C</td>
<td>120 h</td>
</tr>
</tbody>
</table>

Litter mesh bag

Known amount of cellulose was used for establishing cellulose activity during the whole
experiment. Cellulose was put into plastic bag (10 cm × 5 cm) and the bag was applied in the depth of
0–10 cm below surface. After the end of experiment the mesh bag was removed and the weight was
measured. The loss of cellulose was established by annealing. The analysis was performed based on Bocock and Gilbert (1957).

RESULTS AND DISCUSSION

Microbiological analysis

Dilution plate method was evaluated. Out results can be seen in Table 3 and Figures 2 to 6. We wanted to compare the results among different variants and results within the individual container (the two separate parts).

Table 3 Results of microbial analysis among different variants

<table>
<thead>
<tr>
<th></th>
<th>TNM – Total number of microorganism (CFU)</th>
<th>Fungi (CFU)</th>
<th>Nitrogen – fixing bacteris (CFU)</th>
<th>Actinomycetes (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km</td>
<td>670000</td>
<td>28200</td>
<td>1012</td>
<td>1400</td>
</tr>
<tr>
<td>Ks</td>
<td>421000</td>
<td>21300</td>
<td>950</td>
<td>1350</td>
</tr>
<tr>
<td>Am</td>
<td>676700</td>
<td>28400</td>
<td>12650</td>
<td>2800</td>
</tr>
<tr>
<td>As</td>
<td>590900</td>
<td>22050</td>
<td>10200</td>
<td>2195</td>
</tr>
<tr>
<td>Bm</td>
<td>422800</td>
<td>28850</td>
<td>1720</td>
<td>2270</td>
</tr>
<tr>
<td>Bs</td>
<td>1199600</td>
<td>51900</td>
<td>17450</td>
<td>4290</td>
</tr>
</tbody>
</table>

As Table 3 shows variant which was maintained in ideal water conditions has high colonization, on the other hand the variants which were stressed by drought resulted in decreased colonization. The only exception was found in the variant with biochar. Water is necessary for microorganism growth. According to many studies (Karhu et al. 2011, Laird et al. 2010, Ahmad et al. 2010) biochar can modify soil hydrolimits and because of that the stress by drought could not have effect on TNM. The highest colonization was found in the Bs variant, lowest in the Ks.

For Fungi, Nitrogen-fixing bacteria and Actinomycetes the same trend was observed. The highest production was indicated in Bs variant, the lowest in Ks variant.

Figure 2 Results in Control variants

A) TNM
B) Fungi
C) Nitrogen-fixing bacteria
D) Actinomycetes

Legend: Ks – Control (Dry condition), Km – Control (Wet conditions). Different colours means different part of containers.

For control variant, higher colonization was measured in containers with moisture treatment than in containers stressed by drought. The only exception were the Actinomycetes (Figure 2).

This group has got a different survival strategy, Actinomycetes can use water and nutrients from larger environment then the other groups.

Figure 3 Results in Variant with biochar and wet condition

A) TNM
B) Fungi
C) Nitrogen-fixing bacteria
D) Actinomycetes

Legend: Bm – Biochar (Wet conditions), Bm k – Biochar (Wet conditions – Control). Different colours mean different part of containers.
If we compare colonization in the part of container where biochar was applied and where there was maintained the ideal moisture, with the control in the same container, we can see the higher number in biochar treatment for all groups of microorganisms (Figure 3).

**Figure 4 results in Variant with biochar in condition stress by drought**

A) TNM  
B) Fungi  
C) Nitrogen-fixing bacteria  
D) Actinomycetes

Legend: Bs – Biochar (Dry condition), Bs k – Biochar (Dry condition – Control). Different colours mean different part of containers.

In variants stressed by drought where one part was treated with biochar and the other was control, our results show lower colonization for biochar in TNO and higher for Fungi, Nitrogen-fixing bacteria and for actinomycetes as well (Figure 4).

**Figure 5 results in Variant with active carbon and wet condition**

A) TNM  
B) Fungi  
C) Nitrogen-fixing bacteria  
D) Actinomycetes

Legend: Am – Active carbon (Wet conditions), Am k – Active carbon (Wet conditions – Control). Different colours mean different part of containers.

This set of samples was in ideal moisture and consists of control and the part with active carbon. The highest colonization was measured for active carbon in all groups except Actinomycetes (Figure 5).

**Figure 6 results in Variant with active carbon in condition stress by drought**

A) TNM  
B) Fungi  
C) Nitrogen-fixing bacteria  
D) Actinomycetes

Legend: As – Active carbon (Dry condition), As k – Active carbon (Dry condition – Control). Different colours mean different part of containers.

In containers stressed by drought, which contained active carbon, the same trend was observed as for the variant not stressed by drought (Figure 6).
Figure 7 Decomposition of cellulose—Litter mesh bag results

A) Decomposed cellulose – Active carbon, moist condition (g)

0.120
Am

0.125
Am

B) Decomposed cellulose – Active carbon, dry condition (g)

1.365
As

1.335
As

C) Decomposed cellulose – Biochar, moist condition (g)

Bm

0.885
Bm

0.991
Bm

D) Decomposed cellulose – Biochar, dry condition (g)

Km

1.171
Km

1.175
Ks

E) Decomposed cellulose – Control (g)

Legend: Am – Active carbon (Wet conditions), Am k – Active carbon (Wet conditions – Control), Active carbon (Dry condition), As k – Active carbon (Dry condition – Control), Bm – Biochar (Wet conditions), Bm k – Biochar (Wet conditions – Control), Bs – Biochar (Dry condition), Bs k – Biochar (Dry condition – Control), Ks – Control (Dry condition), Km – Control (Wet conditions). Different colours mean different part of containers.

Figure 7 shows, stress by drought did not significantly affect cellulose decomposition. The difference in decomposition was measured only in control variants. In the containers with ideal moisture faster decomposition was observed.

According to our results biochar or active carbon can mitigate the effect of stress by drought for most group of microorganisms. We also indicated that these amendments could affect the ability to maintain the decomposition of soil even in times of drought. Many studies found biochar or active carbon can keep hydroclimates in soil (Kammann et al. 2011). But as O’Neill and Nicholson-Cole (2009), Liang et al. (2010) indicates especially biochar is very diverse and for each experiment it is very important to know as much as possible about the character of using feedstock and pyrolysis process. Many works show that biochar should only be applied in really small amounts (1–10 t) and in this case biochar does not affect soil microbiota in a bad way (Hossain et al. 2010). In our work we used higher concentration of biochar and our results show highest colonization, especially in variants with biochar stressed by drought.

CONCLUSION

Appropriate application of biochar depends on many factors such as climate, soil character, feedstock for the biochar etc. According to our result additives such as biochar or activated carbon can promote the development of microbial communities in the period of drought. Repeating the experiment under field conditions is necessary for supporting our hypothesis.
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