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EFFECT OF STERILIZATION ON MINERALIZATION OF STRAW AND BLACK CARBON

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ABSTRACT

The study was aimed at investigating the role of microorganisms in the degradation of BC (black carbon). CO₂ evolution was measured under sterilized and non-sterilized soil using BC and straw amendments. Black carbon and straw were produced from homogenously ¹⁴C labelled roots of barley (Hordeum vulgare) with a specific activity 2.9 MBq g⁻¹C. Production of BC was implemented at 300 ºC for 24 h in a muffle oven, incubated in soil and ¹⁴C in the evolved CO₂ was measured after 0.5, 1, 2, 4, 8, 16, 26 and 40 days. BC showed much lower and slow evolution of CO₂ than the plant material which refers to high resistance of BC to microbial degradation. The difference between soil respiration in sterilized and non-sterilized soil with plant material was visible from the beginning of the experiment, unlike with BC amendments where differences only occurred after some days. In addition, the CO₂ evolution from the plant material proceeded with a lag phase while CO₂ evolution from the charcoals showed no lag phase. This indicates that microorganisms are not involved in the initial flush of carbon emitted from the BC. We suggest that an alternative source may be carbonates on the surfaces of the BC, but another abiotic source must also be present perhaps abiotic mineralization of labile BC components.

KEYWORDS:
black carbon, sterilized soil, non-sterilized soil, CO₂ evolution

1 INTRODUCTION

Black carbon (BC) may act as an important long-term carbon sink because its microbial decomposition and chemical transformation is apparently very slow [1]. BC is formed in natural and human induced fires in many regions of the world [2]. BC is intimately tied to the global carbon and oxygen cycles, has a large bearing on organic matter burial rates in aquatic environments, is both a source and sink for atmospheric carbon dioxide, and is anticipated to persist in the environment over geologic time-scales [3]. Understanding the role of BC in nutrient cycling and carbon sequestration is vital for understanding the role and minimizing the impact of agriculture on global change [4]. Although BC is often considered to be biologically inert, it is clear that it is oxidized and finally mineralized to CO₂ over long periods of time [5]. Some authors [6, 7] have pointed out, that charcoal can enhance plant growth by supplying, retaining nutrients and improving soil physical and biological properties. There are only few studies estimating process rates connected with BC inertness for biological and chemical reactions, especially oxidation [8]. Microorganisms cannot use BC as an effective energy source and as a result, charcoal does not contribute to soil biological activity or soil organic matter formation. The study of Kuzyakov [1] and Bruun et al. [9] showed direct incorporation of C from BC into microbial biomass that was very small. This indicates an extremely low microbiological availability of BC and indirectly confirms that BC will be decomposed mainly by co-metabolism and is of negligible importance as a C source for microorganisms. Shneour [10] found that over a 96 day period, 2% of artificial graphitic carbon was oxidized to carbon-14 dioxide in non-sterile soil and showed that CO₂ evolution was lower in sterilized soil than non-sterilized. Stevenson and Verburg [11] found, that the rate of CO₂ production in a calcareous and non-calcareous soil was decreased (36 % - 87 %) by different sterilization treatments. They emphasized, that sterilization had no significant effect on isotopic composition of respired CO₂ values in the non-calcareous soil and in the calcareous soil as compared to their respective non-sterilized soil.

The purpose of this research was to assess the role of microorganisms on the degradation of black carbon by testing whether CO₂ evolution is lower in sterilized or non-sterilized soil amended with ¹⁴C labelled biochar and compare it with soil amended with ¹⁴C labelled straw.

2 MATERIAL AND METHODS

To obtain high sensitivity for the method to measure CO₂ evolution, we incubated ¹⁴C labelled BC. The soil
was collected from the experimental site at Taastrup, Denmark (55° 40' 6" N, 12° 18' 14" E) from the 0-0.20 m layer. It was a sandy loam soil that contained 16.7 % clay (< 2 μm), 17.7 % silt (2-20 μm), 64.1 % sand (20-200 μm), 131 % total C and 0.14 % total N. Soil reaction in 0.01M CaCl\textsubscript{2} is 6.0. A portion of 1 kg soil was sterilized by γ-radiation at a dose of 25 kGy. The use of gamma γ-irradiation as a method of soil sterilization has been recommended over other sterilization techniques [12]. Sterility of the irradiated soil was confirmed by suspending the soil in sterilized distilled water (5 g of soil in 50 ml of water) and planting on nutrient agar. Microbial growth was not present after incubating the plates at 24 °C for 4 days.

2.1 Black carbon and straw production

Black carbon and straw was produced from homogeneously \textsuperscript{14}C labelled roots of barley (Hordeum vulgare) with a specific activity 2.9 MBq g \textsuperscript{-1}C. The straw was produced by growing barley in a closed chamber with an atmosphere enriched in \textsuperscript{14}C [13] 3.5 g of ground plant material was spread equally on a glass Petri dish and dried at 70 °C. BC was produced by placing the Petri dish in a muffle oven at 300 °C for 24 h. After cooling to room temperature in a desiccator, the weight loss was determined by weighing the material. The amounts of \textsuperscript{14}C in the charcoal and straw produced were determined via dry combustion of approximately 10 mg on a sample oxidizer (Model 307, Packard, Downers Grove, Illinois) and the \textsuperscript{14}C activity of the evolved CO\textsubscript{2} was determined using scintillation liquid (Winspectral 1414 LSC, Wallac). To estimate carbonate formation during BC and straw production, approximately 10 mg of BC and straw were mixed separately with 15 ml 0.1 M HCl in a tube with a base trap containing 2 ml 1 M NaOH for 24 h. The bases were then mixed with 8 ml scintillation liquid (Ultima Gold, Perkin Elmer) and the \textsuperscript{14}C activity of the trapped CO\textsubscript{2} was determined by counting with a scintillation counter (Winspectral 1414 LSC, Wallac). The \textsuperscript{14}C counting efficiency was about 85%.

2.2 Incubation

Incubation of straw and BC in soil and soil without amendments were carried out for sterilized soil in triplicate and for non-sterilized soil in six replicates. The 0.2 g of straw, dried at 70 °C±5 °C and the equivalent amount of BC were incubated in 50 g of soil at 25 °C±1 °C. The amount of charcoal equivalent to 0.2 g before thermal treatment produced at 300 °C was 0.082306 g. The water content was adjustable to 15 % water which is approximately 80 % of field capacity. During the incubations, the water content was kept constant by checking the weight of the tubes regularly and adding deionized water. The incubations were carried out in the closed glass jars with a base trap containing 2 ml 1M NaOH. After 0.5, 1, 2, 4, 8, 16, 26 and 40 days, the CO\textsubscript{2} trap was taken out and replaced with new NaOH. The NaOH sample was mixed with 8 ml scintillation liquid (Ultima Gold, Perkin Elmer) and counted for 10 min with the scintillation counter (Winspectral 1414 LSC, Wallac).
### TABLE 1 - Characteristics of plant material and BC used in the incubation experiments

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Production/drying temperature °C</th>
<th>Remaining mass %</th>
<th>C content %</th>
<th>N content %</th>
<th>C/N ratio</th>
<th>14C activity MBq/g</th>
<th>Fraction of 14C in carbonates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley straw</td>
<td>300</td>
<td>41</td>
<td>58.2</td>
<td>3.02</td>
<td>19.3</td>
<td>2.02</td>
<td>0.054</td>
</tr>
</tbody>
</table>

**FIGURE 1** - Fraction of added C evolved as CO₂ from barley straw (a) and from straw derived BC (b) in sterilized and non-sterilized Taastrup soil determined as the difference in ¹⁴C activity of released CO₂ from amended and unamended soils.

CO₂ evolution from Day 26 to 40 being significantly higher in the non-sterilized soil (Tukeys HSD test p=0.030). However, the lag phase was not discernable as a phase with low emissions in the beginning followed by a phase of higher emissions in the non-sterilized soil in the same way as for the straw. This was ascribed the initial flush of CO₂ evolution from the BC caused by abiotic processes, which was large compared to the small evolution of CO₂ from the slow mineralization of BC caused by microorganisms, thus obscuring the lag phase. In fact the emissions from the sterilized and non-sterilized BC were quite similar in the beginning which is also what we would expect of an abiotic process. Carbonates constituted 0.054 % of total C in the straw BC (Table 1). This could explain a significant fraction of the CO₂ emitted during the initial flush of CO₂ evolution, but some abiotic emissions from other sources in the initial phase must have occurred, most likely from mineralization of easily degradable BC components. This source of CO₂ could be abiotic oxidation of the BC surfaces [14, 15].

Our understanding of the abiotic processes leading to CO₂ evolution from BC including carbonates and abiotic mineralization is incomplete and further investigations BC is needed.

Former investigations of CO₂ evolution from BC using sterile and non-sterile soils have found higher CO₂ evolution under non-sterile conditions [10, 11]. This indicates that the processes are microbially mediated. This is in agreement with our observations that there is a lag phase before the CO₂ evolution in the non-sterilized treatment becomes greater then the sterilized. This is corroborated by the observations of the straw treatments, where there was a distinct lag phase in the non-sterilized treatment which was absent in the sterilized. The CO₂ evolution from the sterilized treatment was surprisingly high amounting to 54 % of the emissions in the non-sterilized. However this is in line with other studies that have observed substantial respiration after soils have been sterilized with γ-radiation [12, 16]. Lag phases has been observed in incubations of BC in some former studies [17-19] and not in other studies [1, 9, 20, 21]. Whether a lag phase is discernable or not probably depends very much on the time resolution of the measurements, but also on the degree to which carbonates obscure the lag phase. Hilscher et al. [18] made observations of CO₂ emissions after incubation of BC with a very high resolution in time and found two peaks in the CO₂ evolution rates one at 1-10 hours after addition and one from 20-50 hours after addition. The first peak was higher for a BC produced by heating for 4 minutes than for a BC heated for 1 minute. Therefore, it is very likely that this first peak of CO₂ evolution from the BC is derived from carbonates.

**4 CONCLUSION**

BC showed much lower and slow evolution of CO₂ than the plant material which refers to high resistance of BC to microbial degradation. The difference between soil respiration in sterilized and non-sterilized soil with plant material was visible from the beginning of the experiment, unlike with BC amendments where differences only occurred after some days. In addition, the CO₂ evolution from the plant material proceeded with a lag phase while CO₂ evolution from the charcoals showed no lag phase. This indicates that microorganisms are not involved in the initial flush of carbon emitted from the BC. We suggest that an alternative source may be carbonates on the sur-
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