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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). CO_2 evolution was measured under sterilized and non-sterilized soil using BC and straw amendments. Black carbon and straw were produced from homogenously ^{14}C labelled roots of barley (*Hordeum vulgare*) with a specific activity 2.9 MBq g $^{-1}$C. Production of BC was implemented at 300 ºC for 24 h in a muffle oven, incubated in soil and ^{14}C in the evolved CO_2 was measured after 0.5, 1, 2, 4, 8, 16, 26 and 40 days. BC showed much lower and slow evolution of CO_2 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 CO_2 evolution from the plant material proceeded with a lag phase while CO_2 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, CO_2 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 CO_2 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 CO_2 evolution was lower in sterilized soil than non-sterilized. Stevenson and Verburg [11] found, that the rate of CO_2 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 CO_2 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 CO_2 evolution is lower in sterilized or non-sterilized soil amended with ^{14}C labelled biochar and compare it with soil amended with ^{14}C labelled straw.

2 MATERIAL AND METHODS

To obtain high sensitivity for the method to measure CO_2 evolution, we incubated ^{14}C labelled BC. The soil

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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), 1,31 % total C and 0.14 % total N. Soil reaction in 0.01M $CaCl_2$ 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 homogenously ^{14}C labelled roots of barley (*Hordeum vulgare*) with a specific activity 2.9 MBq $g^{-1}C$. The straw was produced by growing barley in a closed chamber with an atmosphere enriched in ^{14}C [13]. 3.5 g of ground plant material was spread equally on a glass Petri dish and dried at 70 ºC. BC was produced by placing the Petri dish in a muffle oven at 300 ºC for 24 h. After cooling to room temperature in a desiccator, the weight loss was determined by weighing the material. The amounts of ^{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 ^{14}C activity of the evolved CO_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 ^{14}C activity of the trapped CO_2 was determined by counting with a scintillation counter (Winspectral 1414 LSC, Wallac). The ^{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 ºC±5 ºC and the equivalent amount of BC were incubated in 50 g of soil at 25 ºC±1 ºC. The amount of charcoal equivalent to 0.2 g before thermal treatment produced at 300 ºC 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_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).

2.3 Statistical analysis

The effect on sterilization from a soil amended with straw and BC was tested by two-way ANOVAs using a post-hoc Tukeys HSD test. All statistical tests were done in R version 2.9.2 (R Development Core Team, 2010).

3 RESULTS AND DISCUSSION

The fraction of added C that was evolved from straw and straw derived BC in sterilized and non-sterilized soil is shown in Fig 1. When comparing the fraction of C evolved as CO_2 in the total incubation period, there was a highly significant effect of material (ANOVA, $p=2.2\times10^{-16}$) and sterilization (ANOVA, $p=6.6\times10^{-11}$), and interaction between the two (ANOVA, $p=8.6\times10^{-11}$). In the last part of the incubation (Day 26 to 40), there was a highly significant effect of material (ANOVA, $p=7.3\times10^{-11}$) and a significant effect of sterilization (ANOVA, p=0.034), but not a significant interaction between the two (ANOVA, p= 0.051). The fraction of CO_2 evolved as CO_2 from BC (0.66 % and 0.76 % from the sterilized and non-sterilized treatments) was as expected much smaller than from straw (24.7 % and 45.4 % from the sterilized and non-sterilized treatments) demonstrating that BC is much more recalcitrant than non-charred plant material.

Sterilization had a strong effect on the amounts of CO_2 emitted from a soil amended with barley straw (Fig 1a, Tukeys HSD test $p=<1\times10^{-8}$). Usually a delay in microbial activity is observed, because the microbes have to synthesize the enzymes systems and possibly multiply before the activity can proceed. Therefore BC mineralization would also be characterized by a lag phase if the process was microbially mediated. If the CO_2 evolved derived from abiotic processes such as carbonates or chemical oxidation of BC, a lag phase would not be expected. In the non-sterilized soil amended with straw, there was a lag phase during which the evolution of CO_2 was from the non-sterilized and sterilized soil was the same. After this, there was a sharp incline in CO_2 evolution which could be explained by microbial activity. Basically the microbial community needs time in order to multiply and synthesize the enzymes needed for straw degradation and is in accordance with the CO_2 evolution from the sterilized soil where no lag phase was discernable. In the sterilized soil, CO_2 evolution from non-charred straw was substantial amounting to 25 % of the added carbon; substantially more than carbon contained in carbonates (0.046 %; Table 1). Therefore, some mechanism must be responsible for mineralization of straw in the soil which was not related to living organisms.

The effect of sterilization on CO_2 evolution from BC was much less pronounced (Fig 1b) than for non-charred straw. During the first five days, there was an initial flush of CO_2 evolution from both soils and there were a phase with no discernable differences between the sterilized and non-sterilized soil followed by a phase where more CO_2 was slowly being emitted from the non-sterilized soil with

TABLE 1 - Characteristics of plant material and BC used in the incubation experiments

Feedstock	Production/drying temperature °C	Remaining mass %	C content %	N content %	C/N ratio	^{14}C activity MBq/g	Fraction of 14C in carbonates %
Barley straw	300	41	58.2	3.02	19.3	2.02	0.054

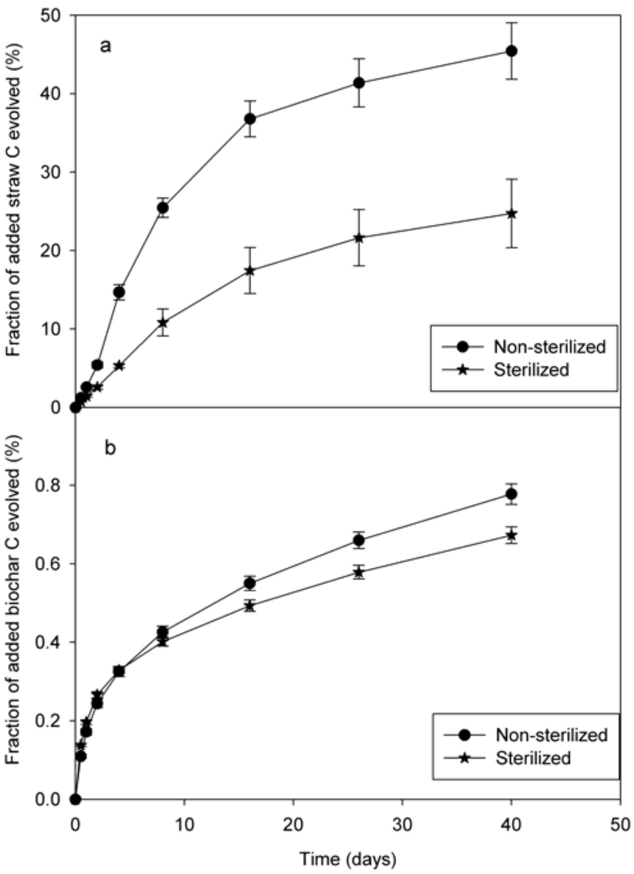

FIGURE 1 - Fraction of added C evolved as CO_2 from barley straw (a) and from straw derived BC (b) in sterilized and non-sterilized Taastrup soil determined as the difference in ^{14}C activity of released CO2 from amended and unamended soils.

CO_2 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 CO_2 evolution from the BC caused by abiotic processes, which was large compared to the small evolution of CO_2 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 CO_2 emitted during the initial flush of CO_2 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 CO_2 could be abiotic oxidation of the BC surfaces [14, 15].

Our understanding of the abiotic processes leading to CO_2 evolution from BC including carbonates and abiotic mineralization is incomplete and further investigations BC is needed.

Former investigations of CO_2 evolution from BC using sterile and non-sterile soils have found higher CO_2 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 CO_2 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 CO_2 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 CO_2 emissions after incubation of BC with a very high resolution in time and found two peaks in the CO_2 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 CO_2 evolution from the BC is derived from carbonates.

4 CONCLUSION

BC showed much lower and slow evolution of CO_2 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 CO_2 evolution from the plant material proceeded with a lag phase while CO_2 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.

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