Effect of Biochar and Earthworm on Organic Matter Mineralization in Topsoil and Deep Soil

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Abstract

In recent years, biochar has been widely used for soil remediation because of its good soil amendment efficacy, but the effect of biochar addition on mineralization of soil organic matter (SOM) is still controversial. Earthworms, as common soil macrofauna, may change the effect of biochar on soil carbon stabilization. Therefore, 0.5% (w/w) corn biochar was added to top and deep soils respectively in the presence or absence of earthworms for 43 days of incubation experiment. The CO$_2$ release rates were monitored on the 2nd, 8th, 15th, 22th, 29th, 36th, and 43th days, differences in soil respiration rates and cumulative emissions were compared between biochar, earthworm and mixed culture groups, and changes in SOM composition were measured by DOC and 3D fluorescence before and after the culture groups.

The results showed that the addition of biochar reduced the SOM mineralization, and the presence of earthworms significantly increase the soil respiration rate and soil carbon emission. Compared to deep soil, earthworms had a stronger respiration effect on top soil. In the short term, the addition of biochar stimulated the mineralization, especially in the topsoil, from earthworms. However, in the long time, the addition of biochar was beneficial to the reduction of the mineralization of SOM.

1. Introduction

Mineralization is the process of the decomposition of soil organic matter (SOM) into inorganic compounds and release of carbon dioxide under the action of soil microorganisms (Kuzyakov et al. 2000), which is a key link between soil carbon stabilization and carbon loss processes, and is important for the accumulation and stabilization of soil organic carbon (Guenet et al. 2010). Mineralization of SOM widely occurred in natural environments. For instance, when the ground surface is covered by exogenous organic matter such as leaves, decaying fruits or even burned herbs, organic matter mineralization will be enhanced because the original microorganisms at the surface will boost their metabolism (Wang et al. 2013). The effect of exogenous organic matter on soil microorganisms is an intrinsic driver of mineralization. The main mechanisms by which exogenous organic matter promotes or inhibits organic matter mineralization include: promoting synergism between microorganisms and their secreted extracellular enzymes (Geisseler et al. 2011, Li et al. 2017), promoting the use of more limited resources by microorganisms (Paterson & Sim 2013, Wang et al. 2015), and leading to selective use of decomposable substrates by microorganisms (Miao et al. 2017). Thus, SOM mineralization were closely related to quality of exogenous organic matter and microorganism activity.

Biochar is the product of thermochemical transformation of biomass under anoxic or low oxygen conditions (Wang et al. 2013). Most studies suggested that the addition of biochar can reduce carbon emission and increase SOM as well as improve soil quality effectively (Ameloot et al. 2014, Lu et al. 2014). However, some studies have found that biochar can promote carbon emissions under certain circumstances, for example, some studies have reported that biochar can promote soluble organic matter solubilization, enhance microbial activity, and promote SOM mineralization (Luo et al. 2011, Zimmerman
et al. 2011). Some studies also reported that the addition of biochar promoted higher carbon mineralization was attributed to fast utilization of a small labile component of biochar (Major et al. 2010). Therefore, several studies have pointed out that the effect of biochar on SOM mineralization is related to the unstable OM content and the natural SOM state (Purakayastha et al. 2016). However, few studies focused on the response of the biotic factor (macrofauna) to the application of biochar in carbon stabilization and remediation.

Earthworms, common soil animals, are suitable to be used widely in farms due to their combined advantages including the traits of omnivorousness, quick reproduction, a long life cycle compared to other soil animals (Angst et al. 2019). Earthworms play a critical role in soil ecology because they have influence on many aspects consisting soil properties, soil morphology, and global carbon cycle through various activities such as feeding, digestion, mucus secretion, excretion, and burrowing (Coq et al. 2007). In addition, it can also act as a microbial "reactor", interacting with microorganisms and changing the community structure of soil microorganisms (Jiang et al. 2018), which makes earthworms closely related to SOM mineralization. For example, earthworms can influence soil microorganisms communities and increase microbial biomass, promoting carbon metabolic activity and accelerating carbon mineralization (Hoang et al. 2017). Earthworms also broke up and decomposed plant litter to produce wormcast, which affects the turnover and stability of organic carbon (Garg et al. 2012, Suthar 2008). During the movement of earthworms, their epidermis secreted mucus acts as a mucilaginous agent, which accelerated formation of organo-mineral complexes (Shipitalo & Protz 1989), while earthworm mucus is also able to stimulate dormant microorganisms and accelerate SOM turnover (Lavelle et al. 1995). On the other hand, earthworms can promote the formation of macro-aggregates and micro-aggregates, thus stabilizing the organic carbon (Speratti & Whalen 2008). Therefore, the effect of earthworms on SOM mineralization remain unknown.

Earthworm abundance obviously increase with intensified organic fertilisation or conservation tillage, which amplify their importance to SOM turnover in agroecosystems (Angst et al. 2019, Lubbers et al. 2013). Biochar was widely used in soil remediation because of its good soil amendment efficacy in recent years. However, the studies on the joint effects of earthworms and biochar on SOM turnover are poorly understood. Therefore, this study focused on the effect of exogenous biochar on SOM mineralization under the presence of earthworms, in order to clarify the following problems: 1) The effect of biochar on SOM mineralization; 2) The effect of earthworms on SOM mineralization; 3) The effect of biochar on SOM mineralization in the presence of earthworms.

2. Materials And Methods

2.1 Sample collection and its processing

Corn straw, located in Kunming, Yunnan Province, was collected as a source of biochar. It was not made into biochar until being air-dried, ground and pyrolyzed at 550°C for 4 h, and then it was further ground and sieved through 100 mesh. The experimental soil was collected in Loyuhe Park next to Kunming
University of Science and Technology in Chenggong District, Kunming City, Yunnan Province (N: 24°51′38.53″, E: 102°52′1.99″), the land of which was used for rice cultivation 20 years ago and is always fertile. When we sampled, the ground surface was covered with a large amount of dead leaves and trees had a well-developed root system. A plot of land was randomly selected, with a 2×2×1 m soil pit excavated, and the sampling depths 10–20 cm and 60–80 cm from the surface, which were marked as top soil (B) and deep soil (S) respectively. After the removing of the vegetation apoplast and plant roots form the surface soil and the deep soil respectively, the two were stored in sterile sealed bags in the laboratory at -40 ºC in a freezer, and then were taken out to get air-dried and a 100-mesh sieve to measure their physicochemical properties. The earthworm used in the experiment was Aisheng earthworm Taiping No. 3, which was soaked firstly to exclude the soil from its body and then was washed.

2.2 Soil culture experiment

For this experiment, 150 g of dry soil and 0.75 g (0.5% of total weight) of biochar was added to each group of flasks in a sealed indoor culture, with the soil moisture maintained at about 30%. Eight culture groups were set up with three replicates (N = 3): top soil (B), top soil with biochar (B-BC), top soil with earthworms (B-E), top soil with biochar and earthworms (B-E-BC), deep soil (S), deep soil with biochar (S-BC), deep soil with earthworms (S-E), deep soil with biochar and earthworms (S-E-BC).

2.3 Detection of CO₂ release

CO₂ was detected by GC-MS with the following parameters: CH₄ (10.10%); CO (10.00%); CO₂ (9.93%); air pressure (101 Kpa); temperature (273 K + 20 ºC). The CO₂ was removed from the incubation vial in advance by nitrogen blowing, and the vial was subsequently sealed with a sealing film. Meanwhile, the release of CO₂ was measured with a GC detector for the corresponding period of time, with each injection of 1 ml and each measurement performed three times.

2.4 Three-dimensional fluorescence measurement

The measured TOC concentration was diluted to below 10 mg/l and measured with a Perkin Elmer LS55 instrument with the excitation wavelength (EX) range of 200–550 nm and excitation sampling interval of 10 nm, and the emission wavelength (Em) range of 200–550 nm and emission sampling interval of 10 nm. The excitation and emission slit widths were 200 nm; the scanning speed was 8000 nm-min⁻¹; the number of times was 70; and the three-dimensional fluorescence was measured at a voltage of 680 V.

According to the previous method (Gao et al. 2016, Li et al. 2019), the excitation-emission matrix was divided into five regions, I, II, III, IV and V, according to the distribution of different components of soluble organic matter in the excitation/emission (Ex/Em) wavelength region of the fluorescence spectrum. The region I (220 ~ 250 nm/280 ~ 330 nm) is a tryptophan-like protein, region III (220 ~ 250 nm/380 ~ 480 nm) is a fulvic acid-like protein, region IV (250 ~ 360 nm/280 ~ 380 nm) is a soluble microbial metabolite, and region V (250 ~ 420 nm/380 ~ 520 nm) is a huminic acid-like protein. The emission wavelength 520 nm was selected, and the excitation wavelength 300–500 nm was scanned to obtain the excitation
spectrum, and the fluorescence intensity ratio at 436 and 383 nm (I\textsubscript{436}/I\textsubscript{383}) was calculated (Provenzano et al. 2004).

## 2.5 Sample characterization experiments

The prepared biochar materials and cultured samples were characterized for their physicochemical properties, mainly including determination of pH (pHS-3, water-soil ratio 2.5: 1), Dissolved organic carbon, DOC (ELEMENTAR, Germany), elemental analysis (VARIO MAX, Elementar, Germany), Fourier Transform Infrared Spectrometer, FTIR (is10, Thermo Electron Corporation) to determine their elemental content, soil carbon content before and after incubation, and surface functional groups.

## 2.6 Data Analysis

Statistical analyses were performed using the SPSS 16.0 software package for Windows (SPSS Inc., Chicago, IL, USA). One-way analysis of variance with 5% significance level was used to detect significant differences in soil CO\textsubscript{2} emission rates, and cumulative CO\textsubscript{2} emissions. The data of pH, elemental analysis, DOC, the emission rate and cumulative emission of soil CO\textsubscript{2} were averaged for this experiment.

The relationships between cumulative soil respiration \( C\textsubscript{(d)} \): gCO\textsubscript{2} g\textsuperscript{-1} soil and days (d) and soil respiration rate \( C\textsubscript{(S)} \): g CO\textsubscript{2} g\textsuperscript{-1} soil\textsuperscript{-1} d\textsuperscript{-1} were:

\[
C\textsubscript{(d)} = 24 \times C\textsubscript{(S)} + C\textsubscript{(l)}
\]

where \( C\textsubscript{(l)} \) represents the accumulation of the previous soil respiration and the first \( C\textsubscript{(l)} \) value is 0.

## 3. Results And Analysis

### 3.1 Changes in elemental composition and DOC of the soil before and after the incubation experiment

The analysis of the elemental composition of the soil before and after the incubation are shown in Table 1, the carbon content of the topsoil was slightly higher than that of the deep soil. After the incubation, except for the addition of biochar (mainly because of the addition of biochar), their contents reduced to some extent, especially significantly in the presence of earthworms, while the N content did not decrease but even increased, which may be related to the secretion of nitrogenous compounds by microorganisms during the incubation process. Therefore, the C/N ratio after the incubation significantly decreased.
Table 1
Changes in soil element composition before and after the incubation

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C%</td>
<td>1.33</td>
<td>1.27</td>
<td>1.61</td>
<td>0.73</td>
<td>1.42</td>
<td>1.22</td>
<td>1.18</td>
<td>1.63</td>
<td>0.84</td>
<td>1.36</td>
</tr>
<tr>
<td>N%</td>
<td>0.11</td>
<td>0.09</td>
<td>0.11</td>
<td>0.12</td>
<td>0.12</td>
<td>0.07</td>
<td>0.09</td>
<td>0.09</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>C/N</td>
<td>14.11</td>
<td>16.46</td>
<td>17.08</td>
<td>7.10</td>
<td>13.81</td>
<td>20.33</td>
<td>15.30</td>
<td>21.12</td>
<td>7.54</td>
<td>12.21</td>
</tr>
</tbody>
</table>

YB Original top soil, B topsoil, B-BC Top soil with biochar, B-E top soil added with earthworms, B-E-BC Top soil added with biochar and earthworms, YS Original deep soil, S deep soil, S-BC deep soil with biochar, S-E deep soil added with earthworms, S-E-BC deep soil with biochar and earthworms

The changes of pH and DOC content of soil before and after incubation are shown in Table 2. The soil pH ranged from 6.27–7.12, which basically showed a neutral bias. The addition of biochar increased the soil pH, while the presence of earthworms decreased the pH slightly. The pH of biochar was about 10.12, which is alkaline, so its addition in small amounts increased the soil pH to some extent. Most studies indicated that the presence of earthworms can stimulate microbial population and activity, and that they themselves and microorganisms may secrete some organic acids, leading to a decrease in soil pH (Valdez-Perez et al. 2011). This result was consistent with our findings.

Additionally, DOC content in soils decreased after the incubation and showed the following trend: B/SBC > B/S > B/S-E-BC > B/S-E. Obviously, the most significant decrease was observed in the presence of earthworms alone, earthworms accelerated the decomposition of DOC through the indirect or direct stimulation of microbial activity (Egert et al. 2004, Suthar 2008). However, the degree of decrease in DOC content with the addition of biochar was the lowest. On the one hand, this phenomenon may be due to the leaching of DOC from biochar itself; On the other hand, it was possible that the adsorption of DOC by biochar inhibited the degradation of DOC. Considering that the biochar was repeatedly washed before addition and added in a smaller amount, DOC released from biochar may contribute less to its DOC in short incubation time. Therefore, it was more likely that the small reduction in DOC content with biochar addition was due to its adsorption by biochar, which inhibited its degradation and thus retained more DOC content. This result was further confirmed by the release of CO$_2$. Compared to top soils, the DOC content of deeper soils, generally with higher mineral fractions and relatively lower microbial activity, was reduced to a lesser extent after the incubation (Rukshana et al. 2013, Zhao et al. 2018). Moreover, the composition structure of DOC may further influence this phenomenon, which would be further elucidated from the 3D fluorescence map of DOC. Thus, this may led to a relatively slow decomposition of DOC in deep soils, which would be further evidenced based on the release of CO$_2$. 
### Table 2
Changes in soil DOC and pH before and after the incubation

<table>
<thead>
<tr>
<th>Name</th>
<th>TOC (mg/g)</th>
<th>pH</th>
<th>Number of surviving earthworms (only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YB</td>
<td>0.2256</td>
<td>6.83</td>
<td>/</td>
</tr>
<tr>
<td>B</td>
<td>0.1551</td>
<td>6.98</td>
<td>/</td>
</tr>
<tr>
<td>B-BC</td>
<td>0.1724</td>
<td>7.12</td>
<td>/</td>
</tr>
<tr>
<td>B-E</td>
<td>0.1221</td>
<td>6.56</td>
<td>7</td>
</tr>
<tr>
<td>B-E-BC</td>
<td>0.1557</td>
<td>6.50</td>
<td>8</td>
</tr>
<tr>
<td>YS</td>
<td>0.2017</td>
<td>6.96</td>
<td>/</td>
</tr>
<tr>
<td>S</td>
<td>0.1594</td>
<td>6.79</td>
<td>/</td>
</tr>
<tr>
<td>S-BC</td>
<td>0.1737</td>
<td>7.11</td>
<td>/</td>
</tr>
<tr>
<td>S-E</td>
<td>0.1413</td>
<td>6.50</td>
<td>6</td>
</tr>
<tr>
<td>S-E-BC</td>
<td>0.1474</td>
<td>6.27</td>
<td>7</td>
</tr>
</tbody>
</table>


### 3.2 Analysis of three-dimensional fluorometry before and after the incubation experiment

To further investigate the difference in DOC composition, this study analyzed its composition with three-dimensional fluorescence spectroscopy (Fig. 1). Before the incubation, both DOC in top and deep soils were mainly dominated by tyrosine and tryptophan. Among them, the response degree of tyrosine and tryptophan was greater in the top soil. In general, the tyrosine and tryptophan are regarded as easily decomposed components (Tremblay et al. 2007). Thus, the top soil had more easily decomposed substances compared to the deep soil. In contrast, the deep soil DOC had a certain response at 420 nm, which belonged to the fulvic acid region, and its large molecular weight was more difficult to degrade compared with the easily degradable tyrosine and tryptophan, further indicating that the composition of DOC in the deep soil was more difficult to degrade than that in the top soil.

After the incubation, the DOC compositions of top and deep soils were mainly dominated by fulvic acids, which indicated that tyrosine and tryptophan were depleted during the incubation. Notably, the response intensity of the fulvic acid region of the topsoil DOC was higher than that of the deep soil (about three times higher than that of the deep soil), indicating that the top soil contained more fulvic acid fractions after the incubation. Specifically, it may be because the topsoil SOM was more easily decomposed into soluble fulvic acid by microorganisms. Furthermore, the fulvic acid response was more pronounced in the
presence of earthworms, and the fulvic acid fraction of topsoil DOC was more significantly increased (about 5 times more than that of the deep soil) than that of the deep soil. This result further demonstrated that earthworms can promote the decomposition of SOM and conversion to soluble DOC fractions. In contrast, after the addition of biochar, the tryptophan, tyrosine and fulvic acid remained have a strong response in soil DOC. Combined with the earlier results showing that DOC content decreased less after biochar addition, this result could further indicate that the addition of biochar inhibited the decomposition of easily degradable fractions.

3.3 Effect of Earthworms/Biochar on Mineralization of SOM

3.3.1 CO$_2$ release rate

According to the changes in soil respiration rate, it is clear that the soil CO$_2$ respiration rate shows a decreasing trend with increasing incubation time, which is mainly for the rate of CO$_2$ emission gradually decreases with time due to the decrease in microbial activity resulting from the reduction of food sources and the absence of the addition of foreign organic matter. There was a decrease in soil respiration rate after the addition of biochar, most DOC of which had been removed before the application, proving that SOM was absorbed and protected by the biochar, as was supported by the previous fluorometric measurements. In addition, as can be seen from Table 4, the respiration rate of the topsoil was slightly higher than that of the deeper soil after the addition of biochar. On the one hand, it is perhaps because the soil microorganisms decompose less organic matter in order to maintain a C/N ratio suitable for their survival under the circumstances that the C/N is greater than 20 with more pronounced nitrogen fixation (Sigua et al. 2014). On the other hand, it is clear from fluorescence that deep soils have a lower content of readily decomposable organic matter.

Regarding the CO$_2$ respiration rate, it was much higher in the presence of earthworms than that of the earthworm-free treatment group. Particularly at the beginning of the incubation, the CO$_2$ respiration rate could reach 100 g·g$^{-1}$·d$^{-1}$. On the one hand, the earthworm itself has a certain respiratory effect to emit CO$_2$ (Binet et al. 1998); On the other hand, the addition of earthworms increased the number and activity of microorganisms in the culture group, resulting in the mineralization of most of the organic matter in the soil (Hoang et al. 2017). Moreover, considering the significant decrease of easily degradable DOC after the addition of earthworms as earlier reported, thus the addition of earthworms accelerated the mineralization of SOM especially for easily degradable DOC. It is worth noting that during the experimental period, the topsoil had a higher respiration rate than the deeper soil, which may be that the higher organic matter content of the topsoil and the higher number of microbial populations (Zhao et al. 2018) facilitate the induction of a dominant community by earthworm (Barois & Lavelle 1986) as well as the acceleration of SOM mineralization, while the deeper SOM is more stable and more difficult to be utilized. In contrast, it can be seen that the addition of biochar can inhibit the rate of earthworm CO$_2$ release as the incubation time increases, mainly thanks to the action of the biochar as an agglomerate in the soil (Wang et al. 2017). In despite of the disruption of the formation of agglomerates from earthworm
activity, it was observed that earthworm activity decreased with incubation time as the experiment proceeded, causing that the mineralization of the experimental group where earthworms were co-cultured with biochar was reduced relative to the experimental group where only earthworms were added.

### 3.3.2 Cumulative release of CO$_2$

The cumulative release of CO$_2$ after earthworms’ treatment was significantly higher than that without earthworms’ treatment, which was consistent with the release rate of CO$_2$. In addition, in earthworm-free treatment, the addition of biochar did not change the cumulative release of soil CO$_2$. However, under earthworms’ treatment, the addition of biochar had lower cumulative release of soil CO$_2$ than earthworm activity alone and was more pronounced with increasing incubation time. Most studies suggested that biochar had insignificant effects on soil carbon sequestration in the short term or may even have positive priming effects (Major et al. 2010). However, our study found that biochar could inhibit the release of CO$_2$ in the short term in the presence of earthworms, especially for deeper soils with lower organic matter content. For one thing, it may be because earthworms would promote the interaction between biochar and SOM, thus avoiding the further decomposition of SOM by adsorption and even formation of agglomerates, which complied with the composition of three-dimensional fluorescent DOC; For another, biochar may also have some toxicity to earthworms (Sohi et al. 2010), and in this experiment, it was found that earthworms had some tendency to avoid biochar at the beginning, and the earthworm activity was weaker in the group of earthworms with biochar added, which may also contribute to the reduction of cumulative soil respiration.

### 4. Conclusion

In summary, the soil supplemented with biochar in our study retained more DOC and readily decomposable organic matter after 43 days of incubation, demonstrating the role of biochar in adsorbing and protecting SOM during the incubation process. The highest fulvic acid response and the highest cumulative CO$_2$ emissions of the soil after earthworm culture indicated that the addition of earthworms significantly enhanced the SOM mineralization. Notably, the soil respiration rate of the earthworm culture group after the addition of biochar decreased with time and there was a partial tryptophan and tyrosine response at the end of the experiment, indicating that biochar reduced the mineralization caused by earthworms protecting the easily decomposable organic matter in the soil. In addition, the response of the results of the topsoil in this experiment was more pronounced than that of the deep soil experiment. This study investigated the effects of earthworms and biochar addition on SOM mineralization and demonstrated the important role of biochar in soil carbon sequestration. In the future, the dynamic effects of biochar addition in the presence of earthworms on SOC, including changes in the number and composition of soil aggregates and changes in soil microorganisms, need to be clarified.

**Declarations**
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Data Availability All relevant data are within the manuscript and available from the corresponding author upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate All authors were participated in this work

Consent to publish All authors agree to publish.

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Figures
Figure 1

Three-dimensional fluorescence map after incubation experiment.

YB=Original top soil, B=top soil, B-BC=Top soil with biochar, B-E=top soil added with earthworms, B-E-BC=Top soil added with biochar and earthworms, YS=Original deep soil, S=deep soil, S-BC=deep soil with biochar, S-E=deep soil added with earthworms, S-E-BC=deep soil with biochar and earthworms.
Figure 2

Histogram of soil respiration rate and cumulative soil carbon emissions in the experimental group of biochar addition culture in the presence of earthworms.

Significance analysis compares the results of each test for different incubation groups. YB=Original top soil, B=top soil, B-BC=top soil with biochar, B-E=top soil added with earthworms, B-E-BC=Top soil added with biochar and earthworms, YS=Original deep soil, S=deep soil, S-BC=deep soil with biochar, S-E=deep soil added with earthworms, S-E-BC=deep soil with biochar and earthworms. Different letters indicate significant differences (P<0.05) in the single CO₂ emission rates of different incubation groups.

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