Research Article

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Soil organic carbon mineralization in relation to microbial dynamics in subtropical red soils dominated by differently sized aggregates

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Abstract: The dynamics of eroded and retained soil organic carbon (SOC) may provide critical clues for evaluating impacts of soil erosion on global carbon cycling. Distribution patterns of soil aggregates in eroded and deposited environments are shaped by selective transport of water erosion. Therefore, detecting the pattern of SOC mineralization in soils dominated by aggregates of different sizes is essential to accurately explore the dynamics of eroded and retained SOC in eroded and deposited environments. In the present study, the characteristics of SOC mineralization and its relationship to microbial dynamics in subtropical red soils dominated by different sizes of soil aggregates were investigated. The results demonstrated that the SOC mineralization rate of soils dominated by graded aggregates were significantly different, indicating that

SOC mineralization in eroded and deposited environments are shaped by selective transport of water erosion. The highest mineralization rate was found in soils containing 1–2 mm aggregates at the initial stage of the experiment, and the daily average mineralization rate of the < 0.5 mm aggregates was significantly higher than that of the 2–3 mm aggregates. During the incubation, fungal communities exhibited a low dynamic character, whereas the composition of bacterial communities in all treatments changed significantly and had obvious differences relative to each other. Bacterial species diversities and relative abundances in the <0.5mm and the 2–3mm aggregates showed opposite dynamic characteristics. However, there were no statistical interactions between the dynamics of microbial communities and the changes of SOC or soil water content. Changes in bacterial community structure had no significant impact on the mineralization of SOC, which might be related to the quality of SOC or the specific utilization of carbon sources by different functional groups of microorganisms. Mineralization of the eroded and retained SOC with specific qualities in relation to their functional microorganisms should be further explored in the future.

Keywords: Water erosion; Soil organic carbon; Mineralization; Bacteria; Fungi.

1 Introduction

Observed and projected increases in greenhouse gas emissions and their associated effects on global warming and rising of sea levels have heightened interest in identifying mitigation options [1]. The soil organic carbon (SOC) pool is considered a potential major factor driving global climate change because it contains twice the amount of carbon as does the atmospheric pool [2]. Even a relatively slight variation in soil carbon content

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because of changes in land use, management practices, or natural disturbances may result in a significant net exchange of carbon between the soil carbon reservoir and the atmosphere [3]. As the most widespread form of soil degradation and the major inducer of SOC dynamics across terrestrial landscapes [2], soil erosion has significant implications for atmospheric dioxide (CO_2) and climate warming [4]. Thus, intensive discussions on evaluating the direction and magnitude of an erosion-induced change in the global carbon balance have been conducted [3]. However, much debate still exists concerning whether soil erosion is responsible for the increases or decreases in atmospheric carbon [5-6]. One of the major uncertainties related to this debate is the fate of eroded and retained SOC. Elucidating the mechanism explaining how these SOC are degraded to CO_2 is critical to improving our knowledge of the role of soil erosion in global carbon cycling.

In fact, the fate of eroded and retained SOC is closely related to their erosional or depositional environments. Whereas erosion and deposition result in redistribution of sediment and SOC (lateral flux) on the landscape scale, SOC is affected differently within each phase while moving through the landscape, which can cause changes in carbon mineralization [7-8]. The selective transport of soil by water erosion plays an important role in creating erosional and depositional features [9-10]. Studies [11-13] have shown that due to selective migration via erosion, fine particles (e.g. primary fine particles and micro-aggregates) are transported first, while large ones (e.g. sand gravel and macro-aggregates) are left in situ, resulting in erosion and depositional environments dominated by different soil textures. In eroded environment, as large particles are left in situ, the soil porosity becomes larger, water holding capacity turns weaker and the permeability gets stronger. On-site mineralization might increase due to enlarged exposure resulting in changed oxygen availability, temperature and moisture conditions [8]. On the contrary, the water holding capacity of the soil in depositional environments is enhanced by the enrichment of fine particles, which reduce the permeability and some other favorable physicochemical parameters of the soil for carbon mineralization. Finally, the biodiversity of the erosional and depositional environments might be changed [14] and environmental conditions set the stage on which all other interactions among SOC, microorganisms and soil minerals take place. Studies [15-18] have shown that soil humidity, aeration and temperature are key variables determining microbial activity and hence SOC mineralization. Therefore, it could be concluded that

the considerable difference of carbon mineralization pattern in eroded/deposited environments is essentially related to the selective migration of soil during water erosion. To detect the pattern of SOC mineralization in soils dominated by different sizes of soil aggregates, it is essential to accurately explore CO_2 emission mechanisms in erosion and sedimentary areas.

Due to the difficulty of field observation, previous studies on erosion-induced SOC mineralization could not provide direct evidence regarding whether the eroded and retained SOC act as sinks or sources of atmospheric carbon. Based on the fact that the distribution patterns of soil aggregates in eroded and deposited environments are shaped by selective transport during water erosion, the present research investigated the characteristics of SOC mineralization and its relationship to microbial dynamics in subtropical red soils dominated by different sizes of soil aggregates. The objectives of this study were to (i) detect the SOC mineralization characteristics in soils dominated by different size of soil aggregates; (ii) investigate the dynamics of microbial community structure in the above soil systems; and (iii) explore the interaction mechanism of microorganism and SOC mineralization shaped by soil erosional and depositional patterns. The results of this research will lead to new insights toward a better understanding of the fate of eroded and retained SOC in global carbon cycling.

2 Materials and methods

2.1 Experimental Materials

Samples of soft, fertile soil were taken from a community vegetable land in Changsha city of Hunan Province in China (**Figure 1**). The soil in this area is classified as Quaternary red soil, which is heavily weathered and has inherently low SOC. After a simple mechanical crushing procedure, the acquired soil samples were separated by a dry-sieving method into five fractions: <0.5 mm, 0.5-1 mm, 1-2 mm, 2-3 mm, and >3 mm. All soil samples were naturally dried at room temperature and preserved at -20°C . The soil microbial sources in all treatments were inoculated with fresh soil samples that were collected at the same location within one day before the experiment. After mechanical crushing and removing of large debris with a 2 mm sieve, the sieved samples were preserved as the inoculation soil. In order to exclude the influence of non-controlling parameters of the soil microbial growth, organic cabbage

Figure 1: Location of the study area and sampling sites.

was provided as a nitrogen source. The cabbage leaves were chopped into pieces of 2-3 mm in diameter and then stored at 4°C.

2.2 Experimental design

Three types of soil aggregates with the sizes of <0.5 mm, 1-2 mm, and >2 mm were selected as materials that would correspond to three groups of experimental treatments. For each experimental treatment, 90 g soil aggregates of a specific size, 30 g organic nitrogen source (cabbage pieces), and 30 g inoculation soil were placed into a 500 ml beaker and mixed well. The moisture content of each mixture was adjusted to 60% [19], which was in the range (50%-70%) for suitable microbial growth and activity [20]. To prevent leakage, the beakers containing the initial samples were immediately sealed with two-layer cling wrap, and loosely tied with elastic to leave enough expansion space for the subsequent gas production. Each group of treatments was repeated 3 times. Therefore, each group of the treatments

contained 12 sets of sample reaction systems (12 beakers) (**Figure 2a**). Due to the 12 beakers containing the same proportion of the substances in the same group of treatments, the initial conditions of the incubation were identical. The sealed beakers were placed in an incubator with a constant temperature of 25°C, and the incubation time was determined by the actual sampling time.

2.3 Sample collection

During the 14 days of the experiment, samples were taken at four times. The first sampling was obtained before the incubation, and the next three samples were taken from the incubator on the 4th, 8th, and 14th days after the start of the incubation (**Figure 2b**). The observation period of the experiment was determined by the results of gas chromatography. When the test result showed that the oxygen (O_2) in the beaker was depleted, the sampling procedure would cease. In the sampling procedure, two duplicate samples were removed simultaneously.

Figure 2: Incubation of different size aggregates (a) and sampling scheme (b) .

The sample was then frozen at -16°C to stop the normal microbial activity, in preparation for subsequent determination of the soil physicochemical parameters.

2.4 Sample analysis

A gas chromatograph (Clarus 500, USA, P.E.) with a thermal conductivity detector (TCD) was used to determine the CO₂ content immediately after the above sampling. Before the gas extraction, it was necessary to shake the beaker gently until the gas inside was well mixed. An equal amount of the sampled gas was then extracted directly through the cling wrap by a 10 μL autosampler syringe and injected into the gas chromatograph. A conventional packed column (HAYESEP DB100/120, 30'×1/8') was used for gas chromatographic analysis under the following conditions: temperature of injector +150°C; temperature of detector +180°C; hydrogen as a carrier gas; flow of the carrier gas 2 ml/min; the temperature environments of chromatographic analysis were as

follows: an initial temperature of the column of 34°C and a heating rate of the column of 30°C/min up to 150°C; the bridge current of TCD maintained at 3 milli-amperes; and sample volume 10 μl.

SOC was determined using the dichromate oxidation method of **Walkley and Black** [21]. Soil moisture was determined by vacuum freeze drying of soil samples at -60°C and 6 Pa for 36 h. Total DNA extraction, polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis for detection of microbial communities were conducted using the method described by **Huang et al.** [22] and **Nakasaki et al.** [23].

2.5 Data processing and analysis

According to the external standard method used in the quantitative analysis of gas chromatography [24], the peak area in the chromatogram corresponding to a gas component in the mixture has a linear relationship with its actual content. The linear relationship can be estimated by

the standard curve fitting method based on determination of the external standards of known components. If equal quantities of a gas mixture are injected every time, the trend of the peak area variation of one gas component in the chromatogram is equivalent to the content variation of this component in the gas mixture, indicating a positive correlation. Our study focused only on the variation of the cumulative CO_2 production without any quantitative analysis. Therefore, evaluation of the peak area of CO_2 in the chromatogram was feasible for determination of the variation trend of the cumulative CO_2 production, instead of the CO_2 content.

The software SPSS18.0 (SPSS, Chicago, IL) was used to carry out single factor analysis of variance (ANOVA) based on parameters such as moisture content, SOC, and the cumulative CO_2 production in the different treatments, and the analysis determined whether these parameters in the different treatments or at different times within the same treatment revealed a significant difference ($P < 0.05$) [25-26]. The software Quantity One was applied to quantitatively analyze DGGE profiles and to produce the microbial community structure matrix. The environmental factor matrix, composing of SOC, moisture content, and the cumulative CO_2 production, was also used to calculate the Shannon diversity index of microbial communities in each sample. The redundancy analysis (RDA) [27], based on the software Canoco, was used to explore the interaction mechanism between the variation of microbial community structure and three parameters including SOC, moisture content, and CO_2 production. A manual selection procedure in the RDA was used to determine which of the environmental factors had significant interactions with microbial community structure. Monte Carlo reduced model tests with 499 unrestricted permutations were used to evaluate the significance of the first canonical axis and of all canonical axes. All of the analyses were conducted at the $P < 0.05$ level.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Variation of SOC and moisture content

Previous studies suggested that among soil physicochemical parameters, temperature and moisture content were two of the most important environmental factors that affect microbes and the process of carbon mineralization [3,24]. Since all of the samples in

Figure 3: Variation of soil organic carbon (a) and moisture content (b).

this experiment were incubated at 25°C, the effect of temperature in the different treatments could be ignored. Therefore, this study focused on the observation and analysis of variation of SOC and moisture content.

Although the content of SOC in the initial state decreased with increasing aggregate size, most of the SOC content revealed no significant differences among the different treatments at the same time in the subsequent incubation processes (Figure 3a). The SOC content over time in the <0.5 mm and the 1-2 mm soil aggregates showed a decrease trend as time progressed. On the other hand, the SOC content in the treatment with the aggregates of 2-3 mm increased significantly at the end of the incubation and was even higher than that of aggregates of < 0.5 mm. The variation of SOC content in the different treatments correlated with its output and supplement mechanism, where the output mainly indicated organic carbon mineralization and the supplement correlated

with the decay rate of the external organic matter in this experiment. The difference of the carbon mineralization rate and level in samples with different size aggregates would inevitably result in the differences in consumption of stored SOC, and thus affect the organic carbon content in soil. In this experiment, cabbage leaf pieces were supplied as the nitrogen source, but the cabbage pieces as organic matter would also affect the SOC content, and the degree of influence closely correlated with the decay rate and level under the microbial activity. It was observed that the degradation rate of the cabbage leaves was much slower in the treatment with smaller size aggregates, which might be an important reason for the difference of carbon content between soil and air in the different treatments.

The initial moisture content in all treatments with different size aggregates was adjusted to a constant 60%, and the moisture content in the incubation is higher than the initial state (**Figure 3b**). However, there was no significant difference of moisture content among the different treatments. This phenomenon might result from two factors: (1) The capacity difference among the different size aggregates to retain water, because the large size aggregate will produce high porosity, which allows the water to evaporate easily. It was observed that more moisture condensed on the beaker wall and cling wrap surface in the treatments with large aggregates; (2) The supplementary mechanism of SOC, because the external organic matter was rich in moisture, and its degradation could supply moisture content in soil. Therefore, the decay rate of the external organic matter was closely correlated with the moisture supplement in soil.

3.2 Variation of microbial communities

Prior to the formal DGGE testing for the presence of bacteria and fungi in the samples, purified total DNA was first amplified by PCR based on the specific primers in order to produce the material for subsequent DGGE analysis. Target fragments were obtained for the amplification of bacterial DNA (**Figure 4a**). The amplification of fungi did not achieve results similar to those in the bacterial amplification (**Figure 4b**), and failed to get a single bright band. These results show that the number and species of fungi in the samples are very small. This phenomenon may be related to the soil source.

The original soil samples used in this experiment were acquired from long-term fertile vegetable land, and the soil organic matter content was higher than the general type of soil. It was found that the soil basal respiration

Figure 4. Agarose gel electrophoresis of PCR products based on the specific primers for bacteria (a) and fungi (b), respectively.

and the quantity of bacteria increased as the organic matter content increased, while the number of fungi in soil decreased as the organic matter content increased. The higher the organic matter content, the lower the number of fungi [28]. Target fragments of fungi in all samples during the incubation were not present according to the electrophoresis diagram of the fungal PCR products after amplification. It was also noted that the activity and function of fungi were limited in all treatments of this experiment. Therefore, this study only conducted a DGGE analysis on the variation of bacterial community structure that had a short-term and direct effect on carbon mineralization.

The DGGE profiles of bacterial community structure are shown in **Figure 5**. According to the quantitative analysis of the DGGE profiles using the software QuantityOne 2.0, ten bacterial bands were present in twelve samples from three sets of the treatments at four observation times. In the initial state, the number and brightness of bands indicated that the diversity and relative abundance of bacterial species in different size aggregates were significantly different. When the size increased, the number and brightness of bands increased, and the species and relative abundance of bacterial became much richer. This phenomenon might result from two factors: (1) The distributions of bacteria could be different in different

Figure 5: DGGE profiles of amplified 16S rDNA fragments (bacteria) from different size aggregates during the incubation. Experiment treatments are designated by 1, 2 and 3 before the hyphen“-” for <0.5mm, 1-2mm, and 2-3mm soil aggregates, respectively. The numbers following the hyphen“-” refer to the sampling days.

Figure 6: The cumulative CO₂-evolution of different size aggregates during the incubation.

size aggregates [29]; (2) The soil aggregates with different sizes had different abilities to protect the bacteria living under long-term drying stress [30].

During the incubation, the distribution and variation of the bacterial bands in the samples from the different treatments revealed obvious differences. In the treatments with the soil aggregate size of < 0.5 mm, the number and brightness of the bacterial bands increased significantly, and the whole community structure showed a gradually active feature that continued until the end of the experiment. In the treatments with the soil aggregate size of 1-2 mm, the number and brightness of the bacterial bands increased significantly during the early phase of incubation, but then decreased gradually.

In the treatments with the soil aggregate size of 2-3 mm, the number and brightness of the bacterial bands always revealed a continuous downward trend during the incubation period and almost disappeared at the end of period. The results indicated that the microbial ecological patterns in different size aggregates showed a significantly different response to the wetting. Therefore, it can be further deduced that the soil microbial community will reveal the different response characteristics under the environmental disturbance, especially in the various surface soil systems dominated by the different aggregates that were caused by the selective migration of hydraulic erosion. Finally, it would result in the different patterns of organic carbon mineralization in these surface soil systems.

3.3 Organic carbon mineralization characteristics of in different size Aggregates

There are significantly different patterns of carbon mineralization in the soil systems with the different size aggregates (**Figure 6**). Within the first 4 days of the incubation, the CO₂ contents in the soil aggregates of < 0.5 mm and 1-2 mm significantly increased by 39 times ($P>0.05$) and 25 times ($P>0.05$), respectively, but there was no significant difference between them ($P>0.05$). Meanwhile, the CO₂ content in the soil aggregates of 2-3 mm did not significantly change during the same period, and showed relatively slow response characteristics.

During the second four days (4th-8th days) of the incubation, the CO₂ content in the soil aggregates of < 0.5 mm increased continuously and was 79.6% more than that on the 4th day; the CO₂ content in the soil aggregates of 1-2 mm remained almost constant during the same period, while the CO₂ content in the soil aggregates of 2-3 mm increased significantly. At the end of incubation, there were significant differences of the CO₂ content among the three groups of treatments, and the CO₂ content decreased with the increased aggregate size. Considering that the different groups of treatments had different carbon mineralization rates during the whole incubation, the average mineralization rate of CO₂ per day first revealed a decrease, followed by an increasing trend for two groups of treatments with the aggregate size of < 0.5 mm and 1-2 mm. Overall, the group of treatments with the aggregate size of 1-2 mm had the highest carbon mineralization rate among three groups of treatments excluding the period of the 4th-8th days. In addition, the release rate of CO₂

rose steadily but was still relatively low during the whole incubation in the treatments with the aggregate size of 2-3 mm.

Although the release characteristics of CO_2 in the different treatments had different response rates and levels after the soil wetting, the CO_2 levels in three groups of treatments all increased significantly during the whole incubation period of 14 days. It has been proposed that carbon mineralization rates are significantly different in samples with differently sized aggregates, and that aggregates of 1-2 mm correlate with the highest rates [31], and our study is consistent with these results. However, we noted that the organic carbon average mineralization rate per day of the small-aggregate (<0.5 mm) was higher than the large-aggregate (2-3 mm), which was opposed to the results based on the pre-incubated soil aggregates in the aforementioned studies [31]. This difference may be related to the content of the active organic carbon in the different size of aggregates, or to a different protective mechanism of organic carbon inside under the wetting stress.

Other researchers found that large amounts of organic carbon were stored in the soil aggregates of 0.25-2 mm [32], and that the levels of active organic carbon in soil would increase as the size decreased [33-34]. The same results were found in this study based on the organic carbon analysis in soil aggregates after the separation by the dry-sieving. Furthermore, the process of carbon mineralization after the moisture adjustment in the aggregates that we observed was totally different from the aforementioned studies which took place after the pre-incubation of the aggregates [35]. The dry aggregates with different sizes after the wetting might result in different mineralization patterns. This study also found that the average carbon mineralization rate per day in the medium and small aggregates first revealed a decrease and then an increase during the incubation, which was related to the quantity composition of the organic carbon inside and the degradation of the external organic matter.

All of the organic carbon storage in the aggregates is composed of active and inert components [36]. At the beginning of the incubation, the active component was easily and quickly degraded by microbes, and the amount of the organic carbon for mineralization gradually decreased, which led to a decreased rate of the organic carbon mineralization. With the external organic matter degrading, large amounts of light-fraction organic carbon were supplied into the soil, and the carbon mineralization rate slowly returned to normal. Due to the intrinsically low carbon mineralization rate in the large aggregates with size of 2-3 mm, the consumption of the easily

Figure 7: The overall RDA of the correlation between bacterial community structure, the cumulative CO_2 -evolution, and soil properties by Canoco. Symbols of circle, diamond, and star represent samples from the treatments of <0.5mm, 1-2mm, and 2-3mm size aggregates, respectively. Environmental parameters (i.e. cumulative CO_2 -evolution, SOC and moisture) are indicated by triangles or solid lines with filled arrows. Species parameters are shown using the symbols of triangle, and each species indicated by a underlined number.

mineralized components in soil would be slow, and then the relatively sufficient mineralized active component and the following-up supplement supported a steady rise of the carbon mineralization rate.

3.4 Microbial community structure variance and carbon mineralization mechanism

As shown in **Figure 7**, the two-dimensional (2D) sorting map of redundancy analysis (RDA) shows the relationships among soil bacterial structure, soil moisture content, SOC, and CO_2 mineralization production in the treatments with different size aggregates. The environmental factor matrix composed of SOC and moisture content could not significantly explain the variance ($P=0.358$) of the bacterial community structure. Furthermore, the environmental factor matrix composed of soil moisture content, SOC, and CO_2 mineralization production also could not explain the variance ($P = 0.668$) of the bacterial community structure. The 2D sorting map indicates that the vectorial angle between CO_2 production and any one of the other two parameters (SOC and moisture content)

approaches 90°, which also confirmed the non-significant correlation between CO₂ production and the other two parameters. According to the results based on the manual selection procedure in RDA, it was found that none of SOC ($P = 0.092$), moisture content ($P = 0.998$), and CO₂ cumulative production ($P = 0.886$) could significantly explain the variance of microbial community structure. In this study, based on the microbial structure matrix and the environmental factor matrix composed of three groups of treatments as the independent variables, the relationship model between microbial species and environment was built and analyzed by RDA, with results indicating that the proposed matrix could significantly explain the variance of microbial community structure.

In conclusion, although the variance of bacterial community structure in the treatments with different aggregates revealed the different dynamic patterns, these patterns had no consequent interaction with SOC and moisture content. This result might be related to several factors: (1) the contents of SOC in whole treatments did not show any significant variance during the incubation, and the moisture content was always in the ideal range for microbial life; therefore, the carbon source and moisture did not become the limiting factors for bacterial reproduction and activity. This study also revealed that the variance of bacterial community structures could not significantly affect the process of organic carbon mineralization, which might be related to the quality of organic carbon in the original soil samples, according to the subsequent analysis. Although the content of SOC in all soil aggregates was relatively high, the SOC fractions might be relatively simple because the samples were from the long-term fertile vegetation land. Because it was unnecessary for various bacterial species with different physiological and biochemical functions to participate in organic carbon mineralization and degradation, the diversity variance of bacterial community structure did not have a direct effect on carbon mineralization. (2) The DGGE profiles indicated that some dominant species existed in all soil samples simultaneously, were always in the samples, and had a large relative abundance during the incubation. Because the SOC mineralization might always be dominated by these species, the carbon mineralization could have a relationship with the dynamics of these dominant species, and not the other species. In view of this, the quality of SOC should be taken into consideration in future studies on the carbon mineralization regularity and mechanism. In addition, the physiological and biochemical functions of specific microbial species in soil should also be further studied.

4 Conclusions

Based on the fact that the distribution patterns of soil aggregates in eroded and deposited environments are shaped by selective transport of water erosion, this research investigated the characteristics of SOC mineralization in relation to microbial dynamics in subtropical red soils dominated by different size of soil aggregates. Our findings suggested that the SOC mineralization rate of soils dominated by graded aggregates were significantly different, which reinforced the fact that SOC mineralization in eroded and deposited environments are significantly shaped by selective transport of water erosion. During the incubation, the fungal communities exhibited a low dynamic character whereas the composition of bacterial communities in all treatments changed significantly and differed obviously between each other. However, there were no statistical interactions between the dynamics of microbial communities and the changes of SOC as well as soil water content. Changes in bacterial community structure had no significant effect on the mineralization of SOC, which might be related to the quality of SOC or the specific utilization of carbon sources by different functional groups of microorganisms. Mineralization of the eroded and retained SOC with specific quality in relation to its functional microorganism should be further explored in the future.

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Conflict of interest: Authors declare no conflict of interest.

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