



Article

Evaluation of the Influence of Individual Clay Minerals on Biochar Carbon Mineralization in Soils

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Abstract: Although association between mineral and biochar carbon have been speculated in some studies, still there is no direct evidence for the influence of individual clay minerals on the mineralization of biochar carbon in soils. To address this, we conducted an incubation study using monomineralic soils constituted by separately mixing pure minerals, i.e., smectite, kaolinite, and goethite, with a sandy soil. Switch grass biochar (400 °C) was added to the artificial soils and samples were incubated for 90 days at 20 °C in the laboratory. The CO₂-C mineralized from the control, and biochar amended soil was captured in NaOH traps and the proportion of C mineralized from biochar was determined using δ¹³C isotopic analysis. The clay minerals significantly decreased the cumulative total carbon mineralized during the incubation period, whereas biochar had no effect on this. The least amount of total C was mineralized in the presence of goethite and biochar amended soil, where only 0.6% of the native soil organic carbon (SOC) (compared to 4.14% in control) and 2.9% of the biochar-C was mineralized during the 90 days incubation period. Native SOC mineralization was significantly reduced in the presence of biochar and the three minerals. Goethite was most effective in stabilizing both biochar and the native soil organic carbon. The short-term data from this study demonstrate that biochar application in Fe oxide rich soils may be an effective strategy to sequester biochar carbon, as well as to stabilize native soil carbon.

Keywords: biochar; organic carbon; carbon mineralization; clay minerals

1. Introduction

In recent years many studies have been conducted on biochar application to the soil to evaluate its potential multiple benefits, not only for increasing soil carbon storage, and therefore mitigating climate change, but also improving soil quality, and promoting and sustaining crop production [1–3]. Woolf et al. [4] modelled that biochar has the potential to substantially reduce greenhouse gas emissions, in the order of 1.8 Pg CO₂-C per year. This potential is, however, largely dependent on the stability of biochar-C in the soil.

There is ample evidence from several incubation studies and historical field samplings that biochar can persist in soils for a long time, with estimated mean residence time generally well over 100 years [5,6]. The persistence of biochar in soils depends on several factors including the pyrolysis temperature of biochar, biochar feedstock, environmental conditions and soil properties [6]. Several researchers have evaluated biochar mineralization in soils, particularly in relation to the influence of biochar addition on the stability of native soil organic carbon (SOC), referred to as “priming”. Positive, negative and neutral priming effects of biochar have been reported on the mineralization of native SOC [5,7–11].

Clay minerals have a major role in the stabilization of natural organic matter in soils [12–14]. Chemical interactions of soluble organic compounds with mineral surfaces, and incorporation of soil organic matter into soil aggregates are the main mechanisms for the preservation of organic matter in soils. Similarly, the stabilization of biochar-C has been postulated to be based on the association of significant amounts of biochar-C with mineral fractions or microaggregates in soils [15–19]. There is some evidence that clay minerals in soils decrease the mineralization of biochar-C; for example, the mineralization of barley root biochar-C decreased with increasing clay content in three Danish soils [20]. Nevertheless, there is limited research on the role of specific clay minerals in the stabilization of biochar carbon. For example, Fang et al. [21] observed a substantially lower mineralization of biochar-C in an Oxisol, where Fe and Al oxides dominated the clay fraction, compared to other soils that contained phyllosilicates in the clay fraction. Consequently, the mean residence time of the biochar was 22–35 percent longer in the Oxisol than in the other soils. Similarly, the mineralization of a pine wood biochar was approximately half when incubated in an andesitic soil, which contained short-range order clay minerals, compared to a granitic soil [9]. However, due to the co-existence of several minerals in soils in previous studies, it is difficult to isolate and quantify the role of individual clay minerals in the preservation of biochar-C.

We aimed to unravel the role of specific clay minerals in influencing the mineralization of biochar-C in soil. We hypothesized that clay minerals will have varying effects on biochar-C mineralization, depending on their charge characteristics, i.e. variable and permanent charge, and that the mineralization of biochar-C will decrease with increasing specific surface area of the mineral. To test our hypothesis, we measured the mineralization of biochar-C in artificial soils in the presence of three clay minerals, i.e., kaolinite, smectite and goethite, using an incubation experiment. Additionally, we measured the mineralization of native soil carbon to evaluate the effects of clay minerals and biochar on the stability of native soil carbon.

2. Materials and Methods

2.1. Soil, Biochar, and Minerals

A sandy surface soil (0–15 cm, Inceptisol) was collected from a farm (30°49′18″S, 116°40′56″E) near Wongan Hills in Western Australia, Australia. The soil was air-dried and sieved through a 2 mm mesh sieve for the incubation experiment. This soil was the basis for the soil mixtures with biochar and specific minerals (specified under 2.2). A switch grass (*Panicum virgatum* L.) biochar produced at 400 °C using slow pyrolysis [22] was used in the experiment. Some important properties of the soil and biochar are presented in Table 1.

Table 1. Some relevant properties of the soil and biochar used in the incubation experiment.

	Organic Carbon (%)	pH (1:5 H ₂ O)	Sand (%)	Silt (%)	Clay (%)	δ ¹³ C (‰)
Soil (Inceptisol)	1.48 (±0.08)	5.70 (±0.01)	97.5(±0.1)	1.2 (±0.1)	1.3 (±0.1)	−28.15 (±0.02)
Biochar	74.3 (±0.11)	7.46 (±0.18)	–	–	–	−13.75 (±0.06)

The numbers in the parentheses are the standard error of the mean (n = 4). “–” means it was not applicable.

Three clay minerals, i.e., kaolinite, smectite and goethite were used to constitute artificial soils for the incubation experiment. Kaolinite and smectite are phyllosilicate minerals. Kaolinite is a variable charge mineral and has relatively low specific surface area, whereas smectite has permanent charge and has high specific surface area (including internal surface area). Goethite is an iron oxide with variable surface charge and greater specific surface area than kaolinite. Kaolinite (KGa-1b) and smectite (Swy-2) were obtained from the Source Clays Repository of the Clay Minerals Society at Purdue University, USA. Goethite was synthesized using a procedure described by Schwertmann and Cornell [23]. Briefly, 100 ml of 1 M Fe(NO₃)₃ solution was prepared freshly by dissolving unhydrolyzed Fe(NO₃)₃·9H₂O in ultrapure water. The solution was transferred into a 2 L polyethylene flask, and 180 ml of 5 M KOH solution was added rapidly while stirring. The resulting suspension was made to volume (2 L) with

ultrapure water, and the closed flask was kept at 70 °C for 60 h. The contents were then centrifuged to separate the precipitate, followed by repeat washings with ultrapure water to remove free ions. Finally, the residue was dried at 50 °C and the mineral composition was confirmed by X-ray diffraction analysis. The specific surface areas of kaolinite, smectite and goethite, measured by N₂ adsorption method, were 23.5, 31.8 and 30 m² g⁻¹, respectively.

2.2. Laboratory Incubation

Eight treatments were designed for the incubation experiment. Soil treatment (S) with soil alone (100 g) was kept as a control, soil-biochar mixed treatment (S+B) consisted of 2% w/w biochar mixed in the soil (100 g soil and 2 g biochar), and soil-mineral mixed treatments (100 g soil and 10 g mineral) were 10% w/w of kaolinite (S+Ka), or 10% w/w smectite (S+Sm) or 10% w/w goethite (S+Go), mixed separately in the soil. Soil-biochar-mineral treatments consisted of 2% w/w biochar mixed separately with 10% kaolinite (S+B+Ka), or smectite (S+B+Sm) or goethite (S+B+Go) constituted soil. The soil and soil-biochar, soil-mineral, soil-biochar-mineral mixtures were placed into 500 ml plastic jars sealed with lids at 20 °C for 90 days. Each treatment was replicated three times. Two beakers, one beaker containing 30 ml of 2.0 M NaOH to trap the CO₂ produced from carbon mineralization and another beaker containing 10 ml deionized water to maintain a constant humidity, were placed in each incubation chamber. To account for headspace CO₂, three blank incubation chambers (i.e., no soil) were also set up in the same manner as the treatments.

A nutrient solution containing approximately 328 mg N, 54 mg P, 212 mg K, 59 mg S (per kg soil) and required amounts of trace elements was uniformly mixed with each treatment including control to support microbial growth during the incubation period. A microbial inoculum was prepared and mixed in with each treatment [6]. The CO₂ traps were changed at days 1, 4, 8, 15, 30, 45, 60, 75 and 90 for total CO₂-C and δ¹³C analyses.

2.3. Mineralization of Biochar-C and Native SOC

A titration method was used to determine the amount of CO₂-C trapped in the NaOH solution [6,21]. Carbon isotopic composition of the trapped CO₂ from various treatments was determined by δ¹³C analysis of SrCO₃ precipitates. The precipitates were obtained by reacting equal volumes of NaOH (CO₂-C trapped) and 1.25 M SrCl₂ solutions [6].

Carbon isotopic analyses was used to determine the proportion of biochar-C (C_B) mineralised using the following equation:

$$C_B(\%) = \frac{(\delta_T^{13}\text{CO}_2 - \delta_{\text{SOC}}^{13}\text{CO}_2)}{(\delta_B^{13}\text{C} - \delta_{\text{SOC}}^{13}\text{CO}_2)} \times 100 \quad (1)$$

where δ_T¹³CO₂ is the δ¹³C value of the total CO₂-C mineralized from biochar-soil-mineral treatments, δ_{SOC}¹³CO₂ is the δ¹³C value for the CO₂-C evolved from native SOC and δ_B¹³C is the δ¹³C value of the fresh biochar. The amount of native soil C mineralized was determined from the difference between total C and biochar-C mineralization data for each of the treatments.

2.4. Mean Residence Time of Biochar-C and Native SOC

The mean residence time (MRT) of native SOC and biochar-C was estimated using the two-pool exponential model as given below [6,21]:

$$C_t(\%) = C_L \times (1 - e^{-k_L t}) + (100 - C_L) \times (1 - e^{-k_R t}) \quad (2)$$

where C_t (%) is the cumulative proportion of C mineralized at a given time (t), C_L is the proportion of labile native SOC or biochar-C, and k_L is the mineralization rate constant of the labile carbon, (100-C_L) are the proportions of recalcitrant native SOC or biochar-C, and k_R is the mineralization rate constant

of the recalcitrant pool. Mean residence time (MRT) of recalcitrant portion of native SOC or biochar-C is the inverse ($1/k_R$) of the mineralization rate constant of the recalcitrant C pool.

2.5. Statistical Analyses

Differences in cumulative total C mineralized, cumulative native SOC and biochar mineralized in different soil mixtures, and incubation time were analysed with the general linear model procedure using SAS (Version 8.0). Difference in the MRT of native SOC was analysed with a factorial ANOVA procedure using SAS. A one-way ANOVA analysis was performed for cumulative total C, native SOC and biochar-C mineralized at each sampling time of the incubation experiment and the estimated MRT of biochar-C. All means were tested for significance at 0.05 probability level.

3. Results

3.1. Total Carbon Mineralization

We determined the total C mineralized from all treatments based on both soil mass ($\mu\text{g CO}_2\text{-C}$ produced per g soil) and soil organic carbon mass ($\text{mg CO}_2\text{-C}$ produced per g soil organic carbon) basis. However, only the soil mass basis data are presented here because the native organic carbon concentration was the same in all treatments and the total C mineralized data on soil mass basis better reflect the effects resulting from different mineral compositions, which is the main objective of this research.

Biochar had no significant influence on the cumulative total C mineralized ($\text{CO}_2\text{-C}$ produced per unit soil mass basis) after 90 days of incubation (Table 2, Figure 1).

In the absence of biochar, goethite and kaolinite significantly decreased cumulative C mineralized, compared with no mineral treatment (i.e., S treatment), whereas smectite showed no effect (Figure 1c, Table 2). In the absence of biochar, goethite and kaolinite exhibited significantly better capability in reducing cumulative C mineralized than smectite, and there was no significant difference between goethite and kaolinite. In the presence of biochar, goethite significantly decreased cumulative mineralized C compared with no mineral added treatment (i.e., S+B treatment), whereas smectite and kaolinite significantly increased cumulative C mineralized (Table 2).

Table 2. T-test (LSD) for cumulative C mineralized ($\mu\text{g C g}^{-1}$ soil) after 90 days of incubation using a linear model for fixed terms of treatment and incubation time and their associated interactions ($p < 0.05$).

Treatment	n	Mean	t Grouping ¹
S+B+Sm	27	357.65	a
S+B+Ka	27	339.44	a,b
S	27	325.69	b,c
S+Sm	27	316.52	c
S+B	27	316.38	c
S+Go	27	285.54	d
S+Ka	27	278.89	d
S+B+Go	27	243.14	e

¹ Means with different letters are significantly different from each other.

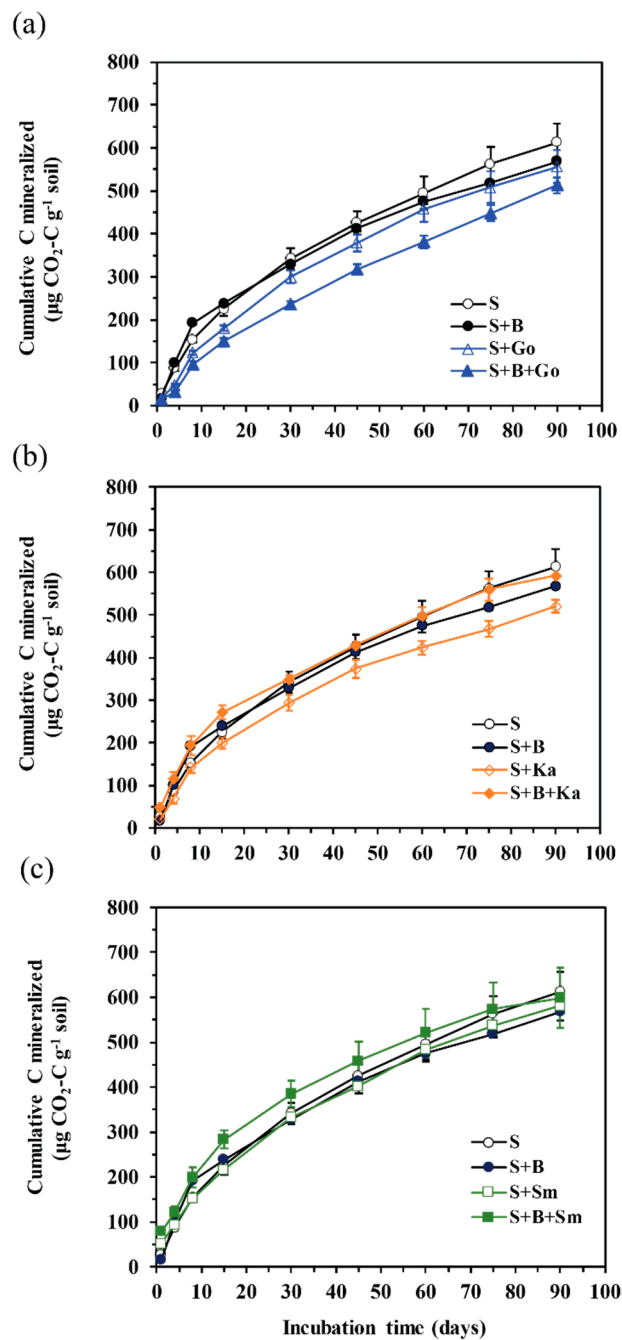


Figure 1. Cumulative carbon (C) mineralized ($\mu\text{g C g}^{-1}$ soil) during 90 days incubation at 20 °C. The plotted values are averages of three replicates, and the standard errors are represented by error bars. (a) Soil, soil + biochar, soil + goethite and soil + biochar + goethite; (b) soil, soil + biochar, soil + kaolinite and soil + biochar + kaolinite; and (c) soil, soil + biochar, soil + smectite and soil + biochar + smectite.

The effects of biochar and mineral on total C mineralization varied with incubation time (Figure 1). For example, significantly more cumulative C was mineralized in the S+B+Sm treatment than in the S+Sm treatment on days 1, 4, 8, and 15. Similarly, S+B+Ka treatment significantly increased cumulative mineralized C compared to the S+Ka treatment from day 1 to day 30. The presence of goethite (S+Go), smectite (S+Sm) and kaolinite (S+Ka) resulted in a progressive decrease in cumulative mineralized C as compared to soil (S) alone during the incubation period; however, significant differences occurred only on two sampling days, i.e. days 4 and 15 in the S+Go treatment, whereas no significant differences

were found for the S+Sm and S+Ka treatments. The cumulative mineralized C during the 90 days incubation period in all treatments followed the order: S+B+Sm > S+B+Ka > S > S+Sm > S+B > S+Go > S+Ka > S+B+Go.

3.2. Mineralization of Native SOC and Biochar-C

Biochar significantly reduced the cumulative native SOC mineralized ($\mu\text{g CO}_2\text{-C}$ produced per g soil) in the absence and the presence of clay minerals (Table 3, Figure 2).

Table 3. T-test (LSD) for native SOC mineralized ($\mu\text{g C g}^{-1}$ soil) after 90 days incubation using linear model for the fixed terms of treatment and incubation time and their associated interactions ($p < 0.05$).

Treatment	n	Mean	t Grouping ¹
S	27	325.69	a
S+Sm	27	316.52	a
S+Go	27	285.54	b
S+Ka	27	278.90	b
S+B	27	76.46	c
S+B+Sm	27	66.28	d, c
S+B+Ka	27	59.19	d, e
S+B+Go	27	46.71	e

¹ Means with different letters are significantly different from each other.

Goethite and kaolinite significantly reduced the cumulative native SOC mineralized both in the presence and absence of biochar, compared to S+B and S treatments, respectively (Table 3); however, smectite showed no significant effect. In the absence of applied biochar, the influence of goethite and kaolinite in decreasing cumulative native SOC mineralized was significantly greater than with smectite (Table 3). In the presence of biochar, goethite was most effective among the clay minerals in reducing the native cumulative SOC mineralized.

The proportion of native SOC mineralized of the total native SOC after the 90 days incubation period was largest in the control treatment (S), where it reached to 4.14% of the initial native SOC, whereas in the S+B+Go treatment it was smallest, where only 0.6% of the initial native SOC was mineralized.

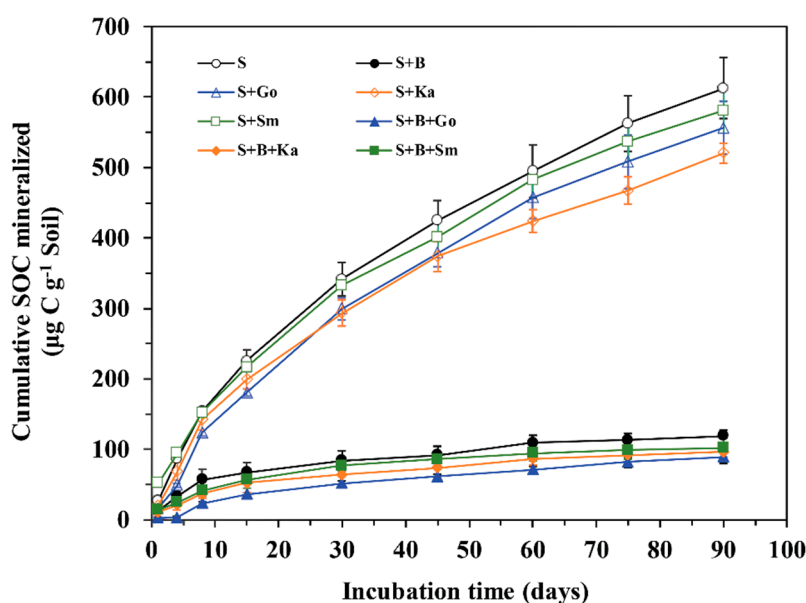


Figure 2. Cumulative native mineralized SOC during 90 days incubation at 20 °C. The plotted values are averages of the of three replicates, and the standard errors are represented by error bars.

After the 90 days of incubation, biochar (S+B treatment), mineral treatments (averaged) and biochar + mineral treatments (averaged) reduced the cumulative native SOC mineralized by 80.6, 10.3 and 83.7% as compared with the soil alone (S). Overall, the cumulative native SOC mineralized in all treatments over the incubation period followed the order: S > S+Sm > S+Go > S+Ka > S+B > S+B+Sm > S+B+Ka > S+B+Go.

Minerals significantly affected the cumulative biochar-C mineralized over the 90 day incubation period (Figure 3). Goethite was the most effective in reducing the mineralization of biochar-C compared to the S+B treatment during the incubation period (Figure 3). However, in the presence of smectite and kaolinite, the amount of cumulative biochar-C mineralized was significantly greater than in the S+B treatment. There was no significant difference in the cumulative mineralized biochar-C between smectite and kaolinite treatments. After 90 days of incubation, 2.89 to 3.38% of the total biochar-C added was mineralized from all treatments.

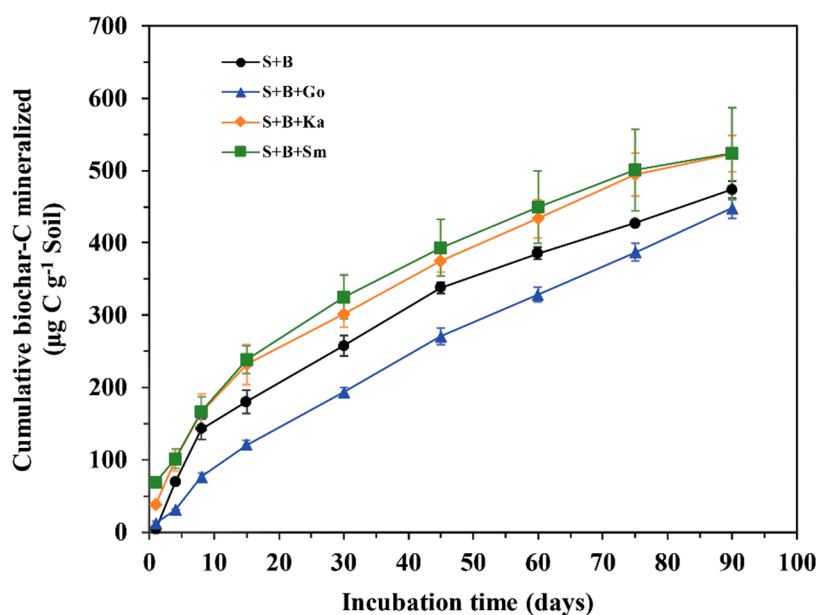


Figure 3. Cumulative mineralized biochar-C during 90 days incubation at 20 °C. The plotted values are averages of three replicates, and the standard errors are represented by error bars.

3.3. Mean Residence Time (MRT) of Biochar-C and Native SOC

The estimated MRT of recalcitrant biochar-C ranged from 86.7 to 138.6 years, and there was no significant difference between various treatments.

The estimated MRT of the recalcitrant fraction of the native SOC was 9.5 years in the control (S) treatment, between 10.0 and 13.4 years in the soil-mineral mixed treatments, 68.4 years in the S+B treatments, and between 88.6 and 107.1 years in the soil-biochar-mineral mixed treatments (Table 4). Biochar significantly increased the MRT of native SOC as compared with the soil only treatment, but minerals had no effect on the MRT of native SOC both in the absence and presence of biochar. The estimated proportion of the labile C in native SOC was 1.6% for S treatment, which was reduced to 0.3–0.5% in biochar amended samples and remained similar (1.5–1.9%) to the control in mineral amended soil. The labile biochar-C fraction was 12% in the S+B treatment and varied from 8.6 to 15.7% in mineral and biochar amended treatments.

Table 4. Mean residence time of native SOC and biochar-C in the two-pool exponential model fitted to the cumulative C mineralized over 90 days.

Treatment	Native SOC		Biochar-C	
	Labile C (Days)	Recalcitrant C (Years)	Labile C (Days)	Recalcitrant C (Years)
S	12.3(±1.5)	9.5(±0.8)	-	-
S+Sm	10.8(±1.2)	10.0(±1.2)	-	-
S+Ka	13.1(±0.9)	12.2(±1.0)	-	-
S+Go	22.7(±3.4)	13.4(±2.3)	-	-
S+B	5.7(±0.5)	68.4(±11.3)	11.0(±1.4)	125.0(±0.0)
S+B+Go	15.9(±3.3)	88.6(±21.7)	21.9(±7.9)	122.6(±12.4)
S+B+Sm	8.6(±0.9)	88.9(±12.8)	9.1(±0.9)	138.6(±32.2)
S+B+Ka	7.0(±2.7)	107.1(±46.5)	14.1(±3.6)	86.7(±38.3)

¹ The mean residence time is the inverse ($1/K_L$ or $1/K_R$) of the mineralization rate constant. The numbers in parentheses are the standard error of the mean (n = 3).

4. Discussion

4.1. Effects of Clay Minerals on Native SOC and Biochar-C Mineralization

Clay minerals can influence the mineralization of both native SOC and biochar-C, and thus the total C mineralization in the soil. Soil organic matter may be physically protected from decomposer organisms or their extracellular enzymes in micropores formed by soil aggregates [12,14]. Furthermore, chemical interaction of soil organic matter (SOM) via complexation reactions on surfaces of Fe and Al (hydr-) oxides and phyllosilicates can protect native SOC against microbial mineralization [14,24,25]. Smectite with a relatively larger specific surface area ($31.8 \text{ m}^2 \text{ g}^{-1}$) exhibited a weaker capability in reducing SOC mineralization than goethite ($30 \text{ m}^2 \text{ g}^{-1}$) and kaolinite ($23.5 \text{ m}^2 \text{ g}^{-1}$) in the absence of biochar. This finding is consistent with Bruun et al. [24] who reported that smectite has weaker capability of reducing SOC mineralization than kaolinite. Goethite and kaolinite were equally effective in reducing the mineralization of native SOC. The interaction of native SOC with variable charged minerals, i.e., goethite and kaolinite, involving ligand exchange reactions, may have caused a decrease in the mineralization of native SOC [26,27].

Mineralization of biochar-C was lowest in the presence of goethite, which is consistent with the involvement of ligand exchange reactions between biochar and goethite surfaces as postulated by Fang et al. [21,28]. The involvement of ligand exchange reactions in clay-organic matter interactions has been frequently suggested [13,24–27]. Such reactions occur between carboxyl and phenolic groups of organic matter and hydroxyl groups on the surfaces of Fe and Al oxides and at the edges of kaolinite and other phyllosilicates. Decreased mineralization of biochar-C in the S+B+Ka, compared with S+B treatment, suggest the involvement of ligand exchange reactions between biochar and kaolinite surfaces. However, these interactions are stronger on goethite surfaces than kaolinite surfaces, possibly due to greater specific surface area and a greater number of sites per unit surface area in goethite than kaolinite. Fernández-Ugalde et al. also observed metal oxides and oxyhydroxides, and vermiculite contribute to the stabilization of biochar-C and native SOC [18].

4.2. Effects of Biochar on Native SOC Mineralization

Total C mineralized in biochar amended treatments consisted of C mineralized from the native SOC and biochar-C. Variable and sometimes contrasting effects of biochar on the total soil C mineralization have been observed in the literature. Researchers have reported that biochar had no effect [29–32], increased [5,8] or decreased [33] total C mineralization from soil. Our results illustrate that the addition of 400°C switch grass biochar had no effect on cumulative C mineralization in the absence of added clay mineral to the soil. However, variable effects were observed in the presence of clay minerals, with increased CO_2 evolution in the presence of smectite and kaolinite, and decreased mineralization in the presence of goethite.

The priming effect of biochar on native soil carbon depends on several factors, including the duration of incubation experiment, and biochar and soil characteristics [10]. In our study, the presence

of biochar significantly decreased the mineralization of native SOC both in the absence and presence of clay minerals. Our results are in contrast with Fang et al. [10], who observed a positive priming effect of biochar on native SOC in the same soil, an Inceptisol. The contrasting results might be due to different biochar used in the two studies. Fang et al. [10] used biochars pyrolyzed from Eucalyptus woody biomass at 450 °C or 550 °C, whereas the biochar in this study was produced at lower pyrolysis temperature (400 °C) from switch grass. The switchgrass biochar contained a lower proportion of C as stable polyaromatic C, and a lower proportion of total OC as BPCA-C than the biochars used by Fang et al [10]. The presence of a relatively large amount of labile carbon in the switchgrass biochar possibly served as a source of energy for soil microbes, and was used in preference to the native C, particularly during the short incubation time. This mechanism has been described as substrate switching [34]. Additionally, the applied biochar possibly had a stabilizing effect on the native SOC via soil aggregation, a mechanism suggested by Fang et al [10]. Keith et al. [6] observed a similar effect in an incubation study where biochar addition resulted in a net negative priming effect on the mineralization of added labile organic C.

4.3. Interactive Effects of Biochar and Clay Minerals on Native SOC

No significant interactive effect of biochar and minerals on native SOC mineralization was observed in this study, though the minerals exhibited different patterns in reducing native SOC mineralization with biochar addition. Generally, the effect of combined addition of biochar, and a mineral in reducing the cumulative amount of native SOC mineralized was less than the sum of individual effects of biochar and a mineral. The effect of biochar was a dominant factor in this regard; however, the results of this short-term incubation study should be verified with long-term experiments, preferably under field conditions.

4.4. Mean Residence Time of Native SOC and Biochar-C

The MRT estimated for native SOC in soil alone (S treatment) is rather short (9.5 years), indicating the relatively labile nature of the native SOC. Since the control soil was almost pure sand, the SOC was not associated with any clay minerals. Hence, the free native SOC would be expected to mineralize relatively fast after the addition of nutrient solutions and microbial culture used in the experiment. The stabilizing effect of biochar on the native SOC is obvious with a significant increase in the MRT of the native SOC in the presence of biochar. The MRT of biochar in our study (about 86.7 to 138.6 years) is consistent with the short-term incubation experiment and low temperature grass biochars [21,35].

5. Conclusions

The biochar used in our study showed a good potential to reduce the mineralization of native SOC in soils, added alone or in combination with each of the three common soil clay minerals. Our results illustrate that biochar addition is most beneficial in soils containing Fe oxides (rather than phyllosilicates), as (1) biochar addition stabilized native SOC, and (2) added biochar-C has the potential to be stable for long periods. For future experiments, biochar properties such as feedstock and pyrolysis temperature, as well as climatic conditions should be considered, particularly for long-term incubation experiments and under field conditions.

Author Contributions: B.S. and Q.Z. conceptualized the incubation experiment; B.S. obtained the biochar, soil and mineral samples; Q.Z. conducted the laboratory work; C.K. did the isotopic analysis; Q.Z. did the data analysis, all three authors contributed to manuscript writing.

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Conflicts of Interest: The authors declare no conflict of interest.

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