



Biochar-induced negative carbon mineralization priming effects in a coastal wetland soil: Roles of soil aggregation and microbial modulation



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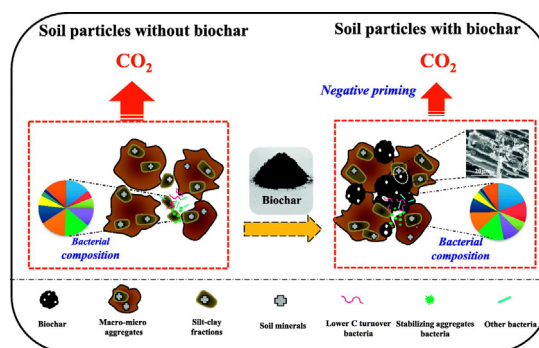
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HIGHLIGHTS

- Biochar had negative priming effect in macro-micro aggregates and silt-clay fractions.
- Enhanced stability of soil aggregate fractions was due to associations of minerals and biochars.
- Biochar increased microbial biomass C and C use efficiency.
- Biochar shifted bacterial community towards to low C turnover bacteria taxa.
- Biochar promisingly enhanced “blue C” sink in the degraded coastal wetland.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar can sequester carbon (C) in soils and affect native soil organic carbon (SOC) mineralization via priming effects. However, the roles of soil aggregation and microbial regulation in priming effects of biochars on SOC in coastal wetland soils are poorly understood. Thus, a coastal wetland soil ($\delta^{13}\text{C} = -22\%$) was separated into macro-micro aggregates (53–2000 μm , MA) and silt-clay fractions (<53 μm , SF) to investigate the priming effect using two ^{13}C enriched biochars produced from corn straw ($\delta^{13}\text{C} = -11.58\%$) at 350 and 550 °C. The two biochars induced negative priming effect on the native SOC mineralization in the both soil aggregate size fractions, attributed to the enhanced stability of the soil aggregates resulting from the intimate physico-chemical associations between the soil minerals and biochar particles. Additionally, biochar amendments increased soil microbial biomass C and resulted in a lower metabolic quotient, suggesting that microbes in biochar amended aggregates could likely incorporate biomass C rather than mineralize it. Moreover, the biochar amendments induced obvious shifts of the bacterial community towards low C turnover bacteria taxa (e.g., *Actinobacteria* and *Deltaproteobacteria*) and the bacteria taxa responsible for stabilizing soil aggregates (e.g., *Actinobacteria* and *Acidobacteria*), which also accounted for the negative priming effect. Overall, these results suggested that biochar had considerable merit for stabilizing SOC in the coastal soil and thus has potential to restore and/or enhance “blue C” sink in the degraded coastal wetland ecosystem.

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1. Introduction

Coastal wetlands (e.g., salt marshes and mangrove swamps), constituting only 0.22–0.34% of the Earth's land surface (Fennessy, 2014), can substantially sequester 13–17.2 Pg (billion tons) of carbon (C) in soils and vegetation (Hiraishi et al., 2014). Thus, coastal wetlands acting as significant natural C sinks play crucial roles in the global C cycling. The C stocks of coastal wetlands are increasingly referred to as “blue C” (Fennessy, 2014; Mcleod et al., 2011), compared to the carbon stored in the terrestrial ecosystems, referred to as “green C” (Mackey et al., 2008). Yet the “blue C” sinks have sharply decreased over the past century because the coastal wetlands have experienced dramatic losses and deterioration due to anthropogenic disturbances (e.g., farming and oil exploration) (Mcleod et al., 2011). The land-use conversion and degradation of coastal wetlands may result in 0.15–1.02 Pg of CO₂ released annually, equivalent to 3.0–19% of those from global deforestation, and have resulted in economic damages of \$US 6–42 billion annually (Pendleton et al., 2012). Thus the “blue C” plan, an international project to conserve C sequestration abilities of the coastal wetlands, has been proposed (Mcleod et al., 2011; Vaidyanathan, 2011). As natural C storage reservoirs, wetland soils accumulated over 90% of total wetland C stocks and directly affected the capacity of coastal wetlands as “blue C” sinks through regulating organic matter (OM) production and decomposition (Howard et al., 2014). Consequently, reclaiming the degraded wetland soils is an urgent task to enhance the “blue C” sink. However, the conventional strategies (e.g., recovering wetland vegetation and returning straw into soils) for promoting soil C accumulation (Luo et al., 2017) could only possess short-term benefits because of their lability (Keith et al., 2011). Therefore, answers for how to restore and elevate the C storage capacity of the wetlands soils need to be addressed.

Biochar has been widely proposed as one of the promising options for enhancing soil C sink due to its resistance to abiotic and biotic degradation (Fang et al., 2014; Singh et al., 2012), as well as its potential of decreasing CO₂ efflux from soil organic matter (SOM) (Lu et al., 2014; Rittl et al., 2015). However, intense studies have been focused on the effects of biochar on C sequestration in agricultural (Cross and Sohi, 2011; Liu et al., 2016), pasture (Rittl et al., 2015) and forest ecosystems (Mitchell et al., 2015), and little is known about the C sequestration potential of biochar in coastal wetland ecosystems (Luo et al., 2016; Sun et al., 2014). As an exogenous organic matter, biochar may increase, decrease or have no effect on soil organic carbon (SOC) mineralization rates, termed as positive (Singh and Cowie, 2014), negative (Lu et al., 2014; Rittl et al., 2015) and no “priming” (Liu et al., 2016), respectively. These uncertainties on SOC mineralization induced by biochar amendments could have resulted from the differences in the nature and properties of the biochar and soils (Whitman et al., 2014), as well as their complicated interactions (Joseph et al., 2010; Luo et al., 2016). Generally, physical protection of SOM by macro-micro aggregates (>53 μm) and chemical stabilization of SOM on mineral surfaces within the silt-clay fractions (<53 μm) are the dominant mechanisms responsible for SOM stabilization and accumulation (Lutzow et al., 2006, 2008), and the structural stability of the two soil aggregate size fractions strongly affect SOC bioavailability (Six and Paustian, 2014). In the Yellow River Delta wetlands, Luo et al. (2016) reported that the peanut shell biochar application into a bulk (non-fractionated) coastal soil resulted in a negative priming effect, but the underlying mechanisms in the roles of soil aggregation were not clear. Therefore, to understand the effect of biochar on the structural stability of different aggregate size fractions is necessary to elucidate the potential of biochar in C sequestration in the coastal wetland ecosystems. Previous studies documented that biochar application into bulk soils promoted the stability of aggregate fractions, because of increased SOM and mineral contents, and formation of cationic bridges between biochar and soil minerals (Gul et al., 2015; Soinnie et al., 2014). However, whether the effect of biochar on the stability of aggregate fractions could determine the direction and extent of

the priming effect on native SOC, is poorly understood. Carboxylic and phenolic functional groups and polyvalent cations (e.g., Ca²⁺ and Al³⁺) in biochars were reported to be fundamental in binding minerals and SOM (Archanjo et al., 2015; Lin et al., 2012), and the intimate associations between biochar and organo-mineral complexes could ultimately contribute to soil aggregation (Gul et al., 2015). Therefore, we hypothesized that biochar addition could decrease SOC mineralization in the coastal soils through promoting the stability of soil aggregate fractions.

Additionally, biochar amendment can also change microbial biomass abundance and community structure due to its influence on substrate (e.g., C and N) availability (Farrell et al., 2013; Whitman et al., 2016) and soil physico-chemical properties (Quilliam et al., 2013; Watzinger et al., 2014), thus affecting SOC cycle. However, majority of these studies focused on the bulk (non-fractionated) soils (Farrell et al., 2013; Lu et al., 2014), scarce attention was paid to the different aggregate size fractions. Limited studies indicate that distinct bacterial communities distributed within different aggregate size fractions may be due to the unique SOM chemical composition, structural stability, and size of aggregate fractions (Davinic et al., 2012). These specific conditions within different aggregate size fractions could affect the composition of soil microbial communities and influence their functions on C dynamics (Gupta and Germida, 2015; Zhang et al., 2013). Therefore, the biochar-induced alteration in the composition of soil microbial communities among various aggregate size fractions could be crucial to illustrate the underlying mechanisms responsible for the biochar induced priming effect in different aggregate size fractions (Davinic et al., 2012; Whitman et al., 2016). Mitchell et al. (2015) found that adding biochar significantly promoted the ratios of Gram-positive/Gram-negative bacteria, which potentially leading to an increased CO₂ fluxes. Moreover, the increased abundance of the low C turnover taxa (e.g., *Actinobacteria* and *Acidobacteria*) have been demonstrated for the reduced SOC mineralization rate in the biochar amended soils (Singh and Cowie, 2014; Trivedi et al., 2013). Therefore, we hypothesized that the biochar addition could elevate the abundance of bacterial taxa responsible for the C accumulation in the soil aggregate fractions.

To test these hypotheses, a soil sample collected from a coastal wetland in the Yellow River Delta was separated into two aggregate size fractions, i.e. macro-micro aggregates (53–2000 μm) and silt-clay fractions (<53 μm), to investigate the roles of soil aggregation and microbial regulation in the biochar induced priming effect. The specific objectives were to: 1) measure the priming effect of biochar amendment on native SOC mineralization within different aggregate size fractions; 2) investigate the relationship between the stability of soil aggregate fractions and native SOC mineralization; and 3) distinguish the bacterial community responsible for the biochar-enhanced C sequestration in the different soil aggregate size fractions.

2. Materials and methods

2.1. Soil aggregate fractions preparation

The surface soils (0–20 cm) were randomly collected from Dongying Halophytes Garden (118.67°N, 37.42°E), located in the Yellow River Delta, China. Okra (*Abelmoschus esculentus* L.) has been cultivated in the sampling fields over the past years without the fertilizer application before. This silty clay soil (2.2% sand, 79.3% silt, and 18.5% clay) was classified as a Fluvisol (FAO, 2000). The selected properties of the soil are given in Table S1. The field-moist soil samples were thoroughly mixed and gently passed through a 5-mm sieve, with minimal disruption of the soil aggregates structures, and then the air dried bulk soil samples (<5 mm) were physically fractionated using the wet-sieving method (Six et al., 1998). Briefly, a 20-g air dried soil sample was re-wetted via capillary action by submerging it in 200 mL deionized water on the top of the 2000-μm sieve for 5 min at room temperature (20 ± 2 °C), and then was separated by moving the sieve up and down 50 times in

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