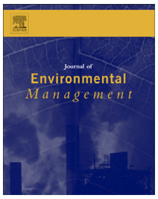
Journal of Environmental Management 182 (2016) 29–36

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman



Research article

Alteration of extracellular enzyme activity and microbial abundance by biochar addition: Implication for carbon sequestration in subtropical mangrove sediment

Ling Luo [a, b, **], Ji-Dong Gu [b, *]

[a] College of Environmental Sciences, Sichuan Agricultural University, Huimin Road, Chengdu, Sichuan Province, People's Republic of China
[b] Laboratory of Environmental Microbiology and Toxicology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China

ARTICLE INFO

Article history:
Received 13 March 2016
Received in revised form
6 July 2016
Accepted 14 July 2016

Keywords:
Biochar
Extracellular enzyme
Microbial abundance
Nutrient availability
C sequestration
Mangrove ecosystem

ABSTRACT

Biochar has attracted more and more attention due to its essential role in adsorbing pollutants, improving soil fertility, and modifying greenhouse gas emission. However, the influences of biochar on extracellular enzyme activity and microbial abundance are still lack and debatable. Currently, there is no information about the impact of biochar on the function of mangrove ecosystems. Therefore, we explored the effects of biochar on extracellular enzyme activity and microbial abundance in subtropical mangrove sediment, and further estimated the contribution of biochar to C sequestration. In this study, sediments were amended with 0 (control), 0.5, 1.0 and 2.0% of biochar and incubated at 25 °C for 90 days. After incubation, enzyme activities, microbial abundance and the increased percentage of sediment organic C content were determined. Both increase (phenol oxidase and β-glucosidase) and decrease (peroxidase, N-acetyl-glucosaminidase and acid phosphatase) of enzyme activities were observed in biochar treatments, but only peroxidase activity showed statistical significance (at least $p < 0.01$) compared to the control. Moreover, the activities of all enzymes tested were significantly related to the content of biochar addition (at least $p < 0.05$). On the other hand, bacterial and fungal abundance in biochar treatments were remarkably lower than control ($p < 0.001$), and the significantly negative relationship ($p < 0.05$) between bacterial abundance and the content of biochar was found. Additionally, the increased percentage of organic C gradually increased with biochar addition rate, which provided evidence for applying biochar to mitigate climate change. Given the importance of microorganisms and enzyme activities in sediment organic matter decomposition, the increased C sequestration might be explained by the large decrease of microbial abundance and enzyme activity after biochar intervention.

1. Introduction

Biochar (charcoal), ubiquitous in the environment, is a solid carbonaceous product of pyrolysis, i.e., the thermal degradation of organic materials in the absence of oxygen (Lehmann et al., 2011; Mašek, 2013). In recent years, the application of biochar in the environment has been widely studied since biochar is considered as an effective sorbent for organic contaminants and heavy metals, an amendment for improving soil conditions and nutrients, as well as an agent of carbon (C) sequestration to mitigate climate change. Due to these desirable properties, biochar is highly described as an environmental friendly material which provides a lower-risk strategy than other materials to reduce contamination and mitigate climate change (Ameloot et al., 2013; Bailey et al., 2011; Harvey et al., 2012; Keith et al., 2011; Kuzyakov et al., 2009; Lehmann, 2007; Lou et al., 2011; Steinbeiss et al., 2009; Woolf et al., 2010; Zimmerman et al., 2011). To our knowledge, a large body of studies are focused on the sorption of organic pollutants, and suggest that the general reasons for biochars as effective sorbents are mainly ascribed to their high surface areas, microporosity, special functional groups, and charge characteristics (Ahmad et al., 2014; Kookana et al., 2011; Mohan et al., 2014). However, the influences of biochar on microbial abundance and extracellular

---

[*] Corresponding author.
[**] Corresponding author. College of Environmental Sciences, Sichuan Agricultural University, Huimin Road, Chengdu, Sichuan Province, People's Republic of China.
E-mail addresses: luoling@sicau.edu.cn (L. Luo), jdgu@hku.hk (J.-D. Gu).

http://dx.doi.org/10.1016/j.jenvman.2016.07.040
0301-4797/

enzyme activity are still poorly documented.

Recently, a topic of growing interest in biochar is its influence on microbial activity and community structure in soil/sediment through altering the physicochemical parameters and improving the availability of micronutrients (Oleszczuk et al., 2013, 2014; Quilliam et al., 2013). For instance, enhanced microbial reproduction rates were reported to increase in some biochar-amended soils (Hamer et al., 2004; Pietikäinen et al., 2000; Prayogo et al., 2014), but decreased microbial biomass or relative population were also observed after addition of biochars (Chen et al., 2013; Dempster et al., 2012). It is noteworthy that the positive or negative effects of biochar on microorganisms often depend on the differences of biochars, soils/sediments as well as microbes (Kookana et al., 2011; Lehmann et al., 2011). On the other hand, the cycling of C and nutrients are mainly driven by heterotrophic decomposing microorganisms, which mineralize biomass of plants and animals, and soil/sediment organic matter (SOM) (Sinsabaugh et al., 2009). This mineralization process is widely recognized to be rate-limiting as it is strongly mediated through extracellular enzymes secreted by bacteria and fungi according to the nutritional conditions of soil/sediment. (Allison et al., 2007; Burns et al., 2013). Specifically, extracellular enzyme activities are intrinsically linked to environmental conditions and indigenous microbes, thus providing valuable information about the responses of C and nutrients cycling to natural and anthropogenic perturbations (Arnosti et al., 2014; Ayuso et al., 2011; Geisseler and Horwath, 2009; Penton and Newman, 2007; Sinsabaugh, 2010). And unfortunately, the relative information is still less known, and even the contradictory results have been reported (Bailey et al., 2011; Demisie et al., 2014; Paz-Ferreiro et al., 2012; Sun et al., 2014).

Concerning the importance of microbes and enzymes in SOM decomposition, it is deduced that biochar probably could influence C storage by adjusting the activities of extracellular enzymes and microbes. Therefore, it is necessary to explore the effects of biochar on microbial and enzyme activity in soils/sediments in order to further understand the role of biochar in C sequestration in terrestrial ecosystems. To date, no clear conclusion has been reached due to the different biochars and environmental variables of the ecosystems involved (Bailey et al., 2011; Ippolito et al., 2012; Kuzyakov et al., 2009; Oleszczuk et al., 2013; Paz-Ferreiro et al., 2012; Quilliam et al., 2013; Steinbeiss et al., 2009; Zimmerman et al., 2011). Biederman and Harpole (2013) summarised 114 published papers to evaluate and summarize responses of multiple ecosystems to biochar application with a meta-analysis of 371 independent studies, and found that biochar was a positive solution to C storage and ecosystem function. However, impact of biochar on microbes and enzymes in subtropical mangrove ecosystems remains unknown, as well as the role of biochar in C sequestration. Mangrove, located between terrestrial and marine ecosystems, is frequently affected by ocean tides and anthropogenic inputs of contaminants from land sources (Kristensen et al., 2008; Li et al., 2011). It contains approximately 2.2% of the global C storage, but exports 10% of the global terrestrial flux of dissolved organic C (DOC) to coastal oceans (Bergamaschi et al., 2012; Donato et al., 2012; Tue et al., 2012). Thus, studies on C sequestration in mangrove ecosystems are meaningful.

In this study, we aimed to determine the potential influences of biochar addition on the decomposition of SOM in a subtropical mangrove ecosystem through observing the changes of microbes and enzymes. Therefore, the scope of this work was to investigate (i) the concentration of soluble phenolics, which is reported to play an important role in the activities of hydrolase involved in C, N and P cycling in peatlands (Freeman et al., 2001), (ii) microbial abundance including bacteria and fungi, which constitute approximately 91% of the total microbial biomass in mangroves (Holguin et al., 2001), (iii) the activities of extracellular enzymes involving in C, N and P cycling, as well as the enzymatic ratios, which could indicate the acquisition of nutrients (Moorhead et al., 2013). Moreover, the increase of C content caused by biochar addition was estimated to further understand the importance of biochar on C storage in this ecosystem.

2. Materials and methods

2.1. Characteristics of sediment and biochar

The sediment sample used in this study was collected from Mai Po Nature Reserve (22°29′N to 22°31′N and 113°59′E to 114°03′E), an intertidal estuary of the Pearl River Delta in Southeast China. This reserve is the largest remaining coastal wetland and located at the northwestern corner of the New Territories of Hong Kong (Li et al., 2011). Sediment samples were taken from three randomly chosen locations for the surface layer (0–2 cm) of intertidal zone and mixed thoroughly to form a composite sample. The physicochemical properties were analysed in our previous study (Luo and Gu, 2015). Total C, N and P of sediment were 15.9, 1.70 and 1.39 mg/g, respectively. The water content was 63.2%, and the pH value was 7.30, close to neutral. The biochar used in this study was made by pyrolysis of bamboo residues at 600 °C for 2 h, and its characteristics were determined before (Xu et al., 2012). The surface area of this biochar was 332 $m^2\ g^{-1}$ and the content of C and N 67.48% and 0.77%, respectively (Xu et al., 2012). In this study, biochar was ground to pass through 250 μm mesh sieve before use.

2.2. Incubation experiment

A set of 40 g (wet weight) of sediment samples were spiked with biochar in sterile 50 mL centrifuge tubes to yield the final concentration of 0%, 0.5%, 1% and 2% (dry weight basis) (Demisie et al., 2014; Fox et al., 2014; Prayogo et al., 2014). The sediments with biochar were thoroughly mixed with stainless spatula. Afterward, the tubes were sealed with caps to prevent water loss, and then incubated at 25 °C for 90 days (Lou et al., 2012). Three replicates were prepared for each concentration of biochar, and the sediment with 0% biochar was used as control.

At the end of incubation, sediment organic C (SOC) and TN were analysed by elemental analyzer (Eurovector EA3028, UK). TP was measured according to an analytical protocol developed by the Standards Measurements and Testing Program of the European Commission (SMT protocol) (Ruban et al., 1999). Besides, the soluble phenolics, inhibitors of microbes and hydrolases activities, were extracted by distilled water (2 g wet sediment: in 10 mL deionized water) and determined by Folin Ciocalteu Method (Toberman et al., 2008).

2.3. Enzyme assay

A series of extracellular enzymes, involved in C, N and P cycling, were assayed for biochar-amended sediments. Phenol oxidase (PHO), an enzymic ‘latch’ of C storage, has been considered to regulate hydrolases (Fenner et al., 2005; Freeman et al., 2001; Toberman et al., 2008). Peroxidase (POD) could oxidize many organic molecules and contribute to lignin degradation (Arora et al., 2002; Stursova and Sinsabaugh, 2008). β-glucosidase (GLU), hydrolysing cellulose and polymeric saccharides to glucose, is the most commonly measured indicator for C dynamics. *N*-acetyl-glucosaminidase (NAG) and acid phosphatase (ACP) often serve as indicators for N and P acquisition, respectively (Moorhead et al., 2012; Sinsabaugh and Moorhead, 1994). The substrate and buffer used for enzyme assays are listed in Table 1, and all enzymes were