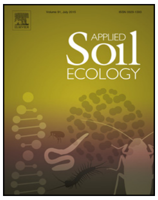
Applied Soil Ecology 91 (2015) 68–79

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China

Junhui Chen [a,b], Xiaoyu Liu [b], Lianqing Li [b], Jinwei Zheng [b], Jingjing Qu [b], Jufeng Zheng [b], Xuhui Zhang [b], Genxing Pan [b,*]

[a] *Zhejiang Provincial Key Laboratory of Carbon Cycling in Forest Ecosystems and Carbon Sequestration, School of Environmental and Resource Sciences, Zhejiang A & F University, Lin'an, Hangzhou 311300, China*
[b] *Institute of Resource, Ecosystem and Environment of Agriculture, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China*

ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form 26 February 2015
Accepted 28 February 2015
Available online 10 March 2015

Keywords:
Biochar
Rice paddy
Abundance and diversity
Pyrosequencing
Functional group
Bacterial community

ABSTRACT

Biochar functionality related to soil microbial community changes has not yet been fully understood. In this study, we present a cross site field experiment on bacterial community changes of rice paddies among three sites (Jiangxi province, JX; Hunan province, HN; and Sichuan province, SC) from South China with biochar amended (BSA) at 0, 20 and 40 t ha^{-1} before rice plantation in 2010. Changes in bacterial abundance and diversity of topsoil (0-15 cm) sampled at rice harvest were assessed. Increases in soil pH, soil organic carbon, total N, soil microbial biomass, as well as bacterial gene copy numbers and diversity indices (phylogenetic diversity, Shannon, Chao1 and OTU richness) were consistently observed under BSA at 40 t ha^{-1}, though generally insignificant at 20 t ha^{-1} across the sites. Cluster analysis of both terminal restriction fragment length polymorphism (T-RFLP) profiles and pyrosequencing of the 16S gene indicated a strong impact of biochar on bacterial community composition, though the changes were variable across the sites. In particular, BSA at 20 and 40 t ha^{-1} greatly increased the relative abundance of Betaproteobacteria (by 54% and 80%) and Deltaproteobacteria (by 164% and 151%) in JX while decreased Betaproteobacteria (by 46% and 52%) and increased Chloroflexi (by 27% and 61%) in SC site, respectively. However, no significant changes were detected in HN site. In addition, some significant but variable changes were observed in the abundance of nitrifying, denitrifying and N-fixing bacteria groups with biochar addition among sites. This study suggested a potential role of biochar in enhancing bacterial abundance, community diversity and modifying the community compositions, particularly of the bacteria involved in N cycling. However, changes in soil microbial structure and functioning related to biochar treatment deserve further studies.

1. Introduction

Incorporating of biochar of crop residues via pyrolysis into croplands has been considered a promising option to enhance soil organic carbon (SOC) sequestration and sustain crop productivity (Lehmann, 2007; Sohi, 2012). In comparison to field burning of crop residues, this approach could allow better controlled and cleaner combustion, reduce CO_2 emission, improve the recycling of nutrients and offer renewable energy (Knoblauch et al., 2011; Clare et al., 2014). The role of biochar soil amendment (BSA) had been well addressed in reducing non-CO_2 greenhouse gas (GHG) emissions in a number of field experiments (Hammond et al., 2013; Liu X.-y. et al., 2012; Zhang et al., 2010, 2013). Being usually alkaline, high in negatively-charged surface area and rich in recalcitrant carbon with highly porous structure, biochar could generally enhance crop productivity (Jones et al., 2012; Major et al., 2010) by a single or a combination of liming, moistening, aggregating and possible nutrient enhancing effects (Liu et al., 2013, 2014). However, biochar's functionality related to soil biochemical process associated with microorganisms is still poorly understood (Lehmann et al., 2011).

Functioning and sustaining soil fertility is known to be governed largely by the activity of soil microorganisms (Anderson,

---

Abbreviations: BSA, biochar soil amendment; SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; T-RFLP, terminal restriction fragment length polymorphism; qPCR, quantitative real-time PCR.

* Corresponding author at: Institute of Resource, Ecosystem and Environment of Agriculture, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China. Tel.: +86 25 8439 6027; fax: +86 25 8439 6027.
E-mail address: pangenxing@aliyun.com (G. Pan).

http://dx.doi.org/10.1016/j.apsoil.2015.02.012
0929-1393/© 2015 Elsevier B.V. All rights reserved.

2003). Changes in crop productivity and reduction of GHG emissions could be regulated by the changes in microbial communities. Constituting more than 90% of the total soil microbial biomass, bacteria together with fungi are the key regulators of soil organic matter dynamics and nutrient transformation (Six et al., 2006). Community diversity and activity of bacteria are considered more sensitive than other biota in response to soil condition changes such as pH, aeration and C and N status (Griffiths et al., 2001). Biochar amendment could alter both biotic and abiotic soil properties, which could potentially modify either microbial biomass or community composition or both in biochar amended soils (Lehmann et al., 2011). For example, increase in gram-negative bacterial biomass was observed in soils with addition of glucose and yeast derived biochar in a greenhouse experiment via phospholipid fatty acid analyses (Steinbeiss et al., 2009). With the help of nano-scaled electron microscopy, soil microorganisms were shown in close association with and/or colonizing on biochar particles in amended soil (Jin, 2010; Lehmann and Joseph, 2009). Moreover, improved crop productivity and soil quality with BSA could be related to an alteration in bacterial community composition and abundance (Lehmann et al., 2011; Graber et al., 2010; Kolton et al., 2011; Steiner et al., 2008). In particular, Terra preta, an anthropogenic dark earth rich in black carbon in the Amazon basin, has shown greater bacterial biomass and diversity than the adjacent soils which generally showed poor fertility (Kim et al., 2007). In a work by Graber et al. (2010), enhanced pepper plant development with BSA could be partly explained by the presence of some plant growth promoting and/or bio-controlling bacteria in the biochar-treated pots. Recently, there have been increasing observations of biochar induced systemic resistance (ISR) to pathogens of plants with plant growth-promoting rhizobacteria between plant roots in pot experiments (Elad et al., 2010; Meller Harel et al., 2012). However, robust changes in bacterial abundance and functional diversity under BSA have not yet been well addressed in field conditions.

Rice paddy soils were characterized by low carbon turnover with a dominance of anaerobic microbial communities under mostly anoxic conditions (Conrad and Klose, 1999; Lu et al., 2004). Covering approximately 20% of the world total irrigated croplands (Frolking et al., 2002), rice paddy soils of China are also important due to their high C storage and sequestration potential (Pan et al., 2003). BSA has been suggested to be effective in reducing N_2O emissions from rice paddies in field experiments across sites of China (Zhang et al., 2010; Liu X.-y. et al., 2012), but biochar's potential role to induce changes in microbial communities related to C and N cycling processes are still poorly understood. In particular, BSA even caused reduction in CH_4 emission from rice paddy soils (Feng et al., 2012; Liu et al., 2011), suggesting potential changes in functioning of taxa-specific soil bacterial/archaeal community in biochar-treated soils. Increased bacterial but declined fungal gene abundance with shifted community structure as a consequence of BSA amendment was also observed in a slight acid rice paddy from South China (Chen et al., 2013). So far, the consistence of these changes with the same biochar across soils has not yet been assessed.

In-depth understanding of the changes in soil bacterial community composition following BSA with powerful tools would be crucial for biochar application in agriculture. Next-generation sequencing technology, such as 454 pyrosequencing, is capable to massively deep-sequence microbial communities in environmental samples without the time-consuming cloning procedure (Sundberg et al., 2013), and has been widely used in microbial ecology studies in various environments including soils (Roesch et al., 2007) and sewage treatment systems (Zhang T. et al., 2012). In a study with biochar amendment in pot experiment, Anderson et al. (2011) assigned the T-RFLP peaks to a database obtained with 454 pyrosequencing. In their work, some specific groups of bacteria involved in N cycling processes were found positively influenced by biochar, including Bradyrhizobiaceae of the class Alphaproteobacteria, Hyphomicrobiaceae of the order Rhizobiales within the class Alphaproteobacteria and Streptosporangineae as a suborder of *Actinomycetales* within the phylum Actinobacteria. Using pyrosequencing of 16S rRNA fragments from root-associated bacterial communities also in a pot experiment, Kolton et al. (2011) reported an improved growth of sweet pepper with BSA due to an augment of a few genera-specific bacteria in the rhizophere (eg. *Flavobacterium* and *Chitinophaga* affiliated with phylum Bacteroidetes, possessing an arsenal of extracellular enzymes involved in organic matter mineralization). Therefore, these shifts in groups of bacterial community revealed by pyrosequencing technology could provide fundamental information of biochar's role in modifying soil microbial community abundance and composition, and thus mediating the biogeochemical cycling of C and N in amended soils.

The aim of the present study was to explore the potential impact of BSA on bacterial abundance, diversity and community composition in rice paddies and the consistency across sites and soil conditions. Here, a randomized block design with and without BSA was laid out across three sites to assess the effects of biochar, site and their interaction. Chemical and microbiological analyses were conducted on topsoil samples collected from rice paddies under BSA (0, 20 and 40 t ha^{-1}) after a whole rice growing season across three sites from South China. The impact of biochar on bacterial abundance and diversity was evaluated using quantitative real-time PCR (qPCR), T-RFLP and 454 pyrosequencing technologies.

2. Materials and methods

2.1. Sites and field experiment

A cross-site field experiment with BSA to rice paddy was conducted in the typical rice production areas of South China (Liu X.-y. et al., 2012). Included in this study, the three sites were respectively JX at the Experimental Farm of Jiangxi Institute of Red Soils (28°15′N, 116°20′E) in Jinxian County of Jiangxi Province, HN at Ganshan Township (28°08′N, 113°12′E) in Changsha Municipality of Henan Province, and SC at Xigao Township (31°03′N, 104°10′E) in Guanghan Municipality in Sichuan Province, of China. Site conditions and soil properties of the three rice paddies are listed in Table 1.

The biochar used in this study was produced in a biochar plant, with a business scale technology of continuous pyrolysis using a vertical kiln (Pan et al., 2011) at Sanli New Energy Company Henan, China. With this technology, wheat straw was continuously pyrolysed at 350–550 °C in a vertical kiln to produce tons of biochar in a day, which was aggregated and homogenized to a large biochar stock. Biochar material from the same stock was ground by machine to pass through a 2 mm sieve and used consistently across sites when the rice paddy field amendment was operated. The basic properties of biochar were measured of repeated samples of the stock before the field use and were widely reported in our previous studies (Zhang A. et al., 2012; Zhang et al., 2010, 2013; Liu X.-y. et al., 2012). On average, the biochar material contained on dry base organic carbon of 467 g kg^{-1}, total N of 5.9 g kg^{-1}, ash of 20.8% and had a surface area of 8.92 m^2 g^{-1} with a bulk density of 0.59 g cm^{-3} and pH (H_2O) of 10.42 as well as a cation exchange capacity of 21.7 cmol kg^{-1}.

The set-up and performance of the field experiment has been previously described in detail (Liu X.-y. et al., 2012). Briefly, biochar was amended at rates of 0 (C0, control), 20 (C1) and 40 (C2) t ha^{-1} before rice (*Oryza sativa* L.) transplantation in May 2010. The biochar material was spread to soil surface and tilled into a depth of approximately 0–12 cm by raking. Urea was applied for rice