Science of the Total Environment 562 (2016) 379–390

Contents lists available at ScienceDirect

Science of the Total Environment

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

Soil biochar amendment shapes the composition of N_2O-reducing microbial communities

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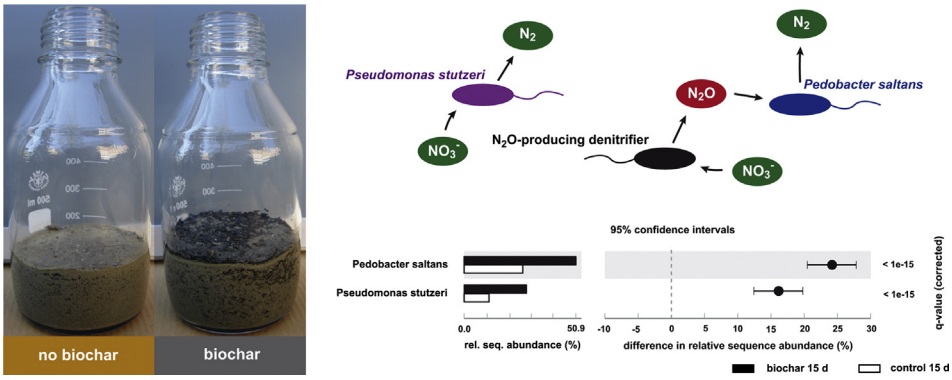
HIGHLIGHTS

- Biochar promoted anaerobic, alkalinity-adapted, and polymer-degrading microbial taxa.
- Biochar fostered the development of distinct N_2O-reducing microbial taxa.
- Taxonomic shifts among N_2O-reducing microbes might explain lower N_2O emissions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 January 2016
Received in revised form 24 March 2016
Accepted 28 March 2016
Available online xxxx

Editor: Simon Pollard

Keywords:
Nitrogen cycle
Biochar
Nitrous oxide emission
nosZ genes
Soil microbial community
454 amplicon pyrosequencing

ABSTRACT

Soil biochar amendment has been described as a promising tool to improve soil quality, sequester carbon, and mitigate nitrous oxide (N_2O) emissions. N_2O is a potent greenhouse gas. The main sources of N_2O in soils are microbially-mediated nitrogen transformation processes such as nitrification and denitrification. While previous studies have focused on the link between N_2O emission mitigation and the abundance and activity of N_2O-reducing microorganisms in biochar-amended soils, the impact of biochar on the taxonomic composition of the *nosZ* gene carrying soil microbial community has not been subject of systematic study to date. We used 454 pyrosequencing in order to study the microbial diversity in biochar-amended and biochar-free soil microcosms. We sequenced bacterial 16S rRNA gene amplicons as well as fragments of common (typical) *nosZ* genes and the recently described 'atypical' *nosZ* genes. The aim was to describe biochar-induced shifts in general bacterial community diversity and taxonomic variations among the *nosZ* gene containing N_2O-reducing microbial communities. While soil biochar amendment significantly altered the 16S rRNA gene-based community composition and structure, it also led to the development of distinct functional traits capable of N_2O reduction containing typical and atypical *nosZ* genes related to *nosZ* genes found in *Pseudomonas stutzeri* and *Pedobacter saltans*, respectively. Our results showed that biochar

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http://dx.doi.org/10.1016/j.scitotenv.2016.03.220

amendment can affect the relative abundance and taxonomic composition of N_2O-reducing functional microbial traits in soil. Thus these findings broaden our knowledge on the impact of biochar on soil microbial community composition and nitrogen cycling.

1. Introduction

Biochar is a carbon-rich solid produced by thermal decomposition of organic material under low oxygen conditions. Biochar has recently gained a lot of attention as soil additive because of its soil quality enhancing properties (Atkinson et al., 2010; Lehmann et al., 2011). Biochar production and soil amendment has been discussed as one way to address environmental issues related to current agricultural practices and mankind's use of fossil energy sources, such as problems associated with the excessive use of synthetic fertilizers and greenhouse gas emission from combustion processes (Atkinson et al., 2010; Clough and Condron, 2010; Sohi, 2012). For example, it has been shown that the physicochemical properties and reactivity of biochar in soil can help decrease nutrient leaching and greenhouse gas emission. Biochar's physicochemical properties vary widely with the type of feedstock and pyrolysis condition but the majority of biochars share common characteristics, such as a high content of aromatic carbon structures, an elevated pH, and a large surface area (Atkinson et al., 2010; Singh et al., 2010). Despite variations in physicochemical properties among different biochars, several studies showed that soil biochar amendment does reduce soil nitrous oxide (N_2O) emissions and leads to shifts in the soil microbial community composition (Cayuela et al., 2014; Khodadad et al., 2011; Kuzyakov et al., 2014). However, if and how these are linked has not yet been intensively studied.

N_2O is a potent greenhouse gas with an atmospheric lifetime of 114 years and an almost 300-fold greater global warming potential compared to CO_2 (Thomson et al., 2012). Microbially-mediated nitrogen transformation reactions in soils represent world's largest sources of atmospheric N_2O (Thomson et al., 2012). In soils, denitrification represents one of the major N_2O producing pathways. N_2O is an obligate intermediate of microbial denitrification, which occurs frequently in oxygen-limited soil horizons and anoxic microsites, especially in the presence of high amounts of nitrogen fertilizer (Braker and Conrad, 2011; Pfab et al., 2011; Philippot et al., 2009). Denitrification describes the stepwise reduction of nitrate (NO_3^-) to dinitrogen gas (N_2) and is performed by many facultative and strict anaerobic chemoorganotrophic bacteria of which many belong to the phylum Proteobacteria (Braker and Conrad, 2011; Philippot et al., 2007). The enzymes catalyzing these stepwise reduction reactions are encoded by the functional genes *narG* and *napA* (nitrate reductases), *nirK* and *nirS* (nitrite reductases), *norB* (nitric oxide reductase), and *nosZ* (nitrous oxide reductase) (Philippot et al., 2007; Richardson et al., 2009). Due to the fact that some denitrifiers lack a functional *nosZ* gene and the high oxygen and pH sensitivity of N_2O reductases, denitrification is often incomplete and N_2O is released and not reduced to N_2 (Bakken et al., 2012; Mckenney et al., 1994; Philippot et al., 2011). Besides denitrification, other microbial processes also contribute to the formation of N_2O (e.g. nitrifier denitrification, nitrification) under certain environmental conditions. However, the only known biological sink for N_2O is the reduction of N_2O to N_2 by N_2O-reducing microorganisms (Thomson et al., 2012).

Many "classical" denitrifiers belonging to the Proteobacteria contain a typical *nosZ* gene and synthesize Z-type nitrous oxide reductases (Philippot et al., 2007; Zumft and Körner, 2007). Recently the existence of a second clade of N_2O reducers comprising microorganisms from several different phyla has been demonstrated. These organisms possess a phylogenetically distinct atypical *nosZ* gene (Jones et al., 2013; Philippot et al., 2007; Sanford et al., 2012; Zumft and Körner, 2007). About half of the atypical *nosZ* gene containing microorganisms do not carry the functional genes encoding nitrate, nitrite and nitric oxide reductases and are thus only capable of reducing N_2O to N_2 (Jones et al., 2014; Orellana et al., 2014). Interestingly, atypical *nosZ* gene carrying N_2O reducers outnumber typical *nosZ* gene carrying microorganisms in many environments suggesting that the ratio of typical to atypical *nosZ* gene carrying microbial populations might impact soil N_2O emissions (Jones et al., 2014; Orellana et al., 2014).

To date, most studies on the effect of soil biochar amendment on the microbial community composition used molecular fingerprinting techniques such as PLFA (Gomez et al., 2014), DGGE (Chen et al., 2013), or T-RFLP (Anderson et al., 2011) to analyze community shifts. Other studies relying on next generation sequencing approaches mainly targeted the bacterial 16S rRNA gene to describe the microbial community composition (Chen et al., 2015; Kolton et al., 2011). According to these studies, soil biochar amendment increases the ratio of bacteria to fungi, the proportion of gram-negative over gram-positive bacteria, and the relative abundance of bacterial taxa capable of degrading aromatic hydrocarbons and transforming (in)organic nitrogen species (Anderson et al., 2011; Chen et al., 2013; Chen et al., 2015; Gomez et al., 2014; Kolton et al., 2011). Biochar-induced taxonomic shifts among nitrogen-transforming functional groups of microorganisms, especially microbial taxa capable of nitrous oxide reduction, might directly affect soil N_2O release, because microbial nitrous oxide reduction is the main biotic sink of atmospheric N_2O (Thomson et al., 2012).

It is known from several studies that soil biochar amendment can change the abundance and activity of denitrifiers, especially of N_2O-reducing microorganisms possessing a typical *nosZ* gene (Ducey et al., 2013; Harter et al., 2014; Van Zwieten et al., 2014). An increased abundance and activity of typical *nosZ* gene containing N_2O reducers is thought to be one of the reasons for the frequently observed decreased N_2O emissions from biochar-amended soils (Harter et al., 2014; Van Zwieten et al., 2014). However, the impact of biochar on the taxonomic composition of typical and atypical *nosZ* containing N_2O-reducing microbial communities has not been studied in detail yet. Since it has been shown that individual N_2O-reducing taxa can differ considerably in N_2O reduction activity, taxonomic shifts among typical and atypical *nosZ* gene carrying N_2O-reducing microbial communities might affect N_2O reduction rates and net soil N_2O release (Cavigelli and Robertson, 2001; Tago et al., 2011).

In order to investigate biochar-induced shifts in the taxonomic composition and structure of the soil bacterial community, as well as shifts among the typical and atypical *nosZ* gene carrying microbial communities, we performed 454 amplicon pyrosequencing on soil samples collected from a previously conducted soil microcosm experiment described in detail in Harter et al. (2014). The main objectives of this study were to i) describe biochar-induced taxonomic shifts among the soil bacterial community, to ii) investigate if soil biochar amendment can affect the composition of the typical and atypical *nosZ* gene carrying microbial communities, and to iii) evaluate if changes in the functional community composition might help to explain the observed reduction in N_2O emissions after biochar amendment in the soil microcosm experiment performed by Harter et al. (2014).

2. Material and methods

The soil samples analyzed in this study were collected from a microcosm experiment previously described by Harter et al. (2014). We briefly describe the experimental setup and sample collection below. A detailed description of the experimental setup and the geochemical