European Journal of Soil Biology 74 (2016) 1–8

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure

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A R T I C L E I N F O

Article history:
Received 22 July 2015
Received in revised form
16 February 2016
Accepted 17 February 2016
Available online 11 March 2016

Handling Editor: C.C. Tebbe

Keywords:
Biochar
Nitrogen leaching
Microbial biomass
Bacterial community structure
High-throughput sequencing

A B S T R A C T

Previous studies already demonstrated that biochar addition reduces nitrogen (N) leaching in soil, but little information is available about its effects on N leaching and bacterial community structure under the application of organic N. This study investigated the effects of corn-straw biochar under the application of urea (250 kg N ha^{-1}) in layered soil columns. The PCR-amplified partial 16S rRNA genes in soil were sequenced before and after biochar treatment in order to assess the change of bacterial diversity and community structure utilizing the Illumina technology. With the application of 2% (B2), 4% (B4) and 8% (B8) biochar (mass ratio), the cumulative amount of total leached nitrogen was reduced by 18.8%, 19.5% and 20.2%, respectively ($P < 0.05$). More than 90% of the total nitrogen leaching was in the form of nitrate, and increasing amount of biochar resulted in reduced amount of N leaching. The water holding capacity, microbial biomass, pH, electrical conductivity, net N mineralization and respiration rate of the soil were all increased under biochar treatments, except that the B8 treatment decreased soil respiration rate and net N mineralization in comparison with B4. Bacterial diversity increased in biochar-amended soil and was positively correlated with the addition ratio of biochar. Dominant phyla across all samples were *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Bacteroidetes*, *Actinobacteria*, *Nitrospirae* and *Gemmatimonadetes*. The relative abundance of *Acidobacteria*, *Chloroflexi* and *Gemmatimonadetes* decreased under biochar treatments, while that of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* increased. Overall, biochar increased water holding capacity, enhanced microbial biomass and changed bacterial community structure of the soil which may all have contributed to the reduction of nitrogen leaching.

1. Introduction

Excessive and/or unbalanced application of nitrogen fertilizers has caused the translocation of nitrogen (N) from farmlands into aquatic systems. Nitrogen, especially in the form of nitrate, is easily soluble in soil pore water, and readily infiltrates beneath the active soil layer with crop root. The N leaching may deplete soil fertility, accelerate soil acidification and reduce crop yields [1]. Moreover, N leaching is regarded as a major contributor to the eutrophication of surface and ground water [2].

Recently, the interest in applying biochar in soil has grown, which is due to the dual benefits of biochar on both climate change mitigation and positive soil amendment [3,4]. Biochar is a solid carbon-rich organic material generated by heating biomass under condition of limited or no oxygen [5]. Previous studies already demonstrated that biochar addition reduces N leaching. This could be attributed to the increase of cation and anion exchange capacities (CEC, AEC) of soil by the biochar material [6,7]. Another reason could be the physical retention of available N dissolved in the soil solution, as the water holding capacity also increased in biochar-amended soil [6]. Although organic N, like urea, is widely used in agriculture, little information is available about the effect of biochar on N leaching and biological interactions under the application of organic N.

Soil amendment with biochar could modify physical and chemical properties of the habitat for microbial colonization, and therefore affect soil microbial activity and community structure [8,9]. Transformation of microbial communities can be associated

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http://dx.doi.org/10.1016/j.ejsobi.2016.02.004
1164-5563/

with the change of nutrient turnover and utilization after the addition of biochar [10]. The carbon-rich “Amazonian dark soils” (Arthrosols) are evidence, which host distinct microbial communities in comparison with adjacent carbon-poor soil, and have higher microbial biomass and diversity as well [11]. Various methods have been used to investigate the microbial communities in biochar-amended soils. The bacterial community composition in Brazilian anthrosols and adjacent soils was investigated by using traditional culturing [11]. Some studies adopted phospholipid fatty acids analysis to describe soil microbial communities responding to biochar [12,13]. PCR-denaturing gradient gel electrophoresis method was also widely deployed to analyze the changes of microbial structure under the addition of biochar [14,15]. However, most of these studies were conducted under a constant incubation condition, instead of the simulated N leaching condition which would be better. Also, the previous studies have only considered the dominant microbial taxa, while next-generation DNA-sequencing technologies of PCR products now offer the potential to also detect less abundant taxa and thus giving a more complete picture of the microbial communities [16].

The main objectives of the present work were thus to (i) study the effect of biochar on N leaching through different soil layers during the application of organic N fertilizer to agricultural soil in layered columns; and (ii) to investigate the effect of biochar on soil bacterial community structure under simulated leaching condition via high-throughput sequencing method.

2. Materials and methods

2.1. Soil and biochar materials

Plow layer soil was collected from a farmland at the fluvo-aquic soil test base of Chinese Academy of Agricultural Sciences, Changping County, Beijing, China. The soil was air-dried and passed through a 2 mm nylon sieve and mixed to get a homogeneous soil sample before use. Corn straw (*Zea mays* L.) was oven dried (80 °C) and converted into biochar through slow pyrolysis using a furnace (Olympic 1823HE) in a N_2 environment at 500 °C for 1.5 h. Biochar samples were ground and sieved to get <1 mm sized particles.

Basic properties of the tested soil and corn straw biochar are presented in Table 1. The soil had a high pH value due to the presence of many coral limestone fragments that released calcium ions. Compared to soil, biochar had a higher pH (10.0) and electrical conductivity (EC, 1319 μS cm^{-1}). The pH and EC of soil and biochar (1:5 and 1:10 w/v, respectively) were measured in deionized water using a pH meter (Mettler Toledo Delta 320) and an electrical conductivity meter (DDS-307A), respectively. The concentrations of soil ammonium and nitrate were determined using a flow injector auto analyzer (Auto Analyzer 3, High Resolution Digital Colorimeter, Germany) in 1 M KCl extract (1:10 w/v) [17]. The CEC of soil and biochar was measured with the ammonium-acetate compulsory displacement method [18].

Ash content was determined by combusting the biochar at 750 °C for 6 h in open crucibles on a dry weight basis. The carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) contents of biochar were measured using an elemental analyzer (vario PYRO cube, Germany). Brunauer–Emmett–Teller specific surface area of the biochar was determined using nitrogen gas on a Micrometrics ASAP 2010 system (Micrometrics, Norcross, GA, USA).

2.2. Leaching experiment

Layered soil column was constructed according to previous methods [19,20] as depicted in Fig. S1. This device consisted of three separated sections which were well joined and sealed throughout the experiment. Soil column with dimension of 10 cm inner diameter and 42 cm length were constructed with polymethyl methacrylate pipes and fitted with polyvinyl chloride end-cap. There were three sampling openings on the column, i.e. a small hole drilled on the sidewall at three different heights, representing soil depths of 10, 20, and 30 cm along the soil profile. The sampling opening was for extraction of soil leachate from different layers in the profile. A 5-cm thick layer of coarse sand was placed at the bottom of each column to further prevent soil loss. A simple water container was used for supplying deionized water to the column to simulate leaching conditions. About three kilograms of prepared soil were packed into the columns to achieve an initial bulk density of about 1.3 g cm^{-3}. The top 10 cm soil in the column was subjected to thorough mixing with 0.325 g urea (equal to 250 kg N ha^{-1}) and biochar with four different application rates of 0, 2, 4 and 8% (mass ratio of biochar/soil, equivalent to 0, 40, 80 and 160 t ha^{-1}), which were designated as CK, B2, B4 and B8, respectively. Three replicates were conducted for each treatment. The columns were kept in an artificial greenhouse at 25 ± 2 °C and a relative humidity of 65%.

Before starting the leaching experiment, about 1200 mL deionized water was added from the top of each column over a period of 7 days for the initiation of ammonification and nitrification in soil. During the leaching period, deionized water was added slowly into each column. Around 10 mL leachate was sampled from the openings at 10, 20 and 30 cm depth along the soil column respectively. Once the leachate volume approached 10 mL, the addition of deionized water was suspended, and the total volume of leachate was measured. All the sampling openings were sealed when not sampling. Leachate was sampled at an interval of half a month during the first three months and of one month during the last two months for a total period of five months. The leachate samples were filtered through disposable 0.45 μm pore-size filters (Whatman, Clifton NJ, USA) and analyzed for pH, EC, nitrate, ammonium and total N according to the methods mentioned above.

Table 1
Basic physiochemical properties of the tested soil and corn straw biochar.

Soil		Corn straw biochar	
pH	8.1	pH	10.0
Organic matter (g kg^{-1})	16.4	C (%)	58.0
Ammonium N (mg kg^{-1})	0.8	H (%)	2.7
Nitrate N (mg kg^{-1})	5.7	O (%)	21.5
Soil bulk density (g cm^{-3})	1.58	N (%)	2.3
Cation exchange capacity (cmol kg^{-1})	17.4	Ash content (%)	16.7
Electrical conductivity (μS cm^{-1})	141.4	Cation exchange capacity (cmol kg^{-1})	23.8
Field moisture capacity (%)	24.8	Electrical conductivity (μS cm^{-1})	1319
		Specific surface area (m^2 g^{-1})	14.7