Soil Biology & Biochemistry 91 (2015) 151–159

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Impact of long-term N additions upon coupling between soil microbial community structure and activity, and nutrient-use efficiencies

Yangquanwei Zhong, Weiming Yan, Zhouping Shangguan*

State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, PR China

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form
15 August 2015
Accepted 21 August 2015
Available online 13 September 2015

Keywords:
Bacterial community
Fungal community
Nitrogen addition
CUE
NUE

ABSTRACT

Many soil ecosystems receive elevated inputs of nitrogen (N) from anthropogenic sources, and it is critical to understand how these increases in N availability affect soil microbial communities. In this study, we investigated the changes of soil physical-chemical parameters and structural shifts in the microbial community after 10 years of N addition (urea fertilizer), in a wheat field with five rates of N application (0, 90, 180, 270 and 360 kg N ha^{-1}), and conducted a laboratory incubation experiment to detect microbial community activity. Our results showed that the soil physical–chemical properties were changed after long-term N fertilization, and these changes correlated with microbial community structure, associated with changes in abundance of certain microbial species. The resulting shift in the community was associated with concomitant changes in microbial activities, which showed different carbon-use efficiency: nitrogen-use efficiency (CUE:NUE) ratios. A threshold rate of 180 kg ha^{-1} N fertilization per year caused the decline of microbial activity.

1. Introduction

Worldwide, many soil ecosystems are receiving large amounts of nitrogen (N) from anthropogenic activities (Ramirez et al., 2012). In particular, large quantities of N (frequently >100 kg N ha^{-1}) are applied directly to soils as fertilizer in farmland ecosystems each year (Fierer et al., 2011). Soil microbes exhibit diverse metabolic functions that affect the soil nutrition cycle and plant health (Kennedy, 1999). The addition of N has potentially important impacts on the diversity and activity of microbial communities, which are mediated in belowground carbon (C) and N dynamics (Fierer et al., 2011; Philippot et al., 2013).

For decades, microbial activity has been studied by many methods that measure soil enzymes, respiration rates, microbial biomass and other indexes (Gallo et al., 2004; Ramirez et al., 2012). Strong reductions in soil microbial activity caused by N addition have been documented in both field and lab-based studies, and the magnitudes of reduction in soil respiration and microbial biomass are strongly related to the duration and amount of N added to the ecosystem (Treseder, 2008; Janssens et al., 2010; Ramirez et al., 2010). A microbial activity shift led by N addition could lead to changes in C dynamics, as reported by Mooshammer et al. (2014), who suggested that microbial communities have the ability to regulate nitrogen-use efficiency (NUE) and carbon-use efficiency (CUE) to manage resource imbalances. For decades, hypotheses were proposed to explain the paradox of declining soil microbial activity with the addition of N, with some based on nutritional theory (Gallo et al., 2004; Moorhead and Sinsabaugh, 2006; Craine et al., 2007; Meier and Bowman, 2008) and others based on the copiotrophic hypothesis, which states that a shift in the microbial community causes changes in the activity of the community (Fontaine et al., 2003; Ramirez et al., 2010; Fierer et al., 2011). With the development of high-throughput sequencing technologies, a more comprehensive understanding of microbial community structure can be obtained, which will help to further reveal the relationships between community structure and changes in activity, and might yield support for the copiotrophic hypothesis (Ramirez et al., 2012). The effects of N addition on the composition of the microbial community are variable (Williams et al., 2013), and the primary factors that cause the changes in the microbial community and in the relationships between the different microbial taxa and the changes in activity are not clear (Fierer et al., 2011). Moreover, there may a threshold for the effect of N fertilization on the microbial structure and activity, as reported by Yao et al. (2014) in a grassland, and whether the N addition rate has a more general

* Corresponding author. Xinong Rd. 26, Institute of Soil and Water Conservation, Yangling, Shaanxi 712100, PR China. Tel.: +86 29 87019107; fax: +86 29 87012210.
E-mail address: shangguan@ms.iswc.ac.cn (Z. Shangguan).

http://dx.doi.org/10.1016/j.soilbio.2015.08.030
0038-0717/

threshold above which changes occur to the microbial community remains unclear. Therefore, a comprehensive approach that considers microbial respiration and CUE:NUE could be beneficial and used to evaluate the activity of microbes altered by changes in the community.

In our study, we analyzed the soil microbial community structure and activity in a controlled experimental plot that received 10 years of N fertilization with five levels of N, in addition to a bare land without crops and fertilizer inputs. The Illumina MiSeq sequencing was used to determine the phylogenetic structure of the soil bacterial communities, and the nutritional stoichiometry was used to determine the activities and the effects on the microbial C and N dynamics. Our aim was to detect the major soil parameters affected by N addition that cause changes in community structure in both bacteria and fungi. We hypothesized that a threshold N rate may need to be reached before community enhancement shifts to community impairment (H1) and that microbial diversity and community structure shifts correlated with changes in activity (H2). Our results could provide insight into how N enrichment effects communities and causes changes in C and N dynamics.

2. Materials and methods

2.1. Experimental site and climatic conditions

The study was conducted in an experimental field of the Institute of Soil and Water Conservation at Northwest A & F University, Yangling, Shaanxi (34°17′56″ N, 108°04′7″ E) beginning in October 2004. The experimental site, which is located on the southern boundary of the Loess Plateau, experiences a temperate, semi-humid climate, a mean annual temperature of 13 °C and mean annual precipitation of 632 mm, with approximately 60% occurring between July and September. The soil type is Lou soil (Eum-Orthic Anthrosol).

2.2. Experimental design

The study involved a randomized block design and six three-replicate treatments, five N treatments and a bare land treatment (BL). N was applied at the following rates: 0, 90, 180, 270 and 360 kg N ha^{-1} (termed N0, N90, N180, N270 and N360 hereafter), and only winter wheat (*Triticum aestivum* L. cv., Changhan No. 58) was cultivated. Each plot had an area of 2 × 3 m and contained 20 15-cm-spaced rows of wheat, which were sown with 90 seeds. N was applied in the form of urea, and P was applied in the form of super phosphate (33 kg P ha^{-1}); applications were the same across all treatments. During the study, the soil was not irrigated, weeds were regularly removed, and no tillage occurred during the growth stage. The BL treatment received no fertilization and had no crops, all other field management practices were identical to those of the treated plots.

2.3. Soil sampling

In the 2014 harvest stage, which represented the 10th year of N application, nine soil samples were sampled from the 0–20 cm soil layer in each treatment plot using the diagonal method and a soil-drilling sampler (5-cm inner diameter). All of the samples were sieved through a 2-mm screen, and the roots and other debris were removed. A portion of the soil was stored at 4 °C for analyses of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). The remaining soil samples from each plot were combined into single samples, and a portion of each soil sample was collected in a 50 mL centrifuge tube, placed in an icebox and transferred to the laboratory. The tubes were archived at −80 °C until soil DNA extraction. The remaining soil was used for incubation and measurement of the soil physicochemical properties (soil pH, soil organic carbon (SOC), soil total nitrogen (TN), soil ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen, soil dissolved organic carbon (DOC) and organic nitrogen (DON)).

2.4. Soil physicochemical measurements

The soil pH was measured using a pH meter after shaking the soil–water (1: 2.5 w/v) suspension for 30 min. The SOC content was assayed via dichromate oxidation (Nelson et al., 1982). The soil TN content was assayed using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil NH_4^+ and NO_3^- nitrogen were extracted by vigorously shaking the sample with 50 mL of 1.0 mol l^{-1} KCl for 30 min; the extract was then filtered, and the NH_4^+ nitrogen and NO_3^- nitrogen concentrations were then measured using a continuous flow analytical system (Autoanalyzer 3, Bran + Luebbe, Germany). The soil MBC and MBN were estimated using the chloroform-fumigation extraction methods that were reported by Brookes et al. (1985) and Wu et al. (1990). The soils were suspended in water (1: 2 soil: water) for 30 min and filtered through 0.45-μm membranes to determine the soil DOC and DON. The organic C in the extracts was determined using an automated total organic C (TOC) analyzer (Shimadzu, TOC-Vwp, Japan), and the N was detected by the Kjeldahl method. Correction factors of 0.38 (K_C) (Vance et al., 1987) and 0.45 (K_N) (Brookes et al., 1985) were used for the calculated MBC and MBN values, respectively. All these soil physicochemical analyses were performed in duplicate.

2.5. Soil incubation and respiration measurements

From each plot, 1 kg of fresh soil collected from the nine soil cores using the diagonal method was weighed into pre-weighed 20-cm diameter and 10-cm tall PVC cores that were fitted with coarse filter paper on the bottom (Tiemann and Billings, 2011). The soil water content was measured using the oven-drying method. All samples were adjusted to 50% water-holding capacity and incubated at 25 °C in a humid environment, and the soil moisture was held constant throughout the incubation in a dark, enclosed climate chamber (AGC-D001P, Qiushi Corp., China). The soil water contents were adjusted by the weighing method. Soil respiration was measured once every two days over the 60-day incubation period using an LI-8100A soil respiration analyzer (Li-Cor, Lincoln, NE, USA). After the 60-day incubation, the MBC, MBN and other soil physicochemical properties that were measured before incubation were measured again.

2.6. DNA extraction and PCR amplification

Microbial DNA was extracted from 0.5 g soil samples using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The V4–V5 region of the bacterial 16S ribosomal RNA gene and the V4 region of the fungal 18S rRNA gene were amplified by PCR (95 °C for 2 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) by using primers 515F 5′-barcode-GTGCCAGCMGCCGCGG-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′ for 16S and primers 0817F 5′-barcode-TTAGCATGGAA-TAATRRAATAGGA-3′ and 1196R 5′-TCTGGACCTGGTGAGTTTCC-3′ for 18S, where the barcode is an eight-base sequence that is unique to each sample. The PCR reactions were performed in triplicate 20 μL mixtures, each containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM deoxynucleotide triphosphates (dNTPs), 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template