European Journal of Soil Biology 74 (2016) 121–127

Contents lists available at ScienceDirect

European Journal of Soil Biology

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

Original article

Responses of ammonia-oxidizing bacterial communities to land-use and seasonal changes in Mollisols of Northeast China

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ARTICLE INFO

Article history:
Received 18 October 2015
Received in revised form
29 March 2016
Accepted 30 March 2016
Available online 17 April 2016

Handling Editor: C.C. Tebbe

Keywords:
Cultivated
amoA gene
Fertilizer
Season
Community structure

ABSTRACT

In this study, AOB community was investigated over a 2-year period in cultivated Mollisols (0–20 cm) with three fertilizer treatments [no fertilizer (NoF), chemical fertilizer (CF) and chemical fertilizer plus manure (CFM)] and in an adjacent plot under natural restoration (NR). The crop rotation of maize, soybean and wheat was repeated every three years. The NR plot was abandoned agricultural land that was naturally revegetated with grasses. The AOB communities were characterized by clone sequencing of *amoA* gene targets, which encode ammonia monooxygenase subunit A. Phylogenetic analyses showed that the AOB communities in the Mollisols of Northeast China were related to defined groups, including clusters 1, 2, 3a, 3b, 9, and 10 in *Nitrosospira* and cluster 7 in *Nitrosomonas*. *Nitrosospira* cluster 3 was the most frequently recovered AOB in all treatments, and cluster 3a.2 and 3a.2-related sequences were common in cultivated soils. However, 12–27% of sequences were not related to any known AOB species, indicating a high diversity of AOB communities in Mollisols. There was a distinct AOB community in the NR plot and the cultivated plots, consistent with the hypothesis that land-use alters AOB community, and this appeared to be related to the soil pH. Cultivated soils with added N fertilizer had a greater frequency of clones in the *Nitrosospira* cluster 10 sequence type, but suppressed clones in the *Nitrosospira* cluster 3a.1 sequence type, whereas *Nitrosomonas* sequences were only recovered from the CFM treatment. The AOB clone frequency differed between the growing and non-growing seasons, possibly because the AOB communities were favoured by warm, moist soil conditions or the active plant rhizosphere during the growing season. The ecological implications of land-use change and seasonality on the contribution of these diverse AOB communities to primary production in Mollisols merits further attention.

1. Introduction

Soil microorganisms are responsible for the decomposition and recycling of nutrients from organic residues, which supports primary production in natural grasslands. Land-use changes from grassland to agriculture generally reduce soil microbial biomass and alter the soil microbial community structure of Mollisols [1,2]. One of the reasons this occurs is because cultivation reduces the soil organic carbon (SOC), which contains the organic substrates that sustain microbial life. For example, the conversion of Mollisols from grassland to growing annual crops such as cereals and soybeans has resulted in a 55% loss of the original SOC content over the past 60 years [3,4]. Those cultivated Mollisols had 35–49% lower microbial biomass and less microbial diversity [5]. Another consequence of land-use change from natural grassland to agriculture is that cereal crops require inorganic NPK fertilizers, whereas leguminous crops receive inorganic PK fertilizers to support the nutrient demands for high yield. Since fertilization shifts the fungal: bacterial ratios by reducing the symbiotic fungi associated with the crop and stimulating the growth of fast-growing rhizobacteria, the application of inorganic NPK/PK fertilizers can induce changes in soil microbial community composition, as documented by Gu et al. [6] and Marschner et al. [7]. The impact of fertilization on soil microorganisms can be mitigated by applying a combination of inorganic and organic fertilizers, where the organic fertilizer provides carbon-rich and organic substrates that are metabolized by saprophytic bacteria and fungi.

Although populations of heterotrophic bacteria are dependent on organic substrates associated with SOC and organic fertilizer inputs, the chemolithotrophic bacteria derive energy and electrons

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http://dx.doi.org/10.1016/j.ejsobi.2016.03.012
1164-5563/

from inorganic compounds and are expected to be more resilient to the effects of land-use change and fertilization on SOC. For example, the ammonia-oxidizing bacteria (AOB) are ubiquitous soil chemiolithotrophs that are responsible for catalysing the transformation of NH_3 to NO_2^-, a precursor to NO_3^- generated by nitrifying bacteria in aerobic soils or subject to nitrifier-denitrification in temporarily or permanently waterlogged soils [8,9]. As NO_3^- is plant-available, the AOB-mediated reaction is important for plant N nutrition in cultivated and natural ecosystems. Still, the AOB community contains strains adapted to a variety of environmental conditions. Phylogenetic analysis partitioned the AOB community into several clusters [10] with specific clusters associated with particular environmental conditions, *e.g.*, acid soils typically favoured *Nitrosospira* cluster 2 [11], whereas *Nitrosospira* cluster 3 dominated in agricultural fields [12], and *Nitrosospira* clusters 1 and 4 were found predominantly in soils from cold-temperature areas [10].

Land-use change and fertilization is expected to impact the AOB community by altering NH_4^+ availability and environmental conditions. First, compared with natural grasslands, agricultural soils are tilled and have more N-rich crop residues when legumes and organic fertilizers are applied, which permits organic N mineralization to NH_4^+, an indirect substrate for ammonia oxidation. This is consistent with the observation that soils receiving balanced fertilization from combined organic-inorganic fertilizer inputs had greater abundance and diversity of AOB [13]. Second, the tillage of agricultural soils alters the water content, temperature, aeration, structure and depth of crop residue mixtures through the soil profile [14], whereas ammonia-rich fertilizers (*e.g.*, urea, liquid animal manure) tend to reduce the soil pH. A lower abundance of AOB was attributed to the significant decrease in soil pH associated with ammonia-based N fertilizer [15]. Therefore, it is reasonable hypothesize that the diversity of AOB communities in Mollisols will differ between natural ecosystems and cultivated, fertilized farmland because these agricultural practices change the NH_3 concentration and environmental conditions that influence AOB growth and survival.

The Mollisols of Northeast China occur in a climate with extreme seasonal variation in temperature and precipitation; the winter months are cold and dry whereas the summer is hot and rainy. It is well known that seasonal fluctuations in soil temperature, soil water content and plant cover during the growing season modulate soil microbial communities including nitrifiers [16,17], and our previous work in the Mollisols of Northeast China also revealed a seasonal change in soil bacterial communities associated with plant growth [5]. While AOB communities probably show a seasonal variation similar to other soil bacteria, this has not been documented in the Mollisols of Northeast China. The temporal variation in AOB communities should provide insight into when they make a relatively larger contribution to soil N cycling and crop N nutrition. We aimed (1) to test whether land-use and fertilization could alter the diversity of AOB communities in naturally restored grassland and on continuously cultivated farmlands on Mollisols, and (2) to examine how seasonality modulated the diversity of AOB communities.

2. Material and methods

2.1. Experimental sites and soil sampling

Soils were taken from a long-term experimental field established in 1985 at the Hailun Agro-Ecological Experimental Station (47°26′ N, 126°38′ E), Heilongjiang Province, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The experimental design and soil sampling were described previously [5]. Briefly, the 3 treatment plots (each 224 m^2) were ploughed to a depth of 20 cm and planted in spring with one phase of a maize-soybean-wheat rotation (soybean was the crop grown during this study). Fertilizers were applied to the treatment plots as no fertilizer (NoF), chemical fertilizer (CF: 113 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, 30 kg K_2O ha^{-1}) and chemical fertilizers plus organic pig manure (CFM). The pig manure was supplied at 15,000 kg ha^{-1} on straw bedding that contained average total N, P and K concentrations of 22.1, 2.6 and 2.4 g kg^{-1}, respectively. Adjacent to the cultivated plots was one unreplicated natural restoration plot (NR) with a total area of approximately 1000 m^2. The NR plot had no human disturbance (i.e., no fertilizer inputs or tillage), allowing the grasses to grow naturally, with the primary species being *Cares* sp., *Equisetum arvense* and *Leymus chinesis*.

Soil samples (0–20 cm) were collected with a soil auger from the three fertilized plots and one NR plot on 15 October 2006 (after maize harvest), 23 March 2007 (after snow melt), 29 April 2007 (before soybean seeding), 28 June 2007 (vegetative growth stage) and 28 August 2007 (reproductive stage). Soil samples were collected from five randomly selected sites in the plot, mixed together to form a single composite sample (one replicate per plot, $n = 4$ samples), placed in a polyethylene bag, and transported to the laboratory at 4 °C. Soon after arrival at the laboratory, the soils were sieved (<2 mm), and approximately 2 g of each composite soil sample was placed in an autoclaved microcentrifuge tube (2 mL) and stored at −80 °C for molecular microbial analysis. The remaining soil was air-dried for soil physiochemical analysis. Soil NH_4^+ and NO_3^- were measured using a flow injection auto-analyzer (SKALAR, San++, Netherlands), for determination of total C and N contents by Elemental analyzer (VarioEL III, Germany), and for soil pH by a pH meter in a water suspension (soil: water, 1: 2.5). The mean values of soil physiochemical properties from the five sampling dates are shown in Table 1.

2.2. DNA extraction and PCR amplification

Soil DNA was extracted from 0.5 g of frozen soil with a Fast DNA SPIN Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted DNA was diluted in 20 μL TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer and stored at −20 °C until use. The PCR was conducted in a total volume of 50 μL. The primers used for *amoA* PCR amplification were *amoA*-F (5′- GGG GTT TCT ACT GGT GGT-3′) [18] and *amoA*-R (5′-CCC CTC GGG AAA GCC TTC TTC-3′) [19]. The thermal program of the PCR was described previously [20]. The PCR products were checked for the expected size (450 bp) on 1.5% agarose gel stained with ethidium bromide under UV light.

2.3. Cloning and sequencing

We conducted two cloning experiments: one was to test whether the AOB community composition was affected by land-use (cultivated soils vs. NR) and fertilization (among the NoF, CF and CFM treatments), and the other was to test whether the AOB community changed seasonally. In the first experiment, the PCR products obtained from each of the five sampling times for each treatment were composited into a single sample; thus we generated four samples for constructing the clone library for each of the treatments (NoF, CF, CFM and NR). In the second experiment, the PCR products generated from the NR treatment sampled on 15 October 2006, 23 March, 29 April, 28 June and 28 August 2007 were used to make five clone libraries designated NR1, NR2, NR3, NR4 and NR5, respectively.

The PCR products were purified using a QIAEX II Gel Extraction Kit (Cat. No. 20021). The purified DNA was ligated into a pMD18 T-