Environmental Pollution 222 (2017) 583–591

Contents lists available at ScienceDirect

Environmental Pollution

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

Dynamic interplay between microbial denitrification and antibiotic resistance under enhanced anoxic denitrification condition in soil☆

Mingming Sun [a, b, *], Mao Ye [b], Kuan Liu [a], Arthur P. Schwab [c], Manqiang Liu [a], Jiaguo Jiao [a], Yanfang Feng [d], Jinzhong Wan [e], Da Tian [a], Jun Wu [a], Huixin Li [a], Feng Hu [a, **], Xin Jiang [b]

[a] Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[c] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 88743, USA
[d] Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[e] Nanjing Institute of Environmental Science, Ministry of Environmental Protection of China, Nanjing 210042, China

ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form
22 September 2016
Accepted 6 October 2016
Available online 10 January 2017

Keywords:
Antibiotic-resistant genes
Denitrifying genes
Enhanced anoxic denitrification
Mutual dynamic interplay
Denitrifiers

ABSTRACT

Mixed contamination of nitrate and antibiotics/antibiotic-resistant genes (ARGs) is an emerging environmental risk to farmland soil. This is the first study to explore the role of excessive anthropogenic nitrate input in the anoxic dissipation of soil antibiotic/ARGs. During the initial 10 days of incubation, the presence of soil antibiotics significantly inhibited NO_3^- dissipation, N_2O production rate, and denitrifying genes (DNGs) abundance in soil ($p < 0.05$). Between days 10 and 30, by contrast, enhanced denitrification clearly prompted the decline in antibiotic contents and ARG abundance. Significantly negative correlations were detected between DNGs and ARGs, suggesting that the higher the DNG activity, the more dramatic is the denitrification and the greater are the antibiotic dissipation and ARG abundance. This study provides crucial knowledge for understanding the mutual interaction between soil DNGs and ARGs in the enhanced anoxic denitrification condition.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Soil denitrification is an important component of the global, geochemical nitrogen cycle (Ollivier et al., 2011; Brankatschk et al., 2013; Lancaster et al., 2016). During the past few years, despite the inhibition of antibiotic use in agriculture in some countries, the overuse of antibiotics in the livestock industry has resulted in the ever-increasing application of manure with residual antibiotics to farmland (Marti et al., 2014; Lin et al., 2016). Consequently, a new mixture of contaminants has emerged with the simultaneous detection of nitrate and antibiotics in arable soil (Roose-Amsaleg and Laverman, 2016). High concentrations of antibiotics significantly inhibit the activity of indigenous soil denitrifiers and restrict anoxic denitrification (Guo et al., 2013; Yan et al., 2013). Although nutrients, micropollutants, and antibiotic-resistant microorganisms can be attenuated in wastewater effluent (Anderson et al., 2015), little attention has been paid to the effect on anoxic denitrification caused by antibiotics in soil.

Anaerobic dissipation of organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs), is enhanced in the presence of NO_3^- and a biosurfactant (Sun et al., 2014a,b; Ye et al., 2014). Nitrate acts as an electron acceptor (an alternative to oxygen in anaerobic environments) and prompts the electron transfer from electron donors (PAHs and OCPs). Similarly, water-soluble antibiotics as the polar organic chemicals are more likely to act as the electron donors (Dwyer et al., 2014) than nonpolar PAHs and OCPs. However, the extent of electron transfer between soil antibiotic dissipation and denitrification process remains to be elucidated.

Despite the relative shorter half-lives (hours to months) of antibiotics in aerobic or anoxic soils compared to the half-lives of years for hydrophobic, persistent organic pollutants (Sun et al., 2014a,b; Pan and Chu, 2015; Solliec et al., 2016), antibiotics are continually added to soil through the routine use of livestock

☆ This paper has been recommended for acceptance by Prof. von Hippel Frank A..

* Corresponding author. Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China; Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China.
** Corresponding author.
E-mail addresses: sunmingming@njau.edu.cn (M. Sun), fenghu@njau.edu.cn (F. Hu).

http://dx.doi.org/10.1016/j.envpol.2016.10.015
0269-7491/© 2016 Elsevier Ltd. All rights reserved.

manure and biosolids from wastewater treatment as soil amendments (Sandberg and LaPara, 2016; Luby et al., 2016). Thus, antibiotics can be prevalent pollutants in soil and can exert selective pressure on the indigenous bacteria, magnify the abundance of antibiotic-resistant bacteria, and stimulate the overexpression of associated antibiotic-resistant genes (ARGs) (Ross and Topp, 2015; Aydin et al., 2016; Cheng et al., 2016). Consequently, ARGs have been accepted as novel, emerging pollutants in the environment (Pruden et al., 2012).

Horizontal gene transfer for denitrification has been observed to occur (Heylen et al., 2006) as has horizontal gene transfer for antibiotic resistance (Pitta et al., 2016). With the assistance of various mobile genetic elements (plasmids, transposons, and integrates), the ARG and DNG dissemination through horizontal gene transfer among different species can be enhanced, posing a threat to human health (Udikovic-Kolicet et al., 2014). Therefore, the possibility exists that a single strain of microorganism could possess, and perhaps express, genes for both nitrification and antibiotic resistance.

The present work investigated the role of excessive anthropogenic nitrate input in the anoxic dissipation of soil antibiotic/ARGs, and the dynamic interplay between soil DNGs and ARGs in the denitrification-dominated process. Subsurface farmland soils with high content of antibiotics were collected in the vicinity of digestion tanks near a dairy farm in the Yangtze River Delta, China, and used to establish an anoxic microcosm. Nitrate was applied to the soil to initiate denitrification. The objectives of this study were to: (1) investigate the effect of denitrification on the dissipation of different fractions of soil antibiotics and associated ARGs; (2) explore the possible presence of denitrifiers harboring both DNGs and ARGs and their role in the denitrification process and antibiotic dissipation; and (3) understand the mutual relationships between the fluctuation of DNG and ARG abundance. The obtained results contribute to the further understanding of the antibiotic/ARG dissipation in anoxic denitrification-dominated conditions, and provide practical reference for the environmental management to mitigate ARG dissemination in the nitrogen non-point-source contamination.

2. Materials and methods

2.1. Test soil and microcosm design

Soils were sampled in September 2015 at Liang Yu dairy farms in Nanjing, eastern China (32°01′03′ N, 119°08′1′ E). There are more than 5000 cows in the dairy farm, which has a history of veterinary antibiotic usage (2000–2015) and produces approximately 20 tons of fresh cattle manure per day. Although most of the cattle manure is transported to a fertilizer plant to produce organic fertilizer immediately, tons of fresh manure have been directly dumped into the four anoxic digestion tanks (length × width × height = 4 m × 4 m × 1 m) for temporary storage. Consequently, high concentrations of nitrate and antibiotics could coexist in the tanks and surrounding farmland soils. Subsurface mixed polluted soil samples (15–30 cm, about 20 kg in total, each 5 kg) close to the anoxic digestion tanks were collected around the farm. Subsurface farmland soils in the adjacent area without detection of antibiotics and ARGs were collected as clean soil in this study. The polluted and clean soil samples were homogenized and grounded to pass a 2.0-mm sieve. Detailed information about the physiochemical properties of soils is presented in Table S1, supplementary information (SI). Antibiotics detected in polluted soil were sulfadiazine (SD), sulfamethoxazole (SMX), florfenicol (FF), and chloramphenicol (CAP) at the concentration of 352.1 ± 12.3, 112.2 ± 8.2, 163.6 ± 11.4, and 67.8 ± 5.4 μg kg^{-1}, respectively.

All soil microcosm experiments were conducted in an anaerobic glove box (Anaerobic System, 855-ACB, PLAS&LABS, INC) filled with 5% CO_2, 5% H_2, and 90% N_2 gases. Oxygen traces were removed from the atmosphere by a palladium wafer that catalyzed the reaction of O_2 and H_2 to form water. One hundred grams of soil was placed in a 250-mL glass bottle with a Teflon-lined cap, to which 80 mL of either deionized water ("untreated" CS and PS) or 80 mL of 10 mM KNO_3 ("treated" CN and PN) (Chang et al., 2002; Ambrosoli et al., 2005) was added. The four soil treatments established in triplicates were (1) CS: clean soil without KNO_3 addition, (2) PS: antibiotic-polluted soil without KNO_3 addition, (3) CN: clean soil with KNO_3 addition, and (4) PN: antibiotic-polluted soil with KNO_3 addition. KNO_3 was added to the treated soils only at day 0. Each treatment was sampled 12 times using the destructive sampling method within 0–30 days of incubation. There were three replicates at each sampling time. A total of 36 glass bottles were prepared for each treatment (triplicate bottles for each of the 12 sampling times). All solutions were carefully deoxygenated prior to use. The soil samples were placed in a small plastic bag at 4 °C for subsequent analysis.

2.2. Analysis of denitrification process and associated denitrifiers

Soil nitrate was quantified by extracting 10 g of soil with 2 mol L^{-1} KCl (50 mL), and a subsequent ion chromatographic analysis (Dionex, GP 50) (Ambrosoli et al., 2005). For the detection of N_2O production, the headspace gas in the glass bottles was extracted using a syringe at each sampling time and quantified using gas chromatography (Shimadzu GC-14B) with a detection limit of 0.1 μg L^{-1} for N_2O (Hou et al., 2015). Soil nitrate reductase and nitrite reductase were quantified based on nitrite production after nitrate addition, and changes in $NaNO_3$ amount during the incubation of buffered soil suspension (Abdelmagid and Tabatabai, 1987).

Total cultivable soil bacteria were extracted by purified water under the anoxic condition, and the extracts were streaked on nutrient agar plates. The plates were then placed into anoxic bags and incubated at 27 °C (Davidson et al., 1984; Reis et al., 2014). The cultivable denitrifiers were determined by counting positive tubes that accumulated gas bubbles in the inverted Durham tubes based on the DT-MPN method according to our previous study (Sun et al., 2014a,b). Each isolate was incubated separately in universal tubes at 27 °C on a shaker at 150 rpm for 24 h. The cells were harvested by centrifugation and resuspended in 50 μL sterile distilled water, boiled at 100 °C for 5 min, and centrifuged at 12,000 rpm for 10 min. Genomic DNA from pure cultures of denitrifiers was extracted as described by Braker and Tiedje (2003). Ten microliter lysate was used for polymerase chain reaction (PCR) to amplify the 16S rRNA gene. When the amplified 16S rRNA gene fragment from each isolate was finalized, the obtained sequence was subjected to Blast analysis against the collection of the nonredundant nucleotide sequence database of NCBI (Heylen et al., 2007; Woo et al., 2008).

2.3. Soil antibiotic and DNG/ARG quantification

The extraction procedures for soil SD, SMX, FF, and CAP followed the methods described by Sun et al. (2015). The soil antibiotics were fractionated into water-soluble, exchangeable, loosely bound, and tightly bound fractions (Rosendahl et al., 2011; Liu et al., 2015). A soil sample (1.0 g) was placed in a 50-mL centrifuge tube and extracted with 40 mL of extract solution [H_2O-, 0.01 mol L^{-1} $CaCl_2$–, and McIlvaine- (0.1 mol L^{-1} citric acid: 0.1 mol L^{-1} disodium phenyl phosphate = 1000:625 (*v/v*))]. All the tubes were sonicated for 20 min and centrifuged at 5000 rpm at 4 °C for 4 min.

Download English Version:

https://daneshyari.com/en/article/5749053

Download Persian Version:

https://daneshyari.com/article/5749053

Daneshyari.com