Ecotoxicology and Environmental Safety 161 (2018) 474–481

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effect of the nitrification inhibitor (3, 4-dimethylpyrazole phosphate) on the activities and abundances of ammonia-oxidizers and denitrifiers in a phenanthrene polluted and waterlogged soil

Zhi-Feng Zhou*, Ze-Yu Zhang, Ming-Xia Wang, Ya-Min Liu, Jun-Shuai Dai

College of Resources and Environment, Southwest University, Chongqing 400716, China

ARTICLE INFO

Keywords:
Nitrification inhibitors
DMPP
Phenanthrene-polluted soil
Ammonia-oxidizers
Denitrifiers

ABSTRACT

Through a 60-day microcosm incubation, the effect of 3, 4-dimethylpyrazole phosphate (DMPP) on the activities and abundances of ammonia-oxidizers and denitrifiers in phenanthrene-polluted soil was investigated. Five treatments were conducted for clean soil (CK), phenanthrene added (P), phenanthrene and DMPP added (PD), phenanthrene and urea added (PU), and phenanthrene, urea, and DMPP added (PUD) soils. The results indicate that the potential nitrification rate (PNR) in the P treatment was significantly higher than that in the PD treatment only on day 7, whereas the PNR in the PU treatment was significantly higher than that in the PUD treatment on each sampling day. The abundance of soil ammonia-oxidizing bacteria (AOB) in the PU treatment was significantly higher than that in the PUD treatment on each sampling day. Moreover, the abundance of AOB but rather than the ammonia-oxidizing archaea (AOA) had significantly positive correlation with soil PNR ($P < 0.05$). DMPP showed no obvious effect on the soil denitrification enzyme activity (DEA), which could have inhibited the abundances of denitrification-related *narG*, *nirS*, and *nirK* genes. The results of this study should provide a deeper understanding of the interaction between soil polycyclic aromatic hydrocarbons (PAH) contamination, ammonia oxidization, and denitrification, and offer valuable information for assessing the potential contribution of denitrification for soil PAH elimination.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH), a group of typically and widely distributed organic pollutants, could pose a serious threat to the environment and human health owing to their intensive carcinogenicity, teratogenicity, and mutagenicity (Xue and Warshawsky, 2005; Couling et al., 2010). Soil has been considered as an important natural conserver for PAH because it has a strong ability to adsorb and degrade organic pollutants; accordingly, high contents of PAH also have been detected in soil (Tang et al., 2005; Nam et al., 2008). PAH in soil could enable long-term retention owing to their stable polycyclic structures and strong hydrophobicity, and their intensive non-polar and lipophilic properties allow easy transference through the food chain (Van-der-Oost et al., 2003; Xue and Warshawsky, 2005). Thus, soil PAH pollution is of global concern because it poses a huge threat to the soil ecosystem.

Soil microorganisms are the primary actors for natural elimination of soil PAH, and their activities and community structures can be influenced by PAH contamination (Cebron et al., 2015; Abbasian et al., 2016; Chen et al., 2016; Wang et al., 2016a, 2016b; Zhou et al., 2017a). Of these microbes, soil denitrifiers, as the primary drivers of the soil denitrification process, could participate in PAH degradation by coupling the reduction of electron acceptors (NO_3^- or NO_2^-) with the oxidation of PAH under an anoxic environment (Mihelcic and Luthy, 1988a, 1988b; Ridgway et al., 1990; Mcnally et al., 1998; Rockne and Strand, 2001; Ambrosoli et al., 2005; Lu et al., 2011; Liang et al., 2014). Thus far, several investigators have studied the relationship between soil denitrifiers and PAH contamination (Guo et al., 2011; Yang et al., 2013; Sun et al., 2014; Zhou et al., 2017b). For example, Guo et al. (2011) investigated the toxicity of pyrene on the activity and abundance of soil denitrifiers through a soil microcosm experiment and found a clear dose-response relationship between pyrene concentration and the abundance of soil denitrifiers. Additionally, it has been reported that the amendment of nitrate, which is an electron acceptor for denitrification, showed a positive effect on the degradation of PAH under an anaerobic condition (Yang et al., 2013; Sun et al., 2014). Therefore, PAH could affect the activities and community structures of soil denitrifiers, and the degradation of soil PAH might be coupled with the soil denitrification process. Unlike denitrifiers, soil ammonia oxidizers (ammonia-oxidizing archaea, AOA; ammonia-oxidizing bacteria, AOB), are usually neglected in PAH contaminated soil because of their

* Corresponding author.
E-mail address: zhouzhf@swu.edu.cn (Z.-F. Zhou).

https://doi.org/10.1016/j.ecoenv.2018.06.030
Received 5 February 2018; Received in revised form 9 May 2018; Accepted 10 June 2018
0147-6513/ © 2018 Elsevier Inc. All rights reserved.

obligate autotrophic characteristics and insensitivities to organic matter (Kusian and Bowien, 1997; Blainey et al., 2011). However, it has been repeatedly confirmed that direct inhibition of soil nitrification by nitrification inhibitors (NIs) could directly or indirectly restrain the activities of soil denitrification, particularly under certain conditions favoring denitrification (Menendez et al., 2012; Di et al., 2014; Ruser, Schulz, 2015; Friedl et al., 2017; Liu et al., 2017; Shi et al., 2017a; Wang et al., 2017; Wu et al., 2017). This implies a complex crosslink among soil ammonia-oxidizers, denitrifiers, and PAH pollution. However, few studies have reported the effect of NIs on ammonia oxidizers and denitrifiers in PAH contaminated soil.

Although it has been reported that various types of soil microbes, such as bacteria, fungi, and algae, could completely degrade PAH with high efficiency and a fast degradation rate under aerobic conditions (Haritash and Kaushik, 2009, 2016), anaerobic degradation of PAH might also intensively occur in soil generally containing a large amount of micro-aerobic or anaerobic micro-environments (Zhang and Bennett, 2005; Johnsen et al., 2005). Denitrification, as one of the potential pathways for anaerobic PAH degradation in soil, widely exists in soil environments. However, the extent to which it can contribute to soil PAH degradation remains unknown, which is very difficult to investigate by direct methods. Thus, if the inhibition effect of NIs on soil denitrification can be confirmed in PAH contaminated soil, the relationship between soil PAH elimination and the denitrification process should be further studied to provide a possible measure for assessing the potential role of denitrification in soil PAH elimination.

This study selected phenanthrene, a typical PAH with three benzene rings listed in the 16 priority contaminants by the United States Environmental Protection Agency. Through a laboratory soil incubation experiment under waterlogged conditions favoring denitrification, the effect of the representative NI 3, 4-dimethylpyrazole phosphate (DMPP) was investigated on the activities and abundances of soil ammonia-oxidizers (*amoA*: ammonia monooxygenase A) and denitrifiers (*narG*: membrane-bound nitrate reductase gene; *nirS*: cd_1-nitrite reductase gene; *nirK*: *Cu*-nitrite reductase gene) in phenanthrene-polluted soil. The objective of this study was to explore the effect of DMPP on the nitrification and denitrification processes in PAH-polluted soil under conditions favoring denitrification, which should supply some useful information for deeper understanding of the correlation among soil ammonia oxidization, denitrification, and PAH pollution, and be helpful for further investigation of the potential contributions of denitrification to soil PAH elimination.

2. Materials and methods

2.1. Soil sampling and experiment design

For the soil incubation experiment, soil samples were collected from the topsoil (0–20 cm in depth) of a wheat-rice rotated field (N30°42′, E113°30′) in Hanchuan city (Hubei Province, China) in April 2017. The soil is classified as fluvo-aquic with some basic properties including pH, 7.58; NH_4^+, 3.58 mg kg^{-1}; NO_3^-, 4.26 mg kg^{-1}; organic matter, 21.86 mg kg^{-1}; available phosphorus, 15.94 mg kg^{-1}; and PAH, 0.00 mg kg^{-1}. After passing through a 2-mm sieve, the soil sample was stored at 4 °C until the following incubation experiment.

To investigate the effect of DMPP on the activities and abundances of ammonia oxidizers and denitrifiers in phenanthrene-polluted soil under waterlogged conditions, five different soil treatments were conducted in triplicates with varied combinations of 50 mg kg^{-1} phenanthrene, 429 mg kg^{-1} urea (200 mg urea-N kg^{-1}), and 20 mg kg^{-1} DMPP (10% amount of urea-N addition) in clean soil (CK), phenanthrene-polluted soil (P), phenanthrene-polluted and DMPP-added soil (PD), phenanthrene-polluted and urea-added soil (PU), and phenanthrene-polluted and urea plus DMPP added-soil (PUD). The brief procedure for the P, PD, PU, and PUD treatments was as follows. First, the fresh soil was evenly spiked with a phenanthrene solution dissolved in acetone for the target concentration and was then placed in an airing chamber for the volatilization of acetone. After aging for one week, the phenanthrene-polluted soil was evenly divided into four parts. Three of these parts were randomly selected, and DMPP, urea, and urea plus DMPP were added for the target concentration, respectively. Each 80 g soil sample for each treatment was placed in a plastic tube 12 cm in height and 5 cm in diameter, and deionized and distilled water was poured into the tube until the soil surface was completely covered. Finally, each tube was incubated in a dark incubator at 25 °C for 60 days, and the soil samples were collected by non-destructive sampling on days 7, 14, 28, and 60 for measuring NH_4^+, NO_3^-, potential nitrification rate (PNR), denitrification enzyme activity (DEA), and DNA extraction.

2.2. Measurement of soil NH_4^+, NO_3^-, PNR, and DEA

Soil nitrate and ammonium were extracted with 2 mol L^{-1} KCl, and their content was determined by using a Continuous Flow Analyzer (SAN^{++}, Skalar, Holland).

The soil PNR was measured by using the chlorate inhibition method according to Kurola et al. (2005). Briefly, $(NH_4)_2SO_4$ and $KClO_3$ were added to catalyze the nitrite formation and to inhibit the last step in which the nitrite is transformed to nitrate, respectively. Subsequently, the PNR value was determined by the accumulation of nitrite.

For the measurement of soil DEA, 5 g of fresh soil was placed in a glass serum bottle with 5 ml sterile distilled water that was sealed with rubber septa and an aluminum crimp cap. The headspace was replaced with high-purity N_2 gas to achieve an anaerobic condition, and approximately 15% (*V/V*) of the headspace N_2 was replaced with acetylene gas (C_2H_2) to inhibit the transformation of N_2O to N_2. After being shaken on a longitudinal shaker for 30 min to evenly distribute the C_2H_2 throughout the soil slurry (White and Reddy, 1999), the bottle was incubated in the dark at 25 °C for 12 h. Finally, the headspace gas sample was taken and analyzed for N_2O by gas chromatography (Agilent Technologies 7890 A, USA).

2.3. Soil DNA extraction and real-time PCR assay

The soil total DNA was extracted from 0.5 g of fresh soil by using a Fast DNA® SPIN Kit for Soil (Q BIOgene Inc, Carlsbad, CA, USA) according to the manufacturer's protocol and was checked by using 1% agarose gel electrophoresis. The extracted DNA was stored at − 20 °C for the following real-time PCR assay.

After the preparation of standard curves using purified plasmid containing each target gene according to the method of He et al. (2007), real-time PCR was performed on an ABI 7500 thermocycler (Applied Biosystems, USA). In a 25-μL reaction mixture by using SYBR® Premix Ex Taq™ following the manufacturer's instructions (Takara, Japan), the amplification was performed with the original DNA as a template in each reaction mixture. The primer sets including AOA: Arch-*amoA*F/Arch-*amoA*R; AOB: *amoA* − 1F/*amoA* − 2R; *narG*: 1960F/2650R; *nirS*: cd3aF/R3cd; *nirK*: *nirK*1F/*nirK*5R and the thermal profiles used in the amplification of each target gene are listed in Table 1. Following thermal profiling, melting curve analysis was conducted to assess the specificity of the PCR products for each real-time PCR amplification by measuring the fluorescence continuously as the temperature increased from 55 to 95 °C. Finally, data analysis was conducted with 7500 software (version 1.0.6) to obtain the parameter C_t (threshold cycle) and to calculate the copy numbers of each target gene in each sample.

2.4. Data analysis

To check for quantitative differences between soil treatments on each sampling day, a one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls test was performed by using SPSS 11.5 (SPSS, USA), and $P < 0.05$ was considered to be significant. The effects

Download English Version:

https://daneshyari.com/en/article/8853471

Download Persian Version:

https://daneshyari.com/article/8853471

Daneshyari.com