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Degradation and bound-residue formation of nonylphenol in red soil and the effects of ammonium

Jie Liu [a], Jun Shan [a], Bingqi Jiang [a], Lianhong Wang [a], Bin Yu [a], Jianqiu Chen [b], Hongyan Guo [a,c], Rong Ji [a,c,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Xianlin Avenue 163, 210023 Nanjing, China
[b] Department of Environmental Science, China Pharmaceutical University, Tongjia Alley 24, 210009 Nanjing, China
[c] Institute for Climate and Global Change Research, Nanjing University, Hankou Road 22, 210093 Nanjing, China

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ABSTRACT

Fate of nonylphenol (NP) in soils and the effects of nitrogen fertilizers are unclear. Using ^{14}C-tracer, we studied the aerobic and anaerobic degradation of 4-NP_{111} in a paddy red soil amended without and with ammonium chloride. Under oxic conditions, 4-NP_{111} had a half-life of 16.1 ± 1.6 days and minor mineralization (3.84 ± 0.02%), forming no extractable metabolite but abundant bound residues (60.9 ± 1.7%, mostly bound to humin) after 49 days of incubation. The ammonium amendment (8 mmol/kg soil) significantly inhibited the degradation (half-life of 68.0 ± 7.7 days), mineralization (2.0 ± 1.1%), and bound-residue formation (23.7 ± 0.2%). Under anoxic conditions, 4-NP_{111} did not degrade during 49 days of incubation and the ammonium amendment (40 mmol/kg soil) did not affect its persistence. Our results demonstrate that bound-residue formation was a major mechanism for NP dissipation in the red soil under oxic conditions and that chemical nitrogen fertilizer at average field application rate may already considerably increase NP recalcitrance in agricultural soils.

1. Introduction

Nonylphenol (NP) is a typical endocrine-disrupting pollutant and is ubiquitous in the environment (Soares et al., 2008; Ying et al., 2002). The major source of NP is the anaerobic degradation of nonylphenol polyethoxylates (NP*n*OEs), which are widely used as non-ionic surfactants in industry and households (Soares et al., 2008; Ying et al., 2002). When NP*n*OEs are released to sewage treatment plants, their degradation product NP tends to adsorb on the sludge at concentrations varying from a few mg NP/kg sludge up to several thousand mg NP/kg sludge, owing to the high hydrophobicity of NP (Log $K_{OW} = 4.48$) (Das and Xia, 2008; Hesselsoe et al., 2001; Soares et al., 2008). The agricultural application of biosolids (i.e., processed sludge) introduces NP into soils (Das and Xia, 2008; Giger et al., 1984; Shan et al., 2010). High concentrations of NP (up to several hundred mg/kg dry weight) have been detected in soil and sediment samples (Das and Xia, 2008), which causes a great potential risk for crop uptake of NP (Cai et al., 2012; Sjöström et al., 2008).

The fate of NP in the environment has raised great public concerns, since NP is able to disrupt the endocrine system of higher organisms (Soares et al., 2008; Ying et al., 2002). Degradation of NP in the environment has been observed under both oxic and anoxic conditions (e.g., Chang et al., 2007; De Weert et al., 2011; Ekelund et al., 1993; Liu et al., 2008; Shan et al., 2011; Telscher et al., 2005; Topp and Starratt, 2000; Ying and Kookana, 2003; Ying et al., 2003; Zhang et al., 2009), suggesting that microbial degradation of NP is ubiquitous.

For rice cultivation in some regions with intensive agricultural activities in China, high rates of chemical nitrogen fertilizer (average 300 kg N/ha soil) are excessively applied to paddy soils to increase the crop production (Lin et al., 2007), which has caused serious surface water eutrophication problems in these areas (Qiao et al., 2012). Urea and ammonium carbonate are the main nitrogen fertilizer applied into the paddy soils and the application introduces high concentration of ammonium into the soils (Lin et al., 2007; Qiao et al., 2012). The addition of ammonium to soils could shift the microbial community, e.g. increasing the activity of denitrifier community (Avrahami et al., 2002) and decreasing the functional diversity of soil microflora (Saratncnadra et al., 2001), which may have great effects on the fate of pollutants in soils.

Contradictory effects of ammonium amendment on degradation of pollutants in soils have been observed. While biostimulation by

* Corresponding author.
E-mail address: ji@nju.edu.cn (R. Ji).

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ammonium for degradation of petroleum hydrocarbon and methyl bromine in soils has been observed (Bento et al., 2005; Ou et al., 1997), high concentrations of nitrogen (NH_4NO_3) have been shown to inhibit mineralization of the herbicides atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D) (Entry et al., 1993; Entry, 1999) and degradation of the insecticide cypermethrin (Xie et al., 2008) in soils. However, there is no report about the fate of NP in soil under ammonium-rich conditions.

Ammonia-oxidizing bacteria play a role in the degradation of phenolic pollutants, including alkylphenols, bisphenol A, and estrogens (Roh et al., 2009; Shi et al., 2004; Skotnicka-Pitak et al., 2009; Sun et al., 2012). Amendment of soil with ammonium may increase activity of ammonia-oxidizing microorganisms (Avrahami et al., 2002), which are abundant in rice paddy soils (Chen et al., 2008). Therefore an increase of the degradation of NP in the paddy soils with ammonium amendment could be expected. Previous studies have shown that NP degradation in soil and sediment samples could form one nitrified metabolite (nitro-NP) (De Weert et al., 2010; Telscher et al., 2005; Zhang et al., 2009) and this metabolism was related to microbial ammonium-oxidization (De Weert et al., 2010).

NP isomers with different nonyl chain structures have different environmental behaviors and endocrine disrupting activity (Gabriel et al., 2005a; Gabriel et al., 2008; Preuss et al., 2006; Shan et al., 2011). The isomer 4-NP_{111} (4-[3′,5′-dimethyl-3′-heptyl]-phenol) is the major component of technical NP (t-NP), accounting to 20% of the weight (Eganhouse et al., 2009), and has been studied for its degradation and bound-residue (or non-extractable residue) formation under different environmental conditions (Liu et al., 2008; Riefer et al., 2011a; Riefer et al., 2011b; Shan et al., 2011). Red soil is a typical paddy soil in China with 102 million hectare and has commonly nutrient deficiencies of both major and minor elements (Wilson et al., 2004). Owing to the characteristic biological and physico-chemical properties of red soil, adsorption and microbial degradation of pollutants in red soil are quite different from those in other soils (Jia et al., 2008; Li et al., 2008; Liu et al., 2008). Using ^{14}C-tracer, we investigated the fate of 4-NP_{111} in a rice paddy red soil amended with and without ammonium during 49 days of incubation to elucidate 1) the different fates of NP in paddy red soils under oxic and anoxic conditions, in terms of mineralization, degradation, and bound-residue formation, and 2) the effects of ammonium amendment on the fate of NP in paddy soils.

2. Materials and methods

2.1. Chemicals

[Uniformly ring-^{14}C]-labeled 4-NP_{111} (^{14}C-4-NP_{111}) with an initial specific activity of 305.6 MBq/mmol and 99% radiochemical purity was synthesized via Friedel–Crafts alkylation of [uniformly ring-^{14}C]-labeled phenol (Shan et al., 2011). All other chemicals were chromatographic or analytical grade. A stock solution of ^{14}C-4-NP_{111} was prepared in methanol with a radioactive concentration of 437 KBq/ml and a chemical concentration of 1.57 mg/ml.

2.2. Soil

A typical Chinese rice paddy soil, red soil, classified as gleyic hydrageric Anthrosols, was collected from the Yingtan Red Soil Experimental Station of Chinese Academy of Sciences in Jiangxi Province, China (28°15′N, 116°55′E). This paddy soil contained 1.8% total organic carbon, 31.6% clay, 46.7% silt, and 21.7% sand (clay loam texture) and had a pH of 5.2 (Liu et al., 2008). The soil was air-dried, passed through a 20-mesh (0.9 mm) sieve, and stored at room temperature shortly before use.

2.3. Oxic incubation

The oxic incubation was conducted in glass vials containing 2.0 g soil. The ^{14}C-4-NP_{111} stock solution (16 μl) was spiked onto the soil to a NP concentration of 12.6 mg/kg soil (dry weight) and a radioactive concentration of 3.4 MBq/kg soil (dry weight). The soil was left for 2 h to allow the evaporation of methanol and then mixed thoroughly by magnetic stirring. About 0.8 ml distilled water (in the case of control) or ammonium chloride solution (in the case of ammonium treatment, 20 mM) was added to the soil to adjust the moisture to 70% of the maximal water-holding capacity. The final concentration of ammonium was 8 mmol/kg soil (dry weight) (equivalent 224 kg N/ha field soil at 0–20 cm depth), which was about the average fertilizer usage rate (228 kg N/ha) per crop in China (Qiao et al., 2012) and was supposed to be the optimal economical application rate to rice field in the Taihu Lake region of China (Lin et al., 2007). The wet soil was homogenously mixed with stainless spatulas and the vials were closed with rubber stopper, from the bottom of which a plastic vial containing 1 M NaOH (1 ml) was suspended in order to absorb the $^{14}CO_2$ released from the soil owing to mineralization of ^{14}C-4-NP_{111}. The glass vials were incubated at 20 °C in the dark. The glass vials were opened for about 0.5 min every two days to exchange the headspace with fresh air. At incubation times 0, 5, 10, 15, 20, 29, 34, 42, and 49 days, three vials were sacrificed for analysis of ^{14}C-4-NP_{111} residues and possible metabolites (see below). Controls were performed with sterilized soil that had been autoclaved three times at 121 °C each for 30 min on three consecutive days (Shan et al., 2011).

2.4. Anoxic incubation

The anoxic incubation was conducted in Hungate tubes (16 mm × 125 mm) under N_2:CO_2 (90:10; v/v) gas protection. Two grams of the air-dried paddy red soil was weighed into the tubes and submerged by 3 ml oxygen-free distilled water. Resazurin was added to three of the tubes at a concentration of 20 mg/L to indicate the formation of anoxic state. The tubes were sealed with butyl rubber stoppers and were incubated at 20 °C in the dark. When the resazurin color changed from pink to colorless, 16 μl ^{14}C-4-NP stock solution and 1 ml distilled water or ammonium chloride solution (80 mM) were added to the tubes under N_2:CO_2 (90:10; v/v) protection. The final concentration of ammonium in the soil–water system was about 40 mmol/kg dry weight. The tubes were refilled with N_2:CO_2 (90:10; v/v) and were further incubated at 20 °C in the dark. At incubation times 0, 7, 14, 21, 35, and 49 days, three tubes were sacrificed for analysis of ^{14}C-4-NP residues and possible metabolites (see below). Controls were sterilized soil that had been autoclaved three times at 121 °C each for 30 min on three consecutive days.

2.5. Soil extraction with organic solvents

For the anoxic incubation, 2 ml headspace gas of the glass vials was taken out by a syringe with a lock valve and injected into 1 ml NaOH to absorb $^{14}CO_2$. The NaOH traps from both anoxic and oxic treatments were mixed with 2 ml of the scintillation cocktail Lumasafe Plus (Lumac LSC, Groningen, The Netherlands) in 6 ml plastic scintillation vials. Water layer in anoxic samples was decanted into 20 ml plastic scintillation vials after centrifugation and mixed with 4 ml scintillation cocktail. Radioactivity was quantified by a liquid scintillation counter (LSC, LS6500; Beckman Coulter, U.S.) with internal standards.

The soil from the oxic incubation and the soil pellets from the anoxic incubation were freeze-dried and extracted by 6 ml methanol (twice) and 6 ml ethyl acetate (once). For each extraction process, the samples were horizontally shaken for 40 min (200 rpm; LSHZ-300; TaiCang Experimental Equipment Factory, China) and treated by ultrasound for 20 min. Organic extracts were collected after centrifugation (720 *g*, 10 min; LD5-2B; Beijing Jingli Co., Ltd, China). Preliminary experiments showed that 84% of ^{14}C-4-NP in soil could be extracted by this procedure. The organic extracts were combined and 0.5 ml was used for determination of radioactivity by LSC. The residual extracts were evaporated to almost dryness using a vacuum rotary evaporator at 40 °C (Rotavapor R210; Büchi Labortechnik AG, Switzerland) and analyzed for metabolites by thin layer chromatography (TLC) followed by autoradiography.

Autoradiography was performed according to Shan et al. (2011). Briefly, the concentrated organic extracts (about 10 μl) was spotted on silica gel plates (GF254, 200 mm × 200 mm × 0.2 mm; Qingdao Haiyang Chemical Co., Ltd, China) and separated using a mixture of *n*-hexane:ethyl acetate:formic acid (100:20:1.5, by vol) as the eluent. The developed plates were exposed to a storage phosphor screen, which was scanned on an imaging scanner (Typhoon Trio+; GE Healthcare, U.S.). The bands on the plates were quantitatively analyzed using the software ImageQuant supplied with the scanner. The R_f values for 4-NP_{111} and its nitrified metabolite were 0.38 and 0.75, respectively.

2.6. Fractionation of bound residues

The organic extract contained the extractable residues and the residues retained in the soil after the organic solvent extraction were operationally defined as bound residues or non-extractable residues (Riefer et al., 2013; Shan et al., 2011).

The bound residues of ^{14}C-4-NP_{111} in the soil were further fractionated into fulvic acids (FA), humic acids (HA), and humin according to Shan et al. (2011). Briefly, the residual soil after organic solvent extraction was air-dried in a fume hood to evaporate the residual organic solvents and was extracted with 0.1 M oxygen-free NaOH (8 ml) for 24 h by horizontal shaking at 200 rpm. The alkaline extract was separated by centrifugation (11 000 *g*, 20 min). Aliquots of the supernatants were fractionated into FA and HA by acidification to pH 1 with 6 M HCl. The alkaline non-extractable soil residues were humin fraction. Radioactivities in the FA and HA fractions were determined by LSC. About 0.5 g humin samples were combusted on a Biological Oxidizer (OX-500; Zinsser Analytic, Germany). The samples were combusted under 900 °C for 4 min with O_2 supply. The produced $^{14}CO_2$ was absorbed by