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# Nonylphenol biodegradation in river sediment and associated shifts in community structures of bacteria and ammonia-oxidizing microorganisms

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ABSTRACT

Nonylphenol (NP) is one of commonly detected contaminants in the environment. Biological degradation is mainly responsible for remediation of NP-contaminated site. Knowledge about the structure of NP-degrading microbial community is still very limited. Microcosms were constructed to investigate the structure of microbial community in NP-contaminated river sediment and its change with NP biodegradation. A high level of NP was significantly dissipated in 6–9 days. Bacteria and ammonia-oxidizing archaea (AOA) were more responsive to NP amendment compared to ammonia-oxidizing bacteria (AOB). *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes* were the largest bacterial groups in NP-degrading sediment. Microorganisms from bacterial genera *Brevundimonas*, *Flavobacterium*, *Lysobacter* and *Rhodobacter* might be involved in NP degradation in river sediment. This study provides some new insights towards NP biodegradation and microbial ecology in NP-contaminated environment.



## 1. Introduction

Nonylphenol polyethoxylates (NPE) are widely used non-ionic surfactants. Nonylphenol (NP) is one of major degradation products when NPE are discharged to sewage treatment plants or directly released to natural ecosystems. Consequently NP can accumulate in aquatic and terrestrial ecosystems. Half-lives of NP in the environment can range between a few days and almost one hundred days (Mao et al., 2012), which poses a potential threat to human health and aquatic and terrestrial ecosystems, due to its toxicity and endocrine activity (Fujii et al., 2000; Toyama et al., 2011). Microbial degradation mainly contributes to the removal of NP in polluted site, which can abate its ecotoxicological risk (De Weert et al., 2010). To date, bacterial degradation of NP under aerobic conditions has been well-documented (De Weert et al., 2010; Toyama et al., 2011).

The characterized NP-degrading bacterial isolates are affiliated with *Acidovorax* (Watanabe et al., 2012), *Pseudomonas* (Soares et al., 2003; Yuan et al., 2004; Watanabe et al., 2012), *Bacillus* (Chang et al., 2008), *Stenotrophomonas* (Soares et al., 2003), *Sphingomonas* (Fujii et al., 2001; Corvini et al., 2005), and *Sphingobium* (Gabriel et al., 2005). Most of them can use NP as energy and carbon sources (De Weert et al., 2010). However, molecular biology techniques, instead of traditional culture-dependent approaches, can enable the identification of NP-degrading microbial community. Fox example, a recent work using clone library analysis suggested that NP biodegradation could be carried out by a wide variety of bacterial species that were not related to the previous reported NP-degrading microorganisms (De Weert et al., 2010).

Organic contaminants can usually affect bacterial community structure (Xie et al., 2013; Yang et al., 2014). There have been few reports on the change of bacterial community structure associated with NP (or other alkylphenols) amendment or/and biodegradation (Jontofsohn et al., 2002; Zhang et al., 2008; De Weert et al., 2010). In contrast, nitrification has been more widely accepted as a sensitive indicator to assess the ecotoxicological effects of xenobiotic pollutants on natural ecosystems (Malkomes, 1992). Nitrifiers' community structures can be a useful metric of contaminant impacts on microbial ecology. Previous researches indicated that community structures of ammonia-oxidizing bacteria (AOB) and archaea (AOA) could be affected by the amendment of a variety of xenobiotic pollutants and their subsequent biodegradation (Wan et al., 2013, 2014). It could be assumed that ammonia-oxidizing community would be affected by NP amendment and subsequent biodegradation. However, to date, information on the impact of NP

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on ammonia-oxidizing community is still lacking. There has been no report on the shift in AOA and AOB community structures in response to NP attenuation.

NP is frequently detected in river sediment (Petrovic et al., 2002; Lacorte et al., 2006; Soares et al., 2008; De Weert et al., 2010). Aquatic sediment can harbor a huge amount of viable microorganisms that might participate in various biogeochemical processes (Cheng et al., 2013). Sediment has been found to be more effective than water to biodegrade NP (Writer et al., 2011). Identification of NP-degrading bacterial community and its change in sediment can aid in our understanding of NP biodegradation in aquatic ecosystem (De Weert et al., 2010). In addition, the abundance of both AOA and AOB has been found in river sediment (Liu et al., 2013). The aim of the present study was to carry out an investigation on the shift in structures of bacterial, AOA and AOB communities in response to NP attenuation in river sediment.

## 2. Materials and methods

### 2.1. Microcosm set-up

Sediment (0–10 cm depth) used for microcosm construction was collected from the Wenyu River (Beijing) which was mainly impacted by pollutants from urban run-off and domestic sewage. At the time of sediment collection, the total NP was below 1 $\mu g\ g^{-1}$ dry weight sediment, without the detection of linear NP. In this study, linear 4-NP (99 percent; Adamas Reagent Co., Ltd.) was purchased for the biodegradation experiments. The culture medium used in the experiments was prepared according to the literature (Chang et al., 2008). Sediment microcosms were constructed using 250-mL jars with 100 g river sediment (dry weight) and 70 mL medium. Three different sets of treatments in triplicate were carried as follows: (A) sterilized sediment + 150 $\mu g\ g^{-1}$ NP; (B) sediment; and (C) sediment + 150 $\mu g\ g^{-1}$ NP. Each microcosm was incubated on a horizontal shaker (120 rpm) at 25 °C. Sediment samples were collected at a 3-day interval to measure the NP concentration. Dissolved oxygen (DO) levels in liquids were above 3.5 $mg\ L^{-1}$ during the whole 9-day incubation period.

### 2.2. Chemical and molecular analysis

The residual NP in sediment was extracted as previously described (Wang et al., 2014; Yang et al., 2014). The analysis of NP concentration was conducted with a high-performance liquid chromatography apparatus, using methanol–water (90:10) as the mobile phase at a flow rate of 1 $mL\ min^{-1}$ (Wang et al., 2014). NP was monitored by absorbance at 276 nm with the retention time of 6.7 min.

Sediment DNA was extracted and subject to further terminal restriction fragment length polymorphism (TRFLP) analysis according to the literatures (Zhang et al., 2012; Feng et al., 2012; Wan et al., 2013, 2014). Briefly, bacterial 16S rRNA genes and the *ammonia monooxygenase A* (*amoA*) genes of AOA and AOB were amplified using primers 27F-FAM/1492R, Arch-*amoA*F/Arch-*amoA*R, and *amoA*-1F/*amoA*-2R, respectively. Purified amplicons were digested with *Hha*I and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems). Software PRIMER 5.0 was applied for sample clustering using the UPGMA method (Clarke and Warwick, 2001).

For bacterial clone library analysis, the unlabeled forward primer 27F was used. The obtained sequences at a 3 percent difference level were clustered into one operational taxonomic unit (OTU). OTUs and OTU-based rarefaction curve and Shannon community diversity index were obtained using the DOTUR program (Schloss and Handelsman, 2005). The taxonomic identities of bacterial sequences were identified using the Ribosomal Database Project analysis tool "classifier" (Wang et al., 2007). The bacterial sequences obtained in this study were submitted to GenBank, under accession numbers KF155522–KF155690.

## 3. Results and discussion

### 3.1. Biodegradation

Fig. 1 illustrates the patterns of the residual 4-NP in the microcosms with treatments *A* and *C* during the 9-day experimental time. On day 6, a significant decline of 4-NP (average 54.7 percent reduction) in the non-sterilized sediment was observed, but the decline was limited in the autoclaved control (average 13.1 percent decrease). On day 9, an average of 93.2 percent reduction

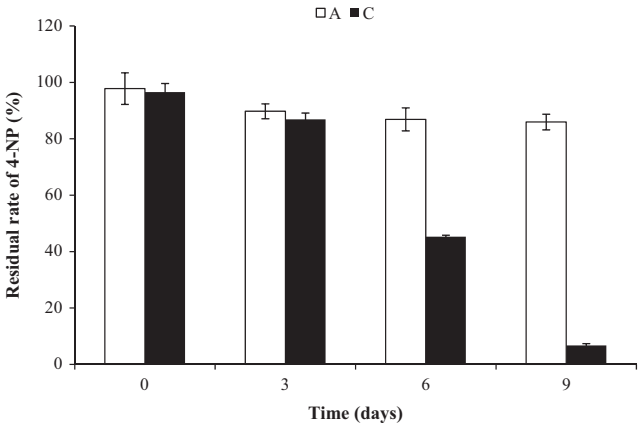


**Fig. 1.** Percentage of residual 4-NP in microcosms with treatments *A* and *C*. Treatment *A*: sterilized sediment + 150 $\mu g\ g^{-1}$ NP; and treatment *C*: sediment + 150 $\mu g\ g^{-1}$ NP. Values are the average of three independent experiments. Vertical bars indicate standard deviations.

occurred in the non-sterilized sediment, but only an average of 14 percent in the autoclaved control. These results indicated a biological attenuation of 4-NP in the NP-amended microcosm.

Natural attenuation of NP can occur in both water and sediment (Writer et al., 2011, 2012). The potential of aerobic NP biodegradation may be widespread in river ecosystem (Bradley et al., 2008). However, little is known about the biodegradation rate of NP in river sediment. Yuan et al. (2004) found that NP (2 $\mu g\ g^{-1}$) in NP-acclimated sediment was completely degraded in 28 days. De Weert et al. (2010) found that biodegradation of branched NP ($14 \pm 1.5\ \mu g\ g^{-1}$) in polluted river sediment started after a lag phase of 2 days and a removal rate of 95 percent occurred within 8 days. The same river sediment was used in our previous study to investigate the biodegradation of bisphenol A. A quick depletion of a high level of bisphenol A in river sediment was found (Yang et al., 2014).

NP with a linear alkyl chain (4-*n*-NP) was usually used as a model for biodegradation of NP isomeric mixture (Bradley et al., 2008; Rozalska et al., 2010; De Weert et al., 2011; Shan et al., 2011). In this study, linear-4-NP was also used for biodegradation test in river sediment. Interestingly, 4-NP (150 $\mu g\ g^{-1}$) in river sediment was also significantly removed in few days. Prior exposure to NP has been shown to reduce the length of the acclimation period associated with NP degradation and increase the rate of NP degradation (Yuan et al., 2004; De Weert et al., 2010). However, isomer-specific degradation of 4-NP has been reported in a variety of ecosystems (Das and Xia, 2008; Hao et al., 2009; Shan et al., 2011). Therefore, further efforts will be necessary to investigate the environmental conditions affecting NP biodegradation in river sediment.

### 3.2. Change of bacterial community structure

In this study, the analysis of bacterial communities in the microcosms with treatments *B* and *C* was performed by TRFLP. The dendrogram constructed for bacterial community structure is illustrated in Fig. 2. For either treatment, the sample on day 0 was distantly separated from the ones on either day 6 or day 9. Moreover, on either day 6 or day 9, the sample in the NP-amended microcosm (with treatment *C*) was also distantly separated from that in the unamended microcosm (with treatment *B*). These results suggested that NP amendment induced a significant shift in sediment bacterial community structure during incubation.

In this study, clone library analysis was used to further characterize the bacterial community compositions of sediment samples in the NP-amended microcosm (with treatment *C*). The sediment