Effects of bioaugmentation in *para*-nitrophenol-contaminated soil on the abundance and community structure of ammonia-oxidizing bacteria and archaea

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Abstract *Pseudomonas* sp. strain WBC-3 mineralizes the priority pollutant *para*-nitrophenol (PNP) and releases nitrite (NO_2^-), which is probably involved in the nitrification. In this study, the rate of PNP removal in soil bioaugmented with strain WBC-3 was more accelerated with more NO_2^- accumulation than in uninoculated soils. Strain WBC-3 survived well and remained stable throughout the entire period. Real-time polymerase chain reaction (real-time PCR) indicated a higher abundance of ammonia-oxidizing bacteria (AOB) than ammonia-oxidizing archaea (AOA), suggesting that AOB played a greater role in nitrification in the original sampled soil. Real-time PCR and multivariate analysis based on the denaturing gradient gel electrophoresis showed that PNP contamination did not significantly alter the abundance and community structure of ammonia oxidizers except for inhibiting the AOB abundance. Bioaugmentation of PNP-contaminated soil showed a significant effect on AOB populations and community structure as well as AOA populations. In addition, ammonium (NH_4^+) variation was found to be the primary factor affecting the AOB community structure, as determined

by the correlation between the community structures of ammonia oxidizers and environmental factors. It is here proposed that the balance between archaeal and bacterial ammonia oxidation could be influenced significantly by the variation in NH_4^+ levels as caused by bioaugmentation of contaminated soil by a pollutant containing nitrogen.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Bioaugmentation · Multivariate analyses · *para*-nitrophenol · *Pseudomonas* sp. strain WBC-3

Introduction

Ammonia oxidation is performed by both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Liu et al. 2013; Prosser and Nicol 2012). The *amoA* gene encodes the α subunit of ammonia monooxygenase which performs the first and rate-limiting step of the nitrification process in both groups. Quantitative real-time PCR of the *amoA* gene has indicated that AOA are abundant in a wide range of habitats and may play a greater role in the nitrification process (Prosser and Nicol 2012). However, the magnitudes of the relative contributions of AOB and AOA, which have ecologically equivalent functions, to ammonia oxidation are still largely unknown. It has been suggested that many environmental factors including pH (Nicol et al. 2008), salinity (Mosier and Francis 2008), NH_4^+ (Hofferle et al. 2010; Martens-Habbena et al. 2009; Verhamme et al. 2011), and dissolved oxygen concentration (Abell et al. 2011) may play a critical role in their individual dominance and the niche segregation of ammonia oxidizers. These findings have stimulated a reassessment of ammonia-oxidizer community ecology, and consequently, the nitrification process in agricultural ecosystems

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should be re-evaluated. Therefore, an understanding of the response of AOA and AOB to particular abiotic and biotic factors within the environment, in particular, the responses of AOA and AOB to environment changes are indispensable for the prediction and management of ecosystem functions (Liu et al. 2013).

para-nitrophenol (PNP), one of the three isomeric mononitrophenols, enters the environment through industrial release and the degradation of parathion-based pesticides (Zhang et al. 2009). PNP has been cataloged as priority pollutant by United States Environmental Protection Agency (USEPA) (Keith and Telliard 1979). PNP is highly water soluble and potentially teratogenic, carcinogenic, and mutagenic to many organisms (Guo et al. 2004). It has been reported that polycyclic aromatic hydrocarbons can reduce soil nitrification (Sverdrup et al. 2002) and that nitrobenzene can inhibit nitrification in soil by acting as the ammonium monooxygenase's suicide substrate (Keener and Arp 1994; McCarty 1999; Zhao et al. 2009). In particular, cyclic N compounds are an important class of nitrification inhibitors because of their N-ring structure (McCarty 1999). In this way, the presence of cyclic N compounds, such as PNP, in soil is likely to affect the nitrification of ammonium oxidizers.

Bioaugmentation refers to the use of bacterial cultures to speed up the rate of degradation of a contaminant. This is generally regarded as a promising way to remove contaminants from soils (Dechesne et al. 2005). It has been used previously for remediating PNP-contaminated soil (Labana et al. 2005). The soil bacterium *Pseudomonas* sp. strain WBC-3 [China Center for Type Culture Collection (CCTCC) M201027] can utilize PNP as the sole source of carbon, nitrogen, and energy, with the concomitant release of NO_2^- (Zhang et al. 2009). NO_2^- was once thought to be a determining factor in the niche variation between AOA and AOB (Sahan and Muyzer 2008). High NO_2^- concentrations may be toxic to AOA (Wuchter et al. 2006). Possibly because of the release of NO_2^- , a dynamic bioaugmentation process of PNP-contaminated soil inoculated with strain WBC-3 may interfere with nitrification processes and the nitrogen cycling in the contaminated sites. For this reason, the response of AOB and AOA to PNP contamination and the bioaugmentation process is an important avenue of investigation.

To date, most studies on the factors affecting the niche separation of nitrifying bacteria and archaea have focused on specific soil properties such as soil pH value (Nicol et al. 2008) and dissolved oxygen (Abell et al. 2011), but there is little information available regarding the response and adaptation of AOB and AOA to dynamic bioaugmentation processes when the elements involved are converted from one substance to another, particularly when the elements are channeled into the nitrogen cycle. Information regarding the effects of such a dynamic bioaugmentation process on ammonia-oxidizer community composition and activity will be important for

understanding their niche specialization. For this reason, the current study aimed to (i) investigate the responses of the abundance and community structure of AOB and AOA to persistent PNP contamination and dynamic bioaugmentation processes and (ii) determine the primary abiotic and biotic factors within the environment that result in the niche differentiation of AOB and AOA in soil.

Materials and methods

Soil sample collection and characteristics

Soil samples were taken from the paddy experimental field (30° 28' 09" N, 114° 20' 54" E) in Huazhong Agricultural University, Wuhan, China, which had no history of PNP contamination. This field was established using a rice-rape rotation system, in which the first growing season, forrice, was from May to August and the second season, forrape, was from September to May of the next year. The top layer (0–15 cm) of the soil was sampled in October of rape planting season in 2010 when the soil was well-drained and the weather was fine. The soil samples were sieved (≤ 2 mm) and kept at 4 °C. Soil total carbon content was 0.88 ± 0.10 %, and total nitrogen content was 0.09 ± 0.01 %. The soil pH was 7.11 ± 0.12 , and water content was 18.11 ± 0.43 %.

Preliminary experiments

Initially, five different concentrations (0.10, 0.25, 0.50, 0.75, and 1.00 mg g^{-1} dry weight (dw)) of PNP were set up in both contamination and bioaugmentation treatments. It was shown that PNP had been removed completely when the concentration reached 0.50 mg g^{-1} dw or lower. In all subsequent experiments, PNP at a final concentration of 0.40 mg g^{-1} dw was selected and added to soil samples.

Soil microcosm experiments

Soil samples corresponding to 100 g dry weight were sterilized in glass bottles (250 mL) with tightened caps using three rounds of autoclaving (20 min at 121 °C) on three consecutive days. Four treatment groups were established: (T1) natural soil, (T2) natural soil with PNP added, (T3) natural soil with PNP added and strain WBC-3 (CCTCCM201027) inoculated and, (T4) sterile soil with PNP added. All subsequent settings of different treatments were prepared as described by Chi et al. (2013).

Chemical analysis

PNP concentration was monitored using high-performance liquid chromatography (HPLC) as described by Chi et al.

Using Canoco software (version 4.5), redundancy analysis (RDA) was used to evaluate the correlation between the AOB/AOA community structures and the environmental parameters. The environmental parameters were divided into two groups of variables: (1) treatment variables (PNP concentration, the abundance of strain WBC-3, NH_4^+ , NO_3^- , and NO_2^- , and the nominal variables of treatments T1, T2, and T3) and (2) time variables. Standard RDA was used to investigate the variance in species composition with the two groups of environmental parameters as the explanatory variables. Partial RDA (pRDA) was then used to assess the variance explained by each parameter group (i.e., time or treatment parameters) by taking the other group of environmental parameters as covariates. The significance of the models was examined using Monte Carlo permutation at the 5 % level (with 499 unrestricted permutations). Both environmental and species numbers were log-transformed for subsequent analyses (Zhao et al. 2009).

Sequence analyses of *amoA* genes from DGGE bands

The marked bands with numbers and the other bands with the same position on the DGGE gels were excised. The corresponding *amoA* genes were extracted for cloning and sequence analysis as described previously (Schwieger and Tebbe 1998). The recovered DNA was re-amplified using the primer pairs (amoA1F-GC and amoA2R for the AOB *amoA* gene, Arch-amoAF and Arch-amoAR-GC for AOA *amoA* gene) and then confirmed by DGGE. After that, the amplified products of purified *amoA* gene were ligated with the pGEM-T vector (Promega) and transformed into *Escherichia coli* DH5 α for sequencing.

Phylogenetic analyses of sequences

BLASTN (<http://www.ncbi.nlm.nih.gov>) was used to investigate the sequences, determine sequence homology, and identify closest relatives. MEGA 5.02 was used for phylogenetic analyses. The neighbor-joining trees were constructed using the Kimura two-parameter distance, and bootstrap values were produced using 1000 replicates (Shen et al. 2008). All of the *amoA* gene sequences were deposited under GenBank accession nos. KM055656–KM055684.

Results

Bioaugmentation of soil contaminated with PNP

The PNP concentration in different treatments was determined using HPLC. PNP was removed more rapidly in bioaugmentation treatment T3 (native soil + PNP +

WBC-3) than in both uninoculated treatments T2 (native soil + PNP) and T4 (sterile soil + PNP) (Fig. 1a). In T3, all PNP added ($0.04 \mu\text{g g}^{-1} \text{ dw}$) was completely removed after 14 days. In contrast, PNP concentration showed no significant changes in either T2 or T4 during the 28-day experimental period ($P>0.05$) (Fig. 1a).

Because NO_2^- was released via PNP oxidation by strain WBC-3, the NO_2^- -N concentration was also monitored in the bioaugmentation treatment T3 (native soil + PNP + WBC-3) and in the other treatment groups. As shown in Fig. 1b, in T3, the NO_2^- -N concentration increased gradually to $7.96 \pm 2.59 \mu\text{g g}^{-1} \text{ dw}$ in the first 8 days. It subsequently decreased to the original concentration by day 22. The molar proportion of PNP degradation to the accumulation of NO_2^- -N was about 3:1 on day 8. In all other uninoculated treatment groups, the NO_2^- -N concentration appeared to remain constant across the 28 days (Fig. 1b).

In the bioaugmentation treatment group T3 (native soil + PNP + WBC-3), the NH_4^+ -N concentration increased rapidly to a maximum concentration of $37.99 \pm 2.22 \mu\text{g g}^{-1} \text{ dw}$ (Fig. 1c) contaminant with the release of NO_2^- during PNP degradation (Fig. 1b). After the complete removal of PNP on day 14, NH_4^+ -N began to decrease gradually to $25.78 \pm 15.26 \mu\text{g g}^{-1} \text{ dw}$ on day 28, still far above the initial level as of the beginning of bioaugmentation ($2.53 \pm 0.79 \mu\text{g g}^{-1} \text{ dw}$) (Fig. 1c). In contrast, its NO_3^- -N concentration kept increasing for the entire period, reaching to $65.49 \pm 8.54 \mu\text{g g}^{-1} \text{ dw}$ (Fig. 1d). In T2 (native soil + PNP), although the NH_4^+ -N concentration increased gradually to $25.45 \pm 3.14 \mu\text{g g}^{-1} \text{ dw}$, it remained lower than in T3 at all times except the first 4 days (Fig. 1c). The NO_3^- -N concentration in T2 decreased steadily to $16.61 \pm 4.17 \mu\text{g g}^{-1} \text{ dw}$ at the end of the experimental period (Fig. 1d). In treatment T1 (native soil), the NO_3^- -N concentration increased gradually to $36.28 \pm 8.04 \mu\text{g g}^{-1} \text{ dw}$ but at a much lower rate than in T3 (Fig. 1d). However, the NH_4^+ -N concentration in T1 showed no obvious change during the entire period and remained at an almost constantly lower level than those in T2 and T3 (Fig. 1c). The high initial NH_4^+ -N concentration in T4 came largely from the autoclaving process of the soil samples (Chi et al. 2013) (Fig. 1c).

The abundance of the inoculated strain WBC-3 in bioaugmentation treatment T3 was quantified using the copy numbers of the *pnpA* gene as previously described by Chi et al. (2013). In the first 8 days of PNP removal in T3, the copy numbers of *pnpA* decreased by approximately two orders of magnitude (from $1.67 \pm 1.08 \times 10^8$ on day 0 to $5.48 \pm 5.68 \times 10^6$ per gram of dry soil) (Fig. 2). As PNP degraded further, its copy numbers increased to $2.57 \pm 3.12 \times 10^7$ per gram of dry soil on day 14 at which time PNP had been removed completely (Fig. 2). It later decreased again by about one order of magnitude to $1.13 \pm 0.91 \times 10^6$ per gram of dry soil at day 18 and then remained constant until the end of the experimental period (Fig. 2).

Fig. 1 Chemical analysis during the bioaugmentation process. **a** Removal of PNP, **b** NO₂⁻-N concentration, **c** NH₄⁺-N concentration, and **d** NO₃⁻-N concentration. Values are means±SD (error bars) ($n=3$). (NS natural soil, SS sterile soil)

Fig. 2 Quantification of *pnpA* gene (strain WBC-3) in inoculated soil microcosm by real-time PCR. Values are means±SD (error bars) ($n=3$). (NS natural soil)

Impact of pollution and bioaugmentation on abundance of AOB and AOA

The abundance of AOA and AOB in non-sterile treatments was quantified using real-time PCR targeting *amoA* gene. The initial AOA copy numbers of *amoA* gene in the original soil sample were $1.13 \pm 0.91 \times 10^6$ g⁻¹ dw (Fig. 3a), one order of magnitude lower than that of the AOB *amoA* gene ($4.00 \pm 3.49 \times 10^7$ per gram of dry soil) (Fig. 3b). During the 28-day experimental period, the AOA *amoA* gene abundance in native soil treatment T1 and PNP contamination treatment T2 showed no significant variation ($P>0.05$) (Fig. 3a). In contrast, the differences in the abundance of the AOB *amoA* gene abundance in treatments T1 and T2 was statistically significant ($0.01 < P < 0.05$) (Fig. 3b). AOA *amoA* gene abundance in treatment T2 was significantly higher than in T3 ($P<0.01$) (Fig. 3a). Similarly, the abundance of AOB *amoA* gene in T3 was also very significantly lower than in T2 ($P<0.01$) (Fig. 3b), suggesting that bioaugmentation of PNP-contaminated soil reduced the abundance of AOA and AOB greatly.

Fig. 3 Quantification of *amoA* gene in different soil microcosms by real-time PCR. **a** AOA *amoA* and **b** AOB *amoA*. Values are means $\pm$ SD (error bars) ($n=3$). (NS natural soil, SS sterile soil)

Impact of PNP contamination on community structure of AOB and AOA

The effects of PNP contamination and bioaugmentation on community structure of AOB and AOA were examined by DGGE profiles targeting the *amoA* genes in the 0-, 8-, and 28-day samples. The presence of PNP did not bring any evident change in the species richness of AOA community between treatments T1 and T2 (Fig. 4a). The correlations between the community structure of AOA and environmental parameters were analyzed by multivariate analysis (RDA and pRDA). Standard RDA indicated that 31.9 % of the variance in species could be explained by environmental parameters (both treatment and time) (Fig. 4b). However, the model did not significantly explain the variation of AOA community structure ($P>0.05$). This indicates that PNP contamination did not significantly influence AOA community structure.

Variation partitioning was also used to evaluate the impact of environmental parameters on AOB community structure in native soil T1 and contamination treatment T2. As shown in Fig. 5b, 56.4 % of the variance in species was explained by standard RDA in which treatment and time both served as explanatory variables ($P<0.05$). However, pRDA (explanatory variable: time, covariable: treatment) indicated that time explained 21.6 % of the variance in species and was statistically significant ($P<0.05$). pRDA (explanatory variable: treatment, covariable: time) showed that 25.3 % of the variance in species could be explained, but this was not statistically significant ($P>0.05$) (Fig. 5b), suggesting that the community structure of AOB was significantly affected by the cooperation of both time and treatment (PNP contamination, NH_4^+ , NO_3^- , NO_2^- , etc.). However, the variation in AOB community structure was caused mostly by time, not by PNP contamination.

Impact of bioaugmentation on community structure of AOB and AOA

Analysis of AOA community structure in treatment groups T2 and T3 showed that 55.4 % of the variance in species could be explained by standard RDA in which time and treatments all served as explanatory variables ($P<0.05$) (Fig. 6b). pRDA (explanatory variable: time, covariable: treatment), however, suggested that 10.5 % of the variance in species was caused by time and that this variance was not statistically significant ($P>0.05$). pRDA (explanatory variable: treatment, covariable: time) indicated that 20.8 % of the variance was due to species variation, and this variance was not statistically significant ($P>0.05$) (Fig. 6b). These results indicated that the community structure of AOA was significantly affected by a combination of both time and soil treatment (PNP contamination, strain WBC-3, NH_4^+ , NO_3^- , and NO_2^- , etc.). Figure 6b shows the correlation between environmental factors and AOA community structure. The first axis of RDA explained 36.2 % and the second 8.8 % of the variance in species, respectively. The first two axes explained 81.1 % of the cumulative variance of the environment-species correlation.

For the AOB community treatment T2 and treatment T3 analyses, standard RDA (both treatment and time as explanatory variables) showed that 72 % of the variance was due to species variation ($P<0.05$) (Fig. 7b). However, time explained 6.7 % of the variance in species, as determined by pRDA (explanatory variable: time, covariable: treatment), but this variance was not statistically significant ($P>0.05$) (Fig. 7b). pRDA (explanatory variable: treatment, covariable: time) showed that treatment explained 59.1 % of the variance in species ($P<0.05$).

Fig. 4 **a** Analyses of AOA community structure in control T1 and pollutant T2 based on PCR-DGGE profiles (0, 8, and 28 days). Bands with arrows were excised and then sequenced. **b** Ordination biplots based on the RDA analyses of DGGE patterns. Solid arrows represent species; empty arrows represent environmental variables. Empty triangles represent T1; solid triangles represent T2. Qualitative variables which

were plotted according to their centroid location in the graph. The cosine of the angle between the respective arrows indicates the correlation between species composition and environmental parameters. The length of arrow suggests its involvement in the model. The sequenced bands are shown in brackets with band numbers (A01–A09: GenBank accession nos. KM055675–KM055683)

Fig. 5 a Analyses of AOB community structure in control T1 and pollutant T2 based on PCR-DGGE profiles (0, 8, and 28 days). Bands with arrows were excised and then sequenced. **b** The description is the

same as in Fig. 4b, and the sequenced bands are shown in brackets with band numbers (B01–B10: GenBank accession nos. KM055656–KM055665)

(Fig. 7b). It can therefore be inferred that the changes in community structure of AOB were significantly affected by bioaugmentation treatment. As shown by the biplots in Fig. 7b, the first axis of pRDA (explanatory variable: treatment, covariable: time) accounted for 63.6 % and the second axis for 1.6 % of the variance in species. The first two axes explained 96.1 % of the cumulative variance of

the environment-species correlation (Fig. 7b). NH_4^+ was the primary factor among the studied treatment variables (PNP, NH_4^+ , NO_2^- , NO_3^- , and strain WBC-3) and affected the AOB community structure as shown by pRDA analysis. NH_4^+ was found to be positively correlated with both NO_2^- and NO_3^- but negatively correlated with both strain WBC-3 and PNP. pRDA showed that inoculation with

Fig. 6 **a** Analyses of AOA community structure in pollutant T2 and bioaugmentation T3 based on PCR-DGGE profiles (0, 8, and 28 days). Bands with arrows were excised and then sequenced. **b** The description is

the same as in Fig. 4b, and the sequenced bands are shown in brackets with band numbers (A01–A10: GenBank accession nos. KM055675–KM055684)

strain WBC-3 is also an important determining factor (next to NH_4^+) affecting the AOB community structure variation.

Genetic profiling of the *amoA* genes of AOB and AOA

The same numbers were used to mark bands that shared the same position in the DGGE gels, and those bands were excised for subsequent phylogenetic analysis. DGGE profiles of AOA showed that all 10 bands (A01–A10, GenBank

accession nos. KM055675–KM055684) of AOA *amoA* genes belonged to cluster S (groups dominated by sequences extracted from soil) designated previously (He et al. 2007; Shen et al. 2008) (Figs. 4a and 6a), and cluster M refers to groups dominated by sequences extracted from marine environments (Fig. 8). The most similar sequences in GenBank to the 10 band sequences were mostly from flooded soil or bioreactors. The soil samples in this work were taken from a paddy soil that is flooded with water for several months every year. All AOB sequences (B01–B17-2, GenBank accession nos.

Fig. 7 **a** Analyses of AOB community structure in pollutant T2 and bioaugmentation T3 based on PCR-DGGE profiles (0, 8, and 28 days). Bands with arrows were excised and then sequenced. **b** The description is the same as in Fig. 4b, and the sequenced bands are shown in brackets with band numbers (B11–B17; GenBank accession nos. KM055667–KM055673)

KM055656–KM055674) were affiliated with the genus *Nitrosospora* and grouped into clusters 3a, 3c, 4, and 9 (Figs. 5a, 7a, and 9), as indicated by phylogenetic analyses of AOB *amoA* gene sequences (2 % nucleotide cutoffs). The AOB *amoA* clusters were classified as described in previous studies (Avrhami and Conrad 2003; He et al. 2007).

Discussion

When PNP is degraded by strain WBC-3 with subsequent NO_2^- release in soil, it is probable that PNP contamination and bioaugmentation could be involved in the nitrification process and the nitrogen cycling. This involvement may initiate a corresponding response of AOB and AOA to the environmental change and their new niche partitioning. Up to present, there has been only a single example of the influences of bioaugmentation processes on AOB community structure in soil (Zhao et al. 2009), but at present, the corresponding

responses of significant groups of AOA have not been reported. The current study focused on the effect of PNP contamination and bioaugmentation on the populations and community structures of both groups of AOB and AOA to determine the respective dominance and niche segregation of ammonia oxidizers under the above dynamic biological conditions.

Both the catabolic capability of the inoculated strains and their existence in polluted soil were essential to successful bioaugmentation (Cunliffe and Kertesz 2006; Singer et al. 2005). The inoculated strain WBC-3 used in the current study was found to play a critical role in the removal of PNP from soil, as indicated by the much higher rate of PNP removal and greater accumulation of NO_2^- in treatment T3 than in treatment groups T2 and T4. It is generally accepted that poor survival of adventitious degraders may cause bioaugmentation to failure (Bouchez et al. 2000). In this study, changes in the abundance of strain WBC-3 during the degradation period and its subsequent stabilization afterwards suggested that the inoculated strain WBC-3 could still use local resources in soil after PNP had been completely depleted, after which a new community developed.

Nitrification is essential to the nitrogen cycling in agricultural paddy soils. In the original soil sample used, the abundance of AOB was two orders of magnitude higher than that of AOA. This indicated that AOB predominated over AOA

Fig. 8 Phylogenetic relationships of archaeal *amoA* sequences retrieved from current study with those from archaea in GenBank. The name of each sequence is followed by a GenBank accession number, and those

from current study are marked by an asterisk (A01–A10; GenBank accession nos. KM055675–KM055684). Bootstrap value (>48 %) are shown at nodes

Fig. 9 Phylogenetic relationships of bacterial *amoA* sequences retrieved from current study with those from bacteria in GenBank. The name of each sequence is followed by a GenBank accession number, and those

from current study are marked by an asterisk (B01–B17-2: GenBank accession nos. KM055656–KM055674). Bootstrap value (>50 %) are shown at nodes

and so played a greater role in nitrification than AOA. It has previously been reported that, in acidic soils, ammonia oxidation may be largely attributable to AOA, but AOB performs better in alkaline or neutral soils (Nicol et al. 2008). DNA-stable isotope probing has provided direct evidences that ammonia oxidation in acidic soils is driven by AOA rather than AOB (Zhang et al. 2012). In the current study, the original paddy soil had a neutral pH. The observed dominance of AOB over AOA was consistent with the previous findings.

Organic pollutants, especially cyclic N compounds, have been reported to reduce soil nitrification (Keener and Arp 1994; McCarty 1999; Zhao et al. 2009). In the current study, PNP contamination did not significantly alter the abundance and community structure of ammonia oxidizers except for inhibiting the AOB abundance. These results suggest that the AOA group is not significantly sensitive to PNP toxicity. However, the decrease in NO_3^- contaminant with the increase in NH_4^+ in treatment T2 implied that nitrification had been inhibited. The reduction of AOB abundance indicated that AOB were primarily responsible for the nitrification process in this habitat. This conclusion was consistent with the predominance of AOB population over AOA in the original soil samples.

In contrast, bioaugmentation of PNP-contaminated soil significantly affected the population and community structure of AOB and the population of AOA. However, there was no significant impact on AOA community structure. This was

most likely due to significant variations in NH_4^+ concentration. Multivariate analysis showed NH_4^+ to be the primary factor affecting the community structure of ammonia oxidizers. Several reports have stated the importance of NH_4^+ in nitrification. For example, the kinetics of ammonia oxidation was reported to determine the niche partitioning of AOB and AOA (Martens-Habben et al. 2009). NH_4^+ concentration can also determine the differential growth of AOB and AOA in soil in that AOA prefers a less NH_4^+ (Verhamme et al. 2011). An increase in NH_4^+ concentration is indicative of a reduced nitrification efficiency (Abell et al. 2011). In treatment T3 in the present study, the NH_4^+ concentration increased as that of NO_2^- increased after PNP degradation by strain WBC-3. This was apparently responsible for the significant inhibition of AOB and AOA abundance and significant effects on the AOB community structure. The increase in NH_4^+ during the increase in NO_2^- in group T3 could be attributable to the release of NH_4^+ by the inoculated strain WBC-3 and indigenous microorganisms during growth or that some indigenous microorganisms can also utilize PNP but via a reductive pathway which releases NH_4^+ .

In the original soil sample, AOB predominated over AOA and was considered the primary contributor to the nitrification process. In the presence of PNP toxicity, the AOB population was significantly inhibited, but the less active AOA group was unaffected by the disturbance. Furthermore, only when the

NH_4^+ (as the substrate of ammonia oxidation) concentration changed greatly in treatment T3 was AOA abundance reduced significantly. Its community composition was not influenced in any visible way. These results indicated that AOA is probably more stable under environmental stresses introduced than AOB and adapted more readily to severe environments. Previous reports have indicated that the AOA could grow well in nutrient-insufficient environments while AOB favored high-energy environments (Hofferle et al. 2010). It has also been demonstrated that AOB and AOA may be active under the same conditions, with both groups competing against each other. This may be due to functional redundancy or fundamental physiological differences that produce distinct ecological niches (Verhamme et al. 2011). In the current study, when oxygen supply and ammonia oxidation were adequate during the rape planting season, which did not involve flooding, the AOA were less active than the AOB. However, the current study showed that the balance between archaeal and bacterial ammonia oxidation can be affected by other factors, such as NH_4^+ variation.

In the current study, a laboratory experiment, rather than a field experiment, was performed so that the responses of AOB and AOA to PNP contamination and bioaugmentation processes would be assessed in a controllable environment. However, the results of the laboratory experiment may not coincide exactly with those collected in real field environments. This current study may provide some knowledge of AOB and AOA responses to PNP in the paddy soil used. More soil types and the real field experimental settings are necessary to indicate the responses more accurately and comprehensively.

In addition, information regarding the diversity and community structure analyzed from DGGE approach in the current study is limited, but this may be rectified using next-generation sequencing techniques. Next-generation sequencing techniques have been developing rapidly and are being considered for use in assessments of the effects of PNP or other similar compounds on microbial communities and the studying microbial degradation of pollutants. Next-generation sequencing techniques based on the 16S rRNA gene and special functional genes (such as *amoA* gene in the current study) may facilitate more abundant and representative studies of these species and their functional genes. The response of microbial community structure and diversity to the PNP and other similar compounds could be evaluated comprehensively. It is possible that some new functional species capable of degrading the pollutants or actively responsible to the pollutants will be found.

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Conflict of interest We declare that we have no conflicts of interest.

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