Effects of pH and polycyclic aromatic hydrocarbon pollution on thaumarchaeotal community in agricultural soils

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Abstract

Purpose Thaumarchaeota is an ecologically relevant archaeal phylum which may significantly contribute to global nitrogen cycling. Thaumarchaeotal abundance, composition, and activity can be changed by soil pH and pollutants such as toxic metals. This study aims to examine the responses of thaumarchaeotal community to soil pH variation and polyclic aromatic hydrocarbon (PAH) pollution which may co-occur in agricultural soils.

Materials and methods Field soil samples were collected from agricultural land impacted by both acidification and PAH contamination. Thaumarchaeotal abundance and composition were assessed using molecular approaches targeting 16S rRNA or *amoA* genes and were linked to environmental factors by correlation and canonical correspondence analysis (CCA). To evaluate the short-term responses of Thaumarchaeota to PAHs, additional soil microcosms amended with either three selected PAHs were established.

Changes in thaumarchaeotal communities during the incubation were monitored.

Results and discussion A significant correlation between thaumarchaeotal gene abundance and soil pH was observed within field samples, with the I.1a-associated group enriched when pH <5.0. CCA suggests that the community variation was primarily related to soil pH. In contrast, the effects of PAHs were minimal. In soil microcosms, high concentrations of PAHs persisted after the 4-week incubation. Independent of the PAHs added, thaumarchaeotal *amoA* abundance slightly increased and the compositions were stable at the end of the incubation. This might be associated with the pollutants bioavailability and potential microbe-PAH interactions in the soil.

Conclusions Soil pH variation strongly shapes the agricultural soil thaumarchaeotal community, whereas PAH effects appear to be marginal even in the presence of high concentrations of pollutants. The complicated interaction between soil matrix, pollutants, and Thaumarchaeota requires further study.

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1 Introduction

Thaumarchaeota, previously referred to as the mesophilic Crenarchaeota (Brochier-Armanet et al. 2008), are widespread and are often the most abundant archaea in terrestrial and marine environments. Some thaumarchaeotal clades, including the I.1a, I.1a-associated, and I.1b groups, possess genes encoding putative ammonia monooxygenase and thus may play a crucial role in global nitrogen transformation (Prosser and Nicol 2008). In addition to these potential ammonia-oxidizing Thaumarchaeota (AOA), there are other

thaumarchaeotal groups including the groups I.1c, ALOHA, and pSL12 that can be dominant in specific habitats but appear to be ammonia oxidation independent (i.e., non-AOA) (Weber et al. 2015).

Because of their potential contribution to ammonia oxidation, the first and rate-limiting step of nitrification, ammonia-oxidizing Thaumarchaeota in agricultural soils have been extensively studied since their discovery. It is presently known that AOA are influenced by multiple edaphic factors, among which the most significant are substrate supply, organic carbon, and pH (Prosser and Nicol 2012; Shen et al. 2014). The pH was of great concern because of the wide variation of soil acidity across the world (He et al. 2012). Additionally, evidence indicates that there is a niche differentiation of Thaumarchaeota along a pH gradient (Gubry-Rangin et al. 2011). For example, *Nitrosotalea devanaterra*, the only enrichment of the I.1a-associated group, is capable of optimal growth at pH values between 4.0 and 5.5 (Lehtovirta-Morley et al. 2011), while pure cultures of the groups I.1a and I.1b prefer neutral conditions (Könneke et al. 2005, Tourna et al. 2011). Indeed, the I.1a-associated group appears to be dominant and dictates ammonia oxidation in many acidic soils (Nicol et al. 2008; Yao et al. 2011; Zhang et al. 2012), although growth of the I.1b AOA at low pH is not unusual (Wu and Conrad 2014). Other acidophilic thaumarchaeotal clades include I.1c and I.3, which mainly thrive under strongly acidic conditions (Oton et al. 2016; Weber et al. 2015). In contrast, the I.1b Thaumarchaeota are dominant in neutral soils, as revealed by large-scale meta-analysis (Gubry-Rangin et al. 2011; Oton et al. 2016).

Thaumarchaeota can be sensitive to inorganic and organic xenobiotics. It was demonstrated in soil microcosms that the toxic metals Cu, Zn, Hg, and As considerably reduced the abundance, composition, and activity of AOA (Mertens et al. 2009; Liu et al. 2010; Mertens et al. 2010; Subrahmanyam et al. 2014a). As for organic pollutants, *Nitrosopumilus maritimus*, a cultivated representative of the group I.1a, was inhibited by the addition of low concentration of organic pollutants to the medium (Urakawa et al. 2012; Sipos and Urakawa 2016). Industrial effluents containing mixed organic compounds also change the soil AOA community (Subrahmanyam et al. 2014b). However, the interactions between organic compounds and Thaumarchaeota are complex. The thaumarchaeotal ammonia monooxygenase belongs to the copper-containing membrane-bound monooxygenase (CuMMO) family, which has a wide substrate range that includes aromatic hydrocarbons (Pester et al. 2011). Furthermore, the growth of Thaumarchaeota could be greatly enhanced by the presence of small organic molecules such as pyruvate (Tourna et al. 2011), which is among the final metabolites of bacterial polycyclic aromatic hydrocarbon (PAH) degradation

(Juhasz and Naidu 2000). In view of the vast diversity of organic pollutants, however, the thaumarchaeotal responses to these compounds are poorly understood. For instance, little is known about the effects of PAHs on Thaumarchaeota, although PAHs might be the most widely distributed persistent organic pollutants in the environment (Harvey 1991).

Soil acidification and pollution pose major risks to crop production and can potentially change the characteristics of the soil microbiota. It is well known that the intensive use of mineral nitrogen fertilization may cause considerable pH decline of cropland soil (Guo et al. 2010), which in turn changes the habitat of Thaumarchaeota and the reaction balance of ammonia oxidation (He et al. 2012), the energy source of AOA. On the other hand, PAH pollution of agricultural soils is common in some rapidly industrialized areas due to their continuous emission (Xu et al. 2006; Ping et al. 2007). As a result, the co-occurrence of soil acidification and PAH pollution might not be unusual. In this study, we examined the thaumarchaeotal communities in agricultural soils impacted by both fertilization-induced acidification and PAH pollution, as well as in soil microcosms exposed to a high concentration of selected PAHs. The aims of the study were therefore (1) to reveal the effects of soil acidification and PAH pollution on the in situ thaumarchaeotal community and (2) to assess the effects of PAHs on soil Thaumarchaeota under controlled conditions.

2 Material and methods

2.1 Site description and field sampling

The field under study is located adjacent to Hua-Xi (HX) village in the outskirts of Nanjing, Jiangsu Province, China (31°51′57″N, 118°35′58″E). The soil was classified as an Argosols according to the Chinese Soil Taxonomy. Two sets of experiments, i.e., an in situ survey and an amendment experiment, were performed to reveal the responses of Thaumarchaeota to long- and short-term PAH pollution. For the in situ survey, 20 composite surface soils (0–10 cm, HX-1 to HX-20) were randomly collected on November 13, 2014, from the field of vegetable cultivation. Each sample was obtained by mixing five 50-g subsamples from a 10×10 m grid. The soils were immediately transported to the laboratory after sampling, and a 50-g subsample of each soil was stored at -20 °C for molecular analysis. The rest of the soil was air-dried, homogenized, and stored at 4 °C for physicochemical analysis. For the PAH amendment experiment, a bulk soil sample was collected from the field, air-dried, sieved through a 2-mm mesh, and stored at 4 °C before use.

2.2 PAH amendment experiment

The soil used in the PAH amendment experiment had a neutral pH of 7.4. Soil microcosms were established with amendment of either anthracene (three-ring), benz(a)anthracene (four-ring), or benzo(a)pyrene (five-ring) (Sigma-Aldrich). Specifically, PAH solutions in acetone were added to air-dried soils which were then placed in a fume hood overnight to allow the volatilization of the solvent. The PAH-spiked soils were well mixed, and a 5-g fraction was added to serum bottles containing 15-g clean soil, giving the final PAH concentrations of approximately 150 (for anthracene) and 50 mg kg^{-1} (for benz(a)anthracene and benzo(a)pyrene). After adjusting to 60 % water-holding capacity (soil moisture approximately 19 %), bottles were capped with butyl stoppers and statically incubated in the dark at 25 °C for up to 4 weeks. During the incubation, the microcosms were kept aerobic by flushing the headspaces with air regularly. All treatments were performed in triplicate, and destructive sampling took place at the beginning of the experiment and the first, second, and fourth weeks for the molecular and PAH analyses described below.

2.3 Analysis of soil chemical characteristics

The soil pH was determined with a soil:water solution (1:2.5). Total carbon (TC) and total nitrogen (TN) were measured with a Vario Max element analyzer (Elementar), and the C/N ratio was calculated by directly dividing TC by TN. Total phosphorus (TP) and total potassium (TK) were analyzed by standard methods (Lu 2000). Inorganic N species (NH_4^+, NO_3^-, and NO_2^-) were extracted with 2 mol l^{-1} KCl and analyzed on a continuous flow analyzer (SA1000, Skalar Analytical).

2.4 PAH analysis

Soxhlet extraction was performed with 10.0 g (field sample) or 1.0 g (microcosm sample, see "2.2") air-dried and homogenized soils for 24 h. Prior to the ultra-fast liquid chromatography (UFLC) analysis, dichloromethane extracts were concentrated on a rotary evaporator and purified by column chromatography with activated silica gel as described previously (Wu et al. 2008). Fifteen out of the 16 EPA priority PAHs were determined with a Shimadzu UFLC-20 system (Shimadzu, Kyoto, Japan) equipped with a reversed phase C18 column (Shim-pack XR-ODSII, Kyoto, Japan) (Zeng et al. 2013). All PAH concentrations are presented based on the soil dry weight.

2.5 Soil DNA extraction and quantitative PCR

DNA was extracted from 0.5 g of each fresh soil sample with the FastDNA SPIN Kit for soil (MP Biomedicals) following the manufacturer's instruction. The quality and quantity of DNA were checked by electrophoresis and by a spectrophotometer (NanoDrop 1000). A tenfold dilution of the DNA showed no inhibition of PCR and was thus used in the downstream molecular analysis.

Gene copies were determined by quantitative PCR (qPCR) based on SYBR Green chemistry using a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories) as previously described (Wu and Conrad 2014). The primer sets 771F/957R (Ochsenreiter et al. 2003) and CamoA-19f/CamoA-616r (Pester et al. 2012) were used to quantify the thaumarchaeotal 16S ribosomal RNA (rRNA) and ammonia monooxygenase (amoA) genes, respectively. The qPCR standards were generated using linearized plasmid DNA from single clones containing the genes. A dilution series of the standard template across seven orders of magnitude for each gene was used in each assay. The blank was run with water as the template instead of DNA extract. Tenfold diluted extracts containing 3.7–10.8 ng DNA were added to the reaction mixture in each assay. The qPCR amplification efficiency for the thaumarchaeotal 16S rRNA gene was 85.4 % with an r^2 value of 0.999; for the thaumarchaeotal amoA gene, the efficiency was 79.0 % and the r^2 was 0.993. Abundance of ammonia-oxidizing bacteria (AOB) was evaluated by qPCR amplification of the bacterial amoA gene with the primer set amoA-1F/amoA-2R (Rotthauwe et al. 1997); this assay had an efficiency of 82.2 % and an r^2 of 0.998. All reactions were performed in triplicate for each assay.

2.6 Terminal restriction fragment length polymorphism

Terminal restriction fragment length polymorphism (T-RFLP) of the thaumarchaeotal 16S rRNA gene was performed following the methods described elsewhere with minor modification (Wu and Conrad 2014). Briefly, an 833-bp fragment was amplified with the primers A109F (Großkopf et al. 1998) and 957R, with Cy5 fluorescent dye attached to the forward primer. After purification, approximately 100 ng of PCR product was digested by two units each of AluI and MseI (New England Biolabs) in a 20-μL volume at 37 °C for 1.5 h. These two enzymes were selected based on an in silico restriction site analysis of available representative sequences from major thaumarchaeotal groups. The reactions were then desalted using the SigmaSpin Post-Reaction Clean-up Columns (Sigma-Aldrich), and 2 μL of the desalted fragment was used in the length polymorphism analysis (CEQ 8000 Genetic Analysis System, Beckman-Coulter). Peaks with relative abundance of >1 % were used to calculate the relative terminal restriction fragment (T-RF) abundance.

For the assignment of major T-RFs, the sequences retrieved in the clone library analysis (see "2.7") were virtually digested (http://insilico.ehu.es/restriction/main/index.php) and

manually assigned to specific T-RFs according to the length of the terminal fragment.

2.7 Cloning, sequencing, and OTU classification

Triplicate PCR amplicons of the 832-bp thaumarchaeotal 16S rRNA gene fragment were pooled, gel purified, and cloned into the pGEM-T Easy Vector (Promega). The recombinant plasmid was transferred into competent *E. coli* JM109 cells, and ~30 positive clones were randomly selected and sequenced (MajorBio, Shanghai).

After removing vector and primer sequences, chimeric sequences in each library were identified by Bellerophon (Huber et al. 2004) and excluded from the following analysis. The sequences were aligned against a representative selection of thaumarchaeotal reference sequences and classified into operational taxonomic units (OTUs) at the 95 % identity level using MOTHUR (Schloss et al. 2009).

2.8 Phylogenetic analysis

Representatives for each OTU, their closest hits in the GenBank database obtained by the BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the reference sequences of major thaumarchaeotal clades were used to reconstruct the phylogenetic tree. Neighbor-joining tree and bootstrap support were calculated with MEGA version 4 (Tamura et al. 2007) using the Jukes–Cantor model.

2.9 Canonical correspondence analysis

Canonical correspondence analysis (CCA) was performed with OTUs or T-RFs as species inputs to reveal the regulation of thaumarchaeotal community structure by environmental factors (ter Braak and Šmilauer 2002). CCA was performed using CANOCO 4.53 with default settings (SCIENTIA Software). A binary matrix was constructed by scoring the relative abundance of particular OTUs or T-RFs, and the matrix was used for CCA.

2.10 Statistics

The coefficient of variation (*Cv*) was calculated by dividing the standard deviation by the mean (SD/mean). Pearson's correlation analyses were performed to evaluate the relationships among data sets.

2.11 Nucleic acids accession number

Thaumarchaeotal 16S rRNA gene sequences representative of each OTU were deposited with GenBank under the accession numbers of KT449785-KT449799.

3 Results

3.1 Soil characteristics and PAH level

Eight soil characteristics including pH, total carbon, nitrogen, phosphorus, potassium, and inorganic N content were determined (Table 1). The soil pH varied between 4.26 and 8.05 and showed a mosaic spatial distribution. The more variable edaphic factors included ammonium and nitrate, as suggested by high values of *Cv*, implicating differential fertilization levels during the cultivation of vegetables. In contrast, soil contents of carbon, nitrogen, phosphorus, and potassium were less variable. The sum of 15 PAHs (ΣPAHs) in the soils varied between 0.18 and 20.7 mg kg^{-1} dry weight (*d.w.*) (Table 1). There were multiple correlations between the soil characteristics; for example, pH was inversely correlated with the ammonium level in the soils ($r = -0.517$, $P < 0.05$, $n = 20$).

3.2 Abundance of Thaumarchaeota and ammonia-oxidizing bacteria

Soil thaumarchaeotal and AOA abundances were evaluated by qPCR targeting the 16S rRNA and *amoA* genes, respectively. The copies of these two thaumarchaeotal genes across all soils were directly correlated ($r = 0.902$, $P < 0.01$, $n = 20$), with the 16S rRNA gene varying from 1.58×10^7 to 8.17×10^8 copies g^{-1} *d.w.* and the *amoA* gene varying between 1.98×10^6 and 1.45×10^8 copies g^{-1} *d.w.* (Table S1, Electronic Supplementary Material). The abundances of both thaumarchaeotal genes were significantly correlated with pH and C/N (Table 2). Additional correlations were observed between ammonium, PAHs and the thaumarchaeotal *amoA* gene.

The copy number of the bacterial *amoA* gene varied from 5.68×10^4 to 2.42×10^7 g^{-1} *d.w.*, and in most samples were less than that of thaumarchaeotal *amoA* genes, resulting in thaumarchaeotal:bacterial *amoA* gene ratios (AOA/AOB) of between 0.9 and 60.5 (Table S1, Electronic Supplementary Material). Bacterial *amoA* gene abundance was mainly correlated with carbon, nitrogen and, in particular, nitrate (Table 2). The ratios of AOA/AOB were inversely correlated to nitrate levels.

3.3 Community composition of Thaumarchaeota

The composition of the thaumarchaeotal community in the soils was examined by both clone library and T-RFLP analysis of an 832-bp 16S rRNA gene fragment. After removal of chimeras, 575 thaumarchaeotal 16S rRNA gene sequences from 20 libraries were retrieved (Fig. 1a). The sequences were classified into 24 OTUs at 95 % identity, with 9 singleton OTUs. Assignment of representatives from each OTU showed that all sequences were affiliated with the phylum Thaumarchaeota, with 77.7, 19.8, and 1.0 % corresponding

Table 1 Soil characteristics and PAH concentrations in the vegetable land soils

	pH	Total C (%)	Total N (%)	C/N	Total P (g kg^{-1})	Total K (%)	NH$_4^+$ (mg N kg^{-1})	NO$_2^-$ (mg N kg^{-1})	NO$_3^-$ (mg N kg^{-1})	ΣPAHs (mg kg^{-1})
HX-1	6.56	2.27	0.23	9.96	1.52	1.78	28.3	0.13	136.6	3.14
HX-2	5.52	2.47	0.29	8.61	1.22	1.97	68.0	0.14	154.4	4.41
HX-3	4.26	1.73	0.20	8.69	1.10	1.98	79.4	0.08	51.9	6.32
HX-4	4.36	1.85	0.20	9.07	1.09	2.04	36.8	0.10	108.4	3.34
HX-5	7.89	2.12	0.19	11.22	1.78	1.98	9.0	0.12	6.6	6.93
HX-6	4.89	2.60	0.27	9.56	2.89	2.12	72.8	0.15	98.7	11.87
HX-7	5.88	1.88	0.19	9.69	1.91	1.99	36.6	0.12	40.7	5.57
HX-8	8.05	2.21	0.18	12.08	1.68	2.11	8.8	0.19	7.1	20.68
HX-9	5.87	2.62	0.26	10.19	1.04	2.00	18.4	0.13	73.1	18.47
HX-10	4.45	1.53	0.17	9.05	1.57	2.22	42.1	0.07	19.8	5.32
HX-11	4.47	1.21	0.14	8.71	1.70	2.20	45.2	0.07	16.0	1.05
HX-12	4.40	1.19	0.14	8.69	1.76	2.40	16.3	0.06	11.1	1.07
HX-13	4.94	1.28	0.15	8.71	1.45	1.76	84.1	0.12	18.3	1.70
HX-14	4.86	1.19	0.16	7.58	1.88	2.32	168.1	0.07	16.1	0.78
HX-15	6.76	0.62	0.11	5.90	0.62	2.23	10.2	0.22	4.2	0.18
HX-16	6.69	0.79	0.12	6.90	0.69	2.06	10.8	0.18	8.2	0.27
HX-17	6.16	0.66	0.11	6.21	0.56	2.13	18.0	0.08	9.4	0.21
HX-18	7.06	0.67	0.12	5.45	0.69	2.25	10.9	0.17	6.5	0.30
HX-19	6.05	0.63	0.10	6.32	0.62	2.14	17.5	0.13	14.8	0.22
HX-20	6.64	0.99	0.15	6.81	1.17	2.45	66.9	0.37	30.7	0.58
Cv	0.20	0.46	0.32	0.21	0.43	0.09	0.92	0.52	1.13	1.29

to groups I.1b, I.1a-associated, and I.1a, respectively. Additional 1.4 % was affiliated with the group I.1c. A few OTUs that were closely related to *Nitrososphaera*, *Nitrosotalea*, soil metagenome fragments 29i4, and 54d9 were abundant (>5 % of the total number of sequences).

With T-RFLP, 10 major terminal restriction fragments (T-RFs) were produced by the combined digestion of AluI and MseI (Fig. 1b). The T-RFs of 102, 103, 280, 310, and 458-bp were affiliated with the group I.1b, as suggested by a virtual enzymatic digestion of library sequences. Among them, T-RF 102 bp was exclusively clustered with the soil fosmid 54d9; T-RF 144 was mostly related to the OTU-5 that corresponds to the I.1a-associated group, while T-RFs 292 and 62 were associated with groups I.1a and I.1c, respectively. The phylogeny of T-RF 459 was not clear because no such T-RF was obtained

from retrieved sequences, but the virtual restriction pattern suggested T-RF 459 was very likely related to the I.1a-associated group. It should be noted that the virtual digestion of some sequences from the most acidic samples (pH < 5.0) produced a T-RF of 24 bp, which was too short to be captured with the current T-RFLP method. These sequences comprised OTU-5 and were related to *N. devanaterra*, a cultivated representative of the I.1a-associated group (Fig. 2).

3.4 Correlation between thaumarchaeotal community composition and environmental factors

To explore the correlation between environmental factors and the thaumarchaeotal community, CCA was performed with OTUs or T-RFs (Fig. 3). The first two CCA axes explained

Table 2 Correlation between the abundance of nitrifiers and edaphic factors

	pH	Total C	Total N	C/N	Total P	Total K	NH$_4^+$	NO$_2^-$	NO$_3^-$	ΣPAHs
Thaumarchaeotal 16S	*0.58*	0.41	0.24	**0.54**	0.23	−0.39	−0.33	0.04	0.06	0.31
Thaumarchaeotal *amoA*	*0.73*	0.42	0.23	**0.56**	0.08	−0.34	**−0.44**	0.23	0.06	**0.47**
AOB *amoA*	0.21	**0.48**	**0.50**	0.29	0.16	−0.42	−0.07	0.26	*0.73*	0.09
AOA/AOB	0.25	−0.20	−0.34	0.06	0.00	0.43	−0.41	0.19	**−0.56**	0.46

The values in bold indicate a $P < 0.05$ and the values in italics indicate $P < 0.01$

AOA ammonia-oxidizing Thaumarchaeota, *AOB* ammonia-oxidizing bacteria

38.1 and 52.8 % of the variance in the OTU and T-RF compositions, respectively. Among the nine variables tested, pH was the only factor that significantly contributed to the thaumarchaeotal community composition ($P < 0.01$) regardless of the analysis methods. In contrast, the impact of PAHs on thaumarchaeotal community was minimal.

Moreover, pH was directly correlated with the abundance of specific thaumarchaeotal lineages. For instance, the sum of OTU-3 and 5, both of which are affiliated with the I.1a-associated group, declined along the pH gradient; these OTUs were only highly abundant (>25 %) in samples of pH <5.0. A similar trend was observed for T-RFs 144, 438, and 459. These I.1a-associated lineages were minimal in neutral or alkalic soils (pH >6). In contrast, most group I.1b-related OTUs or T-RFs were enriched in soils with the increased pH (Fig. S1, Electronic Supplementary Material).

3.5 PAH amendment experiment

After a 4-week incubation, no significant pH variation was observed. Anthracene was transformed considerably, although the residual concentration (approximately 100 mg kg^{-1}) was still much higher than the background (Fig. S2, Electronic Supplementary Material). Negligible dissipation of benz(a)anthracene and benzo(a)pyrene was observed at the end of the experiment, suggesting the persistence of these

PAHs in the respective soil microcosms. There was no net increase in nitrate in all four treatments during the entire incubation period, but a significant NO_3^- accumulation could be observed in anthracene-spiked microcosms from the first to the fourth week (data not shown). The copies of soil thaumarchaeotal 16S rRNA and *amoA* genes in the control microcosms declined from $6.57 \pm 1.10 \times 10^7$ and $2.75 \pm 0.26 \times 10^6$ at the beginning to $4.89 \pm 0.27 \times 10^7$ and $1.58 \pm 0.48 \times 10^6$ after the 4-week incubation, respectively. None of the amended PAHs reduced the thaumarchaeotal gene abundances (Fig. 4). After the decrease in the first week, significantly increased thaumarchaeotal *amoA* gene abundance was observed in the benzo(a)pyrene-supplemented microcosms. Meanwhile, the thaumarchaeotal community compositions were stable after the incubation as revealed by the T-RFLP analysis of 16S rRNA genes (Fig. 5).

4 Discussion

Argosols is typically weakly acidic to neutral; however, significant acidification may occur in cropland Argosols due to the intensive use of mineral N fertilizers (Guo et al. 2010). This is demonstrated by the soil pH variation observed in the field samples (Table 1). Long-term vegetable cultivation may result in the accumulation of

Fig. 1 Thaumarchaeotal community composition along the pH gradient as revealed by **a** clone library and **b** T-RFLP analysis. The values under the bottom panel show the pH of each sample
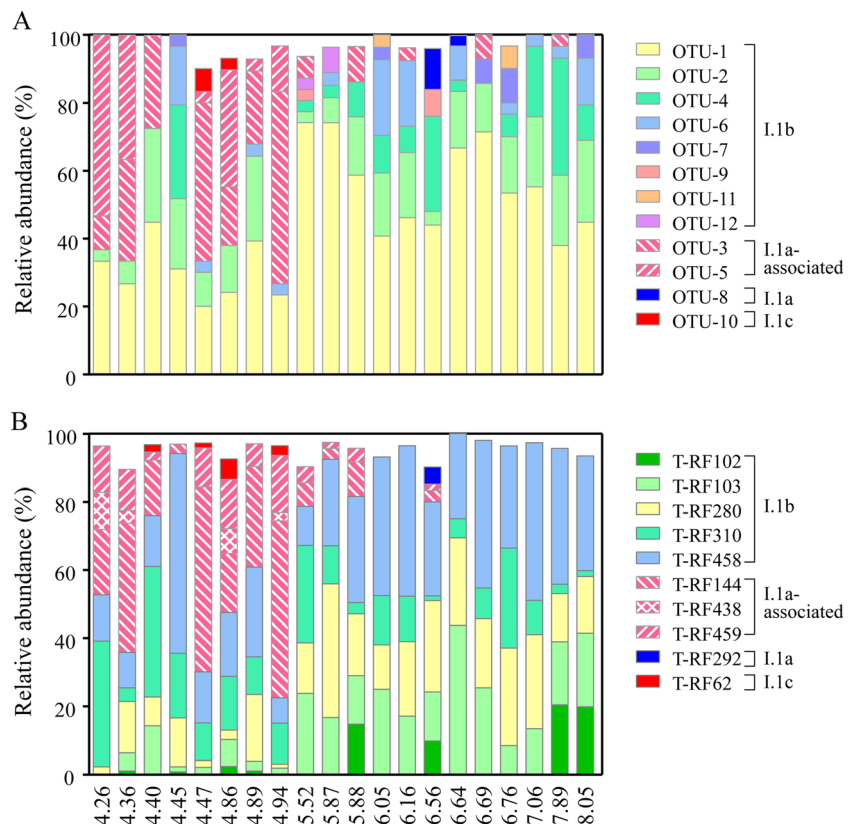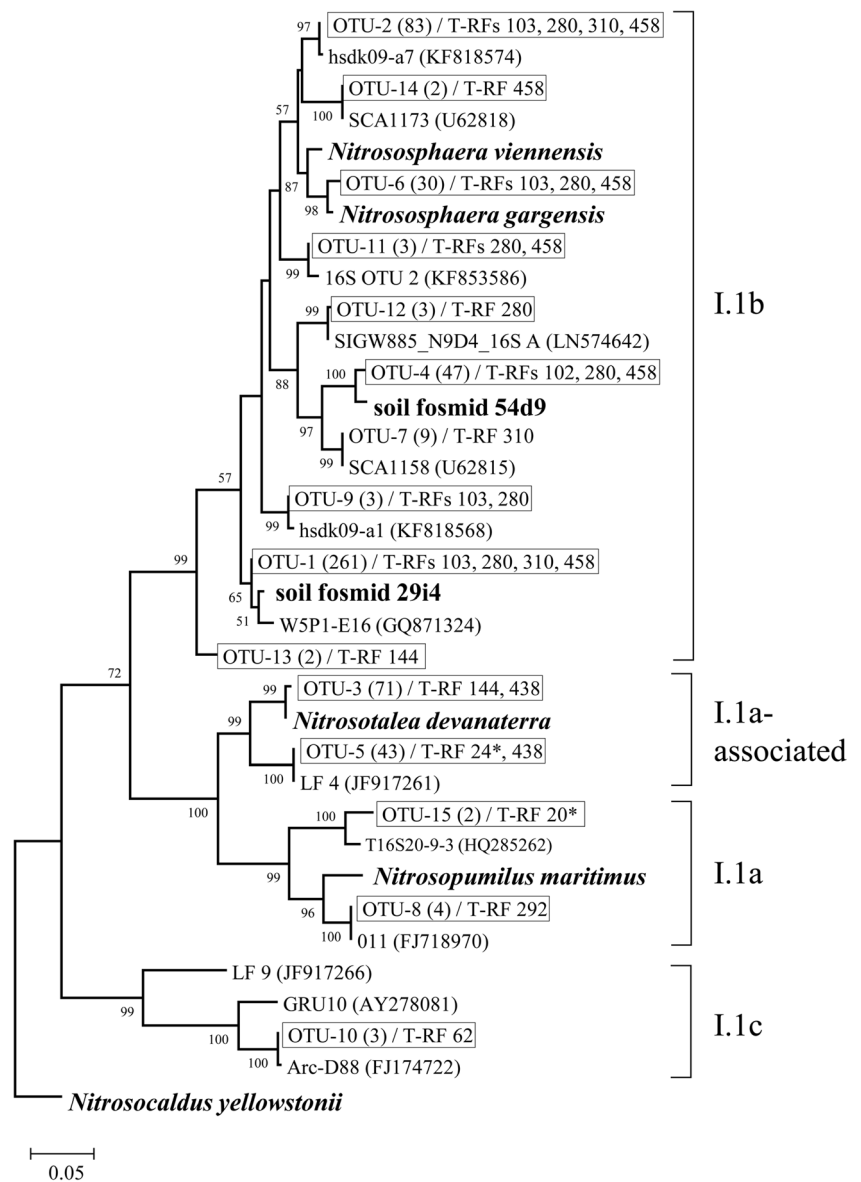
Fig. 2 Neighbor-joining tree of thaumarchaeotal 16S rRNA genes. The representative sequences retrieved in this study for each OTU (in *box*), key reference sequences (in *bold*), and closest matched sequences were included in the tree. The corresponding T-RFs are listed after the OTU. Only bootstrap values greater than 50 % are shown near nodes

inorganic N. As such, the neutral pH may be close to the background of Argosols in this area, while the strong soil acidification was very likely due to the excess use of mineral N fertilizers. This was further supported by the negative correlation between soil inorganic N (NH_4^++ NO_2^- + NO_3^-) and pH ($r = -0.48$, $P < 0.05$, $n = 20$).

Fig. 3 Canonical correspondence analysis (CCA) biplot of **a** clone library and **b** T-RFLP of thaumarchaeotal 16S rRNA genes in the top soils showing regulating factors and samples. The factor of significance is shown by the *red line*
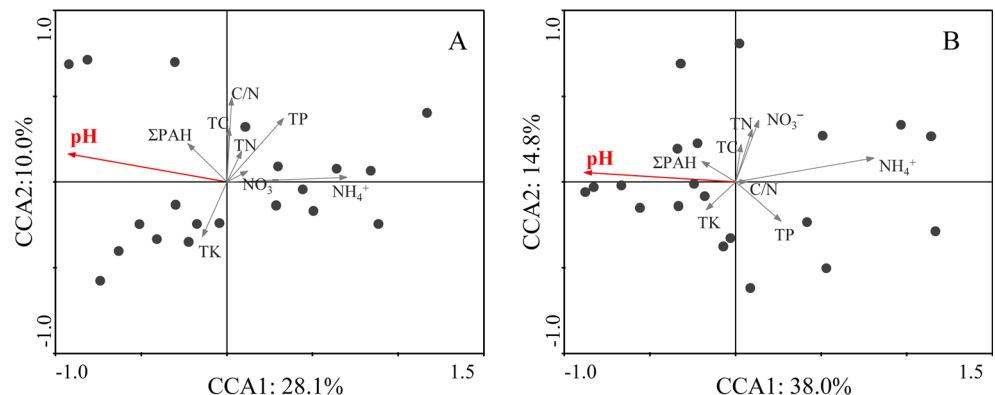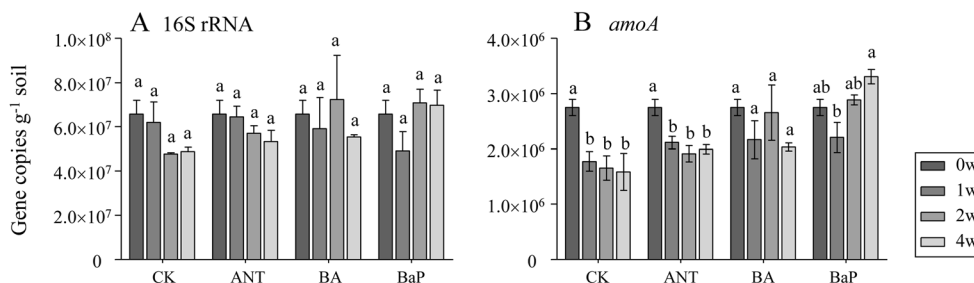
Fig. 4 Changes of thaumarchaeotal **a** 16S rRNA and **b** amoA gene copy numbers in the soil microcosms during the 4-week incubation. The results represent the mean ± SE of three replicate microcosms. CK, ANT, BA, and BaP indicate the control, anthracene-, benz(a)anthracene-, and benzo(a)pyrene-amended microcosms, respectively. *Bars* with the same *lowercase letter* on top were not significantly different ($P > 0.05$)

pH and substrate supply are major factors controlling the thaumarchaeotal community in terrestrial environments (Gubry-Rangin et al. 2011; Prosser and Nicol 2008). Our findings demonstrate a strong association between thaumarchaeotal communities and soil pH in the investigated soils. Indeed, a decline in the soil pH reduced the abundance of Thaumarchaeota or AOA (Table 2), and the enrichment of *Nitrosotalea*-related lineages in soils of pH < 5.0 suggests the I.1a-associated group is more adapted to acidic environments. In contrast, most I.1b group taxa favored neutral soils (Fig. S1, Electronic Supplementary Material). N fertilization showed marginal effects on Thaumarchaeota despite the wide variation in inorganic N concentrations (Table 1), whereas the abundance of AOB could be strongly correlated with nitrate (Table 2). Overall, these and previous observations (Wu et al. 2011) suggest that pH rather than inorganic N is a determinant of the thaumarchaeotal communities in these agricultural soils affected by fertilization-induced acidification.

In addition to pH, the effects of PAH pollution on soil Thaumarchaeota were explored in this study. Two orders of magnitude gradient of PAHs were observed across the soils, with the ΣPAHs in some samples higher than normally found in contaminated agricultural soils from this region (Yangtze River Delta, east China) (Ping et al. 2007), suggesting serious soil pollution. Nevertheless, no stress of the natural occurrence of PAH contamination on soil Thaumarchaeota was found (Table 2 and Fig. 3), although the inhibition of soil nitrification activity by PAH contamination was widely recognized. To avoid the confounding influence of other soil factors, short-term incubation of soil microcosms spiked with individual PAH was carried out. Limited transformation of PAHs, indicative of persistent PAH stress across the incubation period (Fig. S2, Electronic Supplementary Material), was observed. The decreased abundance of thaumarchaeotal genes (Fig. 4) in the control microcosms might be due to the degradation of free DNA molecules or dead microbial cells. Four-week exposure to 50–150 mg kg^{-1} PAHs, which is rarely observed in real situations, did not show any adverse effect on soil Thaumarchaeota.

The absence of response may be mainly associated with the bioavailability of PAHs in soil, because the saturated concentration of PAHs in pore water should be extremely low due to their hydrophobicity and the aging process in soil (Alexander 2000). Most PAH molecules may be tightly attached to soil particles, making them inaccessible and thus less toxic to microbes. This is supported by the high effect concentration of PAHs on soil nitrification activity. For example, significant nitrification inhibition was observed at approximately 1000 mg kg^{-1} of benzo(a)pyrene, one the most toxic PAHs (Sverdrup et al. 2007). Nevertheless, cultivated Thaumarchaeota could be quite sensitive to organic pollutants presented in the medium. *N. maritimus*, a cultivated I.1a Thaumarchaeota, was inhibited by low concentration of crude oil and methylene blue (Sipos and Urakawa 2016; Urakawa et al. 2012). We also observed a growth inhibition of one I.1b thaumarchaeotal enrichment by benzo(a)pyrene (unpublished data).

The enhanced growth of soil ammonia-oxidizing Thaumarchaeota in the presence of benzo(a)pyrene is interesting (Fig. 4). In a previous study (de Menezes et al. 2012), phenanthrene amendment increased the relative abundance of thaumarchaeotal transcripts in soil. These observations implicate complicated interactions between Thaumarchaeota and
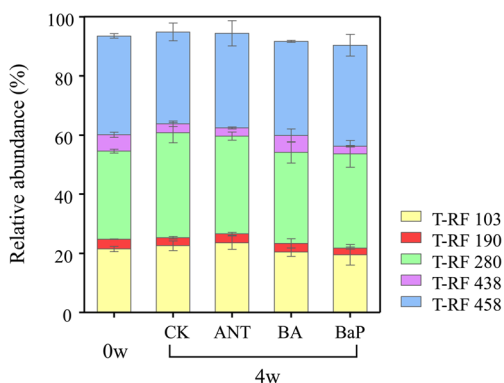

Fig. 5 Thaumarchaeotal communities before and after the incubation as revealed by the T-RFLP of the 16S rRNA gene. The results represent the mean ± SE of three replicate microcosms. CK, ANT, BA, and BaP indicate the control, anthracene-, benz(a)anthracene-, and benzo(a)pyrene-amended microcosms, respectively

PAHs in soil, and many possibilities exist. First, PAH degradation by soil microbes produces small organic molecules (Juhasz and Naidu 2000), which may improve thaumarchaeotal growth even at a low concentration (Tourna et al. 2011). Second, the changes in the soil microbial community caused by PAH stress may in turn affect Thaumarchaeota (Zhalnina et al. 2013). Third, *amoA* belongs to a family of copper-containing membrane-bound monooxygenases (CuMMO), which has a wide substrate range including aromatic hydrocarbons (Pester et al. 2011). PAH degradation has been demonstrated with AOB (Chang et al. 2002), but whether Thaumarchaeota have a similar function merits further study.

The gene *amoA* has often been used as a marker to reveal the community characteristics of thaumarchaeotal ammonia oxidizers due to its taxonomic coincidence with the 16S rRNA gene (Pester et al. 2012). However, *amoA*-based analysis cannot cover the entire phylum of Thaumarchaeota because some clades appear to lack the function of ammonia oxidation (Weber et al. 2015). In this study, the presence of the I.1c group in some of the most acidic samples (pH <5.0), as revealed by clone library and T-RFLP analysis of the 16S rRNA gene (Fig. 1), confirms its preference for acidic conditions (Oton et al. 2016). Moreover, limited by the resolution of these two community analysis methods, no other thaumarchaeotal clades than AOA and I.1c were retrieved; however, these groups could be rare in soil (Oton et al. 2016). Application of next generation sequencing technologies may be helpful to reveal the vast diversity of soil Thaumarchaeota.

5 Conclusions

Taken together, we examined the thaumarchaeotal community in agricultural soils impacted by both acidification and PAH pollution. The results demonstrate a major influence of pH on both thaumarchaeotal abundance and composition. PAHs, even at a high concentration, did not inhibit soil Thaumarchaeota in these aged contaminated soils or short-term exposed microcosms. The influence of PAH on growth demonstrates the complexity of the interactions between Thaumarchaeota and organic pollutants, which should be addressed in future studies.

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