Microbial Ecology (2018) 76:1041–1052
https://doi.org/10.1007/s00248-018-1183-3

SOIL MICROBIOLOGY

Different Recovery Processes of Soil Ammonia Oxidizers from Flooding Disturbance

Fei Ye[1,2] · Mao-Hua Ma[1] · Huub J. M. Op den Camp[3] · Antonis Chatzinotas[4,5] · Lei Li[6] · Ming-Quan Lv[1,2] · Sheng-Jun Wu[1] · Yu Wang[1]

Received: 29 December 2017 / Accepted: 26 March 2018 / Published online: 11 April 2018

Abstract

Understanding how microorganisms respond to environmental disturbance is one of the key focuses in microbial ecology. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are responsible for ammonia oxidation which is a crucial step in the nitrogen cycle. Although the physiology, distribution, and activity of AOA and AOB in soil have been extensively investigated, their recovery from a natural disturbance remains largely unknown. To assess the recovery capacities, including resistance and resilience, of AOA and AOB, soil samples were taken from a reservoir riparian zone which experienced periodically water flooding. The samples were classified into three groups (flooding, recovery, and control) for a high-throughput sequencing and quantitative PCR analysis. We used a relative quantitative index of both the resistance (RS) and resilience (RL) to assess the variation of gene abundance, alpha-diversity, and community composition. The AOA generally demonstrated a better recovery capability after the flooding disturbance compared to AOB. In particular, AOA were more resilient after the flooding disturbance. Taxa within the AOA and AOB showed different RS and RL values, with the most abundant taxa showing in general the highest RS indices. Soil NH_4^+ and Fe^{2+}/Fe^{3+} were the main variables controlling the key taxa of AOA and AOB and probably influenced the resistance and resilience properties of AOA and AOB communities. The distinct mechanisms of AOA and AOB in maintaining community stability against the flooding disturbance might be linked to the different life-history strategies: the AOA community was more likely to represent r-strategists in contrast to the AOB community following a K-life strategy. Our results indicated that the AOA may play a vital role in ammonia oxidation in a fluctuating habitat and contribute to the stability of riparian ecosystem.

Keywords Archaea · Ammonia-oxidizing communities · Response · Resistance · Resilience · Riparian zone

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-018-1183-3) contains supplementary material, which is available to authorized users.

✉ Sheng-Jun Wu
wsj@cigit.ac.cn

✉ Yu Wang
wangyu@cigit.ac.cn

[1] Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Department of Microbiology, IWWR, Radboud University Nijmegen, Nijmegen 6525 AJ, the Netherlands

[4] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research-UFZ, 04318 Leipzig, Germany

[5] Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany

[6] Beijing Academy of Science and Technology, Beijing 100048, China

Introduction

Disturbance is defined as a discrete event, force, or process, of either abiotic or biotic origin, that results in changes in the relative abundance and diversity of organisms, or in their community composition [1, 2]. Disturbances occur at different scales, frequencies, intensities, and periodicities [3]. They can be divided into "pulse" or "press" disturbance according to their duration and impact. In general, pulse disturbances are relatively discrete, short-term events, whereas presses are long term or continuous [4]. In natural ecosystems, disturbances frequently come from regime shifts such as fire or flooding cycles [2, 5, 6], which cause changes in community composition arising from a shift between alternative stable states.

Microbial communities show different strategies in responding to environmental disturbances. In some cases, microbial groups show a high degree of metabolic flexibility and

physiological tolerance which makes them resistant against changing environmental conditions [7]. Resilient microbial communities may experience changes in composition in response to unfavorable conditions; however, they may still recover quickly, whether by fast growth rates, physiological adaptation, or rapid evolution [8]. Whether or to what extent a community could recover from a disturbance depends on the strength and duration of the disturbance [9, 10]. Understanding how microbial guilds respond to cycles of disturbance and the recovery process can reveal important relationships between community structure and ecosystem functions, especially in the context of global climate change with predictable increasing in extreme drought or precipitation events [11].

Ammonia oxidation, which is often the rate-limiting step of nitrification in a wide variety of environments [12], is a critical ecosystem process. It has been long assumed that autotrophic ammonia oxidation is exclusively performed by bacteria (ammonia-oxidizing bacteria (AOB)); however, the isolation of marine ammonia-oxidizing archaea (AOA), Candidatus '*N. maritimus*' [13], initiated extensive studies on the physiology [14], distribution [15–17], and role of *Archaea* in ammonia oxidation [18, 19]. The distributions and relative roles of AOA and AOB are often differentiated by environmental conditions, which is probably due to the distinct physiologies between *Archaea* and *Bacteria*. For instance, AOA tend to dominate at low pH (3.7–5.8) [16, 20], and are nearly ten times more abundant than AOB in a low-oxygen (0.1–0.2 mM) subterranean estuary [21]. Other key factors affecting the relative importance of these two groups include ammonium level [13, 14], temperature [22], and salinity [21].

More recent studies have been paying attention to the recovery of ammonia oxidizers after environmental disturbances. A microcosm-based study showed that the AOA abundance and community composition were less resistant to changes than AOB, and niche differentiation of AOA and AOB associated with ammonia concentration was proposed [23]. Compared to AOB, the AOA had better adaptability to oxygenated/hypoxic alternant conditions as evidenced by the increased AOA operational taxonomic unit numbers and the higher AOA/AOB ratios of abundance [24]. The recovery abilities of AOA and AOB are thus key traits in determining their roles in ammonia oxidation in the environment, since most habitats are subject to disturbances over time. However, the recovery capacities, including resistance and resilience, of AOA and AOB remain largely unclear.

Here, we investigated the recovery capacities of AOA and AOB in soils from a riparian zone of the Three Gorges Reservoir (TGR) in China. The Three Gorges Dam is the world's largest hydroelectric project. The riparian area of the TGR is directly affected by the water-level fluctuation ranging from 145 m in summer up to 175 m in winter since the Dam started full operation in 2010 [25]. The frequent and dramatic water fluctuation turns the original terrestrial ecosystem into a seasonal wetland ecosystem. The original vegetation species failed to survive under the prolonged submergence condition during the high water-level stage and died out [26]. Therefore, the TGR riparian zone is currently at an early stage of secondary succession. So far, studies have focused mainly on vegetation restoration [27] and nutrient turnover [28], but relatively little attention has been paid to the recovery of soil microorganisms which is essential in restoring and supporting the riparian ecosystem functions.

We specifically hypothesized that (i) due to niche differences, the AOA and AOB might structure differently in their response to flooding disturbance, and (ii) the differences in the community structure of AOA and AOB communities might lead to different recovery processes after disturbance. To test these hypotheses, we showed the responses and recovery processes of the AOA and AOB communities using qualitative (high-throughput sequencing) and quantitative (qPCR) analyses of archaeal and bacterial *amoA* genes against flooding disturbance. Furthermore, we studied the link between community structure and recovery capacities by identifying the key taxa of the AOA and AOB communities.

Materials and Methods

Study Site and Sampling Strategy

The riparian zone undergoes periodically water flooding within a year. Accordingly, the riparian soil is flooded for 39–273 days at different elevations, while it is re-exposed to atmosphere and returns to dry state during the rest of the year (the rotations of flooding and exposure, and the respective durations are listed in Table S1).

Four field sampling surveys were conducted in the riparian zone of Baijiaxi (31° 09′ 02″ N, 108° 33′ 45″ E) which is located in a wetland nature reserve with limited impact of human activities (Fig. 1). The zonal soil type is mainly purple soil developed from purple gritstone, and the soil texture is dominated by silt clay. The climate of the study area is characterized by a humid mid-subtropical monsoon type. The annual mean air temperature is 10.8–18.5 °C, and the annual precipitation is about 1200 mm with a rainy season for about 6 months.

The water level of the study area rises periodically to the highest level (174–175-m elevation) in November and then decreases to the lowest level (at 150 m) in May of the following year. Sampling sites were set at 5-m intervals in one transect along the elevation gradient. Samples were taken on 10 October 2013 and 1 April 2014, 23 September 2014, and 6 May 2015, representing the beginning and later stages of

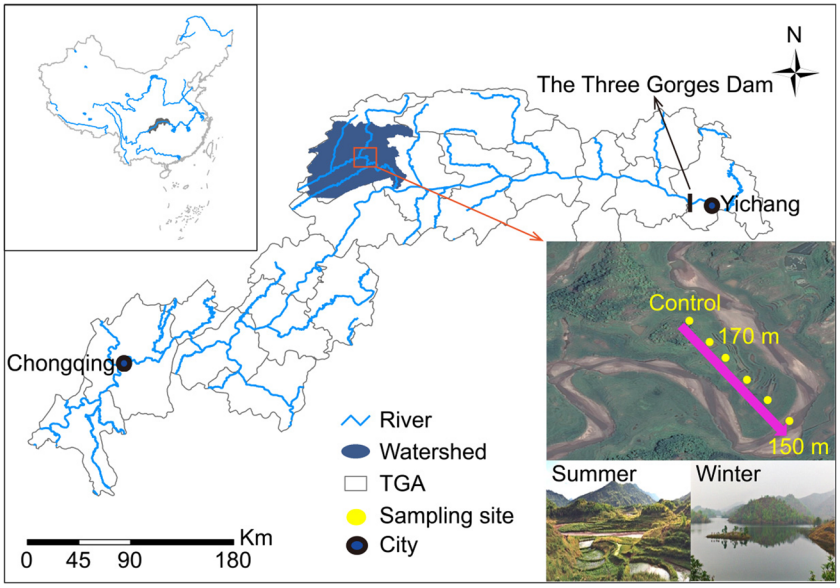

Fig. 1 Location of the study area in the Three Gorges Area (TGA), China. Along an elevation gradient, sampling sites were set up at 5-m intervals ranging from 170 to 150 m in elevation. Samples at 175.5-m elevation (unflooded during the study period) were used as a control. Respective views in summer and winter of sampling location are also presented

water flooding within two inundation cycles, respectively (Fig. 2). A site at 175.5 m, which had never been flooded in recent years, was used as a control site.

Sampling and Sample Classifications

Soil samples (0–10-cm depth) above water level were collected using a stainless steel core sampler, while samples below water level were collected with a Petersen grab. Soil samples were taken away from plant stands to minimize the influence of vegetation. Three parallel subsamples were collected and mixed thoroughly to form one composite sample from each sampling site. Each collected composite sample was divided into two portions. One was stored at 4 °C for physicochemical analysis, and the other one was stored at − 20 °C for DNA extraction and downstream molecular analysis.

Three groups of samples were formed according to the state of the flooding: unflooded sites were used as control group (4 soils), sites below the water-level at the sampling date (flooding group, 13 soils), and sites where the water was receded at the sampling date (recovery group, 7 soils) (Table S1).

Analytical Procedures of Physicochemical Properties

Soil properties of the pH and concentrations of NH_4^+, NO_3^-, organic matter (OM), total carbon (TC), total nitrogen (TN), total sulfur (TS), ferrous iron (Fe^{2+}), and ferric iron (Fe^{3+}) were analyzed. The C/N ratio was calculated from the TC and TN results. The Fe^{2+}–Fe^{3+} system often acts as an electron carrier; therefore, the Fe^{2+}/Fe^{3+} ratio was employed as an indicator to estimate the redox conditions in soil [29, 30]. The soil pH was measured after shaking with MilliQ water at a soil/water ratio of 1:5. The NH_4^+ and NO_3^- were determined by flow injection analysis (FIA Star 5000, FOSS Tecator, Sweden) after extraction with a 2 M KCl solution. The soil OM was measured according to LOI_{550} (loss on ignition at 550 °C) [31]. The contents of TC, TN, and TS were measured with an Element Analyser (Vario EL cube, Elementar, Germany). The Fe^{2+} was measured by o-phenanthroline

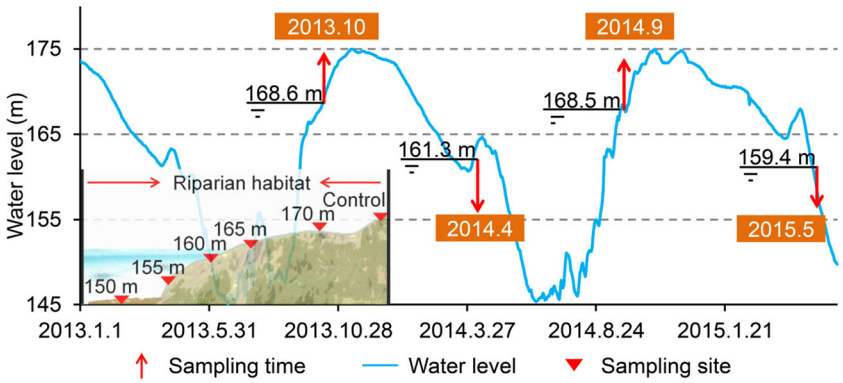

Fig. 2 Water-level fluctuation and sampling site distribution at six elevations within a riparian zone. Sampling dates are marked by arrows with corresponding water levels. Daily data of water level were obtained from the China Three Gorges Corporation (http://www.ctg.com.cn/)

spectrophotography at an absorbance of 520 nm (UV-1750, Shimadzu, Japan) after extraction with a 0.1 M $Al_2(SO_4)_3$ solution (pH = 2.5). Fe^{3+} was determined from the subtraction of the measured total iron (the reduced Fe^{2+} by hydroxylamine hydrochloride and inherent Fe^{2+} in the extract solution) and inherent Fe^{2+} measured directly by o-phenanthroline spectrophotography [32]. All the analyses were carried out in triplicate.

DNA Extraction and Quantitative PCR

Soil DNA was extracted according to the manufacturer's instruction using the PowerSoil DNA Isolation Kit (Mobio, USA). The DNA concentration was determined using a NanoVue Plus Spectrophotometer (GE Healthcare, UK), and the DNA quality was checked by 1% (weight/volume) agarose gel electrophoresis.

The abundances of archaeal and bacterial *amoA* genes were measured by quantitative PCR (qPCR) using primer sets Arch-amoAF/Arch-amoAR [15] for AOA and amoA-1F/amoA-2R [33] for AOB. Standard curves were constructed with plasmid DNA containing archaeal and bacterial *amoA* genes fragments. The 20-μL reaction mixtures contained 10 μL SYBR® Premix Ex Taq™ II (Takara, Japan), 0.4 μL ROX Reference Dye (Takara, Japan), 0.3 μL (10 pmol μL^{-1}) of both forward primer and reverse primers, 8 μL sterilized water, and 1 μL template DNA (ca. 1 ng). Quantitative PCR assay was performed in a ABI ViiA™ 7 (Applied Biosystems, USA) using an initial denaturation of 95 °C for 2 min, 40 cycles of 20 s at 95 °C, 30 s at 55 °C for AOA or 59 °C for AOB, and 1 min at 72 °C. The specificity of each amplicon was checked by melting-curve analysis. The qPCR efficiencies were 92–108% ($R^2 > 0.991$) for AOA and 91–101% ($R^2 > 0.996$) for AOB, respectively.

Amplification and High-Throughput Sequencing

The *amoA* genes were amplified using the same primer sets as mentioned above with appropriate barcodes (10 bp). The amplification was carried out using an ABI GeneAmp® 9700 with the programs identical to the above mentioned. The PCR products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen, USA). Prior to sequencing, the DNA concentration of each PCR product was measured by the QuantiFluor™-ST blue fluorescence system (Promega, USA). Subsequently, purified amplicons were pooled in equimolar amounts and paired-end (PE) sequenced (2 × 300) on an Illumina MiSeq PE300 platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China (http://www.Majorbio.com).

Sequence Analysis

Raw fastq files were demultiplexed and quality-filtered using Trimmomatic (version 0.30). The sequences with low quality (average quality score < 20 and length < 50 bp) were removed. Sequences exactly matching their barcode and primers were kept, and barcode and primer sequences were removed. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) using the Usearch pipeline (version 7.0, http://drive5.com/uparse/) at 85% identity for AOA [34] and 88% identity for AOB [35], which equals about 97% identity of 16S rRNA genes [36]. The representative sequence of each OTU was blasted against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to exclude the non-*amoA* gene OTUs. Neighbor-joining phylogenetic trees were constructed by aligning representative sequences together with reference sequences using 1000 bootstrap replicates using MEGA 5 [37]. The reference sequences were retrieved from the NCBI database by their accession numbers as obtained from previous studies [36, 38–40]. The raw data of the high throughput sequencing were deposited in NCBI Sequence Read Archive (SRA, http://trace.nvbi.nlm.nih.gov/Traces/sra/sra.cgi) under the accession number PRJNA416222.

Quantification of Resistance and Resilience

A relative quantitative measurement of resistance and resilience was used to compare the response of ammonia-oxidizing communities to disturbance. The resistance (RS) index was calculated for ammonia-oxidizing *amoA* gene abundances, alpha-diversity, and the relative abundance of key ammonia-oxidizing taxa, accounting for the differences in the amount of change that a disturbance could cause. The equation for RS index was as follows [41]:

$$\mathrm{RS} = 1 - \frac{2|C_0 - P_0|}{(C_0 + |C_0 - P_0|)}$$

C_0 and P_0 are the values of variables in the control soil and flooding affected soil, respectively. The RS is bounded by − 1 and + 1, with + 1 showing that the water flooding had no effect (maximal resistance) and lower values showing stronger effects (less resistance).

The resilience (RL) index was calculated as follows [41]:

$$\mathrm{RL}\ (x) = \frac{2|C_0 - P_0|}{(|C_0 - P_0| + |C_x - P_x|)} - 1$$

C_0 and P_0 are the values of variables in the control soil and flooding affected soil, respectively. C_x and P_x are the values of ammonia oxidizers relevant variables in the control soil and recovery soil, respectively. In this study, the value of C_0 was equal to C_x. The RL is also bounded by − 1 and + 1, where + 1

indicates complete recovery (maximal resilience) and lower values represent a slower rate of recovery.

Statistical Analysis

Alpha-diversity indices of Shannon diversity, Chao1 richness, and Shannon-based community evenness were calculated with OTU data. Alpha-diversity and rarefaction analysis were calculated using the program Mothur (version v.1.30.1). The difference of AOA and AOB *amoA* gene abundances, alpha-diversity, and relative abundances of AOA and AOB taxa among the three sample groups were calculated based on independent sample *t* test and repeated measures ANOVA (IBM SPSS Statistics 20.0 for Windows). Pearson correlations were used for archaeal abundance and soil properties (IBM SPSS Statistics 20.0 for Windows). A two-way permutational multivariate analysis of variance (PerMANOVA) was calculated on OTU level to imply the dissimilarity of AOA and AOB community compositions between the sample groups based on Bray-Curtis distance with 999 permutations in the R platform (version 3.0.1, vegan package). Community structures and correlation with physicochemical properties were analyzed with redundancy analyses (RDA; 999 Monte Carlo permutation tests) using CANOCO 5 software. Graphs were generated using ORIGIN 9.0 software.

Results

Physochemical Characteristics of Sampling Sites

Clear influences from water flooding were observed on soil NH_4^+, NO_3^-, pH, TS, and Fe^{2+}/Fe^{3+}. The lowest NH_4^+, pH, TS, and Fe^{2+}/Fe^{3+} were observed in the control group, and the highest values were observed in the flooding group (Fig. S1). Contrarily, the lowest NO_3^- concentration was observed in the flooding group, being significantly lower compared to the control group ($P < 0.05$). For the parameters of OM, TC, and TN, minor variations were observed between the three groups.

Community Structures of Ammonia Oxidizers Responding to Flooding Disturbance

Abundance of AOA and AOB

The copy numbers of AOA and AOB *amoA* genes ranged from 1.3×10^6 to 6.6×10^8 and 1.6×10^4 to 1.5×10^7 copies (g d.w.s, gram dry weight soil)$^{-1}$, respectively (Fig. 3). The control group showed the highest abundance of AOA and AOB at 2.0×10^8 and 4.3×10^6 copies (g d.w.s)$^{-1}$, respectively. Since the abundance of AOA and AOB decreased respectively to 5.9×10^7 and 2.1×10^6 copies (g d.w.s)$^{-1}$ after flooding, water flooding appeared to have a negative effect on the abundance of both AOA and AOB in the riparian soil. Significant differences in the AOA abundances were observed between the flooding and recovery groups ($P < 0.05$) (Fig. 3). Significant differences ($P < 0.01$) were found comparing AOA and AOB from the recovery and flooding groups (Fig. 3).

Alpha-Diversity of AOA and AOB

Because rhythm of flooding fluctuation at level of 160 m is almost identical with that at 165 m, the samples collected at 160-m elevation were excluded from analysis, resulting 20 samples for both AOA and AOB. A total of 239,920 high-quality sequences (6519–17,421 sequences per sample) for AOA and 435,119 (11,803–32,325 sequences per sample) for AOB were obtained across the 20 samples, respectively. Sequences were rarified to an even sequencing depth of 6500 per sample according to the minimum sequences of samples. In total, 15 and 17 unique OTUs for AOA and AOB were obtained, respectively. The Good's coverages of the *amoA* gene were around 99.9%, indicating that the sequencing effort covered a significant amount of the richness in the examined samples (Table S2).

Alpha-diversity indices including Shannon diversity, Chao1 richness, and Shannon evenness of AOA and AOB community were assessed based on the *amoA* gene-targeted high-throughput sequencing data (Fig. 4).

In the AOA community, the highest values in all alpha-diversity indices were observed in the flooding group and the lowest values in the control group. Statistical differences between the flooding and recovery groups were observed on Shannon diversity and Chao1 richness ($P < 0.05$), indicating the Shannon diversity and Chao1 richness in the recovery group were more close to those of the control group (Fig. 4).

In the AOB community, we did not observe any alpha-diversity gradient like in the AOA community (Fig. 4). The highest values of the three indices were also observed in the flooding group, but the lowest ones were observed in the recovery group. Statistically significant differences between the recovery and flooding groups were observed on all the three indices ($P < 0.01$). In addition, the Shannon diversity and evenness in the recovery group were significantly lower than those of the control group ($P < 0.05$).

Shannon diversity and Chao1 richness of AOA were positively correlated with soil NH_4^+ ($P = 0.012$ and 0.005, respectively) (Table S3). Moreover, the Chao1 richness of AOA was also positively correlated with the Fe^{2+}/Fe^{3+} ratio ($P = 0.000$) and negatively correlated with soil NO_3^- ($P = 0.008$). In contrast, the Shannon evenness of AOA did not show significant correlation with any of the measured soil properties. Similarly, the Shannon diversity and Chao1 richness of AOB showed

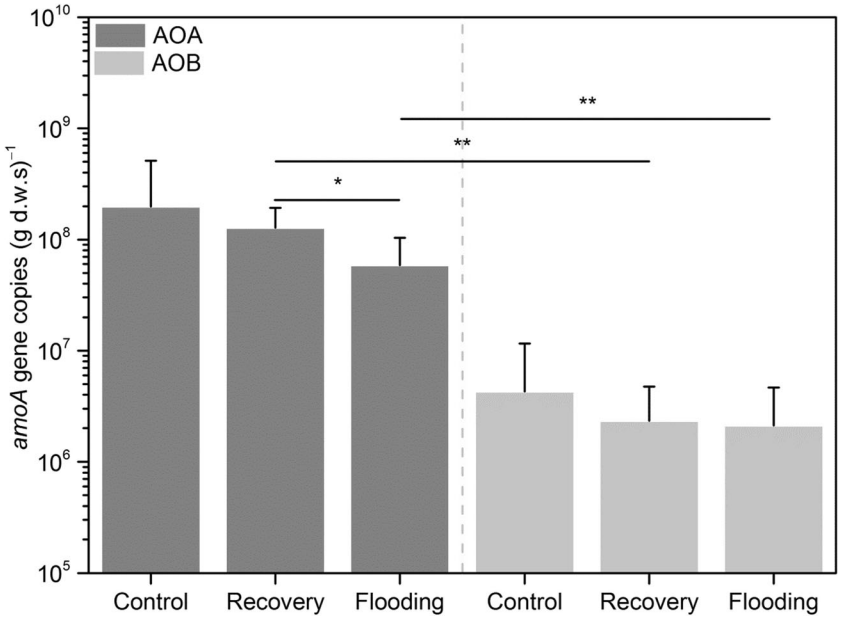

Fig. 3 Abundance of AOA and AOB *amoA* genes among the three sample groups. Error bars represent standard error of the mean ($n = 4$, 7, and 13 in control, recovery, and flooding groups, respectively); "*" and "**" indicate significant differences at $P < 0.05$ and 0.01 based on an independent sample *t* test, respectively

positive correlations with NH_4^+ ($P = 0.001$ and 0.000, respectively) and Fe^{2+}/Fe^{3+} ($P = 0.014$ and 0.000, respectively). The AOB Chao1 richness was also negatively correlated with NO_3^- ($P = 0.042$). In contrast to the Shannon evenness of AOA which did not correlate with any of the measured soil properties, the Shannon evenness of AOB was positively correlated with NH_4^+ ($P = 0.008$), and negatively correlated with TS ($P = 0.018$).

Community Compositions of AOA and AOB

A redundancy analysis (RDA) was performed to illustrate the AOA and AOB community structures of each site (Fig. 5). The distribution of the AOA community showed a gradient from the flooding group to the recovery and control groups representing a recovery process. Besides, the AOA community

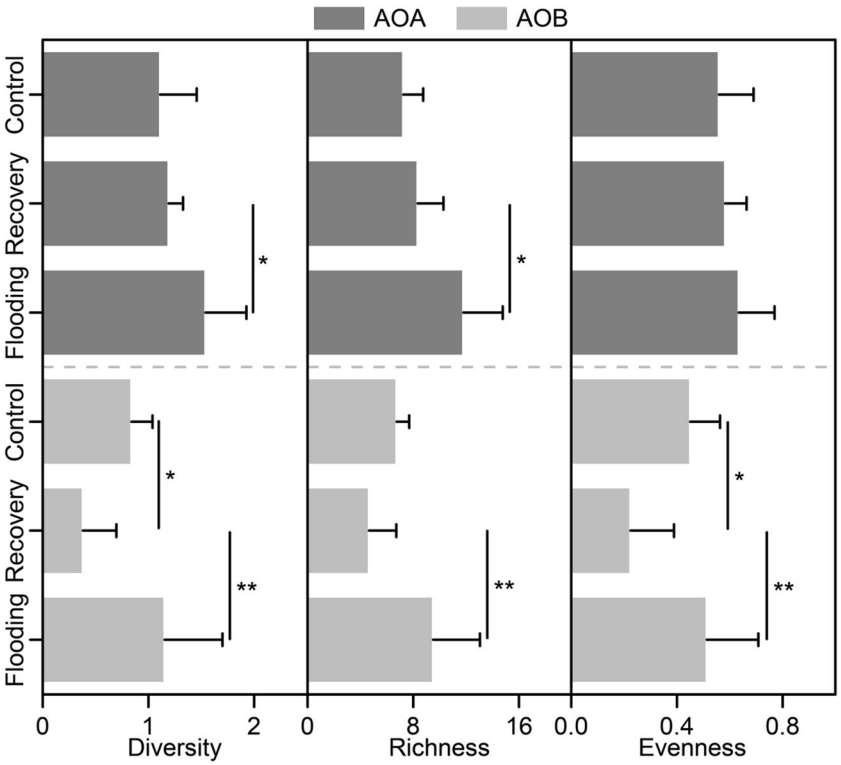

Fig. 4 Alpha-diversity of AOA and AOB across the three sample groups. Error bars represent standard error of the mean ($n = 4$, 7, and 13 in control, recovery, and flooding groups, respectively); "*" and "**" indicate significant differences at $P < 0.05$ and 0.01 based on independent sample *t* test, respectively. Diversity: the Shannon index; richness: the Chao1 estimator; evenness: a Shannon index-based measure of evenness

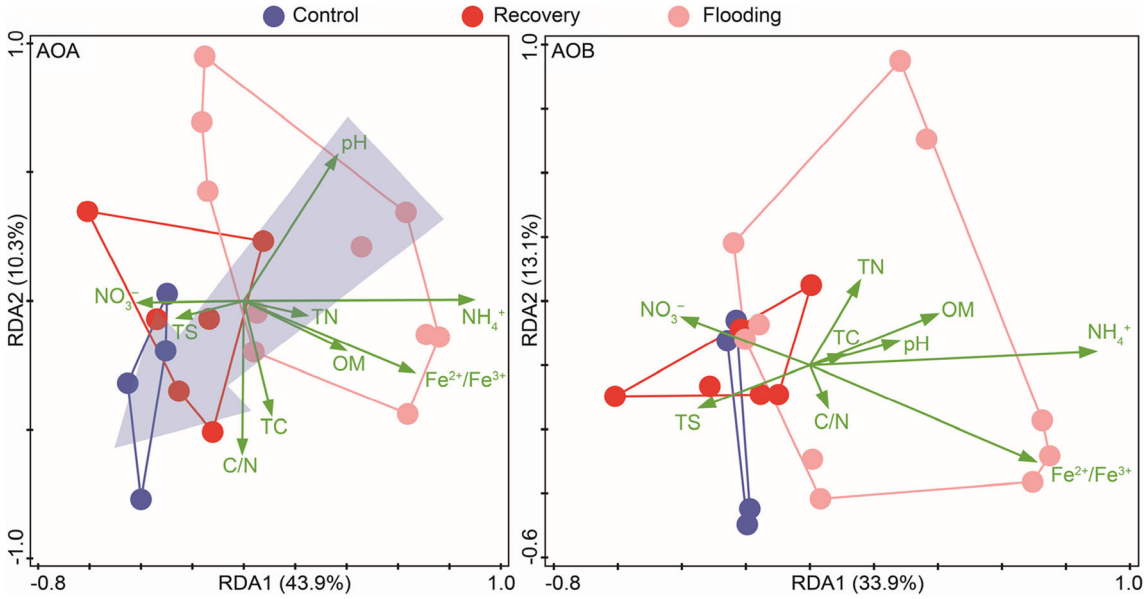

Fig. 5 Redundancy analysis of AOA and AOB community structures and soil properties. Soil groups are marked with colors: blue-control, red-recovery and pink-flooding. The green arrows indicate the soil properties. The blue arrow in the background of AOA plot indicates a gradient from flooding group to the recovery and control groups

structure and the recovery appeared to be governed by the soil NH_4^+ (pseudo $F = 9.7$, $P = 0.001$) and Fe^{2+}/Fe^{3+} (pseudo $F = 4.7$, $P = 0.005$) (Fig. 5). The soil NH_4^+ (pseudo $F = 6.7$, $P = 0.001$) and Fe^{2+}/Fe^{3+} (pseudo $F = 4.1$, $P = 0.004$) were also key variables for the AOB community structure, but no gradient was observed.

Two-way PerMANOVA was conducted to assess the dissimilarities of AOA and AOB community structures among the flooding classifications. Between the control and flooding groups, no significant difference was observed (Table 1). However, judging from the values of R^2, the AOB community ($R^2 = 0.035$) was more similar between the control and flooding groups than the AOA community ($R^2 = 0.114$), suggesting a heavier impact of flooding on the AOA community. When comparing the recovery and control groups, only a relatively minor difference was observed between the AOA communities ($P = 0.684$), while a significant dissimilarity was found for the AOB. Similarly, the AOB community ($P = 0.025$) from the recovery and flooding groups significantly differed, compared to the relatively similar AOA communities from the two groups ($P = 0.059$).

Recovery of Ammonia Oxidizers After Flooding Disturbance

Resistance and Resilience of AOA and AOB

The resistance and resilience of ammonia oxidizers were assessed based on the abundance (i.e., copy numbers) and the alpha-diversity (using Shannon diversity, Chao1 richness, and Shannon evenness) of the *amoA* genes. The RS index of the AOB abundance was higher than that of AOA (0.33 vs. 0.17), suggesting that AOB abundances were more resistant than those of AOA (Fig. 6). In contrast, the RL of AOA *amoA* abundance was much higher than that of AOB (0.33 vs. 0.05) (Fig. 6).

The RS index of AOB Shannon diversity and Chao1 richness was 0.48 and 0.35, respectively, which were both higher than those of AOA. Only the RS index of AOB evenness was lower than that of AOA (Fig. 6). In contrast, the RL values for the AOA diversity indices (with the exception of the Chao 1 richness) were higher than those of AOB.

Resistance and Resilience of Key Taxa

Phylogenetic analysis showed that the obtained AOA sequences were mainly grouped into *Nitrososphaera* subclusters 1, 2, 4, 8, and the clusters *Nitrosotalea*, *Nitrosopumilus*, and *Nitrosocaldus* (Fig. S2). More than 60% of the AOA

Table 1 Dissimilarity of AOA and AOB community compositions among the three sample groups based on two-way PerMANOVA

Comparison	AOA		AOB	
	R^2	P	R^2	P
Control vs. flooding	0.114	0.145	0.035	0.750
Recovery vs. control	0.070	0.684	*0.447**	*0.014*
Recovery vs. flooding	0.137	0.059	*0.250**	*0.025*

Two-way PerMANOVA was calculated on OTU level based on Bray-Curtis distance with 999 permutations implying the dissimilarity between two groups. Significant values are shown in italics; *$P < 0.05$

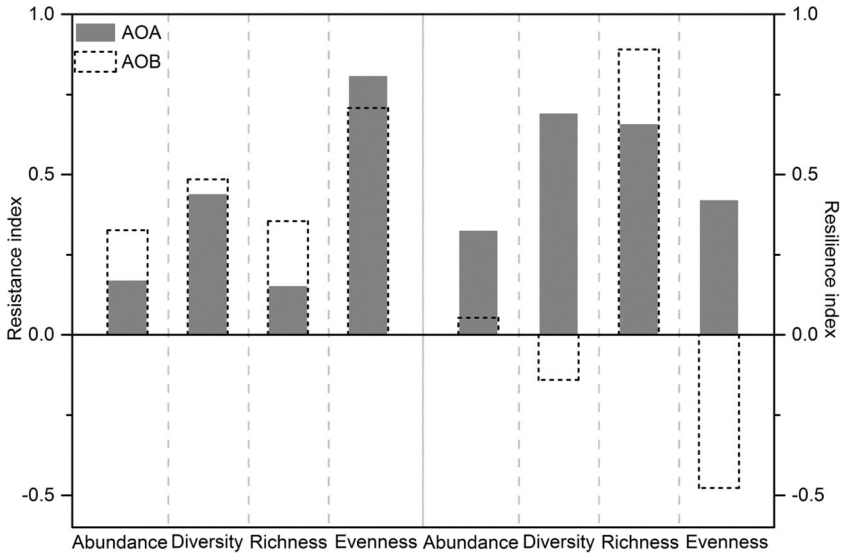

Fig. 6 Resistance (RS) and resilience (RL) indices of abundance and alpha-diversity of AOA and AOB. The alpha-diversity was calculated as diversity (Shannon index), richness (Chao1 estimator), and evenness (Shannon index-based measure of evenness)

sequences grouped within the *Nitrososphaera* subcluster 4 (60.7%) (Fig. S3). The AOB sequences mostly fell into the clusters *Nitrosospira* 3a, 3b, 4, *Nitrosomonas europaea/Nitrosococcus mobilis* and *Nitrosomonas marina* (Fig. S2), with the *Nitrosospira* cluster 3a being the most dominant one (71.7%) (Fig. S3).

The RS and RL indices for taxa with relative abundance > 0.1% were calculated for both AOA and AOB using the variation of relative abundance among groups. For AOA, the *Nitrososphaera* subcluster 4, 1, and 8 had higher RS indices (0.53–0.75), while the *Nitrosopumilus* and *Nitrososphaera* cluster 2 had very low or negative RS values (Fig. 7). Similarly, among the AOB, three clusters (*Nitrosospira* cluster 3a, 3b, and *Nitrosomonas europaea/Nitrosococcus mobilis* cluster) showed higher RS indices (0.42–0.81), while the other two AOB clusters had lower RS values (Fig. 7). With the exception of *Nitrososphaera* subcluster 4 and *Nitrosotalea*, both AOA and AOB clusters with higher RS indices showed lower RL values and vice versa.

RDA was performed to evaluate the relationships of the dominant AOA and AOB taxa (relative abundance > 0.1%) with soil properties (Fig. 8). The measured soil variables explained 56.3 and 49.4% of the total variance on the key taxa distribution for AOA and AOB, respectively. Among the soil variables, NH_4^+ (pseudo $F = 9.4$, $P = 0.001$; pseudo $F = 4.5$, $P = 0.002$, respectively) and Fe^{2+}/Fe^{3+} (pseudo $F = 5.9$, $P = 0.002$; pseudo $F = 2.7$, $P = 0.038$, respectively) had the highest explanatory power. For AOA, soil NH_4^+ and Fe^{2+}/Fe^{3+} were mainly positively correlated with the *Nitrosopumilus* cluster. The two most abundant AOA taxa of *Nitrososphaera* subclusters 4 and 8 showed negative correlations with soil pH, which explained 12.9% of the distribution (pseudo $F = 2.7$, $P = 0.055$). For AOB, soil NH_4^+ and Fe^{2+}/Fe^{3+} were negatively correlated with the dominant taxon *Nitrosospira* cluster 3a and positively correlated with *Nitrosomonas europaea/Nitrosococcus mobilis* cluster.

Discussion

Faster Recovery of AOA than AOB

In this study, the recovery of AOA and AOB after disturbance was assessed with respect to gene abundance, community diversity, and composition. In general, the AOA showed higher ability to recover from flooding than AOB.

Disturbance by flooding exhibited a predictable negative effect on both AOA and AOB abundance. However, the abundance of AOA and AOB showed a tendency to return back to pre-disturbance levels after the flooding receded. It was noteworthy that a significant increase of AOA abundance in the recovery group was observed comparing with the flooding group. This was not observed for AOB, suggesting a better recovery of AOA abundance. Unlike the negative effect on gene abundance, the flooding increased the alpha-diversity. The highest values for both AOA and AOB were observed in the flooding group, which was in accordance with previous studies showing that a certain level of disturbance could result in a higher diversity [9, 42]. The Shannon diversity and Chao1 richness of AOA decreased significantly in the recovery group, which means this group was closer to the values of the control groups. Contrarily, the alpha-diversity of AOB did not show a gradient among the three groups. The distinct responses of community diversity also implied that AOA had a better recovery than AOB after flooding. Although flooding showed a heavier impact on the community composition of AOA than AOB, the community composition of AOA in the recovery group was similar with that of the control group than to the flooding group. Here again, the AOB in the recovery group was similar with that of the flooding group, suggesting that the community composition of AOA recovered faster than that of the AOB.

The better recovery of AOA might be due to their better adaptation to a broader range of habitats, and the more

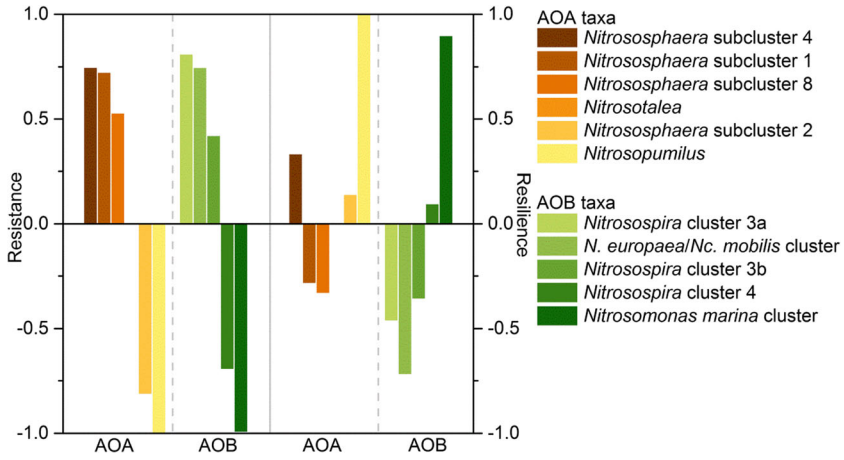

Fig. 7 Resistance (RS) and resilience (RL) indices of the AOA and AOB taxa with relative abundance > 0.1%. *N.europaea/Nc.mobilis cluster*: *Nitrosomonas europaea/Nitrosococcus mobilis* cluster. The RS and RL values of *Nitrosotalea* were close to zero

versatile metabolism which could allow mixotrophic growth [16]. Moreover, compared to AOB, AOA had shown higher affinity for ammonia [43], which indicates that the AOA would outcompete the AOB in a nutrient limiting environments [44, 45]. Notably, the alpha-diversity of AOA in the flooding group was generally higher than that of AOB. Previous studies had shown that the probability of a community to recover from disturbance was increased with high alpha-diversity [9, 46]. A low diversity reduced the resilience of soil microbial community to disturbances [47]. Hence, the higher diversity of AOA could be another reason explaining the higher ability to recover.

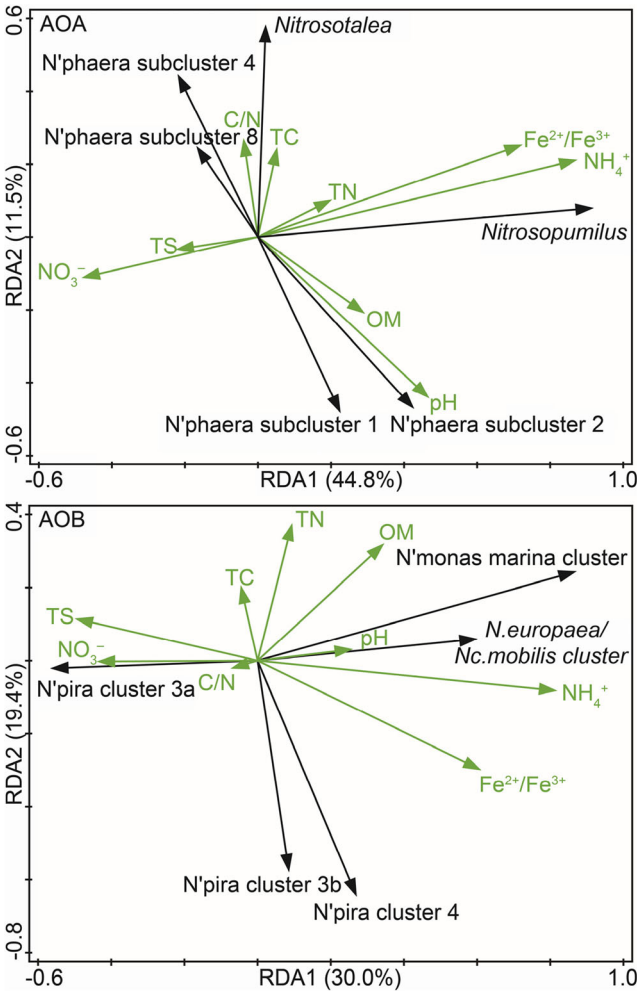

Fig. 8 Redundancy analysis of the dominant AOA and AOB taxa (relative abundance > 0.1%) with soil properties. AOA and AOB taxa are indicated by black arrows and soil properties by green arrows. N'phaera: *Nitrososphaera*; N'pira: *Nitrosospira*; N'monas: *Nitrosomonas*; *N.europaea/Nc.mobilis*: *Nitrosomonas europaea/Nitrosococcus mobilis*

Resistance and Resilience of AOA and AOB

Resistance and resilience are two fundamental properties of ecological stability [48], which were pervasive in evaluating the recovery of microbial community from environmental disturbance [23, 49, 50]. The resistance of a microbial community can be explained as a high metabolic flexibility and broad physiological tolerance to changing environments [7]. Resilient microbial communities seemed to be linked with high abundances, widespread dispersal, and high growth rates [51]. In our study, AOA may fit well into a resilient type of microbial guilds with high abundance and high growth rates (especially in low ammonia environments [52–54]). This assumption was further supported by the RS and RL indices which showed that both the abundance and diversity of AOA were more resilient than those of AOB. Adversely, the AOB was more resistant than AOA to flooding. This is in agreement with previous studies reporting that AOA was less resistant than AOB to drought stress, and that AOA might be more resilient than AOB after alternating wetting-drying cycles [23, 49]. The enhanced resilience of soil microbial communities is often accompanied by higher diversity or species richness [55]. It may be caused by the presence of species with traits or interspecific interactions which may potentially be relevant during and after the disturbance [56]. This probably explains why the AOA, with higher alpha-diversity, exhibited a higher resilience than AOB. Our study also suggested that

the higher resilience of AOA resulted in a better ability to recover from flooding disturbance.

The resistance and resilience of the microbial community may specifically be exhibited by the key taxon of the community [9, 55]. Generally, taxa with better resistance were usually less resilient to disturbances and vice versa, which may be due to a trade-off between resistance and resilience [48, 55, 57]. The majority of the AOA and AOB key taxa followed this basic pattern in our study, but the most abundant AOA taxon (*Nitrososphaera* subcluster 4) had the highest RS index as well as a relatively high RL index. This subcluster might thus be the key taxon preserving the stability of the AOA community. The AOB taxon *Nitrosospira* cluster 3a, constituting the main part (71.7%) of the AOB community, was the most resistant taxon with a relatively low RL, which may explain the overall high resistance ability of the AOB community. Several environmental variables could influence the RS and RL by controlling the relative abundance of the key taxa. For instance, the *Nitrososphaera* subcluster 4 was negatively correlated with soil pH, suggesting a high pH would reduce the relative abundance of members in this cluster, and subsequently the resilience of the AOA community. Similarly, the RDA indicated that a high NH_4^+ would reduce the relative abundance of *Nitrosospira* cluster 3a and thus the resistance of AOB community.

Differential Life-Strategies of AOA and AOB

From the ecophysiological point of view, microorganisms can be characterized by their life-history strategies (i.e., r or K-strategies), indicating that the genetic differences between microorganisms were responsible for their abilities to resist and recover in disturbed environments [58, 59]. Typically, the r-strategists show high growth rates and low efficiency of resource use, the reverse being true for K-strategists [55]. Compared to AOB, the AOA were more likely to be r-strategists. The higher gene abundance of AOA in this study implied potentially higher growth rates than AOB. A previous study also demonstrated growth of AOA in a wide range of ammonium concentrations, while the AOB seemed to be restricted in high ammonium concentration in a microcosm setting [60]. Moreover, there was evidence showing that r-strategists were more resilient but less resistant to disturbances compared to K-strategists in soil microbial communities [61, 62]. In our study, the AOA showed a high resilience and the AOB had high resistance with respect to gene abundance, community diversity and community composition which supported their respective roles as r- and K-strategists.

Ecological Implications

Since the Three Gorges Dam was operated, the riparian zone of the TGR has been confronting with periodic disturbance of water-level changes, leading to serious environmental issues, for instance, the nitrogen pollution. Ammonia oxidation is the rate-limiting step of nitrogen cycle which alleviates ammonia and nitrogen pollution and the serious environmental consequences associated therewith [63, 64]. The presence of AOA and AOB, both of which perform similar function in ammonia oxidation, supports functional redundancy in reducing ammonia pollution in riparian zone ecosystem. In contrast to the AOB which dominate the ammonia oxidation process in stable habitats [18, 52], the AOA are more adaptable to highly variable habitats shaped by the periodic flooding disturbance, and thus, maintain the function against the disturbance, as shown in this study.

In the riparian zone of the TGR, the NH_4^+ in soil increased during flooding (Fig. S1), which might be caused by the decomposition of dead plants and other sediment particles in water. However, the NH_4^+ decreased and the NO_3^- increased in soil after the water receded, and the TN concentration stayed stable in different groups, suggesting that the function of ammonia oxidation was less influenced by the variable habitats of ammonia oxidizers. Therefore, the regime shift did not lead to an accumulation of nitrogen or disturb the nitrogen cycle. The strong recovery capability of AOA may contribute greatly to the stability in the riparian ecosystem of the TGR.

Conclusions

The AOA community showed better ability to recover from flooding disturbance with respect to the community abundance, diversity, and composition. Moreover, AOA were more resilient than AOB after flooding but less resistant against flooding than AOB, which might be associated with the higher environmental adaptability and diversity of AOA. The different mechanisms of AOA and AOB to maintain their community stability in response to flooding disturbance might be linked to their respective r and K life-history strategies. The AOA would be of greater significance in ammonia oxidation in a changing environment and preserving the ecosystem stability.

Acknowledgements This work was supported by the National Natural Science Foundation of China [41303053, 41571497, 41301540]. We are grateful to the Kaizhou Science & Technology Commission for the assistance in sampling and background data collection.

References

1. Rykiel EJ (1985) Towards a definition of ecological disturbance. Aust. J. Ecol. 10:361–365. https://doi.org/10.1111/j.1442-9993.1985.tb00897.x

2. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JBH, Matulich KL, Schmidt TM, Handelsman J (2012) Fundamentals of microbial community resistance and resilience. Front Microbiol 3:417. https://doi.org/10.3389/fmicb.2012.00417
3. Paine RT, Tegner MJ, Johnson EA (1998) Compounded perturbations yield ecological surprises. Ecosystems 1:535–545. https://doi.org/10.1007/s100219900049
4. Bender EA, Case TJ, Gilpin ME (1984) Perturbation experiments in community ecology: theory and practice. Ecology 65:1–13. https://doi.org/10.2307/1939452
5. Collie JS, Richardson K, Steele JH (2004) Regime shifts: can ecological theory illuminate the mechanisms? Prog. Oceanogr. 60: 281–302. https://doi.org/10.1016/j.pocean.2004.02.013
6. Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 35:557–581. https://doi.org/10.1146/annurev.ecolsys.35.021103.105711
7. Meyer AF, Lipson DA, Martin AP, Schadt CW, Schmidt SK (2004) Molecular and metabolic characterization of cold-tolerant alpine soil Pseudomonas sensu stricto. Appl. Environ. Microbiol. 70: 483–489. https://doi.org/10.1128/AEM.70.1.483-489.2004
8. Allison SD, Martiny JBH (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. PNAS 105: 11512–11519. https://doi.org/10.1073/pnas.0801925105
9. Griffiths BS, Philippot L (2013) Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37:112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x
10. Karakoç C, Singer A, Johst K, Harms H, Chatzinotas A (2017) Transient recovery dynamics of a predator–prey system under press and pulse disturbances. BMC Ecol. 17:13. https://doi.org/10.1186/s12898-017-0123-2
11. IPCC (2007) Climate Change 2007: synthesis report. In: Pachauri, RK and Reisinger, A (eds) Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Changed. IPCC, Geneva, Switzerland, pp 104
12. You J, Das A, Dolan EM, Hu ZQ (2009) Ammonia-oxidizing archaea involved in nitrogen removal. Water Res. 43:1801–1809. https://doi.org/10.1016/j.watres.2009.01.016
13. Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546. https://doi.org/10.1038/nature03911
14. Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H, Wagner M (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. PNAS 105:2134–2139. https://doi.org/10.1073/pnas.0708857105
15. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102:14683–14688. https://doi.org/10.1073/pnas.0506625102
16. Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809. https://doi.org/10.1038/nature04983
17. Yang F, Liu WW, Wang J, Liao L, Wang Y (2012) Riparian vegetation's responses to the new hydrological regimes from the Three Gorges Project: clues to revegetation in reservoir water-level-fluctuation zone. Acta Ecol. Sin. 32:89–98. https://doi.org/10.1016/j.chnaes.2012.02.004
18. Jia ZJ, Conrad R (2009) Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ. Microbiol. 11:1658–1671. https://doi.org/10.1111/j.1462-2920.2009.01891.x
19. Nicol GW, Schleper C (2006) Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? Trends Microbiol. 14:207–212. https://doi.org/10.1016/j.tim.2006.03.004
20. He JZ, Shen JP, Zhang LM, Zhu YG, Zheng YM, Xu MG, Di HJ (2007) Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9:2364–2374. https://doi.org/10.1111/j.1462-2920.2007.01358.x
21. Santoro AE, Francis CA, De Sieyes NR, Boehm AB (2008) Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environ. Microbiol. 10:1068–1079. https://doi.org/10.1111/j.1462-2920.2007.01547.x
22. de la Torre JR, Walker CB, Ingalls AE, Könneke M, Stahl DA (2008) Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. Environ. Microbiol. 10:810–818. https://doi.org/10.1111/j.1462-2920.2007.01506.x
23. Thion C, Prosser JI (2014) Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying-rewetting stress. FEMS Microbiol. Ecol. 90:380–389. https://doi.org/10.1111/1574-6941.12395
24. Liu S, Hu BL, He ZF, Zhang B, Tian GM, Zheng P, Fang F (2015) Ammonia-oxidizing archaea have better adaptability in oxygenated/hypoxic alternant conditions compared to ammonia-oxidizing bacteria. Appl Microbiol Biotechnol 99:8587–8596. https://doi.org/10.1007/s00253-015-6750-7
25. China Three Gorges Corporation (2017) Brief introduction of Three Gorges Corporation. http://www.ctgpc.com/en/_304051/_304525/index.html. Accessed 1 Mar 2017
26. Bao YH, Gao P, He XB (2015) The water-level fluctuation zone of Three Gorges Reservoir—a unique geomorphological unit. Earth-Sci. Rev. 150:14–24. https://doi.org/10.1016/j.earscirev.2015.07.005
27. Wen Z, Ma M, Zhang C, Yi X, Chen J, Wu S (2017) Estimating seasonal aboveground biomass of a riparian pioneer plant community: an exploratory analysis by canopy structural data. Ecol. Indic. 83:441–450. https://doi.org/10.1016/j.ecolind.2017.07.048
28. Ye C, Li SY, Yang YY, Xiao S, Zhang JQ, Zhang QF (2015) Advancing analysis of spatio-temporal variations of soil nutrients in the water level fluctuation zone of China's Three Gorges Reservoir using self-organizing map. PLoS One 10:e0121210. https://doi.org/10.1371/journal.pone.0121210
29. Stumm W, Sulzberger B (1992) The cycling of iron in natural environments: considerations based on laboratory studies of heterogeneous redox processes. Geochim Cosmochim Acta 56:3233–3257. https://doi.org/10.1016/0016-7037(92)90301-X
30. Zheng GD, Takano B, Kuno A, Matsuo M (2001) Iron speciation in modern sediment from Erhai Lake, southwestern China: redox conditions in an ancient environment. Appl. Geochem. 16:1201–1213. https://doi.org/10.1016/S0883-2927(01)00016-6
31. Dean WE (1974) Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J. Sediment. Res. 44:242–248. https://doi.org/10.1306/74D729D2-2B21-11D7-8648000102C1865D
32. Bao SD (2000) Chemical analysis for agricultural soil. China Agriculture Press, Beijing (In Chinese)
33. Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63:4704–4712
34. Pester M, Rattei T, Flechl S, Gröngröft A, Richter A, Overmann J, Reinhold-Hurek B, Loy A, Wagner M (2012) *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of *amoA* genes from soils of four different geographic regions.

Environ. Microbiol. 14:525–539. https://doi.org/10.1111/j.1462-2920.2011.02666.x
35. Norton JM, Alzerreca JJ, Suwa Y, Klotz MG (2002) Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177:139–149. https://doi.org/10.1007/s00203-001-0369-z
36. Xiang XJ, He D, He JS, Myrold DD, Chu HY (2017) Ammonia-oxidizing bacteria rather than archaea respond to short-term urea amendment in an alpine grassland. Soil Biol. Biochem. 107:218–225. https://doi.org/10.1016/j.soilbio.2017.01.012
37. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739. https://doi.org/10.1093/molbev/msr121
38. Chen YL, Xu ZW, Hu HW, Hu YJ, Hao ZP, Jiang Y, Chen BD (2013) Responses of ammonia-oxidizing bacteria and archaea to nitrogen fertilization and precipitation increment in a typical temperate steppe in Inner Mongolia. Appl. Soil Ecol. 68:36–45. https://doi.org/10.1016/j.apsoil.2013.03.006
39. Ouyang Y, Norton JM, Stark JM, Reeve JR, Habteselassie MY (2016) Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biol. Biochem. 96:4–15. https://doi.org/10.1016/j.soilbio.2016.01.012
40. Purkhold U, Pommerening-Röser A, Juretschko S, Schmid MC, Koops HP, Wagner M (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. Appl. Environ. Microbiol. 66:5368–5382. https://doi.org/10.1128/Aem.66.12.5368-5382.2000
41. Orwin KH, Wardle DA (2004) New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biol. Biochem. 36:1907–1912. https://doi.org/10.1016/j.soilbio.2004.04.036
42. Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302–1310. https://doi.org/10.1126/science.199.4335.1302
43. Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying archaea and Bacteria. Nature 461:976–979. https://doi.org/10.1038/nature08465
44. Ke X, Lu Y (2012) Adaptation of ammonia-oxidizing microorganisms to environment shift of paddy field soil. FEMS Microbiol. Ecol. 80:87–97. https://doi.org/10.1111/j.1574-6941.2011.01271.x
45. Xie Z, Le Roux X, Wang CP, Gu ZK, An M, Nan HY, Chen BZ, Li F, Liu YJ, Du GZ, Feng HY, Ma XJ (2014) Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows. Soil Biol. Biochem. 77:89–99. https://doi.org/10.1016/j.soilbio.2014.06.024
46. Lee SH, Sorensen JW, Grady KL, Tobin TC, Shade A (2017) Divergent extremes but convergent recovery of bacterial and archaeal soil communities to an ongoing subterranean coal mine fire. ISME J 11:1447–1459. https://doi.org/10.1038/ismej.2017.1
47. Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sørensen SJ, Bååth E, Bloem J, Ruiter PCD (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90:279–294. https://doi.org/10.1034/j.1600-0706.2000.900208.x
48. Pimm SL (1984) The complexity and stability of ecosystems. Nature 307:321–326. https://doi.org/10.1038/307321a0
49. Chen J, Nie YX, Liu W, Wang ZF, Shen WJ (2017) Ammonia-oxidizing archaea are more resistant than denitrifiers to seasonal precipitation changes in an acidic subtropical forest soil. Front Microbiol 8:1384. https://doi.org/10.3389/fmicb.2017.01384
50. Ng EL, Patti AF, Rose MT, Schefe CR, Smernik RJ, Cavagnaro TR (2015) Do organic inputs alter resistance and resilience of soil microbial community to drying? Soil Biol Biochem 81:58–66. https://doi.org/10.1016/j.soilbio.2014.10.028
51. Fenchel T, Finlay BJ (2004) The ubiquity of small species: patterns of local and global diversity. Bioscience 54:777–784. https://doi.org/10.1641/0006-3568(2004)054[0777:Tuossp]2.0.Co;2
52. Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat. Geosci. 2:621–624. https://doi.org/10.1038/ngeo613
53. Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. 72:386–394. https://doi.org/10.1111/j.1574-6941.2010.00861.x
54. Offre P, Prosser JI, Nicol GW (2009) Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. FEMS Microbiol. Ecol. 70:99–108. https://doi.org/10.1111/j.1574-6941.2009.00725.x
55. de Vries FT, Shade A (2013) Controls on soil microbial community stability under climate change. Front Microbiol 4:265. https://doi.org/10.3389/fmicb.2013.00265
56. Fetzer I, Johst K, Schäwe R, Banitz T, Harms H, Chatzinotas A (2015) The extent of functional redundancy changes as species' roles shift in different environments. PNAS 112:14888–14893. https://doi.org/10.1073/pnas.1505587112
57. de Vries FT, Liiri ME, Bjørnlund L, Bowker MA, Christensen S, Setälä HM, Bardgett RD (2012) Land use alters the resistance and resilience of soil food webs to drought. Nat. Clim. Chang. 2:276–280. https://doi.org/10.1038/nclimate1368
58. De Leij FA, Whipps JM, Lynch JM (1994) The use of colony development for the characterization of bacterial communities in soil and on roots. Microb Ecol 27:81–97. https://doi.org/10.1007/BF00170116
59. Langer U, Böhme L, Böhme F (2004) Classification of soil microorganisms based on growth properties: a critical view of some commonly used terms. J Plant Nutr Soil Sci 167:267–269. https://doi.org/10.1002/jpln.200421362
60. Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J 5:1067–1071. https://doi.org/10.1038/ismej.2010.191
61. Bapiri A, Baath E, Rousk J (2010) Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb. Ecol. 60:419–428. https://doi.org/10.1007/s00248-010-9723-5
62. Lennon JT, Aanderud ZT, Lehmkuhl BK, Schoolmaster DR (2012) Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology 93:1867–1879. https://doi.org/10.1890/11-1745.1
63. Wang YF, Gu JD (2013) Higher diversity of ammonia/ammonium-oxidizing prokaryotes in constructed freshwater wetland than natural coastal marine wetland. Appl. Environ. Microbiol. 97:7015–7033. https://doi.org/10.1007/s00253-012-4430-4
64. Wang SY, Wang Y, Feng XJ, Zhai LM, Zhu GB (2011) Quantitative analyses of ammonia-oxidizing archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. Appl Microbiol Biotechnol 90:779–787. https://doi.org/10.1007/s00253-011-3090-0