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Vertical niche differentiation of comammox *Nitrospira* in water-level fluctuation zone of the Three Gorges Reservoir, China

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Abstract

Comammox (CMX) *Nitrospira* bacteria (NB) can accomplish ammonia oxidation independently, and their niche differentiation holds promise for their ecological and survival functions. In this work, the vertical niche differentiation of CMX NB was investigated in the soils of 6 water-level fluctuation (WLF) zones (both natural and artificial) in the Three Gorges Reservoir (TGR) region. The results demonstrated that the level of clade A *amoA* was obviously reduced with increasing soil depth in the natural WLF zones and one of the artificial WLF zones. However, in the other two artificial WLF zones, the abundance of this gene was not dramatically reduced with depth. The level of clade B *amoA* did not markedly decrease with increasing soil depth in most WLF zones and remained stable in the three WLF zones. Total nitrogen (TN) had the most significant effect on the abundance of CMX NB. Clade A.1, clade A.2.1, clade A.2.2, clade A.3, and clade B of CMX NB co-occurred simultaneously in all WLF zones. The number of operational taxonomic units (OTUs) of clade A in the two types of WLF zones first increased and then decreased with increasing depth, whereas the number of OTUs of clade B continuously increased with depth in the artificial WLF zone. Total carbon (TC) and pH, as environmental factors, affected the community structure of CMX NB. This study confirmed the vertical differentiation of the abundance and diversity of CMX NB in the WLF zones of the TGR region, and the artificial restoration of the WLF zones affected the niche differentiation of CMX NB to a certain degree.

Keywords: Three Gorges water-level fluctuation zone, Comammox *Nitrospira*, Ecological niche, Ammonia-oxidizing archaea, Ammonia-oxidizing bacteria

Introduction

Nitrification is a critical link in the nitrogen biogeochemical cycle, and the nitrogen flux in nitrification in terrestrial ecosystems is 330 Tg [1]. Nitrification is a biological process that converts ammonia to nitrate through microorganism catalysis and plays a vital role in global ecosystems. Nitrification mainly consists of two processes, namely, ammonia oxidation and nitrite oxidation [2]. Specifically, ammonia is first oxidized into nitrite by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) [3], and then nitrite is oxidized into nitrate by nitrite-oxidizing bacteria (NOB) [4]. Daims et al. [5] and Van Kessel et al. [6], respectively, discovered a new microorganism for the direct oxidization of

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ammonia to nitrate, which is called comammox (CMX) *Nitrospira* bacteria (NB). These bacteria are widely present in artificial engineering systems, including sewage treatment systems [7] and nitrification reactors [8], and they are also found in natural ecosystems such as salt marshes [9], agricultural soils [10], riparian soils [11], lake sediments [12], and forest soils [13].

All known CMX NB belongs to *Nitrospira sublineage II*, which can be further divided into clade A and clade B [5]. In 2018, Xia et al. firstly proposed dividing clade A of CMX into clade A.1 and clade A.2 [7]. In 2021, clade A.2 was divided into clade A.2.1 and clade A.2.2 [14]. A new clade, A.3, was discovered in 2019 [15]. There are certain differences in the physiological properties of clade A and clade B [16]. The genes coding for formate dehydrogenase are present in CMX clade B but not in clade A [17, 18], which makes the distribution of the two clades in the oxic–anoxic transition zones different. These differences result in spatial and temporal differentiation [9, 19] to adapt to different environmental conditions.

Soil physicochemical properties change with increasing soil depth, which often leads to the corresponding changes in some soil microbial community structures. Previous studies showed that total nitrogen, inorganic nitrogen, organic matter, TC, and dissolved oxygen generally decreased with increasing soil depth, while pH did not change significantly [20–22]. Various ammonia-oxidizing microorganisms have different adaptabilities to ammonia and oxygen, which contribute to bacterial differentiation with increasing soil depth, and this differentiation enables ammonia-oxidizing microorganisms to occupy a wider range of ecological niches. Previous research demonstrated that the average abundance of all AOA, AOB, and CMX was dramatically reduced with increasing soil depth in forests, grasslands, and farmlands [23]. However, in the typical purple paddy soil in Beibei district, Chongqing, China, the abundance of AOA and AOB was dramatically reduced along the soil depth direction, and the abundance of CMX clade A was raised significantly with increasing soil profile depth, but the abundance of clade B exhibited no obvious change trend with increasing soil depth. The abundance of the AOA gene was shown to decrease with depth, while that of the AOB gene decreased in semi-arid soils in southern Australia [24]. In the sediments of a high-altitude freshwater wetland in Yunnan Province, China, AOA diversity decreased with increasing sediment depth, whereas AOB diversity was not significantly correlated with sediment depth [25]. The CMX diversity in bottom sediments (5–10 cm) was higher than that on the surface (0–1 cm) and in middle sediments (1–5 cm) from the tidal flats of the Yangtze Estuary in China [26]. These phenomena indicate that ammonia-oxidizing microorganisms have

the ability to vertically differentiate in different habitats to adapt to diverse natural environments.

The Three Gorges Dam is the largest water conservancy project with the largest comprehensive benefit in the world [27]. After the completion of the Three Gorges Dam, the water level in the reservoir region is 175 m in winter and 145 m in summer with a periodic flooding-exposure water-level fluctuation (WLF) area of 348 km^2 on both sides of the reservoir region. After the Three Gorges began impounding water in 2003, the original vegetation in the WLF zones could not survive. Some zones where new vegetation can be restored naturally are often referred to as natural WLF zones [28]. Other zones where vegetation can only be restored by artificial planting are artificial WLF zones. Numerous studies showed that periodic flooding exposure can change the material transformation process in the soil in the WLF zone and increase the content of organic matter in the soil [29]. The oxygen content in the soil, especially in the topsoil, changes dramatically during the flooding-exposure period, thus affecting the growth of CMX NB, making them more prone to vertical differentiation.

In this study, the natural and artificial WLF zones of the Three Gorges Reservoir (TGR) region were chosen to assess the diversity of ammonia-oxidizing microorganisms and the abundance of AOA, AOB, and CMX NB in the soil. The aim of this research was to (a) reveal the vertical differentiation of CMX NB with soil depth in the WLF zones; (b) analyse the main environmental variables driving such differentiation; and (c) explore the adaptation strategies of different clades of CMX NB to depth changes.

Materials and methods
Study sites and sample collection
In this study, Fuling, Wanzhou, and Zigui were selected as sampling areas representing the upper, middle, and lower reaches of the TGR region, with both natural and artificial WLF zones included. A total of 6 sampling zones were as follows: Fuling natural WLF zone (FL-N), Fuling artificial WLF zone (FL-A), Wanzhou natural WLF zone (WZ-N), Wanzhou artificial WLF zone (WZ-A), Zigui natural WLF zone (ZG-N), and Zigui artificial WLF zone (ZG-A) (Additional file 1: Fig. S1). The vegetation in the natural WLF zone mainly includes naturally grown bermudagrass (*Cynodon dactylon* (L.) Pers.), Polygonum (Polygonum L.), and Arthraxon hispidus (*Arthraxon hispidus* (Trin.) Makino). The vegetation in the artificial WLF zone mainly consists of bermudagrass (*Cynodon dactylon* (L.) Pers.), artificially planted willow (*Salix babylonica* L.), and Zhongshan fir (*Taxodium "Zhongshansha"*). The sampling time was August 2021. Soil profile samples were collected at four altitudes of 150, 160,

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170, and 175 m, and the sampling depths at each altitude sampling site were 0–5, 5–10, 10–20, 20–30, and 30–40 cm. The collected specimens were kept in an ice box and transported back to the laboratory as soon as possible. Some of the soil specimens were stored at 4 ℃ in the laboratory for the subsequent determination of physicochemical properties. Other samples were frozen at – 80 ℃ for DNA isolation and molecular biology tests.

Physicochemical analysis

Then, 25 mL of KCL solution (1 mol L^{-1}) was added to 5 g of soil at a KCL solution/soil ratio of 5:1, and the pH value was measured with a digital acidity metre (METTLER TOLEDO, Switzerland) after leaching for 30 min. The air-dried, ground, and sieved soil specimens were isolated with 1 mol L^{-1} KCl. The ammonia (NH_4^+–N), nitrate (NO_3^-–N), and nitrite (NO_2^-–N) concentrations in the extract were detected. After the freeze-dried samples passed through a 200-mesh sieve, the total nitrogen (TN) and total carbon (TC) in the soil were determined using an elemental analyser (Elementar Vario ELIII analyser, Germany). After drying to constant weight at 105 ℃, the moisture content (MC) was measured.

DNA extraction and quantitative real-time PCR

The total bacterial genomic DNA of soil specimens was isolated with a Fast DNA Spin Kit for Soil (MPbio, USA). The purity and yield of the DNA extracts were detected using a NanoPhotometer-N60 spectrophotometer (IMPLEN, Germany).

AOA *amoA*, AOB *amoA*, CMX clade A *amoA*, and CMX clade B *amoA* genes were determined by qRT-PCR assay using QuantStudioTM 6 Flex quantitative PCR instrument (Thermo-Fisher-Scientific, Singapore). The primer pairs used for PCR were Arch-amoAF/Arch-amoAR [30], amoA-1Fmod/GenAOBR [31], CA377f/C576r [32], and CB377f/C576r [32]. PCR was performed on a 10 μL reaction system containing T5 Fast qPCR Mix (5.0 μL), 10 μM of each primer (0.4 μL), ROX Reference Dye II (0.2 μL), template DNA (1.0 μL), and ddH$_2$O (3.0 μL). The primers and amplification conditions are presented in Additional file 1: Table S1.

Amplicon sequencing and phylogenetic analysis

The high-throughput sequencing primer of CMX NB was comamoAF/R [33]. The Illumina NovaSeq PE250 (Shanghai Personal Biotechnology) sequencing platform was used for sequencing. Vsearch (v2.13.4_linux_x86_64) was used to process raw data [34]. The specific processing procedures were as follows. First, the primer fragment of the sequence was excised using cutadapt (v2.3), and the sequence of the unmatched primer was discarded. Then, sequence splicing, quality control, and deduplication

were conducted using Vsearch. Next, high-quality sequences with 97% nucleic acid similarity were clustered into operational taxonomic units (OTUs) with chimaeras removed, and the singleton OTUs and their corresponding sequences (in the OTU table) were removed. Last, based on the seed protein sequences of the CMX *amoA* gene, insertion and deletion errors in the OTU sequences were corrected using RDP FrameBot (v1.2) [35]. The obtained gene sequences were submitted to the NCBI (https://www.ncbi.nlm.nih.gov/) database with the accession number ON677371-ON677405.

The top 35 OTUs with the largest number of OTUs were selected to analyse the representative sequences of each OTU using the BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST), and the closest similar sequences were selected from GenBank. Based on the selected sequences, phylogenetic trees were constructed using MEGA 7.0, and their reliability was assessed through 1000 bootstrap replicates.

Statistical analysis

One-way ANOVA was conducted using SPSS 25.0, and multiple comparisons were performed by the Duncan method to examine the differences in soil physicochemical indices and gene abundance. Redundancy analysis (RDA) was performed using CANOCO v5.0 to assess the association between gene abundance and environmental variables. Phylogenetic trees were constructed using MEGA7.0, and heatmaps of OTU numbers were plotted using "pheatmap" in R. The Mantel test was used to evaluate the correlation between CMX NB and environmental parameters. Spearman correlation analysis and plotting were performed using the packages "Hmisc" and "Corrplot" in R (version 3.6.1) to assess the correlation between diversity, abundance, and physicochemical factor parameters.

Results

Soil properties of the WLF zone in the TGR region

In the natural WLF zone of the TGR region, the difference in NO_3^- content among various soil depths was significant ($P < 0.05$), and the content of the surface layer (0–10 cm) was significantly higher than that of the bottom layer (30–40 cm), at 17.85–4.79 mg/kg (Table 1). No significant differences were found for other indicators ($P > 0.05$). In the artificial WLF zone, the difference in NO_3^- content among different soil depths was significant ($P < 0.05$), and the NO_3^- content at 0–5 cm was remarkably higher than that at 30–40 cm, which was 18.59–8.38 mg/kg. The differences in other indicators were not significant ($P > 0.05$). The pH of the natural WLF zone was significantly lower than that in the artificial WLF zone ($P < 0.05$).

Table 1 Soil properties at various profile depths in the WLF zone of the TGR region

|	Depth (cm)	pH	TN (g/kg)	TC (g/kg)	NH$_4^+$ (mg/kg)	NO$_3^-$ (mg/kg)	NO$_2^-$ (mg/kg)	MC (%)	C/N
Natural WLF zone	0–5	6.79 ± 0.32a	0.80 ± 0.09a	13.07 ± 5.11a	1.76 ± 0.33a	17.85 ± 13.42a	0.05 ± 0.01a	17.00 ± 2.34a	16.85 ± 8.03a
	5–10	6.65 ± 0.43a	1.03 ± 0.34a	12.22 ± 5.24a	1.89 ± 0.10a	17.53 ± 2.12a	0.05 ± 0.02a	16.87 ± 2.07a	13.38 ± 7.77a
	10–20	6.60 ± 0.50a	0.95 ± 0.53a	10.57 ± 3.20a	1.95 ± 0.06a	8.78 ± 2.56ab	0.07 ± 0.02a	17.57 ± 0.74a	14.32 ± 9.02a
	20–30	6.47 ± 0.51a	0.58 ± 0.14a	8.67 ± 3.53a	1.77 ± 0.22a	4.45 ± 1.19b	0.05 ± 0.03a	15.97 ± 1.24a	16.14 ± 9.04a
	30–40	6.31 ± 0.80a	0.50 ± 0.09a	8.13 ± 4.26a	1.56 ± 0.16a	4.79 ± 1.25b	0.05 ± 0.02a	15.02 ± 0.46a	17.88 ± 11.99a
Artificial WLF zone	0–5	7.14 ± 0.54a	0.83 ± 0.48a	12.94 ± 1.00a	1.99 ± 0.39a	18.59 ± 12.58a	0.08 ± 0.05a	18.61 ± 3.41a	21.62 ± 16.14a
	5–10	6.85 ± 0.62a	0.75 ± 0.34a	11.82 ± 2.55a	1.86 ± 0.08a	10.82 ± 2.97ab	0.07 ± 0.04a	16.97 ± 1.26a	20.68 ± 16.23a
	10–20	6.84 ± 0.66a	0.68 ± 0.34a	10.19 ± 2.21a	1.89 ± 0.28a	6.12 ± 1.46ab	0.06 ± 0.03a	14.70 ± 2.30a	19.74 ± 14.31a
	20–30	6.73 ± 0.76a	0.61 ± 0.22a	8.65 ± 2.47a	1.79 ± 0.11a	5.43 ± 2.95ab	0.06 ± 0.03a	17.76 ± 3.90a	16.49 ± 9.37a
	30–40	6.47 ± 0.85a	0.67 ± 0.19a	8.74 ± 3.05a	1.96 ± 0.19a	8.38 ± 6.45b	0.05 ± 0.04a	17.46 ± 4.17a	13.88 ± 6.06a

Different lowercase letters indicate a significant difference (*P* < 0.05) using ANOVA and Duncan's test

Abundance of CMX NB, AOA, and AOB in the WLF zone of the TGR region

In the natural WLF zone, the level of clade A *amoA* markedly decreased with soil depth (Fig. 1), and its abundance in the 0–5 cm soil surface layer was 1–3 orders of magnitude higher than that at 30–40 cm. In the artificial WLF zone, the level of clade A *amoA* significantly decreased in Zigui with increasing soil depth, but in Fuling and Wanzhou, the abundance of this gene exhibited no obvious downwards trend. The level of clade B *amoA* in the other five fluctuation zones except the Fuling natural WLF zone did not decrease

significantly with increasing soil depth, and the abundance remained stable in the 3 WLF zones. In addition, in the two artificial WLF zones of Fuling and Zigui, the abundance value was highest at 10–20 cm.

Overall, the level of AOA *amoA* decreased with increasing soil depth in the natural WLF zones. In the artificial WLF zone, the level of AOA *amoA* was dramatically reduced with soil depth at the three sites. In all the WLF zones except the Wanzhou natural WLF zone, the AOB abundance showed a downwards trend with increasing soil depth (Fig. 1).
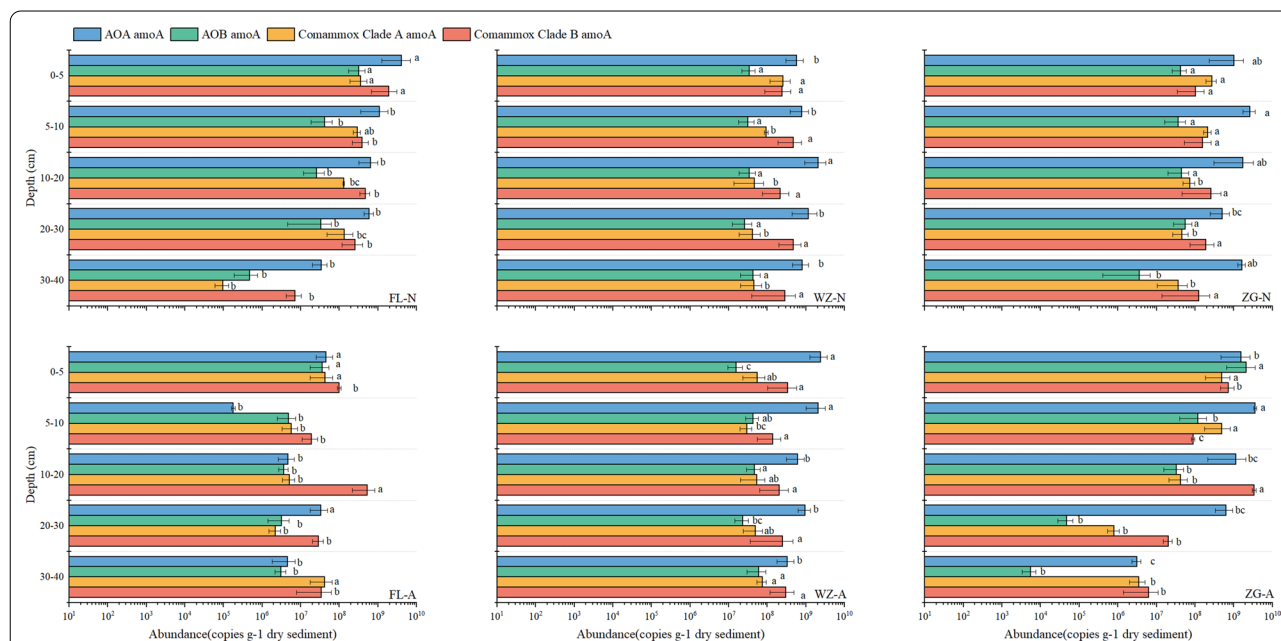
Fig. 1 Distribution of *amoA* gene abundance in ammonia-oxidizing microorganisms at different soil depths: **a** Fuling natural WLF zone; **b** Wanzhou natural WLF zone; **c** Zigui natural WLF zone; **d** Fuling artificial WLF zone; **e** Wanzhou artificial WLF zone; and **f** Zigui artificial WLF zone

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In the natural WLF zone, the average abundance of the *amoA* gene of CMX clade A, CMX clade B, AOA, and AOB was 1.51×10^8 copies/g dry sediment, 3.82×10^8 copies/g dry sediment, 1.46×10^9 copies/g dry sediment, and 5.00×10^7 copies/g dry sediment, respectively. In the artificial WLF zone, the average abundance of these four genes was 1.05×10^8 copies/g dry sediment, 4.00×10^8 copies/g dry sediment, 9.61×10^9 copies/g dry sediment, and 1.45×10^8 copies/g dry sediment, respectively. There was a significant difference in the levels of the AOA gene between the natural WLF zone and the artificial WLF zone ($P < 0.05$), while the *amoA* gene abundance of the other three ammonia-oxidizing microorganisms exhibited no difference ($P > 0.05$).

The average abundance of the AOA *amoA* gene was 1.11×10^9 copies/g dry sediment, which was higher than that in CMX clade B (3.91×10^8 copies/g dry sediment) and CMX clade A (1.14×10^8 copies/g dry sediment). The average level of the AOB *amoA* gene was relatively low (1.10×10^8 copies/g dry sediment).

Co-existence characteristics of ammonia-oxidizing microorganisms and their correlation with environmental factors

Redundancy analysis (RDA) was performed to reveal the correlations between environmental factors and the *amoA* gene abundances of 4 types of ammonia-oxidizing microorganisms (CMX clade A, CMX clade B, AOA, and AOB) in the WLF zones of the TGR region using CANOCO v5.0. The first two axes of RDA accounted for 48.44% of the cumulative variance explanation rate (Fig. 2). Clade A was positively correlated with AOA or AOB. The abundance of the *amoA* gene of comammox clade A was negatively correlated with pH, NO_2^-, and the C/N ratio and positively correlated with TN, TC, NH_4^+, NO_3^-, and MC. The abundance of the *amoA* gene of CMX clade B was negatively correlated with pH, NO_2^-, C/N, NH_4^+, NO_3^-, and MC but was positively correlated with TN.

In the artificial WLF zone, the *amoA* gene level of CMX clade A was positively correlated with that of CMX clade B ($P < 0.01$) (Fig. 3b). However, in the natural WLF zone, there was no correlation in the levels of the *amoA* gene between the two microorganisms (Fig. 3a). The level of the *amoA* gene in CMX clade A was positively correlated with that of AOB in both natural and artificial WLF zones ($P < 0.05$) (Fig. 3). In the natural WLF zone, the *amoA* gene abundance of CMX clade A was negatively correlated with soil depth ($P < 0.01$). In the artificial WLF zone, the abundance of the AOB *amoA* gene was also negatively correlated with soil depth ($P < 0.05$).
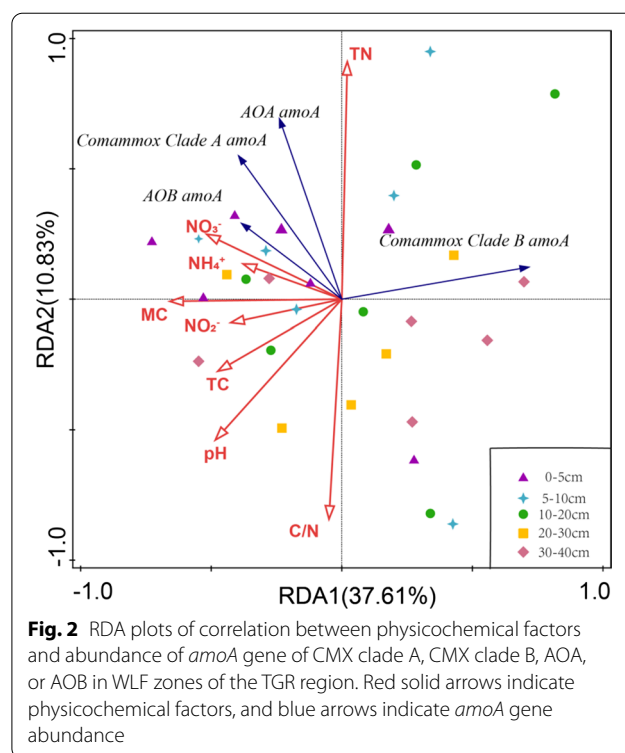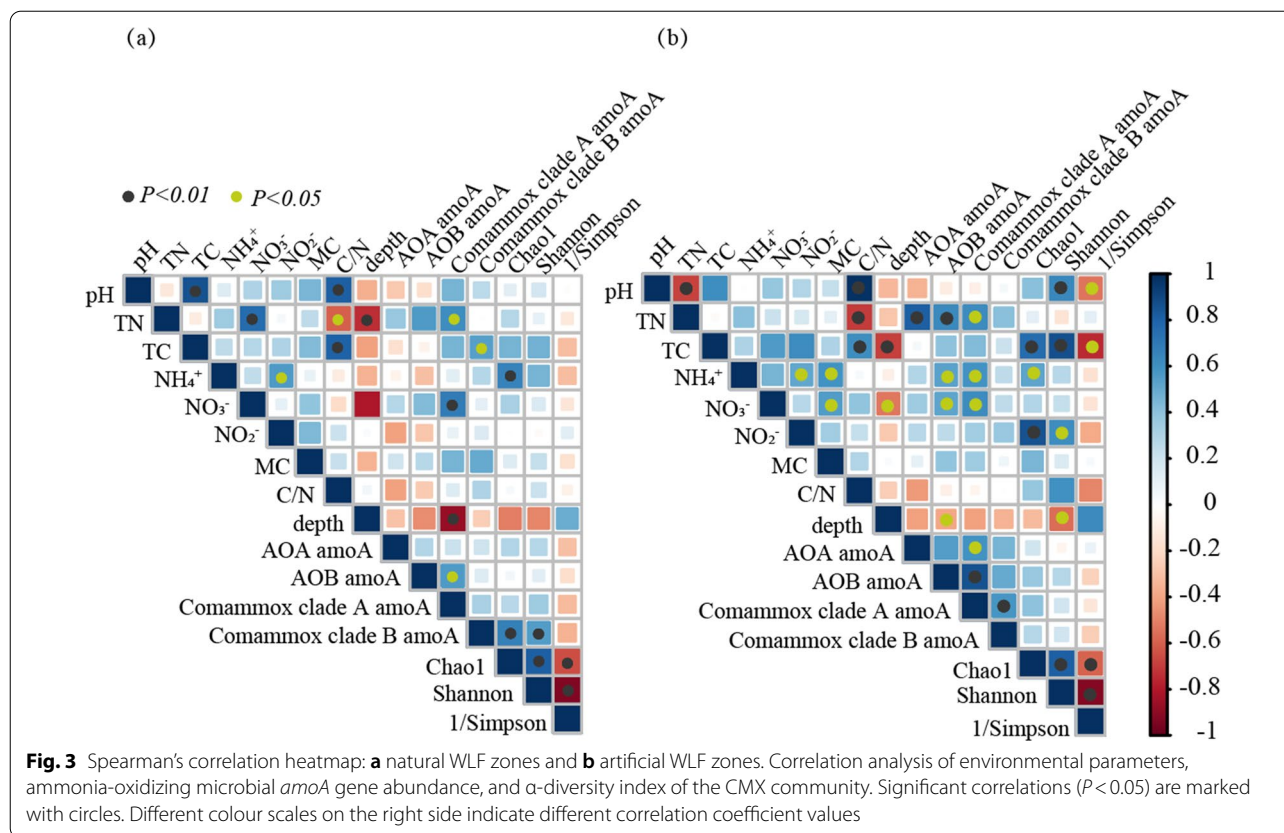

Fig. 2 RDA plots of correlation between physicochemical factors and abundance of *amoA* gene of CMX clade A, CMX clade B, AOA, or AOB in WLF zones of the TGR region. Red solid arrows indicate physicochemical factors, and blue arrows indicate *amoA* gene abundance

Biodiversity of CMX NB

A total of 35,740 high-quality CMX NB *amoA* gene sequences were detected from the WLF zone of the TGR region and were clustered into 14,813 OTUs with 97% nucleic acid similarity. The selected top 35 OTUs sequences with a relative abundance of OTUs greater than 0.5% and similar sequences from the NCBI database were applied to build a phylogenetic tree via the neighbour-joining method (Fig. 4). The 35 selected OTUs accounted for 65.71% of the CMX *amoA* gene sequences. The phylogenetic tree contained two clades, of which clade A contained 17 OTUs and clade B contained 18 OTUs. Clade A was further divided into clade A.1 (1 OTU), clade A.2.1 (13 OTUs), clade A.2.2 (2 OTUs), and clade A.3 (1 OTU).

The distribution of 35 OTUs is shown in Fig. 5. In both the natural WLF zone and artificial WLF zone, at the five soil profile depths, clade B was the most dominant species, followed by clade A.2.1.

As the soil depth increased, the number of OTUs in clade A in the two types of WLF zones increased first and then decreased, with the largest OTU number in the 20–30 cm soil layer (Fig. 6). The OTUs number of clade B had no obvious distribution regularity in the natural WLF zone but showed an increasing trend with depth in the artificial WLF zone. In the natural WLF zone, the number of OTUs of clade A.1 increased along the soil depth from 0 to 30 cm. In both types of

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Fig. 3 Spearman's correlation heatmap: **a** natural WLF zones and **b** artificial WLF zones. Correlation analysis of environmental parameters, ammonia-oxidizing microbial *amoA* gene abundance, and α-diversity index of the CMX community. Significant correlations ($P < 0.05$) are marked with circles. Different colour scales on the right side indicate different correlation coefficient values

WLF zones, the OTU number of clade A.2.1 exhibited a decreasing trend along the vertical direction. In the artificial WLF zone, the OTU number of clade A.2.2 increased along the soil depth from 0 to 30 cm, while in the natural WLF zone, it exhibited no obvious change. Clade A.3 had a tendency to gradually increase in OTU number with depth in the artificial WLF zone. The OTU number of clade B was significantly larger at 30–40 cm.

The connections of CMX at different depths were explored by co-occurrence network plots (Fig. 7). Among all samples, clade B possessed the highest number of nodes (49.05%–53.61%), followed by clade A.2 (34.71–40.82%). However, the average degree of clade A.2 was greater than that of clade B. The membership relationships at different depths graphs mainly showed positive interaction connections (0–5 cm: 57.27%; 5–10 cm: 64.46%; 10–20 cm: 62.73%; 20–30 cm: 58.71%; 30–40 cm: 72.26%). In the two types of zone samples, artificial WLF zones possessed more positive interaction connections than natural WLF zones (artificial: 65.02%; natural: 59.53%) (Additional file 1: Fig. S2).

Correlation between community structure of CMX NB and physicochemical factors

In the natural WLF zone, the number of OTUs of clade A.1 was remarkably correlated with NO_3^- and the C/N ratio ($P < 0.05$) (Fig. 8a), but in the artificial WLF zone, the number of OTUs of clade A.1 was markedly correlated with NO_2^- and MC ($P < 0.05$) (Fig. 8b). In the natural and artificial WLF zones, the number of OTUs of clade A.2 was correlated with both pH and TC ($P < 0.05$) (Fig. 8), but in the artificial WLF zone, the number of OTUs of clade A.3 was correlated with pH, TN, and TC ($P < 0.05$). In the natural and artificial WLF zones, the number of OTUs of clade B was correlated with pH, TC, and C/N ($P < 0.05$) (Fig. 8). In the artificial WLF zone, the Chao1 index and Shannon index, which characterize community abundance and diversity, respectively, were positively correlated with TC ($P < 0.01$).

Discussion

Effect of vertical depth of soil on abundance of CMX NB

Our results demonstrated that the level of the CMX clade A *amoA* gene decreased with increasing soil depth in the WLF zone of the TGR region, and this gene abundance
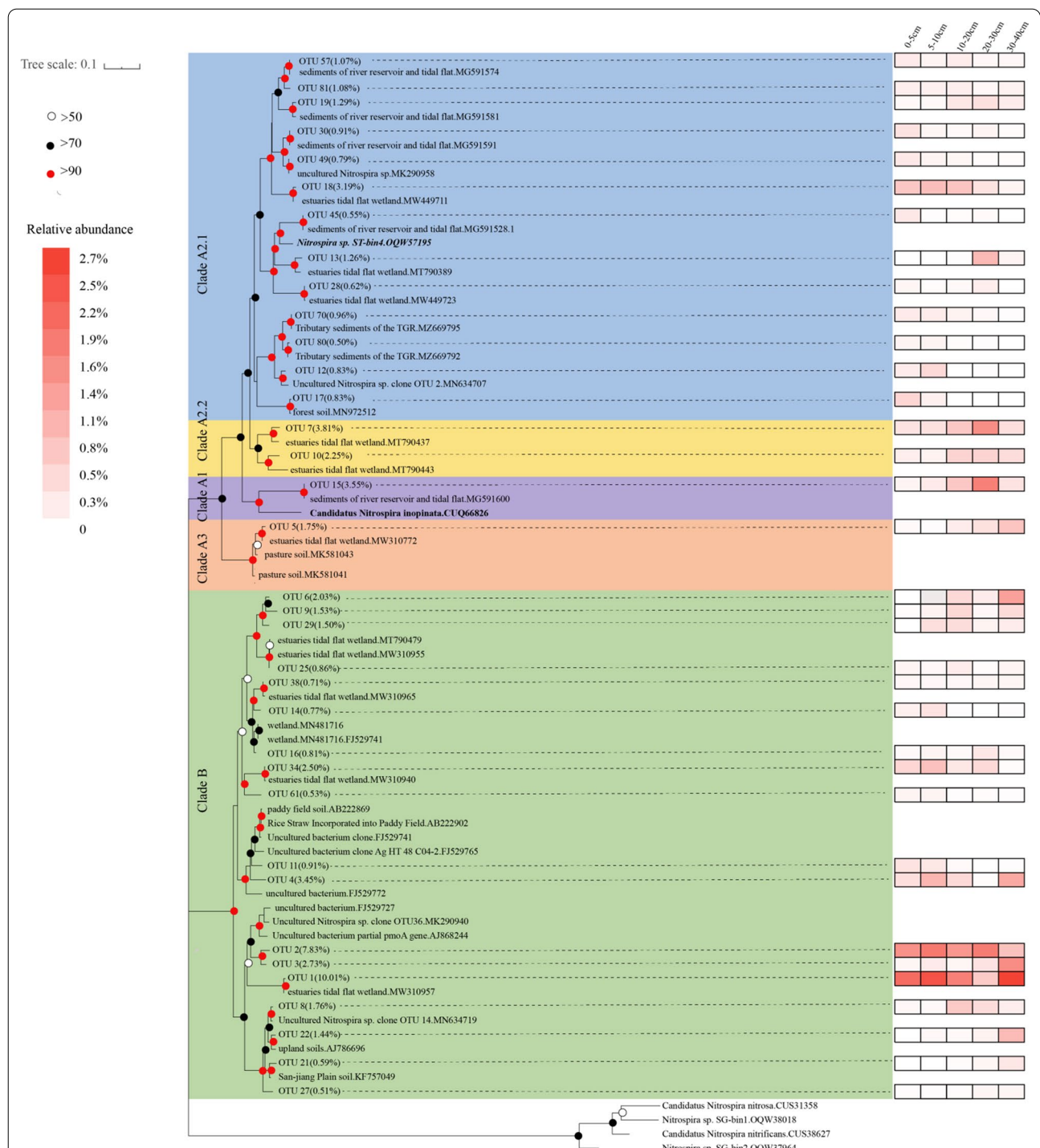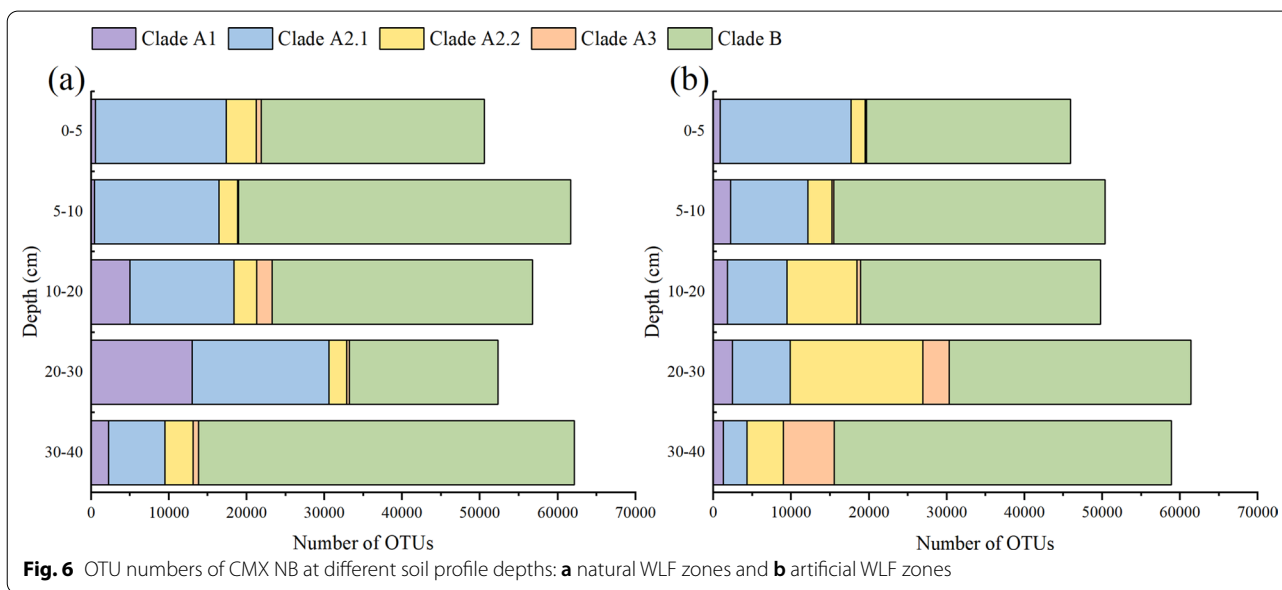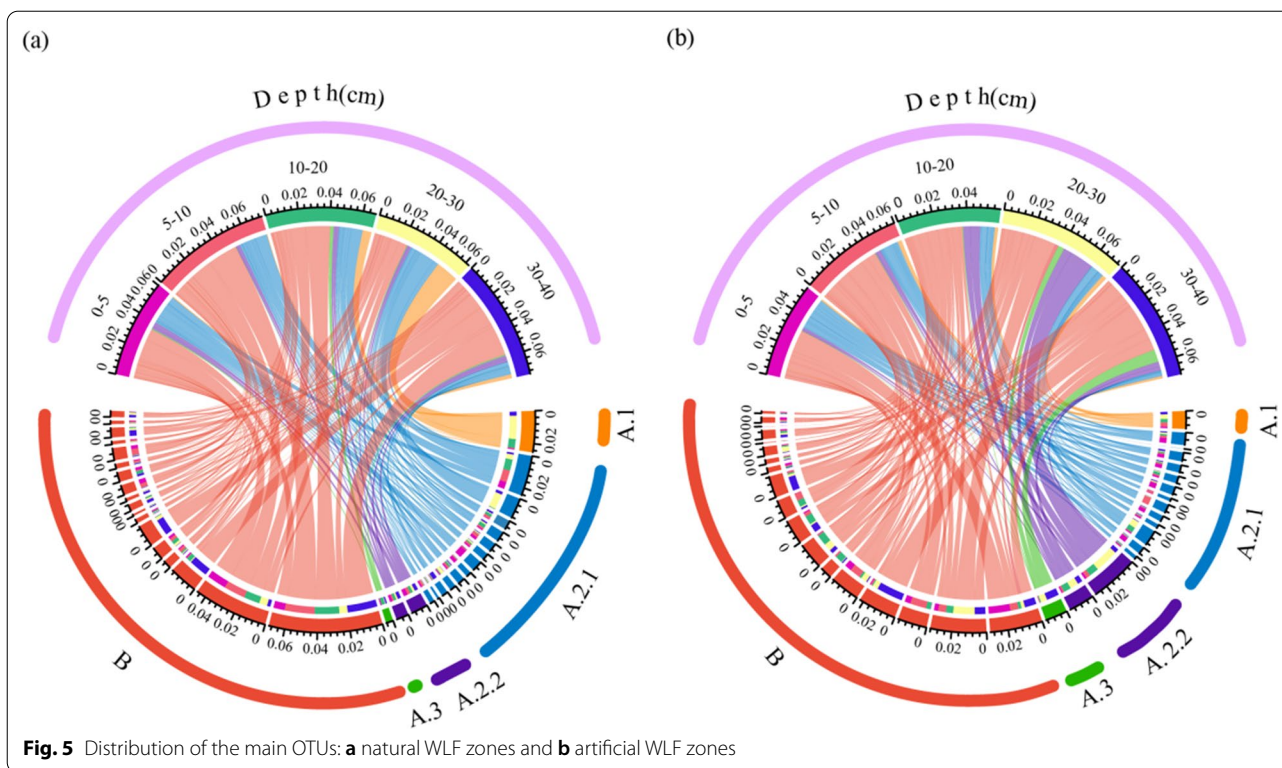
Fig. 4 Phylogenetic analysis of the *amoA* gene of CMX NB. The letters and numbers after the bacterial names indicate the submitted sequence numbers. Different colours of the branch nodes in the phylogenetic tree indicate different evolution distances. Percentages in brackets following the OTUs indicate the percentage of each OTU in the total CMX *amoA* gene sequences

in the surface soil was remarkably higher than that in the deep soil. However, the abundance of the CMX clade B *amoA* gene did not decrease significantly. This indicated that the two CMX clades were differentiated with depth and that these two clades exhibited different adaptability to depth, with clade B having the higher adaptability.
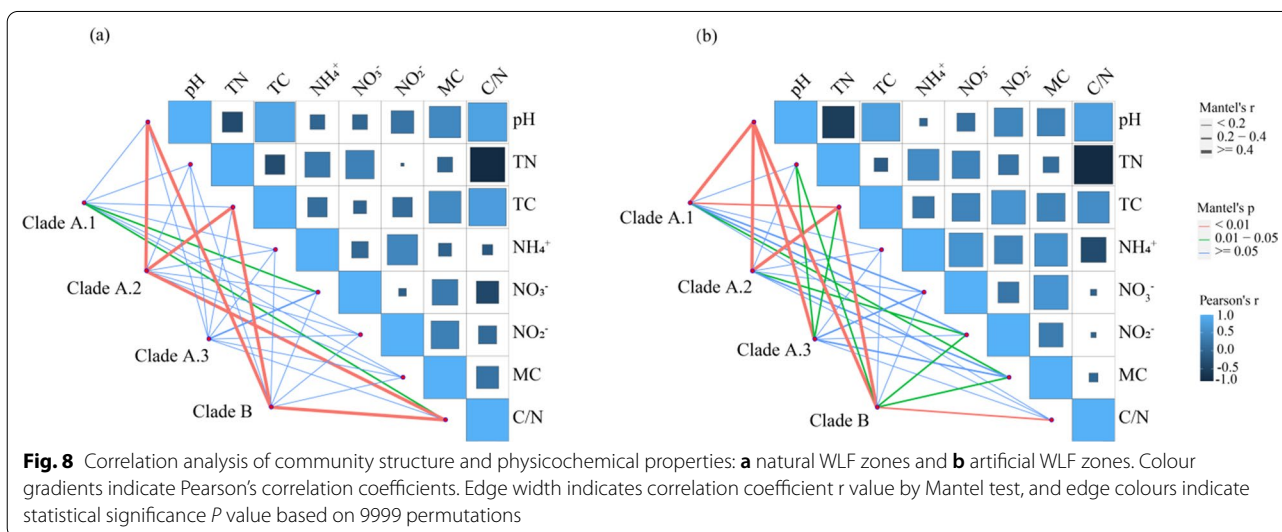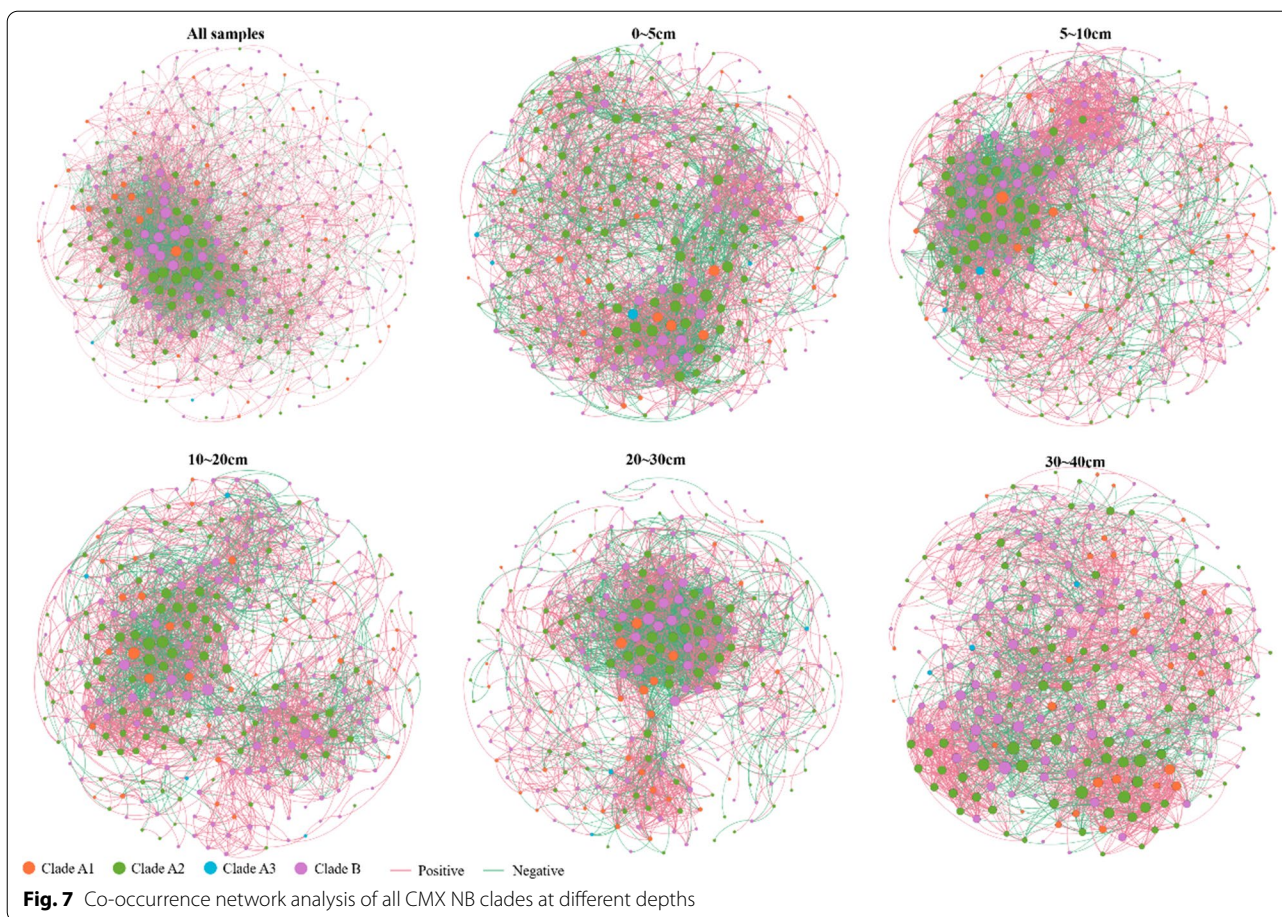
Fig. 5 Distribution of the main OTUs: **a** natural WLF zones and **b** artificial WLF zones

Fig. 6 OTU numbers of CMX NB at different soil profile depths: **a** natural WLF zones and **b** artificial WLF zones

The abundance of the *amoA* gene of CMX (3.58×10^8–4.00×10^{13} copies/g dry sediment) in this study was higher than that in plain wetland ecosystems and Yangtze River estuary regions [9, 14]. Soil in the WLF zone is flooded at high water levels, which gives it some similarity to wetland soil. However, different from other

wetland soils, due to the influence of periodic inundation, the nutrient composition in the Three Gorges WLF zone showed a general downwards trend during the inundation period [36, 37]. Some studies showed that CMX are more adaptable to low-nutrient environments [2, 38]. The soil oxygen content will increase with the decrease in soil

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Fig. 7 Co-occurrence network analysis of all CMX NB clades at different depths

Fig. 8 Correlation analysis of community structure and physicochemical properties: **a** natural WLF zones and **b** artificial WLF zones. Colour gradients indicate Pearson's correlation coefficients. Edge width indicates correlation coefficient r value by Mantel test, and edge colours indicate statistical significance *P* value based on 9999 permutations

moisture during the outcropping period, which is also conducive to the growth of CMX. These two factors may be the main reason for the higher abundance of CMX in the Three Gorges WLF zone than in the wetland.

The soil oxygen content in the WLF zone, especially in the topsoil, changed drastically during the flooding-exposure period, thus inevitably affecting the growth of CMX NB. Previous studies showed that clade A had

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higher metabolic diversity than clade B [39], and clade A not only used ammonia as a metabolic substrate but also utilized urea, cyanate, and hydrogen as substrates for nitrification [6, 18, 40]. However, the evolution rate of ammonia oxidation-related proteins was faster in clade B genomes than in clade A genomes [18]. It may be this advantage that enables clade B to respond more quickly to environmental conditions with frequently changing water levels, which might explain why clade B maintained relatively stable abundances in 3 WLF zones, even reaching the highest abundance at a depth of 10–20 cm in both natural and artificial WLF zones.

Soil vertical depth affects the community structure of CMX NB

With increasing depth, soil environmental conditions change, thus affecting microbial community structure [41]. Previous studies also reported depth-dependent changed in the community structure of typical ammonia-oxidizing microorganisms in lake or wetland sediments [42]. AOA diversity was reduced with increasing sediment depth, while AOB diversity was not significantly related to sediment depth [25]. In subtropical estuarine wetlands, the relative abundance of CMX clade A.2.2 was reduced with depth, while clade A.3 exhibited the opposite trend [43]. In Chongming eastern tidal flat sediment samples, the abundance of CMX clade A.1 in shallow surface sediments (1–5 cm) was higher than that in deep layer sediments (5–10 cm) in summer and winter, while that of clade A.2 exhibited the opposite trend [44].

Our results showed that the number of OTUs in clade A first increased and then decreased with increasing depth. Clade A.2.1 showed a decreasing trend along the vertical depth, the number of OTUs in clade A.2.2 increased along the soil depth direction from 0 to 30 cm in the artificial WLF zone, and clade A.3 in the artificial WLF zone exhibited an increasing trend with depth. However, the number of OTUs of clade B in the artificial WLF zone showed an increasing trend with depth, and clade B was dominant in the 30–40 cm soil layer. These results showed that in the Three Gorges WLF zone, clade A.2.1 preferred an environment with relatively high oxygen content and richer nutrients, while clade A.2.2, clade A.3, and clade B were more suitable for the hypoxic and oligotrophic environments in the WLF zone.

The results also showed that clade B was predominant in the CMX community in the WLF zone of the TGR region, followed by clade A.2.1. Clade B was the dominant CMX species in low-ammonia soils [10], which might mainly be because clade B adopted an Amt-type ammonia transporter, whereas clade A possessed an Rh-type ammonia transporter similar to betaproteobacterial AOB. In environments with a large ammonia concentration fluctuation [29], clade B employing the Amt-type transporter is more competitive [38]. This might be an important reason why clade B was predominant in the WLF zone in this study.

Physicochemical factors affecting the community structure of CMX NB

TN is a pivotal factor affecting the level of the CMX *amoA* gene [43]. In this work, TN was positively correlated with the level of the CMX clade A gene. The microcosm experiment showed that under the condition of insufficient external ammonia supply, CMX NB also had continuous *amoA* gene transcription [7], indicating that CMX NB had a competitive advantage over other ammonia-oxidizing microorganisms under low ammonia conditions. This might be because the slow organic nitrogen mineralization process provided sufficient ammonia for the growth of CMX NB with a high ammonia affinity [45, 46].

In the natural WLF zone of this study, the community structures of clade A.2 and clade B were significantly affected by pH and TC. In the artificial WLF zone, the community structures of clades A.1, A.2, A.3, and B were significantly affected by pH and TC. This suggested that pH and TC were important environmental drivers of niche differentiation for CMX, AOB, and AOA in soil habitats.

CMX NB had a greater competitive advantage over other ammonia-oxidizing microorganisms in acidic soils than in neutral and alkaline soils. This might be because CMX NB had a high affinity for ammonia nitrogen. Acidic soils are oligotrophic environments with very low concentrations of free ammonia, and pH strongly affects substrate availability for CMX NB through ammonia dissociation equilibrium [47, 48]. Another reason might be that the CMX NB genome (such as *Nitrospira inopinata*) consists of a Kdp potassium uptake system encoding KdpABC and kdpDE gene clusters. This system is similar to the pH balance system in AOA, and it is crucial for low pH adaptation. This system is involved in the uptake of potassium and generation of a reverse membrane potential to maintain equilibrium under low pH conditions [49].

In the artificial WLF zone of the TGR, a significant positive correlation was found between the soil TC content and the Chao1 index or the Shannon index of CMX NB. The abundance of the *amoA* gene in both clade A and clade B was significantly positively correlated with soil TC content. These findings indicated that TC could promote the growth of CMX NB in the WLF zone of the TGR region. TC might affect ammonia oxidation by modulating the concentrations of organic carbon. A relatively high organic carbon concentration

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was reported to enhance ammonia oxidation [50]. Sun et al. also found that organic carbon content affected the community structure of CMX NB [51]. In addition, high total carbon content was beneficial to improving the soil C/N ratio. CMX NB in Chinese agricultural soils was found to prefer soils with a high C/N ratio [52]. However, the abundance of CMX NB in US forest soils was negatively correlated with the soil C/N ratio [53]. These different results showed that the growth of CMX NB was not only related to the content of TC and TN but also to their existing forms in the soil.

Conclusion

Our results showed that CMX NB widely existed in the WLF zones of the TGR region of the Yangtze River in China, and these WLF zones were relatively rich in the CMX community and that clade A.1, clade A.2.1, clade A.2.2, clade A.3, and clade B coexisted. The *amoA* gene abundances of AOA, AOB, and CMX clade A showed a significant decreasing trend with soil depth. However, the abundance of clade B did not decrease significantly, indicating that clade B was more adaptable to depth changes. The number of OTUs of clade A.1, A2.2, A3, and B in the soil all showed an increasing trend with soil depth, while clade A2.1 exhibited a decreasing trend with vertical depth. This study confirmed the niche differentiation phenomenon of CMX NB in the WLF zones of the TGR region.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-022-00700-5.

Additional file 1. Table S1. qPCR primers and amplification protocols. **Figure S1.** Soil sampling sites. **Figure S2.** Co-occurrence network analysis of all CMX NB clades in natural and artificial water-level fluctuation zones.

Author contributions
M.M. Hu and J.W. Zhao contributed to conceptualization; H. Ding and M.Z. Zhou contributed to data curation; Y.C. Wang and M.M. Hu were involved in funding acquisition; M.M. Hu and J. Wen performed investigation; M.Z. Zhou and J. Wen were involved in methodology; M.Z. Zhou and Y.F. Bao, and J.W. Zhao performed project administration; Y.C. Wang was involved in supervision; M.Z. Zhou and Y.F. Bao contributed to visualization; H. Ding wrote the original draft; S.Z. Li and J.W. Zhao wrote the review & editing. All the authors read and approved the final manuscript.

Declarations

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