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Temporal and spatial distribution of functional nitrogen genes associated with thermal stratification in the subtropical reservoir

Zhihong Yang (Syangzh22@shu.edu.cn)

Shanghai University https://orcid.org/0000-0002-1721-9696

Ming Yang Shanghai University Chuanzhu Bai Shanghai University Huanan Rong Cixi Fishers Technology Extension Center Yihong Yue

Shanghai University https://orcid.org/0000-0002-9562-9009

Research Article

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Abstract

Seasonal thermal stratification is an important hydrological feature of subtropical reservoirs and affects the biogeochemical cycle of nitrogen, which is highly dependent on microbial processes. However, the influence of seasonal thermal stratification on the temporal and spatial distribution of nitrification and denitrification microorganisms is still not clear. This study conducted a detailed survey of a large subtropical reservoir (Xin'anjiang reservoir, XAJR) in eastern China across time (April, July, October 2021, and January 2022) and space (four sampling points with different water depths, covering the epilimnion, thermocline, and isothermal). The research showed that XAJR was a typical subtropical monomer model. Thermal stratification had spatial distribution of gene abundance of bacteria and archaea (16S rRNA), ammonia-oxidizing archaea (*amoA-AOA*), ammonia-oxidizing bacteria (*amoA-AOB*), and denitrifying bacteria (*nirS*) by quantitative PCR. We found that the abundance of nitrogen functional genes also showed seasonal stratification in the vertical profile, and the vertical changes of the relative abundance of *amoA-AOA / archaea, amoA-AOB / bacteria*, and *nirS / bacteria* genes were highly similar to the corresponding changes of single functional genes. The quantitative distribution of *amoA* and *nirS* genes had seasonal differences. The copies number of *amoA* gene in the stratified stage were greater than that in the mixed stage, while the copies number of the *nirS* gene in the early and mixed stages of thermal stratification were greater than that in the estration analysis showed the analysis showed that a variety of environmental factors affected the seasonal distribution of *amoA* and *nirS* gene abundance, which may be due to environmental changes caused by thermal stratification. Our results emphasized the importance of seasonal thermal stratification in the reservoir and expanded the understanding of the temporal and spatial distribution patterns of nitrifying and denitrifying microorga

1. Introduction

Reservoirs are semi-natural or artificial water bodies created by damming natural rivers and lakes. They provide human society with comprehensive ecological services such as water supply, flood control, hydropower generation, irrigation, and navigation, which promote social and economic development. However, the construction of dams changes the continuity and hydrological conditions of the natural water bodies, and interferes with the biogeochemical cycles of biogenic substances, including carbon, nitrogen, phosphorus, and silicon, which causes a wide range of ecological and environmental problems, such as eutrophication and greenhouse gas emissions (Wang et al. 2020; Han et al. 2018; Huang et al. 2019; Maavara et al. 2015; Maavara et al. 2017; Barros et al. 2011; Deemer et al. 2016). The hydrodynamic force of large deep-water reservoirs is weakened and the hydraulic retention time is prolonged, which is easy to produce water stratification similar to natural lakes (Yang et al. 2021; Nilsson et al. 2005; Wang et al. 2015; Yigzaw et al. 2019; Yu et al. 2019). The sediment releases a lot of nutrients due to a lack of oxygen, which affects the water quality at the bottom, but the layered structure limits the mixing of water from above and below, reducing the spread of pollution (Zhang et al. 2015). When the thermal stratification disappears, the water body is mixed, and a large number of nutrients released by the sediment will enter the upper water body, resulting in water eutrophication (Yu et al. 2014). A Subtropical deep-water reservoir is a typical seasonal stratified reservoir, which affects the chemical parameters of the vertical water body and the biogeochemical cycle of biogenic substances. It also affects the ecological health of the aquatic ecosystem and the water quality of the reservoir to a certain extent. Therefore, the research on seasonal thermal stratification of the subtropical reservoir is of great significance.

In aquatic ecosystems, microbial communities are generally considered to be important organisms indicating environmental changes (Zhang et al. 2020). Microorganisms are affected by a variety of environmental factors, such as temperature, pH, DO, and nutritional status (Zhang et al. 2018; Zhang et al. 2015; Kuypers et al. 2018). In addition, studies have shown that microorganisms drive nutrient circulation and affect water quality to maintain the structure and function of the ecosystem (Salmaso et al. 2018; Yin et al. 2020; Wang et al. 2015). Nitrogen is the main driving factor of water eutrophication. Nitrogen pollution has become a serious environmental problem in the global aguatic ecosystem (Cai et al. 2019). Nitrogen pollution in water includes endogenous pollution, atmospheric deposition, point source pollution, and non-point source pollution (Small et al. 2013; Miyazako et al. 2015; Li et al. 2014; Taylor et al. 2005). These combined effects will increase the complexity of the nitrogen cycle in the reservoir. Nitrification and denitrification are important in the nitrogen cycle. Nitrification is to convert ammonium (NH₄⁺) into nitrate (NO₃⁻) under aerobic conditions. Ammonia oxidation is the first step of nitrification, which limits the reaction speed of the nitrogen cycle (Wankel et al. 2011). Ammonia-oxidizing bacteria (amoA-AOB) and ammonia-oxidizing archaea (amoA-AOA) are the main groups of ammonia-oxidizing microorganisms involved in this process. They can oxidize NH_4^+ in the environment through ammonia monooxygenase (AMO). The abundance of amoA-AOB is significantly different from that of amoA-AOA due to the influence of environmental factors. The denitrification process refers to the reduction of nitrate and nitrite to nitrous oxide (N₂O) and Nitrogen (N₂) under anoxic conditions, which are discharged into the atmosphere. Denitrification will cause 87% nitrogen loss in water, reduce nitrogen load in water and alleviate environmental pressure (Xu et al. 2012). However, N₂O, one of the main products of the denitrification process, is a greenhouse gas in the atmosphere, which can destroy the ozone layer in the lower stratosphere (Bergaust et al. 2010). An important step in the denitrification process is the production of nitric oxide (NO) by nitrite (NO₂⁻) under the action of nitrite reductase, which is nirS and nirK (Zumft et al. 1997). These two enzymes have the same physiology and function, but different structures. Generally, the nirS gene is often used as a molecular marker to explore the denitrifying bacterial community in the environment, because many studies have shown that nirS gene is more widely distributed than nirK gene (Fan et al. 2015; Nogales et al. 2002; Santoro et al. 2006; Priemé et al. 2002). Although a great deal of research has been done on the microorganisms in sediment, attention should also be paid to the functional microorganisms in water (Webb et al. 2019; Zhou et al. 2016). It has been pointed out that the structure and composition of microbial communities are affected by stratification and mixing in stratified reservoirs, but the role of nitrogen functional genes in the process of nitrogen transformation in stratified reservoirs is still poorly studied (Zhou et al. 2020). Therefore, considering the complexity of the nitrogen cycle in reservoirs and the impact of nitrogen pollution on drinking water reservoirs, it is very necessary to study the transformation of subtropical seasonally stratified reservoirs.

In this study, the Xin'anjiang reservoir (XAJR) in the Qiantang River Basin in eastern China was taken as the research object. This reservoir is a typical subtropical artificial deep-water reservoir, which has the characteristics of large storage capacity, strong regulation capacity, and long hydraulic retention time.

The objectives of this study are to 1) explore the impact of seasonal stratification on the water quality of XAJR; 2) study the temporal and spatial changes of nitrogen function genes in stratified reservoir water.

2. Materials And Methods

2.1. Studied reservoir

The Xin'anjiang Reservoir (E118°42'-E118°59', N29°28'-N29°58', Fig. 1), also known as Qiandao Lake, located in Chun'an County, Zhejiang Province, at the junction of Qiantang River and Anhui Province, China, was a large valley-type deep-water reservoir formed after the completion of the dam of Xin'anjiang hydropower station in 1959. The hydraulic retention time is about two years. The climate is a typical subtropical monsoon climate with the warm climate and abundant rainfall. The annual average temperature is 17.2°C (-5°C-36°C) and the annual average precipitation is 1500 mm. The length from the dam to the entrance of XAJR is 150 km; the widest part of the water surface is about 50 km; the average water depth is 31 m, and the maximum water depth is 117 m. When the water level reaches the normal pool level of 108m, the water surface area is 58 km² and the storage capacity is 178.4 ×10⁸ m³ (Zhang et al. 2014). XAJR is the "ecological source" of the Qiantang River and an important drinking water source in Hangzhou and even Zhejiang Province. Although the average water quality of XAJR is good, with the continuous social and economic development of the water body has changed significantly, and there are obvious signs of eutrophication in some water areas.

2.2. Sampling and determination of physicochemical properties

In this study, the sampling points were located in four state-controlled points of XAJR (Table 1): JK (riverine zone), XJS (transitional zone), STD (transitional zone), and DBQ (lacustrine zone). JK is the upstream arm of the XAJR, accounting for approximately 60% of the total flow into the reservoir. As a result, soil erosion and human activities have a great impact on this point. XJS and STD are the central areas, accounting for about 25% of total water input, connecting the two surface runoff areas with another tributary of the reservoir. Located in the southeast of the lake, DBQ is the lacustrine zone and the holding area. Sampling was conducted in XAJR during four field campaigns, April 2021, July 2021, October 2021, and January 2022. Water samples from the vertical profiles at four points were collected at a range of depths using a Niskin water sampler (Model 1010, General Oceanics, USA). Among the four points, JK, XJS, and STD were sampled every other meter from 2 meters to 10 meters. About 500ml water samples were filtered through a 0.22 µm sterilized filter membrane (MF-Millipore, USA) and stored in 50 mL sterile centrifuge tubes at – 80°C for long-term storage.

While collecting water samples, we use the automated multiparameter profiler (model YSI EXO) to measure water temperature (T), total dissolved solids (TDS), conductivity (Cond), pH, dissolved oxygen (DO), and chlorophyll-a (Chla) in situ at each sampling point.

Sites	Depth (m)	Longitude	Latitude	Information	Sampling depth (m)
JK	20	118°43'25"E	29°43'33"N	riverine zone	0,2,3,4,5,6,7,8,9,10
XJS	47	118°55'59"E	29°37'6"N	transitional zone	0,2,3,4,5,6,7,8,9,10,15,30,40
STD	66	118°57'49"E	29°31'27"N	transitional zone	0,2,3,4,5,6,7,8,9,10,15,30,60
DBQ	>80	119°12'27"E	29°29'15"N	lacustrine zone	0,5,10,15,30,50

2.3. DNA extraction and quantitative real-time PCR

Genomic DNA was extracted from the filter using the FastDNA SPIN kit for soil (MP biochemicals, USA). The genomic DNA was examined by 1.2% (w/v) agarose gel electrophoresis. The absolute abundance of ammonia-oxidizing bacteria (*amoA-AOB*), ammonia-oxidizing archaea (*amoA-AOA*), and denitrification gene (*nirS*) were measured by quantitative real-time PCR (qPCR) using specific primer pairs. Primers used for the amplification of these genes are listed in Supplementary Table 1 (Yue et al. 2021). Then, the correct PCR product was linked with the pMD18-T vector to form a plasmid which was measured by Qubit (Thermo Fisher Scientific, USA). The plasmid diluted in 10-fold gradient was used as the standard for qPCR. The PCR reaction mixtures contained 10 μ L PowerTrack[™] SYBER[™] Green Master Mix (Thermo Fisher Scientific, USA), 0.5 μ L Yellow Sample Buffer (Thermo Fisher Scientific, USA), 1 μ L (10 pmol/ μ L) of both forward and reverse primers and 2 μ L template DNA in a final volume of 20 μ L. All qPCR procedures were carried out in the Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, USA) with an initial denaturation at 95°C for 2 min, followed by 40 cycles lasting 15 s each at 95°C, and 60 s at 60°C. After PCR amplification, the specificity of each amplicon was detected by melt curve analysis. The standard curve was constructed by drawing the logarithmic curve between the relative fluorescence unit and the copy number of standard plasmid DNA under the threshold fluorescence value (CT). The standard curve is shown in Supplementary Fig. 1. The amplification efficiency range of qPCR was 80%~90% (R² > 0.99). Each DNA standard and DNA sample analysis were made in duplicate.

2.4 Data Analysis

ArcGIS10.7 software was used to draw the schematic diagram of sampling points of Xin'anjiang Reservoir. Surfer11 was used for Kriging interpolation to draw a contour map and the spatial distribution between water temperature, dissolved oxygen, pH, and the abundance of gene (*amoA-AOA*, *amoA-AOB*, *nirS*, *amoA-AOA* / *archaea*, *amoA-AOB* / *bacteria*, and *nirS* / *bacteria*), and water depth. The correlation between N-related functional gene abundance and environmental factors was analyzed by Pearson correlation with SPSS25 software. The significance level was set as follows: extremely significant correlation, p < 0.01; significant correlation, 0.01 ; no significant correlation, <math>p > 0.05. Redundancy analysis (RDA) explored the relationship between N-functional gene

abundance and environmental variables. Arrows indicate environmental factors or gene abundance. The length of the straight line between the arrow and the origin represents the impact of environmental factors or gene abundance on the sampling point. The longer the line, the greater the correlation, and vice versa. The included angle between the arrows is positive correlation and negative correlation (acute angle is positive correlation, obtuse angle is negative correlation). Project the sample point to the arrow, and the distance from the projection point to the origin indicates the influence of environmental factors on the sample. In this paper, the data were mapped by GraphPad Prism5.0 to demonstrate the spatial distribution of N-functional gene abundance and water depth.

3. Results

3.1 Vertical variation and seasonal stratification of water temperature (T), dissolved oxygen (DO), and pH

The physical and chemical parameters of the water body were measured by YSI. The seasonal vertical changes of T, DO, and pH at the four points of XAJR from April 2021 to January 2022 were shown in Fig. 2. As shown in Fig. 2a, for JK, the highest temperature in October was 29°C and the lowest temperature in January was 14°C. Although there was no obvious vertical change in the temperature of JK in four months, there was an obvious time change, with distinct temperature changes in four seasons. For XJS, STD, and DBQ, with the rise of temperature and the rapid enhancement of solar radiation in April, the surface temperature began to rise gradually; the water temperature changed little after 25 m in the lower layer; the thermal stratification of water began to form. In July and October, with the further increase in temperature, the heat received by the surface layer diffused to the deep-water layer, and the positive stratification of XAJR was more significant and stable. The surface layer to about 15 m was epilimnion; 15 m-30 m was thermocline; below 30 m was isothermal. In October, the surface water temperature reached the peak (> 30°C), and the difference between the surface and bottom water temperatures was the largest (20°C), forming a relatively stable "upper high and lower low" water temperature layered structure, and limiting the exchange between the upper and lower water bodies to a certain extent. January was the coldest season. Affected by the temperature drop, the water column of the reservoir was well mixed, and the difference between the surface and bottom water temperatures was very small.

The stratification of the DO was also developed in the XAJR (Fig. 2b). The DO of JK had no significant vertical stratification in April, July, and January, but the DO change at depth of 10 m in October, forming a narrow thermocline from 10 m to 14 m. For XJS and STD, the difference of DO between the bottom and the surface in July and October was larger than that in January and April, especially in July, there was a significant abrupt layer, namely "oxycline". In July, the DO saturation of the surface layer reached 160%, and the DO saturation of 30 m-40 m in deep water was less than 50%. In October, the thermal stratification was the most significant, thermocline appeared at 15 m, DO also mutated at 15 m, and DO decreased sharply at 15 m-30 m. For DBQ, as a deep-water point, the highest surface DO saturation occurred in July, only 120%, which was similar to JK; In October, the thermocline appeared at 15 m, and DO also changed, but different from XJS and STD, the DO at 15 m-30 m first decreased and then increased.

The pH change of the XAJR longitudinal water body was shown in Fig. 2c. April was the formation period of thermal stratification, and the changing trend of pH was consistent with that of water temperature. The pH stratification was the most obvious in July. For JK, the pH gradually decreased with the water depth from the surface. For XJS, STD, and DBQ, the pH at 0–7 m remained unchanged, and the pH at 7 m-40 m decreased with the increase of water depth. The pH was generally high in October, and the pH of surface water reached 9.7. In January, the pH changed little during the mixing period of the reservoir. In general, the surface water of XAJR was alkaline and the bottom water was slightly acidic.

In conclusion, seasonal stratification occurred in XAJR, and it was more obvious in deep water than in shallow water. The temperature was the most direct indicator of water mixing, while dissolved oxygen and pH were both important water quality indicators to measure the water environmental quality of the reservoir. JK, located in the northwest region of XAJR with shallow water depth, had no significant vertical stratification of temperature, dissolved oxygen, and pH in four months. However, XJS, STD, and DBQ with deep water depth, temperature, dissolved oxygen, and pH all showed seasonal stratification of water bodies with similar change rules.

3.2 Vertical variation of ammonia oxidation gene abundance and denitrification gene abundance

The vertical profiles of ammonia oxidation gene abundance and denitrification gene abundance at the four sampling points from April to January were shown in Fig. 3. (Fig. 3a) For JK, the gene abundance of *amoA-AOA* showed obvious temporal changes, and there was no significant change on the vertical profile, except that it decreased significantly at 4 m and 9 m in October. For XJS, the surface gene abundance was the lowest in July; for STD and DBQ, the surface gene abundance was the lowest in April and July. When the thermal stratification was mature, the gene abundance in the thermocline increased with water depth at the three sampling points; the gene abundance in the isothermal layer had little change with water depth. (Fig. 3b) For JK, the gene abundance of *amoA-AOB* had no vertical profile change. When the thermal stratification matured in October, for XJS and STD, the gene abundance was low in the epilimnion, and they increased with water depth in the thermocline, showing similar spatial changes. (Fig. 3c) For JK, the *nirS* gene abundance also had no vertical profile change. In October, for XJS, STD, and DBQ, gene abundance had little difference in the thermocline, and increased with water depth in isothermal.

By mapping the relative abundance vertical profiles of *amoA-AOA / archaea, amoA-AOB / bacteria*, and *nirS / bacteria* genes (Supplementary Fig. 2), it was found that the vertical changes were very similar to the changes of single functional genes. The results showed that thermal stratification affected not only the vertical distribution of functional genes, but also archaea and bacteria, and their vertical changes were very similar.

3.3 Seasonal variation of ammonia oxidation gene abundance and denitrification gene abundance

Seasonal changes of ammonia oxidation gene abundance and denitrification gene abundance were shown in Fig. 4. For JK, although the gene abundance had no vertical variation, they had seasonal variation. The copies numbers of *amoA* and *nirS* genes were higher in spring and summer. For the other three points, the gene abundance of *amoA*-AOA first increased and then decreased, and the peak appeared in autumn; The copies number of *amoA*-AOB was higher in spring and summer than in autumn and winter, but that of DBQ in autumn was very high. Different from the change of *amoA* gene abundance, the copies number of *nirS* gene in spring and winter were greater than that in summer and autumn, but for DBQ, the gene abundance was only higher in spring, which may be related to DBQ as the outbound section of XAJR.

Comparing the relative abundances of *amoA-AOB / amoA-AOA* (Fig. 5a), it was found that for JK, the relative abundances had no time change, and the ratio was high in July. For XJS, STD, and DBQ, the ratio was high in April and July. (Fig. 5b) For JK, the *amoA-AOB / nirS* was very low. For the other three sampling points, the gene abundance of *nirS* was greater than that of *amoA-AOB* at most different depth points.

3.4 Seasonal variation of ammonia oxidation gene abundance and denitrification gene abundance in longitudinal rivers

The impounding of the reservoir transforms the dynamic river system into a static lake reservoir system, thus forming the river area (JK), transition area (XJS and STD), and lake facies area (DBQ) in the longitudinal direction. Three special points were selected: the surface layer (0 m), the interface between epilimnion and thermocline (15 m), and the interface between thermocline and isothermal (30 m) to analyze the variation trend of gene abundance in different seasons (Fig. 6). JK, as the river area of XAJR, had a relatively large gene abundance of *amoA* and *nirS*. Compared with the thermal stratification in the warm season, the longitudinal difference of gene abundance in the mixed period of the cold season was not significant. In thermal stratification, the variation of *amoA* gene abundance in the surface layer and 15 m was quite different. Different from the variation of *amoA* gene abundance, *nirS* gene abundance had a larger trend in the surface layer and 30 m.

3.5 Relationship between nitrogen functional gene abundance and environmental factors

Using RDA to explore the main environmental factors affecting gene abundance (Fig. 7). The RDA of JK, XJS, and STD explained 76.12%, 52.67%, and 50.92% of the data respectively, indicating that there was a strong seasonal clustering between environmental factors and nitrogen functional genes. According to the sequence diagram, for XJS, and STD, the abundance of *amoA-AOB* and *nirS* were mainly distributed in April and July; the abundance of *amoA-AOA* was mainly distributed in October and January. The points of XJS and STD in January were closed to the origin, indicating less affected by environmental factors; However, the points in April, July, and October were scattered far away from the origin, indicating greatly affected by environmental factors. In general, most environmental factors such as Chla, DO and pH were the key factors affecting nitrogen functional gene abundance.

Study on the correlation between gene abundance and environmental parameters based on Pearson correlation analysis (Table 2). For JK, there was no thermal stratification throughout the year, the vertical changes of physical and chemical parameters were small, and the correlation between gene abundance and environmental parameters was little significant. For XJS and STD, the occurrence of thermal stratification in July and October affected the physical and chemical factors of the water environment in the vertical profile. The gene abundance in July and October was significantly correlated with a large number of environmental physical and chemical factors. However, for DBQ, the significant correlation between gene abundance and environmental factors was different from the other two points. Only some environmental factors were significantly correlated with gene abundance.

Table 2 Pearson correlation analysis of ammonia-oxidizing archaea abundance, ammonia-oxidizing bacteria abundance, denitrification gene abundance, and rese parameters at four sampling points (JK, XJS, STD, and DBQ) during April (2021), July (2021), October (2021) and January (2022).

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AOA JK					ZJS				STD		DBQ			
month	4	7	10	1	4	7	10	1	4	7	10	1	4	7
Depth	-0.019	-0.254	-0.271	-0.202	0.252	0.961**	0.843**	0.771**	0.819**	0.517**	0.680**	0.526**	0.874**	0.972
Т	0.237	0.551*	0.145	0.031	-0.069	-0.910**	-0.893**	-0.568**	-0.105	-0.940**	-0.936**	0.514*	-0.583	-0.88
Sal	-0.419	b	-0.266	b	-0.254	0.836**	0.882**	0.903**	b	0.927**	0.918**	b	b	b
pН	-0.402	0.375	0.143	0.273	0.333	-0.625**	-0.797**	-0.477*	-0.162	-0.599**	-0.843**	0.183	-0.719*	-0.74
Chla	0.203	-0.162	0.047	0.248	0.353	-0.591**	-0.697**	-0.589**	-0.342	-0.580**	-0.775**	-0.180	-0.436	-0.65
DO	0.023	-0.398	0.074	0.315	0.089	-0.583**	-0.728**	-0.457*	-0.071	-0.584**	-0.796**	0.045	-0.824**	-0.64

AOB	JK				XJS				STD	DBQ				
month	4	7	10	1	4	7	10	1	4	7	10	1	4	7
Depth	0.568**	0.141	0.132	0.284	0.791**	0.986**	0.751**	0.377	0.786**	0.888**	0.910**	0.350	0.938**	0.8
Т	-0.543*	-0.109	-0.184	-0.283	-0.744**	-0.979**	-0.809**	-0.570**	-0.151	-0.853**	-0.852**	-0.063	-0.601	-0.8
Sal	0.969**	b	-0.192	b	-0.001	0.687**	0.880**	0.512**	b	0.567**	0.890**	b	b	b
pН	0.728**	-0.163	-0.096	-0.104	-0.484*	-0.831**	-0.632**	-0.022	-0.201	-0.790**	-0.697**	-0.466*	-0.733*	-0.8
Chla	0.338	0.282	-0.201	-0.249	-0.435*	-0.802**	-0.550**	-0.626**	-0.400	-0.702**	-0.620**	-0.110	-0.457	-0.8
DO	-0.353	-0.110	-0.137	-0.057	-0.553**	-0.799**	-0.642**	0.038	-0.135	-0.734**	-0.631**	-0.421*	-0.835**	-0.9

nirS	JK				ZJS				STD	DBQ				
month	4	7	10	1	4	7	10	1	4	7	10	1	4	7
Depth	0.118	0.650**	0.148	-0.197	-0.275	0.297	0.856**	0.764**	0.306	0.642**	0.832**	0.866**	0.250	-0.6
Т	-0.063	-0.252	-0.309	0.075	0.176	-0.388	-0.901**	-0.660**	-0.646**	-0.325	-0.937**	0.192	0.650*	0.5
Sal	-0.076	b	0.014	b	0.015	-0.156	0.895**	0.995**	b	0.132	0.883**	b	b	b
pН	-0.050	-0.372	-0.281	-0.131	0.450*	-0.598**	-0.807**	-0.437*	-0.701**	-0.605**	-0.891**	-0.696**	0.447	0.1
Chla	0.126	0.436	-0.311	-0.137	0.564**	-0.594**	-0.718**	-0.648**	-0.487*	-0.635**	-0.846**	0.227	0.241	0.42
DO	-0.101	-0.246	-0.281	-0.166	0.307	-0.566**	-0.756**	-0.455*	-0.676**	-0.576**	-0.856**	-0.663**	0.620	0.12
Bolded	values rep	resent sian	ificant co	rrelations	between the	e variables.	* <i>p</i> < 0.05: **	<i>p</i> < 0.01.						

4. Discussion

4.1 Seasonal thermal stratification of XAJR

Water temperature was the main driving factor for the change of the water environment in the reservoir (Noori et al. 2021). The study has shown that the thermal stratification of reservoirs essentially depends on the physical properties of water: when the temperature is above 4°C, the water body expands with heat and contracts with cold (Yang et al. 2020). The water at the bottom cannot be affected by external solar radiation, resulting in low water temperature and high density. The lighter surface water cannot be exchanged with the heavier bottom water, forming thermal stratification on the vertical section. With the weakening of solar radiation, the temperature of surface water decreases; the density difference between upper and lower water disappears, and the thermal stratification of the reservoir also disappears. The annual water temperature at the four sampling points of the XAJR in the study area was above 4°C, and the temperature showed obvious seasonal variation (Fig. 2a). The temperature was the highest in summer and autumn, and the lowest in winter. The water depth of JK was about 15 m. Generally, water depth above 10 m was prone to thermal stratification. JK had no thermal stratification throughout the year, mainly because JK, as the entry section of XAJR, was vulnerable to water disturbance, resulting in a small temperature difference between upper and lower water temperatures and good mixing of water throughout the year. The water depth of the other three points was greater than 40 m, and the water body was not easy to be disturbed by the wind. Moreover, XAJR was surrounded by mountains and many islands, which inhibited the development of wind and waves (Zhang et al. 2015). The thermal stratification of XJS, STD, and DBQ formed in spring, matured in summer and autumn, and disappeared in winter. This is a typical seasonal thermal stratification and a typical Asian tropical monomer model (Tafas et al. 1997; Zhang et al, 2014). This model is consistent with research on other subtropical deep reservoi

4.2 Environmental impacts of thermal stratification

Seasonal thermal stratification is an important hydrological feature of subtropical reservoirs (Zhang et al. 2021; Li et al. 2018; Noori et al. 2019). Previous studies have shown that lake thermal stratification affects a series of environmental factors, such as DO, pH, and so on, by limiting migration or interacting with other physical and chemical factors in the reservoir vertical profile (Liu et al., 2019; Coats et al., 2006; North et al., 2014). In this study, the water temperature was significantly correlated with DO and pH (Table.S2), and DO and pH also had seasonal stratification (Fig. 2b, Fig. 2c). Study has confirmed that the stratification of DO in the reservoir largely depends on the thermal stratification model (Zhang et al. 2015). In the epilimnion layer of XAJR, the increase of surface water temperature was conducive to the growth of phytoplankton (Wang et al. 2018). Phytoplankton absorbed CO₂ and released O₂ through photosynthesis, which led to sufficient or even supersaturation of surface dissolved oxygen, while the absorbed CO₂ broke the balance of CO₂ / CO₃²⁻ / HCO₃⁻ and made the pH of water slightly alkaline (Aviles and Niell. 2007; Han et al. 2018). In the process of thermal stratification, with the increase of water depth, the light intensity decreases, and photosynthesis weakens. The thermocline prevents the transport of dissolved oxygen from the upper water body to the lower water body. The isothermal layer cannot supplement oxygen. The mineralization and degradation of sediment organic matter and the death and sinking decomposition of a large number of sedimentary organisms consume a large amount of dissolved oxygen in the water body, forming an anoxic environment makes facultative anaerobic bacteria active. They produce carbon dioxide in the process of self-metabolism, mineralization, and degradation of organic matter in water. Since light cannot penetrate the thermocline, CO₂ will not be consumed by

photosynthesis, resulting in CO_2 accumulation and reducing the pH value of the water body (Lee et al. 2010). With the disappearance of thermal stratification, the upper and lower water bodies are mixed, resulting in little difference in dissolved oxygen and pH between the upper and lower water layers. Therefore, the water temperature and thermal stratification of XAJR are the key factors affecting the distribution of DO and pH in the vertical profile of the water body.

4.3 Effect of seasonal thermal stratification on the distribution of N-functional genes

In this study, the distribution of ammonia oxidation and denitrification genes in XAJR showed that: (1) there was a stratification phenomenon in gene abundance in the vertical profile; (2) there were seasonal differences in gene abundance distribution.

This study found that the nitrogen functional gene abundance showed seasonal stratification in the vertical profile due to seasonal thermal stratification. It has been proved that temperature and dissolved oxygen stratification change the form and transformation of nitrogen in the stratified lake (Wang et al. 2020; Su et al. 2019). For JK, *amoA* and *nirS* gene abundance had no obvious vertical profile changes (Fig. 3); and interestingly, their temperature and dissolved oxygen also had no obvious vertical changes (Fig. 2). XJS, STD, and DBQ have seasonal stratification. The distribution of *amoA* gene and *nirS* gene changed with depth during stratification. The copies number of *amoA* was high in the thermocline, and the gene distribution in the isothermal layer changes little with depth. The thermocline had a large temperature and dissolved oxygen gradient, and the combined action of NH₄⁺ from the lower layer and oxygen from the upper layer provided the best conditions for the nitrification of this group. Other studies also found that *amoA* gene was abundant in the thermocline (Pouliot, Galand, Lovejoy, &Vincent. 2009; Lliros et al. 2010; Auguet et al. 2011). Some studies have confirmed that in the natural environment, denitrification is often combined with nitrification, and nitrS in this study showed that nitrification and denitrification were significantly correlated (Table S3). Falk et al. (2007) found that the abundance of denitrification genes was the highest in deep water with low dissolved oxygen concentration. Similarly, Kim et al. (2010) and He et al. (2019) also reported that higher denitrification gene abundance was found in the bottom waters.

In terms of seasonal patterns, there are seasonal differences in the distribution of amoA and nirS genes. Because the ammonia-oxidizing microbes of archaea and bacteria need to compete for the same resources, coexistence patterns generally do not occur (Liu et al. 2015). As electron donors and acceptors, oxygen and ammonia are necessary for ammonia oxidation. AOA and AOB have different affinities for ammonia and oxygen (Jung et al. 2011; Kim et al. 2012; Lam et al. 2007; Leininger et al. 2006). Our study found that there were seasonal differences in amoA gene distribution patterns in XAJR, and there were also seasonal differences in the relative abundance distributions of amoA-AOB and amoA-AOA. However, the copy number of genes in the stratified stage was greater than that in the mixed stage. A previous study showed that the amoA gene copy numbers were greater in summer than in winter in Catalina Harbor (Beman et al. 2012). It was found that amoA gene was dominant in the stratification period and was low in the mixing period in the alchichica lake (Pajares et al. 2017). Studies have shown that a variety of environmental factors such as Sal, pH, DO and NH₄⁺ have been determined to affect the abundance of AOA and AOB (Verhamme et al. 2011; Molina et al. 2010; Gubry-Rangin et al. 2011; Hu et al. 2010). In our study, RDA and correlation analysis showed that no single environmental factor affected the seasonal distribution of amoA abundance, which seemed to be affected by a variety of environmental factors. This may be due to thermal stratification resulting in a variety of environmental changes. Different from the seasonal variation of the ammonia oxidation gene, the gene copy number of denitrification in the early and mixed stages of thermal stratification was greater than that in the mature stage of stratification. In spring and winter, there was no restriction of stratification structure, and a large number of nutrients at the bottom were released into the upper water body. Sufficient nutrients and low dissolved oxygen concentration were conducive to the growth of denitrifying bacteria. Studies have shown that nirS gene is more common in a hypoxic environment (Knapp et al. 2009; Tatariw et al. 2013). It is also found that denitrification genes have high abundance in the mixing stage in Lake alchichica (Pajares et al. 2017).

Conclusion

In summary, this study found that the thermal stratification of Xin'anjiang Reservoir had obvious seasonal characteristics, and the DO and pH also showed similar stratification phenomena under the influence of thermal stratification. We revealed the temporal and spatial distribution patterns of nitrifying and denitrifying microorganisms affected by seasonal thermal stratification. The abundance of nitrogen functional genes also showed seasonal stratification in the vertical profile. During the stratification period, the larger temperature and dissolved oxygen gradient in the thermocline made the copy number of *amoA* gene change greatly; the lower dissolved oxygen concentration and more sufficient nutrients in the isothermal layer stimulated the increase of denitrification gene abundance. Interestingly, the vertical changes in the relative abundance of *amoA-AOA / archaea, amoA-AOB / bacteria*, and *nirS / bacteria* genes were highly similar to the corresponding changes in a single functional gene. The quantitative distribution of *amoA* and *nirS* genes had seasonal differences. The environmental changes caused by thermal stratification lead to the seasonal distribution of gene abundance affected by various environments. In conclusion, these results provide a new understanding of the temporal and spatial distribution of nitrification and denitrification by seasonal thermal stratification.

Declarations

Supplementary information This manuscript has accompanying supplementary file.

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Statements and Declarations

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Figures



Figure 1

Geographic location of the Xin'anjiang Reservoir and the sampling sites. (JK: Jiekou, XJS: Xiaojinshan, STD: Santandao, DBQ: Dabaqian).



Figure 2

Longitudinal changes of (a) water temperature (T), (b) dissolved oxygen saturation (D0%), and (c) pH at four sampling points of XAJR in April 2021, July 2021, October 2021, and January 2022 (vertical bars represent parameter values).



Vertical changes of the abundance of (a) *amoA-AOA*, (b) *amoA-AOB*, and (c) *nirS* at four sampling points of XAJR in April 2021, July 2021, October 2021, and January 2022 (vertical bars represent parameter values).



Figure 4

Four sampling sites, the abundance of (a) *amoA-AOA*, (b) *amoA-AOB*, and (c) *nirS* in April 2021 (spring), July 2021 (summer), October 2021 (Autumn), and January 2022 (winter). Numbers in a rank with different letters indicate a significant difference (Duncan's Test, *p* < 0.05).



Figure 5

Four sampling sites, the relative abundance of (a) *amoA-AOB / amoA-AOA*, (b) *amoA-AOB / nirS* in April 2021 (spring), July 2021 (summer), October 2021 (Autumn), and January 2022 (winter)



Longitudinal variation of (a) *amoA-AOA*, (b) *amoA-AOB*, and (c) *nirS* gene abundances at 0 m, 15 m, and 30 m along water flow at four sampling sites in different seasons. Numbers in a rank with different letters indicate a significant difference (Duncan's Test, *p* < 0.05).



Figure 7

RDA analysis between *amoA-AOA, amoA-AOB, nirS*, and environmental factors at four sampling points during the four sampling periods. The red arrow represented environmental factors, the blue arrow represented nitrogen functional genes. The dots of four colors represent four sampling times.

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