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Distribution characteristics of nitrogen and the related microbial community in the surface sediments of the Songhua River†

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Nitrogen in surface sediments is becoming an ecological risk to the river environment and it is essential to clarify the relationship between the different forms of nitrogen and related microorganisms. A survey was conducted to analyze the distribution characteristics of the nitrogen and related microbial community in the sediments of the Songhua River during normal season and dry season. In the surface sediments of the Songhua River, no total nitrogen (TN) pollution risk was observed according to the U.S. EPA assessment criteria (1000 mg kg^{-1}) for sediment contamination, but TN in several sampling sites (554.9–759.7 mg kg^{-1}) exceeded the alert values (550 mg kg^{-1}) should be concerned according to the guidelines issued by the Ministry of Environment and Energy of Ontario, Canada. The average TN, NH_4^+–N, NO_3^-–N and total organic nitrogen (TON) in the surface sediments of the Songhua River during normal season were higher than those in the dry period. TON was the main form of nitrogen in the sediment of Songhua River, NO_2^-–N content was lowest and no obvious difference was observed between normal and dry seasons. The highest average NH_4^+–N of both seasons occurred in the Nenjiang River, and the highest average NO_3^-–N of both seasons were found in the main stream of the Songhua River. The community abundance of AOB genes (1.1×10^7 to 2.5×10^8 copies per g soil in normal season, 7.2×10^5 to 3.3×10^8 copies per g soil in dry season) was higher than that (1.2×10^6 to 9.7×10^7 copies per g soil in normal season, 6.6×10^4 to 3.2×10^7 copies per g soil in dry season) of AOA genes in both normal and dry seasons. The denitrifying *nirS* genes were predominant in both seasons, and their abundance (1.8×10^6 to 8.0×10^8 copies per g soil) in dry season was higher than that (9.7×10^5 to 4.6×10^8 copies per g soil) in normal season. Moreover, the moisture concentration, pH, dissolved oxygen and different formation of nitrogen were key factors affecting the variation of nitrogen-transformation microorganisms during normal and dry seasons. This research could help to explain the relationship between nitrogen transformation and the related microbial community in the surface sediment, which could provide a scientific basis for water ecological restoration and water environment improvement of Songhua River.

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1. Introduction

Nitrogen is a core substance maintaining the ecological balance of water bodies. However, with the rapid social and economic development and the improvement in people's living standards, large amounts of domestic, industrial, and agricultural wastewater with nitrogenous substances are discharged into lakes and rivers, which leads to serious eutrophication and causes potential harm to human and animal health and leads to further ecological deterioration.[1–3] Nitrogen pollution of water bodies has become one of the most urgent water environmental issues worldwide.[4]

However, most of the nitrogen in a water body will be mineralized into ammonia nitrogen by sediment microorganisms and then adsorbed on the sediments.[5] Sediment is the main nitrogen reservoir for deposited gaseous nitrogen and organic nitrogen, which is essential for natural water

environment management.[6,7] As the sink and source of nitrogen, the sediments will release nutrients to the overlying water with the changes of ambient conditions as temperature, dissolved oxygen (DO), pH, heavy metal[8–10] and water disturbance, and then cause secondary pollution.[11] The nitrogen forms in sediments are generally divided into total organic nitrogen (TON) and total inorganic nitrogen (TIN), while TON is predominant.[12] Nitrogen distribution in the sediments varies greatly due to the different degrees of mineralization for TON,[13] whose morphology mainly consists of animal and plant residues or humus, and the nitrogen cycle does not include all of the nitrogen forms in the sediments.[14]

Fixed ammonium, i.e., non-exchangeable ammonium, which refers to the $NH_4^+–N$ in the 2 : 1 aluminosilicate crystal layer of minerals and accounts for more than 80% of TIN and 10% of the total nitrogen (TN), cannot be exchanged with neutral salts.[15] According to Huo,[16] the fixed ammonium in lake sediments ranged from 99.45 to 329.02 mg kg^{-1}, and it had negative correlation with nitrate nitrogen. Fixed ammonium and exchangeable nitrogen were mainly resulted from TON mineralization. In recent years, studies on sediment TON in lakes and reservoirs,[16] oceans,[17] estuarine wetlands[17] and rivers[18] are mainly focused on the exchangeable nitrogen, and that about river sediment is limited.[18]

Microorganisms in the sediment can convert nitrogen into different forms by nitrification, denitrification and anammox process.[19,20] The nitration process mainly consists of two steps, i.e., oxidation of ammonia and nitrite oxidation, and it is the first and foremost stage of nitrogen cycle. The microorganisms of ammonia oxidation archaea (AOA), ammonia oxidation bacteria (AOB) and nitrosation bacteria (NOB) work together to ensure the smooth progress of nitrification.[21,22] AOB and AOA are considered as the most important bacteria and archaea for ammonia oxidation.[23–25]

AOA and AOB abundance varies in different environments, and AOB communities are dominant in river sediments,[26] coastal wetlands[27] and cultivated farmland.[28] The number of amoA genes of AOA is higher than that of AOB in the hydrothermal spring sediments[29] and marine environment.[30] It is reported that low ammonia nitrogen concentration, low pH and anaerobic condition is suitable for AOA growth.[31] When the circumstances are suitable for the growth of both AOA and AOB, the growth rate of AOB is much higher than that of AOA.[32] In the nitrite oxidation stage, NO_2^- produced by ammonification is oxidized to NO_3^- by NOB, which interacts with AOB to promote the nitrification process. NOB concentration in different ecosystems changes obviously.[33,34] In river sediments, nitrification bacteria are always dominated by Nitrospira group, whose abundance is greater than that of AOB.[35] The role and concentration of AOA, AOB and NOB in river sediments, as well as their cooperative effects and impacting factors need to be further studied.

Denitrification is recognized as the best way to remove nitrogen from rivers,[36] and the sediment and water interface is very suitable for the growth of denitrification microorganisms. Denitrification process consists of four consecutive steps, i.e., $2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2$, and nitrate reductase (encoding genes are mainly narG and napA), nitrite reductase

(nirS and nirK), NO reductase (norB), and N_2O reductase (nosZ) are essential to catalyze these steps. NarG, nirS, nirK and nosZ genes are the most important functional genes in the denitrification microorganisms,[37] and nirS has higher genetic diversity than nirK in nitrite reductase related genes.[38] It is reported that nirS related bacteria played more important role than nirK related bacteria in the denitrification process of San Francisco Bay estuary sediments, and the denitrification potential rate is positively correlated with nirS gene abundance.[39] The investigation of denitrification rate and dissimilation efficiency of microorganisms is a hot issue.[40]

Previous studies on nitrogen in sediments are mostly focused on the sediments of sea, estuary and coastal zones, while the distribution characteristics of nitrogen in river sediments were seldom reported. Also, few analyses about the nitrogen morphology and denitrification related microorganisms were conducted. In this study, we seasonally collected surface sediment samples from main stream and tributaries along Songhua River. The characteristics of nitrogen distribution, related microbial community and their correlation in the surface sediments of the Songhua River during normal and dry seasons were extensively investigated in this paper, and the sediment TN pollution risk was also assessed, thus provided scientific basis for the nitrogen source analysis, water quality improvement and water ecological restoration.

2. Materials and methods

2.1 Studying region and site sampling

Located in the northeast of China, the Songhua River basin covers 556 800 km^2, which accounts for about 60% of the whole northeast China region. The river basin is surrounded by mountains on three sides, i.e., the Changbai Mountains in the east and southeast, the Greater Khingan Mountains in the west and northwest and the Lesser Khingan Mountains in the north, and the mountainous area accounts for more than 60%. The Songhua River has two headstreams, i.e., the Nenjiang River (NR) originated from the Greater Khingan Mountains and the Second Songhua River (SSR) originated from the Changbai Mountains, which converge at the Sancha River mouth and finally join the Heilong River (HR).[41] Heilong River marks the border between China and Russia. In Songhua River basin, rainfall mainly occurs in July and August, and the average annual precipitation ranges from 400 mm to 700 mm. September and March were selected as the representative month of the normal season and the dry season respectively.[42] In the normal water season of September, 2016, 43 surface sediment samples were obtained from the Songhua River basin, i.e., 8 from NR (NS1–NS8), 8 from SSR (DS1–DS6), 27 from main stream of Songhua River (MSSR) (SS1–SS27), and 2 from the HR (HS1–HS2), which is the reference sampling sites Fig. 1(a). 19 samples were taken in the dry season of March, 2017, i.e., NM2, NM4, NM6, NM8 from NR, DM1, DM2, and DM6 from SSR, SM1, SM2, SM6, SM7, SM8, SM9, SM10, SM18, SM20, and SM21 from MSSR, and HM1 and HM2 from HR (Fig. 1(b)), which were coincided with the corresponding sites in September, 2016. The sampling sites information is shown in the ESI Table S1,† and
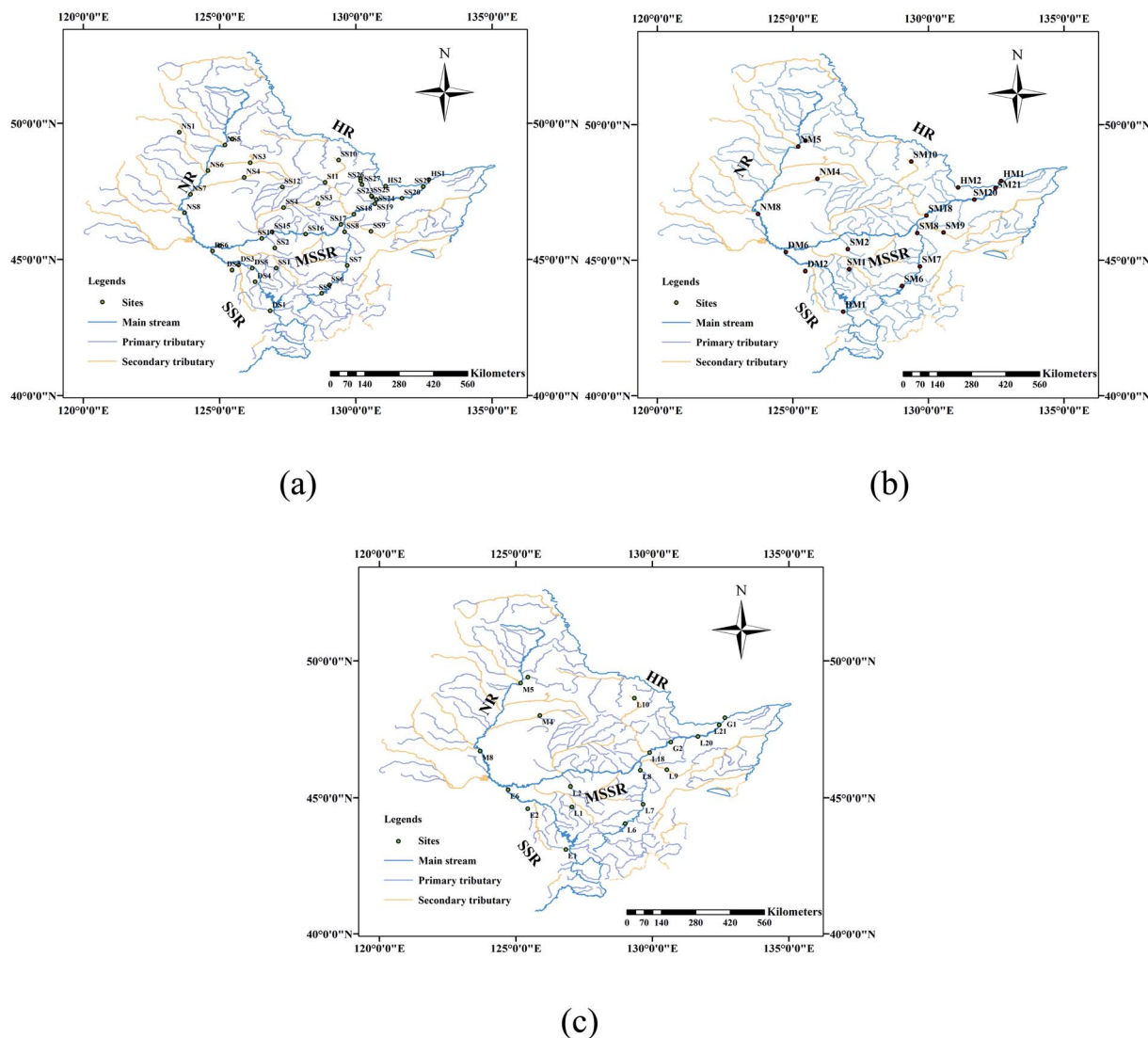
(a)

(b)

(c)

Fig. 1 Distribution diagram of sampling sites in the Songhua River (a) September, (b) March, (c) comparative analysis sites.

the same sample sites of normal and dry season (Fig. 1(c)) were selected for comparative analysis. The sites basically covered the entire river basin and the important cities along the route, and some of the sites located at the positions of structures such as bridges on the river which can change the flow conditions.

2.2. Sample collection and pretreatment

After the removal of the gravel, plant roots, *etc.*, the surface sediments (from the 20 cm upper part) were packed into poly-ethylene self-sealing bags and transported to the laboratory with ice bags as soon as possible.[43] The samples were frozen at −20 °C, dried by freeze-dryer, grounded by mortar, and then passed through 100-mesh nylon sieve for storage. The samples for molecular biological analysis were stored at −80 °C for DNA extraction.

2.3 Analytical method

2.3.1 Chemical analysis method. Moisture concentration (MC) and total phosphorus (TP) in surface sediments was

determined by Standards Measurements and Testing (SMT) method,[43] and TN was determined by alkaline potassium per-sulfate oxidation ultraviolet spectrophotometer. The NO_2^-–N and NO_3^-–N were analyzed by spectrophotometry. Specific test steps of pH were referred to Jiang.[44]

2.3.2 qPCR method for molecular analysis. Total DNA was extracted with genomic DNA kit (Tiangen Biotech(Beijing), Co., LTD., China). As shown in Table 1, specific primers of AOA, AOB and denitrification bacteria (*narG*, *nirK*, *nirS* and *nosZ*) genes were used to perform absolute quantitative PCR analysis on ABI7500 quantitative PCR instrument (Applied Biosystems Inc., USA) by SYBR-Green method.

qPCR reaction conditions were as follows: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 10 s, total 40 cycles, and finally extension at 60 °C for 34 s. PCR reaction mixtures con-tained 16 μL SYBR GreenI (GENEary, GK8020), 1 μL positive primers (10 pmol μL per a), 1 μL reverse primers (10 pmol μL per a) and 2 μL DNA template. The 10^{-2} to 10^{-6} diluents of AOA and AOB standard, *narG*, *nirS* and *nosZ* standard, and 10^{-3} to 10^{-7}

Table 1 Primer sets of AOA, AOB genes and *narG*, *nirK*, *nirS* and *nosZ* genes for qPCR

Gene	Primers	Primer sequence	References
AOA *amoA*	Arch-*amoA*26F	GACTACATMTTCTAYACWGAYTGGGC	45
	Arch-*amoA*417R	GGKGTCATRTATGGWGGYAAYGTTGG	
AOB *amoA*	*amoA*-1F	GGGGTTTCTACTGGTGGT	45
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
narG (f/r)	*narG*-F	TCGCCSATYCCGGCSATGTC	46
	narG-R	GAGTTGTACCAGTCRGCSGAYTCSG	
nirK	*nirK*-583F	TCATGGTGCTGCCGCGKGACGG	47
	nirK-909R	GAACTTGCCGGTPGCCCAGAC	
nirS	*nirS*-cd3af	GTSAACGTSAAGGARACSGG	47
	nirS-R3cd	GASTTCGGRTGSGTCTTGA	
nosZ	*nosZ*-1527F	CGCTGTTCHTCGACAGYCA	47
	nosZ-1773R	ATRTCGATCARCTGBTCGTT	

diluents were selected to prepare the standard curve through pre-experiment. All the standard curves showed excellent correlations between the DNA template concentration and the crossing point with high coefficients of determination ($R^2 > 0.99$).

2.4 The relationship between microorganisms and environmental factors

The relationship between microorganisms and environmental factors were analysed using Canoco 4.5 analysis software, and redundancy analysis (RDA) mainly included environmental and microbial data matrix. The microbial data matrix de-trend correspondence analysis (DCA) was performed, and then the sorting method was determined according to the gradient in the axis. In this study, the calculated length of axial gradient was less than 3, so RDA was chosen to analyze the relationship between microorganisms and environmental factors,[48] and SPSS 22 was used for correlation analysis. The graphics were drawn using ArcGIS (Version 10.2, ESRI).
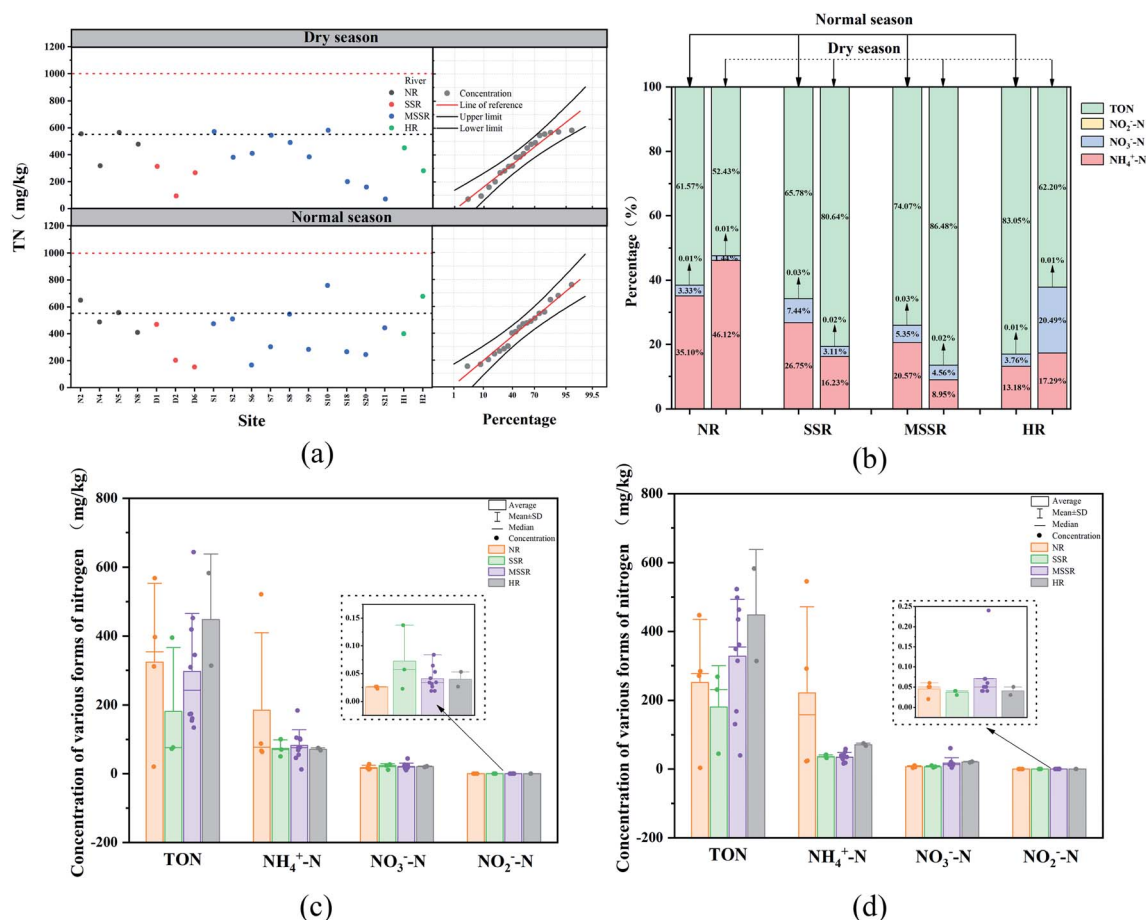

Fig. 2 Nitrogen forms and concentration in surface sediments of the Songhua River during different water seasons (a) TN, (b) the ratios of different nitrogen forms in the 4 basins, (c) 4 forms of nitrogen in normal season, (d) 4 forms of nitrogen in dry season.

3. Results and discussion

3.1 Temporal and spatial distribution of nitrogen in the Songhua River sediments

3.1.1 Temporal and spatial distribution of TN. TN in the sediments of the Songhua River during normal and dry season is shown in Fig. 2(a). In normal season, the TN concentration were 153.3–759.7 mg kg^{-1} with the average value of 421.7 mg kg^{-1} in the sediments. The average TN in NR, SSR, MSSR and HR sediments were 526.3 mg kg^{-1}, 275.2 mg kg^{-1}, 400.3 mg kg^{-1}, and 539.6 mg kg^{-1}, respectively. During the dry season, the sediment TN was 70.5–582.0 mg kg^{-1} with the average value of 374.5 mg kg^{-1}, and the average sediment TN in NR, SSR, MSSR, and HR were 479.2 mg kg^{-1}, 224.1 mg kg^{-1}, 379.4 mg kg^{-1}, and 366.2 mg kg^{-1}, respectively. It can be seen from Fig. 2(a) that the TN concentration sequence in normal season is HR > NR > MSSR > SSR, and that in dry season is NR > MSSR > HR > SSR. Except for HR, TN concentration in NR was the highest, followed by that in MSSR, and TN in SSR was the lowest in both normal and dry periods. According to our investigation, nitrogen in NR mainly came from urban sewage and human and animal excreta input. At the same time, the river had wide surface and low flow rate, and the sediment was more than that of the main stream and other tributaries. The nitrification and denitrification of microorganisms in the water also had a certain influence.[3] In September, TN concentration in the surface sediments of all sampling sites was higher than that in March, which was related to the higher TN load resulted from the relatively heavy non-point sources and soil erosion in July and August, agriculture fertilizes in northeast of China mainly during this period,[49,50] which caused farmland water back, and these nitrogen elements were stored in sediments and released during the normal period. According to the U.S. EPA Sediment Pollution Assessment Standard,[51] the average TN of surface sediments in both water periods in the Songhua River was quite low (TN < 1000 mg kg^{-1}). According to the guidelines issued by the Department of Environment and Energy of Ontario, Canada,[52] the maximum sediment TN with the lowest level of ecological risk effect was 550 mg kg^{-1}, therefore, the average TN of the surface sediments in the Songhua River had no ecological risk. In September, the sediment TN in NR and HR was close to that of the lowest level of ecological risk effect, so more attention should be paid to the nitrogen pollution prevention and

control of NR and the whole Songhua River basin, thus to reduce the nitrogen flux in water and the TN concentration in the HR sediments.

TN variation in different sampling sites may be related to the different sources of nitrogen pollution and human activities along the Songhua River. TN concentration of the 8 sites in NR changed slightly with quite high value, which may be attributed to the numerous farmlands and its non-point source discharge along the river, moreover, industrial wastewater containing nitrogen might be discharged from Qiqihar and other industrial cities in NR basin. Besides the upstream inflow, the nitrogen pollution of MSSR was from Harbin, Kiamusze and other industrial cities, and the plenty of farmland non-point source in the river basin. Because of the water conservancy projects, the velocity of the main stream slows down, which leads to the decrease of dissolved oxygen (DO) and then the weakening of nitrogen purification capacity. Therefore, the sediment nitrogen in MSSR is rather high. Quite a lot petrochemical enterprises and heavy industries were unevenly distributed in SSR basin, so the sediment TN was quite different. Moreover, TN in SSR was lower than that in NR and MSSR, which indicated that non-point source pollution in SSR is not the main influencing factor.

Table 2 shows the TN concentration of surface sediments at different sampling sites in both dry and normal seasons in the Songhua River basin, from which the TN in normal season is higher than that in dry season. Compared with the sediment TN of lakes and rivers at home and abroad as Taihu Lake,[53] Xuanwu Lake,[54] Erhai Lake,[11] Chaohu Lake,[55] Han River[56] and Tigris River,[57] TN in the surface sediments of the Songhua River in both normal and dry season was lower. As a whole, surface sediments of the Songhua River were TN-pollution-free, but some sections need to be concerned.

3.1.2 Temporal and spatial distribution of other nitrogen forms. TN in the sediments can reflect the pollution status of waterbodies in a certain period. Different forms of nitrogen have different ecological effects. Fig. 2(b) shows the concentration and proportion of different forms of nitrogen in each waterbody. It can be concluded form Fig. 2(b) that the main pollutant was TON (52.43–86.48%), and the proportion of NO_2^-–N was very low (0.01–0.03%).

(1) NH_4^+–N. NH_4^+–N in the surface sediments of the Songhua River is shown in Fig. 3(a), which ranged from 12.2 mg kg^{-1} to 521.3 mg kg^{-1} with the average of 101.3 mg kg^{-1} in normal season. NH_4^+–N in the surface sediments of NR, SSR, MSSR, and HR were 184.7 mg kg^{-1}, 73.6 mg kg^{-1}, 82.4 mg kg^{-1}, and 71.1 mg kg^{-1} respectively. During dry season, NH_4^+–N were changed between 15.8 mg kg^{-1} and 545.6 mg kg^{-1} with the average value of 76.8 mg kg^{-1}, and NH_4^+–N in the surface sediments of NR, SSR, MSSR, and HR were 221.0 mg kg^{-1}, 36.4 mg kg^{-1}, 33.9 mg kg^{-1}, and 63.3 mg kg^{-1} respectively. In general, sediment NH_4^+–N in normal season was higher than that in dry season, and the highest value appeared in Nenjiang River, which was resulted from the overlap of cultivation, surface runoff and increase utilization of nitrogenous fertilizer.[58] Also, a higher concentration of NH_4^+–N in the surface sediment suggested that ammonification was stronger in the

Table 2 Sediment TN of different waterbodies in different water periods

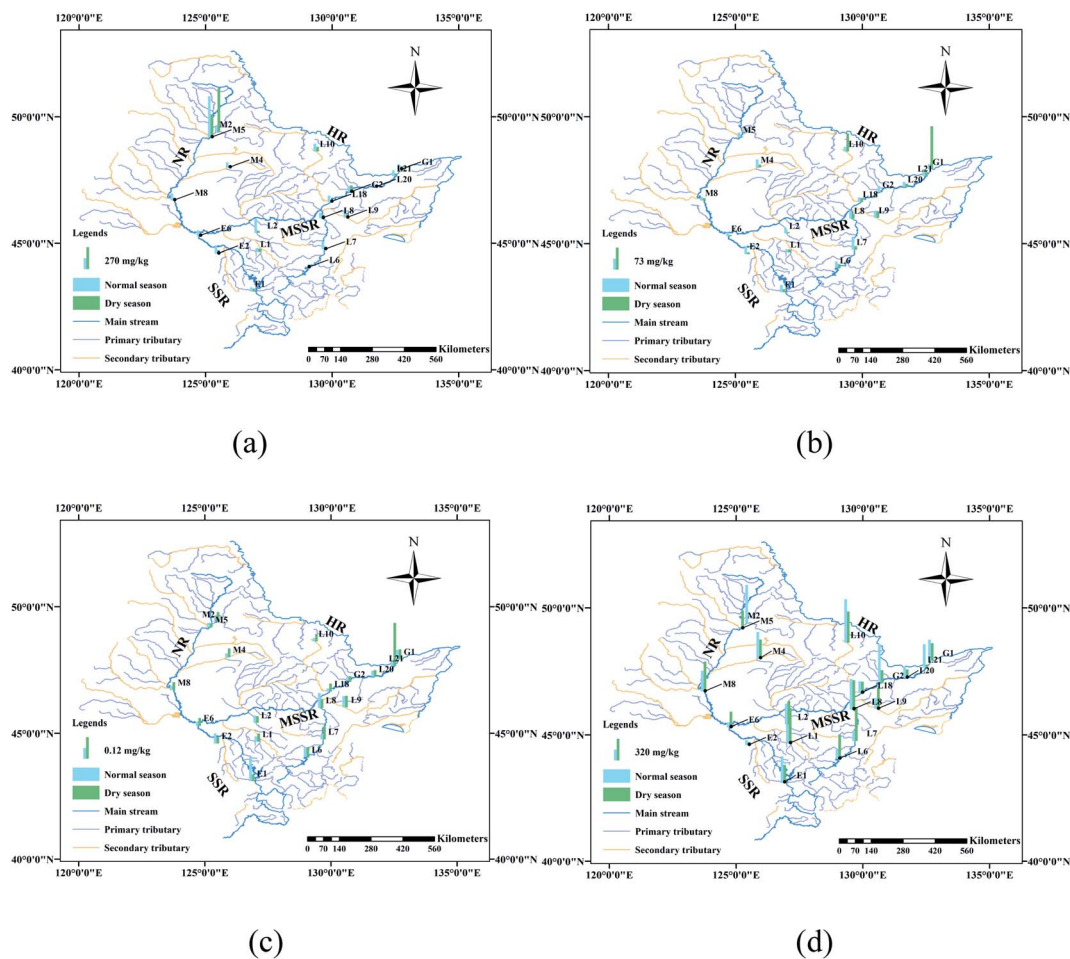| River basin	Water period	TN (mg kg^{-1})
Nenjiang River	Normal season	410.6–649.0
	Dry season	318.2–565.3
Second Songhua River	Normal season	153.3–469.2
	Dry season	93.0–312.8
Main stream of Songhua River	Normal season	168.5–759.7
	Dry season	70.5–582.0
Heilong River	Normal season	400.3–678.8
	Dry season	281.2–451.1

Fig. 3 Different forms of nitrogen in surface sediments of the Songhua River basin (a) NH_4^+-N, (b) NO_3^--N, (c) NO_2^--N, (d)TON.
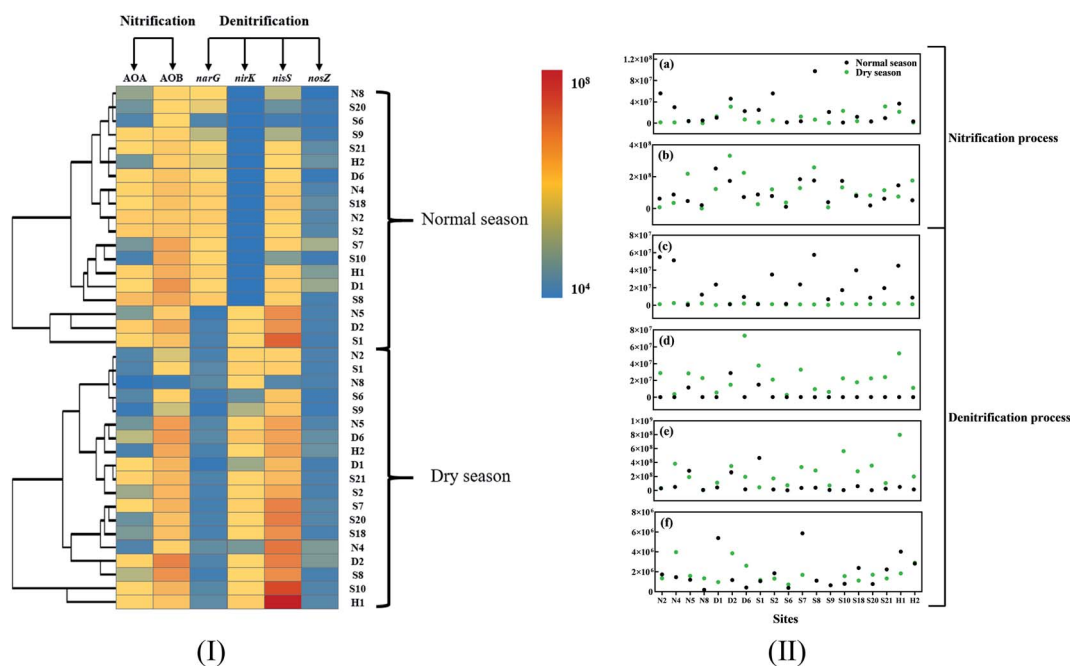

Fig. 4 (l) Relative abundance and concentration of selected functional genes in the sediments (copies per g soil) and (II) (a) AOA (b) AOB genes copies in nitrification process and (c) narG, (d) nirK, (e) nirS, (f) nosZ in denitrification process during dry and normal seasons.

oxic surface sediment, which carried out by most heterotrophic bacteria.[59]

(2) NO_3^-–N. As shown in Fig. 3(b), the sediment NO_3^-–N of the Songhua River were between 10.1 mg kg^{-1} and 44.1 mg kg^{-1} during normal season with an average of 20.3 mg kg^{-1}, and the average NO_3^-–N in the surface sediments of NR, SSR, MSSR, and HR were 17.5 mg kg^{-1}, 20.5 mg kg^{-1}, 21.4 mg kg^{-1}, and 20.3 mg kg^{-1} respectively. In dry season, sediment NO_3^-–N ranged from 3.2 mg kg^{-1} to 146.5 mg kg^{-1} with an average of 19.6 mg kg^{-1}, and NO_3^-–N in the surface sediments of NR, SSR, MSSR, and HR were 6.9 mg kg^{-1}, 17.3 mg kg^{-1}, 7.0 mg kg^{-1}, and 75.0 mg kg^{-1}. The concentration of NO_3^-–N in normal season was higher than that in dry season.

(3) NO_2^-–N. The mean value of sediment NO_2^-–N was 0.04 mg kg^{-1} (ranged from 0.02 to 0.14 mg kg^{-1}) in normal season, and the average NO_2^-–N in the surface sediments of NR, SSR, MSSR, and HR were 0.03 mg kg^{-1}, 0.07 mg kg^{-1}, 0.04 mg kg^{-1}, and 0.04 mg kg^{-1}. In dry season, the sediment NO_2^-–N ranged from 0.02 mg kg^{-1} to 0.24 mg kg^{-1} with a mean value of 0.06 mg kg^{-1}, and NO_2^-–N in the surface sediments of NR, SSR, MSSR, and HR were 0.04 mg kg^{-1}, 0.04 mg kg^{-1}, 0.07 mg kg^{-1}, and 0.04 mg kg^{-1} respectively. Generally, the nitrite concentration in the surface sediments of the Songhua River is low, and its difference between the two water periods was not significant. Surface sediments were characterized by lower concentrations of nitrite and nitrate, which meant that bacterial assimilation and dissimilation (*e.g.*, denitrification) contributes significantly to nitrogen removal,[60] the nitrite or nitrate that were transformed to N_2 by coupled nitrification and denitrification.[61]

(4) TON. Sediment TON was relatively high in normal season with an average of 300.0 mg kg^{-1} (ranged from 20.3 mg kg^{-1} to 644.2 mg kg^{-1}), and the mean value in the surface sediments of NR, SSR, MSSR and HR were 324.0 mg kg^{-1}, 181.0 mg kg^{-1}, 296.5 mg kg^{-1}, and 448.1 mg kg^{-1} respectively. Average TON was 278.1 mg kg^{-1} (ranged from 3.4 mg kg^{-1} to 522.7 mg kg^{-1}) in dry season, which were 251.2 mg kg^{-1}, 180.7 mg kg^{-1}, 328.1 mg kg^{-1}, and 227.7 mg kg^{-1}, respectively. It can be seen from Fig. 4(d) that the sediment TON in HR was relatively high in normal season, but no significant TON difference was observed between normal and dry season for NR, SSR, and MSSR. Moreover, the range of TON concentration was quite wide in the whole Songhua River basin. TON was the main form of nitrogen in the sediment of Songhua River, which was consistent with Lu.[12]

3.2 Distribution characteristics of microbes related to nitrogen transformation in sediments

The relative abundance of the functional genes involved in main nitrogen cycles at all sampling sites are shown in Fig. 4. The abundance and distribution of nitrogen transformation related functional genes varied remarkably between normal and dry seasons, based on which the 19 sampling sites were classified into 3 distinct clusters by hierarchical cluster analysis and then illustrated as a dendrogram (Fig. 4), and then the same dominant microbe in different sites can distinguished.

3.2.1 Distribution characteristics of nitrification microorganisms. Nitrification process mainly includes ammonia oxidation and nitrite oxidation steps, which initiates and drives the effective operation of the whole nitrogen cycle, and the main related functional microorganisms are AOA and AOB.[25]

Fig. 4(a) shows the relevant microbial biomass during the nitrification process in both normal and dry seasons of the Songhua River, from which it can be seen that the sediment number of AOA genes (1.2×10^6 to 9.7×10^7 copies per g soil) in normal season was significantly higher than that (6.6×10^4 to 3.2×10^7 copies per g soil) in dry season. There was not much difference between AOB genes in dry season (ranged from 7.2×10^5 to 3.3×10^8 copies per g of soil) and those in normal season, *i.e.*, from 1.1×10^7 to 2.5×10^8 copies per g of soil. Through the comparison of the quantities of sediment AOA and AOB genes, it can be concluded that AOB were the main ammonia-oxidation bacteria during the nitrification process in the sediments of the Songhua River. Considering the frozen water surface and low DO in dry season and the strong affinity of AOB with oxygen,[62] the AOB genes had relatively high abundance in this research.

NH_4^+–N is a major factor affecting ammonia oxidation process, and AOA grow better than AOB in low NH_4^+–N environment.[11] According to the analysis in Section 3.1, among all the nitrogen forms, TON had the highest concentration, followed by NH_4^+–N in the surface sediments of the Songhua River basin. Therefore, the relatively high sediment NH_4^+–N was conducive to the growth of AOB.

3.2.2 Distribution characteristics of denitrification microorganisms. Denitrification bacteria are the main denitrification microorganisms in the environment, which are very important for the community characteristics and ecological balance. *NarG*, *nirK*, and *nirS* gene related microorganisms play major roles in the nitrate and nitrite reduction, and *nosZ* gene related ones are dominant in the N_2O reduction process.[63]

The number of microorganisms related to denitrification in normal and dry sesaons of the Songhua River basin were illustrated in Fig. 4. The number of *narG* genes in the sediments during normal season (4.3×10^5 to 5.8×10^7 copies per g soil) was greater than that in dry season (3.2×10^5 to 2.5×10^6 copies per g soil). The amount of *nirK* and *nirS* genes (4.3×10^4 to 2.9×10^7 copies per g soil and 9.7×10^5 to 4.6×10^8 copies per g soil) in normal season was much less than that during dry season (2.7×10^6 to 7.3×10^7 copies per g soil and 1.8×10^6 to 8.0×10^8 copies per g soil). The number of *nosZ* genes in dry season (1.8×10^5 to 5.9×10^6 copies per g soil) had slight difference with that in normal season (6.4×10^5 to 4.0×10^6 copies per g soil).

The microorganisms related with *nirS* genes were dominant in both normal and dry water periods, and the microorganisms related with *narG* genes were 1 to 2 orders of magnitude lower than those related with other genes, which was corresponding to the low sediment nitrite in the Songhua River according to the analysis in 3.1.2 section. The number of *narG* genes in normal season was significantly higher than that in dry season, which might be due to the higher DO caused by water flow and natural aeration during normal season, previous study also

(a)

(b)

	AOA	AOB	narG	nirK	nirS	nosZ
TN	0.05	0.21	0.30	-0.20	0.11	0.29
NO_2^--N	0.30	0.14	-0.07	-0.13	0.01	0.09
NO_3^--N	0.03	0.33	0.19	-0.15	0.07	0.41
NH_4^+-N	-0.04	0.03	-0.13	0.22	0.03	0.11
TON	0.11	0.31	0.50*	-0.37	-0.04	0.24
TP	0.13	0.28	0.11	0.07	0.17	0.43
pH	-0.21	-0.01	-0.50*	0.13	-0.07	-0.12
MC	0.09	0.23	-0.19	0.30	0.23	-0.05
DO	0.18	-0.23	-0.34	0.53*	0.04	-0.16

(c)

(d)

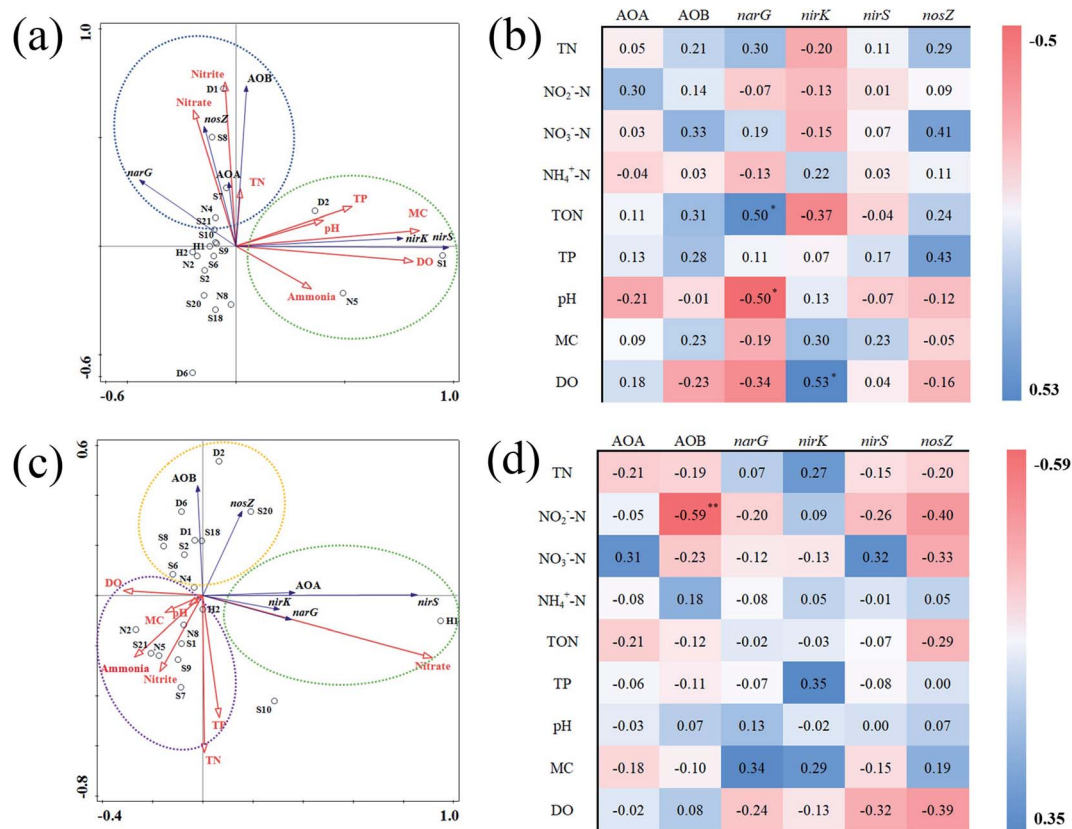|	AOA	AOB	narG	nirK	nirS	nosZ
TN	-0.21	-0.19	0.07	0.27	-0.15	-0.20
NO_2^--N	-0.05	-0.59**	-0.20	0.09	-0.26	-0.40
NO_3^--N	0.31	-0.23	-0.12	-0.13	0.32	-0.33
NH_4^+-N	-0.08	0.18	-0.08	0.05	-0.01	0.05
TON	-0.21	-0.12	-0.02	-0.03	-0.07	-0.29
TP	-0.06	-0.11	-0.07	0.35	-0.08	0.00
pH	-0.03	0.07	0.13	-0.02	0.00	0.07
MC	-0.18	-0.10	0.34	0.29	-0.15	0.19
DO	-0.02	0.08	-0.24	-0.13	-0.32	-0.39

Fig. 5 The RDA analysis ordination chart of the microbial environmental factors and Spearman correlation analysis during normal season ((a) RDA analysis, (b) Spearman analysis) and dry season ((c) RDA analysis, (d) Spearman analysis). ** is shown as significant correlation at the level of 0.01 (bilateral). * is shown as significant correlation at the level of 0.05 (bilateral).

found that oxygen is often considered as a significant factor controlling the nitrogen loss processes in sediment.[61] Since nitrification is oxygen consumption process, it provides sufficient NO_3^-–N for the denitrification process in case of sufficient DO. In dry season, the sediment surface was covered by 1 meter-thick ice, which leads to the decrease of DO, and then results in the conversion of NO_3^-–N to N_2O or N_2 by denitrifying bacteria.[64] The number of *nirS* genes in N5, D2 and S1 was one order of magnitude higher than that in other sampling sites during normal season.

3.3 Relationship between nitrogen transformation microorganisms and physical, chemical factors

3.3.1 Relationship during normal season. RDA analysis is mainly used for the investigation of the relationship of environmental factors and microorganisms, thus to obtain the impact of specific environmental factors on certain species.[65] TP in the surface sediments ranged from 389.5 mg kg^{-1} to 1598.2 mg kg^{-1}, pH varied from 5.69 to 6.75, MC changed from 10% to 90%, and DO in water varied from 4.6 mg L^{-1} to 10.6 mg L^{-1} during normal season in the whole Songhua River basin. The RDA analysis showed that AOA and AOB were positively correlated with NO_2^-–N and NO_3^-–N, and negatively correlated with NH_4^+–N in the surface sediments of the Songhua River basin during normal water season (Fig. 5(a)). *NirK*

gene abundance was related to water DO concentration. As shown in Fig. 5(b), the Spearman correlation analysis confirmed the RDA analysis results mentioned above, indicated the significant negative correlation between *narG* gene abundance and pH ($p < 0.05$) and the significant positive correlation between *nirK* gene abundance and DO ($p < 0.05$).

It can be seen from Fig. 5(a) that sediment MC and water DO were positively correlated with nitrite reductase coding genes *nirS* and *nirK*, and DO and *nirK* gene abundance were remarkably correlated ($p < 0.05$) in normal season. Sediment MC and water DO are the key influencing factors on the redox potential, and high redox potential facilitates nitrification while low redox reduction potential promotes denitrification. The alternation of nitrification and denitrification achieves the purification of nitrogenous sediment pollution. Denitrification rate increases sharply when the soil moisture exceeds 70–75% of the soil water-filled pores, and slight variation in water concentration will change the denitrification rate,[66] which is consistent with the results of this study. As for DO, the water presented aerobic state at high DO levels (DO > 0.5 mg L^{-1}), and nitrifying bacteria could carry out nitrification and convert most ammonia nitrogen in the water to nitrate nitrogen, which increased the concentration of nitrate nitrogen in the water, and the abundance of denitrifying genes was relatively high. The pH and *narG* gene abundance showed obvious negative correlation ($p < 0.05$), which might be due to the decrease of sediment nitrogen

mineralization caused by pH reduction.[67,68] pH affects the microbial activities, and the optimal pH for denitrification is 7.0–8.0.[69] The pH of the sediments in the Songhua River was between 6 and 8 in normal season, which was consistent with the reported researches. The ammonification and nitrification of nitrogen are generally called nitrogen mineralization, which requires the participation of a variety of microorganisms. The produced ammonia will be consumed by microorganisms and plants, fixed by clay minerals, or converted to nitrate by autotrophic or heterotrophic nitrification.[70,71]

3.3.2   Relationship in dry season. In dry season, the surface sediment TP ranged from 472.1 mg kg^{-1} to 1497.6 mg kg^{-1}, pH varied from 6.5 to 8.1, MC ranged from 20% to 60%, and DO changed between 2.8 mg L^{-1} and 9.6 mg L^{-1}. It can be seen from RDA analysis (Fig. 5(c)) that the AOA $amoA$ gene abundance was positively correlated with all environmental factors except NO$_3^-$–N. AOB $amoA$ gene abundance was positively correlated with NH$_4^+$–N, pH and DO, but negatively correlated with NO$_2^-$–N to great extent ($p < 0.05$). $NarG$ and $nirK$ gene abundance was positively correlated with MC and TN.

No significant correlation could be observed between the nitrogen conversion related microorganisms and water content, TP, pH and DO (Fig. 5(c)). Previous studies reported that DO was positively correlated with AOB $amoA$ gene abundance and negatively correlated with AOA $amoA$ gene abundance, which might be resulted from the far less water DO in dry season than that in normal season.[72] AOA and AOB had strong affinity and high abundance in suitable low oxygen environment, while AOA could tolerate even lower DO than AOB. The related $narG$, $nirS$, $nirK$, and $nosZ$ genes were also affected by the low DO concentration in dry season. As shown in Fig. 5(c), pH was negatively correlated with AOA and positively correlated with AOB in the nitrification process. The sediment pH of the Songhua River in dry season ranged from 6.4 to 7.7, which was relatively moderate in the environment, and the nitrification rate increased with the increase of pH within this range.[73]

4.   Conclusion

Comprehensive study about the distribution of different nitrogen forms and the characteristics of relative microbial community was carried out about the surface sediments of the Songhua River in dry and normal seasons. TN (70.8–678.8 mg kg^{-1}) in several sampling sites (678.8–759.7 mg kg^{-1} in normal season and 554.9–678.8582 mg kg^{-1} in dry season) exceeded the alert values 550 mg kg^{-1}, TN, NH$_4^+$–N, NO$_3^-$–N, and TON in normal season were higher than those in dry season. TON was identified as the main form of nitrogen in the sediments of the Songhua River basin during both dry and normal seasons, and the highest concentration of which was 328.1 mg kg^{-1} in MSSR in dry period, and the second largest value was 324.0 mg kg^{-1} in NR during normal season. As for bacteria communities and functional genes structures in the sediments, AOB was dominant for the nitrification process in both dry and normal seasons. AOB genes abundance (7.2 × 10^5 to 3.3 × 10^8 copies per g soil) in dry season was higher than that (1.1 × 10^7 to 2.5 × 10^8 copies per g soilin normal season), while AOA $amoA$ genes

abundance had the reverse distribution (1.2 × 10^6 to 9.7 × 10^7 copies per g soil in normal season and 6.6 × 10^4 to 3.2 × 10^7 copies per g soil in dry season). The denitrification $nirS$ genes were predominant in both dry and normal seasons, the abundance (1.8 × 10^6 to 8.0 × 10^8 copies per g soil) of which in dry season was higher than that (9.7 × 10^5 to 4.6 × 10^8 copies per g soil) in normal season. Physical and chemical factors such as pH, DO, MC, TP and different forms of nitrogen in the sediments had great influence on the profiles of functional genes, bacterial communities, and co-occurrence patterns between the functional genes and bacterial taxa. This study is beneficial to explain the influence of nitrogen forms on the nitrogen transformation microbial community of the sediment in Songhua River, which could afford the technical support for the water ecological restoration of the Songhua River Basin.

Author contributions

Congyu Li: conceptualization, methodology, investigation, validation, writing – original draft. Zhen Zhong: conceptualization, methodology, validation, supervision, resources. Wenfu Wang: supervision. Haiyan Wang: investigation, validation, writing – review & editing. Weiyang Dong: resources. Guokai Yan: validation, conceptualization, resources. Zhaosheng Chu: supervision, resources. Huan Wang: supervision validation. Yang Chang: supervision, validation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

References

1 E. B. McAleer, C. E. Coxon, K. G. Richards, M. M. R. Jahangir, J. Grant and P. E. Mellander, *Sci. Total Environ.*, 2017, **586**, 372–389.

2 W. W. Wong, C. Greening, G. Shelley, R. Lappan, P. M. Leung, A. Kessler, B. Winfrey, S. C. Poh and P. Cook, *Sci. Total Environ.*, 2021, **790**, 147749.

3 D. Deng, Y. Pan, G. Liu, W. Liu and L. Ma, *Sci. Total Environ.*, 2020, **737**, 140253.

4 J. N. Galloway, A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger and M. A. Sutton, *Science*, 2008, **320**, 889.

5 Y. C. Ren, S. L. Dong, F. Wang, Q. F. Gao, X. L. Tian and F. Liu, *Aquacult. Res.*, 2010, **42**, 14–21.

6 S. H. Cui, Y. L. Shi, P. M. Groffman, W. H. Schlesinger and Y. G. Zhu, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 2052.

7 D. M. Orihel, H. M. Baulch, N. J. Casson, R. L. North, C. T. Parsons, D. C. M. Seckar and J. J. Venkiteswaran, *Can. J. Fish. Aquat. Sci.*, 2017, 1–25.

8 V. Turan, *Ecotoxicol. Environ. Saf.*, 2019, **183**, 109594.

9 V. Turan, *Chemosphere*, 2020, **245**, 125611.

10 V. Turan, *Physiol. Plant.*, 2021, 1–12.

11 Z. Ni and S. Wang, *Ecol. Eng.*, 2015, **79**, 42–53.

12 R. K. Lu, *China Agric. Sci. Technol.*, 2000, 106–310.

13 A. L. Herczeg, A. K. Smith and J. C. Dighton, *Appl. Geochem.*, 2001, **16**, 73–84.

14 W. S. Gardner, L. Yang, J. B. Cotner, T. H. Johengen and P. J. Lavrentyev, *J. Great Lakes Res.*, 2001, **27**, 84–97.

15 D. Cavalli, G. Consolati, P. Marino and L. Bechini, *Geoderma*, 2015, **259–260**, 116–125.

16 S. L. Huo, J. T. Zhang, B. D. Xi, F. Y. Zan, J. Su and H. Yu, *Environ. Earth Sci.*, 2014, **71**, 2167–2175.

17 S. M. Liu, B. D. Zhu, J. Zhang, Y. Wu, G. S. Liu, B. Deng, M. X. Zhao, G. Q. Liu, J. Z. Du, J. L. Ren and G. L. Zhang, *Mar. Pollut. Bull.*, 2010, **60**, 1591–1599.

18 Q. J. Guo, C. Y. Wang, R. F. Wei, G. X. Zhu, M. Cui and C. P. Okolic, *Ecotoxicol. Environ. Saf.*, 2020, **195**, 110436.

19 R. J. Bernard, B. Mortazavi and A. A. Kleinhuizen, *Biogeochemistry*, 2015, **125**, 47–64.

20 A. K. Hardison, C. K. Algar, A. E. Giblin and J. J. Rich, *Geochim. Cosmochim. Acta*, 2015, **164**, 146–160.

21 G. Kowalchuk and J. Stephen, *Annu. Rev. Microbiol.*, 2001, **55**, 485–529.

22 C. Okkyoung and H. Zhiqiang, *Environ. Sci. Technol.*, 2008, **42**(12), 4583–4588.

23 C. A. Francis, K. J. Roberts, J. M. Beman, A. E. Santoro and B. B. Oakley, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**(41), 14683.

24 J. C. Venter, K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers and H. O. Smith, *Science*, 2004, **304**(5667), 66.

25 M. Könneke, A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury and D. A. Stahl, *Nature*, 2005, **437**(7058), 543–546.

26 Y. Wang, G. B. Zhu, L. Ye, X. J. Feng, H. J. M. Op den Camp and C. Q. Yin, *J. Environ. Sci.*, 2012, **24**, 790–799.

27 X. Y. Wang, C. Wang, L. L. Bao and S. G. Xie, *Ann. Microbiol.*, 2015, **65**, 1411–1418.

28 X. Y. Wang, C. Wang, L. L. Bao and S. G. Xie, *Appl. Microbiol. Biotechnol.*, 2014, **98**, 1883–1892.

29 C. L. Zhang, Q. Ye, Z. Huang, W. Li, J. Chen, Z. Song, W. Zhao, C. Bagwell, W. P. Inskeep, C. Ross, L. Gao, J. Wiegel, C. S. Romanek, E. L. Shock and B. P. Hedlund, *Appl. Environ. Microbiol.*, 2008, **74**, 6417.

30 S.-J. Park, B.-J. Park and S.-K. Rhee, *Extremophiles*, 2008, **12**, 605.

31 T. H. Erguder, N. Boon, L. Wittebolle, M. Marzorati and W. Verstraete, *FEMS Microbiol. Rev.*, 2009, **33**, 855–869.

32 M. Herrmann, A. Scheibe, S. Avrahami and K. Küsel, *Appl. Environ. Microbiol.*, 2011, **77**, 1896–1899.

33 R. D. Chen and T. M. LaPara, *Process Biochem.*, 2008, **43**, 33–41.

34 J. Ji, Y. Peng, X. Li, Q. Zhang and X. Liu, *Water Res.*, 2020, **175**, 115690.

35 Y. Nakamura, H. Satoh, T. Kindaichi and S. Okabe, *Environ. Sci. Technol.*, 2006, **40**, 1532–1539.

36 E. W. Boyer, R. W. Howarth, J. N. Galloway, F. J. Dentener, P. A. Green and C. J. Vörösmarty, *Global Biogeochem. Cycles*, 2006, **20**, GB1S91.

37 L. Philippot and S. Hallin, *Curr. Opin. Microbiol.*, 2005, **8**, 234–239.

38 A. Priemé, G. Braker and J. Tiedje, *Appl. Environ. Microbiol.*, 2002, **68**, 1893–1900.

39 A. Mosier and C. Francis, *Environ. Microbiol. Rep.*, 2010, **2**(5), 667–676.

40 I. J. Washbourne, C. Crenshaw and M. Baker, *Aquat. Microb. Ecol.*, 2011, **65**, 55–64.

41 Y. Yang, H. Wang, Y. Chang, G. Yan, Z. Chu, Z. Zhao, L. Li, Z. Li and T. Wu, *Mar. Pollut. Bull.*, 2020, **152**, 110923.

42 T. Li, Z. Zhou, Q. Fu, D. Liu, M. Li, R. Hou, W. Pei and L. Li, *J. Water Clim. Change*, 2019, **11**, 839–864.

43 V. Ruban, J. F. López-Sánchez, P. Pardo, G. Rauret, H. Muntau and P. Quevauviller, *J. Environ. Monit.*, 2001, **3**, 121–125.

44 X. Jiang, *Handbook of Sediment Quality Survey and Assessment*, Science Press, 2012, (in Chinese).

45 G. D. Ji, W. Zhi and Y. F. Tan, *Ecol. Eng.*, 2012, **44**, 269–277.

46 A. Vilar-Sanz, S. Puig, A. García-Lledó, R. Trias, M. Balaguer, J. Colprim and L. Bañeras, *PLoS One*, 2013, **8**, e63460.

47 I. Throbäck, K. Enwall, A. Jarvis and S. Hallin, *FEMS Microbiol. Ecol.*, 2004, **49**, 401–417.

48 E. V. Etten, *Austral Ecol.*, 2005, **30**, 486–487.

49 W. Jiang, Y. Xing, X. Wang, X. Liu and Z. Cui, *Sustainability*, 2020, **12**, 1–12.

50 L. Yuan, X. Chen, J. Jia, H. Chen, Y. Shi, J. Ma, C. Liang, Y. Liu, H. Xie, H. He, X. Zhang, X. Peng and C. Lu, *Agric., Ecosyst. Environ.*, 2021, **312**, 107360.

51 S. Cieniawski, D. Macdonald and C. Ingersoll, An Ecosystem-Based Framework for Assessing and Managing Contaminated Sediments, *A Guidance Manual to Support the Assessment of Contaminated Sediments in Freshwater Ecosystems*, 2020, **vol. 1**.

52 H. V. Leland, W. E. Dean, A. Mudroch and J. M. Azcue, *J. North Am. Benthol. Soc.*, 1995, **16**, 296.

53 S. Y. Lu, Y. Yuan, X. C. Jin, W. Jiao, Y. J. Wu, D. Y. Ren, Y. H. Zhou and L. Chen, *Environ. Sci.*, 2012, **33**, 1497–1502(in Chinese).

54 J. Zeng, L. Y. Yang, H. W. Du, L. Xiao, L. J. Jiang, J. Wu and X. L. Wang, *World J. Microbiol. Biotechnol.*, 2009, **25**, 763–772.

55 Y. Y. Fan, B. B. Li, Z. C. Yang, Y. Y. Cheng, D. F. Liu and H. Q. Yu, *Environ. Pollut.*, 2019, **250**, 233–240.

56 D. H. Li, M. Kim, W. J. Shim, U. H. Yim, J.-R. Oh and Y.-J. Kwon, *Chemosphere*, 2004, **56**, 1–6.

57 M. Varol and B. Şen, *Catena*, 2012, **92**, 1–10.

58 E. D. Ongley, Z. Xiaolan and Y. Tao, *Environ. Pollut.*, 2010, **158**, 1159–1168.

59 C. L. Ettinger, S. L. Williams, J. M. Abbott, J. J. Stachowicz and J. A. Eisen, *PeerJ*, 2017, **5**, e3674.

60 Y. Li, C.-X. Li, W. Lin, S.-S. Wang, W.-X. Zhang, Y.-M. Jiang, Y. Zhang, H. Zhang and Y.-H. Han, *Environ. Technol. Innovation*, 2021, **23**, 101731.

61 J. Wu, Y. Hong, X. Liu and Y. Hu, *Environ. Pollut.*, 2021, **286**, 117267.

62 Y. W. Yang, X. L. Gao and P. M. Li, *Environ. Sci.*, 2012, **33**(10), 3449–3456.

63 A. L. W. Kemp and A. Mudrochova, *Limnol. Oceanogr.*, 1972, **17**, 855–867.

64 X. Q. Li, C. Q. Lu, Y. F. Zhang, H. D. Zhao, J. Y. Wang, H. B. Liu and K. D. Yin, *Mar. Pollut. Bull.*, 2020, **151**, 110814.

65 H. W. Chen, H. C. Lin, Y. H. Chuang, C. T. Sun, W. Y. Chen and C. Y. Kao, *Ocean Coast. Manage.*, 2019, **169**, 37–49.

66 F. P. Vinther, *Biol. Fertil. Soils*, 1992, **14**, 43–48.

67 A. M. Blackmer and J. M. Bremner, *Soil Biol. Biochem.*, 1978, **10**, 187–191.

68 D. J. Mladenoff, *Ecology*, 1987, **68**, 1171–1180.

69 M. Šimek, L. Jišova and D. W. Hopkins, *Soil Biol. Biochem.*, 2002, **34**, 1227–1234.

70 M. Akcura, V. Turan, K. Kokten and M. Kaplan, *Ind. Crops Prod.*, 2019, **128**, 140–146.

71 V. Turan, P. Schröder, S. Bilen, H. Insam and M. Fernández-Delgado Juárez, *Sci. Rep.*, 2019, **9**, 15178.

72 S. Tomoko, *Microbes Environ.*, 2012, **1**, 61–66.

73 Y. Y. Li, S. J. Chapman, G. W. Nicol and H. Y. Yao, *Soil Biol. Biochem.*, 2018, **116**, 290–301.