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Relationship between nitrogen transformation and its related genes: comparison among riparian, marsh, and full-scale constructed wetlands

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ABSTRACT

Wetlands are known as effective ways for nitrogen pollutants removal. Three types of wetlands (riparian, marsh, and full-scale constructed wetlands (CWs)) were investigated in this study. Research endeavor was focused on: (1) the abundances and distribution of functional microbes in different kinds of wetlands, and (2) the relationship between nitrogen transformation and its related genes. Results from incubation experiments showed that the topsoil (0–20 cm) of riparian wetlands was more efficient for reducing ammonium, with a rate of 1.50 μg g^{-1} h^{-1}, than the subsurface (20–40 cm). It was also found that full-scale CWs performed most effectively for the removal of nitrite (1.14/1.13 μg g^{-1} h^{-1}) and nitrate (3.77/3.44 μg g^{-1} h^{-1}). According to quantitative real-time PCR and principal component analysis, the highest NH_4^+-N transformation rate in the topsoil (0–20 cm) of riparian wetlands can be mainly attributed to the *amoA* and *Nitrospira* 16S rRNA genes. The similar transformation rates between two depths in CWs can be well explained by the similar abundances of all seven tested genes. The nitrogen transformation rates were similar between two depths of marsh wetlands, regarding the significant differences of tested genes. This is probably due to that the abundances of functional microbes in both depths were similar for the nutrient limits. Furthermore, the absolute abundances of the related genes were found to be influenced by the content of nitrogen and carbon in soil.

Keywords: Nitrogen transformation rates; Nitrogen transformation genes; Quantitative real time-PCR; Constructed wetlands; Natural wetlands

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1. Introduction

Wetland is one of the most crucial ecosystems for improving global biogeochemical cycles of carbon and nitrogen and preserving surface water quality [1]. In particular, natural wetlands, such as river marginal wetlands [2] and marsh [3], play the most important roles. Designed from the principle of natural wetlands, constructed wetlands (CWs) attracted increasingly attention in wastewater treatment, due to their easy construction, low cost, and high purification capacity [4]. It has been reported that the nitrification rates in tidal freshwater marsh wetlands varied from 0.40 to 0.67 μmol L^{-1} h^{-1} [5], the nitrate depletion rates in riparian wetlands varied from 1.2 to 4.7 g ha^{-1} d^{-1} [6], and the nitrate removal rates in CWs varied from 2.4 to 3.1 g N m^{-2} d^{-1} [7]. Apparently, nitrogen transformation rates significantly varied in different types of wetlands, which is worth further investigations.

In either natural or CWs, the microbes are essential in nitrogen cycling processes, such as ammonia-oxidizing bacteria (AOB) [8], nitrite oxidizing bacteria (NOB) [9], denitrifying bacteria [10], and anammox [11]. Furthermore, the microbial population structure and abundance can affect the transformation of nitrogen in wetlands [12,13]. More recently, it was demonstrated that the functional genes can affect nitrogen removal. Zhi and Ji [14] investigated the relationships between nitrogen transformation rates and nitrogen functional genes in a tidal flow CW under C/N ratio constraint, and proved the important role of specific genes. Similar conclusion was later reported that ammonium removal rate was collectively controlled by *amoA*, *nxrA*, and *amx* gene, while nitrate removal rate was governed by *nxrA* and *narG* gene [15]. Thus, further studies should be carried out to find out how the above genes influenced nitrogen transformation, especially in the natural riparian, marsh wetlands, and CWs.

Besides, various parameters were found to affect the nitrogen removal performance in wetlands. Okano et al. [16] applied real-time PCR and found that AOB population size was significantly greater in annually fertilized soil compared with unfertilized one, which was attributed to the different concentration of nutrients, including ammonium. Dalsgaard and Thamdrup [17] found that the distributions of anaerobic ammonium oxidation bacteria in marine sediments were dependent on the temperature, and concentrations of nitrite and organic matter. Thus, physical and chemical factors of substrates appeared to be the key factors that can influence the microbial quantity, which should be taken into account in the investigation.

To investigate the relationship between nitrogen transformation and its related genes in three different wetlands, the incubation experiments were conducted to evaluate the nitrogen transformation rates, while quantitative real-time PCR was applied to quantify the related genes. Then, their relationships were obtained based on the principal component analysis (PCA) results using canoco 4.5. Furthermore, the influences of soil parameters on the microbial quantity were also investigated. Finally, the strategies were discussed regarding improving the inorganic nitrogen removal efficiency, especially in CWs.

2. Materials and methods

2.1. Sample sites and collection

Three sampling sites, located in the north temperate region in the Shandong Province, eastern China, were selected for the present study. They were Yellow River delta area (YR), Xiao Mei River marginal wetland (XM), and Dong Wen River CWs (DW) (Fig. 1), and representing three types of wetlands, i.e. marsh wetland, riparian wetland, and CWs, respectively. YR, more than 160 years, is one of the most important marsh wetlands in China. XM, more than 560 years, is on both sides of Xiao Mei River, which is covered with various types of grass. DW have been operated for around 3 years for further purification of the effluent from wastewater treatment plant (WWTPs) that met the 1A discharge standard of GB18918-2002 (COD < 50 mg L^{-1}, BOD < 10 mg L^{-1}, NH_4^+-N < 5 mg L^{-1}). The three sampling locations were chosen based on the different land use, pollution sources, and aquatic conditions. Substrate samples were collected at three locations on 1 December 2014.

Three substrate cores of each location were collected at each site within a 1 m diameter area. Each substrate core was 20 cm in diameter and 40 cm in depth. Each sediment core was sectioned into two slices (0–20 and 20–40 cm) [18] and then put into sterile containers separately with three replicates per depth, and transported in ice boxes to the lab. The sample was then sieved (1 mm mesh) and stored at 4°C for further analysis. Chemical analysis of the sediment was conducted within 24 h during the entire experiments.

2.2. Analysis of environmental parameters

The pH and dissolved oxygen (DO) values in wetlands overlying water were determined using a pH and DO analyzer (HQ30d 53LEDTM, HACH, USA) on the spot. Water content of the substrate samples were

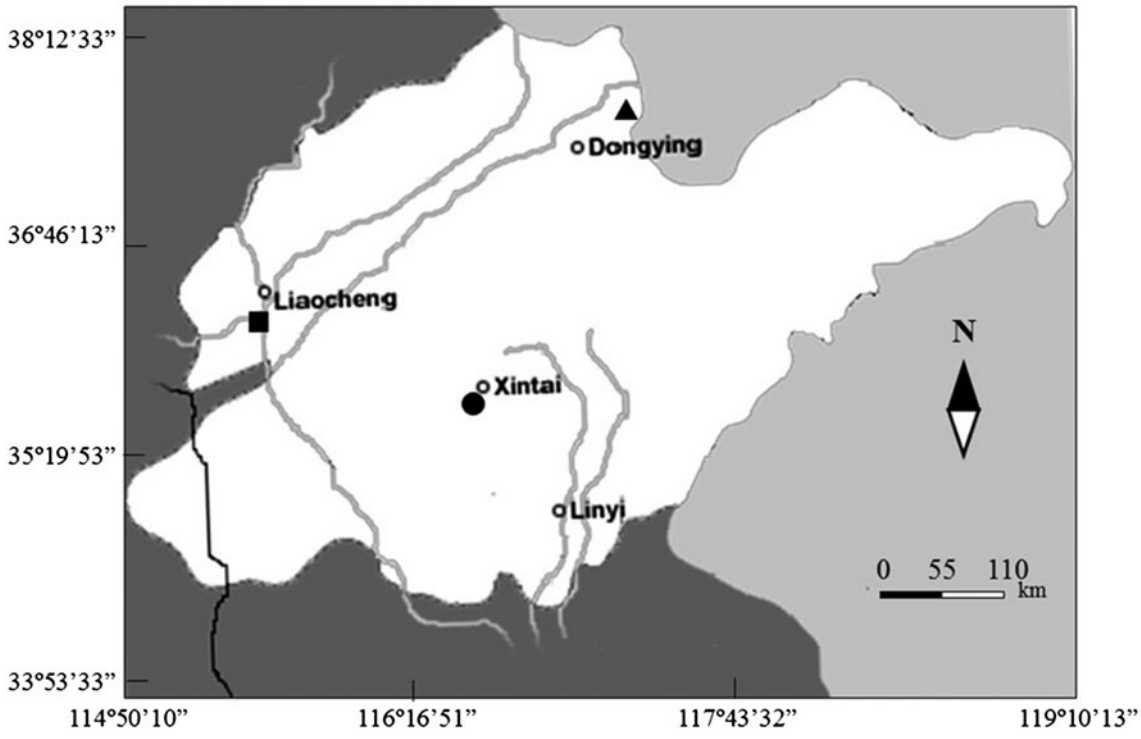

Fig. 1. Sampling sites. ■: Xiao Mei riparian wetlands (XM), ●: Dong Wen CWs (DW), ▲: yellow river delta area (YR).

analyzed based on the Standard Methods [19]. The total organic carbon (TOC) of substrates was obtained after pretreatment by acidification for inorganic carbon removal, using the method as Byers et al. described [20]. Substrate samples were extracted with water (1:1) and the concentrations of NO_3^--N, NO_2^--N, and NH_4^+-N were determined using the methods described by Ryan et al. [21]. Total inorganic nitrogen (TIN) was calculated by adding the concentration of NH_4^+-N, NO_2^--N, and NO_3^--N together.

2.3. Incubation experiments

Nitrogen transformation rates were examined by incubation experiments based on Højberg reported [22]. NH_4^+-N transformation rates were measured as follows: substrate samples (10 g) were mixed with 100 mL medium containing 0.5 mM $(NH_4)_2SO_4$, in 250 mL beakerflask. The flasks were closed with sterilized tampon (aerobic) and held in horizontal position on a vapor-bathing constant temperature vibrator at 25°C and 130 rev min^{-1}. 15 mL solution was quickly removed from each flask with a syringe and transferred to 50 mL eppendorf vials at 0, 4, 16, and 40 h, respectively. After centrifuged, the supernatant was stored in 4°C for later analysis of the NH_4^+-N, NO_2^--N, and NO_3^--N content.

NO_2^--N and NO_3^--N transformation rates were measured under anaerobic condition according to Højberg reported [22]. Substrates samples (10 g) were mixed with 100 ml medium containing 1 mM KNO_3, 100 μM $NaNO_2$ (imitating denitrification process) and 0.1% glucose in 250-ml beakerflask. Argon gas was added into the flasks. Samples were incubated and sampled as described for the ammonia transformation assay.

Pollutant (NH_4^+-N, NO_2^--N, and NO_3^--N and TIN) transformation/removal efficiency was calculated based on percentages of the declined content divided by the original content. Pollutant transformation rate (abbreviated as *R*), of which the unit was μg N g^{-1} dry soil h^{-1} (μg g^{-1} h^{-1} for short), was defined as the difference in the pollutant content between the influent (*I*) and the effluent (*E*) divided by the weight of tested soil (*M*) and incubation time (*T*) [23]. The calculation equation was as follows:

$$R\frac{|I-E|}{M\times T} \quad (1)$$

2.4. DNA isolation and quantitative real-time PCR

Three replicates of DNA for each substrate slice were extracted from 500 mg substrate samples using

the power soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA) as described in the manufacturer's instructions. The concentration of the extracted DNA was measured using a Nano-drop spectrophotometer (Nano-Drop Technologies, USA) at 260 nm.

Quantitative real-time PCR (qPCR) was conducted using the isolated DNA samples. Table S1 in the Supplementary Materials lists the information on the primers selected for amplification of the 16S rRNA fragment of *Nitrospira* (*nobL*) and anammox (*amx*), and the target fragments of the following functional genes: ammonia monooxygenase (*amoA*), cd1-containing nitrite reductase (*nirS*), copper-containing nitrite reductase (*nirK*), Nitrous oxide reductase (*nosZ*), and nitrate reductase (*narG*), which are involved in nitrification (*amoA*, *nobL*), denitrification (*nirS*, *nirK*, *nosZ*, *narG*), and anaerobic ammonium oxidation (*amx*). Triplicates for each DNA sample were processed and then averaged to get a single gene copy number. The qPCR was carried out in LightCycler480 with Sequence Detection Software v1.4 (Applied Biosystems, USA). Each qPCR mixture (20 μL) was composed of 10 μL of SYBR Premix Ex Taq TM II (2×), 0.4 μL, 10 nM of each forward and reverse primers, 8.2 μL ddH_2O and 1.0 μL of template DNA (TaKaRa Biotechnology, Japan). The temperature program of qPCR was shown in Table S2 in the Supplementary Materials. A melting curve analysis for SYBR Green assay was prepared after amplification to distinguish the targeted PCR product from the non-targeted PCR product. Standard curves for real-time PCR were produced based on a serial dilution of known copies of PCR fragments of the respective related genes generated using M13 PCR from clones. All the standard curves showed excellent correlations between the DNA template concentration and the crossing point with high coefficients of determination ($R^2 > 0.99$). The qPCR efficiency was in the range from 80.66 to 98.52%.

2.5. Data analysis

PCA was conducted by canoco 4.5 to analyze the relationship among nitrogen transformation rates, physical parameters, and the absolute abundances of nitrogen transformation genes. Abundance of tested genes was normalized by Log transformation. A one-way analysis of variance was performed using SPSS v20 to access the significant differences of target genes (*amoA*, *nirS*, *nosZ*, *narG*, *nobL*, and *amx*), nitrogen transformation rates, and environmental parameters between samples.

3. Results and discussion

3.1. Environmental conditions of sampling sites

Table 1 shows the basic information of the three wetlands, including their coordinates, DO, water temperature, pH value, and soil types. The water temperatures of three wetlands were all around 10˚C and the soils were slightly alkaline. The DO of surface water in YR was the highest (i.e. 12.6 mg L^{-1}), while that in XM was only 4.32 mg L^{-1}. The XM and YR were formed more than 100 years ago, while the DW was constructed only for 2–3 years. The soils in XM and YR were dense clay loam, whereas those in DW were sandy loam with good ventilation and uniform distribution of soil DO [24].

Table 2 lists the concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, and TOC in the substrate and flow water of all samples. It can be seen that the TOC showed no difference between different depths of substrate of each wetland ($p > 0.05$). However, the TOC in DW appeared to be quite low, only 1.39–1.95%. This may be attributed to that the effluents from WWTPs have already met the GB18918-2002 1A discharge standard (COD < 50 mg L^{-1}). Besides, the concentrations of NH_4^+-N in XM 0–20 cm substrates were much high than that in DW, probably because DW was constructed with extra primary treatment where most ammonium was reduced. As for TIN in substrates,

Table 1
Characteristics of the three study sites

	XM	DW	YR
Coordinates	36˚27′53′′N, 115˚24′50′′E	35˚58′33′′N, 116˚34′11′′E	37˚45′44′′N, 117˚2′28′′E
DO (mg L^{-1})	4.32	9.02	12.6
Water temperature (˚C)[a]	10.3	11.2	10.5
pH values of water[a]	8.27	8.29	8.58
Soil types[b]	Clay loam	Sandy loam	Clay loam

[a]Values of water DO, temperature, and pH value were tested on the spot.
[b]Soil types were determined based on the soil texture.

Table 2
Concentrations of inorganic nitrogen compounds (NH_4^+, NO_2^-, and NO_3^-) in substrate and surface water of three sites

Sites	XM		DW		YR	
Depths (cm)	0–20	20–40	0–20	20–40	0–20	20–40
TOC (%)	2.35 ± 0.11	2.29 ± 0.06	1.39 ± 0.05	1.95 ± 0.07	2.30 ± 0.04	2.65 ± 0.06
Soil (μg g^{-1})						
NH_4^+	19.42 ± 0.01	4.87 ± 0.02	6.55 ± 0.03	8.23 ± 0.04	5.50 ± 0.08	6.55 ± 0.04
NO_2^-	0.00 ± 0.02	4.04 ± 0.02	1.80 ± 0.09	2.17 ± 0.12	1.05 ± 0.02	0.67 ± 0.03
NO_3^-	1.10 ± 0.06	0.39 ± 0.02	1.04 ± 0.04	1.33 ± 0.03	0.58 ± 0.02	0.42 ± 0.01
Water (mg L^{-1})						
NH_4^+	1.85 ± 0.09		2.47 ± 0.01		1.31 ± 0.01	
NO_2^-	0.04 ± 0.01		0.14 ± 0.01		0.00 ± 0.01	
NO_3^-	9.39 ± 0.01		17.49 ± 0.05		0.37 ± 0.01	

NH_4^+-N content accounted for over 50% in all substrates, and among three sites, substrate TIN of YR is the smallest for its low nitrogen input/output [12]. However, nitrate content accounted for the majority of TIN in water, which was owing to the low activity of denitrification under aerobic conditions in surface water [25].

3.2. Nitrogen transformation performance

The potential rates of the substrates can be used to calculate actual rates of nitrification and denitrification [23]. Fig. 2 shows a comparison of NH_4^+-N transformation in the three wetlands. All samples showed NH_4^+-N reduction and NO_2^--N accumulation, to some extent, under aerobic conditions through nitrification [26]. In DW and YR, there was no significant difference between two depths ($p > 0.05$). However, in XM, the rates in 0–20 cm substrate of all wetlands were higher than that in 20–40 cm soil. The trends could be explained by the decline of DO from the top to the bottom [27]. Specially, the accumulation of NO_2^--N in the 0–20 cm substrate of XM was highest with the value of 4.04 ± 0.02 μg g^{-1}.

Fig. 3 shows that the concentrations of both NO_2^--N and NO_3^--N declined after adding 100 μM NO_2^--N, 1 mM NO_3^--N, and 0.1% glucose. Furthermore, the similar NO_2^--N transformations in different depths of DW substrate were probably due to the same air condition caused by the texture of sandy loam [28]. However, clay loam of YR and XM substrates led to a higher NO_2^--N transformation rate with much more anaerobic condition in 20–40 cm soils. Interestingly, the NH_4^+-N concentrations suddenly increased after incubated for 4 h in the samples of XM (0–20 cm) and DW, and then decreased continuously. This indicates that some NH_4^+ released from soil after adding KNO_3, which was called nitrate ammonification [29,30], and then consumed through microbial process.

Fig. 4 presents transformation rates of NH_4^+-N, NO_2^--N, and NO_3^--N of different depths of substrates in the three wetlands. As shown in Fig. 4(A), the NH_4^+-N transformation rate in YR was higher than that of DW for both depths, accounting for the higher TOC in substrate of YR. It is supposed that AOB was likely more metabolically active in organic-rich soils than in mineral nitrogen-rich soils after long-term application [12]. In XM, the NH_4^+-N transformation rate of 0–20 cm was much larger than that of 20–40 cm substrates, probably due to the higher DO in topsoil than in subsoil.

As shown in Fig. 4(B), the NO_2^--N transformation rates of 0–20 cm substrates in three wetlands were 1.14, 1.14, 1.12 μg g^{-1} h^{-1} for XM, DW, and YR, respectively. In 20–40 cm soil, the nitrite transformation rates in DW and YR were almost the same as in topsoil. However, regarding 20–40 cm soil in XM, the rate was significantly smaller than that of the others ($p < 0.05$), which indicated a vertical distribution of nitrite transformation ability in riparian wetlands [31].

NO_3^--N transformation rates in the three wetlands are shown in Fig. 4(C). In XM, the surface soil rate (3.50 μg g^{-1} h^{-1}) was higher than the subsoil (0.31 μg g^{-1} h^{-1}), which proved that denitrification in surface soil was a major route for NO_3^--N removal in riparian wetlands [31]. Also, NO_3^--N transformation rates of different depths were similar in both DW and YR. However, the reasons may be different. In DW, similar distributions of environmental parameters

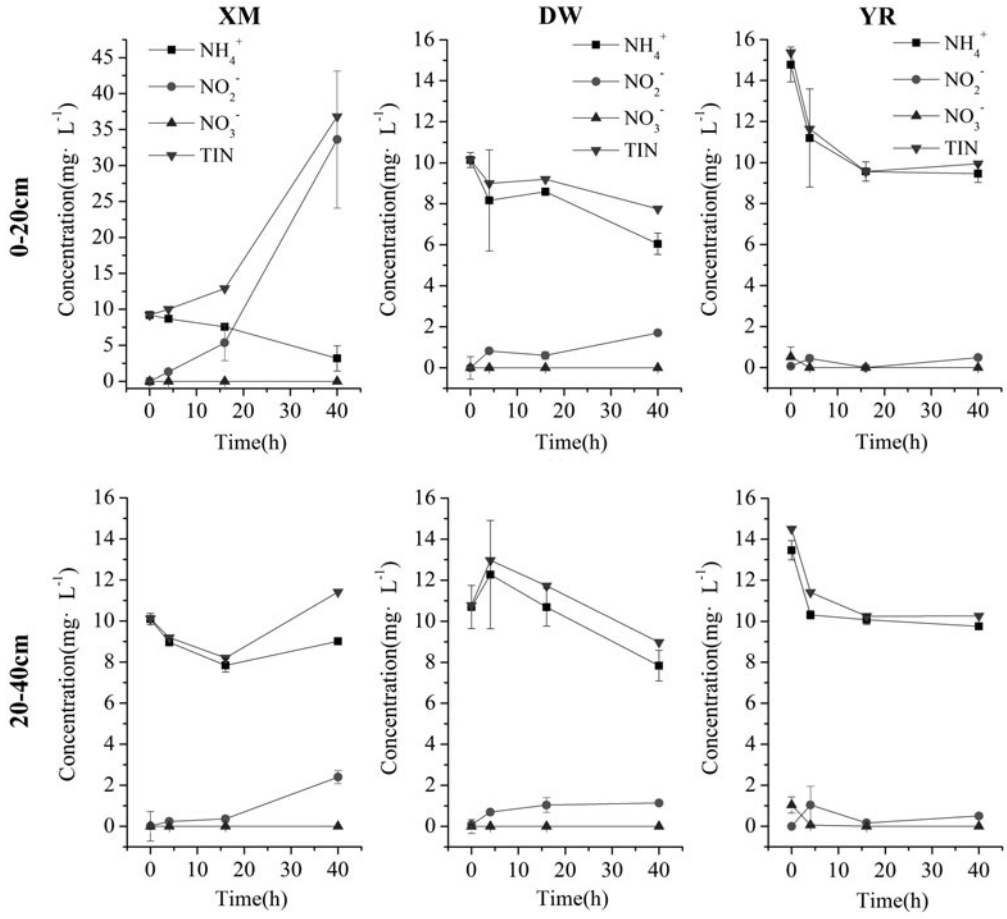

Fig. 2. Comparison of NH_4^+-N, NO_2^--N, NO_3^--N, and TIN in different depths of soil (0–20 cm, 20–40 cm) among three wetlands (XM, DW, and YR) after adding 1 mM NH_4^+-N.

were dominant, whereas in YR the similar rates of NO_3^--N transformation may be resulted from the similar TOC [32]. Besides, NO_3^--N transformation rates in DW were significantly higher than that in YR in both depths because nitrate and nitrite removal would be more rapid in wetlands with mineral soils than organic soils [33].

Most of the nitrate transformation processes included a nitrate "flush" effect, reaching the peak at about 15 h before the expected decline (Fig. 3). This has been reported for many times [6,34] and the possible reason was that the addition of nitrate in the form of KNO_3 caused the K^+ supersede ammonium settled on soil particulate surfaces and slowed down the nitrate transformation [6].

A comparison of TIN removal efficiency in both aerobic and anaerobic conditions is shown in Figs. 2 and 3. The anaerobic TIN removal efficiencies were higher than aerobic ones. As to the anaerobic conditions, the TIN removal efficiencies of DW substrates were quite higher than the others (95.45 and 90.03%). Besides, XM of the 0–20 cm substrate was with 91.29% of TIN accounting for the quick decline of NO_2^--N and NO_3^--N. As to TIN in XM of 0–20 cm substrates, it increased two times after 40 h with accumulation of nitrite. However, TIN of 20–40 cm substrates in XM increased only by 12.89% because the anaerobic processes were inhibited, such as denitrification and anaerobic ammonium oxidation, leading to the large accumulation of NO_2^--N, and finally making TIN increase [35].

3.3. Microbial genes abundances in nitrogen transformation

The absolute gene abundances of *amoA*, *nobL*, *amx*, *nirS*, *nirK*, *nosZ*, and *narG* genes were quantified to evaluate the nitrogen transformation pathways in three wetlands (Fig. 5).

In XM, the NH_4^+-N transformation rates decreased with the decreasing absolute abundance of *amoA* and

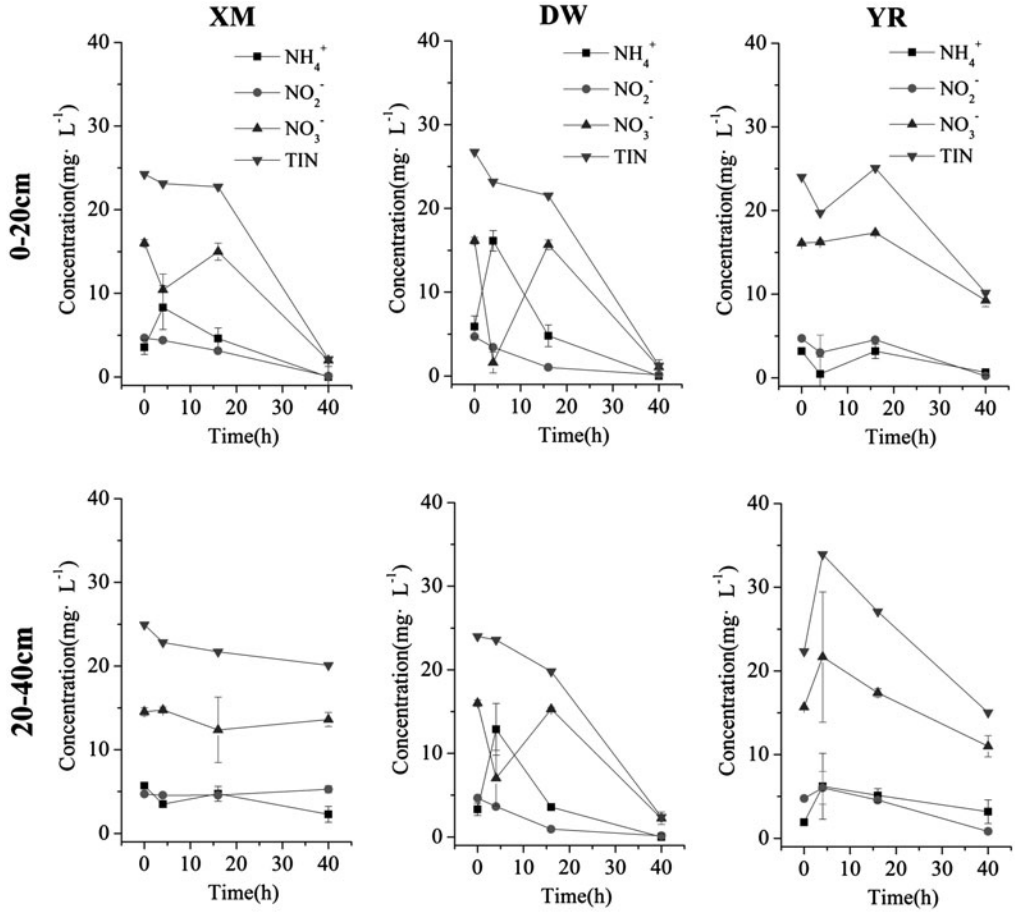

Fig. 3. Comparison of NH_4^+-N, NO_2^--N, NO_3^--N, and TIN in different depths of soil (0–20 cm, 20–40 cm) among three wetlands (XM, DW, and YR) after adding 100 μM NO_2^--N, 1 mM NO_3^--N, and 0.1% glucose.

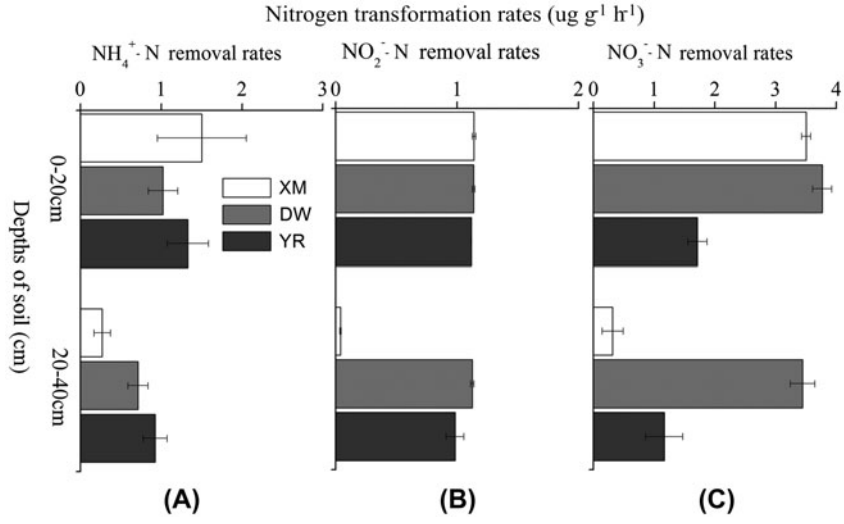

Fig. 4. The transformation rates of ammonium, nitrite, and nitrate by adding extra NH_4^+-N (A), NO_2^--N (B), and NO_3^--N (C) of different depths of Soil (0–20 cm, 20–40 cm) in three types of wetlands (n = 3).

nobL, indicating that the NH_4^+-N was mainly removed by AOB and NOB [9]. However, the accumulation of NO_2^--N was probably due to the aerobic conditions that inhibited the activity of anaerobic microorganisms for reducing NO_2^--N. In addition, under anaerobic conditions, the five genes, *nirS*, *nirK*, *amx*, *nosZ*, and *narG*, were usually used as markers of denitrification, which were all related to NO_2^--N and NO_3^--N transformation in XM [14]. The absolute abundance of amx gene in 0–20 cm was the maximum of all genes with 2.31×10^{10} copies g^{-1}, followed by the *nirS* genes in 0–20 cm soils (1.08×10^{10} copies g^{-1}), suggesting the high anaerobic ammonium oxidation and nitrite reduction activities [36] in XM.

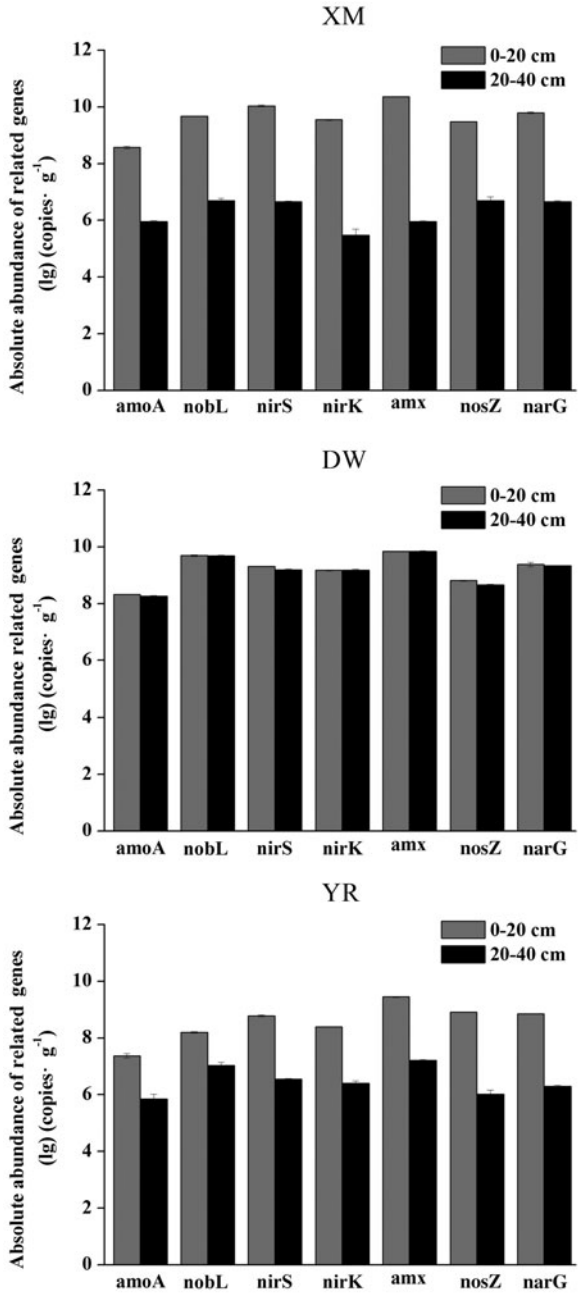

Fig. 5. Absolute abundances of related genes in different depths of the substrate in three wetlands (XM, DW, and YR).

In DW, a full-scale CW, there was no significant difference of all tested genes between 0–20 cm and 20–40 cm substrates ($p > 0.05$), in which *amx* and *nobL* accounting for the majority of all genes with 6.95–6.97×10^9 copies g^{-1} and 4.47–4.91×10^9 copies g^{-1}, respectively. Other studies also reported their abundance in the order of magnitude of 10^8 [14,37]. The similarity indicates that the sandy loam soil in CWs, rather than clay loam soil in the natural wetlands, was fit for these related genes to exist congruously either in surface or subsurface soils [8].

In 0–20 cm soil of YR, the absolute abundances of all tested genes were lower than those in the same depths of XM. The abundance of *amx* genes in 0–20 cm soils was the largest with 2.80×10^9 copies g^{-1}, and decreased significantly to 1.61×10^7 copies g^{-1} in 20–40 cm soils. This was because the more fertilized soil was more suitable for the anammox [38]. Although all the tested genes copies in 0–20 cm were significantly more than that in 20–40 cm substrates, there were no significant differences in nitrogen transformation rates between two depths. The reason could be that the nutrients in the substrates of YR limited the growth of the nitrogen cycle microbes in 0–20 cm substrates, so that the abundance of living microbes were not significantly different with 20–40 cm substrates. This should be further examined by other microbial activity methods, such as fluorescein diacetate (FDA) hydrolysis [39]. In other words, the absolute abundances of tested genes alone could not explain the overall mechanisms of nitrite and nitrate transformation [40].

3.4. Relationship of nitrogen transformation and related genes

To analyse the roles of these related genes in nitrogen removal, PCA was carried out (Fig. 6(A)). The first component accounted for about 97.4% and the second one about 2% of the total variance in the dataset. The approximate correlation between two variables is equal to the cosine of the angle between the corresponding arrows. Thus, the NH_4^+-N removal rates (R_n), NO_2^--N removal rates (R_i), and NO_3^--N removal rates (R_a) were positively correlated with all related genes. Besides, all the genes abundances were

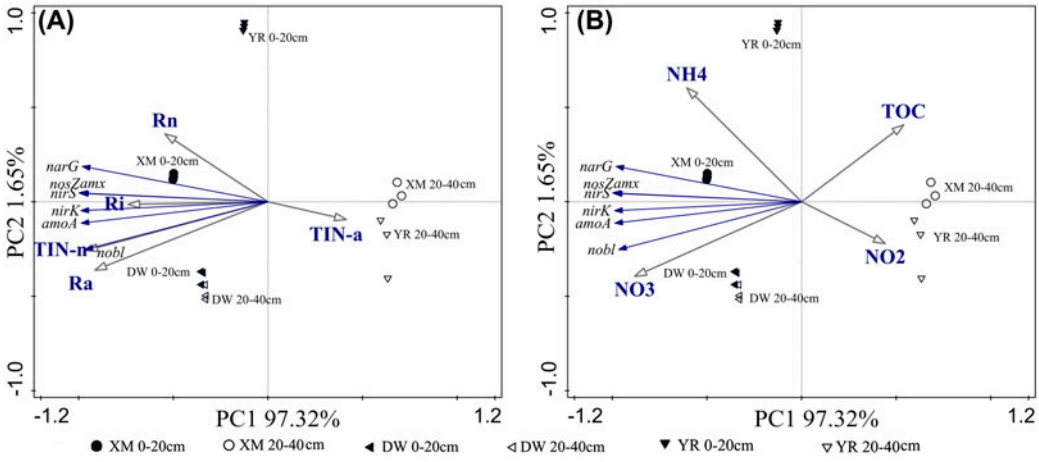

Fig. 6. Principle component analysis of nitrogen transformation rates and related genes, and the effect of different soil parameters on related genes: (A) TIN-a stand for the TIN removal efficiency (%) under aerobic conditions, while TIN-n represents the TIN removal efficiency (%) under anaerobic conditions. R_n, R_i, and R_a are the rates of NH_4^+-N, NO_2^--N, and NO_3^--N removal and (B) TOC stands for the TOC, NH_4^+, NO_2^-, and NO_3^- are the NH_4^+-N, NO_2^--N, and NO_3^--N content in soils.

positively correlated with the TIN removal efficiency (%) in anaerobic (TIN-n). This was because *amx*, *nirS*, *nosZ*, *narG* were the major related genes in TIN-n, while *amoA* and *nobL* genes did not function in the TIN removal under anaerobic conditions [41]. However, *amx*, *nirS*, *nirK*, *nosZ*, and *narG* genes did not function during aerobic conditions [42,43], and were thus negatively correlated with TIN removal efficiency (%) in aerobic (TIN-a). Various environmental parameters (DO and pH) influenced the distribution patterns of nitrifiers (e.g. *amoA* and *nobL*) [44], leading to their negatively correlation to TIN-a. The detailed reason needed further study.

In addition, the samples projecting further from zero in the direction of the arrow are predicted to have above-average abundances, while the sample points projecting in the opposite direction are predicted to have below-average values. Comparing these results with Figs. 2–5, it could be easily found out that *amx*, *nirS*, *nirK*, *nosZ*, and *narG* (XM 0–20 cm substrates) showed close correlation to NO_2^--N removal rates (smaller angles). Besides, the R_a showed a little difference, which was not most correlated with the abundances of *nosZ*, *nirS*, *nirK*, and *narG*, even in substrates with high R_a, such as 0–20 cm of XM, 0–20 cm, and 20–40 cm of DW. This might be due to the complex nature of NO_3^--N transformation processes, such as the nitrate-ammonification [45]. However, R_a presented large correlation with *amx*, which were related to the nitrate re-conversion through anammox processes [17,34].

3.5. The relationships of environmental parameters and nitrogen transformation genes

Fig. 6(B) illustrates the relationships between environmental parameters and related genes. The first component accounted for about 97.4% and the second one about 2%. The six tested genes were positively correlated with NH_4^+-N and NO_3^--N, and negatively with NO_2^--N and TOC.

The related genes presented the positive correlation with NH_4^+-N and NO_3^--N, and change to the opposite with NO_2^--N. The processes could form gaseous nitrogen by denitrification and anammox microbes [46]. The more these microbes existed means the less NO_2^--N was left, in the form of gas, for nitrogen released from the system. In addition, TOC was negatively correlated to the abundances of related genes. It has been known that different categories of organic carbon in soil have different influence on nitrogen transformation. Polysaccharides positively influenced denitrification rate whereas phenolics have negative effect. Thus, this could be the reason (needing further study) why inorganic nitrogen transformation efficiencies (R_n, R_i, and R_a) were negatively correlated to TOC in soil [47].

Furthermore, comparing with Fig. 6(A) and (B), the relationship of related genes with substrate NO_2^--N was similar to that with TIN-a, which was significant in XM substrate with the most amount of NO_2^--N in TIN. This indicates that aerobic processes alone could not remove inorganic nitrogen or transform it into gaseous nitrogen [48]. The

potential nitrogen transformation rates should thus be improved to further characterize the real situation by adjusting the oxygen conditions.

4. Conclusions

The relationship between nitrogen transformation and its related genes among three types of wetlands was established. In particular, it was found that the full-scale CWs maintained higher rates of nitrogen transformation and TIN removal than natural marsh and riparian wetlands. The nitrogen cycle genes could be controlled or adjusted by changing the concentration of soil nitrogen and TOC. Additionally, our results showed that a higher nitrogen removal rates can be achieved by improving the microbial quantity and activity beforehand. It was also suggested that adding specific nitrogen cycle microbes or active sludge to CWs, may lead to more efficient nitrogen pollutant removal.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.10.1080/19443994.2015.1124056.

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