Correlation between Changes of the Abundance of the Nitrification and Denitrification and Quality of the Effluent in Constructed Wetland

Zhu Ying, Tian Chao, Tang Houquan, Shao Yanqiu, and Chen Qingfeng

***Abstract*—Using the constructed wetland of Yan Huang ditch of Qihe County treat wastewater, and the real-time Polymerase Chain Reaction(PCR) technology was used to detect the water abundance of nitrification functional gene amoA and the abundance of denitrification function gene nirS, nirK and nosZ. The results showed that the whole abundance of amoA was low. At each sampling point, nosZ was the highest abundance gene, and nirS had the same trend with nirK, and the abundance of nirS was higher than the abundance of nirK. The study showed that the removal rate of chemical oxygen demand (COD) was relatively high, but the removal rate of ammonia nitrogen was lower, and the effect of nitrogen removal was not obvious, and the end product of denitrification was nitrogen. The content of ammonium nitrogen (NH4+-N) in water had significant negative correlation with the abundance of amoA, but the content of nitrate nitrogen (NO3--N) had no significant correlation with the abundance of nirS, nirK and nosZ, which indicated that there were other nitrogen removal mechanisms in addition to nitrification-denitrification.**

***Index Terms*—Constructed wetland, amoA, nirS, nirK, nosZ.**

I. Introduction

As an ecological and low cost water treatment technology, constructed wetland has been widely used in the treatment of wastewater, and denitrification treatment of wastewater [1]-[3]. There are many kinds of nitrogen removal mechanisms in constructed wetland, in which microbial nitrification-denitrification is considered as the most important nitrogen removal process [4], [5]. The study showed that the removal of nitrogen content by nitrification-denitrification accounts for 60%-86% of total nitrogen removal [6]. Therefore, it has the actual significance to study the community structure and the abundance of nitrogen removal microbial in constructed wetland.

As the limiting step of nitration reaction, ammonia oxidation reaction is the key step of nitrogen removal [7]. As diversity of ammonia oxidizing bacteria molecular markers, *amo*A gene has been widely applied in the study of natural and artificial ecosystems [8]-[10]. Siripong and Rittmann *et al*. [11] showed that *amo*A gene was used to detect the diversity of the bacterial population in the seven water reuse plant, and the hypothesis that the diversity and functional redundancy of the nitrification bacteria in activated sludge with stable and complete nitrification was significantly similar. Mertoglu *et al*. [12] used 16S rDNA and *amo*A gene which based molecular biology techniques in the assessment of the effect of ammonia removal in the landfill bioreactor to determine the nitrification activity. The *amo*A gene was used as phylogenetic relationship of comparative basis to assess the dissolved oxygen of ammonia oxidizing bacteria population in activated sludge in the study of Park and Noguera [13]. Nitrate or nitrite can be reduction into nitrogen or nitrous oxide (N_2O) by denitrification bacteria. This process was accomplished by NO_3^--N reductase (*Nar*), NO_2^--N reductase (*Nir*), NO reductase (*Nor*) and N_2O reductase (*Nos*), in which the *nir*K, *nos*Z, *nir*S and other genes play an important role in the denitrification pathway [14]. At present, denitrification genes' (such as *nir*S, *nir*K and *nos*Z) PCR amplification primer method has been successfully used in the study of diversity of denitrifying bacteria, such as Marine sediments [15], cyanobacteria blooms [16], pollution of groundwater [17] and all kinds of water treatment reactor [18], [19].

Real-time PCR technology which has a higher sensitivity, specificity and greater reliability, can realize multiple reaction and other characteristics, and has been widely applied in all kinds of denitrification function group of bacterial abundance in the environment of study [20]-[22]. At present, the study on the function gene of the constructed wetland is focused on the denitrification gene, but the study of quantitative about genes both the nitrification and denitrification in constructed wetland is rare. In addition, the winter's temperature is lower than other season in northern China, and the temperature is too low will not only affect wetland treatment effect of pollutants, may also cause the water to freeze, low dissolved oxygen content and other adverse consequences, which limits the application of artificial wetland in the north, thus for wetland winter less study. Therefore, through using Real-time PCR technology, this study aimed at *amo*A, *nir*S, *nir*K and *nos*Z genes, to make a quantitative at different levels of nitrifying and denitrifying bacteria in constructed wetland. At the same time, this study determinate content changes of COD, dissolved oxygen (DO), NH_4^+-N, NO_3^--N and total nitrogen (TN) in water, to discuss the relationship between nitrification-denitrification genes abundance variation characteristics of with the quality of the output water.

II. Materials and Methods

Manuscript received December 10, 2015; revised February 10, 2016.

Zhu Ying, Tian Chao, and Shao Yanqiu are with the New Materials Research Institute of Shandong Academy of Sciences, Jinan, 250014, China (e-mail: zhuyingee@163.com, tianchaokwl@163.com, 516729081@qq.com).

Tang Houquan is with the Jinan Environmental Monitoring Center, Jinan, 250014, China (e-mail: 3317446554@qq.com).

Chen Qingfeng is with the Shandong Analysis and Test Center, Jinan, 250014, China (e-mail: chensdcn@163.com).

A. Constructed Wetland Technology

The experiment adopted process as follows: using the undercurrent wetland technology as the mainly pole and combination technology of surface flow constructed wetland as the auxiliary pole. Process is shown in Fig. 1.

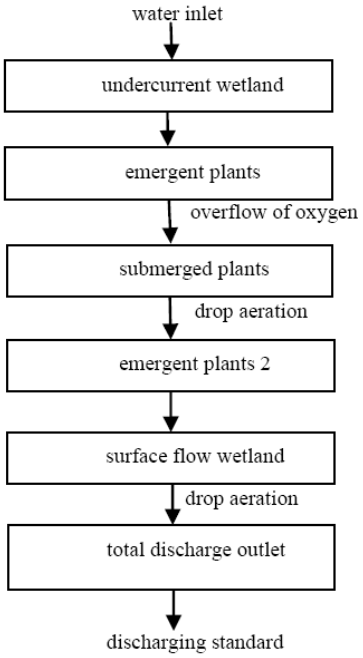

Fig. 1. Process of constructed Wetland.

B. Sample Collection and Water Quality Analysis

Water samples were collected from constructed wetland of yanhuang ditch Qihe County of Shandong Province in December 2014. Set the sampling point at the outlet of each pond, respectively: water inle (A), undercurrent wetland(B), emergent plants (C), submerged plants (D), emergent plants2(E), surface flow wetland (F), total discharge outlet(G). Each sample point was collected from 4 L water, which was quickly transported to the laboratory for 4℃ to freezing. Determinate of water quality indexes (COD, DO, NH_4^+-N, NO_3^--N and TN) content change by using standard methods [23] DNA samples immediately enrichment, extraction, and -20℃ preservation. In order to the stability of the operation, DNA extraction and Real - time PCR test all has three parallel samples

C. DNA Extraction

Using polycarbonate membrane (aperture 0.2μm, diameter of 50 mm, Millipore) vacuum suction filter 50 mL water sample, and then to filter 2 mL sterilization water for washing membrane filter. Cut into pieces, membrane filter in 10 mL of polyethylene centrifuge tube, add 1.8 mL SET buffer (0% sucrose, tendency of 50 mmol/L EDTA, tendency of 50 mmol/L Tris HCl, pH = 7. 6) [24], spiral, - 20 ℃ preservation, for subsequent DNA extraction [25]. Sediment genomic DNA extraction using Fast DNA @ SPIN for soil kit (MP, USA), according to product manuals, with 50μL finally Tris - HCl (pH = 8.0-8.5) dissolve purified DNA.

D. Real-Time Pcr Assays

Amplification of quantitative PCR products was carried out with an ABI Prim SDS 7300 (Applied Biosystems, USA). *amo*A and denitrification function gene (*nir*S, *nir*K and *nos*Z) primers used sequence information shown in Table I [20], [26]-[30], The real-time PCR use 20μL system, components: the PCR mastermix (Applied Biosystems, USA) 10μL; 20μ mol/L upstream and downstream primers each 0.5μL; DNA template 1μL; ddH_2O 8μL. The reaction conditions of PCR: 50^0C for 2 min (enzyme); 95^0C for 10 min;40 cycles , 95^0C for 15 s, annealing 1 min(amoA 54. 5^0C, *nir*S 58^0C, *nir*K 60^0C, nosZ 58^0C), and then 72℃ for 30 s; With the final score of 0.1℃/s rate rising from 60^0C to 95^0C, obtain the solution of the amplified DNA fragments chain curve.

TABLE I: PRIMERS USED IN REAL-TIME PCR

target gene	primers	primer sequences
*amo*A	*amo*A-1F	GGGGTTTCTACTGGTGGT′
	*amo*A-2R	CCCCTCKGSAAAGCCTTCTTC
*nir*S	*nir*S-cd3aF	GTSAACGTSAAGGARACSGG
	*nir*S-R3cd	GASTTCGGRTGSGTCTTGA
*nir*K	*nir*K-876F	ATYGGCGGVAYGGCGA
	*nir*K-1040R	GCCTCGATCAGRTTRTGGTT
*nos*Z	*nos*Z-F	CGYTGTTCMTCGACAGCCAG
	*nos*Z-1622 R	CGSACCTTSTTGCCSTYGCG

E. Statistics and Analysis

Using SPSS18.0 and origin9.0 software analyze the data of environmental factors, nitrification and denitrification, and the correlation.

III. RESULTS AND DISCUSSIONS

A. Purification of Water Quality in Constructed Wetland

COD, DO, NH_4^+-N, NO_3^--N and TN concentrations change of sampling points out-water as shown in Fig. 2 (a) and (b). The content of COD, NH_4^+-N and TN were decreased with the operation process of constructed wetland, and content change of influent and effluent were: 66.9mg/L-15.6mg/L, 5.48mg/L -3.70mg/L and 5.57 mg/L -3.79mg/L. Removal efficiency were 76.68%, 32.48% and 31.96%. The content of DO and NO_3^--N were first increase and after decrease with the operation process of constructed wetland, and the content change of influent and effluent were: 1.44mg/L-6.5mg/L and 0.05mg/L-0.08mg/L.

As is shown in Fig. 2 (b), the content of NH_4^+-N rapidly decreased from water inle to undercurrent wetland, reduce 0.54mg /L, and removal efficiency were 9.85%. The content of NH_4^+-N slowly decreased after water flows through emergent plants, submerged plants, emergent plants2, a decline of only for 0.51mg/L, and removal efficiency were only 9.31%. The content of NH_4^+-N rapidly decreased in surface flow wetland, reduce 0.82 mg/L, and removal efficiency were 14.96%. The content of DO in water has a great influence on nitrification, when the content of DO enough to support the growth of aerobic nitrifying bacteria, nitrification reaction can be carried out smoothly [31], [32]. Study shows that when the content of DO is too low, not only decrease the rate of nitration reaction and removal efficiency of total nitrogen, but also can appear the accumulation of

nitrite, and oxygen is consumed not only of the constructed wetland system in the degradation of nitrogen and phosphorus and other nutrients, also is used in organic material degradation, these make the process of denitrification become more limited [33], [34]. The sampling time is December, after entering the winter, the content of DO in water is all low, but input water contain a certain content of DO which can carry out nitration reaction, so the removal rate of NH_4^+-N is relatively large. After treatment of subsurface flow constructed wetlands, before surface flow wetland, the content of DO in water was lower, and with little change, so NH_4^+-N was removed slow. After several times of drop aeration, to the surface flow wetland, the DO content in water gradually increased, thus of NH_4^+-N removal rate was higher.

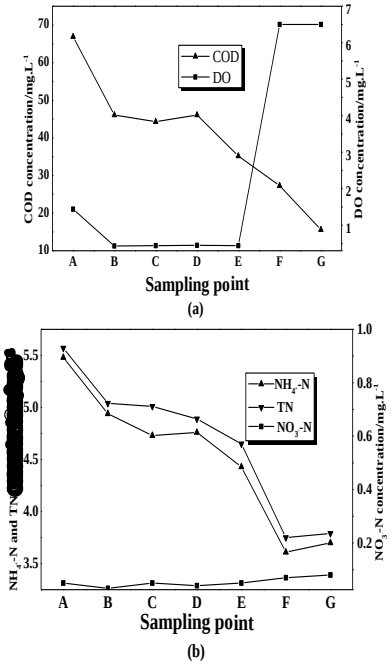

Fig. 2. The results water purification in constructed wetland A: water inle, B: undercurrent wetland, C: emergent plants, D: submerged plants, E: emergent plants2, F: surface flow wetland, G: total discharge outlet.

The Fig. 2(b) shows that the content of NO_3^--N was gradually increasing. The input water content of NO_3^--N was 0.05 mg/L, and the output water the content of NO_3^--N was 0.08 mg/L. The content of NO_3^--N gradually increased in surface flow wetland, This result may be due to there was more DO in water of surface flow wetland, resulting in nitrification was stronger, ammonifier can convert organic nitrogen and ammonia nitrogen to nitrate nitrogen, resulting in content of nitrate nitrogen increased.

Similar to the variation of the changes of TN and NH_4^+-N. But before submerged plants, the decline range of TN was greater than NH_4^+-N. The main reason is that the content of organic nitrogen in influent is relatively large, which provides nitrogen source for ammonia oxidizing bacteria, and ammonia oxidizing bacteria convert organic nitrogen to NH_4^+-N. However, NH_4^+-N affected by DO could not be timely carried out nitrification transformation, resulting in the accumulation of NH_4^+-N, but content of less ammonia oxidizing bacteria in the influent, NH_4^+-N accumulated less.

B. Abundance Changes of Nitrification Microbial Communities in Water

At each sampling point, the quantitative analysis of denitrification gene (*nir*S, *nir*K and *nos*Z)in water results as shown in Fig. 3(b). Overall, the abundance of *nir*S, *nir*K and *nos*Z showed an increasing trend along the process. The abundance of nosZ gene was the higher. The abundance of *nos*Z of the influent was the lowest, only 6.00E+09copies/L, and the surface flow wetland was the highest, which was 1.29E+13copies/L. There *nir*S has increasing trend after the first decreasing. The abundance of *nir*S was lowest in influent, only 4.82E+09copies/L, and was highest in submerged plants, emergent plants 2, surface flow wetland and total discharge outlet followed, respectively 3.50E+12copies/L, 2.97E+12copies/L, 2.55E+12copies/L and 2.40E+12copies/L. *Nir*K had the same trend with *nir*S, and the abundance of *nir*K gene was lower than *nir*S gene at all sampling points. The lowest abundance of *nir*K gene was also in the influent, only 1.15E+09copies/L and reached the highest in the submerged plant, 1.78E+12copies/L. The abundance of *nir*K gene had declined in emergent plants 2, surface flow wetland and total discharge outlet, but did not change significantly.

There are many functional genes involved in denitrification process. Since nitrite is converted to nitric oxide is different from other nitrate metabolism iconic response in denitrification process, and is important in the process of denitrification rate-limiting step. The corresponding nitrite reductase gene (including *nir*S and *nir*K) is often used as a representative molecule denitrifying microorganisms research community structure tag. Jones *et al.* [35] pointed out that although the two kinds of denitrification function of *nir*S and *nir*K genes encode enzymes to exercise the same function in the process of denitrification, and these two genes carrying denitrifying bacteria seem to exist for different micro environmental preferences. Knapp *et al.* [36] pointed out that the abundance of *nir*S is more in anoxic environment and the abundance of *nir*K is more in oxygen-rich conditions. Ligi *et al.* [37] found that the abundance of *nir*S in a riparian zone was higher than that of *nir*K. Zhi *et al.* [38] found that the abundance of *nir*S was also higher than *nir*K in constructed wetland. Similar results were obtained in this study which the abundance of *nir*S was lower in oxygen-rich. *Nir*K had the same trend with *nir*S, and the abundance of *nir*K gene was lower than *nir*S gene at all sampling points. It is indicated that the *nir*S type of the denitrification bacteria can adapt to the water quality environment of the constructed wetland than *nir*K.

NosZ can encode nitrous oxide reductase, and the enzyme N_2O can be reduced to N_2. García-Lledo *et al.* [39] found the abundance of *nir*S was significantly higher than *nos*Z in the study of denitrification function of wetland, and when the abundance of *nos*Z was significantly lower than that of other denitrifying functional gene, N_2O which is denitrification intermediate was cumulated in constructed wetland. The results of this test are opposite, the abundance of *nos*Z is higher than *nir*S, which indicates that the end product of denitrification in the wetland is N_2. In addition, in this test, both abundance of nitrification and denitrification gene were higher in emergent plants 2 and surface flow wetland, which indicates that the environmental conditions of the two sampling points is more appropriate denitrifying bacteria and

anti-nitrifying bacteria growth, and is conducive to nitrification and denitrification common occurrence.

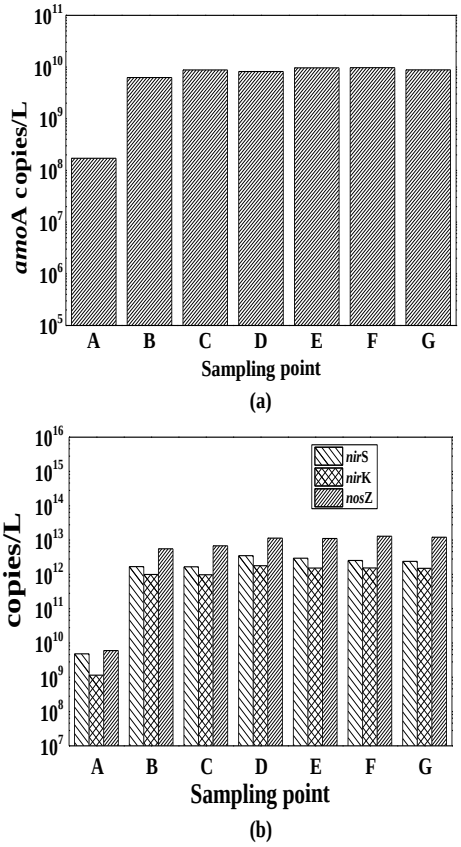

Fig. 3. The abundance of nitrification and denitrification in water A: water inle, B: undercurrent wetland, C: emergent plants, D: submerged plants, E: emergent plants2, F: surface flow wetland, G: total discharge outlet.

C. Abundance Changes of Denitrification Microbial Communities in Water

At each sampling point, the quantitative analysis of denitrification gene (*nir*S、*nir*K and *nos*Z)in water results as shown in Fig. 3(b). Overall, the abundance of *nir*S, *nir*K and *nos*Z showed an increasing trend along the process. The abundance of nosZ gene was the higher. The abundance of *nos*Z of the influent was the lowest, only 6.00E+09copies/L, and the surface flow wetland was the highest, which was 1.29E+13copies/L. There *nir*S has increasing trend after the first decreasing. The abundance of *nir*S was lowest in influent, only 4.82E+09copies/L, and was highest in submerged plants, emergent plants 2, surface flow wetland and total discharge outlet followed, respectively 3.50E+12copies/L, 2.97E+12copies/L, 2.55E+12copies/L and 2.40E+12copies/L. *Nir*K had the same trend with *nir*S, and the abundance of *nir*K gene was lower than *nir*S gene at all sampling points. The lowest abundance of *nir*K gene was also in the influent, only 1.15E+09copies/L and reached the highest in the submerged plant, 1.78E+12copies/L. The abundance of *nir*K gene had declined in emergent plants 2, surface flow wetland and total discharge outlet, but did not change significantly.

There are many functional genes involved in denitrification process. Since nitrite is converted to nitric oxide is different from other nitrate metabolism iconic response in denitrification process, and is important in the process of denitrification rate-limiting step. The corresponding nitrite reductase gene (including *nir*S and *nir*K) is often used as a representative molecule denitrifying microorganisms research community structure tag. Jones *et al*. [35] pointed out that although the two kinds of denitrification function of *nir*S and *nir*K genes encode enzymes to exercise the same function in the process of denitrification, and these two genes carrying denitrifying bacteria seem to exist for different micro environmental preferences. Knapp *et al*. [36] pointed out that the abundance of *nir*S is more in anoxic environment and the abundance of *nir*K is more in oxygen-rich conditions. Ligi *et al*. [37] found that the abundance of *nir*S in a riparian zone was higher than that of *nir*K. Zhi *et al*. [38] found that the abundance of *nir*S was also higher than *nir*K in constructed wetland. Similar results were obtained in this study which the abundance of *nir*S was lower in oxygen-rich. *Nir*K had the same trend with *nir*S, and the abundance of *nir*K gene was lower than *nir*S gene at all sampling points. It is indicated that the *nir*S type of the denitrification bacteria can adapt to the water quality environment of the constructed wetland than *nir*K.

*Nos*Z can encode nitrous oxide reductase, and the enzyme N_2O can be reduced to N_2. Garcí a-Lledo *et al*. [39] found the abundance of *nir*S was significantly higher than *nos*Z in the study of denitrification function of wetland, and when the abundance of *nos*Z was significantly lower than that of other denitrifying functional gene, N_2O which is denitrification intermediate was cumulated in constructed wetland. The results of this test are opposite, the abundance of *nos*Z is higher than *nir*S, which indicates that the end product of denitrification in the wetland is N_2. In addition, in this test, both abundance of nitrification and denitrification gene were higher in emergent plants 2 and surface flow wetland, which indicates that the environmental conditions of the two sampling points is more appropriate denitrifying bacteria and anti-nitrifying bacteria growth, and is conducive to nitrification and denitrification common occurrence.

D. Abundance Variation of Nitrification and Denitrification Functional Geneand Correlation between Water Quality Parameters

Correlation analysis of nitrification functional gene abundance and water quality parameters is shown in Table II. The abundance of *amo*A gene was significantly negatively correlation with the content of NH_4^+-N (P<0.05); there was no significant negative correlation with TN(P>0.05); the abundance of *amo*A gene was positively correlation with DO and NO_3^--N, but the correlation was not significant(P>0.05).

Correlation analysis of denitrification functional gene abundance and water quality parameters is shown in Table III. The abundance of *nir*S and *nir*K were no significantly negatively correlation with the content of NH_4^+-N and TN (P>0.05); the abundance of *nos*Z gene was significantly negatively correlation with the content of NH_4^+-N and TN (P<0.05); the abundance of *nir*S、*nir*K and *nos*Z gene were positively correlation with DO and NO_3^--N, but the correlations were not significant(P>0.05).

Correlation analysis showed that the content of DO didn't exist significantly correlation with the abundance of *nir*S, *nir*K and nosZ, which is same as results about constructed

wetlands by García-Lledó *et al*. [39] studied. Dissolved oxygen can effectively inhibit the denitrification activity of denitrifying bacteria, but does not mean the abundance of denitrification gene function will be affected by oxygen inhibition. Truu *et al*. [40] also pointed out that denitrification activity and abundance of denitrifying functional genes can't be simple linked.

TABLE II: PEARSON CORRELATION ANALYSIS OF ENVIRONMENTAL PARAMETERS AND ABUNDANCE OF NITRIFICATION GENES

Water parameters	C OD	I O	T N	N H_4^+-N	N O_3^--N
*amo*A	- 0.803*	(.385	- 0.711	-0.7 65*	0 .303

Note: *is significant correlation ($P<0.05$).

TABLE III: PEARSON CORRELATION ANALYSIS OF ENVIRONMENTAL PARAMETERS AND ABUNDANCE OF DENITRIFICATION GENES

Water parameters	*nir*S	*nir*K	*nos*Z
COD	0.624	0.747	-0.859*
DO	0.220	0.376	0.578
TN	-0.560	-0.690	-0.844*
NH_4^+-N	-0.584	-0.711	-0.863*
NO_3^--N	0.104	0.232	0.473

Note: *is significant correlation ($P<0.05$).

There was a significant negative correlation between nitrification genes and the content of NH_4^+-N, which was not significant with NO_3^--N. The reason is that with the increase abundance of ammonia oxidizing bacteria in water, NH_4^+-N is oxidized into NO_3^--N or NO_2^--N, which leads to the decrease of NH_4^+-N and increase of NO_3^--N. NO_3^--N is a substrate for denitrification, and it should be closely related to the denitrification bacteria. However, the results showed that there was no significant correlation between denitrification bacteria and NO_3^--N, The results may be that the content of NO_3^--N in influent is less and the quantity of denitrifying bacteria increased along the process, NO_3^--N in water far do not provide sufficient substrate for the denitrification, and the substrate must be another source of denitrification required, Therefore, the low content of NO_3^--N failed to show a significant correlation with the denitrification bacteria.

IV. CONCLUSIONS

The removal of COD by constructed wetland was efficiency, and the removal rate reached 76.68%. The removal rate of TN, NH_4^+-N was lower, and the removal rate was only 31.96% and 32.48%. The removal efficiency of NO_3^--N was not obvious, and the content of NO_3^--N increased slightly.

The whole abundance of *amo*A was low, and that of *amo*A is increasing along the process, which indicates that the initial nitrification ability of the constructed wetland is weak, and there is a gradually increasing trend; the abundance of *nir*K gene was lower than *nir*S gene at all sampling points. But the response of the two types of *nir*-type denitrification bacteria is more consistent with the environmental change, which indicated that the *nir*S type of the denitrification bacteria can adapt to the water quality environment of the constructed wetland. The abundance of *nos*Z was higher than the abundance of *nir*S and *nir*K, which indicated that the end product of denitrification was N_2.

There was significant negative correlation between the content of NH_4^+-N and the abundance of *amo*A, and there was no significant correlation between the content of NO_3^--N and the abundance of *nir*S，*nir*K and *nos*Z, which indicated that the mechanism of nitrification-denitrification in northern winter is not obvious, and there may be other nitrogen removal mechanisms.

ACKNOWLEDGMENT

The work was financially supported by the National Natural Science Foundation of China (51208290) and The Major Science and Technology Program for National Water Pollution Prevention and Control (2012ZX07203-004)

REFERENCES

[1] S. David, F. Lauchlan, B. James *et al*., "Efficiency of small constructed wetlands for subsurface treatment of single family domestic effluent," *Ecological Engineering*, vol. 18, pp. 429-440, 2002.
[2] D. JiG, T. H. Sun, Q. X. Zh *et al*., "Constructed subsurface flow wetland for treating heavy oil-produced water of the Liaohe Oilfield in China," *Ecological Engineering*, vol. 18, pp. 459-465, 2002.
[3] B. C. Braskerud, "Factors affecting nitrogen retention in small constructed wetlands treating agricultural non-point source pollution," *Ecological Engineering*, vol. 18, pp. 459-465, 2002.
[4] J. Vymazal, "Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment," *Ecological Engineering*, vol. 25, no. 5. pp. 478-490, 2005.
[5] C. G. Lee, T. D. Fletcher, and G. Z. Sun, "Nitrogen removal in constructed wetland systems," *Engnieering in Life Sciences*, vol. 9, no. 1, pp. 11-22, 2009.
[6] Y. Ding, X. S. Song, Y. H. Wang *et al*., "Effects of dissolved oxygen and influent COD/N ratios on nitrogen removal in horizontal subsurface flow constructed wetland," *Ecological Engineering*, vol. 46, no. 1, pp. 107-111, 2012.
[7] W. Deboer, P. J. A. K. Gunnewiek, M. Veenhuis *et al*., "Nitrification at low pH by aggregated chemolithotrophic bacteria," *Applied and Environmental Microbiology*, vol. 57, pp. 3600-3604, 1991.
[8] A. Gieseke, U. Purkhold, U. Wagnerx, R. Amann *et al*., "Community structure and activity dynamics of nitrifying bacteria in aphosphate-removing biofilm," *Applied and Environmental Microbiology*, vol. 67, no. 3, pp. 135l-62, 2001.
[9] H. P. Horz, J. H. Rotthauwe, T. Lukow *et al*., "Identification of major subgroups of ammonia-oxidizing bacteria in environmental samples by T-RFLP analysis of amoA PCR products," *Journal of Microbiological Methods*, vol. 39, pp. 197-204, 2000.
[10] J. H. Rotthauwe, K. P. Witzel, and W. Liesack, "The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations," *Applied and Environmental Microbiology*, vol. 63, pp. 4704-4712, 1997.
[11] S. Siripong and B. E. Rittmann, "Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants," *Water Research*, vol. 41, no. 5, pp. 1110-1120, 2007.
[12] B. Mertoglu, B. Calli, B. Inanc *et al*., "Evaluation of in situ ammonia removal in all aerated landfill bioreactor," *Process Biochemistry*, vol. 41, no. 12, pp. 2359-2366, 2006.
[13] H. D. Park and D. R. Noguera, "Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge," *Water Research*, vol. 38, no. 14-15, pp. 3275-3286, 2004.
[14] L. G. Grregory, P. L. Bond, D. J. Richardson *et al*., "Characterization of a nitrate-respiring bacterial community using the nitrate reductase gene (*nar*G)as a functional marker," *Microbiology*, vol. 1, no. 49, pp. 229-237, 2003.
[15] H. Y. Dang, J. Li, X. Zhang *et al*., "Diversity and spatial distribution of amoA-encoding archaea in the deep-sea sediments of the tropical West Pacific Continental Margin," *Applied and Environmental Microbiology*, vol. 106, no. 5, pp. 1482-1493, 2009.
[16] J. Tuomainen, S. Hietanen, J. K. Parinen *et al*. "Baltic sea cyanobacterial bloom contains denitrifieation and nitrification genes,

but has negligible denitrification activity," *FEMS Microbiology Ecology*, vol. 45, pp. 83-96, 2003.
[17] T. Yan, M. W. Fields, L. Wu *et al*., "Molecular diversity and charaeterization of nitrite reductase gene fragments (nirK and nirS) from nitrate-and uranium-contaminated groundwater," *Applied and Environmental Microbiology*, vol. 5, pp. 13-24, 2003.
[18] Y. Sakano, K. D. Piekering, P. F. Strom *et al*., "Spatial distribution of total, ammonia-oxidizing, and denitrifying bacteria in biological wastewater treatment reactors for bioregenerative life support," *Applied and Environmental Microbiology*, vol. 68, pp. 2285-2293, 2002.
[19] S. Tsuneda, R. Miyauehi, T. Ohno *et al*., "Characterization of denitrifying polyphosphate-accumulating organisms in activated sludge based on nitrite reductase gene," *Journal of Bioscience and Bioengineering*, vol. 99, pp. 403-407, 2005.
[20] E. Kandeler, K. Deiglmayr, D. Tscherko *et al*., "Abundance of *nar*G, *nir*S, *nir*K and *nos*Z genes of denitrifying bacteria during primary successions of a glacier foreland," *Applied and Environmental Microbiology*, vol. 72, no. 9, pp. 5957-5962, 2006.
[21] M. D. Wallenstein and R. J. Vilgalys, "Quantitative analyses of nitrogen cycling genes in soils," *Pedobiologia*, vol. 49, no. 6, pp. 665-672, 2005.
[22] J. S. David and J. K. Lee, "Diversity of nitrous oxide reductase (nosZ) genes in continental shelf sediments," *Applied and Environmental Microbiology*, vol. 65, no. 4, pp. 1681-1687, 1999.
[23] APHA, *Standard Methods for the Examination of Water and Wastewater*, 20th ed. Washington, DC. 1998.
[24] C. C. Sommerville, Knightit, W. L. Straube *et al*., "Simple rapid method for direct isolation of nucleic acids from aquatic environments," *Applied and Environmental Microbiology*, vol. 55, no. 3, pp. 548-554, 1989.
[25] Riveraing, Lippek, Gila *et al*., "Method of DNA extraction and application of multiplex polymerase chain reaction to detect toxigenic vibrio cholerae O1 and O139 from aquatic ecosystems," *Environmental Microbiology*, vol. 5, no. 7, pp. 599-606, 2003.
[26] G. A. Kowalchuk, J. R. Stephen, W. Deboer *et al*., "Analysis of ammonia-oxidizing bacteria of the subdivision of the class proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments," *Applied and Environmental Microbiology*, vol. 63, no. 4, pp. 1489-1497, 1997.
[27] A. Hermansson and P. E. Lindgren, "Quantification of ammonia oxidizing bacteria in arable soil by real-time PCR," *Applied and Environmental Microbiology*, vol. 67, no. 2, pp. 972-976, 2001.
[28] J. Geets, M. de Cooman, L. Wittebolle *et al*. "Realtime PCR assay for the simultaneous quantification of nitrifying and denitrifying bacteria in activated sludge," *Applied Microbiology and Biotechnology*, vol. 75, no. 1, pp. 211-221, 2007.
[29] S. Henry, E. Baudoin, J. C. Lopez-Gutierrez *et al*., "Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR," *Journal of Microbiological Methods*, vol. 59, no. 3, pp. 327-335, 2004.
[30] J. Jung, J. Yeom, J. Han *et al*., "Seasonal changes in nitrogen-cycle gene abundances and in bacterial communities in acidic forest soils," *Journal of Microbiology*, vol. 50, no. 3, pp. 365-373, 2012.
[31] G. Maltais-Landry, R. Maranger, J. Brisson *et al*., "Nitrogen transformations and retention in planted and artificially aerated constructed wetlands," *Water Research*, vol. 43, no. 2, pp. 535-545, 2009.
[32] G. Z. Sun and D. Austin, "Completely autotrophic nitrogen-removal over nitrite in lab-scale constructed wetlands: Evidence from a mass balance study," *Chemosphere*, vol. 68, no. 6, pp. 1120-1128, 2007.
[33] A. Sims, J. Horton, S. Gajaraj *et al*., "Temporal and spatial distributions of ammonia-oxidizing archaea and bacteria and their ratio as an indicator of oligotrophic conditions in natural wetlands," *Water Research*, vol. 46, no. 13, pp. 4121-4129, 2012.
[34] H. P. Horz, A. Barbrook, C. B. Field *et al*., "Ammonia oxidizing bacteria respond to multifactorial global change," in *Proc. the National Academy of Sciences of the United States of America*, vol. 101, pp. 15136-15141, 2004.
[35] C. M. Jones and S. Hallin, "Ecological and evolutionary factors underlying global and local assembly of denitrifier communities," *The ISME Journal*, vol. 4, no. 5, pp. 633-641, 2010.
[36] C. W. Knapp, W. K. Dodds, K. C. Wilson *et al*., "Spatial heterogeneity of denitrification genes in a highly homogenous urban stream," *Environmental Science & Technology*, vol. 43, no. 12, pp. 4273-4279, 2009.
[37] T. Ligi, M. Truu, J. Truu *et al*., "Effects of soil chemical characteristics and water regime on denitrification genes (*nir*S, *nir*K and *nos*Z) abundances in a created riverine wetland complex," *Ecological Engineering*, vol. 72, pp. 47-55, 2014.
[38] W. Zhi and G. D. Ji, "Quantitative response relationships between nitrogen transformation rates and nitrogen functional genes in a tidal flow constructed wetland under C/N ratio constraints," *Water Research*, vol. 64, pp. 32-41, 2014.
[39] A. García-Ledó, A. Vilar-Sanz, R. Trias *et al*., "Genetic potential for N_2O emissions from the sediment of a free water surface constructed wetland," *Water Research*, vol. 45, no. 17, pp. 5621-5632, 2011.
[40] M. Truu, J. Juhanson, and J. Truu, "Microbial biomass activity and community composition in constructed wetlands," *Science of the Total Environment*, vol. 407, no. 13, pp. 3958-3971, 2009.

Zhu Ying was born in March 1978. She got her PhD in environmental engineering in 2008 from Tongji University; the master of environmental engineering in 2005 from Shandong University; and the bachelor of environmental engineering in 2000 from Qingdao University. Her research areas are environmental engineering.

She is the head of Environmental Engineering Materials Innovation Team of New Materials Research Institute of Shandong Academy of Sciences. She is a research associate.

Tian Chao was born in September 1989. He got the master of environmental engineering in 2015 from Guilin University of Technology; and the bachelor of environmental engineering in 2012/06 from College of Science & Amp, Arts of Jianghan University. He research areas are environmental engineering.

He is a member of Environmental Engineering Materials Innovation Team of New Materials Research Institute of Shandong Academy of Sciences. He is a research intern.

Tang Houquan got the master of environmental Engineering from Shandong University. His research areas are environmental engineering. He is an environmental engineer.

Shao Yanqiu was born in June 1985. Her education experience is as follows: she got the PhD in environmental engineering in 2015 from University of Chinese Academy of Sciences; the master of environmental engineering in 2011 from Northwest A & F University; and the bachelor of environmental engineering in 2008. Her research areas are environmental engineering.

She is a member of Environmental Engineering Materials Innovation Team of New Materials Research Institute of Shandong Academy of Sciences. She is an assistant researcher.

Chen Qingfeng's research areas are study on key technologies, wetland ecological process and surface source pollution control technology in the construction of multi natural river. He is a PhD in environmental engineering, research associate.