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Nitrification intensity and ammonia-oxidizing bacteria and archaea in different wetland plant rhizosphere soils

Yan Chunni Huang Juan Yang Sisi Cao Chong Peng Cheng Li Runqing

(School of Civil Engineering, Southeast University, Nanjing 210096, China)

Abstract: In order to explore the nitrogen removal process in constructed wetlands (CWs), the moisture, ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrification intensity in three wetland plant rhizosphere soils (*Acorus calamus, Typha orientalis, Iris pseudacorus*) were investigated at a relatively normal temperature range of 15 to 25 ℃. The relative abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were also achieved using fluorescence in situ hybridization (FISH). It is found that *T. orientalis* achieves the highest nitrification intensity of 2.03 mg/(h·kg) while the second is *I. pseudacorus* (1.74 mg/(h·kg)), and followed by *A. calamus* (1.65 mg/(h·kg)) throughout the experiment. FISH reveals that the abundance of bacteria (10^{10} g^{-1} wet soil) is higher than that of archaea (10^9 g^{-1} wet soil), and AOB are the dominant bacteria in the ammonia oxidation process. The abundance of AOB in the rhizosphere soils from high to low are *T. orientalis* (1.88×10^{10} g^{-1}), *I. pseudacorus* (1.23×10^{10} g^{-1}), *A. calamus* (5.07×10^9 g^{-1}) while the abundance of AOA from high to low are *I. pseudacorus* (4.00×10^9 g^{-1}), *A. calamus* (3.52×10^9 g^{-1}), *T. orientalis* (3.48×10^9 g^{-1}). The study provides valuable evidence of plant selection for nitrogen removal in CWs.

Key words: wetland plant rhizosphere; nitrification intensity; ammonia-oxidizing bacteria; ammonia-oxidizing archaea; fluorescence in situ hybridization

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Constructed wetlands (CWs), which are mainly comprised of substrate plants, microorganisms and water, exert a synergistic effect involving physical, chemical and biological mechanisms to remove contaminants and improve the water quality[1-2]. At present, CWs have been widely used to treat various wastewater and restore polluted water bodies[3-4].

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Biographies: Yan Chunni (1989—), female, master; Huang Juan (corresponding author), female, doctor, professor, 101010942@seu.edu.cn.

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Plants are important organic components of CWs for wastewater treatment, which can not only filter and remove suspended solids but also absorb heavy metals from wastewater[5]. The wetland plant also has the functions of oxygen transport and diffusion, hydraulic transmission enhancement and maintenance[6]. For microorganisms, in addition to providing an adhesion interface, the wetland plant can improve microbial activity and ecological distribution by producing root exudates[7].

Nitrification and denitrification promoted by microorganisms are the main nitrogen removal mechanisms in CWs[8]. Nitrification is the process of ammonia oxidation, which includes two stages of ammonia oxidation and nitrite oxidation. Ammonia oxidation, also called sub-nitrification, is not only the first reaction step but also the limited step of nitrification[9]. Studies have shown that ammonia-oxidizing microorganisms are dominant in the ammonia oxidation process. Ammonia-oxidizing bacteria (AOB) were generally regarded as the executors of ammonia oxidation. However, with the development of molecular biology techniques, the researchers have found that ammonia-oxidizing archaea (AOA) also have the capability of ammonia oxidation[10]. Fan et al.[11] inferred that AOA may be more significant than AOB in depleting nutrients in CWs. Caffrey et al.[9] reported that AOA were widely distributed in the soil, hot springs, and especially in marine ecosystems. Much work has been done to study ammonia-oxidizing microorganisms in different ecosystems (rivers[9], oceans[12], lakes[13], other water bodies and sediments[14], grasslands[15], forests[16], rice paddies[17]), but less is known about the ammonia oxidizing microorganisms in CWs.

In this study, ammonia-oxidizing microorganisms in wetland plant rhizosphere soil (*Acorus calamus, Typha orientalis, Iris pseudacorus*) at relatively normal temperatures (15 to 25 ℃) were investigated by measuring moisture, ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrification intensity, along with the relative abundance of AOB and AOA, which was achieved using fluorescence in situ hybridization (FISH). All of these studies aim to provide valuable references for understanding the relationship between ammonia-oxidizing microorganisms and the nitrogen removal mechanism in CWs.

1 Materials and Methods

1.1 Experimental materials

The perennial and emergent water plants of *A. calamus*, *T. orientalis* and *I. pseudacorus* are quite common in Jiangsu Province, China. Due to their strong reproductive capacity and adaptability, they are commonly used in CWs. Therefore, *A. calamus*, *T. orientalis* and *I. pseudacorus* were chosen as test plants in this study. The plants were purchased from Shuyang, Jiangsu Province, and then the same healthy growing plants were chosen to conduct the experiment.

1.2 Experimental design and operation

After being cleaned, different wetland plants were respectively cultivated in three identical devices (The height and diameter of devices were 0.6 m and 0.35 m) which were provided with a 0.2-m-thick gravel layer topped with the same soil to cover the plant root. To meet nutritional conditions for growth, the wetland plants were irrigated every 3 d with a special nutrient solution. The experiment lasted for 45 d. The plants were carefully removed from the system every 7 d, followed by shaking of the roots and obtaining the soil adhered to the root surface, and the air temperature and soil temperature were also recorded. Then, part of soil was used to measure moisture, NH_4^+-N, NO_3^--N and nitrification intensity, and the other part after being pre-treated, was used to detect the abundance of total bacteria, total archaea, AOB and AOA by FISH.

1.3 Determination of physical and chemical properties of soil

Physical and chemical properties of the soil were measured following national environmental standards. The dry method, Nessler's reagent spectrophotometry and phenol two sulfonic acid spectrophotometry were used for moisture, NH_4^+-N and NO_3^--N, respectively. The nitrification intensity was determined by the method of increasing the NO_3^--N content before and after cultivation[18].

1.4 Fluorescence in situ hybridization (FISH) technology procedure

1) Slides and sample pretreatment. After ultrasonic cleaning for adhesion, the slides were soaked for 30 s with the working solution of v(APES)∶v(acetone) = 1∶50 and then preserved in the sterilization box at 4 ℃. For the samples 'pretreatment, after the soil collected in polyethylene pipe, distilled water was added before the Vortex to obtain the soil-microorganisms mixture, 4% paraformaldehyde with volume ratio 1∶1 was then added and fixed at 4 ℃ for 24 h. The treated sample was washed with a phosphate buffer solution (PBS) and centrifuged at 1×10^4 r/min twice. The supernatant was then discarded, and suspension was finally stored in 1 mL of a mixed liquor of v(PBS)∶v(ethanol) = 1∶1.

2) Coating sample, heat fixation and dehydration[19–20]. Before heated at 37 ℃ for 2 h, 10 μL of the treated sample was uniformly coated on the above slide with 10 mm × 10 mm. The sample was dehydrated with an ethanol gradient (50%, 80% and 96%) at room temperature for 3 min, followed by natural drying.

3) Hybridization and staining[21–22]. After putting the hybridization solution containing the probe on the sample, the sample was hybridized at a constant temperature of 46 ℃ (total bacteria for 5 h, the remaining microorganisms for 2 to 3 h), followed by elution for 20 min. 10 uL of 4′, 6-diamidino-2-phenylindole (DAPI) which was diluted with methanol to 2 μL/mL was then coated onto the sample before staining for 20 min. The rinsed and dried sample was then sealed with cover clips. This step should be carried out in a dark environment.

4) Microscopic examination and counting. An OLYMPUS-BX41 fluorescence microscope was used to observe the 20 fields of vision. Count was conducted using Image-Pro Plus 6.0 software. Each sample was counted twice, and the average value was used to calculate the abundance of microorganisms per gram of soil.

Targetting microorganisms in the experiment, the probe sequence and the corresponding hybridization conditions are shown in Tab. 1. The probe was synthesized by the Shanghai Biological Engineering Company and marked by 5'Cy3. The excitation light was red.

Tab. 1 16S rRNA oligonucleotide probe sequence

name	Probe sequence (5'-3')	Formamide concentration/%
Total bacteria-EUB338	GCTGCCTCCCGTAGGAGT	20[23]
Archaea-ARCH915	GTGCTCCCCCGCCAATTCCT	35[24]
AOB-NSO190	CGATCCCCTGCTTTTCTCC	55[21, 25]
AOA-CREN537	TGACCACTTGAGGTGCTG	20[26]

Note: The probe concentration was 50 ng/uL.

1.5 Data processing

Pearson correlation analysis was used to characterize the effect of the soil micro-environment on nitrification. Statistical analyses were performed using SPSS Statistics 19.0 for Windows. The image was conducted with Origin 9.0.

2 Results and Discussion

2.1 Nitrification intensity

Nitrification is always a limited step of nitrogen removal due to insufficient dissolved oxygen in CWs[27]. The performance of nitrogen removal is an important factor for further applications of constructed wetlands[28]. As seen in Fig. 1, for nitrification intensity, *T. orientalis* achieved

the highest nitrification intensity (2.03 mg/(h · kg)) while the second was *I. pseudacorus* (1.74 mg/(h · kg)), followed by *A. calamus* (1.65 mg/(h · kg)) throughout the experiment. It indicates that wetland plant species were one of the decisive factors in the rhizosphere soil nitrification intensity. Although the plants have the functions of oxygen transfer and diffusion[6], the capacity of oxygen transport depends on the plant species[29]. More importantly, different wetland plants further affect the development of bacterial community structure by transporting oxygen and releasing organic carbon[30], which causes differences in nitrification intensity.

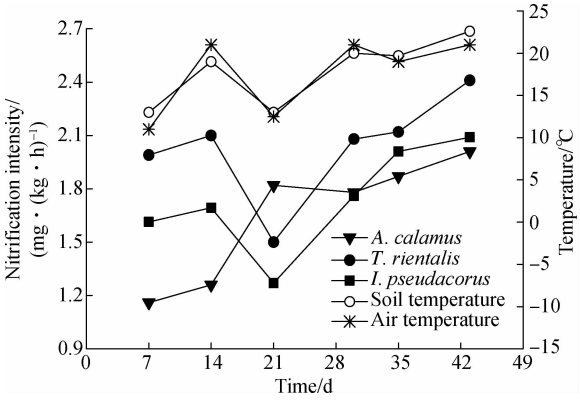

Fig. 1 Nitrification intensity of the plant rhizosphere soil

In addition, the variation in nitrification intensity in all wetlands were basically consistent with the temperature. Early on the experiment (0 to 14 d), the soil temperature increased with the increase of air temperature, and correspondingly, the nitrification intensity showed the same trend. During the following experimental period (14 to 21 d), there was a sharp decline in temperature, significantly inhibiting the nitrification intensities of *T. rientalis* and *I. pseudacorus*, while no effect was found on *A. calamus*. After that, due to the increase in temperature, the nitrification intensity increased steadily and no recurrent case was observed again. The effect of the temperature on the nitrification intensity was obvious and this is in accordance with Huang et al[18].

2.2 Ammonia oxidizing microorganisms in rhizosphere

DAPI staining can eliminate the interference of soil particles and make the microscopy results more accurate. The effect of bacterial DAPI staining is shown in Fig. 2 (a). The fluorescence microscopic images under FISH preliminarily demonstrated that the abundance of AOB was higher than that of AOA (see Figs. 2(b) and (c)). After microscopic counting, the abundances of AOB and AOA with extended time are shown in Fig. 3 and Fig. 4, respectively.

The three plant rhizosphere soils varied greatly in the abundance of AOB and less in the abundance of AOA dur-

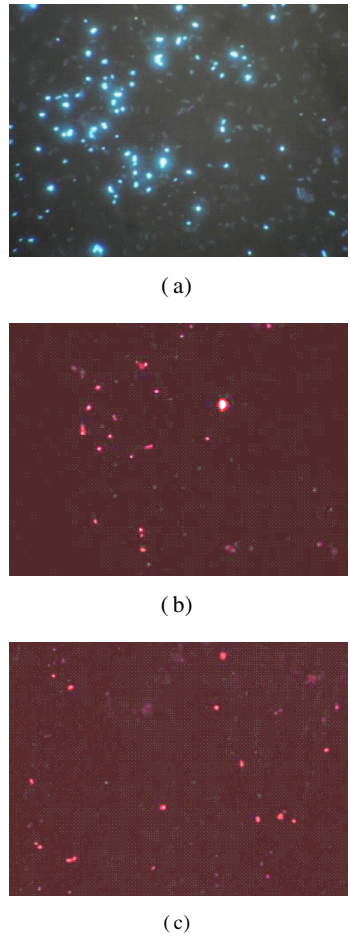

Fig. 2 AOB and AOA in the plant rhizosphere soils under FISH. (a) DAPI staining of bacteria; (b) AOB; (c) AOA

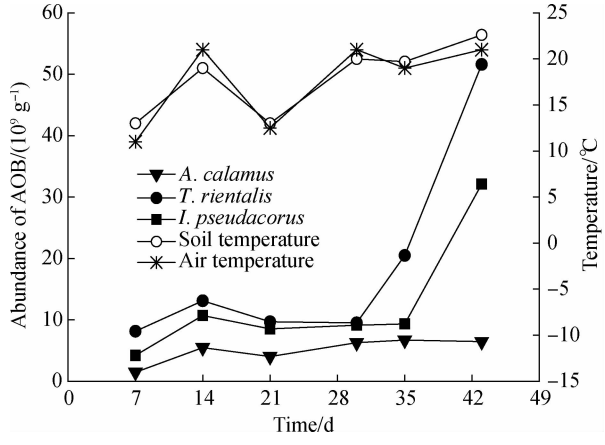

Fig. 3 The abundance of AOB in the plant rhizosphere soil

ing the experiment (see Fig. 3 and Fig. 4). The abundance of AOB in the three plant rhizosphere soils was much higher than that of AOA, revealing that AOB were the dominant microorganisms in the ammonia-oxidizing process. Although AOB and AOA widely cohabit in ecosystems, the growth environment of AOA is much stricter than that of AOB, which leads to significant differences in their relative abundance and contribution. Prosser et al.[31] reported that AOA had a close relationship with ammonia and greater sensitivity to inhibition with a high

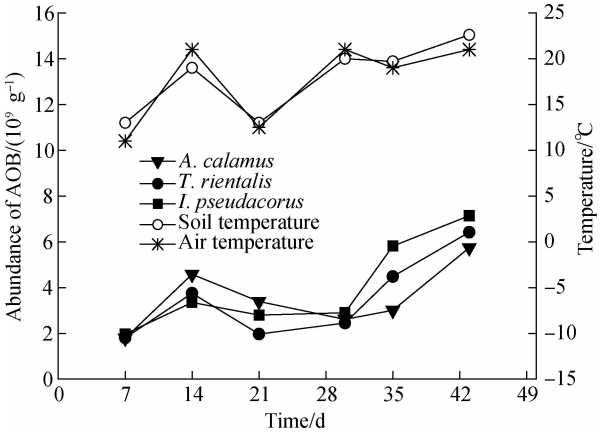

Fig. 4 The abundance of AOA in the plant rhizosphere soil

ammonia concentration. AOA was generally predominant in acidic soils[32−33]. The abundance of AOB from high to low was *T. orientalis, I. pseudacorus, A. calamus*, which was consistent with the sorting of the nitrification intensity. The phenomena demonstrated that the *T. orientalis* rhizosphere soil environment was more in favor of nitrogen removal.

The AOB abundance of *T. orientalis* was the highest and it increased the most quickly with the increase in temperature, revealing that *T. orientalis* rhizosphere at a relatively high temperature was more suitable for ammonia-oxidizing microbial growth. On the other hand, *A. calamus* had the lowest abundance of AOB, compared to the significant increase in abundance of AOB with *T. orientalis* and *I. pseudacorus*. The differences of ammonia-oxidizing microbial abundance in the three plant rhizosphere soils indicated that the wetland plant species had a significant impact on ammonia-oxidizing microbial abundance. This may be due to the fact that AOB are aerobic, while plants species can cause variations in oxygen release[34].

The trends of ammonia oxidizing microbial abundance in different plant rhizosphere soils were basically consistent with the temperature (see Fig. 3 and Fig. 4), which revealed that the effect of temperature on ammonia-oxidizing microbial abundance was also significant. Similar trends of the nitrification intensity with the temperature indicated that ammonia-oxidizing microorganisms decided nitrification intensity and indeed played an important role in the process of NH_4^+-N transformation[35].

2.3 Relative abundance of ammonia-oxidizing microorganisms

The average values of bacteria, archaea, AOB and AOA throughout the experimental period are shown in Fig. 5. Error bars represent the standard deviations (n = 6). For *I. pseudacorus*, *A. calamus* and *T. orientalis*, the ratios of archaea and bacteria were approximately 1∶11, 1∶6, 1∶17, respectively, indicating that the abundance of archaea was significantly less than that of bacteria. Similarly, the proportions of AOA and AOB were 1∶3, 1∶1.5, 1∶5.4 for three wetlands, respectively, indicating that the abundance of AOB was significantly higher than that of AOA. Strauss et al.[36] reported that AOB were often found to dominate in soil. Although AOB accounted for 11.7%, 9.4% and 14.8% of the total bacteria which was the dominant microorganism in the ammonia oxidation process, AOA accounted for 41.1%, 38.0% and 47.0% of archaea for tree wetlands, respectively, revealing that the contribution of AOA to ammonia oxidation also cannot be ignored.

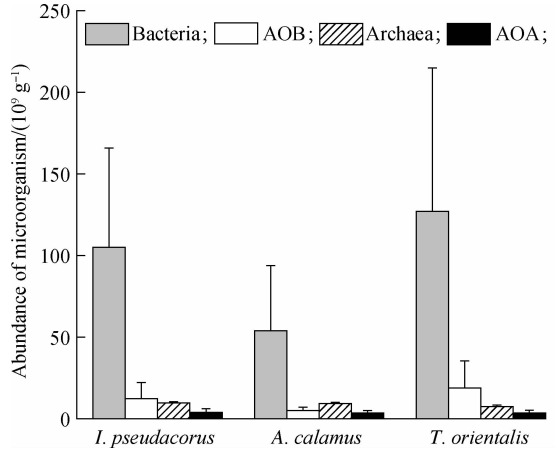

Fig. 5 Relative abundance of ammonia-oxidizing microorganisms in plant rhizosphere soils

Comparing the abundance of different wetland plants, bacteria from high to low was *T. orientalis* (1.27×10^{11} g^{-1}), *I. pseudacorus* (1.05×10^{11} g^{-1}), *A. calamus* (5.39×10^{10} g^{-1}), while archaea was *I. pseudacorus* (9.74×10^9 g^{-1}), *A. calamus* (9.26×10^9 g^{-1}), *T. orientalis* (7.40×10^9 g^{-1}). Meanwhile, the abundance of AOB from high to low was *T. orientalis* (1.88×10^{10} g^{-1}), *I. pseudacorus* (1.23×10^{10} g^{-1}), *A. calamus* (5.07×10^9 g^{-1}), while the abundance of AOA from high to low was *I. pseudacorus* (4.00×10^9 g^{-1}), *A. calamus* (3.52×10^9 g^{-1}), *T. orientalis* (3.48×10^9 g^{-1}). The results indicate that the rhizosphere environment of *T. orientalis* is more suitable for bacteria.

2.4 Effects of soil micro-environment on nitrification intensity and ammonia-oxidizing microorganisms

Some indicators had greater impacts on the wetland plant rhizosphere soil nitrification intensity. The rhizosphere soil nitrification intensities of *I. pseudacorus* and *T. orientalis* had significantly positive correlations with soil temperature ($R = 0.862$, $P = 0.027$; $R = 0.833$, $P = 0.039$). In addition, the rhizosphere soil nitrification intensities of *A. calamus* and *T. orientalis* were also significantly positively correlated with NO_3^--N content ($R = 0.868$, $P = 0.025$; $R = 0.813$, $P = 0.049$). However, the influences of moisture content and NH_4^+-N content on nitrification intensity were not evident.

Tab. 2 The correlation of soil micro-environmental indicators and nitrification in the plant rhizosphere soil

Wetland plant	Correlation	Soil temperature		Moisture content		NH_4^+-N content		NO_3^--N content		Nitrification intensity	
		R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
I. pseudacorus	Nitrification intensity	0.862*	0.027	−0.379	0.458	0.156	0.767	0.454	0.366		
	AOB	0.696	0.124	−0.051	0.923	0.015	0.977	0.686	0.133	0.612	0.197
	AOA	0.769	0.074	−0.362	0.480	−0.039	0.941	0.561	0.247	0.815*	0.048
A. calamus	Nitrification intensity	0.502	0.310	0.439	0.384	0.316	0.541	0.868*	0.025		
	AOB	0.879*	0.021	0.266	0.611	0.636	0.125	0.626	0.184	0.692	0.128
	AOA	0.631	0.179	0.239	0.648	0.395	0.439	0.256	0.625	0.392	0.442
T. orientalis	Nitrification intensity	0.833*	0.039	0.082	0.862	−0.130	0.782	0.813*	0.049		
	AOB	0.688	0.131	0.443	0.132	−0.464	0.397	0.530	0.280	0.685	0.133
	AOA	0.711	0.113	−0.109	0.837	−0.177	0.738	0.354	0.491	0.508	0.303

Notes: *R* represents the correlation coefficient; *P* represents test value; * represents significant correlation at 0.05 level.

The correlations of ammonia-oxidizing microbial abundance and soil temperature indicated that only the AOB abundance in *A. calamus* rhizosphere soil had a significantly positive correlation with soil temperature ($R = 0.879$, $P = 0.021$), while no obvious correlations were observed in *I. pseudacorus* and *T. orientalis* systems. A possible explanation may be that the impact of temperature on ammonia-oxidizing microbial growth and metabolism gradually lost the dominant position under moderate temperatures, and other factors began to become highlighted, making the effect of the environment on ammonia oxidation more complex. As the temperature increased, the activity of ammonia-oxidizing microorganisms was enhanced, while other factors were also affected, such as the reduction in the concentration of dissolved oxygen[37]. Meanwhile, the rapid and abundant reproduction of ammonia-oxidizing microorganisms in soil also leads to a reduction in oxygen concentration, thus resulting in the inhibition of microbial activity. In addition, nitrification was a process that produced acid which can reduce soil pH value. With the increase of soil nitrification intensity, the living environment of ammonia-oxidizing microorganisms was affected, causing the inhibition of the abundance and metabolic activity of ammonia-oxidizing microorganisms.

The correlations of ammonia-oxidizing microbial abundance and nitrification intensity revealed that the AOA abundance in the *I. pseudacorus* rhizosphere had a significantly positive correlation with nitrification intensity ($R = 0.815$, $P = 0.048$), which reflected the unneglectable role of AOA in the ammonia oxidation reaction.

3 Conclusions

1) The wetland plant species had an important impact on ammonia-oxidizing microbial abundance. *T. orientalis* achieved the highest nitrification intensity (2.03 mg/(h·kg)) while the second was *I. pseudacorus* (1.74 mg/(h·kg)), followed by *A. calamus* (1.65 mg/(h·kg)).

2) The rhizosphere environment of *T. orientalis* was more suitable for the growth of bacteria. The abundances of microorganisms in the rhizosphere soils from high to low were bacteria, AOB, archaea and AOA.

3) AOB was the dominant microorganism in the ammonia oxidation process. The abundance of AOB was nearly an order of magnitude higher than that of AOA. The abundance of AOB from high to low was *T. orientalis* (1.88×10^{10} g^{-1}), *I. pseudacorus* (1.23×10^{10} g^{-1}), *A. calamus* (5.07×10^{9} g^{-1}) while the abundance of AOA from high to low was *I. pseudacorus* (4.00×10^{9} g^{-1}), *A. calamus* (3.52×10^{9} g^{-1}), *T. orientalis* (3.48×10^{9} g^{-1}).

References

[1] Saeed T, Sun G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media [J]. *Journal of Environmental Management*, 2012, **112**: 429−448. DOI: 10.1016/j.jenvman.2012.08.011.

[2] Gikas G D, Tsihrintzis V A. A small-size vertical flow constructed wetland for on-site treatment of household wastewater [J]. *Ecological Engineering*, 2012, **44**: 337−343. DOI: 10.1016/j.ecoleng.2012.04.016.

[3] Wu S, Kuschk P, Brix H, et al. Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review [J]. *Water Research*, 2014, **57**: 40−55. DOI: 10.1016/j.watres.2014.03.020.

[4] Badhe N, Saha S, Biswas R, et al. Role of algal biofilm in improving the performance of free surface, up-flow constructed wetland [J]. *Bioresource Technology*, 2014, **169**: 596−604. DOI: 10.1016/j.biortech.2014.07.050.

[5] Zeng F, Chen S, Miao Y, et al. Changes of organic acid exudation and rhizosphere pH in rice plants under chromium stress [J]. *Environmental Pollution*, 2008, **155**(2): 284−289. DOI: 10.1016/j.envpol.2007.11.019.

[6] Peralta R M, Ahn C, Gillevet P M. Characterization of

soil bacterial community structure and physicochemical properties in created and natural wetlands [J]. *Science of the Total Environment*, 2013, **443**: 725 − 732. DOI: 10.1016/j.scitotenv.2012.11.052.

[7] Fang C W, Radosevich M, Fuhrmann J J. Characterization of rhizosphere microbial community structure in five similar grass species using FAME and BIOLOG analyses [J]. *Soil Biology & Biochemistry*, 2001, **33**(4): 679 − 682. DOI: 10.1016/s0038-0717(00)00215-7.

[8] Vacca G, Wand H, Nikolausz M, et al. Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands [J]. *Water Research*, 2005, **39**(7): 1361 − 1373. DOI: 10.1016/j.watres.2005.01.005.

[9] Caffrey J M, Bano N, Kalanetra K, et al. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia [J]. *The ISME Journal*, 2007, **1**: 660 − 662. DOI: 10.1038/ismej.2007.79.

[10] Trias R, Ruiz-Rueda O, Garcia-Lledo A, et al. Emergent macrophytes act selectively on ammonia-oxidizing bacteria and archaea [J]. *Applied and Environmental Microbiology*, 2012, **78**(17): 6352 − 6356. DOI: 10.1128/aem.00919-12.

[11] Fan L, Chen H, Hsieh H, et al. Comparing abundance, composition and environmental influences on prokaryotic ammonia oxidizers in two subtropical constructed wetlands [J]. *Ecological Engineering*, 2016, **90**: 336 − 346. DOI: 10.1016/j.ecoleng.2016.01.034.

[12] Lam P, Jensen M M, Lavik G, et al. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2007, **104**(17): 7104 − 7109. DOI: 10.1073/pnas.0611081104.

[13] Jiang H C, Dong H L, Yu B S, et al. Diversity and abundance of ammonia-oxidizing archaea and bacteria in Qinghai Lake, Northwestern China [J]. *Geomicrobiology Journal*, 2009, **26**(3): 199 − 211. DOI: 10.1080/01490450902744004

[14] Bollmann A, Bullerjahn G S, Mckay R M. Abundance and diversity of ammonia-oxidizing archaea and bacteria in sediments of trophic end members of the Laurentian Great Lakes, Erie and Superior [J]. *Plos One*, 2014, **9**(5): e97068. DOI: 10.1371/journal.pone.0097068

[15] Di H J, Cameron K C, Shen J P, et al. A lysimeter study of nitrate leaching from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea [J]. *Soil Use and Management*, 2009, **25**(4): 454 − 461. DOI: 10.1111/j.1475-2743.2009.00241.x.

[16] Laverman A M, Speksnijder A G, Braster M, et al. Spatiotemporal stability of an ammonia-oxidizing community in a nitrogen-saturated forest soil [J]. *Microbial Ecology*, 2001, **42**(1): 35 − 45. DOI: 10.1007/s002480000038.

[17] Wang Y, Ke X, Wu L, et al. Community composition of ammonia-oxidizing bacteria and archaea in rice field soil as affected by nitrogen fertilization [J]. *Systematic and Applied Microbiology*, 2009, **32**(1): 27 − 36. DOI: 10.1016/j.syapm.2008.09.007.

[18] Huang J, Cai W S, Zhong Q S, et al. Influence of temperature on micro-environment, plant eco-physiology and nitrogen removal effect in subsurface flow constructed wetland [J]. *Ecological Engineering*, 2013, **60**: 242 − 248. DOI: 10.1016/j.ecoleng.2013.07.023.

[19] Lorenzen J, Larsen L, Kjaer T, et al. Biosensor determination of the microscale distribution of nitrate, nitrate assimilation [J]. *Applied and Environmental Microbiology*, 1998, **64**(9): 3264 − 3269.

[20] Domingues M R, Araujo J C, Varesche M B, et al. Evaluation of thermophilic anaerobic microbial consortia using fluorescence in situ hybridization (FISH) [J]. *Water Science and Technology*, 2002, **45**(10): 27 − 33.

[21] Amann R, Fuchs B M. Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques [J]. *Nature Reviews Microbiology*, 2008, **6**(5): 339 − 348. DOI: 10.1038/nrmicro1888.

[22] WiléB M, Onuki M, Hermansson M, et al. Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability [J]. *Water Research*, 2008, **42**(8/9): 2300 − 2308. DOI: 10.1016/j.watres.2007.12.013.

[23] Pernthaler A, Pernthaler J, Amann R. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria [J]. *Applied and Environmental Microbiology*, 2002, **68**(6): 3094 − 3101. DOI: 10.1128/aem.68.6.3094-3101.2002.

[24] Pichon D, Gaia V, Norman M D, et al. Phylogenetic diversity of epibiotic bacteria in the accessory nidamental glands of squids (Cephalopoda: Loliginidae and Idiosepiidae) [J]. *Marine Biology*, 2005, **147**(6): 1323 − 1332. DOI: 10.1007/s00227-005-0014-5.

[25] Schramm A, De B D, Gieseke A, et al. Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm [J]. *Environmental Microbiology*, 2000, **2**(6): 680 − 686. DOI: 10.1046/j.1462-2920.2000.00150.x.

[26] Teira E, Reinthaler T, Pernthaler A, et al. Combining catalyzed reporter deposition-dluorescence in situ hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean [J]. *Applied and Environmental Microbiology*, 2004, **70**(7): 4411 − 4414. DOI: 10.1128/AEM.70.7.4411-4414.2004.

[27] Chang J J, Wu S Q, Dai Y R, et al. Treatment performance of integrated vertical-flow constructed wetland plots for domestic wastewater [J]. *Ecological Engineering*, 2012, **44**: 152 − 159. DOI: 10.1016/j.ecoleng.2012.03.019.

[28] Vymazal J. The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: A review of a recent development [J]. *Water Research*, 2013, **47**(14): 4795 − 4811. DOI: 10.1016/j.watres.2013.05.029.

[29] Wang R, Baldy V, Perissol C, et al. Influence of plants on microbial activity in a vertical-downflow wetland system treating waste activated sludge with high organic matter concentrations [J]. *Journal of environmental management*, 2012, **95**(Suppl): 158 − 164. DOI: 10.1016/j.jenvman.2011.03.021.

[30] Deng H, Ge L, Xu T, et al. Analysis of the metabolic utilization of carbon sources and potential functional diver-

sity of the bacterial community in lab-scale horizontal subsurface-flow constructed wetlands [J]. *Journal of Environmental Quality*, 2011, **40**(6): 17301736. DOI: 10.2134/jeq2010.0322.

[31] Prosser J I, Nicol G W. Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation [J]. *Trends in Microbiology*, 2012, **20**(11): 523 - 531. DOI: 10.1016/j.tim.2012.08.001.

[32] Yao H, Campbell C D, Chapman S J, et al. Multi-factorial drivers of ammonia oxidizer communities: Evidence from a national soil survey [J]. *Environmental Microbiology*, 2013, **15**(9): 2545 - 2556. DOI: 10.1111/1462-2920.12141.

[33] Cubry-Rangin C, Nicol G W, Prosser J I. Archaea rather than bacteria control nitrification in two agricultural acidic soils [J]. *Fems Microbiology Ecology*, 2010, **74**(3): 566 - 574. DOI: 10.1111/j.1574-6941.2010.00971.x.

[34] Vymazal J. Plants used in constructed wetlands with horizontal subsurface flow: A review [J]. *Hydrobiologia*, 2011, **674**(1): 133 - 156. DOI: 10.1007/s10750-011-0738-9.

[35] Oved T, Shaviv A, Goldrath T, et al. Influence of effluent irrigation on community composition and function of ammonia-oxidizing bacteria in soil [J]. *Applied and Environmental Microbiology*, 2001, **67**(8): 3426 - 3433. DOI: 10.1128/aem.67.8.3426-3433.2001.

[36] Strauss S L, Reardon C L, Mazzola M. The response of ammonia-oxidizer activity and community structure to fertilizer amendment of orchard soils [J]. *Soil Biology and Biochemistry*, 2014, **68**: 410 - 418. DOI: 10.1016/j.soilbio.2013.10.016.

[37] Stefanakis A I, Tsihrintzis V A. Effects of loading, resting period, temperature, porous media, vegetation and aeration on performance of pilot-scale vertical flow constructed wetlands [J]. *Chemical Engineering Journal*, 2012, **181**: 416 - 430. DOI: 10.1016/j.cej.2011.11.108.

不同湿地植物根际硝化作用强度及氨氧化细菌和古菌

闫春妮 黄 娟 杨思思 曹 冲 彭 程 李润青

(东南大学土木工程学院, 南京 210096)

摘要: 为探究人工湿地脱氮进程,选用菖蒲(Acorus calamus)、香蒲(Typha orientalis)、黄菖蒲(Iris pseudacorus)三种湿地植物,于常温域 15 ~ 25 ℃下测定其根际土壤含水率、氨氮、硝氮、硝化作用强度,并采用荧光原位杂交(FISH)技术考察植物根际氨氧化细菌(AOB)和氨氧化古菌(AOA)的丰度变化规律. 结果表明,试验期间香蒲根际土壤的硝化作用强度最高,平均为 2.03 mg/(h·kg),其次为黄菖蒲 1.74 mg/(h·kg)和菖蒲 1.65 mg/(h·kg). FISH 技术表明湿地植物根际土壤中的细菌数量(数量级为 10^{10})高于古菌(数量级为 10^{9}),AOB 为氨氧化过程的优势菌群. 3 种湿地植物根际 AOB 的数量从高到低(以湿土计)依次为:香蒲(1.88×10^{10} g^{-1})、黄菖蒲(1.23×10^{10} g^{-1})、菖蒲(5.07×10^{9} g^{-1});AOA 的数量从高到低(以湿土计)依次为:黄菖蒲(4.00×10^{9} g^{-1})、菖蒲(3.52×10^{9} g^{-1})、香蒲(3.48×10^{9} g^{-1}). 该试验结论为人工湿地脱氮的植物选择提供了有价值的参考.

关键词: 湿地植物根际;硝化作用强度;氨氧化细菌;氨氧化古菌;荧光原位杂交

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