

Effects of hydraulic loading rate and aeration mode on nitrogen removal and nitrogen functional gene abundances in subsurface wastewater infiltration systems

Yafei Sun, Jing Pan, Shiyue Qi and Hexin Fei

ABSTRACT

Matrix DO, nitrogen removal and nitrogen functional gene abundances in two artificial aeration modes including continuous aeration (CA) and intermittent aeration (IA) in subsurface wastewater infiltration systems (SWISs) under different hydraulic loading rates (HLRs) were investigated. Aeration not only successfully created aerobic conditions at 50 cm depth, but also did not change anoxic or anaerobic conditions at 80 and 110 cm depths. Meanwhile, aeration significantly enhanced COD, $\text{NH}_4^+\text{-N}$, TN removal and the enrichment of nitrogen removal functional genes (*amoA*, *nxrA*, *napA*, *narG*, *nirK* and *qnorB*) compared to the non-aerated SWIS, especially for high HLRs. IA SWIS (79.7%–85.8%) had a better performance on TN removal contrasted with CA SWIS (73.8%–82.2%) when the HLRs ranged from 0.06 to 0.3 $\text{m}^3/(\text{m}^2 \text{d})$. Intermittent aeration is a sensible strategy to achieve high HLR, good nitrogen removal performance and comparatively low operation cost for SWISs.

Key words | continuous aeration, denitrification, intermittent aeration, nitrification

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INTRODUCTION

Subsurface wastewater infiltration system (SWIS) have obtained increasing attention in recent years due to its high organics and phosphorus removal performance, low construction and operation cost (Li *et al.* 2011; Pan *et al.* 2016). However, nitrogen removal was quite low and pollutant removal decreased obviously under high hydraulic loading rate (HLR) (0.125 $\text{m}^3/(\text{m}^2 \text{d})$), which remained as major challenges for SWISs (Li *et al.* 2012; Pan *et al.* 2015; Wang *et al.* 2015a). Insufficient supply of oxygen is the major cause of limited nitrogen removal in SWISs (Song *et al.* 2016; Yang *et al.* 2016). Artificial aeration has been regarded as an effective way to control DO (Fan *et al.* 2013; Song *et al.* 2016). Many studies have reported that artificial aeration was the effective way to achieve high pollutants removal when dealing with high strength wastewater (Wu *et al.* 2015; Song *et al.* 2016). Fan *et al.* (2013) concluded that intermittent aeration strategy could enhance organics and nitrogen removal in subsurface flow constructed wetlands. Wu *et al.* (2015) evaluated the removal performances of organic pollutants and nitrogen in vertical flow constructed wetlands with and without intermittent

aeration fed with different strengths of influent and Song *et al.* (2016) investigated the effects of COD/N ratios on pollutants removal in the SWISs with/without intermittent aeration. Microbiological nitrification and denitrification are the main mechanisms responsible for nitrogen removal in a SWIS (Li *et al.* 2011, 2012), which involve ammonia monooxygenase (*amoA*), nitrite oxidoreductase (*nxrA*), periplasmic nitrate reductase (*napA*), membrane-bound nitrate reductase (*narG*), nitrite reductase (*nirK/nirS*), nitric oxide reductase (*qnorB*) and nitrous oxide reductase (*nosZ*) functional genes (Ji *et al.* 2012). Unfortunately, so far there have been no reports about the effects of HLR and aeration mode on nitrogen removal and nitrogen functional gene abundances in SWISs.

The main objectives of this paper were: (1) to investigate the characteristics of DO profiles along the SWISs under different aeration modes and HLRs; (2) to evaluate nitrogen removal performance and nitrogen functional genes (*amoA*, *nxrA*, *napA*, *narG*, *nirK*, *nirS*, *qnorB* and *nosZ*) under different aeration modes and HLRs; (3) to identify optimal operation schemes for nitrogen removal.

MATERIAL AND METHODS

System description and operation

Three lab-scale parallel SWISs made of plexiglass vertical tubes (120 cm in height and 50 cm in diameter) were operated under different conditions in a greenhouse. As shown in Figure 1, they were composed of non-aerated (NA) units, continuous aerated (CA) units, and intermittent aerated (IA) units. CA and IA SWISs were composed of aerated units which consisted of air compressors, air tubes and micro-bubble diffusers at a depth of 40 cm. Distributing pipe was installed at 50 cm depth below the surface in each infiltration system. Micro-bubble diffuser and distributing pipe were surrounded by 5 cm of gravel (10–20 mm, diameter) to protect clogging and diffuse air. 10 cm of gravel (10–20 mm, diameter) was prepared at the bottom to support infiltration system and evenly distributed the treated water. The treated wastewater was collected at the bottom of the column near the outlet. DO electrodes were buried at the

midpoint of SWISs at 50, 80 and 110 cm depths to monitor matrix DO of pilot systems. Each infiltration system was filled with the same matrix that consisted of 80% brown soil and 20% coal slag by weight. The brown soil was collected from the top 20 cm from Shenyang Ecological Station, which consisted of 31.3 ± 0.3 g/kg of total organics, 159.2 ± 2.1 m²/kg of surface area, (8.9 ± 0.1) cm/s of hydraulic conductivity. The coal slag purchased from a local market, 4–8 mm in diameter was used to improve the permeability and absorption area of the matrix. The mixed matrix contained total organics 28.1 ± 0.5 g/kg, total nitrogen 0.8 ± 0.2 g/kg, total phosphorus 0.6 ± 0.1 g/kg and pH 7.3.

CA and IA SWISs were subjected to aeration with an airflow rate of 2.0 ± 0.2 L/min. IA SWIS had four aerated/non-aerated (A/N) cycles every day. In each A/N cycle, the system was firstly subjected to aeration for an hour and then had five hours interval without aeration. The aeration would begin at 0:00, 06:00, 12:00 and 18:00, respectively. Influent HLRs of 0.06, 0.18 and 0.3 m³/(m² d) were adopted in this study and the corresponding hydraulic

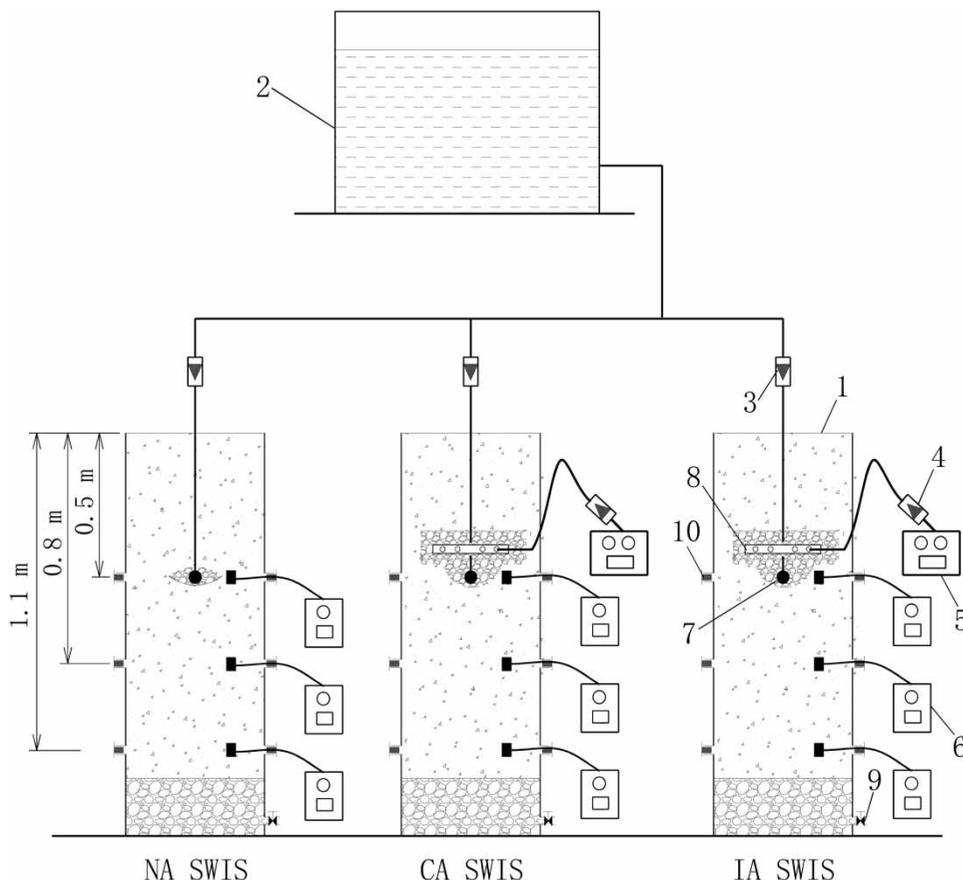


Figure 1 | Schematic diagram of three SWISs. (1) infiltration system body; (2) high-level tank; (3) liquid flow control valve and meter; (4) gas flow meter; (5) air compressor; (6) dissolved oxygen electrode; (7) distributing pipe; (8) micro-bubble diffuser; (9) outlet; (10) sampling port.

retention times were 1.5 d, 0.5 d, 0.3 d, respectively. Wastewater from Shenyang Normal University campus was pretreated in a septic tank prior to being fed into each SWIS continuously. The concentration ranges of wastewater after pretreatment were COD 185.5–261.8 mg/L, $\text{NH}_4^+\text{-N}$ 32.3–43.8 mg/L, TN 36.4–46.7 mg/L, TP 3.2–6.8 mg/L, SS 123–148 mg/L, water temperature 23.2–24.7 °C, pH 7.0–7.4. All SWISs were operated two months before sampling to allow systems mature.

Sample collection and analytical methods

Water samples were taken from influent and effluent every 10 days at 6:00, 12:00, 18:00 and 24:00. Composite samples were used in each analysis. COD, $\text{NH}_4^+\text{-N}$ and TN were analyzed according to standard methods (APHA 2003). Statistical checks were made at significant differences of 0.05 for all analyses using SPSS 12.0 ($n = 10$).

Matrix samples were collected from sampling ports (The samples were labeled 50 cm layer, 80 cm layer and 110 cm layer, respectively.) after each experiment. Four samples were taken from each layer and mixed well (approximately 5.0 g). After each on-site collection, the samples were stored in an ice incubator, and subsequently sent to laboratory for analyses. Functional gene abundances involved in nitrogen removal were quantitated by quantitative polymerase chain reaction (qPCR) technique according to Ji *et al.* (2012). Soil DNA kits (Omega, D5625-01) were used to extract and purify the total

genomic DNA from the samples. Extracted genomic DNA was detected by 1% agarose gel electrophoresis and preserved at $-20\text{ }^\circ\text{C}$ until use. Quantitative analysis was performed on the target fragments of the following functional genes: *amoA*, *nxrA*, *narG*, *napA*, *nirK*, *nirS*, *qnorB* and *nosZ* using the primers listed in Table 1. The primers were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (China). Each primer concentration was 10 pmol/ μL . The plasmids containing the functional genes (*amoA*, *nxrA*, *napA*, *narG*, *nirK*, *nirS*, *qnorB* and *nosZ*) were manufactured by the Sangon Biotechnology Company (Shanghai, China). The protocol and parameters for each target gene are summarized in Table 1. The standard samples were diluted to yield a series of 10-fold concentrations, and were subsequently used for qPCR standard curves. The R^2 values for the standard curves were more than 0.99. qPCR was performed on a Roche Lightcycler 480 Real-Time PCR detection system (Roche Diagnostics, Meylan, France) in final 20 mL volume reaction mixtures containing the following components: 10 mL SYBR Green I PCR master mix (Applied Biosystems, USA), 1 mL template DNA (sample DNA or plasmid DNA for standard curves), forward and reverse primers, and sterile water. qPCR was performed in a three-step thermal cycling procedure. Each qPCR was performed in 40 cycles and followed by a melting curve analysis. Sterile water was used as a negative control and the data obtained from the qPCR were normalized to copies per gram of biological carrier in the SWISs.

Table 1 | Primers and parameters of target genes used in qPCR analysis

Target gene	Primer	Amplification size (bp)	qPCR programs	Reference
<i>amoA</i>	amoA1F amoA2R	491	3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C.	Rotthauwe <i>et al.</i> (1997)
<i>nxrA</i>	F1norA R2norA	323	3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C.	Attard <i>et al.</i> (2010)
<i>narG</i>	1960m2f 2050m2r	100	10 min at 95 °C, 15 s at 95 °C, 45 s at 58 °C and 30 s at 72 °C.	Lopez-Gutierrez <i>et al.</i> (2004)
<i>napA</i>	napAV17F napA4R	152	10 min at 95 °C, 15 s at 95 °C, 50 s at 57 °C and 30 s at 72 °C.	Bru <i>et al.</i> (2007)
<i>nirK</i>	nirK876F nirK1040R	165	3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C.	Henry <i>et al.</i> (2004)
<i>nirS</i>	nirScd3aF nirSR3cd	413	3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C.	Kandeler <i>et al.</i> (2006)
<i>qnorB</i>	qnorB2F qnorB5R	262	5 min at 95 °C, 15 s at 95 °C, 20 s at 56 °C and 8 min at 72 °C.	Gesche & James (2003)
<i>nosZ</i>	nosZ1527F nosZ1773	250	3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C.	Scala & Kerkhof (1998)

RESULTS AND DISCUSSION

DO profiles in an A/N cycle

Anaerobic and aerobic environment can be well distinguished via DO profile in a SWIS (Fan *et al.* 2013). DO concentration profiles of NA, CA and IA SWISs in an A/N cycle are shown in Figure 2. DO concentrations along NA SWIS under different HLRs were below 1.0 mg/L. For different HLRs, DO concentrations detected at 50 cm depth of NA SWIS were in the range of 0.36–0.91 mg/L, and less than 0.26 and 0.03 mg/L at 80 and 110 cm depths, which indicated that NA SWIS was under anoxic or anaerobic conditions at 80 and 110 cm depths, and aerobic conditions were not good at 50 cm depth. Wang *et al.* (2010) showed that the prevailing conditions in SWISs were anoxic or anaerobic below a distribution pipe because air diffusion to the soil matrix was limited. In contrast, artificial aeration enhanced oxygen of the SWISs. DO concentrations at 50 cm depth of IA and CA SWISs were significantly higher than that of NA SWIS ($P < 0.05$), but no significant differences were observed at 80 and 110 cm depths among them ($P > 0.05$). Under different HLRs, DO concentrations were more than 6.52 mg/L during aeration and as high as 2.84 mg/L when supplementary aeration was turned off for IA SWIS at a depth of 50 cm; However, at depths of 80 and 110 cm, DO concentrations were below 0.46 and 0.18 mg/L during aeration, and below 0.38 and 0.09 mg/L without aeration, respectively. High aeration-induced DO concentrations were reduced by the decomposition of nutrients and organic matter, which may explain the decreasing tendency in NA period of IA SWIS. For CA SWIS, DO concentrations were more than 6.52 mg/L at a depth of 50 cm and less

than 0.46 and 0.11 mg/L at depths of 80 and 110 cm under different HLRs, respectively. Aerobic conditions were effectively developed at 50 cm depth and anoxic or anaerobic conditions were not changed at 80 and 110 cm depths within CA and IA SWISs. Sequential aerobic and anaerobic conditions were well developed by intermittent aeration. Zhong *et al.* (2014) reported that aerobic conditions occurred in the top and anaerobic or anoxic conditions occurred in the subsequent sections in matrix, which would favor nitrification and denitrification. Moreover, DO concentrations decreased with increasing HLR in NA, CA and IA SWISs. This phenomenon could be explained by the fact that more oxygen was consumed by oxidation of excessive organic matter and nutrients.

Nitrification and nitrogen removal performance

NH_4^+ -N removal performances are shown in Figure 3. NA SWIS achieved removal rates of NH_4^+ -N, which were 72.4%, 52.9% and 27.4% under the HLR of 0.06, 0.18 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, respectively. Under the HLR of 0.18 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, average NH_4^+ -N concentrations in NA SWIS were 17.94 and 27.66 mg/L, which were higher than the class I value (15 mg/L) according to the discharge standard of pollutant for municipal wastewater treatment plants in China (GB18918-2002). Nitrification, as an aerobic chemo-autotrophic microbial process, plays an important role in nitrogen removal, which occurs only when oxygen is present in a high enough concentration to support the growth of aerobic nitrifying bacteria (Fan *et al.* 2013). Most conventional SWISs fail to fulfill this first step due to insufficient oxygen supply. In order to improve the DO availability in SWISs, artificial aeration appeared to be the most effective alternative to guarantee sufficient oxygen

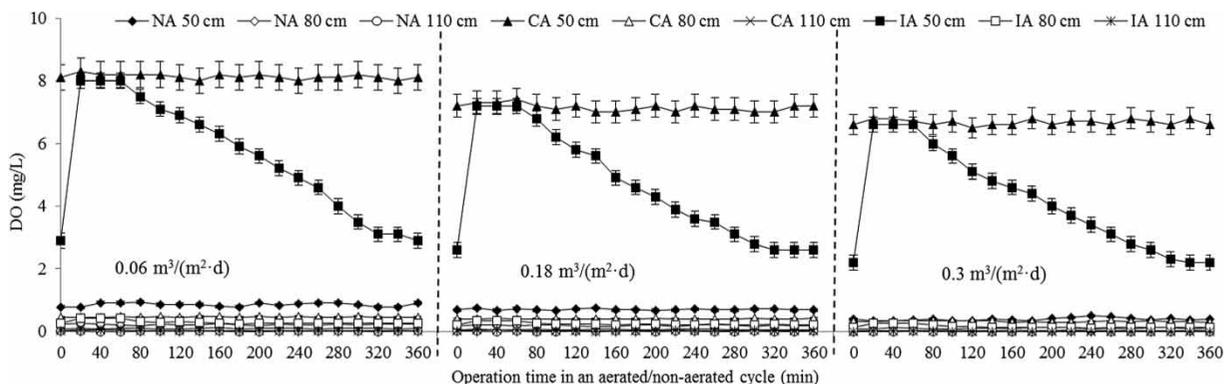


Figure 2 | DO profiles in an A/N cycle in SWISs with intermittent aeration (IA), continuous aeration (CA) and without aeration (NA) under the HLR of $0.06 \text{ m}^3/(\text{m}^2 \text{ d})$, $0.18 \text{ m}^3/(\text{m}^2 \text{ d})$ and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$.

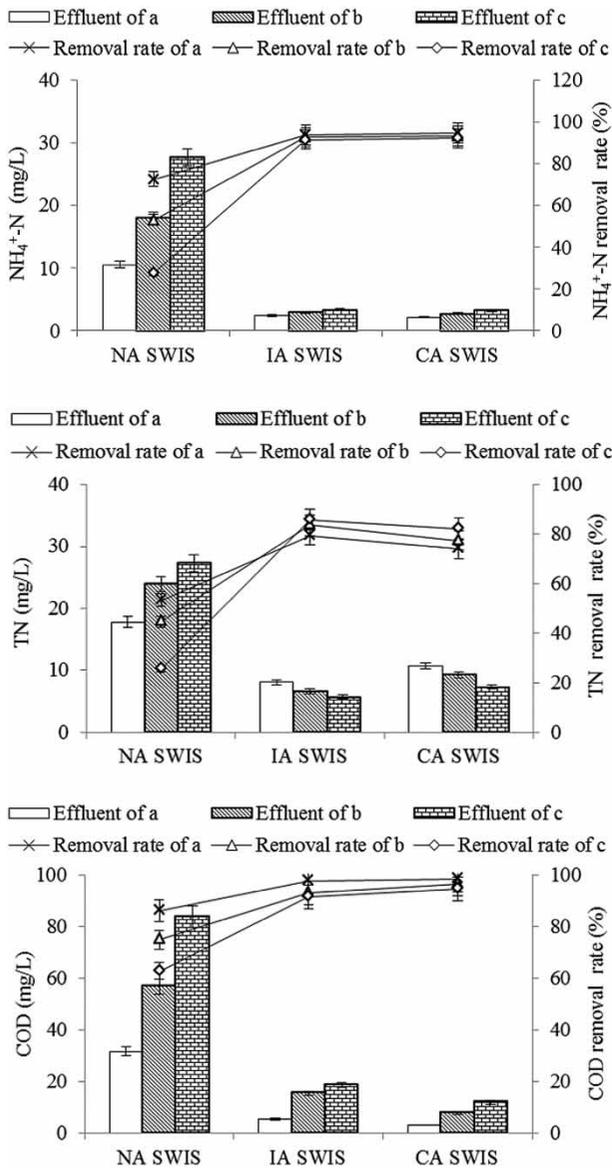


Figure 3 | NH_4^+ -N, TN and COD removal performance in SWISs without aeration (NA), with intermittent aeration (IA) and with continuous aeration (CA) under the HLR a: $0.06 \text{ m}^3/(\text{m}^2 \text{ d})$, b: $0.18 \text{ m}^3/(\text{m}^2 \text{ d})$, c: $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$.

supply (Pan *et al.* 2015). As shown in Figure 3, average effluent NH_4^+ -N concentrations of CA and IA SWISs were significantly lower than that of NA SWIS ($P < 0.05$). There were no significant difference between CA and IA SWISs ($P > 0.05$). It is generally accepted that DO concentrations above 1.5 mg/L are essential for better nitrification (Ye & Li 2009). In NA SWIS, DO concentrations were lower than 1.0 mg/L at all depths, which led to anoxic and anaerobic environment in the matrix, thus NH_4^+ -N removal was seriously limited and average NH_4^+ -N removal rate was below 73% under the HLR of 0.06, 0.18 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$.

HLR had little effect on NH_4^+ -N removal in aerated SWISs within the scope of this study. DO concentrations were above 2.2 mg/L in the upper layers through artificial aeration in CA and IA SWISs, which was in favor of achieving high NH_4^+ -N removal rate above 90% and effluent NH_4^+ -N concentrations below 15 mg/L, even under the high HLR of $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$.

Biological nitrification and denitrification is widely acknowledged to be the major nitrogen removal mechanisms (Wu *et al.* 2015). As shown in Figure 3, average TN removal rates ranged from 25.6%–53.2%, 73.8%–82.2%, 79.4%–85.8% under the HLR ranging from 0.06 to $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$ for NA, CA, and IA SWISs, respectively. These results are consistent with the removal rates reported by Li *et al.* (2012) for conventional SWISs and by Pan *et al.* (2015) and Yang *et al.* (2016) for aerated SWISs. Average TN removal rates in IA and CA SWISs were significantly higher than that in NA ($P < 0.05$), which is in agreement with the studies of Maltais-Landry *et al.* (2009) and Liu *et al.* (2013). Under the HLR of 0.18 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, average effluent TN concentrations in NA SWIS were 21.85 and 29.56 mg/L, which were higher than the class I value (20 mg/L) according to the discharge standard of pollutant for municipal wastewater treatment plants in China (GB18918-2002). The effluents of NA SWIS were still dominated by high concentration NH_4^+ -N due to limited nitrification. NA SWIS could not achieve high NH_4^+ -N removal rate in anoxic or anaerobic conditions, which would greatly inhibit denitrification due to the insufficient supply of NO_3^- -N as electron acceptors. TN removal rates decreased with the increase of HLR in NA SWIS. The result was consistent with previous studies (Zou *et al.* 2009; Li *et al.* 2012). Effluent TN concentrations were between 10.70 and 7.31 mg/L for CA SWIS, while between 8.14 and 5.56 mg/L for IA SWIS. High-efficiency nitrification and denitrification were realized simultaneously in CA and IA SWISs. NH_4^+ -N could be nitrified in aerobic zones and then processed via denitrification in anoxic or anaerobic zones. The needed carbon source was supplied by the continuous feeding, leading to high removal of TN. Average removal rate of TN enhanced with HLR increasing in CA and IA SWISs, which was in accordance with a previous study (Yang *et al.* 2016). Under the same HLR, average COD removal rates of CA and IA SWISs were much higher than of NA SWIS. Decomposition of organic matter took place mainly in the upper matrix of the SWISs (Wang *et al.* 2010). In aerated SWISs, the aerobic conditions were created in the upper matrix by aeration and thus facilitated aerobic removal of organic matter.

Average COD removal rate decreased with the increase of HLR in NA, IA and CA SIWSs (in Figure 3). Under $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, more carbon source in the lower layer was available than under 0.06 and $0.18 \text{ m}^3/(\text{m}^2 \text{ d})$, which led to higher denitrification and resulted in lower effluent TN concentration in IA and CA SWISs under high HLR. Average TN removal rate of IA SWIS was higher than that of CA SWIS under the same HLR because of more carbon source obtained in IA SWIS.

COD, $\text{NH}_4^+\text{-N}$ and TN removal rates of aerated SWISs under the HLR of $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$ were higher than previous studies (Li *et al.* 2011, 2012; Fan *et al.* 2013), though the HLR was nearly 5–10 times higher than the others. Taking TN removal performance, hydraulic efficiency and operation

cost into consideration, intermittent aeration mode and the HLR of $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$ were recommended for SWISs.

Functional genes abundances involved in nitrogen removal

Figure 4 shows the abundances of nitrogen removal functional genes, which are involved in nitrification and denitrification processes. The *amoA* and *nrxA* genes are often regarded as the marker of oxidizing $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$ and oxidizing $\text{NO}_2^-\text{-N}$ to $\text{NO}_3^-\text{-N}$ (Poly *et al.* 2008), respectively. In NA, IA and CA SWISs, the abundances of *amoA* and *nrxA* decreased along the flow direction. This followed the same trend as DO, which showed a decrease

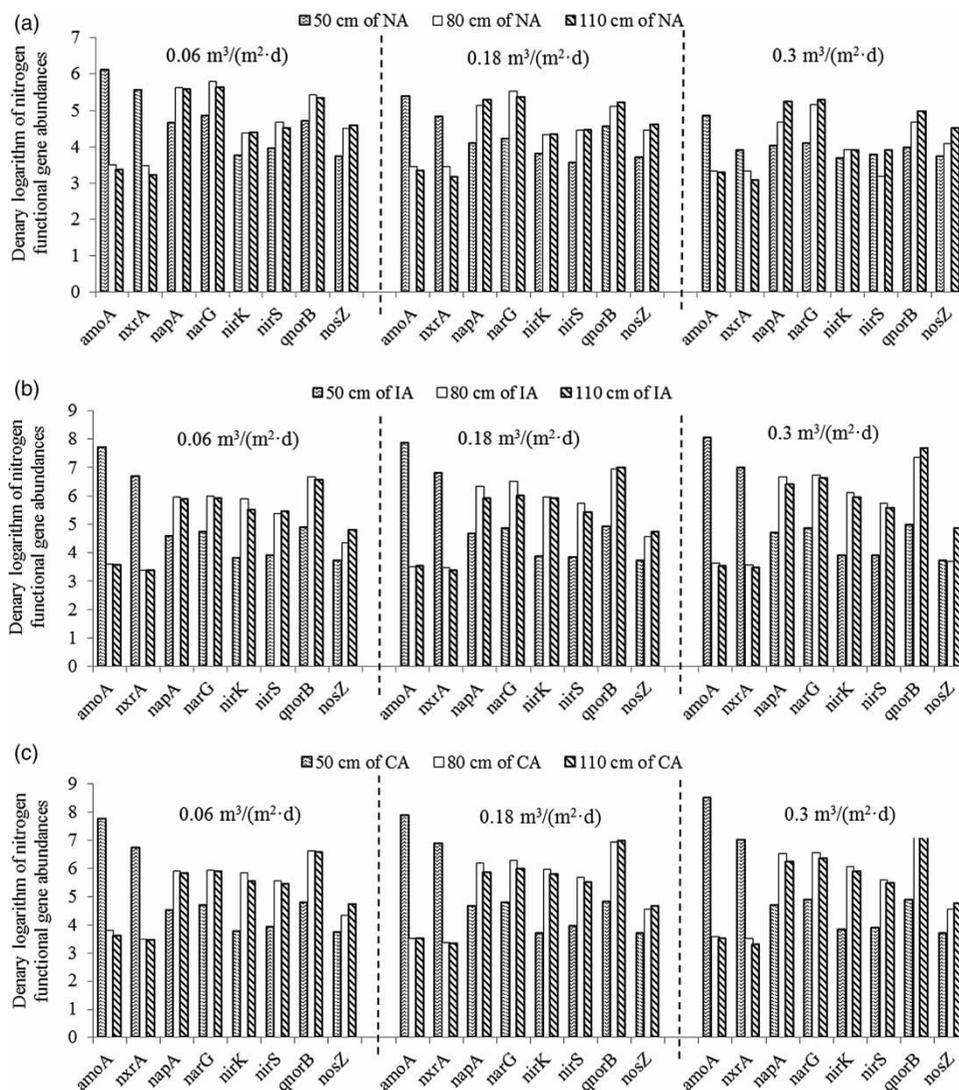


Figure 4 | Abundance of nitrogen functional genes in SWISs without aeration (NA) (a), with intermittent aeration (IA) (b) and with continuous aeration (CA) (c) under the HLR of 0.06, 0.18 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$.

along the flow direction. The metabolic activity, growth and enrichment of ammonia-oxidizing bacteria are related to DO (Wang *et al.* 2015a). DO concentrations ($\text{DO} < 1.5 \text{ mg/L}$) were not in a suitable range for the growth of ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Fan *et al.* 2013). AOB performs $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$ oxidation, providing substrate to NOB for the nitrite oxidation of $\text{NO}_2^-\text{-N}$ to $\text{NO}_3^-\text{-N}$, which might explain the lower abundance of *nxrA* compared to *amoA*. With HLR increasing, the abundances of *nxrA* and *amoA* increased in IA and CA SWISs, and decreased in NA SWIS. The HLR increased by $0.12 \text{ m}^3/(\text{m}^2 \text{ d})$, the abundances of *amoA* and *nxrA* decreased one order of magnitude at 50 cm depth in NA SWIS. Previous studies also found that high HLR reduced the growth of AOB and NOB in conventional infiltration systems (Rothrock *et al.* 2011). In IA and CA SWISs, the abundances of *amoA* and *nxrA* were significantly higher than those of NA SWIS under the same HLR ($P < 0.05$). Sufficient oxygen supply could greatly improve the number of nitrification bacteria and enzyme activities involved in $\text{NH}_4^+\text{-N}$ removal under high HLR (Pan *et al.* 2015). DO concentrations of IA and CA SWISs ($\text{DO} > 2.2 \text{ mg/L}$) were improved through aeration at 50 cm depth, which was favorable for the nitrification bacteria. Former studies also found that aeration obviously enhanced the growth of AOB and NOB by fluorescence *in situ* hybridization analysis infiltration system (Fan *et al.* 2013).

$\text{NO}_3^-\text{-N}$ to $\text{NO}_2^-\text{-N}$ reduction, the first reaction process in denitrification, is catalyzed by the key gene *narG* and *napA*. As seen from Figure 4, the abundances of *napA* and *narG* declined with HLR increasing in NA SWIS similar to other study (Wang *et al.* 2015b). In IA and CA SWISs, the abundances of *napA* and *narG* increased with HLR increasing. Aeration enhanced DO concentrations in IA and CA SWISs which was favorable for nitrification. More NO_3^- as the substrate of anaerobic denitrification of NO_2^- production could be catalyzed, which improved the abundances of *napA* and *narG*. $\text{NO}_2^-\text{-N}$ to NO reduction is the second reaction process in denitrification, which is catalyzed by gene of *nirS* and *nirK* (Kandeler *et al.* 2006). With HLR increasing, the abundance of *nirK* gradually increased in IA and CA SWISs and decreased in NA SWIS. The abundance of *nirS* changed disorderly with HLR (Figure 4). It was reported that the composition of *nirS* genotypes varied greatly and *nirK* was more stable than *nirS* by a previous study (Hallin *et al.* 2006). NO to N_2O reduction is the third process in denitrification, which is catalyzed by *qnorB* (Fujiwara & Fukumori 1996). With HLR increasing, the abundance of *qnorB* gradually

increased in IA and CA SIWSSs, whereas it decreased in NA SWIS (Figure 4). N_2O to N_2 reduction catalyzed by *nosZ* is the last reaction in denitrification process (Wang *et al.* 2015a). The abundance of *nosZ* increased along the flow direction and changed little with HLR increasing in NA, IA and CA SWISs, which consisted with a former report (Wang *et al.* 2015b). Overall, the abundances of *napA*, *narG*, *nirK* and *qnorB* discussed previously, which are intimately involved in denitrification process, tended to gradually decline in NA and increase in IA and CA SWISs when HLR rose. In the HLR range of 0.06 and $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, more organic matter and nutrients provided good nutritional conditions for the growth and enrichment of anaerobic denitrifying bacteria with HLR increasing in IA and CA SWISs after nearly complete nitrification with aeration, in parallel to enhancing the enrichment of the four genes. Under the same HLR, the abundances of *napA*, *narG*, *nirK* and *qnorB* in IA SWIS were higher than those in CA SWIS in the same depth because IA SWIS provided more carbon source. It could further explain high removal of TN in IA SWIS.

The results suggested that a high HLR negatively affected the nitrifying and denitrifying community in conventional SWISs, and aeration improved the abundance of nitrogen functional genes, especially under high HLR, and favored nitrogen removal.

CONCLUSIONS

Aeration strategy successfully created appropriate oxygen conditions for nitrification and denitrification, which increased COD, $\text{NH}_4^+\text{-N}$ and TN removal rates, and enhanced the enrichment of nitrogen removal functional genes (*amoA*, *nxrA*, *napA*, *narG*, *nirK* and *qnorB*). Increasing HLR affected COD, $\text{NH}_4^+\text{-N}$ and TN removal more in NA SWIS than that in intermittent and continuous aerated SWISs. High TN removal rates (85.8%) was obtained in intermittent aerated SWIS under the HLR of $0.3 \text{ m}^3/(\text{m}^2 \text{ d})$, and higher than that in continuous aerated SWIS (82.2%). Intermittent aeration is a reliable strategy to achieve low operation cost, high HLR and TN removal for SWISs.

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