Does influent C/N ratio affect pollutant removal and greenhouse gas emission in wastewater ecological soil infiltration systems with/without intermittent aeration?

Junling Pang, Mo Yang, Deli Tong, Xu Fu, Linli Huang, Bo Sun and Jing Pan

ABSTRACT

Wastewater ecological soil infiltration system (WESIS) is a land treatment technology for decentralized wastewater treatment that has been applied all over the world. In this study, the pollutant removal, emission of greenhouse gases (GHGs) and functional gene abundances with different influent C/N ratios were evaluated in WESISs with/without intermittent aeration. Intermittent aeration and influent C/N ratio affect pollutant removal and GHG emission. Increased influent C/N ratio led to high total nitrogen (TN) removal, low CH_4 and N_2O emission in the aerated WESIS, which was different from the non-aerated WESIS. High average removal efficiencies of chemical oxygen demand (COD) (94.8%), NH_4^+-N (95.1%), TN (91.2%), total phosphorus (TP) (91.1%) and low emission rates for CH_4 (27.2 mg/(m^2 d)) and N_2O (10.5 mg/(m^2 d)) were achieved with an influent C/N ratio of 12:1 in the aerated WESIS. Intermittent aeration enhanced the abundances of bacterial 16S rRNA, amoA, nxrA, narG, napA, nirK, nirS, qnorB, nosZ genes and decreased the abundances of the mcrA gene, which are involved in pollutant removal and GHG emission. Intermittent aeration would be an effective alternative to achieving high pollutant removal and low CH_4 and N_2O emission in high influent C/N ratio wastewater treatment.

Key words | aeration, C/N ratio, greenhouse gas, wastewater ecological soil infiltration system

Junling Pang†
Mo Yang†
Deli Tong†
Xu Fu
Linli Huang
Bo Sun
Jing Pan (corresponding author)
College of Life Science,
Shenyang Normal University,
Shenyang 110034,
China
E-mail: *crystalpan@synu.edu.cn*

†These authors contributed equally to this study and share first authorship.

INTRODUCTION

Wastewater ecological soil infiltration system (WESIS) is a land wastewater treatment that feeds sewage into an underground matrix layer (Wang *et al.* 2010). The sewage first moves up and around under capillary force and then flows into the matrix below under gravity. Finally, the treated sewage is collected by collection pipes at the bottom. Through this process, the pollutants are degraded by the combined actions of soil and microorganisms (Ji *et al.* 2012). Compared with the traditional centralized wastewater treatment methods, it has many advantages: low construction cost, low operation cost, easy management and good treatment function (Wang *et al.* 2010; Yang *et al.* 2016).

During the pollutant removal processes, the WESIS releases many kinds of gas into the atmosphere. Among these gases, CO_2, CH_4 and N_2O are the most relevant for the environment because they are greenhouse gases (GHGs). In the WESIS, CO_2 is produced by the aerobic decomposition of organic matter by microorganisms; CH_4 is generated from the anaerobic degradation of organic matter by methanogens; N_2O is released from nitrification and denitrification by nitrifying and denitrifying bacteria. Itokawa *et al.* (1993) verified the quantities and emission mechanisms of CH_4 and N_2O of the WESIS.

In order to reduce the environmental damage caused by CH_4 and N_2O emissions from WESISs, many studies have been carried out to assess their influencing factors and mechanisms. Kong *et al.* (2002) found that there was a significant positive correlation between oxidation-reduction potential (ORP) and N_2O emission, and CH_4 emission increased with increasing temperature. Li *et al.* (2017) reported that influent hydraulic loading, pollutant loading and drying-wetting ratio impacted N_2O generation. Zheng *et al.* (2018) reported that the combined application of intermittent aeration and influent shunt distributing wastewater reduced N_2O emission in the WESIS.

Influent C/N ratio is the key factor in organic matter and nitrogen and therefore has a direct effect on CO_2,

doi: 10.2166/wst.2020.141

CH_4 and N_2O emission and nitrogen removal from biological organic matter (Wu *et al.* 2009; Li *et al.* 2018). Unfortunately, the correlations between C/N ratio and GHG emissions are controversial in wastewater treatment. Kong *et al.* (2016) found that N_2O emission increased with increasing influent C/N in the WESIS. Wu *et al.* (2009) concluded that N_2O emission is lowest under a C/N ratios of 5 compared with C/N ratio of 8 and 10 in wetlands. Zhao *et al.* (2009) also reported enhanced GHG emissions with increases in the influent C/N ratio in vertical-flow constructed wetlands. However, some researchers have opposing opinions. Zheng *et al.* (2018) reported that the N_2O emission rate decreased with increasing C/N ratio in a biochar-sludge amended WESIS. Lv *et al.* (2018) found the increase in influent C/N ratio reduced GHG emissions during vermicomposting of sewage sludge. So far, few studies have focused on three GHGs (CO_2, CH_4 and N_2O) emitted WESISs with/without intermittent aeration with different influent C/N ratios.

The aim of this work was to investigate the effect of influent C/N ratio on pollutant removal and GHG (CO_2, CH_4 and N_2O) emission in WESISs with/without intermittent aeration. The abundances of bacteria and functional genes involved in the removal of chemical oxygen demand (COD) and nitrogen, and the emission of CO_2, CH_4 and N_2O were also studied. Hopefully, this study will provide a reference for the application of intermittent aeration WESISs in treating high influent C/N ratio wastewater.

MATERIAL AND METHODS

WESISs description and operation

The schematic diagram of two pilot-scale WESISs is shown in Figure 1. Each pilot-scale WESIS, made of a polyvinyl chloride column with a height of 120 cm and internal diameter of 30 cm, was constructed in Shenyang Normal University of China. The interior was filled with 110 cm of mixed matrix (80% brown earth and 20% coal slag by mass) over 10 cm of gravel (5–10 mm in diameter). Wastewater was distributed through an influent pipe at the depth of 50 cm. The effluent was collected by an outlet pipe at the bottom. Matrix sampling ports were at depths of 50, 80 and 110 cm from the top, and were used to assess bacteria and functional genes involved in pollutant removal and GHG emission. The WESIS with intermittent aeration (WESIS B) contained an air compressor, air tube and micro-bubble diffuser at a depth of 40 cm for oxygen supply. Gravel with diameter of 10–20 mm surrounded the micro-bubble diffuser and distributing pipe to enable the air to be well distributed air and to prevent clogging. The conventional WESIS (WESIS A) did not have an air compressor, air tube or micro-bubble diffuser.

In this study, influent total nitrogen (TN) concentration remained stable and influent C/N ratio was regulated by changing the amounts of glucose to produce four influent C/N ratios. Accordingly, synthetic wastewater was made up using

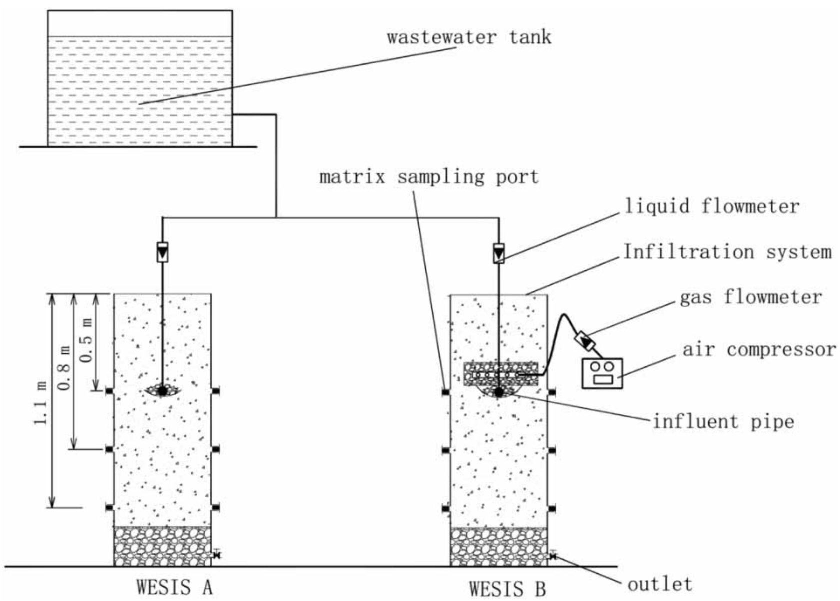

Figure 1 | Schematic diagram of two wastewater ecological soil infiltration systems (WESISs), named WESIS A (without aeration) and WESIS B (with intermittent aeration).

the following components and tap water to create influent C/N ratios of 3:1, 6:1, 9:1 and 12:1 as follows: 768 mg/L, 1.5 g/L, 2.4 g/L or 3 g/L glucose and 188 mg/L $(NH_4)_2SO_4$, 12 mg/L $MgSO_4$, 12 mg/L $MnSO_4$, 12 mg/L $CaCl_2$, 12 mg/L KH_2PO_4, 12 mg/L $NaNO_3$, 12 mg/L $ZnSO_4 \cdot 7H_2O$, 12 mg/L $FeSO_4$. The influent concentrations of the synthetic wastewater with different influent C/N ratios are shown in Table 1. Synthetic wastewater was continuously fed into each WESIS under the hydraulic loading of 0.08 $m^3/(m^2\ d)$ in a greenhouse with a temperature of 22 ± 1 °C. Influent C/N ratio was raised from 3:1 to 6:1, 9:1 and 12:1. Each C/N ratio experiment lasted for 100 days. WESIS B was aerated with an airflow rate of 3.5 ± 0.2 L/min and had four aerated/non-aerated cycles a day. In each cycle, the system was aerated for an hour and then had 5 hours without aeration. Aeration began at 1:00, 7:00, 13:00 and 19:00.

Sampling and analytical methods

Every 10 days, 100 ml of the influent and effluent water were collected. COD, TN, ammonium nitrogen (NH_4^+-N) and total phosphorus (TP) were measured by standard methods according to APHA (2005).

Gas emissions from WESISs were collected in a static stationary chamber (40 cm in height and 15 cm in diameter). On the top of each chamber, a battery-driven fan circulated the air inside the chambers to ensure the gas samples were well mixed. The gases were collected from the air outlet at the middle part of each chamber by gas sampling bags with a mini gas pump. Five gas samples were collected at 0, 1, 3, 5 and 7 hours after on the surface of the matrix at the same time of day between 13:00 and 19:00 every 10 days. The CO_2, CH_4 and N_2O concentrations were analyzed by Agilent 6890N gas chromatography equipped with a thermal conductivity detector, flame ionization detector and electron capture detector, respectively. The details of the CO_2, CH_4 and N_2O measuring methods can be found in previous studies (Picek et al. 2007; Zheng et al. 2018). The CO_2, CH_4 and N_2O emission rates were calculated using the following equation after determining the concentrations of CO_2, CH_4 and N_2O (Chiemchaisri et al. 2009).

$$\text{Greenhouse gas emission rate} = \frac{V}{A}\frac{dC}{dt}$$

where V is the volume of the gas collection chamber (m^3); A is the section area of the gas chamber (m^2); *dC/dt* represents the slope of the best-fit line for the plot of gas concentration inside the chamber and time data points ($mg/m^3 \cdot h$). The greenhouse gas conversion ratio is the percentage of influent total nitrogen or total organic carbon converted to N_2O or CO_2 and CH_4 as appropriate.

In order to measure bacterial and functional gene abundances involved in COD and nitrogen removal, and CO_2, CH_4 and N_2O emission in WESIS A and B, matrix soils were sampled from the sampling ports at depths of 50, 80 and 110 cm after each influent C/N ratio experiment. Quantitative analysis was carried out by quantitative polymerase chain reaction (qPCR) on the 16S rRNA fragment of bacteria, the target fragment of mcrA (methyl coenzyme M reductase A) gene and the target fragments of amoA (ammonia monooxygenase), nxrA (nitrite oxidoreductase), narG (membrane-bound nitrate reductase), napA (periplasmic nitrate reductase), nirS/nirK (nitrite reductase), qnorB (nitric oxide reductase) and nosZ (nitrous oxide reductase) genes. The mcrA gene participates in COD removal and CH_4 production. The amoA, nxrA, narG, napA, nirS/nirK, qnorB and nosZ genes are involved in nitrogen removal and N_2O emission. The primers for the above fragments were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd in China, with a concentration of 10 pmol/L. The detailed qPCR processes can be found in Ji et al. (2012) and Steinberg & Regan (2009).

All experimental data in this study were processed with SPSS 11.0 software. Two-way analysis of variance (ANOVA) was used to evaluate the significance of differences, which were considered statistically significant when $P < 0.05$.

Table 1 | Influent characteristics of two WESISs with different influent C/N ratios

	Influent C/N ratio			
Parameters	**3:1**	**6:1**	**9:1**	**12:1**
COD (mg/L)	138.8 ± 8.3	243.5 ± 15.1	360.8 ± 13.3	485.2 ± 21.7
NH_4^+-N (mg/L)	38.8 ± 2.1	37.5 ± 1.8	38.0 ± 2.4	37.8 ± 2.6
NO_3^-–N (mg/L)	2.2 ± 0.5	2.6 ± 0.2	2.7 ± 0.5	2.5 ± 0.1
TN (mg/L)	41.8 ± 0.7	40.6 ± 0.8	41.1 ± 1.1	40.4 ± 0.7
TP (mg/L)	4.8 ± 0.3	4.6 ± 0.4	4.7 ± 0.5	4.9 ± 0.4

Mean ± SD.

RESULTS AND DISCUSSION

Pollutant removal performance

Table 2 shows the effluent concentrations and removal efficiencies of COD, TP, NH_4^+-N and TN. With influent C/N

Table 2 | Removal performances in WESISs without aeration (WESIS A) and with intermittent aeration (WESIS B)

COD/N ratio in effluent	3:1		6:1		9:1		12:1	
	WESIS A	WESIS B	WESIS A	WESIS B	WESIS A	WESIS B	WESIS A	WESIS B
COD (mg/L)	22.5 ± 1.9	2.8 ± 0.3	48.7 ± 1.6	8.1 ± 1.0	81.1 ± 2.9	13.1 ± 0.9	144.5 ± 2.9	25.3 ± 0.8
Removal efficiency (%)	83.4 ± 1.5	97.9 ± 0.2	80.2 ± 0.7	96.7 ± 0.9	77.6 ± 1.1	96.0 ± 0.2	70.5 ± 0.6	94.8 ± 0.2
NH_4^+-N (mg/L)	11.2 ± 0.8	0.3 ± 0.05	14.6 ± 0.5	0.6 ± 0.06	24.2 ± 1.5	0.9 ± 0.07	28.9 ± 1.6	1.7 ± 0.1
Removal efficiency (%)	63.4 ± 1.6	99.1 ± 0.1	49.5 ± 3.3	98.5 ± 0.2	36.5 ± 3.8	97.7 ± 0.2	24.1 ± 4.0	95.1 ± 0.3
TN (mg/L)	14.8 ± 0.6	32.2 ± 0.6	19.3 ± 0.5	23.1 ± 0.6	25.3 ± 1.7	4.7 ± 0.3	30.2 ± 1.3	3.4 ± 0.2
Removal efficiency (%)	62.5 ± 1.6	18.5 ± 1.5	49.5 ± 3.3	41.9 ± 1.4	35.1 ± 4.3	88.1 ± 0.7	22.1 ± 3.5	91.2 ± 0.2
TP (mg/L)	0.25 ± 0.02	0.21 ± 0.01	0.34 ± 0.02	0.26 ± 0.02	0.41 ± 0.02	0.33 ± 0.02	0.46 ± 0.02	0.42 ± 0.02
Removal efficiency (%)	93.8 ± 0.4	94.5 ± 0.4	92.7 ± 0.5	93.3 ± 0.4	91.2 ± 0.3	92.4 ± 0.5	90.6 ± 0.3	91.1 ± 0.5

Mean ± SD.

ratios of 3:1, 6:1, 9:1 and 12:1, COD concentrations of the effluent were 22.5 ± 1.9 mg/L, 48.7 ± 1.6 mg/L, 81.1 ± 2.9 mg/L and 144.5 ± 2.9 mg/L, with COD removal efficiencies of 83.4 ± 1.5%, 80.2 ± 0.7%, 77.6 ± 1.1% and 70.5 ± 0.6%, respectively, in WESIS A. High COD removal efficiencies and low effluent COD concentrations were obtained when influent C/N ratios were 3:1 and 6:1. Conventional WESISs are effective in organic matter removal under low influent C/N ratios (Fan *et al.* 2013; Zheng *et al.* 2018). The COD concentrations of the effluent were higher than Class I (≤60 mg/L) of the Chinese Criterion for Water Discharge from Municipal Wastewater Treatment Plants (GB18921-2002) with influent C/N ratios of 9:1 and 12:1. This adverse effect of C/N ratio on COD removal was also reported by Song *et al.* (2016) and Fei *et al.* (2017). COD removal efficiencies decreased with increasing influent C/N ratio.

In WESISs, organic matter is mainly decomposed by aerobic heterotrophic bacteria (Wang *et al.* 2010). Influent organic matter concentration increased with increasing influent C/N ratio in the non-aerated WESIS, which led to an insufficient oxygen supply and resulted in COD removal efficiency decreasing. However, COD removal efficiencies were 97.9 ± 0.2%, 96.7 ± 0.9%, 96.0 ± 0.2% and 94.8 ± 0.2% with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS B. COD concentrations in the effluent of WESIS A were significantly higher than those of WESIS B with the same influent C/N ratio ($P < 0.05$). Organic matter degradation was hardly affected when influent C/N ratio increased in WESIS B, which was significantly different from that of WESIS A. Intermittent aeration supplied enough oxygen for aerobic biodegradation of organic matter, which improved COD removal. The same results were reported by Ouellet-Plamondon *et al.* (2006) and Yang *et al.* (2016).

As seen from Table 2, TP removal efficiencies were more than 90% and TP concentrations of the effluent were below 0.5 mg/L with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1 in WESIS A and B. TP removal efficiencies decreased with increasing influent C/N ratio due to more organic matter competing with TP in adsorption sites. TP concentrations of the effluent of WESIS B were a little lower than those of WESIS A. Aeration promoted TP adsorption and precipitation to the matrix (De-Bashan & Bashan 2004). The same results were reported by Dong *et al.* (2012) and Fei *et al.* (2017).

As shown in Table 2, NH_4^+-N concentrations of the effluent were 11.2 ± 0.8 mg/L, 14.6 ± 0.5 mg/L, 24.2 ± 1.5 mg/L and 28.9 ± 1.6 mg/L with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS A. With influent C/N ratios of 9:1 and 12:1, NH_4^+-N concentrations in the effluent were higher than Class I (≤15 mg/L) of the criteria mentioned. Nitrification is an aerobic chemo-autotrophic bioprocess, which needs high oxygen concentrations (Wu *et al.* 2015). Due to insufficient oxygen supply, most conventional WESISs cannot not fulfill nitrification satisfactorily with high influent C/N ratios (Ding *et al.* 2011). NH_4^+-N removal efficiencies decreased with the increase of influent C/N ratio in WESIS A. More organic matter entered with increasing influent C/N ratio in this study, which inhibited the activity of nitrifying bacteria by oxygen competition. Li *et al.* (2018) and Song *et al.* (2016) reported similar results. NH_4^+-N concentrations of the effluent were 0.3 ± 0.05 mg/L, 0.6 ± 0.06 mg/L, 0.9 ± 0.07 mg/L and 1.7 ± 0.1 mg/L with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS B, and NH_4^+-N removal efficiencies were more than 95%. NH_4^+-N removal efficiencies showed no significant differences within the studied influent C/N ratios in WESIS B ($P > 0.05$). Increasing influent C/N ratios hardly affected NH_4^+-N removal, which was different from WESIS A. NH_4^+-N removal efficiencies of WESIS B were significantly higher than those of WESIS A with the same influent C/N ratio ($P < 0.05$). Intermittent aeration significantly enhanced oxygen supply in WESIS B, which promoted nitrification. With adequate oxygen supply, excess organic matter and other oxygen-demanding nutrients were not a limitation for nitrification, which was in agreement with Fan *et al.* (2013).

TN removal efficiencies were 62.5 ± 1.6%, 49.5 ± 3.3%, 35.1 ± 4.3% and 22.1 ± 3.5% with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS A, which were far from satisfactory (in Table 2). TN concentrations of the effluent were 25.3 ± 1.7 mg/L and 30.2 ± 1.3 mg/L with influent C/N ratios of 9:1 and 12:1, respectively, in WESIS A, which were higher than Class I (≤20 mg/L) of the criteria mentioned. NH_4^+-N was the main component of TN in the effluents due to limited nitrification. In conventional WESISs, nitrification is the limiting step of nitrogen removal (Wang *et al.* 2010; Yang *et al.* 2016). They cannot provide an appropriate environment for NH_4^+-N removal, which inhibits denitrification because of insufficient supply of NO_3^--N as electron acceptors (Wu *et al.* 2015). In WESIS A, TN concentrations of the effluent increased with increasing influent C/N ratio due to NH_4^+-N removal. As shown in Table 2, TN removal efficiencies were only 18.5 ± 1.5% and 41.9 ± 1.4% with low influent C/N ratios (3:1 and 6:1) in WESIS B. After effective nitrification under low influent C/N ratios of 3:1 and 6:1, NO_3^--N as electron accepters could not be eliminated. Most of organic carbon was degraded, which led to the carbon source being inadequate as the electron donor. Lee *et al.* (2009) concluded carbon deficiency was the key limiting factor for complete nitrogen removal after highly effective nitrification. TN concentrations of the effluent in WESIS B were significantly lower than those in WESIS A with influent C/N ratios of 9:1 and 12:1 ($P < 0.05$). TN removal efficiencies increased from 41.9 ± 1.4% to 88.1 ± 0.7% and 91.2 ± 0.2%, as influent C/N ratios increased from 6:1 to 9:1 and 12:1, respectively. High influent C/N ratios provided sufficient organic matter for complete denitrification, which resulted in high TN removal in intermittent WESISs. With influent C/N ratios of 9:1 and 12:1, high nitrification and denitrification rates were achieved in WESIS B.

GHG emission

In WESIS A, average CO_2 emission rates were 7.5, 12.8, 17.3 and 20.3 g/(m^2·d) with average CO_2 conversion ratios of 69.4%, 65.7%, 60.3% and 52.3% when influent C/N ratios were 3:1, 6:1, 9:1 and 12:1, respectively (Figure 2). CO_2 conversion ratios decreased with the increase of influent C/N ratio. CO_2 was produced by organic matter aerobic oxidation. In the non-aerated WESIS, air diffusion to the matrix was limited, which created anoxic or anaerobic conditions below the influent pipe. The limitation of oxygen became more obvious with the increase of the influent C/N ratio, which caused a decrease in organic matter aerobic oxidation and resulted in the CO_2 conversion ratio decreasing. Average CO_2 emission rates of WESIS B were 9.9, 17.7, 25.8 and 34.4 g/(m^2·d) with average CO_2 conversion ratios of 91.5%, 90.9%, 89.8% and 88.5% when influent C/N ratios were 3:1, 6:1, 9:1 and 12:1, respectively (Figure 2). Average CO_2 conversion ratios of WESIS B showed no significant difference with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1 ($P > 0.05$). However, average CO_2 emission rates and conversion ratios of WESIS B were significantly higher than those of WESIS A with the same influent C/N ratio ($P < 0.05$). Increasing influent C/N ratios hardly affected CO_2 conversion ratio in WESIS B, which was consistent with COD removal. Adequate oxygen supply by aeration greatly enhanced the performance of organic matter aerobic biochemical oxidation.

It can been seen from Figure 2 that average CH_4 emission rates were 39.9, 83.1, 137.9 and 201.0 mg/(m^2·d) and average CH_4 conversion ratios were 5.2%, 6.0%, 6.8% and 7.3% with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS A. CH_4 emission rates increased with the increase of influent C/N ratio. With influent C/N ratio increasing, more available organic matter might reduce available oxygen, which boosted anaerobic conditions for the growth of methanogenic bacteria (Sánchez-Monedero *et al.* 2010). CH_4 emission rates were less than 1% of CO_2 emission rates with the same influent C/N ratio. This result has also been reported by García *et al.* (2007) in constructed wetlands. However, average CH_4 emission rates and conversion ratios of WESIS B were significantly lower than those of WESIS A with the same influent C/N ratio ($P < 0.05$). Average CH_4 conversion ratios were less than 1.0% with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1, respectively, in WESIS B. Average CH_4 conversion ratios in WESIS B showed no significant difference with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1 ($P > 0.05$). Low emission rates of CH_4 in WESIS B might have been due to aeration, which promoted organic matter aerobic oxidation and resulted in a reduction of substrate available to methanogenic bacteria, which agreed with the reports of Wang *et al.* (2014b) and Yang *et al.* (2017) that

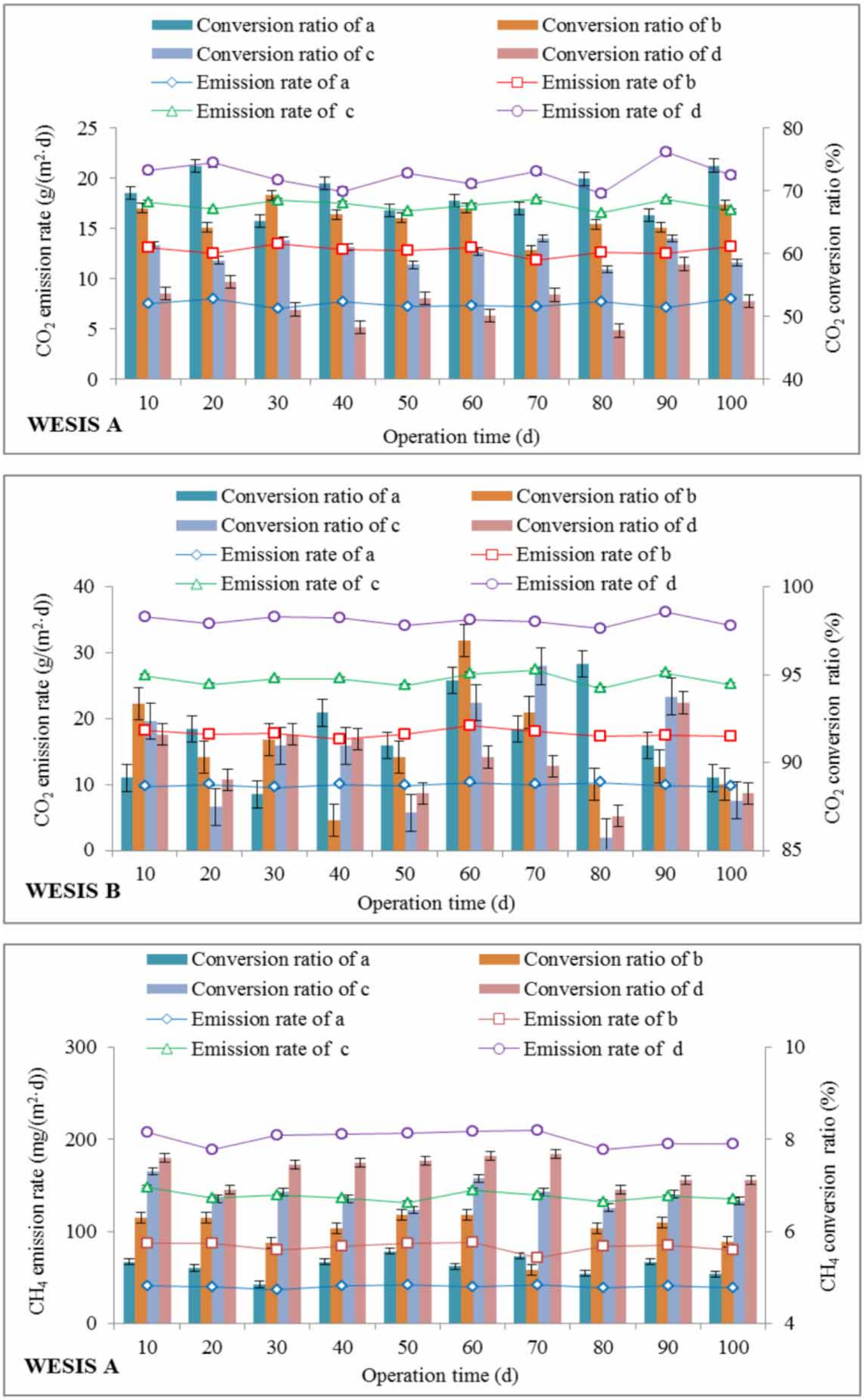

Figure 2 | CO_2, CH_4 and N_2O emission rates and conversion ratios with influent C/N ratios of 3:1 (a), 6:1 (b), 9:1 (c) and 12:1 (d). *(Continued.)*

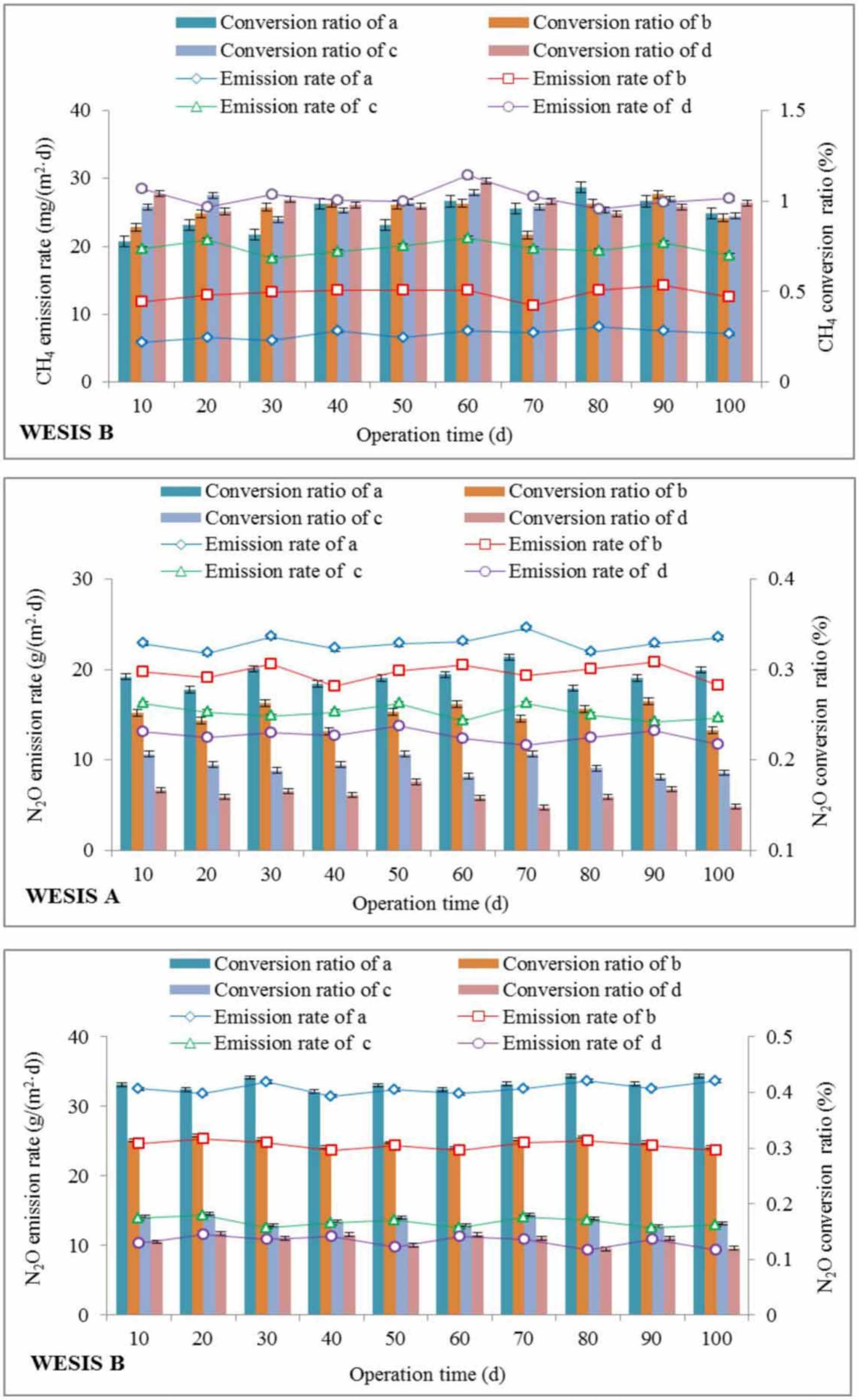

Figure 2 | Continued.

aeration could inhibit the abundance of methanogens and reduce CH_4 production.

Average N_2O emission rates in WESIS A were as follows: 22.9 mg/(m^2 d) with influent C/N ratio of 3:1, 19.7 mg/(m^2 d) with influent C/N ratio of 6:1, 15.2 mg/(m^2 d) with influent C/N ratio of 9:1, 12.7 mg/(m^2 d) with influent C/N ratio of 12:1 (in Figure 2). With the increase of influent C/N ratio, the average N_2O emission rate decreased in WESIS A. High influent C/N ratio meant high concentration of organic matter, which consumed more available oxygen and further restricted the activity of autotrophic ammonia oxidation bacteria. Ultimately, nitrification and denitrification processes were limited in WESIS A, which resulted in a decrease in N_2O emission. Average N_2O emission rates with influent C/N ratios of 3:1, 6:1, 9:1 and 12:1 were 32.6, 24.4, 13.3, 10.5 mg/(m^2 d), respectively, in WESIS B. N_2O emission could be constrained by the availability of degradable carbon sources (Li *et al.* 2018). High influent C/N ratio provided more carbon sources for N_2O to N_2 transformation after efficient nitrification, which led to the N_2O emission rate decreasing in WESIS B. Average N_2O conversion ratios of WESIS A and B were between 0.1% and 0.4%, which were in accordance with the studies of Li *et al.* (2018) and Wang *et al.* (2014a).

Bacteria and functional gene abundances involved in pollutant removal and GHG emission

The abundances of bacteria and functional genes involved in COD removal, nitrogen removal and CO_2, CH_4 and N_2O emission are shown in Figure 3. In a WESIS, organic matter is mainly degraded by bacteria, fungi, actinomyces, and protozoans, etc. (Lance 1986). The abundances of bacterial 16S rRNA declined with increasing matrix depth in both WESISs. With influent C/N ratio increasing, the abundance of bacterial 16S rRNA at 50 cm depth decreased in WESIS A but increased in WESIS B. Oxygen shortage became more obvious with the increase of influent C/N ratio, which led to a decrease of bacteria in WESIS A. With the same influent C/N ratio, the abundances of bacterial 16S rRNA in WESIS B were significantly higher than those in WESIS A at 50 cm depth ($P < 0.05$), which could further explain high COD removal and CO_2 emission in WESIS B. Intermittent aeration provided sufficient oxygen to the upper matrix, which promoted the growth and reproduction of bacteria in WESIS B. The results agreed with the report of Al-Baldawi *et al.* (2013) that supplementary aeration could improve the quantification of bacterial populations and enhance COD removal.

The mcrA gene is involved in CH_4 production (Hallams *et al.* 2003). As can be seen in Figure 3, the abundances of mcrA increased along the influent flow direction in WESIS A and B, which was contrary to the trend of matrix oxygen. Oxygen concentration of the matrix decreased with increasing matrix depth in previous studies (Wang *et al.* 2010; Fei *et al.* 2017). The abundances of mcrA at 80 cm and 110 cm depths increased with increasing influent C/N ratio in both WESISs. Insufficent oxygen became more serious with increasing influent C/N ratio, which favored methanogens in WESIS A and B. Moreover, methanogens could acquire more organic matter with high influent C/N ratios compared with low influent C/N ratios. The abundances of mcrA in WESIS A were significantly higher than those in WESIS B at the same depth and influent C/N ratio ($P < 0.05$), which was consistent with CH_4 emission. Morris *et al.* (2013) concluded there was a significant positive correlation between the abundances of mcrA and CH_4 emission.

The amoA and nxrA genes are involved in nitrification and the abundances of amoA and nxrA decreased along the influent flow direction in WESIS A and B, the same trend as for matrix oxygen (in Figure 3). The abundances of amoA and nxrA declined at 50 cm depth of WESIS A with increasing influent C/N ratio due to insufficient oxygen supply. However, the abundances of amoA and nxrA in WESIS B were hardly affected within the influent C/N ratio range of this study. The abundances of amoA and nxrA at 50 cm depth of WESIS B were significantly higher than those of WESIS A ($P < 0.05$), which followed NH_4^+-N removal.

NO_3^--N to NO_2^--N reduction, NO_2^--N to NO reduction, NO to N_2O reduction and N_2O to N_2 reduction constitute the denitrification process, and are catalyzed by narG and napA, nirS and nirK, qnorB, nosZ, respectively. As can be seen from Figure 3, the abundances of napA, narG, nirS, nirK, qnorB and nosZ decreased at 80 and 110 cm depths in WESIS A and increased at 80 and 110 cm depths in WESIS B with increasing influent C/N ratio. In WESIS A, nitrification was limited by increasing influent C/N ratio, which resulted in low efficiency of denitrification and reduced the enrichment of six functional genes involved in denitrification. In WESIS B, aeration improved the efficiency of nitrification and high influent C/N ratios supplied more carbon for denitrification, which enhanced the enrichment of the six genes. The abundances of napA, narG, nirS, nirK, qnorB and nosZ in WESIS B were significantly higher than those in WESIS A at 80 and 110 cm depths with influent C/N ratios of 9:1 and 12:1 ($P < 0.05$),

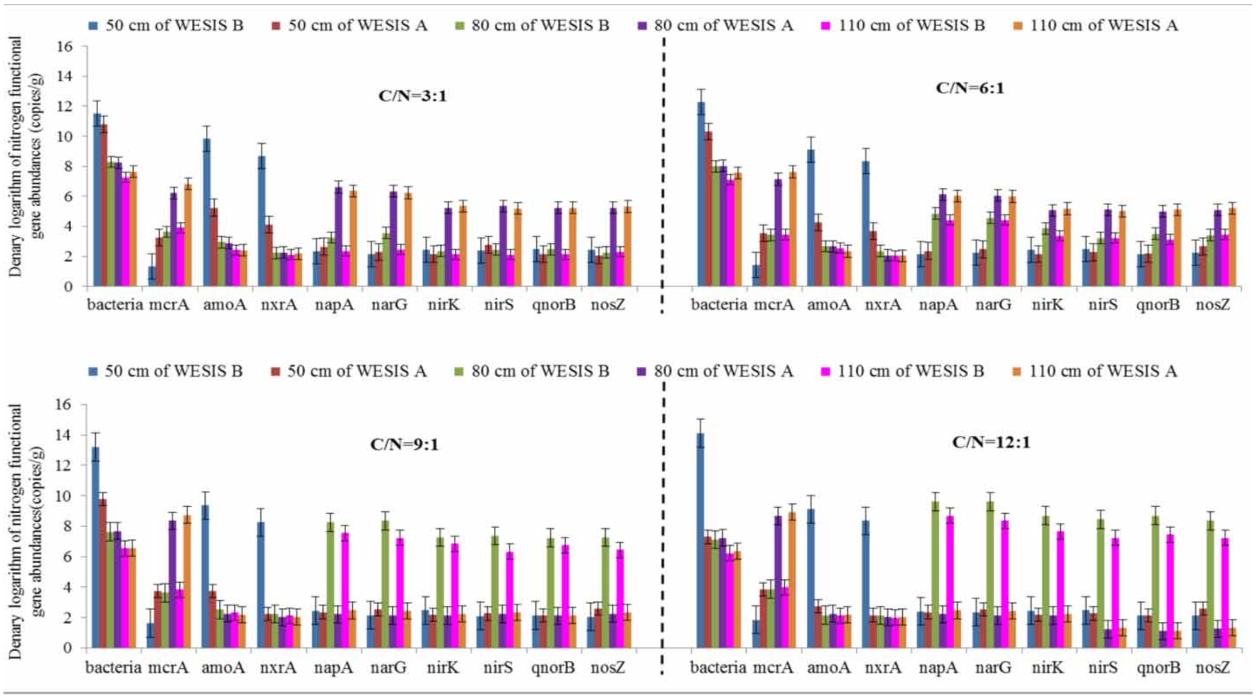

Figure 3 | Functional gene abundances involved in pollutant removal and greenhouse gas emission.

which could further explain high removal of TN and low N_2O emission in WESIS B at high influent C/N ratios.

CO_2 produced by the aerobic decomposition of organic matter can be fixed by photosynthesis, which is deemed to be atmospherically neutral (IPCC 2006). Ignoring CO_2 emissions, the use of WESISs with intermittent aeration is a reliable strategy for treating wastewater with a high influent C/N ratio while simultaneously achieving high COD and TN removal, and low CH_4 and N_2O emission.

CONCLUSIONS

With increasing influent C/N ratio, nitrogen removal increased and CH_4 and N_2O emission decreased in the WESIS with intermittent aeration, which was different from the non-aerated WESIS. Intermittent aeration enhanced the abundances of bacterial 16S rRNA, amoA, nxrA, narG, napA, nirK, nirS, qnorB, nosZ genes and decreased the abundances of the mcrA gene, which achieved high COD (94.8%), NH_4^+-N (95.1%), TN (91.2%), TP (91.1%) removal efficiencies, and low CH_4 (27.2 mg/(m^2 d)) and N_2O emission (10.5 mg/(m^2 d)) with an influent C/N ratio of 12:1. Intermittent aeration is a feasible method for WESISs to obtain high pollutant removal and low N_2O and CH_4 emission when treating wastewater high influent C/N ratios.

ACKNOWLEDGEMENTS

We gratefully thank financial support from the National Natural Science Foundation of China (No. 41471394); Innovative Talents Project of Liaoning Education Department (LR2018080); Liaoning BaiQianWan Talents Program [2018(35)]; Natural Science Foundation of Liaoning (20180550503); Shenyang Science and Technology Project (18-013-0-43); Shenyang Young and Middle-aged Science and Technology Innovation Talents Project (RC20180129); Major Original Program in Shenyang Normal University (ZD201904) and College Students Scientific Research Project (L(B)2019249).

REFERENCES

Al-Baldawi, I. A., Abdullah, S. R. S., Suja, F., Anuara, N. & Mushrifah, I. 2013 Effect of aeration on hydrocarbon phytoremediation capability in pilot subsurface flow constructed wetland operation. *Ecological Engineering* **61**, 496–500.

American Public Health Association (APHA) 2005 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC.

Chiemchaisri, C., Chiemchaisri, W., Junsod, J., Threedeach, S. & Wicranarachchi, P. N. 2009 Leachate treatment and greenhouse gas emission in subsurface horizontal flow constructed wetland. *Bioresource Technology* **100**, 3808–3814.

De-Bashan, L. E. & Bashan, Y. 2004 Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). *Water Research* **38**, 4222–4246.

Ding, Y., Liu, Y. X., Wu, W. X., Shi, D. Z., Yang, M. & Zhong, Z. K. 2011 Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water Air and Soil Pollution* **213**, 47–55.

Dong, H., Qiang, Z., Li, T., Jin, H. & Chen, W. 2012 Effect of artificial aeration on the performance of vertical-flow constructed wetland treating heavily polluted river water. *Journal of Environmental Sciences* **24**, 596–601.

Fan, J., Wang, W., Zhang, B., Guo, Y., Ngo, H. H., Guo, W., Zhang, J. & Wu, H. 2013 Nitrogen removal in intermittently aerated vertical flow constructed wetlands: impact of influent COD/N ratios. *Bioresource Technology* **143**, 461–466.

Fei, H. X., Pan, J., Tong, D. L., Huang, L. L., Yu, L. & Sun, Y. F. 2017 Effect of COD/N ratio on removal performances in two subsurface wastewater infiltration systems. *Water Environment Research* **8**, 693–702.

García, J., Capel, V., Castro, A., Ruíz, I. & Soto, M. 2007 Anaerobic biodegradation tests and gas emissions from subsurface flow constructed wetlands. *Bioresource Technology* **98**, 3044–3052.

Hallams, S. J., Girguis, P. R. & Preston, C. M. 2003 Identification of methyl coenzyme M reductase (mcrA) genes associated with methane-oxidizing archaea. *Applied and Environmental Microbiology* **69**, 5483–5491.

IPCC (Intergovernmental Panel on Climate Change) 2006 Waste. In: *Guidelines for National Greenhouse Gas Inventories*, Vol. 5 (H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara & K. Tanabe, eds). IGES, Japan.

Itokawa, H., Hanaki, K. & Matsuo, T. 1993 Nitrous oxide emission during nitrification and denitrification in a full-scale night soil treatment plant. *Water Science and Technology* **34**, 277–284.

Ji, G. D., Zhi, W. & Tan, Y. F. 2012 Association of nitrogen micro-cycle functional genes in subsurface wastewater infiltration systems. *Ecological Engineering* **44**, 269–277.

Kong, H. N., Kimochi, Y., Mizuochi, M., Inamori, R. & Inamori, Y. 2002 Study of the characteristics of CH_4 and N_2O emission and methods of controlling their emission in the soil-trench wastewater treatment process. *Science of the Total Environment* **290**, 59–67.

Kong, Q., Wang, Z. B., Niu, P. F. & Miao, M. S. 2016 Greenhouse gas emission and microbial community dynamics during simultaneous nitrification and denitrification process. *Bioresource Technology* **210**, 94–100.

Lance, J. C. 1986 Effect of sludge additions on nitrogen removal in soil columns flooded with secondary effluent. *Journal of Environment Quality* **15**, 298–301.

Lee, C. G., Fletcher, T. D. & Sun, G. Z. 2009 Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences* **9**, 11–22.

Li, Y. H., Li, H. B., Xu, X. Y., Xiao, S. Y., Wang, S. Q. & Xu, S. C. 2017 Field study on N_2O emission from subsurface wastewater infiltration system under variable loading rates and drying-wetting cycles. *Water Science and Technology* **76**, 2158–2166.

Li, Y. H., Li, H. B., Xu, X. Y., Wang, S. Q. & Pan, J. 2018 Does carbon-nitrogen ratio affect nitrous oxide emission and spatial distribution in subsurface wastewater infiltration system? *Bioresource Technology* **250**, 846–852.

Lv, B. Y., Zhang, D., Cui, Y. X. & Yin, F. 2018 Effects of C/N ratio and earthworms on greenhouse gas emissions during vermicomposting of sewage sludge. *Bioresource Technology* **268**, 408–414.

Morris, R., Schauer-Gimenez, A. & Bhattad, U. 2013 Methyl coenzyme M reductase (mcrA) gene abundance correlates with activity measurements of methanogenic H_2/CO_2-enriched anaerobic biomass. *Microbiology Biotechnology* **7**, 77–84.

Ouellet-Plamondon, C., Chazarenc, F., Comeau, Y. & Brisson, J. 2006 Artificial aeration to increase pollutant removal efficiency of constructed wetlands in cold climate. *Ecological Engineering* **27**, 258–264.

Picek, T., Cizkova, H. & Dusek, J. 2007 Greenhouse gas emissions from a constructed wetland-plants as important sources of carbon. *Ecological Engineering* **31**, 98–106.

Sánchez-Monedero, M. A., Serramiá, N., Civantos, C. G. O., Fernández-Hernández, A. & Roig, A. 2010 Greenhouse gas emissions during composting of two-phase olive mill wastes with different agroindustrial by-products. *Chemosphere* **81**, 18–25.

Song, S. Y., Pan, J., Wu, S. W., Guo, Y. J., Yu, J. X. & Shan, Q. C. 2016 Effects of COD/N ratios on pollutants removal in the subsurface wastewater infiltration systems with/without intermittent aeration. *Water Science and Technology* **73**, 2662–2669.

Steinberg, L. M. & Regan, J. M. 2009 The mcrA-targeted real-time quantitative PCR method to examine methanogen communities. *Applied and Environmental Microbiology* **75**, 4435–4442.

Wang, X., Sun, T. H., Li, H. B., Li, Y. H. & Pan, J. 2010 Nitrogen removal enhanced by shunt distributing wastewater in a subsurface wastewater infiltration system. *Ecological Engineering* **36**, 1433–1438.

Wang, Z., Liu, C. X., Liao, J., Liu, L., Liu, Y. H. & Huang, X. 2014a Nitrogen removal and N_2O emission in subsurface vertical flow constructed wetland treating swine wastewater: effect of shunt ratio. *Ecological Engineering* **73**, 446–453.

Wang, J. Z., Hu, Z. Y., Xu, X. K., Jiang, X., Zheng, B. H., Liu, X. N., Pan, X. B. & Kardol, P. 2014b Emissions of ammonia and greenhouse gases during combined pre-composting and vermicomposting of duck manure. *Waste Management* **34**, 1546–1552.

Wu, J., Zhang, J., Jia, W. L., Xie, H. J., Gu, R. R., Li, C. & Gao, B. Y. 2009 Impact of COD/N ratio on nitrous oxide emission from microcosm wetlands and their performance in removing nitrogen from wastewater. *Bioresource Technology* **100**, 2910–2917.

Wu, H. M., Fan, J. J., Zhang, J., Ngo, H. H. & Guo, W. S. 2015 Decentralized domestic wastewater treatment using intermittently aerated vertical flow constructed wetlands: impact of influent strengths. *Bioresource Technology* **176**, 163–168.

Yang, Y. Q., Zhan, X., Wu, S. J., Kang, M. L. & Guo, J. A. 2016 Effect of hydraulic loading rate on pollutant removal efficiency in subsurface infiltration system under intermittent operation and micro-power aeration. *Bioresource Technology* **205**, 174–182.

Yang, F., Li, G. X., Zang, B. & Zhang, Z. Y. 2017 The maturity and CH_4, N_2O, NH_3 emissions from vermicomposting with agricultural waste. *Compost Science and Utilization* **25**, 262–271.

Zhao, Y. J., Liu, B. & Zhang, W. G. 2009 Performance of pilot-scale vertical-flow constructed wetlands in responding to variation in influent C/N ratios of simulated urban sewage. *Bioresource Technology* **101**, 1693–1700.

Zheng, F. P., Tan, C. Q., Hou, W. Y., Huang, L. L., Pan, J. & Qi, S. Y. 2018 Does influent COD/N ratio affect nitrogen removal and N_2O emission in a novel biochar-sludge amended soil wastewater infiltration system (SWIS)? *Water Science and Technology* **78**, 347–357.

First received 27 October 2019; accepted in revised form 20 March 2020. Available online 30 March 2020