Pollutants removal, greenhouse gases emission and functional genes in wastewater ecological soil infiltration systems: influences of influent surface organic loading and aeration mode
Jingna Chen, Zefang Jiang, Yue Chen, Yu Qiu, Tingting Tao, Xiaoli Du and Jing Pan

ABSTRACT
The influences of influent surface organic loading rate (SOLR) and aeration mode on matrix oxygen, organic matter, nitrogen, phosphorus removal, greenhouse gases emission and functional gene abundances in lab-scale wastewater ecological soil infiltration systems (WESISs) were investigated. In WESISs, intermittent or continuous aeration improved oxygen supply at 50 cm depth and hardly changed anaerobic condition below 80 cm depth, which enhanced chemical oxygen demand (COD), NH_{4}^{+}-N, total nitrogen (TN) removal, the abundances of bacterial 16S rRNA, amoA, nxrA, narG, napA, nirK, nirS, qnorB, nosZ genes and reduced CH_{4}, N_{2}O conversion efficiencies with SOLR of 16.9 and 27.6 g BOD/(m^{2} d) compared with non-aeration. Increased SOLR resulted in high TN removal, low N_{2}O emission in aeration WESIS, which was different from non-aeration WESIS. High average COD removal efficiency of 90.7%, NH_{4}^{+}-N removal efficiency of 87.0%, TN removal efficiency of 84.6%, total phosphorus (TP) removal efficiency of 93.1% and low average N_{2}O emission rate of 12.8 mg/(m^{2} d) were achieved with SOLR of 16.9 g BOD/(m^{2} d) in intermittent aeration WESIS. However, continuous aeration WESIS obtained high average removal efficiencies of 90.1% for COD, 87.5% for NH_{4}^{+}-N, 84.1% for TN, 92.9% for TP and low average emission rate of 13.1 mg/(m^{2} d) for N_{2}O with SOLR of 27.6 g BOD/(m^{2} d). Aeration could be an optional strategy for WESISs to achieve high pollutants removal and low CH_{4}, N_{2}O emission when treating wastewater with high SOLR.

Key words | aeration mode, greenhouse gas, surface organic loading rate, wastewater ecological soil infiltration system

HIGHLIGHTS
● SOLR and aeration mode significantly affected pollutants removal and GHGs emission.
● Aeration obtained high pollutants removal and low CH_{4}, N_{2}O conversion efficiencies.
● Aeration enhanced the abundances of bacterial 16S rRNA and nitrogen removal genes.
● SOLR of 16.9–27.6 g BOD/(m^{2} d) was suggested for aeration WESISs.
INTRODUCTION

Wastewater ecological soil infiltration system (WESIS) is an optional technology for decentralized wastewater treatment, which has been applied in United States, Russia, Japan, Australia and China (Zhang et al. 2005; Ji et al. 2012). The WESIS has many advantages such as lower cost of construction and operation, lower energy consumption, easier management, better resistance of load shock and better wastewater recovery and reuse compared with conventional biological or chemical wastewater treatment methods (Zheng et al. 2016; Jiang et al. 2021).

CO2, CH4 and N2O are three major gases emitted from wastewater treatment which lead to greenhouse effect by trapping heat from earth surface (Daelman et al. 2013). During wastewater treatment process of WESISs, CO2 emits in aerobic degradation of organic matter by microorganisms; CH4 is produced in the anaerobic decomposition of organic matter by methanogen; N2O emission occurs during nitrogen biological transformation processes by nitrifying and denitrifying bacteria (Kong et al. 2002, 2016). A few studies about greenhouse gases (GHGs) emission have been conducted in WESISs. Kong et al. (2016) found that influent C/N affected N2O emission in the WESIS. Zheng et al. (2018) reported intermittent aeration and influent shunt distributing combined method reduced N2O emission of WESISs. Kong et al. (2002) concluded that CH4 emission increased with the increase of temperature and N2O emission was positively correlated with matrix oxidation reduction potential (ORP). Field study found that influent loading and drying-wetting ratio affected N2O emission in the WESIS (Li et al. 2017). However, few studies have simultaneously investigated three GHGs (CO2, CH4 and N2O) emission of WESISs.

Nitrogen removal of WESISs was unsatisfactory with total nitrogen (TN) removal efficiencies below 50% (Fan et al. 2013). Many strategies have been conducted to improve nitrogen removal, such as adjusting operation parameters (Pan et al. 2020), adjusting configuration styles (Wang et al. 2010; Lloréns et al. 2011) and enhancing oxygen supply by aeration (Yang et al. 2016; Li et al. 2021). Among these studies, aeration was the most effective method to improve nitrogen removal (Pan et al. 2016). Jiang et al. (2017) concluded N2O emission decreased in intermittent aeration WESIS with the increase of influent surface organic loading rate (SOLR) within the range of 5.3 and 16.5 g BOD/(m2 d). Zheng et al. (2018) revealed intermittent aeration WESIS amended with biochar-sludge obtained N2O emission rate of 10.6 mg/(m2 d) under COD/N ratio of 15, which was lower than those in non-aeration WESISs amended with/without biochar-sludge. Sun et al. (2017) reported aeration mode and influent hydraulic loading rate affected TN removal and the abundances of functional gene involved in nitrogen removal in WESISs. So far, there is little information about double effects of influent SOLR and aeration mode on pollutants removal and three GHGs emission in WESISs.

In this study, three pilot WESISs operated with different influent SOLRs and aeration modes were investigated. The main aims of this paper were: (1) to investigate double effects of influent SOLR and aeration mode on pollutants removal; (2) to evaluate double effects of influent SOLR and aeration mode on GHGs emission; (3) to identify spatial distribution of bacteria and functional genes involved in COD, NH4+-N, TN removal and CO2, CH4, N2O emission with different influent SOLRs and aeration modes; (4) to provide useful information for future application of aeration WESISs.

MATERIALS AND METHODS

WESISs description and operation

Soil column experiment is an important research method for WESISs. When the diameter of the soil column is greater...
than 10 cm and the height is greater than 100 cm, the side wall effect can be eliminated (Lloréns et al. 2011). Figure 1 shows the schematic diagram of three lab-scale WESISs. Each WESIS operated in the greenhouse with temperature of $20 \pm 1^\circ C$ in Shenyang Normal University of China, which made of polyvinyl chloride columns with a height of 120 cm and internal diameter of 30 cm. Each WESIS was filled the same matrix, which was 80% of brown earth and 20% of coal slag by mass percentage. Mixed matrix with 110 cm thickness and gravel (5–10 mm in diameter) with 10 cm thickness were arranged from top to bottom. The mixed matrix had hydraulic conductivity of $(1.82 \pm 0.3) \times 10^{-3}$ cm/s, surface area of $210.7 \pm 4.1$ m$^2$/kg and contained total organics of $27.3 \pm 0.8$ g/kg, total nitrogen of $1.1 \pm 0.3$ g/kg, total phosphorus of $0.5 \pm 0.05$ g/kg, pH 7.2. Wastewater was distributed by influent pipe which was installed at 50 cm depth below matrix surface in each WESIS. Water was collected at the bottom by outlet pipe after treatment. Matrix sampling ports were placed at 50, 80 and 110 cm depths from the surface to investigate bacteria and functional genes involved in pollutants removal and GHGs emission. An air compressor, air tube and micro-bubble diffuser constituted an aeration unit. The micro-bubble diffuser was located at 40 cm depth for providing oxygen in WESIS B and C. The micro-bubble diffuser and influent pipe were wrapped up with gravel (10–20 mm in diameter) to prevent clogging and distribute air evenly. WESIS A was without aeration unit which was a conventional WESIS (non-aeration).

Domestic wastewater after being pretreated in a septic tank flowed into each WESIS continuously. In each WESIS, SOLR elevated from 4.4 to 8.5, 16.9 and 27.6 g BOD/(m$^2$ d).

The experimental SOLRs were achieved by adjusting the amount of wastewater treated by WESISs. The SOLR was determined by formula (1):

$$\text{SOLR} = \frac{CQ}{S}$$

where, $C$: BOD concentration in the influent (mg/L); $Q$: wastewater treatment quantity in WESIS (L/d); $S$: the area of WESIS (m$^2$).

Each SOLR experiment lasted for 60 days. WESIS B was with continuous aeration and WESIS C was with intermittent aeration. WESIS C was subjected to aerate for 1 h and then had 5 h interval without aeration. The aeration began at 2:00 AM, 8:00 AM, 14:00 PM and 20:00 PM every day, respectively. Airflow rate of $3.0 \pm 0.2$ L/min was applied in WESIS B and C.

### Sampling and analytical methods

Influents and effluents of the WESISs were sampled every five days. Water samples were analyzed for chemical oxygen demand (COD), NH$_4^+$-N, NO$_3^-$-N, TN, total organic carbon (TOC) and TP according to the Standard Method for Examination of Water and Wastewater (Standard Method for the Examination of Water and Wastewater Editorial Board 2002).

Matrix oxygen concentrations were measured by oxygen electrodes installed at 50, 80, 110 cm depths and were stored in the MDA-501 data storing device (Tuopu Co. Ltd, Shunde, China) every 20 min.

Gases emitted from the WESISs were sampled by the static stationary chambers with 40 cm in height and 15 cm

![Figure 1](http://iwaponline.com/wst/article-pdf/83/7/1619/870831/wst083071619.pdf) **Figure 1** Schematic diagram of three wastewater ecological soil infiltration systems (WESISs), named WESIS A (non-aeration), WESIS B (with continuous aeration) and WESIS C (with intermittent aeration).
in diameter. The details about static stationary chambers were described in previous studies (Li et al. 2017; Zheng et al. 2018). Gas sampling bags were used to collect gases from air outlet at the middle part of chambers. Five gas samples were collected at 0, 1, 3, 5 and 7 h after enclosure at the same time of sampling day between 14:00 PM and 20:00 PM every five days. CH₄, CO₂ and N₂O concentrations of the gas samples were determined using Agilent 6890N gas chromatography with a flame ionization detector, thermal conductivity detector and electron capture detector, respectively. CO₂, CH₄ and N₂O measuring processes were referred to a previous study (Zheng et al. 2018). Emission rates of CO₂, CH₄ and N₂O were calculated by formula (2) (Chiemchaisri et al. 2009).

Greenhouse gas emission rate = \( \frac{V}{A} \times \frac{dC}{dt} \) (2)

where, V: the volume of gas collecting chamber (m³); A: the area of gas collecting chamber (m²); \( \frac{dC}{dt} \): the slope of the best-fit line for the plot of gas concentration inside the gas collecting chamber and time data points (mg/m³ h).

The greenhouse gas conversion efficiency is the mass percentage of influent TN or TOC converted to N₂O or CO₂ and CH₄ within sampling interval, respectively. Conversion efficiencies of CO₂, CH₄ and N₂O were calculated by formula (3).

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\text{Greenhouse gas conversion efficiency (\%)} = \frac{M_1}{M_2} \times 100\% (3)
\]

M₁: CO₂ or CH₄ or N₂O production within sampling interval, mg; M₂: mass of TOC or TN of influent within sampling interval, mg.

In WESISs, COD removal and CO₂ emission relate to bacteria activities. COD removal and CH₄ producing owe to methanogen. Nitrogen removal and N₂O emission mainly attribute to nitrifying bacteria and denitrifying bacteria. These processes involve several functional genes which are mcrA (Methyl Coenzyme M Reductase A), amoA (ammonia monoxygenase), 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 participates in COD removal and CH₄ producing. The amoA and nxrA involve in nitrification process. The narG, napA, nirK, nirS, qnorB and nosZ are six functional genes associated with denitrification (Ji et al. 2012). Matrix samples were collected from sampling ports at 50, 80 and 110 cm depths after each influent SOLR experiment. The abundances of bacteria and functional genes were analyzed by quantitative polymerase chain reaction (qPCR) on 16S rRNA fragment of bacteria, the target fragment of mcrA, amoA, nxrA, narG, napA, nirK, nirS, qnorB and nosZ genes. The primers for above fragments were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd in China with the concentration of 10 pmol/L. The qPCR processes were performed on the basis of Ji et al. (2012) and Morris et al. (2014).

All experimental data were generated from steady state conditions. In this study, statistical analysis used SPSS 12.0 software. Two-way ANOVA was used to evaluate the significance of differences. The level of significance was accepted when P was below 0.05 (n = 12).

**RESULTS AND DISCUSSION**

**Matrix oxygen concentrations in an aeration/non-aeration period**

From Figure 2, it can be seen that average oxygen concentrations were below 0.8 mg/L at all depths in WESIS A with SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d). Non-aeration WESIS A was in anaerobic condition because air diffused into the matrix was limited. Matrix oxygen concentrations at 50 cm depth decreased with SOLR increasing in WESIS A, B and C. With SOLR increasing, more oxygen was depleted by aerobic oxidation of more organic matter. In WESIS B, matrix oxygen concentrations at 50 cm depth were higher than 3.4 mg/L and below 0.33 mg/L at 80, 110 cm depths with SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively. Continuous aeration WESIS was in aerobic condition (oxygen ≥ 2 mg/L) at 50 cm and in anaerobic condition below 80 cm. In WESIS C, matrix oxygen concentrations at 50 cm depth were higher than 3.5 mg/L during aeration and higher than 1.0 mg/L when aeration closed with SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively. However, oxygen concentrations were lower than 0.33 mg/L with/without aeration at 80 and 110 cm, respectively. Oxygen concentrations showed descending trends in non-aeration period owing to aerobic oxidation of nitrogen and organic matter. With SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), average oxygen concentrations were 8.3 mg/L, 7.8 mg/L, 5.3 mg/L, 3.5 mg/L in WESIS B and were 6.2 mg/L, 5.9 mg/L, 5.7 mg/L, 2.3 mg/L in WESIS C at 50 cm, respectively, which were
significantly higher than those of WESIS A \((P < 0.05)\). However, there were no significant differences at 80 and 110 cm \((P > 0.05)\) in WESIS A, B and C. Intermittent or continuous aeration improved oxygen concentrations at 50 cm depth and did not change anaerobic condition below 80 cm depth.

**Pollutants removal performance of three WESISs**

The influent, effluent concentrations and removal efficiencies of COD, \(\text{NH}_4^+\)-N, TN and TP are shown in Table 1.

Organic matter is absorbed by the matrix and then broken down by microbial processes in WESISs (Yang et al. 2019). Although it can be degraded both aerobically and anaerobically, aerobic degradation is usually more important (Li et al. 2021). Oxygen was considered as one of the main rate-limiting factors for organics removal (Wang et al. 2010). Anoxic or anaerobic condition prevails in non-aeration WESIS, which was not conducive to COD removal. The effluent COD concentrations were 41.5 \pm 2.8 mg/L, 57.5 \pm 2.1 mg/L, 146.5 \pm 6.7 mg/L, 211.4 \pm 9.6 mg/L with COD removal efficiencies of 83.8 \pm 0.7\%, 77.5 \pm 1.2\%, 43.5 \pm 2.1\%, 35.1 \pm 2.3\% under influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively. With influent SOLR of 4.4 and 8.5 g BOD/(m² d), COD concentrations of the effluent in WESIS A were below Class I \((\leq 60 \text{ mg/L})\) of Chinese Criterion for Water Discharge from Municipal Wastewater Treatment Plant (GB18921-2002). Jiang et al. (2017) concluded non-aeration WESISs were effective in organic matter removal with low influent SOLR. COD removal efficiencies decreased with influent SOLR increasing in WESIS A. Qi et al. (2018) reported oxygen deficiency developed more obviously with the increase of influent SOLR in non-aeration WESIS, which caused COD removal efficiencies decrease. COD removal efficiencies were 99.4 \pm 0.1\%, 98.4 \pm 0.2\%, 97.9 \pm 0.7\%, 90.1 \pm 0.7\% in WESIS B and were 99.1 \pm 0.2\%,
97.9 ± 0.3%, 90.7 ± 0.3%, 80.2 ± 0.6% in WESIS C with SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), which were significantly higher than those in WESIS A ($P < 0.05$). Aeration supplied extra oxygen for aerobic biodegradation of organic matter, which improved COD removal in WESIS B and C. Liang et al. (2019) concluded that sufficient oxygen supply could greatly improve organic matter aerobic biochemical oxidation. More oxygen was required to degrade organic matter with influent SOLR increasing (Jiang et al. 2011). In this study, aeration could not supply adequate oxygen for organic matter aerobic decomposition with high influent SOLR, which led to COD removal efficiencies decreasing from 98.4 ± 2.7 to 90.1 ± 0.1% for WESIS B and from 97.9 ± 0.3 to 80.2 ± 0.6% for WESIS C as SOLR increasing from 8.5 to 27.6 g BOD/(m² d). Ouellet-Plamondon et al. (2006) and Li et al. (2021) reported the same results, but the research systems were different. One was the wetland, the other was the WESIS.

NH₄⁺-N is removed by plant uptake, ammonia volatilization, microbial nitrification-denitrification and matrix adsorption in the WESISs. Among these, nitrification coupled with denitrification is the main removal process (Yang et al. 2021). NH₄⁺-N elimination is firstly dependent on nitrification. Nitrification is an aerobic chemo-auto-trophic microbial process, which is the limiting step for nitrogen removal (Jia et al. 2019). As can be seen from Table 1, NH₄⁺-N removal efficiencies decreased with influent SOLR increasing in WESIS A, which achieved NH₄⁺-N removal efficiencies of 86.1 ± 2.3, 79.9 ± 1.6, 47.3 ± 5.4 and 19.8 ± 3.2% with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively. The effluent NH₄⁺-N concentrations of WESIS A with influent SOLR of 16.9 and 27.6 g BOD/(m² d) were above Class I (≤15 mg/L) of the criteria mentioned. More organic matter entering with influent SOLR increasing constrained the activity of nitrifying bacteria by oxygen competition which resulted in NH₄⁺-N
Table 1 | Removal performances in WESISs with non-aeration (WESIS A), continuous aeration (WESIS B) and intermittent aeration (WESIS C) under different influent surface organic loading rates (SOLRs)

| Effluent | COD (mg/L) | COD* | NH₄-N (mg/L) | NH₄-N* | TN (mg/L) | TN* | TP (mg/L) | TP* | NO₃-N (mg/L) | DO (mg/L) |
|----------|------------|------|--------------|--------|-----------|------|-----------|------|-------------|-----------|
| Influent | 234.8 ± 41.2 | - | 35.5 ± 3.4 | - | 36.7 ± 5.5 | - | 4.4 ± 1.1 | - | 1.7 ± 0.8 | 1.2 ± 0.4 |
| **Influent SOLR of 4.4 g BOD/(m² d)** | | | | | | | | | |
| WESIS A | 41.5 ± 2.8 | 83.8 ± 0.7 | 5.1 ± 0.7 | 86.1 ± 2.3 | 12.6 ± 0.5 | 68.3 ± 1.2 | 0.26 ± 0.02 | 94.6 ± 1.1 | 3.9 ± 0.3 | 0.23 ± 0.04 |
| WESIS B | 2.1 ± 0.6 | 99.4 ± 0.1 | 0.21 ± 0.2 | 99.4 ± 0.1 | 34.7 ± 1.2 | 12.6 ± 0.3 | 0.21 ± 0.01 | 95.6 ± 0.8 | 32.5 ± 1.2 | 0.25 ± 0.07 |
| WESIS C | 2.4 ± 0.3 | 99.1 ± 0.2 | 0.26 ± 0.2 | 99.3 ± 0.1 | 34.2 ± 0.8 | 13.9 ± 0.5 | 0.24 ± 0.02 | 95.0 ± 0.5 | 31.2 ± 1.0 | 0.23 ± 0.05 |
| **Influent SOLR of 8.5 g BOD/(m² d)** | | | | | | | | | |
| WESIS A | 57.5 ± 2.1 | 77.5 ± 1.2 | 7.4 ± 0.4 | 79.9 ± 1.6 | 14.5 ± 0.3 | 63.5 ± 0.8 | 0.34 ± 0.03 | 92.9 ± 0.7 | 4.7 ± 0.6 | 0.22 ± 0.01 |
| WESIS B | 4.4 ± 0.7 | 98.4 ± 0.2 | 0.40 ± 0.1 | 98.9 ± 0.3 | 23.6 ± 1.2 | 40.6 ± 1.1 | 0.24 ± 0.02 | 95.8 ± 0.3 | 23.1 ± 1.1 | 0.23 ± 0.02 |
| WESIS C | 5.5 ± 0.5 | 97.9 ± 0.3 | 0.44 ± 0.2 | 98.8 ± 0.2 | 23.1 ± 1.0 | 41.3 ± 0.6 | 0.28 ± 0.02 | 94.2 ± 0.8 | 21.5 ± 1.0 | 0.22 ± 0.06 |
| **Influent SOLR of 16.9 g BOD/(m² d)** | | | | | | | | | |
| WESIS A | 146.5 ± 6.7 | 43.5 ± 2.1 | 19.4 ± 2.6 | 47.3 ± 5.4 | 23.8 ± 2.1 | 40.1 ± 0.7 | 0.38 ± 0.01 | 92.1 ± 0.6 | 3.5 ± 0.8 | 0.23 ± 0.03 |
| WESIS B | 5.5 ± 1.2 | 97.9 ± 0.7 | 2.4 ± 0.5 | 93.5 ± 1.5 | 8.2 ± 0.6 | 79.3 ± 0.9 | 0.30 ± 0.01 | 95.8 ± 0.4 | 4.9 ± 0.3 | 0.22 ± 0.01 |
| WESIS C | 24.1 ± 1.1 | 90.7 ± 0.3 | 4.8 ± 0.3 | 87.0 ± 1.2 | 6.1 ± 0.2 | 84.6 ± 0.5 | 0.33 ± 0.02 | 93.1 ± 0.2 | 0.8 ± 0.2 | 0.24 ± 0.04 |
| **Influent SOLR of 27.6 g BOD/(m² d)** | | | | | | | | | |
| WESIS A | 211.4 ± 9.6 | 33.1 ± 2.3 | 29.5 ± 2.1 | 19.8 ± 3.2 | 32.8 ± 2.4 | 17.4 ± 0.8 | 0.46 ± 0.03 | 90.4 ± 0.6 | 2.4 ± 0.5 | 0.21 ± 0.06 |
| WESIS B | 25.6 ± 1.5 | 90.1 ± 0.7 | 4.6 ± 0.4 | 87.5 ± 1.2 | 6.5 ± 0.3 | 84.1 ± 0.6 | 0.39 ± 0.01 | 92.9 ± 0.3 | 0.9 ± 0.3 | 0.23 ± 0.03 |
| WESIS C | 51.5 ± 2.7 | 80.2 ± 0.6 | 7.5 ± 0.7 | 79.6 ± 0.8 | 8.9 ± 0.6 | 77.6 ± 0.5 | 0.41 ± 0.01 | 92.2 ± 0.4 | 1.1 ± 0.2 | 0.24 ± 0.05 |

(mean ± SD) (n = 12).
*removal efficiency, %.
removal efficiencies decreasing in WESIS A. Most conventional WESISs could not finish NH₄⁺-N removal satisfactorily because of insufficient oxygen supply, especially with high SOLR (Yang et al. 2016). NH₄⁺-N removal efficiencies of WESIS B and C were significantly higher than those of WESIS A under the same influent SOLR ($P < 0.05$). NH₄⁺-N removal efficiencies of WESISs with aeration were higher than 97.0% with influent SOLR of 4.4 and 8.5 g BOD/(m² d). Matrix oxygen results showed that the oxidative condition of the matrix was improved by aeration in WESIS B and C, which was favorable for the nitrification process. Average NH₄⁺-N removal efficiencies decreased when influent SOLR increased from 8.5 to 27.6 g BOD/(m² d) in WESIS B and WESIS C. Intermittent and continuous aeration could not provide sufficient oxygen for nitrification with the studied aeration parameters with high SOLR of 27.6 g BOD/(m² d). WESIS C achieved NH₄⁺-N removal efficiency of 79.6 ± 0.8% which was significantly lower than that of WESIS B under influent SOLR of 27.6 g BOD/(m² d) ($P < 0.05$) because continuous aeration supplied more oxygen for nitrification than intermittent aeration.

Nitrification converts nitrogen into various forms and the nitrified nitrogen must be processed via denitrification to be permanently eliminated from WESISs (Pan et al. 2016). Various factors such as insufficient organic carbon source and excess oxygen could limit denitrification (Wang et al. 2010). In WESIS A, TN removal efficiencies decreased with influent SOLR increasing, which were in accordance with NH₄⁺-N removal. TN concentrations of the effluent were 12.6 ± 0.5 mg/L, 14.5 ± 0.3 mg/L, 23.8 ± 2.1 mg/L and 32.8 ± 2.4 mg/L with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d) in WESIS A, respectively (in Table 1). TN concentrations were higher than Class I (≤20 mg/L) of the above criteria with influent SOLR of 16.9 and 27.6 g BOD/(m² d). In non-aeration WESIS A, nitrification process was restricted due to insufficient oxygen supply which could not provide sufficient NO₃⁻-N as electron acceptors, especially with high SOLR. As a result, denitrification process was also inhibited (Pan et al. 2013; Sun et al. 2017). In WESIS B and C, TN in the effluents were dominated by NO₃⁻-N and TN concentrations in the effluents exceeded Class I of the above criteria with influent SOLR of 4.4 and 8.5 g BOD/(m² d). Besides, average TN removal efficiencies were below 40.6% for WESIS B and 41.3% for WESIS C. After effective nitrification under aerobic condition, the NO₃⁻-N as electron accepters could not be removed permanently except sufficient organic carbon was supplied as electron donor. Carbon deficiency was the key limiting factor for TN removal in aerated reactors and NO₃⁻-N accounted for the major part of the effluent TN (Fan et al. 2015). TN removal efficiencies of WESIS B and C were significantly higher than those of WESIS A with influent SOLR of 16.9 and 27.6 g BOD/(m² d) ($P < 0.05$). Average TN concentrations of the effluent were below 8.9 mg/L for WESIS B and C with influent SOLR of 16.9 and 27.6 g BOD/(m² d). High influent SOLR could provide more carbon source as electron donor for denitrification after effective nitrification in aeration WESISs, which achieved high TN removal ultimately. TN removal efficiency of WESIS C was higher than that of WESIS B with influent SOLR of 16.9 g BOD/(m² d) because of more organic matter obtained in WESIS C compared with WESIS B. Fan et al. (2015) confirmed carbon source deficiency was the main limiting factor for TN removal after high effective nitrification. TN removal efficiency of WESIS B was higher than that of WESIS C with influent SOLR of 27.6 g BOD/(m² d) due to more organic matter supply and higher nitrification rate of WESIS B.

In WESISs, physical sedimentation and chemical adsorption are the major ways for phosphorus removal, which complete instantaneously. In addition, phosphorus removal efficiency is positive correlation to the matrix area (Wang et al. 2010). TP concentrations of the effluent were below 0.5 mg/L and TP removal efficiencies were higher than 90% with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d) in WESIS A, B and C. TP removal efficiencies decreased with the increase of influent SOLR because more organic matter competed with TP in adsorption sites in three WESISs. Effluent TP concentrations of WESIS B and C were a little lower than those of WESIS A with the same influent SOLR. Aeration improved contact between phosphorus and matrix near the aeration zone, which was beneficial to physical sedimentation and chemical adsorption for phosphorus removal there (De-Bashan & Bashan 2004). The results were consistent with the study of Dong et al. (2012) and Pan et al. (2016), which reported the wetland and WESIS operated with aeration could enhance TP removal, respectively.

The appropriate SOLR of 4.4–8.5 g BOD/(m² d) was recommended for non-aeration WESISs and 16.9–27.6 g BOD/(m² d) was suggested for intermittent aeration and continuous aeration WESISs according to COD, NH₄⁺-N and TN removal performances.

**GHGs emission**

It can be seen from Figure 3 that CO₂ conversion efficiencies decreased with influent SOLR increasing in WESIS A
Figure 3 | CO₂, CH₄ and N₂O emission rates and conversion efficiencies with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d) in three WESISs.
and average CO₂ emission rates were 3.9, 6.8, 8.6 and 9.1 g/(m² d) with average CO₂ conversion efficiencies of 70.9, 64.2, 40.8 and 26.4% when influent SOLRs were 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively. Air diffusion to the matrix was limited in non-aeration WESISs (Yang et al. 2016). With influent SOLR increasing, lack of oxygen became more serious, which caused organic matter aerobic oxidation decrease and resulted in CO₂ conversion efficiencies reduction. With influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), average CO₂ emission rates and conversion efficiencies of WESIS B and C were significantly higher than those of WESIS A because of the application of aeration (P < 0.05). With influent SOLR increasing from 16.9 to 27.6 g BOD/(m² d), average CO₂ conversion efficiencies of WESIS C decreased from 80.1 to 72.2%. With influent SOLR increasing, more oxygen was required to oxidize excess organic matter. Intermittent aeration could not supply enough oxygen with high SOLR of 27.6 g BOD/(m² d), which inhibited organic matter aerobic oxidation and reduced CO₂ conversion efficiencies in comparison with continuous aeration.

CH₄ conversion efficiencies enhanced with the increase of influent SOLR in WESIS A, which were 1.6, 1.7, 1.8 and 2.0% with average emission rates of 91.6, 185.5, 384.2 and 675.8 mg/(m² d) under the influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d), respectively (in Figure 3). Influent SOLR increasing meant more organic matter, which might consume more oxygen and provide more carbon source for the growth of methanogen (Maucieri et al. 2016). Therefore, CH₄ conversion efficiencies increased with influent SOLR increasing in non-aeration WESIS. Average CH₄ conversion efficiencies were below 0.9% with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d) in WESIS B and C, respectively. Average CH₄ emission rates and conversion efficiencies of WESIS B and C were significantly lower than those of WESIS A with the same influent SOLR (P < 0.05). Average CH₄ conversion efficiencies of WESIS B and C had no significant difference in this study (P > 0.05). Low conversion efficiencies of CH₄ in WESIS B and C might be ascribed to aeration, which boosted organic matter aerobic oxidation and caused a reduction of available organic matter to methanogen. The result was consistent with the study of Wang et al. (2014a) that aeration could reduce CH₄ production.

N₂O is an intermediate product of nitrification and denitrification processes in WESISs. Denitrification is generally regarded as the dominant process responsible for N₂O emission (Kong et al. 2016). N₂O emission rate decreased with influent SOLR increasing in WESIS A. Average N₂O emission rates of WESIS A were 29.5, 27.6, 20.5, 18.9 mg/(m² d) with N₂O conversion efficiencies of 0.24–0.37% under SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d). The SOLR increasing meant more organic matter in the influent, which would consume more available oxygen to degrade it and further limit the autotrophic ammonia oxidation bacteria to oxidize NH₄⁺-N due to oxygen competition. As a result, denitrification next to nitrification process was restricted in WESIS A. Finally, N₂O emission decreased. Average N₂O emission rates of WESIS B and C were 37.7 and 35.5 mg/(m² d) with influent SOLR of 4.4 g BOD/(m² d); 35.6 and 32.4 mg/(m² d) with influent SOLR of 8.5 g BOD/(m² d); 14.6 and 12.8 mg/(m² d) with influent SOLR of 16.9 g BOD/(m² d); 13.1 and 14.2 mg/(m² d) with influent SOLR of 27.6 g BOD/(m² d), respectively. Average N₂O conversion efficiencies were 0.17–0.48% for WESIS B and 0.17–0.45% for WESIS C with SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d). Average N₂O conversion efficiencies of WESIS B and C were higher than those of WESIS A with influent SOLR of 4.4 and 8.5 g BOD/(m² d) because of the application of aeration and low denitrification. Sabba et al. (2015) concluded higher effective nitrification with lower denitrification emitted more N₂O. Sufficient carbon source provided for denitrification could greatly reduce N₂O production after efficient nitrification (Liang et al. 2019). Carbon source of lower part was insufficient which restricted N₂O transforming to N₂ in intermittent aeration and continuous aeration WESISs with low SOLR. Higher SOLR could provide more carbon source for N₂O to N₂ transformation, therefore N₂O conversion efficiency decreased when influent SOLR increased from 8.5 to 16.9 g BOD/(m² d). Similar results were reported by Zhou et al. (2018) and Wang et al. (2014b), although the matrix used were different from this study. Average N₂O conversion efficiency of WESIS C was lower than that of WESIS B with SOLR of 16.9 g BOD/(m² d) and was higher than that of WESIS B with SOLR of 27.6 g BOD/(m² d). The reason was more carbon source was obtained in WESIS C with SOLR of 16.9 g BOD/(m² d). In WESIS B, more carbon source was provided after high efficient nitrification with SOLR of 27.6 g BOD/(m² d) compared with WESIS C, which promoted N₂O to N₂ transformation.

With consideration of CH₄ and N₂O conversion efficiencies, SOLR of 16.9–27.6 g BOD/(m² d) was recommended for intermittent aeration WESIS and continuous aeration WESIS.
Functional gene abundances involved in pollutants removal and GHGs emission

Figure 4 shows the abundances of bacterial 16S rRNA and functional genes involved in COD removal, nitrogen removal and CO₂, CH₄, N₂O emission.

In a WESIS, aerobic decomposition by bacteria is the main way to remove organic matter (Lance 1986). The abundances of bacterial 16S rRNA decreased with the increase of matrix depth in WESIS A, B and C, which followed the same trend of matrix oxygen. The abundances of bacterial 16S rRNA at 50 cm depth decreased in WESIS A and increased in WESIS B, C with influent SOLR increasing. In non-aeration WESIS A, oxygen shortage became more obvious with the increase of influent SOLR, which resulted in a reduction of bacteria. With the same influent SOLR, the abundances of bacterial 16S rRNA of WESIS B and C were significantly higher than those of WESIS A at 50 cm depth (P < 0.05), which could further explain higher COD removal and more CO₂ producing in WESIS B and C. Pang et al. (2013) reported the same result that aeration could improve the richness bacterial 16S rRNA when aeration WESIS treated wastewater with high influent C/N ratio. Aeration provided extra oxygen to the upper matrix and high influent SOLR supply adequate substrate for bacteria which promoted the growth and reproduction of bacteria in WESIS B and C. Al-Baldawi et al. (2015) concluded that supplementary aeration could increase the quantification of bacterial populations and improve COD removal. With influent SOLR of 27.6 g BOD/(m² d), the abundances of bacterial 16S rRNA of WESIS B were significantly higher than those of WESIS C at 50 cm depth due to more oxygen supply by continuous aeration (P < 0.05).

Methanogen belongs to anaerobic bacteria, which could be monitored using mcrA gene (Morris et al. 2014). As can be seen from Figure 4, the abundances of mcrA increased along the wastewater flow direction in three WESISs, which was contrary to the trend of matrix oxygen. The spatial distribution of mcrA in this study was consistent with Pang et al. (2020). The abundances of mcrA at 80 and 110 cm depths increased with influent SOLR increasing in WESIS A. Oxygen insufficiency became more serious with influent SOLR increasing which favored methanogen in WESIS A. Moreover, methanogen could acquire more organic matter with influent SOLR increasing. The abundances of mcrA in WESIS A were significantly higher than those in WESIS B and C with the same depth and influent SOLR (P < 0.05), which was consistent with CH₄ emission. The abundance of mcrA was a significant positive correlation with CH₄ emission (Morris et al. 2014). The abundances of mcrA in WESIS B and C had no significant difference with the same depth and influent SOLR in this study (P > 0.05), which was consistent with CH₄ emission.

Figure 4 | The bacteria and functional gene abundances involved in pollutants removal and greenhouse gases emission with influent SOLR of 4.4, 8.5, 16.9 and 27.6 g BOD/(m² d) in three WESISs.
The oxidation of NH$_4^+$-N to NO$_2^-$-N and NO$_2^-$-N to NO$_3^-$-N are catalyzed by amoA and nxrA genes, respectively (Sun et al. 2017). In three WESISs, the abundances of amoA and nxrA decreased along the wastewater flow direction which concurred with the tendency of matrix oxygen. In non-aeration WESIS A, the abundances of amoA and nxrA decreased at 50 cm depth with influence SOLR increasing due to insufficient oxygen supply, which was in line with NH$_4^+$-N removal. The abundances of amoA and nxrA at 50 cm depth in WESIS B and C increased with influence SOLR increasing. Aeration provided oxygen for nitrification and high influence SOLR supplied extra NH$_4^+$ substrate for nitrifiers which promoted the growth and reproduction of nitrifiers. The abundances of amoA and nxrA at 50 cm depth of WESIS B and C were significantly higher than those of WESIS A (P < 0.05) due to aeration, which followed NH$_4^+$-N removal.

NarG and napA genes catalyze NO$_3^-$-N to NO$_2^-$-N reduction, which is the first process in denitrification. The second reaction process is NO$_2^-$-N to NO transformation catalyzed by nirS and nirK. NO to N$_2$O reduction is the third process catalyzed by qnorB. N$_2$O to N$_2$ reduction catalyzed by nosZ is the last reaction in denitrification. The abundances of narG, napA, nirK, nirS, qnorB and nosZ decreased with the increase of influent SOLR at 80 and 110 cm depths of WESIS A. Nitrification process was restricted with influence SOLR increasing, which led to low efficiency of denitrification and decreased the enrichment of six functional genes involved in denitrification process. When influent SOLRs were 4.4 and 8.5 g BOD/(m$^2$ d), the abundances of six functional genes in WESIS A were higher than those of WESIS B and C. Carbon source insufficiency with low influence SOLR in aeration WESISs decreased the abundances of six functional genes, which was consistent with the study of Qi et al. (2018). The abundances of six functional genes in WESIS B and C were significantly higher than those in WESIS A at 80 and 110 cm depths with influent SOLR of 16.9 and 27.6 g BOD/(m$^2$ d) (P < 0.05), which could further explain high removal of TN and low N$_2$O emission in WESIS B and C with high influence SOLR. Chen et al. (2020) reported the same result. Aeration promoted nitrification and high influence SOLR supplied more carbon source for denitrification, which enhanced the abundances of six genes involved in denitrification.

**CONCLUSIONS**

Aeration mode and influence SOLR had a great influence on organic matter removal, nitrogen removal and GHGs emission. COD, NH$_4^+$-N, TN, TP removal efficiencies, NO$_2$ emission decreased and CH$_4$ emission increased with SOLR increasing in non-aeration WESIS. Aeration provided extra oxygen at 50 cm depth and did not change anaerobic condition below 80 cm depth, which enhanced COD, NH$_4^+$-N, TN, TP removal, the abundances of bacterial 16S rRNA, amoA, nxrA, narG, napA, nirK, nirS, qnorB, nosZ genes and decreased CH$_4$, N$_2$O conversion efficiencies, the abundances of mcrA compared with non-aeration WESIS with influent SOLR of 16.9 and 27.6 g BOD/(m$^2$ d). In light of pollutants removal and CH$_4$, N$_2$O emission, the appropriate SOLR of 4.4–8.5 g BOD/(m$^2$ d) and 16.9–27.6 g BOD/(m$^2$ d) were separately recommended for non-aeration WESISs and aeration WESISs.
Chiemchaisr, C., Chiemchaisri, W. & Junsod, J. 2009 Leachate treatment and greenhouse gas emission in subsurface horizontal flow constructed wetland. Bioresource Technology 100, 3808–3814.

Daelman, M. R. J., van Voorthuizen, E. M., van Dongen, L. G., Volcke, E. I. & Van Loosdrecht, M. C. M. 2015 Methane and nitrous oxide emissions from municipal wastewater treatment results from a long-term study. Water Science and Technology 67, 2350–2355.

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.

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. 2015 Nitrogen removal in intermittently aeration vertical flow constructed wetlands: impact of influent COD/N ratios. Bioresource Technology 145, 461–466.

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

Jia, L. P., Jiang, B. H., Huang, F. & Hu, X. M. 2019 Nitrogen removal mechanism and microbial community changes of bioaugmentation subsurface wastewater infiltration system. Bioresource Technology 294, 122–140.

Ji, Y. Y., Sun, Y. F., Pan, J., Qi, S. Y., Chen, Q. Y. & Tong, D. L. 2017 Nitrogen removal and N2O emission in subsurface wastewater infiltration systems with/without intermittent aeration under different organic loading rates. Bioresource Technology 244, 8–14.

Kong, H. N., Kimochi, Y., Mizuochi, M., Inamori, R. & Inamori, Y. 2002 Study of the characteristics of CH4 and N2O 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 Environmental Quality 15, 298–301.

Li, Y. H., Li, H. B., Xu, X. Y., Xiao, S. Y., Wang, S. Q. & Xu, S. C. 2017 Field study on N2O emission from subsurface wastewater infiltration system under variable loading rates and drying-wetting cycles. Water Science and Technology 76, 2158–2166.

Li, W., Liang, C. L., Dong, L., Zhao, X. & Wu, H. M. 2021 Accumulation and characteristics of fluorescent dissolved organic matter in loess soil-based subsurface wastewater infiltration system with aeration and biochar addition. Environmental Pollution 269, 116100.

Liang, C. L., Li, Y., Chai, B. B. & Wu, H. M. 2019 Evaluating the effects of intermittent aeration and biochar addition on enhancing removal performance of subsurface wastewater infiltration systems with loess soil. Bioresource Technology Report 5, 12–19.

Llorén, M., Pérez-Marin, A. B., Aguilar, M. I., Sáez, J., Ortuño, J. F. & Meseguer, V. F. 2011 Nitrogen transformation in two subsurface infiltration systems at pilot scale. Ecological Engineering 37, 736–743.

Mauclerc, C., Mietto, A., Barbera, A. C. & Borin, M. 2016 Treatment performance and greenhouse gas emission of a pilot hybrid constructed wetland system treating digestate liquid fraction. Ecological Engineering 94, 406–417.

Morris, R., Schauer-Gimenez, A. & Bhattad, U. 2014 Methyl coenzyme M reductase (mcrA) gene abundance correlates with activity measurements of methanogenic H2/CO2-enriched anaerobic biomass. Microbiol Biotechnology 7, 77–84.

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

Pan, J., Yuan, F., Zhang, Y., Huang, L. L., Fei, H. X., Cheng, F. & Zhang, Q. 2016 Nitrogen removal in subsurface wastewater infiltration systems with and without intermittent aeration. Ecological Engineering 94, 471–477.

Pan, W. Y., Huang, Q. Z., Xu, Z. H. & Pang, G. B. 2020 Experimental investigation and simulation of nitrogen transport in a subsurface infiltration system under saturated and unsaturated conditions. Journal of Contaminant Hydrology 213, 103621.

Pang, J. L., Yang, M., Tong, D. L., Fu, X., Huang, L. L. & Sun, B. 2020 Does influent C/N ratio affect pollutant removal and greenhouse gas emission in wastewater ecological soil infiltration systems with/without intermittent aeration? Water Science and Technology 81, 668–678.

Qi, S. Y., Zhao, Y., Wang, S. Y., Zheng, F. P., Pan, J., Fan, L. L., Li, Z. Q., Tan, C. Q. & Hou, W. Y. 2018 Nitrogen removal and N2O emission in biochar-sludge subsurface wastewater infiltration systems. Water Environment Research 9, 800–806.

Sabella, F., Piccioreu, C., Pérez, J. & Nerenberg, R. 2015 Hydroxylamine diffusion can enhance N2O emissions in nitrifying biofilms: a modeling study. Environmental Science Technology 49, 1486–1494.

Standard Method for the Examination of Water and Wastewater Editorial Board 2002 Standard Method for the Examination of Water and Wastewater (Fourth Edition). Environmental Science Press of China, Beijing, China.

Sun, Y. F., Pan, J., Qi, S. Y. & Fei, H. X. 2017 Effects of hydraulic loading rate and aeration mode on nitrogen removal and nitrogen functional gene abundances in subsurface wastewater infiltration systems. Water Science and Technology 76, 201–218.

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, X. Z., Hu, Z. Y., Xu, X. K., Jiang, X., Zheng, B. H., Liu, X. N., Pan, X. B. & Kardol, P. 2014 Emissions of ammonia and greenhouse gases during combined pre-composting and
Wang, Z., Liu, C. X., Liao, J., Liu, L., Liu, Y. H. & Huang, X. 2014b Nitrogen removal and N$_2$O emission in subsurface vertical flow constructed wetland treating swine wastewater: effect of shunt ratio. *Ecological Engineering* **73**, 446–453.

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, S. Y., Zheng, Y. P., Mao, Y. X., Xu, L., Jin, Z., Zhao, M., Kong, H. N., Huang, X. F. & Zheng, X. Y. 2021 Domestic wastewater treatment for single household via novel subsurface wastewater infiltration systems (SWISs) with NiiMi process: performance and microbial community. *Journal of Cleaner Production* **279**, 123434.

Zhang, J., Huang, X. & Liu, C. X. 2005 Nitrogen removal enhanced by intermittent operation in a subsurface wastewater infiltration system. *Ecological Engineering* **25**, 419–428.

Zheng, P., Cui, J. Y., Hu, L., Chen, P. Z., Huang, J. W., Cheng, S. P. & Mu, K. G. 2016 Effect of long-term operation of a subsurface wastewater infiltration system (SWIS) based on the limiting value of environmental carrying capacity. *Ecological Engineering* **92**, 190–198.

Zheng, F. P., Zhao, Y., Li, Z. Q., Tan, C. Q., Pan, J., Fan, L. L., Xiao, L. & Hou, W. Y. 2018 Nitrogen removal and N$_2$O emission by shunt distributing wastewater in aeration or non-aeration subsurface wastewater infiltration systems under different shunt ratios. *Water Science and Technology* **78**, 329–338.

Zhou, X., Jia, L. X., Liang, X. L., Feng, L. K., Wang, R. G. & Wu, H. M. 2018 Simultaneous enhancement of nitrogen removal and nitrous oxide reduction by a saturated biochar-based intermittent aeration vertical flow constructed wetland: effects of influent strength. *Chemical Engineering Journal* **334**, 1842–1850.

Zou, J. L., Dai, Y., Sun, T. H., Li, Y. H., Li, G. B. & Li, Q. Y. 2009 Effect of amended soil and hydraulic load on enhanced biological nitrogen removal in lab-scale SWIS. *Journal of Hazardous Materials* **165**, 816–822.

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