Does intermittent aeration and/or an influent distributary affect nitrogen removal and nitrous oxide emission of an ecological soil wastewater infiltration system?

Yue Zhao, Zhiyu Zhang, Ziqi Li, Shiyao Wang, Chaoquan Tan, Linlin Fan and Jing Pan

ABSTRACT

The effect of intermittent aeration and an influent distributary on NH$_4^+$-N removal, total nitrogen (TN) removal, nitrous oxide (N$_2$O) emission and the abundances of nitrogen removal and N$_2$O emission functional genes in four types of ecological soil wastewater infiltration systems (ESWISs) (which were conventional ESWIS 1 (operated without aeration and influent distributary), ESWIS 2 (operated with intermittent aeration), ESWIS 3 (operated with influent distributary) and ESWIS 4 (operated with intermittent aeration and influent distributary)) were studied. Intermittent aeration in ESWIS 2 and 4 created aerobic conditions above 50 cm depth of the matrix and anoxic or anaerobic conditions in the lower matrix (below 80 cm depth). ESWIS 4 improved NH$_4^+$-N (to 90.1%) and TN (to 87.8%) removal efficiencies and increased the abundances of eight nitrogen removal and N$_2$O emission functional genes (amoA, nxrA, narG, napA, nirS, nirK, qnorB and nosZ) in contrast with other ESWISs. The combination of intermittent aeration and influent distributary achieved the lowest N$_2$O emission rate of 34.7 mg/(m$^2$ d) in ESWIS 4. Intermittent aeration combined with influent distributary was recommended for ESWISs to enhance nitrogen removal and reduce N$_2$O emission.

Key words | aeration, ecological soil wastewater infiltration system, influent distributary, N$_2$O, nitrogen

INTRODUCTION

High construction investment and operation cost of municipal wastewater treatment plants leads to decentralized domestic wastewater in remote districts being discharged into nearby water bodies with only primary treatment or with no treatment in developing countries (Wu et al. 2015). An ecological soil wastewater infiltration system (ESWIS) is recognized as an appropriate and practical choice to treat decentralized wastewater, which has many merits compared with centralized sewage disposal methods, such as lower construction investment, less operation and maintenance cost, and higher organics and phosphorus removal (Wang et al. 2010; Pan et al. 2015; Yang et al. 2016). Nitrogen removal efficiency of ESWISs is low, which becomes the bottleneck to wide application of this technology (Ji et al. 2012). Nitrogen removal by the biological nitrification and denitrification process is the main nitrogen removal mechanism in ESWISs (Lloréns et al. 2011). Various environmental parameters and running conditions affect nitrogen removal. Among these, dissolved oxygen (DO) and carbon source are crucial (Li et al. 2011a; Song et al. 2016). Sequential aerobic conditions in the upper matrix and anoxic or anaerobic conditions in the lower matrix were achieved by intermittent aeration, which promoted organic matter removal and nitrification (Fan et al. 2013a; Pan et al. 2015). However, intermittent aeration caused carbon source shortage in the lower part and resulted in low denitrification rate and total nitrogen (TN) removal (Zou et al. 2009; Fan et al. 2013b). Many studies proved that TN removal was enhanced slightly when an influent distributary providing an extra carbon source from raw domestic wastewater was introduced to the infiltration system, which was ascribed to low nitrification rate (Li et al. 2011b; Pan et al. 2015; Wang et al. 2014; Wu et al. 2015). Nitrogen removal could be increased when the upper matrix of an ESWIS was supplied with enough oxygen and the lower matrix was with
enough carbon source supply (Li et al. 2014b). However, very few studies have investigated the combined use of an influent distributary and intermittent aeration in ESWISs for nitrogen removal.

Nitrous oxide (N₂O) gas is produced by an incomplete nitrification and denitrification process in ESWISs, which has become a research focus during recent years because N₂O’s contribution to the greenhouse effect, ozone depletion and acid deposition (Li et al. 2017b). Nitrogen removal and N₂O emission are closely associated with nitrogen removal functional genes (Wang et al. 2015a, 2015b). The ammonia monooxygenase gene (amoA) oxidizes ammonia nitrogen to nitrite nitrogen, and the nitrite oxidoreductase gene (nxrA) oxidizes nitrite nitrogen to nitrate nitrogen, which makes up the nitrification process. The denitrification process in ESWISs includes nitrate nitrogen to nitrite nitrogen transformation, nitric oxide to nitrous oxide transformation, nitrous oxide to nitrogen transformation, which were catalyzed by narG (membrane-bound nitrate reductase)/napA (periplasmic nitrate reductase), nirS/nirK (nitrite reductase), nrorB (nitric oxide reductase) and nosZ (nitrous oxide reductase) genes, respectively. Nevertheless, there are few reports about the effect of intermittent aeration and/or influent distributary on the abundance of nitrogen removal functional genes and N₂O emission in ESWISs.

Therefore, four batch-operated pilot ESWISs with intermittent aeration, influent distributary, the joint application of intermittent aeration and influent distributary, and without aeration and influent distributary were investigated. The main aims of this study were: (1) to determine DO environment along four types of ESWISs; (2) to assess the effects of intermittent aeration, influent distributary, and the combination of the two strategies on nitrogen removal, nitrogen removal functional genes and N₂O emission in ESWISs; (3) to identify the best running modes for high NH₄⁺-N removal, TN removal and low N₂O emission.

MATERIAL AND METHODS

Laboratory-scale ESWISs description and operation

Four laboratory-scale ESWISs were operated under different conditions named ESWIS 1 (operated without aeration and influent distributary), ESWIS 2 (operated with intermittent aeration), ESWIS 3 (operated with influent distributary) and ESWIS 4 (operated with intermittent aeration and influent distributary). Each ESWIS was made of a Plexiglas column with 50 cm in diameter and 120 cm in height (in Figure 1). Coarse gravel of 5–10 mm diameter (10 cm in thickness) was installed at the bottom of each system to hold up the infiltration matrix and uniform distribution of wastewater, above which was placed a matrix consisting of 20% coal slag and 80% brown earth by mass ratio with thickness of 110 cm. The coal slag was taken from a coal burning boiler, and was 5–10 mm in diameter to enhance the porosity of the mixed matrix. The brown earth came

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**Figure 1** | Schematic diagram of four ecological soil wastewater infiltration systems (ESWISs), named ESWIS 1 (without aeration and influent distributary), ESWIS 2 (with intermittent aeration), ESWIS 3 (with influent distributary) and ESWIS 4 (with intermittent aeration and influent distributary).
from the top 20 cm of an agricultural soil from Xinchengzi District in Shenyang, Liaoning province. The characteristics of the mixed matrix are shown in Table 1. In each ESWIS, the vertical influent pipe was fixed at a depth of 50 cm below the matrix surface and an outlet was installed at the bottom for discharging the effluent. Sampling ports were perpendicular at the depths of 50, 80 and 110 cm. An aerating unit comprising an air pipe and air diffuser was placed at a depth of 40 cm of ESWIS 2 and 4. An influent distributary pipe was laid below the influent pipe at 70 cm depth of ESWIS 3 and 4 according to previous studies (Wang et al. 2010; Pan et al. 2013). Coarse gravel with the diameter of 5–10 mm enwrapped the influent pipe, influent distributary pipe and micro-bubble diffuser to keep from clogging and diffuse air evenly. DO of the matrix was monitored by DO electrodes which were placed at 50, 80 and 110 cm depths. ESWIS 1 was operated without intermittent aeration and influent distributary.

Domestic wastewater was pretreated in a septic tank before being flowed into the four ESWISs continuously. The specific characteristics of wastewater after pretreatment were pH 6.9–7.5, chemical oxygen demand (COD) 187.5–269.8 mg/L, TN 35.2–45.6 mg/L, total phosphorus 3.1–6.6 mg/L and NH4⁺-N 33.7–42.6 mg/L. ESWIS 1 and 2 were operated with hydraulic loading of 0.075 m³/(m² d). ESWIS 3 and 4 were operated with hydraulic loading of 0.05 m³/(m² d). ESWIS 1 and 2 were operated with hydraulic loading of 0.075 m³/(m² d). ESWIS 3 and 4 were intermittently aerated at an air flow rate of 2 ± 0.3 L/min with four aerated/non-aerated cycles (A/N) every day and each cycle lasted for 6 hours. In each A/N cycle, the systems were firstly subject to aeration for an hour and then to a 5 hour interval without aeration. The aeration began at 2 a.m., 7 a.m., 1 p.m. and 6 p.m., respectively. To ensure the maturity of the systems, all ESWISs were run for 2 months prior to this experiment.

Table 1 | The characteristics of mixed matrix in four ESWISs

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic conductivity</td>
<td>(1.27 ± 0.5) × 10⁻³ cm/s</td>
</tr>
<tr>
<td>Surface area</td>
<td>186.4 ± 3.8 m²/kg</td>
</tr>
<tr>
<td>Bulk density</td>
<td>2.5 g/cm³</td>
</tr>
<tr>
<td>Total organics</td>
<td>31.3 ± 1.6 g/kg</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>2.1 ± 0.4 g/kg</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.9 ± 0.2 g/kg</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.5</td>
</tr>
</tbody>
</table>

Sampling and analytical methods

Every 5 days, the influent and effluent of each ESWIS were collected to test COD, NH4⁺-N and TN by standard methods of APHA (2005). Twenty water samples were gathered for a 100 day analysis period. All samples were brought back to the laboratory and measured immediately. Previous studies concluded nitrogen loss through volatilization was negligible when pH was below 9.3 (Li et al. 2011a). TN removed by the matrix was negligible in (Zhang et al. 2005). DO signals were collected online every 20 minutes by an MDA-501 data acquisition system (Tuopu Co. Ltd, Shunde, China). SPSS 12.0 software (Mann–Whitney T test) was used for statistical analysis and significant difference was at P < 0.05.

Gas sampling was done using a closed static chamber. In this study, the chamber made of polymethyl methacrylate was constituted of a box and support section. The box was 20 cm × 50 cm (diameter × height), and there were reserved holes to fix the thermometer and barometer. To make gas samples well-mixed, air inside the box was circulated with battery-driven fans. The support was embedded 20 cm underground to support the box. There was a lap slot at the top of the support to join the box and support. The lap slot was filled with water when collecting gas samples to avoid the gas leaking out from the static chamber. Three gas samples were collected after enclosure at the same time of day from 7 a.m. to 8 a.m., 10 a.m. to 11 a.m., and 12 noon to 1 p.m. every 5 days. The gas emitted from the system was trapped by the chamber, and then sampled from the air outlet at the middle part of each chamber into gas sampling bags by means of a mini gas pump. An Agilent 6890N (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph was used to determine N₂O concentration. The Agilent 6890N was equipped with an electron capture detector and a Poropak Q column and used a carrier gas composed of 95% argon and 5% methane. The temperature of the injection port, detector and oven was set at 105 °C, 300 °C and 120 °C, respectively (Jiang et al. 2017).

N₂O emission rate and conversion efficiency were calculated by following Formula (1) and (2) after determining the concentration of N₂O (Li et al. 2017b), respectively.

\[ \text{N}_2\text{O emission rate} = \frac{H}{\Delta t} (C_2 - C_1) \]  

\[ \text{N}_2\text{O conversion efficiency} = \frac{m_1}{m_2} \times 100\% \]  

where \( H \) is the height of static chamber (m); \( \Delta t \) is interval time of sampling (h); \( C_1 \) and \( C_2 \) are N₂O concentrations.
before and after gas sampling (mg/m³), respectively; \(m_1\) is \(\text{N}_2\text{O}\) emission between sampling interval (mg) and \(m_2\) represents TN quantity of the influent between sampling interval (mg).

Every 10 days, matrix samples of each ESWIS were gathered from three different depths of sampling ports. Soil DNA kits (Omega, D5625-01) were used to extract and purify the total genomic DNA from matrix samples. Extracted genomic DNA was detected by 1% agarose gel electrophoresis and preserved at \(-20°C\) until use. Quantitative analysis was made for the abundances of ammonia monooxygenase (\(amoA\)), nitrite oxidoreductase (\(nirX\)), periplasmic nitrate reductase (\(napA\)), membrane-bound nitrate reductase (\(narG\)), nitrite reductase (\(nirK/nirS\)), nitric oxide reductase (\(qnorB\)) and nitrous oxide reductase (\(nosZ\)) nitrogen removal functional genes by quantitative polymerase chain reaction (qPCR). Primers and parameters of target genes used in qPCR analysis are listed in Table 2. The procedures of qPCR analysis for the infiltration matrices were reported in a former study (Pan et al. 2017).

## RESULTS AND DISCUSSION

### Average DO concentrations in an aerated/non-aerated cycle

Average DO concentration profiles of ESWIS 1, 2, 3 and 4 in an aerated and non-aerated cycle during this experiment are shown in Figure 2. At depths of 50, 80 and 110 cm, DO concentrations were less than 0.87, 0.31 and 0.03 mg/L for ESWIS 1 and were under 1.21, 0.29 and 0.04 mg/L for ESWIS 3, respectively. ESWIS 1 and 3 were under anoxic or anaerobic environment and the influent distributary did not change oxygen conditions in non-aerated ESWISs. In addition, DO concentration decreased with matrix depth. These results of non-aerated ESWISs were consistent with Pan et al. (2015) and Jiang et al. (2017).

For ESWIS 2 and 4, aerobic conditions were achieved at 50 cm depth, with DO concentrations more than 8.0 and 9.45 mg/L, respectively during aerated period and above 2.90 and 3.81 mg/L during non-aerated period, respectively. However, DO concentrations were under 0.42 and 0.06 mg/L all the time for ESWIS 2 and 4, respectively, at both 80 and 110 cm depths, which led to anaerobic and anoxic conditions at greater depths (below 80 cm) of aerated systems. During the non-aerated period, DO concentrations decreased slowly with operation time due to aerobic degradation of pollutants by microorganisms in ESWIS 2 and 4. DO concentrations were a little higher at 50 cm depth of ESWIS 4 with influent distributary compared with ESWIS 2 without influent distributary. The reason was that oxygen consumed to degrade pollutants in ESWIS 4 was less than that in ESWIS 2 because of additional wastewater introduced to the lower matrix by the influent distributary. Furthermore, the aerobic or anaerobic environment was not altered by the influent distributary in aerated and non-aerated ESWISs.

### Table 2 | Primers and parameters of target genes used in qPCR analysis

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
<th>Amplification size (bp)</th>
<th>qPCR programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>amoA</td>
<td>Amo598f Amo718r</td>
<td>120</td>
<td>3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>nirX</td>
<td>F1norA R2norA</td>
<td>323</td>
<td>3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>narG</td>
<td>1960m2f 2050m2r</td>
<td>100</td>
<td>10 min at 95 °C, 15 s at 95 °C, 45 s at 58 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>napA</td>
<td>napAV17F napA4R</td>
<td>152</td>
<td>10 min at 95 °C, 15 s at 95 °C, 50 s at 57 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>nirK</td>
<td>nirK583F nirK909R</td>
<td>165</td>
<td>3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>nirS</td>
<td>nirScd3aF nirScd3cd</td>
<td>413</td>
<td>3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C</td>
</tr>
<tr>
<td>qnorB</td>
<td>qnorB2F qnorB5R</td>
<td>262</td>
<td>5 min at 95 °C, 15 s at 95 °C, 20 s at 56 °C and 8 s at 72 °C</td>
</tr>
<tr>
<td>nosZ</td>
<td>nosZ1527F nosZ1773</td>
<td>250</td>
<td>3 min at 95 °C, 15 s at 95 °C, 20 s at 57 °C and 30 s at 72 °C</td>
</tr>
</tbody>
</table>
NH₄⁺-N and TN removal performance in aeration and/or influent distributary ESWISs

As shown in Figure 3, ESWIS 2 and 4 achieved average NH₄⁺-N removal efficiencies of more than 90% with intermittent aeration. There was no significant difference between ESWIS 2 and 4 for NH₄⁺-N removal efficiency (P > 0.05). Average NH₄⁺-N concentrations in the effluents of ESWIS 1 and 3 were significantly higher than those of ESWIS 2 and 4 (P < 0.05). NH₄⁺-N is converted to NO₃⁻-N by microbial nitrification firstly. Ye & Li (2013) reported DO concentrations of more than 1.5 mg/L were necessary for better nitrification. DO concentrations were below 1.2 mg/L at all depths in the upper matrix of non-aerated ESWIS 1 and 3 in this study, which limited NH₄⁺-N removal and resulted in average NH₄⁺-N removal efficiencies of below 71%. Studies of Fan et al. (2013b) and Yang et al. (2016) have found that most conventional non-aerated ESWISs could not transform NH₄⁺-N to NO₃⁻-N via microbial nitrification with low DO concentration. DO concentrations were more than 2.9 mg/L in the upper matrix of ESWIS 2 and 4 by intermittent aeration, which was propitious to the nitrification process and resulted in NH₄⁺-N concentrations of less than 4 mg/L in the effluents. In aerated ESWISs, average NH₄⁺-N concentration in the effluent increased from 3.5 mg/L in ESWIS 2 to 3.7 mg/L in ESWIS 4. In non-aerated ESWISs, average effluent of NH₄⁺-N concentration increased from 11.1 mg/L in ESWIS 1 to 11.4 mg/L in ESWIS 3, which was consistent with the same trend of aerated systems. The reason was that additional wastewater was introduced by the influent distributary. It can be concluded that intermittent aeration is a practical approach for ESWISs to improve NH₄⁺-N removal and the influent distributary has little influence on NH₄⁺-N removal.
In order to sufficiently eliminate nitrified nitrogen from ESWISs, microbial denitrification must occur (Li et al. 2014a). Denitrification requires anaerobic conditions and sufficient carbon supply (Wang et al. 2013; Li et al. 2014a; Fan et al. 2016a; Wu et al. 2019). As shown in Figure 3, average TN removal efficiencies of aerated systems (ESWIS 2 and 4) were significantly higher than those of non-aerated systems (ESWIS 1 and 3) \((P < 0.05)\). Under anoxic or anaerobic conditions, ESWIS 1 and 3 could not achieve sufficient \(\text{NH}_4^+\)-N removal, which subsequently limited denitrification without available \(\text{NO}_3^-\)-N to function as an electron acceptor. ESWIS 4 achieved the best TN removal efficiency of 87.8%, which was a marked improvement compared to previous studies of 40–60% TN removal efficiency (Wang et al. 2010; Li et al. 2011a; Lloréns et al. 2011; Fan et al. 2013b; Yang et al. 2016). Average TN concentration in the effluent of ESWIS 4 was 4.6 mg/L, which improved to 8.7 mg/L in ESWIS 2. In intermittent aerated ESWIS 2, organic matter is mostly decomposed in the upper aerobic matrix leading to lack of carbon source in the lower part, which resulted in lower denitrification. In this study, the extra carbon source from distributary wastewater from intermittent aeration was recommended for ESWISs to improve nitrogen removal.

### N\(_2\)O emission from aeration and/or influent distributary ESWISs

Average N\(_2\)O emission rate of aeration and/or influent distributary ESWISs was as follows: 50.1 mg/(m\(^2\) d) for ESWIS 1, 86.8 mg/(m\(^2\) d) for ESWIS 2, 69.4 mg/(m\(^2\) d) for ESWIS 3, 34.7 mg/(m\(^2\) d) for ESWIS 4, respectively (in Figure 4). Average N\(_2\)O emission rate was found to be lowest in ESWIS 4. N\(_2\)O conversion efficiencies were 1.3% for ESWIS 1, 2.3% for ESWIS 2, 1.8% for ESWIS 3 and 0.9% for ESWIS 4, which were consistent with the range of 0.02–5.08% reported by previous studies (Jiang et al. 2017; Li et al. 2017a). The low N\(_2\)O emission rate of ESWIS 1 was ascribed to low nitrification, which further limited the denitrification process. Denitrification is widely considered as the primary reaction in charge of N\(_2\)O emission in ESWISs (Fan et al. 2016a). ESWIS 2 obtained the highest N\(_2\)O emission rate. Aerobic biochemical degradation of COD and \(\text{NH}_4^+\)-N nitrification finished completely in the upper matrix of ESWIS 2 due to sufficient oxygen by intermittent aeration, which led to carbon source insufficiency in the lower matrix and restricted transformation of part of N\(_2\)O to N\(_2\) before being emitted to the atmosphere. The nitrification process was poor due to insufficient oxygen supply in ESWIS 3. Although the influent distributary supplied an organic carbon source as electron donor, the enhancement of the denitrification process was limited. Therefore, N\(_2\)O emission rate of ESWIS 3 was slightly higher than that of ESWIS 1. In ESWIS 4, intermittent aeration developed appropriate oxygen conditions. Simultaneously, the extra...
Abundances of nitrogen functional genes in four ESWISs.

Figure 5 | Abundances of nitrogen functional genes in four ESWISs.
carbon source from wastewater of influent distributary fulfilled N2O transformation into N2. So N2O emission rate of ESWIS 4 was the lowest. The result was in agreement with a previous study, which reported that sufficient carbon source introduced from an influent distributary after efficient nitrification greatly reduced N2O emission under appropriate shunt ratio (Wang et al. 2014). Intermittent aeration combined with influent distributary was a valid measure to decrease N2O emission for ESWISs.

Functional genes

The abundances of amoA and nxaA diminished along the wastewater flow direction in each ESWIS, which followed the same trend as DO (Figure 5). At a depth of 50 cm in ESWIS 1 and 3, the abundances of amoA and nxaA were significantly lower than those of ESWIS 2 and 4 (P < 0.05). Intermittent aeration enhanced DO concentrations of ESWIS 2 and 4 at 50 cm depth, which promoted the nitrification process. Previous researches showed similar results (Ji et al. 2012). Fan et al. (2013b) and Wu et al. (2015) concluded that the amount of nitrification bacteria and enzyme activities participating in NH4-N transformation was enhanced through sufficient oxygen supply.

The abundances of napA, narG, nirS, nirK, qnorB and nosZ genes in ESWIS 2 and 4 were significantly higher than those in ESWIS 1 and 3 at the depths of 80 cm and 110 cm (P < 0.05). Nitrification rate of ESWIS 2 and 4 was improved by aeration, which resulted in more NO3 as the substrate for anaerobic denitrification being available and improved the abundances of six genes participating in the denitrification process. The abundance of the qnorB gene in ESWIS 2 was more than that in ESWIS 4 and the abundance of the nosZ gene in ESWIS 2 was less than that in ESWIS 4. It could further explain the highest N2O emission in ESWIS 2. The qnorB and nosZ are two key genes which indicate N2O emits to the atmosphere directly or is converted to N2 (Thomson et al. 2012). More organic matter and nutrients provided appropriate conditions for anaerobic denitrifying bacteria with influent distributary in ESWIS 4 after efficient nitrification with aeration, in parallel to improving the conversion of N2O to N2. It could further illustrate the lowest N2O emission in ESWIS 4. The abundances of functional genes involved in NH4-N and NO3-N transformation could further explain the highest removal efficiency of NH4-N and TN in ESWIS 4.

CONCLUSIONS

The intermittent aeration and influent distributary combined strategy accomplished an efficient nitrification and denitrification process with high NH4-N (90.1%) and TN removal efficiencies (87.8%), which increased the abundances of nitrogen removal functional genes (amoA, nxrA narG, napA, nirS, nirK, qnorB and nosZ) simultaneously. N2O emission rate (34.7 mg/(m2 d)) of the intermittent aeration and influent distributary combined ESWIS was the lowest compared with the conventional ESWIS without intermittent aeration and influent distributary (50.1 mg/(m2 d)), intermittent artificially aerated ESWIS (86.8 mg/(m2 d)) and influent distributary ESWIS (69.4 mg/(m2 d)). The results would be helpful for improving the sustainability of design, operation and nitrogen removal performance and reducing N2O emission of ESWISs.

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