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LETTER

Increased nitrous oxide emissions from intertidal soil receiving wastewater from dredging shrimp pond sediments

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Abstract

The quantities of greenhouse gas emissions and the activity of functional microbes in coastal soils receiving nutrient-rich wastewaters from mariculture activities have seldom been reported. We investigated the effects of wastewater discharge resulting from dredging shrimp pond sediment on the soil fluxes of methane (CH\(_4\)) and nitrous oxide (N\(_2\)O) in intertidal areas and on the functional microorganisms and physio-chemical characteristics of soil. The temporal variations in gas fluxes and soil characteristics following wastewater discharge were also evaluated with the tidal regime on the day of discharge taken into account. The results showed that wastewater discharge immediately resulted in higher levels of ammonia (NH\(_4^+\)-N) deposited and N\(_2\)O emissions from the soil at the discharge site than at the non-discharge site, while the CH\(_4\) flux was not affected. The increase in N\(_2\)O flux lasted for a longer time when the discharge was performed during a neap tide day than when it was performed during a spring tide day. Wastewater discharge also increased the abundance of ammonia-oxidizing bacterial (AOB) \(amoa\) genes and \(nosZ\) genes in soil rather than increasing the abundance of \(narG\) and \(nirK\) genes. The pattern of temporal variations between the N\(_2\)O flux and soil NH\(_4^+\)-N content was similar to that between the flux and the AOB-\(amoa\) gene abundance, suggesting that bacterial nitrification was important for N\(_2\)O production in soil receiving the dredging wastewater. The results suggest that the wastewater discharge impacts nitrogen metabolism processes and causes a significant N\(_2\)O emission problem; therefore, pollutant management is essential in shrimp culturing activities to reduce greenhouse gas emissions into the atmosphere.

1. Introduction

Mariculture is one of the most important economic activities carried out in tropical and subtropical coastal areas, especially in China and other Asian countries (FAO 2018). In addition to the losses of coastal wetlands due to pond construction (FAO 2007), the poor management of pond waste materials is another serious ecological concern for mariculture. During mariculture activities, pond waters are routinely and frequently discharged into adjacent coastal ecosystems; the discharge has been found to impact the bacterial communities and metabolisms of carbon and nitrogen in the adjacent intertidal soil (Sousa \textit{et al} 2006, Molnar \textit{et al} 2013, Suarez-Abelenda \textit{et al} 2014). In addition to routine effluents, at the end of each culture rotation, pond sediments containing harmful pathogenic bacteria and nutrients derived from feed additives (Graslund \textit{et al} 2003, Naylor and Burke 2005) are completely dredged and wastewaters in the form of sludge (hereinafter referred to as ‘dredging wastewater’) are discharged into adjacent ecosystems. Wu \textit{et al} (2014) recently estimated that the inorganic nitrogen load ranged from $-0.06$ to $1.17$ kg N ha\(^{-1}\) yr\(^{-1}\) in pond effluents, and was higher in the dredging wastewaters (3.58 to 14.38 kg N
ha\(^{-1}\) yr\(^{-1}\)), while the latter could have an even higher total nitrogen load (375.2 to 712.8 kg N ha\(^{-1}\) yr\(^{-1}\)). The discharge of this intensive nutrient load into coastal ecosystems consequentially affects carbon and nitrogen metabolism in soils, but this effect is rarely reported.

Intertidal soils have been recognized as sources of nitrous oxide (N\(_2\)O) and methane (CH\(_4\)) (Chen et al 2010) while their fluxes and the microbiological involvement are still poorly quantified in coastal wetlands. The production and emission of these gases are determined by the soil chemical and physical characteristics (Allen et al 2007, Chen et al 2012), and can be greatly enhanced by exogenous nutrient inputs (Alongi et al 2005, Allen et al 2007, Chen et al 2011, Chmura et al 2016, Martin et al 2018). These increases in soil gas emissions can, in some cases, offset the amounts of carbon sequestered in coastal wetlands and may even counterbalance any benefit of carbon storage in regard to climate change mitigation (Suarez-Abelenda et al 2014, Chen et al 2016, Roughan et al 2018).

The production of N\(_2\)O and CH\(_4\) in intertidal soil receiving wastewater inputs is dependent on the form and concentration of carbon and nitrogen in the wastewater (Chen et al 2011). The soils impacted by mariculture pond effluents had a N\(_2\)O flux two times higher than that of the reference soil in a mangrove in NE Brazil, while the CH\(_4\) flux was not affected by the effluent discharge (Queiroz et al 2019). The discharge of dredging wastewater containing a high nutrient load would consequently result in different quantities and emission patterns of greenhouse gases relative to those from soil impacted by typical pond effluents. Moreover, the tidal regime may regulate the retention and metabolism of nutrients in the soil following wastewater discharge. However, there is currently a lack of in situ evidence regarding the impact of wastewater discharge on the quantities and temporal patterns of gas emissions and on the functional microbial groups involved in the gas production in intertidal areas, with tidal regimes taken into account.

The present study thus aims to evaluate the effects and the temporal patterns of discharge of dredging wastewater from shrimp ponds on soil gas fluxes, soil chemical/physical parameters, and the functional gene abundances in intertidal areas. We also tested whether the responses of gas emissions to wastewater discharge varied with the tidal regime on the day of discharge. We hypothesize that (1) the discharge of dredging wastewater would increase the nutrient level in the impacted soil and enhance the gas emissions from soil, and such enhancement would be long-lasting; (2) the tidal regime regulates the emission pattern of the gas flux following wastewater discharge; (3) the wastewater discharge also stimulates the functional microbial abundances involved in the gas production in the impacted soil.

### 2. Materials and methods

#### 2.1. Study area

The present study was conducted in the subtropical Jiulong River Estuary in South China. The highest monthly mean temperature was recorded in July and August (>28 °C), and the lowest monthly mean temperature of ~14.5 °C was recorded in January (Chen et al 2008). Tides are semidiurnal, with an average range of 4 m, and the sea water salinities adjacent to the mangroves range from 12 to 26 (Alongi et al 2005). Most mangrove forests, dominated by Kandelia obovata Sheue, Liu and Yong, in the estuary currently appear as narrow fringing forests because of the destruction of mangrove forests for aquaculture activity and seawall construction. The mangroves are mostly separated from mariculture ponds by a seawall, and dredging wastewaters are intermittently discharged into the mangroves. Two K. obovata-dominated sampling sites ~100 m apart from each other were selected with a similar soil surface elevation along the south bank of the estuary, including a site that frequently receives wastewater from intensive shrimp (Penaeus vannamei) culture ponds and an adjacent site without direct wastewater discharge (additional details of study areas and sampling sites are provided in the Supporting Information (available online at stacks.iop.org/ERL/00/00000/mmedia)). The width of the sampled sites, i.e. the distance between the landward and estuarine edges, were ~25 m.

#### 2.2. Soil to atmosphere greenhouse gas fluxes

The samplings were conducted following two discharge events of dredging wastewater. After discharge, the mangrove floor was covered by a continuous new soft muddy layer (~5 cm) due to the deposition of dredged sludge, and the samplings were done at the middle zone of each site (~12 m away from the landward edge). The 1st discharge occurred during the first low tide period on 30th October 2016 (a spring tide day). Samplings of gas fluxes were carried out during the second low tide period, at 2 pm (~6 h after the discharge finished and the sludge was deposited), 6 pm and 9 pm on the 1st day, and then around the time of the lowest tide during the daytime on the 2nd, 4th, 7th, 14th and 21st days. After the 2nd discharge on 1st June 2017 (a neap tide day when the mangrove floor was not flooded), gas samplings were conducted on the 1st day (at 10 am, 1 pm and 4 pm representing ~6, 9 and 12 h after the discharge event, respectively) and then around the time of the lowest tide on the 2nd, 4th, 8th, 10th, 15th and 20th days.

Gas samplings were done simultaneously at the two sites. At each site, gas fluxes were quantified with 4 (October 2016) or 8 (June 2017) random replicates. Gas samples were collected using a static (closed) chamber and the gas concentrations were analyzed in parallel with a gas chromatography
system (additional details regarding field collection and analysis of gas samples are provided in the Supporting Information).

2.3. Soil sampling and analysis

Soil samples were collected during the first continuous sampling for the analysis of only ammonia (NH$_4^+$-N) and nitrate (NO$_3^-$-N), while a more detailed analysis of soil characteristics was conducted during the second continuous sampling. During the first continuous sampling, top 5 cm of surface soil under each chamber was collected after each gas sampling except for the first two samplings, i.e. from 12 h after discharge on the 1st day. In June 2017, during the second continuous sampling, the redox potential (E$_h$) under the chamber was measured after gas sampling using a pH/E$_h$ meter (WP-81, TPS, Australia) at a soil depth of 5 cm from the surface. After the E$_h$ measurement was performed, the surface soil under each chamber was collected.

Each sample was thoroughly mixed in the laboratory and then separated into two parts, one for microbial analysis and another for the analysis of inorganic nitrogen, organic carbon (OC), total nitrogen (TN) and total phosphorus (TP) contents, as well as grain size distribution (Supporting Information). The contents of OC, TN and TP were analyzed only in the soil samples collected during the 1st gas sampling, because our previous study showed that the temporal changes in their contents in the soil were not significant after 50 d (Chen et al 2011), and changes in their contents in this study were not expected. All of the data were expressed as the dry weight after drying in a 105 °C oven.

2.4. DNA extraction and real-time PCR analysis

The abundances of functional genes involved in CH$_4$ and N$_2$O production were analyzed to elucidate their responses to wastewater discharge. Analysis was performed using the soils collected on the 1st (6 h after discharge), 2nd, 8th and 15th days, as the fluxes measured on these days represented high, medium and low fluxes, respectively. Three replicate DNA extractions were conducted for each soil sample using the PowerSoil® DNA Isolationday Kit (MOBIO, Carlsbad, USA) and the concentration of extracted DNA was measured using a Nanodrop® 2000c spectrophotometer (Thermo Scientific, Delaware, USA). The abundances of ammonia-oxidizing archaeal (AOA) and bacterial (AOB) amoA genes (encoding ammonia monoxygenase subunit A), the narG gene (encoding nitrate reductase), the nirK gene (encoding nitrite reductase) and the nosZ gene (encoding nitrous oxide reductase) were analyzed. For CH$_4$, the pmoA gene encoding the α subunit of the methane monoxygenase enzyme and the mcrA gene encoding the methyl coenzyme M reductase α-subunit were analyzed. Gene abundances were measured by real-time PCR assays following Huang et al (2017) using the respective primers table S1 in the supporting information.

2.5. Statistical methods

The normality of the variables was examined using the Kolmogorov–Smirnov test, and those that did not follow a normal distribution were transformed with the Blom method to improve normality prior to analysis of variance (ANOVA). The main and interactive effects of wastewater discharge and sampling time on gas fluxes and soil parameters were analyzed using parametric two-way ANOVA, with pairwise comparisons of interactive effects conducted using Tukey's post hoc honestly significant difference (HSD) test. When a significant interaction was observed, one-way ANOVA (Tukey’s post hoc test) with a Bonferroni-adjusted alpha level was used to investigate the effect of wastewater discharge at each sampling time and the sampling time effect at each mangrove site. Differences in parameters for the soil samples collected in the 1st gas sampling between the discharge site and the reference site were compared by t-tests. Pearson correlation coefficients were calculated to determine the relationships between soil parameters and gas fluxes. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.).

3. Results and discussion

We found that the discharge of dredging wastewater from shrimp ponds resulted in enriched TN and TP contents in the surface soil (mainly comprised of the deposited sludge) collected 6 h after the discharge at the mangrove forest receiving the wastewater relative to the contents at the non-discharge site (table 1). Wastewater discharge resulted in less acidic soil at the discharge site than at the non-discharge site on the day 1 (figure 1), and a higher silt content and lower clay content was measured at the discharge site than at the non-discharge site (table 1). A more apparent impact than the effect on the TN and TP contents was that the discharge induced a high level of NH$_4^+$–N in the soil at the discharge site (figure 2, table S2), which has been concluded to be the major inorganic nitrogen form in the bottom phases in mariculture systems (Hu et al 2012, Krom et al 2014). The soil NH$_4^+$–N content at the discharge site was ~30 µg g$^{-1}$ on the 1st day during the first continuous sampling, and reached a very high level on the 1st day (~200 µg g$^{-1}$) during the second continuous sampling.

The wastewater discharge immediately and greatly stimulated the N$_2$O emissions from the impacted soils, which reached 100 µmol m$^{-2}$ h$^{-1}$ and 126 µmol m$^{-2}$ h$^{-1}$ on the 1st day during the first and second continuous sampling, respectively (figure 3). The fluxes were much higher than those simultaneously measured at the non-discharge site (mostly <4 µmol m$^{-2}$ h$^{-1}$) in this study (figure 3,
Table 1. Soil organic carbon (OC), total nitrogen (TN), total phosphorus (TP) and grain size at the discharge and non-discharge sites collected on the 1st day after discharge in June 2017.

| Parameters    | Discharge site   | Non-discharge site |
|---------------|------------------|--------------------|
| OC content mg g⁻¹ | 12.1 ± 1.5 a     | 13.0 ± 0.7 a       |
| TN content mg g⁻¹ | 1.24 ± 0.17b     | 0.62 ± 0.21 a      |
| TP content mg g⁻¹ | 1.25 ± 0.14b     | 0.88 ± 0.25 a      |
| Silt content %   | 30.2 ± 2.1b      | 28.1 ± 1.6 a       |
| Clay content %   | 65.7 ± 2.9 a     | 71.1 ± 2.8 b       |
| Sand content %   | 4.1 ± 3.9        | 0.8 ± 1.9          |

Different letters for each parameter indicate a significant difference between the two sampling sites at p < 0.05 according to the t-test.

The increase in N₂O fluxes due to exogenous nutrient inputs was suggested to be temporary (~2 d) in intertidal soils (Chen et al 2011, Moseman-Valtierra et al 2011). This phenomenon was attributed to the rapid microbial assimilation of nitrogen and the reduction of N₂O to N₂ in intertidal soils and the reversion of the microbial population back to the background levels prior to N addition (Lindau and Delaune 1991, Moseman-Valtierra et al 2011). The present study further found that the enhancing effect of discharging dredging wastewater on soil N₂O flux and its duration were related to the tidal regime on the day of discharge. Although the N₂O flux at the discharge site showed a rapid decrease on the 2nd day relative to that on the 1st day in both continuous samplings (figure 3, table S3), a longer time (10 d versus 4 d) was required for the level to recover to that of the non-discharge site when the discharge was performed on a neap tide day (in the first continuous sampling) than on a spring tide day (in the first continuous sampling). We attribute this finding to the fact that the spring tides flooded the forest soil and transported NH₄⁺-N from the discharge site, reducing the substrate availability for microbial N₂O production. The exported nutrients were transported by tides into the nearby non-discharge site and
Figure 2. Temporal variation in the NH$_4^{+}$-N and NO$_3^{-}$-N contents in the soils at the discharge and non-discharge sites during the first (a) and second (b) continuous samplings. The means and standard deviations of four (first continuous sampling) or eight (second continuous sampling) replicates are shown. ns: no significant difference; *, ** and *** indicate significant differences between the two sites according to t-tests at p < 0.05, 0.01 and 0.001, respectively.

stimulated N$_2$O emissions therein, as shown by the higher soil NH$_4^{+}$-N content (>10 µg g$^{-1}$) and N$_2$O flux (>7 µmol m$^{-2}$ h$^{-1}$) at the non-discharge site on the 1st day than on the following days in the first
Figure 3. Temporal variation in soil CH$_4$ and N$_2$O fluxes at the discharge site and the non-discharge site during the first (a) and second (b) continuous samplings. The means and standard deviations of four (first continuous sampling) or eight (second continuous sampling) replicates are shown. ns: no significant difference; *, **, and *** indicate significant differences between the two sites according to t-tests at p < 0.05, 0.01 and 0.001, respectively.

continuous sampling (figures 2 and 3, tables S3 and S4). Although the present study was based on only two continuous samplings, this should not limit our findings that discharge stimulates N$_2$O emissions and
that the tidal regime regulates \( \text{N}_2\text{O} \) emission patterns. This is because our previous studies showed that the response of \( \text{N}_2\text{O} \) emissions to exogenous nutrient input did not substantially vary among different samplings under uniform tidal conditions (Chen et al 2011, 2014). We propose future studies with consideration of impact of wastewater discharge performed in different tide regimes on estuarine water quality and greenhouse gas emission, to develop optimized discharge strategies to reduce the \( \text{N}_2\text{O} \) emissions if the wastewater discharge from aquaculture activities is unavoidable.

The present study recorded both an elevated \( \text{NH}_4^+\text{-N} \) content and AOB abundance in the impacted soil immediately after wastewater discharge, and soil AOB was more abundant than AOA at the discharge site (figure 4). Because the soil samples collected on the first day at the discharge site were mainly comprised of the deposited sludge of wastewater, the nutrient and the microbial characteristics may reflect the nature of the deposited sludge. In contrast, the soil at the non-discharge site had comparable AOA-amoA and AOB-amoA gene copy numbers, indicating that wastewater discharge resulted in a different abundance-ratio of AOB to AOA in the soil. This result is in accordance with previous findings that AOA is taken up in low \( \text{NH}_4^+\text{-N} \) environments (Erguder et al 2009), while AOB prefers to uptake \( \text{NH}_4^+\text{-N} \) as a substrate (Prosser and Nicol 2012). Nevertheless, this effect of wastewater discharge was temporary, as the AOA abundance rapidly recovered to values similar to those measured at the non-discharge site from the 2nd day and beyond when a substantial drop in the soil \( \text{NH}_4^+\text{-N} \) content was observed (figures 2 and 4, table S5). The soil \( \text{narG} \) and \( \text{nirK} \) gene abundances in soils were not significantly affected by wastewater discharge. The abundances of \( \text{nosZ} \) gene encoding reductase of \( \text{N}_2\text{O} \) to \( \text{N}_2 \) at the discharge site were higher in the first two samplings than in the others (figure 4); this may be probably due to the high \( \text{N}_2\text{O} \) production in the first two samplings provided substrate for more \( \text{N}_2\text{O} \) reducers in the soil.

Microbial denitrification and nitrification are both important in \( \text{N}_2\text{O} \) production and cooccur in intertidal soils (Munoz-Hincapie et al 2002, Fernandes et al 2010, Chen et al 2014). The soil \( \text{N}_2\text{O} \) flux during the first continuous sampling was correlated with only the \( \text{NH}_4^+\text{-N} \) content at both sites (\( R = 0.868, p < 0.001 \) for the discharge site; \( R = 0.541, p < 0.05 \) for non-discharge site). The discharge of \( \text{NH}_4^+\text{-N} \)-rich wastewater into the mangrove site also stimulated the abundance of nitrifiers (AOB) and \( \text{N}_2\text{O} \) flux, and the flux showed a significant increase with AOB abundance and with \( \text{NH}_4^+\text{-N} \) content at the discharge site (table 2). Soil \( \text{NO}_3^-\text{-N} \) as a nitrification product was slightly higher at the discharge site than at the non-discharge site during the first continuous sampling, and showed a slight increase with sampling time during the second continuous sampling (figure 2). These results suggest that

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Temporal variations in AOA-amoA, AOB-amoA, \( \text{narG} \), \( \text{nirK} \), and \( \text{nosZ} \) gene abundances in the soils at the discharge and non-discharge sites during the second continuous samplings. The means and standard deviations of eight replicates are shown. ns: no significant difference; * indicates significant differences between the two sites according to t-tests at \( p < 0.05 \).}
\end{figure}
bacterial ammonia oxidation can take place when abundant NH$_4^+$-N is present, contributing to N$_2$O production even under reduced conditions (minus soil E$_h$, figure 1) in this study.

Nevertheless, the soil NO$_3^-$-N concentrations at the discharged site were sustained at levels close to those simultaneously measured at the non-discharge site, which can be attributed to the loss of NO$_3^-$-N through denitrification. The gradual increase in soil pH with sampling time at the discharge site (figure 1) can be evidence of the denitrification process in the discharge soil because soil pH is a function of the balance between nitrification and denitrification processes. Nitrification could release protons into the soil, leading to an acidic pH, while denitrification increases the pH (Bleam 2017). A slight increase in the N$_2$O flux and higher soil narG and nirK gene abundances were observed in the 8-day measurement during the second continuous sampling when the mangrove received more flooding than on the other days (figure 4). Future detailed studies are needed to investigate the contributions of nitrification and denitrification to N$_2$O emission from intertidal soils subjected to dredging wastewater, and the involvement of the biogeochemical processes of nitrogen in soil N$_2$O production.

The soil to atmosphere CH$_4$ flux had a wide range from a negative value (below $-130$ µmol m$^{-2}$ h$^{-1}$) to $>1800$ µmol m$^{-2}$ h$^{-1}$ with high spatial variation among the replicates at each site, and was comparable among the sampling times in both continuous samplings (figure 3, table S2). Unlike the N$_2$O flux, there was no significant effect of wastewater discharge on soil CH$_4$ emissions, which is a similar finding to that reported by Queiroz et al (2019) from a mangrove receiving shrimo pond effluents. However, we found a difference in driving factors for CH$_4$ emissions between the two study sites (table 2). The CH$_4$ flux was increased by a more reduced soil E$_h$ at the non-discharge site, a finding demonstrated in intertidal soils (Allen et al 2007, Livesley and Andrusiak 2012). This phenomenon was also reflected by the higher soil mcrA but lower pmoA gene abundances on the spring tide day (8th day) than on other days during the second continuous sampling (figure 5, table S2). Nevertheless, neither soil chemical/physical parameters nor functional microbial abundance was found to affect the CH$_4$ flux at the discharge site, which suggested that the mechanism was rather complex under wastewater discharge.

Soil NH$_4^+$-N content has been found to enhance CH$_4$ emission into the atmosphere in coastal areas (Allen et al 2007, Chen et al 2010, Irvine et al 2012) because of the competitive inhibition of the methane monoxygenase enzyme by NH$_4^+$-N and nitrite produced by the ammonium oxidation (Bosse et al 1993, Schnell and King 1995). In the present study, the higher NH$_4^+$-N content in the soil immediately after discharge was accompanied by a lower methane oxidizer abundance (indicated by the pmoA gene abundance) at the discharge site and not the non-discharge site. However, there was no significantly different CH$_4$ flux between these two sites. This is probably because of the high spatial variability in the CH$_4$ flux among the replicate plots in this study, preventing the detection of the significant effects of wastewater discharge on the flux. We also suspect that

| Parameter       | Discharge site | Non-discharge site |
|-----------------|----------------|--------------------|
|                 | N$_2$O flux    | CH$_4$ flux        | N$_2$O flux    | CH$_4$ flux    |
| Redox potential | 0.381**        | $-0.191$           | $-0.079$       | $-0.293^*$     |
| pH              | $-0.606^{***}$ | 0.229              | 0.184          | 0.030          |
| NH$_4^+$-N      | 0.760**        | $-0.131$           | 0.015          | 0.075          |
| NO$_3^-$-N      | $-0.418^{**}$  | $-0.158$           | 0.286          | 0.180          |
| mcrA            | $-0.158$       | 0.339              | $-0.082$       |                |
| pmoA            |                | $-0.158$           |                | 0.277          |
| AOA-            | 0.112          |                | 0.138          |                |
| amoA            |                |                    |                |                |
| AOB-            | 0.843**        |                | $-0.104$       |                |
| amoA            |                |                    |                |                |
| narG            | $-0.472^{**}$  |                | 0.006          |                |
| nirK            | $-0.187$       |                | $-0.114$       |                |
| nosZ            | $-0.361^*$     |                | 0.075          |                |

*, ** and *** indicate significant r values at p < 0.05, 0.01 and 0.001, respectively (n = 80 for each soil chemical/physical parameter; n = 32 for each functional gene abundance).

Table 2. Pearson correlation coefficient values between sediment properties and fluxes of CH$_4$ and N$_2$O measured during the second continuous sampling in June 2017.
CH₄ production was limited in the soil when high NH₄⁺-N was present because high NH₄⁺-N was also able to inhibit methanogenesis (Wang et al. 2016). Although no significant difference in soil mcrA gene abundance as found between the two sites, we found a lower mean value of mcrA gene abundance at the discharge site than at the non-discharge site, along with a reduced pmoA gene abundance on the 1st day.

4. Conclusions

We found that the discharge of dredging wastewater from shrimp ponds resulted in nitrogen-rich deposits in the soil and immediately stimulated N₂O emissions. Because the mechanism behind gas production is not addressed in this study and some microbial processes including nitrite oxidation, dissimilatory nitrate reduction to ammonium and nitrifier denitrification which are not considered in this study also play important roles in the gas production (Wragge et al. 2001, Molnar et al. 2013), future detailed studies are needed to investigate the responses of these microbial processes to the wastewater discharge and their contributions to the gas production in the soil.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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