# Simultaneous improvement of waste gas purification and nitrogen removal using a novel aerated vertical flow constructed wetland

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## Abstract

Insufficient oxygen supply is identified as one of the major factors limiting organic pollutant and nitrogen (N) removal in constructed wetlands (CWs). This study designed a novel aerated vertical flow constructed wetland (VFCW) using waste gas from biological wastewater treatment systems to improve pollutant removal in CWs, its potential in purifying waste gas was also identified. Compared with unaerated VFCW, the introduction of waste gas significantly improved $NH_4^+$-N and TN removal efficiencies by 128.48 ± 3.13% and 59.09 ± 2.26%, respectively. Furthermore, the waste gas ingredients, including $H_2S$, $NH_3$, greenhouse gas ($N_2O$) and microbial aerosols, were remarkably reduced after passing through the VFCW. The removal efficiencies of $H_2S$, $NH_3$ and $N_2O$ were 77.78 ± 3.46%, 52.17 ± 2.53%, and 87.40 ± 3.89%,

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respectively. In addition, the bacterial and fungal aerosols in waste gas were effectively removed with removal efficiencies of 42.72 ± 3.21% and 47.89 ± 2.82%, respectively. Microbial analysis results revealed that the high microbial community abundance in the VFCW, caused by the introduction of waste gas from the sequencing batch reactor (SBR), led to its optimized nitrogen transformation processes. These results suggested that the VFCW intermittently aerated with waste gas may have potential application for purifying wastewater treatment plant effluent and waste gas, simultaneously.

**Key words:** Constructed wetland; Waste gas; Nitrogen transformation; Greenhouse gases; Odorous gases.

## 1. Introduction

Constructed wetland (CW) is an environmentally sustainable, socially accepted and cost-effective wastewater treatment technology which shows a strong potential for better secondary effluent treatment (Greenway, 2005). Ávila et al. (2015) found that the effluent treated by hybrid constructed wetlands could meet all the requirements for reclaimed water in Spain. However, the insufficient oxygen supply in traditional CWs often hinders their treatment efficiencies, especially for organic matter and ammonium (Hu et al., 2012). Artificial aeration and tidal flow (the operation of intermittently flood and drain) have been developed as major dissolved oxygen (DO) optimization processes (Liu et al. 2016). Even though artificial aeration is considered to be the most effective method to ensure sufficient oxygen supply, the corresponding operational costs greatly limit its popularity (Zhang et al., 2010). Tidal flow is also a method used to address oxygen transfer limitations (Wu et al. 2011). However, the oxygen supply

efficiency of tidal flow is lower than that of artificial aeration (Wu et al. 2014). Jia et al. (2010) found that removal rates of ammonia nitrogen and chemical oxygen demand (COD) generally increased during the tidal flow operation, although TN removal decreased as a result of the accumulation of nitrate. Hence, it is important to further optimize the wetland oxygen supply strategy.

The seriously deteriorated water environment lead to more stringent wastewater discharge standards. For example, the government released Water Pollution Control Action Plan in April 16, 2015, to guide water pollution control in China. At present, many wastewater treatment plants (WWTPs) in China have been required to meet third-grade surface water standard. However, in most cases, WWTPs are unable to meet the requirements of these new guidelines. Hence, to effectively remove pollutants, such as organic matter and ammonia nitrogen, intensive aeration is implemented in traditional biological wastewater treatment processes, which accounts for 40-60% of the total operating costs of WWTPs (Gu et al., 2008). Additionally, the waste gas produced from aeration often goes directly into the atmosphere, resulting in a nuisance to adjacent populations and serious environmental pollution risks, including odorous gases (Burgess et al., 2001), high emissions of greenhouse gases (mainly nitrous oxide ($N_2O$) (IPCC, 2013)) and microbial aerosols (Brandi et al., 2000). $N_2O$ is an important greenhouse gas that can also cause ozone depletion in the stratosphere. Its 100-year global warming potential is 298 times higher than that of carbon dioxide ($CO_2$). Microbial aerosols have caused concern all over the world. Brenner et al. (1988) reported the distribution of animal viruses, bacteria and phages in the atmosphere

around a sewage irrigation station. In addition, the microorganism aerosol threshold limit value is very important for human health risk assessments (Srikanth et al., 2008). Waste gas emission from biological wastewater treatment has become a serious concern. It should be better controlled or, if possible, utilized to protect the urban environment.

A range of technologies has been developed to purify waste gas, which can be roughly classified as three categories (Burgess et al., 2001; Kennes and Veiga, 2001), biological (biofilters, bioreactors), chemical (chemical scrubbers, thermal oxidation, catalytic oxidation, ozonation), and physical (condensation, activated carbon, clean water scrubbers). However, the present waste gas purification technologies involve long and complex treatment processes, and more important, waste of resources and energy. Few literatures are found to describe how waste gas can be utilized as a kind of "available resource". Therefore, it is of great interest to develop novel aerated CWs, which can efficiently recycle resources and minimize gaseous pollution, simultaneously.

In this study, the vertical flow constructed wetland (VFCW) was aerated by using waste gas from a sequencing batch reactor (SBR). Specific objectives were: 1) to analyze the feasibility of using waste gas to enhance oxygenation of a VFCW; 2) to evaluate the removal efficiency of waste gas ingredients (including odorous gases, greenhouse gas and microbial aerosols) via a VFCW; and 3) to elucidate the mechanisms of pollutant removal in novel aerated VFCW.

## 2. Materials and methods

### *2.1 Experimental system configuration*

The lab-scale systems were located at Shandong University in Jinan, China

(36º40'36"N, 117º03'42"E). The experiment was kept running from March 23, 2016 to November 3, 2016. The systems were composed of a SBR followed by three parallel laboratory-scale VFCWs (System I: unaerated VFCW; System II: VFCW intermittently aerated with air; and System III: VFCW intermittently aerated with waste gas). The schematic diagram of the experimental setup is shown in Fig. 1.

The effective volume of the anoxic/aerobic SBR was 15 L. The internal diameter and working height of the reactor were 25 and 30 cm, respectively. The schematic design of the reactor has been reported by Zhang et al. (2015). The influent wastewater was prepared in a storage tank (100 L) and introduced into the SBR using a peristaltic pump. The DO was supplied by using an air pump through an air diffuser at the bottom of the reactor. The SBR was intermittently aerated at an airflow rate of 0.12 $m^3$/h. The seeding sludge was obtained from the Second Wastewater Treatment Plant of Everbright Water (Jinan) Ltd., China. After one month of operation, the concentrations of pollutants in effluent tended to be stable and the SBR-VFCWs were in a steady state. The mixed liquor suspended solid (MLSS) of the SBR was maintained at 4500-5000 mg/L.

The lab-scale VFCWs (Systems I, II and III) were constructed outdoors (25 cm in length, 25 cm in width and 50 cm in depth) with an outlet at the bottom. A dimensional gradation substrate was adopted, which was made of a 10 cm bottom layer of gravel (3-4 cm in diameter) that served as the supporting layer; silica sand (2-3 cm in diameter) as the main substrate layer that was filled in each wetland, with a depth of 30 cm; and a 10-cm top layer of washed river sand (1-2 mm in diameter) that was added for

facilitating the dispersion of wastewater and growth of plants. To measure various physical and chemical parameters *in situ,* a vertical perforated PVC pipe (60 cm in length and 3 cm in diameter) was inserted into the substrate in the middle of the VFCWs. In System II and System III, the porous air sparger was installed in the bottom supporting layer of each system for oxygen supply. In this study, sweet flag (*Acorus calamus* L.) was selected as the experimental plant. Sweet flag, which is an emergent macrophyte colonizing littoral zones of eutrophic habitats, has been used in naturally occurring and constructed wetlands to treat wastewater (Brändle et al., 1996). Healthy plants with a similar size (approximately 30 cm in height) were weighed and then transplanted into the VFCWs at a density of 20-25 rhizomes per unit. In this study, the size of VFCW units is 25 cm in length and 25 cm in width. According to the density ranges observed in natural aquatic environments, a spacing of sweet flag was set to 5 cm, resulting in 20-25 rhizomes per unit. After transplanting, CW microcosms were fed with the SBR effluent for one month before the experiment started, until wetland plants and microorganisms were well established.

*2.2 Experimental procedure*

The SBR was fed with synthetic wastewater prepared from tap water. The composition of synthetic wastewater is as follows (per liter): sucrose (133.90 mg), starch (115.40 mg), $NH_4Cl$ (114.64 mg), $(NH_4)_2SO_4$ (141.60 mg), $K_2HPO_4•3H_2O$ (18.00 mg), $KH_2PO_4$ (11.00 mg), $CaCl_2•2H_2O$ (10.00 mg), $MgSO_4$ (5 mg), $FeSO4•7H_2O$ (10.00 mg) and a trace element solution (1.0 mL). The composition of the trace mineral solution was derived based on previous literature (Tay et al., 2002).

The COD and $NH_4^+$-N concentrations of the synthetic wastewater were approximately 300 and 60 mg/L, respectively. The influent pH values were adjusted to 7.5-8.0 by adding $NaHCO_3$. The SBR was operated at a volumetric exchange ratio of 50%, with a cycle of 4 h, resulting in a hydraulic retention time (HRT) of 8 h. Each cycle was consisted of 10 min for filling influent, 60 min for the anoxic process, 120 min for the aeration reaction, 30 min for settling, and 20 min for decanting the effluent.

The effluent of the SBR flowed into a setting tank and was subsequently conveyed to the beds of three VFCWs, at a flow rate of 7 mL/min to keep the water level below the sand surface, by using a peristaltic pump. Treated effluent was discharged from the outlet at the bottom of each VFCW. Each wetland was operated continuously at HRT of 1 day. The characteristics of the influent were periodically monitored during the experimental period. Systems II and III were intermittently aerated, which was consistent with the SBR aeration time. System I was operated without aeration.

### *2.3 Sample collection and analysis*

#### *2.3.1 Water quality monitoring*

Water samples were taken from the influent and effluent of the SBR and from the effluent of the three VFCWs every 3 days to analyze the transformation of organic matter (COD), phosphorus (P) and nitrogen (N). Water samples were then analyzed immediately for $NH_4^+$-N, $NO_3^-$-N, $NO_2^-$-N, TP, TN and COD. The DO concentration was determined *in situ* with a DO meter (HQ30d, Hach, USA). The pH value was determined using a pH meter (SG2, METTLER TOLEDO, Switzerland). The oxygen concentrations in air and waste gas were determined by using an $O_2$ meter (CY12C,

KREVOR, China). All analyses were performed according to Standard Methods (APHA, 2005).

*2.3.2 Gas sample and measurement*

The $N_2O$ and carbon dioxide ($CO_2$) emission fluxes of the SBR and the three VFCWs were measured when relatively stable effluent quality was achieved. In each sampling process, an aspirator pump was used to move the emitted gas into gas sampling bags (PV-500 ml; Delin, China) at intervals of 10 min according to a closed static-chamber method (unaerated phase) and 15 min according to using an open-chamber method (aeration phase), respectively (Zou et al., 2016). The open-chamber method means the chamber was always open during sampling process. Subsequently, $N_2O$ concentrations were measured using a gas chromatograph (7890B; Agilent, USA) equipped with an electron capture detector (ECD) and back-flush controlled by a 10-port valve, as described in our previous study (Zhang et al., 2016). A thermal conductivity detector (TCD) measured $CO_2$ simultaneously within the vials. The $N_2O$ emission flux from VFCWs (unaerated phase) was calculated by means of linear increase in the sampling period according to the equation given by Jones et al. (2011). The $N_2O$ emission rate and quantity of SBR were calculated using the equation described by Kong et al. (2013). The $N_2O$ and $CO_2$ emission fluxes of Systems II and III, during aeration phase, were obtained based on the average concentration of $N_2O$ and $CO_2$ by modifying the equation described by Hu et al. (2010), in which sludge volume was replaced by the chamber recovery area.

A six-stage Andersen sampler (Thermo-Andersen, Smyrna, GA, USA) was used to

collect bacterial and fungal aerosols of different sizes from the waste gas of the SBR and System III. The Andersen sampler used here has six stages with different cutoff ($D_{50}$) sizes from high to low: 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65μm, representative of the human respiratory system. Each stage was filled with appropriate agar medium for bacterial and fungal growth. The sampling time was 15 min between 8:00-8:15 AM (the beginning of the aeration phase), with a constant sampling flow rate of 28.3 L/min. At least three independent experiments were selected for both bacterial and fungal aerosols in each environment. Before sampling, the inside of the sampler was disinfected with 70% alcohol. Bacteria from the collected air samples were cultivated in nutrient agar (Hope Biotech Co., China) at 37 $^{o}$C for 24 h. Fungi were incubated in a Rose Bengal Medium at (Hope Biotech Co., China) 28 $^{o}$C for 72 h. After incubation, the numbers of colonies on the plates were determined using the positive-hole correction method (Macher, 1989). The results were calculated as the geometric mean of the replicates and expressed as colony-forming units per cubic meter of air (CFU/$m^3$).

To evaluate the changes of odorous gases in waste gas from the SBR via the VFCW, gas samples were taken at the beginning of the aeration phase. In this study, we have tested the $H_2S$ and $NH_3$ in the waste gas from SBR. Meanwhile, we have also tested the VOCs in the waste gas, but VOCs concentrations were below the detection limit. The sampling time was 40 min at the same time of the day, with a constant sampling flow rate of 1.2 L/min. At least three independent experiments were selected for the analysis of $H_2S$ and $NH_3$ in each environment (SBR and System III). The released $H_2S$ was measured with the methylene blue spectrophotometric method using a suspension

198 formed by adding sodium hydroxide to a solution of zinc acetate as an absorbent and
199 precipitated as zinc sulfide (Fogo et al., 1948). The released $NH_3$ was determined with
200 indophenol blue spectrophotometric method using diluted sulfuric acid as an absorbent
201 (Ivančič et al., 1984).

202 *2.3.3 Plant physiology*

203 In the middle of July, August, September and October, mature leaves of the plants
204 were collected to measure chlorophyll content. The leaf samples were extracted with
205 25 mL 80% acetone in darkness. The contents of total leaf chlorophyll were determined
206 as described in Bruuinsma (1963). Wavelength absorbance was measured at 652 nm,
207 using an ultraviolet spectrophotometer, for the determination of total leaf chlorophyll.
208 The chlorophyll content was expressed as a fresh weight (FW) basis (mg $g^{-1}$). The
209 height of sweet flag was measured every month since the VFCWs were fed with
210 wastewater.

211 *2.3.4 Microbial analysis*

212 At the end of operation, sediment samples were collected from five spots at the
213 same height (approximately 25-30 cm) within each VFCW and mixed as one composite
214 sample. Meanwhile, the mixed liquor sludge (30.0 mL) was sampled from the SBR at
215 the end of the oxic phase. The collected sediment and the mixed liquor were treated
216 with a MOBIO PowerSand™ DNA Isolation Kit to extract the total genomic DNA and
217 stored at -20 °C for further analyses.

218 Microbial processes are known to be important pathways that contribute to nitrogen
219 removal. To assess the removal and transformation microbial mechanisms of nitrogen

220 in CWs, the quantities of functional genes involved in biological nitrogen
221 transformations, i.e., total bacteria (*16S rRNA* gene), nitrifying bacteria (*amoA* and *nxrA*
222 genes) and denitrifying bacteria (*nirK, nirS* and *nosZ* genes) were measured using
223 quantitative polymerase chain reaction (q-PCR) technology, and the detailed
224 information was shown in the Supplementary Materials. Q-PCR has been widely
225 adopted to detect microbes in natural samples without laboratory culture.

226 To obtain the microbial community for each VFCW, Illumina high-throughput
227 sequencing was performed at the Yuanxu Biotechnology Company (Shanghai, China).
228 To minimize the effects of random sequencing errors, the sequences shorter than 250
229 base pairs (bp) in length and with a quality score lower than 30 were removed from the
230 pyrosequencing-derived data sets. The sequence number of each sample was
231 normalized, and the trimmed sequences were grouped into operational taxonomic units
232 (OTUs) at 97% sequence identity by the UCLUST software (Edgar, 2010). Rarefaction
233 analysis, the Simpson diversity index, Chao richness estimations and Good's coverage
234 were calculated by Mothur analysis (http://www.mothur.org) at a 3% distance level.
235 Venn diagrams were generated by R packages Venn diagram.

236 *2.4 Statistic analysis*

237 All statistical analyses were performed by the statistical program SPSS 11.0 (SPSS
238 Inc., Chicago, USA). Two-sample *t*-tests were used to evaluate the significance of
239 differences between means. All tables and figures show the results of averaged data. In
240 all tests, differences and correlations were considered statistically significant when
241 $P<0.05$.

## 3. Results and discussion

### *3.1 Removal of organic and nutrients in different VFCW systems*

After acclimatized for two months, three VFCWs showed stable performance and the plants were flourishing. Figs. 2a and 2b show N removal by different VFCW systems. The effluent $NH_4^+$-N concentrations of Systems I, II and III were 16.32 ± 2.78, 4.91 ± 1.16 and 0.61 ± 0.24 mg/L, respectively and corresponded to $NH_4^+$-N removal efficiencies of 42.83 ± 1.13%, 82.81 ± 2.23% and 97.86 ± 1.92%, respectively. $NH_4^+$-N removal efficiencies in Systems II and III were better than the removal efficiency of System I, indicating that intermittent aeration significantly enhanced $NH_4^+$-N removal.

The $NH_4^+$-N removal difference was attributed to the difference in DO concentration of the three systems (Fig. 2d). More precisely, the decrease of DO concentration in System III was faster than that in System II, suggesting that System III has better nitrification performance (Fig. 2c). However, in the unaerated VFCW, the DO level was always near zero (0.15 ± 0.02 mg/L), causing an anaerobic environment and, thus, may have resulted in negligible nitrification. In VFCWs, TN removal is achieved by nitrification-denitrification, which can be limited by various factors such as excess inorganic N (including nitrate and nitrite) in effluent, excess oxygen and an insufficient organic carbon source (Maltais-Landry et al., 2009c). Accumulations of $NO_3^-$-N were observed in both System II (25.07 ± 3.92 mg/L) and System III (16.53 ± 2.85 mg/L), compared with System I (6.98 ± 2.18 mg/L) because the DO concentrations of aerated systems (Systems II and III) were much higher than the DO concentration in the unaerated VFCW (Fig. 2d). Similar results were reported in other studies

investigating aerated CWs (Maltais-Landry et al., 2009c; Nivala et al., 2007). Throughout the experiment period, $NO_2^-$-N concentration was always below 0.2 mg/L, with no obvious fluctuations.

As shown in Fig. 2b, the TN removal efficiency of System III was 51.88 ± 3.42%, which was higher than the removal efficiency of System I (32.61± 2.35%). However, the TN removal efficiency of System II was 29.04 ± 3.69% lower than that of System I. The best TN removal efficiency was observed in System III, indicating that the introduction of waste gas could effectively develop alternate aerobic and anaerobic conditions in the VFCWs to improve TN removal, which is in accordance with a previous study (Wu et al., 2016). It is worth noting that the VFCW intermittently aerated with air showed the worst TN removal performance (23.14 ± 2.12%), even when nitrification was successful (Fig. 2a), revealing that the denitrification process in System II was limited. As shown in Fig. 2d, the DO concentrations of Systems II and III increased from 0.89 to 3.86 mg/L and 0.65 to 2.65 mg/L, respectively, during the first 0.5 h of aeration time (0 min-30 min). The DO concentration in System II was higher than that in System III during aeration, which was ascribed to the differences in oxygen content between the air (21.00 ± 0.13%) and waste gas (18.96 ± 0.16%). Full denitrification could not be achieved due to excess oxygen in System II. Furthermore, organic carbon sources have an important effect on the denitrification process. Compared with the unaerated VFCW (26.43 ± 1.62%), the introduction of waste gas into the VFCW (49.72 ± 2.13%) greatly improved COD removal efficiency. However, no significant difference in COD removal was observed between Systems II and III,

286 which is likely due to low organic loading. Regarding TP removal, no significant
287 differences were found among the three systems, indicating that artificial aeration had
288 a slight effect on TP removal (from 53.46 ± 3.06% to 58.39 ± 2.89%). Likewise, Tao et
289 al. (2010) and Zhang et al. (2010) found that artificial aeration did not have significant
290 influence ($p > 0.05$) on P removal. The high removal rates of $NH_4^+$-N and TN in this
291 study also showed that intermittent aerated VFCW using waste gas would be a potential
292 choice to intensify nitrogen removal performance for the wastewater. The application
293 of this novel aerated VFCW in full-scale system needs to be further research.

294 *3.2 Plant growth*

295 Wetland plants play an essential role for N and P absorption and the removal of
296 pollutants in CW, which is crucial for its healthy development. Wang et al. (2016)
297 determined that the presence of plants positively affected both microbial abundance and
298 community. Therefore, it is necessary to investigate the growth of wetland plants for
299 each system. Throughout the experiment period, chlorophyll content and plant height
300 in the three systems were investigated, as shown in Fig. 3a. All plants in Systems I, II,
301 and III grew well without any obvious symptoms of toxicity or nutrient deficiency.
302 Although no significant differences in plant growth were observed between Systems II
303 and III ($p > 0.05$), both Systems II and III had higher growth rates during the experiment
304 period (0.13 cm/d and 0.14 cm/d, respectively), compare with the unaerated VFCW
305 (0.06 cm/d), suggesting that aeration would remarkably improve plant growth.
306 Moreover, chlorophyll is the core of photosynthetic activity in vegetation, and it can
307 provide a measure of plant growth conditions from another perspective. The variations

308 in chlorophyll content during the four-month experiment were in accordance with the
309 plant growth rate. The chlorophyll content accumulated in the aerated systems (Systems
310 II and III). Chlorophyll content in System III was significantly higher than the unaerated
311 VFCW, indicating plant growth was improved by artificial aeration because of the
312 enhanced synthesis of chlorophyll. The enhanced synthesis of chlorophyll under aerated
313 conditions can be explained by two reasons: 1) changing oxygen content of the plant
314 root zone affects the growth of plants and long-term anoxic conditions cause death of
315 plants due to alcohol poisoning (Rzewuski and Sauter, 2008); and 2) available nutrients
316 are the key factor influencing the growth of plants. In aerated systems, a high
317 nitrification rate enhances nitrate accumulation and then provides more available
318 nutrients around the surface of the plant root, facilitating its growth. Moreover, $CO_2$
319 fluxes can reflect plant photosynthesis and respiration of aerial parts, and the $CO_2$ total
320 emission fluxes for the three systems are shown in Fig. 3b. The $CO_2$ fluxes in the aerated
321 VFCWs were lower than that in the unaerated VFCW as a result of the larger amount
322 of plant biomass. The result is consistent with the finding of Maltais-Landry et al.
323 (2009a) and Chen et al. (2015), who reported that the biomasses of three woody species
324 (*Ilex aquifolium*, *Rosa chinensis* and *Fatsia japonica*) increased by 381.75, 606.25, and
325 896.88 g/m$^2$ under aerated conditions, respectively.

326 *3.3 Purification of waste gas*

327 To investigate the purification of microbial aerosols in waste gas after passing
328 through the VFCW, the influent and effluent of culturable microbial aerosol
329 concentrations for System Ⅲ were examined, as shown in Fig. 4. A significant

decrease in particle concentrations of bacterial and fungal aerosols was observed. The results showed that bacterial and fungal aerosols in waste gas could be remarkably reduced by 42.72 ± 3.21% and 47.89 ± 2.82%, respectively, after passing through the VFCW. No significant difference in capture efficiency was observed between bacterial and fungal aerosols, as fungi and bacteria have the same maximum diameter size distribution, between 1.1 and 2.1 μm (Sanchez-Monedero et al. 2003). The removal mechanism for microbial aerosols in wetlands mainly depends on the interception and sedimentation of wetland filler. This process in wetlands is similar to biofilters, which have become a widely accepted method for microbial aerosol control in WWTPs (Sanchez-Monedero et al. 2003). Additionally, wetland plants also contribute to the removal of aerosols, which can prevent microbial aerosol diffusion.

Gas ($H_2S$, $NH_3$) odor concentration and greenhouse gas flux (mainly $N_2O$) in different experimental systems were analyzed (Table 1). The pollutants in waste gas could be remarkably reduced via wetland treatment. Approximately 77.78 ± 3.46%, 52.17 ± 2.53%, and 87.40 ± 3.89% of $H_2S$, $NH_3$ and $N_2O$, respectively, could be removed from the waste gas. In this study, the VFCW acted as a biofilter to purify waste gas by substrate adsorption, water dissolution or microbial utilization. The heterotrophic microorganisms on the wetland substrate utilized organic matter from the waste gas as the carbon source for their growth and reproduction, and the organic matter was oxidized to harmless substances, such as $CO_2$ and $H_2O$. Then, $CO_2$ was used as carbon source of autotrophic microorganisms to remove the inorganics ($NH_3$, $H_2S$ and $N_2O$) in the waste gas, which is similar to the photosynthesis of green plants (Kennes

352 and Thalasso, 1998). Thus, the risk of gaseous pollution during biological wastewater
353 treatment could be effectively eliminated. Further investigations should be undertaken
354 regarding the effects of wetland plants on waste gas purification.

355 It was found that intermittent aeration had obvious impact on enhancing $N_2O$
356 emission. More specifically, System II had the highest $N_2O$ emission flux, followed by
357 System III and System I, since $N_2O$ can be produced through both nitrification and
358 denitrification processes. Systems II and III had higher nitrification (Fig. 2a), which
359 accelerated nitrogen transformations, led to high $N_2O$ production and was consistent
360 with other research (Itokawa et al., 2001). However, previous studies reported that
361 lower $N_2O$ emissions could be achieved in CWs through the combination of
362 macrophytes and artificial aeration (Maltais-Landry et al., 2009a), which was different
363 from the present study. The reason is that the influent chemical oxygen
364 demand/nitrogen (C/N) ratio in this study was low (approximately 1~2). Hence,
365 denitrification was inhibited under a low C/N ratio and resulted in more $N_2O$ produce.
366 Furthermore, aerated systems had a better gas flow condition, which is beneficial to the
367 dispersion of produced $N_2O$ into the atmosphere. The highest emission flux detected in
368 System II was approximately 1.36 times higher than that in System III, because System
369 II had the highest $NO_3^-$-N accumulation (Fig. 2c) in treated effluents, indicating that the
370 denitrification process was inhibited, which resulted in more $N_2O$ emissions. Maltais-
371 Landry et al. (2009b) also reported that artificial aeration stimulated $N_2O$ production,
372 potentially via incomplete denitrification.

373 *3.4 Microorganism analysis*

374 *3.4.1 Relationship between nitrogen transformation and related genes*

375 Gene number can reflect the microbe number, which closely corresponded to
376 nitrogen transformations. Fig. 5 gives the relative abundance of bacterial *16S rRNA*,
377 *amoA*, *nxrA*, *nirK*, *nirS* and *nosZ* genes in each system. According to the q-PCR results,
378 there were remarkable differences in the microbial community compositions of the
379 VFCWs. As shown in Fig. 5a, the highest abundance of nitrifying bacteria (AOB, NOB)
380 was detected in System III, followed by System II, while fewer AOB and NOB were
381 detected in System I. The results exhibited that intermittent aeration was beneficial to
382 promote the growth and reproduction of AOB and NOB, and the low DO concentration
383 in the unaerated VFCW seriously limited the growth of nitrifying bacteria, explaining
384 why Systems II and III had higher $NH_4^+$-N removal efficiency than the control, as
385 shown in Fig. 2a. In addition, the gene number of *nirS* and *nirK* demonstrated that the
386 intermittent aeration of the CW with waste gas only inhibited the activity of the
387 denitrifying bacteria but did not eliminate it. The lowest abundance of denitrifying
388 bacteria was detected in the VFCW aerated with air due to its higher DO concentration,
389 which also led to more $N_2O$ emissions (Table 1). However, although denitrification was
390 responsible for $N_2O$ emissions, a negative correlation between denitrifying bacteria
391 numbers and $N_2O$ emissions was detected in System III. Similar result has also been
392 found by Wunderlin et al. (2012). The difference in the *nosZ* gene numbers, a gene
393 encoding nitrous oxide reductase, during denitrification among the three systems also
394 confirmed this result (Fig. 5b). Henry et al. (2006) found that a higher density of *nosZ*
395 gene copies corresponded to more bacteria capable of reducing $N_2O$ to $N_2$. Lower *nosZ*

gene copy numbers were found in System II, indicating a reduced ability to reduce $N_2O$, which led to more $N_2O$ emissions. Furthermore, intermittent aeration with waste gas markedly enhanced the abundance of bacterial *16S rRNA* in the sediment (Fig. 5b). The reason is that the microbe from the SBR was introduced to the CWs by aerating with waste gas.

*3.4.2 Comparative analysis of microbial community structures*

To further study the microbial mechanism of pollutant removal, microbial community structure of each system was determined by using high-throughput sequencing analysis. Across all samples, a total of 142,377 reads were obtained with an average read length of 433 bp to 436 bp. Chao1 and ACE were employed as community richness estimators at 3% dissimilarity. Community diversity was analyzed by calculating the Shannon and Simpson diversity index. Based on these indices, aeration had positive effects on the bacterial community. First, the introduction of waste gas remarkably increased community richness, which was consistent with the q-PCR results (Fig. 5). Second, the VFCWs, intermittently aerated with air or waste gas, had higher microbial diversity than the unaerated VFCW because the aerated VFCWs may provide a suitable environment for various bacteria . Moreover, the microbial diversity in System III dropped slightly compared with System II, which was probably due to a larger amount of microbial species in the air as opposed to the waste gas.

Apart from bacterial community richness and diversity, community structure is also important to understanding the performance of the three VFCWs. The microbial community structures for all the systems at the phylum level were observed, and the

418 major phyla groups in the three systems were uniform. Altogether, 23, 19 and 18
419 bacteria phyla were detected in Systems I, II and III, respectively. System I was mainly
420 dominated by *Proteobacteria* (67.19%), *Bacteroidetes* (14.25%), *Actinobacteria*
421 (5.90%), *Firmicutes* (3.42%), and *Nitrospirae* (1.30%), while System II presented
422 mainly *Proteobacteria* (60.22%), *Bacteroidetes* (16.57%), *Actinobacteria* (7.23%),
423 *Nitrospirae* (5.65%), and *Firmicutes* (2.61%). Similarly, System III contained
424 *Proteobacteria* (58.32%), *Bacteroidetes* (19.31%), *Nitrospirae* (6.82%),
425 *Actinobacteria* (5.59%), and *Firmicutes* (2.01%). The five most dominant phyla made
426 up over 92% of the community in Systems I, II and III. However, the relative sequence
427 abundances of some phyla varied with aeration. For example, *Nitrospirae*'s relative
428 abundance in Systems II and III improved with aeration (76.99% and 80.94% higher
429 than in System I, respectively). The relative abundances of *Proteobacteria*,
430 *Bacteroidetes* and *Firmicutes* showed no significant difference between all the CWs. It
431 has been reported that the three typical phyla of *Proteobacteria*, *Bacteroidetes* and
432 *Firmicutes* strains are crucial for denitrification processes (Miao et al., 2015). Based on
433 the results, nitrifying bacteria were greatly enhanced in the intermittently aerated
434 VFCWs, while denitrifying bacteria experienced no significant difference. These
435 results are consistent with the q-PCR analysis (Fig. 5).

436 The Venn diagram with common and unique OTUs was adopted to describe the
437 difference and similarity among Systems I, II, III and the SBR (Fig. 6). A large part of
438 the OTUs in Systems II and III was unique from the unaerated VFCW, and only 2242
439 and 2209 of the OTUs were shared for those aerated with air or waste gas VFCWs,

respectively. The results demonstrated that the structures of bacterial communities changed during the process of aeration application. Moreover, the numbers of species which were shared between the SBR and Systems I, II, III were 868, 850 and 947, respectively. The VFCW intermittently aerated with waste gas and the SBR had the highest percentage of shared OTUs (19.70%). This result verified that the highest microbial abundance in System III was from the SBR through the introduction of waste gas.

## 4. Conclusions

This work provides an effective method to enhance overall wetland treatment performance and eliminate waste gas pollution during biological wastewater treatment processes. The introduction of waste gas significantly intensified the removal of COD and N. The highest COD, $NH_4^+$-N, and TN removal efficiencies were achieved in the VFCW intermittently aerated with waste gas. The waste gas was also purified after passing through the VFCW (as a biofilter). Furthermore, the abundance of nitrifying bacteria (AOB and NOB), denitrifying bacteria and total bacteria in the VFCWs aerated with waste gas were markedly enhanced due to the introduction of microbes from the SBR by aerating with waste gas. In sum, the VFCW intermittently aerated with waste gas not only optimized the DO distribution but also improved microbial abundance and diversity, which validated the feasibility of using waste gas to enhance the oxygenation of VFCWs and increase resource utilization in biological wastewater treatment systems.

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## Supporting Information

Comparison of phylotype coverage, diversity and richness estimators at a phylogenetic distance of 3% (Table S1), Influent and effluent $NO_2^-$-N concentrations during the experimental period (Fig. S1), Influent and effluent water quality of the different CW systems throughout the experiment: (a) COD and (b) TP (Fig. S2), Relative abundance curves at the phylum level. Sequences that could not be classified into any known group were assigned as unclassified bacteria (Fig. S3), Oxygen content of waste gas profile during the aeration phase (Fig. S4) can be found in the Supporting Information.

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622

**Figure Captions:**

**Fig. 1.** Schematic diagram of the experimental setup (System I: unaerated VFCW; System II: VFCW intermittently aerated with air; System III: VFCW intermittently aerated with waste gas).

**Fig. 2.** Influent and effluent water quality of different VFCW systems, throughout the experiment period: (a) $NH_4^+$-N; (b) TN (c) $NO_3^-$-N and (d) DO (during the aeration phase).

**Fig. 3.** The growth of plants in the three systems, during the experiment period: (a) the chlorophyll content and plant height; (b) $CO_2$ total emission flux in the three systems

**Fig. 4.** Influent and effluent of culturable microbial aerosol concentrations for System Ⅲ: (a) bacterial aerosol and (b) fungal aerosol.

**Fig. 5.** The copy numbers of functional genes related to nitrogen metabolism in each system based on q-PCR analysis: (a) nitrification bacteria and denitrification bacteria; (b) bacterial *16S rRNA* and *nosZ.*

**Fig. 6.** Venn diagrams of different groups. Venn plot showing the shared and unique genera found in each plotted group.

641 **Table 1** $N_2O$ emission flux, $H_2S$ and $NH_3$ concentrations in different experimental

642 systems.

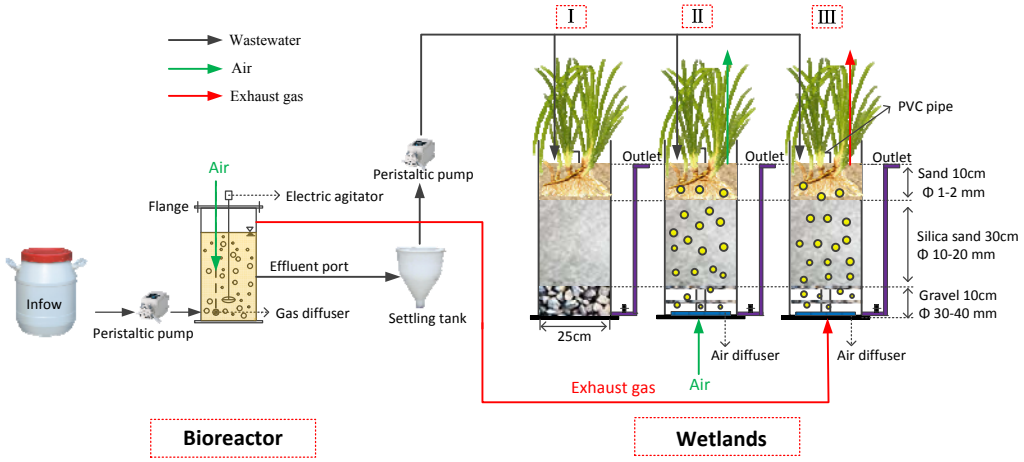
| Experimental systems | Parameters | | |
|---|---|---|---|
| | $N_2O$ ($\mu gN \cdot m^{-2} \cdot h^{-1}$) | $H_2S$ ($mg/m^3$) | $NH_3$ ($mg/m^3$) |
| SBR | 10.58 [c] | 0.0045 ± 0.0006 | 0.23 ± 0.02 |
| Ⅰ | 787.34 ± 23.69 | – | – |
| Ⅱ | 1370.47 ± 37.23 | – | – |
| Ⅲ | 1011.38 ± 30.56 | 0.0010 ± 0.0003[a] | 0.11± 0.01[b] |

643 [a] The concentration of $H_2S$ in waste gas after passing through VFCW (system III)

644 [b] The concentration of $NH_3$ in waste gas after passing through VFCW (system III)

645 [c] $N_2O$ emission quantity during one cycle in SBR (mg)

646




647

648 **Fig. 1.** Schematic diagram of the experimental setup (System I: unaerated VFCW;

649 System II: VFCW intermittently aerated with air; System III: VFCW intermittently

650 aerated with waste gas).

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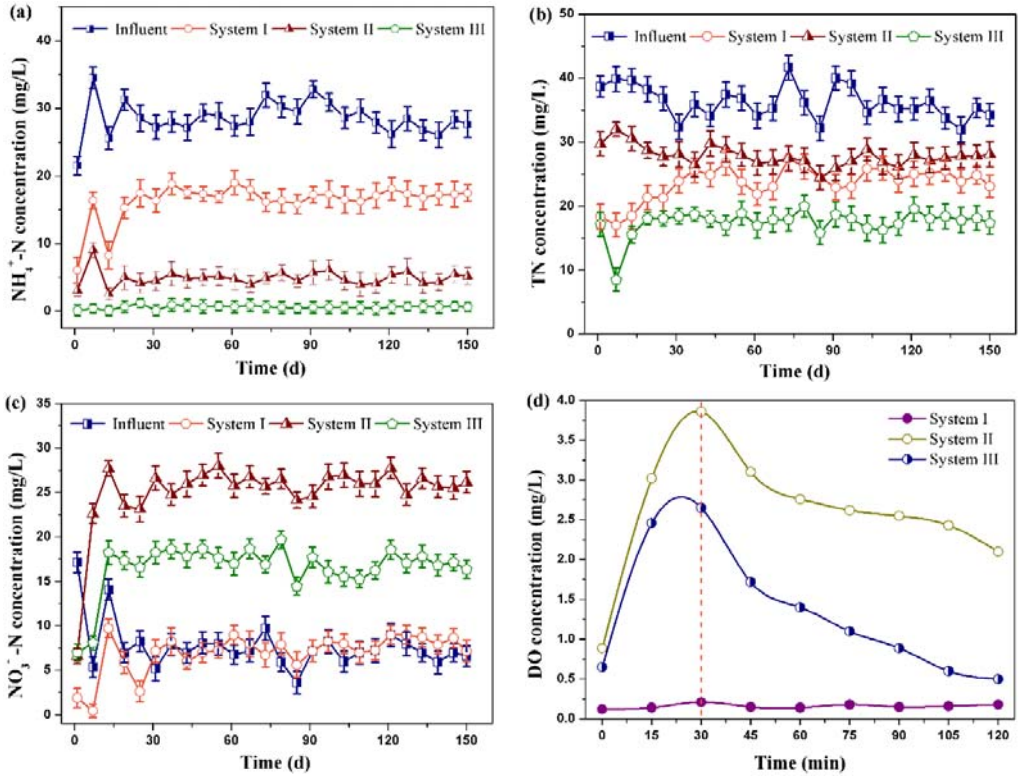


652 **Fig. 2.** Influent and effluent water quality of different VFCW systems, throughout the

653 experiment period: (a) $NH_4^+$-N; (b) TN (c) $NO_3^-$-N and (d) DO (during the aeration

654 phase).

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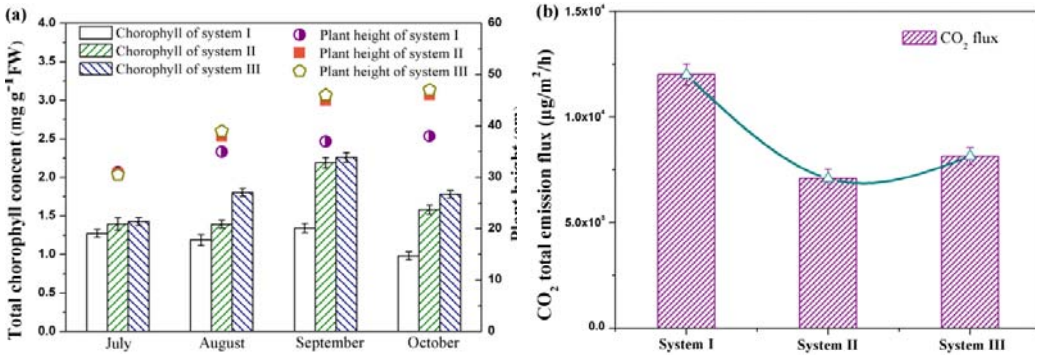


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657 the chlorophyll content and plant height; (b) $CO_2$ total emission flux in the three

658 systems

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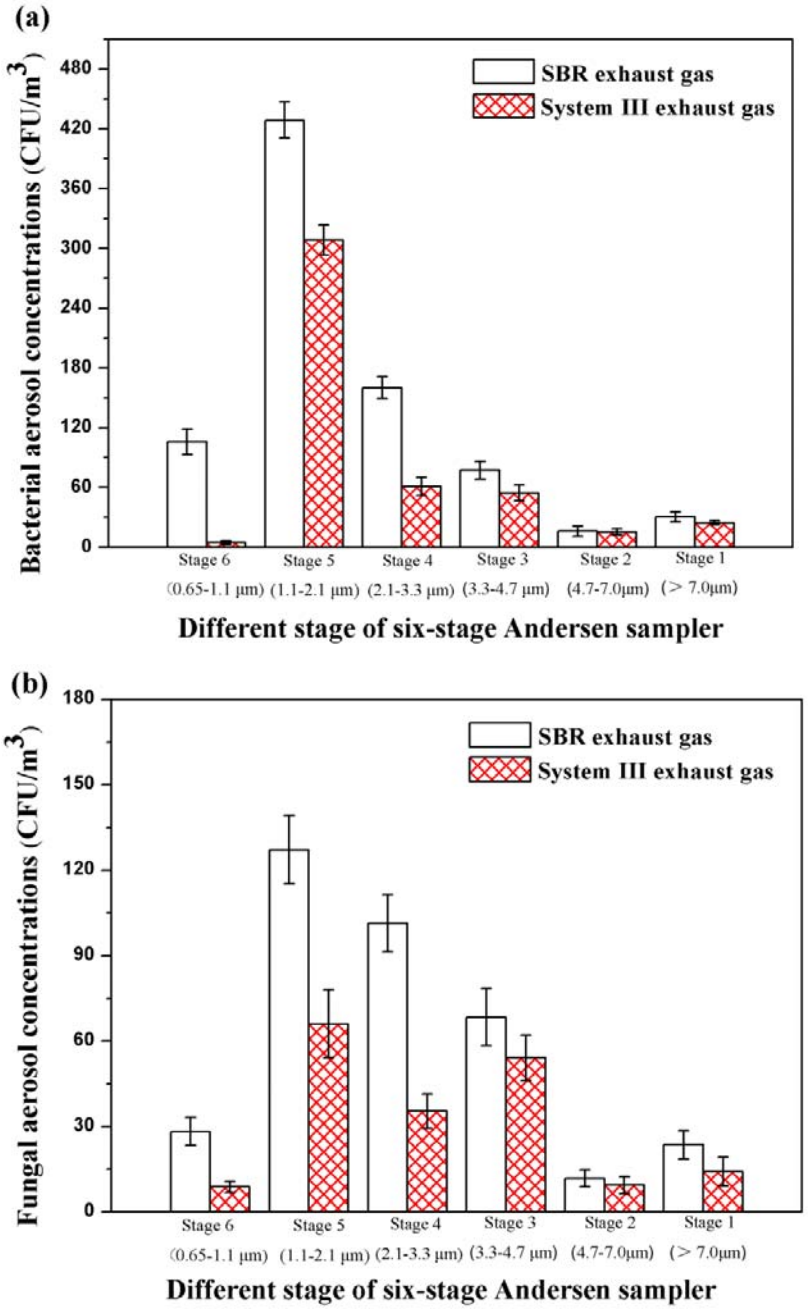


660 **Fig. 4.** Influent and effluent of culturable microbial aerosol concentrations for System

661 III: (a) bacterial aerosol and (b) fungal aerosol.

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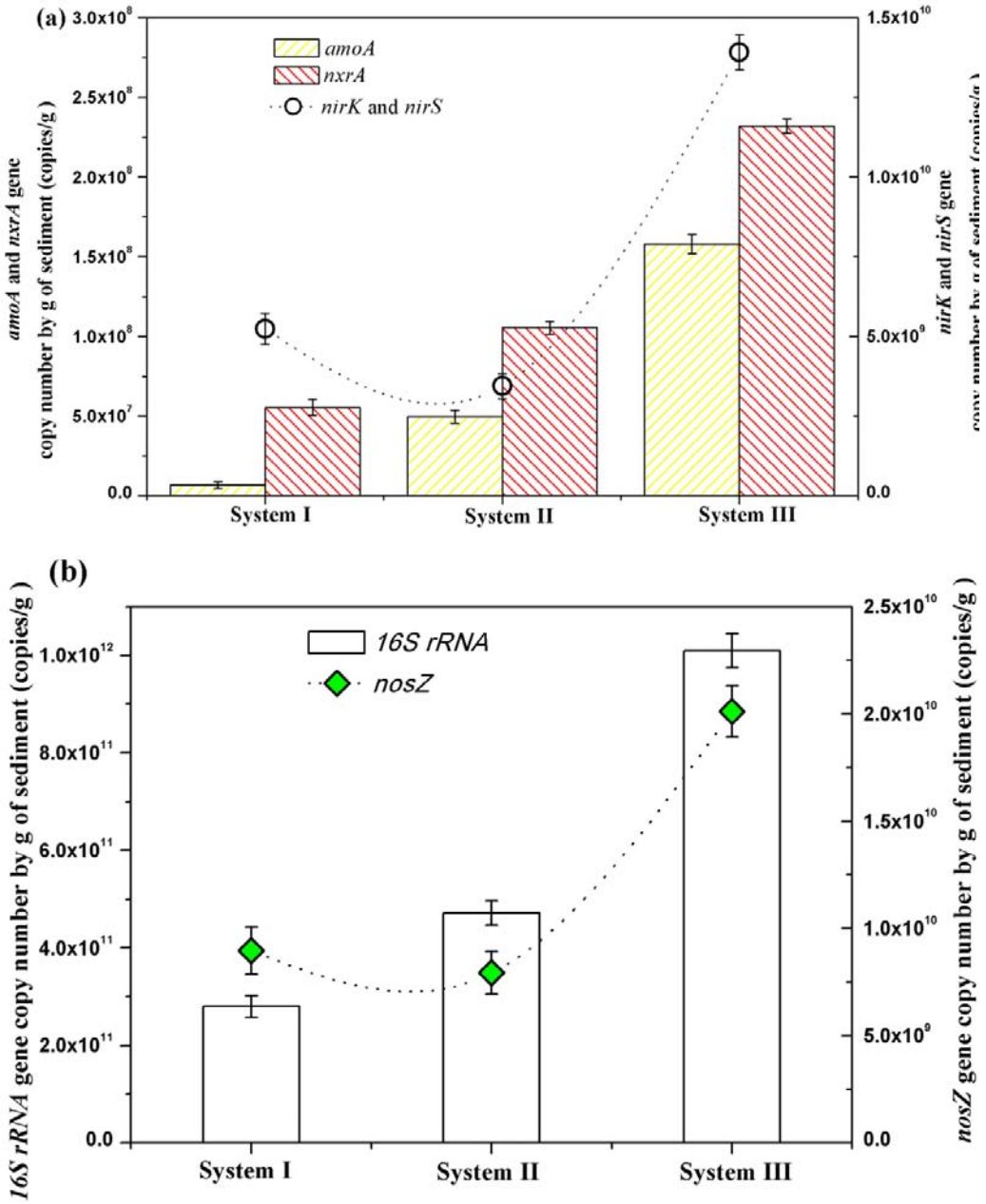


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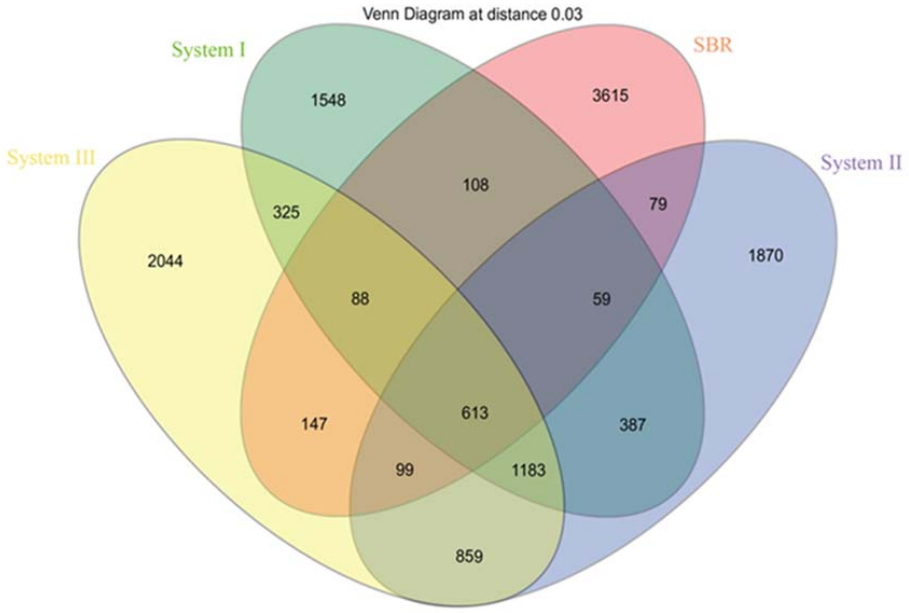


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668 **Fig. 6.** Venn diagrams of different groups. Venn plot showing the shared and unique

669 genera found in each plotted group.

670

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