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Effects of influent C/N ratios on CO_2 and CH_4 emissions from vertical subsurface flow constructed wetlands treating synthetic municipal wastewater

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ABSTRACT

Greenhouse gases (GHG) emissions from constructed wetlands (CWs) can mitigate the environmental benefits of nutrient removal because reduced water pollution could be replaced by emission of GHG. Therefore, the GHG (CO_2 and CH_4) fluxes of vertical subsurface flow constructed wetlands (VSSF CWs) under different influent C/N ratios of synthetic municipal wastewater were analyzed directly by GHG flux measurements, and estimated by carbon mass balance (CMB) over a 12 month period. The VSSF CWs system achieved the highest biological nutrient removal (BNR) efficiency between C/N ratios of 5:1 and 10:1 across all kinds of pollutants. Variation in influent C/N ratios dramatically influenced GHG fluxes from the VSSF CWs system. The GHG flux measured in situ agreed with those predicted by the CMB model and represented relatively low GHG fluxes when C/N ratios were between 2.5:1 and 5:1. It was determined that the optimum C/N ratio is 5:1, at which VSSF CWs can achieve a relatively high BNR efficiency and a low level of GHG flux.

1. Introduction

Constructed wetlands (CWs) are utilized to remove nutrients from wastewaters and to reduce nutrient export to adjacent ecosystems [1,2]. Their use has grown rapidly over recent decades due to cost-effectiveness and efficiency compared to conventional wastewater treatment facilities [3]. CWs used for wastewater treatment can be viewed as combinations of natural wetlands and conventional wastewater treatment plants, where organic compounds are degraded by both aerobic and anaerobic bacteria in the rhizosphere of vegetated beds [4]. They have been successfully utilized to remove organic matter and nutrients from municipal wastewater with different C and N loadings [5]. They have also been utilized as a low-cost and highly efficient option for reducing biological oxygen demand (BOD) [6].

However, when CWs are used for wastewater treatment, higher inputs of N and organic matter may increase productivity of the ecosystem and produce greenhouse gases (GHG) originating from the carbon cycle [7]. The production of GHG compounds, such as carbon dioxide (CO_2) and methane (CH_4), could therefore mitigate the environmental benefits of CWs [8]. CO_2 is produced by the respiration of plants and bacteria, but is also consumed by photosynthetic activity in the CWs [9,10]. CH_4 is produced in CWs by methanogenic bacteria under anaerobic conditions [11]. CH_4 generation is highly variable and regulated by numerous factors, including plant species, temperature and redox potential [12–14].

Although the total GHG emission from CWs worldwide is small compared to that from all industries, the rapid worldwide increase in development of CWs necessitates an understanding of their potential atmospheric impact, especially when environmental regulatory agencies are encouraging their development [15,16]. Because the area covered by CWs is also rapidly increasing all over the world, quantifying the amounts, relative importance and factors controlling GHG production in CWs deserve further attention [17]. Numerous studies addressing emissions and sequestration of CO_2 in CWs suggest that CWs can be sources or sinks of C depending on their meteorological and hydrological conditions [9,18–20].

Given that the main function of CWs is to reduce the levels of nutrients in water bodies and prevent eutrophication, ideally the greatest amount of nutrients should be removed with the lowest rates of GHG emission. The carbon mass balance (CMB) model, nutrient import variation, and various key factors controlling GHG emissions must be evaluated simultaneously in order to determine the best overall performance of a particular CW, e.g., vertical subsurface flow constructed wetlands (VSSF CWs). The CMB model, a simplification of the C cycle model in wetlands with increasing factors of input and output for wastewater, has been

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used in various studies for estimating environmental impacts of CWs systems [21–23]. VSSF CWs are becoming increasingly popular, and are currently subject to intensive research due to their lower land requirements and greater efficiency compared to other types of CWs [3]. In VSSF CWs, wastewater passes the filter in a more or less vertical path, and is activated intermittently. Oxygen-requiring nitrifying bacteria are favored and nitrification can be achieved mostly due to the aerobic conditions in the system [24]. So, any nitrogen-containing gas emissions came from denitrification should be ignored because of the mostly aerobic conditions in the system [25]. Influent water C/N ratio plays a crucial role in wastewater treatment effects [26]. Many studies suggest that higher growth rates are coupled not only to higher N/C or P/C ratios, but also to lower N/P in many heterotrophic organisms that must change their C/N/P stoichiometry as a function of growth rate [5,26,27]. Therefore, prevention of one problem in CWs might lead to a different one. Therefore, reducing water pollution without increasing the emission of GHG, largely relies on knowledge of how the participating organisms in CWs respond to influents with different C/N ratios. However, research on the effects of influent C/N ratios on CO_2 and CH_4 emissions from CWs has rarely been undertaken [21,28,29].

In this study, we focused on the GHG (CO_2 and CH_4) fluxes of VSSF CWs under different influent C/N ratios in synthetic municipal wastewater. The primary purpose was to evaluate the impact of the influent wastewater characteristics on GHG emissions from VSSF CWs during an entire year, and thus to identify optimal C/N ratios and operational parameters for the most efficient nutrient removal with the lowest GHG emission rates. The GHG fluxes at different C or N loadings were also investigated by CMB model in order to determine how such fluxes respond to variation in C/N ratios.

2. Materials and methods

2.1. Description of the wetland

Eighteen pilot-scale VSSF CWs were planted with the rhizomatous herb *Acorus calamus*. Every wetland frame, measuring 100 cm long × 60 cm wide × 80 cm high (Fig. 1a), was made of reinforced cement and filled with gravel (nominal mean diameter of 1.20 cm) up to a depth of 20 cm in the lower layer and slag (mean diameter, 1.50 cm) up to a depth of 25 cm in the upper layer (Fig. 1b). The slag had been cleaned to prevent unfavorable high pH conditions for microbe metabolism and *A. calamus* growth. The influent wastewater was supplied by a 5 cm internal diameter PVC pipe placed on one side of the wetland surface, and perforated with 1.5 mm holes (Fig. 1).

2.2. Experimental procedure

All CWs were planted with *A. calamus* (height 14.50 ± 1.25 cm), using 8–10 rhizomes per wetland on March 5, 2010. CWs were flooded for 30 d with tap water, after which synthetic wastewater was introduced by the PVC pipe with $40\,L\,d^{-1}$ as a single batch. According to the substrate materials, the wetland substrates net void capacity was 60 L and the overall hydraulic retention time was 1.5 d. Then, batch volumes of 200 L were applied weekly to each wetland by gravity for 5 d with the other 2 d remaining as dormant periods. Operation and monitoring of the wetlands were conducted from March 2010 to April 2011. During this period, the range of the water temperature in the CWs was 4.9–43.5 °C, averaging 26.5 °C in the influent.

2.3. Synthetic sewage

For health and safety reasons, as well as for comparison of parallel experiments, the CWs were fed with synthetic wastewater. We adjusted the chemical oxygen demand (COD), and total nitrogen (TN) concentrations in the synthetic wastewater, while maintaining constant total phosphorus (TP) levels. There were two experimental categories (Table 1).

Category 1, C variation treatment, included fixed TN/TP levels at medium strength, and various levels of COD (low, medium, and high). There were three levels of C variation designated C1NP (C:N = 2.5:1), C2NP (C:N = 5:1), and C4NP (C:N = 10:1). The synthetic sewage was prepared prior to each batch feeding by mixing (in tap water) the following components: 100, 200, 400 $g\,m^{-3}$ glucose,

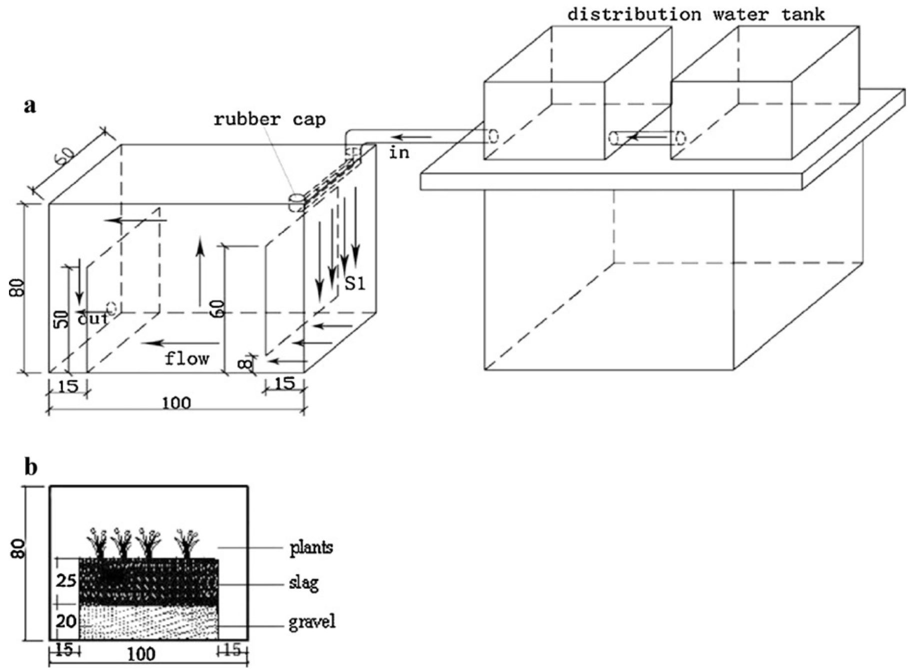

Fig. 1. Diagram of pilot-scale VSSF CWs. (a) Structure, (b) contents and plants (dimensions are in cm).

Table 1
Mean concentrations ± SD of C, N and P in the influent synthetic sewage.

Item	C or N level		C/N treatment	Influent concentration (mg L^{-1})				
				COD	TN	TP	TOC	BOD$_5$
Category 1 C variation treatment	COD level	Low	C1NP (C:N = 2.5:1)	102.32 ± 0.95	40.14 ± 1.24	5.02 ± 0.13	42.67 ± 2.21	66.52 ± 1.02
		Medium	C2NP (C:N = 5:1)	204.71 ± 3.15	41.56 ± 1.13	5.11 ± 0.29	93.01 ± 3.03	116.53 ± 3.23
		High	C4NP (C:N = 10:1)	404.33 ± 4.79	41.26 ± 1.73	5.24 ± 0.53	180.98 ± 3.87	213.38 ± 4.82
Category 2 N variation treatment	TN level	Low	CN1P (C:N = 10:1)	203.56 ± 2.62	22.06 ± 1.44	5.13 ± 0.21	93.57 ± 2.01	115.96 ± 2.71
		Medium	CN2P (C:N = 5:1)	204.71 ± 3.15	41.56 ± 1.13	5.11 ± 0.29	93.01 ± 3.03	116.53 ± 3.23
		High	CN4P (C:N = 2.5:1)	202.64 ± 3.27	81.06 ± 2.53	5.02 ± 0.11	90.63 ± 3.63	115.54 ± 3.94

80 g m^{-3} carbamide, 15 g m^{-3} NaH_2PO_4, 1.5 g m^{-3} KH_2PO_4, 4 g m^{-3} $CaCl_2$, and 2 g m^{-3} $MgSO_4$.

Category 2, N variation treatment, included fixed COD/TP levels at medium strength, and various levels of TN (low, medium, and high). There were three experimental groups for N variation: CN1P (C:N = 10:1), CN2P (C:N = 5:1) and CN4P (C:N = 2.5:1). The following components were used to prepare the synthetic sewage for the N addition treatments: 200 g m^{-3} glucose, 40, 80, 160 g m^{-3} carbamide, 15 g m^{-3} NaH_2PO_4, 1.5 g m^{-3} KH_2PO_4, 4 g m^{-3} $CaCl_2$, and 2 g m^{-3} $MgSO_4$.

All six experimental groups were run in triplicate and a total of 18 pilot-scale VSSF CWs were used for the study. The characteristics of the influent synthetic sewage are given in Table 1.

2.4. Measurement of water quality

During the experimental period, influent and effluent waters in the pilot-scale VSSF CWs were sampled in opaque plastic bottles (100 mL) every two weeks for water quality determinations. Samples obtained from the inlets and outlets of the CWs were analyzed for pH, COD, TN, TP, total organic carbon (TOC), and biochemical oxygen demand after 5 d (BOD_5) by methods described in APHA–AWWA–WPCF [30] (see Eq. (1)). Water temperatures and redox potentials were measured in situ by an Orion 250 Aplus ORP Field Kit.

The treatment effect was calculated as:

$$R = \left(1 - \frac{Ce}{Ci}\right) \times 100 \tag{1}$$

where R is the removal efficiency (%), Ci and Ce are the influent and effluent concentrations (mg L^{-1}), respectively. The mean effluent values for every batch sample over each month were used to calculate removal rates of each parameter.

2.5. Sampling and measurement of GHG

GHG (CO_2 and CH_4) emissions were measured in situ using cylindrical gas collectors made of stainless steel with 10 cm diameter and 35 cm height. There was a measurement port in the upper part of the collector for sampling. The base was inserted into the gravel surface to 5 cm depth and sealed by a water-filled ring on the slag surface. The gas collectors were installed only at the times of measurement by making a hole in the slag and removing all rhizomes and roots of the *A. calamus*. The chambers were placed in the inlet, middle and outlet zones of each experimental VSSF CW.

The GHG (CO_2 and CH_4) emissions were measured every two weeks, between 9:00 and 11:00 a.m. Gas samples were collected into a 10 mL vial by a gas-tight syringe (1010 TLL-SAL 10 mL SYRINGE, Hamilton, Switzerland) from the gas collector at 10 min intervals for 2 h. Then 12 gas samples were collected and their mean value was utilized as the final outcome of this measurement. All gas samples were analyzed by gas chromatography (Hewlett Packard P 5890 Series II, Palo Alto, CA, USA) equipped with a thermal conductivity detector for CO_2, and a flame ionization detector for CH_4 [31]. Gas flux was calculated from the concentration increase in the chamber [32]:

$$F = \frac{V}{A}\frac{dC}{dt} \tag{2}$$

where F is the gas flux (mg m^{-2} h^{-1}), V is the chamber volume (m^3), A is the area enclosed by the chamber (m^2), and dC/dt is the gas concentration gradient (mg m^{-3} h^{-1}).

2.6. Carbon mass balance model

The CMB model is a simplification of the C cycle model in wetlands [21]. Usually, the CMB model is performed as follows [16,29]:

$$(C_{inflow} - C_{outflow}) + C_{photosynthesis} = C_{storage} + C_{emission\text{-}CO_2} + C_{emission\text{-}CH_4} \tag{3}$$

where, C_{inflow} and $C_{outflow}$ are measures of BOD_5 (mg m^{-2} h^{-1}) in the influent and the remaining BOD_5 (mg m^{-2} h^{-1}) in the effluent, respectively [22]; $C_{storage}$ are measures of the 13% of the C_{inflow} (mg m^{-2} h^{-1}); and $C_{photosynthesis}$ is 134.25 mg m^{-2} h^{-1} under C/N at 2:1–10:1 in the influent for *A. calamus* [33,34]. Then, $C_{emission\text{-}CO_2} + C_{emission\text{-}CH_4}$, which provides GHG emission in this study, can be calculated according to the above formula (Fig. 2). The gas flux from the CMB model was calculated as:

$$C_i = \frac{DQ}{A} \tag{4}$$

where C_i is the gas flux (mg m^{-2} h^{-1}), i present inflow, outflow, photosynthesis, storage, emission-CO_2, and emission-CH_4, D is the contaminant concentration (mg m^{-3}), e.g., BOD_5, and A is the area of the VSSF CWs (m^2), and Q is the flow rate (m^3 h^{-1}). In the current work $A = 60\text{cm} \times (100 - 15 - 15)\text{cm} = 4200\text{cm}^2 = 0.42\text{m}^2$ (Fig. 1), $Q = 40\,\text{L}\,\text{d}^{-1} = 40 \times 10^{-3} \times 24^{-1}\,\text{m}^3\,\text{h}^{-1} = 0.0017\text{m}^3\text{h}^{-1}$.

So, Eq. (4) becomes:

$$C_i = 0.004048\ D \tag{5}$$

2.7. Statistical analyses

All statistical analyses were performed using SPSS software [35]. The different effects of variation in carbon or nitrogen concentrations and their interactions on treatment performance, and GHG

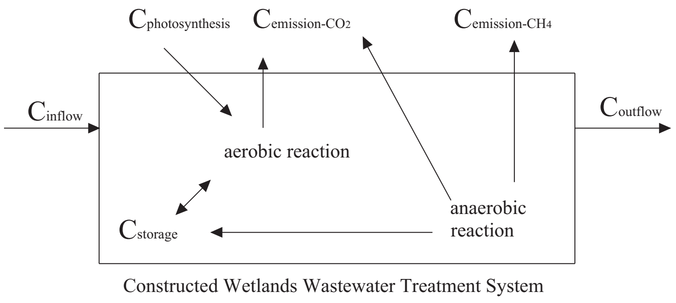

Fig. 2. Conceptual relationships in the static CMB model in the CWs.

Table 2
Mean concentrations ± SD and removal efficiencies of COD, TN, TP, TOC, and BOD_5 in effluent after different C/N treatments. Values with different superscript letters in the same column are significantly different at $P = 0.05$ according to Duncan's multiple range test.

C/N treatment	Effluent concentration (mg L^{-1})					Removal efficiency (%)				
	COD	TN	TP	TOC	BOD_5	COD	TN	TP	TOC	BOD_5
C1NP (C:N = 2.5:1)	44.88 ± 0.55	19.07 ± 1.85	1.06 ± 0.62	35.98 ± 2.11	20.25 ± 1.54	56.14^a ± 22.15	52.47^a ± 15.68	78.69^a ± 12.61	15.67^b ± 6.34	69.55^b ± 15.28
C2NP (C:N = 5:1)	82.17 ± 1.73	21.19 ± 1.04	0.85 ± 0.09	66.84 ± 1.53	24.25 ± 2.01	59.86^a ± 16.24	49.01^a ± 12.15	83.37^a ± 7.34	28.13^a ± 4.54	79.19^a ± 13.29
C4NP (C:N = 10:1)	156.75 ± 2.87	22.16 ± 2.89	1.57 ± 0.48	119.91 ± 3.73	41.48 ± 1.02	61.23^a ± 23.58	46.27^a ± 13.66	69.98^{ab} ± 14.25	33.74^a ± 15.02	80.56^a ± 10.32
CN1P (C:N = 10:1)	88.50 ± 0.78	15.29 ± 3.86	1.17 ± 1.57	66.06 ± 0.42	21.01 ± 1.52	56.52^a ± 21.14	30.65^b ± 11.19	77.08^a ± 9.06	29.40^a ± 10.68	81.88^a ± 12.05
CN2P (C:N = 5:1)	82.17 ± 1.73	21.19 ± 1.04	0.85 ± 0.09	66.84 ± 1.53	24.25 ± 2.01	59.86^a ± 16.24	49.01^a ± 12.15	83.37^a ± 7.34	28.13^a ± 4.54	79.19^a ± 13.29
CN4P (C:N = 2.5:1)	90.64 ± 0.87	37.22 ± 2.75	1.52 ± 2.45	70.54 ± 1.62	39.37 ± 1.62	55.27^a ± 20.88	54.08^a ± 9.36	69.63^b ± 14.21	22.16^a ± 10.25	65.92^b ± 11.28

emission were tested by two-way ANOVA. Duncan's multiple range tests was used to further assess differences among treatment combinations that were significant in ANOVA [10,36]. A probability level of $P = 0.05$ was used as the threshold for significance.

3. Results

3.1. Effects of influent C/N ratios on water characteristics

3.1.1. COD removal efficiency

There were no significant differences in the COD removal rates in all C and N variation treatments (Table 2). Furthermore, the removal efficiencies of COD for both the C and N treatments were quite similar. The most efficient VSSF CWs performances for COD removal were the C4NP (C:N = 10:1) and CN2P (C:N = 5:1) treatments.

3.1.2. TN removal efficiency

There were no significant differences in TN removal efficiency between the three C treatment levels (C1NP, C2NP, and C4NP) (Table 2). Multiple comparisons detected significantly lower TN removal efficiency in the C4NP treatment over the entire study period. For N variation treatments (CN1P, CN2P, and CN4P), differences in the removal efficiency of TN were significant ($P < 0.05$). The CN1P treatment had a much lower TN removal efficiency than the CN2P and CN4P treatments. The CWs performed TN removal most efficiently at C:N = 2.5:1, but there was little decrease TN removal efficiency when C:N increased to 5:1.

3.1.3. TP removal efficiency

C variation treatment (C1NP, C2NP, and C4NP) differences were significant for TP removal efficiency in the case of C4NP compared to other treatments (Table 2). Multiple comparisons detected significantly lower TP removal efficiency in the C4NP treatment over the study period. For the N variation treatments (CN1P, CN2P, and CN4P), there were no significant differences in the TP removal efficiency between the CN1P and CN2P treatments ($P > 0.05$), but the CN4P treatment had a significantly lower TP removal efficiency. The most efficient performance was at C:N = 5:1.

3.1.4. TOC removal efficiency

All three C variation treatments (C1NP, C2NP, and C4NP) differed significantly in TOC removal efficiency (Table 2). Multiple comparisons detected significantly lower TOC removal efficiency in all C1NP treatment comparisons over the study period. For all N variation treatments (CN1P, CN2P, and CN4P), CN4P had lower TOC removal efficiency than the CN1P and CN2P treatments. The VSSF CWs performed most efficiently for TOC removal at C:N = 10:1.

3.1.5. BOD_5 removal efficiency

For C variation treatments (C1NP, C2NP, and C4NP), C1NP differed significantly from the other two treatments (Table 2). Multiple comparisons detected significantly lower BOD_5 removal efficiency in the C1NP treatment over the study period. For the N variation treatments (CN1P, CN2P, and CN4P) there were no significant differences in the BOD_5 removal efficiency between the CN1P and CN2P treatments, but the CN4P treatment had a significantly lower BOD_5 removal efficiency. The VSSF CWs performed most efficiently in BOD_5 removal at C:N = 10:1.

Generally speaking, when the carbon source was low (C:N = 2.5:1), or the nitrogen source was insufficient (C:N = 10:1) there was lower purification ability. Therefore supplementation of the carbon or nitrogen sources, and control of the C/N ratio in the influent may be important for optimizing biological nutrient removal (BNR). The VSSF CWs system in this research achieved the highest BNR efficiency between C/N ratios of 5:1 and 10:1.

Table 3
Mean flux rates ± SD for GHG emission measured in situ under different C/N ratios.

Flux rate ($mg\ m^{-2}\ h^{-1}$)	C/N treatment					
	C1NP (C:N = 2.5:1)	C2NP (C:N = 5:1)	C4NP (C:N = 10:1)	CN1P (C:N = 10:1)	CN2P (C:N = 5:1)	CN4P (C:N = 2.5:1)
CO_2	283.57 ± 2.48	419.19 ± 2.37	457.34 ± 3.16	466.97 ± 3.85	419.19 ± 2.37	396.59 ± 1.38
CH_4	1.36 ± 0.09	2.02 ± 0.07	2.34 ± 0.15	2.86 ± 0.17	2.02 ± 0.07	1.97 ± 0.11

Table 4
Mean ± SD of physico-chemical properties of influents and effluents. Values with different superscript letters in the same column are significantly different at $P = 0.05$ according to Duncan's multiple range test.

C/N treatment	Influent			Effluent		
	pH	E_H (mV)	T (°C)	pH	E_H (mV)	T (°C)
C1NP (C:N = 2.5:1)	7.45^a ± 0.28	79.67^a ± 5.11	26.5 ± 10.5	6.39^a ± 0.56	36.19^a ± 9.58	26.8 ± 10.5
C2NP (C:N = 5:1)	7.41^a ± 0.13	65.84^a ± 6.15	26.5 ± 10.5	6.43^a ± 0.32	40.09^a ± 10.13	26.8 ± 10.5
C4NP (C:N = 10:1)	7.51^a ± 0.25	55.57^b ± 3.07	26.5 ± 10.5	6.61^a ± 0.21	31.15^a ± 13.23	26.8 ± 10.5
CN1P (C:N = 10:1)	7.72^a ± 0.32	82.43^a ± 14.98	26.5 ± 10.5	6.56^a ± 0.33	45.54^a ± 15.29	26.8 ± 10.5
CN2P (C:N = 5:1)	7.41^a ± 0.13	65.84^a ± 6.15	26.5 ± 10.5	6.43^a ± 0.32	40.09^a ± 10.13	26.8 ± 10.5
CN4P (C:N = 2.5:1)	7.61^a ± 0.11	71.86^a ± 6.47	26.5 ± 10.5	6.31^a ± 0.45	43.73^a ± 9.98	26.8 ± 10.5

3.2. In situ GHG emission

Table 3 shows the changes in CO_2 and CH_4 emissions measured in situ in different C/N treatments. For C variation treatments (C1NP, C2NP, and C4NP), the increase in CO_2 gas flux from 283.57 ± 2.48 (C1NP) to 457.34 ± 3.16 $mg\ m^{-2}\ h^{-1}$ (C4NP) was linked with the rise in C loading. The gas flux varied markedly among treatments, and the C4NP treatment achieved the highest CO_2 gas flux. For the N variation treatments (CN1P, CN2P, and CN4P), CO_2 gas fluxes did not differ much, but there was a decreasing trend in CO_2 gas flux from 466.97 ± 3.85 (CN1P) to 396.59 ± 1.38 $mg\ m^{-2}\ h^{-1}$ (CN4P) as the C loading decreased (Table 3). The CN1P treatment achieved the highest CO_2 gas flux. The CH_4 gas flux was much lower than the predicted approximately 5% of the CO_2 gas flux level in each C/N treatment (Table 3). Consequently, the CH_4 emission was negligible. The VSSF CWs in this study produced relatively lower GHG fluxes at C/N ratios between 2.5:1 and 5:1 according to the values measured in situ.

3.3. GHG emission

3.3.1. Effects of influent C/N ratios on physico-chemical characteristics

Water temperatures, pH, and redox potentials (E_H) are given in Table 4. The pH values were about 1 unit higher in influents than in effluents, but were not significantly different across C and N treatments. For C variation treatments of influent (C1NP, C2NP, and C4NP), the only significant difference in E_H was between C4NP and the other treatments (Table 4). In multiple comparisons this significantly lower E_H in the C4NP treatment continued over the entire study period. The average E_H of the effluent for both C and N variation treatments was not significantly different ($P > 0.05$). However, the lack of anaerobic reactions, which are prerequisite for methanogenesis, was in agreement with the E_H measurements, that far exceeded the value of $E_H = -100$ mV [22]. The results indicated that all E_H estimates in this VSSF CWs study were higher than −100 mV (Table 4) and therefore no CH_4 emissions occurred under the aerobic conditions; in theory $C_{emission\text{-}CH_4} = 0$ in this CMB model.

3.3.2. CMB model predictions for GHG emission

The effects of C/N variation on GHG emission are shown in Table 5. For the C variation treatments, the C4NP treatments (717.81 ± 3.16 $mg\ m^{-2}\ h^{-1}$) showed significantly higher CO_2 gas fluxes than the C1NP (286.54 ± 1.12 $mg\ m^{-2}\ h^{-1}$) and C2NP (446.48 ± 2.73 $mg\ m^{-2}\ h^{-1}$) treatments. In summary, the VSSF CWs in this research exhibited relatively low GHG fluxes C/N ratios at between 2.5:1 and 5:1 in the C variation treatments according to the CMB model. For the N variation treatments, only tiny differences for CO_2 gas fluxes were detected among all N variation treatments during the study period. The CN1P and CN4P treatments had the highest (457.59 ± 2.19 $mg\ m^{-2}\ h^{-1}$) and the lowest (381.79 ± 3.01 $mg\ m^{-2}\ h^{-1}$) CO_2 gas fluxes, respectively. However, the predicted values of CH_4 gas fluxes for all C/N variation treatments were negligible (Table 5) because the E_H values higher than −100 mV (Table 4) and indicative of an absence of anaerobic conditions for methanogenesis.

4. Discussion

4.1. Biological nutrient removal

VSSF CWs are complex bioreactors in which the removal of pollutants occur by means of a variety of physical, chemical, and

Table 5
Estimated mean flux rates ± SD for GHG emission at different C/N ratios by the CMB model.

C/N treatment	CMB model parameter ($mg\ m^{-2}\ h^{-1}$)					
	C_{inflow}	$C_{outflow}$	$C_{photosynthesis}$	$C_{storage}$	$C_{emission\text{-}CO_2}$	$C_{emission\text{-}CH4}$
C1NP (C:N = 2.5:1)	269.27 ± 1.02	81.97 ± 1.54	134.25	35.01 ± 1.02	286.54 ± 1.12	0
C2NP (C:N = 5:1)	471.71 ± 2.98	98.16 ± 2.79	134.25	61.32 ± 3.11	446.48 ± 2.73	0
C4NP (C:N = 10:1)	863.76 ± 4.82	167.91 ± 1.02	134.25	112.29 ± 4.82	717.81 ± 3.16	0
CN1P (C:N = 10:1)	469.41 ± 2.71	85.05 ± 1.52	134.25	61.02 ± 2.71	457.59 ± 2.19	0
CN2P (C:N = 5:1)	471.71 ± 2.98	98.16 ± 2.79	134.25	61.32 ± 3.11	446.48 ± 2.73	0
CN4P (C:N = 2.5:1)	467.71 ± 3.94	159.37 ± 1.62	134.25	60.80 ± 3.94	381.79 ± 3.01	0

biochemical processes [1]. The amount of C removed represented by CO_2 emissions was estimated from the BOD_5 data in Table 2. For these calculations, it was assumed that all organic matter for removal was in the form of easily biodegradable substrates, such as glucose. The higher C/N ratio (5:1 or 10:1) treatments were more efficient than lower ones (i.e., C/N = 2.5:1). This was probably because the organic matter could readily be removed aerobically rather than by anaerobic pathways [5]. Adequate supplementation of carbon or nitrogen level, and control of C/N ratios in influents, may be very important for efficient BNR. The VSSF CWs system in this research achieved the highest BNR efficiency between C/N ratios of 5:1 and 10:1.

4.2. GHG gas fluxes

4.2.1. CO_2 gas flux

For the C variation treatments, only the C1NP and C2NP CO_2 gas fluxes measured in situ were in agreement with the values predicted by the CMB model (Tables 3 and 5). The C4NP CO_2 gas flux predicted by the CMB model ($717.81 \pm 3.16\,mg\,m^{-2}\,h^{-1}$) was approximately twice the measured value ($457.34 \pm 3.16\,mg\,m^{-2}\,h^{-1}$). The difference between in situ measured and predicted values may be due to errors in gas measurement linked to problems with the syringes as noted by Garcia et al. [22]. Furthermore, the CMB model utilized to predict the GHG flux was a simplification of the C cycle model, and the parameters for the wastewater had their own shortcomings [21]. The disadvantage of this simplified C cycle also stems from the fact that part of the CO_2 generated may be retained in liquid phase if the influent pH increases under different C/N ratios. This could result in values measured in situ being lower than the actual emission fluxes [37]. On the other hand, decreases in pH lead to larger releases of CO_2 from liquid phase, thereby causing an overestimation of generation rates. Although the mean pH of influents and effluents for each treatment maintained stable values (Table 4), variation during the experimental period could have contributed to variation in CO_2 gas fluxes.

In the present study, we were unable to obtain precise calculations of the CMB model because the parameters used for model building have some limitations. We assumed that $C_{photosynthesis}$ was the same for different C/N variation treatments, and likely ignored influence of the photosynthesis arising from the different inflow treatments [12,27]. The further research about more adequate parameters is needed in our future experiment. We also believe that plant exudates are an important source of C in VSSF CWs not heavily loaded with wastewater. Thus C coming from exudates is further transformed to gaseous forms, adding to the C emissions [38].

For N variation treatments, the CO_2 gas flux measured in situ agreed with the predictions of the CMB model. Although the influent TN content was significantly higher in the CN4P treatment than the CN1P treatment (Table 1), no significant disparity in the predicted CO_2 gas fluxes was found (Table 5). However, $C_{outflow}$ was higher in the CN4P treatment than the CN1P treatment. This difference is hypothesized to be due to the effect of influent TN content on the BOD_5 removal capacity of plants. Thus, while the increasing TN content in the influent may not directly affect the CO_2 gas fluxes, it may increase the BNR capacity.

4.2.2. CH_4 gas flux

Tables 3 and 5 show that the CH_4 gas flux measured in situ, at less than approximately 5% of the CO_2 gas flux in all treatments, agreed with the values predicted by the CMB model. The model indicated that no CH_4 emissions should occur because all E_H estimates were higher than -100 mV (Table 4). This conclusion agrees with literature reports. Søvik et al. [39] suggested that VSSF CWs treated with municipal domestic wastewater can be considered aerobic systems in which methanogenesis should not occur. Furthermore, the results obtained in several reports also indicated that aerobic respiration is one of the most important reactions occurring in different locations of VSSF CWs [40–42].

Although the CMB model cannot be measured directly or estimated precisely, it can be utilized to determine how GHG fluxes respond to variations in C/N ratios. It also helps to improve our understanding of the performance of, and CO_2 emissions from, VSSF CWs, and to improve their operation. The GHG fluxes measured in situ agreed with CMB model predictions provided C/N ratios were not too high (5:1 or 2.5:1). The VSSF CWs in this study had relatively low GHG fluxes at C/N ratios of 2.5:1 and 5:1.

4.3. Optimum C/N ratios

We believe that this research provides evidence and proof to show the importance of influent C/N ratios in the GHG flux of VSSF CWs. It was concluded that the optimum C/N ratio for simultaneously achieving the best BNR efficiency and lowest GHG flux is around C:N = 5:1.

5. Conclusions

The importance of different C/N ratios in influents leading to GHG emission in VSSF CWs has not been studied adequately. In the course of this study, GHG fluxes were not only directly measured in situ but also indirectly estimated by the CMB model. Whereas this can be done very easily, the results are only relative because of the underlying assumptions. Despite the deficiencies, it was quite clear that variation in organic loadings (influent C/N ratios) dramatically influence the GHG fluxes of the system. Appropriate control of the carbon or nitrogen source concentrations and C/N ratios in the influent can achieve optimal conditions for nutrient removal with the lower rates of GHG emissions.

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