Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.22822

127 (2018) 297–308
September

Effect of plant harvesting on greenhouse gas emission from vertical subsurface flow constructed wetlands treating low-strength sewage

Siripoon Nutanong[a], Chart Chiemchaisri[b,*], Wilai Chiemchaisri[b], Suwasa Kantawanichkul[c]

[a]*National Center of Excellence for Environmental and Hazardous Waste Management and Postgraduate Programs in Environmental Management, Chulalongkorn University, Bangkok 10330, Thailand, email: siripoonss@gmail.com*
[b]*Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand, emails: fengccc@ku.ac.th (C. Chiemchaisri), fengwlc@ku.ac.th (W. Chiemchaisri)*
[c]*Department of Environmental Engineering, Faculty of Engineering, Chiangmai University, Chiangmai 50200, Thailand, email: suwasa@eng.cmu.ac.th*

Received 15 March 2018; Accepted 22 July 2018

ABSTRACT

The effect of plant harvesting patterns on CH_4 and N_2O fluxes from vertical flow constructed wetlands was investigated. Four identical constructed wetland units planted with *Cyperus alternifolius* were operated continuously for a period of 8 months during which plant harvesting periods were varied between 2, 4 and 8 months intervals. During the operation, CH_4 and N_2O fluxes were ranged between 1.73 and 3.63 mg C/m^2 h and between 0.049 and 0.157 mg N/m^2 H, respectively. Lowest CH_4 fluxes were detected in the unit without harvesting during 8 months period whereas N_2O fluxes were found lowest in the most frequent harvesting pattern (every 2 months). Analyses of dissolved oxygen and microbial community revealed different level of oxygen availability and greenhouse gas producing microbial population in the root zone between the treatment units.

Keywords: Constructed wetlands; *Cyperus alternifolius*; Domestic sewage; Greenhouse gas emission; Plant harvesting

1. Introduction

Constructed wetland (CW) for wastewater treatment is becoming a popular alternative to conventional wastewater treatment systems. Studies show that CWs can sufficiently remove common contaminants in wastewater with comparable performance to other technologies [1–3]. Moreover, produced biomass from CWs can be harvested and used to produce fodder and fuel [4]. In CW systems, pollutants in wastewater are removed by various mechanisms, predominantly by microbiological processes which produce substantial amount of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), the three major greenhouse gases (GHGs). The rate at which the GHGs are produced is influenced by several factors including water and soil temperature, influent characteristics, hydrological regime and plant conditions.

Plant is one of the most important components in CW systems. The presence of plants and their conditions impact the diversity of microbial species within the system which were found to be the main factors affecting carbon and nitrogen removal efficiencies [5]. Consequently, the rate of greenhouse gas emission from the system. Plant functions include regulating microclimate condition, uptake and storage of nutrient, provide attachable surface for microorganism, and provide additional oxygen to the system via plant aerenchyma [6,7]. In turn, the aerenchyma system can also provide a bypass route for methane (CH_4) and nitrous oxide (N_2O) efflux from the systems [6]. Previous study also reported the effect of plant species on CH_4 emission from CW [8]. In low loaded systems, nitrogen uptake by plants can significantly reduce nitrogen availability, which affects nitrification and denitrification reactions, and thus influencing

* Corresponding author.

nitrous oxide production. Therefore, management of plant in CWs can have a significant impact on GHG emissions from CW systems.

There has been a number of studies which focus on GHG emission from free water surface flow and horizontal subsurface flow (HSSF) systems, yet relatively fewer studies are focusing on vertical flow constructed wetland (VF CW) system despite the fact that VF CWs are more popularly applied in many countries [9]. CWs can treat wastewater more efficiently in warm/tropical climate than in colder climate [10,11]. However, fewer studies have been done on investigation of GHG emission from CWs in tropical climate [9]. Moreover, the effect of plant harvesting during warm climate on the treatment performance and microbial abundance is still unclear [12]. Therefore, this study aimed to investigate effect of plant harvesting patterns on GHG emission and related microbial population of VF CW systems located in tropical climate.

2. Material and method

2.1. Experimental setup

Four identical experimental scale VF CW systems with varied plant harvest conditions were setup and operated simultaneously for the period of 8 months on campus at Chiang Mai University, Thailand (latitude 18°47′ 25.37″ N, longitude: 98°59′ 4.85″ E). The units were placed outdoor and received equal rainfall and sunlight throughout the experiment. The experiment started in January which was a dry/cold period with average daily rainfall and temperature of 0 mm and 21.3°C, respectively. The experiment ended in September when the average daily rainfall and temperature were 5.9 mm and 27.6°C, respectively.

Each VF CW unit consisted of a 0.3 × 0.3 m^2 surface area and 1 m height rectangular tank made of clear acrylic sheets (Fig. 1). All four sides of each tank are covered by black plastic sheets to eliminate the effect of photosynthesis. Each tank was filled with fine sand (0.125–0.25 mm) as the main substrate media and approximately 10 cm thick layers of small gravels (2–5 cm) on the top and the bottom of the tank. Perforated pipes were inserted at 10, 50 and 98 cm depth in each system to measure the changes of carbon, nitrogen and dissolved oxygen (DO) along the vertical profiles. The pipes were tightly sealed at all time except during sampling to ensure that oxygen exchange with the external environment did not occur via the pipes. One of the units was unplanted, whereas the other three units were planted with *Cyperus alternifolius* (umbrella sedge) with harvesting intervals of 2, 4 and 8 months (no harvest during 8-month experimental period), respectively. *C. alternifolius* is a local macrophyte that can be found typically near water and possesses aerenchyma, which allows gases to be transported to and from root zones which allow them to thrive both in saturated and unsaturated soil conditions.

The influent was domestic wastewater obtained from university campus. The influent was obtained every 2 d and stored in a 200 L storage tank. The VF CWs received the influent from the storage tank through a network of perforated pipes spread across the surface with hydraulic loading rate of 10 cm/d. The influent was fed intermittently for 25 s every 4 h at 0.06 L/s, thus the total of 9 L/d. Peristaltic pumps with a timer switch were used to control the inflow rates and the feeding time. The influent was flooded across the entire surface of each unit and seeped vertically slowly through the fine sand media.

2.2. Plant harvesting procedure and analysis

Three of the four experiment units were planted with young *C. alternifolius* with shoot length of approximately 15–20 cm for 2 months prior to this study. During this time, the systems were fed with sewage and dying clumps (damaged root) were removed and replanted. At the start of the experimental period, the average height of the plants in all the three planted units was approximately 50 cm with 20 stems available in each unit. The changes in plant height and density were recorded every 2 weeks during the experimental period. Above ground dry weight for harvested parts was determined after plant harvesting. For each harvest, the stems of the plants were cut to approximately 10 cm above the substrate surface. Harvest was done between 10 and

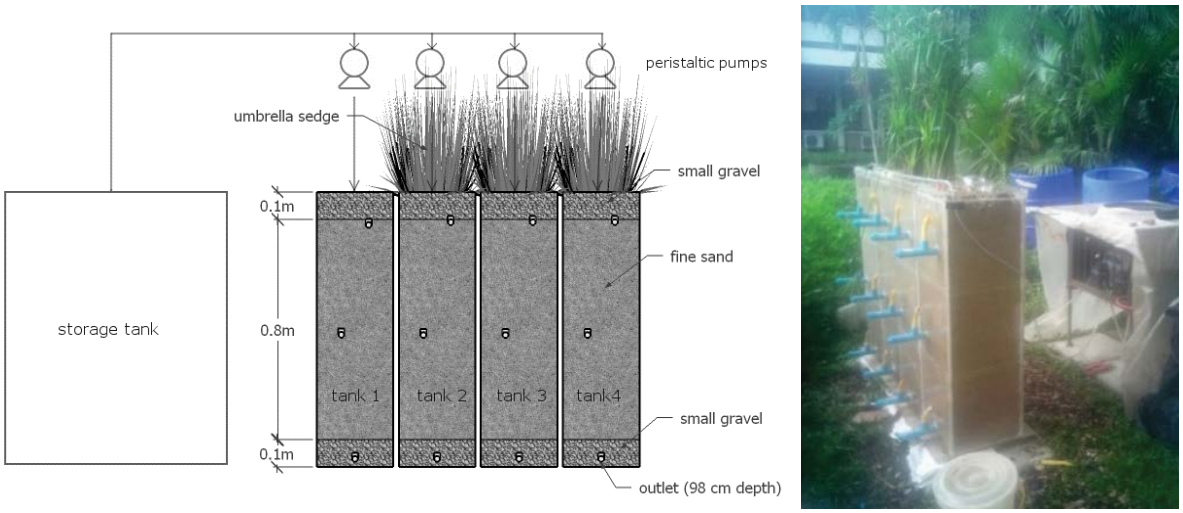

Fig. 1. Experimental setup.

12 am before water and gas sampling. Harvested plant parts were divided into stems and leaves before being oven-dried at 90°C for 2 d. The samples were then grinded and measured for the dry weight. Carbon and nitrogen content at the beginning and at the end of the experiment were determined with Walkley–Black and Macro Kjeldahl methods, respectively.

2.3. Wastewater sampling and analysis

Influent samples were collected at the influent storage tank. The effluents were collected at the 98 cm depth of the VF CWs. Influent and effluent were analysed every 2 weeks throughout the 8-month experimental period with the total of 17 samples. Water samples were collected at various times in the day via the inserted pipes and stored in a 4°C and analysed within 24 h. DO, nitrate (NO_3) and total organic carbon (TOC) were measured at 10 and 50 cm depths. TOC was analysed by a Shimadzu TOC analyser. Total nitrogen (TN) was the sum of total Kjeldahl nitrogen (TKN), nitrite (NO_2-N) and nitrate (NO_3-N). Ammonia nitrogen (NH_3-N), TKN, NO_2-N and NO_3-N were analysed using the Standard Methods for the Examination of Water and Wastewater [13]. DO was measured with a Clean Instrument® DO200 DO meter. Oxidation reduction potential (ORP) was measured with a Clean Instrument® ORP 30 tester.

In this study, percent pollutant mass removal is used to represent treatment performance of the system. This mass removal takes into consideration the differences in water loss through evapotranspiration among the experiment units [14].

$$\%\text{Removal} = 100 \times \frac{Q_i C_i - Q_o C_o}{Q_i C_i} \tag{1}$$

where Q_i is the inflow rate (L/d), Q_o is the outflow rate (L/d) averaged from the effluent daily volumes, C_i is the influent concentration (mg/L), and C_o is the effluent concentration (mg/L).

2.4. Gas sampling and analysis

Gas fluxes emitting from the experimental units were measured between 9 and 11 am once every 2 weeks. Static nonflow-through flux chamber method, which is one of the most used method for measuring soil methane and nitrous oxide fluxes was used to collect gas samples. The chamber was an air tight box made of clear acrylic sheets which is inert to N_2O and sealed with nonreactive sealant with the dimensions of 0.3 × 0.3 × 1.2 m. Gas samplings were performed immediately after flux chamber placement with no gas pressure gradient observed during 30 min sampling period. Air samples were collected from inside the chamber via rubber septa on the top of the chamber. A fan was mounted inside the chamber to ensure even mix of gas within the chamber. A thermometer is also mounted inside the chamber to record temperature inside the chamber during sampling.

Gas chromatography was used to determine CH_4 and N_2O fluxes. Each flux was calculated from four gas samples which were collected at 10-min interval over 30 min. Each sample was contained in a vacuum blood collection tube (BD Vacutainer®) and was analysed by PerkinElmer Clarus 580 GC with Hayesep D column equipped with an autosampler and flame ionization detector and electron capture detector for the detection of CH_4 and N_2O, respectively. The concentration gradients of gases were then plotted against time of sampling (0th, 10th, 20th and 30th min) to get the flux in the unit of mg/m^2 h.

$$F = \left(\frac{V}{A}\right) \times \left(\frac{dC}{dt}\right) \tag{2}$$

where F is flux in mg/m^2 h, C is gas concentration in mg/m^3, V is chamber volume (m^3), A is area enclosed by chamber (m^2) and dC/dt is the change of gas concentration in mg/m^3 over 1 h.

2.5. Microbial community analysis

Sand media and plant root samples were taken from the experimental units at the end of the experiment. Composite samples were prepared from samples collected at different depths, that is, 10, 50 and 98 cm using polyvinyl chloride pipes and stored in polypropylene capped tubes. The samples were analysed for nitrifying, denitrifying and methanogenic bacteria species by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) method.

Genomic DNAs from bacteria are extracted from sand sample following the method by Zhou et al. [15]. The bacterial 16S rRNA genes were amplified by PCR with the primer EUB8F/U1492R in the first round and the specific primer set 338GC-F/518R in the second round. The archaeal 16S rRNA genes were amplified by PCR with the primer A20F/U1492R in the first round and the specific primer set 344GC-F/522R in the second round as described in Khemkhao et al. [16]. Amplification and electrophoresis procedures were the same as described in Boonnorat et al. [17].

Dice index of similarity (Cs) is used to determine the similarities between DGGE fingerprints from each system. Dice index of similarity or Cs = $2j/(a + b)$ where j is the number of bands that are present in both samples A and B, and a and b are the number of bands in sample A and sample B, respectively [18].

Shannon–Weaver's index (H) describing species richness and Simpson's index (D) describing evenness and dominance of microbial species [19] were determined by the following equations:

$$H = -\sum\left[\frac{n_i}{\sum n_i} \times \log_2\left(\frac{n_i}{\sum n_i}\right)\right] \tag{3}$$

$$D = \sum\left(\frac{n_i}{\sum n_i}\right)^2 \tag{4}$$

where n_i is the intensity of the band for each microbial on the DGGE fingerprints. H and 1-D indices were used to determine degree of microbial biodiversity. The DGGE fingerprint images were processed using ImageJ 1.52b (http://rsb.info.nih.gov\ij).

2.6. Statistical analysis

All sample analysis was conducted in triplicate and their average values among the measurements were presented.

SPSS 11.0 (SPSS Inc., Chicago, USA) was used to calculate Pearson's correlation between greenhouse gas fluxes and TOC, TN, organic carbon and nitrate concentrations in the systems as well as other influencing factors such as DO concentration, ORP, water loss and humidity. Differences in emissions and removal efficiencies between unplanted unit and planted units, and between different harvesting interval units were calculated using single factor analysis of variance (ANOVA) ($p < 0.05$) followed by Tukey honestly significant difference post hoc test for any one-way ANOVA results showing at least one pair of significant difference.

3. Results and discussion

3.1. Treatment performance of VF CWs

TOC (Fig. 2) and DO (Fig. 3) concentrations along the depth in VF CWs are monitored. DO in the wastewater increased rapidly from the average influent concentration of 1.4 to 5.04–5.25 mg/L at 10 cm depth. At this depth, the DO concentrations were similar between the planted and unplanted units. However, at 50 cm depth, DO concentration decreased rapidly in the unplanted unit whereas only slight decrease was found in the planted units. The 4- and 8-month harvesting interval units showed higher DO concentrations at the deeper part of units which may be the result of deeper root systems observed in these two units. High organic removal efficiencies in terms of TOC were observed in all VF CW units (89%–94%). Nevertheless, slightly higher removals were observed in the unplanted unit. In the unit with plant harvesting practice, slightly higher organic concentrations in the effluent and reduction in organic removal efficiencies were observed after plant harvesting.

The results suggest that TOC removal efficiencies in the systems in this study were not benefited by plants. Approximately 80% of the feeding organic substances were removed at the shallow zone (0–50 cm depth) in CW media of both planted and unplanted units. According to Kadlec and Wallace [14] aerobic degradation occurs rapidly with sufficient oxygen being supplied during the nonfeeding period of an intermittent loading cycle. In VF CWs, the process commonly occurs at the top 20 cm below the surface [14,20].

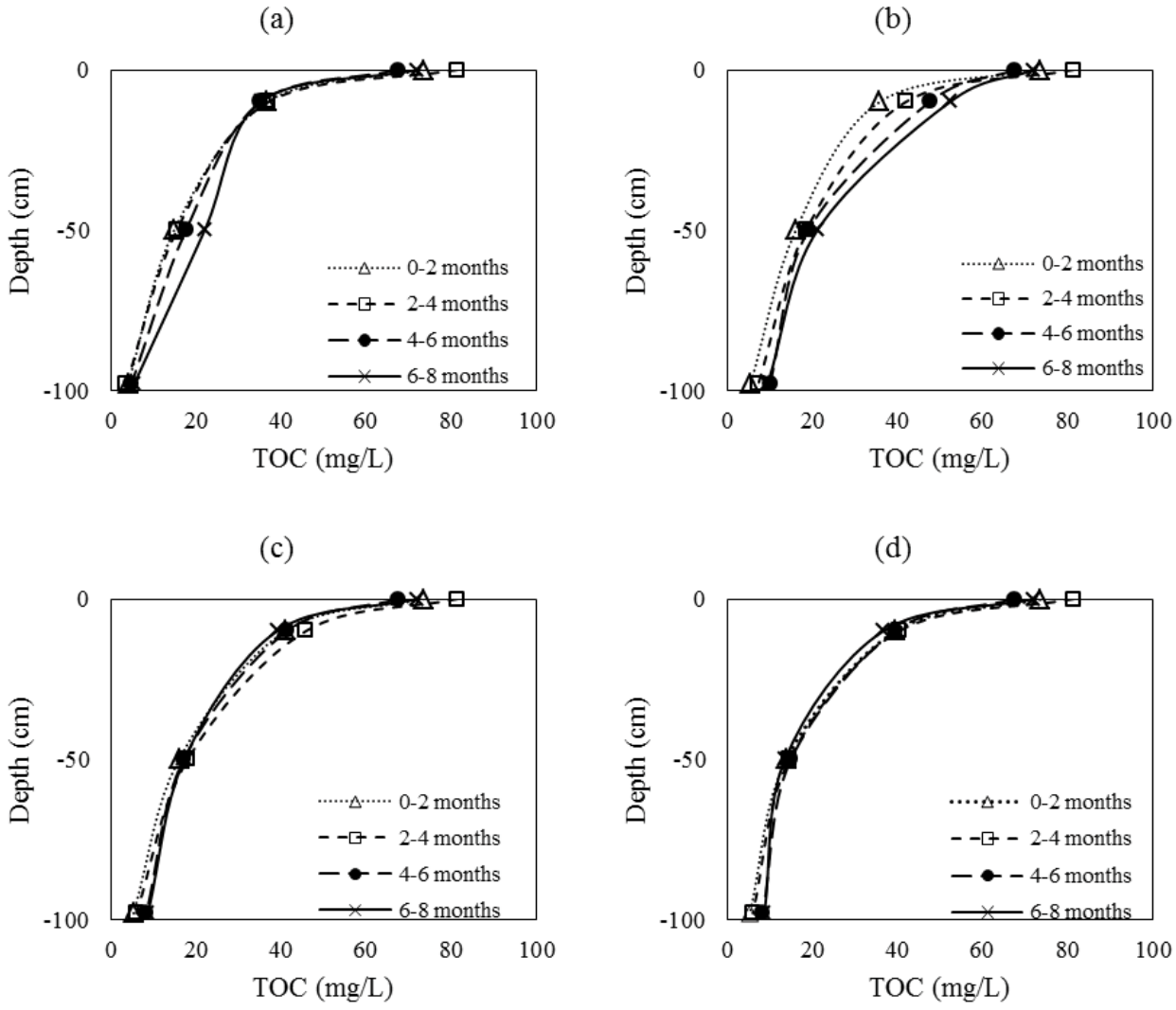

Fig. 2. TOC concentrations at different depth in (a) unplanted, (b) 2-month harvest, (c) 4-month harvest, and (d) no-harvest units.

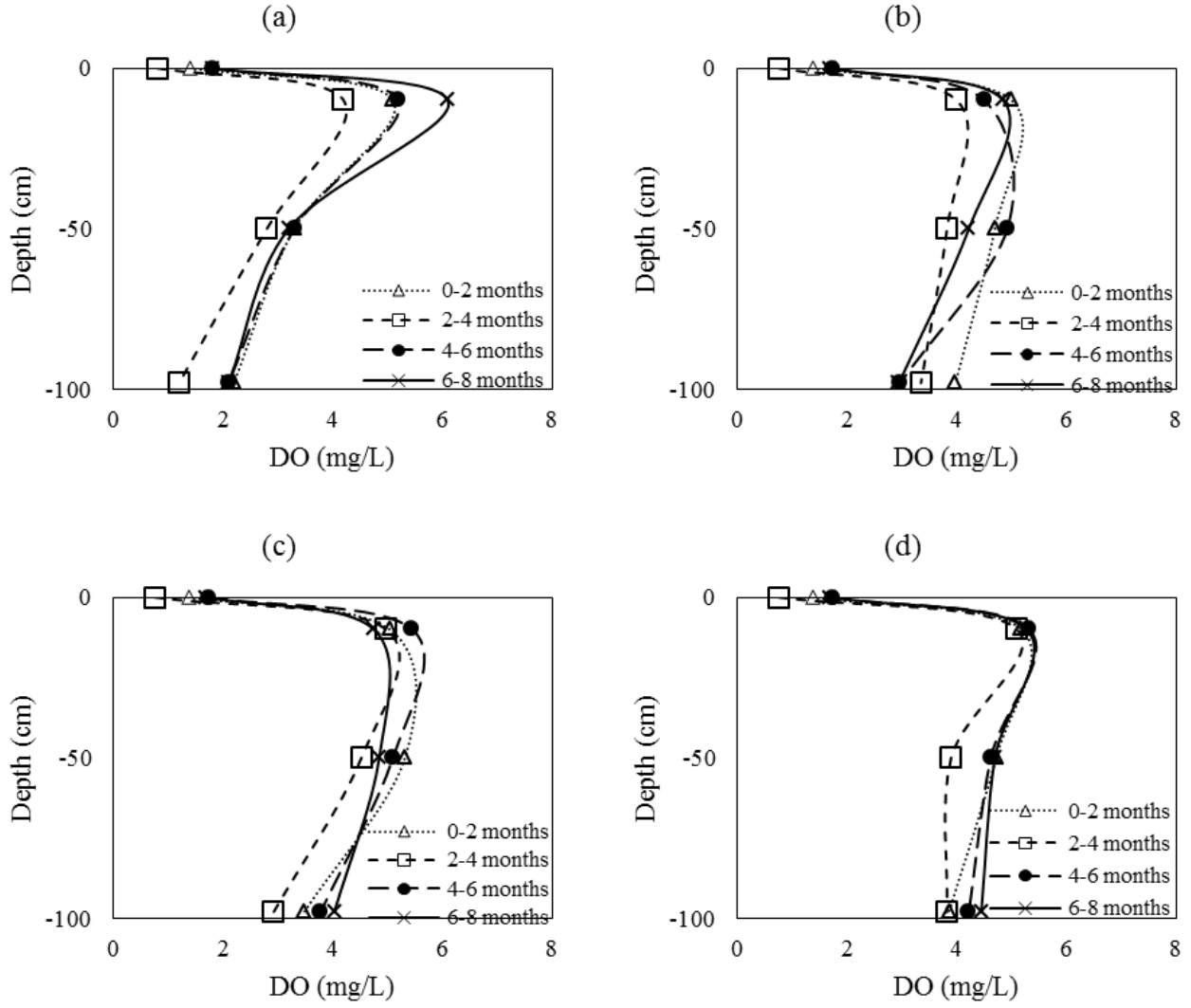

Fig. 3. DO concentrations at different depth in (a) unplanted, (b) 2-month harvest, (c) 4-month harvest, and (d) no-harvest units.

This suggests that there may already be enough oxygen at the shallow part in the units from intermittent loading to carry out most of the aerobic degradation of organic carbon without help from plant oxygen release, which results in similar TOC removal efficiencies between the planted and the unplanted units.

Plant harvest, however, may affect organic removal which is evidenced by the higher reduction of TOC removal efficiencies in the plant harvested units in comparison with the unplanted and the no-harvest units. Even though TOC removal efficiencies by all the units decreased after 4 months of operation (after day 121), larger decrease was evidenced in the two plant harvested units (Table 1). This may be because plant growth was interfered during harvest and the ability of plant aerenchyma to transfer oxygen to the root zone may be compromised [12] which may affect in higher underground dead plant parts as demonstrated by the less dense stems and shallower root zone. The dead plant parts may contribute to additional carbon in the effluent which can be significant in low loading system [21]. Therefore, the results suggest that the presence of plant and plant harvesting did not affect the removal of organic matter by the VF CWs, however, the most frequent harvesting unit showed lower TOC removal efficiencies than other units, which may be due to the additional organic carbon from dead plant biomass.

The VF CWs in this study showed high TKN removal efficiencies whereas NO_3^- concentrations in the effluent from all units were found to be much higher than the influent concentration. The main processes which involve transformation of nitrogen species in CWs are nitrification where ammonia is transformed to NO_3^- in aerobic environment and denitrification where NO_3^- is further reduced to N_2 and N_2O in anoxic environment. In VF CWs, where aerobic condition prevails, nitrification is commonly occurred at much higher rate than denitrification, thus, leaving nitrogen in the form of NO_3^-. This is consistent with results found by Dong et al. [22] where high reductions of NH_3 and accumulations of NO_3^- were detected from different types of CWs. The same study also found that almost complete nitrification was achieved by

Table 1
Water quality parameters

	Influent		Unplanted		2-Month harvest		4-Month harvest		8-Month (or no) harvest	
	Range	Avg ± SD	Avg ± SD	% Removal	Avg ± SD	% Removal	Avg ± SD	% Removal	Avg ± SD	% Removal
Temp (°C)	26.5–29.5	27.9 ± 1.0	26.2 ± 0.7	–	25.8 ± 0.5	–	25.7 ± 0.5	–	25.5 ± 0.4	–
Day 1–120	26.5–29.5	28.2 ± 1.2	26.3 ± 0.9	–	25.7 ± 0.7	–	25.5 ± 0.6	–	25.6 ± 0.6	–
Day 121–243	26.5–28.5	27.6 ± 0.6	26.2 ± 0.4	–	25.9 ± 0.4	–	25.8 ± 0.4	–	25.4 ± 0.3	–
DO (mg/L)	0.5–2.0	1.4 ± 0.5	2.0 ± 0.4	–	3.4 ± 0.6	–	3.6 ± 0.5	–	4.1 ± 0.4	–
Day 1–120	0.5–1.9	1.1 ± 0.5	1.9 ± 0.5	–	3.7 ± 0.4	–	3.6 ± 0.5	–	4.1 ± 0.6	–
Day 121–243	0.9–2.0	1.6 ± 0.4	2.1 ± 0.2	–	3.1 ± 0.1	–	3.8 ± 0.4	–	4.0 ± 0.2	
pH	6.9–7.8	7.3 ± 0.2	6.8 ± 0.3	–	6.7 ± 0.3	–	6.8 ± 0.3	–	6.8 ± 0.3	–
Day 1–120	6.9–7.8	7.3 ± 0.3	6.9 ± 0.3	–	6.7 ± 0.3	–	6.8 ± 0.2	–	6.9 ± 0.3	–
Day 121–243	6.9–7.4	7.2 ± 0.2	6.7 ± 0.2	–	6.7 ± 0.3	–	6.7 ± 0.3	–	6.8 ± 0.3	–
TOC (mg/L)	62.7–89.9	74.5 ± 8.8	4.4 ± 1.3	94	8.2 ± 3.2	89	7.0 ± 2.3	91	5.8 ± 1.3	92
Day 1–120	68.8–89.9	77.3 ± 7.5	3.7 ± 0.5	95	6.3 ± 2.3	93	5.6 ± 0.8	94	5.4 ± 0.7	94
Day 121–243	62.7–85.3	69.9 ± 4.1	5.1 ± 0.9	93	9.6 ± 1.0	88	8.5 ± 0.7	89	6.2 ± 0.7	91
NH_3 (mg/L)	21.7–32.5	27.6 ± 3.3	4.1 ± 0.7	88	2.7 ± 0.7	92	2.5 ± 0.7	93	2.6 ± 0.8	92
Day 1–120	24.7–32.5	29.2 ± 2.7	3.9 ± 0.8	89	2.5 ± 0.9	93	2.6 ± 0.9	93	2.8 ± 1.1	92
Day 121–243	21.7–31.0	27.1 ± 2.7	4.3 ± 0.7	86	2.9 ± 0.4	91	2.4 ± 0.4	93	2.5 ± 0.3	92
TKN (mg/L)	33.3–47.8	39.6 ± 4.1	6.3 ± 1.3	87	3.8 ± 0.7	92	3.5 ± 0.6	93	3.7 ± 0.9	92
Day 1–120	36.6–47.8	42.5 ± 3.5	6.0 ± 1.5	88	3.4 ± 0.7	93	3.8 ± 0.6	93	4.1 ± 1.1	92
Day 121–243	33.3–40.8	36.8 ± 2.2	6.7 ± 1.0	84	4.2 ± 0.5	90	3.3 ± 0.6	93	3.1 ± 0.3	93
NO_3^- (mg/L)	0.03–0.08	0.05 ± 0.02	6.0 ± 0.8	–	5.8 ± 1.3	–	3.3 ± 0.4	–	4.4 ± 1.2	–
Day 1–120	0.04–0.08	0.06 ± 0.02	5.8 ± 1.0	–	5.9 ± 1.5	–	3.2 ± 0.5	–	4.8 ± 1.7	–
Day 121–243	0.03–0.05	0.04 ± 0.01	6.2 ± 0.8	–	5.8 ± 1.1	–	3.3 ± 0.2	–	4.0 ± 0.4	–

Remark: NO_2^- was not detected; No. of samples = 17, No. of samples for day 1–120 = 8, No. of samples for day 121–243 = 9.

the system, which includes VF-CW and HSSF-CW receiving influent with high pH value.

The effects of plants and plant harvesting on nitrogen removal efficiencies in the CWs were clear. TKN removal was much higher in the planted units than the unplanted unit. Nitrification, the main process which removes ammonia, the product from ammonification of TKN in CWs, occurs most intensively around the rhizosphere as root oxygen release can be used for nitrification process in VF CWs [23]. This is also evidenced by the presence of various nitrifying bacteria species in the root samples from the planted units as plant has the direct effect on TKN removal efficiency by providing oxygen for nitrification process. Furthermore, TKN removal efficiencies in each planted unit were consistent with the overserved root depths and densities in each unit. After two harvesting (after day 121), TKN removal efficiency in the 2-month harvesting interval unit became lower than the 4-month harvesting interval unit and 8-month harvesting interval unit. According to the observation on plant growth and root depth, it can be seen that root system in the 2-month harvesting interval unit became shallower than the 4- and 8-month harvesting interval unit after day 150.

Accumulation of NO_3^- was much lower in the planted units than the unplanted unit. This suggests that denitrification also occurs in the units with well-established root systems. This can be linked to the presence of various denitrifying bacteria species and some of the ammonia oxidizing bacteria (AOB) that can carry out denitrification which were also found mostly in the 4- and 8-month harvesting interval units root samples. Nitrifier denitrification, process where TKN can be converted to N_2 and N_2O without NO_3^- formation, which can be carried out by AOB has been suggested to occur in unsaturated soil where the moisture condition is not optimal for denitrification [24], similar to the condition of this study.

pH values remain between 6.7 and 6.8 in all four systems without significant differences between each unit and depth zone is slightly lower than the influent average pH value of 7.3. The effluent flow rates were 7.6, 7.5, 7.4 and 7.4 L/d for the unplanted unit, the 2-month harvesting interval unit, the 4-month harvesting interval unit and the no harvest unit, respectively.

3.2. Plant growth and biomass

Wetland plant, also known as macrophyte, has several important roles in CW treatment mechanisms including root release of oxygen, stabilizing soil hydraulic conductivity, nutrient uptake [6]. Oxygen availability is one of the most important factor for a CW's treatment performance. Wetland plants have multiple air passages in the stems (aerenchyma) where oxygen and other gases can be exchanged between surface and subsurface areas allowing aerobic respiration of organic compounds to occur around the root zone. Macrophyte root systems are known to be able to slow down the flow of water in VF CWs and increase the contact time, which is an important factor determining the treatment capacity, between the wastewater and the microorganism [25]. Furthermore, plant in CW has also been proven to be able to uptake inorganic as well as organic compounds such as phenols [7].

C. alternifolius is a macrophyte belongs to *Cyperus* genus, which is one of the most common genus of wetland plants. According to a study by Kumwimba et al. [26], of the seven wetland plant species, *C. alternifolius* has the second highest nitrogen accumulation in the above ground biomass and much higher than the other five species. Thus, harvesting should remove substantial amount of nitrogen. The oxygen transfer rate by *Cyperus* spp. is found to be significant at high hydraulic loading rate [27]. *Cyperus* spp. has moderate productivity and can survive unpredictable environments [28], which means that it can withstand soil moisture fluctuation, thus, suitable for intermittent loading in VF CWs. In a study by Heritage et al. [29] where treatment performance of VF CWs planted with different plant species were compared, *Cyperus* spp. was found to have higher TN removal efficiency and similar BOD removal efficiency with other plant species.

In the condition of this study, *C. alternifolius* planted reached its final height of approximately 1–1.2 m from young stems of approximately 10 cm tall within 3–4 months (Fig. 4). At the end of the experiment, the 2-month harvesting

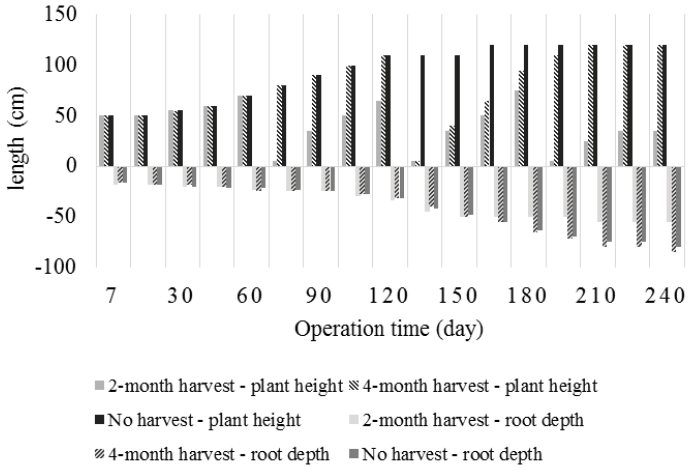

Fig. 4. Plant height and root depth in CW units along the operation.

interval unit had the least total above and below ground biomass, whereas 4-month harvesting interval has slightly higher total above ground biomass than 8-month harvesting interval unit. The initial plant density for the three systems was 222 stems/m^2. At the end of the experiment, plant density in the 2-month harvesting interval unit dropped to 144 live-stems per m^2 whereas the density for the 4-month harvesting interval unit and the 8-month harvesting interval unit increased to 844 and 789 live-stems/m^2, respectively. Increasing number of standing dead stems in the 2-month harvesting interval unit were observed after the second harvest and by the end of the experiment numbers of standing dead stems in 2-month harvesting interval unit, 4-month harvesting interval unit and 8-month harvesting interval unit were 10, 5 and 9 stems which are 43.5%, 6.2% and 11.5% of total stems, respectively.

Below ground biomass measured at the end of the experiment for the 2-month harvesting interval unit was also significantly less than the 4- and 8-month harvesting interval units. Root depth was observed at the end of the experiment and was found that the 4- and 8-month harvesting interval units root densities were high and distributed quite constantly along the vertical profile from the surface to the bottom of the units. However, the 2-month harvesting interval unit was found to have very low root density and only present near the surface. The observable root depth at the end of the experiment for 2-month harvesting interval unit, 4-month harvesting interval unit and 8-month harvesting interval unit were 55, 85 and 80 cm, respectively.

Carbon removal from the system via plant harvest was significantly higher in the 4-month harvesting interval unit than in the 2- and 8-month harvesting interval units. The total carbon content in the harvested biomass from each harvest throughout the experiment from 2-, 4- and 8-month harvesting interval units were 134.5, 177.5 and 82.13 g (2.2, 3.0 and 1.4 kg/m^2/year) respectively. It is also found that substantial amount of nitrogen was removed from the systems via plant harvest, especially by the 4-month harvesting interval unit, as nitrogen is more concentrated in plant leaves than in other parts of the plants. Previous study reported that higher nitrogen concentrations led to higher bioaccumulations of wetland plants but varied significantly between plant species [30]. Nitrogen contents in the plant leaves range between 2.05% and 2.87% of the dry weight whereas only 0.59% and 1.45% and 0.16% and 0.17% were detected in the stems and the roots, respectively. Nitrogen content in the harvested parts of 2.93, 4.86 and 2.99 g were measured from the 2-, 4- and 8-month harvesting interval units, respectively.

3.3. CH_4 and N_2O fluxes

CH_4 fluxes measured from the four units range between 1.73 and 3.63 mg CH_4-C/m^2/h (Fig. 5). The fluxes measured from the unplanted unit, the 2-, 4- and 8-month harvesting interval units were 2.88, 2.35, 1.98 and 2.08 mg C/m^2/h, respectively. The difference between the fluxes from the 4- and 8-month harvesting interval units was not statistically significant. For N_2O, the fluxes range between 0.049and 0.157 mg N_2O-N/m^2/h. The highest flux average from the beginning to the end of the experiment was measured from 4-month harvesting interval unit. The average fluxes for the unplanted unit, the 2-, the 4- and the 8- harvesting interval units were 0.075, 0.079, 0.100 and 0.093 mg N/m^2/h respectively. The differences between N_2O fluxes from the 4- and 8-month harvesting interval units were also not statistically significant.

The lower CH_4 fluxes in the 4- and 8-month harvesting interval units can be related to the deeper and more concentrated roots as plants can transfer oxygen from the atmosphere to the subsurface area [6]. It may be speculated that anaerobic microsites suitable for CH_4 generation by methanogens occur in the deeper part and less anaerobic microsites occur in the units with deeper and more concentrated root systems which results in less CH_4 generation in the 4- and 8-month harvesting interval units. Furthermore, the 2-month

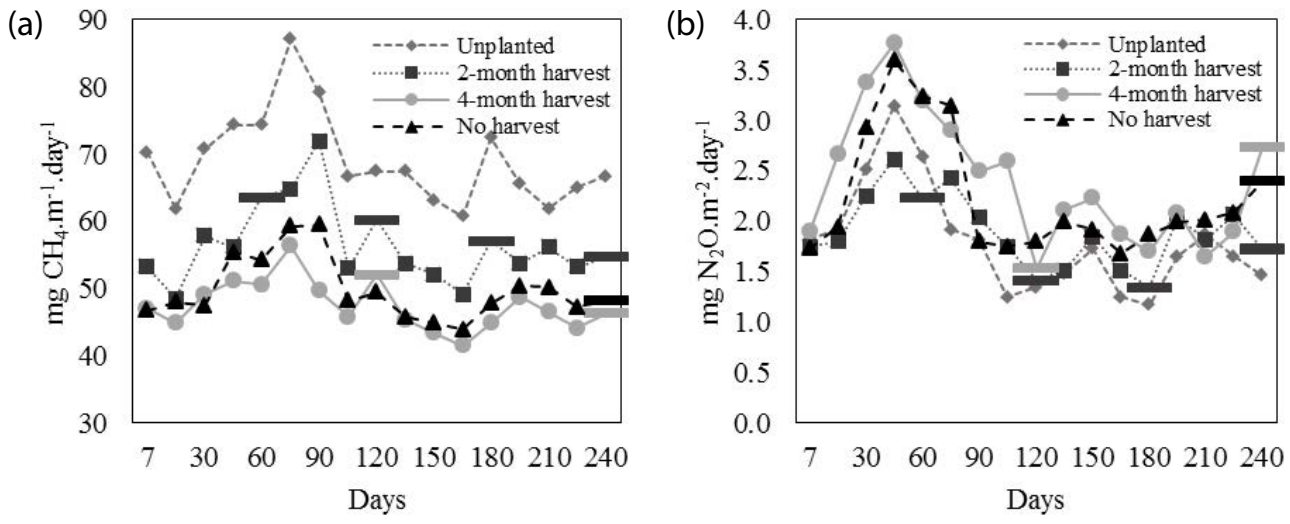

Fig. 5. CH_4 (a) and N_2O (b) fluxes from CW units, horizontal bars indicate fluxes measured after a harvest event.

harvesting interval unit showed significantly less above and below ground plant density than the other two systems after the second harvest, which is corresponding to the increase in the CH_4 fluxes.

An effect of plant roots was also found on N_2O fluxes. The average N_2O fluxes were highest in the 4-month followed by the 8-month harvesting interval units. According to the microbial analysis, more species of both nitrifying and denitrifying bacteria were found in root samples than in the media samples. More species were also found from the 4- and 8-month harvesting interval units root samples than in the 2-month harvesting interval unit root sample which can be linked to the deeper and higher density of the root systems observed in the 4- and 8-month harvesting interval units. As nitrifying and denitrifying bacteria are responsible for the generation of N_2O, it can be suggested that N_2O fluxes would be higher in planted VF CWs with deeper and denser root systems.

3.4. Microbial analysis

Most of the treatment mechanisms occur in CWs are carried out by microorganism. There are various species of bacteria and archaea in CW systems. Methanogens are microorganism that thrives in anaerobic wetland environments which are responsible for CH_4 generation from wetlands. Nitrifying bacteria are responsible for the conversion of ammonia to NO_3 in two-step process where AOB convert ammonia to NO_2 and nitrite oxidizing bacteria (NOB) convert NO_2 to NO_3. Denitrifying bacteria are responsible for the conversion of NO_3 to N_2 and N_2O. Both nitrifying and denitrifying bacteria are recognized as N_2O producers as N_2O can also be generated as a by-product in nitrification process [31]. However, it is suggested that in wet but not water-logged soil, N_2O from denitrification can be more significant [32]. Furthermore, it has been suggested that many AOB can carry out nitrifier denitrification [24], a process in which ammonia is oxidized to NO_2 and the NO_2 is reduced to nitric acid (NO), N_2 and N_2O. Studies suggest that N_2O generated by nitrifier denitrification can be significant in certain conditions such as in low oxygen condition, low carbon content soil and wet (but unsaturated) soil condition [24,33].

Fig. 6 shows methanogen, nitrifying and denitrifying bacteria species found in the systems by PCR-DGGE analysis. Despite the VF CWs having mostly aerobic condition, methanogen species which thrive in anaerobic condition were found in the sand samples from all the four units with more species found in the unplanted unit than the planted unit (Table 2). According to the results from dice similarity index analysis, high similarity between methanogen species in the planted units, especially in the 4- and 8-month harvesting interval units sand samples was found whereas methanogen species found in the unplanted unit have very low similarities to all the three planted units. In term of microbial diversity (Table 3), it is found that methanogen in CW units with plant harvesting was less diverse than the unplanted and unharvested units. However, nitrifying and denitrifying bacteria were found to be most diverse in the 4-month harvest interval unit, followed by the unharvested unit which are the units having highest underground biomass.

Nitrifying and denitrifying bacteria species were clearly more diverse in the root samples than in the sand samples. Among the planted units, more diverse species of nitrifying and denitrifying bacteria including species that can carry out denitrification in the presence of oxygen (*Paracoccus denitrificans*) [34] and those that can carry out nitrification and denitrification simultaneously (*Rhodococcus* sp. and *Bacillus subtilis*) [35] were found in the 4- and 8-month harvesting interval units, the two units with deep and dense root systems. According to the dice similarity index analysis results, the 4- and 8-month harvesting interval units root samples also showed higher similarity in their NOB, AOB and denitrifying bacteria species and much more diverse than 2-month harvesting interval unit. From this result, it may be speculated that the diversity of nitrifying

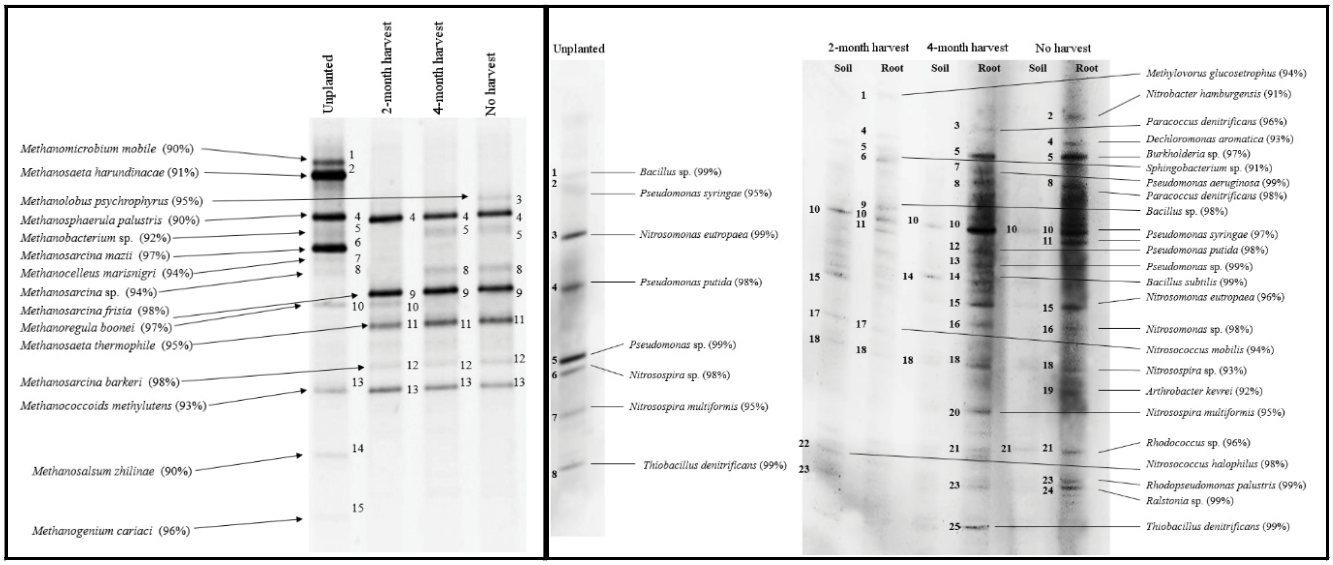

Fig. 6. PCR-DGGE profiles of methanogen (left) and nitrifying and denitrifying bacteria (right).

Table 2
Microbial species found in each unit

	Unplanted	2-Month harvest		4-Month harvest		No harvest		Species
	Sand	Sand	Root	Sand	Root	Sand	Root	
Methanogenic bacteria	✓							*Methanomicrobium mobile*
	✓							*Methanosaeta arundinacae*
						✓		*Methanolobus psychrophilus*
	✓	✓		✓		✓		*Methanosphaerula palustris*
	✓	✓		✓				*Methanobacterium* sp.
	✓							*Methanosarcina mazei*
	✓							*Methanoculleus marisnigri*
	✓			✓		✓		*Methanosarcina* sp.
		✓		✓		✓		*Methanosarcina frisia*
	✓	✓						*Methanoregula boonei*
		✓		✓		✓		*Methanosaeta thermophila*
		✓		✓		✓		*Methanosarcina barkeri*
	✓	✓		✓		✓		*Methanococcoides methylutens*
	✓							*Methanosalsum zhilinae*
	✓							*Methanogenium cariaci*
Nitrifying and denitrifying bacteria			✓					*Methylovorus glucosetrophus*
							✓	*Nitrobacter hamburgensis*
					✓			*Paracoccus denitrificans*
			✓				✓	*Dechloromonas aromatica*
			✓		✓		✓	*Burkholderia* sp.
			✓					*Sphingobacterium* sp.
					✓			*Pseudomonas aeruginosa*
					✓		✓	*Paracoccus denitrificans*
	✓		✓					*Bacillus* sp.
	✓	✓	✓	✓	✓	✓	✓	*Pseudomonas syringae*
	✓				✓			*Pseudomonas putida*
	✓				✓			*Pseudomonas* sp.
				✓	✓			*Bacillus subtilis*
	✓	✓			✓		✓	*Nitrosomonas europaea*
					✓		✓	*Nitrosomonas* sp.
		✓	✓					*Nitrosococcus mobilis*
	✓	✓	✓	✓	✓		✓	*Nitrosospira* sp.
							✓	*Arthrobacter kevrei*
	✓				✓			*Nitrosospira multiformis*
					✓	✓	✓	*Rhodococcus* sp.
		✓						*Nitrosococcus halophilus*
		✓			✓		✓	*Rhodopseudomonas palustris*
							✓	*Ralstonia* sp.
	✓							*Thiobacillus denitrificans*

Table 3
Shannon–Weaver (*H*) and Simpson (1-*D*) diversity indices of CW media

	Methanogens		Nitrifying/denitrifying bacteria	
	H	1-*D*	*H*	1-*D*
No plant	1.87	0.81	1.22	0.55
2-Month harvest (soil)	0.95	0.45	1.54	0.75
2-Month harvest (root)	—	—	1.30	0.59
4-Month harvest (soil)	1.18	0.53	1.08	0.65
4-Month harvest (root)	—	—	2.48	0.91
No harvest (soil)	1.87	0.83	1.05	0.64
No harvest (root)	—	—	2.02	0.80

and denitrifying bacteria in the VF CWs depends largely on healthy root systems and fewer methanogen species can thrive in healthy root systems.

4. Conclusion

This study is based on findings from four laboratory scale VF CWs in tropical climate. It is found that plant harvesting period affects oxygen availability and greenhouse gas production in VF CWs. Observable root depth in the most frequent harvested CW (every 2-month harvest interval) becomes clearly lower than the other planted CWs in the second half of the experiment. During this period, higher oxygen availability in the deeper zone was observed in moderately (every 4 months interval) and least frequent harvested (nonharvesting over 8 months) CWs. More frequent plant harvested CW, that is, every 2 months, with shallower plant root depth and density exhibited lower TKN removal efficiencies after a few plant harvesting times, which likely to be because nitrification was reduced as a result of lower oxygen availability. Less frequent plant harvested CWs (4 and 8 months interval) provided lower CH_4 but higher N_2O emissions during their treatment. Less diversity of methanogen but higher diversity of nitrifying and denitrifying microorganisms was found associated with the reduction in CH_4 emission and higher N_2O emission detected moderately in those systems.

Acknowledgements

This study was financially supported by the Center of Excellence on Hazardous Substance Management (HSM), Chulalongkorn University. The authors are grateful to Department of Environmental Engineering, Chiang Mai University for providing research facilities and postdoctoral fellow at the Department of Environmental Engineering, Faculty of Engineering, Kasetsart University for their support in microbial analyses.

References

[1] N. Mburu, S.M. Tebitendwa, J.J.A. van Bruggen, D.P.L. Rousseau, P.N.L. Lens, Performance comparison and economics analysis of waste stabilization ponds and horizontal subsurface flow constructed wetlands treating domestic wastewater: a case study of the Juja sewage treatment works, J. Environ. Manage., 128 (2013) 220–225.
[2] T. Saeed, G. Sun, A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media, J. Environ. Manage., 112 (2012) 429–448.
[3] C.A. Lutterbeck, L.T. Kist, D.R. Lopez, F.V. Zerwes, Ê.L. Machado, Life cycle assessment of integrated wastewater treatment systems with constructed wetlands in rural areas, J. Cleaner Prod., 148 (2017) 527–536.
[4] C. Avellan, R. Ardakanian, P. Gremillion, The role of constructed wetlands for biomass production within the water-soil-waste nexus, Water Sci. Technol., 75 (2017) 2237–2245.
[5] M. Jahangir, K.G. Richards, M.G. Healy, L. Gill, C. Muller, P. Johnston, Carbon and nitrogen dynamics and greenhouse gas emissions in constructed wetlands treating wastewater: a review, Hydrol. Earth Syst. Sci., 20 (2016) 109–123.
[6] H. Brix, Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol., 35 (1997) 11–17.
[7] U. Stottmeister, A. Wießner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, H. Moormann, Effects of plants and microorganisms in constructed wetlands for wastewater treatment, Biotechnol. Adv., 22 (2003) 93–117.
[8] X. Cao, W. Huang, D. Huang, Y. Tian, X. Jiang, J. Zhang, Effects of plant species on CH_4 emission from integrated vertical subsurface flow constructed wetlands, Desal. Wat. Treat., 85 (2017) 358–364.
[9] Ü. Mander, G. Dotro, Y. Ebie, S. Towprayoon, C. Chiemchaisri, S.F. Nogueira, B. Jamsranjav, K. Kasak, J. Truu, J. Tournebize, W.J. Mitsch, Greenhouse gas emission in constructed wetlands for wastewater treatment: a review, Ecol. Eng., 66 (2014) 19–35.
[10] P. Molle, A. Liénard, C. Boutin, G. Merlin, A. Iwema, How to treat raw sewage with constructed wetlands: an overview of the French systems, Water Sci. Technol., 51 (2005) 11–21.
[11] D. Konnerup, T. Koottatep, H. Brix, Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with Canna and Heliconia, Ecol. Eng., 35 (2009) 248–257.
[12] Z. Yang, Q. Wang, J. Zhang, H. Xie, S. Feng, Effect of plant harvesting on the performance of constructed wetlands during summer, Water, 22 (2015) 7476–7484.
[13] American Public Health Association (APHA), Standard Methods of the Examination of Water and Wastewater, New York, 2002.
[14] R.H. Kadlec, S. Wallace, Treatment Wetlands, CRC Press, Boca Raton, 2008.
[15] J. Zhou, M.A, Bruns, J.M. Tiedje, DNA recovery from soils of diverse composition, Appl. Environ. Microbiol., 62 (1996) 316–322.
[16] M. Khemkhao, B. Nuntakumjorn, S. Techkarnjanaruk, C. Phalakornkule, UASB performance and microbial adaptation during a transition from mesophilic to thermophilic treatment of palm oil mill effluent, J. Environ. Manage., 103 (2012) 74–82.

[17] J. Boonnorat, C. Chiemchaisri, W. Chiemchaisri, K. Yamamoto, Microbial adaptation to biodegrade toxic organic micro-pollutants in membrane bioreactor using different sludge sources, Bioresour. Technol., 165 (2014) 50–59.
[18] T.M. LaPara, C.H. Nakatsu, L.M. Pantea, J.E. Alleman, Stability of the bacterial communities supported by a seven-stage biological process treating pharmaceutical wastewater as revealed by PCR-DGGE, Water Res., 36 (2002) 638–646.
[19] T.C.J. Hill, K.A. Walsh, J.A. Harris, B.F. Moffett, Using ecological diversity measures with bacterial communities, FEMS Microbiol. Ecol., 43 (2003) 1–11.
[20] G. Langergraber, C. Prandtstetten, A. Pressl, R. Rohrhofer, R. Haberl, Removal efficiency of subsurface vertical flow constructed wetlands for different organic loads, Water Sci. Technol., 56 (2007) 75–84.
[21] T. Picek, H. Cizkova, J. Dusek, Greenhouse gas emissions from a constructed wetland – plants as important sources of carbon, Ecol. Eng., 31 (2007) 98–106.
[22] Y. Dong, M. Scholz, S. Mackenzie, Performance evaluation of representative Wildfowl & Wetlands Trust constructed wetlands treating sewage, Water Environ. J., 27 (2013) 317–327.
[23] M. Nikolausz, U. Kappelmeyer, A. Székely, A. Rusznyák, K. Márialigeti, M. Kästner, Diurnal redox fluctuation and microbial activity in the rhizosphere of wetland plants, Eur. J. Soil Biol., 44 (2008) 324–333.
[24] D.M. Kool, J. Dolfing, N. Wrage, J.W. Van Groenigen, Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil, Soil Biol. Biochem., 43 (2011) 174–178.
[25] A. Stefanakis, C.S. Akratos, V.A. Tsihrintzis, Vertical Flow Constructed Wetlands: Eco-Engineering Systems for Wastewater and Sludge Treatment, Elsevier, Boston, 2014.
[26] M.N. Kumwimba, M. Dzakpasu, B. Zhu, T. Wang, L. Ilunga, D. Kavidia Muyembe, Nutrient removal in a trapezoidal vegetated drainage ditch used to treat primary domestic sewage in a small catchment of the upper Yangtze River, Water Environ. J., 31 (2017) 72–79.
[27] S. Kantawanichkul, S. Kladprasert, H. Brix, Treatment of high-strength wastewater in tropical vertical flow constructed wetlands planted with *Typha angustifolia* and *Cyperus involucratus*, Ecol. Eng., 35 (2009) 238–247.
[28] J.K. Cronk, M.S. Fennessy, Wetland Plants: Biology and Ecology, CRC Press, Boca Raton, 2001.
[29] A. Heritage, P. Pistillo, K.P. Sharma, I.R. Lantzke, Treatment of primary-settled urban sewage in pilot-scale vertical flow wetland filters: comparison of four emergent macrophyte species over a 12 month period, Water Sci. Technol., 32 (1995) 295–304.
[30] Y.C. Chen, G.L. Chiang, S.L. Lo, Effect of pollutant concentrations on growth characteristics of macrophytes in a constructed wetland treating municipal combined sewage, Desal. Wat. Treat., 44 (2012) 289–295.
[31] Y. Schneider, M. Beier, K-H. Rosenwinkel, Nitrous oxide formation during nitritation and nitrification of high-strength wastewater, Water Sci. Technol., 67 (2013) 2494–2502.
[32] D. Linn, J. Doran, Aerobic and anaerobic microbial populations in no-till and plowed soils, Soil Sci. Soc. Am. J., 48 (1984) 794–799.
[33] N. Wrage, G.L. Velthof, M.L. van Beusichem, O. Oenema, Role of nitrifier denitrification in the production of nitrous oxide, Soil Biol. Biochem., 33 (2001) 1723–1732.
[34] J.F. Su, K. Zhang, T.L. Huang, G. Wen, L. Guo, S.F. Yang, Heterotrophic nitrification and aerobic denitrification at low nutrient conditions by a newly isolated bacterium, *Acinetobacter* sp. SYF26, Microbiology, 161 (2015) 829–837.
[35] P. Kundu, A. Pramanik, A. Dasgupta, S. Mukherjee, J. Mukherjee, Simultaneous heterotrophic nitrification and aerobic denitrification by *Chryseobacterium* sp. R31 isolated from abattoir wastewater, BioMed Res. Int., 2014 (2014) 1–12.