GREENHOUSE GAS EMISSIONS FROM VERTICAL AND HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS IN TROPICAL CLIMATE

Miss Siripoon Nutanong

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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By	Miss Siripoon Nutanong
Field of Study	Environmental Management
Thesis Advisor	Associate Professor Chart Chiemchaisri, D.Eng.
Thesis Co-Advisor	Associate Professor Suwasa Kantawanichkul, D.Tech.Sci

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.... Dean of the Graduate School

(Associate Professor Sunait Chutintaranond, Ph.D.)

THESIS COMMITTEE

.... Chairman

(Associate Professor Tawan Limpiyakorn, Ph.D.)

.... Thesis Advisor

(Associate Professor Chart Chiemchaisri, D.Eng.)

.... Thesis Co-Advisor

(Associate Professor Suwasa Kantawanichkul, D.Tech.Sci)

.... Examiner

(Pichet Chaiwiwatworakul, Ph.D.)

.... Examiner

(Associate Professor Ekawan Luepromchai, Ph.D.)

.... Examiner

(Associate Professor Srilert Chotpantarat, Ph.D.)

.... External Examiner

(Boonlue Kachenchart, Ph.D.)

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สิริปุณณ์ นุชอนงค์ : การปล่อยก๊าซเรือนกระจกจากบึงประดิษฐ์แบบไหลใต้ผิวดินในแนวดิ่งและแนวราบในเขตร้อนชื้น (GREENHOUSE GAS EMISSIONS FROM VERTICAL AND HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS IN TROPICAL CLIMATE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ชาติ เจียมไชยศรี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. ศุวศา กานตวนิชกูร, 113 หน้า.

การศึกษานี้ได้ทำการเปรียบเทียบการปล่อยก๊าซมีเทนและไนตรัสออกไซด์จากระบบบึงประดิษฐ์แบบไหลใต้พื้นผิวในแนวนอนและแนวดิ่งในเขตร้อนชื้น และเปรียบเทียบการปล่อยก๊าซมีเทนและไนตรัสออกไซด์จากบึงประดิษฐ์แบบไหลใต้พื้นผิวในแนวดิ่งที่มีช่วงระยะห่างในการเก็บเกี่ยวพืชต่างกัน ผลการเปรียบเทียบการปล่อยก๊าซมีเทนและไนตรัสออกไซด์จากระบบบึงประดิษฐ์แบบไหลใต้พื้นผิวในแนวนอนและแนวดิ่งพบว่าการปล่อยมีเทนในบึงประดิษฐ์แบบไหลใต้พื้นผิวในแนวนอนสูงกว่าในแนวดิ่ง และไนตรัสออกไซด์ในแนวดิ่งสูงกว่าแนวนอน ค่าเฉลี่ยของมีเทนฟลักซ์ในระบบที่มีการไหลในแนวนอนและในแนวดิ่งเท่ากับ 7.1 และ 4.1 mg CH_4-C.m^{-2}.h^{-1} ตามลำดับ ค่าเฉลี่ยของไนตรัสออกไซด์ฟลักซ์ในระบบที่มีการไหลในแนวนอนและในแนวดิ่งเท่ากับ 0.134 และ 0.201 mg N_2O-N.m^{-2}.hr^{-1} ตามลำดับ ซึ่งค่า Total organic carbon (TOC) และ Total nitrogen (TN) ในน้ำเสียเป็นปัจจัยหลักที่ส่งผลกับค่าฟลักซ์ของก๊าซทั้งสอง มีเทนและไนตรัสออกไซด์ฟลักซ์ในช่วงฤดูแล้งสูงกว่าในช่วงฤดูฝนอย่างมีนัยสำคัญ การศึกษานี้ยังพบว่ามีเทนและไนตรัสออกไซด์ลดลงในวันที่มีฝนตก ค่า TOC และ TN เฉลี่ยในน้ำเสียที่ใช้เท่ากับ 80.2 และ 55.9 มิลลิกรัมต่อลิตร ตามลำดับ ในการศึกษาผลกระทบของช่วงเวลาเก็บเกี่ยวพืชที่ต่างกันกับการปล่อยก๊าซมีเทนและไนตรัสออกไซด์โดยใช้น้ำเสียเดียวกันและมีค่า TOC และ TN เฉลี่ยในน้ำเสียเท่ากับ 74.5 และ 40.0 มิลลิกรัมต่อลิตร พบว่าพืชส่งผลให้การบำบัดค่าไนโตรเจนต่างๆและการเพิ่มออกซิเจนในระบบดีขึ้นแต่ส่งผลให้การบำบัดออกานิกคาร์บอนลดลง ซึ่งทำให้มีการปล่อยก๊าซไนตรัสออกไซด์มากขึ้นแต่ลดการปล่อยก๊าซมีเทนลง การเก็บเกี่ยวพืชหลังจากที่พืชโตเต็มที่แล้วช่วยให้การบำบัดไนโตรเจนมีประสิทธิภาพมากขึ้นและลดการเกิดไนเตรทในน้ำออกด้วย ในระบบที่มีการเก็บเกี่ยวพืชหลังจากที่พืชโตเต็มที่แล้วยังพบว่าปริมาณออกซิเจนในน้ำออกสูงกว่าในระบบอื่นและยังมีความหลากหลายของสายพันธุ์ของจุลินทรีย์มากกว่าในระบบอื่นด้วย

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SIRIPOON NUTANONG: GREENHOUSE GAS EMISSIONS FROM VERTICAL AND HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS IN TROPICAL CLIMATE. ADVISOR: ASSOC. PROF. CHART CHIEMCHAISRI, D.Eng., CO-ADVISOR: ASSOC. PROF. SUWASA KANTAWANICHKUL, D.Tech.Sci, 113 pp.

In this study, CH_4 and N_2O fluxes from HSSF and VF constructed wetland operated in tropical climate and the effect of different plant harvesting intervals on CH_4 and N_2O fluxes from VF constructed wetlands were compared. Results show that the average CH_4 fluxe was higher in the HSSF and the average N_2O flux was higher in the VF system. Average CH_4 fluxes of 7.13 and 4.05 mg CH_4-C.m^{-2}.h^{-1} and average N_2O fluxes of 0.13 and 0.20 mg N_2O-N.m^{-2}.h^{-1} were measured from the HSSF and the VF constructed wetland, respectively. CH_4 and N_2O fluxes in the wet period were lower than the dry period for both systems. CH_4 and N_2O fluxes dropped during a rainfall event. Inflow TOC and TN were the dominant factors regulating the fluxes. The average TOC and TN concentrations in the influent were 80.2 and 55.9 mg/L, respectively. The study on the effect of plant on VF systems with average influent TOC and TN of 74.5 and 40.0 mg/L, respectively shows that TN removal, effluent DO concentrations, N_2O flux and the number of nitrifying and denitrifying bacteria in the system increased but TOC removal and CH_4 flux decreased with the presence of plants. Harvesting plants after reaching the final height improved DO concentrations, organic nitrogen and NH_3 removal and lower the effluent NO_3 concentrations and increases N_2O flux. The results suggest that harvesting plants after reaching the final height would increase microbiological processes involving in nitrogen transformation which results in the improvement of nitrogen removal efficiencies but would also increase N_2O flux.

Field of Study:	Environmental Management	Student's Signature	....
		Advisor's Signature	....
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Abbreviation

Abbreviation	Name
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CW	Constructed Wetlands
DGGE	Denaturing Gradient Gel Electrophoresis
DO	Dissolved Oxygen
ECD	Electron Capture Detector
FID	Flame Ionization Detector
FWS	Free Water Surface Flow
GC	Gas Chromatograph
HSSF	Horizontal Subsurface Flow
NH_3	Ammonia
NO_2	Nitrite
NO_3	Nitrate
PCR	Polymerase Chain Reaction
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solid
VF	Vertical Flow

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Chapter 1 Introduction

1.1 Background

Greenhouse gas emissions and their sources is a topic of interest in the 21st century among academics and policy makers. Greenhouse gases (GHGs) are gases that can trap infrared radiation within the earth's atmosphere, thus, increasing the temperature of the earth. Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the three major GHGs. The sources of GHGs can be divided into natural sources and anthropogenic sources with natural sources accounted for approximately 37% and 64% of the total global CH_4 and N_2O emission, respectively (Anderson et al., 2010).

Wetland is the largest natural source for CH_4 emission. It is estimated that approximately 170.3 Tg CH_4/yr was emitted from wetlands which is approximately 82% of the total natural CH_4 emission and 30% of the total global CH_4 emission (Anderson et al., 2010). For N_2O emission, the largest natural source is upland and riparian areas where the soil is more aerated and/or subjected to frequent fluctuation in water tables due to tides. It is estimated that 6.6 Tg N/yr of N_2O is emitted from upland and riparian zone which is approximately 55% of total natural N_2O emission and 35% of the total global N_2O emission. The processes which occur in natural wetlands and riparian areas are adopted in constructed wetland as its key treatment mechanisms, making CH_4 and N_2O emission from constructed wetlands a topic of interest.

Constructed wetland is a promising alternative for wastewater treatment, however, like other biological wastewater treatment technology, it also generates substantial amount of greenhouse gases. Constructed wetland is considered a part of wastewater treatment sector which currently contribute to 3%-5% of the total global anthropogenic CH_4 emission (El-Fadel and Massoud, 2001) and 3.2% of the total global anthropogenic N_2O emission (Mosier et al., 1999). Even though, contribution percentage of constructed wetland emission in comparison to natural wetland is small due to much larger area covered by natural wetland, areal fluxes from constructed wetlands are considerably higher (Maltais-Landry et al., 2009).

Constructed wetlands can be categorized into free water surface flow (FWS), horizontal subsurface flow (HSSF) and vertical flow (VF) constructed wetlands. Key treatment mechanisms in each type of constructed wetlands differ considerably. This study focuses on HSSF and VF constructed wetlands which are subsurface flow systems. Typical VF constructed wetlands rely on aerobe to carry out the key treatment mechanisms whereas HSSF constructed wetlands rely on anaerobe to carry out the key treatment mechanisms.

A key component in constructed wetland is plants. Wetland plant, or macrophyte, play several important roles in the treatment process. One of the most important role of macrophytes in constructed wetland is oxygen and gas transportation from the atmosphere to the subsurface area and vice versa, via the aerenchyma. Harvesting plant regularly improves accessibility which is important for system maintenance and improve the system's aesthetic value. However, harvesting would affect the gas transportation mechanism directly.

1.2 Motivation

Constructed wetlands typically require larger land areas with high spatial heterogeneity in the microbiological processes, which are the key treatment mechanism, than conventional wastewater treatment system and constructed wetlands involve complex and interlaced influencing factors for those processes which makes greenhouse gas more difficult to quantify with high accuracy. However, due to its simplicity, low resources and energy requirements, low operation and maintenance requirements, life cycle assessment showed that constructed wetland has lower impact on climate change than other technologies (Fuchs et al., 2011) which therefore, makes constructed wetland a promising alternative to wastewater treatment. Nevertheless, comparing to conventional wastewater treatment systems such as activated sludge, constructed wetland has higher gaseous emission per unit of treatment volume because it is harder to control the optimum condition for nitrification and denitrification (Fuchs et al., 2011). Furthermore, studies so far show large range of emission measured from different constructed wetland with different conditions (Mander et al., 2014). A comprehensive review on greenhouse gas emission by constructed wetlands by Mander et al (2014) showed that there is still a lack of data from tropical areas. Therefore, to fill out this gap of knowledge, more data from especially tropical climate is needed.

VF constructed wetland is relatively new compared to FWS and HSSF (Kadlec and Wallace, 2008). In Europe, VF constructed wetlands are gaining popularity because VF systems commonly require less land (1-3 m^2/PE as compared to 5-10 m^2/PE for HSSF systems). Both surface and subsurface constructed wetlands have been successfully implemented in in Thailand (Zhang et al., 2014). Surface flow constructed wetland has been successfully implemented in Bangkok (Boonsong and Chansiri, 2008) as well as subsurface and hybrid systems in Phuket which were used to treat municipal wastewater in Tsunami effected areas (Brix et al., 2011).

Many studies comparing HSSF and VF treatment performances found that VF systems perform better for most pollutants (Yalcuk and Ugurlu, 2009, Zurita et al., 2009, Pandey et al., 2013). In term of CH_4 and N_2O emissions, relatively few studies have compared VF emissions with other constructed wetland systems in the tropical climate. In Thailand, there is a study comparing greenhouse gases from a surface flow constructed wetland and a subsurface flow constructed wetland where the greenhouse gas fluxes from the subsurface flow system were found to be significantly lower than the fluxes from the surface flow system (Chuersuwan et al., 2014). However, there has not yet been a study comparing a HSSF and a VF constructed wetlands despite the fact that HSSF and

VF shares similar characteristics with natural wetlands and riparian areas, the two major natural sources for CH_4 and N_2O, respectively.

Plant is an important component of constructed wetlands and is a major source of available carbon in the system (Picek et al., 2007) which is one of the main factor influencing CH_4 generation by methanogenesis process. Plant aerenchyma is the main oxygen transportation mechanism in constructed wetlands (Brix, 1997, Stottmeister et al., 2003, Yang et al., 2013). Harvesting has been suggested as a method to increase nutrient removal efficiency of a constructed wetland system. As, in low loading condition, plant uptake can be a significant nutrient removal mechanism (Brix, 1997). There have been studies focusing on greenhouse gas emissions from systems with and without plants (Søvik et al., 2006). However, there is still lack of data which compare emissions from constructed wetland systems with different plant harvest patterns.

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1.3 Objectives

This study has three main objectives.

1. To provide constructed wetlands greenhouse gas emission data in tropical climate.
2. To investigate the mechanisms and influencing factors in which CH_4 and N_2O are emitted from constructed wetlands for wastewater treatment in tropical

climate by comparing N_2O fluxes from subsurface horizontal and vertical flows constructed wetlands.

3. To investigate the effect of plant harvesting intervals on CH_4 and N_2O fluxes from constructed treatment wetlands.

1.4 Hypothesis

1. Constructed wetlands with subsurface vertical flow system generate more N_2O and less CH_4 than horizontal flow systems.
2. Plant harvest effects in lower N_2O and CH_4 fluxes

1.5 Scope

This study focuses on 2 of the major long-lived greenhouse gases, CH_4 and N_2O. Constructed wetland designs in the scope of this study follow typical horizontal subsurface flow constructed wetland design and typical vertical subsurface flow constructed wetland designs as suggested in Constructed Wetland for Pollutant Controls (Kadlec et al., 2000) and Treatment Wetlands (Kadlec and Wallace, 2008). The study locates in Chiang Mai, Thailand which is in tropical climate zone.

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Chapter 2 Literature Review

2.1 Constructed Wetlands - Definition, Functions and History

The definition of wetlands as given by Ramsar Convention on Wetlands (Matthews, 1993) is as follow:

"Wetlands are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters"

Ramsar convention defines wetlands for the purpose of policy making which may not be specific enough to differentiate wetlands from other ecosystems. In general wetlands are commonly referred to places that can support both aquatic and terrestrial species in the same area. It is commonly characterized by having permanently or occasionally saturated soil and with presents of vegetation that can thrive in saturated soil condition (Kadlec and Wallace, 2008).

In the past, sewage and secondary wastewater were discharged directly to natural wetlands in some places as naturally occurring biological processes in wetland ecosystems decompose the organic pollutants in the discharged water. CH_4 and N_2O from wetlands and upland and riparian zone are produced predominantly by these microorganism processes which are the key treatment mechanisms and are crucial to the nitrogen cycle in the ecosystem.

Constructed wetland is a human-made ecosystem, designed and built to replicate functions of natural wetlands. It possesses similar biological processes as in natural wetlands and natural soil systems. Constructed wetlands are built for many different purposes such as to restore natural habitat or to protect shoreline. However, constructed wetlands, in general, refer to wetlands that are constructed for the purpose of pollutant control and waste management. By adjusting the system configuration, wastewater that can be treated successfully by constructed wetlands for wastewater treatments have proven to be very versatile. Apart from municipal and domestic wastewater, animal wastewater with BOD (Biochemical Oxygen Demand), TSS (Total Suspended Solid), and ammonia above 100 mg/L (Kadlec and Wallace, 2008) and strong loading influent such as landfill leachate (Bulc et al., 1997, Chiemchaisri et al., 2009) have also been successfully treated by constructed wetlands. However, with the typical designs, TSS levels are usually kept to minimum to avoid clogging which leads to over flow especially in HSSF constructed wetland system (Kadlec et al., 2000).

Constructed wetlands have been a popular alternative to wastewater treatment systems, especially for decentralize systems due to the economic value and operational simplicity. Constructed wetlands have been used in European and North American countries for several decades (Kadlec and Wallace, 2008). However, the technology is relatively new in Asia countries which started around 1990s (Juwarkar et al., 1995). Since then, there have been several currently operated constructed wetlands throughout Asia.

2.2 Constructed Wetlands Configurations

Three common types of constructed wetlands (CW) for wastewater treatment are free water surface flow constructed wetland (FWS), horizontal subsurface flow constructed wetland (HSSF) and vertical flow constructed wetland (VF). Each type is suitable for wastewater with different characteristics and can be combined to further optimize the treatment processes. FWS CW very closely mimics natural wetlands and is similar to facultative lagoons. Plants can be submersed, emergent or floating. FWS CW commonly requires larger space than subsurface flow systems and the design depends largely on land space requirement and the type of macrophytes being used. Substrate is necessarily if submersed or emergent plants are used. However, most treatment processes in FWS CWs occur in the water column. The deeper part of FWS CW is commonly anaerobic where aerobic processes occur in the shallow zone. FWS constructed wetland is commonly designed to treat secondary treated wastewater or storm water. Due to the risk of human and animal exposure to pathogenic organisms, FWS CW is not suitable for primary or secondary treatments.

For secondary treatment of wastewater, subsurface flow systems are commonly used as water is not exposed to the environment and the substrates in subsurface area provide additional sites for microbial attachment thus increases treatment via microbiological processes which allow subsurface flow systems to handle influent with higher organic loading. The two main types of subsurface flow constructed wetlands which are HSSF and VF constructed wetlands are differ mainly in the water regime. As the name suggested, horizontal subsurface flow constructed wetlands are designed so that the water travel through the substrate media and the root zone horizontally and the water level is kept below the substrate surface. It is also a common design that the system is permanently saturated during the operating period. However, some resting periods where the water is drained out from the system can applied. Vertical subsurface flow constructed wetlands, on the other hand, are designed so that the influent is fed intermittently. Feeding is commonly done by flooding the influent across the entire system surface and allowed the influent to slowly seep through the substrate and the root zone vertically. Sand is the most effective filtering media for VF CWs with the effective filtering depth of 1 meter (Brix and Arias, 2005).

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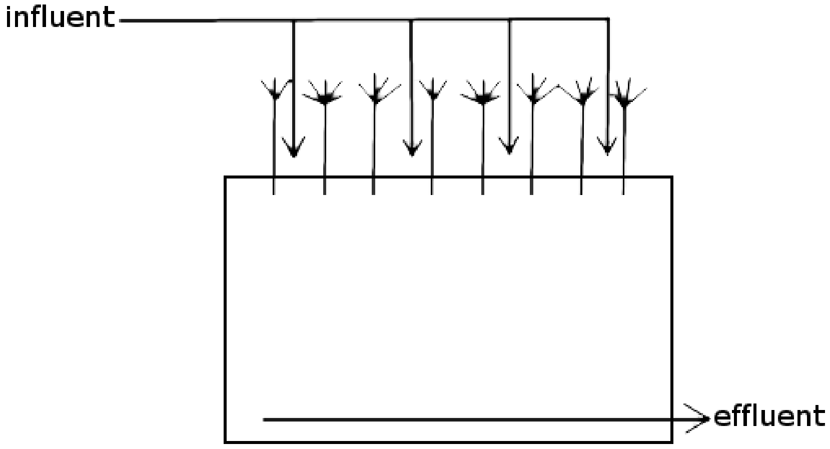

Figure 2.1 a typical VF constructed wetland configuration

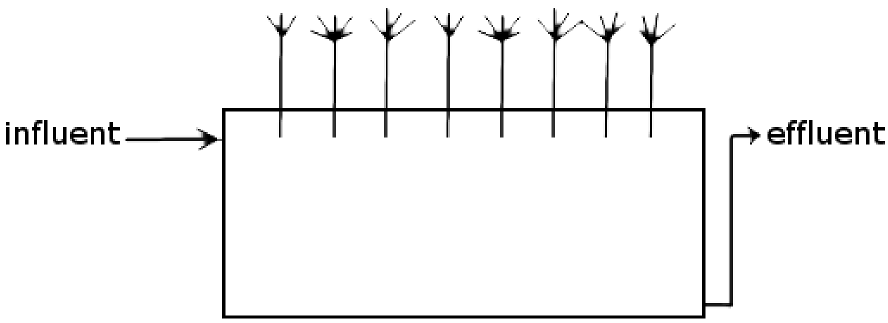

Figure 2.2 a typical HSSF constructed wetland configuration

In natural wetlands, plants and microorganism species and the relevant processes are predominantly defined by the quantity and the quality of the water and its flow regime where soils and nutrients are also influenced by the water condition. In constructed wetlands, systems are typically designed accordingly to the characteristic of the influence and the treatment requirement. Sizing and flow regime for a constructed wetland can be determined by loading rate and treatment requirement. One of the most popular method for sizing a constructed wetland is called the prescriptive criteria method.

However, a limitation of this method is that it relies on data from other constructed wetlands with similar conditions, including climate condition (Kadlec and Wallace, 2008). Scaling method is another popular method for designing a constructed wetland. This method is also widely used in VF system with intermittent loading (Kadlec and Wallace, 2008). Table 2.1 shows examples of scaling rules suggested by different studies for VF CWs. It should also be noted that most of the studies were conducted in temperate/boreal climates. The following equation is used for sizing based on the scaling rules provided.

$$A = mP^b \qquad \textit{(Equation 2.1)}$$

Where

A = area of bed required (m^2)

b = exponent

P = population equivalent

m = scaling factor

Table 2.1 Scaling rules for VF CW (Modified from: Kadlec and Wallace, 2008)

Sources	Country	M	b
Cooper P.F. (1996)	U.K.	1	1.0
Cooper P.F. (1996)	U.K.	2	1.0
Weedon (2003)	U.K.	5.4	0.60
Weedon (2003)	U.K.	2.4	0.85
Boutin and Lienard (2003)	France	2.5	1.0
Molle et al. (2005)	France	2	1.0
Langergraber et al. (2007)	Austria	4	1.0

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2.3 Constructed Wetland Component – Plants and Substrates

Vegetation is a crucial component in wetlands. It is one of the characteristics which defines wetlands from other ecosystems. There are several species of vegetation found in wetlands, however, the most common characteristics is the ability to survive in saturated conditions which are commonly referred to as wetland plants (Cronk and Fennessy, 2016). Larger wetland plants are commonly called macrophytes. The stem of a macrophyte consists of several hollow tubes called aerenchyma (figure 2.3) which allow oxygen to be transported from the atmosphere to the root zone. This is the main mechanism which allows macrophyte to thrive under saturated soil condition.

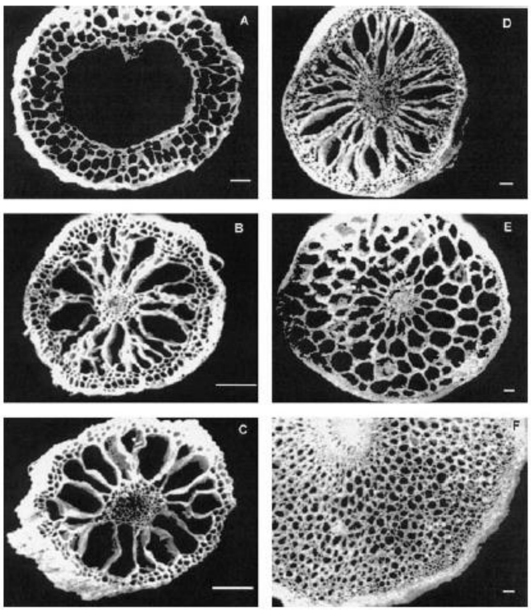

Figure 2.3 Aerenchyma of different macrophyte (A) Isoetes lacustris, (B) Littorella uniflora, (C) Luronium natans, (D) Nymphoides peltata, (E) Nymphaea alba, (F) Nuphar lutea. Bars represent 100 μm. (Cronk and Fennessy, 2016)

The vital roles macrophyte has in wetland ecosystem include the ability to stabilize soil surface and loosen the subsurface soil. In FWS systems, the submerged part of the plants also provides surfaces for microbial growth. These physical effects are crucial as they can reduce the fluctuations of the treatment processes that may be effected by factors such as wind and air temperature and, prevent clogging which is another common operational failure.

One of the most mentioned importance of macrophyte is its ability to transport oxygen from the atmosphere to the rhizosphere. Stems of macrophytes process air channels called aerenchyma which enable them to thrive in saturated soil condition. The aerenchyma does not only benefit the plant itself but also the microorganisms that live in the rhizosphere which are importance to wetland ecosystem as most the treatment processes are carried out by the microorganism. Another important role of macrophytes in constructed wetland is the ability to uptake nutrients. Macrophytes take up nutrient directly from their root system which nitrogen removed by plant can be substantial in low loading constructed wetlands (Brix, 1997).

Dominant plant species in wetlands varies by climate, hydrological regime, carbon and nutrient availability and, soil and substrate characteristic. Species belong Poaceae (grasses), Cyperaceae (sedge), Juncaceae (rushes) and Typhaceae (cattail) families are commonly dominant in wetland ecosystem worldwide (Cronk and Fennessy, 2016). Cyperus species is often used for constructed wetlands for wastewater treatment in Thailand (Kantawanichkul et al., 1999, Perbangkhem and Polprasert, 2010). *Cyperaceae*'s advantage over other wetland plant families is its very high nitrogen uptake rate. Primary production of *Cyperus Papyrus*, a *Cyperus species* in a tropical wetland can be up to 2200-3100 gram dry weight/m^2 within 2 months (Perbangkhem and Polprasert, 2010). Cyperus Papyrus is also shown to have higher nitrogen and phosphorus uptake rates than other *Miscanthidium* species, a wetland plant of Poaceae family (Kyambadde et al., 2004).

Cyperus alternifolius is a perennial plant that can be grown in saturated soil or substrate with high water table fluctuation. *Cyperus alternifolius* roots can penetrate several meters underground through the substrate. It has high toleration to changes in environmental conditions and can multiply quickly and easily. Cyperus alternifolius' advantage compared with other plants like *Miscanthidium Violaceumis* is that it eliminates nutrients of the wastewater (Ebrahimi et al., 2013). According to Ebrahimi et al. (2013), *Cyperus alternifolius* can substantially improve COD, NO_3^-, NH_4 and PO_3 removal efficiencies. For these advantages, *Cyperus alternifolius* has been used in many subsurface flow constructed wetlands, especially vertical flow constructed wetlands, in different studies (Cheng et al., 2002, Iamchaturapatr et al., 2007, Kantawanichkul et al., 2009, Cui et al., 2009, Soda et al., 2012, Leto et al., 2013, Shahi et al., 2013).

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Various type of substrates has been used in constructed wetland for wastewater treatment. As plants usually receive sufficient carbon and nutrient from the influent, sand and gravel are commonly used. In addition to increasing the depth the CW, sand or small particle substrates are commonly used for vertical flow system as to slow down the flow and allow enough time for the essential microbiological reactions. However, small particles in horizontal flow is likely to create surface flow and large particles tend to discourage root propagation thus, medium size particle such as gravel is commonly used (Kadlec et al., 2000).

2.4 Treatment processes

Constructed wetland relies primarily on physical and biological processes for the removal and storage of pollutants in the wastewater. Constructed wetland treatment processes differ significantly between different types of constructed wetlands. This study focuses on subsurface flow systems with both anaerobic and aerobic conditions.

Suspended solid can be filtrated, or settled into stagnant micropockets within the substrate pores. Suspended solid is subjected to physical interaction with the substrate in several ways. These interactions are collectively termed granular medium filtration (Metcalf et al., 1991). Settable organics are removed predominantly by deposition and filtration whereas soluble organic compounds are removed predominantly by heterotrophic microorganisms. Aerobic heterotrophic bacteria can degrade large amount of organic pollutants in the influent with the following reaction:

$$(CH_2O) + O_2 \longrightarrow CO_2 + H_2O.$$

In anaerobic condition, facultative or obligated anaerobic heterotrophs can remove organic pollutants in a series of processes from fermentation, nitrate, iron, sulfate reduction and, methanogenesis which can be simplified in the following reaction:

$$C_6H_{12}O_6 \longrightarrow 3CO_2 + 3CH_4$$

Organic pollutants which contain nitrogen can be removed under aerobic conditions in a series of microbiological processes.

Nitrogen compound in wetlands exist in different oxidation states. The most reduced forms of nitrogen in wetland environment is organic nitrogen and ammonia with the oxidation state of -3. Ammonification oxidize the organic nitrogen to hydroxylamine and nitrite. Nitrite can then be oxidized further to nitrate, with the oxidation state of +5, in nitrification process. High concentration of nitrate in the water is harmful to human and animal health, and the environment. High concentration of nitrate in a water body can cause eutrophication and high concentration of nitrate in drinking water can cause baby blue syndrome in infants. however, it can be removed in anaerobic condition by denitrification process where nitrogen gas, which has neutral oxidation state, is the

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product and can be released safely to the atmosphere. These reactions rely on 3 types of microbial which are ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and denitrifying bacteria. More recent studies also include anaerobic ammonium oxidation (Anammox) where ammonia is oxidized to dinitrogen in anaerobic condition by anammox-capable bacteria. There are other minor microbiological processes that involve in wetland nitrogen cycle such as Dissimilatory Nitrate Reduction to Ammonium (DNRA) and denitrifier nitrification. Other physical processes which also involve in nitrogen transformation in wetland ecosystem are ammonia volatilization and plant uptake and assimilation.

Soils and biota in wetlands is a suitable short-term and long-term phosphorus storage. Adsorption is the main phosphorus removal process in wetlands. For sustainable removal processes, accretion of new substrate may be needed (Kadlec et al., 2000)

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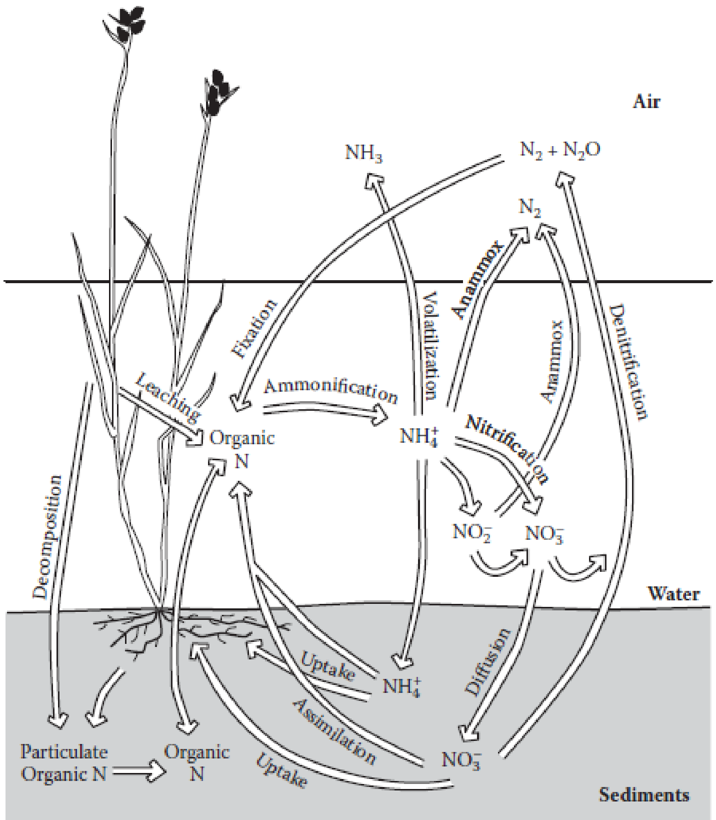

Figure 2.4 Nitrogen cycle in a wetland ecosystem (Kadlec and Wallace, 2008)

Zero-order reaction is the most simplistic quantitative model for determining the system's removal rate. However, contaminant concentration is found to be an important factor for determining the system's removal rate. Therefore, for a rough estimation of the system's removal rate, a first order removal model is widely (Goulet et al., 2001, Kadlec and Wallace, 2008) as the following equation;

$$J = kC \qquad \textit{(Equation 2.2)}$$

Where

C = concentration (g/m^3)

J = removal per unit area, or load removed, (g.m^{-2}.d^{-1})

K = rate coefficient (m/d)

2.5 Greenhouse Gases from Constructed Wetlands

Among the greenhouse gases emitted by constructed wetland, methane and nitrous oxide are of concern due to their persistence in the atmosphere and their much higher impacts on global warming in relative to carbon dioxide (CO_2), as measured by their global warming potential (GWP) values. CH_4 has the GWP of 25 over a time horizon of 100 years and can remain in the atmosphere for 12 years once emitted. N_2O has the GWP of 298 over a time horizon of 100 years and can remain in the atmosphere for 115 years (Ramaswamy et al., 2001). Atmospheric methane emission has increased from around 200 Tg year^{-1} in the 18th century to between 400 to 600 Tg year^{-1} in the last decade (IPCC, 2007).

CH_4 and N_2O are produced in constructed wetlands for wastewater treatment predominantly via microbial processes which involve in the treatment of wastewater. CH_4 is generated in the methanogenesis process. The process is carried out by methanogen, archaea that lives in anaerobic environment which prevails in saturated soil/substrate conditions such as in FWS and HSSF constructed wetlands. In FWS and HSSF wetlands, methanogensis occurs in most part of the substrate except around the root spheres. However, CH_4 can be consumed in constructed wetlands by methanotroph in aerobic condition. CH_4 production rate depend on several factors such as soil conditions and DO and ORP within the system. In HSSF systems, these conditions differ substantially from

VF systems due to the hydrological regime. Naturally HSSF with saturated soil condition, thus, more anaerobic would produce higher CH_4 fluxes. Literatures comparing CH_4 fluxes from HSSF and VF constructed wetlands also found higher CH_4 fluxes from HSSF than VF constructed wetlands (Gui et al., 2007, Liu et al., 2009) However, the ranges of emissions from other studies comparing CH_4 fluxes from HSSF and VF constructed wetlands have found to be very large and differ quite substantially between different climate conditions. Literatures reported, CH_4 fluxes from HSSF range from 0.76 to 17.5 mg m^{-2} h^{-1}(Gui et al., 2007, Picek et al., 2007) whereas CH_4 fluxes from VF CWs range from 0.3 to 3 mg m^{-2} h^{-1} (Teiter and Mander, 2005, Søvik et al., 2006, Gui et al., 2007, Mander et al., 2008). In comparison, to other conventional wastewater treatment systems, CH_4 and N_2O emission from both HSSF and VF constructed wetlands are found to be smaller (Fuchs et al., 2011). It is estimated that N_2O from wastewater treatment plants ranges between 0.67 to 4 mg.m^{-2}.hr^{-1} (Fuchs et al., 2011). However, studies found that aerated wastewater treatment processes emit between 0.83-75 mg.m^{-2}.hr^{-1} (Sümer et al., 1995) and non-aerated process would emit 0.42-1.7 mg.m^{-2}.hr^{-1} of N_2O (Benckiser et al., 1996).

Macrophyte is also found to effect methane emission from wetlands substantially (Laanbroek, 2010). Macrophyte in wetland can directly affect methane emission as one of the main transportation mechanism from the subsurface to the atmosphere. It can also effect methane emission indirectly by increasing oxygen availability in the subsurface which increases methane oxidation rates. Furthermore, plants can fuel microbial metabolism by providing microbes with additional carbon source (Picek et al., 2007)

Nitrous oxide emission comes from several microbiological processes with nitrification and denitrification as the main processes. It is estimated that approximate 70% of global N_2O emissions comes from nitrification and denitrification processes (Ussiri and Lal, 2013). These processes are critical to constructed wetland treatment processes. Organic nitrogen and ammonia nitrogen are removed primarily by nitrification. Nitrate nitrogen, even though only presents in domestic and municipal wastewater in small concentration, is a product of nitrification processes and is harmful to human. Nitrate can be removed in denitrification process which occur in anaerobic condition. Plants also directly affect N_2O emission from constructed wetlands in similar ways as CH_4 emission. Plant can also uptake NO_3 which may result in less N_2O production via denitrification.

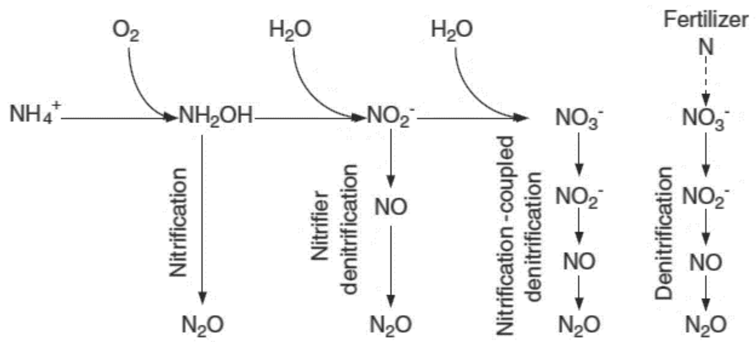

Figure 2.5 Simplify nitrous oxide production pathway (Ussiri and Lal, 2013)

The following table shows the effect of these factors on CH_4 and N_2O emissions from different studies. Effect of plants to CH_4 and N_2O fluxes differ between different studies which may be different by plant species and other environmental factors.

Table 2.2 CH_4 and N_2O influencing factors (IPCC, 2013b)

Increase/presence of factors/processes	CH_4	N_2O
Water/soil/air temperature	Increase in most cases[1-6]	No clear relationship[1-4,7,8]
Influent loading	Clear increase[9,10]	Decrease[9,10]
Aerenchymal plants	Increase[14-16] Decrease[17]	Increase[16,18] Decrease[16,19]
Pulsing hydrological regime	Clear decrease[9,20]	Increase[9,21,22] Decrease in some SF CWs[23]
Depth of water	Decrease[9,10]	Increase[9,10]
Source: [1] (Mander et al., 2003); [2](Mander et al., 2005); [3] (Teiter and Mander, 2005); [4](Søvik et al., 2006); [5] (Kayranli et al., 2010); [6] (VanderZaag et al., 2010); [7] (Søvik and Kløve, 2007); [8](Fey et al., 1999); [9] (Mander et al., 2011); [10] (Yang et al., 2013); [11] (Tanner and Kloosterman, 1997); [12] (Tai et al., 2002); [13] (Hunt et al., 2009); [14] (Inamori et al., 2007); [15] (Inamori et al., 2008); [16] (Wang et al., 2008); [17] (Maltais-Landry et al., 2009); [18] (Rückauf et al., 2004); [19] (Silvan et al., 2005); [20] (Altor and Mitsch, 2008); [21] (Jia et al., 2011); [22] (Van de Riet et al., 2013); [23] (Hernandez and Mitsch, 2006)		

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2.6 Measurement of Soil Gas Fluxes

The most common measurement technique is enclosure method (Livingston and Hutchinson, 1995, Ussiri and Lal, 2013). Enclosure or chamber method is based on the changes of gas concentration within the enclosed space over a short sampling period. Longer sampling period will suppress the fluxes as pressure builds up within the enclosed space (Ussiri and Lal, 2013). The advantage of enclosure technique is its low cost and the simplicity of operation. Several studies have used chamber methods to measure CH_4 emission in both surface flow constructed wetlands (Gui et al., 2007, Johansson et al., 2003, Johansson et al., 2004, Mander et al., 2003, Søvik et al., 2006, Liikanen et al., 2006) and subsurface flow constructed wetlands (Tanner and Kloosterman, 1997, Mander et al., 2003, Teiter and Mander, 2005, Søvik et al., 2006, Gui et al., 2007, Liu et al., 2009)

Static chamber method is also commonly used for nitrous oxide measurement (Ussiri and Lal, 2013). However, as nitrous oxide is usually measured in lower concentrations than methane, accurate determination of fluxes using static chamber method may require adjustment in sampling frequency. Other soil gas measuring methods include Sub-surface method, Mass balance approach and micrometeorological approach.

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Chapter 3 Materials and Methods

3. 1 Experiment Design

The experiment was conducted in 2 parts. Part 1 consists of 2 experimental-scale constructed wetlands, a vertical subsurface flow unit (VF) and a horizontal subsurface flow unit (HSSF), operated and monitored simultaneously. This part was designed to answer the first hypothesis which stated that constructed wetlands with subsurface vertical flow system generate more N_2O and less CH_4 than horizontal flow systems. The second part consisted of 4 experimental-scale VF units with vary plant harvest patterns. The second part was designed to test the second hypothesis which stated that plant harvesting effects in lower annual nitrous oxide and methane fluxes

3.1.1 Part 1 Experimental Setup

In part 1, a vertical subsurface flow (VF) and a horizontal subsurface flow (HSSF) constructed wetland units were built at Environmental Engineering Department, Chiang Mai University, Thailand. Both units operated simultaneously from August 2013 to May 2014 with the same cross section areas, hydraulic loading rate and plant density.

The VF unit has internal dimension of 1.2-meter deep with a rectangular surface area of 1.4 x 1 m^2 with wall thickness is approximately 10 cm. The unit was filled with fine sand with thin layers of 10 cm of small gravel at the top and the bottom. The unit was planted with 12 clumps of umbrella sedge with approximately 5 stems per clumps initially.

Figure 3.1 The VF CW system

Inflow distribution pipes consist of networks of perforated PVC pipes with internal diameter of 18 mm. The inflow pipes were placed above thin layers of small gravels and can be removed during gas sampling. The outlet pipes also consist of perforated PVC pipes of 18 mm diameter. The outlet pipes were placed horizontally at the bottom of the tank buried in a 10-cm layer of small gravel. A layer of geotextile fabric was placed on top of the gravel layer to prevent plant roots and sand from clogging and damaging the outlet pipes. The pipes were made perforated by drilling small holes of 2 mm diameter on 4 sizes around the pipes.

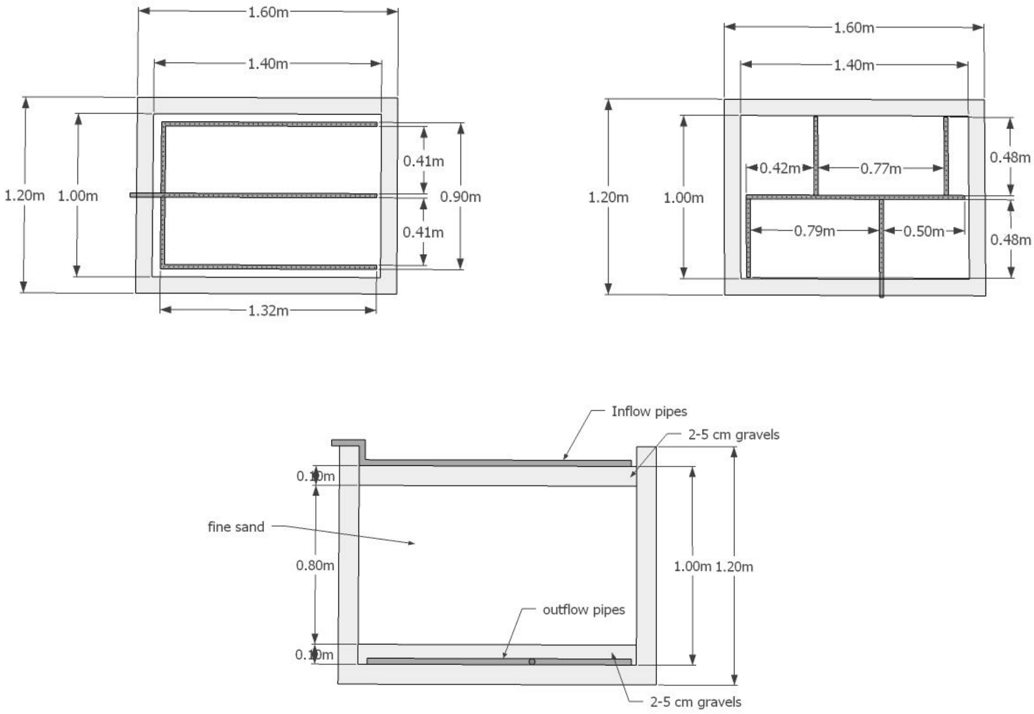

Figure 3.2 Top view of the VF inflow (top left) and outflow piping networks (top right) and, side view of the VF system (bottom)

The HSSF unit is 0.6 m depth and 0.6 m width and 2.3 m in length. Small gravel is used as the media in the vegetation zone. The inlet and the outlet zones were filled with large gravels with approximate size of 5 cm.

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Figure 3.3 The HSSF CW system

Influent entered the unit via a 0.5 m long perforated PVC pipe with 18 mm diameter. The effluent flow out of the unit at the opposite side along the 2.3 m length of the inlet via another 0.5 m long perforated pipe. All inflow and outflow pipes were made perforated by drilling holes at approximately every 5 cm along the pipes on four sides around the pipes using 5 mm drill bit. Three 70 cm long pipes were inserted vertically into the media at the inlet zone, vegetation zone and the outlet zone. The bottom 60 cm of the pipes were under the media surface with the top 10 cm stick above the media. The top ends of the pipes were sealed at all time except during sampling.

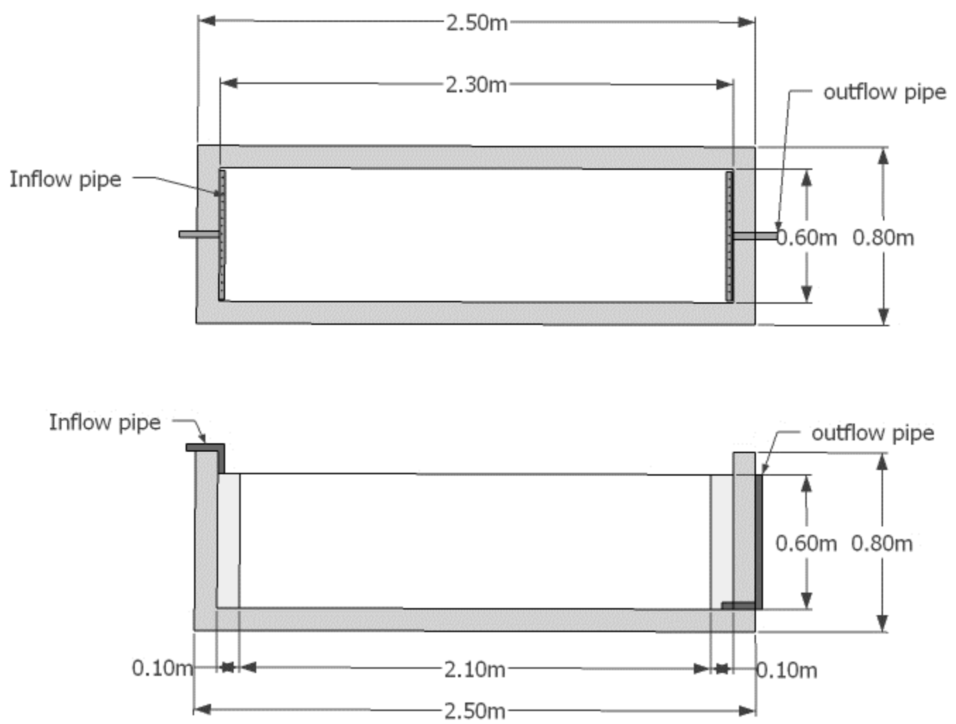

Figure 3.4 Top view (top) and side view (bottom) schematic drawing of the HSSF system

Table 3.1 shows the characteristics of wastewater used in this study. Municipal wastewater obtained from the equalizing tank from Chiang Mai university wastewater treatment facility was used. The wastewater collection system was a combined sewage system which also receives storm water. According to Thailand's Pollution Control Department (PCD), the wastewater average TN concentration of 28 mg/L was above the municipal effluent discharge standard of 20 mg/L. The average COD concentration of 126.8 mg/L also exceeded the maximum discharge standard. The maximum discharge standard for was given as BOD of 20 mg/L. According to PCD, typical COD to BOD ratio for municipal wastewater in Thailand is approximately 2-4. Therefore, BOD concentration of 20 mg/L can be approximated to COD concentration of 40 to 80 mg/L. Thus, the influent used in this study could be considered as low strength municipal wastewater which required to be treated before being discharged.

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Approximately 400 liters of wastewater was delivered to the site every 2-3 days and was stored in two 200-L storage tanks. Peristaltic pumps were used to deliver the wastewater from the storage tank to the units at the controlled rate. The VF system was fed intermittently every 4 hours and the HSSF system received wastewater continuously. The peristaltic pump connected to the VF unit was switched on and off every 4 hours using a timer switch. Hydraulic loading for both units was at 5 cm day^{-1}. HSSF has the hydraulic retention time (HRT) of approximate 11.8 days. The influent has the following characteristics.

*Table 3.1 Part 1 Influent characteristics (n = 20, *n=8)*

	range	mgL^{-1} ±SD
COD	89.0 - 182.3	126.8±31.4
TOC*	55.2-105.6	77.9±16.4
SS	30.0-92.0	53.3±26.5
TKN	20.7 - 36.9	27.9±4.8
NH_3	14.6-29.7	23.45±5.6
NO_3	0.00-0.10	0.06±0.02

Both units were planted with umbrella sedge (*Cyperus alterniforlius* L.) at 34.3 plants per square meter. Umbrella sedge is a common local macrophytes found naturally near water with large air passages in the stems which allowed the plant to be able survive in saturated soil condition.

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3.1.2 Part 2 Experimental Setup

For the part 2 experiment, 4 experimental scale constructed wetlands were setup outdoor at Chiang Mai University from January to September 2014. The four systems varied plant conditions and had the same system configurations and operational setups.

The dimensions of each unit are identical with 0.3 x 0.3 m^2 surface area and 1 m tall rectangular tank. The tank made of clear acrylic sheet and covered with black plastic sheets. The tanks were filled with sand as the media. Layers of small gravels (2-5 cm) were placed on the top and the bottom of each tanks. Perforated pipes were inserted horizontally at 10 cm, 50 cm and 98 cm depth in all the 4 units so that changes of carbon, nitrogen and DO, ORP and pH levels along the vertical profiles can be measured. The pipes were tightly sealed at all time except during sampling. The first tank was unplanted. The second, third and fourth tank were planted with *Cyperus alternifolius* L. (umbrella sedge) with 2-months, 4-month and 8-month harvesting intervals respectively.

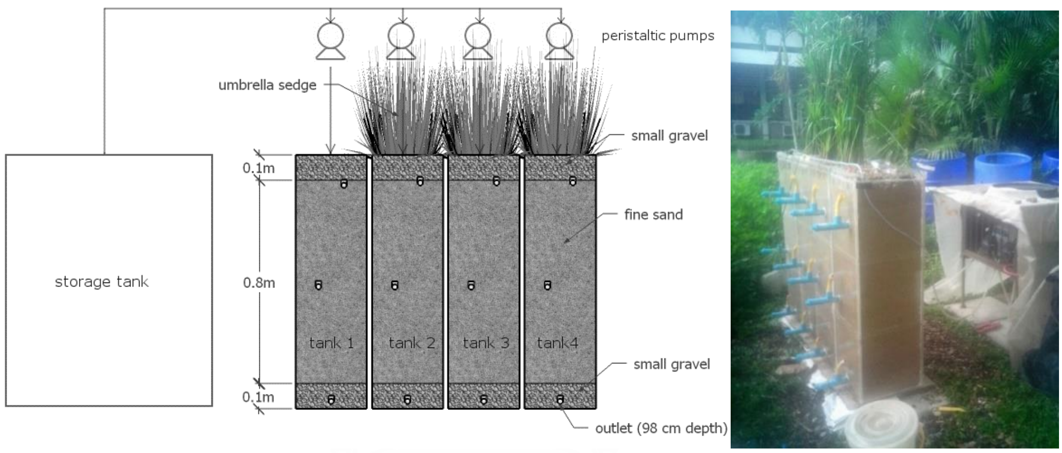

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Figure 3.5 Schematic drawing of the experiment, consists of an influent storage tank, 4 peristaltic pups and 4 experimental scale vertical flow constructed wetlands (left), actual system setup (right)

Wastewater used in the experiment was obtained from Chiang Mai University wastewater treatment facility's equalizing tank every 2 - 3 days. The wastewater was then stored in a storage tank next to the constructed wetland tanks. 9 liters of wastewater was fed to each system every day at 4-hour interval. Each feed, 1.5 liter of wastewater was flooded across the entire surface of each tank and seeped slowly through the system vertically. Peristatic pumps with a timer switch were used to control the inflow rates and the feeding time. Effluent was collected at the bottom (98 cm depth) of the systems. The characteristics of influent used in this part 2 experiment are shown in Table 3.2.

Table 3.2 Influent Characteristics (n=17)

	Range (mg L^{-1})	Mean (mg L^{-1})	±SD
TOC	62.7-89.9	74.5	±8.8
TN	34.3-55.8	40.0	±6.2
Organic-N	5.4-22.0	11.5	±4.1
NH_3	21.69-32.50	27.6	±3.3
NO_3	0.03-0.08	0.05	±0.02

3.2 Water sampling and analysis

In the 2 parts of this study, COD, TOC, TKN, NH_3, NO_2, NO_3, DO, pH and ORP were monitored. TOC was analyzed by a Shimadzu TOC analyzer while COD, TKN, NH_3, NO_2 and NO_3 were analyzed by the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Dissolved oxygen (DO) was measured simultaneously with gas sampling, using Clean Instrument® DO200 DO meters. Redox potential (OPR) was measured with Clean Instrument® OPR 30 tester.

For part 1, influent entered the HSSF tank at point 1 and the effluent was collected at point 5. TOC and DO were also monitored at inlet, middle and outlet zones (points 2, 3 and 4 in figure 3.7). For the VF unit, influent samples were collected at the surface and the

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effluent samples were collected at the bottom of the tank. Both influent and effluent samples were collected every week during the first 4 months of the experiment and once every 2 weeks during the last 4 months of the experiment. The samples were stored at 4 degrees Celsius and analyzed within 1 day.

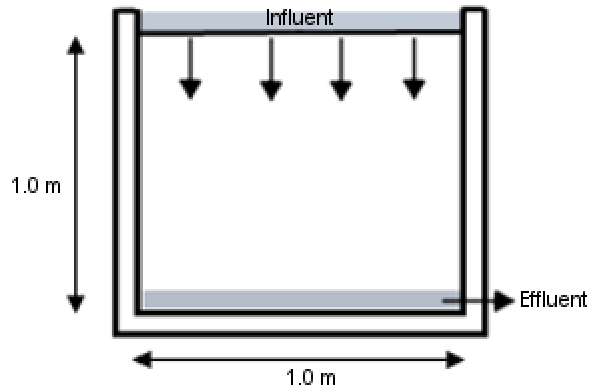

Figure 3.6 side view of VF water and gas sampling points

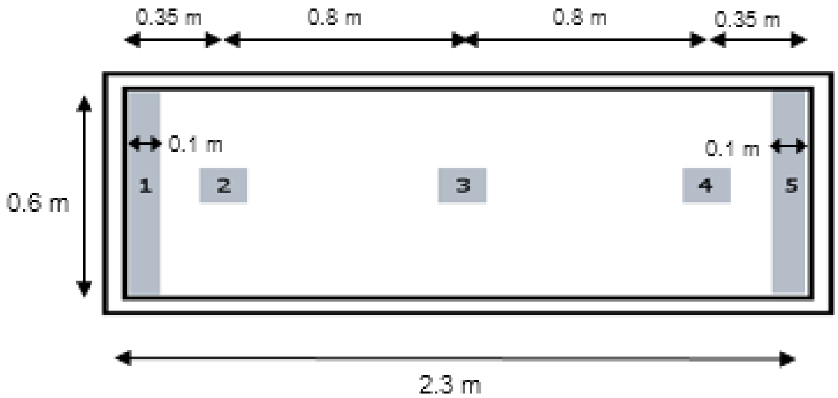

Figure 3.7 top view of HSSF water and gas sampling points

Water samples for the 4 VF tanks in part 2 were analyzed every 2 weeks. Influent samples were collected from the 200-L influent storage tank. The outlet for the effluent of the 4 VF tanks locate at the bottom of the tank which was 98 cm below the substrate surface. Each tank has 2 additional sampling point at 10 cm and 50 cm depth which allow for the monitoring of changes in water quality along the vertical profile.

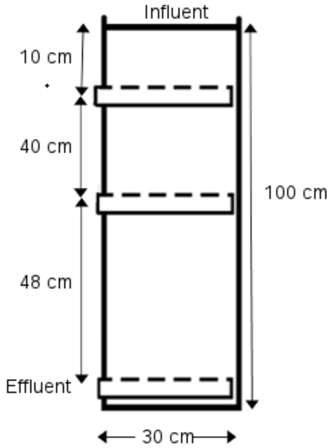

Figure 3.8 Piping diagram and water sampling points

Percent pollutant mass removal is used to represent water treatment performance of the CWs which considers the differences of water loss in each tank (Kadlec, 2009).

$$\%\text{mass removal} = 100 \times \frac{Q_iC_i\text{-}Q_oC_o}{Q_iC} \qquad \textit{(Equation 3.1)}$$

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Where Q_i is inflow rate (liter per day), Q_o is the outflow rate (liter per day), C_i is the influent concentration and C_o is the effluent concentration.

Since the influent total nitrogen concentration is approximately the sum of organic and ammonia nitrogen, the nitrified nitrogen (NITR) and denitrified nitrogen (DENITR) were calculated by the following equations:

(NITR) = (Influent TN) – (Effluent TKN) *(Equation 3.2)*

(DENITR) = (Influent TN) – (Effluent TKN) *(Equation 3.3)*

The percentages of nitrification (%NITR) and denitrification (%DENITR) were calculated by determining the ratio between nitrified nitrogen and the influent total nitrogen and the ratio between denitrified nitrogen and the influent total nitrogen with the assumption that the nitrogen accumulated in the systems were negligible.

(% NITR) = (NITR)/(Influent TN) *(Equation 3.4)*

(% DENITR) = (DENITR)/(Influent TN) *(Equation 3.5)*

3.3 Gas sampling and analysis

3.3.1 Gas sampling procedures

Methane and nitrous oxide fluxes were measured by static chamber method (Ussiri and Lal, 2013). Two identical chambers of 0.3 x 0.3 x 1 m^3 (figure 3.10) made of acrylic sheet were used in this study. A thermometer and a fan were glued on the internal wall of each chamber. The fan was connected to a 9-volt battery and was switched on during sampling to ensure through mixed of gas inside the chamber (Tai et al., 2002). A 63.5 mm diameter hole was cut on the acrylic sheet at the top of the chamber to make an airtight socket fitted with a rubber septum. Bases, also known as anchors, were used to keep the enclosed space inside the chamber sealed without disturbing the substrate. The height of the bases used in part 1 were 15 cm with the bottom 10 cm anchored into the substrates at each sampling point. The bases made of clear acrylic with narrow grooves around the top rim so that the chamber can be placed and sealed with water during sampling.

Figure 3.9 Pre-inserted chamber base

Figure 3.10 The static closed flux chambers used in this study

Methane and nitrous oxide fluxes were measured between 9 am to 11 am, every 2 weeks from January to April 2014 and from January to September 2014 for part 1 and part 2 respectively. To estimate the daily flux, sampling was conducted during the hours which the flux is believed to represent the daily mean as fluxes are influenced by ambient temperature (Livesley et al., 2008, Alves et al., 2012). For this condition, it is suggested that 9 am to 10 am is the most suitable time (Alves et al., 2012). Part 1's VF unit was measured at a single point in the middle of the unit's surface area. The HSSF unit was measured at 3 different locations, the inlet zone, the middle zone and the outlet zone (sampling points 2, 3 and 4 in figure 3.7, respectively) which are the same location for DO monitoring. For part 2, the anchors were embedded into the tanks and the entire surface area of the tanks was enclosed.

For each flux measurement, air samples were collected from inside the chamber 4 times at 10 min interval (at 0th, 10th. 20th and 30th minute) via the rubber septum fitted at the top of the chamber by using a syringe. The samples were then stored in 10 mL BD Vacutainer® rapid serum tube (figure 3.11).

Figure 3.11 Vacuum blood collection tubes were used as gas sample containers

The stored samples were taken to Kasetsart University, Department of Environmental Engineering's laboratory to be analyzed by Perkin Elmer Clarus 580 gas chromatograph (GC), equipped with auto sampler and flame ionization detector (FID) and electron capture detector (ECD) installed with Heyesep D column with helium as the carrier gas. CH_4 and N_2O were detected by FID and ECD respectively. The GC was pre-calibrated for the areas under the graphs with the associated gas concentrations. The standards of 10 ppm and 0.99 ppm were used for CH_4 and N_2O respectively prior to each analysis.

3.3.2 Flux calculation

The concentrations of CH_4 and N_2O obtained from the GC were in part per million (ppm) which need to be converted to $mg.m^{-3}$ first before plotting the linear regression graph to obtain the flux. The conversion can be done by multiplying the concentration in ppm with the molecular mass for each gas (16.04 g/mol for CH_4 and 44.01 g/mol for N_2O) and divided by gas volume at STP (0ºC at 1 atm = 22.45 liters/mol).

The concentration at 0^{th}, 10^{th}, 20^{th} and 30^{th} minute of each flux measurement were plotted against time of sampling in a linear regression graph where the flux equals to the change of the concentrations over time.

$F = V/A \times dC/dt \times k$ *(Equation 3.6)*

Where

F = flux ($mg.m^{-2}.hr^{-1}$)

C = gas concentration ($mg\ m^{-3}$)

V = chamber volume (m^3)

A = area enclosed by chamber (m^2)

t = time of sampling (30 mins)

k = time conversion factor

CH_4 and N_2O fluxes in this study are presented as mg CH_4-C.$m^{-2}.hr^{-1}$ and mg N_2O-N.$m^{-2}.hr^{-1}$, respectively. CH_4-C and N_2O-N can be converted from CH_4 and N_2O with the following equations (IPCC, 2013a):

mg.CH_4-C = mg.CH_4 x (12/16) *(Equation 3.7)*

mg.N_2O-N = mg.N_2O x (28/44) *(Equation 3.8)*

Carbon dioxide (CO_2) equivalents were calculated as follow:

1 kg N_2O = 298 kg CO_2 equivalents

1 kg CH_4 = 25 kg CO_2 equivalents

Emission factors were also calculated as follow (Mander et al., 2014)

$EF_{CH4} = (CH_4\text{-}C_{emission}/TOC_{in}) \times 100(\%)$ *(Equation 3.9)*

$EF_{N2O} = (N_2O\text{-}N_{emission}/TN_{in}) \times 100(\%)$ *(Equation 3.10)*

TOC_{in} and TN_{in} are cumulative inflow TOC and TN in g/m^2 from the beginning to the end of the experiment. CH_4 $C_{emission}$ and N_2O $N_{emission}$ are cumulative of the daily fluxes throughout the experimental period of 8 months. In the 8-month experimental period, CH_4-

C and N_2O-N fluxes were determined every two weeks, thus, the total of 17 measurements from each tank. CH_4-$C_{emission}$ and N_2- $N_{emission}$ were estimated from the 14-days cumulative values of 17 measurements.

3.4 Plant Harvest

Plant in tank 2 was harvested every 2 months and every 4 months for tank 3. Each harvest was done 1 day prior to the scheduled bi-weekly gas sampling. Harvest was done by cutting the stems of the plant by a glass clipper at approximately 3-5 cm above the substrate surface. At the end of the experiment which was after tank 2's 4^{th} harvest, tank 3's 2^{nd} harvest, all plants in tank 2, 3 and 4 were removed and analyzed for dry weight, carbon content and nitrogen content.

Figure 3.12 Tank 2 approximately 1 week after the first harvest (left), the 2nd harvest (middle) and the 3rd harvest (right)

Figure 3.13 Tank 3 approximately 1 week after harvest

3.5 Plant biomass

In part 1, each system was planted with 12 clumps of umbrella sedge. Each clump consisted of 5-7 stems with similar height (20 to 30 cm). At the beginning of the experiment, the initial plant dry weight was estimated from the sample of 18 stems, taken randomly from the prepared plants to be used in the experiment. At the end of the experiment, plants were harvested and the total dry weight of the harvested plants were determined for each system.

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In part 2, tank 2, 3 and 4 were planted with 4 clumps of 5-6 stems of similar height umbrella sedge (20-30 cm) each where tank 1 was unplanted. Samples of 9 stems were taken randomly from the prepared plants. The samples were separated into root samples, stem samples and leave samples before oven dried and weighed. After each harvest, the harvested parts were separated into leaves and stems before measuring the dry weight. At the end of the experimental period all plants in tank 2, tank 3 and tank 4 were harvested and the dry weights were estimated for root, stem and leave samples for each tank.

Plant dry weight was determined by drying the plant parts in an oven at 90°C for 2 days before grinding and weighed. Carbon and nitrogen contents in plants were determined for plants

3.6 Microbial Analysis

Media samples were taken from 4 reactors and plant root samples were taken from reactor 2, 3 and 4. At the end of the experiment, composite substrate samples were taken from mixed depth within the systems from 3-4 random position with a PVC pipes and stored in polypropylene tubes. Plants were removed from the tanks at the end of the experiment and root samples from composite depths were collected and stored in polypropylene tubes. The samples were analyzed for nitrifying, denitrifying and methanogenic bacteria species by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) method at the Department of Environmental Engineering laboratory at Kasetsart University, Bangkok.

Genomic DNAs from bacteria are extracted from soil sample following the method by Zhou et al. (1996). 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 (Khemkhao et al., 2012). Amplification and electrophoresis procedures were the same described in Boonnorat et al. (2014).

Dice index of similarity (Cs) is used to determine the similarities between DGGE fingerprints from each system. Dice index of similarity (Cs) can be determined from the following equation:

$$Cs = 2j/(a+b) \qquad \textit{(Equation 3.11)}$$

where j is the number of bands that present in both samples A and B, and a and b are the number of bands in sample A and sample B respectively (LaPara et al., 2002). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram is used to represent the clustering of the samples.

3.7 Statistical Analysis

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.

Chapter 4 Comparison between HSSF and VF Systems

4.1 Treatment performance of CWs

Both systems were monitored and the treatment performances were analyzed for the total of 9 months (from August 2013 until April 2014). Removal efficiencies for both systems were unstable in the first 4 months of the study and became more stable in the last 5 months of the operation.

Table 4.1 shows the influent and effluent characteristics from HSSF and VF systems during the study. The VF system shows better removal efficiency for TOC and COD with the average of 94.4% and 88.1% for COD and 86.6% and 83.1% for TOC for the VF system and the HSSF system respectively. Total nitrogen (TN) was also removed with higher efficiency in the VF system at 92.0%, the HSSF total nitrogen removal efficiency is 84.4%. NO_3 concentration increases in the VF system whereas HSSF effluent concentration remains approximately the same as the influent concentration.

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Table 4.1 Part 1 Influent and Effluent Characteristics

	Influent		VF Effluent		HSSF Effluent	
	range (mgL^{-1})	average (mgL^{-1}±SD)	range (mgL^{-1})	average (mgL^{-1}±SD)	range (mgL^{-1})	average (mgL^{-1}±SD)
COD	89.0 - 182.3	126.8±31.4	4.2 - 17.0	9.0±4.8	12.7 - 33.9	21.2±8.8
TOC	55.2-105.6	77.9±16.4	9.2-15.8	12.9±2.2	12.1-21.1	17.3±2.9
SS	30.0-92.0	53.3±26.5	0.4-2.5	1.1±0.7	1.0-4.0	2.3±1.1
TKN	20.7 - 36.9	27.9±4.8	0.11 - 0.56	0.5±0.3	1.68 - 5.04	2.7±1.1
NH_3	14.6-29.7	23.45±5.6	0.30-0.56	0.5±0.1	0.45-3.02	1.8±0.9
NO_3	0.00-0.10	0.06±0.02	1.90-5.75	4.2±0.60	0.01-0.14	0.06±0.03
DO	0.1-0.3	0.2±0.08	2.1-3.8	2.9±0.61	1.6-3.8	2.5±0.81

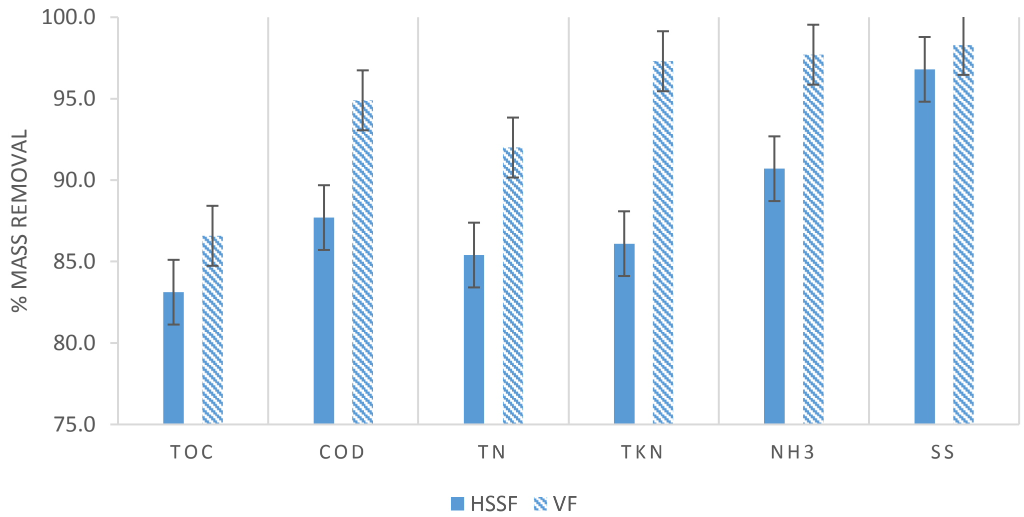

Figure 4.1 Part 1 removal efficiencies

TOC and COD removal efficiencies for both systems fall in the same ranges with other studies of similar conditions. The HSSF's removal efficiency of 87.7% is close to results from other studies of 75.5-77.2% (Zurita et al., 2009) and 81.1% (Abou-Elela et al., 2013). The VF shows slightly higher removal efficiency than other studies with 94.4%

comparing to 92.9% by Abou-Elela et al. (2013) and 88.9% by Zurita (2009). However, the VF CWs in both studies were slightly shallower (0.7 meter and 0.85 meter) and both used gravels as the media. The height of the VF CW and the media used in this study may contribute to system' better filtration ability. Furthermore, the removal efficiencies from Abou-Elela and Zurita's studies were averaged from the samples measured over 36 and 9 months respectively which were longer than the operational period in this study. According to Zurita (2009) the VF system can remove organic carbon (measured as COD, BOD and TOC) more efficiently due to high oxygen transfer rate in the system and the filtration capability, especially in new systems (Zurita, 2009).

For convenient comparison with other studies, the ratio between TOC and COD were determined for the both systems based from 4 samples measured weekly from 2nd to 28th of January, 2014. The average ratios were 2.5, 2.4 and 2.3 for the influent, the VF effluent

Table 4.2 COD treatment performance (n=8)

	Influent	VF	HSSF
Range (mg/l)	89.0 - 182.3	4.2 - 17.0	12.7 - 33.9
Mean (mg/l)	126.8	9	21.2
SD	31.4	4.8	8.8
Removal rate ($g/m^2.d$)	-	4.65	3.89
Removal efficiency (%)	-	94.4	87.7

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Table 4.3 TOC treatment performance (n=8)

	Influent	VF	HSSF
Range (mg/l)	55.2-105.6	9.2-15.8	12.2-21.8
Mean (mg/l)	77.9	12.9	17.3
SD	16.4	2.18	2.92
Removal rate (g/m^2.d)	-	3.38	3.26
Removal efficiency (%)	-	86.6	83.2

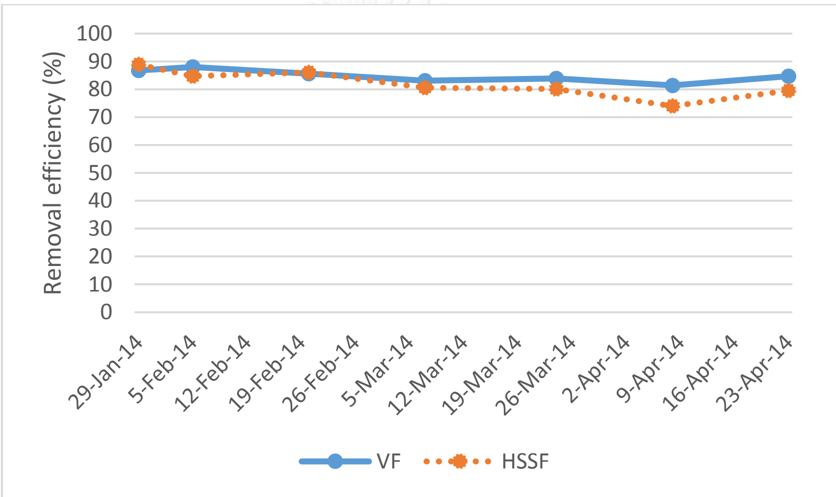


Figure 4.2 COD removal efficiency of the VF and the HSSF units (n=8)

TOC concentrations were also measured along the horizontal distance from the inlet in the HSSF system. The results show that most organic carbon was removed at the vegetation zone and where more than 44.8 percent of the influent concentration was removed.

Table 4.4 Average TOC concentration reduction along the distance from the inflow in the HSSF system (n=8)

	range	average (mg/L)	±SD
Influent	55.2-105.6	77.87	±16.4
HSSF inlet	50.6-91.7	72.63	±15.8
HSSF middle	24.3-50.7	37.84	±9.5
HSSF outlet	18.8-27.5	22.45	±2.8
HSSF effluent	12.15-21.1	17.29	±2.9

Total nitrogen (TN) was removed with slightly higher efficiency in the HSSF unit than the VF unit. However, TKN and NH_3 removal efficiencies are higher in the VF unit where there was a large increase in NO_3 concentration in the effluent from the VF system.

Table 4.5 Total nitrogen treatment performance (n=8)

	Influent	VF effluent	HSSF effluent
Range (mg/l)	40.4-67.8	4.46-5.86	2.21-7.24
Mean (mg/l)	51.4	5.41	4.57
SD	9.1	0.54	1.69
Removal rate (g/m^2.d)	-	2.30	2.34
Removal efficiency (%)	-	93.5	91.7

TKN was removed at a better rate by the VF unit than the HSSF unit. TKN concentration in the HSSF effluent was higher and more fluctuated than the VF system. Higher oxygen availability in the VF unit can be the main factor for the higher TKN removal efficiency by the VF system. TKN is measured as a sum of organic nitrogen and ammonia nitrogen. Organic nitrogen and ammonia nitrogen are removed predominantly during ammonification and nitrification respectively. These two processes are aerobic processes which means that oxygen is crucial and can be responsible for the higher TKN removal efficiency in the VF system.

Table 4.6 TKN treatment performance (n=8)

	Influent	VF effluent	HSSF effluent
Range (mg/l)	20.72-36.96	0.11-1.24	1.68-5.04
Mean (mg/l)	27.93	0.5	2.68
SD	4.79	0.32	1.08
Removal rate ($g/m^2.d$)	-	1.4	1.3
Removal efficiency (%)	-	98.6	93

Table 4.7 NH_3 treatment performance (n=8)

	Influent	VF effluent	HSSF effluent
Range (mg/l)	14.56-30.80	0.30-0.57	0.45-3.02
Mean (mg/l)	23.45	0.46	1.83
SD	5.55	0.08	0.85
Removal rate ($g/m^2.d$)	-	1.2	1.1
Removal efficiency (%)	-	98.45	94.3

Most studies also found an increase in NO_3 concentration in the effluent from VF CWs (Vymazal, 2006; Abou-Elela, 2013) as VF systems are commonly unable to promote denitrification where NO_3 is reduced to N_2 because of the high level of dissolved oxygen within the system. In this study, NO_3 increased from a trace amount of 0.05 mg/L in the influent to the average of 4.2 mg/L in the VF system and remain 0.05 mg/L in the HSSF effluent. The high NO_3 concentration in the VF effluent can also be supported by the higher percent nitrification, where ammonia nitrogen is oxidized to NO_3, whereas percent denitrification, where NO_3 is reduced to N_2, for both systems are approximately the same (table 4.9).

High effluent nitrate concentration in the VF systems can be reduced in hybrid systems that can further introduce anoxic condition to the effluent. However, in this study, despite the increase in the effluent nitrate concentration, the concentrations of 3.5-6 mg/L are still well below the PCD's standard limit for drinking water obtained from groundwater of 45 mg/L.

Table 4.8 NO_3 treatment performance (n=8)

	Influent	VF effluent	HSSF effluent
Range (mg/l)	0.03-0.09	3.53-4.96	0.01-0.08
Mean (mg/l)	0.05	4.45	0.05
SD	0.02	0.6	0.03

Table 4.9 Percent nitrification and percent denitrification between the two systems (total operation = 238 days)

	HSSF		VF	
TNin	900.1	g	900.1	g
TNout	63.7	g	69.4	g
TKN out	31.4	g	6.4	g
Nitri	836.4	g	830.7	g
Denitri	868.7	g	893.7	g
%nitri	96.5	%	99.3	%
%denitri	92.9	%	92.3	%

Total nitrogen's removal efficiency in this study are higher than other studies (Vymazal, 2006; Zurita, 2009) but still within the range reported by Stefanakis et al. (2014). This may be because of the low HLR of 5 $cm.day^{-1}$ which is lower than other studies and another reason may be that the system locates in the warmer climate. According to Tunçsiper (2009) lower HLR and warmer climates are two of the main factors that can significantly increase total nitrogen removal efficiency in constructed wetlands.

DO, pH, temperature and daily flow rates were being measured for both systems. The effluent from the VF unit had higher DO concentration and slightly more acidic than the effluent from the HSSF system. The HSSF had higher water loss which partly contributed to evapotranspiration as well as taken up by plants and microorganism.

Table 4.10 Comparison of DO, Temperature, pH and flow rate between the VF and HSSF system

	Influent	VF effluent	HSSF effluent
DO (mg/L)	0.2	2.9	2.5
Temperature (°C)	25.6	27.4	25.9
pH	7.29	6.56	7.13
daily flow rate (liter/day)	70	55.3	50.9

In addition to the effluent concentration, DO concentrations along the distance from the inflow in the HSSF unit were also measured. DO concentrations increase almost linearly from the inlet zone (0.1 m from the inflow) to the outflow (2.3 m) with R^2 of 0.95.

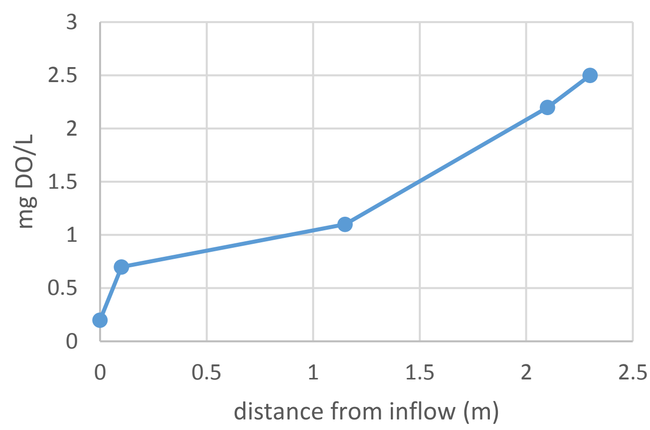


Figure 4.3 DO concentrations along the distance from the inflow

4.2 Greenhouse gas emission

The average CH_4 flux from 3 sampling points in the HSSF system is 7.13 mg CH_4-C.m^{-2}.hr^{-1} and the average CH_4 of 4.05 mg CH_4-C.m^{-2}.hr^{-1} was found from the VF system. N_2O fluxes of 0.133 and 0.201 mg N_2O-N.m^{-2}.hr^{-1} were measured from the HSSF CW and the VF CW respectively. CH_4 flux measured near the inlet zone was the highest and the flux measured from the outlet zone was the lowest. There was no significant different between N_2O fluxes measured in the inlet zone and the middle zone. There was also no significant difference between N_2O fluxes measured from HSSF outlet zone and the VF system.

CH_4 can be generated during methanogenesis where organic carbon is consumed by microbial and emit gaseous methane. Methanogenesis occurs in anaerobic condition and is prohibited by oxygen in aerobic condition. Therefore, higher CH_4 flux from the HSSF system can be due to the system's anaerobic environment which is also supported by the lower DO concentration in the HSSF effluent. N_2O can be produced in both aerobic (nitrification process) and anaerobic (denitrification process) (Ussiri and Lal, 2013), which may be one of the reasons that no significance differences between fluxes from different CW types were found in most studies (Mander et al., 2014).

Table 4.11 Average CH_4 during wet and dry periods

	Dry*			Wet**		
	Range	Average	SD	Range	Average	SD
HSSF Inlet	7.34-13.33	10.56	2.16	4.04-4.44	4.19	0.22
HSSF Middle	6.71-12.44	9.84	2.85	2.78-4.34	3.64	0.79
HSSF Outlet	5.32-11.44	7.66	2.50	2.17-2.75	2.40	0.31
HSSF Average	6.45-11.63	9.35	2.09	3.05-3.84	3.41	0.40
VF	3.24-6.65	4.85	1.28	2.53-2.81	2.71	0.16

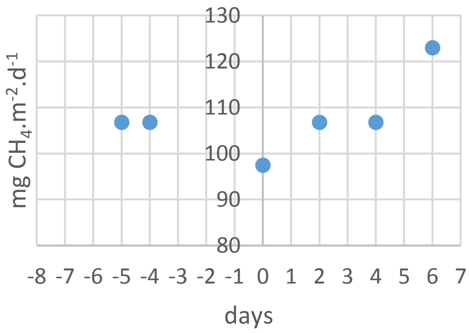
**n=5, **n=3*

Table 4.12 Average N_2O during wet and dry periods

	Dry*			Wet**		
	Range	Average	SD	Range	Average	SD
HSSF Inlet	0.115-0.161	0.134	0.023	0.066-0.100	0.078	0.019
HSSF Middle	0.062-0.226	0.147	0.068	0.064-0.067	0.066	0.002
HSSF Outlet	0.146-0.251	0.206	0.047	0.072-0.136	0.113	0.036
HSSF Average	0.117-0.203	0.163	0.031	0.067-0.101	0.086	0.017
VF	0.093-0.313	0.235	0.102	0.132-0.171	0.146	0.022

**n=5, **n=3*

During the dry period, when there was absolutely no rainfall at all for the entire period, both CH_4 and N_2O fluxes were found to be higher than the wet period which cover the later period of the experiment. The difference in the fluxes between dry and wet periods were significantly larger in the HSSF system than the VF systems for both CH_4 and N_2O emission. The average CH_4 fluxes measured during the wet period were 63.5% and 44% less than the dry period and N_2O fluxes in the wet period were 47% and 38% less than the dry period for HSSF and VF systems, respectively. CH_4 fluxes measured on the day with rain were found to be lower than the fluxes measure a few days before and after a rainy day (figure 4.4). However, the same effect was not found in N_2O fluxes from both systems (figure 4.5).



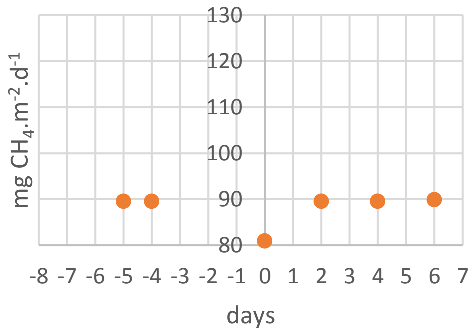
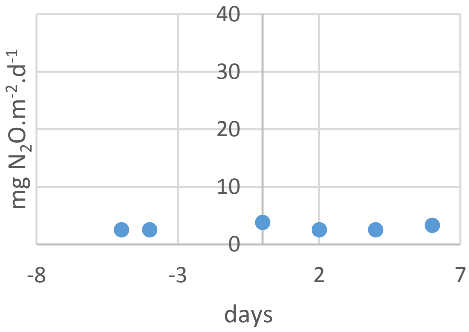


Figure 4.4 CH_4 fluxes measured days before (negative numbers) and after (positive numbers) rain in HSSF (left) and VF systems (right)



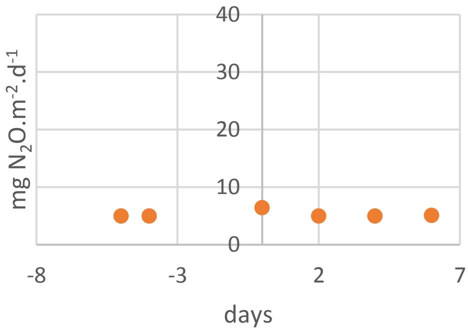


Figure 4.5 N_2O fluxes measured days before (negative numbers) and after (positive numbers) rainfall in HSSF (left) and VF systems (right)

TOC and TN concentrations in the influent were found to affect CH_4 and N_2O fluxes substantially as the drop in the CH_4 and N_2O fluxes significantly correlate the influent TOC and TN concentration. Pearson's R between influent TOC and CH_4 fluxes are 0.75 and 0.87 for HSSF and VF respectively. The same values for TN and N_2O correlations are 0.76 and 0.81 for HSSF and VF respectively with P-value less than 0.05. The effect also found in studies measuring CH_4 and N_2O fluxes from subsurface flow CWs in temperate and warm climates (Gui et al., 2007; Lui et al., 2009). According to Luo (2013), available C and N was suggested to be a significant and a more direct control of CH_4 and N_2O flux where the effect from other factors such as temperature and moisture can be more complicated. Similar TOC to CH_4 and TN to N_2O ratios were found between this study and other studies in different climate. These suggest that despite climate condition, effect of C and N availabilities on CH_4 and N_2O prevail.

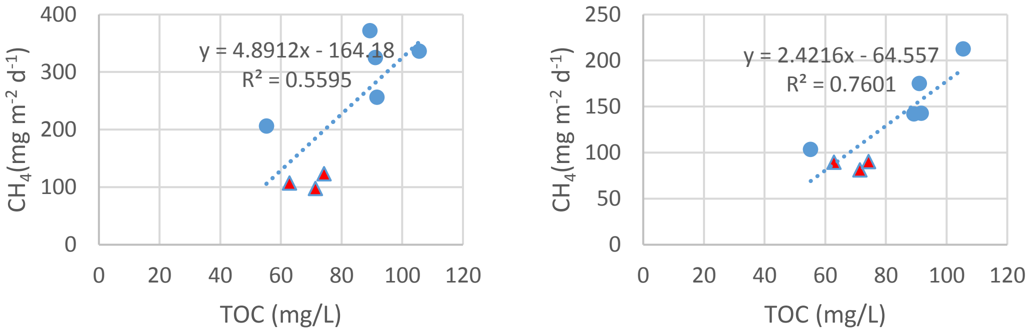


Figure 4.6 TOC and CH_4 relationship in HSSF (left) and VF (right)

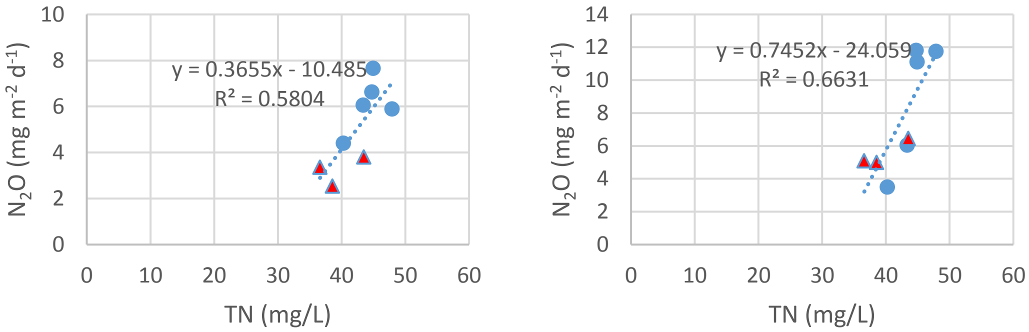


Figure 4.7 TN and N_2O relationship in HSSF (left) and VF (right)

A review on other studies measuring CH_4 and N_2O fluxes from VF and HSSF CWs in different climate zones shows that the fluxes measured in this study were slightly higher than fluxes measured in colder climate conditions. However, as suggested earlier that available C and N are a dominant factor effecting the fluxes, CH_4 and TOC and N_2O and TN ratios were compared. The CH_4 to TOC ratios from this study for both HSSF and VF CWs were similar to studies in temperate/warm climate which are higher than studies from boreal climate which suggests that higher CH_4 flux for the same TOC concentration in the influent can be expected in warmer climates.

Table 4.13 CH_4-C fluxes from different studies

Climate	CH_4-C ($mg.m^{-2}\ h^{-1}$)	CH_4/TOC (%)	Plants	CW type	Reference
temperate/ boreal	3	0.88	P. australis	VF	Teiter and Mander (2005), Søvik et al. (2006) and Mander et al. (2008)
boreal	5.4	0.38	No vegetation	VF	Søvik et al. (2006)
boreal	7.1	9.6	No vegetation	HSSF	Søvik et al. (2006)
temperate/warm	0.3	1.68	P. australis	VF	Gui et al. (2007)
temperate/warm	0.76	4.3	P. australis	HSSF	Gui et al. (2007)
temperate/warm	3	1.73	P. australis	VF	Liu et al. (2009)
temperate/warm	7	4	P. australis	HSSF	Liu et al. (2009)
tropical	2.9	-	Cyperus spp	SF	Chuersuwan (2014)
tropical	3.4-8.9	1.9	Cyperus alternifolius L.	VF	This study
tropical	4.6-15.5	2.8	Cyperus alternifolius L.	HSSF	This study

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Table 4.14 N_2O fluxes from different studies

Climate	N_2O-N ($mg.m^{-2}\ h^{-1}$)	N_2O/TN (%)	Plants	CW type	Reference
temperate/ boreal	0.225	0.021	P. australis	VF	Teiter and Mander(2005), Søvik et al. (2006) and Mander et al. (2008)
boreal	0.200	0.011	No vegetation	VF	Søvik et al. (2006)
boreal	0.894	3.01	No vegetation	HSSF	Søvik et al. (2006)
temperate/warm	0.123	0.096	P. australis	VF	Gui et al. (2007)
temperate/warm	0.073	0.042	P. australis	VF	Liu et al. (2009)
temperate/warm	0.4	0.23	P. australis	HSSF	Liu et al. (2009)
tropical	1.0	-	Cyperus spp	SF	Cheursuwan (2013)
tropical	0.07-0.20	0.22	Cyperus alternifolius L.	VF	This study
tropical	0.09-0.31	0.15	Cyperus alternifolius L.	HSSF	This study

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Chapter 5 : Effect of Harvest Patterns

5.1 Treatment Performance

The present of plants and the harvest patterns effect most monitored parameters in this study. Overall performance shows that the unplanted tank was able to removal organic carbon more efficiently whereas planted tanks could remove organic nitrogen and ammonia nitrogen more efficiently. Nitrate nitrogen increased in all four systems. The highest nitrate effluent concentration was measured from the unplanted tank. DO and ORP increases from the influent values which suggests that the four systems were in aerobic condition. DO concentrations increased more noticeably with the present of plants. pH values, however, remain between 6.7 to 6.8 in all four systems. Removal efficiencies are presented as percent mass removal (see section 3.1) in table 5.3. Total organic carbon (TOC) was removed most efficiently in the unplanted tank than the three planted tanks. Among the planted tanks, tank 2 where plant was harvested most frequently shows the lowest TOC removal efficiency. There is no statistically different between TOC removal efficiencies in tank 3 and tank 4, where plants were harvested at 4-month and 8-month intervals.

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Table 5.1 Influent and effluent DO, ORP, pH and temperature (average ±SD)

	influent	Effluent			
		tank 1	tank 2	tank 3	tank 4
DO (mg/l)*	1.39±0.49	2.01±0.39	3.35±0.48	3.80±0.56	4.02±0.47
ORP (mV)*	81.65 ±63.43	136.02 ±30.31	181.35 ±26.17	199.00 ±25.7	180.94 ±26.26
pH*	7.3±0.22	6.8±0.25	6.7±0.27	6.8±0.25	6.8±0.28
temperature (^{o}C)**	28.7	28.2	27.8	27.6	27.5

* n = 17, ** n = 18

Table 5.2 Influent and Effluent characteristics (average ±SD, n=18)

	Influent	Effluent			
		Tank 1	Tank 2	Tank 3	Tank 4
TOC (mg/l)	74.5±8.8	4.4±1.1	8.2±2.4	7.0±1.7	5.8±0.8
TN (mg/l)	39.7±6.2	12.3±1.8	9.6±1.0	6.8±0.7	8.4±2.0
Organic-N (mg/l)	11.5±4.1	2.22±0.9	1.10±0.7	1.03±0.9	1.08±0.9
NH_4^+ (mg/l)	27.6±3.3	4.13±0.9	2.65±0.7	2.50±0.7	2.63±0.8
NO_3 (mg/l)	0.05±0.02	6.0±0.9	5.83±1.3	3.28±0.4	4.43±1.2
Flow volume (l/day)	9.0±0.0	7.56±0.90	7.47±0.81	7.35±0.68	7.40±0.72

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Table 5.3 Removal efficiency (%)

	Tank 1	Tank 2	Tank 3	Tank 4
TOC	94.8	90.7	91.9	93.4
TN	73.8	79.9	85.9	83.1
Organic-N	80.8	91.0	92.6	91.4
NH_3	85.3	90.6	91.1	90.7
NO_3	-10×10^3	-9.6×10^3	-5.3×10^3	-7.2×10^3

DO concentration in the effluent increased considerably from the influent concentrations in all four systems. The increase is significantly higher in the planted tanks than the unplanted tank (figure 5.1). The effluent DO concentrations dropped slightly during the summer months (between 1st and 2nd harvest) which coincides with the influent DO concentrations. The increase of the effluent DO concentrations from tank 1 throughout the experiment period was minimal compared to tank 2, 3 and 4 where plants were present. In contrast to tank 1, 3 and 4 where the effluent DO concentrations slightly increased after the summer months, tank 2 effluent DO concentrations were significantly dropped below other systems' values.

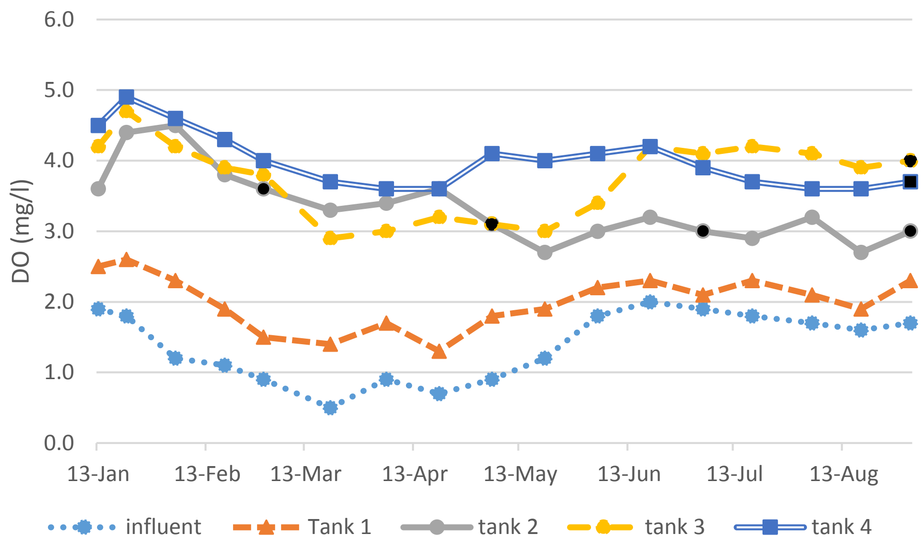

Figure 5.1 Influent and effluent DO concentrations (black dots represent data measured after each harvest)

DO concentrations increased to almost 5 times the influent concentration at the top 10 cm depth from the substrate surface. The difference between DO concentrations from the substrate surface to 50 cm depth in the planted and unplanted tanks was small. At the deeper zone, however, the DO concentrations were found to be significantly higher in the planted tanks (figure 5.2). The results are similar to Yang (2016) which found that DO concentration is higher in a harvested subsurface flow constructed wetlands than the unharvested system.

Figure 5.2 shows the changes of DO concentrations along the vertical profile of the systems. DO levels increased significantly after the influence was flooded across the entire system surface in all four tanks. The concentrations measured at the 10-cm depth below the surface were approximately 3 mg/L higher than the influence concentration. A slight decreased was found in the DO concentration measured at 50-cm depth with only small range between each system. DO concentrations at the effluent outlet however, differ considerably between systems. Only slight decrease of DO concentrations between the 50-cm depth and the effluent samples were found in the planted systems where a large drop was found in the unplanted system.

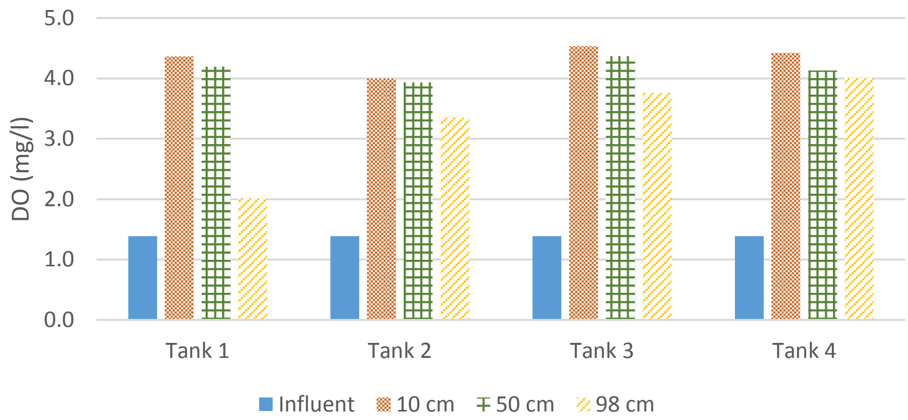

Figure 5.2 DO concentrations along the vertical profile

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ORP measured ranges between 65 to 315 mV. ORP comparison between tanks are concurring to DO concentrations where the lowest ORP value was measured from the unplanted system where the values for the 3 planted systems were similar. ORP values also reduces along the depth of the substrate. pH values reduce slightly from the influent level. There was no significant different between the values from the planted and unplanted system and at different depth.

TOC removal efficiency is significantly higher in tank 1 than the 3 planted units. Among the three planted units, TOC removal efficiencies are clearly affected by different harvest interval. The more frequent harvest pattern results in lower TOC removal efficiency. Tank 2 where plants was harvested every 2 months show the lowest TOC removal capability followed by tank 3 and tank 4.

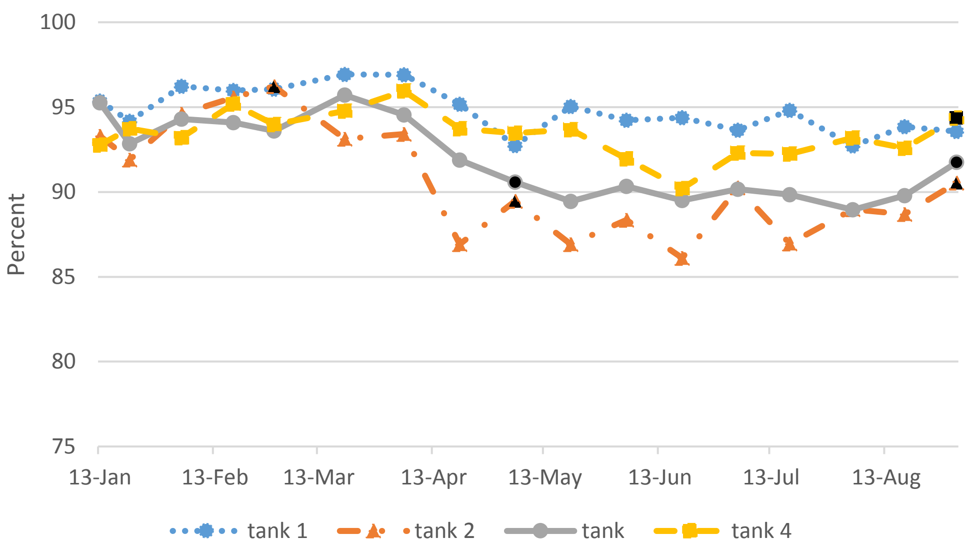

Figure 5.3 TOC percent mass removal (black dots represent data measured after each harvest)

Tank 2 removal efficiencies dropped dramatically after the first harvest and remain between 86% to 90% throughout the rest of the experiment. Apart from having the lowest removal efficiency, tank 2 TOC effluent concentrations are more dispersed than the other tanks. Tank 4 where plants were harvested at the end of the 8-month experimental period shows the most stable removal efficiency. Percent mass removal for each tank decreased after 2 months of operation. The decreasing rates are higher in the planted tanks than the unplanted tank. The highest decline was seen in tank 2 and tank 3. Tank 2 removal efficiency dropped after the 7th sampling which was right after the first harvest. This suggests that plant harvest also results in more fluctuation as well as lower the system removal efficiency.

Figure 5.4 shows the changes in TOC concentrations along the vertical profile. It can be seen than the majority of the reduction occurred between the surface to the 50 cm depth with approximately the same rate for the all of the four tanks which suggests that harvest has no effect on the rate of TOC removal along the vertical profile.

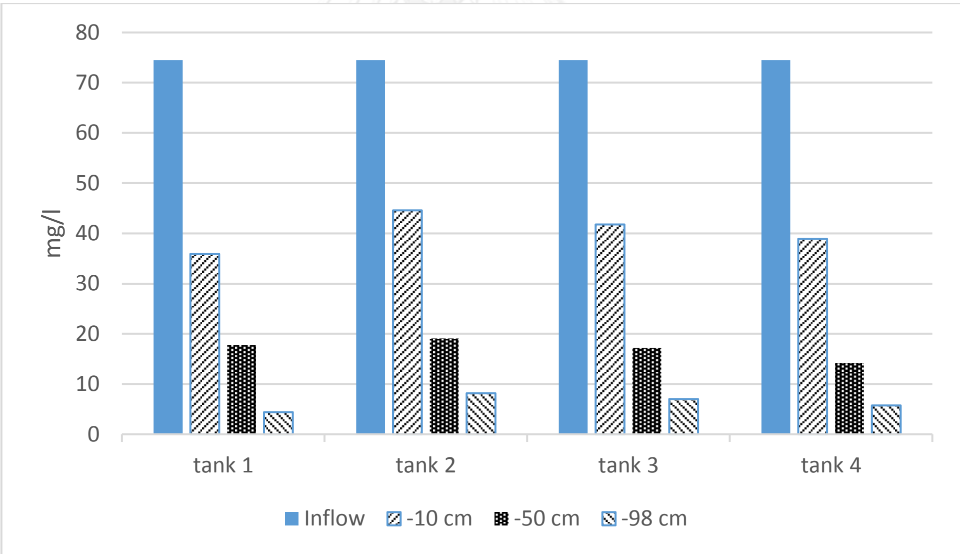

Figure 5.4 TOC concentrations along the vertical profile

TOC removal rates in a constructed wetland depend on several factors including DO concentrations, temperature and carbon availability. However, despite having the highest average DO concentration in the effluent, TOC removal efficiency in tank 2 was significantly lower than the other 3 tanks. According to Picek, et al. (2007), significant amount of carbon in constructed wetland comes from plants and may contribute to additional carbon in the effluent. This is supported by the decrease of live shoots in tank 2 (see appendix). Furthermore, results from Yang (2015) shows that COD removal efficiencies were higher in the harvested system as there were less dead plant parts which could contribute to the additional organic carbon in the system. Yang's study shows that COD removal efficiency was lower in the harvested system. Furthermore, statistical analysis shows that there was no statistical difference between the removal efficiencies for the planted tanks before the first harvest. After the first harvest, tank 2 removal efficiency became significantly lower than tank 3 and tank 4.

Total nitrogen was measured as the total sum of TKN, NO_2 and NO_3. NH3 was measured which allows organic carbon to be determined. The systems show high total nitrogen removal ability. Total nitrogen characteristic in the effluent is presented in table 5.4. Large amount of total nitrogen concentration in the influent was removed by all tanks.

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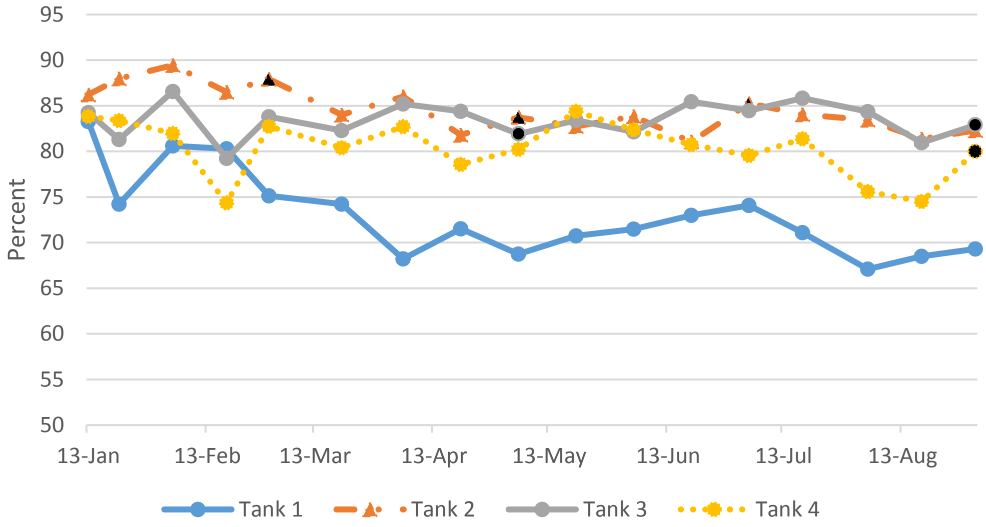

Figure 5.5 TN removal efficiency (black dots represent data measured after each harvest)

Table 5.4 Influent and effluent TN concentrations (mg/L, n=17)

	range	mean	SD
Influent	33.3-47.9	39.72	4.1
Tank 1	8.0-14.9	12.34	1.8
Tank 2	5.9-12.8	9.24	1.5
Tank 3	5.7-8.0	6.81	0.7
Tank 4	6.4-14.6	8.14	2.0

Tank 3 where plants were harvested every 4 months show the highest removal efficiency for total nitrogen. According to Zheng (2015), plant harvest at 12-month interval would significantly increase total nitrogen removal on the first harvest. However, the total experiment period of this study was 8 months. Nevertheless, results from this study shows that plant harvested at 4-month period can increase total nitrogen removal efficiency, when compare to the more frequently harvested tank and the unharvested tank.

Organic nitrogen was removed most efficiently by tank 3. However, there was no significant different between organic removal efficiencies of the three planted tanks. Tank 1 shows the lowest organic nitrogen removal efficiency. Organic nitrogen was removed in ammonification process as well as other physical processes. The results show that the most frequently harvested tank could remove organic nitrogen most efficiently.

Ammonium nitrogen was removed at higher efficiency by the planted systems than the unplanted system. According to ANOVA analysis, significant difference was found between the removal efficiencies of the systems with plants and the system without plant (alpha = 0.05). However, the differences between different harvest patterns were found to be insignificant.

The average nitrate concentration in the effluent increased from the average influent concentration which is concurring to other studies measuring nitrate concentration from vertical flow constructed wetland effluent (Vymazal, 2006; Abou-Elela, 2013) as VF systems are commonly unable to promote denitrification. NO_3 was produced during nitrification process where ammonia nitrogen was converted to NO_3 in aerobic condition. This is supported by DO and ORP results discussed earlier in this section.

More than half of the total nitrogen concentration in the influent was removed before 50 cm depth but increased at the bottom of the systems. However, when looking at the changes of each nitrogen constituent at different depth, organic nitrogen and ammonia nitrogen decreased along the vertical profile. However, nitrate nitrogen increases significantly from 50 to 98 cm depth which contributes to the increase of total nitrogen.

Table 5.5 Total nitrogen concentration (mg/L) along the vertical profile (mean ±SD, n=17)

	Influent	10 cm	50 cm	98 cm
Tank 1	39.7±4.1	18.4±3.0	13±2.6	12.3±1.8
Tank 2	39.7±4.1	18.6±3.3	6.4±1.0	9.2±1.0
Tank 3	39.7±4.1	16.2±3.5	8.8±2.3	6.8±0.7
Tank 4	39.7±4.1	18.6±1.9	10.5±1.7	8.1±2.0

Table 5.6 Organic nitrogen concentration (mg/L) along the vertical profile (n=17)

	Influent	10 cm	50 cm	98 cm
Tank 1	11.52±4.1	5.0±3.3	7.0±2.7	2.2±0.9
Tank 2	11.52±4.1	6.3±2.6	1.7±1.3	1.10±0.7
Tank 3	11.52±4.1	6.0±2.8	3.7±2.59	1.03±0.93
Tank 4	11.52±4.1	4.4±2.16	4.3±1.63	1.08±0.86

Table 5.7 NH_4 concentration (mg/L) along the vertical profile (n=17)

	Influent	10 cm	50 cm	98 cm
Tank 1	28.15±2.8	13.3±2.2	5.2±1.45	4.1±0.9
Tank 2	28.15±2.8	12.2±2.3	3.9±1.2	2.7±0.71
Tank 3	28.15±2.8	10.2±1.8	4.6±1.1	2.5±0.72
Tank 4	28.15±2.8	14.1±1.7	5.4±1.5	2.6±0.8

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Table 5.8 NO_3 concentration along the vertical profile (n=17)

	Influent	10 cm	50 cm	98 cm
Tank 1	0.05±0.02	0.05±0.02	0.77±0.18	6.0±0.88
Tank 2	0.05±0.02	0.05±0.04	0.78±0.25	5.83±1.29
Tank 3	0.05±0.02	0.05±0.01	0.63±0.15	3.28±0.4
Tank 4	0.05±0.02	0.04±0.02	0.82±0.15	4.43±1.24

Table 5.9 Percent nitrification and percent denitrification

Tank 1	Influent TN	97.2	g	Influent TN	97.2	g
	Plant accumulation	0	g	Plant accumulation	0	g
	Effluent TKN	15.6	g	Effluent TN	30.3	g
	NITR	81.6		DENITR	66.9	
	%Nitrification	83.95	%	%Denitrification	68.82	%
Tank 2	Influent TN	97.2	g	Influent TN	97.2	g
	Plant accumulation	2.9	g	Plant accumulation	2.9	g
	Effluent TKN	9.2	g	Effluent TN	23.6	g
	NITR	85.1		DENITR	70.7	
	%Nitrification	90.24	%	%Denitrification	74.97	%
Tank 3	Influent TN	97.2	g	Influent TN	97.2	g
	Plant accumulation	4.9	g	Plant accumulation	4.9	g
	Effluent TKN	8.7	g	Effluent TN	16.7	g
	NITR	83.6		DENITR	75.6	
	%Nitrification	90.57	%	%DENITR	81.91	%
Tank 4	Influent TN	97.2	g	Influent TN	97.2	g
	Plant accumulation	3	g	Plant accumulation	3	g
	Effluent TKN	9.1	g	Effluent TN	20	g
	NITR	85.1		DENITR	74.2	
	%Nitrification	90.34	%	%DENITR	78.77	%

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5.2 Greenhouse Gas Emission

CH_4 and N_2O fluxes measured from the four tanks range between 55.5 to 116.1 mg/m^2 d and 1.86 to 5.91 mg/m^2 d respectively. The highest average CH_4 flux comes from the unplanted tank whereas the lowest flux comes from tank 3. However, at alpha = 0.05, the difference between the fluxes from tank 3 and tank 4 was not statistically significant. For N_2O, the highest flux average was from tank 3, followed by tank 4, tank 2 and tank 1.

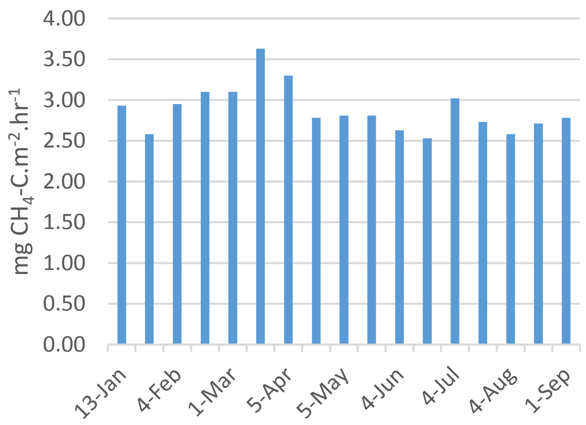
The unplanted system had highest methane flux average and the system with 4-month harvest interval had the lowest flux average. However, opposite results are found between methane and nitrous oxide fluxes. The highest nitrous oxide flux was found in the system with 4-month harvest interval and the lowest from the system without plants.

Table 5.10 CH_4-C and N_2O-N fluxes from each system (mg.m^{-2}.hr^{-1})

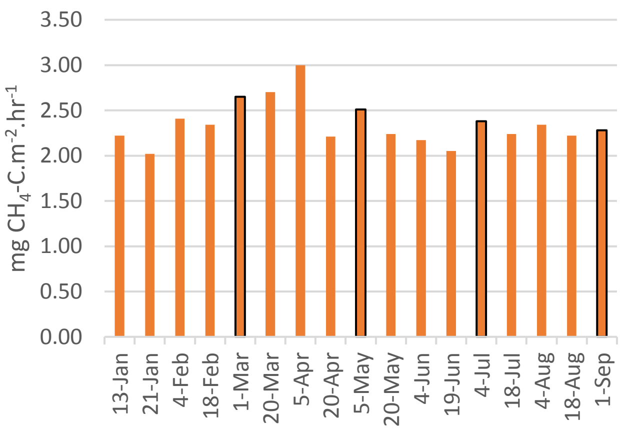
	CH_4-C		N_2O-N	
	Range	mean ±SD	Range	mean ±SD
Tank 1	2.53-3.63	2.88 ±0.29	0.049-0.131	0.075±0.022
Tank 2	2.02-3.00	2.35 ±0.25	0.056-0.109	0.079±0.015
Tank 3	1.73-2.35	1.98 ±0.15	0.064-0.157	0.100±0.027
Tank 4	1.83-2.48	2.08 ±0.19	0.070-0.150	0.093±0.026

Figure 5.6 shows temporal changes of the methane fluxes. The highest fluxes from all the four tanks were measured in late March and early April which are the hottest time of the year (see appendix for climate data). ANOVA single factor comparison also shows that the fluxes are significantly lower (at alpha = 0.05) in the planted systems than the

unplanted system. Furthermore, the fluxes in tank 3 and tank 4 are also significantly lower than tank 2 where plants were harvested most frequently. However, the analysis shows that there was no significant different between tank 3 and tank 4 where plants were harvested every 4 months and 8-month respectively.



(a)



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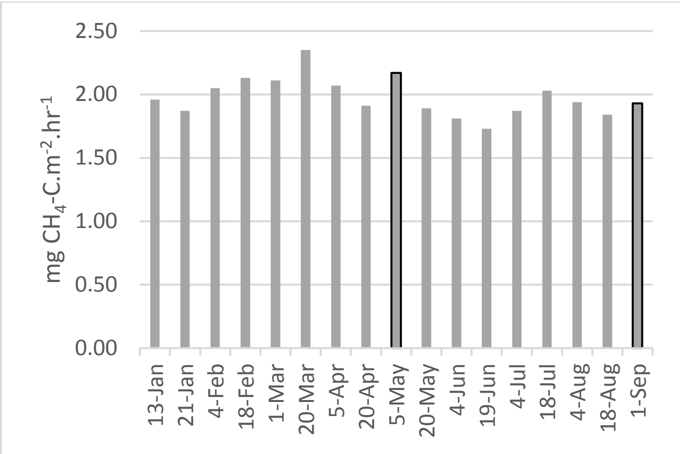

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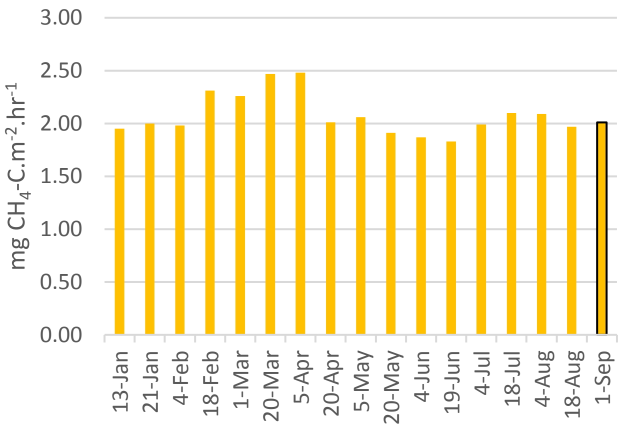
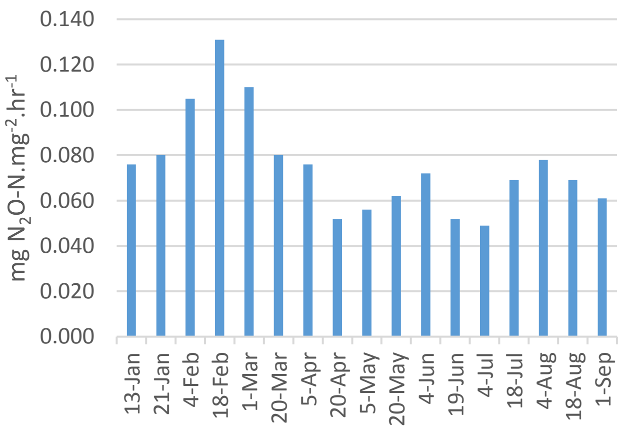


(d)

Figure 5.6 CH_4 flux throughout the 8-month experimental period for tank 1 (a), tank 2 (b), tank 3 (c), tank 4 (d). The fluxes measured after each harvest are outlined.

Temporal changes of nitrous oxide fluxes from the four tanks are presented in figure 5.7. The highest nitrous oxide fluxes were also measured from the hottest period which is the beginning of the summer after a period of long dry months with no rainfall. The first rain arrived in the middle of April where significant decrease in the fluxes are shown especially in the unplanted system.

It can be seen in tank 2 that nitrous oxide fluxes dropped after each harvest, however, the same drops are also found in the unplanted tank which suggest that plant harvest is not the cause of the low fluxes. However, the increase in the fluxes are found after the harvest in tank 3 and tank 4, in which the increase is not visible in the unplanted system. This can suggest that there's an increase in the flux after a harvest.



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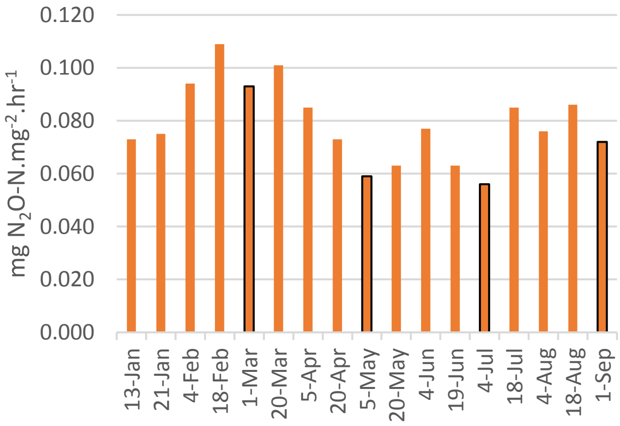

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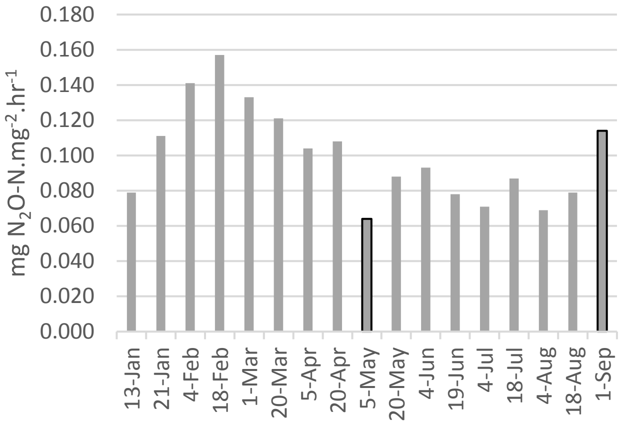

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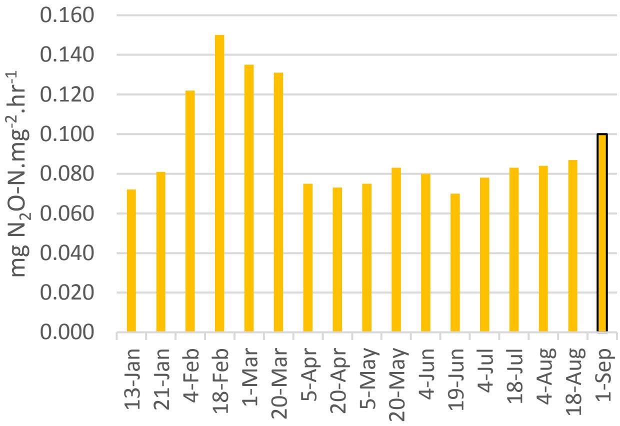

(d)

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Figure 5.7 N_2O flux throughout the 8-month experimental period for tank 1 (a), tank 2 (b), tank 3 (c), tank 4 (d). The fluxed which measured after each harvest are outlined.

Methane fluxes measured from the four tanks fall in the same range as other studies of the similar conditions. Table 5.11 shows methane fluxes measured by other studies from subsurface flow constructed wetlands treating domestic wastewater. The unplanted tank in this study also shows higher fluxes than the planted tanks which is concurring to results from other studies. Nitrous oxide fluxes from this study are slightly higher than fluxes from VF constructed wetland treating domestic wastewater reported by other studies (table 5.12).

Table 5.11 Methane fluxes (mg CH_4-C.m^{-2} h^{-1}) from different studies

	CH_4	Plants	Harvest	Climate
Teiter and Mander(2005), Søvik et al. (2006) and Mander et al. (2008)	3	P. australis	No	temperate/boreal
Søvik et al. (2006)	5.4	No vegetation	No	boreal
Gui et al. (2007)	0.3	P. australis	No	temperate/warm
Liu et al. (2009)	3	P. australis	No	temperate/warm
Zhu (2007)	0.0012-0.42	Phragmites communis	Yes (unknown interval)	temperate
Picek (2007)	17.5	P. australis	Yes (at final height)	temperate
This study	3.4-4.8	No vegetation	No	tropical
This study	2.7-4.0	Cyperus alternifolius L.	Yes (2-month interval)	tropical
This study	2.3-3.1	Cyperus alternifolius L	Yes (4-month interval)	tropical
This study	2.4-3.3	Cyperus alternifolius L	No	tropical

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Table 5.12 Nitrous oxide fluxes (mg N_2O-N.m^{-2} h^{-1}) from different studies

	N_2O	Plants	Harvest	Climate
Teiter and Mander(2005), Søvik et al. (2006) and Mander et al. (2008)	0.225	P. australis	No	temperate/ boreal
Søvik et al. (2006)	0.222	No vegetation	No	temperate/ boreal
Gui et al. (2007)	0.123	P. australis	No	temperate/ warm
Liu et al. (2009)	0.073	P. australis	No	temperate/ warm
This study	0.075	No vegetation	No	tropical
This study	0.079	Cyperus alternifolius L.	Yes (2-month interval)	tropical
This study	0.100	Cyperus alternifolius L.	Yes (4-month interval)	tropical
This study	0.093	Cyperus alternifolius L.	No	tropical

Pearson's correlation between TOC concentrations and the fluxes were very high for other three systems except for the 4-month harvest system with significant correlation but still noticeably less than the other three. Correlations between nitrous oxide fluxes and total nitrogen concentrations in the influent were significant for all four systems (>0.6). According to Picek (2007), methane can be produced at much higher rate when plants are present and become the main carbon source for methanogenesis processes. Therefore, the absent of plants in tank 1 may be responsible for the higher flux than in other systems. Highest nitrous oxide flux is from tank 4 which according to the PCR-DGGE fingerprints, has the highest nitrifying and denitrifying bacteria.

According to Gui et al. (2007) and Liu et al. (2009) TOC concentration in the influent can affect methane flux, where, TN concentration in the influent effect nitrous oxide flux. These relationships are also clearly visible in this study. In this study, inflow TOC, inflow TN, TOC removal and TN removal also show correlation with methane and nitrous oxide fluxes respectively. Higher nitrous oxide fluxes in tank 3 and tank 4 is agreeing with the microbial analysis which shows larger nitrifying and denitrifying bacteria in the roots of the plants in these two systems.

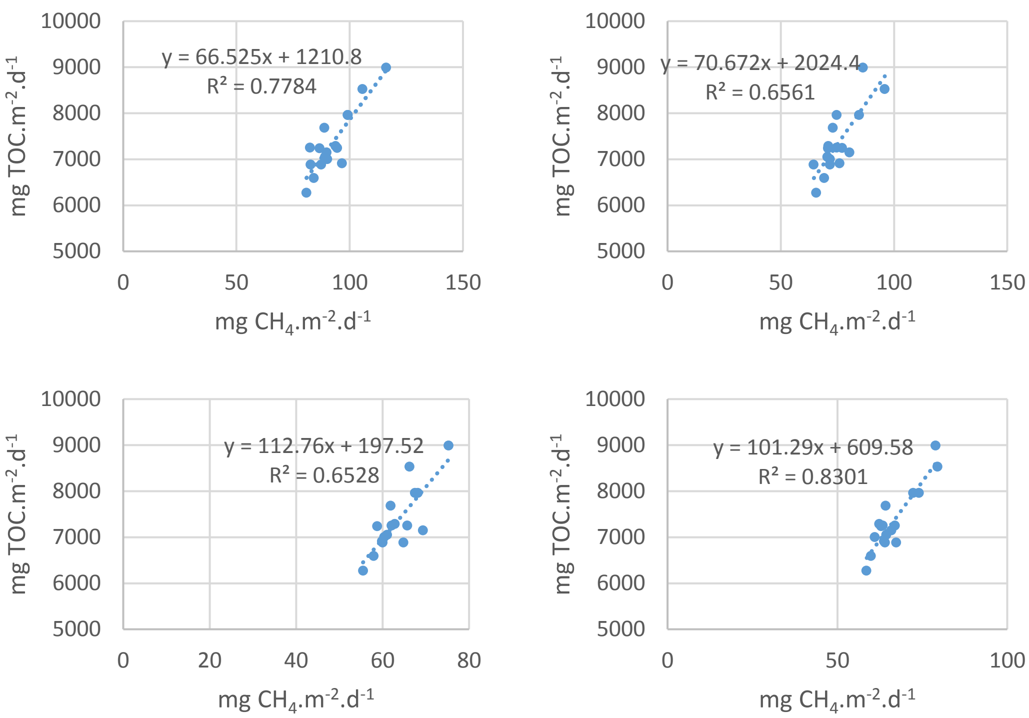

Figure 5.8 CH_4 and TOCin relationship in tank 1 (top left), tank 2 (top right), tank 3 (bottom left) and tank 4 (bottom right).

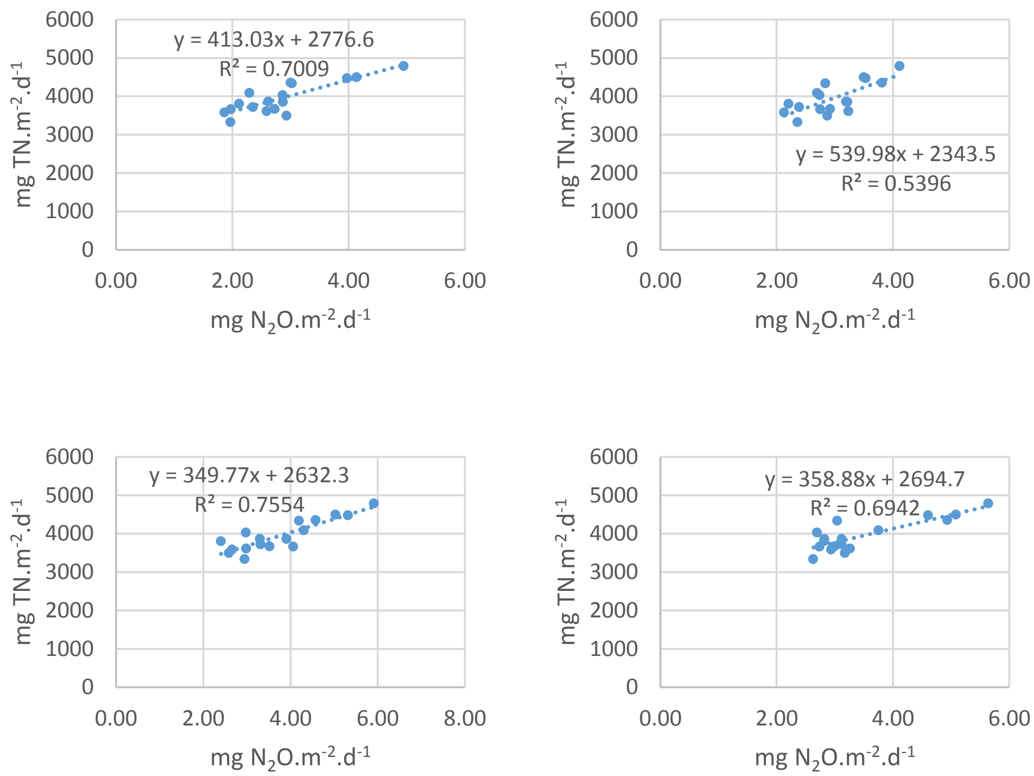

Figure 5.9 N_2O and TNin relationship in tank 1 (top left), tank 2 (top right), tank 3 (bottom left) and tank 4 (bottom right).

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The flux increased temporary which responds to the immediate drop in DO concentration after harvest. This effect is also found in a study by Zhu (2007) in both surface flow and subsurface flow systems. However, as suggested earlier that the main control factors for CH_4 and N_2O fluxes were the inflow TOC and TN respectively, temporal changes between CH_4:TOC_{in} ratio and N_2O:TN_{in} ratios for each flux from each tank were plotted in figure 5.10 and 5.11 where the same effect for CH_4 was also visible. However, this effect may cause by the immediate changes in the convection property of plants as well as the sudden drop in dissolved oxygen availability in the system. N_2O, on the other hand, can be produced by both anaerobic and aerobic condition. In contrast of CH_4 fluxes,

N_2O fluxes dropped immediately after harvest show suggests that convection property may not play an important role in methane fluxes after harvest.

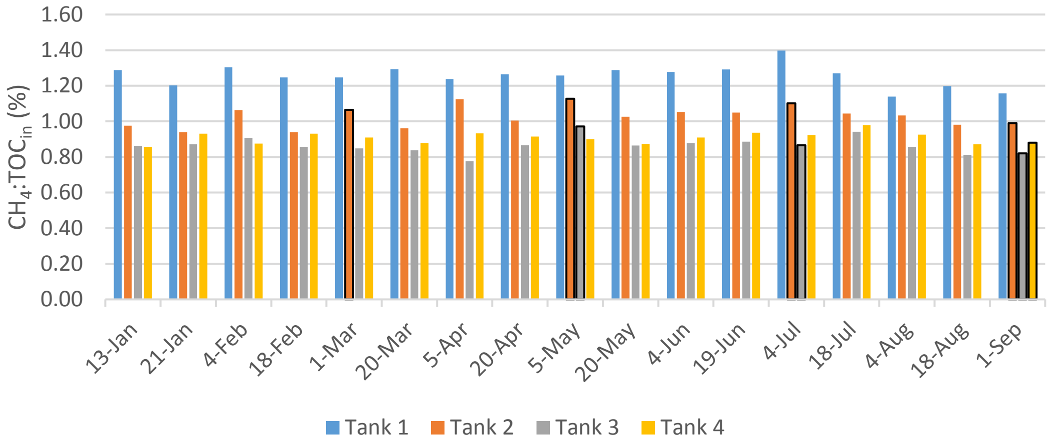

Figure 5.10 Temporal changes in CH_4:TOC_{in} ratio

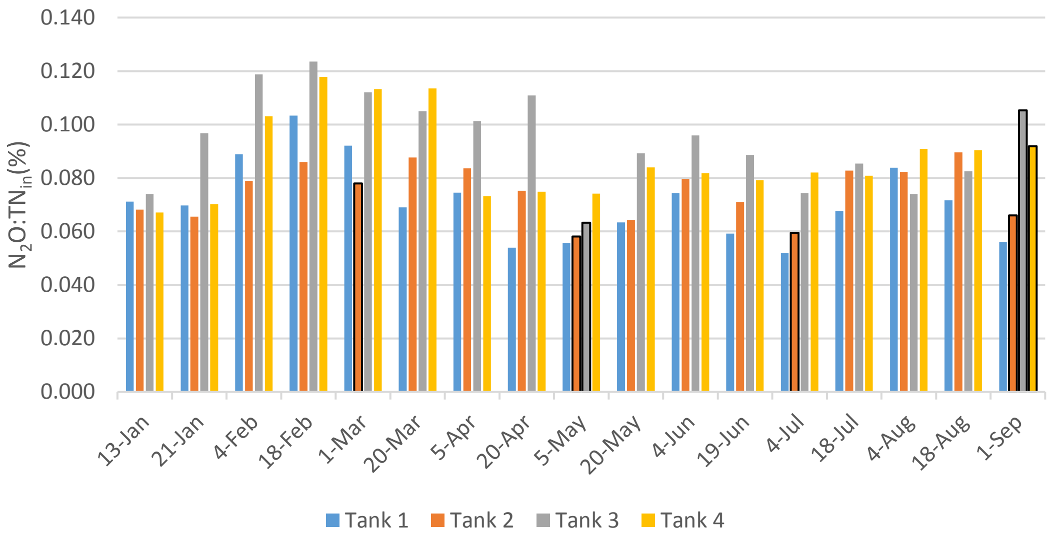

Figure 5.11 Temporal changes in N_2O:TN_{in} ratio

Methane emission factor from the unplanted tank was significantly higher than the planted tank which suggests that plant could reduce CH_4 fluxes. However, N_2O emission factors were slightly higher in the planted tanks which suggests that plants result in higher N_2O flux.

Table 5.13 Emission Factors comparison

sources	EF_{CH4-C}	EF_{N2O-N}	Plant species	Climate Zone
This study	0.94	0.025	No plant	Tropical
This study	0.77	0.026	Cyperus Alternifolius*	Tropical
This study	0.65	0.033	Cyperus Alternifolius**	Tropical
This study	0.68	0.030	Cyperus Alternifolius	Tropical
Teiter and Mander (2005), Søvik et al. (2006) and Mander et al. (2008)	0.88	0.021	P. australis	Temperate/Boreal
Sovik et al. (2006)	0.38	0.011	No plant	Temperate/Boreal
Gui et al. (2007)	1.68	0.096	P. australis	Temperate/Warm
Liu et al. (2009)	1.73	0.042	P. australis	Temperate/Warm

*2-month harvest, ** 4-month harvest

The highest N_2O emission from tank 3 may also be explained by percent nitrification and percent denitrification as shown in table 5.9. Tank 3 also has the highest number of nitrifying and denitrifying species presented in the system. This suggests that in the condition of this study, plant harvested every 4 months would enhance nitrification and denitrification processes results in higher nitrogen removal efficiency thus results higher N_2O fluxes.

Table 5.14 CO_2 equivalent values for each system ($mg.m^{-2}.d^{-1}$)

	CH_4	N_2O	CO_2 eq
Tank 1	92.17	2.84	3980.1
Tank 2	75.3	2.97	3445.26
Tank 3	63.3	3.91	3317.38
Tank 4	66.5	3.5	3304

Overall average fluxes from each tank were compared as CO_2 equivalents. Despite emitting higher N_2O fluxes than other systems, tank 3 and tank 4 where plants were harvested after reaching the final height and not harvested showed the lowest emissions in term of CO_2 equivalent. For the reason that tank 3 has the highest overall treatment performance (TN, organic nitrogen, NH_3 and NO_3 and nitrogen removal via plant harvest) and better TOC treatment performance than tank 4, harvesting plants after reaching the final height is recommended.

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5.3 Microbial Analysis

Microbial community in the system was determined by PCR-DGGE method. Methanogen, nitrifying bacteria and denitrifying bacteria were found in both planted and unplanted systems. However, different species are detected in the planted and unplanted systems.

By performing PCR-DGGE on the substrate samples, it is found that there are variety of methanogen found in the four systems. According to the dice similarity index analysis from the PCR-DGGE fingerprints, methanogen population in tank 1 has the least similarity with other systems in this study whereas methanogen in tank 3 and 4 have very high similarity.

Nitrifying and denitrifying bacteria are more concentrated in the plant roots in tank 3 and tank 4 where plants were only harvested once every 4 months and 8 months, respectively. However, tank 2 root sample, where plants were harvested every 2 months also show low variety of nitrifying and denitrifying bacteria than tank 3 and tank 4. Less variety of nitrifying and denitrifying bacteria were found from the substrate samples than from the root zone. Table 5.15 shows number of methanogen, ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and denitrifying bacteria species in the systems.

Table 5.15 Number of different species in each system

	Tank 1	Tank 2 soil	Tank 2 root	Tank 3 soil	Tank 3 root	Tank 4 soil	Tank 4 root
Methanogen	11	6	0	7	0	8	0
Nitrifying and Denitrifying bacteria							
AOB	1	2	1	0	3	1	2
NOB	3	1	4	2	3	0	1
Denitri	3	1	4	1	5	1	6
undefied	0	2	0	0	4	0	4
total	7	6	9	3	15	2	13

Dice similarity index analysis from the PCR-DGGE showed that methanogen presented in the 3 systems with plants show high similarity between them whereas, the system without plants show different methanogen community. Nitrifying and denitrifying bacteria are more concentrated in the plant roots in tank 3 and tank 4 where plants were only harvested once every 4 months and 8 months (no harvest), respectively.

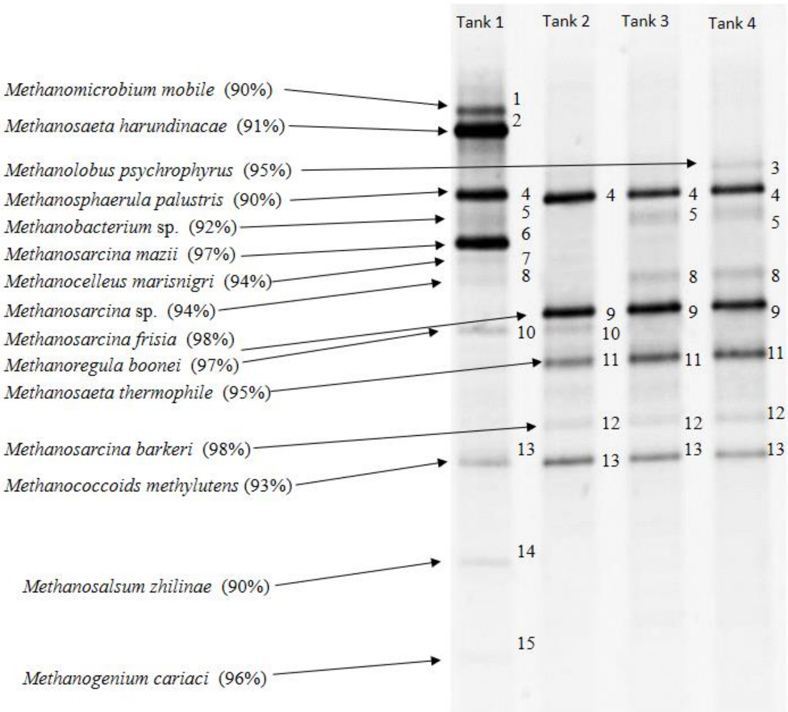

Figure 5.12 Methanogen PCR-DGGE fingerprint

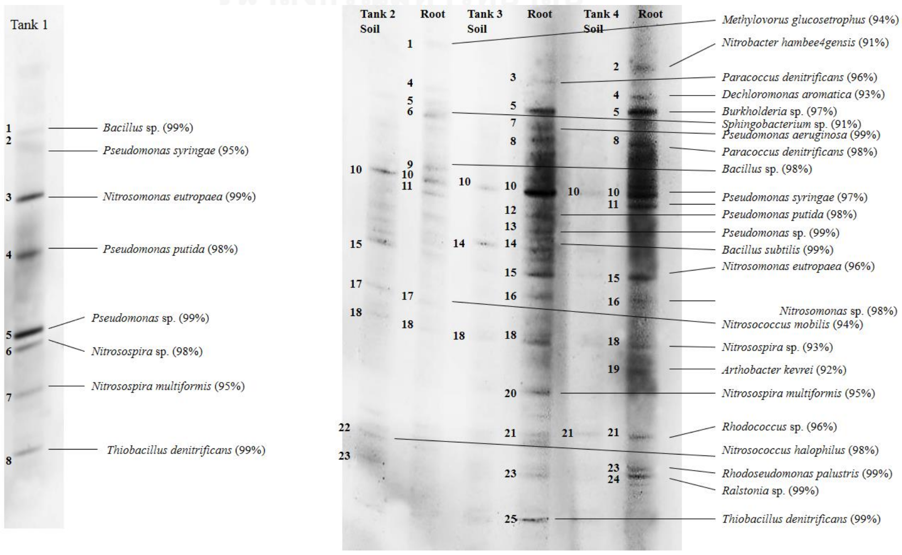

Figure 5.13 Nitrifying and denitrifying PCR-DGGE fingerprint

Table 5.16 Dice similarity index for PCR-DGGE methanogen fingerprint

	Tank 1	Tank 2	Tank 3	Tank 4
Tank 1	1			
Tank 2	0.35	1		
Tank 3	0.44	0.77	1	
Tank 4	0.42	0.71	0.93	1

Table 5.17 Dice similarity index for PCR-DGGE nitrifying and denitrifying bacteria fingerprint

		Tank 1	Tank 2		Tank 3		Tank 4	
			media	root	media	root	media	root
Tank 1		1.00	0.00	0.47	0.00	0.35	0.00	0.38
Tank 2	media	0.00	1.00	0.40	0.44	0.38	0.25	0.42
	root	0.47	0.40	1.00	0.33	0.25	0.18	0.45
Tank 3	media	0.00	0.44	0.33	1.00	0.33	0.40	0.25
	root	0.35	0.38	0.25	0.33	1.00	0.24	0.57
Tank 4	media	0.00	0.25	0.18	0.40	0.24	1.00	0.27
	root	0.38	0.42	0.45	0.25	0.57	0.27	1.00

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Number of methanogen species found in the unplanted tank was higher than the plant tanks. This corresponds to the higher methane flux in the unplanted tank. This suggests, despite the pulse (intermittent) influent feeding strategy, higher anaerobic zones may be found in VF systems without plants comparing to systems with plants. This also supports by the effluent DO concentration.

Total nitrogen, organic nitrogen and NH_3 removal efficiencies were the highest in tank 3. This corresponds the higher number of nitrifying and denitrifying bacteria species found in Tank 3 and tank 4. Furthermore, N_2O fluxes from tank 3 and tank 4 were found to be significantly higher than tank 1 and tank 2.

According to Truu et al. (2009), the presence and the condition of plants as well as the hydraulic condition, substrate used, wastewater characteristics and environmental condition determine the great deal the microbial populations within the systems. As mentioned earlier, microorganism is the main driver for greenhouse gas emission from constructed wetland systems. The presence of plants, their condition and plant diversity impact the diversity of microbial species within the system which were found to be the main factors for carbon and nitrogen removal ability of the system (Jahangir et al., 2016) and thus, which links directly to the rate of greenhouse gas emission from the system. In this study, more diverse species of especially nitrifying and denitrifying bacteria were found in tank 3 and tank 4. Species that were only present in tank 3 and tank 4 were found to be those *that can carry out denitrification in the presence of oxygen (paracoccus denitrificans) (Su et al., 2015) and those* that can carry out nitrification and denitrification simultaneously (*Rhodococcus* sp. and *Bacillus subtilis) (Kundu et al., 2014).* This suggests that despite being more aerobic, tank 3 and tank 4 where plants were allowed to grow to their final height attracts were able to perform denitrification by attracting microbial species which were able to denitrify in aerobic conditions which are supported by the lower nitrate concentrations in the effluent.

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Chapter 6 Conclusion

Methane emission from HSSF CW was clearly higher than the VF system. The fluxes in the HSSF CW shows large spatial heterogeneity. The fluxes measured near the outlet zone were almost the same as fluxes measured from the VF system. Nitrous oxide fluxes were higher in the VF system. Nitrification and denitrification occurred at high rates in both system where rainfall caused temporary drops in fluxes.

Planted systems showed higher removal efficiencies than the unplanted system in almost all the parameters except for organic carbon. Even though plants may increase oxygen availability in the system thus enhancing the degradation of organic matter, additional carbon from plants may lower the removal efficiency. The unplanted system showed lower dissolved oxygen concentration in the effluent, suggesting that the system was more anaerobic than the planted systems which results higher methane emission.

Plant harvest clearly improves treatment performance by increasing DO availability in the system. However, the harvesting interval should be carefully determined. In this study, the system where plants were allowed to grow to the final height before harvested and the unharvested system show the highest DO concentration in the effluent.

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Table 6.1 Effect of different plant harvesting patterns

	No plant	Harvest before final height	Harvest after final height	No harvest
CH_4	Highest	2nd highest	Lowest	3rd highest
N_2O	Lowest	3rd highest	Highest	2nd highest
Effect of inflow TOC to CH_4 emission	Strong	Strong	Strong	Strongest
Effect of inflow TN to N_2O emission	Strong	Strong	Strongest	Strong
Effect of harvest on DO concentration	-	Strong	No effect	-

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The results suggest that above ground and below ground plant density, which decreased considerably if plants were harvested before reaching the final height, effect oxidation rate in the system. Chemical and biological processes require aerobic condition occurred more rapidly in tank 3 and tank 4 where plants were harvested after reaching the final height and not harvested throughout the 8-month experiment period which is implied by the higher reduction of organic nitrogen and ammonia nitrogen and high concentration of nitrate nitrogen in the effluent. This is also supported by the larger number of nitrifying and denitrifying microbial species found in tank 3 and 4. It is also suggested that nitrification and denitrification occurred simultaneously in all system and that nitrous oxide was more likely to be produced during nitrification process than denitrification process. Harvesting plants after allowing them to reach the final height results in higher dissolved oxygen availability in the system which increases percent nitrification and percent denitrification which increases the removal efficiencies of nitrogen pollutants. However, higher nitrifying and denitrifying bacteria activities also results in higher nitrous oxide emissions.

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APPENDIX

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Table A1 CH_4 fluxes (mg CH_4-C.m^{-2}.h^{-1}) for part 1 (HSSF and VF systems)

	HSSF inlet	HSSF middle	HSSF outlet	HSSF	VF
06-Jan-14	7.34	6.71	5.32	6.45	3.24
21-Jan-14	10.10	11.47	8.94	10.17	5.47
05-Feb-14	11.04	6.77	6.21	8.01	4.47
20-Feb-14	13.33	11.79	6.40	10.51	6.65
07-Mar-14	11.00	12.44	11.44	11.63	4.44
24-Mar-14	4.09	2.78	2.27	3.05	2.53
08-Apr-14	4.04	3.80	2.17	3.34	2.80
23-Apr-14	4.44	4.34	2.75	3.84	2.81
average	8.17	7.51	5.69	7.13	4.05
SD	3.68	3.89	3.32	3.46	1.47

Table A2 N_2O fluxes (mg N_2O-N.m^{-2}.h^{-1}) for part 1 (HSSF and VF systems)

	HSSF inlet	HSSF middle	HSSF outlet	HSSF	VF
06-Jan-14	0.117	0.062	0.171	0.117	0.093
21-Jan-14	0.115	0.153	0.213	0.161	0.16
05-Feb-14	0.12	0.098	0.251	0.156	0.312
20-Feb-14	0.156	0.226	0.146	0.176	0.313
07-Mar-14	0.161	0.197	0.251	0.203	0.295
24-Mar-14	0.1	0.067	0.136	0.101	0.171
08-Apr-14	0.066	0.064	0.072	0.067	0.132
23-Apr-14	0.069	0.066	0.132	0.089	0.135
average	0.113	0.116625	0.1715	0.13375	0.201375
SD	0.03492	0.06641	0.062944	0.047252	0.090287

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Table A3 CH_4 fluxes (mg CH_4-C.m^{-2}.h^{-1}) for part 2 (comparing plant harvest effect)

	Tank 1	Tank 2	Tank 3	Tank 4
13-Jan	2.93	2.22	1.96	1.95
21-Jan	2.58	2.02	1.87	2.00
4-Feb	2.95	2.41	2.05	1.98
18-Feb	3.10	2.34	2.13	2.31
1-Mar	3.10	2.65	2.11	2.26
20-Mar	3.63	2.70	2.35	2.47
5-Apr	3.30	3.00	2.07	2.48
20-Apr	2.78	2.21	1.91	2.01
5-May	2.81	2.51	2.17	2.06
20-May	2.81	2.24	1.89	1.91
4-Jun	2.63	2.17	1.81	1.87
19-Jun	2.53	2.05	1.73	1.83
4-Jul	3.02	2.38	1.87	1.99
18-Jul	2.73	2.24	2.03	2.10
4-Aug	2.58	2.34	1.94	2.09
18-Aug	2.71	2.22	1.84	1.97
1-Sep	2.78	2.28	1.93	2.01
average	2.88	2.35	1.98	2.08
min	2.53	2.02	1.73	1.83
max	3.63	3.00	2.35	2.48
SD	0.29	0.25	0.15	0.19

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Table A4 N_2O fluxes (mg N_2O-N.m^{-2}.h^{-1}) for part 2 (comparing plant harvest effect)

	Tank 1	Tank 2	Tank 3	Tank 4
13-Jan	0.076	0.073	0.079	0.072
21-Jan	0.080	0.075	0.111	0.081
4-Feb	0.105	0.094	0.141	0.122
18-Feb	0.131	0.109	0.157	0.150
1-Mar	0.110	0.093	0.133	0.135
20-Mar	0.080	0.101	0.121	0.131
5-Apr	0.076	0.085	0.104	0.075
20-Apr	0.052	0.073	0.108	0.073
5-May	0.056	0.059	0.064	0.075
20-May	0.062	0.063	0.088	0.083
4-Jun	0.072	0.077	0.093	0.080
19-Jun	0.052	0.063	0.078	0.070
4-Jul	0.049	0.056	0.071	0.078
18-Jul	0.069	0.085	0.087	0.083
4-Aug	0.078	0.076	0.069	0.084
18-Aug	0.069	0.086	0.079	0.087
1-Sep	0.061	0.072	0.114	0.100
average	0.075	0.079	0.100	0.093
min	0.049	0.056	0.064	0.070
max	0.131	0.109	0.157	0.150
SD	0.022	0.015	0.027	0.025

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Table A5 Plant height (cm)

	tank 1	tank 2	tank 3	tank 4
1-Jan	0	50	50	50
13-Jan	0	50	50	50
21-Jan	0	55	55	55
4-Feb	0	60	60	60
18-Feb	0	70	70	70
28-Feb	0	75	75	75
1-Mar	0	5	80	80
20-Mar	0	35	90	90
5-Apr	0	50	100	100
20-Apr	0	65	110	110
4-May	0	70	110	110
5-May	0	5	5	110
20-May	0	35	40	110
4-Jun	0	50	65	120
19-Jun	0	65	80	120
3-Jul	0	75	95	120
4-Jul	0	5	110	120
18-Jul	0	25	120	120
4-Aug	0	35	120	120
18-Aug	0	35	120	120
31-Aug	0	35	120	120
1-Sep	0	5	5	5

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VITA

Ms. Siripoon Nutanong was born on March 25, 1982 in Ratchaburi Province, Thailand. The author received her Bachelor degree of Industrial Engineering and Engineering Management from Monash University, Australia in 2005. She obtained a Master's Degree in Environmental Engineering form the University of Melbourne in 2006.

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