# ผลของการบำบัดน้ำเสียโดยบึงประดิษฐ์ต่อการปลดปล่อยก๊าซเรือนกระจก
# และแบบแผนของแบคทีเรียในดิน

นางสุกาญดา เชื้อสุวรรณ

# THE EFFECTS OF WASTEWATER TREATMENT BY

# CONSTRUCTED WETLANDS ON GREENHOUSE

# GAS FLUX DYNAMIC AND SOIL

# BACTERIAL PROFILE

**Suknada Chuersuwan**

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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# THE EFFECTS OF WASTEWATER TREATMENT BY CONSTRUCTED WETLANDS ON GREENHOUSE GAS FLUX DYNAMIC AND SOIL BACTERIAL PROFILE

Suranaree University of Technology Council has approved dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

_____

(Dr. Pongrit  Krubphachaya)

Chairperson

_____

(Asst. Prof. Dr. Pongthep  Suwanwaree)

Member (Thesis Advisor)

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สุกาญดา เชื้อสุวรรณ : ผลของการบำบัดน้ำเสียโดยบึงประดิษฐ์ต่อการปลดปล่อยก๊าซเรือน
กระจกและแบบแผนของแบคทีเรียในดิน (THE EFFECTS OF WASTEWATER
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การศึกษานี้มีวัตถุประสงค์เพื่อประเมินปริมาณการปล่อยก๊าซเรือนกระจกจากบึงประดิษฐ์ที่
ใช้บำบัดน้ำเสียชุมชน ศึกษาการผันแปรของการปล่อยก๊าซดังกล่าวในช่วงวันและฤดูกาล รวมทั้ง
ศึกษาความสัมพันธ์ระหว่างชนิดพืช และแบบแผนของแบคทีเรียในดินบึงประดิษฐ์ที่มีผลต่อการ
ปล่อยก๊าซเรือนกระจก บ่อทดลองขนาด 2.0 ม.×0.5 ม.×0.8 ม. (ยาว×กว้าง×ลึก) จำนวน 12 บ่อ ถูก
สร้างขึ้นในมหาวิทยาลัยเทคโนโลยีสุรนารี จังหวัดนครราชสีมา ใช้เป็นพื้นที่ชุ่มน้ำประดิษฐ์แบบ
ไหลบนพื้นผิวและปลูกกก (Cyperus sp.) พุทธรักษา (Canna sp.) และอ้อ (Phragmites sp.) ส่วน
แบบไหลใต้ดินปลูกกกอย่างเดียว ระหว่างปี พ.ศ. 2552-2554 พบว่า พื้นที่ชุ่มน้ำประดิษฐ์แบบไหล
บนพื้นผิวปล่อยก๊าซมีเทน ไนตรัสออกไซด์และคาร์บอนไดออกไซด์ในอัตรา 5.9±9.8, 1.8±2.1 และ
29.6±20.2 มก./ตร.ม./ชม. ตามลำดับ พื้นที่ชุ่มน้ำประดิษฐ์แบบไหลใต้ดินปล่อยก๊าซดังกล่าวใน
อัตรา 2.9±3.5, 1.05±1.7 และ 15.2±12.3 มก./ตร.ม./ชม. ตามลำดับ พบการผันแปรของการปล่อย
ก๊าซเรือนกระจกในช่วงวัน ในช่วงกลางวันก๊าซมีเทนถูกปล่อยสูงกว่าช่วงกลางคืน ซึ่งสัมพันธ์กับ
อุณหภูมิของดินที่เปลี่ยนแปลง เพราะอุณหภูมิที่เพิ่มขึ้นมีผลกับการย่อยสลายของจุลินทรีย์ในดินจึง
ผลิตก๊าซมีเทนมากขึ้น ก๊าซคาร์บอนไดออกไซด์ถูกปล่อยสูงกว่าในช่วงกลางคืนจากกระบวนการ
หายใจ ทั้งนี้ ไม่พบการเปลี่ยนแปลงในช่วงวันของการปล่อยก๊าซไนตรัสออกไซด์ ส่วนการผันแปร
ตามฤดูกาลพบว่า การปล่อยก๊าซมีเทนและไนตรัสออกไซด์สูงสุดในฤดูฝน(มิถุนายน-ตุลาคม)
ขณะที่ก๊าซคาร์บอนไดออกไซด์ถูกปล่อยสูงสุดในฤดูร้อน (มีนาคม-พฤษภาคม) และยังพบว่าพื้นที่
ชุ่มน้ำประดิษฐ์ที่ปลูกพืชแตกต่างกัน มีอัตราการปล่อยก๊าซมีเทนและไนตรัสออกไซด์แตกต่างกัน
อย่างมีนัยสำคัญทางสถิติ (p<0.05)    โดยพื้นที่ชุ่มน้ำประดิษฐ์แบบไหลบนพื้นผิวที่ใช้ต้นอ้อ
ปลดปล่อยก๊าซมีเทนในอัตราสูงที่สุด แต่ปลดปล่อยก๊าซไนตรัสออกไซด์ออกสู่บรรยากาศในอัตรา
ต่ำที่สุด เมื่อเปรียบเทียบกับพืชชนิดอื่น ๆ ที่ใช้ในการศึกษา นอกจากนี้ ประเภทของจุลินทรีย์ที่
เกี่ยวข้องกับการปลดปล่อยก๊าซเรือนกระจกมีความสัมพันธ์กับความลึกของชั้นดินในพื้นที่ชุ่มน้ำ
ประดิษฐ์ ทั้งแบบไหลบนพื้นผิวและไหลใต้ดิน แต่ไม่พบความสัมพันธ์กับชนิดของพืช

สาขาวิชาชีววิทยา                ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2556                 ลายมือชื่ออาจารย์ที่ปรึกษา_____

SUKANDA CHUERSUWAN : THE EFFECTS OF WASTEWATER
TREATMENT BY CONSTRUCTED WELTNADS ON GREENHOUSE
GAS FLUX DYNAMIC AND SOIL BACTERIAL PROFILE. THESIS
ADVISOR : ASST. PROF. PONGTHEP SUWANWAREE, Ph.D. 232 PP.

GREENHOUSE GAS EMISSIONS/DIURNAL VARIATIONS/SEASONAL
VARIATIONS/BACTERIAL PROFILE/CONSTRUCTED WETLANDS/
DOMESTIC WASTEWATER

This study was conducted to quantify $CO_2$, $CH_4$ and $N_2O$ fluxes from wetlands
constructed for wastewater treatment, to estimate diurnal and seasonal fluctuations of
these gases, and to investigate the effects of plant species on microbial distribution
and gas fluxes. The experimental scale constructed wetlands were built in Suranaree
University of Technology, Nakhon Ratchasima province during 2009-2011. Twelve
constructed wetlands were built with identical dimensions of 2.0 m× 0.5 m× 0.8 m
(length×width×depth). Experiments employed two regimes of constructed wetlands,
free water surface flow (FWS) planted with *Phragmites* sp., *Canna* sp. and *Cyperus*
sp. and subsurface flow (SF) planted with *Cyperus* sp. The average $CH_4$, $N_2O$ and
$CO_2$ fluxes from FWS planted with various emergent plants were 5.9±9.8, 1.8±2.1
and 30±20 mg/m$^2$/hr, respectively. In comparison, the average $CH_4$, $N_2O$ and $CO_2$
fluxes from SF planted with *Cyperus* sp. were 2.9±3.5, 1.05±1.7 and 15.2±12.3
mg/m$^2$/hr, respectively. Diurnal fluctuations of greenhouse gas fluxes were observed.
Higher $CH_4$ flux occurred during daytimes while the flux at night was lower. These
diurnal variation patterns were correlated with changes in soil temperatures, since

high temperatures during daytime allow higher microbial activities resulting higher production of methane. Average $CO_2$ flux during nighttime was higher than daytime due to respiration.  However, $N_2O$ fluxes did not show an obvious pattern of diurnal variation.  Seasonal fluctuations of greenhouse gas fluxes were also observed.  The average $CH_4$ and $N_2O$ fluxes were highest in the hot rainy season (July-October), whereas average $CO_2$ flux was highest in summer season (March-May). The means of $CH_4$ and $N_2O$ fluxes from different plants species were significantly different ($p<0.05$).  FWS planted with *Phragmites* sp. emitted the highest $CH_4$ flux, but lowest $N_2O$ flux.  Additionally, the types of microbial communities in both types of constructed wetlands were influenced by the depth of soil, but not by plant species.
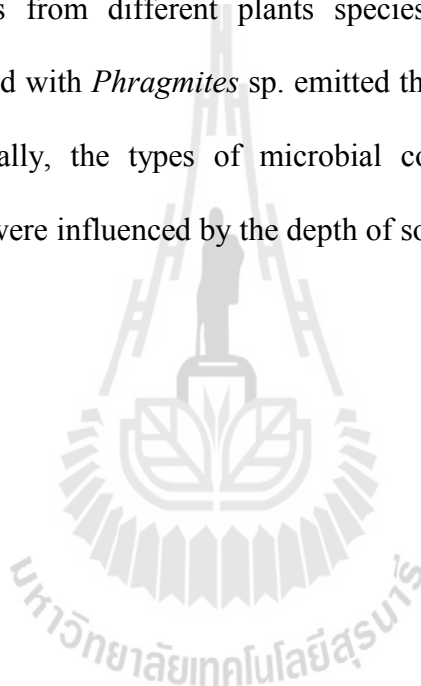
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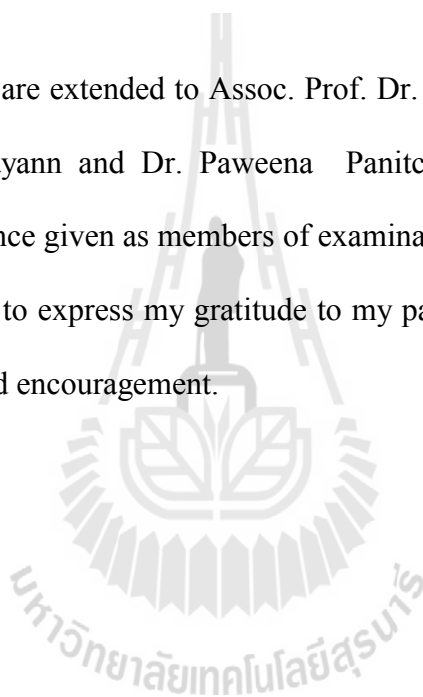
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# LIST OF ABBREVIATION

| | |
|---|---|
| BOD | Biochemical oxygen demand |
| CFCs | Chlorofluorocarbons |
| $CH_4$ | Methane (gas) |
| $cm^3$ | Cubic centimeter |
| $CO_2$ | Carbon dioxide (gas) |
| COD | Chemical oxygen demand |
| CWs | Constructed wetlands |
| DGGE | Denaturing gradient gel electrophoresis |
| DO | Dissolved oxygen |
| FWS | Free water surface (constructed wetland) |
| GWP | Global warming potential |
| hr | Hour |
| HSSF | Horizontal subsurface flow (constructed wetland) |
| IPCC | Intergovernmental Panel on Climate Change |
| $m^2$ | Square meter |
| $m^3/d$ | Cubic meter per day |
| mg | Milligram |
| $mg/m^2/hr$ | Milligram per square meter per hour |
| mL | Milliliter |
| mV | Millivolt |
| $N_2O$ | Nitrous oxide (gas) |

# LIST OF ABBREVIATION (Continued)

| | |
|---|---|
| $NH_3$-N | Ammonia nitrogen |
| $NH_4$-N | Ammonium nitrogen |
| $NO_3$-N | Nitrate nitrogen |
| ORP | Oxidation-reduction potential |
| PCA | Principal component analysis |
| PCR | Polymerase chain reaction |
| PgC | Pico gram carbon |
| ppb | Part per billion |
| ppm | Part per million |
| SF | Subsurface (constructed wetland) |
| SS | Suspended solids |
| Tgr | Tetra gram |
| TP | Total phosphorus |
| VSSF | Vertical subsurface flow (constructed wetland) |
| yrs | Years |

# CHAPTER I

# INTRODUCTION

## 1.1  Rationale

Due in large part to the expectation that climate changes will follow upon an increase in atmospheric concentration of greenhouse gases (e.g. $CO_2$, $CH_4$, $N_2O$, etc.), there is intense interest in the sources and sinks of these gases, and in the strength of their respective emission and consumption (Houghton *et al.,* 2001). Natural sources are investigated to reveal natural fluctuations and magnitudes, while anthropogenic sources are intensively targeted in efforts to cut their emissions and mitigate climate change (Houghton *et al.,* 2001). Natural wetlands, as significant greenhouse gases sources, contribute to the global balance of the key greenhouse gases. They act as sinks for $CO_2$ by photosynthetic assimilation from the atmosphere and sequestration of the organic matter produced in the wetland soil. In contrast, wetlands are sources of $CH_4$ and $N_2O$ (Brix *et al.,* 2001).

Constructed wetlands (CWs) systems are combinations of natural wetlands and conventional wastewater treatment processes and are constructed in order to reduce input of nutrients and organic pollutants to water bodies. Constructed wetland systems, a cost-effective alternative, apply various technological designs, using natural wetland processes, associated with wetland hydrology, soils, microbes and plants. When wetlands are used for purification of wastewater, microbial processes

and gas dynamics are likely to be altered. With increased inputs of nutrients and organic pollutants, the productivity of the ecosystem could increase as well as the production of greenhouse gases, which are by- or end-products of microbial decomposition processes. Constructed wetlands, therefore, can be sources of important greenhouse gases. (Kadlec and Knight, 1996; Mitsch and Gosselink, 2000).

However, there are relatively few studies consider $CO_2$, $N_2O$ and $CH_4$ fluxes from wetlands constructed for water quality controlling purposes. Since total area of CWs worldwide is negligible as compare to all natural wetlands and agricultural areas. But the worldwide increase in the development of CWs necessitates an understanding of their potential atmospheric impact in light of the trend that natural wetlands in many countries are decreasing (e.g., Thailand) while environmental regulatory agencies are trying to stimulate an increase in CW acreage. Thus, comprehensive knowledge to clarify the atmospheric impact of such wetlands is an urgent need.

In constructed wetland microcosm, soil-plant is a highly complex environmental system that acts as a reservoir for microorganisms with their activity varying over space and time. Plants release root exudation, which easily decomposable and preferentially used by microorganisms, increased carbon input into the system (Tanner, 2001). Microbial growth in wetlands soil has been believed to depend upon the plant species and substrate. Furthermore, many species of emergent plants CWs possess a convective flow mechanism; oxygen is transported to the roots and gaseous microbial by-products are emitted from plant roots to the atmosphere (Brix, 1989; Brix *et al.,* 1996). The transport of gases by the convective mechanism is

faster than diffusion through water. The presence of plants in constructed wetland system may increase gas emissions from the soil. Therefore, plant species affect on microbial ecology and gas emission from treatment processes.

Wetland gas dynamics are also greatly affected by climatic and weather conditions, especially by temperature and moisture (McDonald *et al.,* 1998). Rate of photosynthesis (the source of energy and carbon in ecosystems) and microbial activities producing greenhouse gases increase with increasing temperature. Both denitrification and methane formation depend on the oxygen status of the soil or sediment and decomposition rates of organic matter. As a result, the temporal and seasonal variability of fluxes of $CO_2$ (Liikanen *et al.,* 2006) $N_2O$ and $CH_4$ (Inamori *et al.,* 2007) are extremely high resulting from variation in the environmental factors regulating the microbial processes behind the gas fluxes. In some seasons wetland can act as a source or sink for carbon and there can be great differences in the $CH_4$ (Nykanen *et al.,* 1995) and $N_2O$ fluxes (Huttunen *et al.,* 2002). Therefore, prospective studies are needed to obtain a holistic picture of the gas dynamics of constructed wetlands.

Although constructed wetlands can be beneficial for wastewater treatment they may have an unfavorable environmental impact by increasing the fluxes of greenhouse gases to the atmosphere. Further understanding to cut emission from constructed wetland, magnitude and variation of gas fluxes including influential factors should be extremely explored to provide essential knowledge associated major greenhouse gas emission and the benefit of wastewater treatment.

## 1.2  Research objectives

The objectives of this research are as followings.

1) To quantify $CO_2$, $CH_4$ and $N_2O$ fluxes from wetlands constructed for wastewater treatment.

2) To estimate diurnal and seasonal fluctuations of $CO_2$, $CH_4$ and $N_2O$ fluxes from wetlands constructed for wastewater treatment.

3) To investigate the effect of plant species on microbial distribution and $CO_2$, $CH_4$ and $N_2O$ fluxes.

## 1.3  Research hypothesis

The hypotheses of this research are:

1) wetlands constructed for wastewater treatment are sources of $CO_2$, $CH_4$ and $N_2O$;

2) greenhouse gas fluxes from constructed wetlands have diurnal and seasonal fluctuations influenced by several factors such as plant, wastewater characteristics, and some environmental factors; and

3) plant species and rhizobacterium are involved in greenhouse gas production and consumption.

## 1.4  Scope and limitation of the study

This research was performed on experimental scale free water surface flow (FWS) and horizontal subsurface flow (HSSF or SF) constructed wetlands used to

treat artificial domestic wastewater and located in Suranaree University of Technology. Different emergent plants were compared among *Phragmites* sp., *Cyperus* sp., and *Canna* sp..
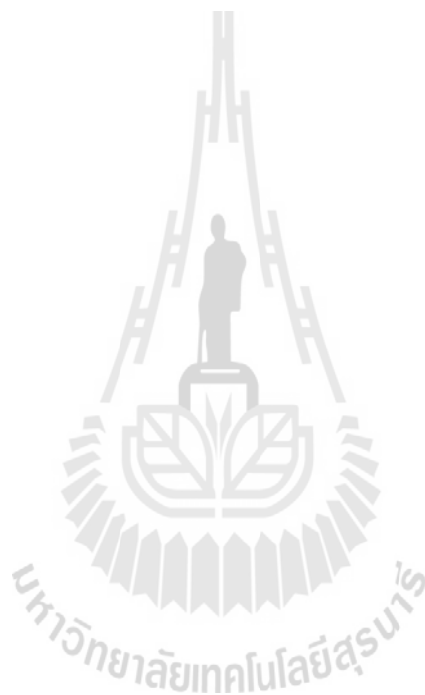
# CHAPTER II

# LITERATURE REVIEW

## 2.1  Global warming and greenhouse gases

### 2.1.1  Global warming and expected effect

Global warming is the increase in the average measured temperature of the Earth's near-surface air and oceans. The average global air temperature near the Earth's surface increased $0.74 \pm 0.18°C$ during the 100 years ending in 2005 (IPCC, 2007). The Intergovernmental Panel on Climate Change (IPCC) concludes that most of the observed increase in globally averaged temperatures since the mid-twentieth century is very likely due to the observed increase in anthropogenic greenhouse gas concentrations via an enhanced greenhouse effect (IPCC, 2007). Although most studies focus on the period up to 2100, warming is expected to continue for more than a thousand years even if greenhouse gas levels are stabilized.

Increasing global temperature is expected to cause sea levels to rise, an increase in the frequency and intensity of extreme weather events, and significant changes to the amount and pattern of precipitation and increased pace of desertification. Other expected effects of global warming include changes in agricultural yields, glacier retreat, reduced summer stream flows, mass species extinctions and increases in the ranges of disease vectors (IPCC, 2007).

### 2.1.2 Cause of global warming : greenhouse gases

The detailed causes of the recent warming remain an active field of research, but the scientific consensus is that the increase in atmospheric greenhouse gases due to human activity caused most of the warming observed since the start of the industrial era.

Greenhouse gases are gaseous constituents of the atmosphere, both natural and anthropogenic, that absorb and emit radiation at specific wavelengths within the spectrum of thermal infrared radiation emitted by the Earth's surface, the atmosphere itself, and by clouds. This property causes the greenhouse effect (IPCC, 2007). Greenhouse gases are essential to maintaining the temperature of the earth; without them the planet would be so cold as to be uninhabitable (Karl *et al.,* 2003). However, an excess of greenhouse gases can raise the temperature of a planet to lethal levels. The most abundant greenhouse gases are, in order of relative abundance: water vapor, carbon dioxide, methane, nitrous oxide, ozone and CFCs. This study focuses on carbon dioxide ($CO_2$) methane ($CH_4$) and nitrous oxide ($N_2O$) since ozone and CFCs are irrelevant to the use of constructed wetland.

#### Carbon Dioxide

Carbon dioxide ($CO_2$) is a colorless, odorless non-flammable gas and is the most prominent greenhouse gas in the earth's atmosphere. It is recycled through the atmosphere by photosynthesis, which makes human life possible. Photosynthesis is the process of green plants and other organisms transforming light energy into chemical energy. Light Energy is trapped and used to convert carbon dioxide, water, and other minerals into oxygen and energy rich organic compounds. Carbon dioxide is emitted into the air as humans exhale, burn fossil fuels for energy,

and deforests the planet. Since the beginning of the industrial revolution, the concentrations of many of the greenhouse gases have increased.

Global atmospheric $CO_2$ concentration has increased by approximately 39% from a pre-industrial value of about 280 ppm to 387 ppm in 2008. The annual $CO_2$ concentration growth rate was larger during the last decade (2000-2009 average: 1.9 ppm/year) than it has been since the beginning of continuous direct atmospheric measurements (1960-2005 average: 1.4 ppm/year), although there is year-to-year variability in growth rates. The main source of $CO_2$ has been from fossil fuel emission and cement production. In 2009, estimated $CO_2$ emission was about $8.4\pm0.5$ PgC which is about 37% higher than the 1990 levels. This is the second highest in human history (highest being 41% in 2008). The mean growth rate of $CO_2$ emissions was 3.2% per year from 2000-2008 (GCP, 2009).

**Methane**

Methane ($CH_4$) is a colorless, odorless, flammable gas. It is formed when plants decay and where there is very little air. It is often called swamp gas because it is abundant around water and swamps. Bacteria that breakdown organic matter in wetlands and bacteria that are found in cows, sheep, goats, buffalo, termites, and camels produce methane naturally. Since 1750, methane has doubled, and could double again by 2050. Each year anthropogenic sources add 350-500 million tons of methane to the air. It stays in the atmosphere for only 12-17 years, but traps 23 times more heat than carbon dioxide.

**Nitrous Oxide**

Nitrous oxide is another colorless greenhouse gas; however, it has a sweet odor. It is primarily used as an anesthetic because it deadens pain. Nitrous oxide is emitted by bacteria in soils and oceans. Agriculture is the main source of human-produced nitrous oxide: cultivating soil, the use of nitrogen fertilizers, and animal waste handling can all stimulate naturally occurring bacteria to produce more nitrous oxide. Nitrous oxide is the main naturally occurring regulator of stratospheric ozone. Considered lifetime over a 100 year period, it has 296 times more impact per unit weight than carbon dioxide. Thus, despite its low concentration, nitrous oxide is a largest contributor to these greenhouse gases. It ranks behind carbon dioxide, and methane. Control of nitrous oxide is part of efforts to curb greenhouse gas emissions. Comparative of greenhouse gases characteristics are shown in Table 2.1.

**Table 2.1** Compare characteristics of three major greenhouse gases.

| Characteristics | $CO_2$ | $CH_4$ | $N_2O$ |
|---|---|---|---|
| Natural source | Respiration | Wetland | Soil, Tropical forest |
| Anthropogenic source | Deforestation, Fossil fuel combustion | Paddy field, Livestock, Biomass burning | Soil fertilization, Land use activity |
| Lifetime[a] | 50-200 yrs. | 12-17 yrs. | 120 yrs. |
| Concentration[a] | 365 ppm | 1,750 ppb | 310 ppb |
| GWP[a] | 1 | 23 | 296 |
| Cause Greenhouse Effect | 49%[b] | 25%[c] | 5%[a] |

Note: [a]IPCC (2001); [b]Lyman (1990); [c]Mosier (1998)

## 2.2 Global carbon budget

Since approximately 1980, researchers have estimated the uptake of carbon by the world's oceans and terrestrial ecosystems at the global level, with an emphasis on terrestrial ecosystems. The world's terrestrial ecosystems were a net source of 40 PgC to the atmosphere over the period 1850-2000. Total emissions to the atmosphere were, thus, 315 PgC (275 PgC from fossil fuels and cement production plus 40 PgC from land), and the airborne fraction, defined relative to total emissions. The flux of carbon from changes in land use depends on the area of land affected, the carbon stocks before and after change, and the rates of decay and recovery following disturbance or management. Over the past 300 years, forests have been replaced with agricultural lands and, thus, the amount of carbon on land has decreased. Although carbon has accumulated on land in some regions (Houghton *et al.,* 1999), the change resulting from direct human activity over the 150-year period from 1850 to 2000 is estimated to have been a release of 156 PgC (Houghton, 2002), shown in Table 2.2.

**Table 2.2** The global carbon budget for 1850-2000 (units are PgC).

| Carbon budget | Year 1850-2000 |
|---|:---:|
| Emissions from fossil fuels and cement production | 275 |
| Atmospheric increase | -175 |
| Oceanic uptake | -140 |
| Net terrestrial flux | 40 |
| Land-use change | 156 |
| Residual terrestrial flux | -116 |

Source: Houghton (2008).

## 2.3 Global methane budget

Wetlands are the most important sources of atmospheric methane as listed in Table 2.3. Although the major source terms of atmospheric $CH_4$ have been identified, many of the source strengths are still uncertain due to the difficulty in assessing the global emission rates of the biospheric sources, whose strengths are highly variable in space and time: e.g., local emissions from most types of natural wetland can vary by a few orders of magnitude over a few meters. Nevertheless, new approaches have led to improved estimates of the global emissions rates from some source types. Attempts have been made to deduce emission rates from observed spatial and temporal distributions of atmospheric $CH_4$ through inverse modeling (e.g., Hein *et al.,* 1997; Houweling *et al.,* 1999). The emissions derived depend on the precise knowledge of the mean global loss rate and represent a relative attribution into aggregated sources of similar properties. The results of some of these studies are included in Table 2.3.

The mean global loss rate of atmospheric $CH_4$ is dominated by its reaction with OH in the troposphere.

$$OH + CH_4 \longrightarrow CH_3 + H_2O$$

This loss term can be quantified based on the mean global OH concentration derived from the methyl chloroform ($CH_3CCl_3$) budget. In addition there are other minor removal processes for atmospheric $CH_4$. IPCC 2nd assessment report estimates a soil sink of 30 Tg/yr. Minor amounts of $CH_4$ are also destroyed in the stratosphere by reactions with OH, Cl and O($^1$D), resulting in a combined loss rate of 40 Tg/yr. Summing these, estimate of the current global loss rate of atmospheric $CH_4$ totals 576 Tg/yr (see Table 2.3).

**Table 2.3** Estimates of the global methane budget in (Tg CH$_4$/yr) from different sources.

| Base year: | 1980s | 1992 | - | 1994 | 1990 | 1980s | 1998 |
|---|---|---|---|---|---|---|---|
| Natural sources | | | | | | | |
| Wetlands | 115 | 225[a] | 145 | | | | |
| Termites | 20 | 20 | 20 | | | | |
| Ocean | 10 | 15 | 15 | | | | |
| Hydrates | 5 | 10 | - | | | | |
| Anthropogenic sources | | | | | | | |
| Energy | 75 | 110 | 89 | | 109 | | |
| Landfills | 40 | 40 | 73 | | 36 | | |
| Ruminants | 80 | 115 | 93 | 80 | 93[b] | | |
| Waste treatment | | 25 | - | 14 | | | |
| Rice agriculture | 100 | | - | 25-54 | 60 | | |
| Biomass burning | 55 | 40 | 40 | 34 | 23 | | |
| Other | - | - | 20 | 15 | | | |
| **Total source** | **500** | **600** | | | | **597** | **598** |
| Imbalance (trend) | +40 | +20 | | | | +37 | +22 |
| Sinks | | | | | | | |
| Soils | 10 | 30 | 30 | 44 | | 30 | 30 |
| Tropospheric -OH | 450 | 510 | | | | 490 | 506 |
| Stratospheric loss | - | 40 | | | | 40 | 40 |
| **Total sink** | **460** | **580** | | | | **560** | **576** |
| **Reference:** | Fung *et al.,* 1991 | Lelieveld *et al.,* 1998 | Houweling *et al.,* 1999 | Mosier *et al.,* 1998 | Olivier *et al.,* 1999 | IPCC 2nd Assessment report | IPCC 3th Assessment report |

Note : IPCC 3th assessment report budget based on 1,745 ppb, 2.78 Tg/ppb, lifetime of 8.4 yr, and imbalance of +8 ppb/yr[a] Rice included under wetlands.

### 2.3.1 Methane emission from wetlands

Methane is produced microbiologically in anaerobic environments where oxygen and sulfate are scare such as natural wetlands, rice fields, enteric fermentation in animals, termites and landfills. The biogenic methane is mostly produced by methanogenic archaea (methanogens) in anaerobic environments. Microorganism can also remove methane from the environment through aerobic (Rudd and Taylor, 1980) and anaerobic (Alperin and Reeburgh, 1984) oxidation by methanotrophs.

**Production of methane**

In anaerobic habitats, organic carbon is converted to $CH_4$ and $CO_2$ by an anaerobic microbial food chain that includes fermentative, acetogenic, and methanogenic bacteria. The methanogens are the terminal bacteria in this food chain. In anaerobic condition, methane diffusion to the atmosphere is slow because a significant fraction may be lost through aerobic and anaerobic oxidation before it leaves the sediment. Although the anaerobic oxidation of methane has been well documented in sulfate-containing sediments and anoxic waters, little is known about organisms that carry out this process (Rogers and Whitman, 1991).

In contrast, much is known about the organisms that oxidize methane in aerobic environments. A substantial part of the gas that diffuses into the aerobic zone is metabolized by organisms, such as the methanotrophic bacteria, that are typically present in large numbers in or at the periphery of anaerobic zones. Methanotrophs can obtain all of their carbon and energy from $CH_4$ under aerobic conditions. For example, 85% of methane produced in deep sediments of freshwater lakes may be consumed by methanothrophs in the overlying water column before it reaches the surface.

The following is an overview of the underlying microbial basis for production, oxidation and emission of methane in natural wetland.

**Methanogens**

Methanogens are strictly anaerobic unicellular organisms originally thought to be bacteria but now recognized as belonging to a separate phylogenetic domain, the *Archae* (Garcia, 1990). Phenotypic characteristics of methanogenic bacteria are listed in Table 2.4.

**Table 2.4** Characteristics of methanogenic and methanotrophic bacteria.

| Characteristics | Methanogens | Methanotrophs |
|---|---|---|
| **Cell form** | rods, cocci, spirilla, filamentous, sarcina | rods, cocci, vibrios |
| **Gram stain reaction** | Gram +/– | Gram – |
| **Classification** | Archaebacteria | Eubacteria |
| **Metabolism** | Anaerobic | Aerobic |
| **Energy and carbon source** | $H_2+CO_2$; $H_2+$ methanol; formate; methylamines; methanol; acetate | methane; methanol; dimethylether; methylformate; dimethylcarbonate |
| **Catabolic products** | $CH_4$ or $CH_4+CO_2$ | $CO_2$ |
| **Typical species** | *Methanobacterium bryanthii* | *Methylosinus trichosporium* |
| | *Methanobrevibacter smithii* | *Methylomonas methanica* |
| | *Methanomicrobium mobile* | *Methylocystis minimus* |
| | *Methanogenium cariaci* | *Methylobacter albus* |

Source: Dubey (2005).

Methanogens can be categorized into three groups. Group I comprises of *Methanobacterium* and *Methanobrevibacter*, Group II contains *Methanococcus,* and Group III comprises of the genera including *Methanospirillum* and *Methanosarcina* (Garcia, 1990). They proliferate in anaerobic freshwater environments, such as sediments and the digestive tract of animals (Topp and Pattey, 1997). In these habitats, methanogens play an important role in the degradation of complex organic compounds. Methanogens mainly use acetate (contributes 80% to $CH_4$ production) as a carbon substrate but other substrate like $H_2/CO_2$ and formats also contribute 10-30% to $CH_4$ production (Dubey, 2005).

**Methanogenesis**

Methane is produced in the anaerobic layers of soil by bacterial decomposition of organic matter. The organic matter converted to $CH_4$ is derived mainly from plant-borne material, and organic manure. The anaerobic degradation of organic matter involves four main steps: a) hydrolysis of polymers by hydrolytic organisms, b) acid formation from simple organic compound by fermentative bacteria, c) acetate formation from metabolites of fermentations by homoacetogenic or syntrophic bacteria, and d) $CH_4$ formation from $H_2/CO_2$, acetate, simple methylated compounds or alcohols and $CO_2$ as shown in Figure 2.1 and Table 2.5.
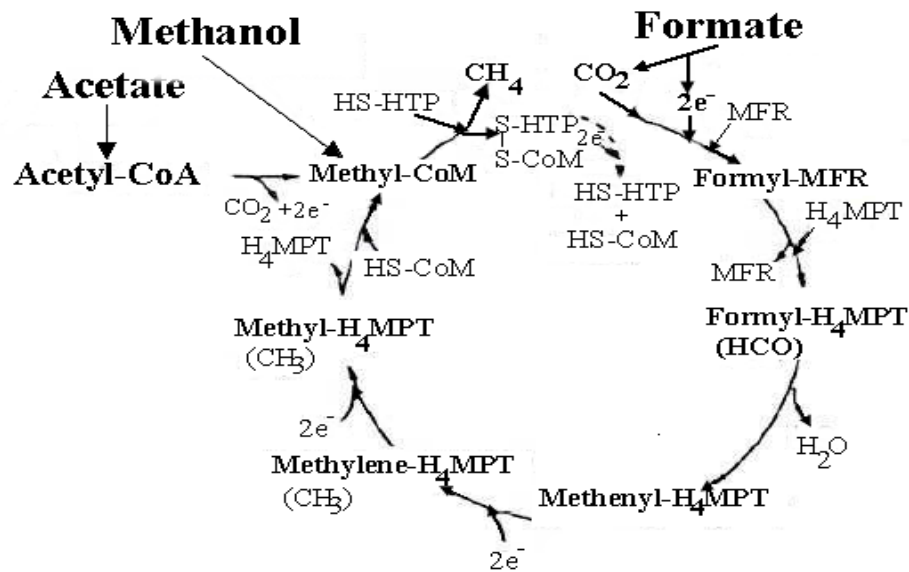
**Figure 2.1** Generalized pathway for methane production from $CO_2$, acetate, methanol, and formate.

Abbreviations: CoM, coenzyme M; $H_4$MPT, tetrahydromethanopterin; MFR, methanofuran; HS-HTP, 7-mercaptoheptanoyltheronine phosphate (Jones, 1991).
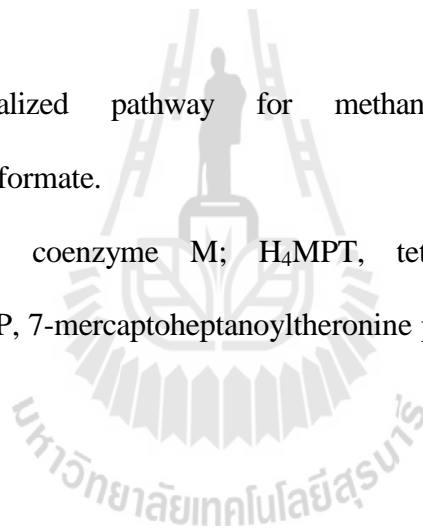
**Table 2.5** Substrates and energetics of methane production.

| Reactions | $\Delta G_0$, (kJ/mol of methane) |
|---|---|
| Hydrogenotrophic reactions | |
| $4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$ | -135.6 |
| $4 \text{ Formate} \longrightarrow CH_4 + 3CO_2 + 2H_2O$ | -130.1 |
| $4(2\text{-proponal}) + CO_2 \longrightarrow CH_4 + 4 \text{ acetone} + H_2O$ | -36.5 |
| Aceticlastic reaction | |
| $\text{Acetate} \longrightarrow CH_4 + CO_2$ | -31.0 |
| Disproportionate reactions | |
| $4\text{-Methanol} \longrightarrow 3CH_4 + CO_2 + 2H_2O$ | -104.9 |
| $4 \text{ Methylamine} + 3H_2O \longrightarrow 3CH_4 + CO_2 + 4NH_4^+$ | -75.0 |
| $2 \text{ Dimethyl sulfide} + 2H_2O \longrightarrow 3CH_4 + CO_2 + H_2S$ | -73.8 |

Source: Jones, 1991.

**Methanotrophs**

Methanotrophs (gram negative, aerobic bacteria belonging to the subset of a physiological group of bacteria known as methylotrophs) oxidize $CH_4$ via methane-monooxygenase (MMO) enzyme. These bacteria are classified into three groups: Type-I, Type-II and Type-X. According to Conrad (1999), all the methanotrophs that have been isolated and described belong to the Proteobacteria, of the γ sub-class (Type I) or α sub-class (Type II). The Type I group is represented by the *Methylomonas, Methylocaldum, Methylosphaera, Methylomicrobium* and *Methylobacter*. The Type-II comprises of *Methylosystis* and *Methylosinus*. The members of the genus *Methylococcus* occupy an intermediate position and have been

kept into a separate group Type-X (Hanson and Hanson, 1996). By using molecular ecology techniques, it has becomeclear that methanotrophs are ubiquitous in nature and well adopted to high or low temperature, pH and salanity. Methanotrophic bacteria are present in the aerobic soil layer, rhizosphere and on the roots and stem bases of flooded plants (Watanabe *et al.,* 1997).

### 2.3.2  Pathways of methane emission

The net amount of $CH_4$ emitted from soil to the atmosphere is the balance of two opposite processes-production and oxidation. Methane, the product of methanogenesis, escapes to the atmosphere from soil via aerobic interfaces where $CH_4$ oxidation takes place. There are three pathways of $CH_4$-transport into the atmosphere-molecular diffusion, ebullition and plant transport (Figure 2.2). Diffusion is not the only mechanism for release of trace gases from anaerobic environments to the atmosphere. Ebullition is also the common and significant mechanism of $CH_4$ flux in natural wetlands (Wassmann and Martius, 1997). Aquatic plants also can provide and important pathway for the transfer of gases between anaerobic environments and the atmosphere. Gas can move from the root zone up through the stems into the atmosphere or from the atmosphere into the root zone.
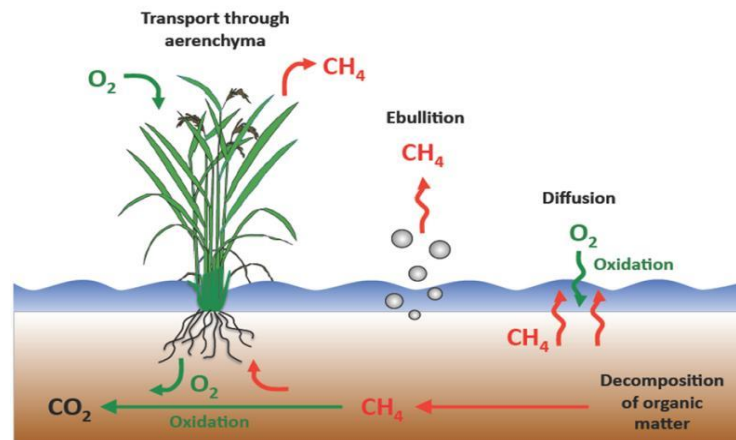
**Figure 2.2** Primary modes of gas transfer to the atmosphere from aquatic environments (Dubey, 2005).

## 2.4 Global nitrous oxide budget

Agricultural soils and wet forest are important sources of $N_2O$ as listed in Table 2.6 with estimates of their emission rates and ranges. As with $CH_4$, $N_2O$ remains difficult to assess global emission rates from individual sources that vary greatly over small spatial and temporal scales. The study calculated values for agricultural $N_2O$ emissions that include the full impact of agriculture on the global nitrogen cycle and showed that $N_2O$ emissions from soils are the largest term in the budget. Emissions from other anthropogenic and natural sources to calculate a total emission is 17.7 TgN/yr for 1994 (see Table 2.6).

The identified sinks for $N_2O$ are photodissociation (90%) and reaction with electronically excited oxygen atoms ($O(^1D)$); they occur in the stratosphere and lead to an atmospheric lifetime of 120 years. The small uptake of $N_2O$ by soils is not included in this lifetime, but is rather incorporated into the net emission of $N_2O$ from soils because it is coupled to the overall N-partitioning (IPCC, 2001).

### 2.4.1 Nitrous oxide emission

The formation of nitrous oxide results from the inefficient conversion of ammonium ion to nitrate or nitrate to molecular nitrogen. Denitrification has been considered the principal source of nitrous oxide to the atmosphere. Nitrification, however, also contributes a significant amount of nitrous oxide to the atmosphere.

### Denitirification

Denitrification is a microbially facilitated process of dissimilatory nitrate reduction that may ultimately produce molecular nitrogen ($N_2$) through a series of intermediate gaseous nitrogen oxide products. This respiratory process reduces oxidized forms of nitrogen in response to the oxidation of an electron donor such as organic matter. The preferred nitrogen electron acceptors in order of most to least thermodynamically favorable include: nitrate ($NO_3^-$), nitrite ($NO_2^-$), nitric oxide (NO), and nitrous oxide ($N_2O$). In terms of the general nitrogen cycle, denitrification performs the opposite function of nitrogen fixation, which converts gaseous nitrogen into a more oxidized and biologically available form. The process is performed primarily by heterotrophic bacteria (such as *Paracoccus denitrificans* and various pseudomonads), although autotrophic denitrifiers have also been identified (e.g., *Thiobacillus denitrificans*). Denitrifiers are represented in all main proteolytic groups.

**Table 2.6** Estimates of the global $N_2O$ budget (TgN/yr) from different sources.

| Base year: | 1994 | range | 1990 | range | 1980s | 1990s |
|---|---|---|---|---|---|---|
| **Sources** | | | | | | |
| Ocean | 3.0 | 1-5 | 3.6 | 2.8-5.7 | 3 | |
| Atmosphere ($NH_3$ oxidation) | 0.6 | 0.3-1.2 | 0.6 | 0.3-1.2 | | |
| *Tropical soils* | | | | | | |
| Wet forest | 3.0 | 2.2-3.7 | | | 3 | |
| Dry savannas | 1.0 | 0.5-2.0 | | | 1 | |
| *Temperate soils* | | | | | | |
| Forests | 1.0 | 0.1-2.0 | | | 1 | |
| Grasslands | 1.0 | 0.5-2.0 | | | 1 | |
| All soils | | | 6.6 | 3.3 -9.9 | | |
| Natural sub-total | 9.6 | 4.6-15.9 | 10.8 | 6.4-6.8 | 9 | |
| Agricultural soils | 4.2 | 0.6-14.8 | 1.9 | 0.7-4.3 | 3.5 | |
| Biomass burning | 0.5 | 0.2-1.0 | 0.5 | 0.2-0.8 | 0.5 | |
| Industrial sources | 1.3 | 0.7-1.8 | 0.7 | 0.2-1.1 | 1.3 | |
| Cattle and feedlots | 2.1 | 0.6-3.1 | 1.0 | 0.2-2.0 | 0.4 | |
| Anthropogenic Sub-total | 8.1 | 2.1-20.7 | 4.1 | 1.3-7.7 | 5.7 | 6.9[a] |
| Total sources | 17.7 | 6.7-36.6 | 14.9 | 7.7 4.5 | 14.7[b] | |
| Imbalance (trend) | 3.9 | 3.1-4.7 | | | 3.9 | 3.8 |
| Total sinks (stratospheric) | 12.3 | 9-16 | | | 12.3 | 12.6 |
| Implied total source | 16.2 | | | | 16.2 | 16.4 |
| **Reference:** | Mosier *et al.,*1998b  Kroeze *et al.,*1999 | | Olivier *et al.,* 1998 | | SAR | TAR |

[a] *SRES 2000 anthropogenic $N_2O$ emissions.*
[b] *N.B. total sources do not equal sink + imbalance*

Generally several species of bacteria are involved in the complete reduction of nitrate to molecular nitrogen, and more than one enzymatic pathway has been identified in the reduction process.

Denitrification takes place under special conditions in both terrestrial and marine ecosystems. In general, it occurs where oxygen, a more energetically favorable electron acceptor, is depleted, and bacteria respire nitrate as a substitute terminal electron acceptor. Due to the high concentration of oxygen in our atmosphere, denitrification only takes place in environments where oxygen consumption exceeds the rate of oxygen supply, such as in some soils and groundwater, wetlands, poorly ventilated corners of the ocean, and in seafloor sediments. Denitrification generally proceeds through some combination of the following intermediate forms:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \text{ (gas)}$$

Denitrification is the second step in the nitrification-denitrification process, the conventional way to remove nitrogen from sewage and municipal wastewater. It is also an instrumental process in wetlands and riparian zones for the removal of excess nitrate from groundwater with excess nitrate levels, commonly by extensive agricultural or residential fertilizer usage.

**Nitirification**

Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite.

In the first step of nitrification, ammonia-oxidizing bacteria oxidize ammonia to nitrite according to Equation (2.1).

$$NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \qquad\qquad \nearrow N_2O \qquad\qquad (2.1)$$
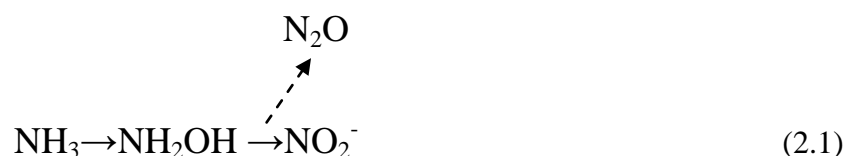
The oxidation of ammonia into nitrite is performed by two groups of organisms, ammonia oxidizing bacteria and ammonia oxidizing *archaea*. Ammonia oxidizing bacteria can be found among the β- and γ-proteobacteria (Purkhold *et al.,* 2000). In soils the most studied ammonia oxidizing bacteria belong to the genera *Nitrosomonas* and *Nitrosococcus*. Although in soils ammonia oxidation occurs by both bacteria and *archaea* in harsher environments like oceans ammonia oxidation is dominated by *archaea* (Treusch *et al.,* 2005). Recent works have shown that certain *archaea* can also oxidize ammonium to nitrite with a metabolism similar to that of bacterial ammonium (Konneke *et al.,* 2005).

The second stage is nitrite oxidation to nitrate, with nitric oxide acting as an intermediate and possible precursor of $N_2O$, according to Equation (2.2).

$$NO_2^- \rightarrow NO_3^- \qquad\qquad \nearrow N_2O \qquad\qquad (2.2)$$

*Nitrobacter* is the most frequently identified genus associated with this second step, although other genera, including *Nitrospina, Nitrococcus*, and *Nitrospira* can also autotrophically oxidize nitrite (Watson *et al.,* 1981).

Both steps are producing energy to be coupled to ATP synthesis. Nitrifying organisms are chemoautotrophs, and use carbon dioxide as their carbon source for growth. Nitrification also plays an important role in the removal of nitrogen from municipal wastewater. For many years, denitrification was thought to be the only source of $N_2O$. However, it is now well recognized that $N_2O$ can be produced during nitrification. Production of $NO_2^-$ and $NO_3^-$ from $NH_4^+$ via nitrification can result from a number of different pathways (Firestone and Davidson, 1989). Chemoautotrophic nitrifying bacteria can obtain energy from the oxidation of $NH_4^+$ to $NO_2^-$ and $NO_3^-$. The most thoroughly investigated of these bacteria are the genera *Nitrosomonas* and *Nitrobacter*. A second important group is the heterotrophic nitrifying bacteria that oxidize ammonium ion at the expense of a carbon substrate. Various groups of heterotrophic bacteria and fungi can also carry out nitrification, although at as lower rate than autotrophic organisms (Watson *et al.,* 1981). Chemoautotrophic nitrifying bacteria have specific activities $10^2$ to $10^3$ greater than heterotrophic nitrifying bacteria; however, heterotrophs vastly outnumber chemoautotrophs in the environment.

## 2.5  Carbon dioxide flux from wetlands

Photosynthesis is the process by which plants fix carbon from the atmosphere for their growth and maintenance. Photosynthesis is therefore the uptake of $CO_2$ by plants by capturing light energy that splits water molecules to produce high energy molecules and energy, after which the reduction of $CO_2$ into carbohydrates occurs (see Equation (2.3)).

$$CO_2 + \text{light energy} + H_2O + CH_2O + O_2 \qquad (2.3)$$
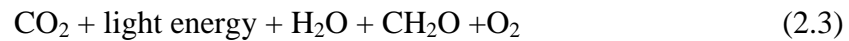
Part of the carbon taken up by plants is returned to the atmosphere as $CO_2$ during maintenance and growth respiration of above and belowground biomass of the plants and their heterotrophic microbial communities. The rest is transformed into plant structures and subsequently deposited as peat. In anoxic layers, this is the carbon that is available for methanogenic bacteria, which produces $CH_4$ as end product. While diffusing upwards, this $CH_4$ is then oxidized by methanotrophic bacteria back into $CO_2$ in upper aerobic peat layers (Sundh *et al.,* 1994). Net primary productivity (NPP) which is the result of gross carbon uptake minus release by plants (autotrophic respiration) explains to a large extent the sink function of $CO_2$ in peat lands since photosynthesis (GPP) usually exceeds plant respiration (Rplant). Thus, NPP = GPP - Rplant is negative. In order to incorporate a better proportion of the ecosystem scale carbon uptake function, it is better to make use of the net ecosystem exchange (NEE), see Figure 2.3, which is the difference between carbon uptake by plants and the total ecosystem carbon loss through plant and soil respiration (Reco). Thus, NEE = GPP-Reco.

Another factor of relevance to the sink function of carbon in subarctic mires would be the export of particulate and dissolved organic carbon to nearby ecosystems which this study does not take into consideration.
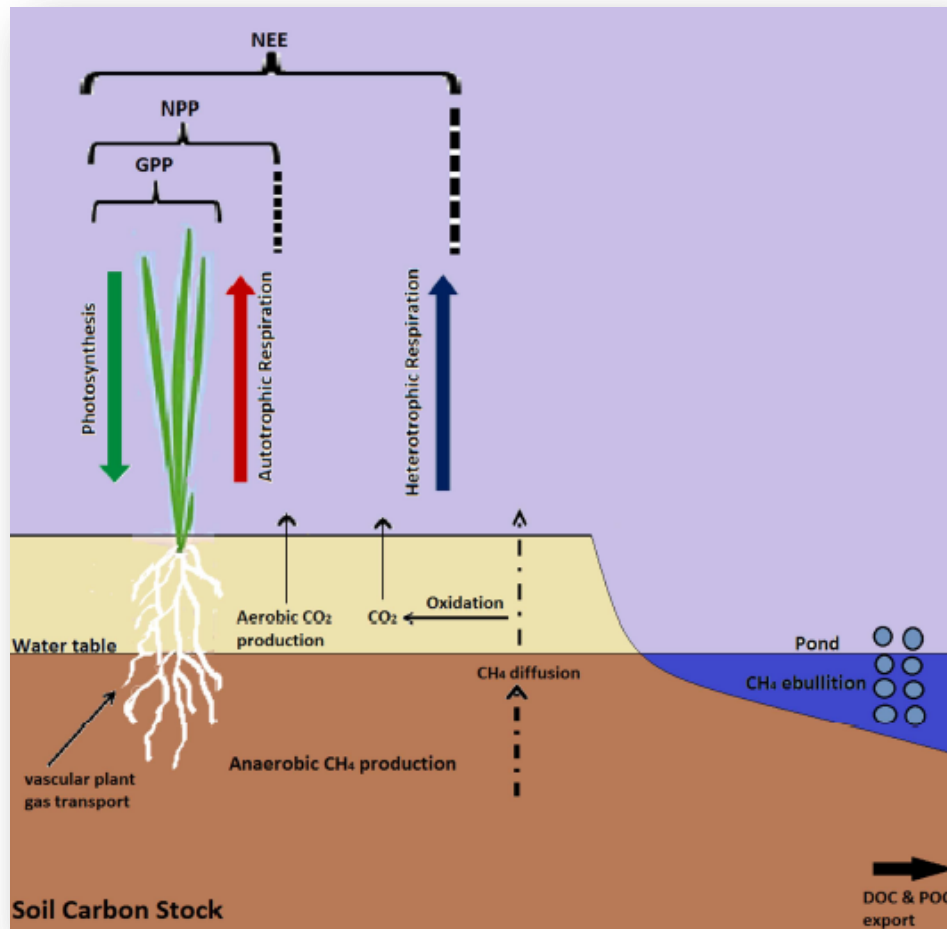
**Figure 2.3** General elements of carbon cycling, i.e. GPP, NPP, NEE and respiration. (Modified from Christensen *et al.*, 2007).

## 2.6  Diurnal and seasonal variations of greenhouse gas fluxes

### 2.6.1  Diurnal variations

Diurnal variation of greenhouse gases are different pattern governed by several factors as described below.

#### CH$_4$ emission

Emission rates of CH$_4$ generally increase after sunrise, reach a peak in the early afternoon then decline at night. Grunfeld and Brix (1999) measured methane

emission from *Phragmites australis* in Denmark. They found that rates of $CH_4$ emission from *Phragmites australis* peaked at midday and were 50-150% higher than the relatively constant rates observed in the morning and during the night. Peaks in emission rates were associated with high solar illumination, high air temperatures and low humidity, factors that are known to stimulate pressurized convective flow in *Phragmites australis* (Brix *et al.,* 1996). Whereas, Yang and Chang (1999) investigated diurnal methane emission from paddy field in Taiwan and found that methane emission rate was high from 12:00-15:00 PM and low from 2:00-5:00 AM. Methane emission showed high correlation coefficient with air temperature.

Whiting and Chanton (1996) studied diurnal pattern of methane emission from swamp in the coastal plain of southeastern Virginia, United States. The wetland was covered by two plants are *Typha lafifolia* and *Peltandra virginica*. The study showed methane emission from *Typha lafifolia* displayed a transient peak between 10:00 and 11:00 AM. This peak was over 50% greater than emission rates determined on either side of this peak period and associated with rising light levels. This pattern of emission was similar to *Typha latifolia* and *Typha domingensis* emissions measured in the Everglades of Florida where emissions peaked about 400% above adjacent base emissions (Chanton *et al.,* 1993). While emission from *Peltandra virginica* revealed a gradual rise throughout the daytime with a peak of emissions during the mid-afternoon (15:00 PM). This pattern corresponded to rising air temperatures throughout the morning and midday with a maximum in the mid-afternoon. Wang and Han (2005) studied diurnal variation in methane emissions from marshes of the Xilin River basin in the eastern Inner Mongolia Plateau. Riparian marshes mainly covered with *Carex* sp*., Juncus* sp*., Glyceria* sp*.,* and *Scirpus* sp. $CH_4$

emissions increased with sunrise and decreased with sunset. The highest flux rates appeared in late afternoon about 12:00-15:00 PM after sunrise.

**N$_2$O and CO$_2$ emission**

Du *et al.* (2006) studied diurnal variation of N$_2$O fluxes from semi-arid temperate native *Leymus chinensis* grassland of inner Mongolia. The peak of N$_2$O flux commonly appeared during daytime, whereas fluxes were low at night, and the timing of the peak flux varied in different growing periods. In addition, Maljanen *et al.* (2002) reported variation of N$_2$O fluxes from cultivated and forest soil in Eastern Finland. The dominant plant comprise of grass (a mixture of *Phelum pretense, Festuca pratensis* and *Trifolium pretense*) and barley. They found a strong diurnal variation in N$_2$O and CO$_2$ fluxes from cultivated and forest soil. The maximum N$_2$O emission from agricultural soil took place during daytime 10:00 AM-16:00 PM but, in forest soil, the maximum N$_2$O emission occurred in early morning. Whereas, the maximum CO$_2$ emission from agricultural and forest soil took place during daytime 10:00 AM-16:00 PM.

Jun *et al.* (2008) studied CO$_2$ efflux on subalpine meadows of Shangri-La, Northwest Yunnan Province, China. The dominant grass species were *Blysmussino compressus* and *Kobresia setchwanensis*. They revealed that ,in summer, highest rates of both ecosystem respiration and soil respiration occurred at 14:00 PM while the lowest rates occurred at 6:00 and 8:00 AM. In winter, the highest rates also occurred at 14:00 while the lowest rates occurred at 2:00 and 6:00 AM. The highest values were more than twice the lowest.

Bolpagni *et al.* (2007) investigated CO$_2$ fluxes across the water-atmosphere interface in a shallow oxbow lake colonized by the water chestnut *(Trapa*

*natans* L.*).* They found that the water chestnut stand was a net sink of $CO_2$ during the day-light period but it was a net source at night.

### 2.6.2 Seasonal variation

Several studies indicated that greenhouse gases in constructed wetland ecosystems showed seasonal variation. As Sovik *et al.* (2006) studied emission of the nitrous oxide and methane from constructed wetlands in Estonia, Finland, Norway, and Poland during winter and summer in horizontal and vertical subsurface flow (HSSF and VSSF), free surface water (FSW) wetlands. They reported that emissions of $N_2O$, $CO_2$ and $CH_4$ were significantly higher during summer season than during winter season. Contrast to Sovik and Klove (2007) investigated emission of $N_2O$ and $CH_4$ from a free surface water wetland polishing chemically treated municipal wastewater in southeastern Norway and consists of three ponds as well as trickling, unsaturated filters with light weight aggregates. They revealed that flux of $N_2O$ has a significant difference between the summer, winter and autumn, with the highest emissions occurring during the autumn. The fluxes of $CH_4$ were, on the other hand, not significantly different with regard to seasons. Both the emissions of $N_2O$ and $CH_4$ were positively influenced by the amount of total organic carbon (TOC).

Inamori *et al.* (2008) studied seasonal $N_2O$ emission from sub-surface flow constructed wetland treating artificial domestic wastewater, established in the National Institute for Environmental Studies of Tsukuba, Japan. The treatment cells (monoculture) were planted to *Phragmites saustralis*, *Typha latifolia* and *Zizania latifolia*. They revealed that $N_2O$ fluxes showed significant differences with seasonal fluctuations. The emission peak appeared in growth seasons (July-September) and the $N_2O$ amount was much higher with variation of years.

Liikanen *et al.* (2006) investigated $CO_2$, $CH_4$ and $N_2O$ from constructed surface and subsurface flow wetland treating peat mining are a situated in Northern Finland. The dominant vegetation species were *Menyanthes trifoliata, Carex lasiocarpa* and *Potentilla palustris.* They found that $CH_4$ fluxes were smallest in winter and highest in autumn. The $N_2O$ fluxes were high in spring and summer, but negligible in autumn and winter. For $CO_2$, release of $CO_2$ varied from a wintertime minimum to summertime maximum.

## 2.7 Factors affecting greenhouse gases emission

Greenhouse gas emission from constructed wetland is controlled by a complex set of parameters such as temperature, plant, soil redox state etc. The emission of $CH_4$, $CO_2$ and $N_2O$ from wetland systems could be explained more precisely by analyzing the methanogenic and methanotrophic microbial populations. On the other hand, the activities of these microbial populations were reported to be influenced by many factors such as temperature, water quality, pH, water table level and redox state of the rhizosphere (Le Mer and Roger, 2001; Yang and Chang, 1998).

### 2.7.1 Soil pH, redox potential and texture

Methane production in flooded soils is very sensitive to pH with an optimum range between 6.7 and 7.1 (Wang *et al.,* 1993). Yagi and Minami (1990) reported that values of redox potential (Eh) varied from -100 to -200 mV for the initiation of $CH_4$ production in paddy soils. All researches found the range of oxidation reduction potential (ORP), that favored both $N_2O$ and $CH_4$ generation, were lower than -100 mV. Some suggested that soils containing greater amounts of readily decomposable

organic substrates (acetate, formate, methanol, methylated amines, etc.) and low amounts of electron acceptors ($Fe^{3+}$, $Mn^{4+}$, $NO3^-$, $SO_4^{2-}$) are likely to show high production of $CH_4$. According to sequential oxidation-reduction order, molecular $O_2$ is the first to be reduced at an Eh of about +30 mV followed by $NO_3^-$ and $Mn_4^+$ at +250 mV, $Fe_3^+$ at +125 mV and $SO_4^{2-}$ at -150 mV (Patrick, 1981). Subsequent to $SO_4^{2-}$ reduction, methanogens will start producing methane. As texture determines various physico-chemical properties of soil, it could influence $CH_4$ production indirectly. Jackel *et al.* (2001) found that rates of $CH_4$ production increased when the aggregate size of the soil increased.

### 2.7.2 Temperature

Methane emission is much more responsive to temperature. Seiler (1984) reported that methane production increased twice when temperature rose from 20°C to 25°C. Sass *et al.* (1991) found that methane production peaks when soil temperature reaches at 37°C. Temperature not only has an effect on methane production itself but also has an effect on the decomposition of organic materials from which the methanogenic substrates are produced. The influence of temperature on $CH_4$ production rates has been reported for several ecosystems. In constructed wetland, seasonal shift strongly related to changes in the surface soil, sediment and water temperature (Johansson, 2004; Picek *et al.,* 2007). Air temperature is another parameter that affects net $CH_4$ flux, by influencing the $CH_4$-oxidizing and $CH_4$-producing microbial community and its level of activity (Moore, 1993). Activity of methanogens has commonly been found to fluctuate in response to temperature (Schultz *et al.,* 1989). Grunfeld and Brix (1999) recorded the highest rates of gas exchange through the plant component during hot and dry summer days, which was

related to the effect of solar radiation on the convective gas flow. The flow rates through the efflux culms were significantly correlated with solar radiation. However, the degree of variation explained by this relationship was fairly limited (Picek *et al.,* 2007). For $N_2O$, nitrification reaction rate depends on temperature and the optimum temperature for nitrifiers' activity is approximately 30°C (Thornley, 1998). Liiakanen *et al.* (1984) reported that $CO_2$ emission from constructed wetland had a strong correlation with soil and air temperatures, but $CH_4$ and $N_2O$ fluxes had weak correlations with surface soil or air temperatures.

### 2.7.3  Water pollutant

Both the emissions of $N_2O$ and $CH_4$ were positively influenced by the amount of total organic carbon (TOC) (Sovik and Klove, 2007) and BOD concentration (Inamori *et al.,* 2007; Sovik *et al.,* 2006). Since, supply of organic matter is essential for $CH_4$ production, even though only a small portion of the organic substrate pool is directly utilized by the methanogens, mainly acetate and $CO_2$ (Oremland, 1988). Comparable to Wang *et al.* (2008) revealed that difference in emission intensity varied with influent pollutants concentrations. Thus, substrate supply and degree of oxidation are the principal controls on $CH_4$ and $N_2O$ fluxes from all soils. For $N_2O$, Sovik *et al.* (2006) indicated that wetlands receiving water with high concentrations of total N (i.e., the wetlands receiving municipal wastewater) are also the wetlands with highest emissions of $N_2O$. Probably both the nitrification and the denitrification processes are causing the high emission rates from constructed wetlands. Nitrification has been found to release $N_2O$ to a large degree in microaerobic conditions, whereas high loading rates of wastewater may give partial anaerobic conditions where nitrate may be reduced to $N_2O$ and $N_2$ gases.

### 2.7.4 Plant

The variations among the methane flux rates depend on plant type 31-88% (Johansson, 2004). As Wang *et al.* (2008) showed that $CH_4$ flux properties, activities of methanogens and methanotrophs and the relationship between $CH_4$ flux rate and some environmental parameters were greatly different in different plant species within constructed wetland systems. It is assumed that aquatic plant oxygen release enhanced $CH_4$ generation. The capabilities of oxygen transportation and carbon accumulation were affected by different aquatic plant species. In addition, rhizosphere structure of wetland aquatic plants had a large effect on the microbial ecology. Inamori *et al.* (2007) found that there was very different rhizosphere structure for the *Zizania latifolia* versus *Phragmites australis* systems. The root of *Zizania latifolia* is shallow, and 90% of the root biomass is concentrated in the upper 10 cm of the experimental unit. Conversely, the root of *Phragmites australis* is deeper and the root biomass more evenly distributed from near the soil surface to the bottom of the rhizosphere. The shallow root of *Zizania latifolia* confines oxygen's availability and the activity of methanotrophs in the upper portion of the soil, while the root of *Phragmites australis* is deeper and can oxidize methane to a greater depth resulting higher $CH_4$ emission from *Zizania latifolia*. For $N_2O$, Inamori *et al.* (2008) revealed that different aquatic plants resulted in different $N_2O$ emission. Since plant root exudates provide a source of reduced carbon, nitrogen and other nutrients for microorganisms. Acting as a conduit for oxygen transportation into and out of the substratum, the areas of active root growth of different plants species have played important roles in $N_2O$ conversion (Tanner, 2001).

The growth state of aquatic plants rhizosphere may be of importance to control greenhouse gases emission. Nouchi *et al.* (1994) indicated that the maximum $CH_4$ emission occurred at the maximum growth phase of plants due to stimulation of methanogenic bacteria by root exudation. Comparable to Wang *et al.* (2008) which revealed that fluxes of $N_2O$ in the growth season were 2-6 fold higher than those of the senescence period. During the maximum growth stage of the vegetation, N mineralization by the roots was accelerated as a result of increased $NH_4^+$-N and $NO_3^-$-N concentration in the soil (Li, 1999). Such an increase in mineral N might promote denitrification in addition to autotrophic and heterotrophic nitrification processes.

Additionally, methane emission was significantly influenced by plant harvest. High methane emission was recorded immediately after harvesting in both wetlands (Zhu *et al.,* 2007).  As in wetlands inhabited by plants, vascular transport is most important for the flux of $CH_4$ (Schutz *et al.,* 1991).The increase in $CH_4$ flux immediately after plants were cut may have been due to the rapid release of $CH_4$ retained inside the vascular systems of the stalks.

## 2.8  Constructed wetland

### 2.8.1  Definition

A constructed wetland is an artificial marsh or swamp, created for anthropogenic discharge such as wastewater, storm water runoff or sewage treatment, and as habitat for wildlife. They have four key components:

- soil and drainage materials (such as pipes and gravel),
- water,
- plants (both above and below the water),

- microorganisms.

Constructed wetlands purify the water that flows through them. Compared to conventional treatment methods, they tend to be simple, inexpensive, and environmentally friendly. Constructed wetlands may be used to treat water from many different sources:

- sewage (from small communities, individual homes, and businesses),
- storm water,
- agricultural wastewater (including livestock waste, runoff, drainage water),
- landfill leachate,
- partially treated industrial wastewater,
- drainage water from mines,
- runoff from highways.

### 2.8.2 Constructed wetland types

Constructed wetlands are of two basic types: free water surface-flow and subsurface-flow wetlands. Free water surface-flow constructed wetlands (FWS) move effluent above the soil in a planted marsh or swamp whereas subsurface-flow constructed wetlands (SSF) move effluent through a medium on which plants are rooted. SSF can be further classified as horizontal sub-surface flow (HSSF) and vertical sub-surface flow (VSSF) constructed wetlands which the effluent may move either horizontally, parallel to the surface, or vertically, from the planted layer down through the substrate and out, respectively. These systems admit variations in the construction criteria and may be operated differently according to several specific designs (Table 2.7).

**Table 2.7** Summary of the characteristics of different constructed wetland types.

| Type | Advantages | Disadvantages |
|------|------------|---------------|
| FWS | - Combine aerobic and anaerobic conditions | - Large extension of land required for construction |
| | - More efficient nitrogen removal than SSF | - High susceptibility to climate conditions |
| | - More suitable in warmer climates | - Potential exposure of water to human contact |
| | - Well suited for small communities | |
| | - Aesthetic appeal, recreational and environmental education activities | |
| HSSF | - Greater treatment surface | - Limited aeration |
| | - High organic consumption rates | - Poor potential for nitrification |
| | - Decreased odor production | - Large extension of land required |
| | - Decreased insect proliferation | |
| | - Higher tolerance to climatic conditions | |
| VSSF | - Minimized treatment area than HSSF | - Higher construction costs, restrict large spatial development |
| | - Better oxygen transfer, higher nitrification than HSSF | - Less efficient in removal of suspended solids than H-SSF |
| VSSF | - Suited to small communities where inexpensive land and low cost media are readily available | - Often need further polishing in HSSF |

Adapted from Kadlec and Knight, 1996.

### 2.8.3 General contaminant removal

Physical, chemical, and biological processes are combined in wetlands to remove contaminants from wastewater. Theoretically, treatment of wastewater within a constructed wetland occurs as wastewater passes through the wetland medium and the plant rhizosphere. A thin aerobic film around each root hair is aerobic due to the leakage of oxygen from the rhizomes, roots, and rootlets. Decomposition of organic matter is facilitated by aerobic and anaerobic micro-organisms present. Microbial

nitrification and subsequent denitrification release nitrogen as gas to the atmosphere. Phosphorus is co-precipitated with iron, aluminum, and calcium compounds located in the root-bed medium. Suspended solids are filtered out as they settle in the water column in surface flow wetlands or are physically filtered out by the medium within subsurface flow wetland cells. Harmful bacteria and viruses are reduced by filtration and adsorption by biofilms on the rock media in subsurface flow and vertical flow systems. Principal contaminant removal and transformation mechanisms in FWS and SSF constructed wetland are summarized in Table 2.8.

**Table 2.8** Description of principal contaminant removal and transformation mechanisms in free water surface (FWS) and subsurface flow (SF) constructed wetlands.

| Contaminant | FWS system | SSF system |
|---|---|---|
| Organic Material | Bioconversion by aerobic facultative, and anaerobic bacteria on plant and debris surfaces (soluble BOD) Adsorption, filtration, and sedimentation (particulate BOD) | Bioconversion by facultative, and anaerobic bacteria on plant and media surfaces Adsorption, filtration, and sedimentation (particulate BOD) |
| Trace organics | Volatilization, adsorption, photolysis, biodegradation | Adsorption, biodegradation |
| Suspended solids | Sedimentation, filtration | Filtration, sedimentation |
| Nitrogen | Nitrification/denitrification, microbial/plant uptake, volatilization | Nitrification/denitrification, microbial/plant uptake, volatilization |
| Phosphorous | Sedimentation, soil sorption, plant and microbial uptake | Filtration , sedimentation, media sorption, uptake |
| Heavy metal | Adsorption of plant and debris surfaces, sedimentation, plant uptake | Adsorption of media surfaces, sedimentation, plant uptake |
| Pathogens | Natural decay, predation, UV irradiation, sedimentation | Natural decay, sedimentation , predation |

Source: Crites and Tchobanoglous, 1998.

### 2.8.4  Roles of vegetation in constructed wetlands

Vegetation selected for the constructed wetland will be emergent hydrophytic plants suitable for local climatic conditions and tolerant of the concentrations of nutrients, pesticides, and other constituents in the storm water. Principal plants to be used include

cattail, maiden cane, bulrush, and reed. Although natural wetlands typically have a wide diversity of plant life, attempts to reproduce the natural diversity in a constructed wetland have proven unnecessary. Cattails alone or in combination with either reeds or bulrushes will often dominate in an established system. Free floating plants, such as water hyacinth and duckweed, have proven useful in municipal treatment systems; however, they are not to be used in constructed wetlands associated with these requirements due to the need for harvesting. For aesthetics and beautification, one should consider blue flag iris, canna lily, ginger lily, and wildflowers on dikes and other disturbed areas which are outside of maintenance activity areas. Nutrient uptake is not a major consideration in plant selection. The roots and stems in the water column serve as a medium for bacterial growth and serve as a media for filtration and adsorption of solids and enhanced settling. The stems and leaves at or above the water surface provide shade and thus reduce growth of algae. Wetland plants provide for the transfer of oxygen to and from the submerged parts of the constructed wetland plants. Plants can be planted with a dibble bar, trencher, or a one-row tree planter and should be established on about 3.0 feet centers. The planting depth will vary, depending on species but all roots should be covered with 2-4 inches of soil mixed with available organic matter. Several experimental results showed that pollutant removal efficiencies of constructed wetland systems, which have different emergent plants, are various. The removal efficiencies of major emergent macrophytes can be summarized in Table 2.9.

**Table 2.9** Comparison of pollutant removal efficiency from different emergent plants.

| Removal efficiency (%) | *Typha* sp. | *Scirpus* sp. | *Phragmites* sp. | *Canna* sp. | *Vetiveria* sp. |
|---|---|---|---|---|---|
| BOD | | > 60[3,4] | 85.8[5] | 90.5±4.8[7] | 97-98[8] |
| COD | 68[1] | | 94.4[5] | 75.5±7.9[7] | |
| T-N | 89[1] | 85-97[3,4] | 64-86[5,6] | 44.3±5.3[7] | 81-82[8] |
| NH$_3$-N | 98[2] | | | 56.9±13.4[7] | |
| T-P | 67[2] | 93-99[3,4] | 17[5] | 56.7±8.2[7] | |
| SS | 86-92[1] | 60[3,4] | | | 93-94[8] |

Note :[1]Kerdsup, 2000;[2]Koanetsuwan, 2001; [3]Kantawanichkul, 2003;[4]Buddhawong ,1996; [5]Panapawuttikul, 1996; [6]Urbance-Bercic and Bulc, 1995;  [7]Saranakomkun, 2005; [8]Wongpankamol, 2005.

The results are not readily comparable due to different constructed wetland designs and operations were used. Since variation of removal efficiency of these emergent plants depending on various factors such as type of constructed wetland, flow rate, media, hydraulic retention time, wastewater strength, pollutant loading etc. However, it can be observed that BOD and COD removal efficiencies of *Phragmites* sp., *Canna* sp., *Vetiveria* sp. are relatively high.

Wetland environments may emit considerable amounts of both CH$_4$ and N$_2$O, gases formed under the anoxic conditions in the sediments of inundated areas. Many of the wetlands have populations of emergent plants that were either deliberately planted or naturally colonized the area (Kadlec and Knight, 1996). These plants are morphologically adapted to growing in anoxic sediments in various ways, including the development of aerenchymous tissues that supply their roots with oxygen. However, this aerenchyma can also act as conduits for CH$_4$  and N$_2$O, thereby increasing the flux strength of these gases from the wetland to the atmosphere. Several researches explored different emergent plants in various constructed wetland systems

emitted fluxes of $CO_2$, $CH_4$ and $N_2O$ with temporal and spatial variations. Greenhouse gases fluxes from various emergent macrophytes can be summarized in Table 2.10.

The rates of greenhouse gases emission for among constructed wetland, rice paddy and natural wetland, at various climates are tabulated in Table 2.10. Most studies were conducted in temperate zone rather than tropical climate. The results show highly fluctuating due to spatial and season variation. However, it can be observed that constructed wetland tend to emit greenhouse gases relatively higher than rice paddy and natural wetland.

Variations of greenhouse gas fluxes from constructed wetlands are influenced by plant species and CW systems. Besides these factors, gaseous fluxes vary by several factors such as seasonal change (Gui *et al.,* 2007; Liikanen *et al.,* 2006; Zhu *et al.,* 2007),  operational design, hydraulic retention time (Kaewkamthong, 2002; Zhu *et al.,* 2007), plant growth rate and plant biomass (Gui *et al.,* 2007; Liikanen *et al.,* 2006).

**Table 2.10** Comparison of greenhouse gas fluxes ($mg/m^2/d$) from FWS and SSF constructed wetland, rice paddy and natural wetland.

| Wetland | Location | Plant | $CO_2$ | $CH_4$ | $N_2O$ | Reference |
|---|---|---|---|---|---|---|
| CW FWS | Finland | *Menyanthes trifoliate* *Carex lasiocarpa* *Potentilla palustris* | 7,270-13,600 | 140 - 400 | 0.34-0.45 | Liikanen *et al.*, 2006[1] |
| | Sweden | *Typha latifolia* *Spirogyra* sp. *Glyceria maxima* | | -375 - 1739 | | Johansson *et al.*,2004 [2] |
| | Thailand | *Digitaria bicoarnis* *Typha angustifoli* L. | | 64.8-1,817 | | Kaewkamthong 2002[3] |
| | Japan | *Phragmites australis* *Zizania latifolia* | | 0-1,560 | 0-3.36 | Inamori *et al.*, 2007[4] |
| | Japan | *Typha latifolia* | | 433-2,540 | | Wang *et al.*, 2007[4] |
| | | *Zizania latifolia* | | 1,621-6,487 | | |
| | | *Phragmites australis* | | 1,063-1,697 | | |
| | Japan | *Phragmites communis* | | 0-4 | | Zhu *et al.*,2007 [3] |
| CW SSF | Czech Republic | *Phragmites australis* | 96-7,416 | 0-2,232 | | Picek *et al.*, 2007 [5] |
| | Estonia | *Phragmites australis* *Typha latifolia* | -146-25,200 | -0.14-2,093 | 0.02-62.4 | Teiter and Mander, 2005[6] |
| | Estonia | *Phragmites australis* *Scirpus sylvaticus* | 600-2,000 | 1.4-4.1 | 1.3-1.4 | Mander *et al.*, 2008[6] |
| Rice paddy | Japan | | 16,264 | | 0.20-0.31 | Raghareutai, 2003 |
| | Thailand | | | 14.4-1,020 | | IPCC, 1995 |
| Natural wetland | USA | *Typha* sp. (Marsh) | | 0-1700 | | Schipper and Reddy, 1994 |
| | Australia | *Eleocharis sphacelata* (Floodplain wetland) | | 4-1056 | | Boon and Sorrell , 1995 |
| | Germany | *Phragmites australis* (Prairie wetland) | | 40-650 | | Kim *et al.*,1998 |

[1] *constructed wetland purify draining waters from the adjacent peat mining area.*

[2] *constructed wetland treating municipal wastewater from sewage treatment plant*

[3] *pilot scale constructed wetland treating domestic wastewater*

[4] *experimental scale constructed wetland treating non-point sewage at the rural area*

[5] *horizontal subsurface flow treating combined sewage and storm water runoff*

[6] *horizontal subsurface flow purify wastewater from a hospital and hybrid system treated municipal wastewater*

# CHAPTER III

# MATERIALS AND METHODOLOGY

Investigating dynamic of the greenhouse gas flux from the treatment of wastewater in constructed wetlands and the affiliation of microbial dissemination demanded experimental scales of constructed wetlands. The constructed wetlands were designed and built according to criteria provided by recommended design from renounce local and international institutions. United States Environmental Protection Agency (U.S. EPA) and Asian Institute of Technology (AIT) provided design criteria and recommendations of using constructed wetlands for municipal wastewater treatment (Asian Institute of Technology, 2007; U.S. EPA, 2000). Then, the constructed wetlands were tested and used to perform their roles in wastewater treatment while greenhouse gaseous emissions were regularly monitored and analyzed throughout the course of this study.

## 3.1 Study site

The experimental scale of constructed wetlands was built outdoor in an open space covered approximately 120 m$^2$ in Suranaree University of Technology, Nakhon Ratchasima province. The climatic conditions in the northeast area were semidry. Statistical data for a long-term ten-year period (1999-2008) from Thai Meteorology Department (TMD) reported that Nakhon Ratchasima had the average rainfall of 1,117.8 ± 198.2 mm/yr. Rainfall found mostly in the rainy season starting from May to October. Average temperature and humidity were in the ranges of 18.9-36.3°C

and 63.1-80.3% respectively. Study site is relatively flat, located approximately at 14°53'24.48"N and 102°00'23.11"E (Figure 3.1).



**Figure 3.1** Location of the study area.

## 3.2 Constructed wetlands design

Twelve constructed wetlands with identical dimensions were built based primarily on criteria of aspect ratio (AR) or length to width of 4:1 to minimize short circuiting and force the flow to move closely to plug flow hydraulic regime (U.S. EPA, 2000). Each wetland had the dimension of approximately 2.0 m×0.5 m×0.8 m (length×width×depth). Figure 3.2 shows a schematic design of the experimental scale constructed wetlands covered with transparent roof. Brick, cement and mortar were the materials used for the construction of the constructed wetlands. Thick mortar was placed both inside and outside of each constructed wetlands to prevent leak. Leak test

was performed subsequently to ensure that the constructed wetlands were water tight by filling all cells with tap water and holding for seven days. Water levels were marked and re-checked every day. Any leaks found were repaired and fixed with cement.
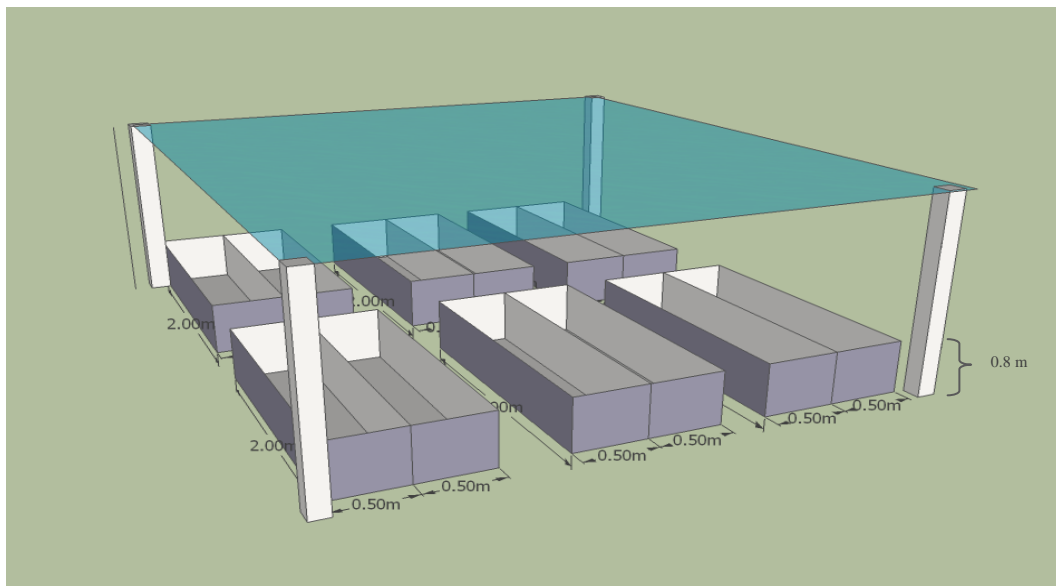


**Figure 3.2** Schematic design of the experimental scale constructed wetlands.



**Figure 3.3** Experimental constructed wetland sites.

A water outlet was placed at about 0.65 m from the bottom of each constructed wetland at the end of the cell and used as water out flow and sampling point while water inlet was placed on top (Figure 3.3). The bottom of constructed wetland cells was built with a gentle slope. A slope of approximately 1% was used at the bottom to facilitate flow in the constructed wetlands from the inlet to the outlet. Layer of coarse sand, agricultural soil in nearby land and No.2 construction gravels were used as media layer. Residues in the agricultural soil were removed using mesh screen prior to be used while construction gravels were clean by rinsing with water.

Since the experiment employed two regimes of constructed wetlands, subsurface flow (SF) and free water surface flow (FWS), all designed criteria were relatively identical, except configuration of the outlet of SF constructed wetlands. For subsurface flow constructed wetlands, perforated 1.27 cm PVC piping was laid at the bottom to form lateral and main drainage system, similar to fish-bone structure but the outlet was placed at about 0.65 m using 90° elbow pipe connector at the bottom connected upright to the designated height (0.65 m) as shown in Figure 3.4. The water in the cells will accumulate and rise up to the height prior to discharge from the constructed wetlands. In FWS constructed wetlands, the water outlet was simpler using a straight pipe.



**Figure 3.4** Water outlet of subsurface flow constructed wetlands.

The media depth in FWS constructed wetlands was approximately 0.45 m in height with the water depth of approximate 0.20 m above the media, 0.65 m all together. The SF constructed wetlands had the media depth of about 0.70 m since the water must filtrate down to the bottom and accumulate until it reached the outlet at 0.65 m. Additional 0.05 m of topsoil (0.70 minus 0.65 m) was used to prevent water level seeping on the surface. Details on technical parameters of both constructed wetlands show in Table 3.1. The flow rate for the constructed wetland units are calculated by Equations (3.1) and (3.2).

**Table 3.1** Technical parameters of the constructed wetlands.

| Parameter | FWS | SF |
|---|---|---|
| Length of each bed (m) | 2 m | 2 m |
| Width of each bed (m) | 0.5 m | 0.5 m |
| L/W ratio | 4:1 | 4:1 |
| Media depth (m) | 0.45 m | 0.65 m |
| Water Depth (m) | 0.20 m | 0.55 m |
| Average flow (m$^3$/d) | 0.04 | 0.04 |
| Hydraulic retention time (d) | 9 | 4.5 |

Free water surface flow system (FWS)

From
$$HRT = \frac{LW(d_m n + d_w)}{Q} \qquad (3.1)$$

(Lim and Polprasert, 1996)

where;    HRT  =  hydraulic retention time, (d)

L    =  basin length, (m)

W    =  basin width, (m)

$$d_m \quad = \quad \text{media depth, (m)}$$

$$d_w \quad = \quad \text{water depth from media surface, (m)}$$

$$n \quad = \quad \text{void fraction in the media (as a decimal fraction)}$$

$$Q \quad = \quad \text{average flow through the unit, (m}^3\text{/d)}$$

Sub-surface flow system

From $\quad$ HRT $\quad = \dfrac{\text{LWnD}}{\text{Q}}$ $\qquad$ (3.2)

(Metcalf and Eddy,1991)

where; $\quad$ HRT $\quad$ = $\quad$ hydraulic retention time, (d)

$$L \quad = \quad \text{basin length,(m)}$$

$$W \quad = \quad \text{basin width,(m)}$$

$$D \quad = \quad \text{depth of basin, (m)}$$

$$n \quad = \quad \text{porosity of the bed}$$

$$Q \quad = \quad \text{average flow through the unit, (m}^3\text{/d)}$$

Permanent transparent roof made from clear plastic was also constructed to prevent rain getting into the experiment setup and allow direct sun light exposure (Figure 3.5).



**Figure 3.5** Transparent roof above the constructed wetlands.

## 3.3  Vegetation in the constructed wetlands

Three emergent plants were used in this study according to their locally available, capability to treat wastewater and aesthetic aspects. All constructed wetlands were monoculture with *Phragmites* sp*.*, *Cyperus* sp. and *Canna* sp. (Figure 3.6)*.* Each had two replicate cells. This study also used two replicates non-vegetation control (CL) for each type of constructed wetlands. Diagram of all twelve constructed wetland units are shown in Figure 3.7.



(a)    (b)

(c)    (d)
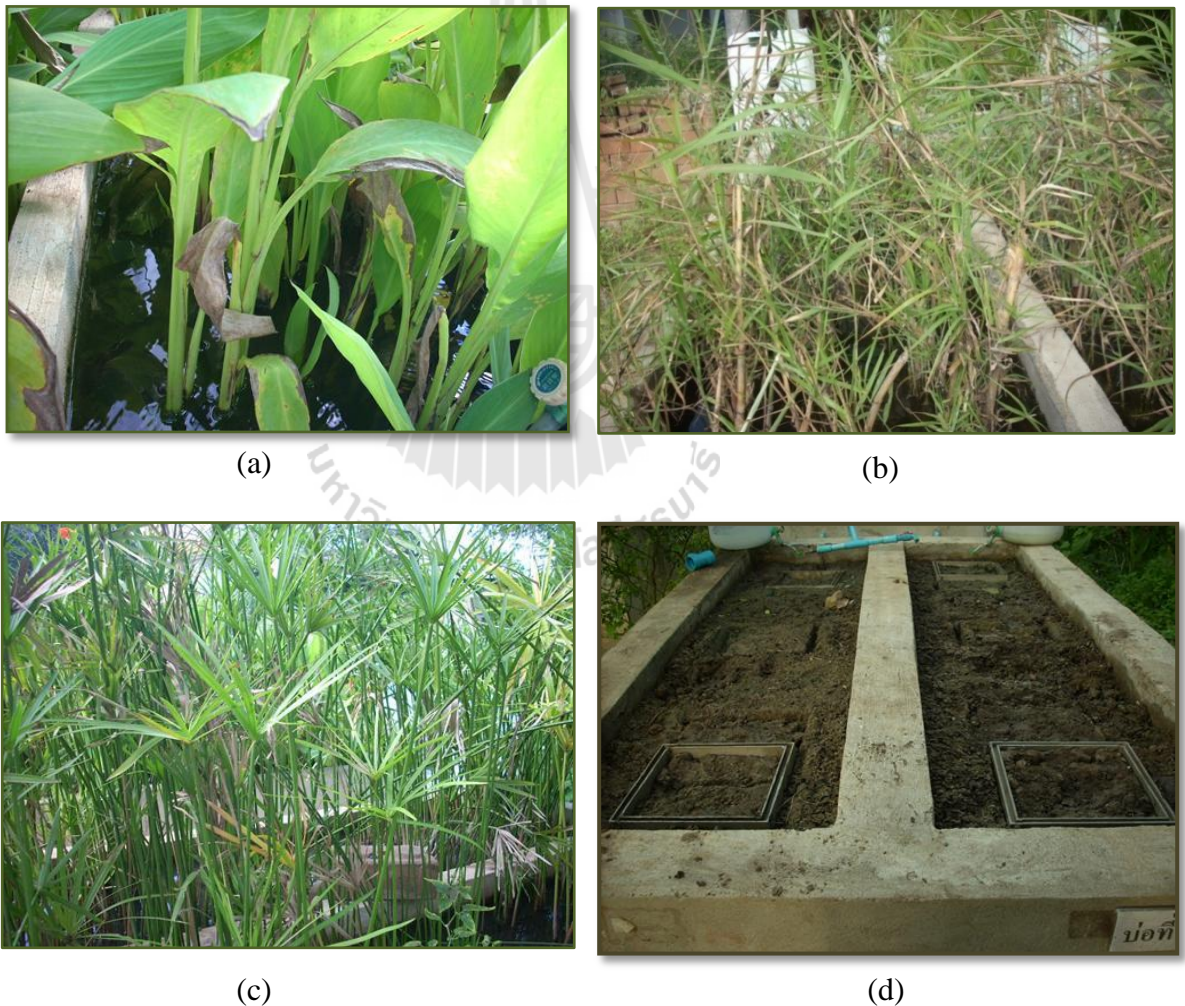
**Figure 3.6** Vegetation used in the constructed wetlands (a) *Canna* sp. (b) *Phragmites* sp*.*, (c) *Cyperus* sp. and (d) non-vegetation control.
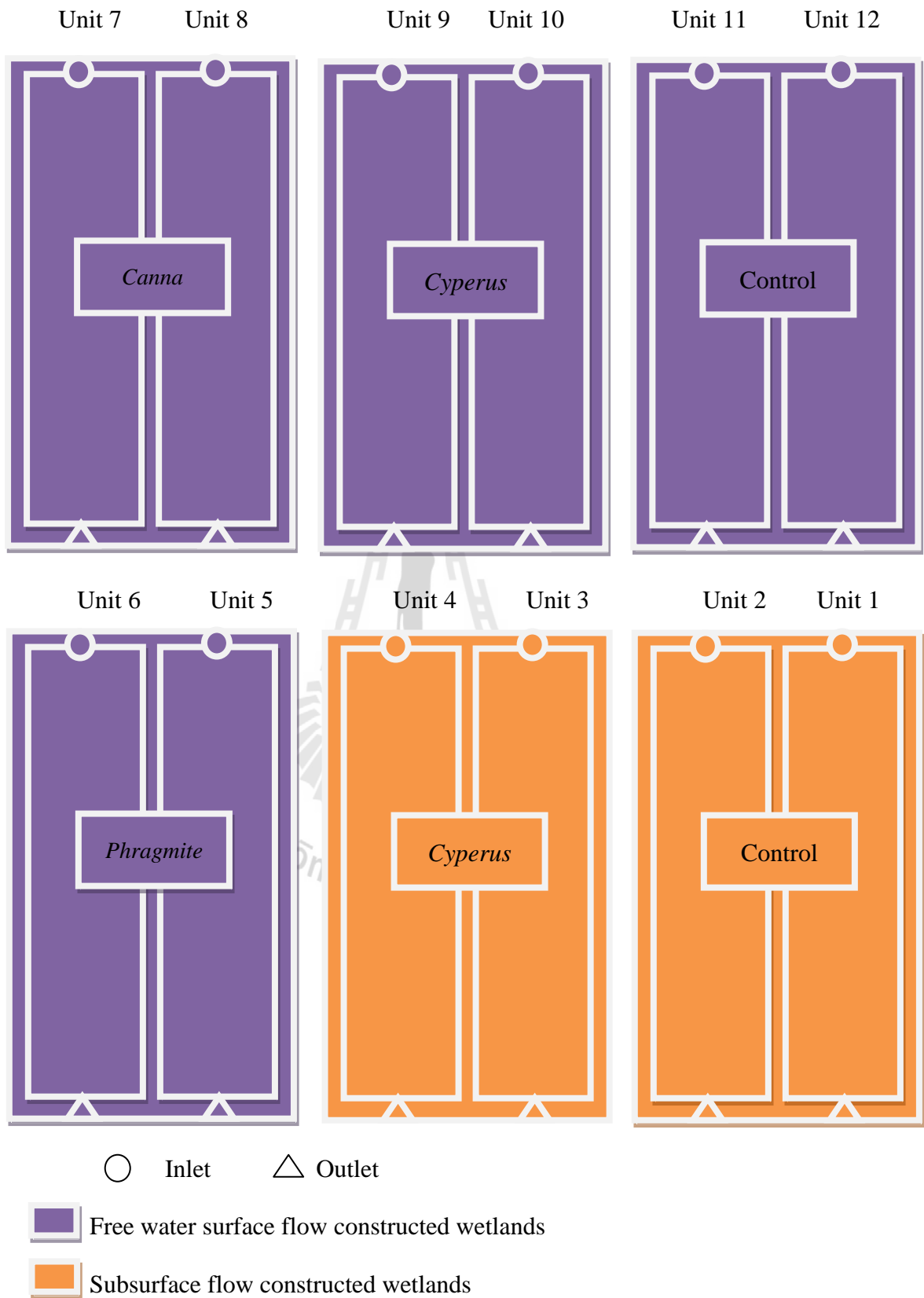
**Figure 3.7** Overall constructed wetland units with plants and controls.

Young emergent plants were acquired locally and planted inside the constructed wetlands. Plants were allowed to familiar with the systems for about three weeks prior to the experiment to achieve steady-state conditions. Only tap water was fed during this time and visual inspection was performed daily to observe the plant conditions. Three rows of plant were cultivated across the width of each constructed wetland with approximately 0.25 m apart throughout the length, except at the middle and the control wetlands. In the middle of each constructed wetland, a space was reserved for non-vegetation gas flux measurement (Figure 3.8). Any invasive plants found in the constructed wetlands were removed daily. At the beginning of the experiments, the average height of the cultivated was about 1.5 m above the water level. After achieving steady-state conditions, the first run with twelve concurrent experiments were started.



**Figure 3.8** Vegetation configuration in the middle of cell to compare gas flux.

## 3.4 Wastewater and its characteristics

Due to the intrinsic nature of wastewater that varies temporally and spatially, it was essential to this study to control variability of wastewater strength. Thus, synthetic wastewater was used in the experiment. The compositions of synthetic domestic wastewater consisted of glucose, $FeCl_3$, $NaHCO_3$, $KH_2PO_4$, $MgSO_4 \cdot 7H_2O$, and urea (Sirianuntapiboon, 2000), similar to the domestic wastewater from Thailand's Housing Estates. The concentration of each component is described below.

| | | | | | |
|---|---|---|---|---|---|
| Glucose | 190 | mg/L | $FeCl_3$ | 0.31 | mg/L |
| $NaHCO_3$ | 6.7 | mg/L | $KH_2PO_4$ | 6.0 | mg/L |
| $MgSO_4 \cdot 7H_2O$ | 3.9 | mg/L | Urea | 9.0 | mg/L |

Synthetic domestic wastewater was fed daily into the constructed wetlands using gravimetric flow and the flow was controlled by needle valves. The characteristics of the prepared synthetic wastewater are shown in Table 3.2. However, synthetic wastewater with high concentrations of BOD was also fed to further observe larger flux of greenhouse gases.

**Table 3.2** Characteristics of synthetic wastewater.

| Parameter | Range (mg/L) | Average (mg/L) |
|---|---|---|
| BOD | 115-235 | 163 |
| COD | 17-23 | 20 |
| TP | 0.03-0.33 | 0.14 |
| TKN | 0.28-0.35 | 0.32 |

## 3.5 Experimental stages

The experiment comprises of two part. First part was to compare greenhouse gas flux emitted from FWS and SF constructed wetlands. The experiment beds consisted of four FWS beds, which had two replicates of plant and non-plant (control) and four SF beds, which are also 2 replicates of plant and non-plant (control). The emergent plant for this stage is *Cyperus* sp. The second stage was to investigate the effect of plant species on greenhouse gas emission from free-water surface flow constructed wetlands. Emergent plants for this stage were *Phragmites* sp. and *Canna* sp. The experiment system was initially built in the late 2009 and examined for leaks including preliminary tests. Intensive experiments were performed during 2010 and 2011.

### 3.5.1 Tracer study for determining actual retention time

Salt tracer experiment is a cost-effective tool widely used in studies of flow and transport in free surface flows. Chloride represents a useful tracer, since it is relatively inert and not used by biota in a great degree. Thus, actual retention time can establish using the salt tracer based on the difference in the electric conductivity (EC). Breakthrough curves were measured by electric conductivity probes, and the electric conductivity values obtained were then converted to chloride concentrations by using calibration curves. The concentration versus conductivity relationships proved quite stable, with very little scatter ($R^2 \approx 0.999$) (Schmid *et al.,* 2004).

In this study, research grade sodium chloride (NaCl) was used as a salt tracer to evaluate the flow pattern in experimental constructed wetland units. Concentration of 0.6 g/L was prepared during the tracer study and was fed constantly into all constructed wetland units. The tracer study was conducted one month after all units

were well established. Conductivity was monitored, with calibrated conductivity probe (Jenway, UK), prior to the release of tracer and the background conductivity was monitored. Conductivity probe was submerged in the water sample and conductivity values were recorded. The tracer response curve was displayed during passage and after return to the background conductivity. Then, the procedure was stopped and the response curve was evaluated. Tap water was used to flush sodium chloride residue remained in the constructed wetlands for two days after the tracer study to minimize the stress of the system.

### 3.5.2 Diurnal study of gas fluxes

Diurnal study were made on $CH_4$, $N_2O$ and $CO_2$ fluxes over a daily cycle. The experiment design for diurnal $CH_4$, $N_2O$ and $CO_2$ were done over 24 hours with 3 hours interval in each constructed wetland unit (Figure 3.9) on 28-29 November 2010 and 27-28 March 2011. At each sampling occasion, the chamber was enclosed for 45 min. Samples were collected at 06:00, 09:00, 12:00, 15:00, 18:00, 21:00, 24:00 and 03:00, respectively. In addition, soil temperatures were measured at 5 cm depth.

### 3.5.3 Seasonal study of gas fluxes

Seasonal variations in $CH_4$, $N_2O$ and $CO_2$ fluxes from constructed wetland units were investigated during June 2010 to May 2011. In the seasonal variation study, one sampling was conducted each month. At each sampling occasion, soil temperature (5 cm depth), soil ORP, and soil pH were collected simultaneously.

**Figure 3.9** Diurnal study over 24 hours.

## 3.6 Gas fluxes measurement

The gas emissions were measured using a static chamber method described by Hutchingson and Moiser (1981). The chambers consist of two parts. The upper part was constructed from 3.0 mm clear acrylic sheet and made gas-tight by heated glue doubling with silicon sealant (Figure 3.10). The chamber included two gas sampling points, a thermometer, and a fan. Size of the acrylic chamber was 0.25 m×0.25 m×1.5 m (width×length×height). A small electronic fan was to ensure a thorough gas mixing inside the chamber during the measurement. The bottom part was made from aluminum rod (Figure 3.11). This frame was used as a base for the upper part. Four-side groove was made with 4.0 mm trench to accommodate the 3.0 mm acrylic

chamber during the gas sampling. The aluminum frame was firmly inserted into the top soil overnight prior the measurement. During the measurements, the chamber was placed on top of the aluminum frame and water was filled in the groove to prevent gas leakage (Figure 3.12). In each constructed wetlands, three chambers were installed; two chambers at the entry and exit points of wastewater and the vegetation was included within the chamber (Figure 3.13) and another chamber was installed in no vegetation area at the middle of cell.



**Figure 3.10** Acrylic chamber for gas sampling.



**Figure 3.11** Aluminum base for acrylic chamber.

**Figure 3.12** Water fill in the groove of the aluminum chamber.



**Figure 3.13** Placement of sampling locations.

Gas flux measurements were performed at three locations in each constructed wetlands, inlet, middle and outlet. The acrylic chambers were placed on the aluminum bases and gas measurements began at 0, 15, 30 and 45 minutes intervals. A polypropylene syringe was used for gas withdrawal (30 cm$^3$) from the chamber. The gas samples were collected into vacuum glass vials (butyl-gum capped) fitted with a rubber stopper. They were packed in the cool box and transferred to the

laboratory for analysis. Typical sampling time for monthly schedule started about 9:00 AM. These data provided seasonal variations of gas fluxes over one year. During diurnal study, the measurements were3-hour for 24-hour period. Three major greenhouse gases, methane, nitrous oxide and carbon dioxide, were measured and analyzed. During gases sampling, chamber and ambient air temperatures were also recorded along with soil temperatures and soil ORP.

Carbon dioxide was real-time measured with $CO_2$ gas analyzer (LI-820 model from LI-COR, Inc., USA). Data were recorded in computer and retrieved for later analysis (Figure 3.14). The instrument used non-dispersive infrared (NDIR) method to determine the concentration of carbon dioxide.
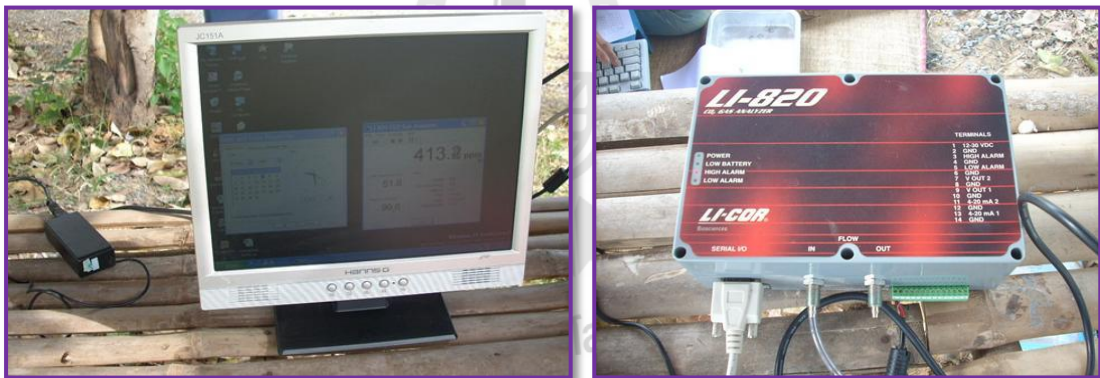


**Figure 3.14** $CO_2$ gas analyzer (LI-COR, Inc., USA).

While carbon dioxide was measured real-time in the field, methane and nitrous oxide samples were needed to collect from the chamber and were analyzed later in laboratory with gas chromatography (GC) instrument. Gas samples were taken from each chamber with polypropylene syringes and transferred to evacuated glass vial. Air inside the glass vials were evacuated and create negative pressure prior to

sampling. Vials were overfilled in order to minimize potential diffusion across the septa. All the samples were labeled and kept cool in ice-packed cooler immediately after the sampling (Figure 3.15). Then, the samples were transport to a freezer, <4°C, waiting for later GC analysis.



**Figure 3.15** Glass vials and preservation of samples.

The gas samples were analyzed in a laboratory using two gas chromatographs (Agilent GC 6890, USA) equipped with a flame ionization detector for $CH_4$, an electron capture detector for $N_2O$. All gas samples were analyzed in duplicate.

The $CH_4$ concentrations were determined by means of gas chromatograph (Agilent GC 6890, USA) equipped with a flame ionization detector (FID) and a Poraplot Q capillary column (10 m×0.32 mm ID). Column, injector and detector temperatures were 40°C, 250°C and 300°C, respectively, split ratio 0.7:1 and split flow 15.0 mL/min, with $N_2$ gas as carrier. Flow rate of 20 mL/min was maintained during analysis. The concentration of methane in gas samples was extrapolated by comparing chromatogram (peak area) of gas sample with standard methane gas.

The N$_2$O concentrations were determined using an HP 6890 gas chromatograph (Agilent, USA) equipped with a 0.53 mm×15 m HP-Plot Q column and an electron capture detector ($\mu$ECD). The temperatures of $\mu$ECD and the column were 300 and 180°C, respectively. Nitrogen was supplied as the carrier gas at a flow rate of 15 ml/min. The sensitivity of N$_2$O detection was 0.2 ppmv. Data were analyzed by Agilent ChemstationA.08.03 software (Agilent, USA).

**Gas flux analysis**

Emission rates were calculated based on the linear change of gas concentration over time as shown in Figure 3.16. The gas concentration for each sample is plotted in a concentrations time graph. The derivative represents the gas emission rate (ppm/hr) of the series, which were converted to flux rates (mg m$^{-2}$ hr$^{-1}$) and corrected for chamber volume and temperature (Healy $et$ $al.,$ 1996). Regressions were performed on each flux rate in Microsoft Excel to determine linearity of flux. If the increase/decrease in the gas concentration was non-linear ($r^2 < 0.85$) the measurement was rejected (Altor and Mitsch, 2006).
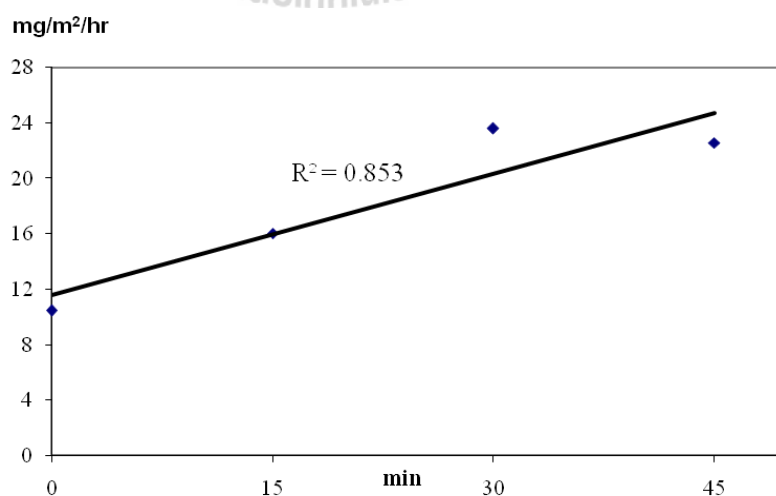


**Figure 3.16** Sample linear change of gas concentration over time.

Gas flux (mg m$^{-2}$hr$^{-1}$) was calculated by the following equation:

$$E = \frac{XhM}{RT}$$

where  E = Emission on the aerial basis (mg m$^{-2}$hr$^{-1}$)

X = Gas concentration increase in chamber (ppm hr$^{-1}$)

h = height of chamber (m)

M = Molecular weight

R = Gas constant = 0.0821 (atm K$^{-1}$ mol$^{-1}$)

T = Absolute temperature (K)

Air pressure = 1 atm.

### Environmental factors monitoring

During the gas sampling period, soil temperature at the 5-cm depth was also continuously monitored, whereas soil pH, soil ORP (at 5-cm depth) were measures by pH/ORP meter.

## 3.7 Wastewater analysis

Daily wastewater samples were collected from the influent and effluent points, and analyzed for BOD concentration until the steady-state conditions reached. After the steady-state conditions, the parameters analyzed in influent and effluent wastewater samples included BOD, NH$_3$-N, TP for the period of once a month. Details of analyses are given in Table 3.3.

**Table 3.3** Method of analysis.

| Parameter | Method |
|-----------|--------|
| $BOD_5$ | Dilution method |
| COD | Open Reflex method |
| $NH_3$ | Phenate method |
| TP | Ascorbic Acid method |

Source:  APHA, AWWA and WEF, 2005.

## 3.8  Soil sampling and analysis

Soil samples from all twelve plots were sampled and analyzed to identify microbial community.  The litter on the soil surface was removed prior to sampling. Soil at the depths of 10, 20 and 30 cm below the surface were taken using clean PVC tubes, diameter of 1.27 cm. To ensure the representative of soil samples, three replicate tubes per experiment cell were taken. All samples were protected from sunlight by wrapping with aluminum foil and put in nitrogen purged plastic bag immediate after the sampling to minimize the contact with oxygen.  Ice packed cooler was used to store the samples during the transport to laboratory for further analysis. These samples were used for chemical analysis and DNA extraction. For chemical analysis, sampled soil in the same cell was mixed together and stored immediately in a cooler with ice. After sieving the soil were stored at -20°C until processed further.

## 3.9  Data analysis

Statistical analysis was done with SPSS and Microsoft Excel for Windows. All data entering statistical comparisons were tested for homogeneity of variance and normal distribution using  Levene and Kolmogorov-smirnov Test. If assumptions were fulfilled, one-way ANOVA analyses with following Tukey Post-hoc test or independent-Samples t-test were carried out. Otherwise non-parametric Chi-Square ($\chi^2$) Test (Kruskal Wallis) or Mann-Whitney U Test were used instead, followed by Mann-Whitney as Post-hoc test. Spearman Correlation was performed to analyze correlations between gaseous fluxes and environmental parameters.  Figures were drawn using Microsoft Excel®.  In all analysis, $p<0.05$ was considered as statistically significant.

## 3.10  DNA extraction, isolation and PCR amplification of universal 16S  rRNA

### 3.10.1  DNA extraction and PCR

The first step involved DNA extraction from core soil samples.  The soil samples were analyzed separately for each depth, 10, 20 and 30 cm.  Target groups of microbs were eubacteria, archeobacteria, ammonium-oxidizing bacteria, methanogenic archaeobacteria, and methanotrophic archeobacteria.  DNA extraction from the core soil samples was performed using the Ultra Clean Soil DNA kit (MoBio Laboratories, Solana Beach, California, USA). A portion of 0.25 g of Chinese kale rhizosphere was processed according to the protocol provided by the manufacturer with an additional bead-beating step using as cell homogenizer (Braun,

Melsungen, Germany) to achieve a harsh cell lysis. Primer and target group used in this study are summarized in Table 3.4.

**Table 3.4** Primers used in PCR.

| Target group | Primer | |
|---|---|---|
| | **Forward** | **Backward** |
| Eubacteria | F984 | R1378 |
| Archaeobacteria | PARCH340F | PRAH519R |
| Ammonium-oxidizing bacteria | Arch-amoAF | Arch-amoAR |
| Methanogenic  Archaeobacteria | MG357F | MG0691R |
| Methanotrophic Archaeobacteria | Parch519F | Parch519R |

a) Eubacteria

In the process of eubacterial amplification, 16S rRNA gene was performed using universal primers set, known as F984 as forward primer and R1378 as backward primer.  The 40-bp long GC-clamps (5'-CCC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC G-3') (Costa *et al.,* 2005) was added to the 5'end of the forward primer to avoid complete separation of DNA strands during denaturing electrophoresis.  The PCR reaction contained 50 ng of DNA from soil samples, 0.5 µmol of each primer, 0.2 mMdNTP, 1x PCR buffer, 3 mM $MgCl_2.2H_2O$ and 0.05 U Taq DNA polymerase (Promega, USA). The thermal cycler was performed using a Perkin Elmer, GeneAmp PCR System 2400 under the following reaction conditions: 94°C for 5 min (1 cycle), followed by 94°C for 30 s, 55°C for 30 s, 72°C for 30 s (35 cycles), and final extension step at 72°C for 10 min (1 cycle).

b) Archaeobacteria

For Archaeobacteria amplification, archaeal 16S rRNA gene was amplified by using the forward primer PARCH340F and a reverse primer PRAH519R which yielded products of approximately 200 base pairs (Moeseneder *et al.,* 1999). The GC-clamp (Costa *et al.,* 2005) was added to the 5'end of the forward primer. The PCR reaction contained 50 ng of DNA from soil sample, 0.5 µmol of each primer, 0.2 mMdNTP, 1x PCR buffer, 3 mM $MgCl_2.2H_2O$, and 0.05 U Taq DNA polymerase (Promega, USA). The PCR amplifications were performed using a Perkin Elmer, GeneAmp PCR System 2400 under in the following conditions: 94°C for 5 min, followed by 30 cycles of 95°C for 45 s, 53.5°C for 45 s, 72°C for 2 min, and a final extension step at 72°C for 10 min.

The PCR products of Eubacteria and Archaeobacteria were separately subjected to denaturing gradient gel electrophoresis (DGGE) analyses. PCR product (50 µL) was loaded onto 10% (w/v) polyacrylamide (Acrylamide: Bisacrylamide ratio, 37.5:1) gel in 1.0 strength Tris-acetate-EDTA (TAE, pH 8.5) buffer. The polyacrylamide gel was prepared with a denaturing gradient ranging from 30% to 70%. DGGE was performed at 60°C. The electrophoresis was run for 12 h at 120 V. Subsequently, the gel was stained with SYBR Green solution and documented on gel documentation and analysis.

c) Ammonium-oxidizing archaeobacteria

The ammonium-oxidizing primers, Arch-amoAF and Arch-amoAR were used in the amplification processes. The GC-clamp was attached to the 5' end of the Arch-amoAF primer. The DNA was diluted 10-fold and bovine serum albumin (BSA) was added to reduce the interference of humic acid in the PCR. ArcheobacterialamoA

genes were quantified using the primers Arch-amoAF and Arch-amoAR with HotStarTaq DNA Polymerase (Qiagen, Valencia, CA) in a 25 μL reaction mixture containing 1×PCR buffer, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 2.5 U HotStarTaq DNA polymerase. The PCR amplifications were performed in the following conditions: 5 min at 94°C, followed by 30 cycles of 94°C for 45 s, 53.5°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min. Denaturing gradient gel electrophoresis was carried out using a D-Code universal mutation detection system (Bio-Rad Laboratories) according to the instruction manual, and 6% (w/v) polyacrylamide [acrylamide-bisacrylamide (37.5:1)] gels containing denaturing gradients of 40-60% (100% denaturant containing 7 M urea and 40% formamide) for separation of PCR products. Subsequently, the gel was stained with SYBR Green solution and documented on gel documentation and analysis.

d) Methanogeic archaeobacteria

The communities of methanogenic Archae were analyzed using MG357F and MG0691R. The GC-clamp was attached to the 5' end of the MG357F primer. The reaction medium consisted of 5 μL of PCR buffer (Roche, 100 mMTris-HCl, pH 8.3, 500 mMKCl, 15 mM $MgCl_2$), 1 μL of dNTP (10 mM), 1 μM of each primer, 1 μL of GC-rich solution for enhancing PCR efficiency (Roche, France), 500 ng of Bovine Serum Albumin (Fermentas, Italy), 0.5 μL of Taq-polymerase (5 Units/μL, Roche, France) and 2 μL of genomic DNA (10 ng) brought to a final volume of 50 μL. The amplification program consisted of an initial cycle of denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 53°C for 60 s and extension at 72°C for 2 min. The amplification concluded with a final elongation step at 72°C for 8 min. For DGGE analyses, 50 μL of PCR products were loaded into a

polyacrylamide gel (8%) with a 30-60% denaturing gradient for 16 h at 100 V and 60°C Gels were stained with SYBR$^{®}$ Green (1:10000) and visualized under UV light.

e) Methanotrophic archaeobacteria

Methanotrophic archaeobacteria 16S rRNA gene fragments that were suited for DGGE was conducted by using a primer set Parch519f, which is complementary to reverse sequence of primer Parch519r. The stability of the archaeal 16S rRNA gene fragments in the DGGE was obtained by attaching a GC-clamp to the 5'-end of the ARC915r primer. PCR conditions included an initial denaturation step of 4 min at 96°C, followed by 35 cycles including a denaturation step for 30 s at 94°C, a primer annealing step for 40 s at 57°C, and a primer extension step for 40 s at 72°C. A final extension was performed for 10 min at 72°C. PCR samples were applied directly onto 6% (wt/vol) polyacrylamide gels (acrylamide/*N*,*N*'-methylene bisacrylamide ratio, 37: 1 [w/w]) in 1×TAE buffer (pH 8.3), which had been prepared from sterile solutions and casted between sterilized glass plates. The gels contained a linear gradient of denaturant from 20-70% (100% denaturant is 7 M urea plus 40% [v/v] formamide). Electrophoresis proceeded for 5 h at 200 V and 60°C. Subsequently, the gel was stained with SYBR Green solution and documented on gel documentation and analysis.

### 3.10.2 Cloning and sequencing

The microbial community composition in DGGE gel was analyzed by cloning and partial sequencing of the 16S rDNA and 18S rDNA genes. Interested bands from DGGE gel were used as a DNA template in PCR reactions as following by Prakamhang *et al*. (2009). 16S rDNA genes were amplified using the primers pair described in section 3.6.2. The PCR products were purified using the QIA quick PCR

purification kit (Qiagen, Hilden, Germany). The amplicons were ligated into the pGEM®-T Easy Vector System (Promega, USA) and then further transformed into *E.coli* DH5∞ competent cells, following the manufacturer's protocol. Cells were grown overnight at 37°C on Petri plates containing S-gal®/LB agar blend (Sigma-Aldrich) supplemented with 100 µg/mL ampicillin (Sigma-Aldrich). White colonies (transformants) were picked randomly from the plates for colony PCR using the SP6 and T7 primers (Promega, USA). Twenty-five microliters of PCR reactions containing 0.1 U/µL GoTaq DNA Polymerase (Promega, USA), 1×PCR buffer and 1.5 mM $MgCl_2$ supplied with the enzyme, 0.2 mM dNTPs, and 0.2 µM of each primer were performed using and Perkin Elmer, GeneAmp PCR System 2400 under the following reaction conditions: 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 48°C for 45 s, 72°C for 45 s, and a final extension step at 72°C for 10 min. PCR products were evaluated by running a small volume of product in an agarose gel. DNA sequencing was performed by MACROGEN company (Korea). The DNA sequences were generated and the most closely related sequences were obtained from the NCBI database.

# CHAPTER IV

# RESULTS AND DISCUSSION

In this study, experimental observation and results of greenhouse gas flux dynamic emitted from domestic wastewater treatment constructed wetlands (CW) and microbial identification were separately discussed in six parts as the followings.

  4.1  Tracer study

  4.2  Performance of pollutant removal

  4.3  Diurnal variation of greenhouse gas fluxes

  4.4  Seasonal variation of greenhouse gas fluxes and environmental factors

  4.5  Influence plant species on pollutant removal and greenhouse gas fluxes

  4.6  Identification of microbial community in constructed wetlands soils

## 4.1  Tracer study

A tracer study was carried out at the initial step of the experiment to evaluate the flow pattern in four experimental units with different in CW types and plants (P3, P5, P7, P9).  Analytical-grade NaCl solution of 600 mg/L was prepared and fed into the inlet of the constructed wetlands. The effluents were continuously determined for chloride levels.  Two flow rates were used, 58.0 and 30.25 L/hr for free water surface (FWS) and subsurface flow wetland systems (SF), respectively.  Equations (4.1) to (4.4) were applied to determine the dispersion number and actual hydraulic retention time (HRT) (Mattaraj, 1995).  Raw data of tracer study ise given in Tables A.1-A.4 in Appendix A.  Figures 4.1-4.4 show the results of the tracer experiments.

Mean HRT (actual), $\qquad t = \dfrac{\sum t_i C_i}{\sum C_i}$ (4.1)

Standard Deviation, $\qquad \acute{o}^2 = -t^2 \dfrac{\sum t_i^2 C_i}{\sum C_i}$ (4.2)

Then $\qquad \acute{o}_e^2 = \dfrac{\acute{o}^2}{t^2} = 2d + 8d^2$ (4.3)

The dispersion number of flow, d, can be expressed as:

$$d = \frac{D}{uL_1}$$ (4.4)

Where, $D$ = the longitudinal or axial dispersion coefficient characterizing

the degree of back mixing during flow,

$u$ = the flow velocity (m/s),

$L_1$ = the length of fluid travel path from influent to effluent (m).

The condition of dispersion number $\left(\dfrac{D}{uL_1}\right)$ can be characterized as follows:

$\left(\dfrac{D}{uL_1}\right)$ = 0, is plug flow condition (negligible dispersion),

$\left(\dfrac{D}{uL_1}\right)$ = 0.002, is small amount of dispersion,

$\left(\dfrac{D}{uL_1}\right)$ = 0.025, is intermediate amount of dispersion,

$\left(\dfrac{D}{uL_1}\right)$ = 0.2, is large amount of dispersion,

$\left(\dfrac{D}{uL_1}\right)$ = α is mixed flow condition (large dispersion).
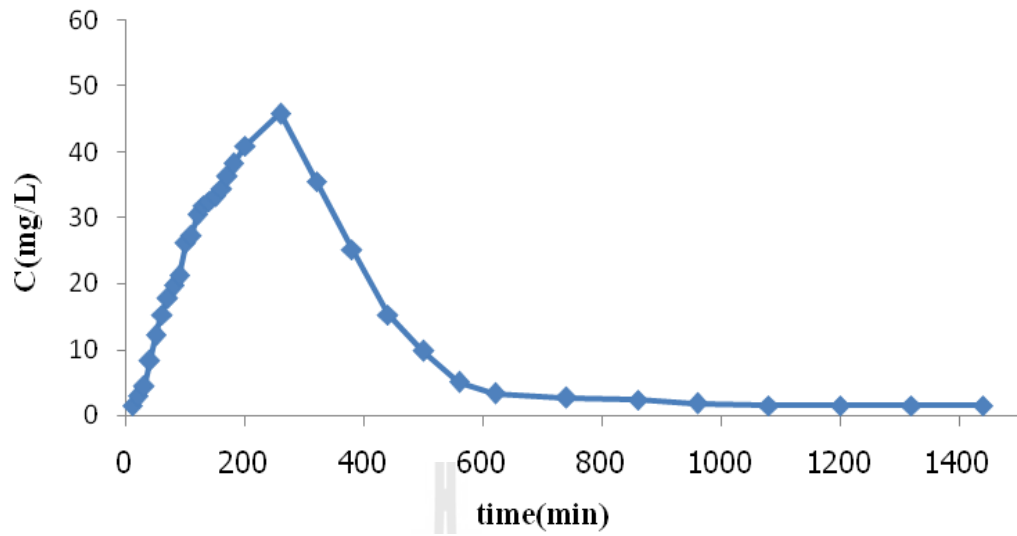
**Figure 4.1** Chloride concentrations versus time in the effluent of subsurface flow (SF) constructed wetland planted with *Cyperus* sp. (P3).
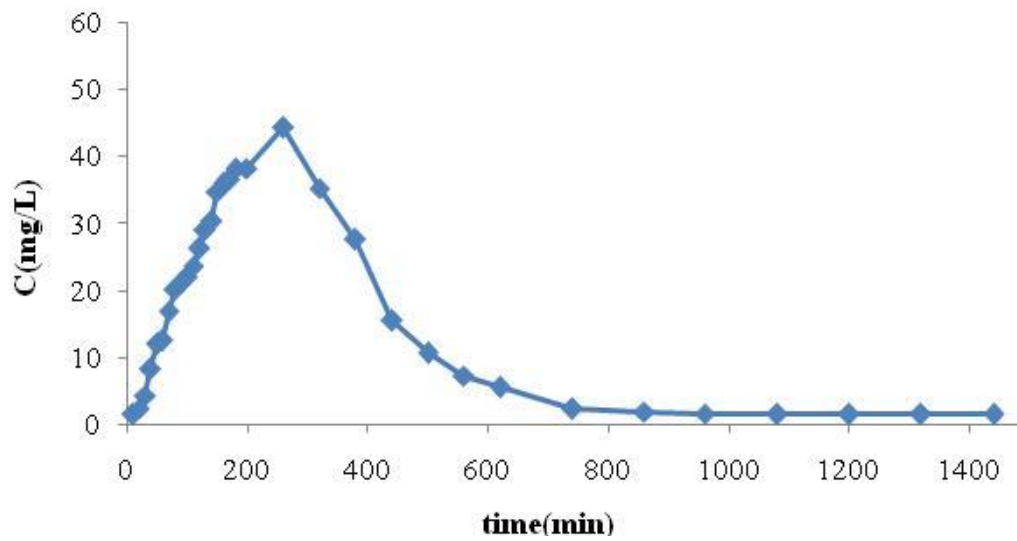


**Figure 4.2** Chloride concentrations versus time in the effluent of free water surface flow (FWS) constructed wetland planted with *Phragmites* sp. (P5).
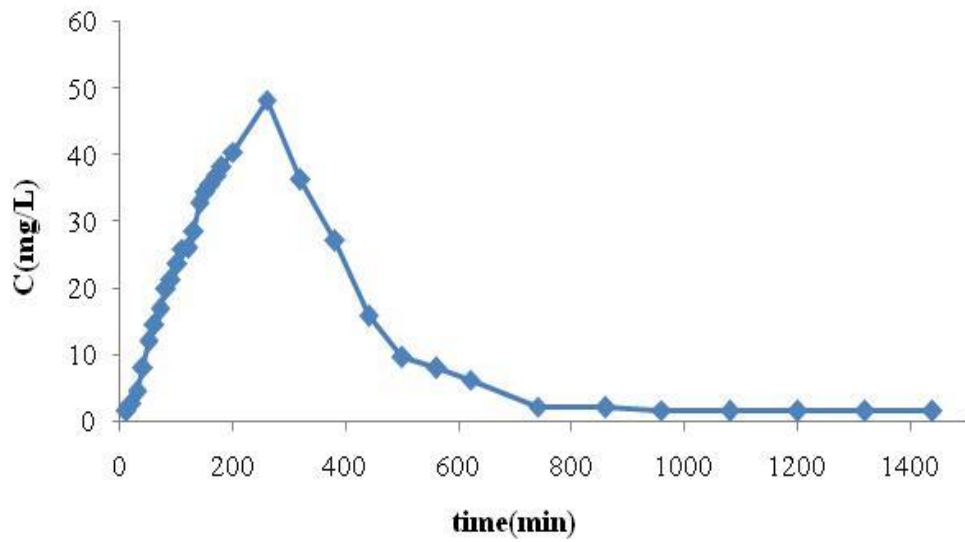
**Figure 4.3** Chloride concentrations versus time in effluent of free water surface flow (FWS) constructed wetland planted with *Canna* sp. (P7).
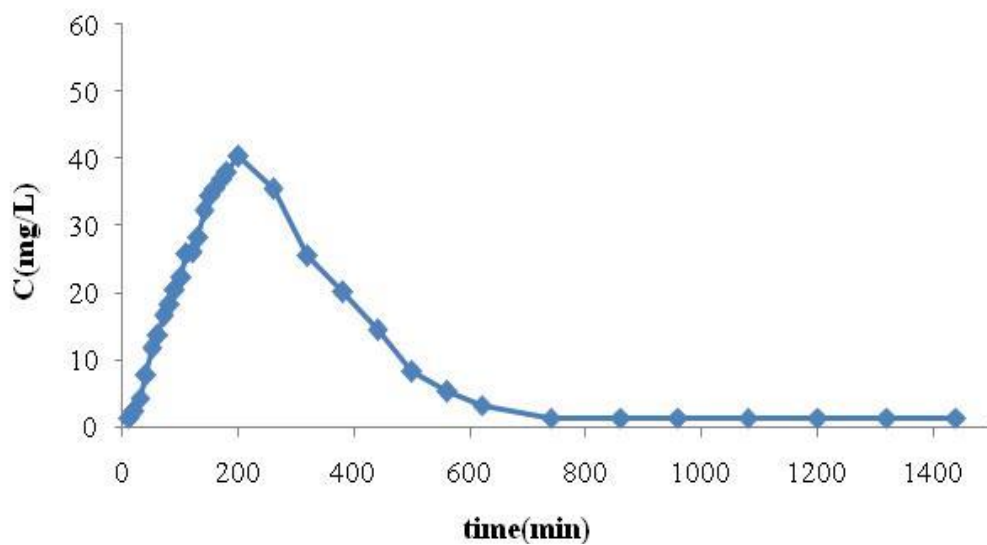


**Figure 4.4** Chloride concentrations versus time in effluent of free water surface flow (FWS) constructed wetland planted with *Cyperus* sp. (P9).

From Figures 4.1-4.4, the results show that chloride in the effluent were noticeably observed with increased concentrations after sodium chloride solution was introduced into the constructed wetlands until it reached the peak values several hours later. After reaching the peak, chloride concentrations subsequently decreased with time and the experiment was conducted until the concentrations went back to background levels, approaching zero. Collected data were used to calculate actual hydraulic retention time (HRT) and dispersion number of the constructed wetlands (Table 4.1).

**Table 4.1** Actual HRT and dispersion numbers from the tracer study.

| CW unit | HRT (hr) | Actual HRT (hr) | Dispersion Number (d) |
| --- | --- | --- | --- |
| P3 | 6.0 | 5.88 | 0.18 |
| P5 | 6.0 | 5.96 | 0.17 |
| P7 | 6.0 | 5.94 | 0.17 |
| P9 | 6.0 | 5.83 | 0.19 |

*Note:* *P3 = SF with Cyperus* sp.     *P5 = FWS with Phragmites* sp.
        *P7 = FWS with Canna* sp.     *P9 = FWS with Cyperus* sp.

The dispersion numbers, determined by Equation (4.4) were in the range of 0.17 to 0.19, which were slightly difference in FWS and SF including plant species. These values showed small flow dispersion in the constructed wetland units. At low dispersion number (d), the flow characteristic of all of constructed wetland units could be classified as approaching plug flow pattern meaning that the wastewater flow in the experimental units which indicated the wastewater directionally move from inlet to outlet successively.

## 4.2  Performance of pollutants removal

During the operation of constructed wetland units, the degree of removal of BOD, COD, TN, TP and $NH_3$-N was investigated, from July 2010 to May 2011. Water samples were taken monthly.

Average removal rates of BOD, COD, $NH_3$ and TP were in the range 57-7, 49-67, 25-41 and 39-48%, respectively (Figure 4.5).  The removal efficiencies varied in different constructed wetlands. High BOD removal rate occurred in free water surface flow constructed wetland (FWS) planted with *Phragmites* sp. and low removal rate occurred in FWS planted with *Canna* sp. High COD removal rate occurred in FWS planted with *Phragmites* sp. while removal rate was low in subsurface flow constructed wetland (SF) planted with *Cyperus* sp.  $NH_3$ was removed in FWS planted with *Canna* sp. more efficient than FWS planted with *Phragmites* sp. TP removal rate was high in SF planted with *Cyperus* sp. and was low in FWS planted with *Cyperus* sp..
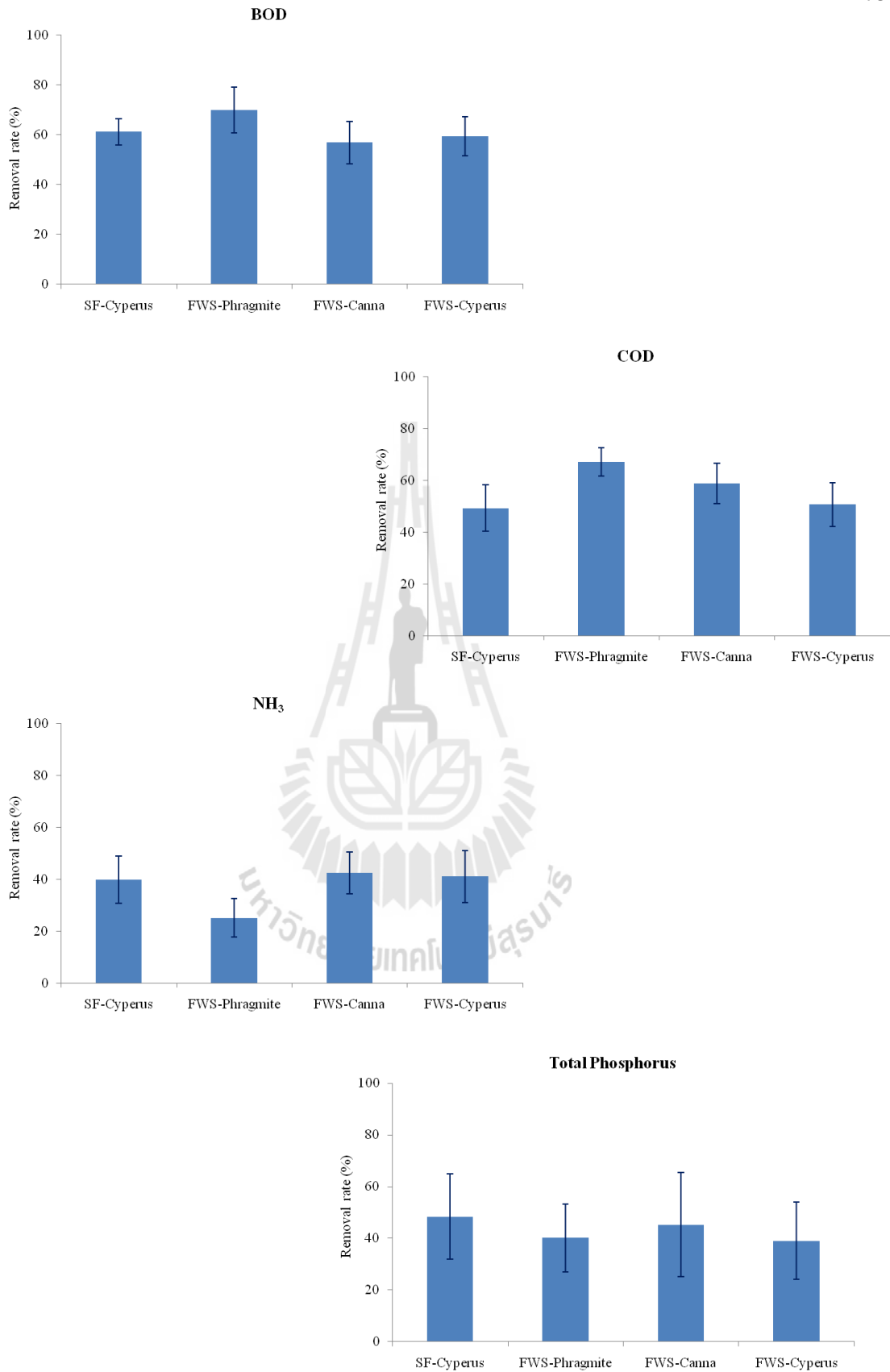
**Figure 4.5** Pollutant removal efficiencies of constructed wetlands.

Descriptive statistics for pollutants removal rates of all constructed wetlands are shown in Tables 4.2 and 4.3. Since these data were normal distribution and have homogeneity of variances, t-test and one-way ANOVA tests are suitable for the analysis of variation of pollutants removal rates from different constructed wetlands. When compare pollutant removal efficiency between SF and FWS constructed wetlands, planted with *Cyperus* sp., pollutant removal efficiencies of both types constructed wetlands are not significantly different (p>0.05) as shown in Table 4.2.

**Table 4.2** Descriptive statistics and comparison of pollutants removal rates between SF and FWS constructed wetlands using t-test.

| Pollutant/CW | | Pollutants removal rates (%) | | | t-test | |
|---|---|---|---|---|---|---|
| | | N | Mean | S.D. | Mean difference | Sig. (2-tailed) |
| BOD | SF *Cyperus* sp. | 12 | 61.26 | 5.26 | 1.73 | 0.531 |
| | FWS *Cyperus* sp. | 12 | 59.53 | 7.83 | | |
| COD | SF *Cyperus* sp. | 12 | 49.26 | 8.98 | -1.45 | 0.688 |
| | FWS *Cyperus* sp. | 12 | 50.71 | 8.41 | | |
| NH$_3$ | SF *Cyperus* sp. | 12 | 39.92 | 9.05 | -1.22 | 0.757 |
| | FWS *Cyperus* sp. | 12 | 41.13 | 9.94 | | |
| TP | SF *Cyperus* sp. | 12 | 48.42 | 16.48 | 9.37 | 0.159 |
| | FWS *Cyperus* sp. | 12 | 39.05 | 14.97 | | |

**Table 4.3** Descriptive statistics of pollutants removal rates in FWS with different plant species.

| Pollutant /CW | | Pollutants removal rates (%) | | | | |
|---|---|---|---|---|---|---|
| | | N | Mean | S.D. | Min | Max |
| BOD | FWS *Phragmites* sp. | 12 | 69.97 | 9.26 | 54.80 | 81.90 |
| | FWS *Canna* sp. | 12 | 56.85 | 8.45 | 43.40 | 68.70 |
| | FWS *Cyperus* sp. | 12 | 59.53 | 7.83 | 46.80 | 68.40 |
| | Total | 36 | 62.11 | 10.08 | 43.40 | 81.90 |
| COD | FWS *Phragmites* sp. | 12 | 67.17 | 5.54 | 58.16 | 75.04 |
| | FWS *Canna* sp. | 12 | 58.83 | 7.91 | 45.79 | 70.80 |
| | FWS *Cyperus* sp. | 12 | 50.71 | 8.41 | 37.37 | 64.61 |
| | Total | 36 | 58.90 | 9.90 | 37.37 | 75.04 |
| NH$_3$ | FWS *Phragmites* sp. | 12 | 25.24 | 7.44 | 9.57 | 36.29 |
| | FWS *Canna* sp. | 12 | 42.52 | 8.08 | 29.30 | 59.82 |
| | FWS *Cyperus* sp. | 12 | 41.13 | 9.94 | 28.65 | 59.86 |
| | Total | 36 | 36.30 | 11.50 | 9.57 | 59.86 |
| TP | FWS *Phragmites* sp. | 12 | 40.16 | 13.09 | 22.18 | 57.43 |
| | FWS *Canna* sp. | 12 | 45.23 | 20.09 | 19.79 | 78.56 |
| | FWS *Cyperus* sp. | 12 | 39.05 | 14.97 | 19.75 | 67.89 |
| | Total | 36 | 41.48 | 16.08 | 19.75 | 78.56 |

The mean of pollutant removal efficiency of different plants species within FWS constructed wetland were compared by one-way ANOVA, followed by LSD's post hoc test. Means of BOD, COD and $NH_3$ removal rates of different plants species were significant differences, while TP removal rates indicated no significant difference (Table 4.4). Results from LSD's post hoc test indicated that FWS planted with *Phragmites* sp. had the highest removal efficiency of BOD and COD but had lowest efficiency on $NH_3$. However, FWS planted with *Cyperus* sp. or *Canna* sp. had the highest removal efficiency on $NH_3$ (Table 4.5).

**Table 4.4** Descriptive statistics and comparison of pollutants removal rates among FWS with different plant species using one-way ANOVA.

| Pollutant/CW | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| BOD | Between Groups | 1152.92 | 2 | 576.46 | 7.914 | 0.002 |
| | Within Groups | 2403.82 | 33 | 72.84 | | |
| | Total | 3556.74 | 35 | | | |
| COD | Between Groups | 1626.85 | 2 | 813.42 | 14.875 | 0.000 |
| | Within Groups | 1804.62 | 33 | 54.69 | | |
| | Total | 3431.46 | 35 | | | |
| $NH_3$ | Between Groups | 2212.44 | 2 | 1106.22 | 15.111 | 0.000 |
| | Within Groups | 2415.75 | 33 | 73.20 | | |
| | Total | 4628.19 | 35 | | | |
| TP | Between Groups | 260.97 | 2 | 130.48 | 0.490 | 0.617 |
| | Within Groups | 8788.59 | 33 | 266.32 | | |
| | Total | 9049.56 | 35 | | | |

**Table 4.5** LSD Post Hoc test for multiple comparison of pollutants removal rates among FWS with different plant species.

| Pollutant | (i) Plant | (j) Plant | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| BOD | *Phragmites* sp. | *Canna* sp.* | 13.12 | 3.484 | 0.002 |
| | | *Cyperus* sp.* | 10.4 | 3.484 | 0.014 |
| | *Canna* sp. | *Phragmites* sp.* | -13.12 | 3.484 | 0.002 |
| | | *Cyperus* sp. | -2.68 | 3.484 | 0.725 |
| | *Cyperus* sp. | *Phragmites* sp.* | -10.44 | 3.484 | 0.014 |
| | | *Canna* sp. | 2.68 | 3.484 | 0.725 |
| COD | *Phragmites* sp. | *Canna* sp.* | 8.35 | 3.019 | 0.024 |
| | | *Cyperus* sp.* | 16.47 | 3.019 | 0.000 |
| | *Canna* sp. | *Phragmites* sp.* | -8.35 | 3.019 | 0.024 |
| | | *Cyperus* sp.* | 8.12 | 3.019 | 0.029 |
| | *Cyperus* sp. | *Phragmites* sp.* | -16.47 | 3.019 | 0.000 |
| | | *Canna* sp.* | -8.12 | 3.019 | 0.029 |
| NH$_3$ | *Phragmites* sp. | *Canna* sp.* | -17.28 | 3.493 | 0.000 |
| | | *Cyperus* sp.* | -15.89 | 3.493 | 0.000 |
| | *Canna* sp. | *Phragmites* sp.* | 17.28 | 3.493 | 0.000 |
| | | *Cyperus* sp. | 1.39 | 3.493 | 0.917 |
| | *Cyperus* sp. | *Phragmites* sp.* | 15.89 | 3.493 | 0.000 |
| | | *Canna* sp. | -1.39 | 3.493 | 0.917 |

*\* The mean difference is significant at the 0.05 level*

This study also observed seasonal changes in BOD, COD, NH$_3$ and TP removal (Figures 4.6 and 4.7). The percentage of pollutant removal in all constructed

wetlands began to decrease from December 2010 to February 2011. Seasonal changes of COD removal were hardly observed.

**Seasonal variation of BOD removal**



(a)

**Seasonal variation of COD removal**
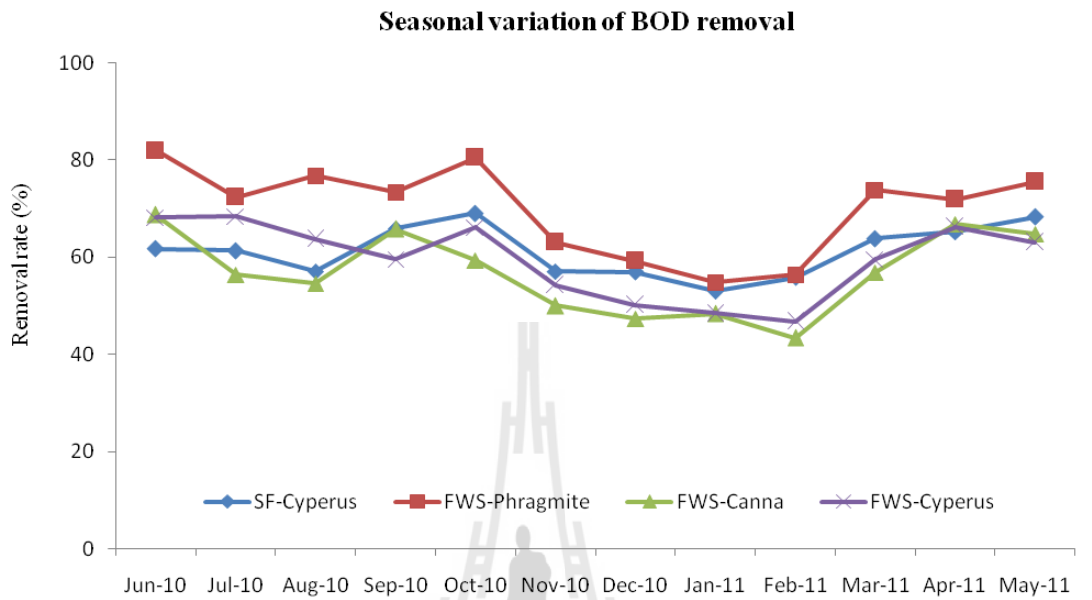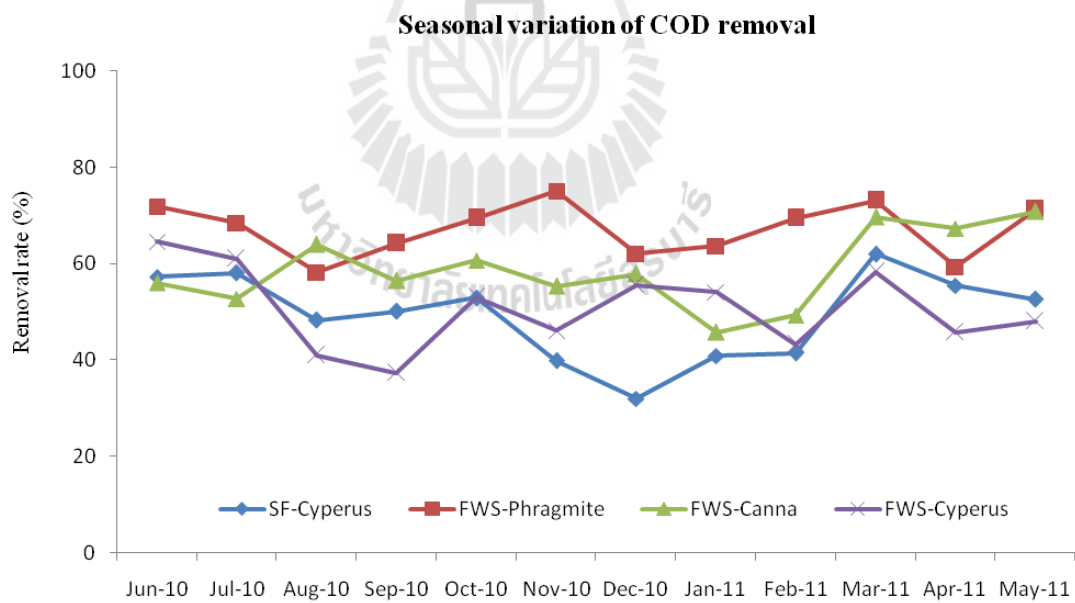


(b)

**Figure 4.6** Seasonal changes of (a) BOD and (b) COD removal rate (%) among different constructed wetlands.

Seasonal variation of NH₃ removal

(a)

Seasonal variation of TP removal

(b)
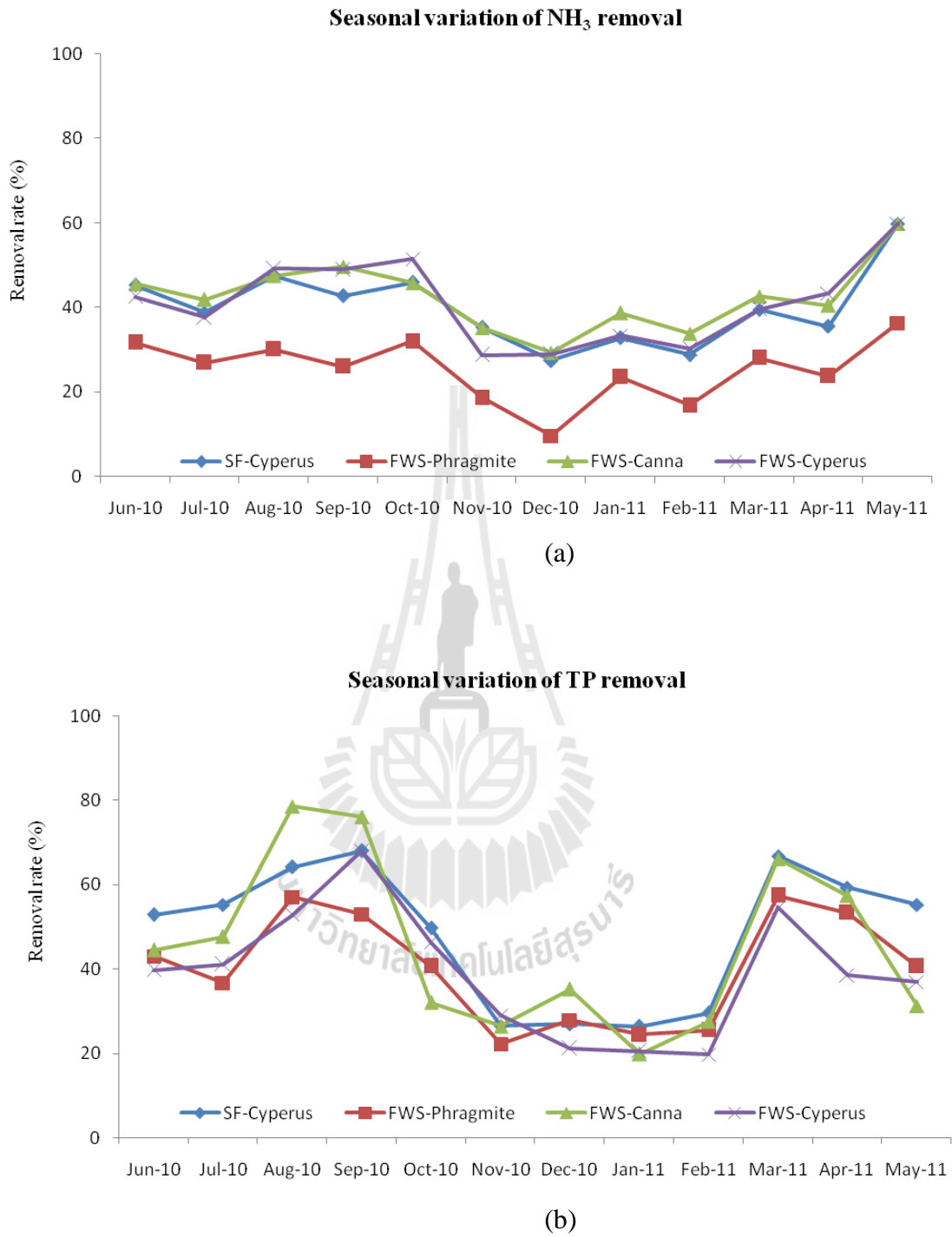
**Figure 4.7** Seasonal changes of (a) NH$_3$ and (b) TP removal rate (%) among different constructed wetlands.

## 4.3  Diurnal variation of greenhouse gas fluxes

Diurnal variation studies of greenhouse gas fluxes were primarily investigated on $CH_4$, $N_2O$ and $CO_2$ fluxes.  The studies were examined twice during the experiments, on 28-29 November 2010 and 27-28 March 2011.  Greenhouse gases were measured eight times over 24-hr periods.  Greenhouse gas fluxes were measured at three locations (namely A, B and C) in each experimental constructed wetland. The distances of points A, B and C measured from the entry point of the inlet were 0.5, 1.0 and 1.5 m, respectively. Point B was set in the middle and without plant in the plot as a control within the experimental unit.

Diurnal variation of greenhouse gas fluxes were studied at three hours cycles within 24 hours resulting in about 1,344 samples of gas samples collected totally. Comparisons of greenhouse gas fluxes from different constructed wetlands were conducted in eight experimental units as follow:

- P3 and P4: subsurface flow constructed wetland (SF) planted with *Cyperus* sp.,

- P5 and P6: free water surface flow constructed wetland (FWS) planted with *Phragmites* sp.,

- P7 and P8: free water surface flow constructed wetland (FWS)  planted with *Canna* sp., and

- P9 and P10: FWS planted with *Cyperus* sp..

In addition, this study also monitored three greenhouse gas fluxes from two non-plant SF units and two-non plant FWS units, or control units.  Results from these units were used as background data of the emissions.

### 4.3.1 Diurnal variation of methane flux

The averages of methane flux over diurnal cycle for each 3-hour period exploring from the 1$^{st}$ and 2$^{nd}$ experiments (November 2010 and March 2011) were illustrated in Figures 4.8-4.11. These figures show methane fluxes from different constructed wetlands and soil temperatures as an environmental factor. It is important to note that a large amount of methane produced in the soil was likely to be oxidized by methanotrophic bacteria. The methane gas captured in the chambers during the experiments was the net emission of methane.

Methane flux from SF constructed wetlands with *Cyperus* sp. showed higher fluxes at the inlets than the outlets while the control location was the lowest. The methane fluxes from inlets and outlets increased during the daytime and peaked at 15:00 in the afternoon for both sampling periods. However, the maximum diurnal rates were different during both periods. On 29 November 2010, the rates at the inlets and outlets were 7.4 and 12.5 mg/m$^2$/hr, respectively. Lower rates were observed on 28 March 2011 at about 1.8 and 2.3 mg/m$^2$/hr at inlets and outlets, respectively. The emission rates decreased at nighttime and reached the minimum values at 03:00 AM and regained the rate in the morning. The minimum diurnal rates at inlets and outlets were 0.7 and 2.3 mg/m$^2$/hr on 29 November 2010, and 0.2 and 0.3 mg/m$^2$/hr on 28 March 2011 (Figure 4.10).
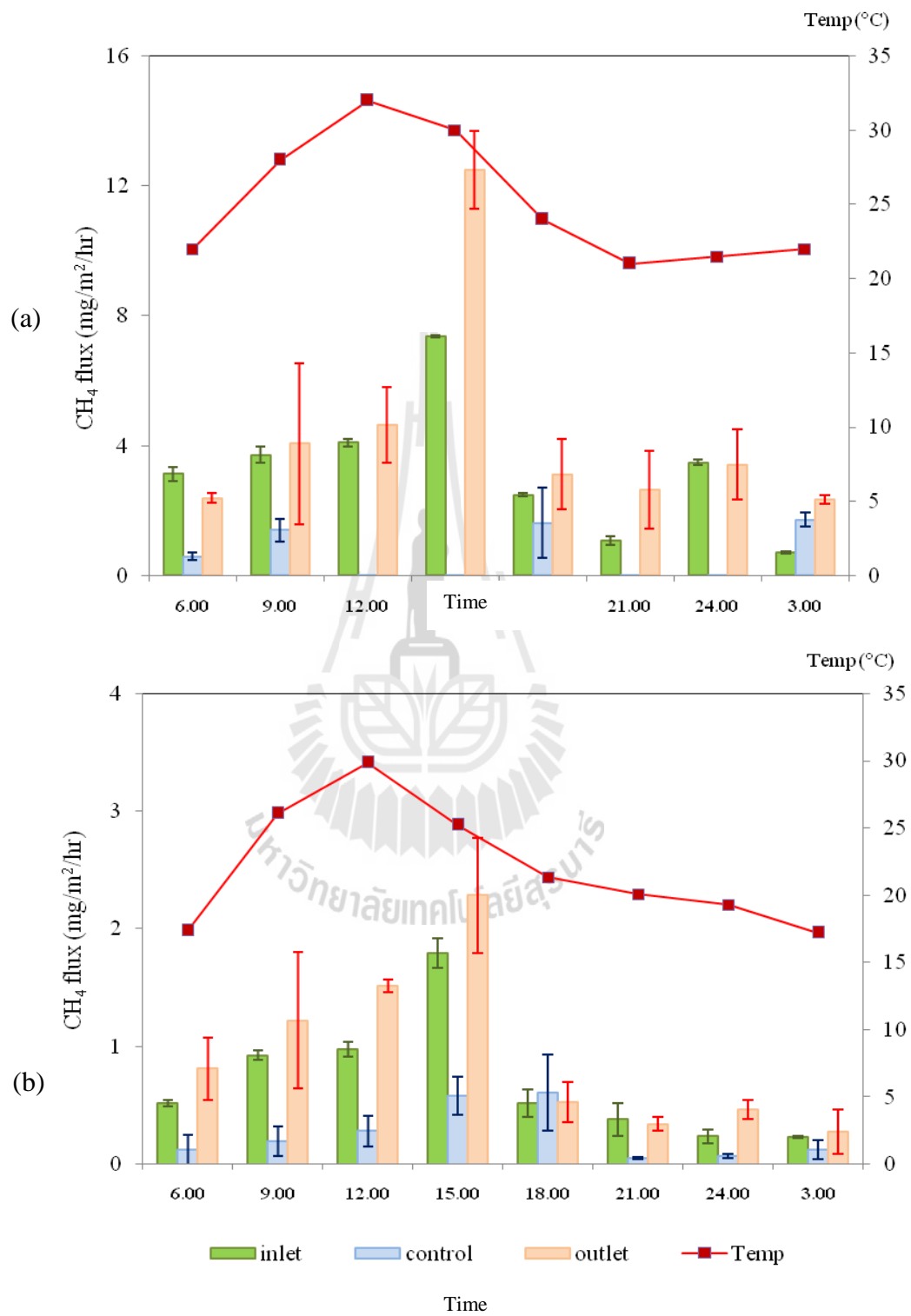
**Figure 4.8** Diurnal variation of methane fluxes and soil temperature at SF constructed wetlands during (a) 28-29 November 2010 (b) 27-28 March 2011.

In FWS constructed wetlands planted with *Phragmites* sp., methane fluxes at the outlet points were also higher than the inlets during November 2010 experiment while the fluxes at the inlet were higher that the outlets during March 2011 experiment. Lowest methane flux occurred at control chamber in middle point for both experiments. Methane fluxes at inlets and outlets increased during the daytime and peaked at 15:00 in the afternoon for both experimental periods. Maximum diurnal fluxes at inlets and outlets were 54.7 and 67.8 mg/m$^2$/hr, respectively, on 28 November 2010, and 13.9 and 18.5 mg/m$^2$/hr, respectively on 27 March 2011. The emission rates decreased during nighttime and reached the lowest rates at 24:00-03:00 AM prior to regained higher flux in the morning. Minimum diurnal rates at inlets and outlets were about 3.6 and 8.1 mg/m$^2$/hr on 29 November 2010 and 4.7 and 0.4 and mg/m$^2$/hr on 28 March 2011, respectively (Figure 4.11).
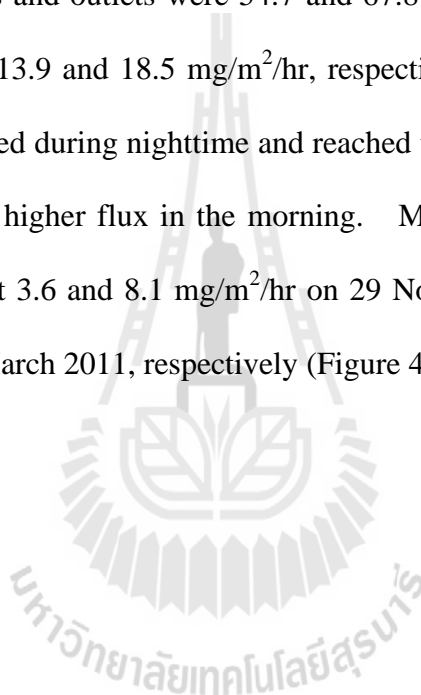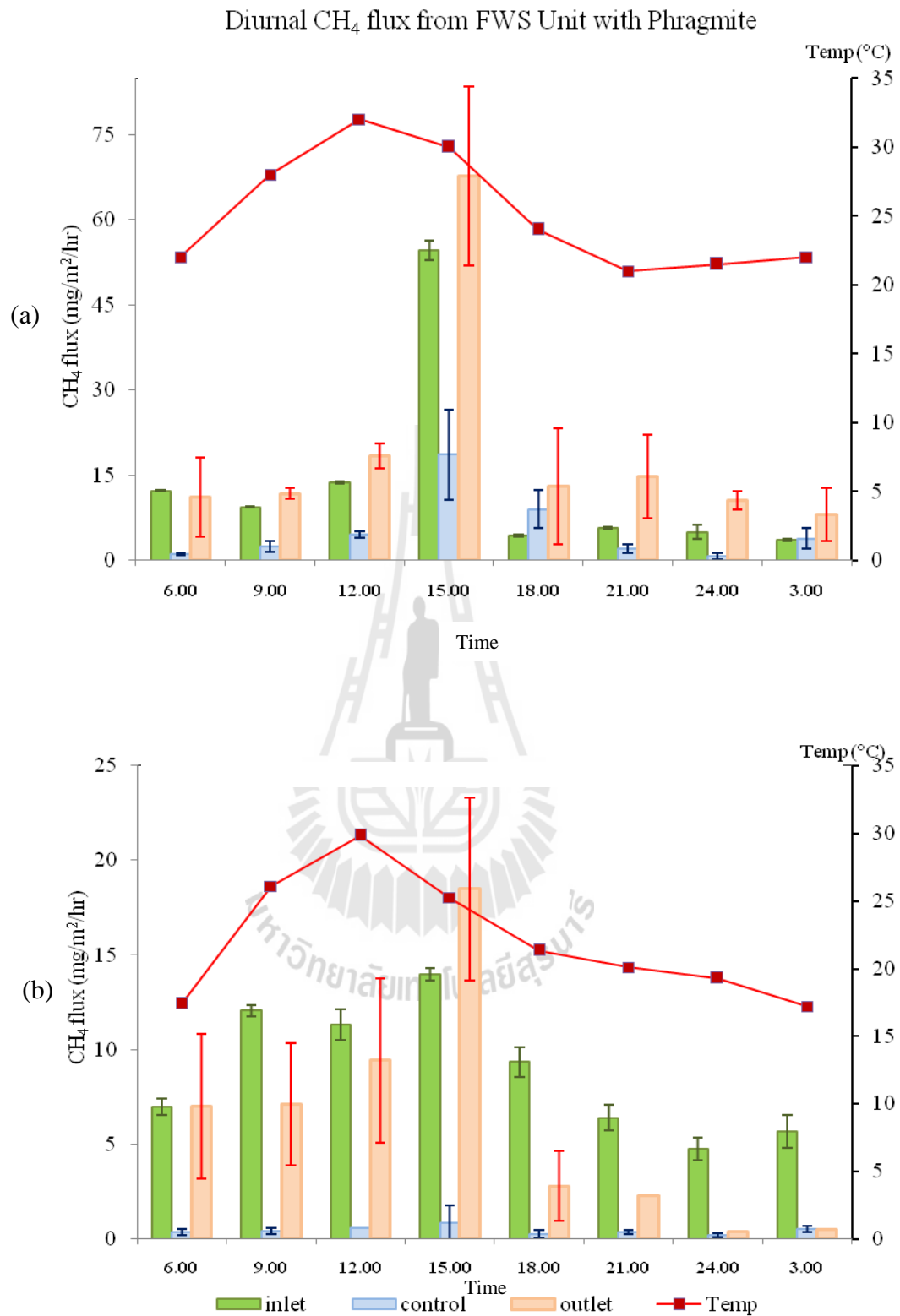
**Figure 4.9** Diurnal variation of methane fluxes and soil temperature at FWS constructed wetlands with *Phragmites* sp. during (a) 28-29 November 2010 (b) 27-28 March 2011.

At the FWS constructed wetland planted with *Canna* sp., when comparing methane fluxes among three points, found that methane flux from inlet point was higher than outlet point and lowest at control chamber in middle point. In FWS constructed wetlands planted with *Canna* sp., methane fluxes from the inlets were higher than outlets and the lowest fluxes found at control chamber in middle locations. Methane fluxes at outlets and inlets increased during daytime and peaked at 15:00 in the afternoon for both experiment periods. Maximum diurnal flux at outlets and inlets were 9.5 and 8.0 mg/m$^2$/hr on 28 November 2010, 1.8 and 2.2 mg/m$^2$/hr on 27 March 2011. The emission rate decreased at night to the minimum at 24:00-03:00 AM prior to regained higher flux in the morning. Minimum diurnal rates at outlets and inlets were 4.5 and 3.0 mg/m$^2$/hr on 29 November 2010, 0.7 and 0.3 mg/m$^2$/hr, respectively on 28 March 2011 (Figure 4.12).

**Figure 4.10** Diurnal variation of methane fluxes and soil temperature at FWS constructed wetlands with *Canna* sp. during (a) 28-29 November 2010 (b) 27-28 March 2011.

In FWS constructed wetlands planted with *Cyperus* sp., methane fluxes from inlets were higher than outlets and the lowest fluxes found at control chamber in middle locations. Methane fluxes at outlets and inlets increased during daytime and peaked at 15:00 in the afternoon for both experiment periods. Maximum diurnal fluxes at inlets and outlets were 20.8 and 20.3 mg/m$^2$/hr, respectively, on 28 November 2010, and 5.5 and 1.9 mg/m$^2$/hr, respectively, on 27 March 2011. The emission rates decreased at night and reached the minimum at 03:00 AM prior to regained higher flux in the morning. Minimum diurnal rates at inlets and outlets were 2.4 and 4.5 mg/m$^2$/hr, respectively, on 29 November 2010, and 1.7 and 0.3 mg/m$^2$/hr, respectively, on 28 March 2011 (Figure 4.13).

In SF and FWS beds with non-plants or control units, the results showed that high methane fluxes occurred during daytime. The fluxes peaked around 15:00 in the afternoon and subsequently decreased at night prior to reach the minimum values at 03:00 AM and regained higher flux in the morning (Figures 4.12-4.13). For control chambers, methane fluxes were relatively stable and relatively low comparing to inlets and outlets. In addition, diurnal variation could be observed. High methane fluxes occurred during daytime and low fluxes occurred during nighttime.

The results show that methane flux from constructed wetlands treated municipal wastewater has diurnal variations. Higher methane flux can be observed during daytime and peaked in the afternoon, around 15:00 while the flux at night is lower. Plants assist the emission of methane from the constructed wetlands since the results from un-planted location, or control chamber, within the experimental beds clearly show lower methane flux. Methane flux from control chambers are relatively less fluctuated compared to inlet and outlet locations.

Descriptive statistics for diurnal methane fluxes of two types of constructed wetlands including soil temperatures are shown in Table 4.6. Diurnal measurements performed on 28-29 November 2010 had higher methane fluxes in all experiment plots than the measurements on 27-28 March 2011. Daily soil temperature ranged from 21°C to 32°C during 28-29 November 2010 and 17 to 30°C during 27-28 March 2011. Since temperature is an important environmental factor influencing microbial activities in soil, emissions of methane are inevitably affected by changing temperature (U.S.EPA, 2010). Average methane flux between daytime and nighttime, calculated from two experiments on 28-29 November 2010 and 27-28 March 2011, are showed in Table 4.6.

Since these data were not a normal distribution and did not have homogeneity of variances, Mann-Whitney U tests are suitable for analysis diurnal variation of methane flux from different constructed wetlands. The results showed that the differences of methane flux between daytime and night time are significant (p-value < 0.05) at all constructed wetlands, as shown in Table 4.6. Average methane fluxes during daytime are about 1.7-10.6 times higher than during nighttime.

**Figure 4.11** Diurnal variation of methane fluxes and soil temperature at FWS constructed wetlands with *Cyperus* sp. during (a) 28-29 November 2010 (b) 27-28 March 2011.
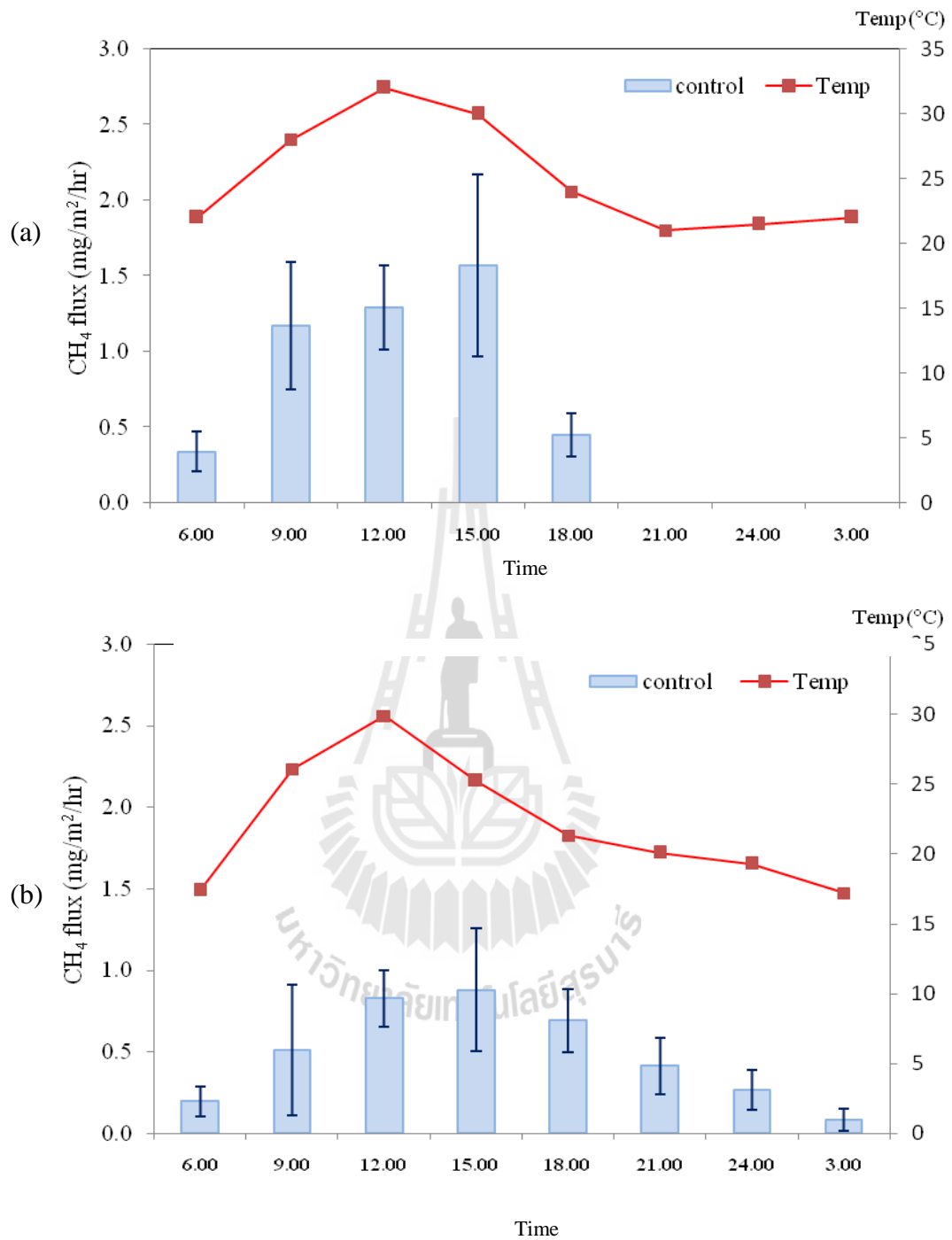
**Figure 4.12** Diurnal variation of methane fluxes and soil temperature at SF constructed wetlands with non-plants (control unit) during (a) 28-29 November 2010 (b) 27-28 March 2011.
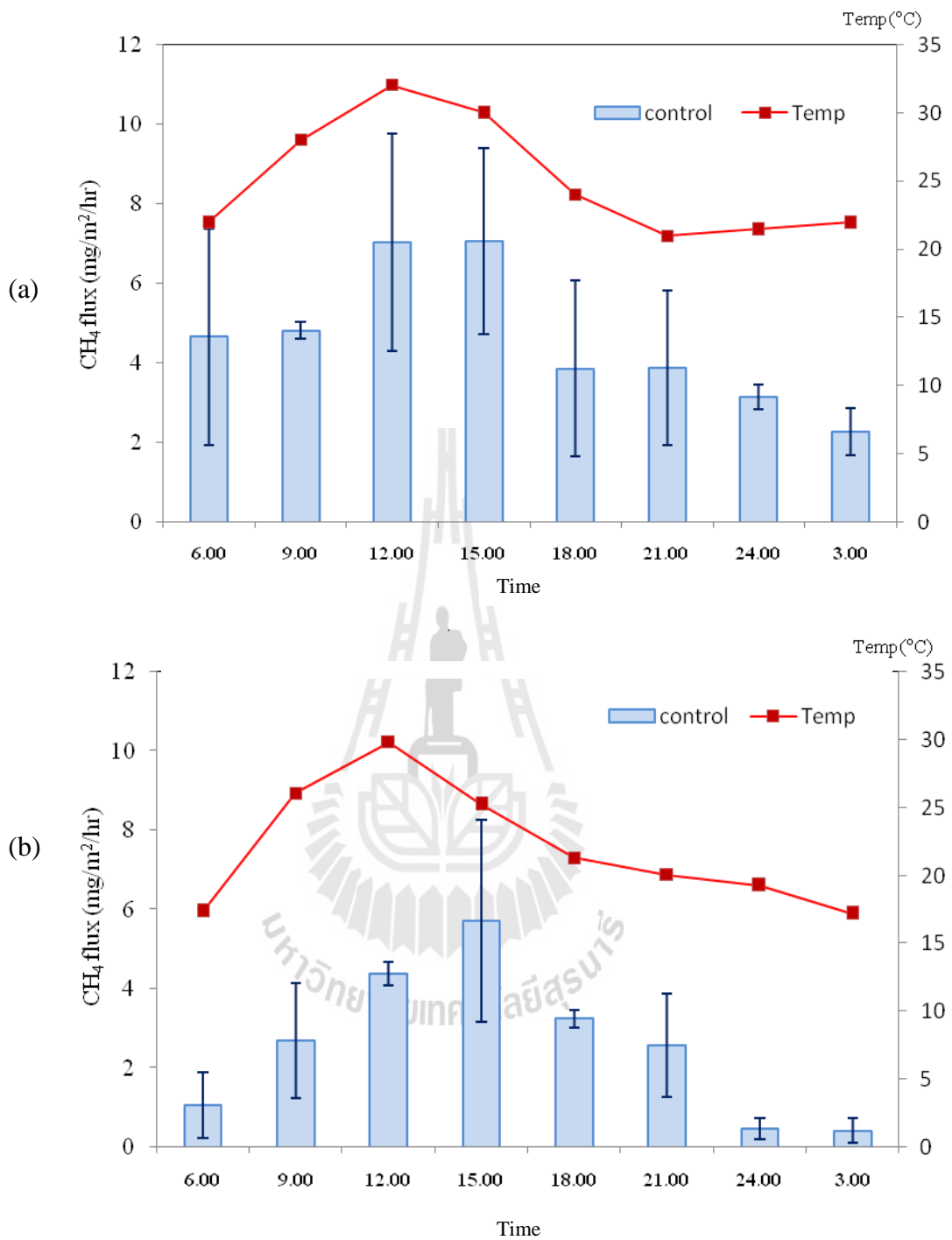
**Figure 4.13** Diurnal variation of methane fluxes and soil temperature at FWS constructed wetlands with non-plants (control unit) during (a) 28-29 November 2010 (b) 27-28 March 2011.

**Table 4.6** Descriptive statistics of daily methane fluxes (mg/m$^2$/hr) and soil temperature on 28-29 November 2010 and 27-28 March 2011 at different constructed wetlands. (n=16)

| Date | | 28-29 Nov. 2010 | | | | 27-28 Mar. 2011 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average | S.D. | Max | Min | Average | S.D. | Max | Min |
| SF *Cyperus* sp. | Inlet | 3.26 | 1.98 | 7.38 | 0.68 | 0.70 | 0.51 | 1.88 | 0.20 |
| | Control | 0.66 | 0.81 | 2.38 | 0.00 | 0.47 | 0.84 | 3.49 | 0.04 |
| | Outlet | 6.32 | 7.11 | 30.0 | 2.22 | 0.93 | 0.85 | 3.48 | 0.14 |
| SF (control) | Control | 1.85 | 3.24 | 8.27 | 0.00 | 0.48 | 0.39 | 1.50 | 0.03 |
| FWS *Phragmites* sp. | Inlet | 13.60 | 11.45 | 55.88 | 3.44 | 8.81 | 3.28 | 14.22 | 4.65 |
| | Outlet | 5.30 | 7.36 | 29.81 | 0.28 | 0.44 | 0.32 | 1.49 | 0.13 |
| | Control | 19.81 | 12.78 | 100.87 | 4.77 | 5.99 | 5.27 | 21.78 | 0.35 |
| FWS *Canna* sp. | Inlet | 7.15 | 1.57 | 9.68 | 4.35 | 1.44 | 0.56 | 2.28 | 0.64 |
| | Control | 2.13 | 2.09 | 8.51 | 0.15 | 0.18 | 0.15 | 0.61 | 0.02 |
| | Outlet | 4.96 | 2.77 | 11.33 | 0.58 | 1.01 | 0.54 | 2.39 | 0.23 |
| Soil Temp (°C) | | 25.06 | 4.31 | 32.00 | 21.00 | 22.04 | 4.54 | 30.00 | 17.20 |

**Table 4.6** (Continued).

| Date | | 28-29 Nov. 2010 | | | | 27-28 Mar. 2011 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average | S.D. | Max | Min | Average | S.D. | Max | Min |
| FWS *Cyperus* sp. | Inlet | 8.68 | 6.52 | 21.28 | 0.50 | 3.33 | 1.31 | 5.58 | 1.52 |
| | Control | 2.29 | 1.81 | 6.51 | 0.19 | 0.36 | 0.30 | 1.25 | 0.02 |
| | Outlet | 8.14 | 6.85 | 31.38 | 3.27 | 1.28 | 0.84 | 3.30 | 0.18 |
| FWS (control) | Control | 7.16 | 9.27 | 39.31 | 1.09 | 2.81 | 2.80 | 10.94 | 0.20 |
| Soil Temp (°C) | | 25.06 | 4.31 | 32.00 | 21.00 | 22.04 | 4.54 | 30.00 | 17.20 |
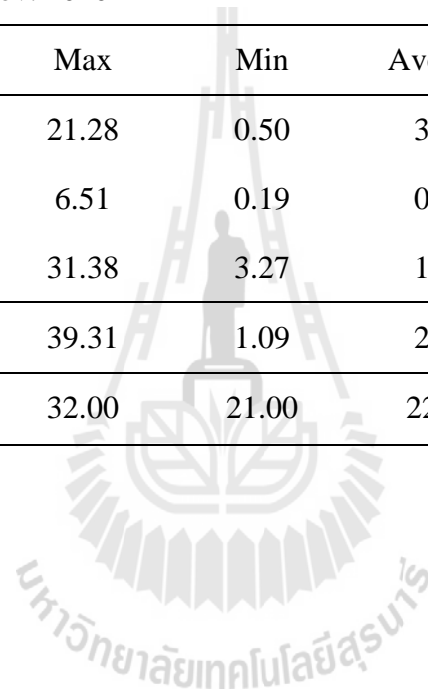
**Table 4.7** Comparison of diurnal methane fluxes (mg/m$^2$/hr) from different constructed wetlands using Mann-Whitney U Test.

| CW/ Time period | | N | Mean | Std. Deviation | Z | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|---|
| SF-*Cyperus* sp. | Day | 40 | 2.96 | 2.26 | -2.624 | 0.009 |
| | Night | 40 | 1.15 | 1.32 | | |
| | Total | 80 | | | | |
| FWS-*Phragmites* sp. | Day | 40 | 13.23 | 11.54 | -3.591 | 0.000 |
| | Night | 40 | 4.76 | 4.55 | | |
| | Total | 80 | | | | |
| FWS-*Canna* sp. | Day | 40 | 3.47 | 3.26 | -2.426 | 0.015 |
| | Night | 40 | 2.15 | 1.89 | | |
| | Total | 80 | | | | |
| FWS-*Cyperus* sp. | Day | 40 | 5.96 | 7.01 | -3.202 | 0.001 |
| | Night | 40 | 2.06 | 1.88 | | |
| | Total | 80 | | | | |
| SF-Control | Day | 16 | 2.13 | 3.104 | -2.193 | 0.028 |
| | Night | 16 | 0.20 | 0.249 | | |
| | Total | 32 | | | | |
| FWS-Control | Day | 16 | 7.41 | 9.360 | -2.374 | 0.018 |
| | Night | 16 | 2.56 | 1.921 | | |
| | Total | 32 | | | | |

### 4.3.2 Soil temperature influence on diurnal methane flux

Results from data analyses showed that average soil temperature during November 2010 and March 2011 experiments were 25.06°C (S.D.= 4.31) and 22.04 (S.D.= 4.54), respectively (Table 4.6). Almost all constructed wetlands, with the exception of SF and FWS control units, there were significant correlations between soil temperature and methane emissions from both experiments (Table 4.8). It can be

concluded that methane emissions had diurnal variations at all units and diurnal methane flux was correlated with changing in soil temperature.

**Table 4.8** Correlation between methane fluxes ($mg/m^2/hr$) and soil temperature (°C) in different plants and constructed wetland types.

| Correlated Dimension | Pearson Correlation | | |
|---|---|---|---|
| | 1st experiment | 2nd experiment | Total |
| SF *Cyperus* sp | 0.13 | 0.30* | 0.23* |
| FWS *Phragmites* sp. | 0.44** | 0.39** | 0.43** |
| FWS *Canna* sp. | 0.48** | 0.40** | 0.50** |
| FWS *Cyperus* sp. | 0.55** | 0.34* | 0.51** |

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

### 4.3.3 Diurnal variation of nitrous oxide flux

The averages of nitrous oxide flux over diurnal cycle for each 3-hour period during the 1st and 2nd experiments (November 2010 and March 2011) were illustrated in Figures 4.14-4.18. These figures show nitrous oxide fluxes from different constructed wetland and soil temperature, as an environmental factor. Descriptive statistics for diurnal nitrous oxide fluxes from different constructed wetlands and soil temperatures are shown in Table 4.9.

In SF constructed wetlands, nitrous oxide fluxes from two experimental periods were different. On 28-29 November 2010, nitrous oxide fluxes were increased in the morning and peaked at 9:00 AM. Subsequently, the fluxes decreased continuously and reached minimum value at night, around 21:00-24:00. Then, they

regained higher fluxes in the morning. Maximum and minimum daily fluxes were 26.2 and 0.0 mg/m$^2$/hr, respectively (Figure 4.14a). For the second experiment on 27-28 March 2011, nitrous oxide fluxes during the 21:00-03:00 period were higher than other periods. Maximum and minimum daily fluxes were 4.9 and 0.05 mg/m$^2$/hr, respectively (Figure 4.16b).

In FWS constructed wetlands planted with *Phragmites* sp., nitrous oxide fluxes between two experimental periods showed different pattern of diurnal variations. On 28-29 November 2010, nitrous oxide fluxes were high during 06:00-12:00 period. Maximum daily flux was 27.1 mg/m$^2$/hr whereas minimum daily flux was 0.05 mg/m$^2$/hr (Figure 4.15a). For the second experiment on 27-28 March 2011, nitrous oxide fluxes during 24:00-03:00 period were higher than other periods. Maximum daily flux was 13.6 mg/m$^2$/hr whereas minimum daily flux was 0.03 mg/m$^2$/hr (Figure 4.17b).

In FWS constructed wetland planted with *Canna* sp., nitrous oxide fluxes between two experimental periods also had different pattern of diurnal variations. On 28-29 November 2010, nitrous oxide fluxes were high during 06:00-09:00 period. Maximum daily flux was 24.6 mg/m$^2$/hr whereas minimum daily flux was 0.1 mg/m$^2$/hr (Figure 4.16a). For the second experiment on 27-28 March 2011, nitrous oxide fluxes during 24:00-03:00 period were higher than other periods. Maximum daily flux was 6.2 mg/m$^2$/hr whereas daily minimum flux was 0.0 mg/m$^2$/hr (Figure 4.18b).
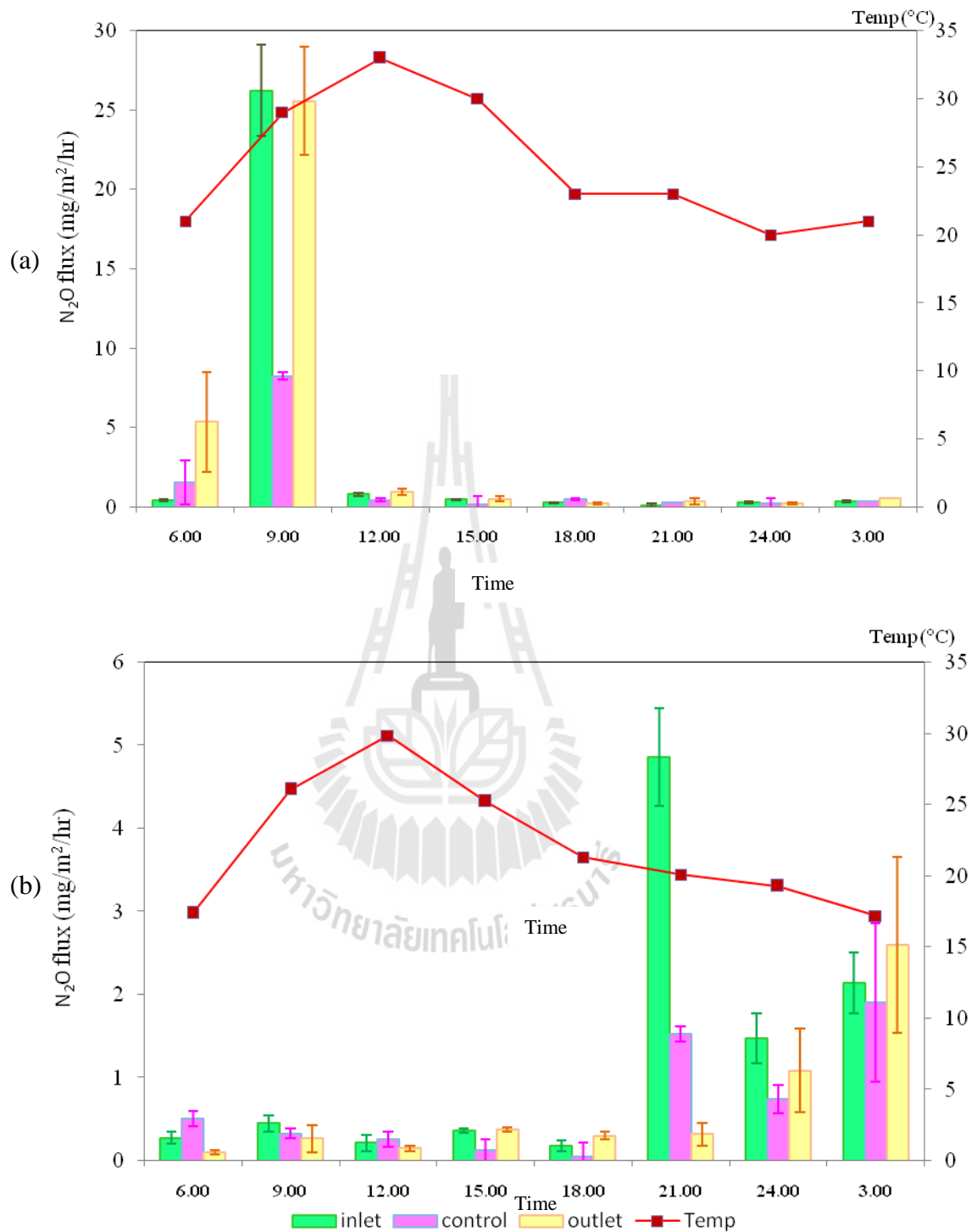
**Figure 4.14** Diurnal variation of nitrous oxide and soil temperature of SF constructed wetlands during (a) 28-29 November 2010 and (b) 27-28 March 2011.
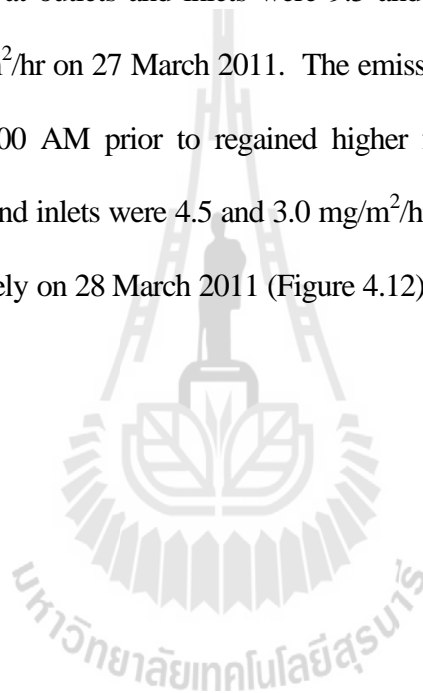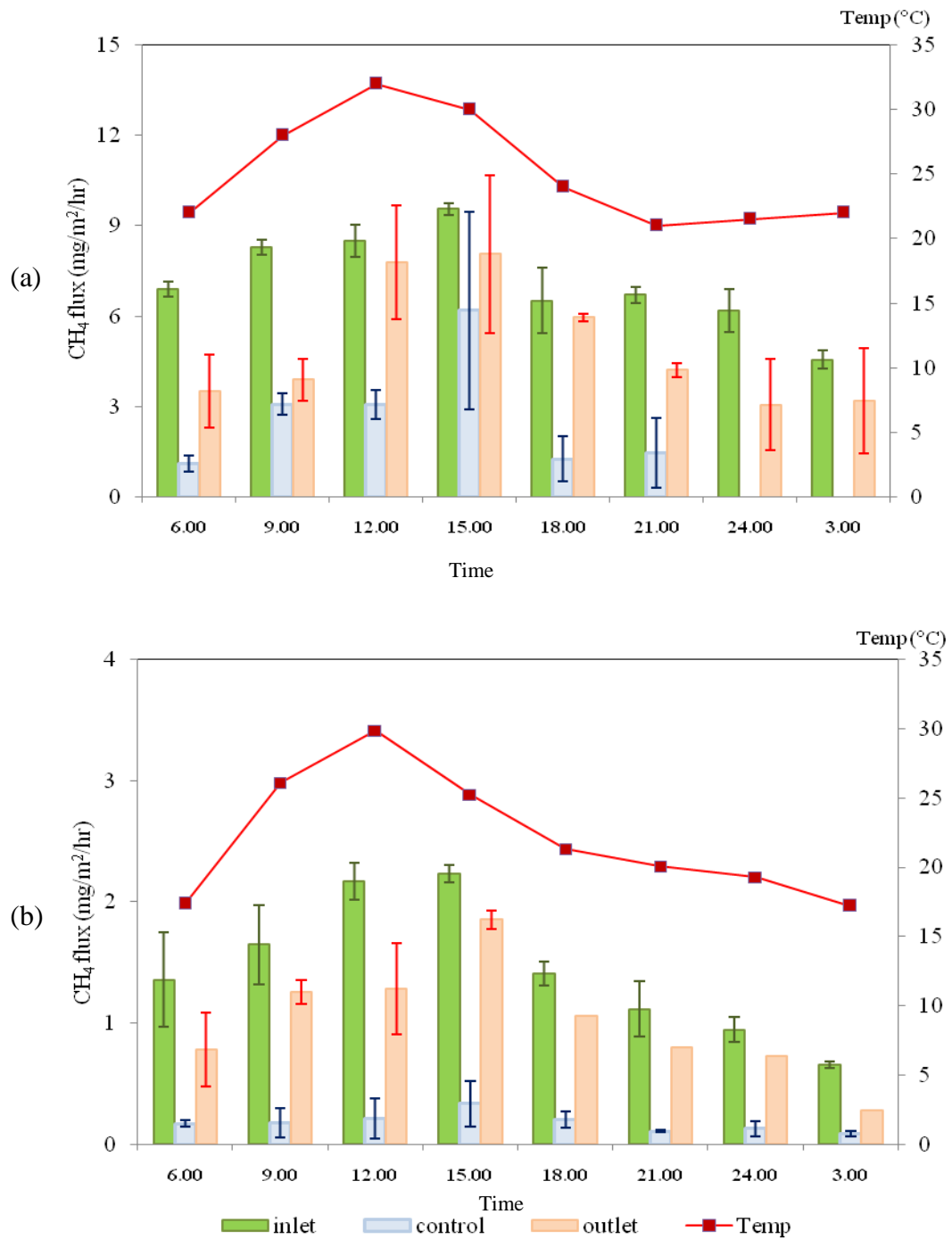
**Figure 4.15** Diurnal variation of nitrous oxide and soil temperature of FWS constructed wetlands with *Phragmites* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.
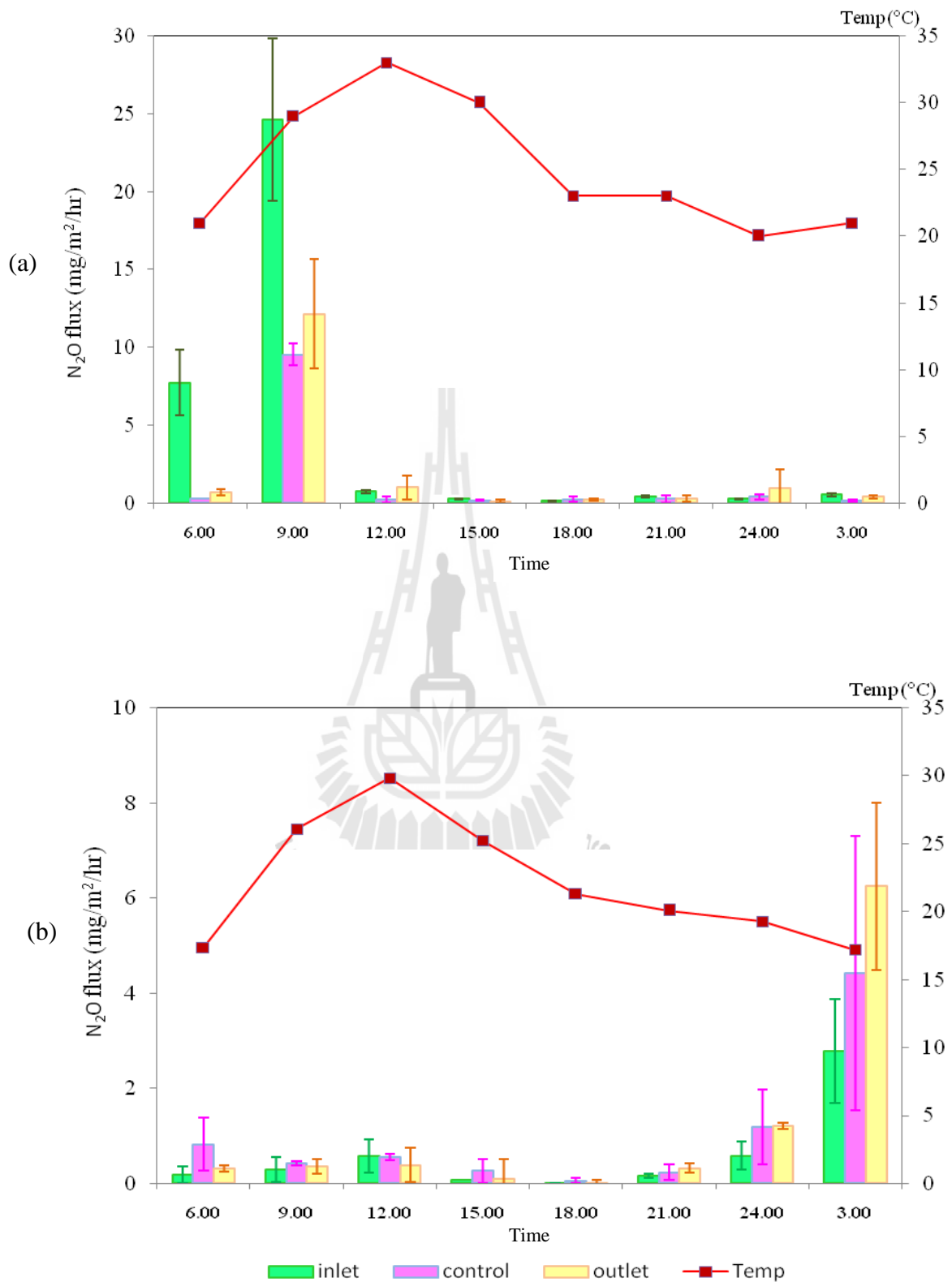
**Figure 4.16** Diurnal variation of nitrous oxide and soil temperature of FWS constructed

wetlands with *Canna* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.
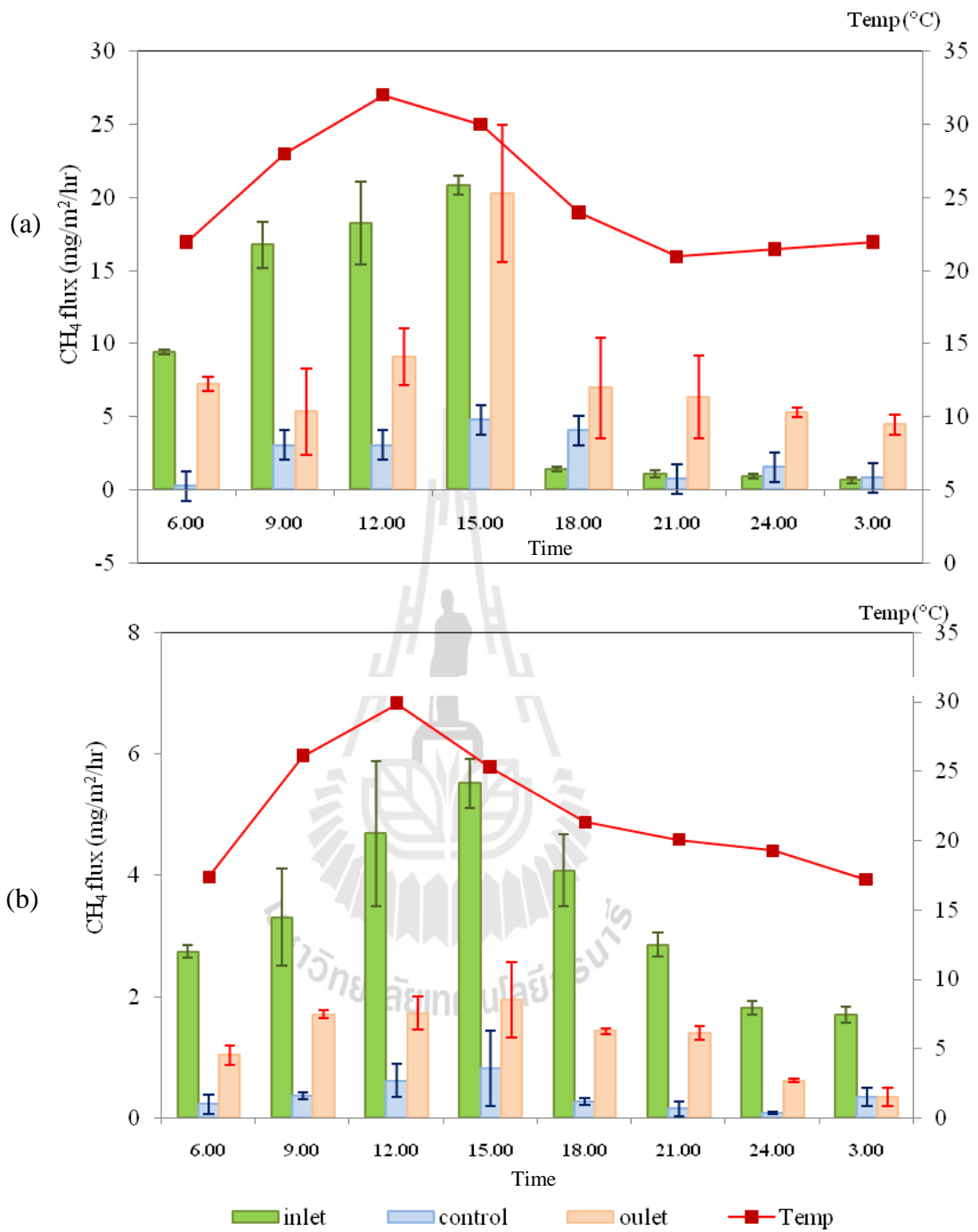
**Table 4.9** Descriptive statistics of daily nitrous oxide fluxes and soil temperature on 28-29 November 2010 and 27-28 March 2011 at different constructed wetlands (n=16).

| Date | | | Average | S.D. | Max | Min |
|---|---|---|---|---|---|---|
| 28-29 Nov. 2010 | SF *Cyperus* sp. | Inlet | 3.63 | 8.86 | 28.28 | 0.08 |
| | | Control | 1.48 | 3.30 | 13.52 | 0.03 |
| | | Outlet | 4.23 | 8.69 | 28.01 | 0.19 |
| | SF (control) | Control | 4.31 | 10.91 | 39.49 | 0.04 |
| | FWS *Phragmites* sp. | Inlet | 4.34 | 8.98 | 28.08 | 0.03 |
| | | Control | 0.81 | 1.26 | 4.07 | 0.06 |
| | | Outlet | 3.67 | 8.35 | 30.75 | 0.02 |
| | FWS *Canna* sp. | Inlet | 4.34 | 8.38 | 25.40 | 0.10 |
| | | Control | 1.42 | 24.61 | 10.06 | 0.09 |
| | | Outlet | 1.98 | 4.10 | 14.65 | 0.02 |
| | FWS *Cyperus* sp. | Inlet | 4.01 | 7.40 | 25.22 | 0.08 |
| | | Control | 0.52 | 0.71 | 2.80 | 0.00 |
| | | Outlet | 2.81 | 5.47 | 19.33 | 0.00 |
| | FWS (control) | Control | 3.29 | 5.99 | 18.05 | 0.00 |
| 27-28 Mar. 2011 | SF *Cyperus* sp. | Inlet | 1.24 | 1.58 | 5.28 | 0.13 |
| | | Control | 0.68 | 0.93 | 3.00 | 0.00 |
| | | Outlet | 0.65 | 0.99 | 4.06 | 0.08 |
| | SF (control) | Control | 0.29 | 0.68 | 2.78 | 0.00 |

**Table 4.9** (Continued).

| Date | | | Average | S.D. | Max | Min |
|---|---|---|---|---|---|---|
| 27-28 Mar. 2011 | FWS *Phragmites* sp. | Inlet | 1.41 | 2.58 | 8.09 | 0.16 |
| | | Control | 0.50 | 0.38 | 1.23 | 0.08 |
| | | Outlet | 2.07 | 4.87 | 18.57 | 0.01 |
| | FWS *Canna* sp. | Inlet | 0.58 | 0.88 | 2.88 | 0.00 |
| | | Control | 1.37 | 2.75 | 11.03 | 0.03 |
| | | Outlet | 1.13 | 2.08 | 7.50 | 0.00 |
| | FWS *Cyperus* sp. | Inlet | 1.31 | 1.36 | 4.08 | 0.18 |
| | | Control | 1.21 | 1.64 | 6.48 | 0.00 |
| | | Outlet | 1.97 | 3.70 | 12.94 | 0.01 |
| | FWS (control) | Control | 0.98 | 1.23 | 4.34 | 0.00 |

In FWS constructed wetland planted with *Cyperus* sp., nitrous oxide fluxes between two experiment periods had different diurnal variation patterns. On 28-29 November 2010, nitrous oxide fluxes were high at 06:00-09:00 period. Maximum daily flux was 22.2 mg/m$^2$/hr, whereas daily minimum flux was 0.0 mg/m$^2$/hr (Figure 4.17a). For the second experiment on 27-28 March 2011, nitrous oxide fluxes at 3:00 AM were higher than other periods. Maximum daily flux was 11.2 mg/m$^2$/hr whereas minimum daily flux was 0.06 mg/m$^2$/hr (Figure 4.19b).

In SF and FWS beds with non-plants or control units, nitrous oxide fluxes between two experiment periods found that pattern of diurnal variation was different. On 28-29 November 2010, nitrous oxide fluxes at 09:00 AM were higher than other periods. Maximum daily rate for SF and FWS beds were 31.1 and 16.6 mg/m$^2$/hr, respectively. Minimum daily rate for SF and FWS beds were 0.14 and 0.0 mg/m$^2$/hr, respectively (Figures 4.18-4.19). For the second experiment on 27-28 March 2011, two peaks of nitrous oxide fluxes occurred during 09:00-15:00 and 24:00-3:00 in SF beds and 06:00-12:00 and 21:00-3:00 periods in FWS beds. Maximum daily rate for SF and FWS beds were 1.5 and 3.8 mg/m$^2$/hr, respectively. Minimum daily flux for SF and FWS was 0.0 mg/m$^2$/hr for both SF and FWS beds (Figures 4.20-4.21).

Nitrous oxide fluxes at control chambers within the beds were relatively fluctuated but the fluxes were low compared to both inlets and outlets. Moreover, the fluxes did not show an obvious pattern of diurnal variation.
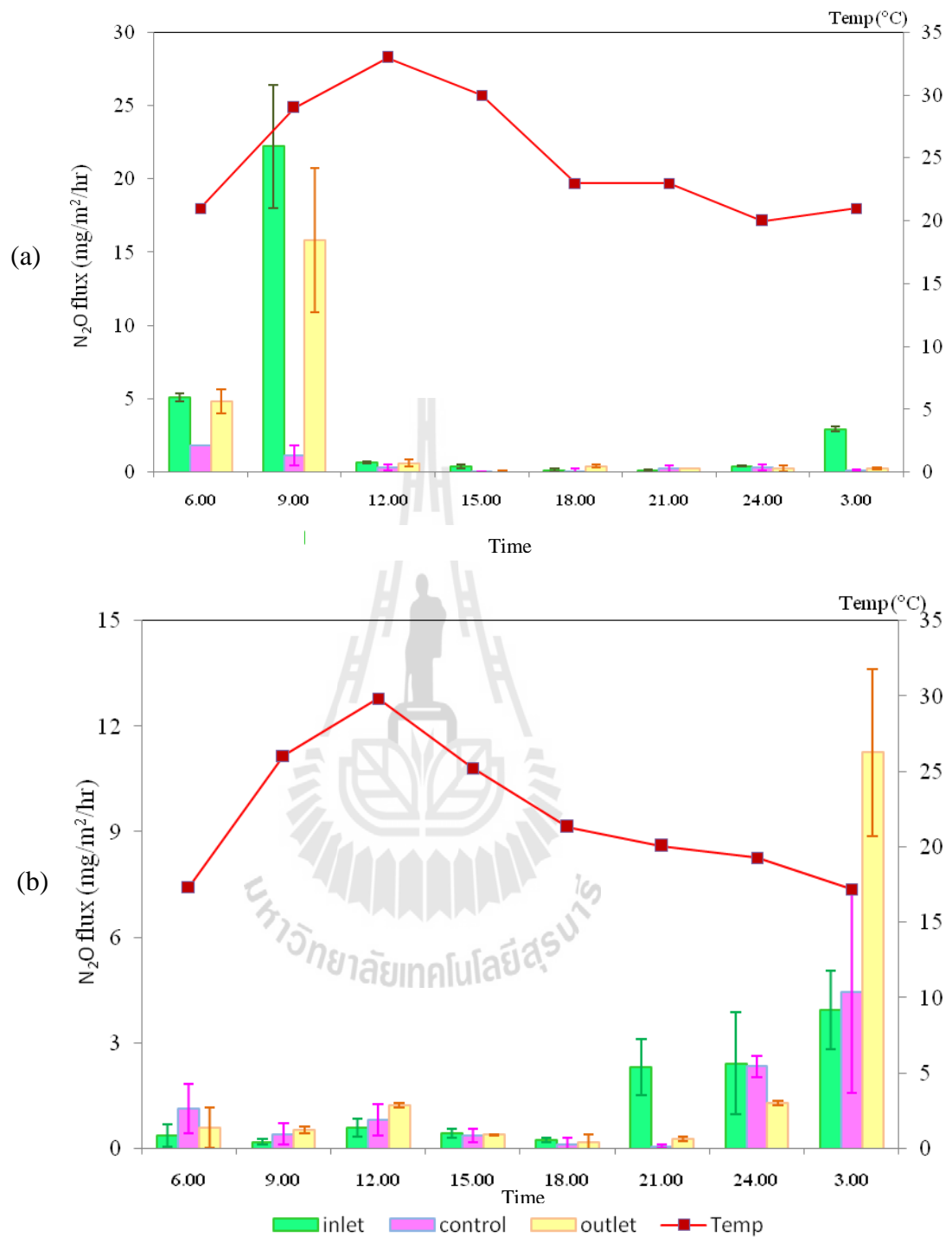
**Figure 4.17** Diurnal variation of nitrous oxide and soil temperature of FWS constructed wetlands with *Cyperus* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.
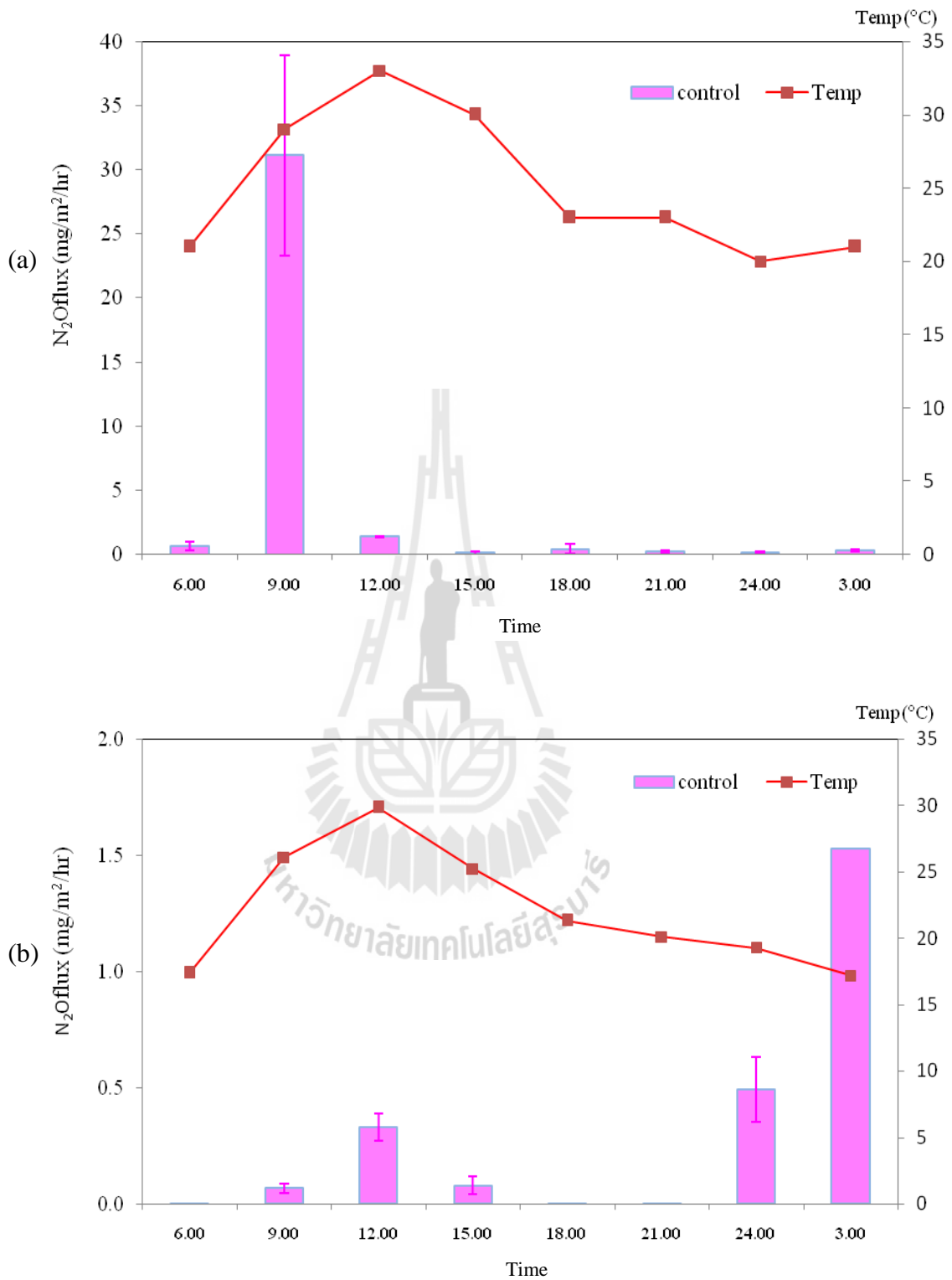
**Figure 4.18** Diurnal variation of nitrous oxide and soil temperature of SF beds with

non-plants (control unit) during (a) 28-29 November 2010 and (b) 27-28 March 2011.
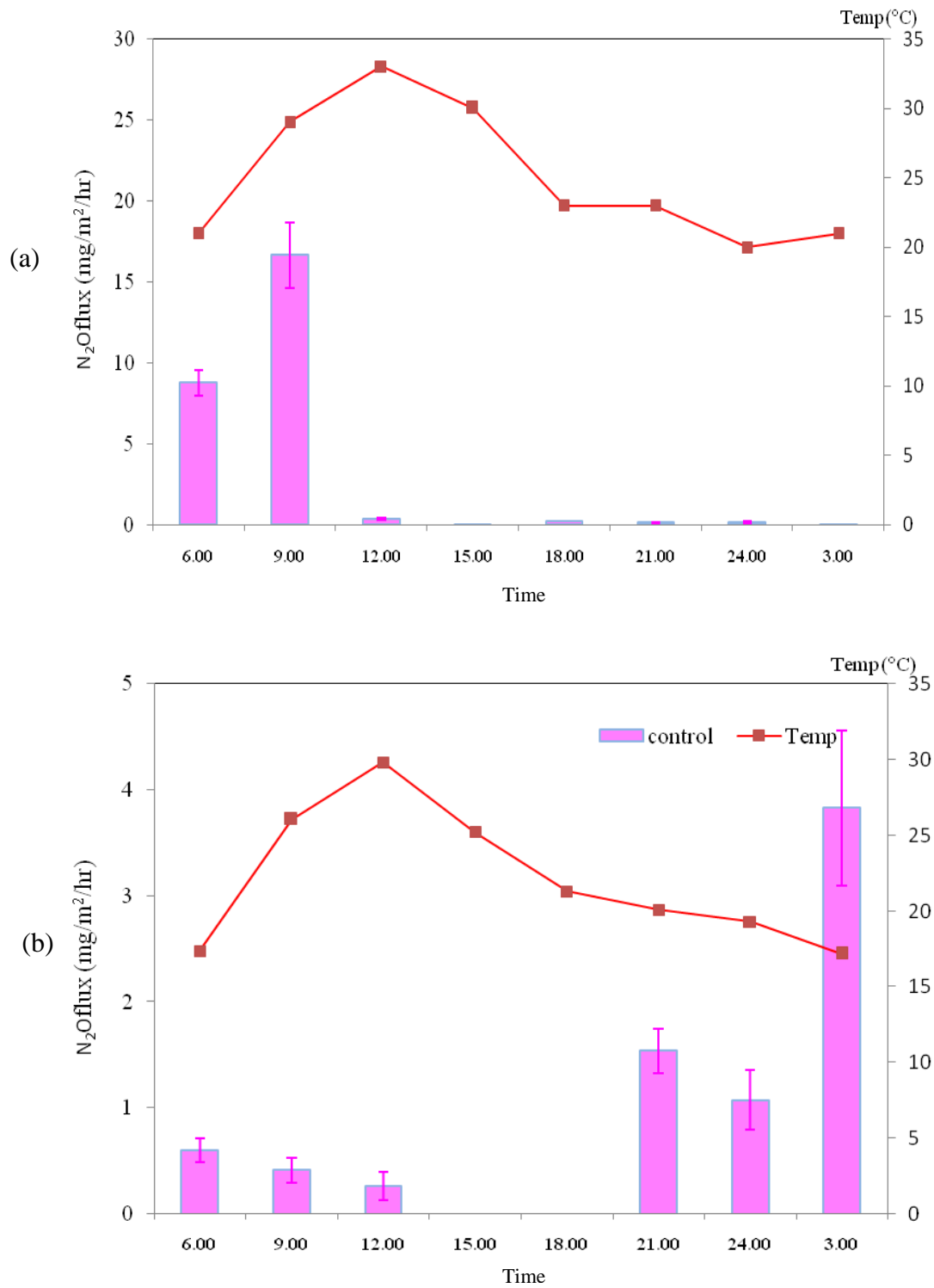
**Figure 4.19** Diurnal variation of nitrous oxide and soil temperature of FWS beds with non-plants (control unit) during (a) 28-29 November 2010 and (b) 27-28 March 2011.

When compare diurnal nitrous oxide fluxes between two experiments on 28-29 November 2010 and 27-28 March 2011, nitrous oxide fluxes during November experiment were higher than March experiment at almost all constructed wetlands. Daily soil temperature ranged from 21°C to 32°C during 28-29 November 2010 and 17 to 30°C during 27-28 March 2011. At this point, nitrous oxide flux variation may possibly be the result of differences in soil temperature during both experimental periods. Average daytime and nighttime nitrous oxide fluxes between two experiments are shown in Table 4.10.

Since these data were neither a normal distribution nor homogeneity of variances, Mann-Whitney U tests are suitable for the analysis of diurnal variation of nitrous oxide flux from different constructed wetlands. The results showed that the differences of nitrous oxide flux between daytime and nighttime are not significant ($p > 0.05$) at almost all constructed wetlands, except FWS planted with *Phragmites* sp. and *Cyperus* sp. as shown in Table 4.10. However, average nitrous oxide fluxes during daytime are about 1.6-10.6 times of those of nighttime. The results indicated that nitrous oxide fluxes did not show an obvious pattern of diurnal variation.

**Table 4.10** Comparison of diurnal nitrous oxide fluxes from different constructed wetlands using Mann-Whitney U Test.

| CW/ Time period | | N | Mean | Std. Deviation | Z | Asymp. Sig(2-tailed) |
|---|---|---|---|---|---|---|
| SF-*Cyperus* sp. | Day | 48 | 3.09 | 7.36 | -0.550 | 0.583 |
| | Night | 48 | 0.88 | 1.20 | | |
| | Total | 96 | | | | |
| FWS-*Phragmites* sp. | Day | 48 | 3.02 | 7.11 | -2.942 | 0.003* |
| | Night | 48 | 1.25 | 3.21 | | |
| | Total | 96 | | | | |
| FWS-*Canna* sp. | Day | 48 | 2.58 | 5.67 | -1.363 | 0.173 |
| | Night | 48 | 1.03 | 2.05 | | |
| | Total | 96 | | | | |
| FWS-*Cyperus* sp. | Day | 48 | 2.51 | 5.37 | -1.931 | 0.053 |
| | Night | 48 | 1.44 | 2.51 | | |
| | Total | 96 | | | | |
| SF-Control | Day | 16 | 4.23 | 10.94 | -.944 | 0.345 |
| | Night | 16 | 0.38 | 0.67 | | |
| | Total | 32 | | | | |
| FWS-Control | Day | 16 | 3.40 | 5.94 | -0.945 | 0.344 |
| | Night | 16 | 0.88 | 1.29 | | |
| | Total | 32 | | | | |

*significant at the 0.01 level*

### 4.3.4 Soil temperature and diurnal nitrous oxide flux

Measurements of soil temperature showed that average temperature in the first experiment in late November 2010 was 25.06°C (S.D.=4.31) while the second experiment in late March 2011 was lower, 22.04°C (S.D.=4.54). Statistical test indicated no significant correlations between soil temperature and nitrous oxide fluxes during late November 2010. However, results from statistical test of the second experiment showed that soil temperature and nitrous oxides fluxes were significantly correlated in all types of constructed wetlands (Table 4.11). Therefore, this study did not have clear evidence to conclude that diurnal nitrous oxide fluxes were influence by soil temperature in the constructed wetlands used to treated municipal wastewater. U.S EPA (2010) reported that environmental controls may result in non-linear responses to small changes which mean that a change in temperature may change the balance between production and consumption of gas emissions.

**Table 4.11** Correlation between $N_2O$ fluxes (mg/m$^2$/hr) and soil temperature (°C).

| Correlated Dimension (analysis in pair) | Pearson Correlation | | |
|---|---|---|---|
| | 1$^{st}$ experiment | 2$^{nd}$ experiment | Total |
| SF *Cyperus* sp. | 0.23 | -0.37** | 0.18 |
| FWS *Phragmites* sp. | 0.25 | -0.32* | 0.11 |
| FWS *Canna* sp. | 0.21 | -0.38** | 0.10 |
| FWS *Cyperus* sp. | 0.15 | -0.40** | 0.01 |

*\* Correlation is significant at the 0.05 level (2-tailed).*

*\*\* Correlation is significant at the 0.01 level (2-tailed).*

### 4.3.5 Diurnal variation of carbon dioxide flux

The averages of carbon dioxide fluxes over diurnal cycle for each 3-hour period exploring from the 1st and 2nd experiment (November 2010 and March 2011) were illustrated in Figures 4.20-4.24. These figures show carbon dioxide fluxes from different constructed wetlands and soil temperature as an environmental factor. Descriptive statistics for carbon dioxide fluxes from different constructed wetlands and soil temperatures are shown in Table 4.12.

In SF constructed wetlands planted with *Cyperus* sp., diurnal variations of carbon dioxide fluxes between two experimental periods were slightly different. Carbon dioxide fluxes showed positive values during 12:00-03:00 period and peaked at 18:00 during the experiment on 28-29 November 2010. Negative fluxes occurred during 06:00-09:00 period after that carbon dioxide flux increased slightly until peaked at 18:00 period. Maximum daily rate was estimated at 100.4 mg/m$^2$/hr whereas minimum daily rate was -283.8 mg/m$^2$/hr (Figure 4.22a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive values during 18:00-06:00 period and maximum value occurred at 03:00 period. Negative fluxes occurred during 09:00-15:00 period and reached the bottom at 09:00 period. Maximum daily rate was about 77.0 mg/m$^2$/hr where as minimum daily rate was -41.0 mg/m$^2$/hr (Figure 4.22b).
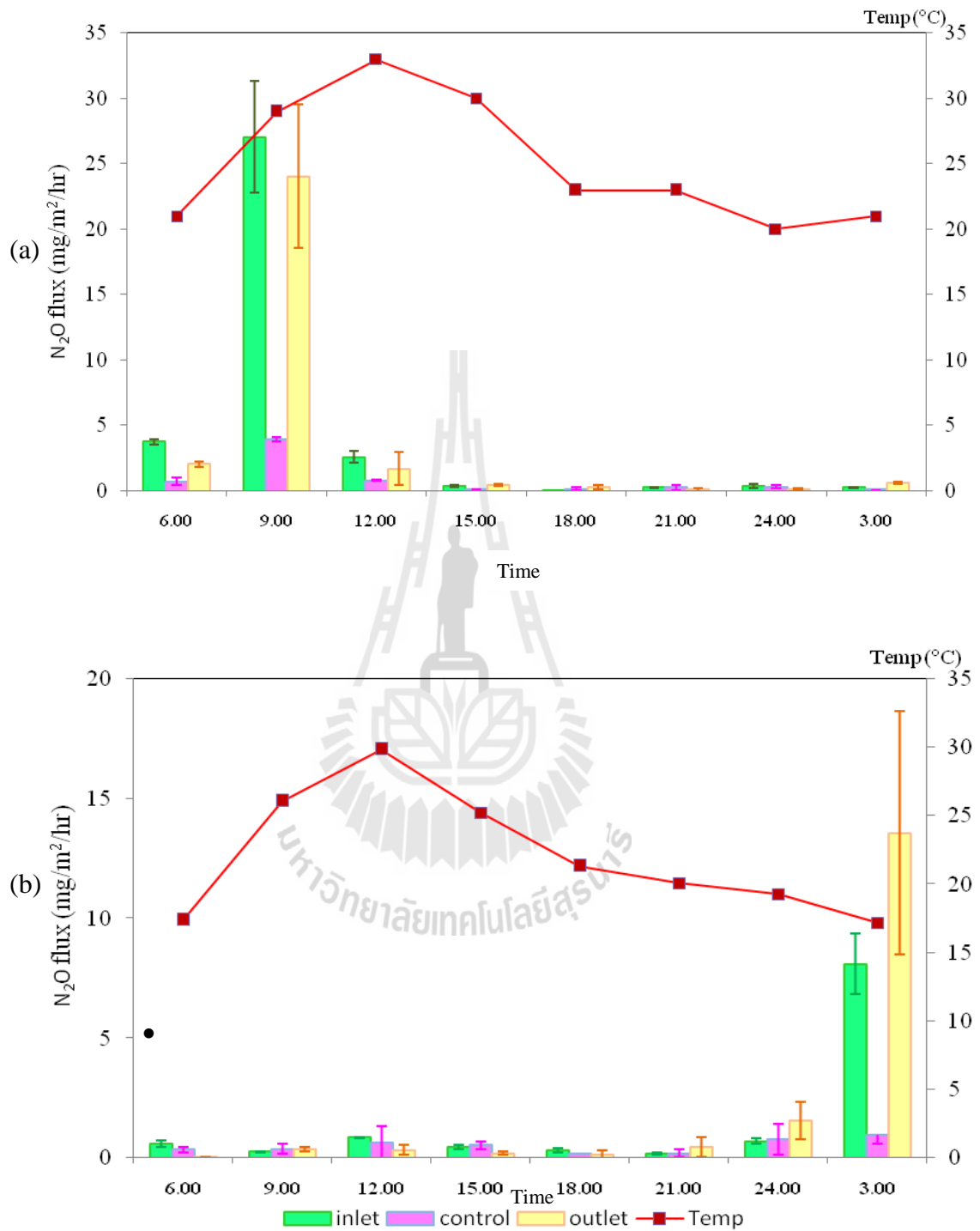
In FWS constructed wetlands planted with *Phragmites* sp., carbon dioxide fluxes between two experimental periods had difference diurnal variations. During 28-29 November 2010, carbon dioxide fluxes showed positive values during 12:00-03:00 period and peaked at 21:00 period. Negative fluxes occurred during 06:00-09:00 period. Maximum daily rate was 94.4 mg/m$^2$/hr whereas minimum daily rate

was -369.3 mg/m$^2$/hr (Figure 4.23a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive values during 18:00-06:00 period and maximum value occurred at 03:00 period. Negative fluxes occurred during 09:00-15:00 period. Maximum daily rate was 135.2 mg/m$^2$/hr whereas minimum daily rate was -153.7 mg/m$^2$/hr (Figure 4.23b).

**Table 4.12** Descriptive statistics of daily $CO_2$ flux and soil temperature on 28-29 November 2010 and 27-28 March 2011 at different constructed wetlands (n=16).

| Date | | | Average | S.D. | Max | Min |
|---|---|---|---|---|---|---|
| | SF *Cyperus* sp. | Inlet | 0.11 | 114.14 | 102.88 | -288.00 |
| | | Control | 0.26 | 126.27 | 228.97 | -322.47 |
| | | Outlet | 21.63 | 102.23 | 200.47 | -279.23 |
| | SF (control) | Control | 24.09 | 66.51 | 138.73 | -140.55 |
| | FWS *Phragmites* sp. | Inlet | -9.85 | 138.86 | 98.28 | -388.28 |
| | | Control | -8.35 | 147.29 | 125.47 | -396.52 |
| 28-29 Nov. 2010 | | Outlet | -4.51 | 141.88 | 131.72 | -388.28 |
| | FWS *Canna* sp. | Inlet | 22.71 | 73.16 | 25.40 | -118.80 |
| | | Control | 25.48 | 45.99 | 10.06 | -37.76 |
| | | Outlet | 10.94 | 84.05 | 14.65 | -196.47 |
| | FWS *Cyperus* sp. | Inlet | 15.95 | 54.00 | 25.22 | -88.00 |
| | | Control | 25.14 | 54.01 | 2.80 | -55.00 |
| | | Outlet | 15.26 | 38.80 | 19.33 | -50.79 |
| | FWS (control) | Control | 44.74 | 26.65 | 18.05 | 1.79 |
| | Soil Temp ($^o$C) | | 25.06 | 4.31 | 32.00 | 21.00 |

**Table 4.12**  (Continued).

| Date | | | Average | S.D. | Max | Min |
|---|---|---|---|---|---|---|
| 27-28 Mar.2011 | SF *Cyperus* sp. | Inlet | 6.95 | 16.65 | 40.20 | -14.20 |
| | | Control | 5.86 | 36.68 | 111.36 | -48.49 |
| | | Outlet | 11.01 | 35.19 | 104.75 | - 47.27 |
| | SF (control) | Control | 9.79 | 31.98 | 69.26 | -37.91 |
| | FWS *Phragmites* sp. | Inlet | 23.81 | 36.40 | 88.80 | -18.28 |
| | | Control | 9.02 | 76.26 | 172.64 | -123.25 |
| | | Outlet | 10.29 | 87.46 | 200.83 | -171.39 |
| | FWS *Canna* sp. | Inlet | 29.13 | 68.73 | 118.80 | -48.00 |
| | | Control | -4.50 | 94.04 | 248.99 | -152.86 |
| | | Outlet | -10.79 | 73.38 | 98.95 | -148.70 |
| | FWS *Cyperus* sp. | Inlet | 24.47 | 87.48 | 148.80 | -128.00 |
| | | Control | 1.61 | 74.06 | 136.17 | -172.23 |
| | | Outlet | -7.21 | 77.31 | 10.75 | -133.51 |
| | FWS (control) | Control | 24.77 | 100.20 | 188.34 | -132.74 |
| | Soil Temp ($^{o}$C) | | 22.04 | 4.54 | 30.00 | 17.20 |
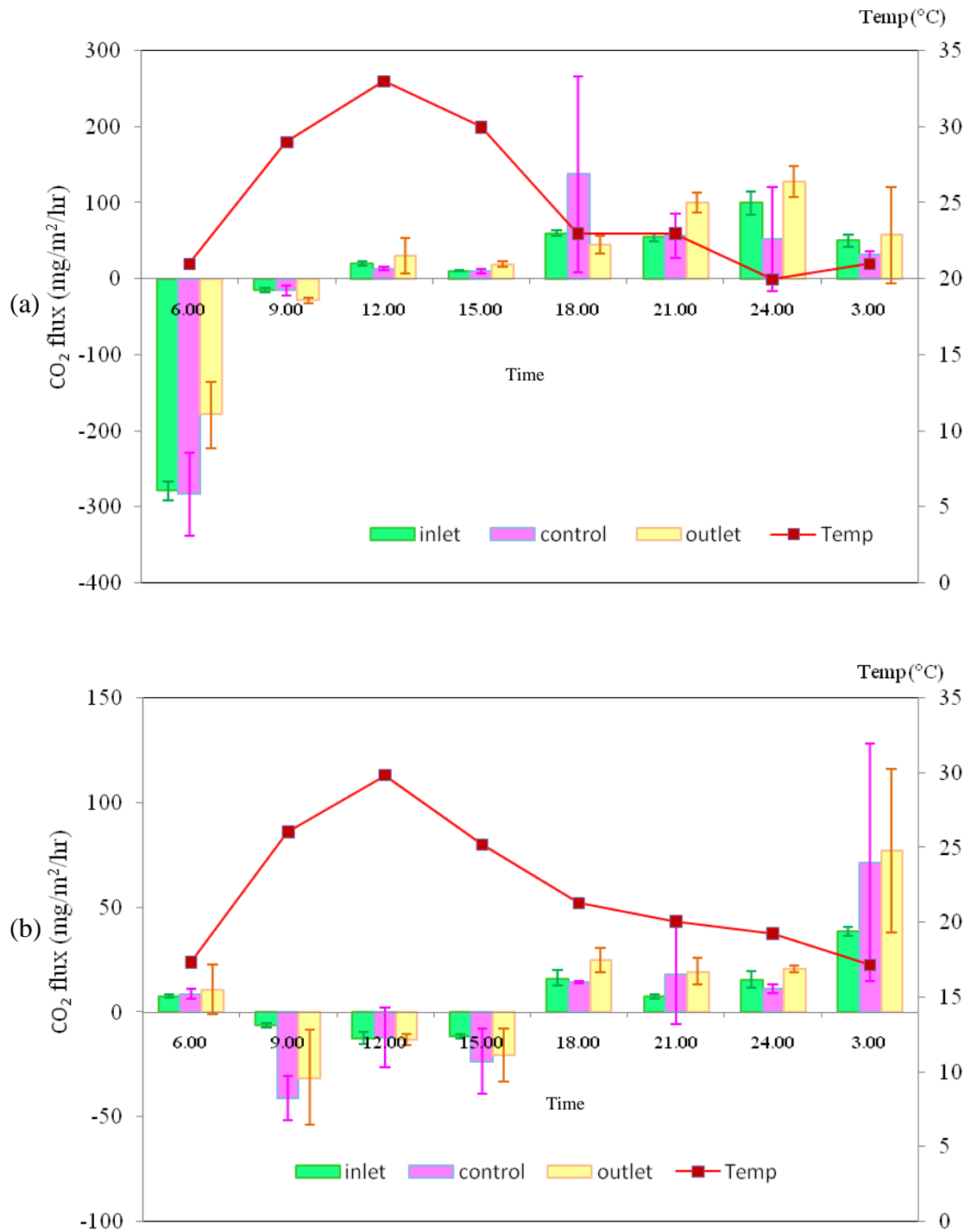
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.20** Diurnal variation of carbon dioxide and soil temperature of subsurface flow constructed wetland during (a) 28-29 November 2010 and (b) 27-28 March 2011.
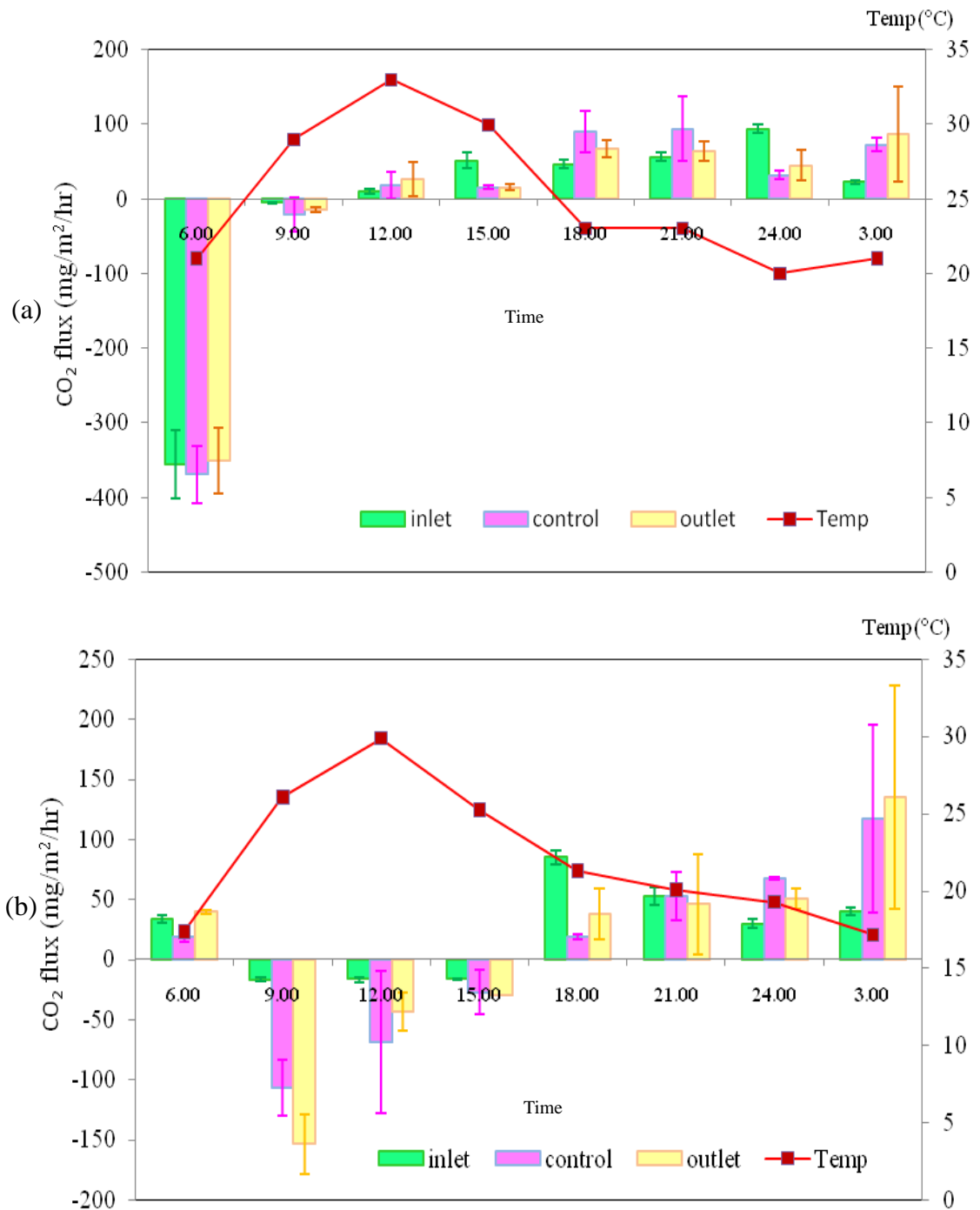
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.21** Diurnal variation of $CO_2$ and soil temperature of FWS constructed wetlands with *Phragmites* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.

In FWS constructed wetlands planted with *Canna* sp., carbon dioxide fluxes had difference diurnal variation between two experimental periods. Carbon dioxide fluxes showed positive values during 15:00-03:00 period and maximum value occurred at 21:00 period during on 28-29 November 2010. Negative fluxes occurred during 06:00-12:00 period and minimum value occurred at 06:00 period. Maximum daily rate was 119.1 mg/m$^2$/hr whereas daily minimum rate was -168.3 mg/m$^2$/hr (Figure 4.23a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive value during 18:00-03:00 period and maximum value occurred at 21:00 period. Negative fluxes occurred during 06:00-15:00 period and minimum value occurred at 09:00 period. Maximum daily rate was 138.4 mg/m$^2$/hr whereas minimum daily rate was -145.9 mg/m$^2$/hr (Figure 4.23b).

In FWS constructed wetlands planted with *Cyperus* sp., carbon dioxide fluxes had difference diurnal variation between two experimental periods. Carbon dioxide fluxes showed positive values during 15:00-03:00 periods and maximum value occurred at 21:00 period during 28-29 November 2010. Negative fluxes occurred during 06:00-12:00 period and minimum value occurred at 06:00 period. Maximum daily rate was 98 mg/m$^2$/hr where as minimum daily rate was -85.1 mg/m$^2$/hr (Figure 4.24a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive values during 18:00-03:00 period and maximum value occurred at 18:00 period. Negative fluxes occurred during 06:00-15:00 period and minimum value occurred at 06:00 period. Maximum daily rate was 135.9 mg/m$^2$/hr, whereas minimum daily rate was -118.7 mg/m$^2$/hr (Figure 4.24b).
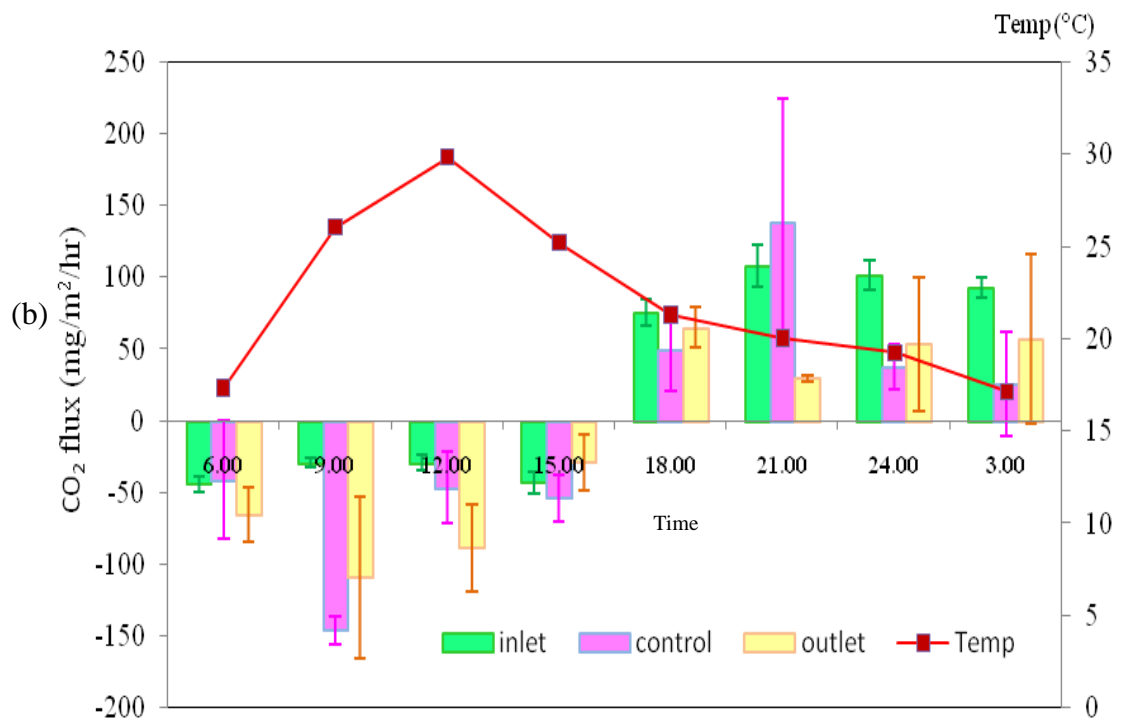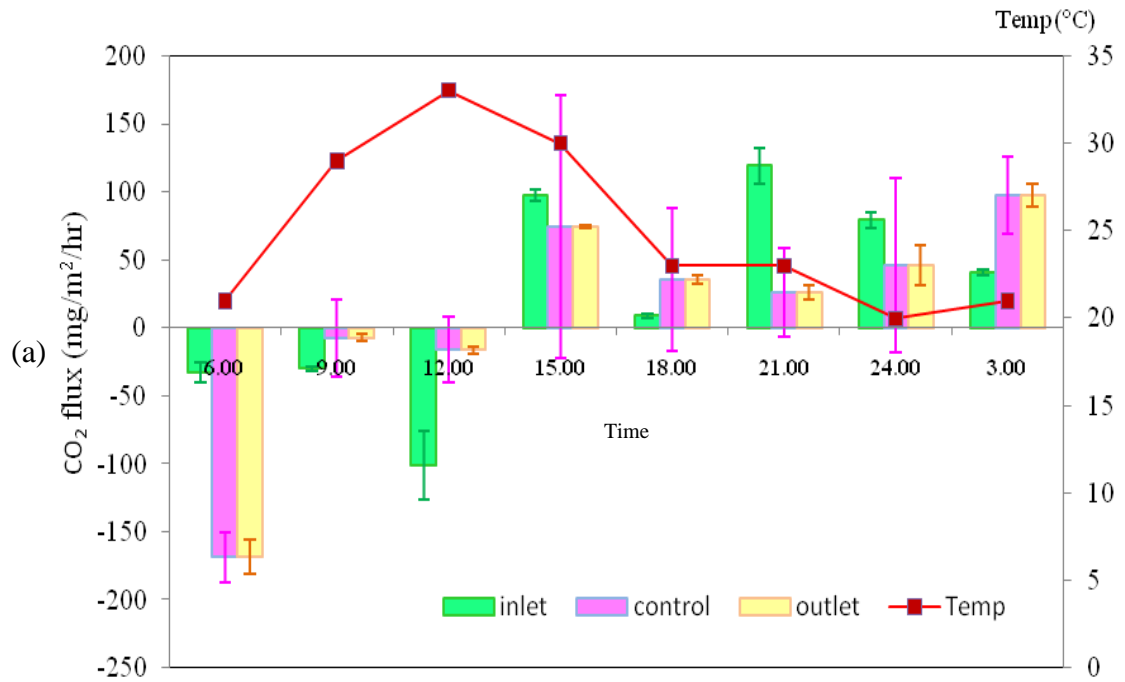
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.22** Diurnal variation of $CO_2$ and soil temperature of FWS constructed wetlands with *Canna* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.
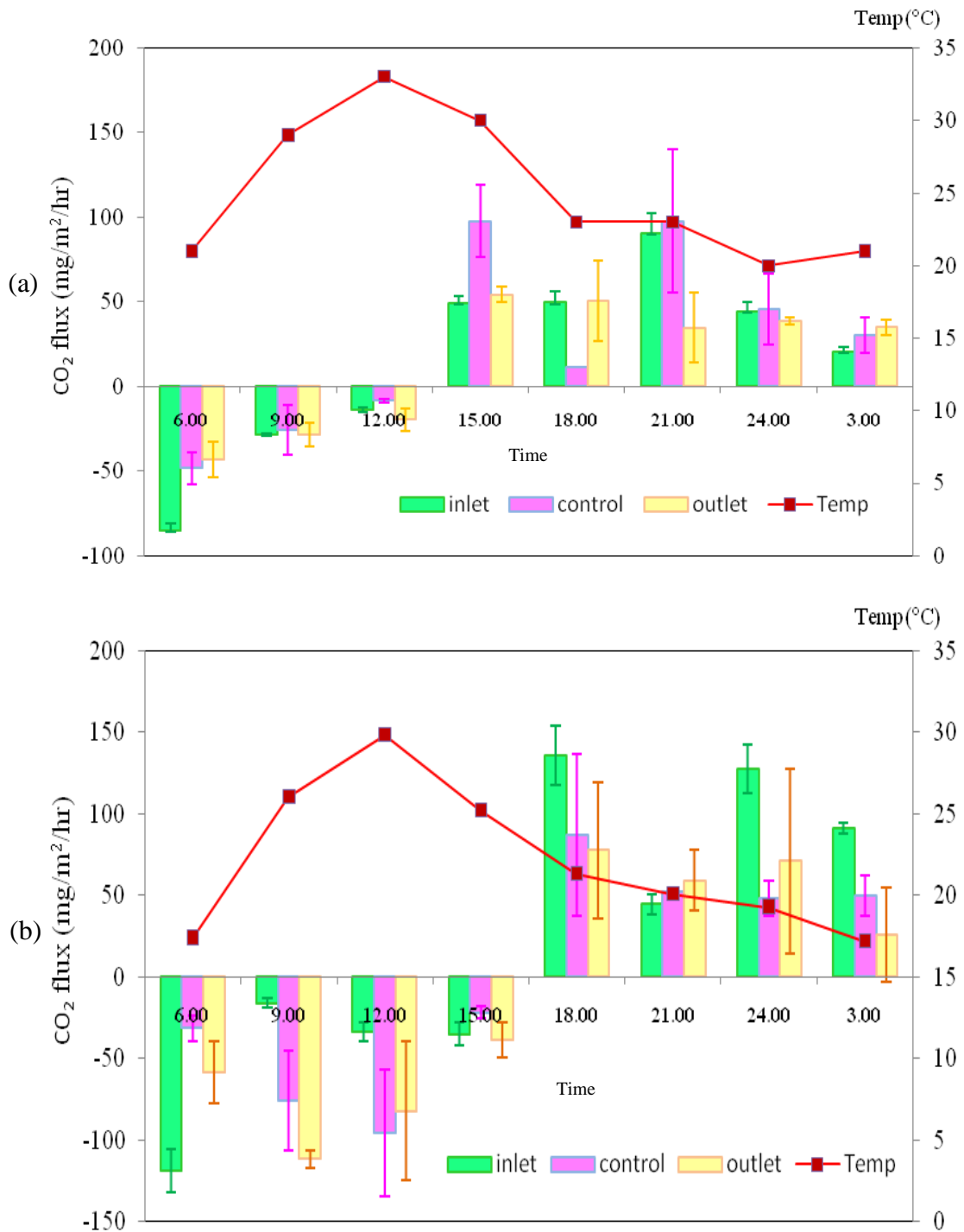
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.23** Diurnal variation of $CO_2$ and soil temperature of FWS constructed wetlands with *Cyperus* sp. during (a) 28-29 November 2010 and (b) 27-28 March 2011.

In SF beds with non-plants or control units, carbon dioxide fluxes showed difference diurnal variation between two experimental periods. During 28-29 November 2010, carbon dioxide fluxes showed positive values during 12:00-03:00 period and maximum value occurred at 24:00 period. Negative fluxes occurred during 06:00-09:00 period and minimum value occurred at 06:00 period. Maximum daily rate was 85.8 $mg/m^2/hr$, whereas minimum daily rate was -97.0 $mg/m^2/hr$ (Figure 4.26a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive values during 18:00-06:00 period and maximum value occurred at 03:00 period. Negative fluxes occurred during 09:00-15:00 periods and minimum value occurred at 12:00 period. Maximum daily rate was 59.78 $mg/m^2/hr$, whereas minimum daily rate was -31.8 $mg/m^2/hr$ (Figure 4.26b).

In FWS beds with non-plants or control units, carbon dioxide fluxes had difference diurnal variation between two experimental periods. During 28-29 November 2010, carbon dioxide fluxes showed positive values at all periods. Maximum carbon dioxide flux occurred at 15:00 period with 81.6 $mg/m^2/hr$. Minimum carbon dioxide flux occurred at 12:00 period with 3.22 $mg/m^2/hr$ (Figure 4.27a). For the second experiment on 27-28 March 2011, carbon dioxide fluxes showed positive values during 18:00-03:00 period and maximum value occurred at 21:00 period. Negative fluxes occurred during 06:00-15:00 period and minimum value occurred at 06:00 period. Maximum daily rate was 135.9 $mg/m^2/hr$, whereas minimum daily rate was -118.7 $mg/m^2/hr$ (Figure 4.27b).
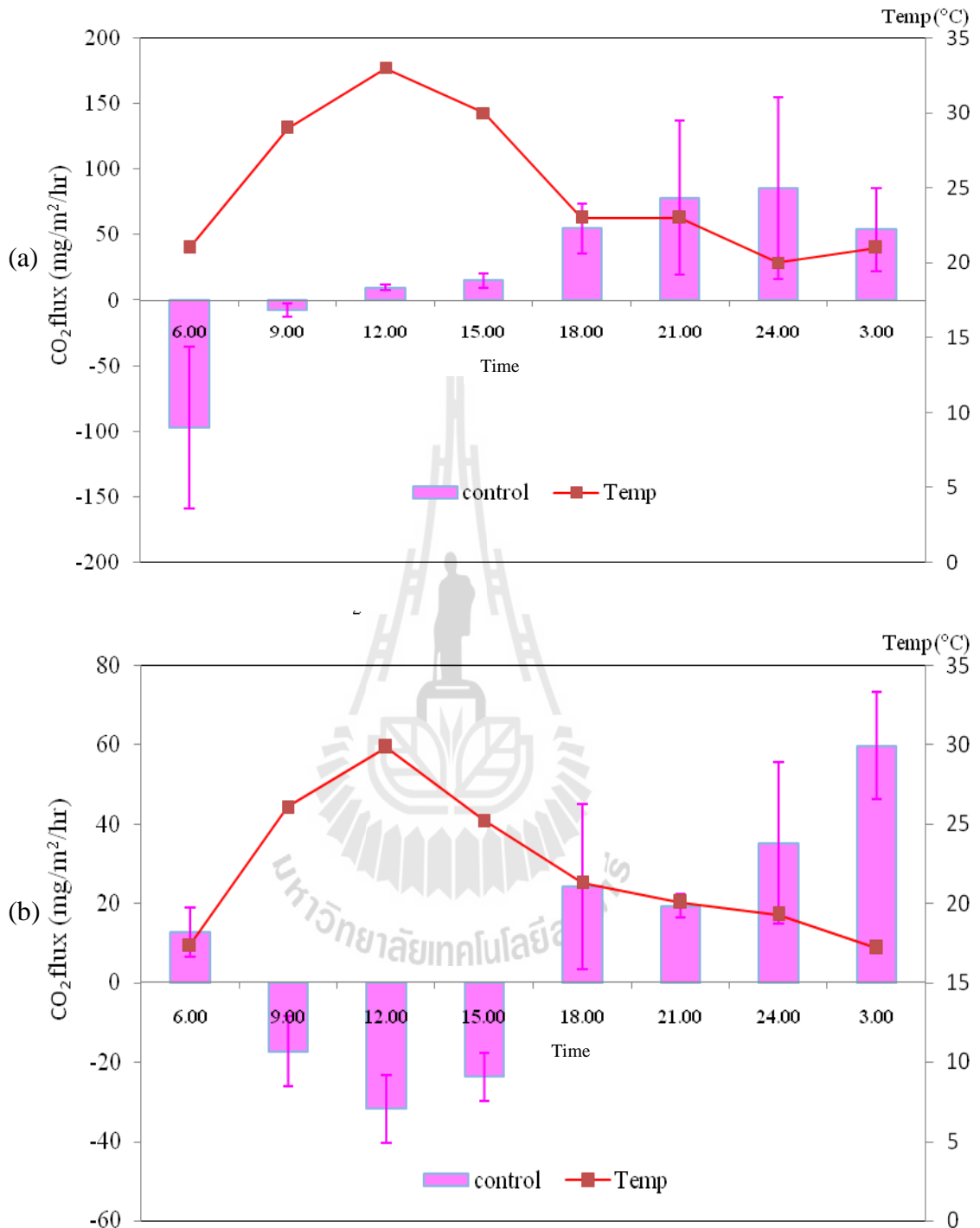
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.24** Diurnal variation of $CO_2$ and soil temperature at SF beds with non-plants
(control unit) during (a) 28-29 November 2010 and (b) 27-28 March 2011.
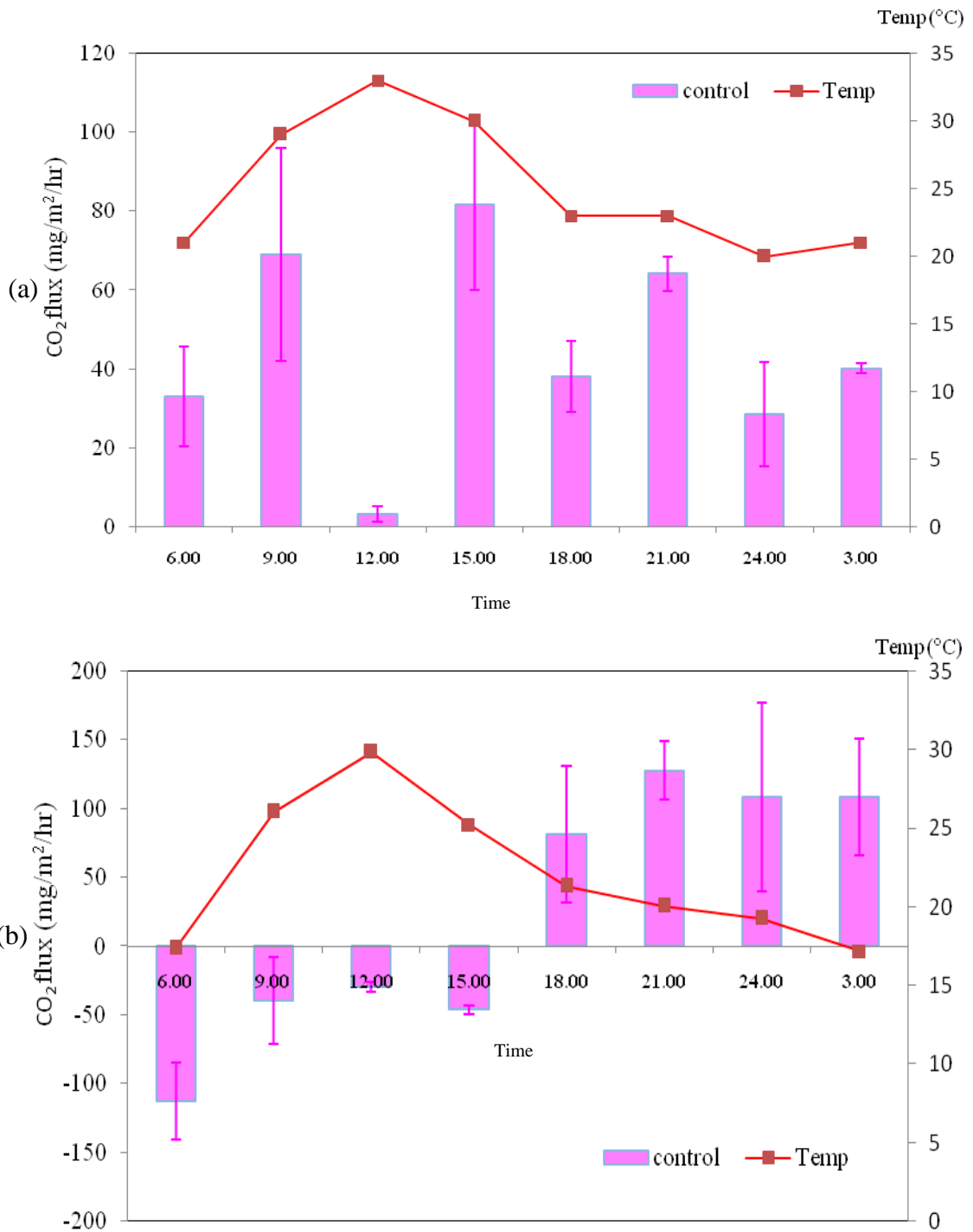
Note: The negative values indicate consumption of carbon dioxide

**Figure 4.25** Diurnal variation of $CO_2$ and soil temperature of FWS beds with non-plants (control unit) during (a) 28-29 November 2010 and (b) 27-28 March 2011.

In control chambers with no plant, the measurements were the rate of soil respiration. The results revealed that carbon dioxide was unstable and had relatively higher value comparing to other points. However, carbon dioxide fluxes did show a pattern of diurnal variation. Negative fluxes usually occurred during daytime and positive fluxes occurred during nighttime.

When compare diurnal flux of carbon dioxide between two experiments on 28-29 November 2010 and 27-28 March 2011, the results showed that carbon dioxide fluxes during late March 2011 experiment were higher than late November 2010 experiment at almost all constructed wetlands. Daily soil temperature ranged from 21°C to 32°C during 28-29 November 2010 and 17 to 30°C during 27-28 March 2011.

The pattern of carbon dioxide variation was observed but it did not correlate with changing in soil temperature. Average carbon dioxide fluxes between daytime and nighttime are given in Table 4.13. Since these data were neither a normal distribution nor homogeneity of variances, Mann-Whitney U tests are suitable for analysis diurnal variation of carbon dioxide fluxes from different constructed wetlands. Average carbon dioxide fluxes during nighttime were higher than daytime. The results showed that the differences of carbon dioxide fluxes between daytime and night time were statistically significant ($p<0.01$) at all constructed wetlands as shown in Table 4.13. It indicated that carbon dioxide fluxes did show diurnal variation pattern.

**Table 4.13** Comparison of diurnal $CO_2$ flux from different constructed wetlands using Mann-Whitney U Test.

| CW/ Time period | | N | Mean | Std. Deviation | Z | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|---|
| SF-*Cyperus* sp. | Day | 48 | -35.06 | 87.93 | -7.339 | 0.000** |
| | Night | 48 | 50.32 | 46.37 | | |
| | Total | 96 | | | | |
| FWS-*Phragmites* sp. | Day | 48 | -55.94 | 126.24 | -7.430 | 0.000** |
| | Night | 48 | 62.74 | 37.30 | | |
| | Total | 96 | | | | |
| FWS-*Canna* sp. | Day | 48 | -36.77 | 64.69 | -6.822 | 0.000** |
| | Night | 48 | 61.10 | 45.62 | | |
| | Total | 96 | | | | |
| FWS-*Cyperus* sp. | Day | 48 | -34.10 | 54.29 | -7.064 | 0.000** |
| | Night | 48 | 59.17 | 36.54 | | |
| | Total | 96 | | | | |
| SF-Control | Day | 16 | -17.51 | 39.00 | -4.598 | 0.000** |
| | Night | 16 | 51.38 | 38.77 | | |
| | Total | 32 | | | | |
| FWS-Control | Day | 16 | -5.18 | 64.50 | -2.902 | 0.004** |
| | Night | 16 | 74.69 | 58.33 | | |
| | Total | 32 | | | | |

*** significant at the 0.01 level*

### 4.3.6 Soil temperature and on diurnal carbon dioxide flux

Measurements of soil temperature showed that average temperature in the first experiment in late November 2010 was 25.06°C (S.D.=4.31) while the second experiment in late March 2011 was lower, 22.04°C (S.D.=4.54). Statistical test indicated no significant correlations between soil temperature and carbon dioxide fluxes during late November 2010. However, results from statistical test of the second experiment showed that soil temperature and carbon dioxides fluxes were significantly correlated in all types of constructed wetlands (Table 4.14). Therefore, this study did not have clear evidence to conclude that diurnal carbon dioxide fluxes were influence by soil temperature in the constructed wetlands used to treated municipal wastewater.

**Table 4.14** Correlation between $CO_2$ flux (mg/m$^2$/hr) and soil temperature (°C).

| Correlated Dimension (analysis in pair) | Pearson Correlation | | |
|---|---|---|---|
| | 1$^{st}$ experiment | 2$^{nd}$ experiment | Total |
| SF *Cyperus* sp. | 0.08 | -0.68** | 0.11 |
| FWS *Phragmites* sp. | 0.02 | -0.72** | 0.17 |
| FWS *Canna* sp. | -0.07 | -0.52** | -0.31** |
| FWS *Cyperus* sp. | -0.01 | -0.48** | -0.30** |

*\* Correlation is significant at the 0.05 level (2-tailed).*

*\*\* Correlation is significant at the 0.01 level (2-tailed).*

## 4.4 Seasonal variation of greenhouse gas fluxes and environmental factors

The study on seasonal variation of greenhouse gas fluxes emitted from different constructed wetlands was performed monthly from June 2010 to May 2011. Measurements of greenhouse gas fluxes were conducted between 09:00 and 15:00 periods. Gas fluxes data were analyzed seasonally. Northeastern Thailand has three-season monsoonal climate, with a relatively cool dry season from November to late February, followed by a hot dry season from March to May and then a hot rainy season from June to October.

### 4.4.1 Seasonal variation of environmental factors in constructed wetlands

Seasonal variations were observed from atmospheric temperature differences at study site. Monthly average of air temperature was maximum in September 2011 (36.7°C) while the lowest mean was found in March (24.7°C) (Figure 26a). There were statistically significant of air temperatures differences among three seasons (p<0.01). The highest air temperature occurred during hot rainy season (July-October) with the average of 33.1±3.9°C and lowered found during cool season (November-February) with the average of 30.9±4.6°C. However, the lower air temperature in March was unusual but it occurred in March 2011 during the experiment. Lower temperature in supposed to be summer months (mid-March-May) had the average of 28.6±3.1°C (Table 4.15).

**Table 4.15** Comparison of seasonal variation of environmental factors in constructed wetlands using Kruskal Wallis test.

| Factor/ Season | N | Mean | S.D. | Min | Max | $\chi^2$ | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|---|---|
| Air Temp. (°C) | | | | | | | |
| - Hot rainy | 30 | 33.10 | 3.91 | 24.3 | 37.7 | 15.59 | 0.001** |
| - Cold | 24 | 30.97 | 4.58 | 24.6 | 41.4 | | |
| - Summer | 18 | 28.59 | 3.12 | 23.6 | 33.4 | | |
| Soil Temp. | | | | | | | |
| - Hot rainy | 30 | 26.80 | 0.52 | 25.8 | 27.5 | 0.82 | 0.663 |
| - Cold | 24 | 25.63 | 3.54 | 19.3 | 30.4 | | |
| - Summer | 18 | 25.87 | 2.68 | 21.5 | 28.6 | | |
| Soil pH | | | | | | | |
| - Hot rainy | 30 | 7.27 | 0.45 | 6.1 | 7.8 | 0.44 | 0.801 |
| - Cold | 24 | 7.43 | 0.12 | 7.2 | 7.6 | | |
| - Summer | 18 | 7.38 | 0.16 | 7.0 | 7.6 | | |
| Soil ORP | | | | | | | |
| -Hot rainy | 30 | -227.22 | 24.57 | -248.5 | -174.0 | 20.19 | 0.001** |
| - Cold | 24 | -221.01 | 28.76 | -257.6 | -176.8 | | |
| - Summer | 18 | -172.57 | 46.28 | -237.3 | -124.6 | | |
| Water Temp. | | | | | | | |
| - Hot rainy | 30 | 26.71 | 0.76 | 25.3 | 27.6 | 1.83 | 0.401 |
| - Cold | 24 | 25.51 | 3.98 | 19.1 | 30.0 | | |
| - Summer | 18 | 25.66 | 3.23 | 21.0 | 28.5 | | |
| Water pH | | | | | | | |
| - Hot rainy | 30 | 7.34 | 0.67 | 6.1 | 8.3 | 2.43 | 0.297 |
| - Cool | 24 | 7.65 | 0.20 | 7.4 | 8.0 | | |
| - Summer | 18 | 7.68 | 0.26 | 7.2 | 8.0 | | |

*** significant at the 0.01 level*

Soil temperatures at all constructed wetlands were measured at 5 cm depth. The graphic shown in Figure 26b illustrates that soil temperatures increased during hot rainy season, peaked in September 2010. Then, soil temperatures sharply decreased during cool season and reached its minimum value in January followed by March before it increased again in summer season. However, there was no statistically significant in the soil temperature among three seasons ($p>0.05$).

Likewise, there were no statistically significantly differences of soil pH across the seasons ($p>0.05$, Figure 27a), with relatively narrow range, 7.27 (S.D.=0.45) -7.43 (S.D.=0.12). However, soil pH during cold season (November-February) was higher than that measured in hot rainy season (July-October) and summer season (March-May).

There were significant differences in soil oxidation-reduction potential (ORP) across the seasons ($p<0.01$, Figure 27b). The lowest soil ORP occurred during hot rainy season (July-October) with the average of $-227.2\pm24.6$ mV whereas the highest soil ORP occurred during summer season (mid March-May) with the average of $-172.57\pm46.3$ mV (Table 4.15).

In addition, water temperature and water pH were measured in FWS constructed wetland to observe any variation. There were no significant differences in water temperature and water pH in three seasons ($p<0.05$, Figures 28a and 28b). Water temperature during hot rainy season (July-October) was higher than that measured in cold season (November-February) and summer season (March-May). For water pH, the lowest value occurred during hot rainy season (July-October) with the average of $7.4\pm0.7$ whereas highest water pH occurred during summer season (March-May) with the average of $7.7\pm0.2$.
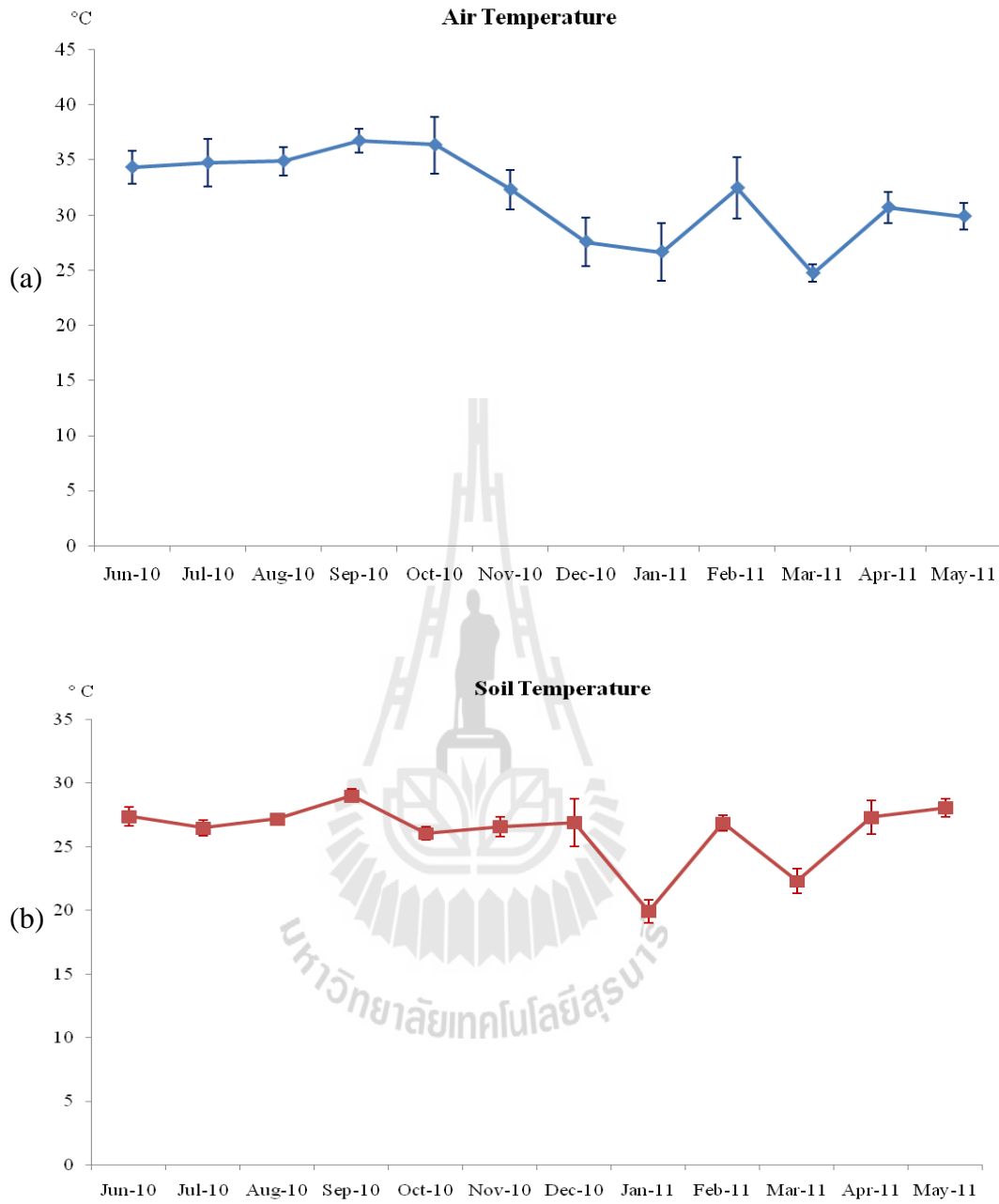
**Figure 4.26** Seasonal variations of environmental factors in constructed wetlands (a) air temperature and (b) soil temperature.
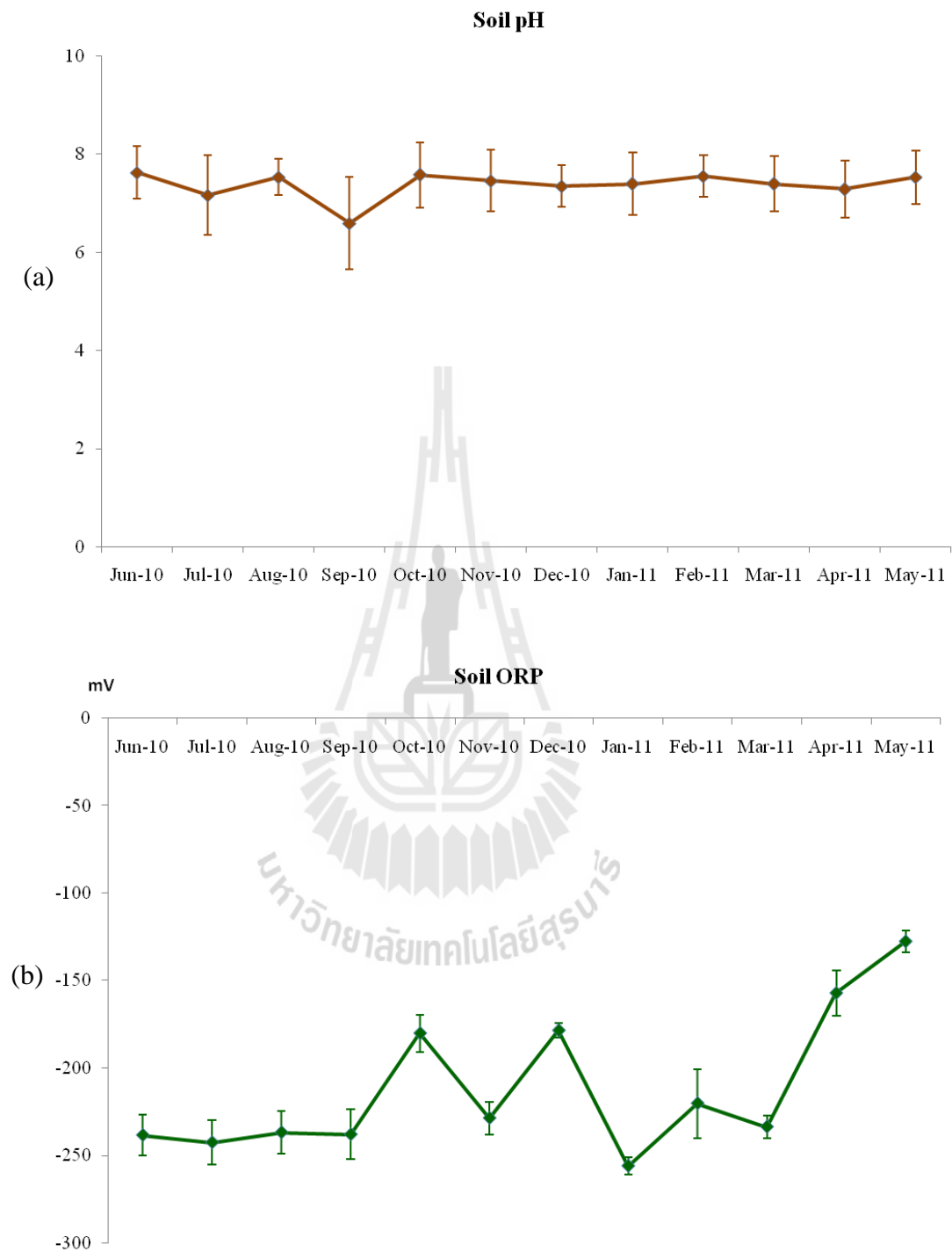
**Figure 4.27** Seasonal variations of environmental factors in constructed wetlands

(a) soil pH and (b) soil ORP.
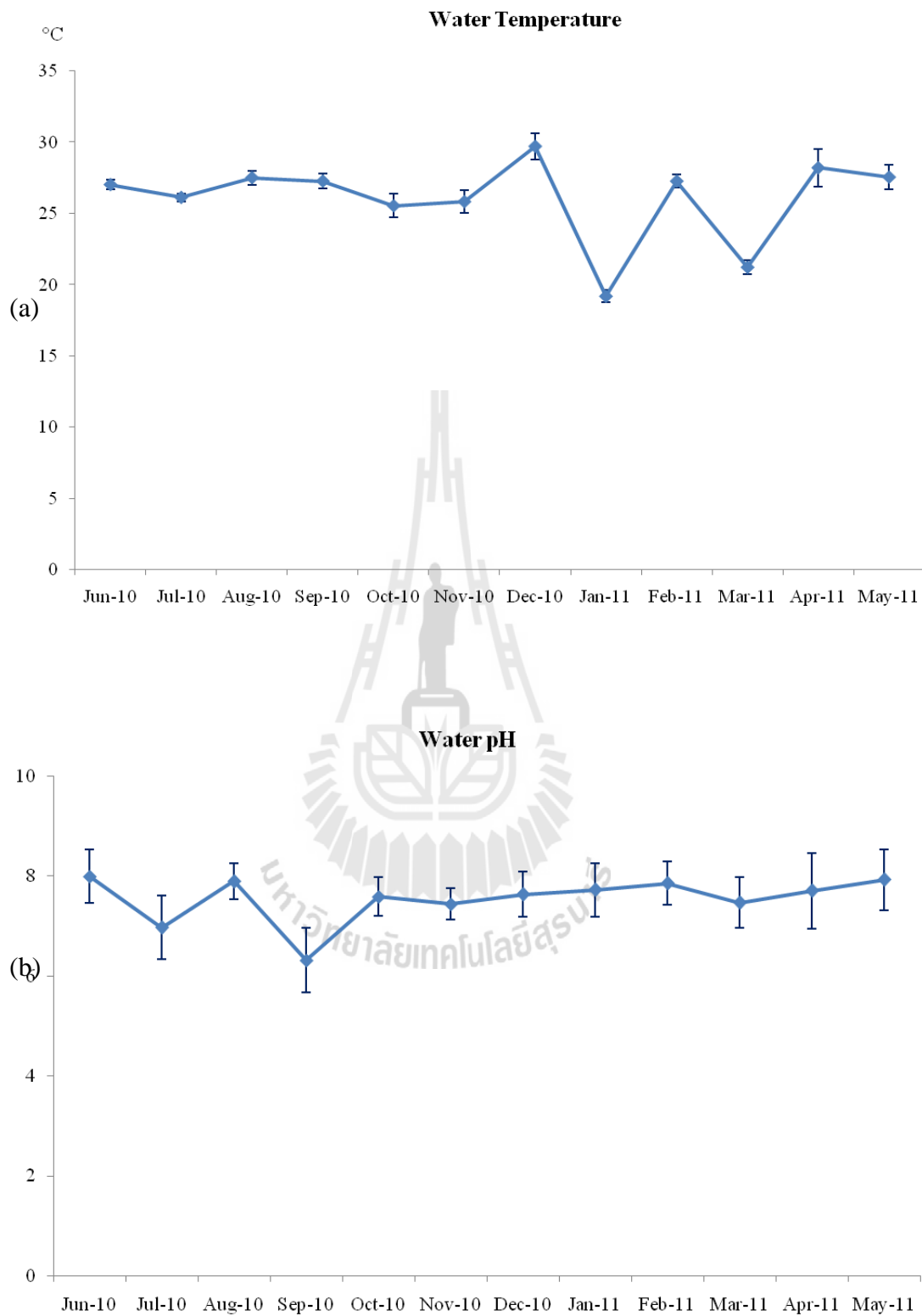
**Figure 4.28** Seasonal variations of environmental factors in constructed wetlands

(a) water temperature and (b) water pH.

### 4.4.2 Seasonal methane flux from different constructed wetlands

#### 4.4.2.1 Subsurface flow constructed wetlands (SF) planted with *Cyperus* sp.

Average methane fluxes from SF constructed wetlands planted with *Cyperus* sp. was in the range of 0.8-10.5 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during August and October were noticeable higher than those in other months. The highest and lowest methane fluxes occurred in August and May, respectively (Figure 4.29a). When seasonal variation was taken into account, there were significant differences in the methane fluxes (p<0.01). The methane flux was highest in hot rainy season (July-October) with the average of 5.2±4.5 mg/m$^2$/hr, followed by cold season (November-February) with the average of 1.5±1.2 mg/m$^2$/hr and lowest in summer season (March-May) with the average of 1.0±0.5 mg/m$^2$/hr (Table 4.16). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in August (hot rainy season) and the lowest methane flux occurred in May (summer season) as shown in Figure 4.29b.
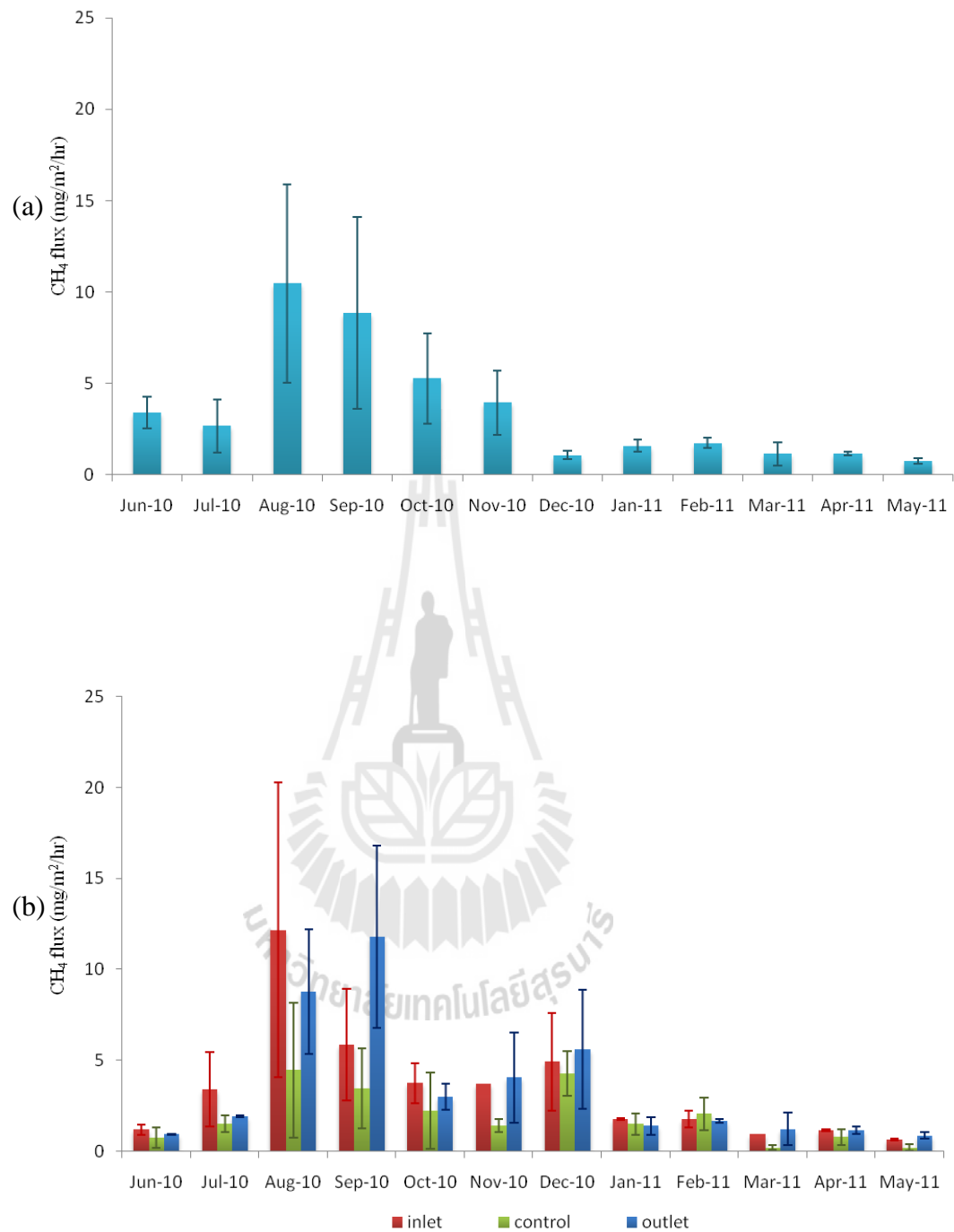
**Figure 4.29** Seasonal variation of methane fluxes in SF constructed wetland planed with *Cyperus* sp. (a) average fluxes from inlets and outlets (b) monthly fluxes from different points.

**4.4.2.2 Free water surface (FWS) constructed wetlands planted with *Phragmites* sp.**

Average methane fluxes from FWS constructed wetlands planted with *Phragmites* sp. was in the range of 1.8-54.1 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April to November were considerably higher than those in other months. The highest and lowest methane fluxes occurred in September and December, respectively (Figure 4.30a). When seasonal variation was taken into account, there were significant differences in the methane fluxes (p<0.01). The methane flux was highest in hot rainy season (July-October) with the average of 19.9±21.2 mg/m$^2$/hr, followed by summer season (March-May) with the average of 6.3±6.4 mg/m$^2$/hr and lowest in cold season (November-February) with the average of 3.9±5.3 mg/m$^2$/hr (Table 4.16). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in September (hot rainy season) and the lowest methane flux occurred in December (cold season) as shown in Figure 4.30b.
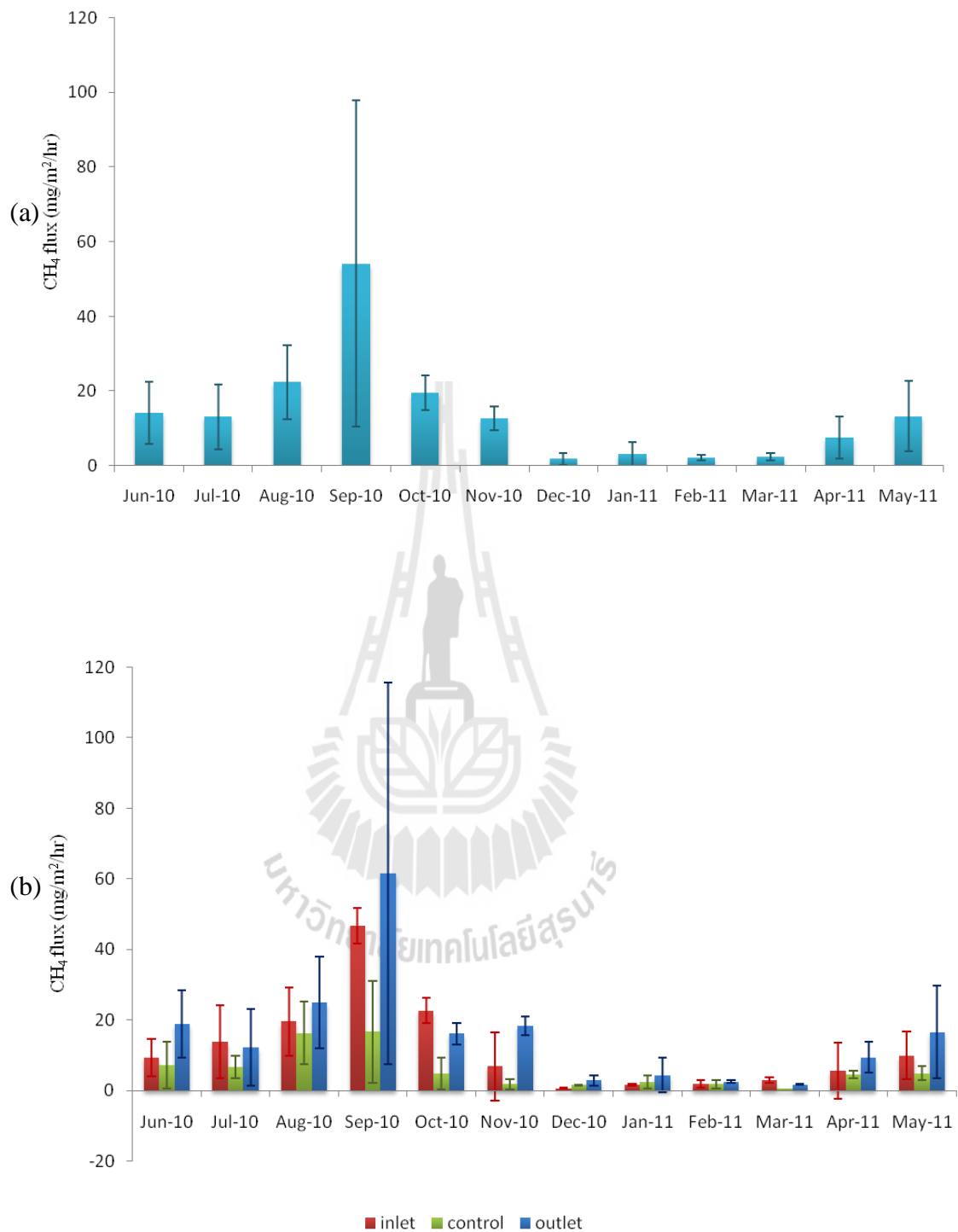
**Figure 4.30** Seasonal variation of methane fluxes in FWS constructed wetlands with *Phragmites* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

### 4.4.2.3 Free water surface (FWS) constructed wetlands planted with *Canna* sp.

Average methane fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 1.2-16.1 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during May to November were considerably higher than those in other months. The highest and lowest methane fluxes occurred in September and April, respectively (Figure 4.31a). When seasonal variation was taken into account, there were significant differences in the methane fluxes ($p<0.01$). The methane flux was highest in hot rainy season (July-October) with the average of 10.3±7.7 mg/m$^2$/hr, followed by summer season (March-May) with the average of 3.7±4.7 mg/m$^2$/hr, and lowest in cold season (November-February) with the average of 2.4±2.5 mg/m$^2$/hr (Table 4.16). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in September (hot rainy season) and the lowest methane flux occurred in April (summer season) as shown in Figure 4.31b.
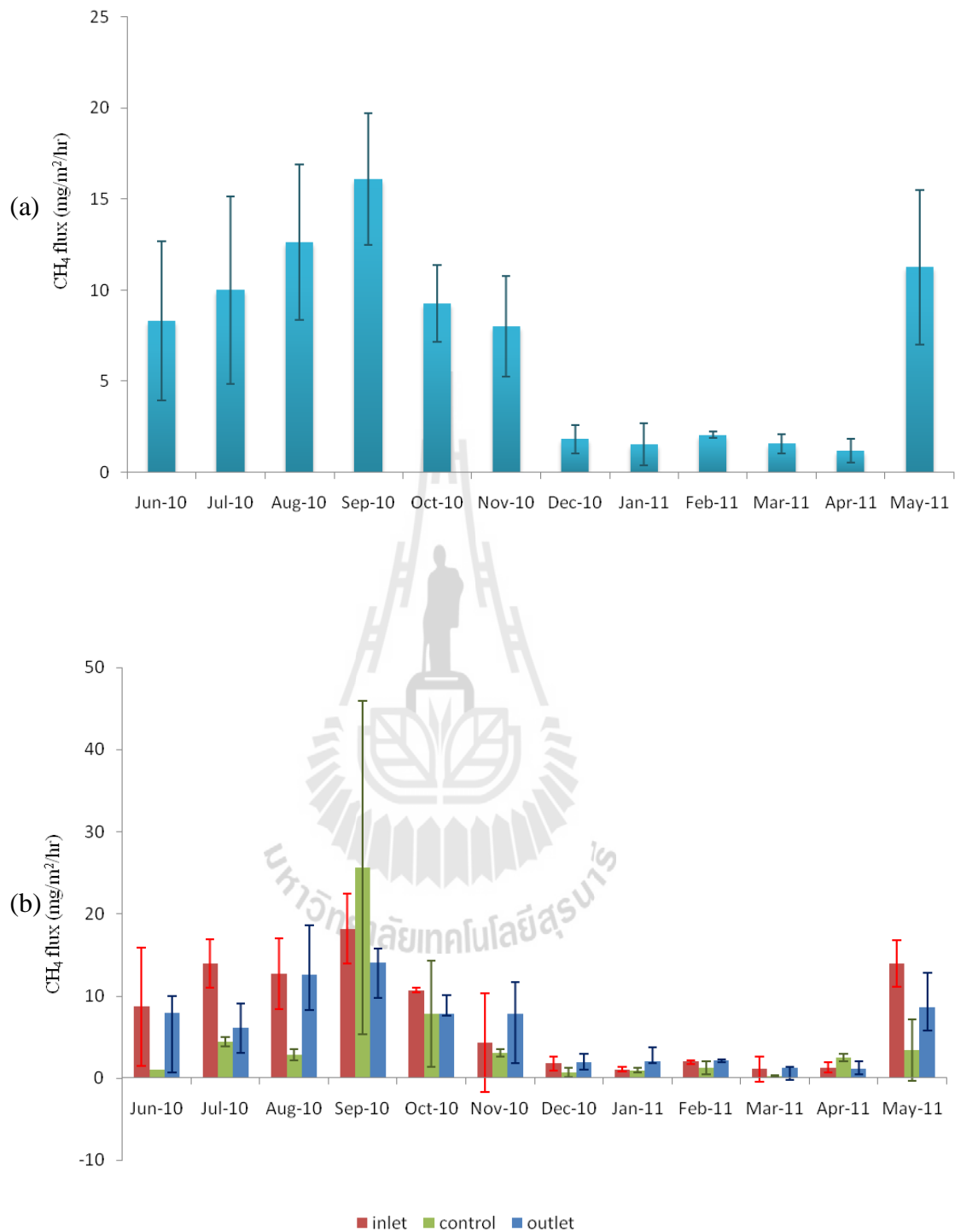
**Figure 4.31** Seasonal variation of methane fluxes in FWS constructed wetland planted with *Canna* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.2.4 Free water surface (FWS) constructed wetlands planted with *Cyperus* sp.**

Average methane fluxes from FWS constructed wetlands planted with *Cyperus* sp. was in the range of 1.0-15.3 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during June and August to November were considerably higher than those in other months. The highest and lowest methane fluxes occurred in October and December, respectively (Figure 4.32a). When seasonal variation was taken into account, there were significant differences in the methane fluxes ($p<0.01$). The methane flux was highest in hot rainy season (July-October) with the average of 10.8±13.5 mg/m$^2$/hr, followed by cold season (November-February) with the average of 2.6±3.1 mg/m$^2$/hr and lowest in summer season (March-May) with the average of 2.1±1.4 mg/m$^2$/hr (Table 4.16). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in September (hot rainy season) and the lowest methane flux occurred in December (cold season) as shown in Figure 4.32b.

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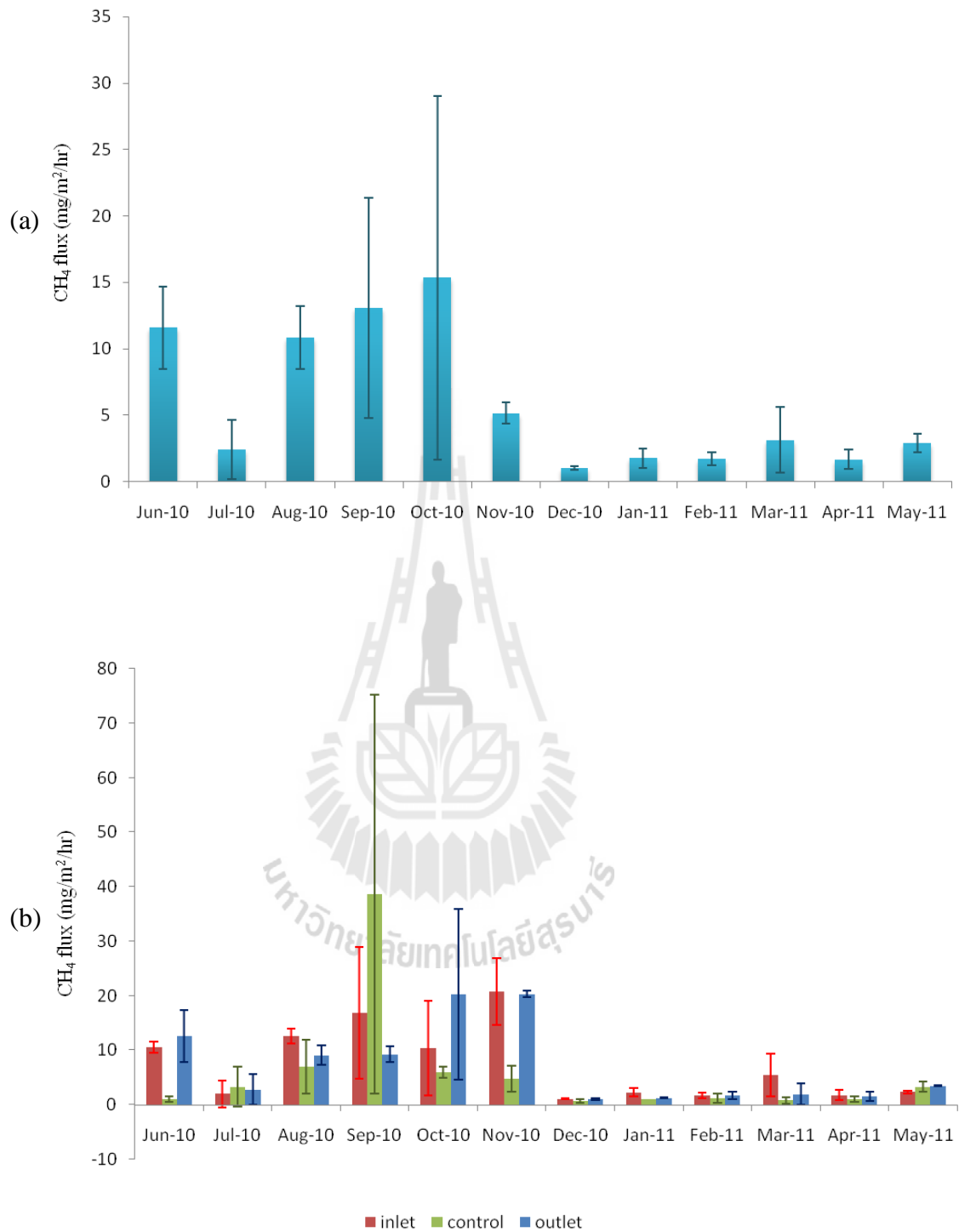
**Figure 4.32** Seasonal variation of methane fluxes at FWS constructed wetland planted with *Cyperus* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

### 4.4.2.5 Subsureface flow (SF) beds with non-plants (control units)

Average methane fluxes from SF constructed wetlands with non-plant varied within the range of 0.5-4.3 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during May and September were higher than those in other months. The highest and lowest methane fluxes occurred in September and March, respectively (Figure 4.33a). When seasonal variation was taken into account, there were significant differences in the methane fluxes ($p<0.01$). The methane flux was highest in hot rainy season (July-October) with the average of $5.2 \pm 4.5$ mg/m$^2$/hr, followed by cold season (November-February) with the average of $1.5 \pm 1.2$ mg/m$^2$/hr and lowest in summer season (March-May) with the average of $1.0 \pm 0.5$ mg/m$^2$/hr (Table 4.14). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in September (hot rainy season) and the lowest methane flux occurred in March (cold temperature month in 2011) as shown in Figure 4.33b.
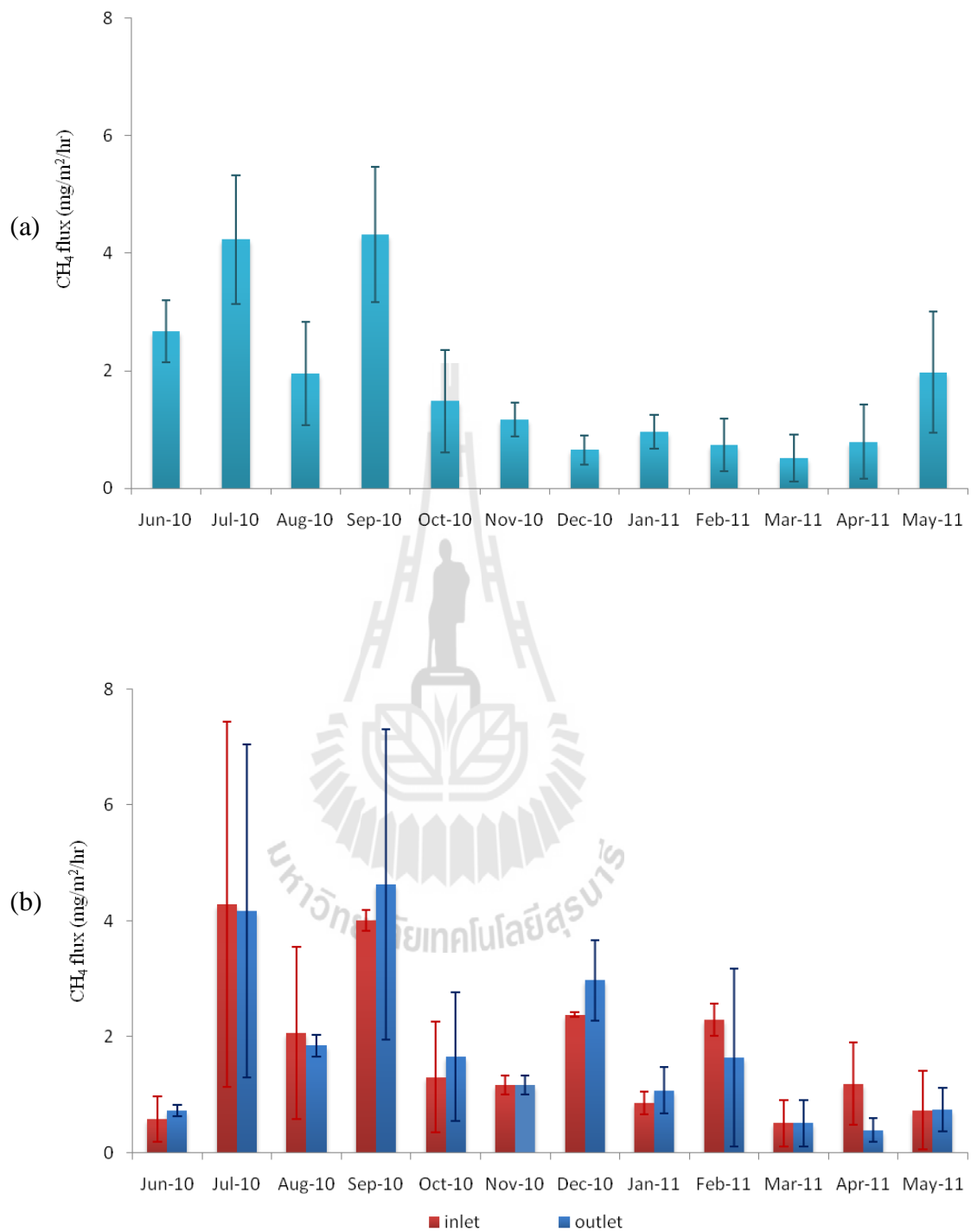
**Figure 4.33** Seasonal variation of methane fluxes at SF beds with non-plant (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

### 4.4.2.6 Free water surface flow (FWS) beds with non-plants (control units)

Average methane fluxes from FWS constructed wetlands with non-plant varied within the range of 1.1-36.0 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during May and October were higher than those in other months. The highest and lowest methane fluxes occurred in September and December, respectively (Figure 4.34a). When seasonal variation was taken into account, there were significant differences in the methane fluxes (p<0.01). The methane flux was highest in hot rainy season (July-October) with the average of 23.5±18.7 mg/m$^2$/hr, followed by summer season (March-May) with the average of 4.9±5.3 mg/m$^2$/hr and lowest in cold season (November-February) with the average of 2.0±1.4 mg/m$^2$/hr (Table 4.16). However, similar patterns of seasonal variations were observed at all measurement locations (inlet, outlet and control) within the plot constructed wetlands. The highest methane flux occurred in September (hot rainy season) and the lowest methane flux occurred in December (cold season) as shown in Figure 4.34b.
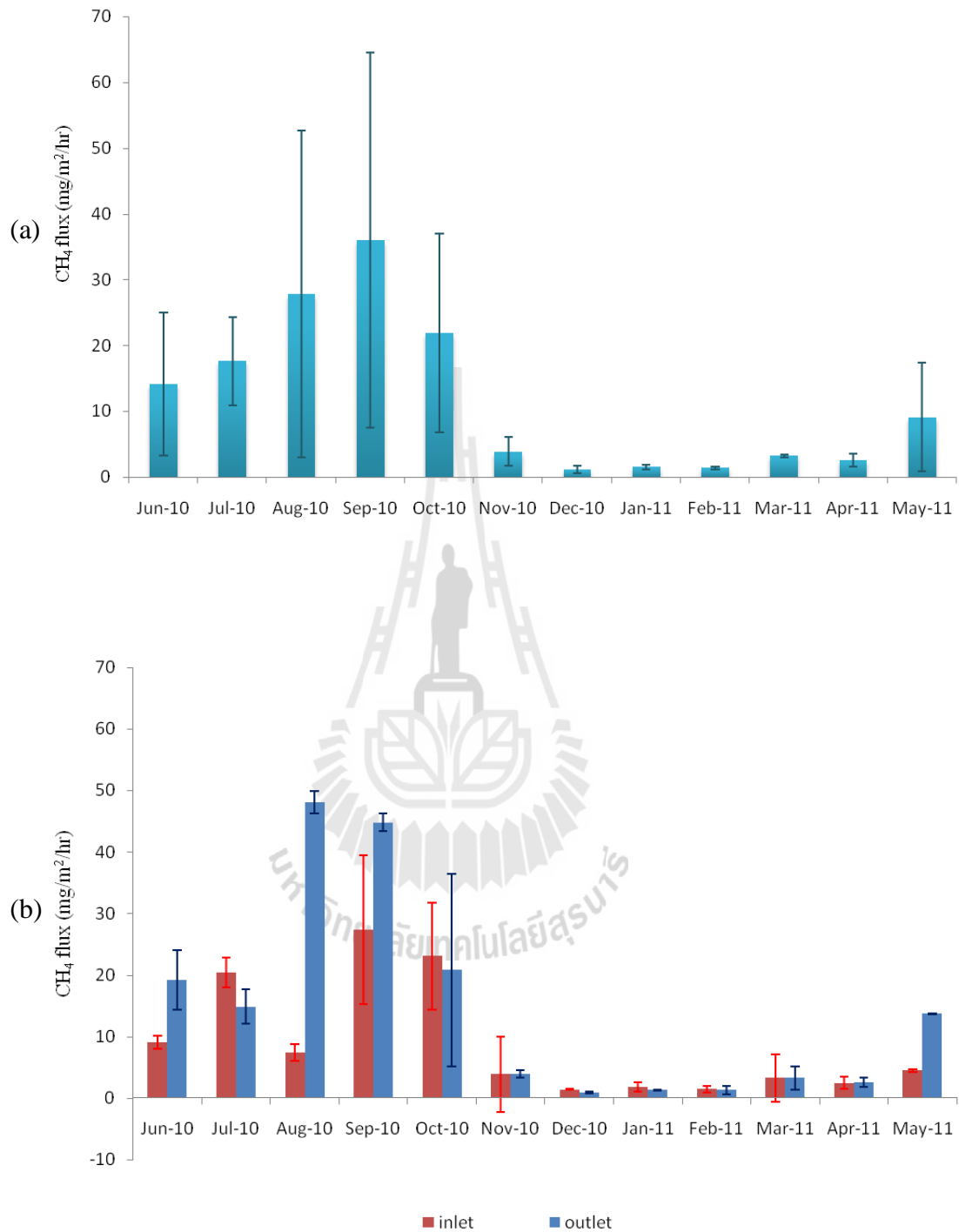
**Figure 4.34** Seasonal variation of methane fluxes at FWS beds with non-plant (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

Measurement data clearly demonstrated seasonal variations of methane fluxes across in different constructed wetlands (Table 4.16). The data were neither a normal distribution nor homogeneity of variances. Thus, Chi-Square is suitable for the analysis differences in methane fluxes among three seasons. The results showed that the differences of methane fluxes among three seasons are significant ($p<0.01$) in all constructed wetlands.

### 4.4.2.7 Seasonal variation of methane fluxes and environmental factors

Seasonal methane fluxes were investigated to observe the correlation between the fluxes and environmental factors that were measured during the sampling period, i.e., air, soil and water temperature, soil and water pH, and soil oxidation-reduction potential (ORP).

**Temperature**

Air, soil and water temperatures were corresponded to seasonal differences during the study period. Average air temperatures were highest during hot rainy season and were lowest during summer season in all constructed wetlands (Table 4.17).

Air temperatures correlate well with soil temperatures because both are determined by the energy balance at the ground surface. The daily amplitude of soil temperatures at the surface are greater than the daily amplitude of air temperatures for clear days and are less for cloudy days. A depth of 5 cm was selected for soil temperatures because most soil ecosystem processes occur within the top layers of soil (Buringh, 1984; Pritchett and Fisher, 1987).

**Table 4.16** Comparing seasonal variation of methane fluxes using Kruskal Wallis test.

| CW/Season | N | Mean | S.D. | $\chi^2$ | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|
| SF with *Cyperus* sp. | | | | | |
| - Hot rainy | 30 | 5.17 | 4.48 | 38.54 | 0.001** |
| - Cool | 24 | 1.48 | 1.22 | | |
| - Summer | 18 | 0.96 | 0.55 | | |
| FWS with *Phragmites* sp. | | | | | |
| - Hot rainy | 30 | 19.88 | 21.23 | 29.44 | 0.001** |
| - Cool | 24 | 3.92 | 5.32 | | |
| - Summer | 18 | 6.29 | 6.42 | | |
| FWS with *Canna* sp. | | | | | |
| - Hot rainy | 30 | 10.29 | 7.67 | 27.38 | 0.001** |
| - Cool | 24 | 2.40 | 2.48 | | |
| - Summer | 18 | 3.71 | 4.72 | | |
| FWS with *Cyperus* sp. | | | | | |
| -Hot rainy | 30 | 10.83 | 13.58 | 18.25 | 0.001** |
| - Cool | 24 | 2.65 | 3.07 | | |
| - Summer | 18 | 2.12 | 1.47 | | |
| SF beds-control | | | | | |
| -Hot rainy | 20 | 2.94 | 2.25 | 14.21 | 0.001** |
| - Cool | 16 | 0.89 | 0.34 | | |
| - Summer | 12 | 1.09 | 0.93 | | |
| FWS beds-control | | | | | |
| -Hot rainy | 20 | 23.50 | 18.74 | 33.99 | 0.001** |
| - Cool | 16 | 1.98 | 1.43 | | |
| - Summer | 12 | 4.95 | 5.32 | | |

*** significant at the 0.01 level*

**Table 4.17** Comparison of average methane fluxes and environmental factors from different constructed wetlands among three seasons.

| CW/ Season | Air Temperature (°C) | Soil Temperature (°C) | Water Temperature (°C) | Soil pH | Water pH | Soil ORP (mV) |
|---|---|---|---|---|---|---|
| SF with *Cyperus* sp. | | | | | | |
| - Hot rainy | 33.10 | 26.55 | NA | 7.91 | NA | -226.64 |
| - Cool | 30.97 | 25.54 | NA | 8.02 | NA | -223.03 |
| - Summer | 28.59 | 25.69 | NA | 7.96 | NA | -176.66 |
| FWS with *Phragmites* sp. | | | | | | |
| - Hot rainy | 33.10 | 27.08 | 27.35 | 6.70 | 7.25 | -229.02 |
| - Cool | 30.97 | 26.25 | 26.33 | 7.09 | 7.85 | -226.59 |
| - Summer | 28.59 | 26.33 | 26.65 | 7.13 | 8.03 | -168.81 |
| FWS with *Canna* sp. | | | | | | |
| - Hot rainy | 33.10 | 26.65 | 26.36 | 6.82 | 7.07 | -221.53 |
| - Cool | 30.97 | 25.20 | 24.94 | 7.04 | 7.32 | -217.44 |
| - Summer | 28.59 | 25.56 | 25.01 | 6.88 | 7.17 | -169.48 |
| FWS with *Cyperus* sp. | | | | | | |
| - Hot rainy | 33.10 | 26.68 | 26.36 | 7.11 | 7.31 | -232.34 |
| - Cool | 30.97 | 25.35 | 25.14 | 7.06 | 7.38 | -215.17 |
| - Summer | 28.59 | 25.47 | 25.02 | 6.94 | 7.27 | -171.01 |
| Average all | | | | | | |
| - Hot rainy | 33.10 | 26.80 | 26.71 | 7.27 | 7.34 | -227.22 |
| - Cool | 30.97 | 25.63 | 25.51 | 7.43 | 7.65 | -221.01 |
| - Summer | 28.59 | 25.87 | 25.66 | 7.38 | 7.68 | -172.75 |

Soil temperatures play an important role in controlling the magnitude of methane production. Low soil temperatures reduce methane production by decreasing the activity of methanogens (Le Meramd Roger, 2001). A large part of the seasonal variation of methane production could be ascribed to change of sediment temperature. Dunfield *et al*. (1993) reported a remarkable dependence of methane production on temperature with the optima between 25 and 30°C and very low production rates at low temperatures.

In this study, soil temperatures were in the optimum range and changed in a narrow-range during three seasons, particularly between cold and summer seasons (Table 4.17). The correlation values among seasonal methane fluxes and environmental factors are illustrated in Table 4.18. For temperature, there were significantly positive correlation ($p<0.05$, $r=0.39$) between air temperatures and methane fluxes, which mean that methane fluxes increase when air temperatures increase. However, change in soil and water temperatures did not relate to seasonal variation of methane fluxes, which may because temperatures of soil and water changed in narrow range, and soils were thought to be favorable for optimum growth of methanogenic bacteria. Therefore, soil temperatures might not responsible for low or high fluxes of methane in different seasons.

**pH**

Methanogens are pH-sensitive microorganisms. Most of methanogens grew over a relatively narrow pH range of about 6-8 and the optimal pH was about 7 (Alexander, 1977; Oremland, 1988). In this study, average soil pH and water pH during the experiments ranged between 6.70-8.02 and 7.07-8.03, respectively, indifferent constructed wetlands (Table 4.17). Soil pH remained in the optimum

range. There were significantly negative correlation between soil pH and seasonal variation of methane fluxes (p<0.01, r=0.50, Table 4.17). As a result, methane fluxes increased with decreased soil pH. For water pH, as an indirect influencing factor of seasonal methane flux, there were significantly negative correlation between water pH and seasonal variation of methane fluxes (p<0.01, r=0.56, Table 4.18). Thus, methane fluxes increased with decreased water pH.

### Soil oxidation-reduction potential (ORP)

Soil oxidation-reduction potential (ORP) measures the ability of a soil environment to supply electrons to an oxidizing agent, or to take up electrons from a reducing agent. Methanogenesis occurs only at strict anaerobic conditions. Methane production is closely related to the soil oxidation-reduction status. The thermodynamic energy yield of the oxidation of organic matter coupled to the reduction of various electron acceptors decreases in the order of $O_2>NO_3^->Mn^{+4}>Fe^{+3}>SO_4^{-2}>CO_2$. After submergence of the soil, the small amount of $O_2$ dissolved in the floodwater will be consumed quickly. The need for electron acceptors by facultative anaerobic and true anaerobic organisms results in the reduction of several oxidized components. Reductions of $NO_3^-$ to $NO_2^-$, $Mn^{+4}$ to $Mn^{+2}$, $Fe^{+3}$ to $Fe^{+2}$, $SO_4^{-2}$ to $S^{-2}$ and $CO_2$ to $CH_4$ occur sequentially in the soil according to their thermodynamic principles, as long as an available C sources exists (Wang *et al.*, 1996). The critical soil ORP for initiation of methane production observed was lower than -150 mV (Wang *et al.*, 1993). Lemer and Roger (2001) reported that methanogenesis requires low soil ORP <200 mV. This study found that soil ORP values were consistency low in the range of -168 to -232 mV (Table 4.18). At these levels, soil environment was in favor of methanogenesis, which might result

in higher methane fluxes. However, there were no correlations between soil ORP and seasonal variation of methane fluxes (Table 4.18). Soil ORP might not strongly influence the variations of methane fluxes each season in this experiment. However, was thought to be essential for methanogenesis.

**Table 4.18** Correlation between seasonal methane flux (mg/m$^2$/hr) and temperature (°C).

| Correlated Dimension | Pearson Correlation (r) | | | | | |
|---|---|---|---|---|---|---|
| | Air Temp | Soil Temp | Water Temp | Soil pH | Water pH | Soil ORP |
| SF *Cyperus* sp. | 0.26* | 0.14 | - | -0.09 | - | -0.22 |
| FWS *Phragmites* sp. | 0.33** | 0.10 | 0.16 | -0.26* | -0.46** | -0.15 |
| FWS *Canna* sp. | 0.35** | 0.29* | 0.26* | -0.38** | -0.37** | -0.10 |
| FWS *Cyperus* sp. | 0.27* | 0.16 | 0.11 | -0.24* | -0.40** | -0.18 |
| Average | 0.39** | 0.22 | 0.22 | -0.50** | -0.56** | -0.16 |

*Not: excluded CH$_4$ fluxes from control unit*
*\* Correlation is significant at the 0.05 level (2-tailed).*
*\*\* Correlation is significant at the 0.01 level (2-tailed).*

### 4.4.3 Seasonal nitrous oxide fluxes from different constructed wetlands

#### 4.4.3.1 Subsurface flow constructed wetlands (SF) planted with *Cyperus* sp.

Average nitrous oxide fluxes from SF constructed wetlands planted with *Cyperus* sp. were in the range of 0.3-5.3 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April and October were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in

April and January, respectively (Figure 4.35a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the all seasons ($p < 0.01$). The nitrous oxide flux was highest in summer season (March-May) with average of $2.0 \pm 3.1$ mg/m$^2$/hr, followed by hot rainy season (July-October) with average of $1.0 \pm 0.7$ mg/m$^2$/hr, and lowest in cold season (November-February) with average of $0.4 \pm 0.1$ mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber found the similar pattern of seasonal variation that the highest nitrous oxide flux occurred in April (summer season) and the lowest nitrous oxide flux occurred in January (cold season) as shown in Figure 4.35b.
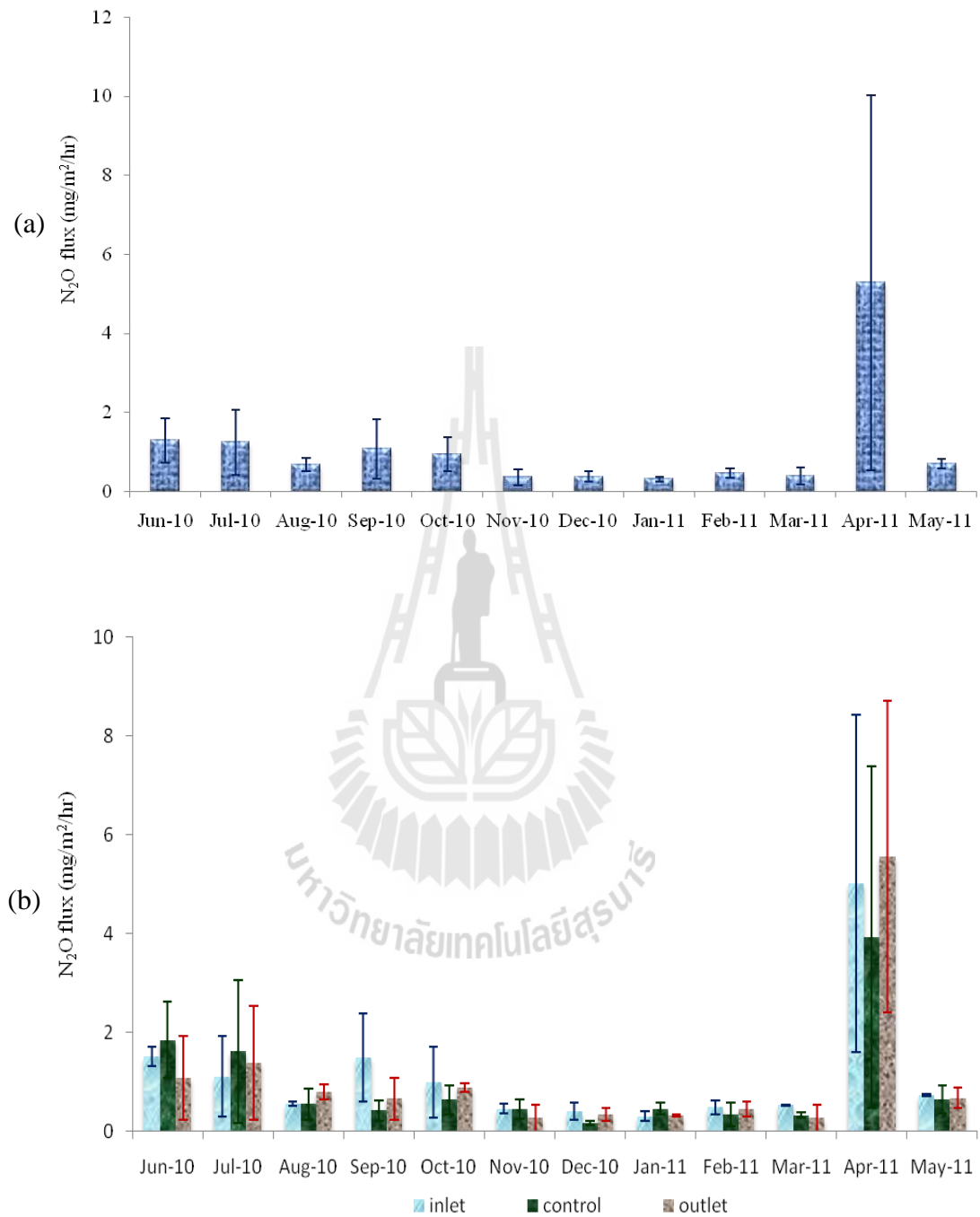
**Figure 4.35** Seasonal variation of $N_2O$ fluxes at SF constructed wetland with *Cyperus* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.3.2 Free water surface (FWS) constructed wetlands planted with *Phragmites* sp.**

Average nitrous oxide fluxes from FWS constructed wetlands planted with *Phragmites* sp. was in the range of 0.3-4.2 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April and June were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in June and January, respectively (Figure 4.36a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the seasons (p<0.01). The nitrous oxide flux was highest in hot rainy season (March-May) with average of 1.3 ± 1.5 mg/m$^2$/hr, followed by summer season (March-May) with average of 0.9 ± 0.9 mg/m$^2$/hr, and lowest in cold season (November-February) with average of 0.3 ± 0.1 mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber found the similar pattern of seasonal variation that the highest nitrous oxide flux occurred in June (hot rainy season) and the lowest nitrous oxide flux occurred in January (cold season) as shown in Figure 4.36b.

**Figure 4.36** Seasonal variation of $N_2O$ fluxes at FWS constructed wetland with *Phragmites* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.3.3 Free water surface (FWS) constructed wetlands planted with *Canna* sp.**

Average nitrous oxide fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 0.2-3.4 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April and October were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in April and November, respectively (Figure 4.37a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the seasons (p<0.01). The nitrous oxide flux was highest in summer season (March-May) with average of 1.42±1.3 mg/m$^2$/hr followed by hot rainy season (July-October) with average of 1.41±1.4 mg/m$^2$/hr, and lowest in cold season (November-February) with average of 0.3±0.1 mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber, the highest nitrous oxide flux occurred in April, September, and June and the lowest nitrous oxide flux occurred in January (cold season) as shown in Figure 4.37b.
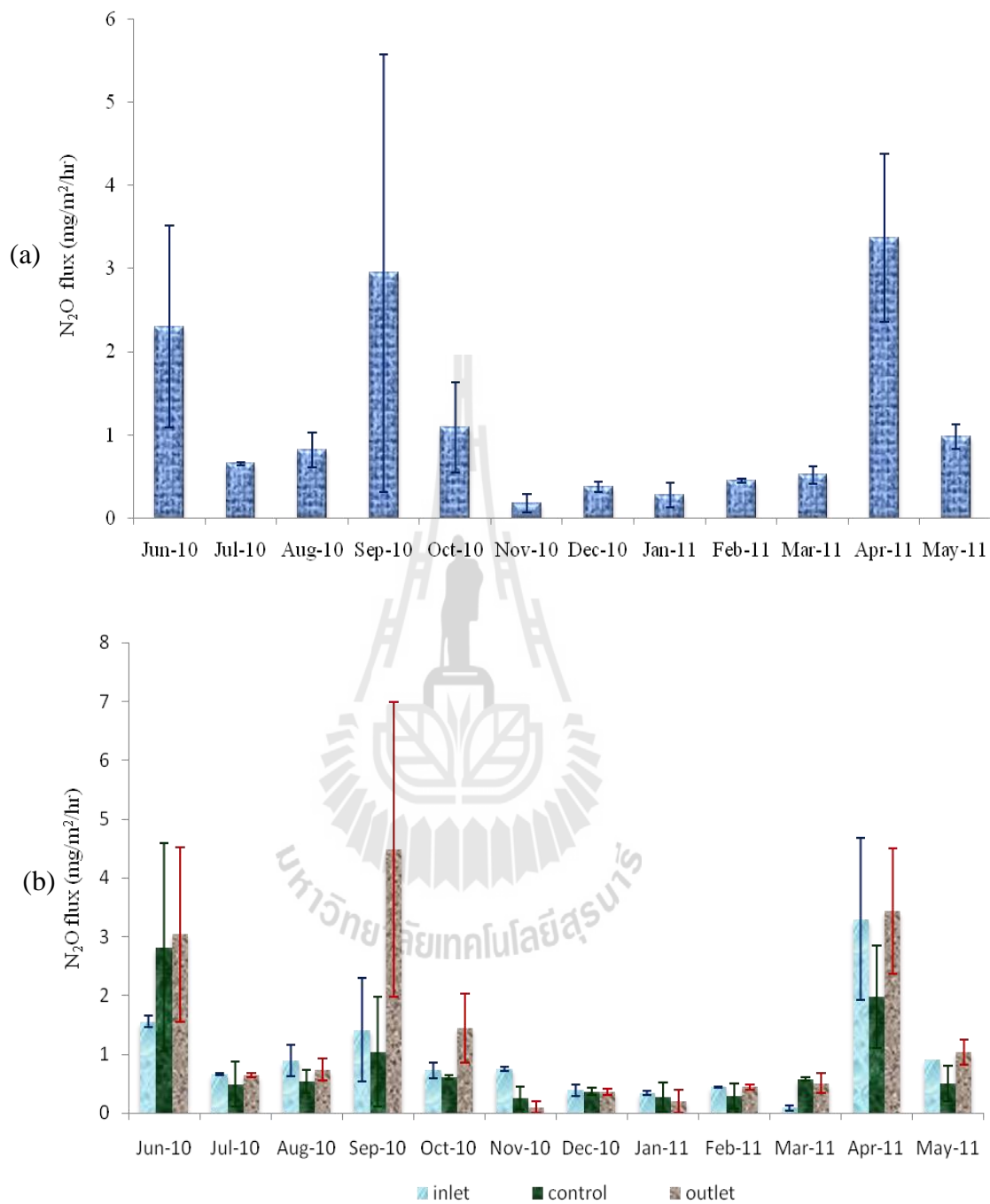
**Figure 4.37** Seasonal variation of N$_2$O fluxes at FWS constructed wetland with *Canna* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.3.4 Free water surface (FWS) constructed wetlands planted with *Cyperus* sp.**

Average nitrous oxide fluxes from FWS constructed wetlands planted with *Cyperus* sp. were in the range of 0.2-6.9 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April and September were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in August and December, respectively (Figure 4.38a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the seasons ($p < 0.01$). The N$_2$O flux was highest in hot rainy season (July-October) with average of 2.6±2.4 mg/m$^2$/hr, followed by summer season (March-May) with average of 2.5±1.7 mg/m$^2$/hr and lowest in cold season (November-February) with average of 0.3±0.1 mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber, the highest nitrous oxide flux occurred in August (hot rainy season) and the lowest nitrous oxide flux occurred in December (cold season) as shown in Figure 4.38b.
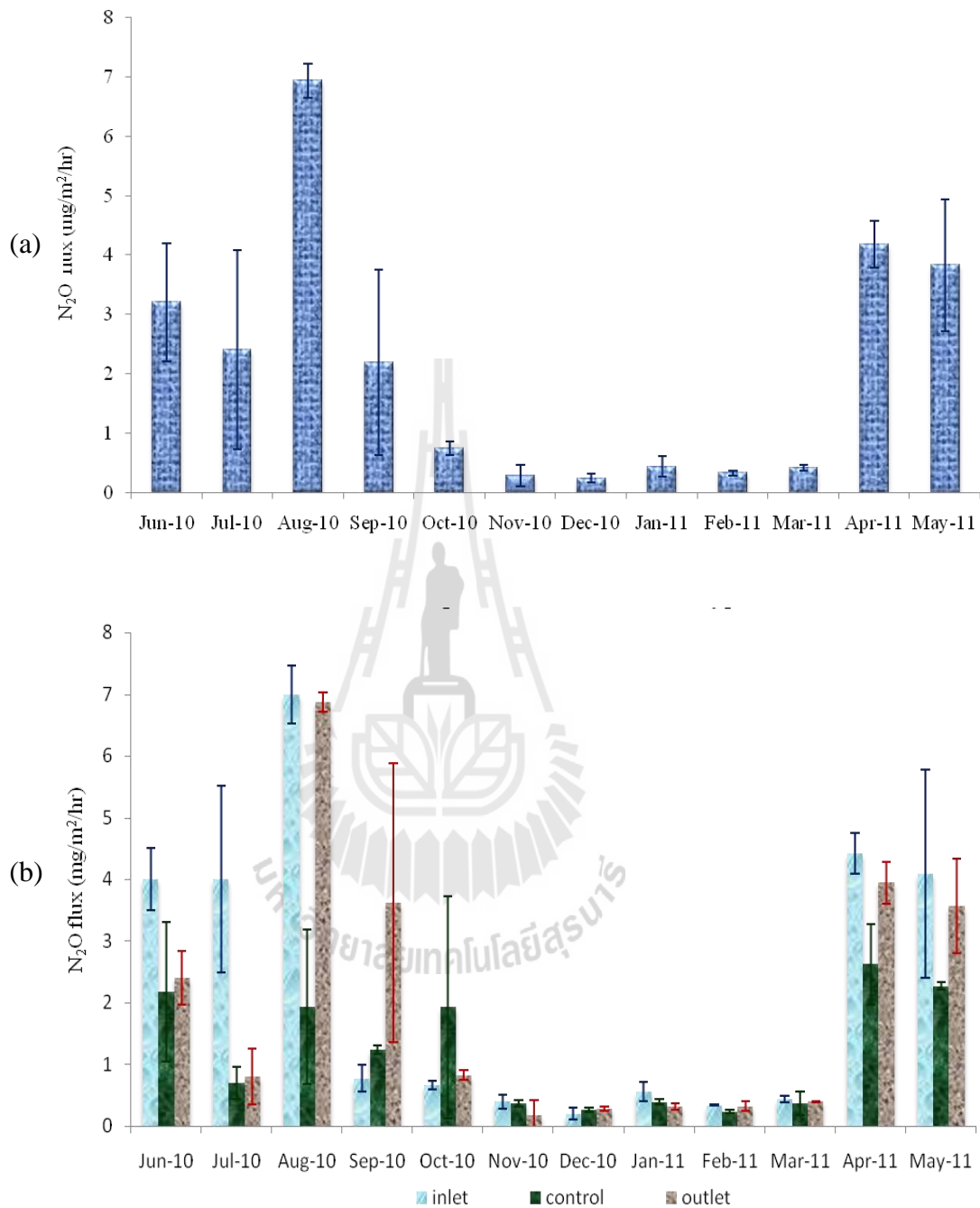
**Figure 4.38** Seasonal variation of N$_2$O fluxes at FWS constructed wetland with *Cyperus* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

### 4.4.3.5 Subsurface flow (SF) beds with non-plants (control units)

Average nitrous oxide fluxes from SF constructed wetlands with non-plant (control units) were in the range of 0.2-1.9 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April and October were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in June and November, respectively (Figure 4.39a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the seasons ($p<0.01$). The nitrous oxide flux was highest in hot rainy season (July-October) with average of 1.1±1.5 mg/m$^2$/hr, followed by summer season (March-May) with average of 1.0±1.0 mg/m$^2$/hr and lowest in cool season (November-February) with average of 0.2±0.1 mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber, the highest nitrous oxide flux occurred in September (hot rainy season) and the lowest nitrous oxide flux occurred in November (cold season) as shown in Figure 4.39b.
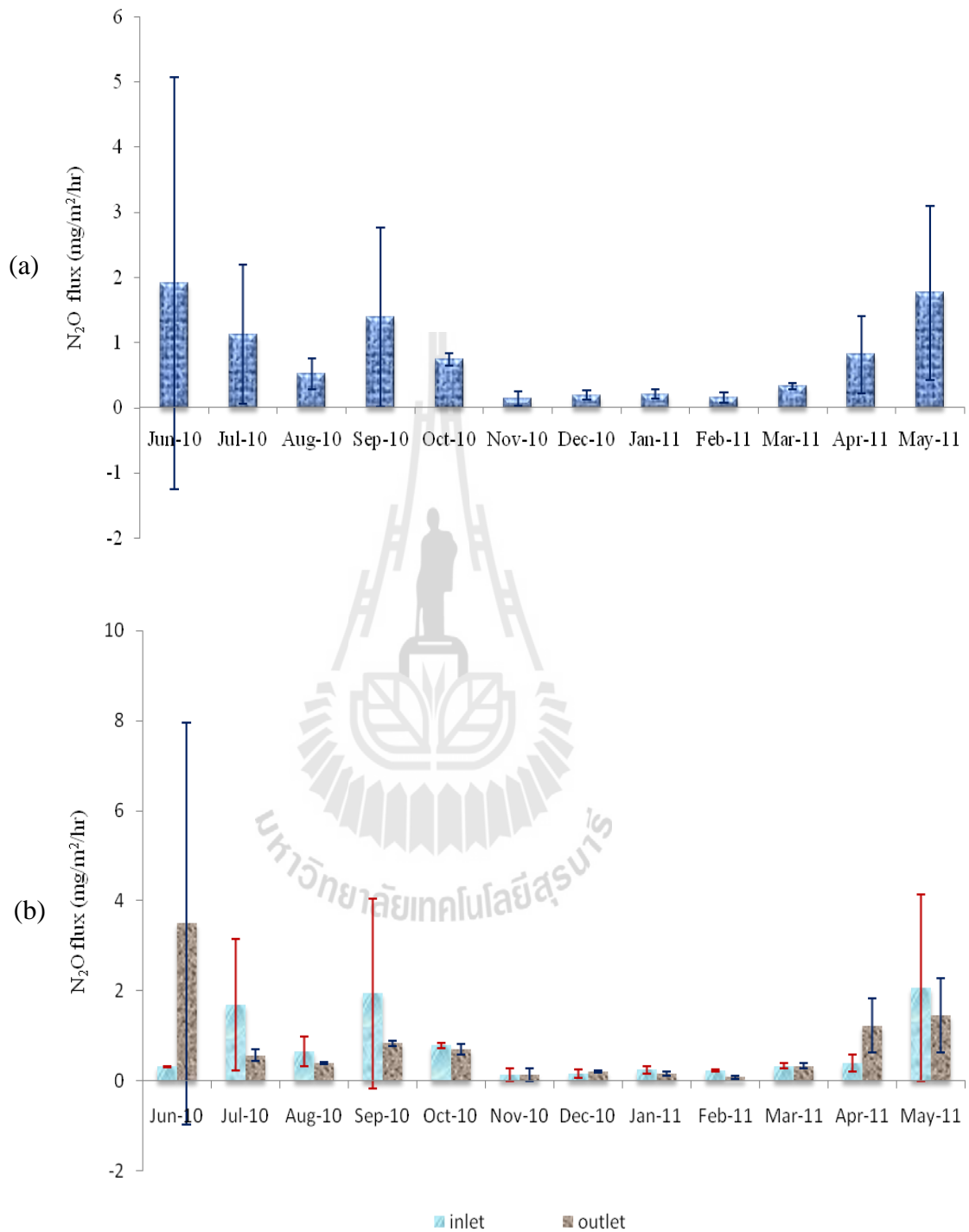
**Figure 4.39** Seasonal variation of N$_2$O fluxes at SF beds with non-plant (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.3.6 Free water surface (FWS) beds with non-plant (control units)**

Average nitrous oxide fluxes from FWS constructed wetlands with non-plant (control units) were in the range of 0.3-3.9 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during May and October were noticeably higher than those in other months. The highest and lowest nitrous oxide fluxes occurred in September and November, respectively (Figure 4.40a). If consider in terms of three seasonal periods, there were significant differences in the nitrous oxide flux among the seasons (p<0.01). The nitrous oxide flux was highest in hot rainy season (July-October) with average of 2.3±1.6 mg/m$^2$/hr, followed by summer season (March-May) with average of 1.2±1.2 mg/m$^2$/hr and lowest in cool season (November-February) with average of 0.3±0.2 mg/m$^2$/hr (Table 4.19). When comparing among inlet point, outlet point and control chamber, the highest nitrous oxide flux occurred in September (hot rainy season) and the lowest nitrous oxide flux occurred in November (cold season) as shown in Figure 4.40b.
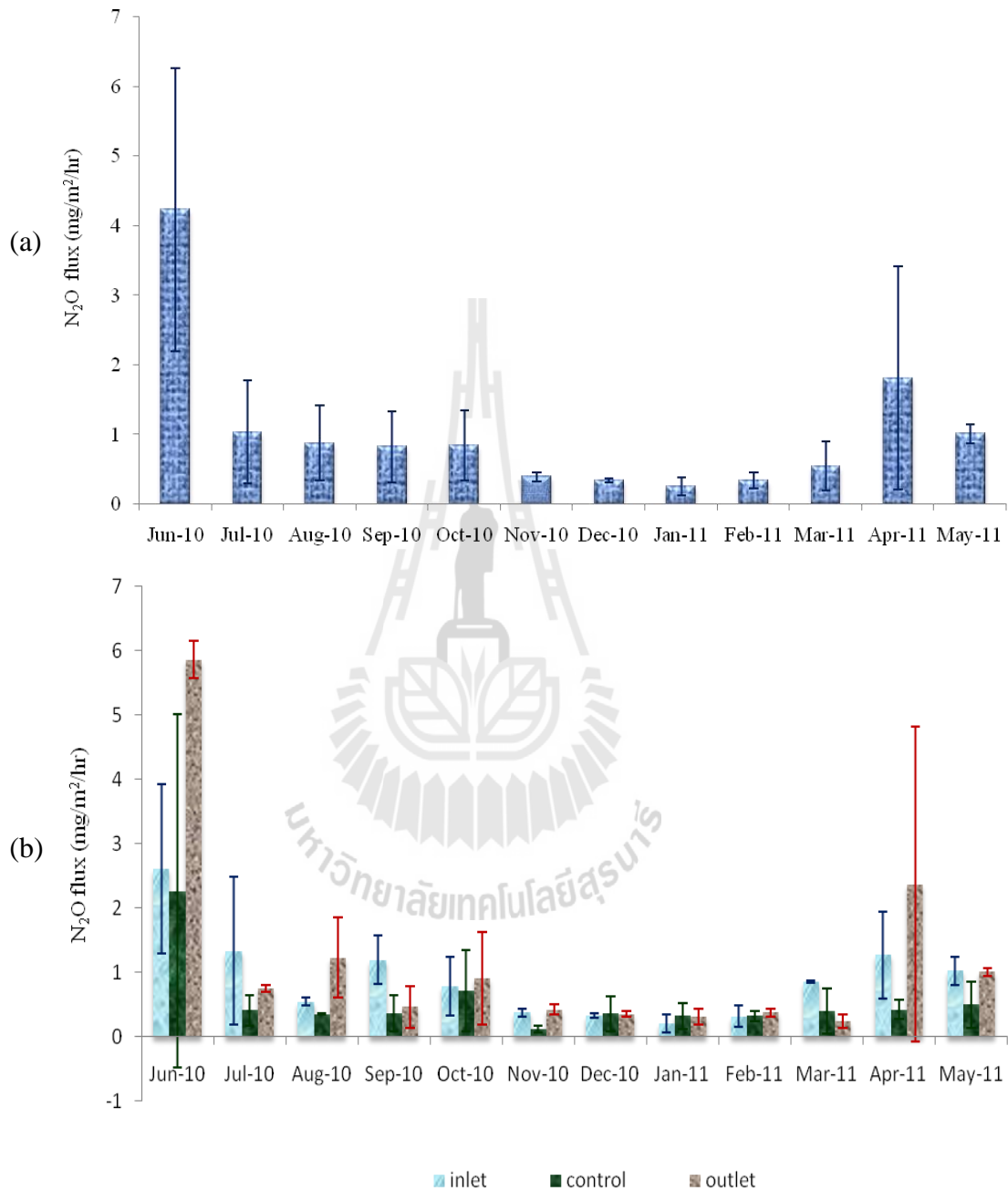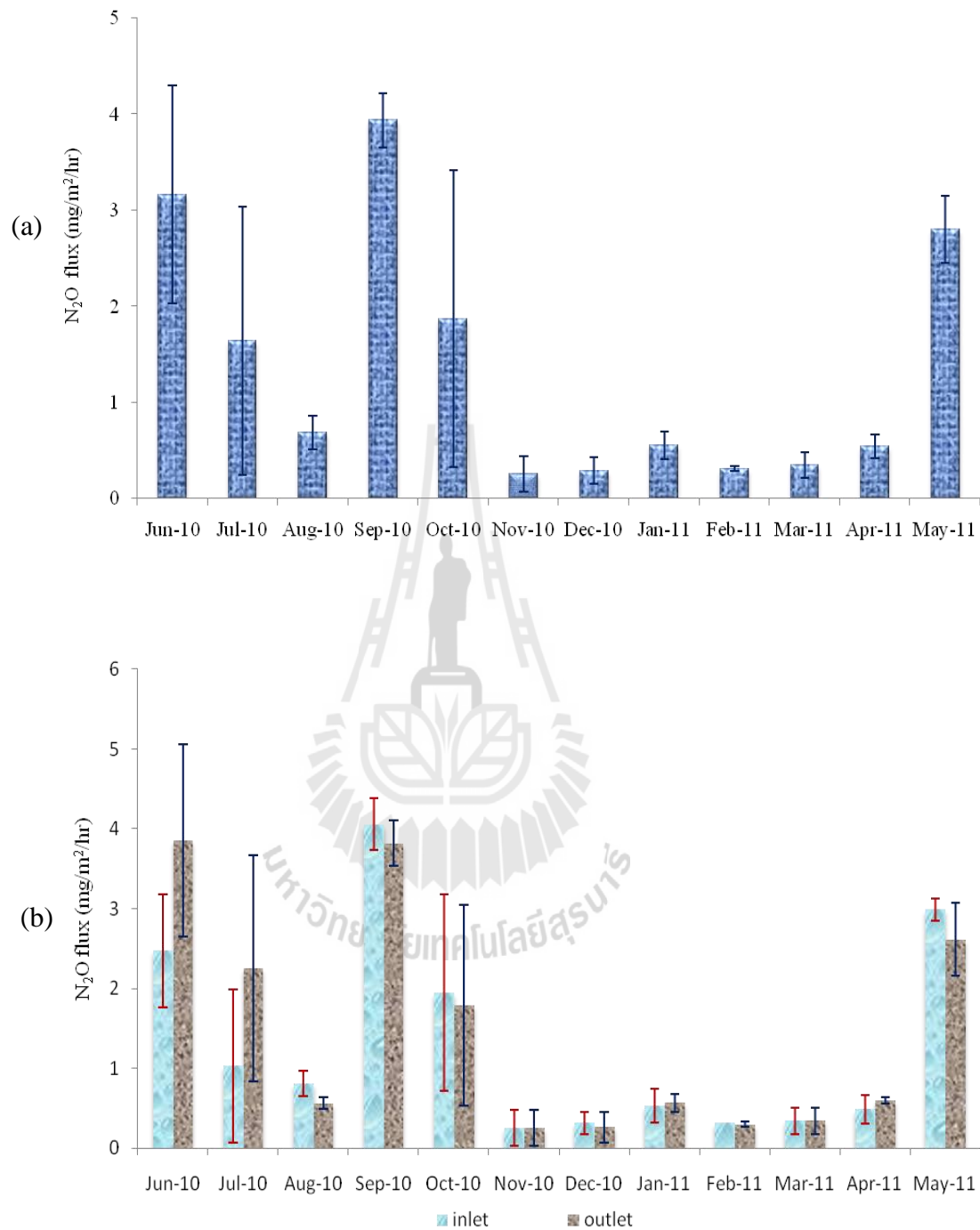
**Figure 4.40** Seasonal variation of $N_2O$ fluxes at FWS beds with non-plant (a) average

fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.3.7  Comparison of nitrous oxide fluxes among three seasons**

Seasonal variation of nitrous oxide fluxes across different constructed wetlands, illustrated in Table 4.19, can be clearly observed.  Since these data were neither a normal distribution nor a homogeneity of variances. Thus, Chi-Square is suitable for analysis differences of nitrous oxide fluxes among three seasons.  The results showed that the differences of nitrous oxide fluxes among three seasons are significant (p<0.01) in all constructed wetlands.

**4.4.3.8  Seasonal variation of nitrous oxide fluxes and environmental factors**

Seasonal nitrous oxide fluxes were investigated to observe the correlation between the fluxes and environmental factors that were measured during the sampling period, i.e., air, soil and water temperature, soil and water pH and soil oxidation-reduction potential (ORP). Results of seasonal nitrous oxide fluxes and environmental factors are shown in Table 4.17.

**Table 4.19** Comparing seasonal variation of nitrous oxide fluxes using Kruskal Wallis test.

| CW/Season | N | Mean | S.D. | $\chi^2$ | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|
| SF (*Cyperus* sp.) | | | | | |
| - Hot rainy | 30 | 1.03 | 0.70 | 22.35 | 0.000** |
| - Cool | 24 | 0.37 | 0.15 | | |
| - Summer | 18 | 1.96 | 3.11 | | |
| | | | | | |
| FWS (*Phragmites* sp.) | | | | | |
| - Hot rainy | 30 | 1.32 | 1.55 | 20.05 | 0.000** |
| - Cool | 24 | 0.32 | 0.12 | | |
| - Summer | 18 | 0.90 | 0.89 | | |
| FWS (*Canna* sp.) | | | | | |
| - Hot rainy | 30 | 1.41 | 1.39 | 36.33 | 0.000** |
| - Cool | 24 | 0.31 | 0.14 | | |
| - Summer | 18 | 1.42 | 1.26 | | |
| FWS (*Cyperus* sp.) | | | | | |
| -Hot rainy | 30 | 2.60 | 2.44 | 40.51 | 0.000** |
| - Cool | 24 | 0.32 | 0.12 | | |
| - Summer | 18 | 2.46 | 1.71 | | |
| SF beds-control | | | | | |
| -Hot rainy | 20 | 1.14 | 1.52 | 30.83 | 0.000** |
| - Cool | 16 | 0.17 | 0.08 | | |
| - Summer | 12 | 0.97 | 0.98 | | |
| FWS beds-control | | | | | |
| -Hot rainy | 20 | 2.26 | 1.56 | 22.17 | 0.000** |
| - Cool | 16 | 0.35 | 0.17 | | |
| - Summer | 12 | 1.23 | 1.18 | | |

*** significant at the 0.01 level*

**Temperature**

Soil temperature played an important role in controlling the magnitude of nitrous oxide production. Like other biological processes, nitrification and denitrification rates increase with increasing temperature within a certain range. Higher temperature favors a higher ratio $N_2O/NO_3^-$ from nitrification (Good road and Keeney, 1984). As soil temperature increases, $N_2O$ emissions also increase, at least up to 37°C, but $N_2O/N_2$ ratio declines with increasing temperatures above 37°C (Castaldi, 2000; Keeney *et al.,* 1979).

In this study, soil temperatures were in the optimum range and changed in a narrow-range during three seasons, particularly between cold and summer season (Table 4.19). The correlation values among seasonal nitrous oxide fluxes and environmental factors were illustrated in Table 4.20. For temperature, there are significantly positive correlation ($p<0.05$, $r=0.39$) between seasonal variation of nitrous oxide fluxes and temperature of air, soil and water. That means nitrous oxide fluxes increase with increased air, soil and water temperature.

**pH**

Soil pH is a secondary controller of denitrification mainly affecting the nitrification process. The optimal rate for nitrification as well as denitrification occurs at a pH range of about 7-8 (Haynes, 1986). $N_2O$ production is enhanced or even becomes dominant at pH<5.5-6.0 (Weier and Gilliam, 1986). As the pH increase, denitrification products tend more, or completely, towards $N_2$ production (Focht and Verstraete, 1977). In this study average soil pH and water pH during the measuring period ranged between 6.70 to 8.02 and 7.07 to 8.03, respectively, in

different constructed wetlands and each season (Table 4.19). Soil and water pH remained in the optimum range. However, there were no correlations between seasonal variation of nitrous oxide fluxes and pH of soil and water (Table 4.20). This study was found that soil and water pH did not influence nitrous oxide flux variation in each season. Soil and water pH is thought to be essential for denitrification.

### Soil oxidation-reduction potential (ORP)

The significant nitrous oxide accumulation in soil ORP ranged between +120 and +250 mV. Little nitrous oxide emission occurred at soil ORP higher than +250 mV or lower than+120mV. The range of minimum accumulation of both methane and nitrous oxide was generally situated between +120 and -170 mV (Yu *et al.,* 2001). In this study, most soil ORP were out of optimal range except soil ORP during summer season that were in optimal range. However, there were no correlations between soil ORP and seasonal variation of nitrous oxide fluxes (Table 4.21). The effect of this factor on nitrous oxide emission might be relatively small or compensate each other under the conditions of this experiment.

**Table 4.20** Correlation between seasonal $N_2O$ flux (mg/m$^2$/hr) and temperature (°C).

| Correlated Dimension | Pearson Correlation (r) | | | | | |
|---|---|---|---|---|---|---|
| | Air Temp | Soil Temp | Water Temp | Soil pH | Water pH | Soil ORP |
| SF *Cyperus* sp. | 0.12 | 0.26* | - | 0.02 | - | 0.18 |
| FWS *Phragmites* sp. | 0.18 | 0.12 | 0.13 | 0.08 | 0.16 | -0.03 |
| FWS *Canna* sp. | 0.24* | 0.20 | 0.25* | -0.17 | -0.16 | 0.17 |
| FWS *Cyperus* sp. | 0.20 | 0.29 | 0.31** | 0.05 | 0.12 | 0.15 |
| Average | 0.26* | 0.32** | 0.35** | 0.02 | 0.09 | 0.22 |

*Not: excluded $N_2O$ fluxes from control unit*
*\* Correlation is significant at the 0.05 level (2-tailed).*
*\*\* Correlation is significant at the 0.01 level (2-tailed).*

### 4.4.4  Seasonal carbon dioxide fluxes from different constructed wetlands

### 4.4.4.1  Subsurface flow (SF) constructed wetlands planted with *Cyperus* sp.

Average $CO_2$ fluxes from SF constructed wetlands planted with *Cyperus* sp. was in the range of 3.1-32.7 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during March and May including August and October were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in August and February, respectively (Figure 4.41a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes (p<0.01). The $CO_2$ fluxes was highest in summer season (March-May) with average of 21.2±9.4 mg/m$^2$/hr, followed by hot rainy season (July-October) with average of 19.6±13.5 mg/m$^2$/hr, and lowest in cool season (November-February) with average of 5.1±3.3 mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and

outlets, high $CO_2$ fluxes occurred in August (hot rainy season) and May (summer season), respectively. Low $CO_2$ fluxes at inlets and outlets occurred during December to February (cool season) as shown in Figure 4.41b.

### 4.4.4.2 Free water surface (FWS) constructed wetland planted with *Phragmites* sp.

Average $CO_2$ fluxes from FWS constructed wetlands planted with *Phragmites* sp. was in the range of 5.0-61.5 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during March and November were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in March and December, respectively (Figure 4.42a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes ($p<0.01$). The $CO_2$ fluxes was highest in summer season (March-May) with average of $30.8\pm21.5$ mg/m$^2$/hr, followed by hot rainy season (July-October) with average of $28.5\pm17.0$ mg/m$^2$/hr and lowest in cool season (November-February) with average of $11.3\pm11.9$ mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and outlets, high $CO_2$ fluxes occurred in March and October, respectively. Low $CO_2$ fluxes at inlets and outlets occurred during December and January (cool season) as shown in Figure 4.42b.
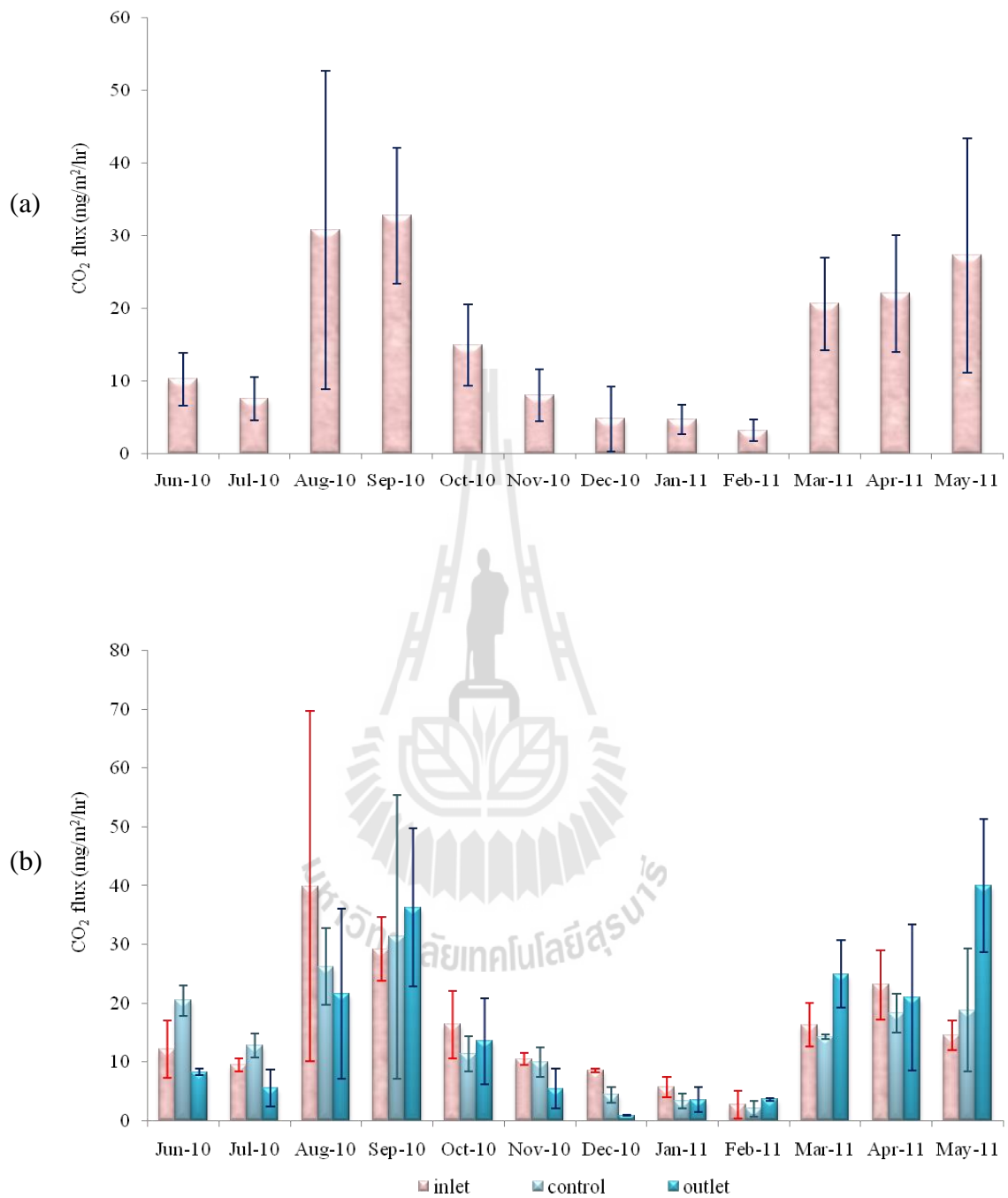
**Figure 4.41** Seasonal variation of $CO_2$ fluxes at SF constructed wetland with *Cyperus* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**Table 4.21** Seasonal variations of $CO_2$ fluxes using Kruskal Wallis test.

| CW/Season | N | Mean | S.D. | $\chi^2$ | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|
| SF with *Cyperus* sp. | | | | | |
| - Hot rainy | 30 | 19.63 | 13.53 | 40.43 | 0.000** |
| - Cold | 24 | 5.06 | 3.31 | | |
| - Summer | 18 | 21.25 | 9.39 | | |
| FWS with *Phragmites* sp. | | | | | |
| - Hot rainy | 30 | 28.46 | 17.04 | 29.15 | 0.000** |
| - Cold | 24 | 11.34 | 11.93 | | |
| - Summer | 18 | 30.82 | 21.56 | | |
| FWS with *Canna* sp. | | | | | |
| - Hot rainy | 30 | 36.62 | 21.13 | 34.84 | 0.000** |
| - Cold | 24 | 11.91 | 11.04 | | |
| - Summer | 18 | 52.64 | 24.84 | | |
| FWS with *Cyperus* sp. | | | | | |
| -Hot rainy | 30 | 28.47 | 14.05 | 29.56 | 0.000** |
| - Cold | 24 | 15.99 | 17.88 | | |
| - Summer | 18 | 49.68 | 15.89 | | |
| SF beds-control | | | | | |
| -Hot rainy | 20 | 27.63 | 15.57 | 16.65 | 0.000** |
| - Cold | 16 | 10.83 | 8.03 | | |
| - Summer | 12 | 23.62 | 15.88 | | |
| FWS beds-control | | | | | |
| -Hot rainy | 20 | 30.27 | 16.40 | 11.77 | 0.003** |
| - Cold | 16 | 14.27 | 14.76 | | |
| - Summer | 12 | 42.02 | 48.80 | | |

*\*\* significant at the 0.01 level*
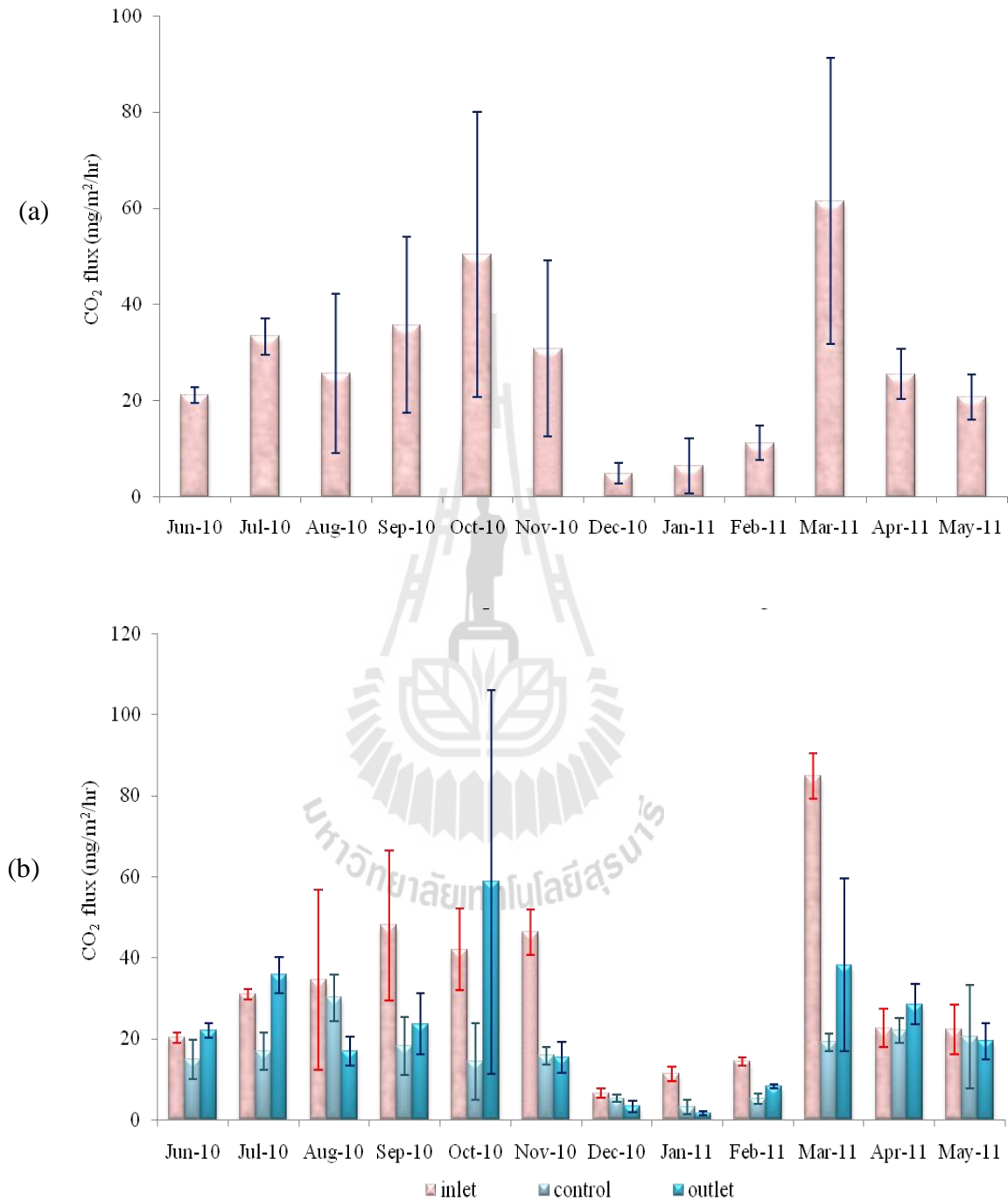
**Figure 4.42** Seasonal variation of $CO_2$ fluxes at FWS constructed wetland with *Phragmites* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

### 4.4.4.3 Free water surface (FWS) constructed wetlands planted with *Canna* sp.

Average $CO_2$ fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 4.5-72.9 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during March and October were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in May and January, respectively (Figure 4.43a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes (p<0.01). The $CO_2$ fluxes was highest in summer season (March-May) with average of 52.6±24.8 mg/m$^2$/hr followed by hot rainy season (July-October) with average of 36.6±21.1 mg/m$^2$/hr, and lowest in cool season (November-February) with average of 11.9±11.0 mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and outlets, high $CO_2$ fluxes occurred in March and October, respectively. Low $CO_2$ fluxes at inlets and outlets occurred during December and February (cold season) as shown in Figure 4.43b.
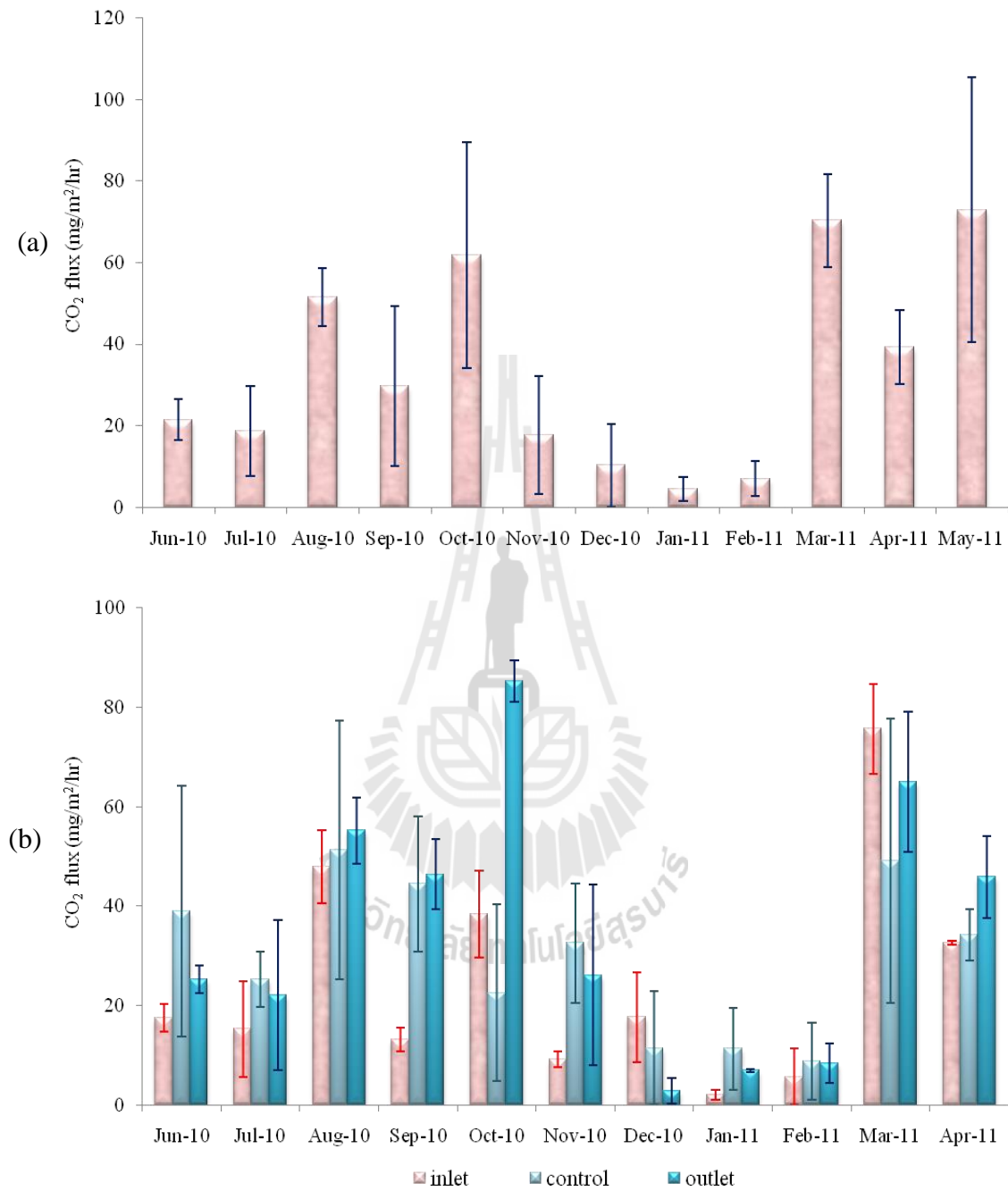
**Figure 4.43** Seasonal variation of $CO_2$ fluxes at FWS constructed wetland with *Canna* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.4.4 Free water surface (FWS) constructed wetlands planted with *Cyperus* sp.**

Average $CO_2$ fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 8.5-60.2 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during March and November were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in March and December, respectively (Figure 4.44a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes (p<0.01). The $CO_2$ fluxes was highest in summer season (March-May) with average of 49.7±15.9 mg/m$^2$/hr, followed by hot rainy season (July-October) with average of 28.5±14.0 mg/m$^2$/hr and lowest in cool season (November-February) with average of 15.6±17.9 mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and outlets, high $CO_2$ fluxes occurred in May and March, respectively. Low $CO_2$ fluxes at inlets and outlets occurred during December and February (cold season) as shown in Figure 4.44b.
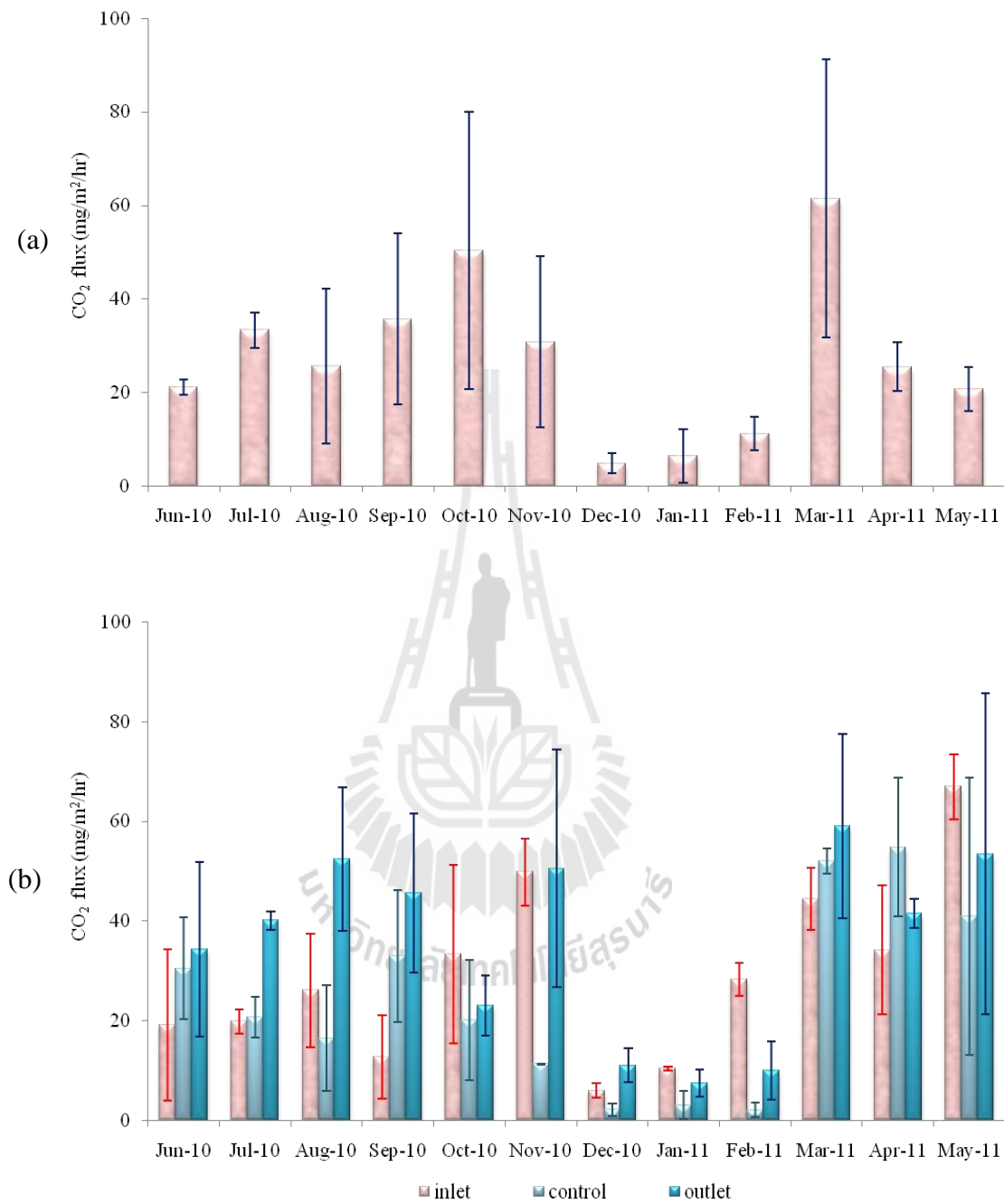
**Figure 4.44** Seasonal variation of $CO_2$ fluxes at FWS constructed wetland with *Cyperus* sp. (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.4.5 Subsurface (SF) beds with non-plants as control units**

Average $CO_2$ fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 2.6-46.1 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during April, September and October were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in September and December, respectively (Figure 4.45a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes (p<0.01). The $CO_2$ fluxes was highest in hot rainy season (July-October) with average of 27.6±15.6 mg/m$^2$/hr, followed by summer season (March-May) with average of 23.6±15.9 mg/m$^2$/hr and lowest in cool season (November-February) with average of 10.8±8.0 mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and outlets, high $CO_2$ fluxes occurred in September and April, respectively. Low $CO_2$ fluxes at inlets and outlets occurred in December (cold season) as shown in Figure 4.45b.
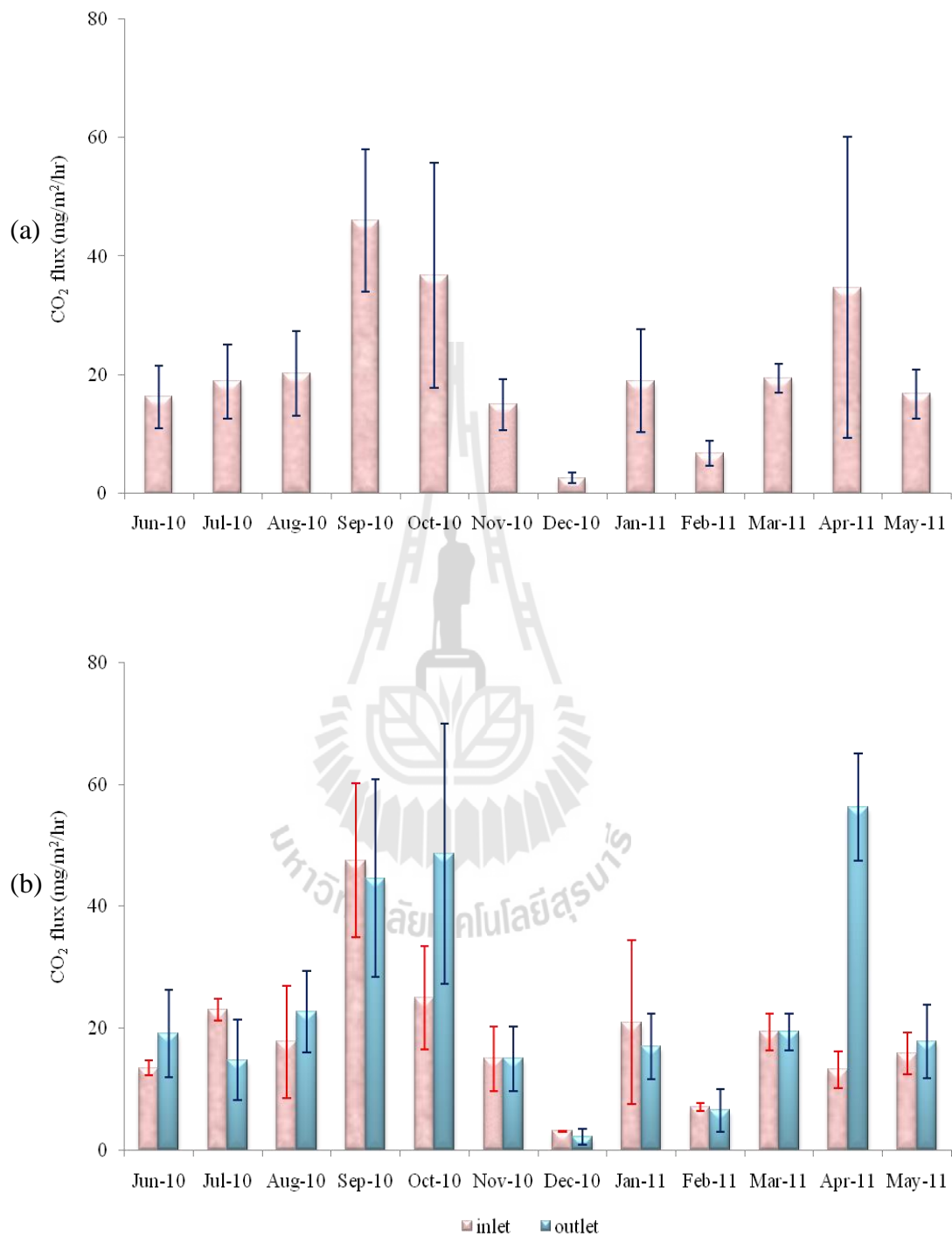
**Figure 4.45** Seasonal variation of $CO_2$ fluxes at SF beds with non-plant (a) average fluxes from inlet and outlet points (b) monthly fluxes from different points.

**4.4.4.6 Free water surface (FWS) beds with non-plants (control units)**

Average $CO_2$ fluxes from FWS constructed wetlands planted with *Canna* sp. was in the range of 4.1-81.5 mg/m$^2$/hr, averaging from inlets and outlets values. The fluxes observed during March and November were noticeable higher than those in other months. The highest and lowest $CO_2$ fluxes occurred in March and December, respectively (Figure 4.46a). When seasonal variation was taken into account, there were significant differences in the $CO_2$ fluxes (p<0.01). The $CO_2$ fluxes was highest in hot rainy season (July-October) with average of 27.6±15.6 mg/m$^2$/hr, followed by summer season (March-May) with average of 42.0±48.8 mg/m$^2$/hr, followed by hot rainy season (July-October) with average of 30.3±16.4 mg/m$^2$/hr and lowest in cool season (November-February) with average of 14.3±14.8 mg/m$^2$/hr (Table 4.22). When the fluxes were compared between inlets and outlets, high $CO_2$ fluxes occurred in March. Low $CO_2$ fluxes at inlets and outlets occurred in December (cold season) as shown in Figure 4.46b.
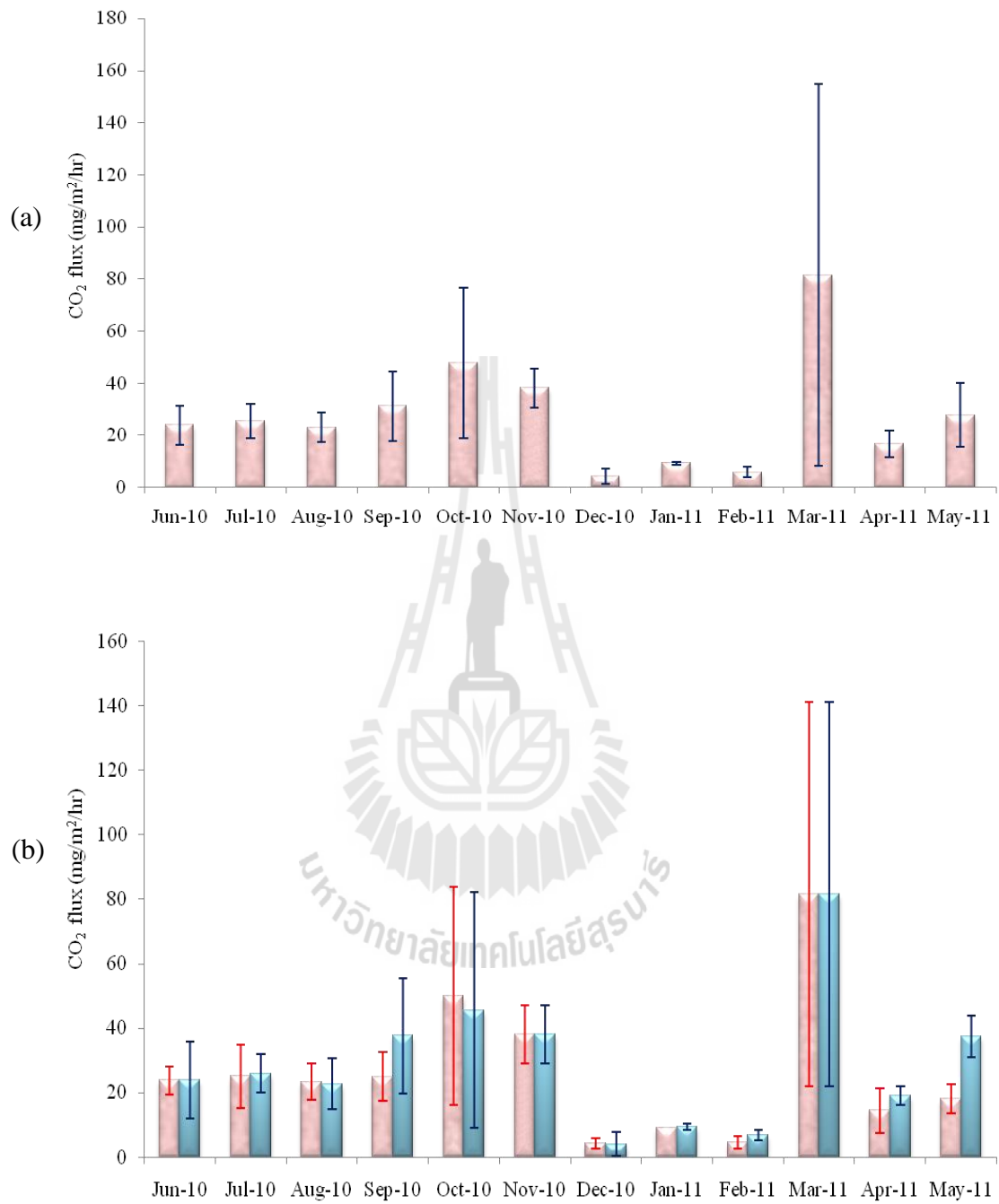
**Figure 4.46** Seasonal variation of $CO_2$ fluxes at FWS beds with non-plant (a) average

fluxes from inlet and outlet points (b) monthly fluxes from different points.

Measurement data clearly demonstrated seasonal variations of $CO_2$ fluxes across in different constructed wetlands (Table 4.22). The data were neither a normal distribution nor homogeneity of variances. Thus, Chi-Square is suitable for the analysis differences in methane fluxes among three seasons. The results showed that the differences of methane fluxes among three seasons are significant ($p<0.01$) in all constructed wetlands.

### 4.4.4.7 Seasonal variation of $CO_2$ fluxes and environmental factors

Seasonal $CO_2$ fluxes were investigated to observe the correlation between the fluxes and environmental factors that were measured during the sampling period, i.e., air, soil and water temperature, soil and water pH, and soil oxidation-reduction potential (ORP). Summary of seasonal $CO_2$ fluxes and environmental factors are shown in Table 4.23.

**Temperature**

Temperature is a primary control of $CO_2$ production in most soils (Kirschbaum, 2000; Rustad and Fernandez, 1998), but not all studies concur (Giardina and Ryan, 2000). As global temperatures rise, any changes in soil $CO_2$ emissions will in part be determined by the temperature dependence of soil $CO_2$ production. Because root and microbial sources of $CO_2$ show increased activity as a function of temperature (Boone *et al.,*1998) and since new studies suggest that global temperature increases are amplified in the ground (Beltrami *et al.,* 2000; Huang *et al.,* 2000), it is critical that the temperature dependence of soil $CO_2$ production be examined (Risk, 2002).

**Table 4.22** Comparison of average methane fluxes and environmental factors from different constructed wetlands among three seasons.

| CW/ Season | Air Temperature (°C) | Soil Temperature (°C) | Water Temperature (°C) | Soil pH | Water pH | Soil ORP (mV) |
|---|---|---|---|---|---|---|
| SF with *Cyperus* sp. | | | | | | |
| - Hot rainy | 33.10 | 26.55 | NA | 7.91 | NA | -226.64 |
| - Cold | 30.97 | 25.54 | NA | 8.02 | NA | -223.03 |
| - Summer | 28.59 | 25.69 | NA | 7.96 | NA | -176.66 |
| FWS with *Phragmites* sp. | | | | | | |
| - Hot rainy | 33.10 | 27.08 | 27.35 | 6.70 | 7.25 | -229.02 |
| - Cold | 30.97 | 26.25 | 26.33 | 7.09 | 7.85 | -226.59 |
| - Summer | 28.59 | 26.33 | 26.65 | 7.13 | 8.03 | -168.81 |
| FWS with *Canna* sp. | | | | | | |
| - Hot rainy | 33.10 | 26.65 | 26.36 | 6.82 | 7.07 | -221.53 |
| - Cold | 30.97 | 25.20 | 24.94 | 7.04 | 7.32 | -217.44 |
| - Summer | 28.59 | 25.56 | 25.01 | 6.88 | 7.17 | -169.48 |

**Table 4.22** (Continued).

| CW/Season | Air Temperature (°C) | Soil Temperature (°C) | Water Temperature (°C) | Soil pH | Water pH | Soil ORP (mV) |
|---|---|---|---|---|---|---|
| FWS with *Cyperus* sp. | | | | | | |
| -Hot rainy | 33.10 | 26.68 | 26.36 | 7.11 | 7.31 | -232.34 |
| - Cold | 30.97 | 25.35 | 25.14 | 7.06 | 7.38 | -215.17 |
| - Summer | 28.59 | 25.47 | 25.02 | 6.94 | 7.27 | -171.01 |
| Average all | | | | | | |
| - Hot rainy | 33.10 | 26.80 | 26.71 | 7.27 | 7.34 | -227.22 |
| - Cold | 30.97 | 25.63 | 25.51 | 7.43 | 7.65 | -221.01 |
| - Summer | 28.59 | 25.87 | 25.66 | 7.38 | 7.68 | -172.75 |

In this study, soil temperatures were in the optimum range and changed in a narrow-range during the experiment, particularly between cold and summer season (Table 4.22). The correlation values among seasonal $CO_2$ fluxes and environmental factors show in Table 4.23. However, statistical analysis did not find correlations between seasonal variation of $CO_2$ fluxes and temperature of air, soil and water.

**pH**

Soil pH is an abiotic factor which affects soil respiration process. The pH influences most of the biochemical reactions, taking place in the soil. Bacterial enzymes synthesis can affect respiration. In the soil, humus absorption of enzymes causes pH to increase and leads to changes of the community structure. Most of the known bacterial species live at pH 4-9. Luo and Zhou (2004) reported a series of quantitative data about $CO_2$ efflux in relation to the different pH values (Cerhanova *et al.,* 2006). They showed that the $CO_2$ quantity produced at a pH of 3 can be up to 12 times smaller than the one produced to a pH of 4. They also observed that once pH increases up to values of 7 the $CO_2$ efflux increased as well. Once the threshold of 7 was over passed, the $CO_2$ production decreased up to 83% when the soil pH reaches the value 10. However, there were no correlations between seasonal variation of $CO_2$ fluxes and pH of soil and water (Table 4.24).

**Soil oxidation-reduction potential (ORP)**

Emission of $CO_2$ from the soil represents the combined effects of root respiration and mineralization of organic matter (Raich and Schlesinger, 1992). Soil oxygenation state is closely related to soil redox transformations and moreover, influence

the type of respiration and microorganism population (Glinski *et al.,* 2000) Soil ORP is an aeration parameter also related to substrate available and energy transformation and so can play a crucial role in maintaining soil microbial abundance, diversity, and community structure (Song *et al.,* 2008). However, it is important to recognize the effect of soil ORP on respiration activity. Although it is still unclear how soil respiration responds soil ORP in the soil environment, this study found that there were no correlations between seasonal variation of $CO_2$ fluxes and soil ORP (Table 4.24).

**Table 4.23** Correlation between seasonal $CO_2$ flux (mg/m$^2$/hr) and temperature (°C).

| Correlated Dimension | Pearson Correlation (r) | | | | | |
|---|---|---|---|---|---|---|
| | Air Temp | Soil Temp | Water Temp | Soil pH | Water pH | Soil ORP |
| SF *Cyperus* sp. | 0.11 | 0.14 | - | -0.33** | - | 0.13 |
| FWS *Phragmites* sp. | -0.07 | -0.10 | -0.11 | -0.13 | -0.19 | 0.04 |
| FWS *Canna* sp. | -0.25* | 0.07 | -0.01 | -0.05 | 0.00 | 0.22 |
| FWS *Cyperus* sp. | 0.00 | 0.08 | 0.00 | -0.04 | -0.12 | 0.22 |
| Average all | -0.11 | 0.04 | 0.05 | -0.05 | -0.06 | 0.22 |

*Not: excluded $CO_2$ fluxes from control unit*

*\*\* Correlation is significant at the 0.01 level (2-tailed).*

*\* Correlation is significant at the 0.05 level (2-tailed).*

The correlation among seasonal variation of $CO_2$ fluxes and the environmental factors were hardly observed. These may because $CO_2$ flux is not the result of a one-factor action but of the interaction of more biotic and abiotic factors.

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## 4.5  Influence of plant species on greenhouse gas fluxes

Data of greenhouse gas fluxes from seasonal study were used to evaluate the influence of plant species within constructed wetlands on greenhouse gas fluxes. Descriptive statistics for greenhouse gas fluxes from FWS constructed wetlands with different plants species are shown in Table 4.25.  Since these data were neither a normal distribution nora homogeneity of variance, Chi-Square is suitable for analysis differences of greenhouse gas fluxes among different plant species within constructed wetlands. The means of greenhouse gas fluxes from different plants species within FWS constructed wetland were compared by Kruskal Wallis followed by Mann-Whitney U Test as post hoc test.  The results showed that the mean of $CH_4$ and $N_2O$ fluxes from different plants species were significantly different ($p<0.05$ Table 4.25) while mean of $CO_2$ fluxes among three plant species did not show significantly different ($p>0.05$). Results from Mann-Whitney U Test as post hoc test indicate that FWS planted with *Phragmites* sp. emitted the highest $CH_4$ flux and lowest $N_2O$ flux while FWS planted with *Cyperus* sp. emitted the highest $N_2O$ flux (Table 4.26).

**Table 4.24** Descriptive statistics and comparison of greenhouse gas fluxes from FWS with different plant species using Kruskal Wallis Test.

| GHG/CW | | GHG flux ($mg/m^2/hr$) | | | | | $\chi^2$ | Asymp. Sig (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| | | N | Mean | S.D. | Min | Max | | |
| $CH_4$ | FWS *Phragmites* sp. | 72 | 11.16 | 16.10 | 0.00 | 113.87 | 9.88 | 0.007** |
| | FWS *Canna* sp. | 72 | 6.01 | 6.70 | 0.00 | 39.96 | | |
| | FWS *Cyperus* sp. | 72 | 5.92 | 9.82 | 0.00 | 71.56 | | |
| | Total | 216 | 7.70 | 11.76 | 0.00 | 113.87 | | |
| $N_2O$ | FWS *Phragmites* sp. | 72 | 0.88 | 1.17 | 0.08 | 6.06 | 7.06 | 0.029* |
| | FWS *Canna* sp. | 72 | 1.04 | 1.20 | 0.02 | 6.67 | | |
| | FWS *Cyperus* sp. | 72 | 1.80 | 2.06 | 0.03 | 7.32 | | |
| | Total | 216 | 1.24 | 1.58 | 0.02 | 7.32 | | |
| $CO_2$ | FWS *Phragmites* sp. | 72 | 23.35 | 18.71 | 1.18 | 92.22 | 5.86 | 0.053 |
| | FWS *Canna* sp. | 72 | 32.39 | 24.96 | 0.93 | 110.60 | | |
| | FWS *Cyperus* sp. | 72 | 29.61 | 20.25 | 0.00 | 76.18 | | |
| | Total | 216 | 28.45 | 21.70 | 0.00 | 110.60 | | |

*significant at the 0.05 level*

**significant at the 0.01 level*

**Table 4.25** Mann-Whitney U Test as pos hoc test for multiple comparison of greenhouse fluxes from different plant species within constructed wetlands.

| GHG | (i) Plant | (j) Plant | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| $CH_4$ | *Phragmites* sp. | *Canna* sp.* | 5.15 | 1.925 | 0.024 |
| | | *Cyperus* sp.* | 5.24 | 1.925 | 0.021 |
| | *Canna* sp. | *Phragmites* sp.* | -5.15 | 1.925 | 0.024 |
| | | *Cyperus* sp. | 0.09 | 1.925 | 1.000 |
| | *Cyperus* sp. | *Phragmites* sp.* | -5.24 | 1.925 | 0.021 |
| | | *Canna* sp. | -0.09 | 1.925 | 1.000 |
| $N_2O$ | *Phragmites* sp. | *Canna* sp. | -0.17 | 0.256 | 1.000 |
| | | *Cyperus* sp.* | -0.92 | 0.256 | 0.001 |
| | *Canna* sp. | *Phragmites* sp. | 0.17 | 0.256 | 1.000 |
| | | *Cyperus* sp.* | -0.76 | 0.256 | 0.010 |
| | *Cyperus* sp. | *Phragmites* sp.* | 0.92 | 0.256 | 0.001 |
| | | *Canna* sp.* | 0.76 | 0.256 | 0.010 |

*. The mean difference is significant at the 0.05 level.

Average methane and nitrous oxide fluxes from FWS constructed wetland planted with various plant species were different. The highest methane fluxes occurred in FWS planted with *Phragmites* sp., but the fluxes from *Cyperus* sp. and *Canna* sp. were not significantly different ($p > 0.05$). Observations of physical appearance of plants and their root systems during the experiment and at the end showed different root system of the

plant within the plots. It is possible that differences in gases fluxes may contributed from root expansion characteristics. Roots of *Phragmites* sp. expanded deeper and more evenly distributed from near the soil surface to the bottom of the rhizosphere. This latter favorable structure was good for reducing methane emissions from wetlands. When macrophytes develop an aerenchymatous structure to avoid deficiency in their roots, the aerenchyme cells then form an important route for the transport of methane from the anaerobic layer into the atmosphere (Shannon *et al.,* 1996). This also will favor the providing easily degradable substrate for anaerobic decomposition, e.g. root litter and exudates from such roots to the rhizospheric bacteria of macrophytes result in enhancing methane emission.

When consider the lowest $N_2O$ flux in FWS planted with *Phragmites* sp., it was possible that *Phragmites* sp. stand generally have great production (Meyerson *et al.,* 2000). They also tend to have deeper roots and rhizomes (Windham, 2001) and longer growing seasons than other plant species. This suggesting that plants might compete for $NO_3^-$ and $NH_4^+$ from soil for growth with microbes (nitrifers/denitrifier), and suppress nitrous oxide emissions from nitrification and denitrification processes (Cheng *et al.,* 2007) in the constructed wetland.

When compare greenhouse gas fluxes from SF and FWS, planted with *Cyperus* sp., mean of $CH_4$ and $CO_2$ fluxes from SF and FWS constructed wetland showed significantly different (p<0.05, Table 4.27). Average $CH_4$ and $N_2O$ fluxes from FWS were significantly higher than the fluxes from SF constructed wetland. However, mean of

N$_2$O fluxes from SF and FWS constructed wetlands did not show significant differences (p>0.05).

**Table 4.26** Comparison of greenhouse gas fluxes from different constructed wetland using Mann-Whitney U Test including descriptive statistics.

| GHG/CW | | GHG fluxes (mg/m$^2$/hr) | | | Mean difference | Sig. (2-tailed) |
|---|---|---|---|---|---|---|
| | | N | Mean | S.D. | | |
| CH$_4$ | SF *Cyperus* sp. | 72 | 2.89 | 3.55 | -3.03 | 0.022* |
| | FWS *Cyperus* sp. | 72 | 5.92 | 9.82 | | |
| | Total | 144 | 4.41 | 7.51 | | |
| N$_2$O | SF *Cyperus* sp. | 72 | 1.05 | 1.70 | -0.76 | 0.108 |
| | FWS *Cyperus* sp. | 72 | 1.80 | 2.06 | | |
| | Total | 144 | 1.42 | 1.92 | | |
| CO$_2$ | SF *Cyperus* sp. | 72 | 15.18 | 12.32 | -14.43 | 0.000** |
| | FWS *Cyperus* sp. | 72 | 29.61 | 20.25 | | |
| | Total | 144 | 22.39 | 18.20 | | |

*significant at the 0.05 level*
*** significant at the 0.01 level*

## 4.6 Estimated global warming potential (GWP) emitted from the constructed wetlands

Since the average CH$_4$, N$_2$O and CO$_2$ fluxes from subsurface flow constructed wetland (SF) were about 2.9, 1.05 and 15.2 mg/m$^2$/hr, respectively, the emissions could

be estimated as global warming potential (GPW). The mean $CH_4$ and $N_2O$ fluxes from SF were approximately 61 and 325 mg $CO_2$ equivalents/$m^2$/hr or approximately 401 mg $CO_2$ equivalents/$m^2$/hr. In free-water surface flow constructed wetland (FWS), the average $CH_4$, $N_2O$ and $CO_2$ fluxes were 5.9-11.2, 0.9-1.8 and 23.3-32.4 mg/$m^2$/hr, respectively, which were corresponded to the average GWP of 124 to 235, and 279 to 558 mg $CO_2$ equivalents/$m^2$/hr for $CH_4$ and $N_2O$ respectively. Thus, GWP of FWS was about 426 to 825 mg $CO_2$ equivalents/$m^2$/hr.

Estimated GWP emitted from the constructed wetland in various seasons could be calculated from the greenhouse gas fluxes measured during the experiment. During hot rainy season (July-October), the average $CH_4$, $N_2O$ and $CO_2$ fluxes were 11.5, 1.59, 28.3 mg/$m^2$/hr, respectively. The GWP for the average of $CH_4$ and $N_2O$ fluxes were about 242 and 493 mg $CO_2$ equivalents/$m^2$/hr, respectively, or approximately 763 mg $CO_2$ equivalents/$m^2$/hr. In cold season (November-February), the average $CH_4$, $N_2O$ and $CO_2$ fluxes were 2.6, 0.33, 11.1 mg/$m^2$/hr, respectively. The GWP was estimated to about 242 and 493 mg $CO_2$ equivalents/$m^2$/hr, for the mean $CH_4$ and $N_2O$ fluxes, respectively, or approximately 168 mg $CO_2$ equivalents/$m^2$/hr. During summer season (March-May), the average $CH_4$, $N_2O$ and $CO_2$ fluxes were 3.3, 1.68, 38.6 mg/$m^2$/hr, respectively. The GWP of the mean $CH_4$ and $N_2O$fluxes was 242 and 493 mg $CO_2$ equivalents/$m^2$/hr. The results indicated that the estimated GWP was high in hot rainy season compared to summer and cool season during the experiment.

Estimated GWP from various plant species found that in FWS constructed wetland planted with *Phragmites* sp., the average $CH_4$, $N_2O$ and $CO_2$ fluxes were 11.16,

0.88 and 23.35 mg/m$^2$/hr, respectively. Calculated GWP from the average of CH$_4$, and N$_2$O fluxes, was about 234 and 273 mg CO$_2$ equivalents/m$^2$/hr or approximately 531 mg CO$_2$ equivalents/m$^2$/hr. In FWS constructed wetland with *Canna* sp., the average CH$_4$, N$_2$O and CO$_2$ fluxes were 6.01, 1.04 and 32.39 mg/m$^2$/hr, respectively. The GWP of the average of CH$_4$, and N$_2$O fluxes, was 126 and 322 mg CO$_2$ equivalents/m$^2$/hr or approximately 481 mg CO$_2$ equivalents/m$^2$/hr. In FWS constructed wetland planted with *Cyperus* sp., the average CH$_4$, N$_2$O and CO$_2$ fluxes were 5.92, 1.80 and 29.61 mg/m$^2$/hr, respectively, Estimated GWP of CH$_4$, and N$_2$O fluxes was 61 and 325 mg CO$_2$ equivalents/m$^2$/hr or approximately 712 mg CO$_2$ equivalents/m$^2$/hr. It can be concluded that the *Cyperus* sp. Was response to high GWP compared to *Phragmites* sp. and *Canna* sp., respectively.

## 4.7  Identification of microbial community in constructed wetlands soils

Soil samples from twelve constructed wetlands were analyzed for microbial community using PCR-DGGE techniques with the help of cluster and principal component analyses for classifying microbial community. Groups of microbial community involved in methane and nitrous oxide source and sink in the constructed wetlands were separately identified at 10, 20 and 30 cm depth, i.e., archaebacteria, eubacteria, ammonia-oxidizing archaebacteria, methanogenicarchaebacteria, and methanotrophicarchaeobacteria. Separation of PCR products in agarose gel of the extracted samples shows distinct nucleic acid bands separated in each lane. The picture taken from actual DGGE of DNA fragments is shown in Figure 4.47 without any label.
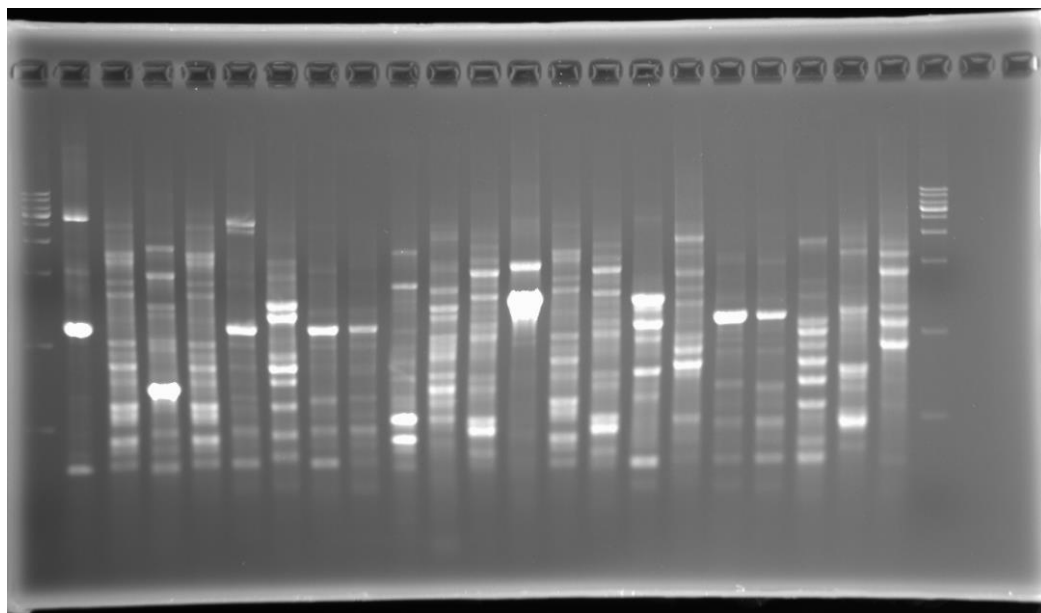
**Figure 4.47** Agarose gel showing total bacteria DNA extracted from soil samples in constructed wetlands of 24 isolates after PCR.

In the comparison of all samples from each plot and depth, all the gel electrophoresis were placed together as of archaebacteria in Figure 4.48 and eubacteria in Figure 4.49. In these figures alphabet "P" represents each soil samples taken from corresponding plot of the constructed wetlands and numbers 10, 20 and 30 cm, represent the depth of soil samples. For example, P1 10 cm represents plot 1 and 10 cm depth. Clear white bands could be observed in each sample after subjected to DGGE analyses indicated that conditions of PCR and DGGE were appropriated in the separation of extracted DNA fragments in the samples. Visual examination of the bands from each lane initially noticed similar patterns of the bands from each depth. For instance, isolates from soil samples at 10 cm had resemble bands patterns which differ from 20 and 30 cm depth

(Figure 4.48). At each depth, some lanes show distinct bands at corresponded size ladder

(bp-basepair) indicating relationships of the DNA at the same depth.  Visual observation,

however, was insufficient to determine possible relationship of the microbial community

in the soil samples obtained from the constructed wetlands. Distinct bands were selected

and sent for DNA sequencing.
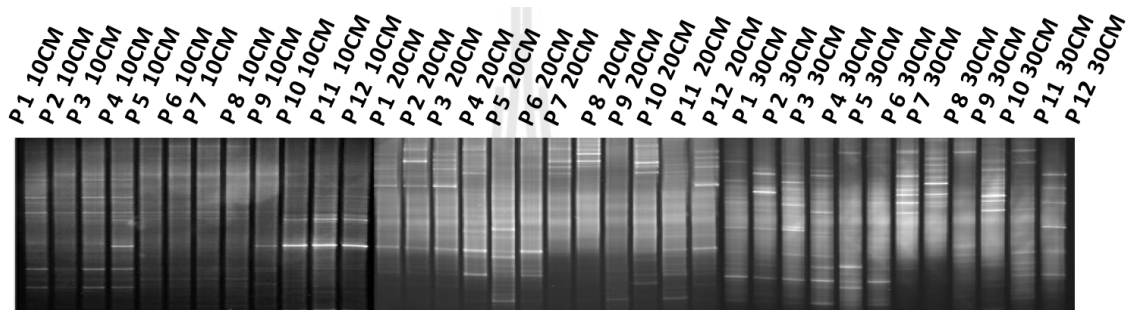


**Figure 4.48** Agarose gel electrophoresis of PCR products from 24 isolates of
archaebacteria.
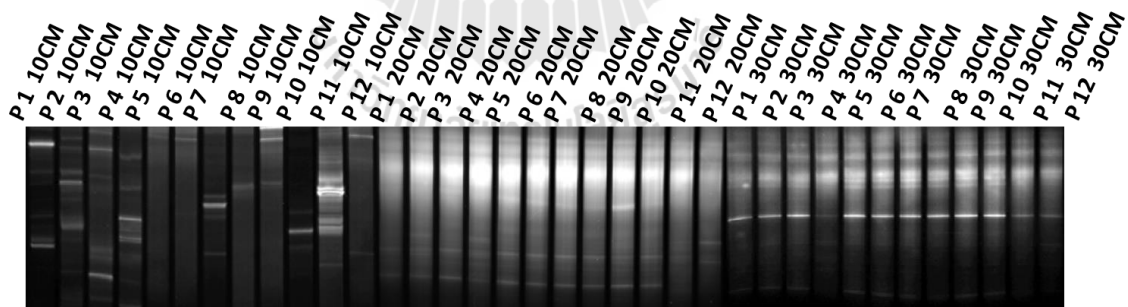


**Figure 4.49** Agarose gel electrophoresis of PCR products from 24 isolates of eubacteria.

The DNA sequences were generated and the relationships were identified based

on the sequences obtained from the NCBI database.  The cluster analysis was used to

distinguish the microorganism relationships in the DNA sequences. Using the presented and absence of bands appeared on agarose gels, the values were assigned, 1 for the present and 0 for the absence of bands, respectively. Similarities of DGGE results in each lane were analyzed with a software, NTSYSpc 2.2 (Exeter Software, USA). Dendogram was produced to associate the similarities based on calculated distance.

The DGGE fingerprints of archaebacteria found in each depth indicated that several bands of ribotype found at all depth (Figure 4.50). Similarity of the ribotype could be grouped according to depth, e.g., group of 10 cm depth and so on. Subsequent sequences showed that archaebacteria groups from constructed wetland plots were the members of the uncultured archaebacteria.

In the analysis of ammonia-oxidizing archaebacteria, the DGGE fingerprints appeared rather different from those of archaebacteria. The lanes from 30 cm depth exhibited visual similar patterns but not all other depth found accordingly (Figure 4.50).

Further analysis, thus, required to enhance the distinction among microbial community existed within the group using the principal component analysis (PCA). The result from PCA plot is able to show three distinct groups according to corresponding depth (Figure 4.51). Subsequent DNA sequences showed that ammonia-oxidizing archaebacteria groups from constructed wetland plots were the members of the uncultured archaebacteria. Krasnits *et al*. (2009) reported similar results that depth was found to have a greater influence on the distribution of the major microbial communities than distance from the inlet. The study used SF constructed wetlands for the treatment of municipal wastewater.
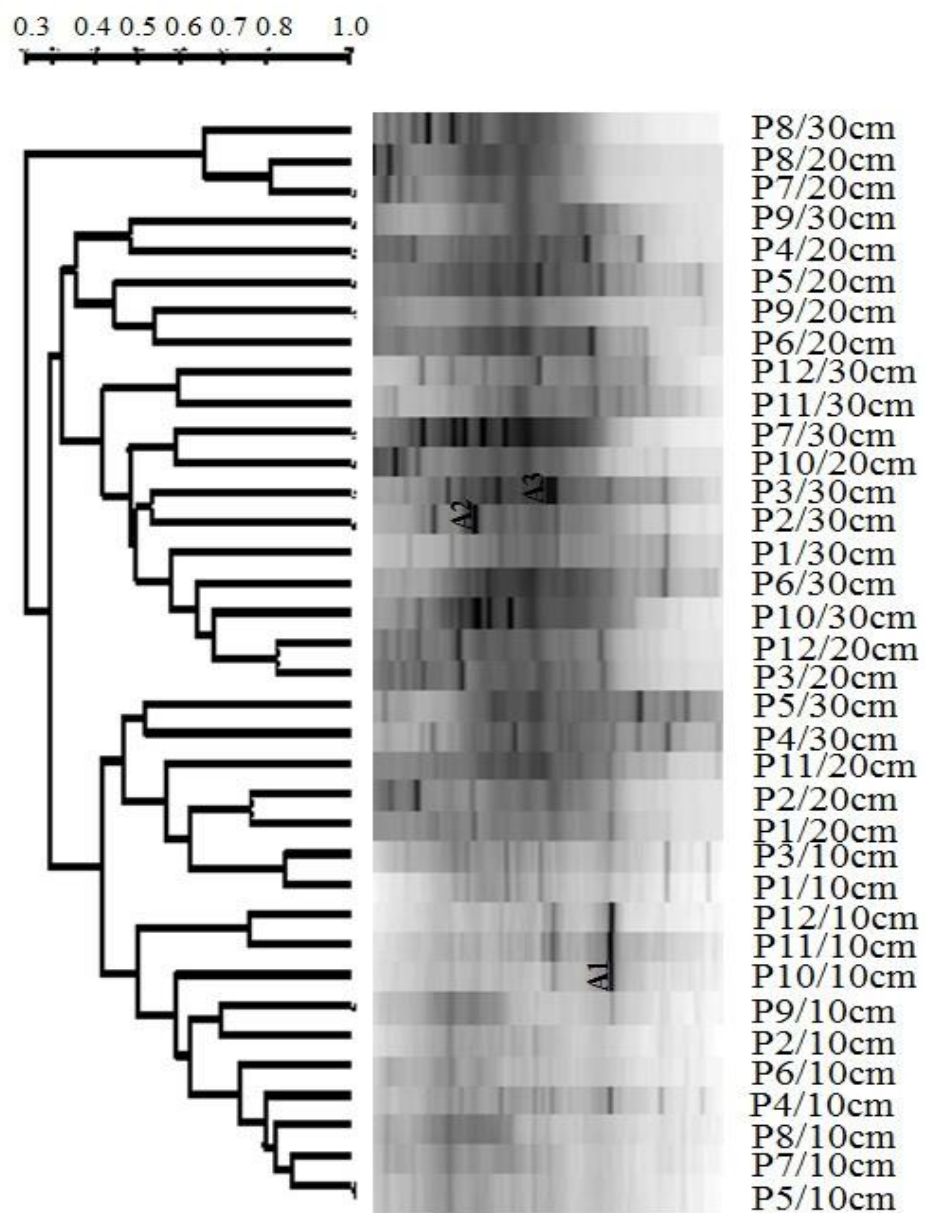
**Figure 4.50** Dendogram from microbial community grouping by cluster analysis archaebacteria.
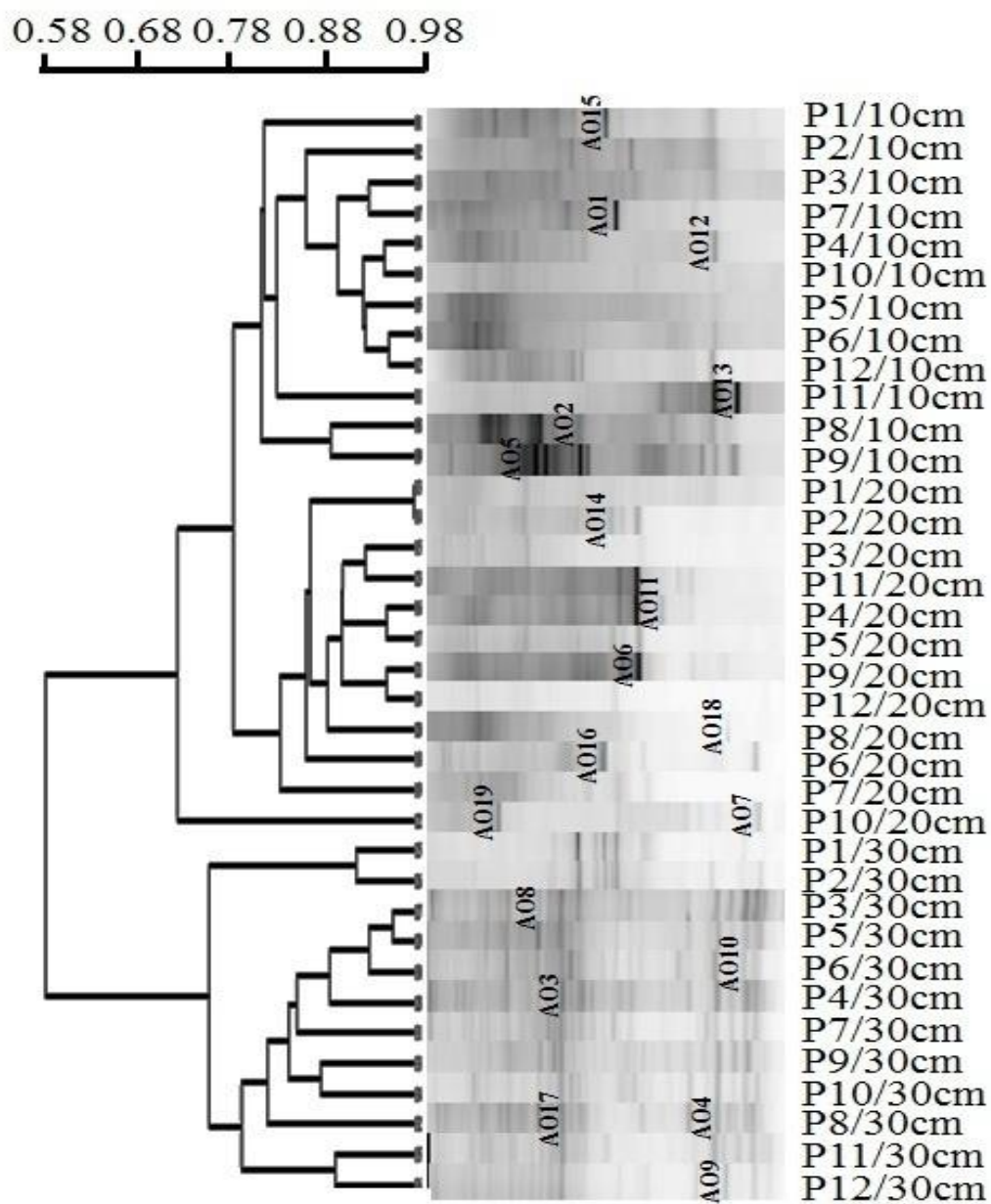
**Figure 4.51** Dendogram from microbial community grouping by cluster analysis

ammonia-oxidizing archaebacteria.

In the analysis of methanogenic archaebacteria, the dendogram from cluster analysis of the DGGE fingerprints are rather scatter but some lanes show similarity in band patterns according to similar depth (Figure 4.52). The PCA plot is able to distinguish group of methanogenic archaebacteria but many lanes are closely related in different depth comparing to ammonia-oxidizing archaebacteria (Figure 4.53). Subsequent DNA sequences showed that methanogenic archaebacteria group from constructed wetland plots were the members of the uncultured bacteria.
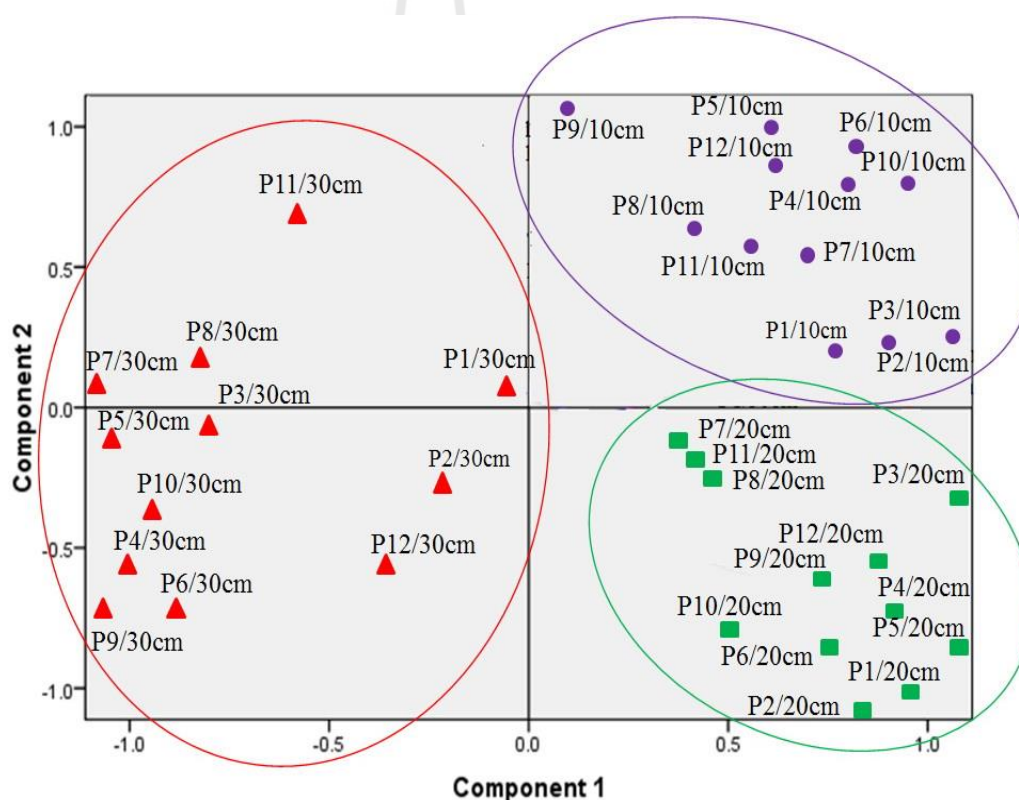


**Figure 4.52** Principal component analysis of ammonia-oxidizing archaebacteria.

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In the analysis of methanotrophic bacteria, the dendogram from cluster analysis of the DGGE fingerprints are less scatter compared to methanogenic bacteria and some lanes show similarity in band patterns according to depth (Figure 4.55).  The PCA plot is able to distinguish group of methanotrophic bacteria related in different depth (Figure 4.56).

The results from microbial community analysis indicated that depth of soil in both type of constructed wetlands had influence on type of microbial communities similar to the results reported by Krasnits *et al*. (2009) and Deng *et al*. (2007). Krasnits *et al*. (2009) reported similar results that depth was found to have a greater influence on the distribution of the major microbial communities than distance from the inlet. The study used SF constructed wetlands for the treatment of municipal wastewater.
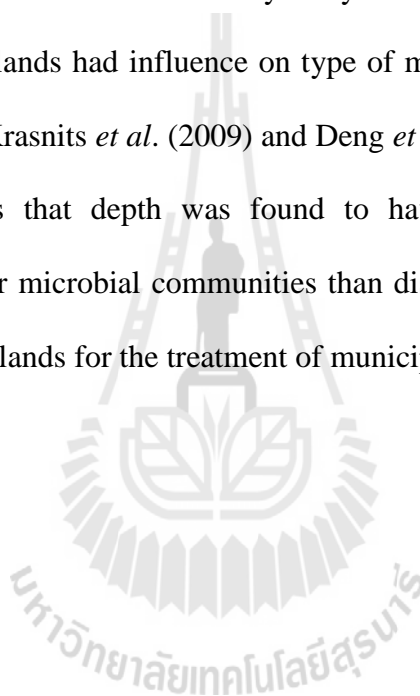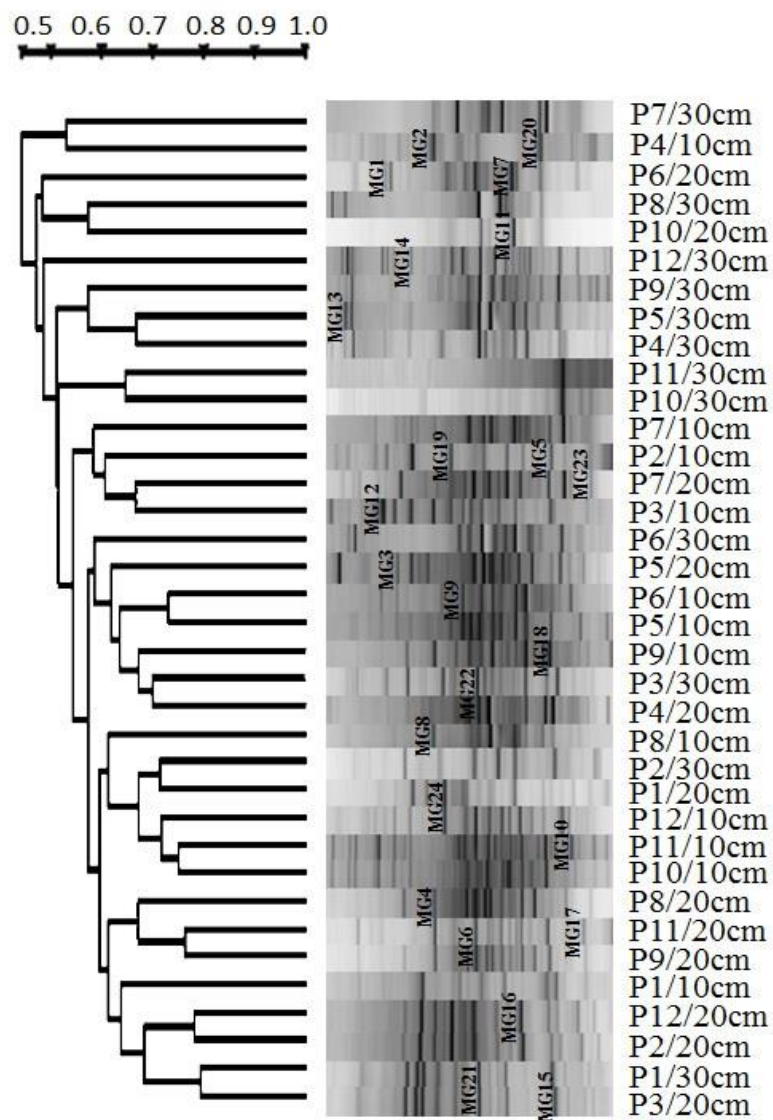
**Figure 4.53** Dendogram of methonogenic archaebacteria community grouping by cluster analysis.
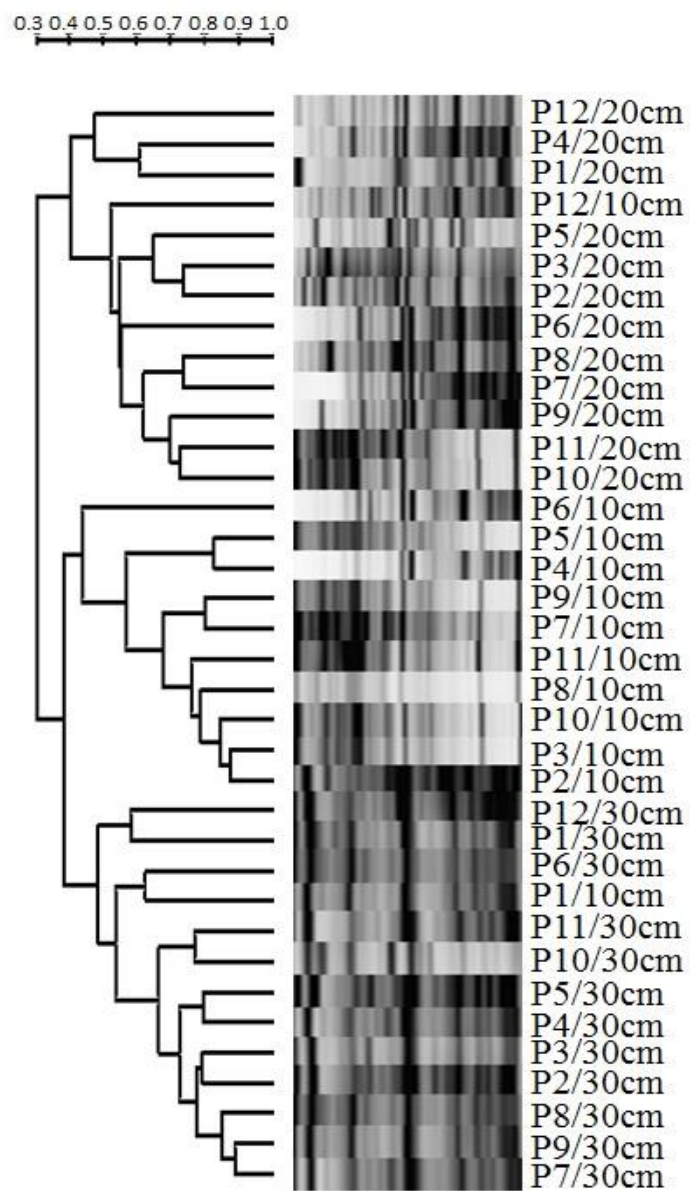
**Figure 4.56** Dendogram of methonotrophic bacteria community grouping by cluster analysis.

# CHAPTER V

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

The results show that overall emissions of greenhouse gas fluxes from constructed wetlands planted with *Canna* sp., *Phragmites* sp. and *Cyperus* sp. were lower than control, indicating the potential sink of the greenhouse gases. On the average, $CH_4$, $N_2O$ and $CO_2$ fluxes from subsurface flow constructed wetlands (SF) planted with *Cyperus* sp., were about $2.9\pm3.5$, $1.05\pm1.7$ and $15.2\pm12.3$ mg/m$^2$/hr, respectively. The range of $CH_4$ fluxes varied from 0 to 20 mg/m$^2$/hr while $CO_2$ fluxes ranged from 0.8 to 61.1 mg/m$^2$/hr. These values were within the range reported by Picek *et al.* (2004), who conducted the experiment on subsurface flow constructed wetland treating municipal wastewater ($CH_4$ ranged from 0 to 93 mg/m$^2$/hr and $CO_2$ ranged from 4 to 309 mg/m$^2$/hr). However, the average $N_2O$ flux from this study was higher, about 1.05 mg/m$^2$/hr (ranging from 0.1 to 9.9 mg/m$^2$/hr), compared to Picek *et al.* (2004).

In free water surface flow (FWS) constructed wetlands with various emergent plants, average $CH_4$, $N_2O$ and $CO_2$ fluxes were $5.9\pm9.8$, $1.8\pm2.1$ and $29.6\pm20.2$ mg/m$^2$/hr, respectively. The $CH_4$ flux ranged from 0.0 to 113.9 mg/m$^2$/hr, which is comparable to a study by Inamori *et al.* (2007) conducted on experimental scale free water surface flow constructed wetlands treating non-point sewage at the rural area ($CH_4$ ranged from 0 to 1,560 mg/m$^2$/hr). However, the $N_2O$ levels, which ranged from 0.02 to 7.3 mg/m$^2$/hr,

were higher than those found in the previous study. Inamori *et al.* (2007) reported $N_2O$

flues varied from 0 to 3.4 mg/m$^2$/hr.  In addition, $CO_2$ fluxes averaged 29.6 mg/m$^2$/hr,

ranging from 0.0 to 110.6 mg/m$^2$/hr which were lower than in a study of Liikanen *et al.*

(2006) conducted on a free water surface flow constructed wetland used to purify

draining waters from the adjacent peat mining area. ($CO_2$ ranged from 7, 270 to 13,600

mg/m$^2$/hr).  These results support the hypothesis that wastewater treatment constructed

wetlands are sources of $CH_4$ and $N_2O$, but the constructed wetlands tend to be sinks of

$CO_2$.

Greenhouse gas fluxes from wastewater treatment constructed wetlands showed

diurnal fluctuations, except $N_2O$. Higher $CH_4$ flux occurred during daytime and peaked in

the afternoon, around 15:00 while the flux at night is lower. Average $CH_4$ flux during

daytime ranged from 2.1 to 13.2 mg/m$^2$/hr while average $CH_4$ flux during nighttime

ranged from 0.2 to 4.8 mg/m$^2$/hr. $CO_2$ fluxes did show a diurnal variation pattern.

Average $CO_2$ flux during nighttime was higher than daytime. The average $CO_2$ flux

during day time ranged from -55.9 to -5.2 mg/m$^2$/hr while average $CH_4$ flux during

nighttime ranged from 51.4 to 74.7 mg/m$^2$/hr.  These diurnal variation patterns were

correlated with changing in soil temperature. However, $N_2O$ fluxes did not show an

obvious pattern of diurnal variation.

Seasonal fluctuations of greenhouse gas fluxes from wetlands constructed for

wastewater treatment could be observed in this study.  The average $CH_4$ flux was highest

in hot rainy season (July-October) with the average of 11.5 mg/m$^2$/hr, followed by

summer season (March-May) with the average of 3.3 mg/m$^2$/hr and lowest in cold season

(November-February) with the average of 2.6 mg/m$^2$/hr. The pattern of seasonal CH$_4$ variation was correlated with changing in air temperature, soil pH and water pH. Average N$_2$O flux was highest in hot rainy season (July-October) with the average of 33.1 mg/m$^2$/hr, followed by cold season (November-February) with the average of 40.0 mg/m$^2$/hr and lowest in summer season (March-May) with the average of 28.6 mg/m$^2$/hr. The pattern of seasonal N$_2$O variation was correlated with changing in temperature of air, soil and water. In addition, average CO$_2$ flux was highest in summer season (March-May) with the average of 38.6 mg/m$^2$/hr, followed by hot rainy season (July-October) with the average of 28.3 mg/m$^2$/hr and lowest in cold season (November-February) with the average of 11.1 mg/m$^2$/hr. However, the seasonal CO$_2$ variation and environmental factors was hardly observed. These results support the hypothesis that greenhouse gas fluxes from constructed wetland have diurnal and seasonal fluctuations and are influenced by some environmental factors.

Investigating the effect of plant species on greenhouse gases fluxes found that the mean of CH$_4$, and N$_2$O fluxes from different plants species show significant differences ($p<0.05$). However, the mean CO$_2$ fluxes among three plant species did not show significant differences ($p>0.05$). FWS planted with *Phragmites* sp. emitted the highest CH$_4$ flux but emitted the lowest N$_2$O flux, while FWS planted with *Cyperus* sp. emitted the highest N$_2$O flux.

The results from microbial community analysis indicated that depth of soil in both types of constructed wetlands had influence on the types of bacterial communities, whereas an influence of plant species on bacterial profile could not observed. These

results did not support the hypothesis that plant species affect the community of bacteria involved in greenhouse gas fluxes. However, plant species affect variation of greenhouse gas fluxes, possibly because of their differences in physiology and phenology.

## 5.2 Recommendations

To better understand greenhouse gas cycles in constructed wetland microcosms, further study should be conducted on factors influencing the rate of greenhouse gas production, entrapment, oxidation and emission. Additionally, greenhouse gas fluxes from constructed wetlands may be influenced by other factors, such as type and amount of substrates, water chemistry (e.g. dissolved $SO_4^{-2}$ or $NO_3^-$) and the activity of bacteria. Nevertheless, the root activity of plants and growth state of plants may also be the important influencing factors. Thus, for propose study the relationship between the root activity and greenhouse gas fluxes should be considered.

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**APPENDIX**

**Table A.1** Data of tracer study of constructed wetland CW3.

| $T_1$(min) | $Dt_1$ | C(mg/L) | $C/C_0 = C_1$ | $C_1Dt_1$ | $t_1C_1Dt_1$ | $t_1^2C_1Dt_1$ |
|---|---|---|---|---|---|---|
| 10 | 10 | 1.46 | 0.00 | 0.02 | 0.24 | 2.43 |
| 20 | 10 | 2.95 | 0.00 | 0.05 | 0.98 | 19.67 |
| 30 | 10 | 4.37 | 0.01 | 0.07 | 2.19 | 65.59 |
| 40 | 10 | 8.26 | 0.01 | 0.14 | 5.50 | 220.17 |
| 50 | 10 | 12.20 | 0.02 | 0.20 | 10.17 | 508.33 |
| 60 | 10 | 15.30 | 0.03 | 0.26 | 15.30 | 918.00 |
| 70 | 10 | 17.80 | 0.03 | 0.30 | 20.77 | 1453.67 |
| 80 | 10 | 19.75 | 0.03 | 0.33 | 26.33 | 2106.67 |
| 90 | 10 | 21.23 | 0.04 | 0.35 | 31.85 | 2866.05 |
| 100 | 10 | 26.20 | 0.04 | 0.44 | 43.67 | 4366.67 |
| 110 | 10 | 27.20 | 0.05 | 0.45 | 49.87 | 5485.33 |
| 120 | 10 | 30.45 | 0.05 | 0.51 | 60.90 | 7308.00 |
| 130 | 10 | 31.90 | 0.05 | 0.53 | 69.12 | 8985.17 |
| 140 | 10 | 32.55 | 0.05 | 0.54 | 75.95 | 10633.00 |
| 150 | 10 | 33.33 | 0.06 | 0.56 | 83.33 | 12498.75 |
| 160 | 10 | 34.45 | 0.06 | 0.57 | 91.87 | 14698.67 |
| 170 | 10 | 36.30 | 0.06 | 0.61 | 102.85 | 17484.50 |
| 180 | 10 | 38.20 | 0.06 | 0.64 | 114.60 | 20628.00 |
| 200 | 20 | 40.96 | 0.07 | 1.37 | 273.07 | 54613.33 |
| 260 | 60 | 45.82 | 0.08 | 4.58 | 1191.32 | 309743.20 |
| 320 | 60 | 35.50 | 0.06 | 3.55 | 1136.00 | 363520.00 |
| 380 | 60 | 25.20 | 0.04 | 2.52 | 957.60 | 363888.00 |
| 440 | 60 | 15.30 | 0.03 | 1.53 | 673.20 | 296208.00 |
| 500 | 60 | 9.82 | 0.02 | 0.98 | 491.00 | 245500.00 |
| 560 | 60 | 5.00 | 0.01 | 0.50 | 280.00 | 156800.00 |
| 620 | 60 | 3.28 | 0.01 | 0.33 | 203.36 | 126083.20 |
| 740 | 120 | 2.67 | 0.00 | 0.53 | 395.16 | 292418.40 |
| 860 | 120 | 2.32 | 0.00 | 0.46 | 399.04 | 343174.40 |
| 960 | 120 | 1.82 | 0.00 | 0.36 | 349.44 | 335462.40 |
| 1080 | 120 | 1.48 | 0.00 | 0.30 | 319.68 | 345254.40 |
| 1200 | 120 | 1.47 | 0.00 | 0.29 | 352.80 | 423360.00 |
| 1320 | 120 | 1.46 | 0.00 | 0.29 | 385.44 | 508780.80 |
| 1440 | 120 | 1.46 | 0.00 | 0.29 | 420.48 | 605491.20 |
| | | | | sum | 8633.06 | 4880545.99 |

**Table A.2** Data of tracer study of constructed wetland CW5.

| $T_1$(min) | $Dt_1$ | C(mg/L) | $C/C_0 = C_1$ | $C_1Dt_1$ | $t_1C_1Dt_1$ | $t_1^2C_1Dt_1$ |
|---|---|---|---|---|---|---|
| 10 | 10 | 1.46 | 0.00 | 0.02 | 0.24 | 2.43 |
| 20 | 10 | 2.42 | 0.00 | 0.04 | 0.81 | 16.13 |
| 30 | 10 | 4.26 | 0.01 | 0.07 | 2.13 | 63.90 |
| 40 | 10 | 8.11 | 0.01 | 0.14 | 5.41 | 216.27 |
| 50 | 10 | 11.90 | 0.02 | 0.20 | 9.92 | 495.83 |
| 60 | 10 | 12.50 | 0.02 | 0.21 | 12.50 | 750.00 |
| 70 | 10 | 16.89 | 0.03 | 0.28 | 19.71 | 1379.35 |
| 80 | 10 | 20.01 | 0.03 | 0.33 | 26.68 | 2134.40 |
| 90 | 10 | 21.11 | 0.04 | 0.35 | 31.67 | 2849.85 |
| 100 | 10 | 22.00 | 0.04 | 0.37 | 36.67 | 3666.67 |
| 110 | 10 | 23.67 | 0.04 | 0.39 | 43.40 | 4773.45 |
| 120 | 10 | 26.22 | 0.04 | 0.44 | 52.44 | 6292.80 |
| 130 | 10 | 28.91 | 0.05 | 0.48 | 62.64 | 8142.98 |
| 140 | 10 | 30.22 | 0.05 | 0.50 | 70.51 | 9871.87 |
| 150 | 10 | 34.66 | 0.06 | 0.58 | 86.65 | 12997.50 |
| 160 | 10 | 35.99 | 0.06 | 0.60 | 95.97 | 15355.73 |
| 170 | 10 | 36.50 | 0.06 | 0.61 | 103.42 | 17580.83 |
| 180 | 10 | 37.99 | 0.06 | 0.63 | 113.97 | 20514.60 |
| 200 | 20 | 38.21 | 0.06 | 1.27 | 254.73 | 50946.67 |
| 260 | 60 | 44.30 | 0.07 | 4.43 | 1151.80 | 299468.00 |
| 320 | 60 | 35.23 | 0.06 | 3.52 | 1127.36 | 360755.20 |
| 380 | 60 | 27.50 | 0.05 | 2.75 | 1045.00 | 397100.00 |
| 440 | 60 | 15.42 | 0.03 | 1.54 | 678.48 | 298531.20 |
| 500 | 60 | 10.67 | 0.02 | 1.07 | 533.50 | 266750.00 |
| 560 | 60 | 7.22 | 0.01 | 0.72 | 404.32 | 226419.20 |
| 620 | 60 | 5.56 | 0.01 | 0.56 | 344.72 | 213726.40 |
| 740 | 120 | 2.25 | 0.00 | 0.45 | 333.00 | 246420.00 |
| 860 | 120 | 1.87 | 0.00 | 0.37 | 321.64 | 276610.40 |
| 960 | 120 | 1.56 | 0.00 | 0.31 | 299.52 | 287539.20 |
| 1080 | 120 | 1.47 | 0.00 | 0.29 | 317.52 | 342921.60 |
| 1200 | 120 | 1.46 | 0.00 | 0.29 | 350.40 | 420480.00 |
| 1320 | 120 | 1.46 | 0.00 | 0.29 | 385.44 | 508780.80 |
| 1440 | 120 | 1.46 | 0.00 | 0.29 | 420.48 | 605491.20 |
| | | | | sum | 8742.63 | 4909044.47 |

**Table A.3** Data of tracer study of constructed wetland CW7.

| $T_1$(min) | $D\,t_1$ | C(mg/L) | $C/C_0 = C_1$ | $C_1Dt_1$ | $t_1C_1Dt_1$ | $t_1^2C_1Dt_1$ |
|---|---|---|---|---|---|---|
| 10 | 10 | 1.46 | 0.00 | 0.02 | 0.24 | 2.43 |
| 20 | 10 | 2.50 | 0.00 | 0.04 | 0.83 | 16.67 |
| 30 | 10 | 4.40 | 0.01 | 0.07 | 2.20 | 66.00 |
| 40 | 10 | 8.00 | 0.01 | 0.13 | 5.33 | 213.33 |
| 50 | 10 | 12.00 | 0.02 | 0.20 | 10.00 | 500.00 |
| 60 | 10 | 14.50 | 0.02 | 0.24 | 14.50 | 870.00 |
| 70 | 10 | 16.80 | 0.03 | 0.28 | 19.60 | 1372.00 |
| 80 | 10 | 19.88 | 0.03 | 0.33 | 26.51 | 2120.53 |
| 90 | 10 | 21.22 | 0.04 | 0.35 | 31.83 | 2864.70 |
| 100 | 10 | 23.55 | 0.04 | 0.39 | 39.25 | 3925.00 |
| 110 | 10 | 25.85 | 0.04 | 0.43 | 47.39 | 5213.08 |
| 120 | 10 | 26.10 | 0.04 | 0.44 | 52.20 | 6264.00 |
| 130 | 10 | 28.44 | 0.05 | 0.47 | 61.62 | 8010.60 |
| 140 | 10 | 32.82 | 0.05 | 0.55 | 76.58 | 10721.20 |
| 150 | 10 | 34.45 | 0.06 | 0.57 | 86.13 | 12918.75 |
| 160 | 10 | 35.50 | 0.06 | 0.59 | 94.67 | 15146.67 |
| 170 | 10 | 36.72 | 0.06 | 0.61 | 104.04 | 17686.80 |
| 180 | 10 | 38.20 | 0.06 | 0.64 | 114.60 | 20628.00 |
| 200 | 20 | 40.42 | 0.07 | 1.35 | 269.47 | 53893.33 |
| 260 | 60 | 48.28 | 0.08 | 4.83 | 1255.28 | 326372.80 |
| 320 | 60 | 36.25 | 0.06 | 3.63 | 1160.00 | 371200.00 |
| 380 | 60 | 27.03 | 0.05 | 2.70 | 1027.14 | 390313.20 |
| 440 | 60 | 15.83 | 0.03 | 1.58 | 696.52 | 306468.80 |
| 500 | 60 | 9.50 | 0.02 | 0.95 | 475.00 | 237500.00 |
| 560 | 60 | 8.02 | 0.01 | 0.80 | 449.12 | 251507.20 |
| 620 | 60 | 6.22 | 0.01 | 0.62 | 385.64 | 239096.80 |
| 740 | 120 | 2.22 | 0.00 | 0.44 | 328.56 | 243134.40 |
| 860 | 120 | 2.00 | 0.00 | 0.40 | 344.00 | 295840.00 |
| 960 | 120 | 1.67 | 0.00 | 0.33 | 320.64 | 307814.40 |
| 1080 | 120 | 1.49 | 0.00 | 0.30 | 321.84 | 347587.20 |
| 1200 | 120 | 1.46 | 0.00 | 0.29 | 350.40 | 420480.00 |
| 1320 | 120 | 1.46 | 0.00 | 0.29 | 385.44 | 508780.80 |
| 1440 | 120 | 1.46 | 0.00 | 0.29 | 420.48 | 605491.20 |
| | | | | sum | 8977.05 | 5014019.90 |

**Table A.4** Data of tracer study of constructed wetland CW9.

| $T_1$(min) | $D\,t_1$ | $C$(mg/L) | $C/C_0 = C_1$ | $C_1Dt_1$ | $t_1C_1Dt_1$ | $t_1^2C_1Dt_1$ |
|---|---|---|---|---|---|---|
| 10 | 10 | 1.46 | 0.00243 | 0.02433 | 0.24335 | 2.43350 |
| 20 | 10 | 2.44 | 0.00407 | 0.04067 | 0.81333 | 16.26667 |
| 30 | 10 | 4.40 | 0.00733 | 0.07333 | 2.20000 | 66.00000 |
| 40 | 10 | 7.75 | 0.01292 | 0.12917 | 5.16667 | 206.66667 |
| 50 | 10 | 11.86 | 0.01977 | 0.19767 | 9.88333 | 494.16667 |
| 60 | 10 | 13.65 | 0.02275 | 0.22750 | 13.65000 | 819.00000 |
| 70 | 10 | 16.68 | 0.02780 | 0.27800 | 19.46000 | 1362.20000 |
| 80 | 10 | 18.22 | 0.03037 | 0.30367 | 24.29333 | 1943.46667 |
| 90 | 10 | 20.51 | 0.03418 | 0.34183 | 30.76500 | 2768.85000 |
| 100 | 10 | 22.25 | 0.03708 | 0.37083 | 37.08333 | 3708.33333 |
| 110 | 10 | 25.85 | 0.04308 | 0.43083 | 47.39167 | 5213.08333 |
| 120 | 10 | 26.00 | 0.04333 | 0.43333 | 52.00000 | 6240.00000 |
| 130 | 10 | 28.28 | 0.04713 | 0.47133 | 61.27333 | 7965.53333 |
| 140 | 10 | 32.28 | 0.05380 | 0.53800 | 75.32000 | 10544.80000 |
| 150 | 10 | 34.48 | 0.05747 | 0.57467 | 86.20000 | 12930.00000 |
| 160 | 10 | 35.66 | 0.05943 | 0.59433 | 95.09333 | 15214.93333 |
| 170 | 10 | 36.99 | 0.06165 | 0.61650 | 104.80500 | 17816.85000 |
| 180 | 10 | 38.00 | 0.06333 | 0.63333 | 114.00000 | 20520.00000 |
| 200 | 20 | 40.35 | 0.06725 | 1.34500 | 269.00000 | 53800.00000 |
| 260 | 60 | 35.50 | 0.05917 | 3.55000 | 923.00000 | 239980.00000 |
| 320 | 60 | 25.50 | 0.04250 | 2.55000 | 816.00000 | 261120.00000 |
| 380 | 60 | 20.20 | 0.03367 | 2.02000 | 767.60000 | 291688.00000 |
| 440 | 60 | 14.48 | 0.02413 | 1.44800 | 637.12000 | 280332.80000 |
| 500 | 60 | 8.28 | 0.01380 | 0.82800 | 414.00000 | 207000.00000 |
| 560 | 60 | 5.34 | 0.00890 | 0.53400 | 299.04000 | 167462.40000 |
| 620 | 60 | 3.33 | 0.00555 | 0.33300 | 206.46000 | 128005.20000 |
| 740 | 120 | 1.46 | 0.00243 | 0.29200 | 216.08000 | 159899.20000 |
| 860 | 120 | 1.46 | 0.00243 | 0.29200 | 251.12000 | 215963.20000 |
| 960 | 120 | 1.46 | 0.00243 | 0.29200 | 280.32000 | 269107.20000 |
| 1080 | 120 | 1.46 | 0.00243 | 0.29200 | 315.36000 | 340588.80000 |
| 1200 | 120 | 1.46 | 0.00243 | 0.29200 | 350.40000 | 420480.00000 |
| 1320 | 120 | 1.46 | 0.00243 | 0.29200 | 385.44000 | 508780.80000 |
| 1440 | 120 | 1.46 | 0.00243 | 0.29200 | 420.48000 | 605491.20000 |
| | | | | sum | 7331.06 | 4257531.38 |

# CURRICULUM VITAE

Sukanda Chuersuwan currently works for Bureau of Research Development and Hydrology, Department of Water Resources, Ministry of Natural Resource and Environment. She received her B.Sc. from King Mongkut's Institute of Technology Landkrabang and M.A. from Mahidol University including M.Phil. from King Mongkut's University Technolgy Thonburi.