EMISSIONS OF GREENHOUSE GASES CARBON DIOXIDE AND METHANE FROM DUCKWEED SYSTEMS FOR STORMWATER TREATMENT

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

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And hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

This study determined the greenhouse gas emission from lab-scale duckweed treatment systems that were used for stormwater treatment. By using the static chamber technique, the fluxes of CO_2 emission from the duplicate duckweed systems were 1472 ± 721 and 626 ± 234 mg m^{-2} d^{-1}, respectively. After the complete removal of duckweeds, CO_2 emission from the systems decreased to 492 ± 281 and 395 ± 53 mg m^{-2} d^{-1}, respectively. A thin-film model was successfully applied to predict the increasing CO_2 concentrations approaching saturation in the static chamber. In contrast, the concentrations of methane in the closed chamber fluctuated a lot with time, which were attributed to complex methane production and consumption reactions at the soil-water interface. The CH_4 flux from the two duckweed systems were 299 ± 74 mg m^{-2} d^{-1} and 180 ± 91 mg m^{-2} d^{-1}, respectively. After the removal of duckweeds, the flux were 559 ± 215 mg m^{-2} d^{-1} and 328 ± 114 mg m^{-2} d^{-1}, respectively. The higher CO_2 emission in the duckweed systems was linked to more biomass debris formation on the soil surface due to duckweed growth and decay. As a result of duckweed growth, the duplicated duckweed systems removed 54 ± 13 % COD, 94 ± 4 % NH_4^+-N, 87 ± 7 % NO_3^--N, 34 ± 7 % PO_4^{3-}-P at the hydraulic retention time of 10 days. When the duckweeds were removed, the nutrient removal efficiencies decreased significantly: 68 ± 3 % for NH_4^+-N, 43 ± 7 % for NO_3^--N, 10 ± 6 % for PO_4^{3-}-P. The COD removal efficiency without duckweeds was 47 ± 6 %, which did not change significantly.

1 INTRODUCTION

1.1 Constructed Wetlands

Wetlands are transitional zone located between terrestrial ecosystems and aquatic ecosystems that saturated with water, and the vegetation that has adapted to the hydric soil distinguishes wetlands from other forms of land or water (Semeniuk and Semeniuk 1995). Wetlands are part of the foundation of water resources and play an important role to the health of waterways and ecosystems. They store floodwaters, supply downstream water requirements, and recharge groundwater. Wetlands are also beneficial by providing recreational services such as fishing, hunting and birds watching.

There are four main types of wetlands in United States —marshes, swamps, bogs, and fens. Marshes are dominated by herbaceous vegetation like grasses, rushes or reeds. Swamps have mostly woody plants; Bogs are mires that accumulate peat, a deposit of dead plant material; Fens are fed by mineral-rich surface water or groundwater and dominated by grasses and sedges, and typically have brown mosses in general (Keddy 2010). Wetlands vary widely because of differences in soils, topography, climate, hydrology, water chemistry, vegetation, and other factors (Carter 1996).

Constructed wetlands (CW) are wetland systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial community to assist in treating wastewater. During the past decades, constructed wetlands have become a preferable solution for stormwater runoff and sewage treatment because of their low costs, energy and maintenance requirements (Vymazal 2010). Constructed wetlands can provide a variety of functions including sediment retention, nutrient removal, and habitat restoration (Jordan et al. 2003). In the wetland treatment process, a combination of hydric soil, aquatic vegetation and hydraulics provides a unique aerobic and anaerobic environment, which is applicable in biological removal of organic contaminants and nutrients (nitrogen and phosphorus) (Cronk 1996). Therefore, constructed wetlands can be accustomed on the base of site selection, composition of substrate, type of vegetation, flow pattern, hydraulic pathways, retention time, etc. (Vymazal and Kröpfelová 2008).

In terms of water flow regime, there are two types of CWs, free water surface (FWS) or surface flow and subsurface flow (SSF), which mainly differ in the presence of a free water flow over the sediment surface (Brix 1994). Subsurface flow systems are designed to create subsurface flow through a permeable medium, keeping the water being treated below the surface, while free water surface systems are more similar to natural wetlands, with shallow flow over the saturated soil surface. Subsurface flow constructed wetlands are further divided into two groups: vertical flow (VF) systems and horizontal flow (HF) systems.

Both SSF and FWS systems are efficient in organic matter removal. SSF systems have higher potential for total organic matter and TSS removal than FWS systems. High effluent total COD and TSS due to the algal growth at spring months in the FWS indicate that subsurface flow systems are more reliable than FWS systems (Naz et al. 2009). On the other hand, FWS systems have better NO_3^--N removal efficiency than SSF systems (Tunçsiper et al. 2006). Also, SSF systems are less sensitive to low temperature and therefore suitable for winter operation (Kadlec 2009).

Three basic processes and mechanisms in constructed wetlands are physical, biological, and chemical removal processes. Some are non-destructive which only relocate the pollutants, such as volatilization, phytovolatilization, plant uptake, phytoaccumulation, sorption, and sedimentation. On the other hand, some are destructive like phytodegradation and microbial degradation (Imfeld et al. 2009). Several pathways are available to eliminate contaminants in a complex constructed wetland system. For instance, a batch-scale study to characterize benzene biodegradation processes revealed that benzene was degraded aerobically, mainly via the monohydroxylation pathway by combining carbon and hydrogen isotope signatures followed by two-dimensional stable isotope analysis. At least 85% of benzene was degraded by this pathway and thus, only a small fraction was removed abiotically or through other mechanisms (Rakoczy et al. 2011).

Constructed wetlands are not only widely used in wastewater treatment (Kadlec and Wallace 2008), they have also been used for stormwater treatment as well in recent years (Mungasavalli and Viraraghavan 2006).

1.2 Stormwater Management

Stormwater is precipitation from rain and snowmelt events. As stormwater runoff flows over impervious surfaces (e.g., parking lots, roads, buildings, and compacted soil), it accumulates pollutants such as nutrients, metals, and other chemicals that could adversely affect water quality and degrade ecosystem health. The runoffs are often classified as nonpoint source pollution that requires treatment. With more stringent regulations, nowadays, most stormwater discharges are considered point sources and require applying for an national pollutant discharge elimination system permit. In the United States, it requires best management practices (BMPs) in stormwater management (USEPA 2005). BMPs can be both structural or engineered control devices and systems to treat or store polluted stormwater, as well as operational or procedural practices. Wetlands are one of the most effective BMPs in terms of pollutant removal and their monetary and entertainment values. As stormwater runoff flows through the wetland, pollutants can be removed through physiochemical and biological means. In the meantime, wetland also provides a significant volume of temporary storage for stormwater influent (Niemczynowicz 1999).

The main pollutants conveyed by stormwater are heavy metals, nutrients, suspended solids, and organic matter. Sedimentation is the dominant removal process for particulate pollutants operating within a stormwater treatment system. It has long been recognized as the principal process in the removal of heavy metals from stormwater in natural or constructed wetlands. However, there are a range of other processes including filtration, adsorption, biological uptake/assimilation, biodegradation, chemical transformation and volatilization that may also play roles (Walker and Hurl 2002). For the removal of suspended solids, the vegetation provides hydraulic resistance, thus facilitating physical filtration, which enhance sediment removal. The root network of the plants helps reducing the potential of particle resuspension. For the removal of heavy metals and other chemical pollutants, adsorption of pollutants to the surfaces of bottom sediments, wetland vegetation and organic detritus could be significant.

Nitrogen is removed in CWs via three major processes: assimilation (also referred to as N uptake), adsorption, and biological nitrification coupled with denitrification (Ye and Li 2009). Major classical nitrogen removal routes in CWs and stormwater retention ponds are shown in Figure 1.1. Higher total nitrogen removal during summer time is attributed to lower dissolved oxygen (DO), higher temperature and increased microbial activity, which likely result in the higher nitrification and denitrification rates (Borne et al. 2013). Higher organic matter availability in the pond due to release of root exudates and supply of detritus from plant decay may have contributed to floc formation in the water column, increasing particulate nitrogen settlement.

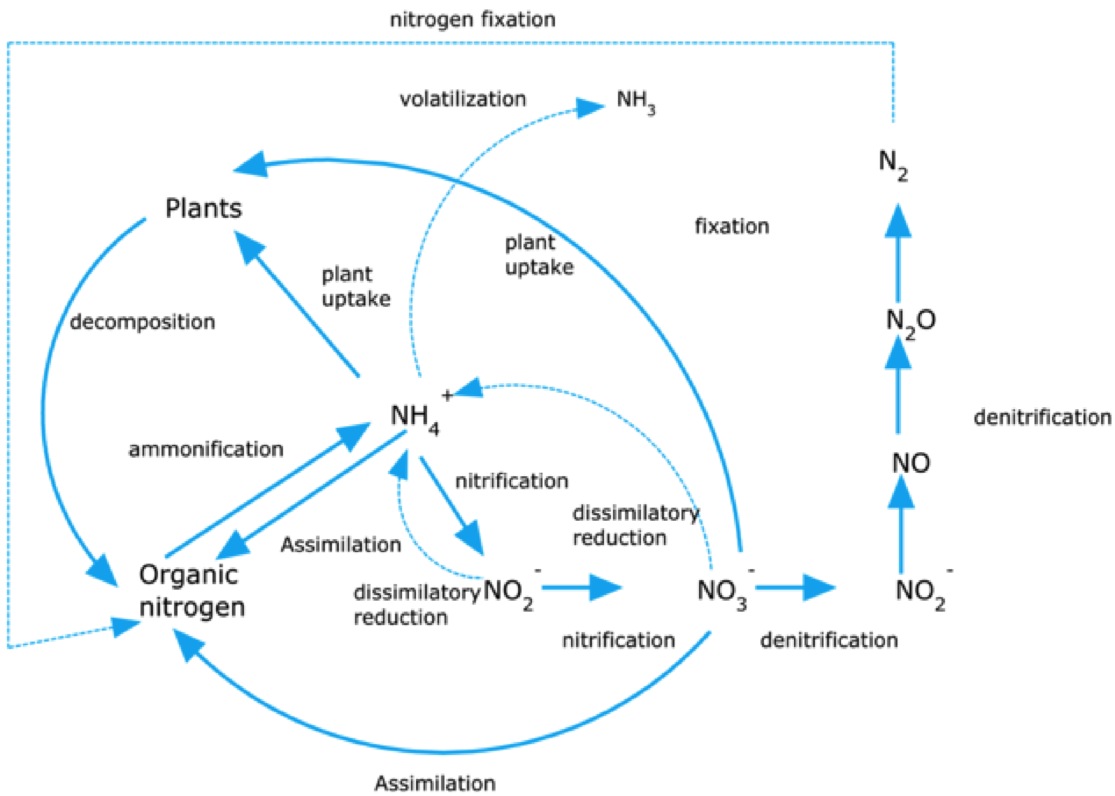


Figure 1.1 Nitrogen cycle and major nitrogen removal pathways in constructed wetlands (Saeed and Sun 2012)

The phosphorus dynamics during stormwater treatment in CWs include adsorption/desorption, precipitation/dissolution, fragmentation/leaching, mineralization, sedimentation and burial, and some of the processes have limited capacity (Mann and Bavor 1993, Pant 2007, Reddy and D'angelo 1997, Sakadevan and Bavor 1998, Song et al. 2007). The major phosphorus removal processes are sorption, precipitation, plant uptake and sedimentation (Vymazal 2007). Adsorption is a key removal process for phosphorus as sediments in the wetlands and freshwater marshes have a great capacity for P adsorption (Lai and Lam 2009).

A variety of biotic and abiotic processes can contribute to the removal of organic substances in stormwater runoff. Microorganisms break down organic matter in order to produce new biomass, reproduce, and sustain life through aerobic, anoxic, and anaerobic degradation. In an aerobic environment, oxygen is present and serves as the terminal electron acceptor. This is the most efficient conversion of starting material to end products. In an anoxic environment nitrates and nitrites serve as the terminal electron acceptor, which are reduced to form nitrogen gas. Anoxic reactions are less efficient than aerobic reactions. In anaerobic environments organisms use sulfates, carbon dioxide and organics as the terminal electron acceptor. The reactions the organisms use to break organics into energy yield energy to support their growth.

1.3 Vegetation

The wetland vegetation is an important component in the treatment process that occurs in constructed wetlands. The presence of macrophytes is one of the most significant features of wetlands and their presence distinguishes CWs from unplanted soil filters or lagoons (Brix 1997, Vymazal 2011).

Plant efficiency in promoting CW performance depends on several factors: CW type (e.g., vertical, horizontal, surface, or subsurface flow, with or without recirculation), quality and quantity of the wastewater loads (Sklarz et al. 2009), plant species and their combinations (Brisson and Chazarenc 2009), climate, medium type, and plant management, such as harvesting regime. Many studies report a significant and positive effect of plants on CW performance. Plants can improve removal efficiency or contribute to the CW in many ways include: filtering effect, provision of surface for microbial attachment, plant uptake and metal phytoremediation (Langergraber 2005, Shelef et al. 2013). Moreover, the richness of plant species increases nitrogen removal in CWs because of the greater nitrogen accumulation in plant tissues at the higher species richness level (Chang et al. 2014, Fargione et al. 2007, Spehn et al. 2005).

Wetland vegetation also plays a vital role in wetland ecology by performing a number of significant functions (Cronk and Fennessy 2001) and may have a direct impact on soil microbial community (Zak et al. 2003). Plant exudation also affects microbial processes and pore water quality. In this way, plants increase the efficiency of nitrogen removal from the wastewater by supporting denitrifying microorganisms with easily decomposable organic matter (Picek et al. 2007).

In FWS systems with shallow water table and low water flow, the system supports the growth of floating, submerged and/or emergent plants. Floating plants have their photosynthetic parts at or just above the water surface with roots extending down into the water column. These roots are an excellent medium for the filtration/adsorption of suspended solids and growth of bacteria, and nutrients are taken up from the water through them. During photosynthesis, floating aquatic plants use atmospheric oxygen and carbon dioxide.

The penetration of sunlight into water is reduced when floating plants exist, in the meantime the gas transfer between water and atmosphere is restricted. Floating plants suppress algae biomass and in turn lead to neutral pond conditions. However, some molecular oxygen produced by photosynthetic tissue is translocated to the roots and keep root microorganisms' growth aerobically, though the surrounding water is anaerobic/anoxic (Papadopoulos and Tsihrintzis 2011).

Duckweeds are tiny free-floating vascular plants found throughout the world. Their morphology is extremely simple as they have no stems or true leaves, and usually consist of a single or a few flat, oval-shaped and small leaf-like fronds. They are classified under the *Lemnaceae* family which consists of about 40 species in five genera; *Spirodela, Lemna, Landolita, Wolffiella* and *Wolffia* (Haustetn et al. 1990).

Duckweeds are often chosen to grow in the constructed wetlands for stormwater treatment ponds because of their fast growth. The plant density on the water surface depends on the availability of nutrients, temperature conditions and the frequency of harvest (Frederic et al. 2006).

The growing plants form a floating mat on the surface of the water and this surface cover minimizes light penetration into the water column, which have been used as a means of impeding light penetration and consequently precluding photosynthetic algal growth in wastewater treatment ponds (Brix 1993). Duckweed coverage on water surface in the CWs creates a low dissoved oxygen environment for microbial growth because of the poor light penetration, which inhibits oxygen production by other phytoplankton such as algae (Sims et al. 2013). Algae elimination also has effects on stabilizing water pH (Zirschky and Reed 1988). Also, the growth of duckweeds form as a mat on the water surface makes it very easy to harvest (Bonomo et al. 1997). On the other hand, duckweeds are also sensitive to inhibition and their growth can be limited by high metal concentrations, presence of PCBs and ethylene, as well as filamentous algae or fungus (Zirschky and Reed 1988).

Nutrient removal in duckweed ponds is mainly through plant uptake (metabolism and bioaccumulation) and subsequent removal from the system by harvesting of the plant biomass (Harvey and Jackson 1973). Nitrogen removal by duckweed depends on the combined action of ammonium transport and duckweed ammonium uptake at the surface (Chaiprapat et al. 2003), with the nitrogen uptake by duckweeds ranging from 0.26 $gN/m^2 \cdot d$ to 0.59 $gN/m^2 \cdot d$. Duckweeds also assimilate phosphorus in the orthophosphate form (Culley et al. 1981). The plants' ability to uptake P depends on the growth rate, harvesting frequency and the available ortho-P (Iqbal 1999). A pilot study of constructed wetlands using duckweeds was operated on domestic primary effluents for water reuse purposes in desert areas, and the nitrogen removal was 10-20% with the influent concentration of 51.0 ± 7.1 mg/L while phosphorus removal was negligible with the influent concentration of 60.5 ± 7.8 mg/L (Ran et al. 2004).

A laboratory scale study by Al-Nozaily and Alaerts was conducted on oxygen balance and organic matter removal from duckweed (*L. gibba*)-covered sewage lagoons. They found out that removal of COD did not differ in duckweed-covered and control reactors, and the role of duckweed cover was marginal in changing the redox potential or the DO in the deeper reactors (Al-Nozaily et al. 2000).

1.4 Greenhouse gas emission from stormwater treatment systems

As a byproduct from the removal of organic matter and nutrients in the CWs, the emission of greenhouse gases (GHG) in such systems for stormwater treatment can be significant but it is still not well studied.

A GHG is a gas in an atmosphere that absorbs and emits radiation within the thermal infrared range. The primary greenhouse gases in the earth's atmosphere are carbon dioxide, methane and nitrous oxide (Robertson et al. 2000), which contribute global warming. Global atmospheric concentrations of carbon dioxide, methane and nitrous oxide have increased remarkably as a result of human activities since 1750 and now far exceed pre-industrial values determined from ice cores spanning many thousands of years (Alley et al. 2007). The global increases in carbon dioxide concentration are due primarily to fossil fuel use and land-use change, while those of methane and nitrous oxide are primarily due to agriculture (Solomon 2007). The global warming potential (GWP) depends on both the efficiency of the molecule as a GHG and its atmospheric lifetime. GWP is measured relative to the same mass of CO_2 and evaluated for a specific timescale. Although CO_2 is often the GHG given the most attention in popular media due to the contributions of human activity to its rising levels, methane (CH_4) has about 25 times the potential for global warming per molecule and nitrous oxide (N_2O) is about 300 as dangerous per molecule as CO_2 (Lashof and Ahuja 1990).

Natural and constructed wetlands affect the global balance of the key greenhouse gases, CO_2 and CH_4. They act as sinks for CO_2 by photosynthetic assimilation from the atmosphere and sequestration of the organic matter produced in the wetland soil. On the other hand, wetlands are sources of CH_4 and N_2O (Sovik and Klove 2007, Uggetti et al. 2012, Wang et al. 2008b). Most wetlands are inherently net sources of gaseous compounds like methane and nitrous oxide, which are of environmental concern due to their rapid accumulation in the atmosphere and their potent global warming capacity (Johansson et al. 2004).

In treatment wetlands, various gaseous substances, such as CO_2, CH_4, N_2, N_2O, and NH_3 are generated through volatilization during organic material and nitrogen removal processes. They are emitted from the soil either by diffusion through the water/air interface or by active transport through the wetland plants. Many species of emergent macrophytes 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 (Picek et al. 2007).

Life cycle assessment (LCA) is a powerful tool to determine the environmental impacts of constructed wetlands. The LCA results suggest that constructed wetlands have less environmental impact compared to different treatment performance scenarios and to conventional wastewater treatment, in terms of resource consumption and greenhouse gas emissions (Fuchs et al. 2011).

A study focused on the carbon balance of North American wetlands stated that North American wetlands contain about 220 Pg C, most of which is in peat (Bridgham et al. 2006). The carbon sink has a small to moderate amount of about 49 Tg C yr^{-1}, although the uncertainty around this estimate is greater than 100% because the role of carbon sequestration by sedimentation in freshwater wetlands is the largest unknown factor. U.S. produces 0.05 Tg yr^{-1} of CO_2 equivalent through peat extraction. It is also estimated that North American wetlands emit 9 Tg methane (CH_4) yr^{-1} with the uncertainty of this estimate is also greater than 100%. CH_4 emissions from wetlands may largely offset any positive benefits of carbon sequestration in soils and plants in terms of climate forcing. The destruction of wetlands through land-use changes has had the largest effects on the carbon fluxes. The primary effects have been a reduction in their ability to sequester carbon, oxidation of their soil carbon reserves upon drainage and reduction in CH_4 emissions, however reduction in methane has far less benefits compared to the loss of wetland functions (Bridgham et al. 2006).

While plants and algae utilize carbon dioxide in photosynthesis, CO_2 is produced by respiration in the root system of plants, and by microbial processes in soils and sediments. Oxidation of carbonaceous components in water also results is CO_2 release. As a result, CO_2 are actively present in wetlands, some as influxes to the green plants, and some as releases (Kadlec and Wallace 2008).

Organic carbon in wetlands can be transformed to CO_2 and other gaseous forms. A horizontal subsurface flow constructed wetland planted with *Phragmites australis*. Treating municipal wastewater had gas emissions ranging from 4 to 309 mg CO_2-C/$m^2 \cdot h$ and from 0 to 93 mg CH_4-C/$m^2 \cdot h$. The amount of C emitted was higher than carbon input in the wastewater; it was calculated that between one fourth and one third of total carbon emissions originated in plants and 10% of total carbon emissions were in the form of CH_4 (Picek et al. 2007).

Temperature, substrate supplies and biochemical environment with different degrees of oxidation are the principal controls on CH_4 fluxes from all soils. The transport of CH_4 from anaerobic sites in wetland soils to the troposphere involves a number of mechanisms including diffusion, ebullition and transport by rooted macrophytes. Organic carbon in wetland soils and water is essential for CH_4 production, even though only acetate and CO_2 can be directly converted to methane by the methanogens (Bryant 1979). In the anoxic environment, when nitrate concentration increases, CH_4 emission decreases due to depression of methanogenesis (Guo et al. 2009). In a study of a constructed wetland purifying peat mining runoff waters, there was a positive correlation between NO_3^- and emission of CH_4. On the other hand, if NH_4^+ concentrations increase, the in situ CH_4 oxidation is more inhibited and as a result CH_4 emission increases (Sovik and Klove 2007).

Vegetation increases the flux of CH_4 as well. Different plant species in the treatment cells affected the CH_4 emission flux. In addition, the CH_4 flux variation is because activities of methanogens and methanotrophs and the relationship between CH_4 flux rate and some important environmental parameters can be quite different in CWs with different plant species. With the same aquatic plant, the GHG emission intensity is related to influent organic pollutant concentrations (Wang et al. 2008a).

The low oxidation-reduction potential (ORP, an important indicator to reflect the oxiation status of soil from aerobic to anaerobic at different depths) value is another important factor affecting CH_4 production and consumption in soil. The ORP values remained high in the upper and plant rhizosphere, reflecting oxidation status in these zones. It is therefore 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 (Wang et al. 2008a).

Nitrous oxide is another important GHG in wetlands has 298 times the global warming potential of CO_2 (Ipcc 2007). N_2O production was positively correlated with nitrate concentration. Carbon dioxide production was also highest at the highest nitrate concentration, which indicates that increased nitrate loading on ponds and wetlands will stimulate organic matter decomposition rates under anoxic conditions due to denitrification (Stadmark and Leonardson 2005).

Denitrification in wetlands and ponds is the primary process in the emission of nitric oxide (NO) and nitrous oxide (N_2O). Denitrification proceeds through a series of reduction processes , ultimately leading to the formation of dinitrogen gas. In addition, partial oxidation of ammonia (partial nitrification) may also contribute to N_2O formation (Chuang et al. 2007). The typical median emission rates of N_2O emission in various CW types like FWS, VSSF and HSSF CWs are 0.09, 0.12, and 0.13 mg N m^{-2} h^{-1} correspondingly (Mander et al. 2014).

Nitrous oxide emission is also seasonal (Sovik and Klove 2007) as denitrification is strongly seasonal, with larger rates in warm seasons, therefore some studies has showed that. The presence and the type of vegetation, mainly due to changes in the sediment carbon and nitrogen content, was discovered to be correlated negatively to the ratio between nitrate and nitrite reducers and positively to the ratio between nitrite and nitrous oxide reducers. These results suggest that the potential for nitrous oxide emissions is higher in vegetated sediments (Garcia-Lledo et al. 2011). Beside, plant species richness can also increase N_2O emissions (Chang et al. 2014).

The GHG emission from wetlands data is summarized by its wastewater type and plant species and shown in Table 1.1

Table 1.1 Literature data of carbon dioxide (CO_2-C), methane (CH_4-C) and Nitrous oxide (N_2O-N) emissions from CWs (Mander et al. 2014)

Waste-water type	Study site, country	Plant species	CO_2-C flux ($mg\ m^{-2}h^{-1}$)	CH_4-C flux ($mg\ m^{-2}h^{-1}$)	N_2O-N flux ($mg\ m^{-2}h^{-1}$)	References
Domestic wastewater	Nykvarn, Sweden	*Typha latifolia*	n.a.	1.9	0.081	(Johansson et al. 2003)
Domestic wastewater	Nykvarn, Sweden	*Phalaris arundinacea*	n.a.	3.6	0.152	(Johansson et al. 2003)
Domestic wastewater	Nykvarn, Sweden	*Glyceria maxima*	n.a.	1.8	0.031	(Johansson et al. 2003)
Domestic wastewater	Nykvarn, Sweden	*Lemna minor*	n.a.	7.7	0.094	(Johansson et al. 2003)
Domestic wastewater	Nykvarn, Sweden	*Spirogyra spp*.	n.a.	1.9	0.036	(Johansson et al. 2003)
Domestic wastewater	Nykvarn, Sweden	Plots without plants	n.a.	2.8	0.192	(Johansson et al. 2003)
Domestic wastewater	Lakeus, Finland	*Phragmites australis, T. latifolia*	108.3	8.4	0.007	(Søvik et al. 2006)
Domestic wastewater	Ruka, Finland	*Carex–Sphagnum*	95.8	4.4	0.106	(Søvik et al. 2006)

Domestic wastewater	Skallstuggu, Norway	*P. australis*	87.5	5.8	0.041	(Søvik et al. 2006)
Agricultural non-point pollution	Hovi, Finland	*T. latifolia, Scirpus sylvaticus, Alisma, plantago-aquatica, P. arundinacea. Filipendula ulmaria, Iris pseudacorus, Juncus conglomeratus*	29.4	1.6	0.001	(Søvik et al. 2006)
Dairy farm wastewater	Ngatea, New Zealand	*Schoenoplectus validus*	n.a.	8.5	n.a.	(Tanner et al. 1997)
Dairy farm wastewater	Truro, Nova	Non-vegetated	n.a.	12.5	0.25	(Tanner et al. 1997)
Dairy farm wastewater	Scotia, Canada	*T. latifolia*	176	10.8	n.a.	(VanderZaag et al. 2010)
Raw municipal wastewater	Jiaonan, China	*P. australis, Acorus calamus L minor*	n.a.	5220.0	0.068	(Tai et al. 2002)

1.5 Microorganisms related to GHG emission

Microorganisms in wetland soils play an important role in organic and nutrient removal and GHG emission. The aerobic microorganisms, like heterotrophs, consume oxygen to degrade organic matter and convert organic carbon to carbon dioxide. A variety of anaerobic microorgansisms, including bacteria and archaea, work synergistically to convert organic matter into methane and carbon dioxide.

Methane is only produced by methanogens, a group of strictly anaerobic archaea. Microbial methanogenesis is the process that methanogenic archaea reduce organic carbon to methane in anaerobic, carbon-rich environments such as ruminant livestock, rice paddies, landfills, and wetlands. About 70% of the methane is formed from acetate through acetoclastic mathogenesis and the remaining 30% from carbon dioxide and hydrogen through hydrogentrophic methanogenesis (Gandy and Gandy 1980). At least three different groups of microorganisms are involved in anaerobic degradation and methane production. They are hydrolytic-fermentative bacteria, acetogenic bacteria and methanogens (Inamori et al. 2007). Not all of the methane produced in wetlands and stormwater treatment systems ends up in the atmosphere because the existence of methanotrophic bacteria, which oxidize methane into CO_2 in the presence of oxygen. An overview of methanotrophs and their metabolic pathways for utilizing methane is shown in Figure 1.2. When methanogens in the soil produce methane faster than the consumption by methanotrophs, methane escapes into the atmosphere (Willey et al. 2008). Methanotrophs are therefore important regulators of methane fluxes in the atmosphere. However they are difficult to isolate due to the firm attachment to soil particles and their slow growth in the nature.

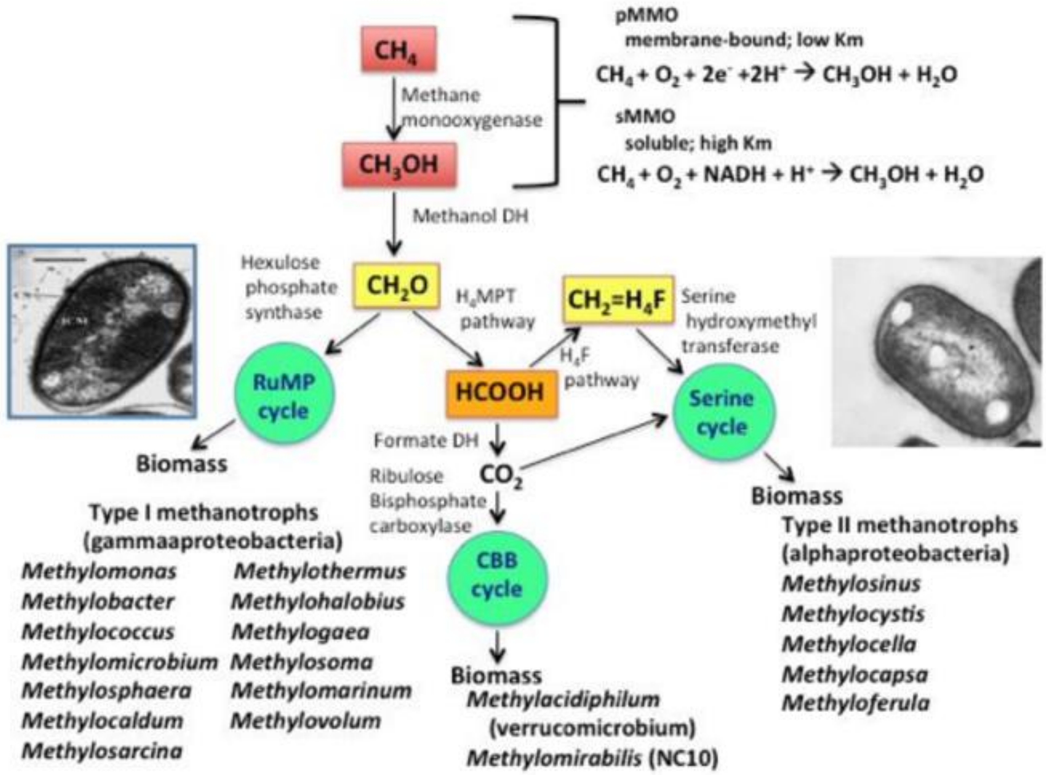


Figure 1.2 The overview of aerobic methanotrophs and their metabolic pathways for methane oxidation.[1]

Methane may be oxidized at the interface between the anoxic and oxic interface, which can be at the surface of soil and in the rhizosphere of aquatic plants. The concentration gradients of methane and oxygen may overlap and methane emission will decrease because of oxygen diffusion through roots (Inamori et al. 2007). The more that CH_4-oxidizing bacteria inhabit the area, the less CH_4 emission occurs. Although the existence of CH_4-oxidizing bacteria in marshes is believed to depend upon the types of plants there, few studies have compared the effect of different plant species on the efficiency of removing specific pollutants, or of CH_4 or N_2O emissions.

[1] Courtesy: http://www.methanotroph.org/wiki/introduction

In addition to CH_4 and CO_2 emission due to microbial activity, many microorganisms are involved in nitrogen cycle in constructed wetlands. N_2O emissions can be affected by the presence or absence of vegetation in a FWS system as incomplete denitrification may result in the net N_2O emission (Garcia-Lledo et al. 2011). In the process of nitrification (during which ammonia is oxidized to nitrate), microbes release NO and N_2O, two critical greenhouse gases, into the atmosphere as intermediates. Ammonia-oxidizing organisms including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are involved in nitrification processes (Sims et al. 2013).

There are many factors affecting microbial activities and ultimately GHG emission. High concentrations and fluxes of dissolved organic matter (DOM) in paddy soils from plant debris trigger microbial activity and thus the emission of GHG. Retention of DOM by soil minerals and its subsequent stabilization against microbial decay depend on the redox state (e.g. DOM precipitation by Fe^{2+} under anaerobic conditions) (Kögel-Knabner et al. 2010). Furthermore, the type of plant had a more important effect on bacterial communities than did hydraulic loadings (Calheiros et al. 2009).

At the soil-water interface, aerobic and anaerobic microbes may live together, each within microsites. The aerobic microbes such as methanotrophs live near the surface of the soil particles and prefer to grow in aerobic microsite through the oxidation of CH_4. The aerobic microbes use up the available oxygen creating an anaerobic environment that favors the growth of anaerobic microbes such as methanogens involved in CH_4 generation. High potential of CH_4 emission is often linked to more abundant methanogens and relatively low amount of methanotrophs (Wang et al. 2008a).

1.6 Duckweed Pond for Stormwater Treatment

Wet detention ponds are common stormwater treatment systems for attenuation of flow and removal of pollutants. One novel approach to improve water quality in a wet detention pond is to retrofit with floating treatment wetlands (FTWs) (White and Cousins 2013). FTWs are composed of emergent aquatic plants grown on a mat floating on the water surface, rather than rooted in the bottom sediments (Tanner and Headley 2011). FTWs have been applied in stormwater treatment in some mesocosm and pilot studies, and the available data on removal of key pollutants such as organic matter, suspended solids, nutrients, and metals show that they can significantly enhance performance of pond systems, and provide similar or better performance than surface flow wetlands (Headley and Tanner 2012). The reason why the inclusion of FTWs in stormwater retention ponds improve N removal is due to two reasons: 1) higher release of plant detritus increases particulate organic nitrogen settlement; 2) lower DO induced by FTWs increases organic carbon availability and microbial activity (Borne et al. 2013).

Duckweed ponds may be viewed as a special type of FTWs, which has a similar floating mat. Duckweed ponds have been used for wastewater treatment and nutrient removal. It shows that duckweed ponds are suited as polishing step for heavy metal removal, especially Cr and Zn at lower concentration, but a pre-treatment step in wastewater treatment is required for the treatment of high loads of heavy metal (Sekomo et al. 2012). They have also been successfully used in swine waste polishing and have a significant improvement in the effluent quality with the removal of 98.0% of the Total Kjeldahl Nitrogen (TKN) and 98.8% of the Total Phosphorous (TP) (Mohedano et al. 2012). Systems consisted of two duckweed ponds in series were found to be suitable for the treatment of domestic pre-settled wastewater, and they demonstrated highly efficient removal of both organic matter and nutrients (Ben-shalom et al. 2014).

Duckweed ponds are the source of GHG emission as well. Although they act as a sink of CO_2 due to plant photosynthesis, duckweed ponds are considered as net sources of GHG including CO_2 and CH_4 (Silva et al. 2012). There was a linear correlation between the organic loading rates and CH_4 emission, and 30% of the COD removed was converted to CH_4 (Hernandez-Paniagua et al. 2014).

Few studies have been reported about the use of duckweed ponds for stormwater treatment (Kerr-Upal et al. 2000, Sims and Hu 2013). In terms of duckweed types, monoculture *Lemna minor* removed the largest amount of ammonia from stormwater and had the largest biomass density. However, a polyculture of *Lemna minor* and *Spirodela polyrhiza* was the most stable nutrient sink and removed the highest amount of phosphorus from stormwater (Perniel et al. 1998).

1.7 Research Objectives

As demands for stormwater treatment is increasing, duckweed ponds which has similar principles as floating treatment wetlands may be an effective solution in organic matter and nutrients removal. However, the process of pollutants removal in duckweed ponds by microorganisms can also produce greenhouse gas such as carbon dioxide, methane, and nitrous oxide. In addition, duckweeds as the primary plant in the systmes create low dissoved oxygen environment and are able to uptake nutrients. With no harvesting, the biomass debris may also involve in the carbon cycle. Therefore, it is important to determine the role of duckweeds in nutrients removal and greenhouse gas emission.

The objectives of this study were:

- To determine greenhouse gas (CH_4, N_2O and CO_2) emissions in duckweed ponds treating stormwater;
- To evaluate the efficiency of duckweed pond in nutrient removal;
- To determine the abundance and role of microorganisms involved in nutrient removal and GHG emissions from the duckweed ponds.

2 MATERIALS AND METHODS

2.1 Duckweed Treatment Pond Design and Operation

Two lab-scale duckweed treatment tanks were set up in parallel with detailed design and operating information described elsewhere (Sims et al. 2013). Briefly, the duplicate systems were made of glass, each with a dimension of 1.0 m (length) $\times$ 0.36 m (width) $\times$ 0.44 m (depth). A layer of gravel (size = 2 cm) was filled at the bottom to a depth of 5 cm and then a 15 cm thick layer of hydric soil collected from a marshland close to the Columbia Water Treatment Plant (Columbia, MO) was topped on them. The synthetic stormwater filled the tanks a height of 42 cm with the water height of 20 cm. Each tank was divided into three cells by two baffles (installed vertically) to prevent short-circuiting of water flow through the system. Both systems were run under almost identical conditions for more than 200 days. Fluorescent lights (40 W) provided artificial illumination (light intensity =37-40 $\mu molm^{-2}s^{-1}$) at the water surface with a light period of 12 hours per day at the room temperature (23$\pm$1ºC). Duckweed seeds (*Lemna minor*) were originally from the wetland soils. During the whole study period, the duckweed fully covered the surface of water in both tanks with an average biomass fresh weight of 1236 ± 3 g /m^2.

Each tank was fed with synthetic stormwater, which contained the following chemicals per liter (details in Table 2.1): 0.05 g glucose, 0.05 g beef extract, 0.001 g glycine (NH_2CH_2COOH), and other macro/micro nutrients, based on similar synthetic stormwater mixtures (Davis et al. 2001, Hatt et al. 2007). Both systems were operated at a hydraulic retention time (HRT) of 10 days.

Table 2.1 Chemical makeup of synthetic stormwater

Water Quality Parameters	Concentration (mg/L)	Source
COD	100	Beef extract and glucose
Ammonium-N	1.4	NH_4Cl
Nitrate-N	2.07	$NaNO_3$
Organic nitrogen	3	Glycine and beef extract
Phosphorus	0.5	Na_2HPO_4
Copper	0.1	$CuSO_4$
Lead	0.1	$PbCl_2$
Zinc	0.1	$ZnCl_2$

On day 176, all duckweeds were removed from the tanks to evaluate the role of surface vegetation (duckweeds) in stormwater treatment and greenhouse gas emission.

2.2 Water Chemical Analysis

Water quality parameters such as influent and effluent chemical oxygen demand (COD), total phosphorus, NH_4^+-N, NO_2^--N and NO_3^--N were determined once a week following the Standard Methods (APHA, 2005). All tests were conducted in duplicate.

2.3 Greenhouse Gas Emission Rate Measurement

The static chamber technique (Uggetti et al. 2011) was adopted for quantification of surface fluxes of greenhouse gases at the water-air interface. Static chambers were sealed gas-tight (Figure 2.1). The funnel-shaped chambers were made of plastic and consisted of two parts. The chamber had a total volume of 4 L with the bottom surface area of 400

cm^2. The chamber was placed in the middle part of each tank and pressed into the soil to a depth of around 5 cm to ensure airtightness.

The flux was estimated by the accumulated concentration of a given gas in the chamber,

$$J = \frac{V}{A}\frac{\mathrm{d}C}{\mathrm{d}t} \tag{2.1}$$

Where:

J= flux of gas, mg $m^{-2}d^{-1}$

V = chamber volume, m^3

A = area of soil surface enclosed by the chamber, m^2

dC/dt = time rate of change of gas concentration in the air within the chamber, mg m^{-3} d^{-1}

Gas samples were collected every 2 days for 14 days through the sampling port using a 250 µL syringe. Carbon dioxide and methane content in the gas sample were analyzed by gas chromatography (GC, Shimadzu 2014) equipped with a thermal conductivity detector (TCD), along with the use of ShinCarbon ST 80/100 Column (Restek, PA) as separation column and helium gas as carrier gas. The GC operating parameters were as follows: injection temperature, 100 ºC; flow rate, 10 ml/min; column temperature, held at 40 ºC for 3 min, then increased to 150 ºC at 10 ºC/min and held for 1 min while the TCD temperature was held at 200 ºC. Other gas components such as hydrogen and N_2O were not detected.

Figure 2.1 A static chamber used to collect gas samples for GHG emission analysis.

2.4 Thin-Film Model for Air-Water Exchange

A thin-film model was applied to understand CO_2 release from water to the atmosphere. Volatile chemicals partition themselves between water and air phases. At equilibrium, the ratio of concentrations from these phases is described by a partitioning coefficient called Henry's Law Constant (H), which is defined as,

$$H = \frac{\text{equilibrium concentration in air}\,[\text{mass / volume air}]}{\text{equilibrium concentration in water}\,[\text{mass / volume water}]} \tag{2.2}$$

The model is based on the assumption that a laminar sub-layer exists on both sides of the air-water interface. A chemical must pass through both an air-side and a water-side laminar sub-layer to move from the air into the water or vice versa. We can estimate the net mass exchange if we assume that the transport through these layers controls the overall flux.

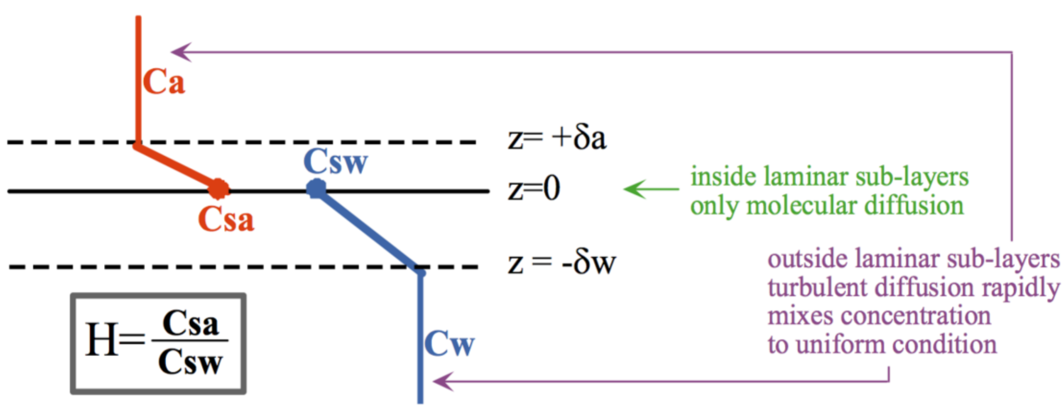


Figure 2.2 The Thin-Film Model describes the exchange of volatile species across the air-water interface under conditions for which transport is limited by diffusion across the laminar sub- layers.

As shown in

Figure 2.2, the depth of laminar sub-layer of water side and air side are $_w$ and $_a$, respectively. Outside the laminar sub-layers turbulent diffusion is sufficient to make the concentrations in the water (C_w) and in the air (C_a) uniform. Within the laminar sub-layers molecular diffusion is functioning, so the concentration profile must be linear under steady-state conditions. We assume that chemical equilibrium exists at the interface ($z = 0$), such that the dissolved phase concentration at the surface (C_{sw}) is in equilibrium with the air phase concentration at the surface (C_{sa}), so $C_{sw} = C_{sa} / H$. As a final constraint, if we assume that there are no sources or sinks of chemical within the laminar sub-layers, then the flux through the water-side boundary layer must equal the flux through the air-side boundary layer. This constraint gives us,

$$\dot{m} = -D_a A \left(\left. \frac{\partial C}{\partial z} \right|_{z=0} \right)_a = -D_w A \left(\left. \frac{\partial C}{\partial z} \right|_{z=0} \right)_w \qquad (2.3)$$

where D_a and D_w represent the molecular diffusion in air and water. Using the end-point concentrations to define the gradients results in

$$D_a A \frac{(C_a \quad C_{sa})}{_a} = \quad D_w A \frac{(C_{sw} \quad C_w)}{_w} \qquad (2.4)$$

Noting that $C_{sw} = C_{sa} / H$, we solve for C_{sa} in (2.4) and use this value in (2.3) to find the flux across air-water interface based on the thin film model,

$$\dot{m} = \frac{\left(C_w - \frac{C_a}{H}\right)A}{\frac{\delta_w}{D_w} + \frac{\delta_a}{HD_a}} \qquad (2.5)$$

We can define two limits of (2.5). If $\frac{{}_w}{D_w} >> \frac{{}_a}{HD_a}$, the second term in the denominator of (2.5) may be dropped, and we arrive at

- Water -side control [typically, $H >> 0.01$]:

$$\dot{m} = D_w A \frac{(C_w - C_a / H)}{\delta_w} \qquad (2.6)$$

This limit is referred to as water-side control, because the water-side boundary layer controls the flux through D_w and ${}_w$. The air side conditions, both ${}_a$ and D_a, have no influence over the flux given in (2.6). At the other limit, $\frac{{}_w}{D_w} << \frac{{}_a}{HD_a}$, the first term in the denominator of (2.5) is dropped, and we arrive at,

- Air -side control [typically, $H << 0.01$]:

$$\dot{m} = D_a A \frac{(HC_w - C_a)}{\delta_a} \qquad (2.7)$$

In this limit the flux depends only on the air-side conditions, through ${}_a$ and D_a, with no dependence on the water-side conditions, specifically D_w and ${}_w$. ${}_w / D_w$ is typically larger than ${}_a / D_a$ by a factor of 100, i.e., $({}_a / D_a)/({}_w / D_w) = 0.01 \quad H_c$. That is, in general, the flux of a chemical with $H >> H_c = 0.01$ is water-side controlled; the flux of a chemical with $H << H_c = 0.01$ is air-side controlled.

Since Henry's law constant for CO_2 is 0.83, which is much greater than 0.01, the flux of CO_2 is controlled by water-side laminar sub-layer and therefore can be modeled with (2.6). The evolution of CO_2 in the chamber can be described by the following equations:

$$\frac{M}{t} = V\frac{C_a}{t} = D_w A\frac{(C_w \quad C_a / H)}{{}_w} \tag{2.8}$$

The CO_2 flux from the stormwater tank to the atmosphere is positive, i.e. directed upward. Since the emission of CO_2 from the pond happens all the time and CO_2 is soluble in the water with the solubility of 1.45 g/L at 25 °C, 100 kPa, we can assume water plays a role as balancing and storing CO_2 and concentration in water is constant and equal to equilibrium concentration at the surface of aqueous phase. Then, (2.8) converts to,

$$\frac{C_a}{t} = D_w A\frac{(C_{sa} \quad C_a)}{VH \;{}_w} \tag{2.9}$$

This is a first-order reaction with a constant rate

$$k = \frac{D_w A}{VH \;{}_w} \tag{2.10}$$

With initial CO_2 concentration inside the chamber $C_a = 0$, the concentration of CO_2 in the static chamber evolves as,

$$C_a(t) = C_{sa}(1 \quad \mathrm{e}^{\quad kt}) \tag{2.11}$$

Based on the equation (2.11) in the air-water exchange model, we did the nonlinear regression by using the Solver in Microsoft Excel to analyze our data sets.

3 RESULTS AND DISCUSSION

3.1 Effluent Water Quality

At the influent COD concentration of about 100 mg/L, effluent COD concentration ranged from 28 ~ 70 mg/L, resulting in the average COD removal efficiencies of 54 ± 13 % and 47 ± 6 % before and after the removal of duckweeds, respectively (Figure 3.1). There was no significant difference in COD removal before and after the removal of duckweeds ($p = 0.07$).

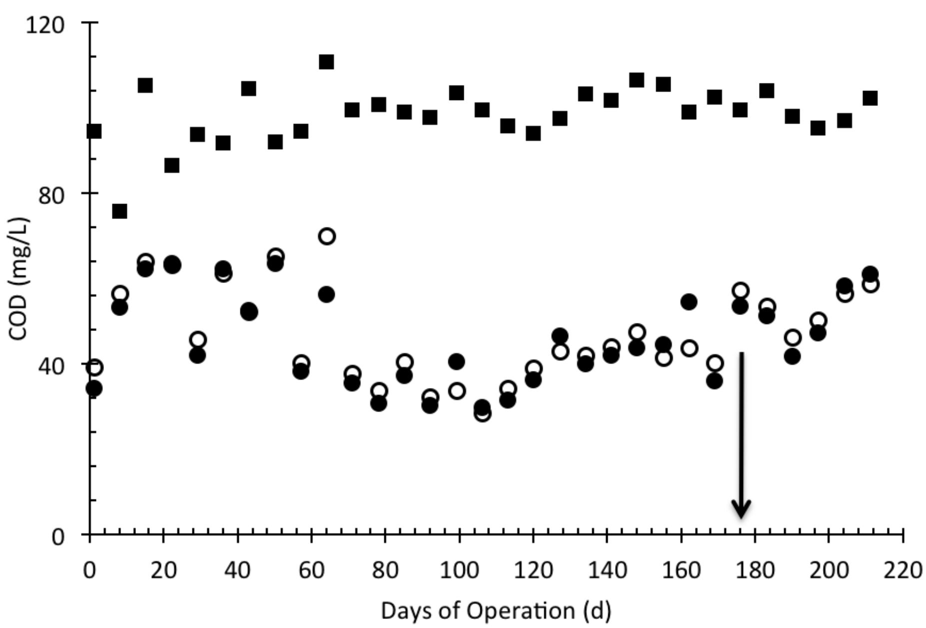
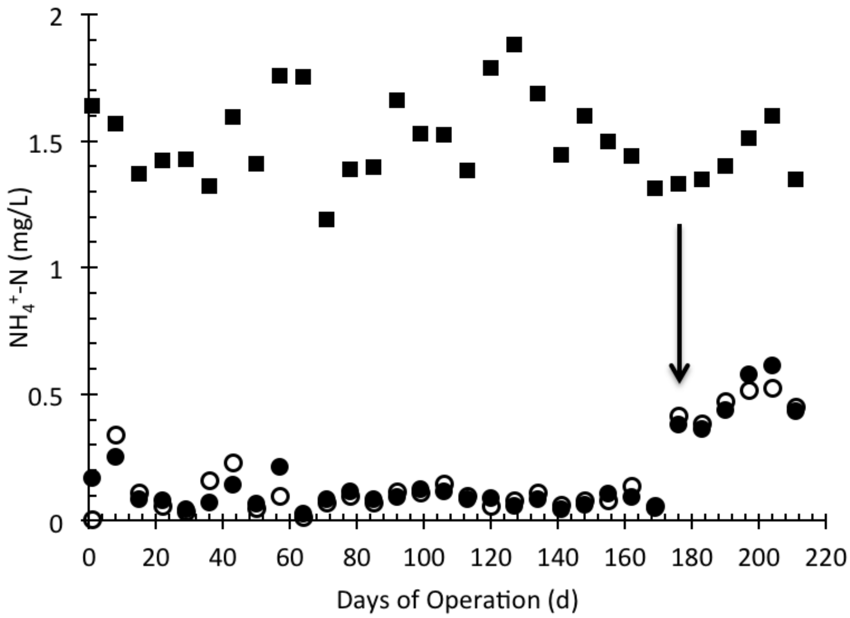


Figure 3.1 Influent (■) and effluent COD concentrations of the stormwater treatment ponds: tank#1 (○), and tank#2 (●). The arrow shows the point when duckweeds were removed.



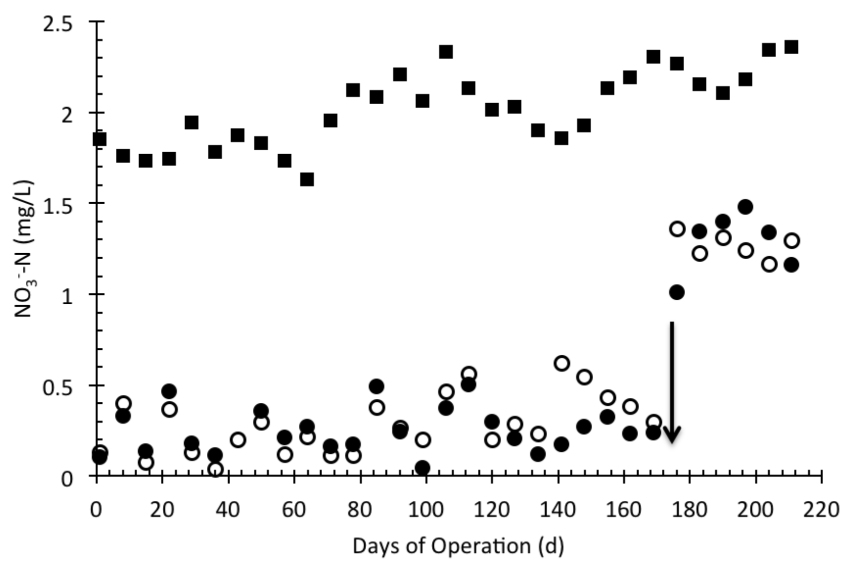


Figure 3.2 Influent (■) and effluent nitrogen concentrations of the stormwa ter treatment systems: tank #1 (○), and tank #2 (●). The arrow shows the point when duckweeds were removed.

However, nitrogen removal efficiency decreased for both tanks after the removal of duckweeds. In the duckweed tanks operated for more than 170 d, the concentrations of NH_4^+-N and NO_3^--N in the effluent were 0.10 ± 0.06 mg/L and 0.26 ± 0.15 mg/L, respectively. After the duckweed removal on day 176 (Figure 3.2), the effluent concentrations of NH_4^+-N and NO_3^--N were 0.46 ± 0.08 mg/L and 1.28 ± 0.12 mg/L, respectively. The NH_4^+-N removal efficiency decreased from 94 ± 4 % to 68 ± 3 % after the removal of duckweeds. Similarly, the NO_3^--N removal efficiency decreased from 87 ± 7% to 43 ± 7%. The results show that duckweeds played an important role in nitrogen removal as was reported in other studies (Lim et al. 2001, Sims and Hu 2013).

Compared to nitrogen removal efficiency, the P removal efficiencies were lower and they were 34 ± 7 % and 10 ± 6 % before and after the duckweed removal, respectively (Figure 3.3).

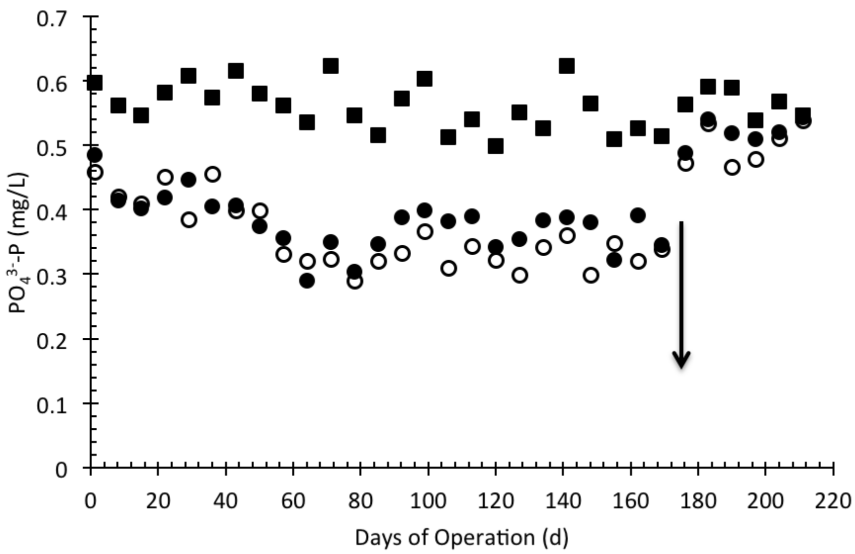


Figure 3.3 Influent (■) and effluent phosphorus concentrations of the stormwater treatment ponds: tank#1 (○), and tank#2 (●). The arrow shows the point when duckweeds were removed.

3.2 GHG Emissions from Stormwater Treatment Systems

Figure 3.4 shows that the total amount of CO_2 emission was high before duckweeds were removed in both tanks. The patterns of cumulative CO_2 concentration profiles in the closed chamber were similar; the CO_2 concentrations increased rapidly at the beginning of sampling and were almost stable at the end of sampling period.

In contrast, the total amount of methane production was higher after duckweeds were removed despite the variance (Figure 3.5). The change in the CH_4 concentration in the closed chamber with time were not the same between the tanks, both having high variation throughout the sampling period. Such large variations could be attributed to the complex interactions of biochemical reactions involving methane production and methane removal by methanogens and methanotrophic bacteria respectively, as described in detail below:

- Hydrogenotrophic methanogenesis: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
- Methane oxidation: $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$

It appeared that high methane concentrations at the soil-water interface might promote methane oxidation by the bacteria, resulting in an irregular repetitive oscillation of the methane concentrations in the closed chamber (Figure 2.1). It remains unknown why more methane was produced after the duckweed removal. One possible explanation is that bacteria could effectively remove methane anoxically or aerobially in the duckweed systems.

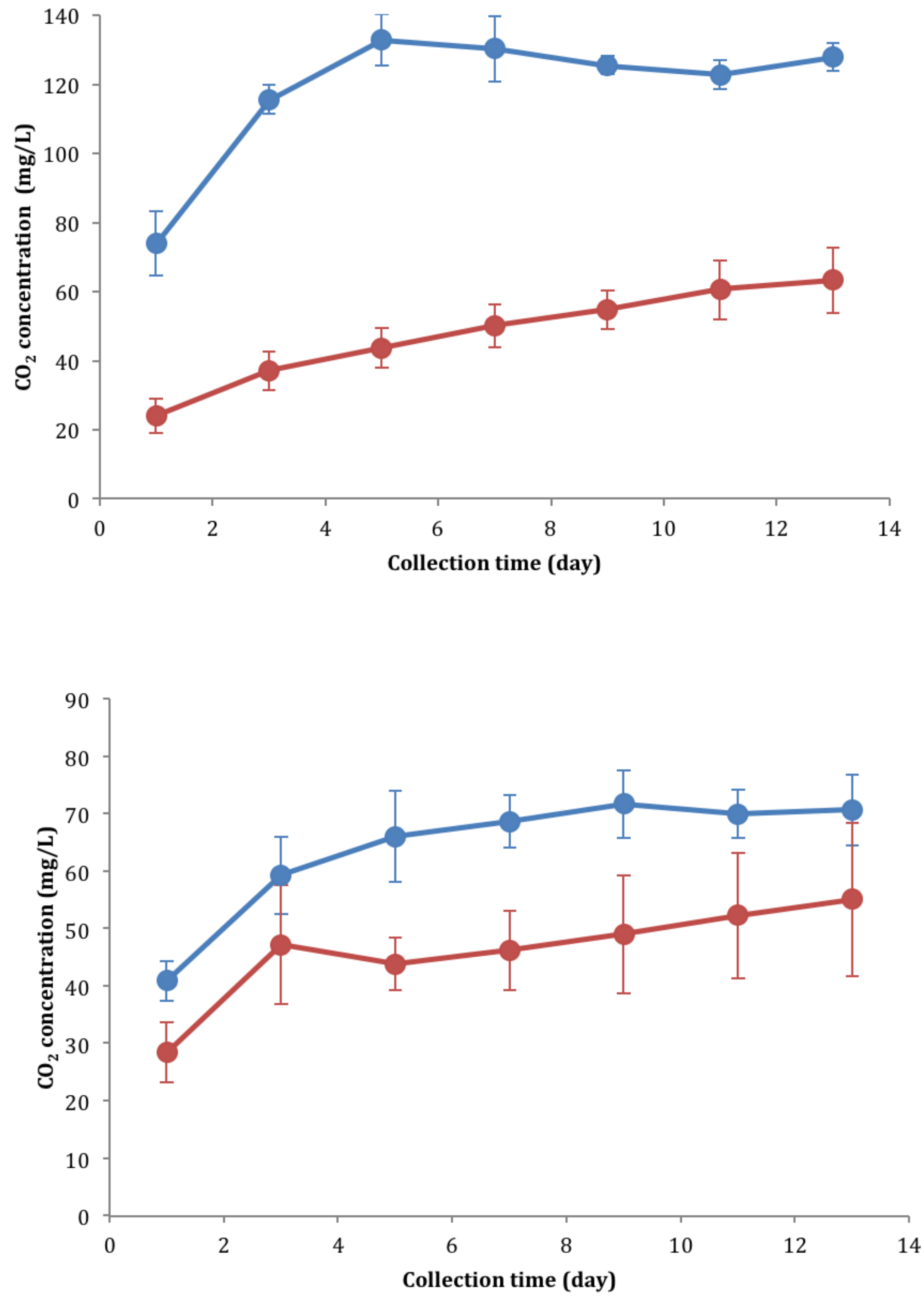


Figure 3.4 Cummulative CO_2 concentrations from tank #1 and #2 before (●) and after (●) the removal of duckweeds.

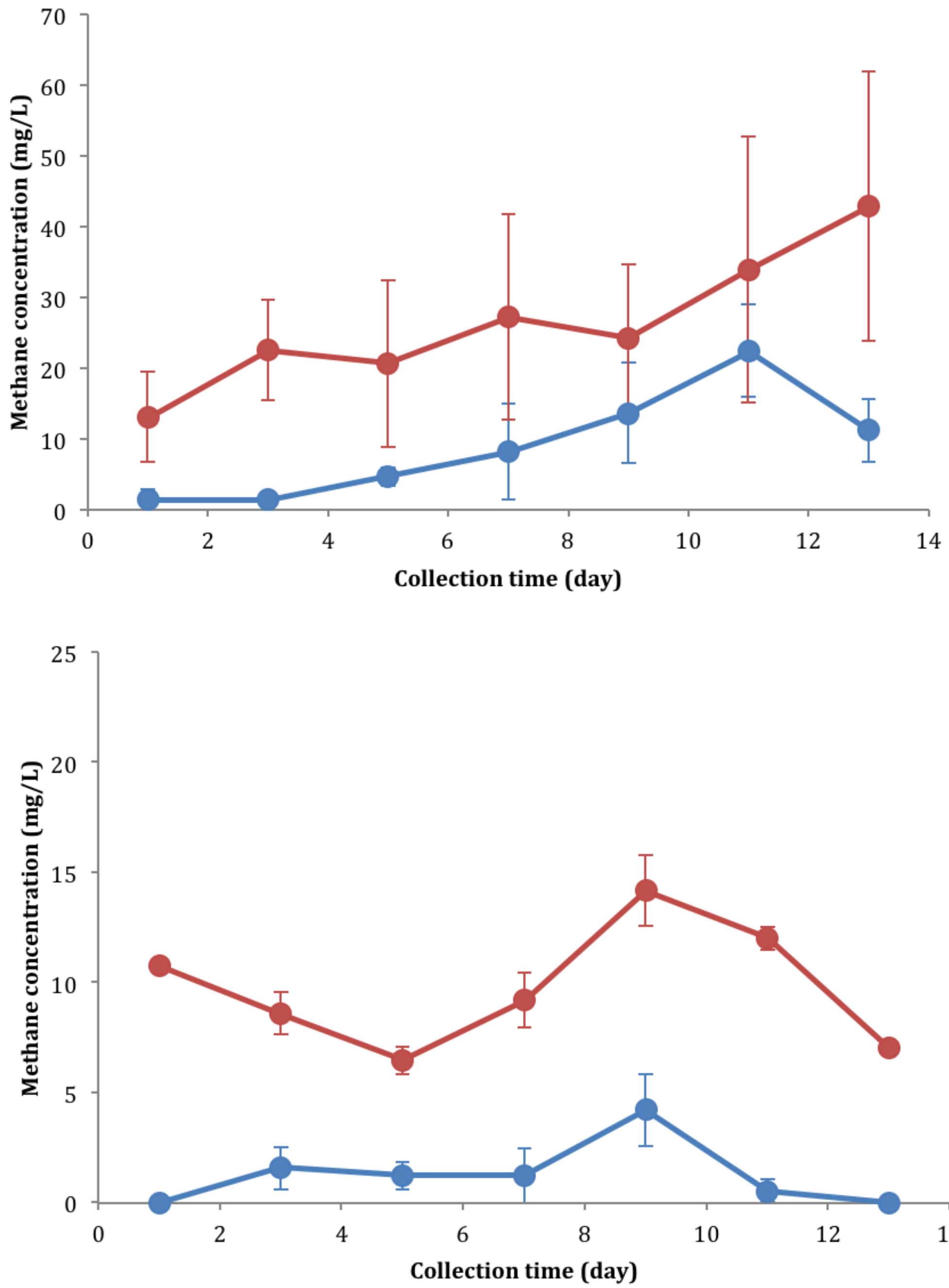


Figure 3.5 Cumulative methane concentrations from tank #1 and #2 before (●) and after (●) the removal of duckweeds.

The rate of CO_2 and CH_4 emission was quite different before and after duckweed removal (**Error! Reference source not found.**). To evaluate the flux of CO_2, we use the first three points to estimate the maximum GHG emission as gases are truly released to the atmosphere in stormwater treatment systems. For methane, situations are more complex; we use the rising parts of the curves to calculate the flux and do the average of three measurements for each situation. After the removal of duckweeds, CO_2 flux was lower for both tanks. However, methane flux was higher without duckweeds. The flux of CO_2 and CH_4 from the duckweed tanks was in the range reported from a previous study (Mander et al. 2014).

Table 3.1 CO_2 and methane flux from two stormwater treatment systems

GHG Type	Duckweed Tank #1	Duckweed Tank #2	After duckweed removal Tank #1	After duckweed removal Tank #2
CO_2 (mg m^{-2} d^{-1})	1472 ± 721	626 ± 234	492 ± 282	395 ± 53
CH_4 (mg m^{-2} d^{-1})	299 ± 74	180 ± 91	559 ± 215	328 ± 114

3.3 Correlation between CO_2 and Methane Emission

There were weak positive correlations between the CO_2 and methane emission profiles. For instance, in the duckweed tanks, the higher the CO_2 emission rate, the higher the methane release (Figure 3.6). After the duckweed removal, the correlation remained with much higher methane emission at the same CO_2 emission rate, indicating the role of microorganisms and unique low DO environment (Sims and Hu 2013) in controlling methane emission .

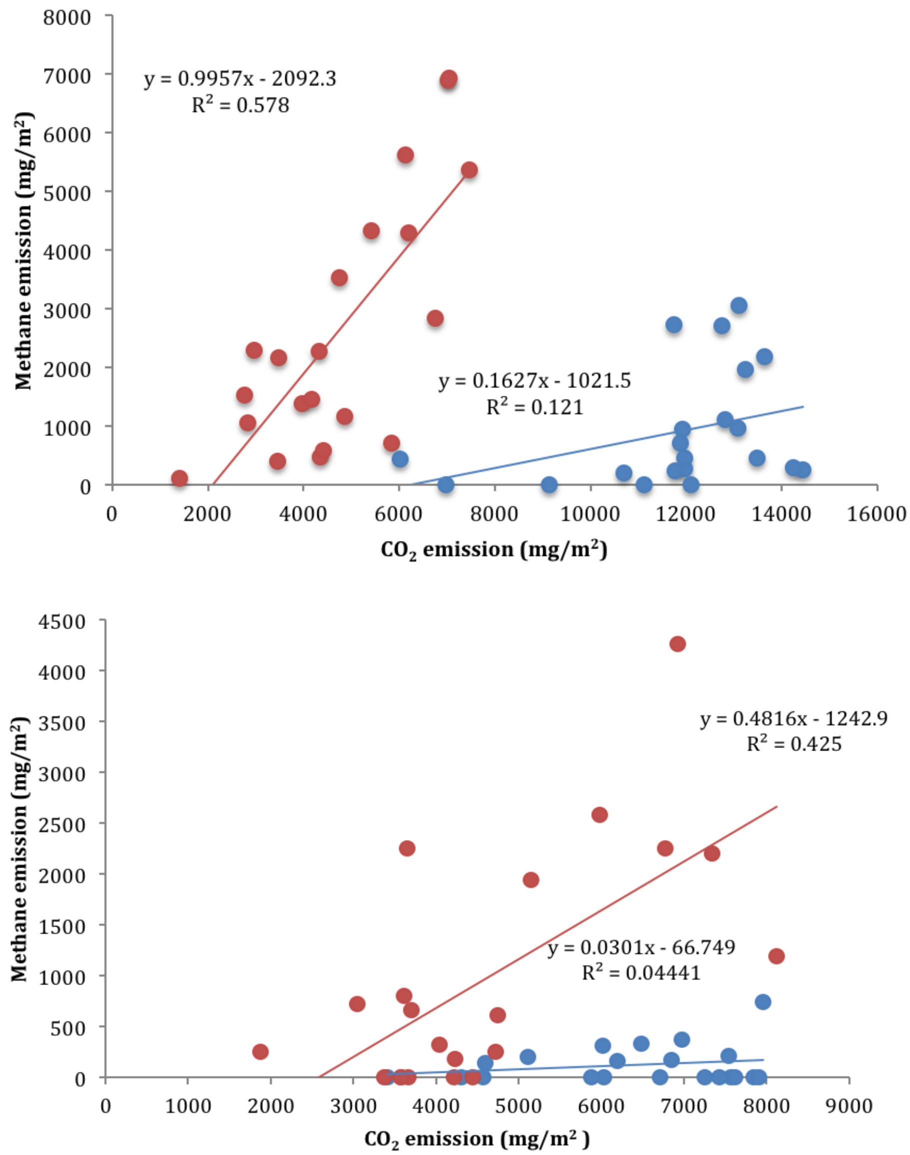


Figure 3.6 The relationship between CO_2 and methane production from tank #1 and #2 before ( )and after ( )the removal of duckweeds.

3.4 CO_2 Release Kinetics and Modeling

The thin-film model was successfully applied to fit the CO_2 concentration data in the closed chambers. The CO_2 concentrations in the duckweed tanks increased quickly in the first three days, then leveled off at about 128 mg/L and 71 mg/L for tank # 1 and # 2, respectively (Figure 3.7).

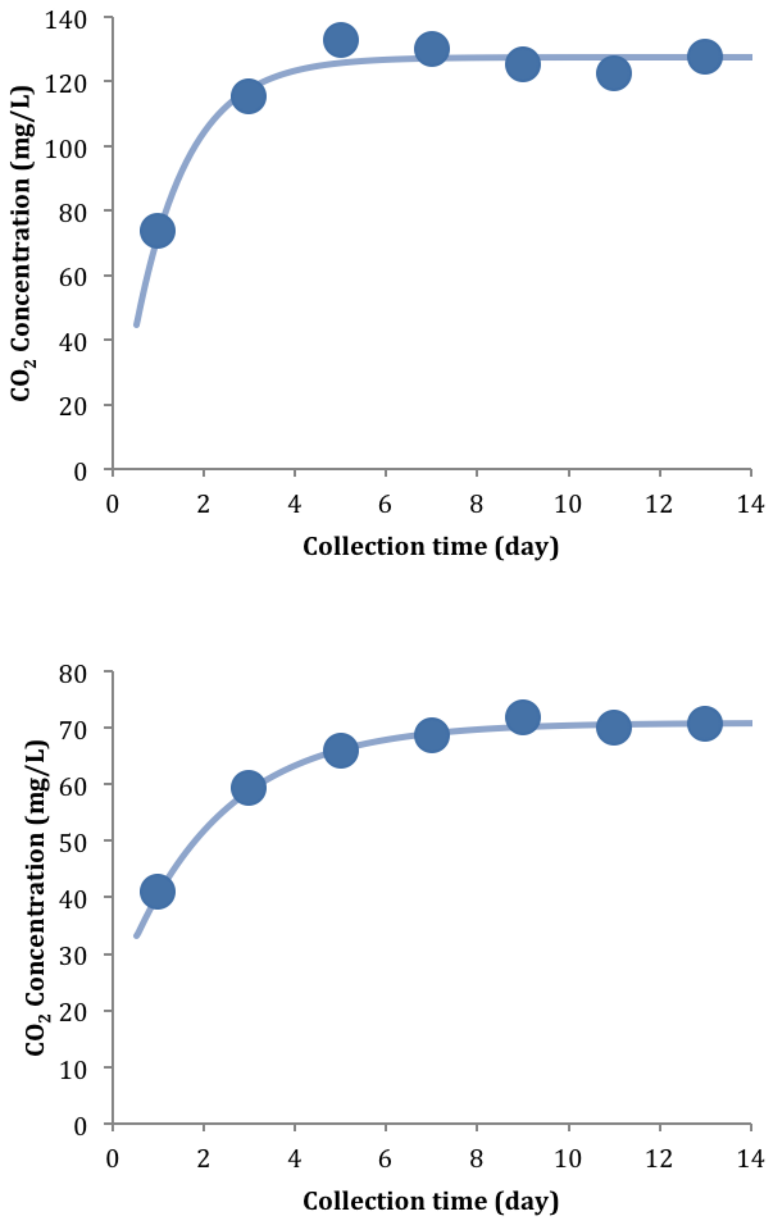


Figure 3.7 The change in CO_2 concentrations in the closed chamber that was submerged in the duckweed tank #1 and #2.

The CO_2 concentrations after removing the duckweeds increased slowly compared with that before during the period. The CO_2 concentrations after removing the duckweeds reached 75 mg/L and 51 mg/L for each chamber (Figure 3.8).

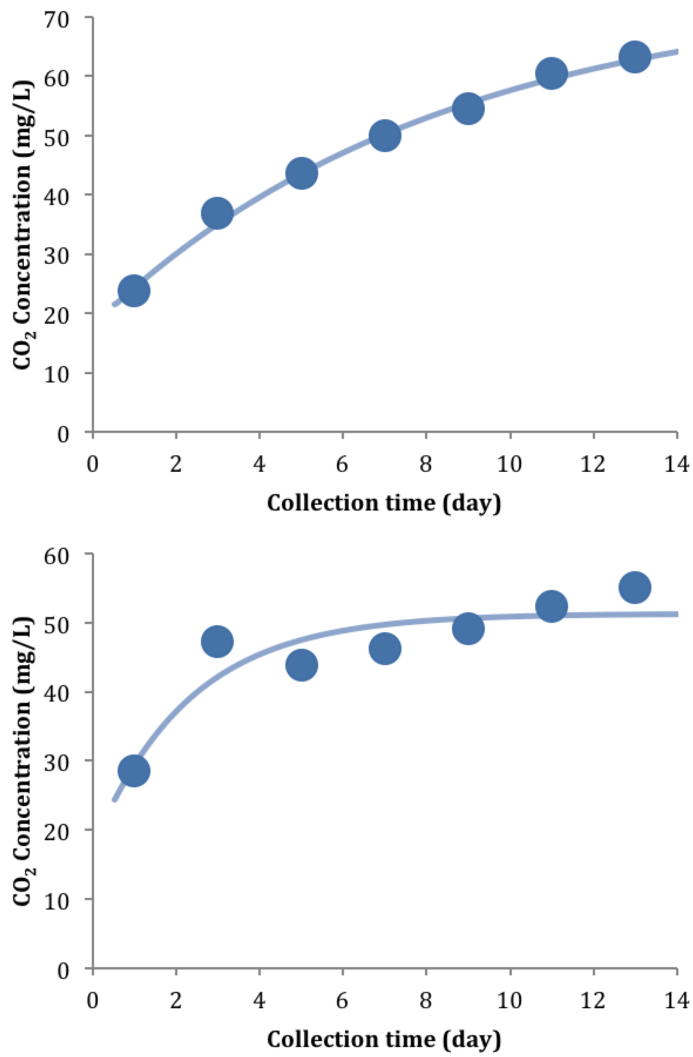


Figure 3.8 The change in CO_2 concentrations in the closed chamber that was submerged in tank #1 and #2 after the removal of duckweeds.

After the removal of duckweeds, both of the tanks have a smaller C_{sa} and k (shown in Table 3.2). The smaller k value is, the longer time it takes to reach the equilibrium concentration. In duckweed systems, the floating plants might increase CO_2 diffusion in water through their root systems. In the meantime, higher C_{sa} may provide a larger concentration gradient, which may also affect the k value. As duckweed biomass debris on the soil surface could be a carbon source to support soil microbial growth in the stormwater treatment ponds, the carbon was further transformed to gaseous forms and increases carbon emissions from the ponds.

Table 3.2 Fitted parameters of the thin-film model

Coefficients	C_{sa} (mg/L)	k (d^{-1})
Duckweed Tank #1	128	0.86
Duckweed Tank #1	71	0.46
Tank #1 after duckweed removal	75	0.12
Tank #2 after duckweed removal	51	0.44

The thin-film model did not apply to methane concentrations in the closed chamber in the study because of complex interactions between methanogens and methanotrophic involved in methane production and methane removal. Moreover, we could not assume the constant methane concentration in aqueous phase by taking account the low methane solubility in water (22.7 mg/L at the temperature of 25 ºC and 1 atm) (Gevantman 2000).

4 CONCLUSIONS

Duckweed treatment ponds removed 54 ± 13 % COD, 94 ± 4 % NH_4^+-N, 87 ± 7 % NO_3^--N, 34 ± 7 % PO_4^{3-}-P at the HRT of 10 days. Nutrients removal efficiency decreased after the removal of duckweeds with a removal efficiency of 68 ± 3 % for NH_4^+-N, 43 ± 7 % for NO_3^--N, 10 ± 6 % for PO_4^{3-}-P, while COD removal did not change significantly (47 ± 6 % removal efficiency). It proves that duckweeds play an important role in removing nutrients in stormwater treatment ponds.

In terms of GHG emission, the flux of CO_2 was lower after the duckweeds were removed. Although photosynthesis of duckweeds can absorb CO_2, the decomposition of biomass debris provide more carbon source for chemoheterotrophs to generate CO_2.

However, the methane emission flux was higher without duckweeds. The significant fluctuation of cumulative methane concentrations in the closed chamber indicates that both the generation and decomposition of methane happen during the GHG emission. The dominant process depends on the availability of electron donors or acceptors and associated biochemical environments.

In summary, although CO_2 emission was higher in duckweed stormwater treatment systems, but methane emission was lower and water quality improved with duckweeds. Therefore, it is better to have duckweeds or other floating plants in the stormwater treatment.

5 FUTURE STUDY

In order to fully understand how methane is produced and decomposed, we plan to use molecular techniques like qPCR or next generation sequencing to analyze the DNA samples of soil in the duckweed ponds which had been extracted under the condition with and without duckweeds cover. The results can show us the abundance of specific microorganisms, specially, methanogens and methanotrophs. The profile of microbial population and taxonomy will give a better explanation to verify our estimated reasons or not.

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APPENDIX

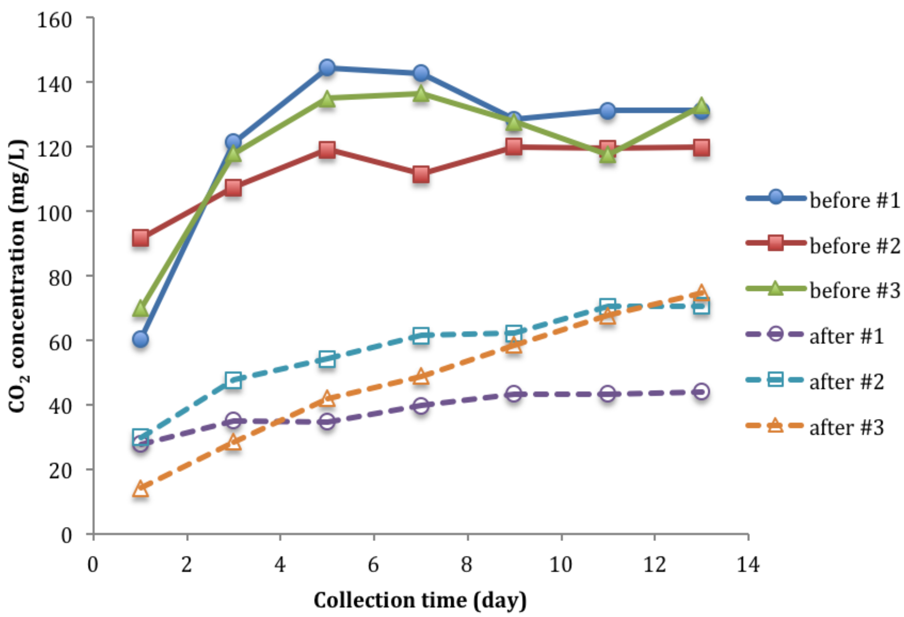

Figure A CO_2 emission of six times measurement for tank #1

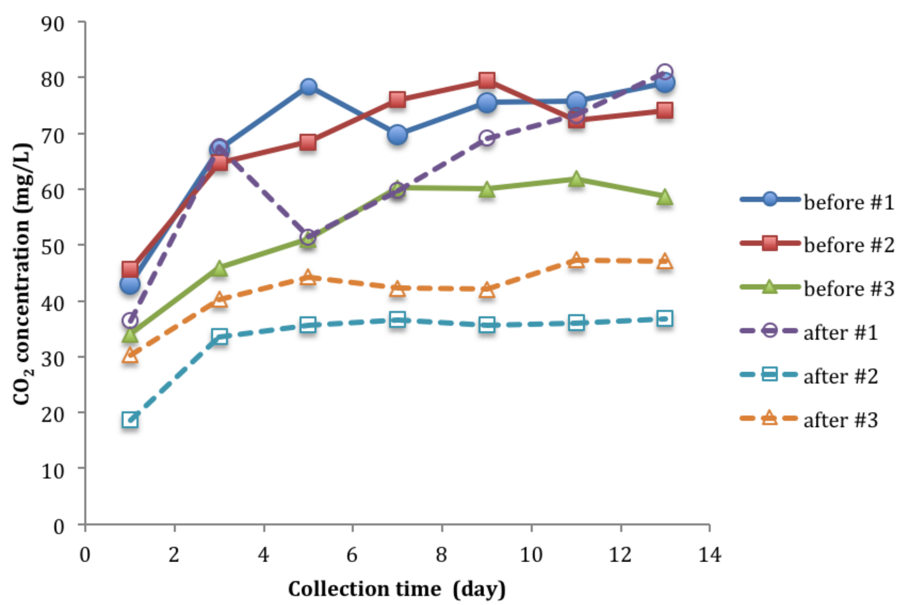

Figure B CO_2 emission of six times measurement for tank #2

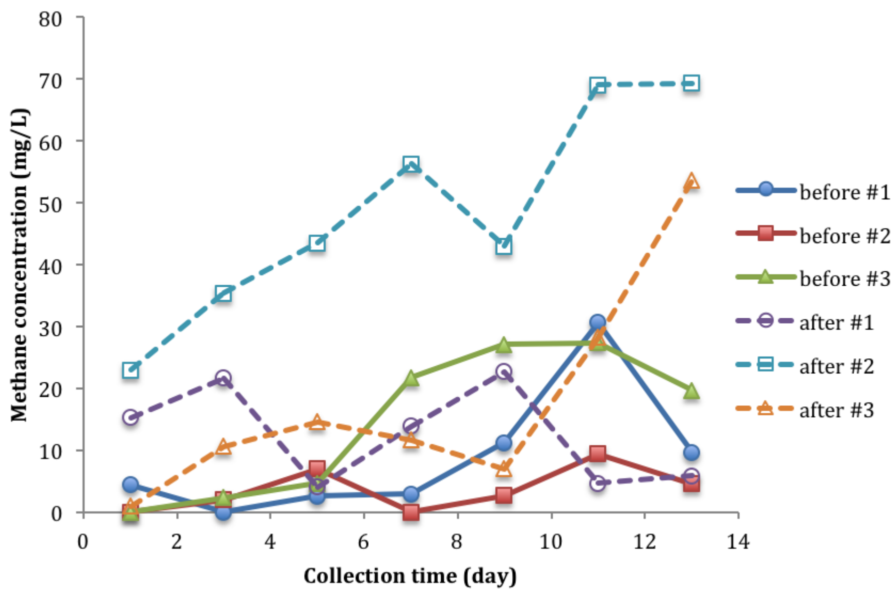

Figure C Methane emission of six times measurement for tank #1

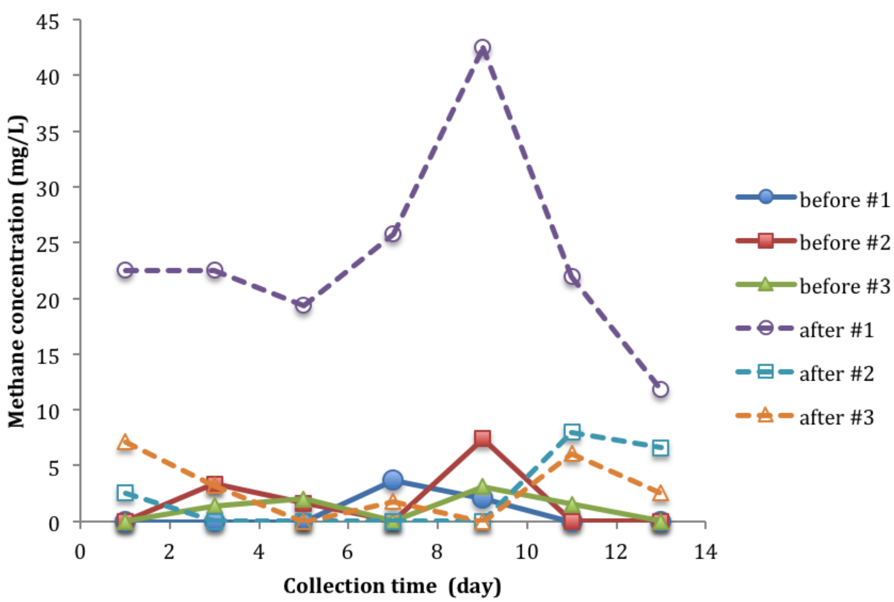

Figure D Methane emission of six times measurement for tank #2