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Nutrient removal and greenhouse gas emissions in duckweed treatment ponds

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ARTICLE INFO

Article history:
Received 24 July 2012
Received in revised form
10 November 2012
Accepted 6 December 2012
Available online 14 December 2012

Keywords:
Duckweed pond
Nitrogen removal
Methanogenesis
Greenhouse gas
Ammonia-oxidizing archaea

ABSTRACT

Stormwater treatment ponds provide a variety of functions including sediment retention, organic and nutrient removal, and habitat restoration. The treatment ponds are, however, also a source of greenhouse gases. The objectives of this study were to assess greenhouse gas (CH_4, CO_2 and N_2O) emissions in duckweed treatment ponds (DWPs) treating simulated stormwater and to determine the role of ammonia-oxidizing organisms in nutrient removal and methanogens in greenhouse gas emissions. Two replicated DWPs operated at a hydraulic retention time (HRT) of 10 days were able to remove 84% (±4% [standard deviation]) chemical oxygen demand (COD), 79% (±3%) NH_4^+-N, 86% (±2%) NO_3^--N and 56% (±7%) orthophosphate. CH_4 emission rates in the DWPs ranged from 502 to 1900 mg CH_4 m^{-2} d^{-1} while those of nitrous oxide (N_2O) ranged from 0.63 to 4 mg N_2O m^{-2} d^{-1}. The CO_2 emission rates ranged from 1700 to 3300 mg CO_2 m^{-2} day^{-1}. Duckweed coverage on water surface along with the continued deposit of duckweed debris in the DWPs and low-nutrient influent water created a low dissolved oxygen environment for the growth of unique ammonia-oxidizing organisms and methanogens. Archaeal and bacterial *amoA* abundance in the DWPs ranged from $(1.5 \pm 0.2) \times 10^7$ to $(1.7 \pm 0.2) \times 10^8$ copies/g dry soil and from $(1.0 \pm 0.3) \times 10^3$ to $(1.5 \pm 0.4) \times 10^6$ copies/g dry soil, respectively. The 16S rRNA acetoclastic and hydrogenotrophic methanogens ranged from $(5.2 \pm 0.2) \times 10^5$ to $(9.0 \pm 0.3) \times 10^6$ copies/g dry soil and from $(1.0 \pm 0.1) \times 10^2$ to $(5.5 \pm 0.4) \times 10^3$ copies/g dry soil, respectively. Ammonia-oxidizing archaea (AOA) appeared to be the dominant nitrifiers and acetoclastic *Methanosaeta* was the major methanogenic genus. The results suggest that methane is the predominant (>90%) greenhouse gas in the DWPs, where the relatively low stormwater nutrient inputs facilitate the growth of *K*-strategists such as AOA and *Methanosaeta* that may be responsible for ammonia removal and greenhouse gas emissions, respectively.

1. Introduction

Stormwater treatment ponds (STPs) retain sediments, remove nutrients, control flooding and help habitat and species conservation (Kadlec, 1999). Duckweeds are often observed in STPs with mostly stagnant water. Because of their fast growth, duckweeds are the primary choice of aquatic vegetation in various lagoons and constructed wetlands for wastewater treatment (Greenway, 1997; Körner et al., 2003).

Coupled aerobic and anaerobic environments form the grounds for the biological/chemical transformation of organic matter and nutrients (nitrogen and phosphorus) (Imfeld et al., 2009). The combined process of aerobic nitrification (Kowalchuk and Stephen, 2001) and subsequent anoxic denitrification

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0043-1354/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2012.12.009

(Risgaard-Petersen, 2003; Zumft, 1997) potentially emits greenhouse gases (GHG) such as nitric oxide (NO) and nitrous oxide (N_2O) (Elgood et al., 2010; García-Lledó et al., 2011). Anaerobic degradation by methanogenic archaea also produces greenhouse gases such as carbon dioxide (CO_2) (Lafleur et al., 1997; Mitsch and Gosselink, 1993) and methane (CH_4) (Inamori et al., 2007; Mander et al., 2011).

Methane has a global warming potential of 23 relative to CO_2 (Solomon et al., 2007) when it is released under strictly anaerobic conditions (Pangala et al., 2010). Methane fluxes were found to be significantly affected by pulsing hydrology and fluctuating water table levels (Altor and Mitsch, 2008). Flooded and anaerobic wetland soils support the production of CH_4 that accounts for 15–30% of the total annual CH_4 emission. Studies have demonstrated that most methane emissions occur when surface water levels are between 30 and 50 cm (Nahlik and Mitsch, 2011) and higher strength influent load gives rise to higher methane flux (Wang et al., 2008). Among other factors, sediment and water temperatures also strongly affect methane emissions (Johansson et al., 2004).

Nitrous oxide (N_2O) acts as a powerful greenhouse gas as it is 298 times more effective than CO_2 and is increasing in the atmosphere at a rate of 0.2–0.3% per year (Anderson et al., 2010; IPCC, 2001). The emission of N_2O gas may occur in denitrification (García-Lledó et al., 2011; Palmer et al., 2012). N_2O emission also occurs when the activities of nitrifying bacteria form a byproduct of nitrification or an intermediate of nitrifier denitrification (Ma et al., 2008). Factors that influence N_2O emission include ammonium concentrations (Galloway and Cowling, 2002), high clay content and anaerobic conditions (Van Groenigen et al., 2004), temperature and water content (Smith et al., 1998), fertilization (Hyde et al., 2006), and soil pH (Lesschen et al., 2011).

CH_4 emission has been measured in various types of natural and engineered systems which include natural wetlands (Heikkinen et al., 2002; Juutinen et al., 2003; Zhang et al., 2012), riparian mashes (Sha, 2011), constructed wetlands (da Cunha de Oliveira Santos Neves et al., 2011; Tanner et al., 1997), riparian buffer zones (Teiter and Mander, 2005), sludge treatment wetlands (Uggetti et al., 2012), anaerobic ponds (Heubeck and Craggs, 2010) and stabilization ponds (Stadmark and Leonardson, 2005). Nitrous oxide emissions have also been studied in denitrifying bioreactors (Elgood et al., 2010), freshwater marshes (Lu et al., 2012), and constructed wetlands (García-Lledó et al., 2011), but rarely in STPs. Because duckweed treatment ponds (DWPs) are increasingly used due to their low cost and effectiveness in organic and nutrient removal (Körner et al., 2003), it is necessary to study the GHG emissions and compare their impact and nutrient efficiency with alternative systems. DWP systems create a unique environment for the adaptation of special microbial population because of their fast-growing coverage of duckweed (*Lemna gibba*) on the water's surface and biomass debris formation in the sediment along with the low nutrient input from stormwater runoff. The objectives of this study were 1) to assess greenhouse gas (CH_4, CO_2 and N_2O) emissions in DWPs treating simulated stormwater and 2) to determine how low stormwater nutrient inputs affect the selection of important microorganisms including ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and methanogenic archaea involved in stormwater nutrient removal and GHG emissions.

2. Materials and methods

2.1. Duckweed treatment pond design and operation

Duplicated lab-scale stormwater treatment ponds (tanks) were made of glass, each having a dimension of 1.0 m (length) $\times$ 0.36 m (width) $\times$ 0.44 m (depth). The STPs were filled with a layer of gravel (size = 2 cm) at the bottom and sand up to a depth of 5 cm and then topped with a 15 cm thick layer of hydric soil that was collected from a marshland close to the Columbia Water Treatment Plant (Columbia, MO). The tanks were then filled with water to a height of 42 cm (water height = 22 cm). Each tank consisted of two end-around baffles to prevent short-circuiting of water flow through the system, thus producing three equidistant 0.12 m wide cells (details described elsewhere) (Sims and Hu, 2012).

The treatment ponds were run in parallel under almost identical conditions for 170 days. Fluorescent lights (300 W) provided artificial illumination (light intensity = 39 $\mu mol\ m^{-2}\ s^{-1}$) with a light period of 12 h per day at the room temperature (23 $\pm$ 1 °C). Synthetic stormwater was prepared every week, which contained the following chemicals per liter (details in Supporting Information, Table S1): 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; Henderson et al., 2007). Both ponds were operated at a hydraulic retention time (HRT) of 10 days.

Duckweeds (*Lemna minor*) originally from wetland soils started to grow in the two ponds, referred hereafter to as duckweed ponds (DWPs), DWP #1 and DWP #2. Occasionally the duckweeds were moved around to cover the water surface evenly. After 20–30 days of operation, both ponds were totally covered by duckweeds, which were left unperturbed while routine monitoring of effluent water quality and microbial analyses continued. On day 170, cattails were planted in DWP #1 but these plants were not sustainable in indoor laboratory environments. On day 210, DWP #1 regained its former setting. DWP #1 and DWP #2 were then run for another 50 days. The experiment ran for a total of 250 days with no duckweed biomass wasted except for minor loss in the DWP effluent.

2.2. Soil and biomass sampling and water chemical analysis

Once the duckweeds fully covered the water surface, a layer of duckweed biomass debris accumulated on the soil surface. The biomass debris samples containing an average organic carbon content of 2.3% were collected from each tank to make a composite sample for microbial population analysis. Similarly, soil samples were taken using a push-core sampler that collected a core of soil from the surface of soils to 15 cm depth from all the three cells in each DWP. Soils in the DWPs were classified as sandy loam with an average organic carbon content of 1.8%. At the completion of this study, duckweed biomass was collected and analyzed.