Ecological Engineering 95 (2016) 390–398

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Effects of plant diversity and sand particle size on methane emission and nitrogen removal in microcosms of constructed wetlands

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 19 May 2016
Accepted 16 June 2016
Available online 4 July 2016

Keywords:
Species richness
Species identity
Greenhouse gases
Ecosystem functioning

ABSTRACT

To find an effective approach that improves nitrogen removal while reducing methane (CH_4) emission during the process of wastewater treatment in constructed wetlands (CWs), we conducted a microcosm experiment simulating CWs with two treatments. The first treatment was plant diversity (species richness and identity), and the second treatment was substrate sand particle size (fine sand and coarse sand). Results showed that, (1) the range of CH_4 emission in fine sand microcosms (−8.46 to 21.03 mg CH_4 $m^{-2}\,d^{-1}$) was much greater than that in coarse sand (5.06 to 6.85 mg CH_4 $m^{-2}\,d^{-1}$); (2) in coarse sand microcosms, plant species richness increased nitrogen removal ($P < 0.05$), but did not affect CH_4 emission ($P > 0.05$); (3) in fine sand microcosms, species identity surpassed species richness as a key driver of CH_4 emission, and the presence of *Oenanthe javanica* significantly decreased CH_4 emission, whereas the presence of *Rumex japonicus* notably increased CH_4 emission ($P < 0.05$, respectively); (4) the nitrogen removal in fine sand microcosms was higher than in coarse sand microcosms ($P < 0.05$). Results also showed that *Phalaris arundinacea* monoculture in fine sand microcosms had a high N removal and low CH_4 emission. Overall, we conclude that the best combination of low CH_4 emission and high N removal rates could be achieved in fine sand microcosms with special plant species as *P. arundinacea*.

1. Introduction

Wastewater is a huge source of methane (CH_4) emission (Johansson et al., 2004; IPCC, 2014). In 2010, for example, CH_4 emission from wastewater treatment has reached 2381.2 Mt, accounting for 8.5% of the global CH_4 emission (IPCC, 2014). Therefore, finding ways to mitigate CH_4 emission in the process of wastewater treatment has drawn more and more attention (Liu et al., 2012). With low operation and maintenance requirements, constructed wetlands (CWs) have relatively lower CH_4 emission per unit nitrogen (N) removal than wastewater treatment plants (WTPs) (Vymazal, 2011; Liu et al., 2012). However, with the increasing amount of wastewater, the CH_4 emission from CWs is quite extensive as these wetlands are expanding dramatically (Vymazal, 2011). Yet, ensuring high nitrogen removal and still reducing CH_4 emissions is a challenge.

CH_4 emission from CWs is affected by the combination of physical structure (e.g., substrate properties) and ecological structure (e.g., plant species identity and community composition) of the CWs (Tanner et al., 1997). Substrate can provide suitable conditions for plant growth as well as the microbial growth that relates significantly to CH_4 emission (Inamori et al., 2007; Lu et al., 2014). Several studies that have been carried out in various substrates, such as sand, peat, gravel, clay, zeolite, and rock (Wagner et al., 1999; Mander et al., 2014; Sakata et al., 2015), found that substrate with smaller particle size have higher surface area to protect microbial growth and provide adsorptive environment for closer interactions of microorganisms and their decomposition products (Ladd et al., 2004), with gravel emitting more CH_4 than sand (Wagner et al., 1999; Le Mer and Roger, 2001). However, to date there is no available information about the effect of sand substrate with different particle sizes on CH_4 emission from CWs.

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http://dx.doi.org/10.1016/j.ecoleng.2016.06.047
0925-8574/

Plants play an important role in ecological structure of CWs in mediating CH_4 production, oxidation, and transportation from CWs (Joabsson et al., 1999). Plants can affect soil methanogenic processes by providing available carbon (Picek et al., 2007; Wang et al., 2013), resulting in increased CH_4 emission (Teiter and Mander, 2005). In addition, as the degradation of organic matter into CH_4 requires anoxic condition and low oxidation-reduction potentials, prevalent oxygen availability reduce CH_4 emission (Johansson et al., 2004; Maltais-Landry et al., 2009). Plant roots can release oxygen that increases CH_4 consumption by altering the soil oxidation-reduction potential status (Whiting et al., 1991). Some plant species have aerenchyma that can supply their roots with oxygen as well as act as a conduit for CH_4 (Inubushi et al., 2003; Bouchard et al., 2007). This suggests that optimizing the ecological structure of CWs is of great importance to reduce CH_4 emission.

Nitrogen removal is one of the major target services of CWs (Liu et al., 2009), which is affected by both substrate and plants (Lu et al., 2014; Ge et al., 2015). Substrate could affect the N removal of CWs directly by adsorbing wastewater compounds (Truu et al., 2009; Ren et al., 2011). The substrate with smaller particles has larger specific surface area and high adsorbing capacity, while it may not be conducive to wastewater treatment due to a poorer permeability (Wu et al., 2015). Previous studies have shown that the ammonium adsorption of zeolite is significantly higher than that of volcanic rock (Huang et al., 2013). Plants influence N removal in CWs in two main ways: enhancing N removal by plant uptake (Tanner, 2001) and enhancing microbial activities indirectly by releasing organic matter or oxygen (Menon et al., 2013). Studies on the relationship between plant diversity and ecosystem functioning have found that plant species richness increases the nitrogen removal efficiency of CWs (Sun et al., 2013; Chang et al., 2014). However, the process of nitrate removal in CWs may promote methane emissions (Wu et al., 2009), as nitrate can constrain methane production by increasing redox potentials (Roy and Conrad, 1999; Stadmark and Leonardson, 2005). In addition, most studies have found a positive effect of plant biomass on CH_4 emission (Whiting and Chanton, 1993; Zhang et al., 2012) due to increased organic matter and gas transportation, while some others have reported either negative or no effect of plant biomass on CH_4 emission (Bouchard et al., 2007; Bhullar et al., 2013). Yet, these impacts on CH_4 emission and N removal from CWs vary greatly among plant species (Zhang et al., 2011; Bhullar et al., 2014). Therefore, to achieve high nitrogen removal and low CH_4 emission, it is necessary to find out the appropriate system structure with suitable substrate and plant diversity.

In this study, we established microcosms with two particle sizes (fine and coarse) of sand substrate to study the effects of plant diversity on CH_4 emission and nitrogen removal. Using these two types of sand substrates, the objectives of this study were to (1) investigate the effects of plant species richness on CH_4 emission and nitrogen removal; (2) evaluate the effects of plant species identity (indicated by a specific species monoculture or community composition) on CH_4 emission and nitrogen removal; and (3) compare the relative importance of plant species richness and species identity on CH_4 emission and nitrogen removal. Overall, the intent of this study was to identify a combination of plants and substrate that provide high N removal and low CH_4 emission in CWs.

2. Materials and methods

2.1. Experimental design

The microcosms that simulated CWs were established in the campus of Zhejiang University (120°05′E, 30°18′N), Hangzhou City, Southeast China. The region has a humid subtropical climate with an average annual temperature of 17 °C and rainfall of 1521 mm. A total of 90 microcosms (51 cm length × 38 cm width × 18 cm height) were divided into two groups: 45 microcosms filled with coarse sand and the other 45 microcosms filled with fine sand. Two size particles were prepared by sieve method, and the mean particle diameter of coarse sand was 0.5–1.0 mm and that of fine sand was 0.25–0.5 mm (Fig. 1). The sands were washed with tap water and then filled at a depth of 15 cm in each microcosm.

Four plant species, *Rumex japonicus* Houtt., *Oenanthe javanica* (Blume) DC., *Phalaris arundinacea* L. and *Juncus effusus* L., which are commonly found in CWs (Chang et al., 2014), were used in the experiment. Seedlings with similar size (initial height 20 cm) rather than seeds were used in order to improve the survival rate. Based on a randomized block design (Hooper and Vitousek, 1997), 45 microcosms were distributed among 5 blocks with a plant species richness treatment (including 1, 3 and 4 species) in both coarse sand and fine sand microcosms, respectively. Each block included four monocultures for each species, four mixed cultures for three-species combination and one mixed culture for four-species combination. The planting density was 12 individuals per microcosm. During the experiment, invasive species were weeded out weekly.

The simulated wastewater was the Hoagland nutrient solution (Hoagland and Arnon, 1950) with minor modification, where NO_3^--N was the sole form of nitrogen with a concentration of 336 mg L^{-1}, plus 272.12 mg L^{-1} sucrose in the solution. Each microcosm received 2352 mg N every 10 days, and the irrigation schedule was repeated eight times. Water levels were kept to 1.5 cm above the sand surface by supplying with tap water daily throughout the experiment.

2.2. Sampling methods

Gas sampling was conducted using closed static chambers (Johansson et al., 2003). We placed a chamber with 31 cm diameter and 32 cm height on the microcosms (Sun et al., 2013). After stabilizing the chamber for 30 min, gas samples were collected using a 50 mLpolyurethane syringe, and then injected into a 100 mL gas sampling bags (Plasticgas, Delin Company, China). Before formal gas sampling, pre-trial for rational sampling time duration was conducted; further details of this preliminary experiment can be found in Chang et al. (2014).

After gas sampling, the effluent samples were collected and stored at −20 °C for nitrogen content and total organic carbon (TOC) analysis (Gagnon et al., 2012). Once the effluent samplings were completed, plant above-ground and below-ground biomasses were obtained by the harvesting method after 90 days growth in microcosms. Plant material was oven dried at 65 °C for 72 h and weighed. Plant dry mass was expressed as g m^{-2} surface of microcosm.

2.3. Parameter analyzes

The CH_4 samples were analyzed using a gas chromatography (Agilent 7820, Agilent Technologies Inc., USA) equipped with a flame ionization detector (FID) and a Poropak Q column (3 m). The calculation of CH_4 emission (mg m^{-2} d^{-1}) was followed according to Cheng et al. (2007).

The water samples were filtered using membrane filters with a pore size of 0.45 μm. The NO_3^--N and NH_4^+-N contents in effluent samples were analyzed using a continuous flow analyzer (SAN plus, Skalar, the Netherlands). Total inorganic nitrogen (TIN) content was calculated as the sum of NO_3^--N and NH_4^+-N contents. The effluent TOC concentration was measured using a total organic carbon analyzer (Torch TOC, Teledyne Tekmar Corporation, USA).