



Plant species diversity impacts nitrogen removal and nitrous oxide emissions as much as carbon addition in constructed wetland microcosms



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ARTICLE INFO

Article history:

Received 14 December 2015

Received in revised form 13 March 2016

Accepted 10 May 2016

Available online 24 May 2016

Keywords:

Species richness

Species identity

Ecosystem functioning

Carbon source

ABSTRACT

With the aim to develop an approach for treating wastewater with low carbon (C) to nitrogen (N) ratio in constructed wetlands (CWs), we compared the effects of C addition and species diversity on N removal and nitrous oxide (N₂O) emissions. Compared with the monocultures without C addition, C addition significantly increased N removal efficiency in monocultures (reached 71% on average); increasing species richness impacted N removal as much as C addition, and the N removal efficiency in the four-species mixture reached 75%. Both C addition and increasing species richness level significantly increased N₂O emission rates (reached 1.26 and 1.40 mg m⁻² d⁻¹, respectively), but had no significant effect on N₂O emissions per unit N removal. The effects of species richness on N removal and N₂O emissions were stronger than species identity. Compared with C addition, assembling plant communities with high diversity in actual applications could be an effective and more economical approach for treating wastewater with low C/N ratio in CWs.

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1. Introduction

With the rapid growth of population and urbanization, the increasing production of wastewater with high-strength nitrogen (N) has become a serious problem impacting water quality, air quality and human health (Galloway et al., 2008; Schwarzenbach et al., 2010). A large amount of nitrous oxide (N₂O) is emitted during wastewater processing (Kampschreur et al., 2009; Huang et al., 2013). Thus, it is of great significance to develop technologies to treat wastewater that has high N removal and low N₂O emissions.

Constructed wetlands (CWs) have been widely used for treating various wastewaters with the benefit of low operation and maintenance requirements (Vymazal, 2007; Liu et al., 2012). Yet, there are great differences in the ratio of C to N (C/N) among different sources of wastewater (Wu et al., 2014; Vymazal, 2014). Wastewater from some high-tech industry sources, such as semiconductors and optoelectronics, has a very low C/N ratio (Kumar et al., 2012).

The influent C/N ratio, representing the relative amount of C source available in a system, plays a crucial role in N removal and N₂O emissions in CWs (Huang et al., 2013). Lack of available organic C could inhibit biological N removal in CWs, cause incomplete denitrification and increase N₂O emissions in CWs at the same time (Kampschreur et al., 2009; Huang et al., 2013). Finding ways to improve the performance of CWs for treating wastewater with a low C/N ratio has been an active area of research.

Adding labile C to wastewater to enhance the denitrification is an approach that can be used to improve the N removal in CWs for treating wastewater with a low C/N ratio (Sirivedhin and Gray, 2006; Wu et al., 2014). However, excessive C availability in wastewater often competes for limited oxygen supply and then inhibits nitrification (Saeed and Sun, 2011). In addition, as C addition obviously increases the cost (Wu et al., 2014), finding the low-cost alternative approaches are needed.

Plants in CWs can provide organic matter as the C sources for denitrification (Picek et al., 2007). The soil C pool in different systems varies with the plant productivity, chemical composition of root exudates, turnover rate of the root, and composition and activity of microbial community in the rooting zones (De Deyn et al.,

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2008). Increasing species richness not only increases the soil C pool (Chang et al., 2014; Lange et al., 2015), but also increases plant productivity and plant N uptake, and then increases the N removal (Ge et al., 2015). In addition, assembling plant communities with several species almost does not raise the cost than monoculture, so increasing plant diversity should be an economical approach for substituting C addition to improving N removal in CWs.

Nitrous oxide is a byproduct of nitrification and an intermediate product of denitrification (Canfield et al., 2010). Regulating the C/N ratio by C addition may be an approach that can be employed to control N_2O emissions. Wu et al. (2009) reported that when influent C/N ratio is 5, N_2O emission from CWs was the minimum. Plant species richness may also affect N_2O emissions by influencing biotic and abiotic factors, such as microbial communities, N concentrations, and availability of C sources (Thomson et al., 2012). In grassland ecosystem, N_2O emissions decreased with species richness (Niklaus et al., 2006) or no response (Abalos et al., 2014) or. In CWs for treating wastewater without extra C input, N_2O emissions increase with increasing species richness (Sun et al., 2013; Chang et al., 2014). In order to determine whether assembling high species richness could replace C addition when treating wastewater with low C/N ratio in CWs, it is important to compare the effects of these two approaches on N_2O emissions.

Species identity (species composition) is an important component of species diversity. In fact, species identity can play a greater role in controlling ecosystem productivity than species richness in grassland and marine communities (Hooper and Vitousek, 1998; Bruno et al., 2005). Abalos et al. (2014) demonstrated that species identity surpasses species richness as a key driver of N_2O emissions in a grassland ecosystem. In CWs, species identity can significantly affect ecosystem productivity (Sun et al., 2013; Ge et al., 2015), N removal (Read et al., 2008; Ge et al., 2015) and N_2O emissions (Sun et al., 2013). As such, it is key to further understand the relative importance of species identity and species richness on N removal and N_2O emissions in CWs.

In this study, microcosms simulating vertical flow CWs were established. These microcosms included monoculture and mixture of four plant species. All plant communities were supplied with a simulated wastewater with or without C (sucrose) addition. The objectives of this study were to (1) investigate the effects of C addition on N removal and N_2O emissions; (2) study the effects of species richness and identity on N removal and N_2O emissions; (3) compare the effects of plant diversity and C addition on N removal and N_2O emissions.

2. Materials and methods

2.1. Experimental design

The study was conducted in 50 simulated vertical flow CWs microcosms in an open field at Zhejiang University (30°18' N, 120°05' E, Hangzhou City, Southeast China). The microcosms were constructed using ceramic tubs (53 cm long × 17.5 cm wide × 18.5 cm high) and filled with coarse sand (0.5–3 mm) to a depth of about 12 cm.

On the basis of the physiological and morphological traits, which influence the resource requirements and growth traits, four common local plant species, *Juncus effusus* L., *Oenanthe javanica* (Blume) DC, *Phalaris arundinacea* L. and *Rumex japonicus* Houtt. were selected for the experiment. The seedlings were transplanted into microcosms with a density of 12 individuals per microcosm in March 2013.

In this study, the simulated wastewater was based on the Hoagland nutrient solution (Hoagland and Arnon, 1950) with minor

modifications. Nitrate-N was the sole N source, and the concentration (336 mg NL^{-1}) was three times higher than that of Hoagland. Four treatments were used here: (1) monocultures supplied with simulated wastewater without C addition was set as the control; (2) monocultures supplied with simulated wastewater with C addition; (3) high species richness (four-species mixture) supplied with simulated wastewater without C addition; and (4) four-species mixture supplied with simulated wastewater with C addition. As shown in Fig. 1, the experiment was arranged in a completely random block design with five replicates laid out in five blocks, totally 50 microcosms.

For the experiment supplied with simulated wastewater with C addition, sucrose was added to create chemical oxygen demand (COD) to N ratio (1:1). The simulated wastewater was supplied once every ten days, for a total of eight times. During the experiment, each microcosm received 18.816 g total inorganic N (TIN). The water level was kept at 1.5 cm above the sand surface of each microcosm, and each microcosm contained 7 L of simulated wastewater. The microcosms were supplied daily with tap water to compensate for evaporation loss.

2.2. Sampling and measurements

Water samples were taken on the eighth day after the last supply of simulated wastewater and then were stored in a freezer at $-20^{\circ}C$. Each water sample was filtered using a membrane syringe filter (pore size 0.45 μm) before analysis. The total organic C concentration (TOC) in water was measured by the non-dispersion infrared method, the nitrate-N and ammonium-N concentrations were measured by spectrophotometric method (Clesceri et al., 1999), although nitrate-N was the sole N source, the ammonium-N may come from the organic N released by plants (Chang et al., 2014). The sum of nitrate-N and ammonium-N concentrations is the TIN concentration. The N removal efficiency (NRE) in the last batch of supplying simulated wastewater was calculated based on mass balance:

$$NRE(\%) = (N_i - N_e) / N_e \times 100$$

where N_i is the amount of influent TIN of each microcosm (mg); N_e is the amount of effluent TIN of each microcosm (mg).

Gas samples were collected on the eighth day after the last supply of simulated wastewater using the static chamber method described in Chang et al. (2014). The PVC static chambers (24 L) were placed on the ground surface of microcosms. The gas collection was carried out from 8:00 to 10:00 am at 30 min intervals. Gas samples were collected in 100 mL gas sampling bags (Plastic gas, Delin Company, China) using 50 mL polyurethane syringes. The air temperature inside the chamber was monitored during gas collection. The N_2O concentration was determined using a gas chromatograph (Agilent-7820, USA). The N_2O emission rate ($mg\ m^{-2}\ d^{-1}$) was calculated according to the equation described in Cheng et al. (2007). Then N_2O -N emission per unit N removal ($mg\ g^{-1}$) was calculated as the N_2O -N emission per day divided by TIN removal per day in the last batch of supplying simulated wastewater.

At the end of the experiment, all plants were harvested by species, cleaned and measured for above- and below-ground biomass after the samples were oven dried at $65^{\circ}C$ for 72 hr. Dry plant tissue materials were ground to the fine powders. Then plant tissue N concentrations and foliar $\delta^{15}N$ value were measured using an isotope ratio mass spectrometer linked to an elemental analyzer (Flash HT2000, Thermo Finnigan, Bremen, Germany). Plant tissue N pools were calculated by multiplying tissue N concentration per species by its tissue biomass. Above- and below-ground N pools were summed to obtain the total N pool. The contribution of plant

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