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Decreases in ammonia volatilization in response to greater plant diversity in microcosms of constructed wetlands

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HIGHLIGHTS

- We first explore the relationship between ammonia volatilization and biodiversity.
- Ammonia volatilization reduced with plant species richness in simulated CWs.
- Ammonia volatilization was more affected by species composition than richness.
- Systems with *Rumex japonicus* had lower ammonia volatilization than others.

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ABSTRACT

Ammonia volatilization from wastewaters with a high concentration of ammonium is a serious environmental and health problem. Constructed wetlands (CWs) are widely used for treating wastewater, and plant diversity clearly improves some functions of ecosystem such as nitrogen removal. However, whether plant diversity can affect ammonia volatilization from wastewater is still unknown. In this study, we conducted a microcosm experiment with different plant diversity treatments using four plant species. Results showed that, (1) ammonia volatilization decreased with increasing plant species richness; (2) ammonia volatilization from systems containing *Rumex japonicus* was lower than other systems; and (3) ammonia volatilization was affected more by species composition than species richness. This paper is the first to report that ammonia volatilization is reduced by plant diversity, and that some plant species combinations are important to reduce ammonia volatilization from CWs when treating wastewater.

1. Introduction

Ammonia (NH_3) volatilization causes broad concern due to its environmental and health impacts (Sutton et al., 2008; Gu et al., 2014), especially its contribution to haze (Kirkby et al., 2011; Shen et al., 2014). Wastewaters with a high concentration of ammonium nitrogen (NH_4^+-N, those from livestock production and agricultural fertilizer application) are the important source of NH_3 volatilization (Reis et al., 2009; Huang et al., 2012). How to deal with wastewaters with a high concentration of NH_4^+-N and reduce NH_3 volatilization has become an urgent challenge.

Constructed wetlands (CWs) have been widely applied to treat wastewater pollution around the world (Liu et al., 2009; Vymazal, 2014). CWs, as an alternative to the wastewater treatment plant, are feasible and suitable to treat the decentralized agricultural wastewaters with low cost (Liu et al., 2012). However, problems still exist in the large amount of NH_3 that volatilizes from wastewater in the treatment process. While there are some abatement methods for reducing NH_3 from wastewater, such as acidification and rapid nitrification by artificial means, these methods are expensive and energy consuming, and therefore are not feasible (Lin et al., 2005; VanderZaag et al., 2015). There is still a lack of appropriate abatement methods, while alternative ecological approach like plants receives less attention.

Plants play an important role in nutrient cycle and physiochemical condition of substrate (Vymazal, 2007) and can affect NH_3 volatilization in wastewater treatment processes in CWs (Mattsson and Schjoerring, 2002; Poach et al., 2002). Some studies have found that *Bromus erectus* monocultures emitted more than twice the NH_3 than *Lolium perenne* monocultures (Mattsson and

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Schjoerring, 2002), while the *Scirpus* sp. and *Typha* sp. monocultures had no difference in mean NH_3 volatilization (Poach et al., 2002). The effect of plant communities with diverse species on NH_3 volatilization has not yet been reported to our knowledge.

Plant diversity (species richness and composition) is a major determinant of ecosystem processes and functions, such as productivity, nutrient retention, microbial activity, greenhouse gas emissions, and nitrogen removal (Tilman et al., 1996; Zhang et al., 2012; Chang et al., 2014; Ge et al., 2015; Grace et al., 2016). In this study, we conducted a microcosm experiment to simulate CWs. We chose four species planted in a gradient of increasing richness from 1 to 4 species and assembled 15 possible species combinations. We measured the NH_3 volatilization flux from each microcosm. The aim of this study was to explore in the wastewater treatment process, (i) the relationship between NH_3 volatilization and plant species richness; (ii) the relationship between NH_3 volatilization and plant species composition; and (iii) the relative importance of the effects of species richness versus species composition on NH_3 volatilization.

2. Materials and methods

2.1. Microcosm design, planting pattern and wastewater irrigation

We set up a microcosm experiment to test the effect of plant diversity on NH_3 volatilization. The study site, located at the campus of Zhejiang University in Hangzhou City (120°05′ E, 30°18′ N), Southeast China, has a humid subtropical climate with an average annual temperature of 18.7 °C and rainfall of 1350 mm. The microcosm simulated a constructed wetland and consisted of a porcelain column (length × wideness × height = 51 cm × 38 cm × 18 cm) filled with sand (particle diameter = 0.2 mm - 3 mm) to a depth of 15 cm. Prior to the experiment, the sand was washed with tap water to ensure there was no plant propagule left.

Four common early-spring species, *Cichorium intybus* L., *Lolium perenne* L., *Medicago sativa* L. and *Rumex japonicus* Houtt., with similar size and vitality were transplanted into the microcosms in March 2014. We planted these species to establish 15 species combination treatments that ranged from a richness gradient of 1–4 species: monocultures of the four species, all possible combinations of two-species, all possible combinations of three-species, and a combination with all species. The experiment was arranged in a complete block design with 6 blocks, ensuring that each block contained one microcosm from each treatment and excluding the occurrence of identical replicates in the same block. Therefore, each treatment had 6 replicates and there were a total of 90 microcosms. Each microcosm had 12 individuals with an equal number of individuals assigned to each species. Weeds were removed by hand during the experiment to maintain the original designed species composition.

The simulated wastewater was the Hoagland nutrient solution (Hoagland and Arnon, 1950) with a minor modification. NH_4^+-N was the sole nitrogen source in the influent, and its concentration was 336 mg L^{-1} (Table 1). The simulated wastewater was supplied once every 10 days, from late March to late June 2014. Each microcosm received 7 L simulated wastewater, and the water level was kept 3 cm below the sand surface.

2.2. Sampling methods

The volatilization of NH_3 was measured once using the static chamber technique (Van der Stelt et al., 2007) one day after wastewater feeding at the end of experiment. The chamber was a square polyvinyl chloride chamber (68 L), and was placed to cover a microcosm, with its opening edge overlapping with the edge of porcelain column. A small flask, containing 20 mL 3.2 *M* H_2SO_4, was placed on the surface of the sand in advance, in order to trap the volatilized NH_3. The gas absorption continued for 16 h and was conducted simultaneously for each microcosm in the same block in order to detect the effect of plant diversity. Immediately after gas collection, the absorption liquid was put into a 100 mL polyethylene plastic bottle, and stored temporarily in a refrigerator at 4 °C. Before gas sampling, 100 mL wastewater was sampled in each microcosm and stored in a refrigerator at −20 °C. The plants were harvested by species after gas sampling. When conducting the harvest, we added water into the microcosms to loosen the sand, so that the whole plants including the aboveground and belowground parts could be taken out of the sand easily.

2.3. Parameter analyses

When the gas was collected, the concentration of NH_4^+-N in the absorption liquid was detected by Nessler's reagent colorimetry. The NH_3 volatilization flux (F, μg N m^{-2} h^{-1}) in the CWs was,

$$F = \frac{C \times V}{T \times S} \tag{1}$$

where C is the concentration of NH_4^+-N in the absorption liquid (μg N mL^{-1}); V is the volume of absorption liquid (mL); T is absorption time (16 h); S is the trapping area of the chamber (0.194 m^2).

The water samples were filtered by membrane (pore size, 0.45 μm) prior to the analysis for NH_4^+-N concentration in wastewater. Plant total biomass was calculated as the dry weight per square meter after the plant samples were oven-dried at 65 °C for 72 h and then weighed.

2.4. Statistical analyses

In order to detect the effect of species richness, the simple linear regression was used to test the relationships between plant species richness and the response variables (NH_3 volatilization flux,

Table 1
Content of nutrient components in the simulated wastewater (modified Hoagland nutrient solution).

Macroelements		Microelements	
Nutrient components	Content (g L^{-1})	Nutrient components	Content(mg L^{-1})
$(NH_4)_2SO_4$	1.58	H_3BO_3	2.86
$CaCl_2 \cdot 2H_2O$	0.74	$MnCl_2 \cdot 4H_2O$	1.81
KH_2PO_4	0.14	$ZnSO_4 \cdot 7H_2O$	0.22
$MgSO_4 \cdot 7H_2O$	0.49	$CuSO_4 \cdot 5H_2O$	0.08
KCl	0.45	$H_2MoO_4 \cdot 4H_2O$	0.09
		$FeSO_4 \cdot 7H_2O$	5.56
		Na_2EDTA	7.44