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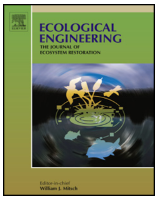

Positive effects of plant diversity on nitrogen removal in microcosms of constructed wetlands with high ammonium loading

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ABSTRACT

Wastewater with a high nitrogen (N) loading rate and high ammonium (NH_4^+-N)/nitrate (NO_3^--N) ratio is increasingly becoming a problem in regional environment. An experimental system was established to test the effects of plant diversity on N removal in microcosms of constructed wetlands. The microcosms were treated with simulated wastewater with NH_4^+-N as the sole chemical form of N, and with mixed NH_4^+/NO_3^-N (1:1) as a control, both at a loading rate of 162 g N m^{-2} yr^{-1}. Results indicated that: (1) high plant species and functional group richness improved N removal efficiency under a high ammonium loading rate, (2) high species richness reduced the difference of N removal between sole NH_4^+-N and mixed NH_4^+/NO_3^--N treatments, (3) the presence of *Coix lacryma-jobi* (C_4 grass) increased both biomass and N removal, whereas the presence of *Aeschynomene indica* (legume) increased N removal but decreased biomass of the communities. Overall, the results showed that high plant diversity enhanced N removal efficiency regardless of the N-form ratios and N level in the ecosystem.

1. Introduction

Anthropogenic nitrogen (N) contamination is increasing rapidly in recent decades (Galloway et al., 2008; Compton et al., 2011; Gu et al., 2013). Excessive N loading to the receiving water bodies may degrade air and water quality and ecosystem function (Galloway et al., 2008). Wastewater from different sources has complex N forms, with the NH_4^+/NO_3^--N ratio ranging from 0.4% to 99.1% (Liu et al., 2009). In domestic and livestock wastewater, NH_4^+-N is the main inorganic N form (Hunt et al., 2002; Zhang et al., 2009). Pollution of NH_4^+-N can place multiple stresses on the environment (Britto and Kronzucker, 2002; Chang et al., 2010). Therefore, it is critical to identify proper treatment technologies to address wastewater with high N loading and NH_4^+/NO_3^--N ratio.

Presently, the artificially enhanced nitrification process is one way to deal with wastewater with high NH_4^+-N concentration. However, the industrialized rapid nitrification process is relatively costly so it can only be used in specific point sources (such as shrimp aquaculture) with high economic value (Lin et al., 2005). As an alternative approach, constructed wetlands (CWs) have been widely used for treating various wastewaters as a cost-effective method with low operational and maintenance requirements (Vymazal, 2007; Wu et al., 2014). Optimizing physical structures can also improve ecosystem N removal efficiency, for example vertical flow CWs enhance nitrification and thus decrease NH_4^+-N concentration (Vymazal, 2007, 2013). Furthermore, optimizing the community structure of ecosystems is another important aspect. Regulating plant community structure may influence the microbial diversity and substrate N process and enhance N removal efficiency in CWs (Knops et al., 2002; Vymazal, 2011).

Plant species may have specific tolerance to and preference for NO_3^--N and/or NH_4^+-N (Britto and Kronzucker, 2002; Nakamura et al., 2010; Gherardi et al., 2013). Such differences may affect ecosystem function such as resource utilization (Tilman, 1997; Hooper and Dukes, 2004; McLaren and Turkington, 2010). Some researchers have found that plant diversity improved ecosystem function under high N concentration with mixed NO_3^-/NH_4^+-N (Zhu et al., 2010; Zhang et al., 2011) or sole NO_3^--N in CWs (Chang

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Table 1
The simulated wastewater (modified Hoagland nutrient solution).

Macronutrients	Stocking solution (mmol L^{-1})		Micronutrients	Concentration (mg L^{-1})
	Sole NH_4^+-N	Mixed NH_4^+/NO_3^--N		
KNO_3	0	0.8	H_3BO_3	2.86
$Ca(NO_3)_2 \cdot 4H_2O$	0	1.6	$CuSO_4 \cdot 5H_2O$	0.08
$CaCl_2 \cdot 2H_2O$	5	3.4	$ZnSO_4 \cdot 7H_2O$	0.22
$(NH_4)_2SO_4$	4	1	$MnCl_2 \cdot 4H_2O$	1.81
KH_2PO_4	1	1	$H_2MoO_4 \cdot 4H_2O$	0.09
$MgSO_4 \cdot 7H_2O$	2	2	Fe-EDTA	7.64
KCl	6	5.2		

et al., 2014). However, whether such effects occur when NH_4^+-N is the sole inorganic N form in wastewater has not been reported to date. NH_4^+-N has a longer and more complex transformation pathway than NO_3^--N, and plant diversity may have complex effects on ecosystem function under sole NH_4^+-N treatment. It is necessary to study the effects of plant diversity on ecosystem function, especially N removal efficiency, when NH_4^+-N is the dominant N form in wastewater.

In this study, microcosms were established for CWs and fed with simulated wastewater with NH_4^+-N as the sole N form and mixed NH_4^+/NO_3^--N (ratio = 1:1) at the same high N concentration in order to investigate the effects of plant diversity on ecosystem function. The objectives were to investigate that, under the two N-form ratios, the effects of (1) plant species and functional group richness on ecosystem N removal efficiency, (2) individual species presence on ecosystem N removal efficiency.

2. Materials and methods

2.1. Experimental platform and design

Microcosms simulating vertical flow in CWs were established at the South Campus of Shaoxing University (29°59′N, 120°34′E, Shaoxing City, Zhejiang Province, China). A total of 198 microcosms using ceramic tubs (53 cm long × 17.5 cm wide × 18.5 cm high) were constructed in an open field in March 2009. Each microcosm was filled with coarse sand (particle size 2–4 mm) to a depth of about 12 cm.

A species pool was established and the species were growing in the wetlands and meadows nearby. On the basis of the physiological and morphological traits, which influence the resource requirements and growth traits (Tilman et al., 1997; Reich et al., 2001; Mokany et al., 2008), eight species with similar size and as the dominate species in the communities were selected as the experimental species. The species belong to four functional groups (C_3 and C_4 grasses, legumes and forbs): *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steudel (C_3 grasses), *Imperata cylindrical* (L.) Beauv. and *Coix lacryma-jobi* L. (C_4 grasses), *Campylotropis macrocarpa* (Bunge) Rehd. and *Aeschynomene indica* L. (legumes), *Canna indica* L. and *Lythrum salicaria* L. (forbs). These four functional groups represent important functional distinctions relevant to production and resource utilization (Wedin and Tilman 1996; Reich et al., 2001). It will also helpful for comparing with other biodiversity and ecosystem functioning experiments that used species belong to the four functional groups.

A two-factor experimental design was used in this study. One factor was the N-form ratio, with 99 microcosms supplied simulated wastewater with sole NH_4^+-N as the treatment. Another 99 microcosms were supplied simulated wastewater with mixed NH_4^+/NO_3^--N (ratio = 1:1) as the control. As NO_3^--N and NH_4^+-N can convert to other N form in microcosms, the N-form ratio in the experiment was just controlled in the influent. The simulated wastewater was based on the Hoagland nutrient solution (Table 1). From mid-May to late-June 2009, the simulated wastewater was added once a week, for a total of seven times. The N loading rate of each microcosm was 162 g N m^{-2} yr^{-1}. During the experiment, each microcosm was irrigated with tap water once a day to compensate for evaporation loss.

Under each N-form ratio treatment (sole NH_4^+-N and mixed NH_4^+/NO_3^--N), plant diversity treatments included five species

Table 2
Design of plant species and functional group diversity in the experiment.

Species richness	Functional group richness	Species composition	Functional group composition
1	1	*Ad,Pa*	C_3
1	1	*Ic, Cl*	C_4
1	1	*Cm, Ai*	L
1	1	*Ci, Ls*	F
2	1	Ad + *Pa*	C_3
2	1	*Ic* + *Cl*	C_4
2	1	*Cm* + *Ai*	L
2	1	*Ci* + *Ls*	F
2	2	*Ad* + *Ic, Ad* + *Cl, Pa* + *Cl*	$C_3 + C_4$
2	2	*Pa* + *Cm, Pa* + *Ai*	C_3 + L
2	2	*Ic* + *Ai, Cl* + *Ai*	C_4 + L
2	2	*Ai* + *Ci, Ai* + *Ls, Cm* + *Ls*	L + F
4	2	*Ad* + *Pa* + *Ic* + *Cl*	$C_3 + C_4$
4	2	*Ad* + *Pa* + *Cm* + *Ai*	C_3 + L
4	2	*Ic* + *Cl* + *Cm* + *Ai*	C_4 + L
4	2	*Cm* + *Ai* + *Ci* + *Ls*	L + F
4	3	*Ad* + *Cl* + *Cm* + *Ai, Pa* + *Ic* + *Cl* + *Cm*	$C_3 + C_4$ + L
4	3	*Pa* + *Ic* + *Ci* + *Ls*, Pa + *Ic* + *Cl* + *Ci*	$C_3 + C_4$ + F
4	3	*Ad* + *Pa* + *Cm* + *Ai, Pa* + *Ai* + *Ci* + *Ls*	C_3 + L + F
4	3	*Ic* + *Cl* + *Ai* + *Ci, Cl* + *Cm* + *Ci* + *Ls*	C_4 + L + F
4	4	*Pa* + *Ic* + *Ai* + *Ci, Ad* + *Cl* + *Cm* + *Ls*	$C_3 + C_4$ + L + F
6	3	*Ad* + *Pa* + *Ic* + *Cl* + *Cm* + *Ai*	$C_3 + C_4$ + L
6	3	*Ad* + *Pa* + *Ic* + *Cl* + *Ci* + *Ls*	$C_3 + C_4$ + F
6	3	*Ad* + *Pa* + *Cm* + *Ai* + *Ci* + *Ls*	C_3 + L + F
6	3	*Ic* + *Cl* + *Cm* + *Ai* + *Ci* + *Ls*	C_4 + L + F
8	4	*Ad* + *Pa* + *Ic* + *Cl* + *Cm* + *Ai* + *Ci* + *Ls*	$C_3 + C_4$ + L + F

Note: *Ad*: *Arundo donax*; *Pa*: *Phragmites australis*; *Ic*: *Imperata cylindrical*; *Cl*: *Coix lacryma-jobi*; *Cm*: *Campylotropis macrocarpa*; *Ai*: *Aeschynomene indica*; *Ci*: *Canna indica*; *Ls*: *Lythrum salicaria*. Plus sign+ means different species or functional groups mixture in a composition; comma , means different species compositions.