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Effects of plant diversity on greenhouse gas emissions in microcosms simulating vertical constructed wetlands with high ammonium loading

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ABSTRACT

Wastewater with relatively high nitrogen concentrations is a major source of nitrous oxide (N_2O) and methane (CH_4) emissions and exerts multiple stresses on the environment. Studies have shown that plant diversity plays an important role in ecosystem functioning. However, the effects of plant species diversity on CH_4 and N_2O emissions under high ammonium (NH_4^+-N) loading rates remain unclear. In this study, a microcosm experiment simulating vertical constructed wetlands supplied with high NH_4^+-N water levels was established. The treatments included four species richness levels (1, 2, 3, 4) and 15 species compositions. There was no significant relationship between species richness and N_2O emissions. However, N_2O emissions were significantly reduced by specific plant species composition. Notably, the communities with the presence of *Rumex japonicus* L. reduced N_2O emissions by 62% compared to communities without this species. This reduction in N_2O emissions may have been a result of decreased N concentrations and increased plant biomass. CH_4 emissions did not respond to plant species richness or species identity. Overall, plant species identity surpassed species richness in lowering N_2O emissions from constructed wetlands with high NH_4^+-N water. The results also suggest that communities with *R. japonicus* could achieve higher N removal and lower greenhouse gas emissions than other wetland species.

Introduction

The amount of wastewater with high-strength nitrogen (N) has been increasing rapidly in recent decades along with economic development (Galloway et al., 2008; Gu et al., 2015). Wastewater from different sources varies in N forms (Liu et al., 2009; Vymazal, 2014). For example, domestic and livestock wastewater contains relatively high ammonium (NH_4^+-N) concentrations (Hunt et al., 2002). Excess NH_4^+-N can exert multiple stresses on the environment, such as eutrophication, resulting in ammonium toxicity for many plant species (Britto and Kronzucker 2002). Wastewater is also a major source of greenhouse gas emissions, including methane (CH_4) and nitrous oxide (N_2O) emissions (IPCC 2014; Yan et al., 2014). Thus, the development of suitable technology to improve NH_4^+-N removal efficiency and lower greenhouse gas emission is critical.

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Constructed wetlands (CWs) are cost-effective systems and have been widely used for treating various wastewaters (Liu et al., 2012; Vymazal, 2014; Wang et al., 2017). Optimizing the physical structural of CWs can improve the N removal efficiency and reduce greenhouse gas emissions. For example, vertical flow CWs increases NH_4^+-N removal through improving the nitrification process (Vymazal 2007) and decreases CH_4 emissions through enhancing CH_4 oxidation (Teiter and Mander 2005; van der Zaag et al., 2010). Furthermore, assembling high plant diversity, with increased species richness and identity, can optimize the ecological structure (community structure) of CWs. Many recent studies have indicated that high plant species diversity enhances N removal efficiency across a wide range of N levels and forms (Fraser et al., 2004; Zhu et al., 2010; Ge et al., 2015).

The effect of plant species diversity on greenhouse gas emissions has received much attention (Tilman et al., 2014; Maucieri et al., 2017). Plants species can differ greatly in the production, consumption, and transport of N_2O and CH_4 (Cheng et al., 2007; Maltais-Landry et al., 2009; Jørgensen et al., 2012). These differences are mainly explained by anatomical and physiological properties of species (Jørgensen et al., 2012; Zhang et al. 2012). Planting different species in an ecosystem can use resources effectively or create competition, as well as have positive or negative effects on ecosystem functioning (Maucieri et al., 2014; Barbera et al., 2015; Jahangir et al., 2016). Increasing plant species diversity likely decreases the CH_4 and N_2O emissions by enhancing plant N uptake (Bouchard et al., 2007; Niklaus et al., 2016; Han et al., 2017). However, some studies have reported high plant species diversity increases CH_4 and N_2O emissions due to plant species with aerenchym (Zhang et al., 2012; Chang et al., 2014), while other studies have shown that species diversity did not affect CH_4 and N_2O emissions (Abalos et al., 2014; Zhao et al., 2016). However, it should be noted that all these studies were conducted in habitats with only NO_3^--N or mixture of NH_4^+-N and NO_3^--N.

The concentration of chemical forms of N (NH_4^+-N, NO_3^--N and the mixture ratio) are expected to influence the CH_4 and N_2O emissions (Kampschreur et al., 2009; Mander et al., 2014). As the size and structure of NH_4^+ are similar to those of CH_4, it is likely to inhibit CH_4 oxidation because of competition for methane oxidase (Schimel 2000). In addition, NH_4^+-N may also influence CH_4 production through influencing plant growth, which influenced carbon supply to methane producers (Schimel 2000; Bodelier and Laanbroek 2004). Furthermore, the high concentration of NH_4^+-N may increase N_2O emissions through enhancing nitrification (Bouwman et al., 2002). Yet, the effects of plant species diversity on CH_4 and N_2O emissions from CWs with high NH_4^+-N loading rate remains unclear.

In this study, we established 90 microcosms to simulate the vertical flow CWs. The systems were supplied with the simulated wastewater with NH_4^+-N as the sole N form. Four species richness levels (1, 2, 3, and 4) and 15 species compositions were assembled. The CH_4 and N_2O emissions, N concentrations in effluent, N accumulations in substrate and plant tissues were measured. The objectives of this study were to (1) investigate the effects of plant species richness on CH_4 and N_2O emissions from CWs with high NH_4^+-N loading; (2) investigate the effects of plant species identity on CH_4 and N_2O emissions; and (3) determine appropriate plant communities with high N removal efficiency and low greenhouse gas emissions in CWs with high NH_4^+-N loading.

1. Material and methods

1.1. Experimental design

The microcosms simulating vertical flow CWs, which had high ammonium removal efficiency (Vymazal 2007), were established in an open field at the campus of Zhejiang University (30°18′ N, 120°05′ E, Hangzhou, China) in March 2014. The microcosms were constructed using ceramic tubs (51 cm long × 38 cm wide × 18 cm high) and filled with sand (particle diameter = 0.5–3 mm) to a depth of 15 cm. The simulated wastewater is dosed onto the surface of substrate and then flows download passing through the substrate (Fig. 1).

Four common herbaceous plants, *Lolium perenne* L., *Cichorium intybus* L., *Medicago sativa* L., and *Rumex japonicus* L. were selected as the experimental species. These four species were mesophytes herbaceous plants, which could growth well in this system with water level was fluctuated and keep constant water level at 3 cm below the substrate surface. Furthermore, the plants could be used as feed for ruminant livestock. They also differ in their functional traits with respect to aerenchyma, shoot/root ratio, and specific leaf area (Table 1). There were four species richness levels (1, 2, 3, 4) and 15 plant community compositions: four monocultures, six two-species mixtures, four three-species mixtures, and a four-species mixture. The planting density was 12 individuals per microcosm (about 60 plants/m^2). During the experiment, invasive species were removed weekly to maintain the original species compositions. The experiment used a randomized complete block design, with six replications laid out in six blocks. In total, there were 90 microcosms in this experiment.

The simulated wastewater was based on the Hoagland nutrient solution (Hoagland and Arnon 1950) with minor modifications (Table 2). The solution ensures the normal growth and development of plants and simplified the experiment condition and focus on the target factor. The solution used as simulated wastewater has been widely used in many experiments (Chaney et al., 2009; Kaokniffin et al., 2011; Du et al., 2018). In simulated wastewater, ammonium-N (NH_4^+-N) was the sole N source, with concentration of 336 mg N/L. Each microcosm was supplied with 7 L of simulated wastewater once simulating the batch water operation mode of CWs (Faulwetter et al., 2009; Du et al., 2018), the simulated wastewater was supplied once every 10 days, for a total of seven times. The total N loading rate of each microcosm was 442 g N/m^2/year. To supplement the water loss by evapotranspiration of the system, tap water was feed intermittently with period two times per day.

1.2. Sampling methods

In June 2014, static chambers were used to collect gas samples (Johansson et al., 2003; Chang et al., 2014). To improve the comparability among 15 plant compositions, a square

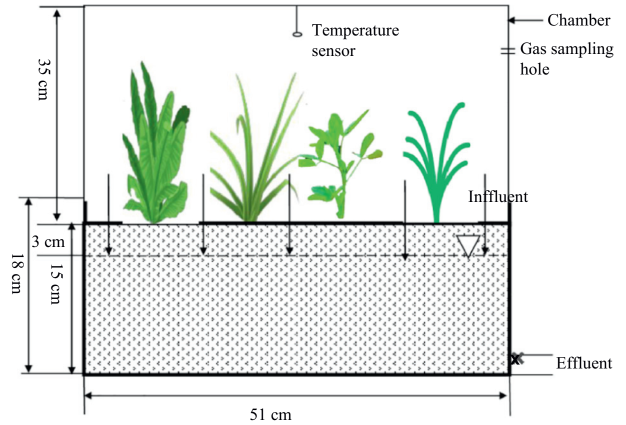

Fig. 1 – Illustration of the microcosms of constructed wetlands. The vertical cross-section of the microcosm (51 cm long × 38 cm wide × 18 cm high) and the sampling chamber (51 cm long × 38 cm wide × 35 cm high).

polyvinyl chloride chamber (51 cm long × 38 cm wide × 35 cm high) was simultaneously placed to cover a microcosm, with its opening edge overlapping with the edge of porcelain column (Fig. 1). After stabilizing the chamber for 30 min, gas samples were collected in 100 mL gas sampling bags (Plastics, Delin Company, China) using 50 mL polyurethane syringes. The air temperature inside the chamber was monitored during gas collection. N_2O and CH_4 concentrations in the gas samples were determined using a gas chromatograph (Agilent - 7820, USA) with a detector (Electron Capture Detector and Flame Ionization Detector, respectively) and a Poropak Q column (3 m). N_2O and CH_4 emissions were calculated according to the equation from Cheng et al., (2007).

Following gas sampling, effluent samples were collected and then were stored in a refrigerator at −20 °C prior to N concentration testing. Each effluent sample was filtered using a membrane syringe filter (pore size 0.45 μm) before analysis. The NH_4^+-N and nitrate-N (NO_3^--N) concentrations in effluent were determined by a spectrophotometric method with a continuous flow analyzer (SAN plus, Skalar, The Netherlands). The total inorganic N (TIN) concentration was calculated as the sum of NH_4^+-N and NO_3^--N concentrations.

Prior to harvesting plants, the five fully expanded leaves from four species monocultures were collected. The leaf area of samples was measured with a leaf area measurement system (WinFOLIA). The samples were dried in a paper envelope at 65 °C for 72 hr and weighed to measure dry weight. These measurements of leaf area and dry weight were used to calculate the specific leaf area.

Table 1 – Plant traits of the species in our species pool.

Plant species	Family	Aerenchyma	Shoot/root ratio	Specific leaf area (cm^2/g)
Lolium perenne	Poaceae	No	3.92 [b]	289.11 [a]
Cichorium intybus	Asteraceae	No	7.43 [a]	232.62 [b]
Medicago sativa	Fabaceae	No	4.56 [b]	209.48 [bc]
Rumex japonicus	Polygonaceae	Yes	2.24 [c]	269.23 [ab]

Note: Different letters within columns indicate significant difference by applying “Dunn’s test” at $P < .05$.

After treated for 70 days, all plants were harvested by species at the stage of their biomass peak. The above- and belowground biomass were measured after the samples were dried at 65 °C for 72 hr. The sum of above- and belowground biomass was the total biomass.

After plant harvesting, the substrate of each microcosm was well homogenized, sampled and stored in a refrigerator at −20 °C waiting for further analysis. A subsample from each substrate sample was used to determine soil water content. Soil bulk density is the ratio of the mass to the bulk volume of soil. The soil mass is determined after drying to constant weight at 105 °C, and the volume (10 cm^3 in this study) is that of the samples as taken in field (Blake and Hartge 1986). To determine substrate N accumulation, 150 g fresh sample was extracted with 2 mol/L KCl at a ratio of 1:1.5 (water volume to substrate weight), and then NH_4^+-N and NO_3^--N concentrations in extract liquid were determined as described above. Substrate N accumulations were calculated based on soil water content and bulk density of the microcosms.

1.3. *Statistical analysis*

Data were examined to investigate the effects of species richness and identity on ecosystem functioning. Initially, the data were analyzed to test for normality (Kolmogorov–Smirnov test) and equality of variance (Levene’s test). As variances were not equal, a non-parametric test (Kruskal–Wallis test) was used to analyze the effects of species richness or species compositions on parameters (N_2O and CH_4 emissions, N concentrations in effluent, N accumulations in substrate, and plant biomass). If the effect proved to be significant, the “Dunn’s test” was used to determine difference among treatments (Dunn 1961). A non-parametric test (Kruskal–Wallis test) was also ran to determine the difference between means of the parameters when a species was present or absent from the system. A diversity effect may be the result of the presence or absence of the four species. Thus, multiple linear regressions were used to test concurrently for the effects of species richness and species identity (presence vs. absence in a community) on the parameters (Engelhardt and Ritchie 2002). Relationships among parameters were analyzed using the Pearson correlation coefficient. All statistical analyses were conducted using software R 3.4.2. The statistical significance level was set at $\alpha = 0.05$.

Table 2 – The simulated wastewater (modified Hoagland nutrient solution).

Macronutrients		Micronutrients	
$(NH_4)_2SO_4$ (g/L)	1.58	H_3BO_3 (mg/L)	2.86
KNO_3 (g/L)	0	$MnCl_2 \cdot 4H_2O$ (mg/L)	1.81
$Ca(NO_3)_2 \cdot 4H_2O$ (g/L)	0	$ZnSO_4 \cdot 7H_2O$ (mg/L)	0.22
$CaCl_2 \cdot 2H_2O$ (g/L)	0.74	$CuSO_4 \cdot 5H_2O$ (mg/L)	0.08
KH_2PO_4 (g/L)	0.14	$H_2MoO_4.4H_2O$ (mg/L)	0.09
$MgSO_4 \cdot 7H_2O$ (g/L)	0.49	$FeSO_4.7H_2O$ (mg/L)	5.56
KCl (g/L)	0.45	Na_2EDTA (mg/L)	7.44

2. Results

2.1. Nitrous oxide and methane emissions in response to species richness and identity

Neither N_2O nor CH_4 emissions respond to species richness (Table 3). Species identity significantly affected N_2O emissions, but not CH_4 emissions. The presence of *R. japonicus* significantly decreased N_2O emissions, which was by 62% compared to communities without this species (Table 4). When accounting for the effects of species richness and the presence of *R. japonicus*, species identity had a significantly negative effect on N_2O emissions while species richness was insignificant (Table 5).

2.2. Nitrogen concentrations in effluent and N accumulations in substrate in response to species richness and identity

The concentrations of NH_4^+-N, NO_3^--N, and TIN in effluent did not respond to plant richness (Table 3). However, species composition significantly affected NH_4^+-N and TIN concentrations in effluent (Tables 3 and 6). The NH_4^+-N and TIN concentrations in effluent of *R. japonicus* monoculture was the lowest among the four monocultures (Table 6), and the presence of *R. japonicus* had a significantly negative effect on N concentrations in effluent. The NH_4^+-N and TIN concentrations in effluent of *M. sativa* monoculture was the highest among the four monocultures (Table 6), and the presence of *M. sativa* had a significantly positive effect on N concentrations in effluent (Table 4).

The accumulations of NO_3^--N, NH_4^+-N, and TIN in substrate did not respond to species richness (Table 3). Species composition significantly affected the NO_3^--N accumulation in substrate (Table 3). The presence of *R. japonicus* decreased the NO_3^--N accumulation in substrate, while the presence of the other three plant species did not affect the N accumulation in substrate (Table 4).

2.3. Plant biomass production in response to species richness and identity

The aboveground-, belowground-, and total plant biomass did not respond to species richness, but species composition significantly affected plant biomass (Table 3). Among the four monocultures, plant biomass of *L. perenne* or *R. japonicas* monocultures were higher than other two species (Table 6), and the presence of *L. perenne* or *R. japonicus* significantly increased plant biomass (Table 4). In contrast, the presence of *C. intybus* significantly decreased plant biomass (Table 4).

2.4. Greenhouse gas emissions in response to N concentrations in effluent and N accumulations in substrate and plant biomass

The N_2O emissions positive correlated to TIN concentrations in effluent (Fig. 2a). In contrast, N_2O emissions negative correlated to plant biomass (Fig. 2b). However, CH_4 emissions not correlated to N concentrations in effluent, N accumulations in substrate, and plant biomass in this study ($P > .05$; data is not shown).

Table 3 – Non-parameter test the effect of species richness and species composition on ecosystem functioning.

	Species richness			Species composition		
	df	χ^2	*P*	*df*	χ^2	*P*
GHG emissions						
N_2O	3	1.33	0.72	14	6.86	0.94
CH_4	3	1.53	0.67	14	16.10	0.31
N concentrations in effluent						
NH_4^+-N	3	1.21	0.75	14	35.22	**<0.01**
NO_3^--N	3	2.81	0.42	14	12.48	0.57
TIN	3	1.10	0.78	14	35.48	**<0.01**
N accumulations in substrate						
NH_4^+-N	3	0.06	0.99	14	8.82	0.84
NO_3^--N	3	4.45	0.22	14	26.36	**0.03**
TIN	3	0.06	0.99	14	7.75	0.85
Plant biomass						
Aboveground	3	0.44	0.93	14	51.29	**<0.01**
Belowground	3	0.96	0.81	14	52.51	**<0.01**
Total	3	0.24	0.97	14	54.56	**<0.01**

Note: Significant *P* values ($P < .05$) are highlighted.

3. Discussion

3.1. Effects of species identity

As previously noted, plants species can differ greatly in mediating the production, consumption, and transport of

Table 4 – The effects of the presence or absence of particular species on ecosystem functioning.

Response variables	Source of variation			
	L. perenne	*C. intybus*	*M. sativa*	*R. japonicus*
GHG emissions (μg/m²/day)				
N_2O	0.900	0.365	0.067	**0.007↓**
CH_4	0.254	0.610	0.785	0.345
N concentrations in effluent (mg/L)				
NH_4^+-N	0.223	**0.020↑**	**0.007↑**	**<0.001↓**
NO_3^--N	0.685	0.412	0.275	**0.011↓**
TIN	0.225	**0.020↑**	**0.006↑**	**<0.001↓**
N accumulations in substrate (g/m)				
NH_4^+-N	0.244	0.449	0.345	0.376
NO_3^--N	0.053	0.160	0.075	**<0.001↓**
TIN	0.254	0.442	0.339	0.359
Plant biomass (g/m)				
Aboveground	**<0.001↑**	**<0.001↓**	0.054	**0.011↑**
Belowground	**0.009↑**	**<0.001↓**	0.051	**<0.001↑**
Total	**<0.001↑**	**<0.001↓**	0.051	**0.001↑**

Note: Arrows indicate significant increase (↑) or decrease (↓) of variables with the presence of certain species. Significant *P* values ($P < .05$) are highlighted.

Table 5 – Effect of species richness and presence of individual species on N_2O emission in multiple linear regressions.

Factor	Overall model		Species presence/absence			Species richness	
	Intercept	*P* value	R^2	Slope	*P* value	Slope	*P* value
R. japonicus	0.078	**0.002**	0.132	−0.099	**<0.001**	0.027	0.080
L. perenne	0.082	0.997	<0.001	0.001	0.969	0.001	0.966
C. intybus	0.082	0.311	0.026	0.044	0.128	−0.010	0.537
M. sativa	0.080	0.208	0.035	0.050	0.077	−0.011	0.498

Note: Significant *P* values ($P < .05$) are highlighted.

N_2O and CH_4 (Cheng et al., 2007; Maltais-Landry et al. 2009; Jørgensen et al., 2012). In this study, the communities with *R. japonicus* present reduced N_2O emissions by 62% compared those communities absent of this species (Tables 4 and 6). Such results may have been due to the high biomass of this species helping to improve the utilization of underground resources (Palmborg et al., 2005), and then reduced N_2O emissions. The presence of *R. japonicas* decreased the N concentration in effluent and increased plant biomass certificated this point (Table 4). In addition, *R. japonicas* has aerenchyma, which may increase the oxygen concentration in the root zone then inhibit the denitrification and decreased N_2O emission (Canfield et al., 2010). It should also be noted that aboveground biomass per individual of this species was relatively higher than expected in mixed communities (Fig. 3), that means this species is consistently dominant in mixed plant communities (Engelhardt and Ritchie 2001). The dominance of this species in terms of biomass under high N availability is also documented in previous studies (Luo et al., 2016; Han et al., 2017). This competitive advantage may improve its performance in mixed microcosms and overall be well suited in CWs for reducing N_2O emissions.

Previous studies have shown that species identity significantly influences CH_4 emissions (Jørgensen et al., 2012; Bhullar et al., 2014). However, species identity did not affect CH_4 emissions in this study (Table 4), this result was consistent with another study conducted under high NO_3^--N conditions (Han et al., 2017). This may have been due to the effects of various factors (i.e., N concentrations in effluent and plant biomass) on CH_4 emissions offset in the present study. For example, the presence of *R. japonicus* likely may have reduced NH_4^+-N concentrations and then stimulated CH_4 oxidation, as many studies have shown a large inhibitory effect of NH_4^+ on CH_4 oxidation (Carlsen et al., 1991; Niklaus et al., 2006). The presence of *R. japonicus* increased plant biomass, as plant biomass often promotes CH_4 emissions (Cheng et al., 2007; Zhang et al., 2012). Other factors, such as temperature, pH, and oxygen level (Le Mer and Roger 2001; Maucieri et al., 2017), may have also affected CH_4 emissions. Therefore, further study is needed to assess the mechanisms of plant species diversity did not affect CH_4 emissions from CWs with high NH_4^+-N loading.

3.2. Effects of species richness

In CWs with high NH_4^+-N loading, plant species richness did not affect N_2O emissions (Table 3). Likewise, Abalos et al. (2014) found that high plant species richness did not affect N_2O emissions from grasslands with low N loading. Previous studies have also reported that high plant species richness increased (Chang et al., 2014) or decreased N_2O emission (Han et al., 2017), although these experiments were conducted

Table 6 – Ecosystem functioning among fifteen species compositions.

Species composition	GHG emissions (mg/m^2/day)		N concentrations in effluent (mg/L)			N accumulation in substrate (g/m)			Plant biomass (g/m)		
	CH_4	N_2O	NH_4^+-N	NO_3^--N	TIN	NH_4^+-N	NO_3^--N	TIN	AGB	BGB	TB
Lp	−0.68	0.06	233.45^{ab}	1.10	234.56^{ab}	27.95	0.13^{ab}	28.09	898.96^{a}	319.76^{ab}	1218.72^{a}
Ci	−0.32	0.13	346.30^{ab}	0.78	347.08^{ab}	27.09	0.35^{ab}	27.43	187.55^{b}	21.04^{b}	208.59^{b}
Ms	−0.64	0.16	635.77^{a}	3.00	638.77^{a}	48.48	0.44^{a}	48.92	29.53^{b}	8.32^{b}	37.84^{b}
Rj	−0.50	0.04	192.67^{b}	0.51	193.17^{b}	25.11	0.15^{ab}	25.26	535.80^{ab}	478.8^{a}	1014.65^{a}
Lp × Ci	−0.27	0.08	432.48^{ab}	0.49	432.97^{ab}	30.15	0.15^{ab}	30.30	423.25^{ab}	226.35^{ab}	649.59^{ab}
Lp × Ms	−0.96	0.10	306.32^{ab}	1.55	307.88^{ab}	23.99	0.26^{ab}	24.26	558.28^{ab}	258.34^{ab}	816.62^{ab}
Lp × Rj	0.37	0.03	214.33^{b}	0.40	214.74^{b}	27.15	0.07^{b}	27.23	654.49^{a}	497.05^{a}	1151.55^{a}
Ci × Ms	−0.48	0.15	419.35^{ab}	2.37	421.72^{ab}	35.84	0.27^{ab}	36.11	76.07^{b}	12.24^{b}	88.31^{b}
Ci × Rj	−0.11	0.02	223.42^{ab}	0.42	223.85^{ab}	42.02	0.11^{ab}	42.13	412.04^{ab}	266.63^{ab}	678.67^{ab}
Ms × Rj	0.56	0.01	216.88^{b}	0.69	217.56^{b}	25.42	0.16^{ab}	25.58	479.56^{ab}	328.32^{ab}	807.87^{ab}
Lp × Ci × Ms	−1.73	0.18	370.27^{ab}	1.95	372.22^{ab}	46.00	0.29^{ab}	46.28	343.99^{ab}	170.86^{ab}	514.85^{ab}
Lp × Ci × Rj	−1.49	0.07	295.15^{ab}	1.04	296.18^{ab}	22.83	0.13^{ab}	22.95	529.05^{ab}	280.40^{ab}	809.45^{ab}
Lp × Ms. × Rj	−0.15	0.05	173.96^{b}	0.63	174.59^{b}	25.82	0.08^{b}	25.90	736.06^{a}	439.91^{a}	1175.98^{a}
Ci × Ms. × Rj	−0.19	0.06	401.03^{ab}	0.35	401.38^{ab}	29.97	0.21^{ab}	30.18	327.43^{ab}	209.91^{ab}	537.34^{ab}
Lp × Ci × Ms. × Rj	−0.56	0.11	315.03^{ab}	0.44	315.47^{ab}	29.12	0.15^{ab}	29.27	480.82^{ab}	192.64^{ab}	673.46^{ab}

Note: Different letters within columns indicate significant difference by applying "Dunn's test" at $P < .05$.

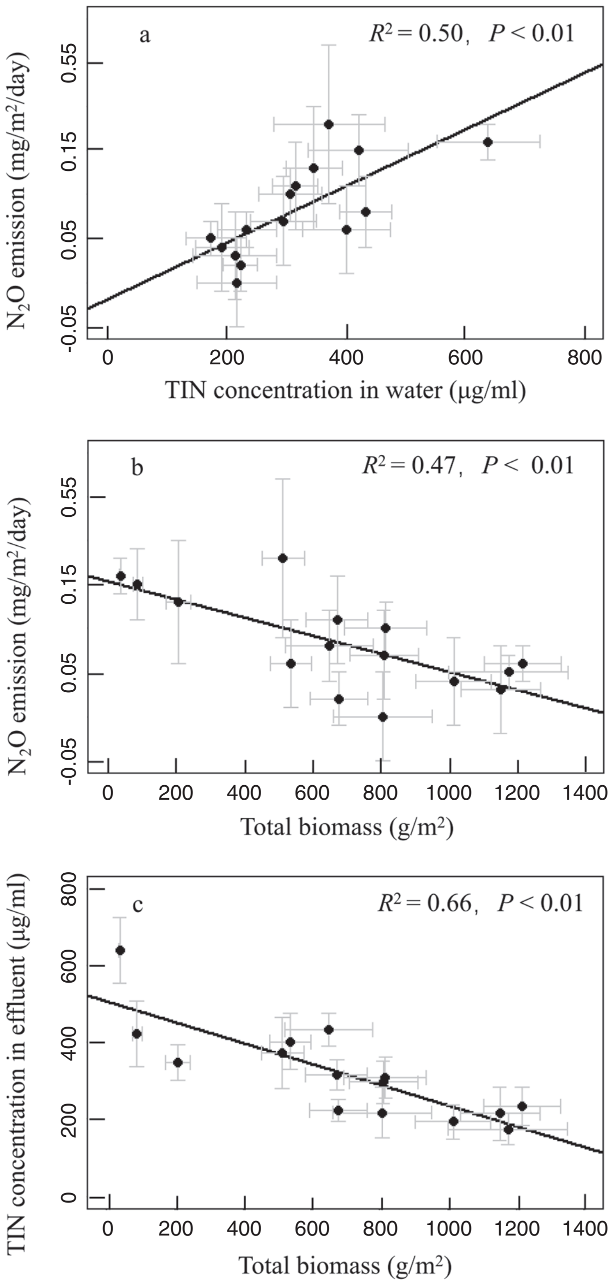

Fig. 2 – Relationship between TIN concentrations in effluent and total biomass with N_2O emissions, and between total biomass with TIN concentrations among treatments. Error bars indicate standard errors.

under relatively high NO_3^--N loading. In this study, high NH_4^+-N loading may have influenced N_2O emissions as well as N concentrations in effluent and plant growth (Britto and Kronzucker 2002; Law et al., 2012), and then impacted the effect of plant species richness on N_2O emissions.

In the system supplied only with NH_4^+-N, it is expected that N_2O is produced by nitrification and denitrification (Canfield et al., 2010; Law et al., 2012). As decreasing N concentrations reduce N_2O emissions (Kampschreur et al., 2009; Mander et al., 2014), Pearson correlation analysis in this study also showed the positive relationship between N_2O emissions and N concentrations in effluent (Fig. 2). Plant species richness did not affect N concentrations in effluent (Table 3), therefor had no significant effect on N_2O emissions. However, previous studies also have demonstrated that high plant species richness decreases N concentrations in effluent (Fraser et al., 2004; Chang et al., 2014), even if treating water in the CW with NH_4^+-N only (Ge et al., 2015). The reason may be that the NH_4^+-N concentration in this study (336 mg N/L) was much higher than that of the previous study (112 mg N/L) (Ge et al., 2015). High NH_4^+-N concentrations are known to inhibit plant growth (Britto and Kronzucker 2002; Cao et al., 2010) and therefore may have influenced the outcome of plant species richness on N concentrations in effluent and N_2O emissions.

In this study, plant species richness also did not affect CH_4 emissions (Table 3), consistent with other previous studies (Mo et al., 2015; Zhao et al., 2016; Han et al., 2017). However, some studies reported that plant species richness increased CH_4 emissions through increasing plant biomass (Zhang et al., 2012). Here, plant species richness did not significantly impact plant biomass (Table 3), which may partially explain why there was no observed effect on CH_4 emissions. NH_4^+-N has a complex effect on CH_4 emissions, in which NH_4^+ is competitively bound to the enzyme catalyzed in the first step of CH_4 oxidation, and thus reduces CH_4 oxidation rates and then increases CH_4 emissions (Carlsen et al., 1991; Schimel 2000); as such, NH_4^+-N may stimulate CH_4 oxidation instead of inhibiting it (Bodelier and Laanbroek 2004). In this study, NH_4^+-N concentrations were not significantly correlated with CH_4 emissions, and plant species richness also did not affect NH_4^+-N concentrations (Table 3). As a consequence, plant species richness did not influence CH_4 emissions. Further studies are needed to investigate more abiotic and biotic factors to better understand the effects of plant species richness on N_2O and CH_4 emissions.

3.3. Relative contribution of species richness and species identity

Some studies have demonstrated that species identity surpassed species richness to affect ecosystem functions of CWs, such as nitrogen removal (Zhao et al., 2016), phosphate removal (Geng et al., 2017), and ammonia volatilization (Luo et al., 2016). In this study, N_2O emissions were more strongly affected by species identity than species richness. When simultaneously accounting for the effects of species richness and species identity (including the presence of a particular species, *R. japonicus* in this study) on N_2O emissions in a multiple linear regression, the effect of species richness was still insignificant, while species identity had a significantly negative effect (Table 5). Similarly, Abalos et al., (2014) also found that species identity surpasses species richness as a key driver of N_2O emissions in grasslands. Furthermore, only species identity significantly affected the N_2O emission when separately considering the effects of species richness and species identity (Table 4). CH_4 emissions did not respond to species richness and species identity (Tables 3 and 4, respectively). Considering these findings, assembling proper species composition may be more important than simply increasing species richness to reduce greenhouse gas emissions. In addition, it should be noticed that only four species

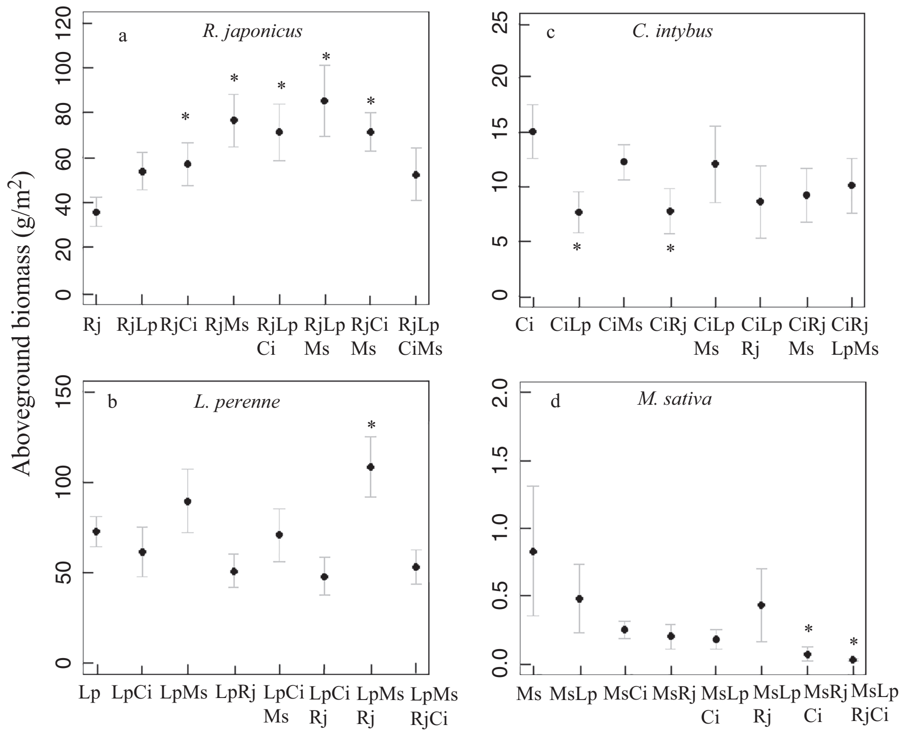

Fig. 3 – Aboveground biomass of particular species in monoculture and mixtures. Rj: *R Rumex japonicus*, Lp: *Lolium perenne*, Ci: *Cichorium intybus*, Ms.: *Medicago sativa*. Shaded area stands for the standard error of monoculture. Asterisks denote significant difference between mixture and monoculture ($P < .05$).

were used in this study, further study should focus on more species to evaluate the effect of species richness on the N_2O and CH_4 emissions.

3.4. *Approach for enhancing N removal and reducing greenhouse gas emissions*

CWs have been widely used for treating various wastewater due to its low cost and easy maintenance (Liu et al., 2009; Vymazal, 2014). However, the benefit of its high N removal efficiency may be offset by the large amount of CH_4 and N_2O emissions (Kampschreur et al., 2009; Mander et al., 2014). As plants are an important component of CWs, assembling the proper diversity should be considered to best enhance ecosystem functioning (Saeed and Sun, 2012; Upadhyay et al., 2016). Previous studies have shown that increasing plant species richness can enhance N removal but also increase N_2O emission in CWs with high NO_3^--N loading (Chang et al., 2014). High species richness may also enhance N removal in CWs with high NH_4^+-N loading (Ge et al., 2015), but such findings were not observed in this study. However, study observations revealed that the presence of *R. japonicus* enhanced N removal efficiency and plant biomass, and simultaneously decreased the N_2O emission (Table 4). Moreover, this species has also been found to reduce N_2O emissions and enhance N removal efficiency and plant biomass in CWs with generally high NO_3^--N loading (Han et al., 2017). Therefore, among the four species used in this study, *R. japonicus* may be a sound option in CWs for cost-effectively treating wastewater with various N forms, particularly for enhancing high NH_4^+-N removal efficiency and providing an opportunity for greenhouse gas mitigation.

4. Conclusions

This study provides an alternative method for enhancing N removal efficiency and reducing potential greenhouse gas emissions from wastewater with high NH_4^+-N concentrations by assembling plant communities in CWs. To our knowledge, this is notably the first study to explore such relationships in CWs with high NH_4^+-N loading. Results suggest that plant species richness had no significant impact on CH_4 and N_2O emissions, while species identity significantly affected N_2O emissions but not CH_4 emissions. The presence of *R. japonicus* also reduced N_2O emissions by increasing plant N uptake and reducing N concentrations in wastewater. It is recommended that future studies investigate other plants to help identify the most suitable species and their composition for ensuring high N removal and low greenhouse emission. Other factors, such as temperature, pH, and microorganisms, also should be considered to more comprehensively understand the effects of plant species diversity on greenhouse gas emissions.

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