



Effects of plant diversity on biomass production and substrate nitrogen in a subsurface vertical flow constructed wetland

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ABSTRACT

Most biodiversity experiments have been conducted in grassland ecosystems with nitrogen limitation, while little research has been conducted on relationships between plant biomass production, substrate nitrogen retention and plant diversity in wetlands with continuous nitrogen supply. We conducted a plant diversity experiment in a subsurface vertical flow constructed wetland for treating domestic wastewater in southeastern China. Plant aboveground biomass production ranged from 20 to 3121 g m⁻² yr⁻¹ across all plant communities. In general, plant biomass production was positively correlated with species richness ($P=0.001$) and functional group richness ($P=0.001$). Substrate nitrate concentration increased significantly with increasing plant species richness ($P=0.046$), but not with functional group richness ($P=0.550$). Furthermore, legumes did not affect biomass production ($P=0.255$), retention of substrate nitrate ($P=0.280$) and ammonium ($P=0.269$). Compared to the most productive of the corresponding monocultures, transgressive overyielding of mixed plant communities did not occur in most polycultures. Because greater diversity of plant community led to higher biomass production and substrate nitrogen retention, thus we recommend that plant biodiversity should be incorporated in constructed wetlands to improve wastewater treatment efficiency.

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1. Introduction

Constructed wetlands (CWs) have recently been widely used around the world to purify wastewater because of their ability to remove pollutants at low cost (Knight et al., 1993; Tanaka et al., 2006; Liu et al., 2009). The nutrient retention function of CWs is closely related with the uptake and immobilization of nutrients by plants, microorganisms and substrate matrixes (Stottmeister et al., 2003; Coleman, 2001; Faulwetter et al., 2009). In addition, some have studied the relationship between biodiversity and CWs' ability to clean wastewater (Engelhardt and Ritchie, 2001, 2002). However, research on plant diversity–ecosystem function relationship in CWs is scarce.

Most studies on the relationship between biodiversity–ecosystem function were conducted in grassland ecosystems (Hector et al., 1999; Tilman et al., 2001; Roscher et

al., 2004), which often have limited soil nitrogen (N) availability (Vitousek and Howarth, 1991; Zanetti et al., 1997). Many of these studies showed that greater plant species richness resulted in higher biomass production, i.e., productivity (Balvanera et al., 2006; Cardinale et al., 2006). When studies on biodiversity–ecosystem function were carried out with fertilization, productivity–species richness relationships were found to be stronger in fertilized than in unfertilized treatments (He et al., 2002; Wacker et al., 2009). Relationships between plant diversity and inorganic soil N availability are variable (Hooper and Vitousek, 1998; Niklaus et al., 2001; Palmberg et al., 2005), and several studies found decreases of substrate nitrate pool size with increased plant species diversity or functional diversity (Niklaus et al., 2001; Scherer-Lorenzen et al., 2003; Palmberg et al., 2005). Others reported that plant functional identity had no effect on N retention in substrates (Symstad et al., 1998). Many studies found that legumes played an important role in forming positive biodiversity–productivity relationships (Lambers et al., 2004; Fargione et al., 2007; Van Ruijven and Berendse, 2009), and complementary effects between legume and non-legume species existed in many grasslands with limited N availability (Tilman et al., 1997; Mulder et al., 2002; Palmberg et al., 2005).

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Table 1
Influent and effluent water quality and percent removal of pollutants in the SVFCW in Zhoushan from November 2006 to October 2007.

Parameters	NH ₄ -N (g N m ⁻² yr ⁻¹)	NO ₃ -N (g N m ⁻² yr ⁻¹)	TN (g N m ⁻² yr ⁻¹)	BOD ₅ (g m ⁻² yr ⁻¹)	COD (g m ⁻² yr ⁻¹)	TP (g m ⁻² yr ⁻¹)
Influent	265.0 ± 26.3 ^a	144.5 ± 39.8	2302.6 ± 123.4	382.9 ± 67.9	736.5 ± 130.5	212.4 ± 57.3
Effluent	46.2 ± 19.3	41.0 ± 18.7	436.4 ± 73.1	87.7 ± 22.8	169.1 ± 43.9	15.2 ± 5.9
% removal ^b	82.6 ± 14.8	71.6 ± 21.2	81.0 ± 18.7	77.1 ± 10.4	77.0 ± 10.7	92.8 ± 28.6

^a Mean ± SD.

^b Percent removal was calculated as ((influent – effluent)/influent) × 100.

Work on grasslands has suggested that greater plant species richness leads to more efficient uptake of nutrients and greater productivity (Tilman et al., 1997; Symstad et al., 1998; Hector et al., 1999). Meanwhile, CWs provide important ecosystem services, such as wastewater purification, which may depend on how plant diversity influences productivity and nutrient retention (Engelhardt and Ritchie, 2001). Philip and Alexander (2000) has reported that a mixture of plant species was more efficient than monocultures in wastewater treatment in CWs, since the former provided better conditions for denitrification. The subsurface vertical flow CW (SVFCW) with unsaturated flow is known to be particularly efficient in treating many types of wastewater (Molle et al., 2006; Tietz et al., 2008; Panuvatvanich et al., 2009; Cui et al., 2009). A better understanding of plant species effects on nutrient retention will greatly help us design CWs with greater removal efficiency of pollutants from treating wastewater. Therefore, a plant diversity experiment was conducted in a full-scale SVFCW system built near Zhoushan City, Zhejiang Province in southeastern China. The main objectives of this paper were to (1) evaluate relationships between plant diversity and productivity under high N input and (2) test effects of plant diversity on substrate N retention.

2. Materials and methods

2.1. Site description and experimental design

A 1000 m² of SVFCW system was built in 2005 at Zhujiajian (29°53' N, 122°23' E), Zhoushan City, Zhejiang Province, in southeast China. The goal was to remove the high level of inorganic nutrients contained in the post-treatment domestic wastewater which had been banned by the local government for release into the sea.

The SVFCW was constructed with a 3-layer filter, and its structure (length = 50 m, width = 20 m, depth = 1.2 m) was described in detail in Zhang et al. (2010). The PVC pipes with spraying holes provided a consistent supply of water to each plot during pulse-irrigation, and ensured that each plant species had a similar growth environment. The irrigation water flooded the CW and the water level was about 30 cm below the sand substrate surface. Therefore, the biodiversity experiment in this study was conducted in a uniform mesophytic habitat.

The irrigation capacity for wastewater in the SVFCW was about 60 thousand tons per year (i.e., about 58.5 m³ water m⁻² yr⁻¹, corresponding to about 6000 mm of precipitation). The average NH₄-N (ammonium) and NO₃-N (nitrate) input in the domestic sewage water was 265.0 and 144.5 g N m⁻² yr⁻¹, respectively (Table 1). And the amount of N supply was about 7 times that of the average rate (64 g N m⁻² yr⁻¹) used in a typical farmland in China between 1992 and 2002 (Hou et al., 2008), and about 18 times (24 g N m⁻² yr⁻¹) that of the biodiversity experiment of Wacker et al. (2009).

The study included 164 plots with 2.0 m × 2.0 m in size (Table 2). Each plot bordered with PVC pipes for irrigation, but otherwise there was no physical barrier to isolate the plots. In April 2006, all plant assemblages were transplanted at a density of 10 seedlings m⁻² following the experimental design described

below, and the initial height of the planted seedlings ranged between 20 and 30 cm. All plots were hand-weeded during the summer to prevent invasion of unwanted species. In addition, the zero species richness treatment (i.e., the 5 unplanted plots) served as controls.

The planted plots were transplanted with 1, 2, 4, 8 or 16 species, which were mainly native to the subtropical region (Table 2), representative of mesophytic species found under subtropical monsoonal climate. The species fall into four functional groups, i.e., C₃ grasses: *Arundo donax* and *Phragmites australis*; C₄ grasses: *Coix lacryma-jobi*, *Imperata cylindrical*, *Miscanthus sinensis*, *Neyraudia montana*, *Saccharum arundinaceum* and *Triarrhena sacchariflora*; legume species: *Campylotropis macrocarpa*, *Cassia tora*, *Lespedeza bicolor* and *Indigofera pseudotinctoria*, and non-legume herbaceous forbs (for simplicity hereafter called forbs): *Canna indica*, *Cyperus alternifolius*, *Lythrum salicaria* and *Thalia dealbata*. Each plot within a diversity level had a different species composition to avoid confusing the effects of diversity with species identity. Fill planting was conducted when a transplanted plant did not survive after the initial planting.

2.2. Measurement

For measurement of plant biomass production, plants were cut at 5 cm above the surface of the substrate at the end of September 2007. The plant material was harvested within a strip of 0.5 m × 2 m in the centre of each plot to avoid edge effect. Samples were sorted to species, dried at 65 °C to constant weight.

Substrate samples were collected from the 0–30 cm depth in most of the plots after biomass sampling. In each plot, five substrate cores were collected using an auger (5 cm diameter) to form a composite sample, and roots were removed by hand once the samples were brought into the laboratory. The samples were air-dried at room temperature for about one week. Then the air-dried samples were extracted with 1 mol L⁻¹ KCl on an orbital shaker (200 rpm for 1 h), filtered by ash-free filter papers (pore size 4.5 μm), and the extracts were analyzed for ammonium and nitrate concentrations. The NH₄-N and NO₃-N concentrations in all extracts were determined using a segmented flow analyzer (SAN plus, Skalar, the Netherlands).

Table 2

Number of plots for different species-functional group richness combinations in the Zhoushan experiment and the number of plots analyzed in this study (in parentheses).

Functional group richness	Number of species per plot				
	1 species	2 species	4 species	8 species	16 species
1	16 (16)	12 (8) ^a	12 (7)		
2		16 (11)	24 (20)	6 (4)	
3			24 (22)	6 (5)	
4			16 (7)	16 (13)	16 (5)
All groups	16 (16)	28 (19)	76 (56)	28 (22)	16 (5)

^a Not all plots were used for analysis due to (1) the exclusion of plots that did not get harvested in September 2007 and (2) the exclusion of 5% of the plots with the highest or lowest biomass.

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