Si-Xi Zhu¹ Peili Zhang¹ Hai Wang¹ Han-Liang Ge¹ Jie Chang¹ Scott Chang^{2,3,*} Zhi Qiu⁴ Hongbo Shao³ Ying Ge¹

 ¹College of Life Sciences, Zhejiang University, Hangzhou, P. R. China
²Department of Renewable Resources, University of Alberta, Edmonton, Alberta, Canada

³The CAS/Shandong Provincial Key Laboratory of Coastal Environmental Processes, Yantai Institute of Costal Zone Research, Chinese Academy of Sciences (CAS), Yantai, P. R. China

⁴Environmental Science Research and Design Institute of Zhejiang Province, Hangzhou, P. R. China

Research Article

Plant Species Richness Affected Nitrogen Retention and Ecosystem Productivity in a Full-Scale Constructed Wetland

The effects of plant species richness (SR; i.e., 1, 2, 4, 8, and 16 species per plot) on substrate nitrate and ammonium retention and ecosystem productivity in a full-scale constructed wetland (CW) with high nitrogen (N) input were studied. Substrate nitrate $(0.1-16.4 \text{ mg kg}^{-1})$ and ammonium concentrations $(1.3-9.2 \text{ mg kg}^{-1})$ in this study were higher than those in other comparable biodiversity experiments. Substrate nitrate concentration significantly increased while ammonium concentration significantly decreased with the increase of plant SR (p = 0.008 and 0.040, respectively). The response of ecosystem productivity to increasing SR was unimodal with four species per plot achieving the greatest productivity. Transgressive overyielding, which was compared to the most productive of corresponding monocultures, did not occur in most polycultures. We conclude that substrate N retention was enhanced by plant SR even with high N input, and plant SR could be managed to improve the efficiency of N removals in CWs for wastewater treatment.

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1 Introduction

The potential impact of global loss of biodiversity on ecosystem functioning has been hotly debated [1]. Plants are major players in key ecosystem functioning such as nutrient cycling. As such, many manipulative field experiments have been investigated the effects of a loss of plant species richness (SR) on nitrogen (N) cycling [2]. In N-limited grasslands experiments, the mechanistic hypothesis was that different plant species could use available nutrients in a complementary way [3]. Therefore, the relationships between biodiversity and ecosystem functioning could be dependent on the availability of resources [1, 4]. Meanwhile, plant SR and ecosystem productivity are strongly affected by N retention and the rates at which limiting nutrients (such as N) are supplied in substrates [5].

Our understanding of the effects of SR on nutrient retention and ecosystem productivity mostly came from experiments conducted in N-limited grassland ecosystems [1, 2]. In these experiments, the relationships between plant SR and plant-available nitrate in the substrate have frequently been found to be negative [6–10], sometimes no relationships were found [11, 12], while others found positive relationships [7, 13], as is detailed in Tab. 1. Meanwhile, in most N-limited ecosystems, SR had positive effects on ecosystem productivity [1, 2]. Other studies found a monotonically increasing relationship between SR and ecosystem functioning that was stronger in nutrient-rich than in nutrient-poor environments [14]. Since most biodiversity experiments were carried out in N-limited grassland ecosystems [1], the relationship between biodiversity and ecosystem functioning in environments with no N limitation is still poorly understood.

Constructed wetlands (CWs) used for treating wastewater (such as the removals of N [15]) are artificial ecosystems that have high N availability, with N input as high as 400 g N m⁻² year⁻¹ in the form of dissolved inorganic N [16], much higher than those used in artificial N deposition (4 g N m⁻² year⁻¹) experiments [4]. Meanwhile, a subsurface vertical flow CW, which was constructed in 2005 in Zhejiang Province, southeast China, provided an opportunity for the biodiversity assemblage experiment [16, 17]. Here, we test the hypothesis that plant SR has positive effects on substrate inorganic N retention and ecosystem productivity in the CW with high N input. This work will enrich the biodiversity–ecosystem functioning literature by providing a case study under a different ecosystem type and N availability. Meanwhile, this work will also explore the potential to improve the efficiency of potential N removal from wastewater in CW by managing plant SR.

2 Materials and methods

2.1 Site description

The field experiment was located at the CW (1000 m^2 , $29^{\circ}53'\text{N}$, $122^{\circ}23'\text{E}$) in Zhoushan City and the CW's structure was described in Zhang et al. [18] and Zhu et al. [16]. Each plant species had a uniform mesophytic growth habitat in the down-flow chambers (i.e., A pond, Fig. 1) of the CW with high N input (about $400 \text{ g N m}^{-2} \text{ year}^{-1}$). Further details of the field experiment were provided in Zhu et al. [16].

Correspondence: Professor J. Chang, College of Life Sciences, Zhejiang University, Hangzhou 310058, P. R. China **E-mail:** jchang@zju.edu.cn

Abbreviations: CW, constructed wetland; SR, species richness

^{*}Additional corresponding author: S. Chang E-mail: scott.chang@ualberta.ca

Location	Community type	Plot size (m)	No. of plots	SR-nitrate	SR-ammonium	Ref.
Cedar Creek, USA	Prairie species	13 imes 13	147	_a)	_	[9]
Cedar Creek, USA	Grasslands	9×9	143	+ ^{b)}	nd ^{d)}	[13]
Manitoba, Canada	Perennial grasses	3×3	110	0 ^{c)}	0	[12]
Northwestern, Switzerland	Grasslands	nd	nd	_	0	[10]
Bayreuth, Germany	Permanent meadows	2 imes 2	64	0	0	[11]
Umeå, Sweden	Vascular plants	2.2 imes 5	68	$-^{e)}$ or $+^{f)}$	$0^{(e)} \text{ or } +^{(f)}$	[7]
Jena, Germany	Mesophilic grassland	20 imes200	86	_	0	[8]
Jena, Germany	Mesophilic grassland	3.5 imes 3.5	206	_	0	[6]

Table 1. Relationships between plant species richness (SR) and substrate nitrate and ammonium in different biodiversity experiments

^{a)} The relationship of SR-nitrate or ammonium was significantly negative.

^{b)} The relationship of SR-nitrate or ammonium was significantly positive.

^{c)} SR had no significant effects on nitrate or ammonium.

d) No data.

^{e)} In plots with pure legumes.

^{f)} In plots with mixed legumes and non-legumes and in plots without legumes.



Figure 1. A schematic drawing of the vertical structure of the CW established in Zhoushan.

2.2 Experimental design

Each plot was 2.0 m × 2.0 m in size and was transplanted at a density of 10 seedlings m⁻² in 2006. The 164 plots were transplanted with 1, 2, 4, 8, or 16 subtropical species (Tab. 2). These species fell into four functional groups, i.e., legumes (*Campylotropis macrocarpa*, *Cassia tora*, *Lespedeza bicolor*, and *Indigofera pseudotinctoria*), C₃ grasses (*Arundo donax* and *Phragmites australis*), C₄ grasses (*Coix lacryma-jobi*, *Imperata cylindrical*, *Miscanthus sinensis*, Neyraudia montana, Saccharum

Table 2. Numbers of transplanted plots and experimental plots (in parenthesis)

Functional		Number of species per plot							
group richness	1	2	4	8	16				
1	16 (16)	12 (9) ^{a)}	12 (8)	_b)	-				
2		16 (16)	24 (22)	6 (4)	-				
3	-		24 (23)	6 (5)	-				
4	-	-	16 (13)	16 (14)	16 (16)				
All groups	16 (16)	28 (25)	76 (66)	28 (23)	16 (16)				

^{a)} Not all plots were used for analysis due to (1) the exclusion of plots that did not get harvested in September 2008, and (2) the exclusion of 5% of the plots with the highest or lowest productivity. arundinaceum, and Triarrhena sacchariflora), and non-legume herbaceous forbs (i.e., forbs, including *Canna indica*, *Cyperus alternifolius*, *Lythrum salicaria*, and *Thalia dealbata*).

2.3 Measurements

The sandy substrate samples (0–30 cm depth in the selected plots) were collected after biomass sampling at the end of September 2008. In each plot, using an auger (5 cm diameter), five substrate cores were collected by the diagonal method. Roots were removed by hand before the substrate samples were brought to the laboratory. After 1 wk at room temperature, the air-dried sandy samples were extracted with $1 \mod L^{-1}$ KCl [8] and the extracts were analyzed for ammonium and nitrate concentrations with a segmented flow analyzer (SAN plus, Skalar, The Netherlands).

For measurement of ecosystem productivity, plants were cut 5 cm above the substrate surface, when plant biomass peaked. Plant material from the selected plots was harvested within a strip of $0.5 \text{ m} \times 2.0 \text{ m}$ in the center of each plot and separated into species. After oven drying (65°C) to constant weight, the plant material from each plot was weighed to obtain dry weights for all species.

2.4 Calculation of transgressive overyielding in ecosystem productivity

We used the proportional deviation index D_{Max} to compare observed and expected total productivity of a polyculture [19], calculated as follows:

$$D_{\text{Max}} = \frac{O_{\text{T}} - \text{Max}_{(\text{Mi})}}{\text{Max}_{(\text{Mi})}}$$

where $O_{\rm T}$ is the observed total productivity of all transplanted species in a plot and ${\rm Max}_{\rm (Mi)}$ is the most productive of the corresponding monoculture. When $D_{\rm Max} > 0$, the polyculture is more productive than any of the species transplanted in the corresponding monoculture (i.e., transgressive overyielding) [19].

2.5 Statistical analysis

To test the effects of presence of legumes, C₃ grasses, C₄ grasses, and forbs, SR, and identities of species in polycultures, data were analyzed with ANOVA using general linear model based on type III sum

of squares by the SPSS software (SPSS 16.0, SPSS Inc., Chicago, IL, USA). Differences among plant SR were tested with a one-way ANOVA and Tukey's HSD test (with $\alpha = 0.05$). All error estimates given in the text and error bars in figures are standard errors of means. All statistical significance was noted with $\alpha = 0.05$ unless otherwise noted.

3 Results

3.1 Relationships between species richness and substrate inorganic N

Across all plots, with increasing plant SR substrate nitrate concentration increased linearly (Fig. 2a) while substrate ammonium concentration decreased linearly (Fig. 2b) in 2008. Functional group did not affect substrate inorganic N (Tab. 3). In addition, nitrate and ammonium concentrations ranged from 0.1 to 16.4 (2.3 ± 0.2 for mean \pm SE, same below) and 1.3– $9.2 \,\mathrm{mg}\,\mathrm{N}\,\mathrm{kg}^{-1}$ dry substrate (2.8 ± 0.1), respectively, for all samplings (Fig. 2a and b).

3.2 Relationships between species richness and ecosystem productivity

The relationship between SR and ecosystem productivity was unimodal (p < 0.05), and the peak value of productivity was at SR level of four species per plot (Fig. 2c). The relationship could be represented by a quadratic equation:

$$y = -10.2x^{2} + 158.9x + 462.1(R^{2} = 0.038)$$

In terms of the effects of functional groups, only the presence of C_3 grasses had a significant effect on productivity (Tab. 3). Productivity in all plots ranged from 13 to $6535 \,\mathrm{g \, m^{-2}}$ (784 \pm 221 g m⁻²).

3.3 Relationships between species composition and substrate inorganic N and ecosystem productivity

The species *M. sinensis* and *N. montana* affected positively on substrate nitrate concentration (p = 0.018 and 0.006, respectively, Tab. 4), while *A. donax*, *C. tora*, and *I. pseudotinctoria* had negative effects on the ammonium concentration (p = 0.049, 0.019, and 0.005, respectively, Tab. 4).

The two species (*A. donax* and *N. montana*) with the highest monocultural productivity had positive effects on ecosystem productivity, while *L. bicolor* had a negative effect (p < 0.01 for all three species, Tab. 4). Moreover, large differences in substrate inorganic N concentrations and productivity were found among treatments with similar levels of SR, indicating that the species composition had an important impact on substrate inorganic N concentrations and ecosystem productivity (Fig. 2a–c).

3.4 Transgressive overyielding

The mean D_{Max} in all polycultures was <0 (p < 0.001, Fig. 2d). And D_{Max} in 84 polycultures was <0 in all 130 polycultures, which means that there were no transgressive overyielding in most polycultures (Fig. 2d).

4 Discussion

4.1 Effects of species richness on substrate inorganic N concentrations

The amount of substrate inorganic N depends on many processes including plant uptake, ammonification, and nitrification, denitrification, and microbial immobilization of N [20–23]. Substrate nitrate and ammonium concentrations in this study were similar to those in plots with pure legumes in the biodiversity experiment in



Figure 2. Relationships of plant SR and substrate nitrate and ammonium ecosystem productivity and $D_{\rm Max}$ in 2008.

Source of variation	df	N	Nitrate		Ammonium		Productivity	
		F	<i>p</i> -Value	F	<i>p</i> -Value	F	<i>p</i> -Value	
Presence of legumes	1	1.46	0.229	1.35	0.247	0.18	0.670	
Presence of C_3 grasses	1	0.29	0.593	0.16	0.695	7.63	0.006 ↑	
Presence of C_4 grasses	1	1.16	0.284	0.49	0.485	1.67	0.198	
Presence of forbs	1	1.64	0.202	0.37	0.544	0.19	0.662	
Species richness (SR)	4	4.38	0.008 ↑	1.98	0.040	1.24	0.297	
$SR \times legumes$	3	0.14	0.937	0.99	0.399	0.23	0.878	
Residual	137							

Table 3. ANOVA data on biodiversity effects on substrate nitrate and ammonium and ecosystem productivity based on the type III sum of square

Arrows indicate significant increase (\uparrow) or decrease (\downarrow) of the measures with species richness or presence of a certain functional group. Significant *p*-values (p < 0.05) are in bold.

Table 4. Direction ("sign") and significance of effects of a particular species on substrate nitrate and ammonium and ecosystem productivity based on the type III sum of square

Taxon	N	itrate	Ammonium		Productivity	
	Sign	<i>p</i> -Value	Sign	<i>p</i> -Value	Sign	<i>p</i> -Value
Arundo donax		0.177	_	0.049	+	0.002
Phragmites australis		0.171		0.478		0.217
Coix lacryma-jobi		0.183		0.255		0.169
Imperata cylindrical		0.447		0.378		0.990
Miscanthus sinensis	+	0.018		0.487		0.302
Neyraudia montana	+	0.006		0.233	+	0.009
Saccharum arundinaceum		0.071		0.073		0.752
Triarrhena sacchariflora		0.129		0.145		0.176
Campylotropis macrocarpa		0.091		0.473		0.061
Cassia tora		0.240	_	0.019		0.505
Indigofera pseudotinctoria		0.064	_	0.005		0.127
Lespedeza bicolor		0.058		0.181	_	0.015
Canna indica		0.255		0.410		0.059
Cyperus alternifolius		0.070		0.426		0.391
Lythrum salicaria		0.091		0.628		0.881
Ťhalia dealbata		0.343		0.127		0.058

"+" or "-"indicate a significant increase or decrease when the species was planted in polycultures. Significant p-values (p < 0.05) are in bold.

Palmborg et al. [7], and was significantly higher than those in N-limited grassland diversity experiments reported in Scherer-Lorenzen [24] and Fornara and Tilman [1] (Tab. 5). Meanwhile, nitrate concentrations in the substrate increased and ammonium concentrations decreased from 2007 to 2008 after the establishment of the experiment (Fig. 3a), indicating that nitrification in plant rhizome became stronger over time in the CW with high nutrient input [25].

Nitrification depends primarily on ammonium concentration and activities of nitrifiers in the substrate [17, 21, 26]. The substrate ammonium and nitrate concentrations were positively related in

Table 5.	Ranges of	substrate	nitrate	concentrations	in KC	l extracts ir	n different	biodiversity	exr	periments
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Location	Community	Site description	Substr	Ref.	
	type		$(\mathrm{mg}\mathrm{N}\mathrm{kg}^{-1})$	(kg N ha^{-1})	_
Minnesota, USA	Savanna-grasslands	Well-drained, fine sand, and N poor	0.2-0.4	nd ^{c)}	[47]
Bayreuth, Germany	Grasslands	Loamy sand or sandy clay and nutrient poor	0-10.0	nd	[24]
Northwestern Switzerland	Grasslands	A nutrient-poor calcareous substrate	0-2.5	nd	[10]
SLU ^{b)} in Umeå, Sweden	Vascular plants	Fine silty sand with no fertilizer	nd	5.0-20.0 ^{d)} 0-7.5 ^{e)}	[7]
Minnesota, USA	Savanna-grasslands	Well-drained, fine sand, and N poor	0-0.9	nd	[1]
Zhejiang, China	Subtropical plants	Sandy substrate with high N input	0.3-5.6	0.6-10.5	[16]
Zhejiang, China	Subtropical plants	Sandy substrate with high N input	0.1-16.4	0.2-30.7	This study

^{a)} Dry substrate at 0–30 cm depth.

^{b)} The Swedish University of Agricultural Sciences.

^{c)} No data.

^{d)} In plots with pure legumes.

e) In plots with mixed legumes and non-legumes and in plots without legumes.

Figure 3. Comparisons and relationships of substrate nitrate and ammonium concentrations in 2007 and 2008.

2007 but not in 2008 (Fig. 3b and c), with the overall ammonium concentrations lower in 2008 than in 2007 [16], indicating strong interannual variations in the studied processes and relationships. In most N-limited grassland experiments substrate nitrate pool size was found to decrease with increasing plant SR [8, 10] (Tab. 1), as plant communities with higher SR can exploit more of the substrate nitrate [8, 11]. However, substrate nitrate concentration was positively correlated with SR under high N input in this study. This may be due to (1) plant SR had positive effects on microbial biomass carbon and N in the rhizosphere [18], for the diverse roots could increase the release of exudates [27], the availability of oxygen in the substrate and microbial activities and nutrient cycling in the CW [23, 28], (2) higher root production in communities with higher plant SR would increase nitrate accumulation through filtering functions [2], and (3) substrate nitrate concentration did not decrease though plant N uptake due to continuous N input from the CW. Therefore, plant communities with higher SR had higher nitrate concentrations in the substrate.

Substrate ammonium concentrations decreased with the increase of SR in 2008 in this study, maybe as a result of increased microbial community diversity and microbial biomass carbon and N and enzyme activities in the substrate with higher SR enhanced nitrification [18, 26]. Meanwhile, the slope of the positive regression between substrate nitrate concentration and SR became steeper in 2008 as compared with that in 2007 (Fig. 2a, [16]), suggesting that microbial composition and their functioning had large interannual variations [25] and that microbial nitrate (belowground biomass) production increased with the growth of plants [29, 30]. Our finding is consistent with the literature reporting higher nitrification rates in communities with higher plant SR [1, 22]. Meanwhile, plants can greatly contribute to N removals in CWs by affecting nitrification and denitrification intensity in the root zones [31–34].

4.2 Effects of species richness on ecosystem productivity

The relationship between SR and ecosystem productivity was unimodal in this study (Fig. 2c), similar to those in the biodiversity experiment reported by Jiang and Zhang [35] and Hughes and Petchey [36]. Our data thus support the single-peak pattern hypothesis about the relationship between SR and productivity [1, 37]. Meanwhile, many grassland biodiversity experiments found that high available nutrients could lead to the decreased competitiveness of subdominant species and increased community productivity, resulting in the unimodal pattern of SR and productivity [38, 39]. In addition, the unimodal pattern illustrated that extortionate species numbers in biodiversity experiments went against attaining higher ecosystem productivity, and that systems with suitable SR could have the highest productivity [5]. In this study, plots with four species had the highest productivity in the CW. Therefore, in the management of SR in wetlands, moderate SR could achieve the maximum ecosystem service functioning of wetlands [40].

Plant functional group composition (such as legumes, C_3 grasses, C_4 grasses, and forbs in this study) is thought to influence ecosystem N dynamics [41]. However, legumes had no significant effects on ecosystem productivity in this study (Tab. 3), indicating that legumes had little effect on ecosystem productivity when external N input was very high [16, 42, 43]. And C_3 grasses significantly affected ecosystem productivity, producing the highest monocultural productivity (i.e., *A. donax*). The results would provide guidance for plant species configurations in CWs and their management. However, inorganic N concentrations in substrates can directly affect ecosystem productivity in wetland ecosystem [32, 33], just as plant species, and plant SR can influence productivity in this study.

4.3 Effects of species composition on substrate inorganic N and ecosystem productivity

Since substrate inorganic N concentrations were affected by plant SR (Fig. 2a and b) and different species had different effects on inorganic N concentrations (Tab. 4), we conclude that plant combinations (such as *A. donax, P. australis, S. arundinaceum, and N. montana, or S. arundinaceum, N. montana, C. lacryma-jobi,* and *T. dealbata*) that had higher substrate inorganic N concentrations and ecosystem productivity should be chosen for planting in the CW.

Species compositions had also affected productivity (Fig. 2c, Tab. 4), due to considerable differences in growth rates, resource utilization abilities, and functional traits of different plant species [44]. Higher productivity could enhance the efficiencies of wastewater removal in CWs [34], and *A. donax, S. arundinaceum*, and *N. montana* had greater productivity and inorganic N retention (Tab. 4). So, these species with higher productivity and inorganic N retention would have important roles to play in wastewater treatment in CWs [45].

4.4 Analysis of transgressive overyielding

Transgressive overyielding was commonly used to identify both selection effects and complementary effects in polycultures [46]. In this study, most polycultures had no transgressive overyielding (Fig. 2d), which meant that selection effects might have a greater impact on productivity than complementary effects. This was possibly related to high N input from the CW, but further research about biodiversity effects on overyielding in polycultures is needed.

5 Conclusions

This study showed that plant SR increased substrate nitrate but decreased ammonium concentrations under high N input from the full-scale CW. The SR effects were positive on nitrate concentrations until reaching 16 species per plot, while the effect on ecosystem productivity was positive until reaching 4 species per plot. Species composition also affected substrate N retention and productivity. Therefore, this study indicated that plant SR should be considered in designing CW to enhance nutrient retention and productivity and improve the removal efficiencies of N from wastewater. This study would offer a case study on the relationship between SR and ecosystem functioning in an environment with high N input.

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References

- D. A. Fornara, D. Tilman, Ecological Mechanisms Associated with the Positive Diversity–Productivity Relationship in an N-Limited Grassland, *Ecology* 2009, 90 (2), 408–418.
- [2] E. M. Spehn, A. Hector, J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. Bazeley-White, C. Beierkuhnlein, et al., Ecosystem Effects of Biodiversity Manipulations in European Grasslands, *Ecol. Monogr.* 2005, 75, 37–63.
- [3] B. J. Cardinale, J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, et al., Impacts of Plant Diversity on Biomass Production Increase through Time Because of Species Complementarity, Proc. Natl. Acad. Sci. USA 2007, 104 (46), 18123–18128.
- [4] P. B. Reich, Elevated CO₂ Reduces Losses of Plant Diversity Caused by Nitrogen Deposition, *Science* 2009, 326, 1399–1402.
- [5] A. J. T. van der Krift, F. Berendse, The Effect of Plant Species on Soil Nitrogen Mineralization, J. Ecol. 2001, 89 (4), 555–561.
- [6] C. Roscher, S. Thein, B. Schmid, M. Scherer-Lorenzen, Complementary Nitrogen Use among Potentially Dominant Species in a Biodiversity Experiment Varies between Two Years, J. Ecol. 2008, 96, 477–488.
- [7] C. Palmborg, M. Scherer-Lorenzen, A. Jumpponen, G. Carlsson, K. Huss-Danell, P. Högberg, Inorganic Soil Nitrogen under Grassland Plant Communities of Different Species Composition and Diversity, *Oikos* 2005, 110, 271–282.
- [8] Y. Oelmann, W. Wilcke, V. M. Temperton, N. Buchmann, C. Roscher, J. Schumacher, E. D. Schulze, et al., Soil, Plant Nitrogen Pools as

- [9] D. Tilman, D. Wedin, J. Knops, Productivity and Sustainability Influenced by Biodiversity in Grassland Ecosystems, *Nature* 1996, 379, 718–720.
- [10] P. A. Niklaus, P. W. Leadley, B. Schmid, C. Korner, A Long-Term Field Study on Biodiversity and Elevated CO₂ Interactions in Grassland, *Ecol. Monogr.* 2001, 71, 341–356.
- [11] M. Scherer-Lorenzen, C. Palmborg, A. Prinz, E. D. Schulze, The Role of Plant Diversity and Composition for Nitrate Leaching in Grasslands, *Ecology* 2003, 84, 1539–1552.
- [12] N. C. Kenkel, D. A. Peltzer, D. Baluta, D. Pirie, Increasing Plant Diversity Does not Influence Productivity: Empirical Evidence and Potential Mechanisms, *Commun. Ecol.* 2000, 1, 165–170.
- [13] R. Dybzinski, J. E. Fargione, D. R. Zak, D. Fornara, D. Tilman, Soil Fertility Increases with Plant Species Diversity in a Long-Term Biodiversity Experiment, *Oecologia* 2008, 158, 85–93.
- [14] L. Wacker, B. Oksana, S. Eichenberger-Glinz, B. Schmid, Diversity Effects in Early- and Mid-Successional Species Pools along a Nitrogen Gradient, *Ecology* 2009, 90 (3), 637–648.
- [15] L. Wang, H. Gan, F. Wang, X. M. Sun, Q. L. Zhu, Characteristic Analysis of Plants for the Removal of Nutrients from a Constructed Wetland Using Reclaimed Water, *Clean – Soil Air Water* 2010, 38, 35–43.
- [16] S. X. Zhu, H. L. Ge, Y. Ge, H. Q. Cao, D. Liu, J. Chang, C. B. Zhang, et al., Effects of Plant Diversity on Biomass Production and Substrate Nitrogen in a Subsurface Vertical Flow Constructed Wetland, *Ecol. Eng.* 2010, 36, 1307–1313.
- [17] D. Liu, Y. Ge, J. Chang, C. H. Peng, B. H. Gu, G. Y. S. Chan, Y. F. Wu, Constructed Wetlands in China: Recent Developments and Future Challenges, Front. Ecol. Environ. 2009, 7, 261–268.
- [18] C. B. Zhang, J. Wang, W. L. Liu, S. X. Zhu, H. L. Ge, S. X. Chang, J. Chang, et al., Effects of Plant Diversity on Microbial Biomass and Community Metabolic Profiles in a Full-Scale Constructed Wetland, *Ecol. Eng.* 2010, 36, 62–68.
- [19] M. Loreau, Biodiversity and Ecosystem Functioning: A Mechanistic Model, Proc. Natl. Acad. Sci. USA 1998, 95, 5632–5636.
- [20] J. P. Schimel, J. Bennett, Nitrogen Mineralization: Challenges of a Changing Paradigm, *Ecology* 2004, 85, 591–602.
- [21] J. H. Bai, H. F. Gao, W. Deng, Z. F. Yang, B. S. Cui, R. Xiao, Nitrification Potential of Marsh Soils from Two Natural Saline–Alkaline Wetlands, Biol. Fertil. Soils 2010, 46 (5), 525–529.
- [22] W. Z. Liu, G. H. Liu, Q. F. Zhang, Influence of Vegetation Characteristics on Soil Denitrification in Shoreline Wetlands of the Danjiangkou Reservoir in China, *Clean – Soil Air Water* 2011, 39 (2), 109–115.
- [23] Y. W. Cui, C. Y. Peng, Y. Z. Peng, L. Ye, Effects of Salt on Microbial Populations and Treatment Performance in Purifying Saline Sewage Using the MUCT Process, *Clean – Soil Air Water* 2009, 37 (8), 649–656.
- [24] M. Scherer-Lorenzen, Effects of Plant Diversity on Ecosystem Processes in Experimental Grassland Communities, Bayreuther Forum Ökologie 75, BITÖK, Bayreuth, Germany 1999.
- [25] Y. Ouyang, S. M. Luo, L. H. Cui, Estimation of Nitrogen Dynamics in a Vertical-Flow Constructed Wetland, *Ecol. Eng.* 2011, 37, 453–459.
- [26] C. B. Zhang, J. Wang, W. L. Liu, S. X. Zhu, D. Liu, S. X. Chang, J. Chang, et al., Effects of Plant Diversity on Nutrient Retention and Enzyme Activities in a Full-Scale Constructed Wetland, *Bioresour. Technol.* 2010, 101, 1686–1692.
- [27] B. C. Braskerud, Factors Affecting Phosphorus Retention in Small Constructed Wetlands Treating Agricultural Non-Point Source Pollution, Ecol. Eng. 2002, 19, 41–61.
- [28] Y. Ge, C. B. Zhang, Y. P. Jiang, C. L. Yue, Q. S. Jiang, H. Min, H. T. Fan, et al., Soil Microbial Abundances and Enzyme Activities in Different Rhizospheres in an Integrated Vertical Flow Constructed Wetland, *Clean – Soil Air Water* 2011, 39, 206–211.
- [29] D. A. Wardle, R. D. Bardgett, J. N. Klironomos, H. Setala, W. H. van der Putten, D. H. Wall, Ecological Linkages between Aboveground and Belowground Biota, *Science* 2004, 304, 1629–1633.

- [30] S. Sugiyama, H. M. Zabed, A. Okubo, Relationships between Soil Microbial Diversity and Plant Community Structure in Seminatural Grasslands, *Grassland Sci.* 2008, 54, 117–124.
- [31] J. H. Bai, W. Deng, Q. G. Wang, B. S. Cui, Q. Y. Ding, Spatial Distribution of Inorganic Nitrogen Contents of Marsh Soils in a River Floodplain with Different Flood Frequencies from Soil-Defrozen Period, Environ. Monit. Assess. 2007, 134, 421-428.
- [32] J. H. Bai, Q. G. Wang, W. Deng, H. F. Gao, W. D. Tao, R. Xiao, Spatial and Seasonal Distribution of Nitrogen in Marsh Soils of a Typical Floodplain Wetland in Northeast China, *Environ. Monit. Assess.* 2011, published online. DOI 10.1007/s10661-011-2037-3
- [33] H. Q. Cao, Y. Ge, D. Liu, S. X. Chang, X. Y. Wang, J. Chang, NH₄⁺/NO₃⁻ Ratio Affect Ryegrass (*Lolium perenne* L.) Growth and N Accumulation in a Hydroponic System, J. Plant Nutr. 2011, 34, 1–11.
- [34] S. S. Parker, J. P. Schimel, Soil Nitrogen Availability and Transformations Differ between the Summer and the Growing Season in a California Grassland, *Appl. Soil Ecol.* 2011, 48, 185– 192.
- [35] X. L. Jiang, W. G. Zhang, Separating Sampling Effect from Complementary Effect in the Annual Plant Communities, Acta Ecol. Sin. 2006, 26 (6), 1896–1902.
- [36] J. B. Hughes, O. L. Petchey, Merging Perspectives on Biodiversity and Ecosystem Functioning, *Trends Ecol. Evol.* 2001, 16, 222–223.
- [37] O. L. Petchey, Integrating Methods That Investigate How Complementarity Influences Ecosystem Functioning, *Oikos* 2003, 101, 323–330.
- [38] A. DiTommaso, L. W. Aarssen, Resource Manipulations in Natural Vegetation: A Review, Vegetation 1989, 84, 9–29.

- [39] J. B. Grace, The Factors Controlling Species Density in Herbaceous Plant Communities: An Assessment, Perspect. Plant Ecol. Evol. Syst. 1999, 2, 1–28.
- [40] K. A. Engelhardt, M. E. Ritchie, Effect of Macrophyte Species Richness on Wetland Ecosystem Functioning and Services, *Nature* 2001, 411, 687–689.
- [41] D. A. Fornara, D. Tilman, S. E. Hobbie, Linkages between Plant Functional Composition, Fine Root Processes and Potential Soil N Mineralization Rates, J. Ecol. 2009, 97, 48–56.
- [42] V. M. Temperton, P. N. Mwangi, M. Scherer-Lorenzen, B. Schmid, N. Buchmann, Positive Interactions between Nitrogen-Fixing Legumes and Four Different Neighbouring Species in a Biodiversity Experiment, Oecologia 2007, 151, 190–205.
- [43] S. von Felten, N. Buchmann, A. Hector, P. A. Niklaus, B. Schmid, M. Scherer-Lorenzen, Belowground Nitrogen Partitioning in Experimental Grassland Plant Communities of Varying Species Richness, *Ecology* 2009, 90 (5), 1389–1399.
- [44] D. Tilman, Distinguishing between the Effects of Species Diversity and Species Composition, Oikos 1997, 80, 185.
- [45] A. García-Lledó, O. Ruiz-Rued, A. Vilar-Sanz, L. Salab, L. Bañerasa, Nitrogen Removal Efficiencies in a Free Water Surface Constructed Wetland in Relation to Plant Coverage, *Ecol. Eng.* 2011, *37*, 678–684.
- [46] J. van Ruijven, F. Berendse, Diversity-Productivity Relationship: Initial Effect, Long-Term Patterns, and Underlying Mechanisms, *Ecology* 2005, 102, 695–700.
- [47] A. J. Symstad, D. Tilman, J. Willson, J. M. H. Knops, Species Loss and Ecosystem Functioning: Effects of Species Identity and Community Composition, *Oikos* 1998, *8*1, 389–397.