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## Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland

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### ABSTRACT

There has been less understanding of relations of microbial community patterns with plant diversity in constructed wetlands. We conducted a single full-scale subsurface vertical flow constructed wetland (SVFCW, 1000 m<sup>2</sup>) study focusing on domestic wastewater processing. This study measured the size and structure of microbial community using fumigation extraction and BIOLOG Ecoplate™ techniques, to examine the effects of macrophyte diversity on microbial communities that are critical in treatment efficiency of constructed wetlands. We also determined the relationship of plant diversity (species richness) with its biomass production under disturbance of the same wastewater supply. Linear regression analysis showed that plant biomass production strongly correlated with plant species richness ( $R=0.407$ ,  $P<0.001$ ). Increase in plant species richness increased microbial biomass carbon and nitrogen ( $R=0.494$ ,  $P<0.001$ ;  $R=0.465$ ,  $P<0.001$ ) and utilization of amino acids on Ecoplates ( $R=0.235$ ,  $P=0.03$ ), but limited the utilization of amine/amides ( $R=-0.338$ ,  $P=0.013$ ). Principal components analysis (PCA) showed that the diversity and community-level physiological profiles (CLPP) of microbial community at 168 h of incubation strongly depended on the presence or absence of plant species in the SVFCW system, but not on the species richness. This is the first step toward understanding relations of plant diversity with soil microbial community patterns in constructed wetlands, but the effect of species diversity on microbial community should be further studied.

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### 1. Introduction

Constructed wetlands (CWs) are a low-cost, natural technology for wastewater treatment, and are emerging as a useful technology for the treatment of a variety of wastewaters and treatment scenarios (Faulwetter et al., 2009). An important part of the treatment performance in these systems is attributable to the presence and activity of plants and microorganisms (Krasnits et al., 2009), and the interactions between plants and microbial assemblages (Aguilar et al., 2008). With limited experimental evidence, the relationship between plant species and microbial community in the treatment process is debatable (Baptista et al., 2008).

Some previous studies found that plants did not have an effect on the structure of the microbial community using length heterogeneity PCR (Ahn et al., 2007); the size and structure of methanotrophic bacterial community identified by competi-

tive quantitative PCR (DeJournett et al., 2007); the diversity of ammonia-oxidizing bacteria (Gorra et al., 2007); and microbial biomass (Tietz et al., 2007a). However, other studies reported that the plants have a major effect on bacterial community profiles on the GN2 Microplates (Collins et al., 2004) and Ecoplates (Osem et al., 2007; Truu et al., 2009); the microbial biomass carbon and enzyme activities (dehydrogenase, urease, protease-BAA and acid phosphatase, Caravaca et al., 2005); the dynamics of bacterial number and bacterial community structure profiles identified by DGGE (Calheiros et al., 2009); and the quantity of ammonia-oxidizing bacteria (*Nitrosomonas* and *Nitrobacter*) and denitrifying bacteria (Kantawanichkul et al., 2009).

Plants and soil biota generally have a strong functional linkage as producers and decomposers of terrestrial ecosystems, e.g. plants provide organic carbons for soil biota through litter and root exudates, whereas soil biota decomposes organic matter and release mineralized nitrogen and phosphorus essential for plant growth (Wardle et al., 2004). Generally speaking, high plant species richness or functional diversity causes high heterogeneity of resource environments, and consequently develop diverse

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microbial groups (Sugiyama et al., 2008). Those relationships have been better studied for terrestrial ecosystems. However, constructed wetlands, often irrigated with wastewater with high COD or BOD loading, are different from both natural and disturbed terrestrial ecosystems. Therefore, understanding the dynamics of soil microbial communities in relation to plant diversity in wetlands would be critical in answering whether the plant-microbial linkage found in terrestrial ecosystems also exists in constructed wetlands.

The subsurface vertical flow constructed wetland (SVFCW) system with unsaturated flow is known to be particularly efficient in treating many types of wastewater (Molle et al., 2005; Brix and Arias, 2005). The SVFCW systems process greater oxygen transport ability than the horizontal subsurface flow beds (Cooper, 2005) and they are more effective for the mineralization of biodegradable organic matter (Kantawanichkul et al., 2009). Therefore, a full-scale SVFCW system was designed and constructed in the vicinity of Zhoushan City, Zhejiang Province of Eastern China, in September 2005, where 5 plant diversity gradients with species richness of 1, 2, 4, 8 and 16 species per treatment were created in April 2006.

In the present study, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were analyzed using the fumigation-extraction method, and BIOLOG Ecoplates were used for characterizing community level physiological profiles (CLPP) to determine differences in microbial community structure and carbon utilization intensity. These plates were developed especially for bacterial community analysis of environmental samples (Insman, 1997) and have already been proven useful for pinpointing differences in microbial activity and community structure among and within wastewater-treatment environments (Victorio et al., 1996; Collins et al., 2004; Hench et al., 2004; Osem et al., 2007; Truu et al., 2009; Faulwetter et al., 2009). The objectives of this paper are: (1) to evaluate the relationships between plant species richness and its biomass under the condition of same wastewater supply; (2) to test effects of plant diversity (species richness) on the size and structure of microbial community.

## 2. Materials and methods

### 2.1. Wetland location and design

Because the post-treated domestic wastewater contains higher level of inorganic nutrients, the local government forbade drainage of the post-treated domestic wastewater into the sea prior to a further treatment. Therefore, a full-scale constructed wetland (29°53'N, 122°23'E) was constructed in the vicinity of Zhoushan city, Zhejiang province in eastern China. This area has a subtropical monsoonal climate with cool, wet winters and hot, dry summers. Mean annual precipitation is 1200–1900 mm, and mean annual temperature is 16–19 °C.

The full-scale SVFCW system (length = 50 m, width = 20 m, depth = 1.2 m) was established with a three-layer filter: 0.5 m of 50–120 mm gravel in the bottom followed by 0.2 m of 6–12 mm gravel in the middle and a 0.4 m layer of coarse 1–2 mm sea sand at the top (Fig. 1A). The treatment system was fed with pre-treated domestic wastewater (BOD<sub>5</sub> = 12.6 mg/l, COD = 13.2 mg/l, total nitrogen content = 39.3 mg/l, total phosphorus content = 3.6 mg/l and total suspended solids (TSS) = 66.0 mg/l) from local wastewater plant near Zhoushan City, and had an ability to treat a loading of 2000 m<sup>3</sup>/d by pulse-irrigation.

The overall plant species pool consisted of 16 species (Table 1), representative of typical water-tolerant species for subtropical monsoonal climate. We created 5 diversity levels with a species richness of 1, 2, 4, 8, and 16 species per plot (2 m × 2 m). Five unplanted plots were also included for comparison purpose

**Table 1**

Plant species used in this study.

Plant Latin names	Abbreviations
<i>Lythrum salicaria</i> Linn.	Ls
<i>Imperata cylindrical</i> (Linn.) Beauv.	Ic
<i>Campylotropis macrocarpa</i> (Bge.) Rehd.	Cm
<i>Indigofera pseudotinctoria</i> Mats.	Ip
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Pa
<i>Cyperus alternifolius</i> Linn.	Ca
<i>Canna indica</i> Linn.	Ci
<i>Thalia dealbata</i>	Td
<i>Triarrhena sacchariflora</i> (Maxim.) Nakai	Ts
<i>Lespedeza bicolor</i> Turcz.	Lb
<i>Cassia tora</i> Linn.	Ct
<i>Miscanthus sinensis</i> Anderss.	Ms
<i>Sapindus mukorossi</i> Gaertn.	Sm
<i>Neyraudia montana</i> Keng.	Nm
<i>Saccharu arundinaceum</i> Retz.	Sa
<i>Arundo donax</i> Linn.	Ad

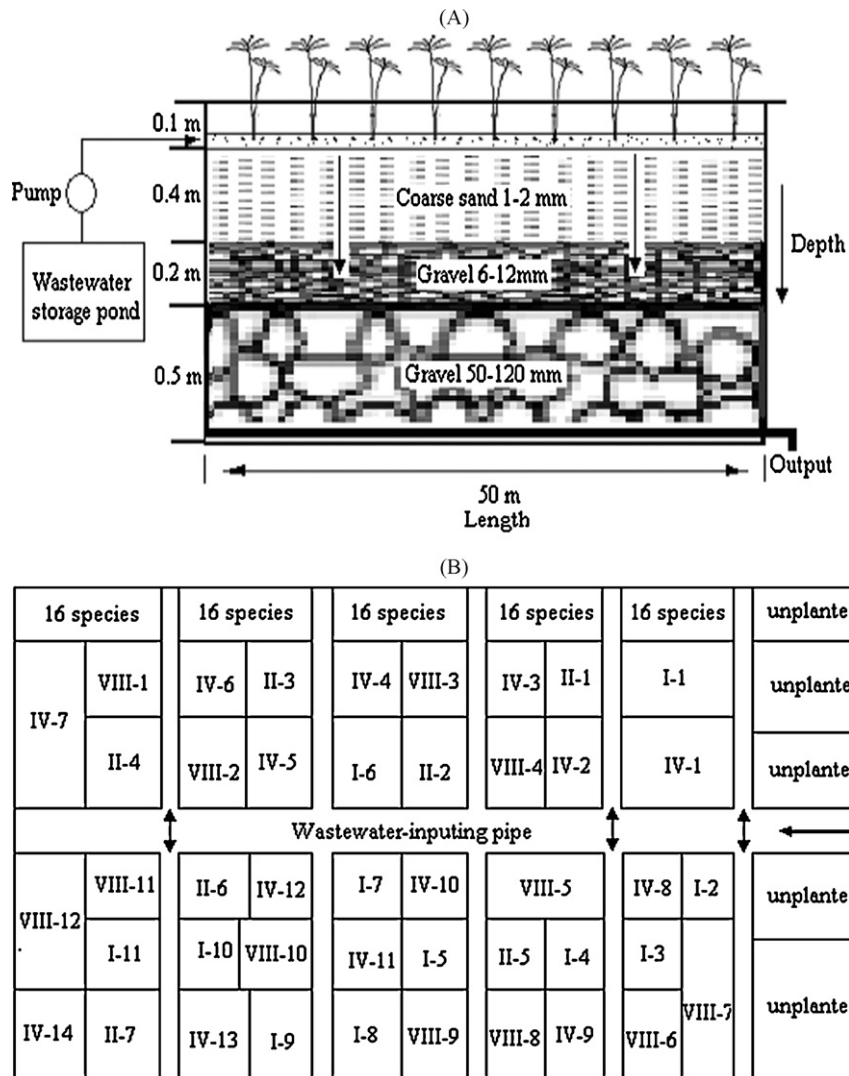
(Fig. 1B). Each site within a diversity level had a different species composition to avoid confounding of the effects of diversity with species identity. We replicated species richness—sixteen mixtures with one species, seven mixtures with two species, fourteen mixtures with four species, twelve mixtures with eight species and five mixtures with sixteen species, making fifty-four plots (including unplanted plots) in total (Fig. 1B). At the higher diversity levels, we used fewer mixtures to reduce overlap in species composition, because we expected less variability in ecosystem processes than among mixtures at lower diversity levels (Doak et al., 1998; Spehn et al., 2000). All plant assemblages were planted at a density of 144 viable seedlings per plot in a substitutive design (i.e. identical total seedling density in each plot, van Ruijven and Berendse, 2003). The mixtures of two, four, eight species were assembled by constraint random selection from the species pool. Selecting a certain composition twice was not allowed in this procedure (van Ruijven and Berendse, 2003). The planting-density for each species was equal in the mixtures of two, four, eight and sixteen species.

### 2.2. Evaluation of the experimental plant communities

During the experiment, all species mixtures that were planted were still present, except for the one-species mixtures, where these mixtures, individually planted with *Thalia dealbata*, *Cassia tora* Linn., *Sapindus mukorossi* Gaertn., *Neyraudia montana* Keng. and *Saccharu arundinaceum* Retz., were not included in the study due to plant fading or withering. Species richness was measured as presence of species in the plots at the time of the harvests. According to difference of plant growth form and density, total aboveground plant biomass was measured by harvesting all plant material in three sub-plots (small plants: 25 cm × 25 cm; large plants: 35 cm × 35 cm) of each plot in order to maintain the routine operation of the constructed wetland. Plants were clipped to 2.5 cm above the soil surface, and newly produced aboveground tissue was oven-dried for at least 48 h at 70 °C, and computed as g/m<sup>2</sup>.

### 2.3. Substrate sample collection

Early August 2007, five rhizosphere samples (from five target plants) were taken using the hand-shaking method (Baudoin et al., 2002) in each plot, and then were mixed into a composite sample. To avoid confounding edge effects between plots, rhizosphere samples were purposely taken near the center of each plot. After roots and macrofauna were removed by hand, the field-moist samples were sieved (2 mm) and immediately were collected in separate plastic Ziploc bags, which were stored in refrigerator at 4 °C for



**Fig. 1.** Dissecting structure (A) of the subsurface vertical flow constructed wetland (SVFCW), and distribution of species mixtures (B) in the SVFCW. On the plot B, I-1–11 represent eleven sites planted with one species, e.g. *Ls* (I-1), *Ic* (I-2), *Cm* (I-3), *Ip* (I-4), *Pa* (I-5), *Ca* (I-6), *Ci* (I-7), *Ts* (I-8), *Lb* (I-9), *Ms* (I-10), *Ad* (I-11); II-1–7 represent seven sites planted with two species, e.g. *Pa-Ts* (II-1), *Ad-Ls* (II-2), *Ic-Ci* (II-3), *Lb-Ct* (II-4), *Nm-Ci* (II-5), *Ts-Sm* (II-6), *Nm-Ic* (II-7); IV-1–14 represent fourteen sites planted with four species, e.g. *Sa-Pa-Lb-Ct* (IV-1), *Sa-Pa-Nm-Ad* (IV-2), *Pa-Lb-Ip-Ci* (IV-3), *Ad-Ic-Sm-Ct* (IV-4), *Pa-Ic-Cm-Ca* (IV-5), *Sa-Ms-Lb-Ls* (IV-6), *Pa-Sm-Cm-Ca* (IV-7), *Lb-Ip-Cm-Ct* (IV-8), *Sm-Ip-Ca-Ci* (IV-9), *Pa-Nm-Ad-Ls* (IV-10), *Ic-Sm-Cm-Td* (IV-12), *Sa-Td-Ca-Ci* (IV-13), *Ad-Ic-Cm-Td* (IV-14). VIII-1–12 represent fourteen sites planted with four species, e.g. *Sa-Pa-Nm-Ad-Ic-Sm-Ip-Ct* (VIII-1), *Sa-Ad-Ip-Cm-Ct-Cs-Ca-Ci* (VIII-1), *Nm-Ad-Sm-Ms-Ip-Cm-Cs-Ci* (VIII-1), *Pa-Ad-Ts-Ic-Sm-Ms-Ip-Ci* (VIII-1), *Sa-Pa-Nm-Ad-Ic-Sm-Ip-Ca* (VIII-1), *Ts-Ic-Sm-Ms-Ct-Td-Ca-Ci* (VIII-1), *Ts-Ic-Sm-Ms-Td-Ls-Ca-Ci* (VIII-1), *Sa-Pa-Nm-Ad-Td-Ls-Ca-Ci* (VIII-1), *Pa-Nm-Ad-Ts-Ms-Lb-Ca-Ci* (VIII-1), *Sa-Pa-Nm-Ad-Ts-Ic-Sm-Ms* (VIII-1), *Ts-Ic-Sm-Ms-Lb-Ip-Cm-Ct* (VIII-1), *Sa-Ad-Sm-Ms-Ip-Cm-Ca-Ci* (VIII-1). The mixture planted with 16 species, see Table 1.

analysis of microbial parameters (biomass and community composition).

#### 2.4. Microbial biomass analysis

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined by the chloroform fumigation extraction technique (Sparling and West, 1988; Amato and Ladd, 1988). Ten grams of fresh sand was fumigated with chloroform for 24 h. Following fumigation, the microbial biomass carbon was extracted with 50 ml 0.5 mol/l  $K_2SO_4$  and was determined using a heated  $K_2Cr_2O_7-H_2SO_4$  digestion. The  $K_{EC}$ -factor was 0.35 which means 35% of organic carbon is converted into microbial carbon (Sparling and West, 1988). The microbial biomass nitrogen was extracted with 50 ml 2 mol/l KCl and was measured photometrically (UV2102-PC photoelectric photometer, UNICO) at 570 nm after the ninhydrin reaction. A  $K_{EN}$ -factor was 3.1 which indicates that 1  $\mu$ g

biomass nitrogen per g dry soil is equivalent to 3.1  $\mu$ g ninhydrin-reactive nitrogen per g of dry sand (Amato and Ladd, 1988).

#### 2.5. Community level physiological profiles (CLPP) analysis

The cell supernatant of sand sample was made using 10 g of sand sample and 0.85% NaCl solution, and was diluted with sterile NaCl solution to a mean inoculum density of  $10^6-10^7$  cells  $ml^{-1}$ . 125  $\mu$ l of the cell suspension was added to each well of the Ecoplates (Biolog, Hayward, CA). The plates were incubated at 28 °C in darkness in plastic boxes containing water-soaked paper towel in order to minimize water evaporation from the wells. The turnover of each carbon source was estimated by measuring the reduction of tetrazolium violet to formazan spectrophotometrically at 590 nm by use of a microplate reader (GENios Pro™, Tecan, Trading AG, Switzerland). The reading ( $A_{590}$ ) for individual substrates was corrected for the background absorbance by subtracting the absorbance of

the control well containing water only. The positive and negative values of the corrected readings were set to  $A_{590} \geq 0.1$  and zero, respectively (Moynahan et al., 2002).

Average well color development (AWCD) of substrate utilization at  $A_{590}$  was calculated as the average optical density across all wells per plate by  $AWCD = (\sum n_i)/31$  (Zak et al., 1994), where  $n_i$  represented the relative optical density of the  $i$ th well which was corrected by color development in the control well. Shannon's diversity in each soil sample was also computed using  $H = -\sum p_i(\ln p_i)$ , where  $p_i$  is the ratio of individual carbon source utilization to the sum of all carbon source utilizations.

In addition to analyze individual substrate utilization patterns, 31 carbon sources were subdivided into six substrate groups as suggested by Zak et al. (1994): carbohydrates (CH), carboxylic acids (CA), amines/amides (AM), amino acids (AA), polymers (PL) and miscellaneous (MS), and average absorbance of all carbon sources within each group was computed, respectively.

## 2.6. Data analysis

In the present study, the significant differences of all measurements were analyzed using one-way ANOVA followed by LSD tests (at  $\alpha = 0.05$  level). Effects of plant parameters (species richness and plant biomass) on microbial parameters (microbial biomass and carbon substrate group utilization and diversity) were tested using the Pearson coefficient. The metabolic profiles pattern and the relationships between microbial and plant parameters were analyzed using principal components analysis (PCA) and redundancy analysis (RDA).

According to the suggestion from Weber et al. (2007), before the PCA was run, AWCD data at 168 h of incubation in the BIOLOG Ecoplates were logarithmically transformed. The PCA profiles were used to project the maximum variance of AWCD data optimally in multiple dimensions (e.g., axis 1 and axis 2), in an unconstrained ordination. In the PCA ordination diagram, samples with similar AWCD datasets are located close to one another, and those dissimilar are located far apart.

In RDA, microbial parameters were used as 'species', and the ordination axes were constrained to be linear combinations of the environmental variables (i.e., plant variables). This analysis, therefore, allowed the relationships between the plant variables and the microbial parameters to be directly compared. With the Monte Carlo permutation test (number of permutations), the significance of the plant variables in accounting for the observed variance of the microbial parameters can be assessed with  $P$ -values. In the RDA diagram, positively correlated variables are shown as arrows pointing in the same direction, negatively correlated variables point in opposite directions, and perpendicular variables are uncorrelated. In addition, the length of the arrow is a measure of the relative importance of the plant variables in explaining variances of the microbial parameters. PCA was run with SPSS 11.0 software (SPSS, Inc.) and RDA were performed with the Canoco 4.5 software (Center for Biometry, Wageningen, Netherlands) as discussed by ter Braak (1994) and Lepš and Šmilauer (2003).

## 3. Results

### 3.1. Plant biomass

An obvious effect of plant species richness on aboveground plant biomass production was observed (Fig. 2,  $R = 0.407$ ,  $P = 0.002$ ). Although plant biomass (means from replicate samples) did not significantly change among 1, 2 and 4 species or 8 and 16 species ( $P > 0.05$ ), but was significantly higher in species richness levels of

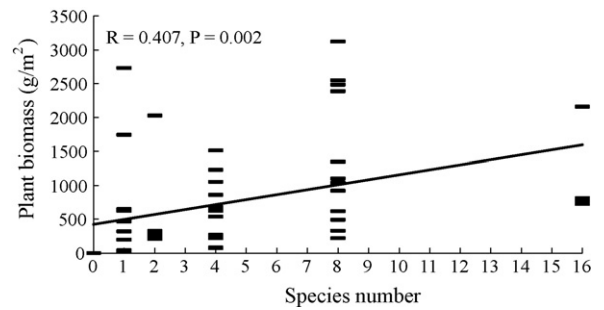


Fig. 2. Relationships between plant species richness and its biomass. The "R" in the plot represents coefficient between plant species richness and biomass, and the "P" indicates the significant level of the R.

8 and 16 species (1382.8 and 1117.0 g/m<sup>2</sup>) than in 1, 2 and 4 species level (622.36, 517.29 and 622.08 g/m<sup>2</sup>, respectively  $P < 0.05$ ).

### 3.2. Microbial biomass

Compared with MBC in the control treatment (52 mg/g dw), MBC significantly increased in the five species treatments (Fig. 3A), with values ranged between 139 and 178 mg C/g dw. Despite the fact that MBC did not significantly change across the species richness levels of 1, 2, 4 and 8 species, the Pearson correlation analysis indicated that plant species richness levels were significantly correlated with MBC ( $R = 0.494$ ,  $P < 0.001$ ). The MBN ranged between 4.1 and 6.0  $\mu$ g/g dw across all treatments (Fig. 3B), as compared with those in the control it did not significantly change across the three treatments of 1, 2 and 4 species levels, but significantly increased at the 8 and 16 species levels, thus indicating the effects of higher species richness level on MBN ( $R = 0.465$ ,  $P < 0.001$ ).

### 3.3. CLPP of microbial community

Plant species richness exhibited different effects on utilizations of six carbon substrate groups in Ecoplates at the end of 168 h of incubation (Fig. 4A and B). The utilization of amino acids consistently increased with species richness level (Fig. 4A,  $R = 0.235$ ,  $P = 0.03$ ), whereas the utilization of the amine/amides signifi-

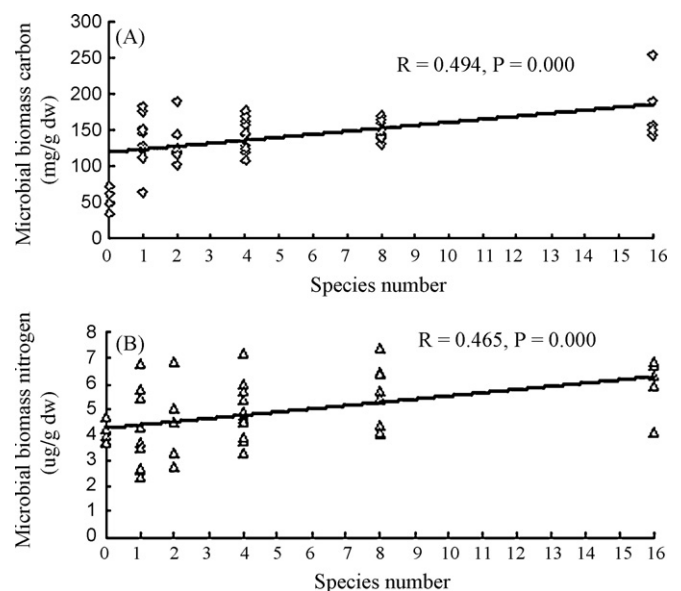
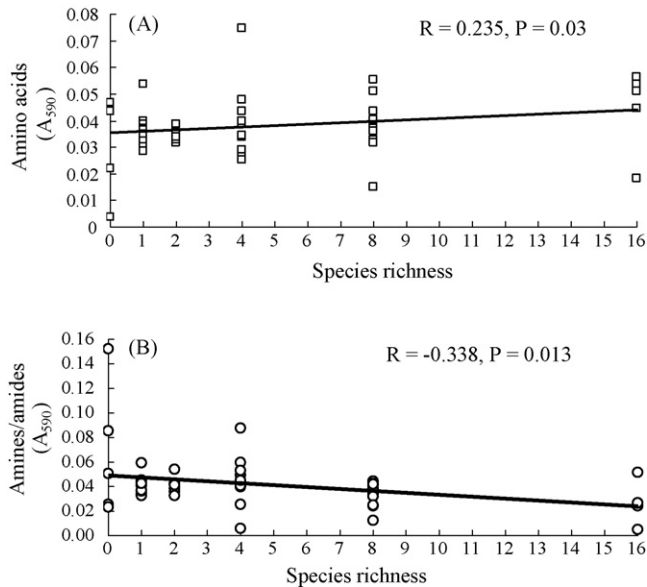


Fig. 3. Relationships between plant species richness and microbial biomass carbon (A) and nitrogen (B). Both "R" and "P" are explained in Fig. 2.



**Fig. 4.** Relationships between plant species richness and AWCD data of carbon substrate groups (A and B) at 168 h in BIOLOG Ecoplates™. Both “R” and “P” are explained in Fig. 2.

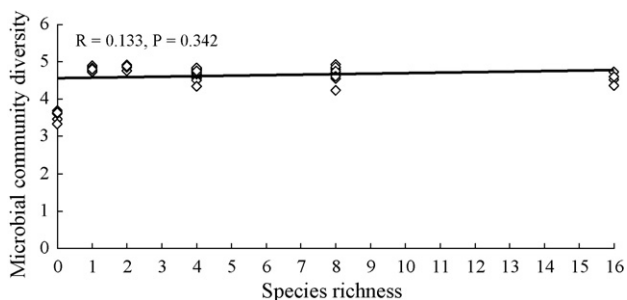
cantly decreased (Fig. 4B,  $R = -0.338$ ,  $P = 0.013$ ). Utilizations of the remaining groups (carbohydrates, carboxylic acids, polymer and miscellaneous) did not significantly change with species richness level (data not shown).

The diversity of microbial community did not change with plant species richness ( $R = -0.133$ ,  $P = 0.342$ , Fig. 5). Nevertheless, compared with those in unplanted treatments, the diversity value significantly increased across the five species richness levels ( $P < 0.05$ ).

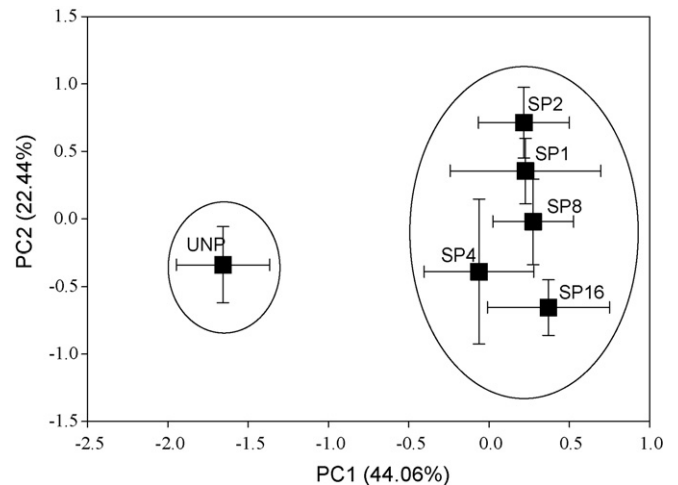
The PCA showed that metabolic profiles from the five plant species treatment (SP1, 2, 4, 8 and 16) were similar to each other (Fig. 6), but significantly distinguished from that of unplanted treatment ( $P < 0.05$ ). The first two PCs (PC1 and PC2) explained 44.06% and 22.44% of the variance in AWCD data. The largest loadings on the PC1 axis were from polymers ( $-0.884$ ), amine/amides ( $-0.868$ ), carbohydrates (0.724) and amino acids (0.705), and on the PC2 axis from carboxylic acids (0.715) and miscellaneous (0.706).

#### 3.4. RDA analysis of microbial and plant parameters

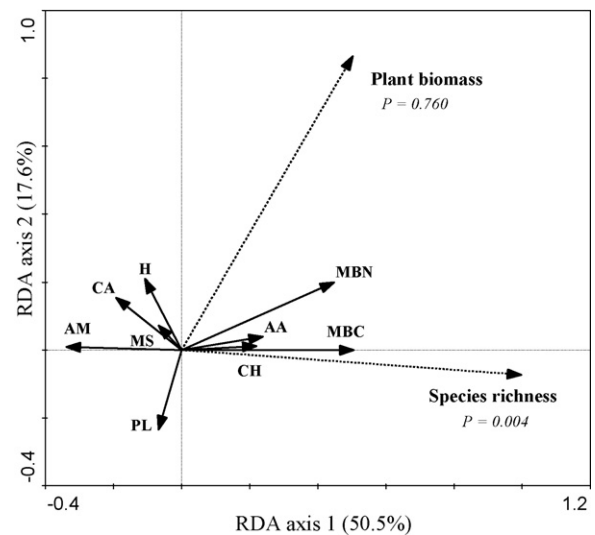
As shown in Fig. 7, RDA, as tested by Monte Carlo permutation, identified plant parameters (species richness and biomass production) significantly correlated with microbial parameters (e.g. six carbon substrate groups, community diversity, microbial biomass carbon and nitrogen). Significant plant parameter only included



**Fig. 5.** Relationships between plant species richness and diversity of microbial community at 168 h in BIOLOG Ecoplates™. Both “R” and “P” are explained in Fig. 2.



**Fig. 6.** PCA-ordination biplot according to AWCD data of carbon substrate groups at 168 h in BIOLOG Ecoplates™. The “UNP” represents the treatment unplanted with plant species, the “SP” 1, 2, 4, 8, 16 represents different species richness level, respectively.



**Fig. 7.** Biplot of the first two RDA axes of microbial (e.g. six carbon substrate groups, community diversity, microbial biomass carbon and nitrogen) and plant parameters (species richness and biomass production) for five planted treatments and unplanted treatments. The microbial parameters (expressed as response variables in the RDA analysis) were presented as *solid line* vectors, and the plant parameters (explanatory variables) were presented as *dotted line* vectors. The plant parameters were labeled with their  $P$ -values, no matter how significant the effects of these plant variables on metabolic profiles. Abbreviations: CH: carbohydrates, CA: carboxylic acids, AM: amines/amides, AA: amino acids, PL: polymers, MM: miscellaneous, H: microbial community diversity, MBC: microbial biomass carbon and MBN: biomass nitrogen.

the plant species richness ( $P = 0.004$ ). MBN, MBC, AA (amino acids) and CH (carbohydrates) utilization were positively correlated with plant species richness and biomass production, while utilizations of CA, AM, PL and MS, as well as functional diversity (H) were negatively correlated with plant parameters.

## 4. Discussion

### 4.1. Relations of plant diversity with its biomass production

A variety of plant diversity–productivity relationships from terrestrial ecosystems have been reported, including positive, neg-

ative, hump-shaped, U-shaped, and flat (non-significant) patterns (Tilman, 1996; Gjerde et al., 2005). No single pattern seems to apply to all spatial scales (Fukami and Morin, 2003). The present study clearly showed the positive relationship of plant species richness with biomass production in the SVFCW system. The positive diversity–productivity relationship most likely benefited from the supply of wastewater high in nutrient concentrations and thus plant competition for nutrients was eliminated (Ahn et al., 2007). On the other hand, the positive diversity–productivity relationship was likely attributed to the complementary resource use in a high species level of community (van Ruijven and Berendse, 2009) or to the selecting effect, e.g. more diverse communities have a higher chance of containing a very productive species that dominates the community (Huston, 1997; Tilman, 1997). Simultaneously, the present study also observed a slight decline in plant biomass production in the 16 species treatment. The lack of effects of diversity beyond the level of certain species was difficult to explain, but reminded us to consider the optimal plant species pattern for maximizing the removal of pollutants by plants in CWs.

#### 4.2. Effects of plant diversity on microbial biomass

Both microbial biomass carbon and nitrogen were significantly higher in five species treatments than in the unplanted treatments, and were strengthened by the species richness and biomass production levels (identified by Pearson coefficients and RDA), indicating effects of plant parameters on the size of microbial community. This is consistent with the findings of Calheiros et al. (2009) who studied the changes in the bacterial community structure in two-stage constructed wetlands with different plants for industrial wastewater treatment, and suggested that both bacterial counts and structure were higher in plant root zone samples than in the filled substrates. Similar results were also observed in studies of the mesocosm treatment wetland (Collins et al., 2004), semiarid Mediterranean salt marsh (Caravaca et al., 2005), a vertical flow constructed wetlands (Kantawanichkul et al., 2009), and a field wetland (Truu et al., 2009). However, some contradictory results were also present in the literature, such as Larsen and Greenway (2004) and Tietz et al. (2007b) found no significant difference of microbial biomass between planted and unplanted gravel beds (EPS) or pilot-scale subsurface vertical flow constructed wetlands (PSCW). Besides, Ahn et al. (2007) and DeJournett et al. (2007) studied the molecular characterization of microbial communities in treatment microcosm wetlands, and found no significant effects of the presence of macrophytes on LH-PCR profiles of soil microbial communities (likely due to low growth of plants and sampling anomalies), and the quantities of either Type I or Type II methanotrophic bacteria using competitive quantitative PCR (likely due to the artifact of the technique or effects of interfered factors). Baptista et al. (2008) also pointed out that the numbers of archaea, bacteria and sulphate reducing bacteria were indistinguishable in both reactors (planted and unplanted horizontal subsurface-flow laboratory-scale constructed wetlands), and concluded that plants did not appear to exert a strong effect on the structure of the microbial communities in the HSCW systems, which was attributed to effects of a common source community assembled randomly.

#### 4.3. Effects of plant diversity on CLPP

Recently, Ros et al. (2008) have found a very low diversity within the Biolog plates. Nevertheless, the procedure may yield a great deal of information about an important functional attribute of microbial communities and has been shown

in some cases to be as sensitive as or more sensitive than measuring microbial biomass and respiration (Johnson et al., 1998), it, therefore, has already proven useful for pinpointing differences in microbial activity and community structure among and within wastewater-treatment environments (Victorio et al., 1996; Collins et al., 2004; Hench et al., 2004; Osem et al., 2007).

PCA of carbon substrate utilization data revealed that the significant change in CLPP profiles of microbial community strongly depended on the presence or absence of plants. This confirmed results from Hench et al. (2004) and Osem et al. (2007) in which the BIOLOG technique was used for analyzing the change in microbial community structure between planted and unplanted wetland systems. Truu et al. (2009) also found significant differences in the bacterial community in planted versus unplanted constructed wetland fed with pre-treated wastewater. This possibly showed that the incorporation of plant roots into substrate of wetland may provide unique attachment sites for certain microbial populations (Osem et al., 2007); or plant growth ‘stimulates’ the formation of certain communities in the wastewater (Vacca et al., 2005). Besides, the release of oxygen from the plant internal root zones was possibly another important cause (Stottmeister et al., 2003; Kantawanichkul et al., 2009), since the root oxygen release can influence the type of microorganisms present in the rhizosphere (Gagnon et al., 2007).

Plants and soil biota have a strong functional linkage as producers and decomposers of terrestrial ecosystems, therefore, high plant species richness or functional diversity can cause high heterogeneity of resource environments, and consequently develop diverse microbial groups (Wardle et al., 2004; Sugiyama et al., 2008). Nevertheless, PCA revealed that there was no significant change in CLPP profiles of microbial community among plant assemblages of our wetland system. This was similar to results of Victorio et al. (1996), i.e. samples from the same wastewater-treatment system (activated sludge and aerated lagoon system) produced similar metabolic fingerprints (CLPP), suggesting that the community and/or activity did not change within treatment over the time period studied. This was most likely attributed to effects of the same supply of wastewater over those of plant composition on structure of microbial community, since the terrestrial ecosystem is largely different from the man-made wetland system often characterized by disturbance of higher nutrients.

The one-way ANOVA showed that the utilization of two out of six carbon groups was significantly impacted by shift of plant species richness and biomass production as demonstrated by both Pearson coefficients and RDA, despite the fact that the diversity of microbial community derived from the utilization of carbon substrates at 168 h of incubation did not significantly change. Of the two carbon groups, the utilization of amino acids significantly increased with species level and biomass production, indicating that plant diversity increased the utilization of amino acids in rhizosphere environments. This in part supported the results of Osem et al. (2007), where kitchen wastewater was treated with hydroponic reactors, and showed stronger utilization of L-asparagine on plant roots than on tuff stones; On the other hand, the strong utilization of phenylethyl amine was also observed in the study of Osem et al. (2007), but the lower utilization of amine/amides in plant rhizosphere occurred in our study. This was possibly attributed to the difference of planting design in two studies. In our study, there were sixteen plant species which were different from the study of Osem et al. (2007), where only *Typha domingensis* and *Cyperus alopecuroides* were used. Meanwhile, wastewater types were also possibly responsible for the difference in utilization of amine/amides in the two studies.

## 5. Conclusions

Plant species richness positively correlated with plant biomass production, and therefore the plant species richness is an important predictor of plant productivity richness in our wetland treatment system. The sizes of microbial community such as biomass carbon and nitrogen were increased by plant species richness, mediated primarily by a raise in plant production. However, changes in the structure of the microbial community strongly depended on presence or absence of plants, but not on the changes in plant richness. Therefore, this study suggests that the increase in plant diversity improved the size of microbial community in wetland treatment system, but did not promote the development of microbial community structure.

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