

Short Communication

# Effects of Emergent Plant Species and Growth Strategy on Microbial Community Structure and Diversity

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## Abstract

A field study was conducted in two adjacent shallow lakes (Aiwan Lake and Qingnian Lake) in Tianjin, China, to investigate the effects of plant species and growth strategy (single or mix) on the microbial community's structure and diversity in the rhizosphere of emergent plants by phospholipid fatty acid (PLFA) methods. The results demonstrated that microbial biomass was higher in the *Typha orientalis* (*T. orientalis*) rhizosphere than that in the *Phragmites australis* (*P. australis*) rhizosphere, whether they grew separately or together. The bacterial population of gram-positive bacteria ( $G^+$ ) was found to be less than that of the gram-negative bacteria ( $G^-$ ) in all samples, and the ratio of  $G^+$  to  $G^-$  in the plant rhizosphere was less than that in the non-rhizosphere. The diversity index of plant rhizosphere was higher than that of the non-rhizosphere, and was higher in the *T. orientalis* rhizosphere than in the *P. australis* rhizosphere. Cluster analysis demonstrated that microbial community structure was more significantly influenced by plant species than by growth strategy.

**Keywords:** phospholipid fatty acid (PLFA), microbial diversity, emergent plant rhizosphere

## Introduction

The microbial community structure and diversity of wetlands is very important for the treatment efficiency of pollutants and ecosystem stability [1, 2]. In recent years research has been carried out on removal efficiency of organic pollutants and nutrients in the constructed wetlands; however, less work has been done on microbial community structure in the rhizosphere of wetland plants [3-5]. Understanding the microbial community structure and diversity is important for studying the degradation and transformation pathways of pollutants. At present, the research of wetland microorganisms is mainly concentrated in culturable microorganisms; the analysis of microbial community structure is still very difficult due to the limitations of traditional methods [6]. Phospholipid fatty acid

(PLFA) is an important part of the microbial cell membrane, and its composition mode can reveal information about the microbial community structure [5, 7].

The aim of this study was to analyze and compare the microbial community structure and diversity in the rhizosphere of two emergent plants and non-rhizosphere by using the PLFA method and provide data support for the remediation of pollutants in water.

## Material and Methods

### Site Description and Sample Collection

A field investigation was carried out in two adjacent shallow eutrophic lakes: Qingnian Lake (QL; 39°06'42" N, 117°10'04" E) and Aiwan Lake (AL; 39°06'27" N, 117°09'44" E) in Tianjin, China. There are large amounts of

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emergent plants, such as *Phragmites australis* (*P. australis*) and *Typha orientalis* (*T. orientalis*), in both lakes. On May 18, 2010 (within the growing season of the two plants), six samples of non-rhizosphere and rhizosphere sediments were collected from the two lakes. *P. australis* and *T. orientalis* grew separately at the sampling sites of QL, while they grew together at the sampling sites of AL. The plant growth rates in the two lakes were similar except that for of *P. australis* in AL, the growth of which was inhibited when growing together with *T. orientalis*, and plant height was only half of that when growing in single. Rhizosphere sediment samples were obtained from all over the root zone by shaking off sediment that was loosely adhering to the roots. Non-rhizosphere sediment samples were collected from the same depth at a distance of 10 m from vegetation. The depth from which the samples were taken was about 30 cm. Sediment samples were sealed in polytetrafluoroethylene bags and cooled in a refrigerator (4°C) during transport to the laboratory.

After transportation to the laboratory, the sediment samples were immediately freeze-dried, ground, and passed through an 80-mesh sieve for analysis of phospholipid fatty acids (PLFAs).

### Extraction and Analysis of PLFAs

PLFAs were extracted in three steps using a modified procedure [8]. In brief, 2.0 g of freeze-dried sediment was extracted with a chloroform-methanol-citrate buffer mixture (1:2:0.8), and the sample was separated and cleaned by a silica gel column (6 mm×100 mm) to get rid of neutral and glycolipids. The phospholipids were subjected to alkaline methanol, and fatty acid methyl esters (FAMES) formed were extracted twice with 2 mL hexane. After evaporation of the solvent under nitrogen gas, the FAMES were re-suspended in 100 µL hexane containing 2 µg nonadecanoic acid methyl ester (Sigma Chemical, Poole, UK) as an internal standard. FAMES were quantified by GC-MS. A splitless injection was employed (injector at 250°C).

The oven temperature was 80°C initially, then increased to 150°C at 30°C/min, followed by an increase to 250°C at 3°C/min and held for 1 min. Finally, the oven temperature was increased to 280°C at 10°C/min and held for 3 min. Helium was used as a carrier gas (1.0 mL/min).

Fatty acids were designated as the total number of carbon atoms: the number of double bonds followed by the position of the double bond from the methyl end ( $\omega$ ) of the molecule. Cis and trans-configurations are indicated by c and t. The prefixes a- and i- indicate anteiso and iso branching; br indicates an unknown methyl branching position; 10Me indicates a methyl group on the 10<sup>th</sup> carbon atom from the carboxyl end of the molecule and cy refers to cyclopropane fatty acids.

Total microbial biomass was estimated by the total amount of extracted PLFAs (nmol/g dry weight). The sum from the following PLFAs was used to measure total bacterial biomass: i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, 17:0, 16:1 $\omega$ 7, 16:1 $\omega$ 9, 18:1 $\omega$ 7, 18:1 $\omega$ 9, cy17:0, and cy19:0 [8]. The peak of PLFA 18:2 $\omega$ 6,9 was used to measure fungal biomass [8]. The sum from the following fatty acids was considered to represent Gram-positive bacteria: i15:0, a15:0, i16:0, i17:0, and a17:0 [9]. The Gram-negative bacteria were represented by the sum of 16:1 $\omega$ 7, 16:1 $\omega$ 9, 18:1 $\omega$ 7, 18:1 $\omega$ 9, cy17:0, and cy19:0 [9].

The diversity of the microbial community was assessed by the Shannon index ( $H'$ ), calculated for each sediment sample using the following formula:

$$H' = -\sum_{i=1}^s pi \ln pi \quad (1)$$

...where  $H'$  is the value of the Shannon index,  $pi$  is the concentration of  $i^{\text{th}}$  individual fatty acid relative to the concentration of all fatty acids, and  $s$  is the number of species found in the community profile.

Cluster analysis of the PLFA profiles of the microbial community was performed using hierarchical clustering according to the between-groups linkage method with the software package SPSS 13.0 for Windows.

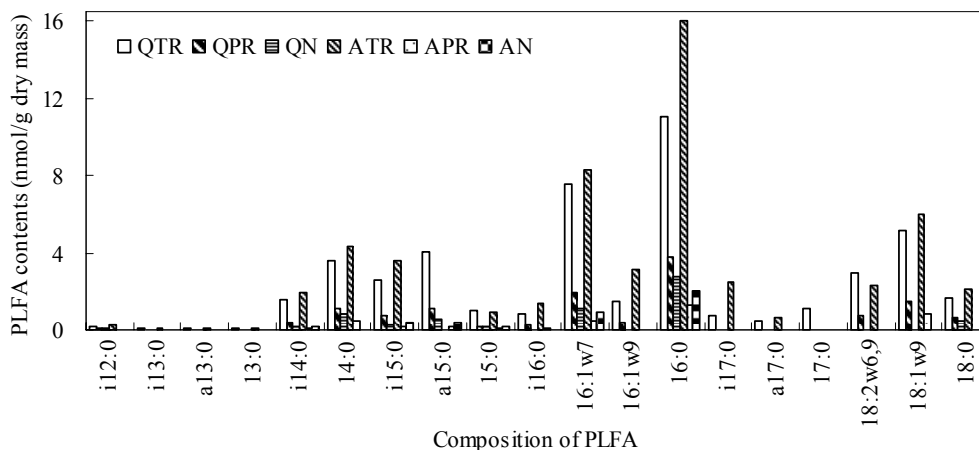


Fig. 1. Composition of PLFA in sediment samples. AN – non-rhizosphere sediments collected from Aiwan Lake, QN – non-rhizosphere sediments collected from Qingnian Lake, APR – *P. australis* rhizosphere sediments collected from Aiwan Lake, QPR – *P. australis* rhizosphere sediments collected from Qingnian Lake, QTR – *T. orientalis* rhizosphere sediments collected from Qingnian Lake, ATR – *T. orientalis* rhizosphere sediments collected from Aiwan Lake.

Table 1 Contents of PLFAs and index of Shanon in sediments.

|   | QL    |       |      | AL    |      |      |
|---|-------|-------|------|-------|------|------|
|   | TR    | PR    | N    | TR    | PR   | N    |
| Total microbial biomass (nmol/g dry mass)         | 44.17 | 12.35 | 5.99 | 51.17 | 3.52 | 3.93 |
| Gram-positive bacteria (nmol/g dry mass)          | 8.65  | 2.17  | 0.83 | 8.11  | 0.46 | 0.70 |
| Gram-negative bacteria (nmol/g dry mass)          | 14.21 | 3.81  | 1.12 | 17.39 | 1.30 | 0.93 |
| G <sup>+</sup> /G <sup>-</sup>                    | 0.61  | 0.57  | 0.74 | 0.47  | 0.35 | 0.75 |
| Total bacterial biomass (nmol/g dry mass)         | 24.91 | 6.20  | 2.10 | 26.40 | 1.86 | 1.78 |
| Fungal biomass (nmol/g dry mass)                  | 2.99  | 0.77  | ND   | 2.35  | ND   | ND   |
| number of species found in the community profiles | 19    | 13    | 9    | 17    | 10   | 6    |
| Shanon index ( <i>H'</i> )                        | 2.39  | 2.20  | 1.72 | 2.25  | 1.79 | 1.35 |

QL – Qingnian Lake, AL – Aiwan Lake, TR – *T. orientalis* rhizosphere sediment, PR – *P. australis* rhizosphere sediment, N – non-rhizosphere sediment; ND – not detected.

## Results

### The Type and Population of Microorganisms in Sediments

The PLFA profiles of the whole microbial community showed that the samples collected from sediment contained a variety of PLFAs composed of saturated, unsaturated, and methyl-branched fatty acids (Fig. 1). The cyclopropane fatty acids cy17:0 and cy19:0, which are commonly found in Gram-negative bacteria, and the actinomycete specific fatty acid 10Me18:0 were not detected. The fatty acid 16:0 was most abundant in all the sediment samples.

The contents (nmol/g dry mass) of total microbial biomass, total bacterial biomass, Gram-positive bacteria and Gram-negative bacteria are shown in Table 1. Total microbial biomass, total bacterial biomass, Gram-positive bacteria, and Gram-negative bacteria were significantly higher in the plant rhizosphere than that of non-rhizosphere, except *P. australis* rhizosphere in Aiwan Lake. The microbial biomass from the rhizosphere sediments was related to plant species. The microbial biomass was significantly higher in *T. orientalis* rhizosphere sediments than that in *P. australis* rhizosphere sediments in both lakes. The Gram-positive bacteria and Gram-negative bacteria were significantly higher in the rhizosphere sediments of *T. orientalis* than in the rhizosphere sediments of *P. australis* in both lakes. The bacterial population of Gram-positive bacteria was found to be less than that of the Gram-negative bacteria in all samples, and the ratio of Gram-positive bacteria to Gram-negative bacteria in the rhizosphere was less than that in the non-rhizosphere.

### Shannon Index in Sediments

The Shannon index (*H'*) that was calculated from the PLFA data is shown in Table 1. The Shannon index in the rhizosphere sediments was higher than that in the non-rhizosphere sediments from the two lakes, and was higher in

the *T. orientalis* rhizosphere than in the *P. australis* rhizosphere, showing that root exudates not only affected the population of rhizosphere microorganisms but also affected microbial community diversity.

### Cluster Analysis of Microorganisms in Sediments

Cluster analysis of PLFA data from the sediment samples is shown in Fig. 2. The non-rhizosphere sediments and *P. australis* rhizosphere sediments from the two lakes were strictly linked together at a low Euclidean distance, while similarities between them and the two *T. orientalis* rhizosphere sediments were found at considerably higher distances.

## Discussion

The factors affecting rhizosphere microorganisms are complicated, including the role of root exudates, the influence of root on the mobility of microorganisms, and nutrient cycle in the rhizosphere [10, 11]. It is well known that

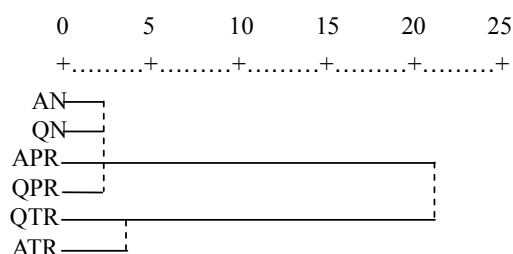


Fig. 2. Cluster analysis of PLFAs extracted from sediment samples. AN – non-rhizosphere sediments collected from Aiwan Lake, QN – non-rhizosphere sediments collected from Qingnian Lake, APR – *P. australis* rhizosphere sediments collected from Aiwan Lake, QPR – *P. australis* rhizosphere sediments collected from Qingnian Lake, QTR – *T. orientalis* rhizosphere sediments collected from Qingnian Lake, ATR – *T. orientalis* rhizosphere sediments collected from Aiwan Lake.

microorganism populations are higher in rhizosphere sediments than in non-rhizosphere sediments because the root exudates can provide carbon and energy for rhizosphere microorganisms [12-14]. Root exudates were also chemotactically active on microorganisms [15]. Jenkins and Lion [16] found that bacteria can be considered as a colloid, and mobile colloids in the pore water may enhance the mobility of bacteria from the non-rhizosphere to the rhizosphere. The microbial biomass of the same plant rhizosphere was influenced by plant growth strategy (single or mix). As shown in Table 1, there was only a minor difference in the microbial biomass of *T. orientalis* rhizosphere because of the similar growth condition (plant height) in the two lakes. When the two plants grew together, the growth of *P. australis* was significantly inhibited, and significantly lower microbial biomass was assayed than that under the separated growing condition.

PLFA analysis reveals the structural characteristics of a living microbial community at the time of sampling and is suitable for detecting rapid changes in microbial communities. The total microbial biomass was significantly higher in the *T. orientalis* rhizosphere than in the *P. australis* rhizosphere in both lakes. The total microbial biomass in the *P. australis* rhizosphere was obviously higher than that in the non-rhizosphere when *P. australis* and *T. orientalis* grew separately, while similar to that in the non-rhizosphere when grown together. Cluster analysis showed that the microbial community structure in *P. australis* rhizosphere sediments was similar to the non-rhizosphere sediments, but obviously different from that in *T. orientalis* rhizosphere sediments. These suggest that a change in the microbial biomass was not always accompanied by a change in the microbial community structure, and the microbial community structure was more significantly influenced by plant species than by growth strategy. This is consistent with previous findings [17, 18].

In this study, Shannon index was higher in rhizosphere sediments than in non-rhizosphere sediments from the two lakes, showing that root exudates not only affected the population of rhizosphere microorganisms but also affected microbial community diversity.

## Conclusions

The microbial biomass was higher in the plant rhizosphere than that in the non-rhizosphere, except *P. australis* rhizosphere in Aiwan Lake, and was significantly higher in *T. orientalis* rhizosphere sediments than that in *P. australis* rhizosphere sediments in both lakes. The microbial biomass of the same plant rhizosphere was influenced by plant growth strategy (single or mix). As for the similar growth conditions (plant height) in the two lakes, there were only minor differences in the microbial biomass of the *T. orientalis* rhizosphere. When the two plants grew together, the growth of *P. australis* was significantly inhibited, and significantly lower microbial biomass was assayed than that under the separated growing condition. The Shannon index in the rhizosphere sediments was higher than that in the non-

rhizosphere sediments from the two lakes, and was higher in the *T. orientalis* rhizosphere than in the *P. australis* rhizosphere. The microbial community structure was more significantly influenced by plant species than by growth strategy.

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