

Variations in rhizosphere microbial community structure of bulrush in Wusong estuarine wetland, Shanghai

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Abstract: In this work, variations in rhizosphere microbial community in Wusong estuarine wetland were studied under simulated conditions through phospholipid fatty acids (PLFAs) analysis. Native plant species, bulrush (*Scirpus tripueter*) was tested in the study. The results indicated the root effect caused a shift in the microbial community structure to more fungi, more GP bacteria and more aerobic bacteria. Total PLFAs to change with seasons with the greater biomass size occurring in summer, and the total PLFAs decreased in later summer. Season is the dominant factor influencing the size of soil microbial biomass and biomass is higher in early summer and early winter. The results illustrated that bulrush and the rhizosphere microorganisms could be appropriately matched to cure and restore the ecological environment similar to Wusong estuary wetland contaminated by petroleum pollutants.

Introduction

The wetland ecological environment near Huangpu-Yangtze River Estuary is deteriorating more and more seriously due to oil spills [1]. Diesel is the main pollutant in Huangpu River sediments. Phytoremediation of organic pollutants depends on plant-microbe interactions in the rhizosphere [2]. The content of TPH in the soils from the estuary could be extremely high when oil spill occurred (10000–20000 mg/kg). Thus native plants are strong phytoremediation ability of oil pollutants [3].

Previous studies have analyzed the differences in microbial community structure between rhizosphere and bulk soil by means of phospholipid fatty acid profiles or by molecular techniques [4]. And how changes in exudate patterns, which result from a variety of environmental stimuli, affect the rhizosphere microbiota has been studied in terms of the quality and quantity of root exudation, soil type, plant age, root herbivory and plant species interaction[5-7]. But only few studies characterized the composition and activity of the soil microbiota along plant growth cycle in estuarine wetland. Thus, in the present study, native plant bulrush (*Scirpus tripueter*) was studied, in order to study the variations of rhizosphere micro-environment during the growth cycle of native plants.

Methods and Materials

Soil samples

Soils were sampled from Wusong estuarine wetland from March to October in 2011. Wusong estuarine wetland is located in the Huangpu-Yangtze River estuary, Shanghai, China. The climate is

subtropical monsoon climate, monsoon season is from June to October. Bulrush (*Scirpus tripueter*) is one of the major plants in Wusong estuarine wetland. It occupies a major portion (about 50%) of the study site. Core samples (5cm depth) adhering to roots were collected as rhizosphere soils. The soils from the tidal flat where no plants were collected as unplanted soils. Samples were taken to the laboratory in cool boxes with ice bags. Roots and stones were removed before the soil samples were freeze-dried and sieved (2mm mesh size). However, it was impossible to remove all the roots from the soil. The samples were stored at -80 °C.

PLFA Analyses

Microbial biomass of the samples was assessed by the total PLFA (phospholipid fatty acid). Measurements of soil PLFAs were performed as described by Bligh and Dyer[8].

Statistical analyses

Data were analyzed by ANOVA, with the type of sample as the main factors. Significant differences in the main effects were further analyzed by paired comparisons, with the Duncan's multiple range tests. ANOVA was conducted using the software packages SPSS 17.0.

Results and Discussion

PLFA diversity

38 PLFAs were detected from the bulrush rhizosphere samples and 30 PLFAs from unplanted soil samples. 6 PLFAs (i14:0, 14:0, i15:0, 16:1 ω 7, 16:0, 18:0) were common to all sampling times. 13 PLFAs (i13:0, 13:0, 16:1 ω 5, i17:1 ω 5, 18:3 ω 5,7,9, 18:2 ω 9,12, 18:1 ω 5, 10Me18:0, 19:1 ω 7, 20:4 ω 6,9,12,15, 20:5 ω 3,6,9,12,15, 20:0 and 23:0) were only detected in bulrush rhizosphere samples, 5 PLFAs (15:1 ω 7, 17:1 ω 9, 18:2 ω 3,6, 19:1 ω 10 and 26:0) were only detected in unplanted soils, respectively. The relative abundance of the diversity index was presented in Fig.1.

It was obvious that the diversity index in soil samples was higher in March and August, lower in April and May, and increased continuously from April to August. In March and April, there was no significant difference between rhizosphere and unplanted soils ($P < 0.05$). After April, then index in rhizosphere soils was significantly higher than unplanted soils ($P < 0.05$) in most months. The index was presenting down after August, while it was also higher in rhizosphere soils and reached 2.33 in October. After May, the index in rhizosphere soils was stable, there was no significant difference among June, August and September ($P < 0.05$).

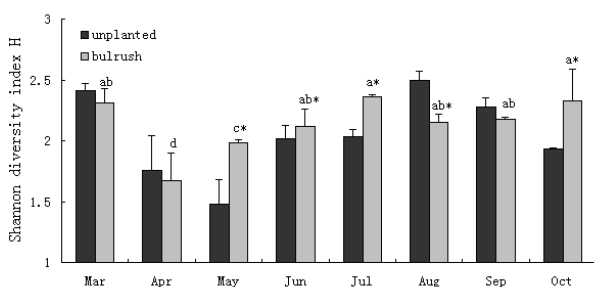


Fig.1 The diversity index in rhizosphere and unplanted soils

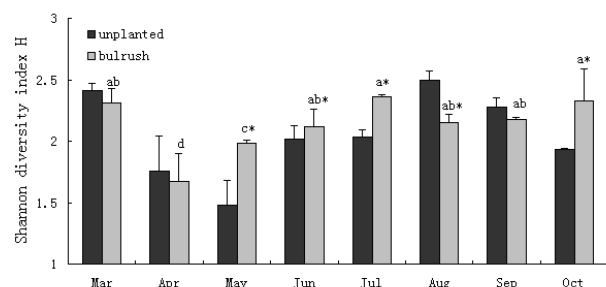


Fig.2 The microbial biomass in rhizosphere and unplanted soils

Microbial biomass

Microbial biomass in terms of total PLFAs was presented in Fig.2. It was obvious that microbial biomass in rhizosphere soils was significantly higher than unplanted soils ($P < 0.05$). Microbial biomass increased linearly from March to May, in May it reached 16.94, twice in March and three times unplanted soils in May, while microbial biomass decreased over time after June. Variation in unplanted soils was not as significant as rhizosphere, but there was no significant difference between rhizosphere and unplanted soils in August, September and October.

Our work also showed total PLFAs to change with seasons with the greater biomass size occurring in summer, and the total PLFAs decreased in later summer. On the other hand, there was no difference between the trend of rhizosphere and unplanted soils. These results were similar to many studies which suggest that season is the dominant factor influencing the size of soil microbial biomass and biomass is higher in early summer and early winter [9].

Microbial community structure

The fatty acids 15:0, 16:0, 17:0, i15:0, i16:0, i17:0, a15:0, a16:0, a17:0, 16:1 ω 7, 18:1 ω 7, 18:1 ω 9, 18:1 ω 10, cy17:0 and cy19:0 were chosen to represent bacterial PLFAs. The polyunsaturated PLFA 18:2 ω 3,6 and 18:2 ω 6,9 were used as a fungal biomarker. The monounsaturated and cyclopropane PLFAs 16:1 ω 7, 17:1 ω 7, 18:1 ω 7, 18:1 ω 9, 18:1 ω 10, cy17:0, cy19:0 were chosen to represent Gram-negative (GN) bacteria. The branched, saturated PLFAs i15:0, i16:0, i17:0, a15:0, a16:0, a17:0 were chosen to represent Gram-positive (GP) bacteria. The ratio between monounsaturated and branched PLFAs was chosen to represent aerobic/anaerobic bacteria ratio. The fungi/bacteria ratio, GP/GN ratio and aerobic/anaerobic ratio were presented in Fig 3-5.

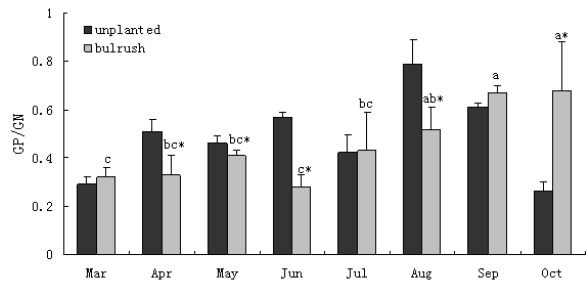
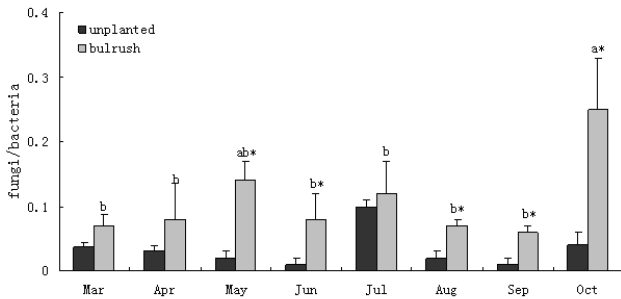


Fig.3 The fungi/bacteria ratio of rhizosphere and unplanted samples

Fig.4 The GP/GN ratio of rhizosphere and unplanted samples

The analyses of variance of the biomarker PLFAs in rhizosphere and unplanted soils indicated that there was a significant difference in community composition between them. It was obvious that the fungi/bacteria ratio was significantly higher in rhizosphere soils than unplanted soils ($P < 0.05$). It was over 3-5 times higher than unplanted soils in most months. The variance of fungi/bacteria ratio in rhizosphere soils was similar to total PLFAs. In October, the ratio reached 0.25, over twice other months.

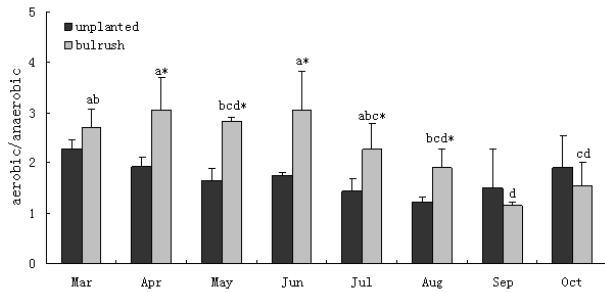


Fig.5 The aerobic/anaerobic ratio of rhizosphere and unplanted samples

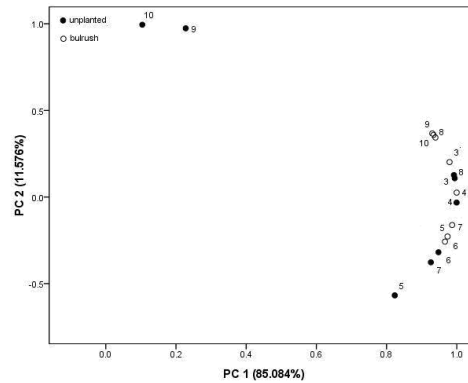


Fig.6 Principle components analysis of PLFAs of rhizosphere and unplanted soils

On the other hand, the GP/GN ratio was higher in unplanted soils than rhizosphere soils, indicating more GN bacteria in rhizosphere soils. But during the whole growth period, the GP/GN ratio was significantly increased over time in rhizosphere soils ($P < 0.05$). It reached 0.68 in October, about twice higher than unplanted soils.

Oppositely, the aerobic bacteria were more common in rhizosphere soils. The aerobic/anaerobic ratio was significantly higher in rhizosphere ($P < 0.05$). In June, it reached 3.06, but it was presenting down since June, and only 1.15 in September, lower than unplanted soils. The ratio in unplanted soils decreased continuously from March to August and then increased.

The PLFA concentrations (mol%) in the 16 soil samples (2 replicates per soil sample) were included in the cluster and principal component analysis. PCA based on PLFA data showed that the exposure to diesel had a distinct soil microbial community composition (Fig.6).

The PC1 of the PLFA data accounted for 85.084% of the variance and the PC2 accounted for 11.576% of the variance. The 16 soil samples were separated into two groups mainly along the PC1. The unplanted soils in August and October were clustered together and had lower scores on the PC1 compared with other soil samples. The rhizosphere soils were separated in 3 groups mainly along the PC2. The rhizosphere soils in August, September and October had higher scores on the PC2, and the soils in May, June and July had lower scores. It indicated that there was a systematic change in the PLFA composition pattern associated with the growth of plants. Difference between rhizosphere and unplanted soils in September and October was more significant than other months.

Conclusion

The microbial community structure in the rhizosphere of bulrush was absolutely different with unplanted soils. Bulrush in study site was mycorrhizal, and the root effect shifted a more GP and aerobic bacterial community. Some earlier studies showed that OC (oxygen content) is a very important factor in the biodegradation of petroleum hydrocarbon. During the degradation process, the production rate of carbon dioxide under aerobic condition was several orders higher than anaerobic condition. Furthermore, bulrush was the dominant plants in Wusong estuarine wetland. The results illustrated that bulrush and the rhizosphere microorganisms could be appropriately matched to cure and restore the ecological environment similar to Wusong estuary wetland contaminated by petroleum pollutants.

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