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Changes in soil microbial biomass and community composition in coastal wetlands affected by restoration projects in a Chinese delta

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ABSTRACT

Severely degraded wetlands due to the lack of freshwater were restored by reintroducing freshwater through the placement of artificial dikes and channels in the Yellow River Delta (YRD) of China. Many studies have evaluated the performances of this restoration projects in aspects of vegetation, soils, hydrology, wildlife, etc. In contrast, limited information was available on the effects of restoration projects on the soil microbial biomass and community composition. To better understand the wetland restoration, differences in soil microbial biomass and community composition between restored tidal wetlands and unrestored wetlands under the disturbance of petroleum exploitation activities or tidal intrusion were assessed. Surface soils (0–10 cm) under three different plant covers (*Phragmites australis*, *Suaeda salsa* and bare land) were collected from each three wetland zones. Chloroform fumigation-extraction (CFE) method and phospholipid fatty acid (PLFA) analysis were used to characterize soil microbial biomass and community composition. Our results showed significantly elevated microbial biomass in soils of the restoration zone, as indicated by both microbial biomass carbon and total PLFAs. Soil microbial community composition in the restoration zone also differed significantly from those in the petroleum exploitation zone and tidal zone. The freshwater input of restoration projects induced a soil microbial community shift to the increased relative abundance of fungi and decreased relative abundance of *Desulfovibrio* bacteria. The significantly promoted fungi in soils under *P. australis* of the restoration zone reflected the necessity of decomposers of the increased surface plant residues after restoration, which in turn contributed to the organic residues accumulation in soils and large aggregates formation by fungal hyphae. Meanwhile the modified soil carbon pool and aggregate structure following restoration of degraded wetland may favor the colonization of soil organisms. These results indicated that freshwater input had strongly altered soil microbial communities in the restored wetland, which may be of great significance in understanding the soil microbial responses to the restoration projects and the underlying mechanisms of wetland restoration.

1. Introduction

Characterized as the youngest and the most extensive new-born wetland ecosystem in China's warm-temperate zone, Yellow River Delta (YRD) was formed of tons of sediment carried by the Yellow River (Bai et al., 2016). In addition to sediment deposition, water supply from the Yellow River was of great significance in maintaining the coastal estuary wetlands of the delta (Li et al., 2009). However, the original hydrologic connections, between the river and wetlands were seriously destroyed recently due to the decreasing runoff of the Yellow River and road constructions, for convenience of petroleum exploitation. Combined with the adverse environmental conditions, such as low precipitation, high soil evaporation and seawater intrusion, human-induced stresses had caused severe soil salinization and wetland degradation (Zhang and Sun, 2005; Cui et al., 2009). To alleviate wetland degradation, flow-sediment regulation had been operated at the Xiaolangdi dam (in the middle stream) to provide sufficient water for downstream wetlands since 2002 (Wang et al., 2016). Simultaneously, wetland restoration projects including digging artificial dikes and channels and delivering the Yellow River water to the degraded wetlands in the wet season were implemented since 2002.

It had been emphasized by many studies the importance of evaluating the success of a wetland restoration project (Kentula, 2000; Theiling et al., 2015; Zhao et al., 2016a). To monitor and assess the effects of wetland restoration projects in YRD, changes of water (Cui et al., 2009), soils (Cui et al., 2009; Wang et al., 2011; Guan et al., 2013; Gao et al., 2014; Yao et al., 2015; Bai et al., 2015; Zhao et al., 2016b; Xiao et al., 2016), vegetation (Cui et al., 2009; Wang et al., 2011; Wang et al., 2012), waterbirds (Cui et al., 2009; Hua et al., 2010; Li et al., 2011) and macrobenthos (Li et al., 2015) had been studied. Among those studies on soils, reduction of soil salinity and accumulation of soil organic carbon were widely

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documented (Cui et al., 2009; Wang et al., 2011). But some adverse effects such as possible heavy metal input along with freshwater were also mentioned (Bai et al., 2015). Additionally, Li et al. (2015) pointed out that the current restoration strategy of reintroducing freshwater primarily focused on the reestablishment of freshwater vegetation, there still were some other aquatic organisms such as macrobenthos did not fully recover in the restored areas. However, monitoring and assessing work should focus not just on these aspects, but also on soil microbes (Sims et al., 2013).

Assessing microbial responses to the restoration projects had been widely put into practice in recent years. Plassart et al. (2008) evaluated the molecular and functional responses of soil microbial communities to the grassland restoration practices in northern France, and found increased total microbial biomass, fungal and bacterial populations in restored meadows, indicating the positive impact of grassland restoration in maintaining the soil status. Martucci do Couto et al. (2016) studied soil microbial changes in restored forests and found significantly higher basal respiration and soil microbial biomass in mixed species reforestation, which had a guiding significance in choosing the forest restoration measures. Wang et al. (2015) investigated the variations of two soil enzyme activities after restoration by completely removing the invasive plants and associated soils in the Florida Everglades and the results showed that the restored soils developed toward the soils in the natural wetlands with time since restoration. As can be seen, using microbial indicators to assess the restoration projects had become an indispensable tool in many districts or ecosystems, but few in the coastal wetlands of China. Moreover, the restoration projects implemented in YRD covered 5023.7 ha coastal wetlands and had pumped 3 million m^3 freshwater to this area each year (Cui et al., 2009), which were far different from those restoration projects conducted in other areas or ecosystems. Therefore, it was necessary to bring soil microbial indicators into consideration in fully assessing the performance of ecological projects in YRD.

Among quantities of microbial indicators having been applied into soil studies, microbial biomass and microbial community composition were two indicators proved to be very sensitive, responsive and relatively convenient. Although accounting for a relatively small pool of nutrients and soil organic matter, soil microbial biomass had been recently reported to respond significantly to the altered aboveground plant productivity (Chen et al., 2015; Deng et al., 2016), soil physical-chemical properties, such as soil texture (Wu et al., 2013), moisture (Poret-Peterson et al., 2007), nutrient contents (Bai et al., 2014; Huang et al., 2016), temperature (Zhang et al., 2015) and so on. Furthermore, as an early indicator of trends, soil microbial community composition was documented to be sensitive to the physical and chemical variations of the soil environment induced by natural and anthropogenic disturbances in many studies (Card and Quideau, 2010; Yang and Zhang, 2014; Deng et al., 2016). Hence, it could be reasonably supposed that the changing soil status and processes induced by petroleum exploitation, restoration measures and salt water intrusion in YRD would be expressed by the differential soil microbial biomass and microbial community composition in different sites concerned. And the potential causes/influences of increased soil organic carbon and other improved soil physical-chemical properties of/on specific microbial groups in restored wetland soils may be expressed by the microbial indicators.

Previous studies involving soil microbes in YRD mainly focused on the variations of microbial community structure in saline-alkali soils and discussed the effects of plant community structure and succession on soil microbes (Liu et al., 2007; Wang et al., 2010; Yu et al., 2012; Cao et al., 2014). Nevertheless, the responses of soil microbes to wetland restoration in YRD had been rarely reported. The overall objectives of this study were to (1) examine the microbial biomass and community composition in restored wetland soils and compare with those in soils of the other two typical zones in YRD—petroleum exploitation zone and tidal marsh zone to evaluate the ecological performances of restoration projects in the aspect of soil microbes; (2) establish their relationship with environmental factors through multivariate statistical analysis to indicate the key factors affecting soil microbial community. Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial community composition as it was a powerful technique to indicate the presence of specific microbial groups (Frostegård et al., 2011). In addition to total PLFAs, chloroform fumigation extraction (CFE) was used to give reliable results for soil microbial biomass (Bailey et al., 2002; Leckie et al., 2004).

2. Materials and methods

2.1. Study area

The study area was located in the YRD (N37°45′ 57.8″, E119°11′ 15.2″) of the eastern part of Shandong Province on the southern bank of the Bohai Gulf. It had a warm temperate monsoon climate, with the annual average precipitation of 551.6 mm and the annual average air temperature of 12.4 °C (Zhao et al., 2016b). The soil in this region was typical Fluvisols developed on the loess material of the Quaternary period, and was carried by the Yellow River water from the Loess Plateau (Bai et al., 2015). The dominant plant species were *Phragmites australis* and *Suaeda salsa* (Yao et al., 2015).

2.2. Soil collection and analysis

Study sites were chosen from three typical zones in YRD: restoration zone (Zone R), petroleum exploitation zone (Zone P), and tidal zone (Zone T) in this study. The sampling sites (37° 44′ 38″ N, 119° 07′ 52″ E) in Zone R with dense vegetation (e.g., *P. australis* and *S. salsa*) were located beside the artificial ditch constructed for inputting freshwater, and sites (37° 45′ 31″ N, 119° 11′ 23″ E) in Zone P were located on the supratidal zone, where *P. australis* and *S. salsa* were sparsely distributed and there were oil derricks nearby. The sites (37° 44′ 29″ N, 119° 11′ 33″ E) in Zone T received both freshwater and tidal flows, and soils under *P. australis* in this zone were waterlogged with 0–2 cm overlying water while the soils under *S. salsa* in this zone were dry and salinized with precipitated white salt on the soil surface.

In each zone, wetland soils from *P. australis* (p), *S. salsa* (s) communities and bare land (b) were collected with triplicates. Each triplicate was a five-multi-point mixed surface (0–10 cm) soil sample and was named after its belonging zone plus its land covers and a number. For example, Rp1, Ts2 and Pb3 meant the first, second or third soil sample collected from *P. australis*, *S. salsa* communities or bare land of the restoration zone, tidal zone, or petroleum exploitation zone, respectively. Thus, total 27 soil samples were collected in May 22 of 2014 and each soil sample was placed in polyethylene bags and then brought to the laboratory using cooler box filled with ice. Before soil analysis, all visible roots, litter materials and macrofauna were removed and each sample was divided into three parts. One part was used to determine the physical-chemical properties of the soils; another one was sieved through 2 mm, and stored at 4 °C for the measurement of microbial biomass within a week; and the last part was freeze-dried and preserved in refrigerator at −80 °C for determination of the content of PLFAs in soil.

The fresh soils were oven dried at 105 °C for 24 h and weighed for calculating soil moisture. Soil organic carbon (SOC) was measured using dried and sieved (<0.149 mm) soils with dichromate oxidation method (Anderson and Domsch, 1989). Soil pH was measured using a HANNA pH meter (Hanna Instruments, Woonsocket, RI, USA) (soil:water = 1:5). Electric conductivity (EC) was determined in the supernatant of 1:5 soil-water mixtures using an EC meter (VWR Scientific, West Chester, PA, USA). The classification of soil aggregates was determined using the wet sieving method. Each soil sample was finally separated into three fractions: macroaggregates (>0.25 mm), microaggregates (0.053–0.25 mm) and silt and clay fractions (<0.053 mm) (Aye et al., 2016; Hontoria et al., 2016). Soil aggregates retrieved at each sieve were carefully backwashed into beakers, oven-