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Comparison of phosphorus fractions and phosphatase activities in coastal wetland soils along vegetation zones of Yancheng National Nature Reserve, China

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ABSTRACT

Phosphorus (P) fractions and phosphatase activities were measured in 22 coastal wetland soils with typical vegetation successions in Yancheng National Nature Reserve, China. P forms and phosphatase activities varied greatly from site to site even under the same vegetation cover. NH_4Cl–P, bicarbonate/dithionite extracted P and NaOH–P were remarkably higher ($p < 0.05$) in soils with exotic invasive plants, *Spartina alterniflora*, than in soils with the native species *Suaeda salsa*, *Scirpus mariquete* and *Phragmites australis*. HCl–P and refractory P showed little variation. No significant differences were detected for either alkaline phosphatase (ALAP) or acid phosphatase (ACAP) among the soils. All of the above properties were much higher in soils with plant growth compared to bare flat soils. Regression analysis demonstrated that organic matter (OM), Al, Ca, Fe and total P (TP) were able to explain more than 70% of the variations in the P fractions (except 29% of NH_4Cl–P), and OM was the most important contributing factor. ALAP and ACAP were irrelevant to P but were significantly related to TOC, suggesting that carbon was a limiting factor for P mineralization in this area. Owing to its huge biomass and densities, *Spartina alterniflora* displayed great potential for carbon input, thus facilitating P mineralization and cycling. The results enhance our understanding of P availability differences in this area covered by invasive and native vegetation.

1. Introduction

Coastal wetland ecosystems are important not only to maintain plant and animal life but also to sustain standards of human living. Soils are important components of coastal wetlands, which serve as the most basic substrate to support biological activities and productivities. Soil nutrients are generally limited and unevenly distributed in the coastal marsh. Therefore, plant survival and succession will crucially depend on a plant's ability to acquire nutrients from the soil or the water. Inversely, plant species could affect the nutrient status in soils through foliar litter decay, root exudates and microbe function (Hobbie, 1992).

It has been well established that nitrogen is a major element limiting the plant community in a salt marsh (Ryan and Boyer, 2012). However, Sundareshwar et al. (2003) noted that phosphorus (P) limitation in the bacterial community would gradually result in P limitation in coastal wetlands due to a decrease in the activities of nitrogen-transforming bacteria. In addition, visible biomass differences among plant species would inevitably link to different ability in P utilization efficiency. It is essential to know how plant types affect the soil status of P, which in turn affects its potential availability. Sequentially extracted P forms in soils, as reviewed by Condron and Newman (2011), provide a methodology to examine P that allows better understanding of P cycling and dynamics in the soil-plant system. In coastal wetlands, the lowland soils are flooded periodically with brackish water, and highland soils may be covered by fresh water of variable depth. Therefore, the redox potential of the top soils varies greatly during flood and drying conditions (Keller et al., 2013). It is well known that iron- and manganese-bound P is sensitive to redox changes. The choice of the P fractionation method should take this point into account (Coelho et al., 2004).

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Yancheng National Nature Reserve (YNNR), established in 1983, is located on the east coast of Jiangsu Province, China. Its aim is to protect the biodiversity and habitat for migrating birds. The YNNR wetland has a typical plant species landward succession: (1) *Spartina alterniflora* (*S. alterniflora*) dominates the elevated part of the intertidal zones; (2) *Suaeda salsa* (*S. salsa*) dominates the high tide zones; and (3) *Phragmites australis* (*P. australis*) is the dominant species in the supratidal zone with other prevalent communities, such as *Scirpus mariquete (S. mariquete*), *Aeluropus littoralis*, *Imperata cylindrical* and *Zoysiam jacrostachys*. *S. salsa* and *P. australis* are the native vegetation types of YNNR (Ke et al., 2011). *S. alterniflora*, the pioneer species to grow on newly alluvial soils, was first introduced to the coastline from the USA in the early 1980s. It has been an invasive plant that shows obviously higher productivity than the native species. Therefore, YNNR provides an ideal region to study P geochemistry behaviors in coastal soils.

In soils, mineral orthophosphate (PO_4^{3-}) is the sole form of P assimilated by microorganisms and plants. However, organic P generally accounts for at least 30% and occasionally up to 80% of the total phosphorus (TP) (Richardson et al., 2005). Organic P is mineralized to PO_4^{3-} so that it can be easily assimilated by higher plants. This process is catalyzed by phosphatase enzymes, which are mostly found in soil and litter microorganisms, plant roots and in extracellular forms in soil (Criquet et al., 2004). Carbon inputs to the soil are reported to be a driver of phosphatase enzyme production in freshwater wetlands (Wang et al., 2010). In YNNR, we want to clarify whether the plant types induce differences in the phosphatase enzyme activities due to the marked differences in aboveground biomass (carbon sources) among the plant species.

Therefore, the objectives of this study are (1) to quantify the distribution of P sediment fractions at representative coastal wetland sites under typical vegetation; (2) to evaluate the response of soil phosphatase enzymes to different plant-soil systems.

2. Materials and methods

2.1. Study site

YNNR spans the five counties from 32°20′N to 34°37′ N and from 119°29′E to 120°16′E. The total area is 453 thousand ha with a 582-km long coastline. The elevation of YNNR ranges from 0 to 4 m, with an average slope of less than 5°. The annual average temperature is 14.5 °C, and the annual precipitation is 980–1070 mm. The northern part of YNNR is frequently eroded by sea waves; thus the north end is narrow. The central and southern parts of YNNR belong to the depositional tidal flat. It is estimated that approximately 900 ha of land area is formed by silt supply and deposition at the coastal edge of YNNR.

2.2. Sampling and analysis

Sampling was conducted in the Xinyanggang estuarine wetland, a core part of YNNR (Fig. S1), in July 2012, during the diurnal ebb-tide. Twenty-two sites were selected along the vegetation succession, including 5 from the *Spartina alterniflora* salt marsh (Sampling No. 1–5), 5 from the *Suaeda salsa* salt marsh (Sampling No. 6–10), 3 from the *Scirpus mariquete* oligohaline wetland (Sampling No. 11–13), 8 from the *Phragmites australis* oligohaline wetland (Sampling No. 14–21), and 1 the from the bare flat as background (Sampling No. 22). The bare flat was closest to the sea. The *S. alterniflora* community in the sample transects have been occupying the bare flat for ten years (Yang et al., 2013). This meant that the *S. alterniflora* closer to the bare flat were the latest invaded community. Subsequent vegetation type succession in the reserve from seaward to upland included the *S. salsa*, *S. mariquete* and *P. australis* communities, which were the native species growing at higher elevations. Surface soils (0–20 cm) were collected using stainless steel shovels and stored in ziplock bags. The soil samples were air-dried at room temperature and were ground through a 2-mm sieve for further analysis. The soil pH of was measured using a pH electrode meter (pHS-3, Leici, Shanghai). Soil electrical conductivity (EC) was determined using a conductivity meter (DDS370, Leici, Shanghai). Soil organic carbon (C) (g kg^{-1} dry soil) was determined using the dichromate digestion method (Kalembasa and Jenkinson, 1973). Total nitrogen (TN) was determined using the method described in Sparks et al. (1996). The Olsen P was extracted using Olsen method (1954). Carbonate was determined using HCl titration method. Total aluminum (Al), calcium (Ca) and iron (Fe) were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Leeman Labs, Profile DV) after digestion. All these analysis were run in triplicate and the coefficients of variation were within 8%.

2.3. P fractionation and phosphatase activities

The P fractionation procedure used in this study principally follows the 5-step procedure developed originally by Psenner et al. (1984) and modified by Hupfer et al. (1995). In the first step, dried soil was extracted with 1.0 M NH_4Cl (pH 7) (NH_4Cl–P). In the second step, the residues were extracted by 0.1 M bicarbonate/dithionite (BD-P). In the third step, 1 M NaOH was added to the residue and extracted for 16 h. One sub-sample was determined as NaOH-extracted total P (NaOH-TP) by autoclaved method. Another sub-sample was detected as NaOH-extracted reactive P (NaOH-RP) after acidified and precipitated. The non-reactive P (NaOH-NRP) was calculated indirectly by the differences of NaOH-TP minus NaOH-RP. In the fourth step, 0.5 M HCl was added to the residue and was extracted to measure the P concentration (HCl–P). In the fifth step, the residue was dried in an oven at 105 °C. Then the dried residue was digested using H_2SO_4 and $HClO_4$ (Res-P). All of the P was determined using spectrophotometric method. We have compared the sum of all P fractions with the TP determined by $H_2SO_4/HClO_4$ digestion method in 5 of samples. The results showed that the recoveries ranged from 92% to 104% (data not list in the paper). So, Total P (TP) was computed based on the sum of the above-mentioned five fractions (Romero-González et al., 2001). Four replicates were carried out throughout these procedures to guarantee precision and further statistical analysis.

The alkaline phosphatase activity (ALPA) and Acid phosphatase activity (ACPA) assay was utilized as described by Eivazi and Tabatabai (1977). All samples were run in triplicate.

2.4. Statistical analysis

Soil P fractions and phosphatase activities within the same vegetation were analyzed using one-way analyses of variance (ANOVA, site differences) by post hoc Tukey's tests ($p < 0.05$). Stepwise multiple regression analyses were conducted using OM, Fe, Al, Ca and TP as independent variables and using P fractions as dependent variables. Due to multicollinearity between Fe and Al ($R^2 = 0.97$), we introduced ridge regression if both Fe and Al significantly contributed to the dependent variable. All statistical methods were analyzed using SPSS 19.0.

3. Results

3.1. Basic properties of the test soils

Soil characteristics are shown in Table 1. pH values varied over a narrow range (8.5–9.6). Large variations in EC were observed in