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Biogeochemical drivers of phosphatase activity in salt marsh sediments

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ABSTRACT

Although nitrogen has become a major concern for wetlands scientists dealing with eutrophication problems, phosphorous represents another key element, and consequently its biogeochemical cycling has a crucial role in eutrophication processes. Microbial communities are a central component in trophic dynamics and biogeochemical processes on coastal systems, since most of the processes in sediments are microbial-mediated due to enzymatic action, including the mineralization of organic phosphorus carried out by acid phosphatase activity. In the present work, the authors investigate the biogeochemical sediment drivers that control phosphatase activities. Authors also aim to assess biogeochemical factors' influence on the enzyme-mediated phosphorous cycling processes in salt marshes. Plant rhizosediments and bare sediments were collected and biogeochemical features, including phosphatase activities, inorganic and organic phosphorus contents, humic acids content and pH, were assessed. Acid phosphatase was found to give the highest contribution for total phosphatase activity among the three pH-isoforms present in salt marsh sediments, favored by acid pH in colonized sediments. Humic acids also appear to have an important role inhibiting phosphatase activity. A clear relation of phosphatase activity and inorganic phosphorous was also found. The data presented reinforces the role of phosphatase in phosphorous cycling.

1. Introduction

Salt marshes are highly productive areas located in the interface between freshwater and marine systems, often subjected to high nutrient loadings due to anthropogenic activity that can lead to systems eutrophication (Tobias et al., 2001). Salt marshes have been regarded as intertidal wastelands, but currently their important role in coastal defense, as a biodiversity pool, as nursing, shelter and feeding areas for several animals and as a source of organic materials and nutrients for marine communities is widely recognized (Boorman, 1999).

Another important function of salt marshes is their role as a sink of pollutants like heavy metals or excess nutrients. Conversely, salt marshes could also become a source of pollutants due to exportation of dead biomass, although the amount of contaminants exported is lower than the amount of contaminants retained (Caçador et al., 2009; Duarte et al., 2008).

Excess nutrients such as nitrogen (N) and phosphorus (P) can have damaging effects, leading to eutrophication of coastal waters (Andrieux-Loyer et al., 2008; Howarth et al., 2011). Although nitrogen is considered to be a major concern when dealing with eutrophication, phosphorus is also considered to be one of the key limiting nutrients to primary productivity and therefore it is one of the nutrients responsible for eutrophication (Correl, 1998; Paerl, 2009). Phosphorous is delivered to aquatic systems in several forms such as longer chain poly-phosphates, pyrophosphates, organic phosphate esters and phosphodiesters, and organic phosphonates. This element may also be delivered both in the dissolved and particulated form being deposited in the salt marsh sediments (Correl, 1998). However, P is only biologically available when it is in the inorganic form, as orthophosphate.

Sediment microbial communities are an essential component in trophic dynamics and biogeochemical processes in coastal ecosystems. The microbial community synthesizes extracellular enzymes that are responsible for the decomposition processes. Phosphatases are extracellular hydrolases that catalyze the mineralization of organic phosphorus into inorganic and more easily metabolized forms of phosphorus. One large and important group of these enzymes are the phosphomonoesterases and their pH-isoforms: alkaline, acid and neutral phosphatases, being therefore active in several kinds of sediments (Alef et al., 1998).

Although phosphatases are not exclusively produced by the microflora (being also produced as exudates by plant roots), microbial growth is favored by salt marsh plants that release oxygen from the root system into the sediments promoting reactions with reduced species (Caetano et al., 2011; Lillebø et al., 2006). In fact, *Spartina maritima* marshes have been shown to have an important role on phosphorus bioavailability decreasing total P, probably due to uptake for growth purposes (Lillebø et al., 2007). Also, due to the release of oxygen by plant root systems into the sediment, P efflux is reduced (Lillebø et al., 2007). Plant roots can also affect microbial communities due to root exudates input to the rhizosphere (Hartman et al., 2009). Plant type has also been shown to alter microbial community structure and function affecting microbial activity (Garbeva et al., 2008).

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Due to the elevation gradient from the upland marsh to the lower mudflats, a physic-chemical gradient is generated allowing the colonization by different halophytic species (*Halimione portulacoides*, *Sarcocornia fruticosa*, *Sarcocornia perennis* and *S. maritima*) providing differential cycling areas along the marsh. This way, in the present work the authors aim to clarify the influence of the biogeochemical marsh environment on the activity extracellular phosphatases and thus how this modulates the phosphorous recycling in marsh sediments.

2. Materials and methods

2.1. Site description and sampling

Rosário (38°40′N, 9°01′W) is a mature salt marsh located in the southern part of the Tagus estuary, in the vicinity of various urbanized and industrialized zones (Valiela et al., 2000). The upper marsh is mainly colonized by *H. portulacoides* (Amaranthaceae) and *S. fruticosa* (Amaranthaceae) and undergoes short submersion episodes during high tide. The middle marsh is colonized mainly by *S. perennis* (Amaranthaceae), which can also be found although in smaller extents in the lower marsh where *S. maritima* (Poaceae) is dominant. Sampling occurred in the end of the growing season (September) as it is the period where the sediment enzymatic activity is highest (Duarte et al., 2008). Three transects were assessed perpendicular to the margin. In each transect five sediment cores were sampled (four in pure stands of each species and one in the bare mudflat below the lower marsh). All the samplings were made during low tide. According to previous studies (Reboreda and Caçador, 2008), the sediment layers between 5 and 10 cm proved to have high extracellular enzymatic activity (EEA). For this reason all analyses were carried out in the sediment samples collected between these depths, and are referred hereafter as rhizosediment. Meteorological data from the period of 2000–2010 was extracted from the National System of Water Resources Information database (www.snirh.pt). During the summer Tagus estuary reaches the highest air temperatures with a maximum temperature of 42.7 °C and an average temperature of 21.8 °C; however the minimum temperature during the summer is 9.3 °C. The lowest average temperature occurs during winter (10.6 °C), that is also the season with the lowest maximum temperature (35 °C), and a minimum temperature of −9.1 °C. During the spring temperatures vary between −5.1 °C and 40.9 °C, with an average temperature of 15.2 °C, while in autumn the temperatures range between −0.1 °C and 42 °C, with an average temperature of 17.4 °C. Winter has the lowest thermal amplitude (21.1 °C), followed by spring (29.9 °C) and autumn (30.7 °C) while spring has the highest thermal amplitude (31.3 °C). Throughout the year average air humidity varies between 63.7% during summer and 80.3% during winter. Spring and autumn had averages of 71.1% and 72.8%, respectively. Average precipitation peaks during autumn and winter with an average of 0.1 mm/day, followed by spring with an average of 0.09 mm/day and the period when there is the lowest precipitation average is the summer when it rains 0.01 mm/day (Duarte et al., 2013a,b).

2.2. Sediment physicochemical characteristics

Sediment pH was measured using a HANNA pH/mV (HI 9025) electrode directly in the sediment. The pH calibration was performed using buffer solutions of pH 4 and pH 7. Organic matter was determined by the loss on ignition (LOI) method by burning 1 g of sediment at 600 °C during 2 h. Sediment water content was determined by drying sediment samples at 60 °C until constant weight. Total and inorganic C and N were determined in air-dried and burned sediment samples, using a CHNS/O analyser (Fisons Instruments Model EA 1108). Organic C and N were determined as the difference between total fraction and inorganic fraction. For phosphorous determinations all lab wares were soaked for two days in HCl (10%) and rinsed with distilled water to avoid contaminations. Inorganic and total phosphorous was extracted according to Ruban et al. (2001) and Ladakis et al. (2006). Briefly, two sub-samples of 200 mg of rhizosediment were used for phosphorous determinations per sample. Sediments were passed through a 2 mm mesh to remove plant and shell detritus. For total phosphorous (TP) analysis one subsample was burned at 450 °C for 3 h and extracted with 20 ml of HCl 3.5 M, overnight (about 16 hours). After this extraction period the slurry was centrifuged at 200 × g for 15 min at 4 °C. The supernatant was frozen in amber glass vials until analysis. A second subsample was used for inorganic phosphorous (IP) extraction with 20 ml of HCl 1 M overnight, after which it was centrifuged as described above for TP. The supernatant was also frozen in amber glass vials until analysis. Phosphorous concentration was measured by the molybdenum-blue colorimetric analysis with a Tecator FIAstar™ 5000 Analyser. Organic phosphorous (OP) was determined as the difference between TP and IP. All phosphorous concentrations were expressed as mg $P–PO_4^{3-}$ per gram sediment dry weight (DW). Humic acids were quantified according to Adani et al. (2006) with some modifications. To extract humic acids, 25 ml of a solution containing 0.1 m NaOH and 0.1 m $Na_4P_2O_7$ was added to 5 g of dried and sieved sediment. The extraction was carried out in an end-to-end shaker for 24 h at 65 °C. Then, the samples were centrifuged at 45,880 ×*g* for 20 min at 4 °C. The supernatant was totally recovered and distilled water was added to the solid residue, which was resuspended and centrifuged again. This operation was repeated until the supernatant was clear. The supernatant solutions were combined and acidified with 50% sulphuric acid to pH < 1.5 to precipitate the humic acids. These were separated by centrifugation as described above, the supernatant completely evaporated at 60 °C until constant weight, and the humic acids weighed.

2.3. Phosphatase activity

All enzymatic determinations were carried out with colorimetric methods and the absorbance was read on a TECAN Absorbance Microplate Reader (SPECTRA Rainbow). Phosphatase activity was assayed according to Ravit et al. (2003) with a modification in the incubation temperature and without dilution of the supernatant. The buffers used were acetate buffer 50 mM (pH 5.0), TRIS buffer 100 mM (pH 8.7) and citrate buffer 50 mM (pH 7.1) respectively for acid, alkaline and neutral phosphatase assays. Briefly, 75 ml of buffer was added to 5 g of fresh sediment, and mixed for 1 min in order to obtain the sediment slurry. Two milliliters of p-nitrophenyl-phosphate 5 mM was added to 2 ml of slurry and incubated at 30 °C with gentle agitation for 30 min. After incubation, samples were centrifuged at 6.530 ×*g* for 15 min, at 4 °C and 0.2 ml of 0.1 N NaOH was added in order to stop the reaction and reveal the p-nitrophenol (pNP) formed. The absorbance of the supernatant was read at 410 nm and compared with the calibration standards for pNP. The phosphatase activity was expressed as μg of pNP released per gram sediment dry weight per hour and normalized for organic carbon content.

2.4. Statistical analysis

Statistical analysis was performed using Statistica Software version 10 from Statasoft Inc. The lack of normality and homogeneity of the data package lead to the application Kruskal–Wallis non-parametrical tests for significance analysis. In order to understand the interaction and the effect of the abiotic factor (sediment physico-chemistry and climate) a principal component analysis (PCA) and a similarity percentage test (SIMPER) were performed using Primer 6 software (Clarke and Gorley, 2006).

3. Results

3.1. Sediment physicochemical characteristics

Bare sediments presented the lowest water, humic acid and organic matter contents, while *S. perennis*, *H. portulacoides* and *S. fruticosa*