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Impact of sediment grain-size and biofilm age on epipelic microphytobenthos resuspension

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ABSTRACT

Intertidal zones are dynamic areas, where tidal currents and wind-induced waves are responsible of resuspension of the sediment and associated microphytobenthos (MPB). Sediment composition (mud–sand mixtures) and MPB biofilm age are two major components involved in resuspension of epipelic microphytobenthos in muddy areas. However, their relative role in resuspension phenomenon must be better understood in controlled conditions. In this study, three mud–sand mixtures (Pure mud M1, 75% mud/25% sand M2 and 50% mud/50% sand M3) were tested with an epipelic MPB biofilm of different ages (3, 6 and 9 days after inoculum) using an erodimeter flume. The biofilm biomass, physiological state, photosynthetic parameters and Extracellular Polymeric Substances (EPS) were surveyed as well as water content and ammonium concentration in the sediment. Chl a content and Suspended Particular Inorganic Matter (SPiM) erodability differed between treatments, biofilm being able to be eroded before sediment when it is well constituted (especially in pure mud M1). Between day 3 and day 9 of culture, biofilm age did significantly affect critical thresholds for Chl a erosion and sediment resuspension for mud–sand mixtures (M2 and M3). Sediment resuspension seemed to be also driven by physical constraints like differential compaction and vertical sand segregation as a function of mud content. Indeed, grain-size was the main factor involved in MPB resuspension phenomenon, with an optimum reached near a equilibrate ratio between mud and sand (50% mud–50% sand). Proteins of the EPS bound fraction (extracted with dowex resin) appeared to have a critical role in the pioneering stages of biofilm installation, allowing its formation in a less favorable environment caused by sand enrichment (mixtures M2 and M3). This effect of bound EPS must be mediated by an increasing cohesion and lowering sediment permeability. Carbohydrate content of the bound EPS fraction was directly related to the sediment (SPiM) erodability, independently from mixture type or biofilm age.

1. Introduction

Macrotidal estuaries are open ecosystems subject to hydrodynamic processes such as wind induced waves and currents generated by tidal rhythm. The stress generated by these physical factors results in a resuspension of the sediment and associated microphytobenthos (de Jonge and van Beusekom, 1995). Microphytobenthic communities inhabiting cohesive sediments are mainly constituted of epipelic benthic microalgae – dominated by diatoms in intertidal mudflats (Smith and Underwood, 1998) – and are able to migrate vertically through the sediment top layer, according to a chronobiological rhythm (Mitbavkar and Anil, 2004). Tidal cycle and light are main factors explaining the migration of epipelic diatoms (Perkins et al., 2001; Blanchard et al., 2004; Mitbavkar and Anil, 2004), migration being mediated by the excretion of carbohydrate-rich heteropolymers called exopolymeric substances (EPS). EPS secretion by epipelic microphytobenthos is under control of abiotic factors such as light (Staats et al., 2000a) and nutrients (Staats et al., 2000b), and there is direct metabolic pathway between photosynthesis and secretion of colloidal EPS (Underwood and Smith, 1998). EPS are also able to stabilize the sediment by limiting the erosion of the latter (Friend et al., 2008; Grant et al., 1986; Holland et al., 1974; Paterson, 1989; Smith and Underwood, 1998, 2001). This microphytobenthos (MPB) biostabilisation of sediment surface is variable upon time, since MPB has its own dynamic and growth cycle. Combination of tidal cycles (McKew et al., 2011), day/night cycles (Cartaxana et al., 2011), biofilm age (Sutherland et al., 1998) and biomass lead to different physiological states of microalgae, thus influencing the sediment erodibility.

The biomass of MPB on intertidal flats is driven by (i) exportation processes such as grazing and resuspension, (ii) factors affecting growth rate and/or health of the MPB such as light, temperature or nutrients and (iii) sediment grain-size, with interaction with both previously mentioned factors (resuspension, nutrient availability). All these factors are drastically regulated by the respective contribution of sand and mud

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proportion (Orvain et al., 2012; Van de Koppel et al., 2001). When factors responsible for MPB losses from sediment (resuspension, grazing) are removed, the growth of the biofilm is known to follow a logistic curve until a maximum value reached at the biotic capacity of the local environment (Blanchard et al., 2001; Orvain et al., 2003a, 2003b). The number of days necessary to reach the biotic capacity differs according to the authors, and has been modeled by Wolf (2007) with an initial lag phase of about 3 days, followed by an exponential growth phase until a pseudo-steady state "mature" phase after approximately 13 days. The physiological state of the biofilm is assumed to change as a function of the biofilm age (Sutherland et al., 1998). Photosynthetic capacity and light use efficiency has been shown to decrease with increasing biofilm development (Morris, 2005; Serôdio et al., 2005), and EPS are more secreted in the late phase of the biofilm development caused by overflow metabolism in case of nutrient limitation (Orvain et al., 2003a, 2003b).

Physical factors are decisive regarding sediment stability against biological ones. Sandy sediments are easily transported by haulage during bed-load transport and exported in the water column during strong hydrodynamic conditions. On the contrary, cohesive sediments resist to erosion but, in the case of harsh conditions such as strong swells, critical thresholds can be transcended leading to significant sediment massive erosion. Numerous experiments focusing on microphytobenthos mediation of sediment erodibility have been done in laboratory conditions, most of the time focusing on homogeneous sandy (De Brouwer et al., 2005; Friend et al., 2008; Lucas, 2003) or muddy sediments (Andersen and Pejrup, 2002; Droppo et al., 2007; Gerbersdorf et al., 2007; Orvain et al., 2004; Spears and Saunders, 2008; Stone et al., 2008; Tolhurst et al., 2003, 2006, 2008; Yallop et al., 2000). However, sand and mud can be intimately mixed in natural intertidal systems, and may exhibit a horizontal gradient, or can be layered in the bed (Le Hir et al., 2011). The mixture behaves mostly like pure sand, but there is a critical mud fraction (typically 30%), above which the mixture behavior is fully cohesive (Le Hir et al., 2011). Below this critical value, the mixture shear strength depends on the relative mud concentration as stated by Migniot (1989) and Waeles et al. (2008). In fact, if physical processes such as local hydrodynamic conditions are responsible for particle grain-size selection, a succession of vertical layers of sediment from different grain-size often occurs in nature. Moreover, biological processes such as bioturbation and sediment reworking can influence the particle mean-size, leading to modify the sediment vertical structure, therefore leading to bulk sediment mixtures (Krantzberg, 1985). As a consequence, intertidal ecosystems are often characterized by mud–sand mixed sediments, with a strong spatial heterogeneity from pure sand to pure mud (Orvain et al., 2012; Ubertini et al., 2012). These mixed sediments must be taken into account in both microphytobenthos development and material export to the water column by erosion processes. Erosion thresholds of sediment mixtures in relation with microbial indices have been studied in situ (Defew et al., 2003; Lelieveld et al., 2003; Ziervogel and Forster, 2006) or by modeling approaches (Le Hir et al., 2011; Paarlberg et al., 2005; Waeles et al., 2008), but rarely in controlled conditions. Van de Koppel et al. (2001) clearly put in evidence the positive effect of mud proportion on the biofilm growth. However, the combined effect of mud–sand proportion and microphytobenthic biofilm age has never been experimented to evaluate the contribution of these 2 factors in the response of sand, mud and chl a erodability.

The objectives of the study were to characterize: 1) the influence of grain-size on a MPB biofilm development within a controlled environment, 2) the tidal currents influence on both epipelic microphytobenthos and sediment resuspension, 3) the relative and interacting effects of sediment grain-size and biofilm age on this phenomenon. In order to do this, mesocosm biofilm cultures were controlled to assess different development stage of the biofilm by regulating emersion-immersion periods under a night and day light cycle.

2. Materials and methods

2.1. Experimental design

We used natural sand and mud sediments respectively coming from a beach located at Luc sur mer and a mudflat located in the Bay of Veys (Basse-Normandie, France). There were taken from the 10 top cm. The 2 stocks of sediment were left outdoors for 1 month in order to remove the bulk of the present MPB. In order to eliminate the macrofauna naturally present, the fresh sediments were sieved with water using a 1 mm mesh size, this mesh size being the minimal size allowing fine sediment to be sieved with the volumes we used. In order to remove most of the organisms ranging from 0.5 to 1 mm, sediments were unused for 1 month. Three cohesive sediment types (Fig. 1) were prepared: one of pure mud (100%, mixture M1) and two mud–sand mixtures (75% mud/25% sand and 50% mud/50% sand respectively mixture M2 and M3). For each of these mixtures, sediment was then dispatched in twelve cores (20 cm in diameter and a depth of 20 cm). The first upper cm was enriched with an epipelic MPB inoculum collected from a mudflat – with typical grain-size corresponding to M1 – located in the Orne estuary (WGS84, 49°16′17.41″N, 0°14′ 7.24″O) in Basse-Normandie during April 2011. It was collected by scratching the sediment surface. The biofilm was mainly composed of pennate diatoms including small *Navicula* sp. (length ~17 μm, >95% of total MPB), *Amphora* sp., *Pleurosigma* sp., *Niztschia* sp. and *Cylindrotheca closterium*. The core surface was then wreathed in order to be uniform as best as possible on the whole surface. The cores were placed in a tidal mesocosm able to simulate a high/low tide alternation every 6 h in order to simulate immersion and emersion phases. A night and day alternation (18 h/6 h) was applied with adapted neon lights, with a light intensity of 1600 μmol photons $m^2.s^{-1}$ (LUMINUX, 36 W Osram). The combination of light intensity and duration was chosen as a function of the photoperiod at the moment of the experiment (April). Each of these sediment series was tested during 3, 6 or 9 days continuous treatment, with a sub-sampling within cores allowing At days 3, 6 and 9, 4 sub-cores were sampled within each culture cylinder, of which 3 were dedicated to sediment and biofilm features analyses and 1 was dedicated to erosion experiments (see Fig. 2 for the experimental design drawing).

2.2. Pigment extraction and analyses

Sediment samplings within the experimental cores were performed at 3, 6 and 9 days at the beginning of diurnal emersion periods in order to access respectively the latency, growth and stationary phases of the biofilm (Orvain et al., 2003a, 2003b; Sutherland et al., 1998). The first upper cm of the sediment was sampled and mixed, and fresh sediments were weighted. This depth was chosen as the maximum depth for diatom vertical migration (Saburova and Polikarpov, 2003). After 3 days in an oven at 60 °C a weight measurement was also done to obtain the water content of the sediment. Microphytobenthos content was assessed by measuring the chlorophyll *a* (Chl a) content following the Lorenzen's method (Lorenzen, 1967). Chloropigments were extracted from 200 mg freeze-dried sediment subsamples with 90% acetone solution for 24 h at 4 °C in the dark. After centrifugation (5 min, 2000 g, 4 °C), fluorescence of the supernatant was measured using a TD-700 Fluorometer (Turner Design, USA) before and after acidification (HCl 0.3 M for 1 mL of supernatant). Total Chl a and pheopigment biomass were calculated according to Lorenzen equations. In order to avoid the dewatering over the emersion period (Perkins, 2003), water content and bulk density of the sediment were used to express the Chl a as a content per m^{-2}. Microphytobenthos physiological state measurements as well as photosynthetically active biomass measurements have been done using a Pulse Amplitude Modulation fluorometer (PAM, Walz-Mess und Regeltechnik, Deutschland, see Section 2.5).