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Structures of benthic prokaryotic communities and their hydrolytic enzyme activities resuspended from samples of intertidal mudflats: An experimental approach ☆

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ABSTRACT

Resuspended sediment can increase plankton biomass and the growth of bacteria, thus influencing the coastal planktonic microbial food web. But little is known about resuspension itself: is it a single massive change or a whole series of events and how does it affect the quantity and quality of resuspended prokaryotic cells? We simulated the sequential erosion of mud cores to better understand the fate and role of benthic prokaryotes resuspended in the water column. We analyzed the total, attached and free-living prokaryotic cells resuspended, their structure and the activities of their hydrolytic enzymes in terms of the biotic and abiotic factors that affect the composition of microphytobenthic biofilm.

Free living prokaryotes were resuspended during the fluff layer erosion phase (for shear velocities below 5 cm · s^{-1}) regardless of the bed sediment composition. At the higher shear velocities, resuspended prokaryotes were attached to particulate matter. Free and attached cells are thus unevenly distributed, scattered throughout the organic matter (OM) in the uppermost mm of the sediment. Only 10–27% of the total cells initially resuspended were living and most of the Bacteria were Cyanobacteria and Gamma-proteobacteria; their numbers increased to over 30% in parallel with the hydrolytic enzyme activity at highest shear velocity. These conditions released prokaryotic cells having different functions that lie deep in the sediment; the most important of them are Archaea. Finally, composition of resuspended bacterial populations varied with resuspension intensity, and intense resuspension events boosted the microbial dynamics and enzyme activities in the bottom layers of sea water.

1. Introduction

Terrestrial and marine influences converge at tidal flats to produce a complex patchwork of habitats. Research on the productivity of coastal systems has established that intertidal flats influence, both physically and trophically, the adjacent marine and land areas. The intense microbial activity due to microalgae and prokaryotes in these areas results in great biological productivity that is essential for aquaculture. Mudflats are therefore socially and economically extremely important (Héral et al., 1989). Most of the primary production in these areas is due to benthic microalgae, mainly epipelic diatoms (Blanchard et al., 1998; Leguerrier et al., 2003) because the turbidity of the waters limits the presence of macrophytes and phytoplankton (McLusky, 1989). The twice-daily immersion in water and the access to daylight stimulate epipelic microalgae to migrate to the surface of the sediment, where they form a continuous biofilm. This biofilm may contain over 20 mg of chlorophyll (Chl *a*) per m^2 and its photosynthetic activity can increase the algal biomass, so doubling the size of the biofilm (Blanchard et al., 2002). This great productivity can lead to depletion of nitrogen or phosphorus, conditions that stimulate microalgae to secrete exopolymeric substances (EPS). These EPS are produced mainly by "overflow metabolism" or other processes like locomotion (Brouwer and de Stal, 2002; Stal, 2003; Orvain et al., 2003). The EPS form a matrix around the microalgae (Paterson and Black, 1999) that is one of the main resources leading to the rapid development of prokaryotes (Goto et al., 2001; Middelburg et al., 2000; van Duyl et al., 1999). Bacterial production can be as high as or even higher than the production of the microphytobenthos (Hamels et al., 2001; Pascal et al., 2009; Van Duyl et al., 1999). While the production of

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bacteria fluctuates widely during the year this does not result in fluctuations in their abundance. The poor correlation between biomass and bacterial production has been classically explained by their "top-down" disappearance (Thingstad, 2000). Several experiments designed to study grazing on mudflats (Brouage, France) (Pascal et al., 2009) throughout the year have shown that the bacteria consumed by larger animals never exceeded 6% of the bacterial production, so having only a limited impact on bacterial production and stock. However, grazers may stimulate bacterial production by disturbing the biofilm which allows the diffusion of oxygen and nutrients throughout the sediment (Alkemade et al., 1992) or by secreting nutrient-rich compounds such as mucus (Riemann and Helmke, 2002). The activity of grazers can also increase the resuspension of sediment leading to the export of benthic prokaryotes to the pelagic environment (Blanchard et al., 1997; Orvain et al., 2006). The free-living or particle-associated prokaryotes transferred to the water column may then find their way into coastal waters (Teal, 1962) where they may be consumed by filter-feeding organisms or pelagic grazers (Guizien et al., 2014; Wainright, 1987).

Both tidal currents and wind-induced waves can cause the resuspension of sediment (Blanchard et al., 2002). Sediment erosion generally increases with the rate of shear or friction (u^* expressed in cm s^{-1}). Erosion rates depend on the balance between shear stress and the critical threshold for bed erosion. This threshold depends on properties of the sediment, and physical, geochemical and biological processes (Grabowski et al., 2011). The EPS matrix secreted by the benthic microbial communities plays a key role in sediment stabilization/destabilization, and hence in the export and retention of microphytobenthic and prokaryotic biomasses (Gerbersdorf et al., 2009; Underwood and Paterson, 2003). The sequential resuspension of microorganisms in a subtidal setting subjected to increasing hydrodynamics energy has been attributed to differences in sediment erodibility and the behavior of microorganisms (Grabowski et al., 2011; Shimeta et al., 2003). This sequential resuspension of microorganisms should be enhanced during the erosion of the biofilms that form on the sediment surface in intertidal flats because the vertical structure of the biota is well defined. Chronic erosion (type 0 erosion) generally occurs and can be defined as a fluff layer erosion (i.e. simple detachment of loose aggregates from the sediment matrix, at low bed shear stress). Orvain et al. (2006) demonstrated the relevance of bioturbation by macrofauna in the creation of such a biogenic fluff layer. This chronic erosion can be followed by a catastrophic erosion (namely mass erosion; type I and/or type II according to the consolidation status of the bed load), which corresponds to the general bed failure that can be achieved only when wind-induced waves produce high values of bed shear stress that can overpass the critical threshold for mass erosion (i.e. resistance force). This defined vertical distribution is accompanied by a spatial structure due to top-down control by benthic herbivores (Weerman et al., 2011). The drivers of sequential erosion and the critical erosion thresholds of the microorganisms that make up a biofilm are analyzed in a companion paper (Dupuy et al., 2014). Resuspension of the top few centimeters of sediment can increase the plankton biomass and bacterial growth (Wainright, 1990). This affects the coastal planktonic microbial food web due to the direct movement of cells from the sediment into the water column so increasing the seston concentration or the mineralizing capacity (Wainright, 1987, 1990). Dissolved nutrients and trophic interactions like bacterivory by nanoflagellates can also have indirect effects (Blanchard et al., 1997; Garstecki et al., 2002). Resuspension has been shown to increase the abundance and volume of bacteria in both field and experimental studies (Ritzrau and Graf, 1992; Wainright, 1987), but most of these early studies considered sediment resuspension to be a massive, homogeneous process. The present study resuspension experiments were done to determine whether the sequential resuspension of prokaryotes under varying environmental conditions could alter the direct and indirect effects on planktonic food-web. We monitored the enrichment of resuspended prokaryotic cells and their structure to assess if there was sequential resuspension under all the environmental conditions tested. We also checked whether prokaryotic cells were alive or dead so as to better identify their indirect effects on the plankton food-web. Lastly, we measured the activities of their hydrolytic enzymes. These enzymes regulate the assimilation of nutrient monomers by microbial cells and therefore play an important part in the cycling of organic matter and remineralization.

2. Material and methods

2.1. Field sampling

Muddy sediment samples were collected from Marennes-Oléron Bay (Atlantic Coast of France) in July 2008 (Fig. 1) (45°54′50″N, 01°05′25″W) during spring tides (17 and 19 July), maximum tides (21 July) and neap tides (22 and 24 July). At each sampling occasion, two 8 cm diameter sediment cores were taken at low tide (middle of the emerged period). Only ridges were sampled since biofilms are not normally found on runnels. The sediment cores were placed in a tank for transport back to the laboratory. The resuspension experiments were done at the time of the immersion period in the field. Three additional 15 cm diameter cores were taken to assess the biotic and abiotic parameters of the top 2 cm of sediment.

2.2. Resuspension experiments and instrumentation

The sediment samples were transferred from the cylindrical cores to the bottom of the flume of an erosion device developed by IFREMER. This was modified to form a straight recirculation flume, named "Erodimetre" (Guizien et al., 2012; Le Hir et al., 2006; Orvain et al., 2007). The flume was filled with filtered artificial seawater (15 L) to obtain a baseline close to 0 for each type of microorganism. The discharge through the erosion device was increased in twenty 5-minute steps to yield bed friction velocities ranging from 0 to 11 cm · s^{-1}. A total of 6 samples (1.5 L each) were taken from the water column of the erodimeter as the flow, and thus shear velocity, was increased. These were used to monitor the resuspension of prokaryotes and the total particle matter (TPM) concentration. The induced bed shear stress was calculated from measurements of the pressure head loss between the upstream and downstream ends of the sample section (Guizien et al., 2012). Friction velocity was calculated as the square root of the bed shear stress divided by the sea water density. Turbidity and fluorescence were continuously recorded and used to measure chl *a* and suspended particular matter. Erosion kinetics were analyzed to evaluate the relationship between bed erodibility (erosion rates of suspended particulate matter and chl *a* biomass) and the sediment properties (abiotic and biotic factors) and their temporal changes over 14 days (Orvain et al., 2014–in this issue). From this long set of experiments across a spring–neap tidal cycle, we selected 4 dates for a detailed analysis of the fate of bacterial and archaeal benthic communities after resuspension. The dates were chosen to evaluate the response of prokaryote resuspension for different biofilm stages of development. The July 17 sample illustrated an increase in tidal range (midtime between neap tides and spring tides, low tide occurs in the morning), those taken on July 20 and 21 (spring tide) corresponded to a maximum tidal range (just before and just after the peak, low tide occurred around noon), while the July 24 sample reflected the response to a decrease in tidal range (midtime between spring tides and neap tides, low tide started after noon).

2.3. Abiotic parameters

2.3.1. Salinity and nutrient (NH_4, NO_3, NO_2, PO_4, silicates) concentrations in the upper layer of the sediment

Pore water was separated from 300 mL of fresh sediment by centrifugation (4 °C, 3000 *g*, 15 min). Several aliquots of interstitial water were obtained by passage through GF/C filters. Salinity was determined with a refractometer.