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# Microbial interactions in marine water amended by eroded benthic biofilm: A case study from an intertidal mudflat

Hélène Montanié [a,*], Pascaline Ory [a], Francis Orvain [b], Daniel Delmas [c], Christine Dupuy [a], Hans J. Hartmann [a]

[a] Littoral, Environnement et SociétéS (LIENSs), Université de La Rochelle, UMR 7266 CNRS-ULR, 2 rue Olympe de Gouges, 17000 La Rochelle Cedex, France
[b] Université de Caen Basse-Normandie, FRE3484 BioMEA CNRS, Esplanade de la Paix, 14032 Caen, France
[c] IFREMER, Dyneco, Laboratoire Pelagos, BP70, 29280 Plouzané, France

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ABSTRACT

In shallow macrotidal ecosystems with large intertidal mudflats, the sediment–water coupling plays a crucial role in structuring the pelagic microbial food web functioning, since inorganic and organic matter and microbial components (viruses and microbes) of the microphytobenthic biofilm can be suspended toward the water column. Two experimental bioassays were conducted in March and July 2008 to investigate the importance of biofilm input for the pelagic microbial and viral loops. Pelagic inocula (<0.6 μ- and <10 μ filtrates) were diluted either with <30 kDa-ultrafiltered seawater or with this ultrafiltrate enriched with the respective size-fractionated benthic biofilm or with <30 kDa-benthic compounds (BC). The kinetics of heterotrophic nanoflagellates (HNF), bacteria and viruses were assessed together with bacterial and viral genomic fingerprints, bacterial enzymatic activities and viral life strategies. The experimental design allowed us to evaluate the effect of BC modulated by those of benthic size-fractionated microorganisms (virus + bacteria, +HNF). BC presented (1) in March, a positive effect on viruses and bacteria weakened by pelagic HNF. Benthic microorganisms consolidated this negative effect and sustained the viral production together with a relatively diverse and uneven bacterial assemblage structure; (2) in July, no direct impact on viruses but a positive effect on bacteria modulated by HNF, which indirectly enhanced viral multiplication. Both effects were intensified by benthic microorganisms and bacterial assemblage structure became more even. HNF indirectly profited from BC more in March than in July. The microbial loop would be stimulated by biofilm during periods of high resources (March) and the viral loop during periods of depleted resources (July).



## 1. Introduction

In shallow estuarine ecosystems, hydrodynamics (tide, waves, wind) induce erosion of particulate material from the sediments into the overlying water column and at the sediment–water interface, the gradients in organic and inorganic nutrients may structure the composition of the microbial communities and their activities (Seymour et al., 2007). Differences in grain size distribution may explain the distribution of dissolved and particulate matter according to the porosity and permeability of the sediment (Pinto et al., 2013). The smaller pore size and higher surface area in clays and silts compared to sand may infer a higher retention of dissolved nutrients in clayed sediment and a higher desorption of organic particles in sandy sediment (Wainright, 1987). Consequently, understanding the pelagic microbial food web dynamics of an intertidal muddy ecosystem requires consideration of the benthic–pelagic coupling. Sediment suspension may be an efficient way to provide inorganic nutrients for phytoplankton and to deliver sedimentary organic matter (Particulate Organic Matter and Dissolved Organic Matter) for pelagic heterotrophic entities (Arfi and Bouvy, 1995; Garsteki et al., 2002; Hopkinson et al., 1998; Wainright, 1987, 1990).

The resuspended benthic organic matter, used by bacteria, is channeled to the higher trophic levels via the heterotrophic nanoflagellate (HNF) bacterivory within the pelagic microbial loop (Azam et al., 1983), while viral lysis tends to maintain the matter at lower levels by shunting those fluxes (Bratbak et al., 1992; Wilhelm and Suttle, 1999). Microorganisms may also be resuspended (Shimeta et al., 2002). In the water column, the dynamics of benthic viruses may be globally governed by the trade-off between their irreversible and non-infective adsorption onto benthic-suspended matter (Suttle and Chen, 1992) and their multiplication at the expense of more active attached bacteria (Kernegger et al., 2009; Riemann and Grossart, 2008). The abundance of HNF depends directly on the resuspension of their benthic representatives and would also, in the case of muddy sediments, indirectly benefit from the increase of their prey (bacteria and pico-, nanoautotrophs), which benefit from the input of benthic nutrients (Garstecki et al., 2002; Guizien et al., 2013). Thus, the microbial interactions include HNF bacterivory, HNF virivory (Bettarel et al., 2005), viral lysis of bacteria, HNF or viruses and coincidental HNF predation of

* Corresponding author at: Littoral, Environnement et SociétéS (LIENSs), Université de La Rochelle, UMR 7266, 2 rue Olympe de Gouges, 17000 La Rochelle Cedex, France. Tel.: +33 546458280.
*E-mail address:* helene.montanie@univ-lr.fr (H. Montanié).

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infected bacteria (Miki and Jacquet, 2008). Apart from these direct links, the availability of nutritive resources, including the feed-back effect of the regenerated products from lysis and predation, and the presence/absence of the respective second member of the predator guild of bacteria (virus, HNF) may influence the expression of the bacterial resistance to predators and the life cycles of temperate viruses (Miki and Jacquet, 2008, 2010; Pradeep Ram and Sime-Ngando, 2010; Winter et al., 2010). The potential size and activity of the bacterial community and consequently the identity of the virus–host systems (VHS) would also be driven by the bioavailability of nutritive resources (Sandaa et al., 2009). The bacterial stock and the number of VHS may depend upon the processes of species-dependent viriolysis and size-selective grazing (Bouvier and del Giogio, 2007; Church, 2008; Ovreas et al., 2003; Thingstad and Lignell, 1997; Winter et al., 2010).

Since imports of benthic organic matter and microorganisms have the potential to modify the dynamics of microbial entities and the functioning of the pelagic microbial food web, previous experiments have already tested the direct and indirect effects of marine sediment resuspension on planktonic bacteria and protozoans by using either intertidal sediment at two macrotidal sites [a marsh system (Hopkinson et al., 1998) and a muddy site (Garstecki et al., 2002)] or 20-m-deep sandy sediment (Wainright, 1987, 1990). But viruses have always been excluded from these investigations. In the present study, we investigated the intertidal mudflats, which cover up to 60% of the macrotidal coastal area of the Marennes-Oleron Bay (France). At low tide, a complex and transient assemblage of both eukaryotic (as mainly epipelic microalgae, protozoans) and prokaryotic cells as well as viruses were associated on and in the surficial sediment in a mucilage matrix, i.e. the microphytobenthic biofilm (Guizien et al., 2013). A rising tide may more or less erode microbes living in this biofilm (Guizien et al., 2013). We designed experimental bioassays to investigate the potential alteration of the microbial interactions in the water column by the suspended biofilm. Using an erosion device (Orvain et al., 2007), the surficial substratum and the associated microorganisms living in the pore water or attached to sedimentary particles were eroded from a sediment core and added to pelagic water inocula. We then compared the temporal dynamics of microbes (virus, bacteria, HNF), bacterial and viral activity, fingerprint diversity and viral life strategies. The objectives were (1) to untangle the effects mediated by benthic organic matter and nutrients on microbial interactions from those of resuspended benthic microorganisms and (2) to examine whether there is a seasonal trend to the induced modification in food web functioning by targeting two seasons with contrasting productivity.

## 2. Materials and methods

### 2.1. Sediment and water sampling

Water column samples were taken with the flat-bottom oceanographic barge "ESTRAN", at the sub-surface (1-m depth) in the Marennes-Oléron Bay, French Atlantic coast, at station E (7–10 m depth range, Fig. 1). A few days before each experiment conducted on 3 March and 14 July 2008 (e.g., on 27 February 2008 and 9 July 2008, respectively), 50 L was collected at high tide, in order to produce <30 kDa ultrafiltered seawater by tangential flow through a 30-kDa polysulfone cartridge (Ultraslice support, Sartorius). This ultrafiltrate was conserved at 4 °C in autoclaved Erlenmeyer flasks until filling the flume of the erosion device and using it as pelagic water diluent in the experimental treatments (Fig. 2B). This ultrafiltrate served as diluent of pelagic inocula, either unenriched (in pelagic control treatments) or enriched with eroded sediment mimicking the erosion influxes (in erosion treatments). Sediment was sampled with an aircraft boat on 3 March and 14 July 2008 at low tide during the emersion of the Brouage intertidal mudflat located at the south end plume of the Charente estuary (Fig. 1). Cores of the 2-cm top surface sediment were collected using a 90-mm diameter manual Polyvinyl Carbonate coring device which

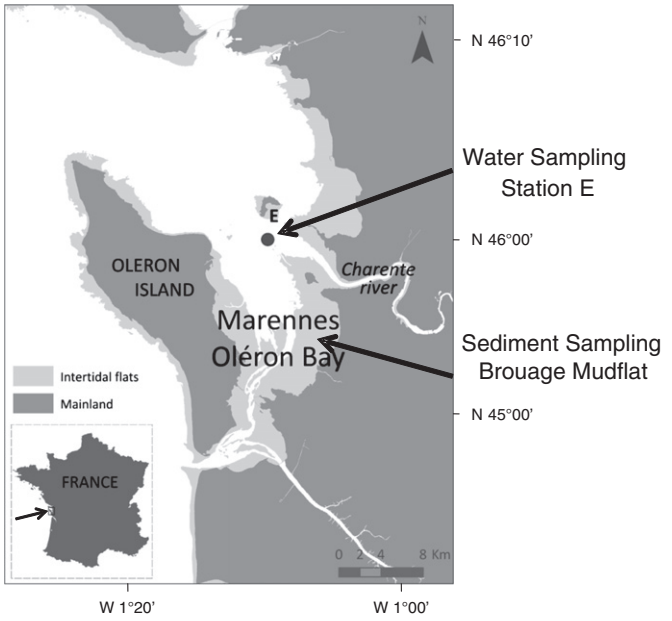


**Fig. 1.** Study area in the Marennes-Oleron Bay (French Atlantic coast, France) characterized by a 2–5 m tidal range. Water sampling was performed at Station E, located in the outer part of the Charente River estuary (7–10 m depth). Sediment coring was conducted on the Brouage mudflat during emersion at 2 km from the beach.

was acid rinsed, ethanol disinfected and rinsed with field sediment before use. Cores were eroded within 30 min after collection. In parallel with the barge "ESTRAN", 25 L of seawater was sampled at mid-rising tide at station E and treated in the laboratory within 2 h to obtain the different pelagic water inocula for the experiments (Fig. 2A).

### 2.2. Erodimetry

An erosion device was deployed near the beach of the Brouage mudflat (4 km) to enrich the ultrafiltered seawater in sedimentary particles, nutrients and biofilm. The erosion device developed by IFREMER and modified for this study is a straight recirculation flume named "Erodimeter" (Orvain et al., 2007). Briefly, two surficial sediment cores (90 mm in diameter) were settled into the eroding unit (a closed circuit which is connected to a pump). The sediment samples were directly transferred from cylindrical cores to the bottom of the flume of the erosion device. The <30-kDa ultrafiltered seawater (free of microorganisms) was used to fill up the flume. Turbidity and fluorescence were recorded continuously to measure chl *a* and suspended particular matter to verify that initial erosion of microphytobenthic biofilm was well underway. In July, the flow discharge was incremented step-by-step with a succession of 20 steps, each one lasting 2 min. This procedure yielded bed friction velocities U* ranging from 0 to 6.6 $cm \cdot s^{-1}$ with an increment of 0.34 $cm \cdot s^{-1}$. In March, only 11 steps were applied to provoke an initial erosion phase (because of a lower critical threshold for biofilm erosion, u*crit) and U* ranged from 0 to 4.1 $cm \cdot s^{-1}$, with an increment of 0.35 $cm \cdot s^{-1}$. The total volume of suspended sediment water (35 L of enriched ultrafiltrate) was taken from the eroding device at the end of the process and directly used for experiments.

### 2.3. Inocula and diluents

Water volumes were systematically prepared by sequential steps, i.e. a prior screening through 250, 25 and 10-μm nylon meshes followed by filtration through 1.2, 0.6 and 0.2-μm membranes (Sartorius, cellulose acetate) and eventually tangential ultrafiltration.

Four types of water served as diluents (Fig. 2B) after equilibration to the ambient seawater temperature: for control treatments the

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