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Surface adhesion of microphytobenthic biofilms is enhanced under *Hediste diversicolor* (O.F. Müller) trophic pressure

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ABSTRACT

In soft-bottom tidal flats, sediment stability is one of the crucial parameters modulating the abundance and composition of benthic assemblages. It is dependent on a wide range of variables, both abiotic and biotic. Investigating how these variables and their interactions influence sediment stability is therefore essential to understand how benthic assemblages are distributed in their environment. In this context, we designed a microcosm study to examine how microorganisms and macrofauna interact to alter sediment stability. We cultured a natural microbial community, enriched with diatoms, both alone and together with the common ragworm *Hediste diversicolor*, and monitored their effects on photosynthetic biomasses, bacterial abundances, exopolymer secretions and sediment stability. We also assessed the consumption of biofilm by worms using fatty acid biomarkers. Our results demonstrate that even if *H. diversicolor* fed on diatoms, they stimulated biofilm development, in terms of photosynthetic biomass and exopolymer production. Also, sediment cohesiveness was enhanced when both diatoms and *H. diversicolor* were cultured together; this result was unexpected since macrofauna, through consumption of microorganisms and modification of sediment properties, is often considered to have a destabilising effect on sediment. Predicting the effect of macrofauna on microphytobenthic biofilms and their associated influence of sediment stability is therefore not straightforward. Similar experiments including different types of organisms or more complex assemblages might help to further characterise the effect of biota on sediment stability.

1. Introduction

Sediment stability is an important feature in ecosystems subjected to physico-chemical gradients, such as intertidal areas or freshwater streams. It determines the occurrence of several macrofaunal and meiofaunal species, which depend, for their settlement and development, on specific environmental conditions (Norkko et al., 2001; Van Colen et al., 2009; Volkenborn et al., 2009). Any parameter promoting either stabilisation or erosion of the sediment may therefore alter the density and the diversity of benthic assemblages.

Sediment stability depends on a large range of variables, both abiotic (grain size and water content for instance) and biotic (Black et al., 2002; Widdows and Brinsley, 2002). Among biotic factors, the presence of microphytobenthic and bacterial biofilms is crucial. Biofilms are formed of an assemblage of microorganisms entangled in a matrix of extracellular polymeric substances (EPS), mainly composed of carbohydrates and proteins. These molecules play many different roles associated with the maintenance of a stable environment, including attachment of microbes to surfaces, protection against high irradiance, UV, pollution and desiccation tolerance (Decho, 1990) and they also have a stabilising effect on the sediment (de Brouwer et al., 2005; van Duyl et al., 2000). EPS can indeed form a network which traps and binds particles together, ultimately increasing sediment adhesion and stability (de Brouwer et al., 2002; Stal, 2010). The EPS composition is highly variable between species, cells, and nutrient availability (Decho, 1990; Stal, 2003) and any change in its arrangement promotes fluctuations in adhesion potential (Sanin et al., 2003).

Another biotic component affecting sediment stability is the presence of macrofaunal organisms. Two kinds of direct effects are well recognised: first, macrofaunal organisms disturb sediment through bioturbation, increasing its water content and surface micro-topography (Andersen, 2001; de Deckere et al., 2001), therefore promoting sediment erosion. On the contrary, most macrofaunal organisms produce mucus in order to facilitate their locomotion and feeding activity, as well as to solidify burrows; this mucus finally consolidates sediments (Murray et al., 2002; Stabili et al., 2011). Macrofauna also has an indirect negative effect on sediment stability through consumption on biofilms (de Deckere et al., 2001); however, their mucus might be used as a food source by bacteria, so that macrofaunal presence may lead to an increase of biofilm development (Coull, 1999; Fernandes et al., 2006) and hence sediment biostabilisation.

Given these complex interactions between organisms, as well as with physical and chemical variables, it is difficult to investigate in the field the effect of living organisms on sediment stability. We

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therefore conducted a microcosm experiment with natural microbial communities and one of their potential consumers, the common ragworm *Hediste diversicolor*, alone or in combination. This species was chosen for its ability to display a diversity of feeding modes including deposit feeding on microbial assemblages (Galván et al., 2008; Riisgård and Larsen, 2010) and also because it produces mucus (Riisgård and Larsen, 2010; Scaps, 2002), which is generally used to construct burrows. We monitored microbial biomasses in terms of pigment content and bacterial cell number, as well as EPS production and sediment surface adhesion. The composition of organic matter in sediment was characterised through its fatty acid (FA) content and we followed the consumption of microorganisms by *H. diversicolor* with FA biomarkers. We tested the hypothesis that the effective consumption of microorganisms by *H. diversicolor* in combination with its bioturbation will decrease sediment surface adhesion, a proxy for sediment stability.

2. Material and methods

2.1. Microphytobenthic culture

Muddy intertidal sediment was sampled at low tide (Chausey archipelago, Normand-Breton Gulf, France), mixed with autoclaved seawater (35 $g \cdot l^{-1}$) and filtered on 5 μm to separate benthic microalgae and bacteria from sediment particles. The filtrate was enriched with Guillard's (f/2) medium to stimulate diatom growth. The culture was grown for 2 weeks at room temperature with natural light.

2.2. Hediste diversicolor

Adult worms (Annelids, Polychaetes) were bought from the "Service d'Expédition de Modèles Biologiques" (CNRS/FR2424), of the biological Station of Roscoff (France). They were starved during 1 week before their use for the experiment.

2.3. Experimental design

The diatom culture and worms were grown both separately and together in microcosms containing 180 g of sterilised marine sand (40 to 100 μm, Fisher Scientific; burned 4 h at 550 °C) and 500 ml of autoclaved seawater. 4 treatments were used (Fig. 1): a control treatment (C), without culture or worms; a diatom treatment (D), inoculated with the diatom culture; a *Hediste* treatment (H), with one worm per microcosm; and a last treatment with both diatoms and worm (DH treatment). Five replicate microcosms were used for each treatment (total of 20 microcosms, 14 × 14 × 7 cm, l × w × h). In control and *Hediste* microcosms, streptomycin (150 $mg \cdot l^{-1}$) and chloramphenicol (20 $mg \cdot l^{-1}$) were added to limit bacterial proliferation. In D and DH treatments, 30 ml of the diatom culture was added to each microcosm. In H and DH treatments, 1 worm was added to each microcosm (equivalent to a density of 51 $ind \cdot m^{-2}$). Microcosms were placed at room temperature (approximately 20 °C) and subjected to a daily 10 h photoperiod throughout the experiment (at about 180 μmol $photons \cdot m^{-2}$, PAR 400 to 700 nm).

2.4. Sampling protocol

Diatom culture and antibiotics were added to microcosms at the beginning of the experiment (T_0). Biofilm growth was allowed during 5 days before microcosms were sampled (T_5) for chlorophyll. Worms were then added to microcosms and the experiment was continued for 14 days, after which microcosms were sampled again (T_{19}) and analysed for sediment surface adhesion. 4 sediment cores (1.2 cm diameter, 2 mm depth) per microcosm were taken to measure chlorophyll concentrations, bacterial cell numbers, EPS concentrations and analyse fatty acid content. They were immediately frozen in liquid nitrogen and stored at −25 °C until analysis. Worms were caught and frozen.

2.5. Chlorophyll concentrations

The sampling depth (2 mm) was chosen to target photosynthetically active biomass only (Black and Paterson, 1996; Paterson et al., 1998). 1.5 ml of 90% acetone was added to each sample for pigment extraction (16 h in darkness). Concentrations of chlorophylls *a*, *b* and *c* were determined by spectrophotometry (Spectronic Genesys 2 spectrophotometer, Milton Roy; Jeffrey et al., 1997).

2.6. Bacterial cell numbers

Sediment cores were fixed with 1.5 ml of 3% formalin in sterile seawater. Bacteria were separated from sediment with one drop of Tween® 80 (Fisher Scientific) in the sediment slurry. Samples were then placed in a sonication bath for 3 min (35 kHz). Samples were

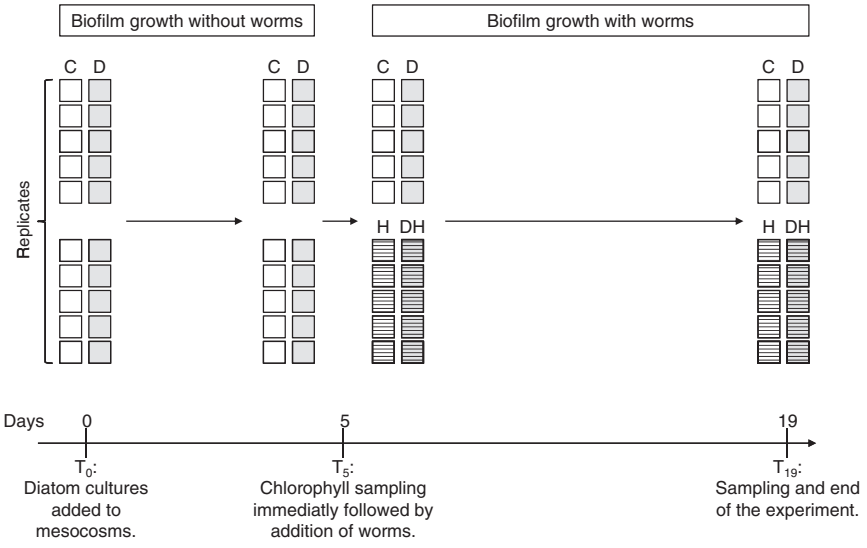

Fig. 1. Experiment and sampling design. C: control treatment (with antibiotics); D: diatom treatment; H: *Hediste* treatment (with antibiotics); and DH: diatoms and *Hediste* treatment.

centrifuged 10 min at 1700 *g* and the supernatant containing bacteria was kept. DAPI (4′,6-diamidino-2-phenylindole dihydrochloride, Sigma-Aldrich) was added to 1 ml of the supernatant, to a final concentration of 2 $\mu g \cdot ml^{-1}$. The mixture was filtered on 0.2 μm. Dried filters were mounted on glass slides and bacteria were counted by epifluorescence microscopy.

2.7. Fatty acid (FA) analysis

Fatty acids analysis was performed following the modified method of Bligh and Dyer (1959) as described in Meziane et al. (2006). 3 sediment cores and 3 worms per treatment were lyophilised overnight; the whole core or individual was weighted and used for analysis. Before extraction, an internal standard (FA 23:0) was added to every sample for quantification purpose. Lipids were extracted with a 20 minute ultrasonication in a mixture of distilled water, chloroform and methanol in ratio 1:1:2 (v:v:v). A mixture of distilled water and chloroform was added to form a two-layer system, and samples were centrifuged 5 min at 2000 g. The lower chloroform phase containing lipids was recovered, and the same volume of clean chloroform was added for a second extraction with ultrasonication and phase separation. The chloroform phase, which contains the lipids, was pooled with the first one. Lipids were concentrated under N_2 flux, and saponified, in order to separate FAs, with a mixture of NaOH (2 $mol \cdot l^{-1}$) and methanol (1:2 v:v) at 90 °C during 90 min. Saponification was stopped with chlorhydric acid; FAs were recovered with chloroform and concentrated under N_2 flux. Samples were incubated with BF_3-methanol (boron-trifluoride methanol) at 90 °C during 10 min to transform FAs into methyl esters, which were re-extracted and kept frozen in chloroform. Just before analysis, samples were dried under N_2 flux and transferred to hexane. 1 μl of the mixture was injected to a gas chromatograph (GC, Varian CP-3800 equipped with flame ionisation detector), which allowed separation and quantification of FAs. Separation was performed with a Supelco OMEGAWAX 320 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) with He as carrier gas. The following temperature programme was used: 60 °C for 1 min, then raise to 150 °C at 40 $°C \cdot min^{-1}$ (held 3 min), then raise to 240 °C at 3 $°C \cdot min^{-1}$ (held 7 min). Peaks of FAs were identified by comparison of the retention time with analytical standards (Supelco™ 37, PUFA-1 Marine Source, and Bacterial Mix, Supelco Inc., USA) and analysis of the sample in a gas chromatograph coupled to mass spectrometer (GC–MS, Varian 450GC with Varian 220-MS). FA concentrations in sediments (C_{FA}, $mg_{FA} \cdot g_{sediment}^{-1}$) were calculated using the area of their peak compared to the one of the internal standard according to the following formula:

$$C_{FA} = \frac{A_{FA}}{A_{23:0}} \times \frac{C_{23:0}}{W},$$

where A_{FA} is the peak area of the FA, $A_{23:0}$ is the peak area of FA 23:0, $C_{23:0}$ is the weight of FA 23:0 in the sample (mg) and W is the dry weight of sediment used for extraction.

FAs are designated as *X*:*Y*ω*Z*, where *X* is the number of carbons, *Y* the number of double bonds and *Z* the position of the ultimate double bond from the terminal methyl. Branched FAs have a second methyl group on the carbon wearing the terminal methyl (iso FA) or on the carbon before (anteiso FA).

2.8. EPS concentrations

Sediment cores were continuously rotated with 2 ml of distilled water for 90 min at room temperature (Horizontal mixer, RM5-30V, Ingenieurbüro CAT), which allowed the extraction of colloidal EPS. Carbohydrates and proteins were analysed according to Dubois method (Taylor and Paterson, 1998) and modified Lowry method (Frolund et al., 1996), respectively. For carbohydrates, 200 μl of the sample was added to 200 μl of 5% phenol and 1 ml of sulphuric acid; the solution was then incubated at 30 °C for 35 min and absorbance was measured at 488 nm. For proteins, 250 μl of the sample was added to 250 μl of 2% SDS (Sodium Dodecyl Sulphate) and 700 μl of a mixture of chemicals as described by Frolund et al. (1996) and incubated 15 min at 30 °C. 100 μl of Folin reagent (diluted 5:6 with distilled water) was added and samples were incubated again 45 min at 30 °C. Absorbance was measured at 750 nm. Calibration curves were prepared with glucose for carbohydrates and bovine serum albumin (BSA) for proteins.

2.9. Sediment surface adhesion

Sediment stability was assessed through the proxy of sediment surface adhesion. The ability of the surface to retain particles was measured by Magnetic Particle Induction (MagPI), a device recently developed by Larson et al. (2009). Briefly, a given amount of ferrous and stained particles were spread onto the sediment surface. Then, the magnetic force needed to detach the particles from the substratum was measured, using a variable electromagnet set at a specific distance from the test surface. The current supplied to the magnet controlled the strength of the magnetic field and the force required to remove the ferrous particles was recorded (Larson et al., 2009). Ferrous particles (diameter >270 μm) were spread onto the sediment surface of the microcosm in a single layer. The electromagnet was set 4 mm away from sediment surface and connected to a variable voltage power supply (HY3005 DC Power Supply, Mastech). Voltage was increased from 0 V by increments of 0.1 V until all particles detached from sediment. This final voltage was recorded and the magnetic force associated was determined using a calibration curve previously established with a gaussmeter (410-HCAT, LakeShore). This magnetic flux (mTa) was used as a measure of surface adhesive capacity. This measurement has been correlated with Cohesive Strength Meter (CSM) measurements (Lubarsky et al., 2010), which assesses sediment resistance to erosion.

2.10. Statistics

Differences in variables in the biofilm were tested using R software with Wilcoxon tests for comparison between 2 treatments and with Kruskal–Wallis (KW) tests between 4 treatments. Post hoc tests (non-parametric SNK) were performed for pairwise multiple comparisons.

For FA compositions of sediments, Bray–Curtis distance between samples was calculated and n-MDS (non-metric MultiDimensional Scaling) was performed using Primer software (PRIMER 5 software, version 5.2.9, 2001, PRIMER-E Ltd; 200 iterations). SIMPER (SIMilarity PERcentages) was thereafter performed to isolate FAs explaining the most dissimilarity between treatments. Permutational Multivariate ANOVA (PerMANOVA) was then used to test for significant differences between treatments in terms of global FA content, using R software, after proper verification for multivariate homogeneity of group dispersion (tested with a permutation-based test). Kruskal Wallis (KW) tests were used to analyse differences between treatments for individual FA in sediments. Non-parametric SNK tests were performed a posteriori for pairwise multiple comparisons. For worms, permutation t-tests were performed to test for differences between individual FA percentages between treatments.

3. Results

3.1. Microbial composition of the biofilm

After 5 days, sediment of microcosms inoculated with diatoms contained more chlorophyll *a* than control microcosms (Wilcoxon test, $p<0.001$); chlorophyll *a* concentrations reached 1.89 ± 0.68 $\mu g \cdot cm^{-2}$

Table 1
Results of Kruskal–Wallis (KW) tests and a posteriori pairwise tests (non-parametric SNK tests) on biofilm variables at T_{19}. The first row displays the p-value of the KW test; the second row displays the differences between treatments demonstrated by pairwise comparison tests (different if p-value<0.05). Chl.: Chlorophyll. C: control treatment; D: diatom treatment; H: *Hediste* treatment; and DH: diatoms and *Hediste* treatment.

	Chl. *a*	Chl. *b*	Chl. *c*	Bacteria	Carbohydrates	Proteins	Stability
KW	0.034	ns	ns	0.039	0.012	ns	0.002
Pairwise comparison	C<DH			C=H<D=DH	C=H=D<DH		C<H<D<DH

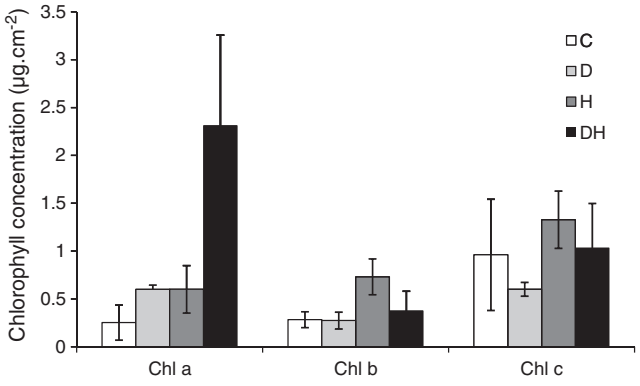

Fig. 2. Concentrations of chlorophylls *a*, *b* and *c* in microcosms at T_{19}. Bars are mean ± SE. C: white, control treatment; D: light grey, diatom treatment; H: dark grey, *Hediste* treatment; and DH: black, diatoms and *Hediste* treatment.

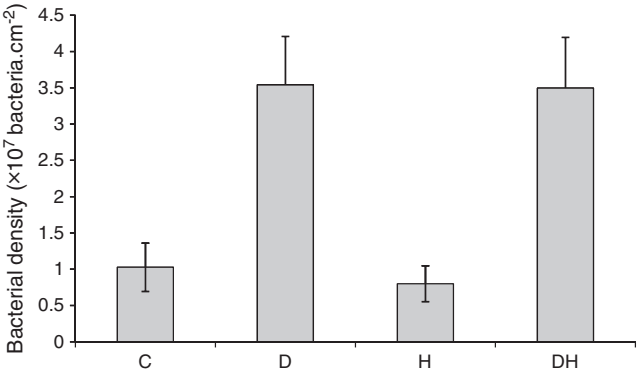

Fig. 3. Bacterial densities in microcosms at T_{19}. Bars are mean ± SE. C: control treatment; D: diatom treatment; H: *Hediste* treatment; and DH: diatoms and *Hediste* treatment.

in microcosms with diatoms. 5 days were therefore enough for biofilm development.

At T_{19}, chlorophyll *a* concentrations in sediment were different between treatments (Table 1, Fig. 2, KW test followed by non-parametric SNK tests, $p<0.05$). Chlorophyll *a* was maximal in treatment DH and minimal in treatment C. Chlorophylls *b* and *c* did not show any differences between treatments.

At the end of the experiment, bacterial abundances were significantly different among treatments (Table 1, Fig. 3, KW test followed by non-parametric SNK tests, $p<0.05$). They were higher in treatments D and DH compared to treatments C and H.

FA extraction and analysis results are displayed in Table S1. Total content of FAs in sediments was significantly different between treatments at the end of experiment (Table 2, Fig. 5a, KW test followed by non-parametric SNK tests, $p<0.05$); they ranged from $1.30 \pm 0.34 \times 10^{-2}$ mg·g^{-1} sediment for treatment C to $20.26 \pm 3.60 \times 10^{-2}$ mg·g^{-1} in treatments DH. FA concentrations were significantly higher when worms were present (Table 2). FA compositions of the sediments also differed significantly between treatments, with sediments with worms being similar (Fig. 4, PerMANOVA, $p<0.001$). Saturated FAs (SFAs; 16:0, 18:0, 14:0 and 12:0) explained most of the dissimilarity among treatments, as well as 16:1ω7 when comparing treatments C or H with D or DH. SFAs were more abundant in treatments with worms compared to other treatments (Table 2, Fig. 5b, KW test followed by non-parametric SNK tests, $p<0.05$), and the ratio between saturated and polyunsaturated FAs (SFA/PUFA) also differed among treatment, being maximal in treatments with worms (Table 2, KW test, $p<0.05$). The concentrations of branched FAs (BFA, sum of concentrations of iso and anteiso FAs) were different among treatments (Table 2, Fig. 5c, KW test followed by non-parametric SNK tests, $p<0.05$); they were higher in treatments D and DH. 16:1ω7 and 20:5ω3 (eicosapentaenoic acid, EPA) concentrations in sediment were different among treatments (Table 2, Fig. 5d and e, KW tests followed by non-parametric SNK tests, $p<0.05$), being significantly higher in treatments D and DH compared to other treatments.

3.2. FA composition of worms

FA compositions of the worms are displayed in Table S2. PUFA were abundant as their percentage was 44.2 ± 0.6% in treatment DH and 48.1 ± 1.7% in treatment D. 16:1ω7 was in significantly higher proportion in worms in treatment DH (Table 3, Fig. 6a, permutation t-test, $p<0.001$), whereas 20:5ω3, or EPA, did not show any differences between treatments (Table 3, Fig. 6b, permutation t-test, $p>0.05$). 18:3ω3 and 20:3ω3 proportions were significantly higher in DH worms compared to H worms (Table 3, Fig. 6c and d, permutation t-test, $p<0.05$).

3.3. EPS and sediment adhesive capacities

Carbohydrates and proteins in colloidal EPS did not show the same pattern at the end of the experiment. Carbohydrates concentrations in sediment were significantly different between treatments (Table 1, Fig. 7, KW test followed by non-parametric SNK tests, $p<0.05$), with concentration being maximal in treatment DH. Proteins did not show any differences between treatments (Table 1, Fig. 7, KW test, $p>0.05$).

Table 2
Results of Kruskal–Wallis (KW) tests and a posteriori pairwise tests (non-parametric SNK tests) on fatty acids weights in sediments at T_{19}. The first row displays the p-value of the KW test; the second row displays the differences between treatments demonstrated by pairwise comparison tests (different if p-value<0.05). C: control treatment; D: diatom treatment; H: *Hediste* treatment; and DH: diatoms and *Hediste* treatment.

	Total	SFA	SFA/PUFA	Branched	16:1ω7	20:5ω3
KW	0.016	0.016	0.022	0.049	0.028	0.033
Pairwise comparison	C<D<H<DH	C<D<H<DH	D<C<DH=H	C=H<D=DH	H=C<DH=D	C=H<D=DH

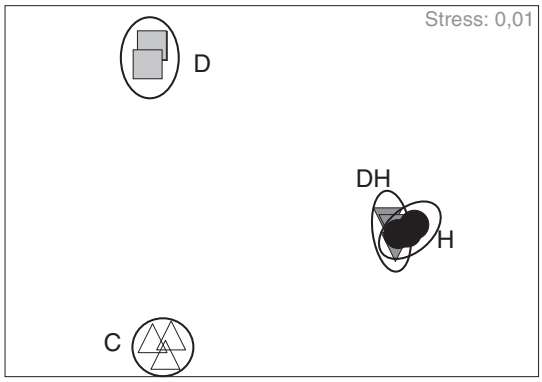

Fig. 4. Non-metric Multidimensional Scaling (n-MDS) plot of Bray Curtis similarities of the fatty acid (FA) compositions of sediment at T_{19}. C: empty triangles, control treatment; D: light grey squares, diatom treatment; H: black circles, *Hediste* treatment; and DH: dark grey triangles, diatoms and *Hediste* treatment. The low value of stress indicates that the grouping representations are meaningful: distances between symbols correlate well with dissimilarities between the sediment composition of FAs.

Sediment surface adhesion, as measured by MagPI device, was significantly different between treatments at T_{19} (Table 1, Fig. 8, KW test followed by non-parametric SNK tests, $p<0.01$). It was maximal in treatment DH and minimal in treatment C.

4. Discussion

4.1. Validation of the experimental procedure

Our experiment displayed a simplified system, with a natural microbial culture and only one species of macrofauna; thus our study does not consider the whole range of interactions that might affect the sediment in the field. Furthermore, in order to control precisely which microorganisms were added to every treatment, sediment was burned to remove OM, and antibiotics were applied to treatments C and H. These steps therefore make the results less applicable to field situation, as *H. diversicolor* usually occur in sediment rich in OM. Our results have therefore to be considered with caution before to be extrapolated to natural systems (Braeckman et al., 2010). Yet, our experiment was appropriate to separate the effect of these groups of interest from other factors, and therefore to test hypothesis of what might be the global influence of these groups in the field.

In the present experiment, microphytobenthic development was assessed through two complementary parameters: chlorophylls and FA concentrations. Each microcosm inoculated with diatoms displayed in its sediment significantly higher chlorophyll *a* concentration than microcosms without diatoms, as well as 16:1ω7 and 20:5ω3 (also called eicosapentaenoic acid, or EPA), which are biomarkers of diatoms in this environment (Arts and Wainman, 1999; Dunstan et al., 1994).

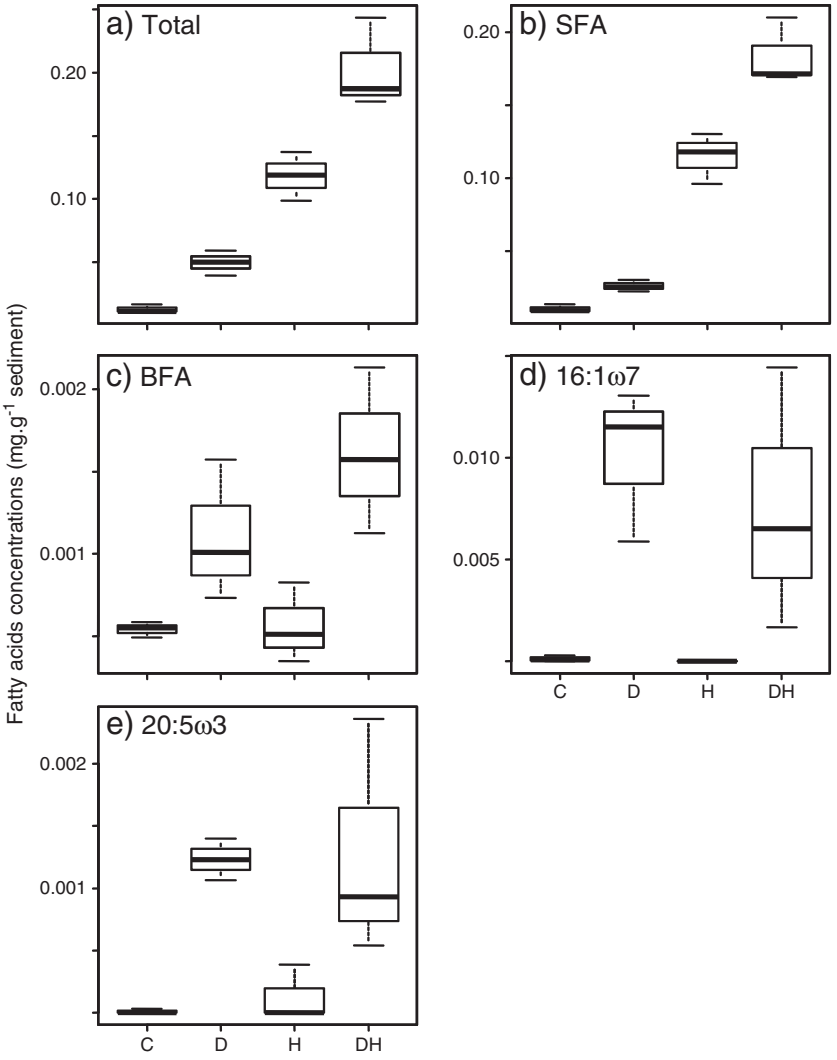

Fig. 5. Fatty acid (FA) concentrations in sediments at T_{19}: a. total FA weight; b. saturated FA (SFA); c. branched FA (BFA); d. 16:1ω7; and e. 20:5ω3. C: control treatment; D: diatom treatment; H: *Hediste* treatment; and DH: diatoms and *Hediste* treatment.

Table 3
Results of permutation t-tests on fatty acids proportions in worms (p-values), between treatments H and DH. The first row displays p-value of the test. The second row displays the direction of variation between treatments (different if p-value<0.05). H: *Hediste* treatment and DH: diatoms and *Hediste* treatment.

	16:1ω7	20:5ω3	18:3ω3	20:3ω3
Perm. t-test	$<2 \cdot 10^{16}$	ns	0.048	0.025
Comparison	H<DH		H<DH	H<DH

The microbial culture and light supply were therefore appropriate to promote microphytobenthic growth in the microcosms.

At the end of the experiment, mean proportions of PUFA in *H. diversicolor* were higher than 40%, thus demonstrating that worms were still in a good shape (Bradshaw et al., 1989; Luis and Passos, 1995; Meziane et al., 1997). The FA compositions of worms also differed between starved individuals and those fed with diatoms; particularly, the proportion of 16:1ω7 was significantly higher in worms fed with diatom. On the contrary, 20:5ω3 (EPA) was in similar proportion in both treatments. EPA, considered as an important FA for this animal (Luis and Passos, 1995; Meziane et al., 1997), was in high proportion in every worm (exceeding 18%), and can be synthesised by them from intermediate FAs such as 18:3ω3 and 20:3ω3 if not abundant in available food sources (Leonard et al., 2004; Sargent et al., 1999; Sprecher, 2000). FAs 18:3ω3 and 20:3ω3 were found in significantly lower proportions in starved worms than in those fed with diatoms, thus suggesting that EPA was synthesised de novo in these animals, whereas it was directly assimilated from diatoms grazing when available. We can therefore conclude than worms actively fed on diatoms in the present experiment.

Bacterial abundances, as well as the contributions of branched FAs in sediments, which are biomarkers of these microorganisms in this environment (Arts and Wainman, 1999), were minimal in both treatments C and H, which confirms that the antibiotic cocktail used was sufficient to inhibit bacterial development.

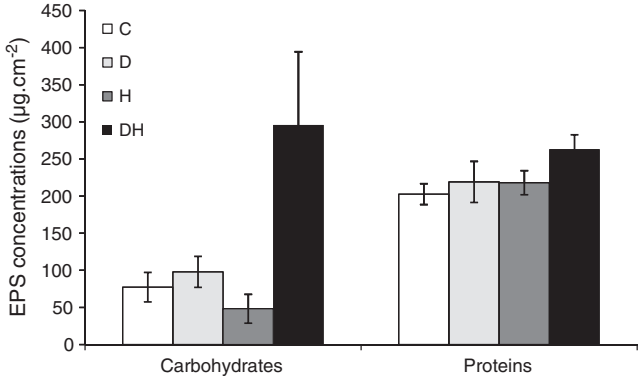

Fig. 7. Concentrations of colloidal EPS in terms of carbohydrates (left panel) and proteins (right panel) in different treatments at T_{19}. Bars are mean ± se. C: white, control treatment; D: light grey, diatom treatment; H: dark grey, *Hediste* treatment; and DH: black, diatoms and *Hediste* treatment.

4.2. Stimulation of microphytobenthic development in presence of worms

Chlorophyll *a* concentrations in sediment were significantly higher when diatoms and worms were present together, compared to the treatment with diatoms only. This was surprising as *H. diversicolor* had in fact assimilated microphytobenthos. Several processes might explain this increase; first, the addition of worms seems to have brought some chlorophyll *a* to microcosm, as the chlorophyll *a* concentrations in treatments H and D were similar. This contamination presumably occurred as worms brought some photosynthetic organisms along. Yet, FA analysis showed that FAs 16:1ω7 and 20:5ω3, or EPA, which are biomarkers of diatoms in this environment (Arts and Wainman, 1999; Dunstan et al., 1994), were virtually absent from sediments in H treatments. Also, as measured in our experiment, chlorophyll *a* concentrations may include degradation products of chlorophyll, named phaeophytin

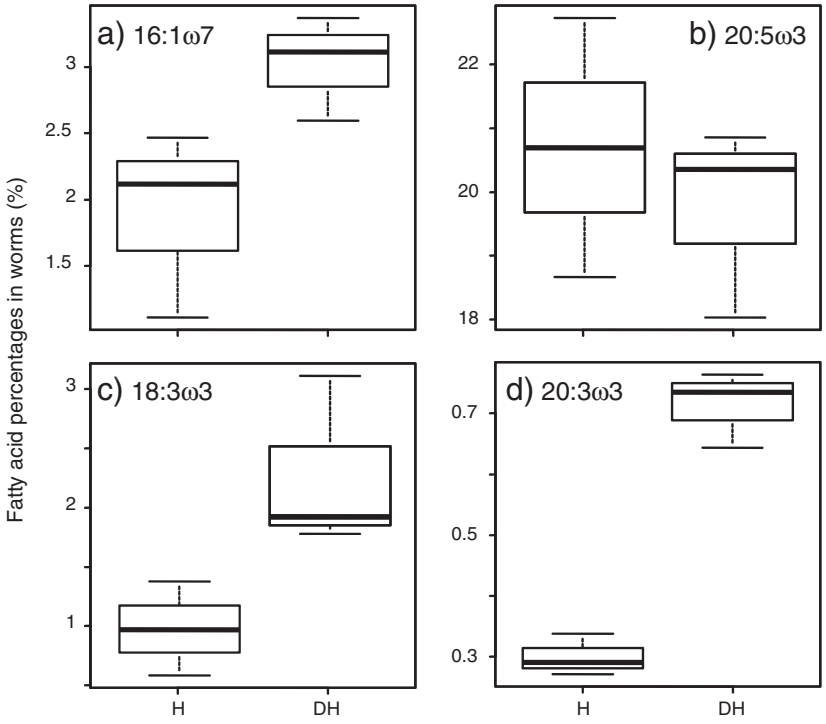

Fig. 6. Percentages of fatty acids in worms at T_{19}: a. 16:1ω7; b. 20:5ω3; c. 18:3ω3; and d. 20:3ω3. H: *Hediste* treatment and DH: diatoms and *Hediste* treatment.

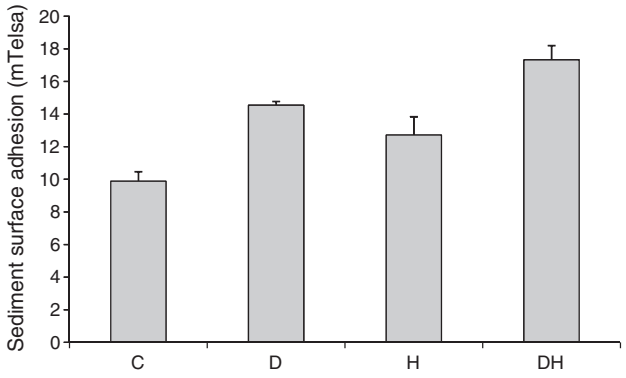

Fig. 8. Sediment surface adhesion as measured by MagPI in different treatments at T_{19}. Bars are mean ± se. C: control treatment; D: diatom treatment; H: *Hediste* treatment; and DH: diatoms and *Hediste* treatment.

(Yentsch and Menzel, 1963). These results suggest that worms did not bring viable diatoms along, and therefore the provision of chlorophyll or phaeophytin by worms cannot explain the threefold increase of chlorophyll *a* concentrations in treatment DH.

Another process to take in consideration is the bioturbation by worms, which is known to enhance the aeration of sediment, thereby allowing the diatoms to settle and photosynthesise deeper than they would without worms (Braeckman et al., 2011; Scaps, 2002). Also, bioturbation increases geochemical fluxes and therefore facilitates the recycling of nutrients in the environment (Braeckman et al., 2010; Coull, 1999; Scaps, 2002). Such nutrients will stimulate diatom proliferation (de Jonge, 1985; Schäfer et al., 2002; Stal, 2003; Underwood and Kromkamp, 1999).

Furthermore, in our experiment, the concentration of saturated FAs (SFA) and their ratio to polyunsaturated FAs (PUFA) increase dramatically in sediments when worms were present. SFA, present in all organisms, are also the end products of organic matter (OM) degradation in sediment (Balzano et al., 2011; Lü et al., 2010; Sun et al., 1997); on the contrary, PUFA are quickly used/degraded as soon as producing organisms die (Grossi et al., 2006). The increase in SFA/PUFA ratio in treatments H and DH demonstrated that OM, particularly worms faecal pellets and dead microflora, was quickly degraded when worms were present. Worms initiate OM degradation through consumption, reducing the size and complexity of molecules which can afterwards more easily be regenerated into nutrients by bacteria, as shown before with nematodes (Hubas et al., 2010) and macrofauna (Werry and Lee, 2005). We suggest that this increase in nutrient recycling, promoted by worms, is another process explaining the stimulation of microphytobenthic growth when these macroorganisms are present.

This stimulation explains the threefold increase of chlorophyll *a* when both diatoms and worms were present together. Simultaneously, the consumption of diatoms by worms in treatment DH maintained the concentration of fatty acid biomarkers at the same level than when diatoms are alone. Indeed, in contrary to chlorophyll *a*, FAs 16:1ω7 and 20:5ω3, or EPA, were of similar abundance in treatments D and DH. Therefore, when worms were present, sediment got enriched by degradation products of chlorophyll and more generally OM, as shown by the total content of FA in sediment (Meziane et al., 1997), which was maximal when both diatoms and worms were present. It is interesting to notice that a similar pattern had already been observed with meiofauna, which was shown to stimulate the development of bacteria and diatoms (Hubas et al., 2010).

4.3. Enhanced sediment adhesives capacity in presence of both diatoms and worms

In addition to bacteria and diatoms, microphytobenthic biofilms are mainly composed of EPS, which are known to be of crucial importance for microorganisms themselves (e. g. protection, attachment), but also for ecosystem functioning (Decho, 1990, 2000). For example, they are able to trap pollutants and provide cues for the settlement of a wide range of organisms (Decho, 1990; Stocum and Plante, 2006; Wotton, 2004). In the present study, colloidal EPS concentrations showed different responses to treatments in terms of protein and carbohydrate contents. Protein levels were similar among treatments: sediment in treatments D, H and DH did not contain more proteins than in the control treatment. We can therefore conclude that neither diatoms, nor bacteria, nor *H. diversicolor* produced a significant amount of extracellular proteins in the time frame of the present experiment.

Moreover, carbohydrate concentrations were significantly higher when both diatoms and worms were present, in comparison to other treatments. This pattern, similar to what was observed for chlorophyll *a* concentrations, suggested that diatoms were the main EPS producers. Indeed, not only diatoms are well known contributors to EPS in soft-bottom environments, but also their EPS are mainly composed of carbohydrates (Hoagland et al., 1993; Underwood et al., 2004). Other possible contributors to EPS secretion in our experiment were bacteria (Decho, 1990; Lundkvist et al., 2007) and also *H. diversicolor*, which produces mucus helping their locomotion and feeding behaviours (Scaps, 2002; Stabili et al., 2011). Yet, bacterial EPS are mainly made of proteins (Bhaskar et al., 2005; Lubarsky et al., 2010), as well as mucus of annelids (Stabili et al., 2011). If one of these organisms was responsible for a significant part of EPS production in our experiment, then an increase in protein concentrations would be expected where these organisms were present, which was not the case in the present study. This confirms that diatoms were the main EPS producers.

Two complementary hypotheses can explain the increase in carbohydrate concentrations when diatoms were cultured with worms. First, an increase of photosynthetic biomass was demonstrated in this treatment; secondly, worms can modulate the EPS secretion by each diatom (Czaczyk and Myszka, 2007; Wotton, 2004). Indeed, EPS production depends on a large range of variables, such as nutrient concentrations in the environment (Decho, 1990; Underwood and Paterson, 2003; Underwood et al., 2004), which was modified when worms were present.

Sediment adhesive ability, as measured by MagPI, is a proxy for sediment stability (Larson et al., 2009; Lubarsky et al., 2010). Sediment adhesive ability varied significantly among treatments at the end of the present experiment, being minimal in control treatment. As demonstrated by treatment H, worms alone increased sediment stability in microcosms, in spite of their expected activity of bioturbation. They are known to compact the sediment and build burrows (Fernandes et al., 2006; Murray et al., 2002), which appeared to increase sediment cohesion in our experiment. Similarly, the development of microphytobenthic biofilms in microcosms intensified sediment adhesive capacities, through EPS production (Stal, 2010; Sutherland et al., 1998; Yallop et al., 2000). These molecules, due to their stickiness, help microorganisms to coagulate with sediment particles, which finally bind these particles together, increasing therefore cohesion inside sediment (de Brouwer et al., 2002, 2005; van Duyl et al., 2000). When both organisms were present, all individual effects added, and stabilisation was further increased as diatom development and EPS secretion were stimulated by worms. Therefore, we need to reconsider our initial hypotheses as worms do not only consolidate sediment by their own secretions; they also stimulate microphytobenthic development, which further increases sediment adhesive ability through extra EPS secretion.

5. Conclusion

In conclusion, our study demonstrates that predicting the effect of macrofauna on microphytobenthic biofilms and their associated influence of sediment stability is not straightforward. Even if the consumption of diatoms by *H. diversicolor* was significant, they stimulated, through an

increased input of nutrients in the environment, the proliferation of microphytobenthos. Biofilm development was also stimulated in terms of secretion of exopolymers, which in turn affected sediment properties such as cohesion. Also, *H. diversicolor* had a dual effect on sediment surface adhesion; directly through the consolidation of sediment, and indirectly through the stimulation of biofilm development. Further investigations may help in elucidating the influence of diverse organisms and their interactions on sediment stability. For instance, although bacteria were not a significant contributor to EPS secretion in our experiment, their presence considerably facilitates the development of microphytobenthic biofilms on sediment (Bruckner et al., 2008; Buhmann et al., 2012; Schäfer et al., 2002). Meiofauna is also known to stimulate microbial development and EPS secretion (Hubas et al., 2010), but the combined effect of meiofauna and macrofauna on biostabilisation has still to be characterised. Analysing how different types of organisms and stabilising mechanisms interact to modulate both the development and functioning of microbial communities may help in understanding the properties determining sediment stability and the ecosystem function of habitat stabilisation (Gingold et al., 2011; Paterson et al., 2009).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2012.10.005.

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