

Trophic role of large benthic sulfur bacteria in mangrove sediment

Pierre-Yves Pascal^{1,*}, Stanislas Dubois², Henricus T. S. Boschker³, Olivier Gros¹

¹Département de Biologie, Université des Antilles et de la Guyane, UMR 7138 UPMC-CNRS-MNHN-IRD, Equipe 'biologie de la mangrove', UFR des Sciences Exactes et Naturelles, BP 592, 97159 Pointe-à-Pitre, Guadeloupe, France

²IFREMER, DYNECO Laboratoire d'Ecologie Benthique, 29280 Plouzané, France

³Royal Netherlands Institute of Sea Research (NIOZ), PO Box 140, 4400 AC Yerseke, The Netherlands

ABSTRACT: Large filamentous sulfur-oxidizing bacteria belonging to the *Beggiatoaceae* family can cover large portions of shallow marine sediments surrounding mangroves in Guadeloupe (French West Indies). In order to assess the importance of *Beggiatoa* mats as an infaunal food source, observations were conducted of the area within mats and at increasing distances from mats. We used natural isotopic compositions and a ¹³C enrichment study. Both revealed an ingestion of bacterial mats by associated meiofauna, dominated by rotifers and to a smaller extent by small polychaetes and nematodes. Compared to adjacent sites, sediment covered by bacterial mats presented a higher abundance of diatoms, whereas the total biomass of bacteria did not vary. This constant bacterial abundance suggests that the proportion of organic matter represented by sulfur bacteria is limited compared to the fraction of total bacteria. There was no significant difference in infaunal abundance in mats, suggesting that the availability of this chemosynthetic food resource had a limited local effect. Grazers presented a $\delta^{13}\text{C}$ value increasing with distance from the mat. However, isotopic composition of phospholipid-derived fatty acids specific for diatoms and bacteria revealed that this change is related to modifications of $\delta^{13}\text{C}$ dietary components rather than to changes in diet composition. These complementary methods revealed that the occurrence of sulfur-oxidizing bacterial mats does not necessarily affect grazer abundance and importance of bacteria in their diet. Despite its wide occurrence, *Beggiatoa* mats would consequently have a minor influence on the structure of the mangrove food web.

KEY WORDS: *Beggiatoa* · Mangrove · Benthic food web · Sulfur bacteria · Meiofauna · Nematode · Rotifers · Stable isotope

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INTRODUCTION

Bacteria are very important for the structure and functioning of all ecosystems due to their role in organic matter degradation and nutrient cycling. Bacteria can also be grazed and may play a major role in food webs as a food source (Sherr et al. 1987). This trophic role has been well established in pelagic environments, but, due to technical difficulties, the trophic role of bacteria is less well known in benthic systems (Kemp 1990). Outside of hydrothermal vent systems, studies focusing on the benthic bacterial

compartment suggest that grazing is <30% of the bacterial production in several marine environments such as intertidal mudflats (van Oevelen et al. 2006a, Pascal et al. 2009), shallow-water sands (Sundbäck et al. 1996) and deep-sea sediments (Gontikaki et al. 2011). The bacterial contribution to grazers' diet has also received little attention. This role is potentially limited as meiofauna would derive <10% of their total carbon demand from bacteria in estuarine (van Oevelen et al. 2006a,b) and deep-sea environments (Gontikaki et al. 2011). The majority of studies suggest a role <11% for macrofauna (van Oevelen et al.

*Corresponding author: pypascal@univ-ag.fr

2006b), even in cases where a contribution of 50% was assessed for deep-sea macrofauna (Gontikaki et al. 2011). Previous grazing experiments performed simultaneously with dual-labeled food items (bacteria and diatoms) allowed the evaluation of ingestion selectivity by meio- and macrograzers; small meiofauna appeared to have a better selection efficiency due to their size and to preferentially ingest benthic microalgae as compared to less selective macrofauna (Pascal et al. 2008, 2013).

Several reasons potentially explain why bacteria do not constitute a major food resource and are not preferentially ingested by benthic browsers. Firstly, bacteria may lack essential components such as fatty acids that are present in diatoms and other microalgae (Zhukova & Kharlamenko 1999). Secondly, bacteria and diatoms can differ in their spatial distribution and ultimately in their availability. Most studies have been performed in the intertidal environment, with benthic microalgae concentrated at the air–sediment interface during low tide, whereas bacteria are distributed more homogeneously over a vertical gradient (Joint et al. 1982). Finally, most benthic bacteria are attached to sediment particles, in contrast to benthic microalgae, and feeding on microalgal biofilm would hence save energy by eliminating the need to (1) select food particles, for selective feeders, or (2) reject non-digestible material, for non-selective feeders. In contrast, feeding on bacteria would cost more energy for all grazer feeding modes.

As they form filaments reaching 200 μm in diameter, the white sulfur-oxidizing bacteria belonging to the family *Beggiatoaceae* are among the largest prokaryotic organisms (Larkin et al. 1994). High concentrations of sulfide are produced in their habitat, and *Beggiatoa* cells obtain their energy from the oxidation of sulfide to sulfate. They can also produce elemental sulfur, stored as internal granules that are located mostly in the periplasm, explaining their white appearance (Schulz & Jørgensen 2001). These bacteria inhabit the interface between anoxic sediments and oxic water and form mats that can reach 3 cm in thickness, are characterized by a patchy spatial distribution (Lloyd et al. 2010) and are typically located in quiet waters, in sediment with high organic matter loading or at sulfide seeps (Montagna & Spies 1985). They are found in a large variety of freshwater as well as marine environments: in deep-sea mud volcanoes and hydrothermal vents, around seeps of hydrocarbons and methane and below productive upwelling areas, and they have also been observed in shallow waters in polar (Van Gaever et

al. 2006) and temperate (Fenchel & Bernard 1995) environments, where they have been regarded as an indicator of organic enrichment (Elliott et al. 2006).

Beggiatoa are highly vacuolated and represent a small amount of dry matter (Bernard & Fenchel 1995). Despite this apparently unfavorable characteristic, *Beggiatoa* seem to play an important role in the food web, as many protozoan species depend on sulfur-oxidizing bacteria for food; ciliates are assumed to be the main grazers of *Beggiatoa* (Bernard & Fenchel 1995). Isotopic composition and high abundances of meiofaunal grazers in bacterial mats both suggest that sulfur bacteria are ingested in numerous deep-sea environments (Spies & DesMarais 1983, Van Gaever et al. 2006, Pape et al. 2011). In contrast to deep-sea environments, the importance of bacterial mats for meio- and macrofauna in coastal food webs remains to be investigated (Bernard & Fenchel 1995).

The aim of the present study was to determine if bacterial consumption by benthic organisms was increased when bacteria were concentrated in a *Beggiatoa* mat. The importance of bacteria as a potential food source for meio- and macrofauna was estimated here in a Caribbean mangrove forest using complementary methods. Abundance of infauna was evaluated, as well as natural carbon and nitrogen isotopic composition of potential grazers and their food source, along a spatial gradient of increasing distance from *Beggiatoa* mats. ^{13}C enrichments were made to enhance differences in isotopic compositions between the *Beggiatoa* mat and other food items. To our knowledge, this study is the first observation of *Beggiatoa* in mangrove environments. Due to their fragility, most meiofaunal taxa do not withstand sieving, fixation, or freezing techniques. However, permanent access to the study site allowed us to work with living animals and to consider those organisms largely neglected in food web studies.

MATERIALS AND METHODS

Study area

'Manche à eau' is a small tropical lagoon connected to the marine channel 'Rivière Salée' separating the 2 main islands of Guadeloupe (French West Indies) (Fig. 1). In this lagoon, tides are semidiurnal, with a mean tidal amplitude of 30 cm (tide gauge of Pointe-à-Pitre, REFMAR®). Temperature and salinity at below 0.5 m depth are relatively constant, with average values of 28°C and 35, respectively.

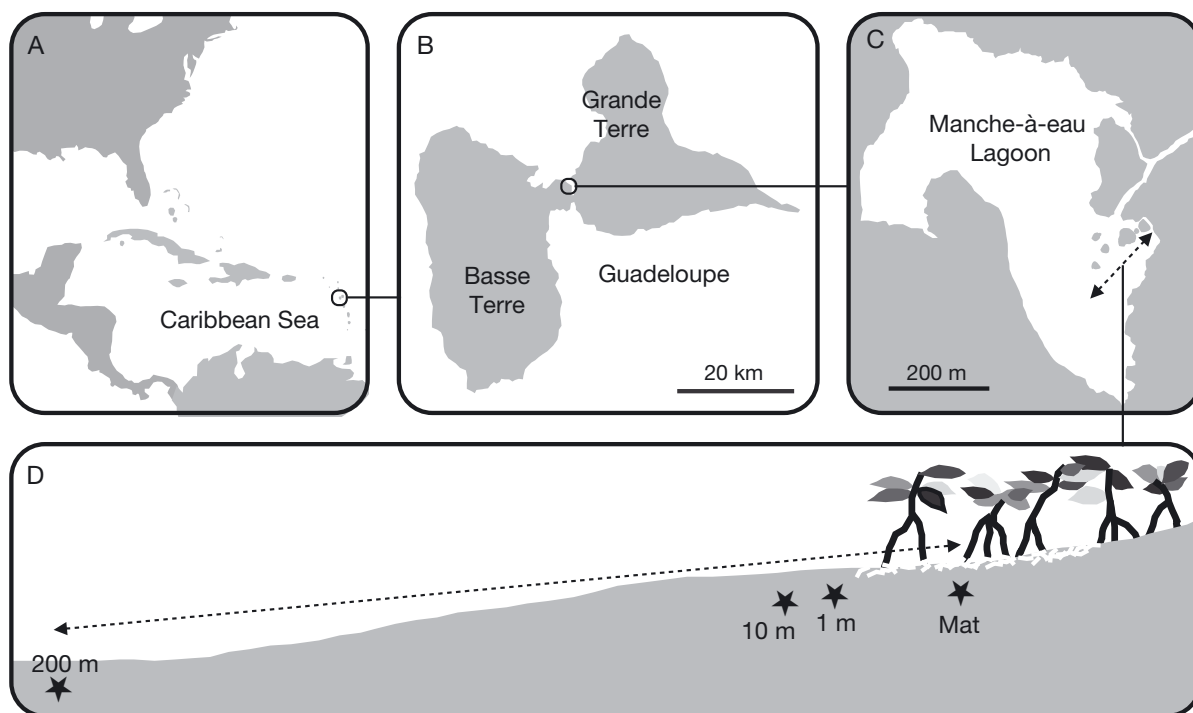


Fig. 1. (A) Location of Guadeloupe Island in the Caribbean Sea, (B) location of Manche-à-eau lagoon on Guadeloupe, (C) location of sampling transect and (D) schematic view of 4 sampling points along the transect, i.e. inside *Beggiatoa* mats and 1, 10 and 200 m away from mats (not drawn to scale)

The lagoon is bordered by a mangrove forest dominated by *Rhizophora mangle*. The sediment (<1 m depth) between mangrove tree roots is anoxic and contains high sulfide concentrations (Maurin 2009). In some places, the sediment is covered by large patches of dense and conspicuous (20 to 60 μm diameter) filamentous white sulfur bacteria, visible with the naked eye (Fig. 2, almost exclusively large *Beggiatoa* spp.). The size of these bacterial patches is temporally highly variable, with a diameter measuring from a few centimeters to several meters. High numbers of interstitial organisms such as ciliates, nematodes and turbellarians are associated with the mats.

Samples were collected by snorkeling in October 2011 at 4 different locations along a transect: inside a *Beggiatoa* mat (hereafter called 0 m station) and 1, 10 and 200 m away from the mats. Water depth along the transect ranged between 0.5 m (at the mat) and 2 m (200 m away) (Fig. 1). When collected, bacterial patches measured approximately 1 m width and were located 1 m away from the edge of the mangrove forest. At each location, 3 replicate samples were randomly collected. For stable isotope and abundance analyses, each sample consisted of 10 pooled cores of the 0–1 cm layer, collected with a



Fig. 2. Mats of white benthic filamentous sulfur bacteria between mangrove tree roots (water depth = 1 m)

syringe pushed gently into the sediment to avoid sediment suspension (inside core diameter = 5.5 cm). For abundance of rotifers, polychaetes, copepods and nematodes, each sample was sieved and the fraction remaining on 63 μm mesh was separated equally into different aliquots using a Motoda splitting box (Motoda 1959). This protocol allows reporting results per unit surface area.

Species identification

The most abundant meiofaunal species were isolated and gathered according to morphology. When morphological traits were inconspicuous using a dissecting scope, species were pooled. This study consequently integrates different taxonomic levels. Abundant colonial ciliates were identified as the family Vorticellidae (hereafter called vortical). Nematodes and copepods were identified to phylum and subclass levels, respectively. Rotifers and gnathostomulida species were identified using morphological traits as *Rotaria* spp. and *Haplognathia ruberrima*. The 2 abundant platyhelminth species were identified using molecular approaches as *Macrostomum* sp. and *Polycanthus* sp.; DNA was extracted from freshly collected specimens using DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions. The 18S rDNA markers were amplified using primers 1F and 5R. PCR products were purified with QIAquick PCR purification kit (Qiagen) and directly sequenced by Genoscreen. The 18S rDNA gene sequences obtained were compared with the National Center of Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

Abundance and isotopic composition

Sediment was freeze-dried, phospholipid-derived fatty acids (PLFA) were extracted and their isotopic composition was determined using a gas-chromatograph combustion-interface isotope-ratio mass spectrometer (GC-c-IRMS) following the protocol in Boschker et al. (1999). Concentrations and $\delta^{13}\text{C}$ PLFA specific to bacteria (i14:0, i15:0, ai15:0, i16:0, C18:1 ω 7c and cy19:0), diatoms (C20:4 ω 6, C20:5 ω 3, C22:5 ω 3 and C22:6 ω 3) and cyanobacteria (C18:2 ω 6c, C18:3 ω 3, C18:4 ω 3) were used to estimate the relative contribution of these groups to the total PLFA pool and their weighted-average $\delta^{13}\text{C}$ composition. The carbon content of bacteria and diatoms was evaluated using carbon PLFA/carbon biomass ratios of 0.056 and 0.035, respectively (Boschker & Middelburg 2002).

Polychaetes, nematodes, copepods and rotifers were extracted from sediment using Ludox HS40 (de Jonge & Bouwman 1977). For abundance evaluations, samples were fixed with 2% formalin and stained with Rose Bengal. For stable isotope analysis, sediment was frozen and 150 *Ceratocephale* sp., 700 nematodes, 100 copepods and 1500 rotifers were haphazardly removed from each sample. Several

protocols were applied to extract potential prey and infauna from the sediment. Sediment sampled from *Beggiatoa* mats was allowed to settle a few minutes in the laboratory until a new bacterial biofilm formed; diatoms and filamentous sulfur-oxidizing bacteria were individually picked alive and cleaned of debris under a dissecting microscope. A similar protocol was used to collect 150 *Macrostomum* sp., 60 *Haplognathia ruberrima* and 2000 vorticels. In sediment without bacterial mats, white specimens of *Polycanthus* sp. were easily identified against the dark sediment, and, for each sample, ≥ 150 specimens were live picked using a glass pipette. Macrofaunal specimens of ragged sea hare (*Bursatella leachii*) were collected in the field and starved overnight to clear gut contents. For each sample, 12 specimens were homogenized using a blender, freeze-dried, and a fraction of the sample was used for stable isotope analyses. The C/N ratio and isotopic composition of bulk sediment containing bacteria and diatoms was determined for each sample from untreated sub-samples for ^{15}N content and from acid (1 M HCl)-treated sub-samples for ^{13}C content. Using mass-balance equations, isotopic compositions and abundances of bacteria and diatoms evaluated with PLFA were used to calculate isotopic composition of detritus free of bacteria and diatoms.

Isotope samples were analyzed at the Isotope Facility at the University of California, Davis, using an elemental-analyzer isotope ratio mass spectrometer. Samples were reported relative to the standards atmospheric N_2 and Vienna PeeDee Belemnite carbon. Stable isotope values are reported in δ notation (in ‰):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Using standards, analytical precision was estimated to 0.2‰ for both ^{13}C and ^{15}N .

Enrichment experiment

To further investigate the consumption of *Beggiatoa* by sediment fauna, we designed a ^{13}C -labeling experiment where chemoautotrophic bacterial mats were selectively labeled in the dark. Sediment from the bacterial mat environment was sampled in January 2012 and placed in 3 circular mesocosms (internal diameter = 23.5 cm with a sediment height of 25 cm). A recirculating system of 4 l water allowed sediment of each tank to be covered by 1 cm of oxygenated lamellar running water. $\text{NaH}^{13}\text{CO}_3$ (>99%

^{13}C -enriched) was added to reach a final concentration of 1 g m^{-2} (Middelburg et al. 2000). Incubations were realized in the dark, at 25°C , salinity 35 and during 4 d. At the end of the incubation, the isotopic compositions of bacteria, nematodes and rotifers were measured with methods previously described.

Data analyses

One-way analysis of variance (ANOVA) was used to test for differences in the C/N ratio of sediment, biomass of bacteria and diatoms and abundance of meiofauna (rotifers, polychaetes, copepods and nematodes). Normality of residuals was tested using Shapiro-Wilk tests before performing ANOVA. When overall ANOVA tests were significant, Tukey tests were used for post hoc comparisons. Unless specified, values are presented as means \pm standard deviations (SD).

A Bayesian isotopic mixing model was used to determine possible contributions of different food items to the diet of infauna found in *Beggiatoa* mats. Isotopic compositions of diatoms and bulk organic matter from sediment were not discriminated from $\delta^{13}\text{C}$ compositions and were averaged into one food source called BOM (bulk organic matter). SIAR (stable isotope analysis in R; Parnell et al. 2010) incorporates the variability of consumers and trophic enrichment factors (TEFs) to produce a mean and a 95% confidence interval of the percent contribution of each source to a consumer. As *Haplognathia ruberrima* graze mainly, if not exclusively on *Beggiatoa* mats (Pascal et al. in press), we used the *a posteriori* isotopic signature of the gnathostomulids to calculate a TEF of $-1.5 \pm 1.0\%$ for $\delta^{13}\text{C}$ of *Beggiatoa* bacteria. For the $\delta^{13}\text{C}$ of BOM, a TEF of $1.1 \pm 0.3\%$ (McCutchan et al. 2003) and, for the $\delta^{15}\text{N}$ of all food sources, a TEF of $3.4 \pm 1.1\%$ were used (Minagawa & Wada 1984).

RESULTS

The *Beggiatoa* mat environment

One species of Platyhelminth was identified as *Macrostomum* sp., as it shows 99.2% similarity with *Macrostomum lignano* (550 bp), and the other species was identified as *Polycanthus* sp., as it presents 98.0% similarity with *Polycanthus torosus* (500 bp). Using morphological traits, the most abun-

dant polychaete was identified as *Ceratocephale* sp. (C. Glasby pers. comm.), which was supported by the 18S rDNA sequence analysis.

Individual weights of infauna were derived from stable isotope samples (Table 1). In the surficial sediment, mean percentage contributions of PLFA specific for bacteria, diatoms and cyanobacteria are presented in Table 2. Expressed in abundances per surface unit, the biomass of bacteria was higher than the biomass of algae (Fig. 3). Meiofauna presented highly variable abundances in the mats' environment (Fig. 4). Among meiofauna enumerated in the samples, rotifers were dominant in biomass ($101.7 \pm 96.1 \text{ mg C m}^{-2}$), followed by *Ceratocephale* sp. ($71.0 \pm 75.6 \text{ mg C m}^{-2}$), nematodes ($15.4 \pm 9.1 \text{ mg C m}^{-2}$) and copepods ($1.5 \pm 2.5 \text{ mg C m}^{-2}$).

Of all food sources, *Beggiatoa* were the most depleted in ^{13}C , whereas diatoms were the most enriched (Fig. 5). Sediment detritus had a carbon isotopic composition close to that of diatoms, and, among potential grazers, *Haplognathia ruberrima* was the most ^{13}C depleted, whereas *B. leachii* was the most enriched. All other meiofaunal members presented a $\delta^{13}\text{C}$ varying between -26.2 and -21.9% . Diatoms were the food source with the lowest $\delta^{15}\text{N}$ value, while detritus and *Beggiatoa* were more enriched in ^{15}N . All fauna were enriched in ^{15}N compared to the food sources, with $\delta^{13}\text{C}$ isotope signatures ranging between 4.06 and 8.94‰ (Fig. 5).

Table 1. Individual infaunal weights derived from the weights of stable isotope samples

	Number of specimens examined (triplicate samples)	Weight per specimen
Rotifers	3 \times 1500	158 \pm 70 ng
Copepods	3 \times 100	685 \pm 233 ng
Nematodes	3 \times 700	789 \pm 241 ng
<i>Macrostomum</i> sp.	3 \times 150	2.79 \pm 0.12 μg
<i>Ceratocephale</i> sp.	3 \times 100	2.98 \pm 0.86 μg
<i>Haplognathia ruberrima</i>	3 \times 60	3.94 \pm 2.65 μg
<i>Bursatella leachii</i>	3 \times 12	2.02 \pm 0.33 g

Table 2. Contributions of phospholipid-derived fatty acids specific for bacteria, diatoms and cyanobacteria to total fatty acid methyl esters along transect stations (% , means \pm SD, n = 3)

	Mat	1 m	10 m	200 m
Bacteria	25.1 \pm 2.0	27.1 \pm 2.6	21.1 \pm 3.2	25.1 \pm 0.4
Diatoms	8.1 \pm 2.0	6.5 \pm 0.8	6.6 \pm 1.3	3.5 \pm 1.0
Cyanobacteria	2.4 \pm 0.3	2.5 \pm 0.2	2.2 \pm 0.2	1.7 \pm 0.0

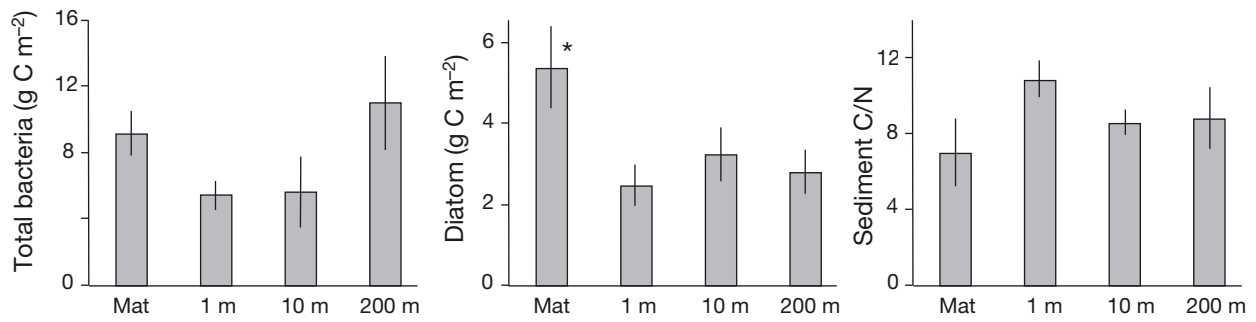


Fig. 3. Biomass of bacteria and diatoms (in g C m^{-2}) and carbon/nitrogen ratio of surficial sediment (1 cm) along transect stations (means \pm SD, $n = 3$). *: significant differences ($p < 0.01$; ANOVA; Tukey test)

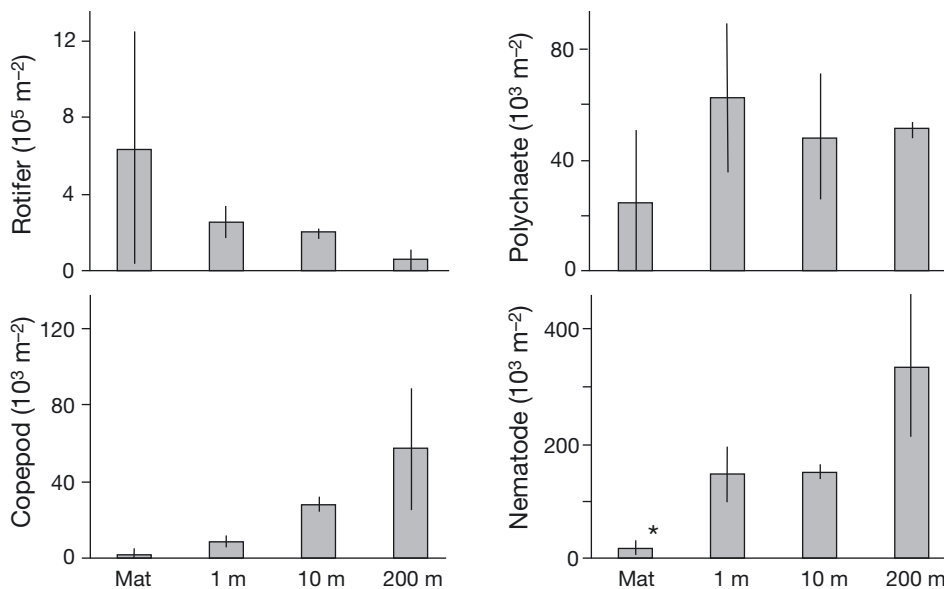


Fig. 4. Abundances of meiofauna (rotifers in 10^5 m^{-2} and polychaetes, copepods and nematodes in 10^3 m^{-2}) in surficial sediment (1 cm) along transect stations (means \pm SD, $n = 3$). *: significant differences ($p < 0.01$; ANOVA; Tukey test)

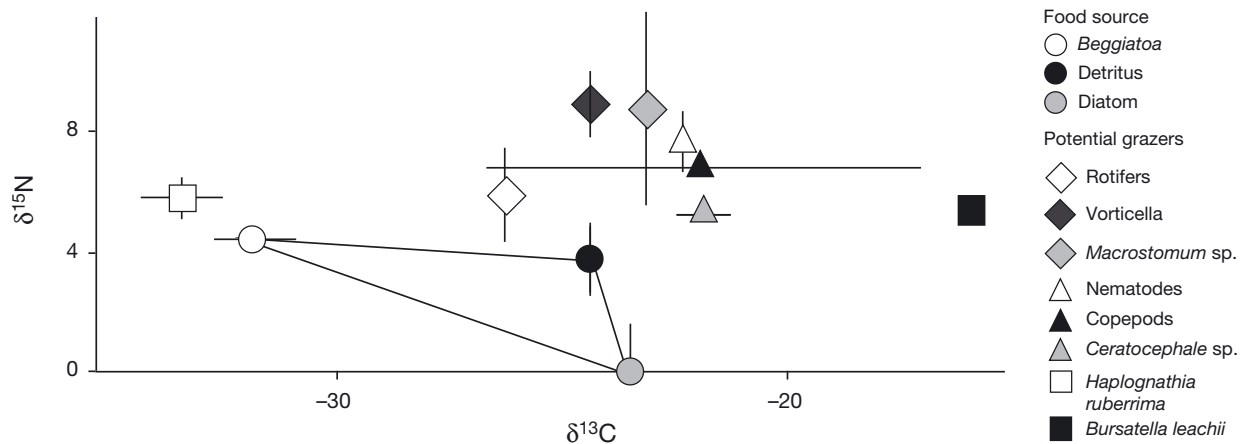


Fig. 5. Natural isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of food sources (*Beggiaia*, diatoms, bulk sediment [detritus]) and meiofaunal (rotifer, vorticel, *Macrostomum* sp., nematode, copepod, *Ceratocephale* sp., *Haplognathia ruberrima*) and macrofaunal (*Bursatella leachii*) potential grazers. Means (\pm SD; $n = 3$) are reported

The diet composition of *B. leachii* could not be resolved based on isotope compositions. SIAR outputs suggested that all other meiofaunal grazers were ingesting *Beggiatoa*, but in different proportions. Grazers with the highest contribution of the bacterial mats in their diet were *H. rubberima*, copepods, rotifers and nematodes, with respective mean percentages of 86, 41, 28 and 22%. *Ceratocephale* sp., vorticels and *Macrostomum* sp. ingested less *Beggiatoa* material with respective mean percentages of 16, 12 and 5% (Fig. 6).

Complementary enrichment experiments with $\text{NaH}^{13}\text{CO}_3$ led to ^{13}C enrichment of *Beggiatoa* with $\delta^{13}\text{C}$ increasing from -31.7 to 1693‰ (Table 3). Nematodes and rotifers incubated with these enriched bacteria both showed an increase in ^{13}C content. Isotopic compositions of potential grazers and food sources in ^{13}C -enriched conditions were also used to run a SIAR mixing model. Model outputs with enrichment conditions confirmed a contribution of *Beggiatoa* in grazer diets. This contribution was analogous in enriched versus control conditions for nematodes (23 vs. 24%) and rotifers (27 vs. 28%). Similarly, contributions of other food items to the diet did not differ between control and enriched conditions (Table 3).

Environments adjacent to *Beggiatoa* mats

Along the transect at 1, 10 and 200 m from *Beggiatoa* mats, the total biomass of bacteria was not significantly affected by the presence of *Beggiatoa* mats (Fig. 3). Conversely, microalgal biomass was significantly higher in *Beggiatoa* mats than at all other stations (Fig. 3). Cyanobacteria were always less abundant than bacteria and diatoms (Table 2). Differences in detritus C/N ratios were not significant between stations.

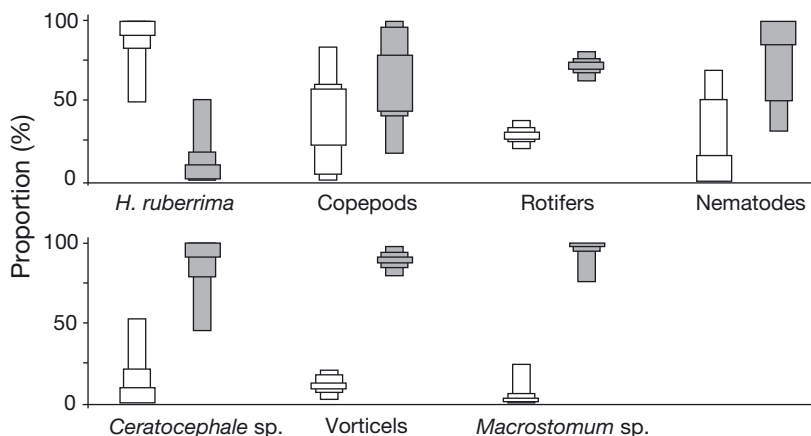


Fig. 6. Contribution (%) of different food items (white: *Beggiatoa*; grey: bulk organic matter) to the diet of meiofaunal grazers (the gnathostomulid *Haplognathia ruberrima*, copepods, rotifers, nematodes, the polychaete *Ceratocephale* sp., vorticels, the plathyhelminth *Macrostomum* sp.). Results were issued with the SIAR (stable isotope analysis in R) mixing model. For each source 95, 75 and 25% credibility intervals of probability distributions were reported

Table 3. Isotopic composition ($\delta^{13}\text{C}$) in the enrichment experiment (means \pm SD). Mean contributions (%) and 95% confidence intervals of different food items (*Beggiatoa*, diatoms and detritus) to the diet of nematodes and rotifers based on the SIAR (stable isotope analysis in R) mixing model

	Control	Enrichment experiment
$\delta^{13}\text{C}$ (‰)		
<i>Beggiatoa</i>	-31.7 ± 0.9	1693.2 ± 275.5
Nematodes	-22.3 ± 0.3	338.8 ± 65.9
Rotifers	-26.2 ± 0.6	389.3 ± 95.9
Nematode diet composition (%)		
<i>Beggiatoa</i>	24 (0–68)	23 (11–38)
Diatoms and detritus	75 (31–100)	77 (62–89)
Rotifer diet composition (%)		
<i>Beggiatoa</i>	28 (18–38)	27 (6–51)
Diatoms and detritus	71 (61–81)	72 (49–94)

Abundances of nematodes and copepods tended to increase, whereas abundances of rotifers tended to decrease with increasing distance from mats. However, none of those differences were significant except that nematodes were significantly less abundant in mats (Fig. 4). The variability in rotifer, polychaete, copepod and nematode abundances was higher in mats, where SD reached 106% of the mean value. Individual biomasses of grazers were derived from weights of stable isotope samples and were not significantly different among stations for nematodes, copepods, *Polycanthus* sp., or *Bursatella leachii*. There was no clear trend in nitrogen isotopic composition of grazers along the transect (Fig. 7). Carbon isotopic compositions in all grazers decreased with increasing distances from mats (Fig. 7). The difference in grazer $\delta^{13}\text{C}$ between 200 m and mat stations was higher for copepods (9.3‰) and nematodes (4.4‰) but was lower for *B. leachii* (0.6‰) (Fig. 7). The isotopic composition of PLFA revealed gradual ^{13}C enrichment of both bacteria and diatoms along

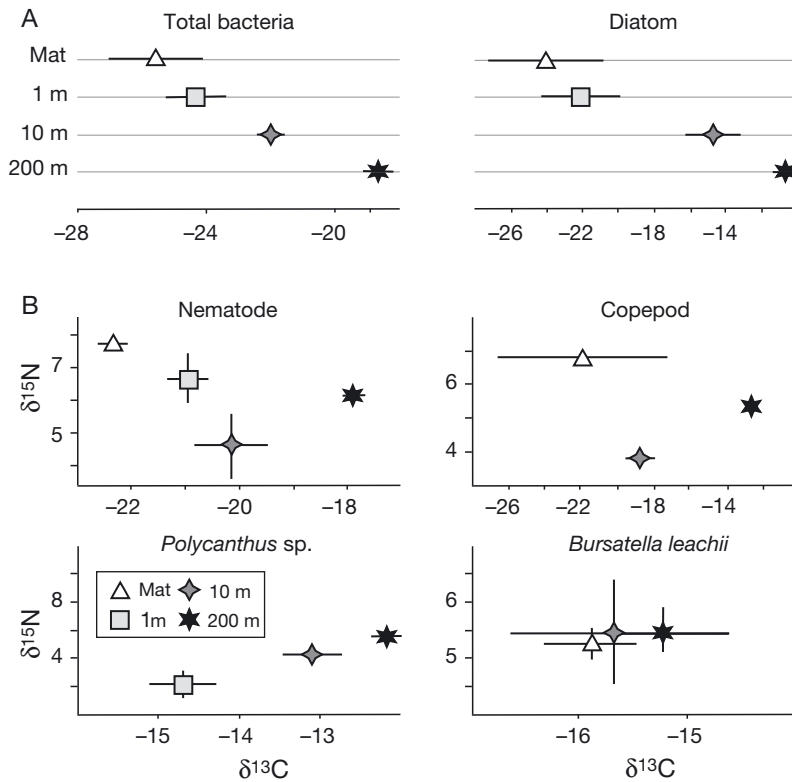


Fig. 7. Natural isotopic composition of (A) bacterial and algal phospholipid-derived fatty acids ($\delta^{13}\text{C}$) and (B) potential grazers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of nematodes, copepods, *Polycanthus* sp. and *Bursatella leachii*), along transect stations (means \pm SD, $n = 3$)

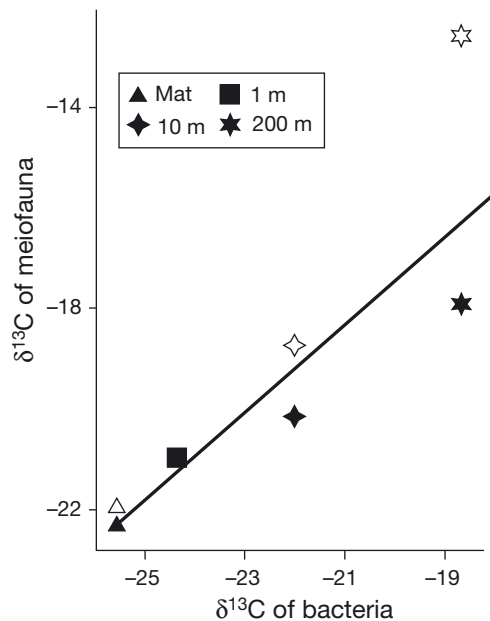


Fig. 8. Carbon natural isotopic compositions of meiofauna (white symbols: copepods; black symbols: nematodes) according to the $\delta^{13}\text{C}$ of bacterial phospholipid-derived fatty acids along transect stations. The theoretical line represents the variation in identical amounts of bacteria and meiofauna

the transect away from the mat (Fig. 7). The decrease in $\delta^{13}\text{C}$ from the 200 m station to the mat station was smaller for bacteria (6.9‰) than for algae (13.3‰) (Fig. 7). The $\delta^{13}\text{C}$ of bacteria and of meiofaunal grazers covaried; both values showing similar increases with respect to distances from the *Beggiatoa* mat (Fig. 8).

DISCUSSION

The simplest approach to determine trophic linkages between bacteria and grazers is to compare their respective distributions in natural environments. Interpretation of these results can be difficult as grazers potentially affect bacterial dynamics through processes other than grazing. For instance, nematodes have been reported to favor development of *Beggiatoa* mats as they increase oxygen penetration and nutrient diffusion into bacterial mats (Salvadó et al. 2004). The use of stable isotopes is an increasingly applied alternative approach to investigate trophic interactions. The full potential of stable isotopes is tightly linked to the

discrimination of potential food sources in terms of isotopic compositions. In the studied mangrove environment, *Beggiatoa* are distinct from diatoms as they present a lower $\delta^{13}\text{C}$ due to their specific pathways for carbon metabolism (Güde et al. 1981). One way to increase the power of stable isotope techniques is to artificially enhance differences in isotopic compositions of food items for better determination of their contribution in food webs (van Oevelen et al. 2006a,b, Galván et al. 2008). In the present study, the sediment contained mangrove tree detritus, mostly coming from *Rhizophora mangle* bordering the mangrove forest seaward, and this tree material is depleted in ^{13}C with values reaching -30‰ (A. Mothet pers. comm.). The $\delta^{13}\text{C}$ of mat bulk sediment does not express variability in the composition of each detrital compound as it presents an average value of -24‰ . Selective ingestion of depleted detritus by browsers would overestimate the contribution of depleted *Beggiatoa* in their diet. To increase the power of discrimination among sources, an enrichment experiment was performed in the dark to modify the isotopic composition of bacteria, but not of diatoms and detritus.

Natural isotopic compositions revealed that *Beggiatoa* was ingested by all studied grazers, with a variable contribution of this food item according to infaunal species. For 2 of those grazers, this conclusion was corroborated by the enrichment experiment, revealing similar contribution rates of *Beggiatoa* in their diets.

Meiofaunal dwelling in *Beggiatoa* mats was largely dominated in both abundance and biomass by rotifers. This dominance was observed throughout a year-round survey (Pascal et al. unpubl. data). This result was unexpected as most species of benthic rotifers were described in freshwater and limno-terrestrial environments (Schmid-Araya 1998), but rotifers in marine environments have received far less attention. Rotifer species were reported in a hypersaline brine channel of Arctic sea ice, suggesting their ability to colonize extreme environments (Friedrich & deSmet 2000). Their occasional occurrence in anoxic and sulfidic marine environments has previously been observed (Fenchel & Riedl 1970, Bernard & Fenchel 1995). More recently high rotifer abundances were observed at 800 m water depth colonizing surficial gas hydrates (Sommer et al. 2003, 2007), where oxidation of methane leads to the production of large amounts of sulfide, which ultimately favors the growth of sulfidic bacteria like *Beggiatoa*. Similar to deep-sea gas hydrates, rotifers in the present study dominated the meiofaunal community when sulfide concentrations were high and presented a high average abundance: 5.3×10^5 (Sommer et al. 2003) versus 6.4×10^5 ind. m^{-2} in the present study. However, other deep-sea investigations of methane seeps revealed absence (Pape et al. 2011) or marginal (Hauquier et al. 2011) density of rotifers. Guilini et al. (2012) considered that rotifers observed in the studies by Sommer et al. (2003, 2007) might have resulted from artificial contamination by tap water. Such contamination was unlikely in the present study as rotifer specimens were observed directly in untreated sediment. Sommer et al. (2007) suggested that high variations in rotifer abundances could be explained by migration between oxygenated surface sediment and deeper zones to escape predation by nematodes. Little is known about the feeding ecology of marine rotifers, and most species have a ventral ciliated field used to scrape the biofilm of bacteria, fungi and diatoms (Schmid-Araya 1998). Uptake of sulfur-oxidizing bacteria by rotifers in sulfidic environments has previously been suggested (Fenchel & Riedl 1970), and their highest abundance in *Beggiatoa* mats has been explained by sulfur-oxidizing bacteria consumption

(Sommer et al. 2007). In our study, isotopic compositions under both natural abundance and enriched conditions suggested a substantial contribution of *Beggiatoa* in the rotifers' diet. *Beggiatoa* would not be an obligatory feeding resource as rotifers were found in environments adjacent to *Beggiatoa* mats where they may depend on organic carbon from the overlying water column (Sommer et al. 2007).

Polychaetes dwelling in *Beggiatoa* mats of mangrove sediments are members of the meiofauna, and their stable isotopic composition also suggests potential ingestion of sulfur-oxidizing bacteria. Consumption of filamentous sulfur-oxidizing bacteria from methane seeps by macrofaunal dorvilleid polychaetes was previously revealed by direct observation of gut content and stable isotope analysis (Levin & Michener 2002).

Nematodes are the third dominant members of the meiofauna in *Beggiatoa* mats. Our stable isotope analyses in natural and enriched conditions supported the consumption of sulfur bacteria by the nematode community. According to previous investigations, nematodes are the dominant taxonomic group in shallow-sediment *Beggiatoa* mats (Montagna et al. 1989, Bernard & Fenchel 1995), as well as in deep-sea environments (Van Gaever et al. 2006, Pape et al. 2011). Ingestion of filamentous sulfur-oxidizing bacteria by nematodes has been observed (Bernard & Fenchel 1995) and also revealed by their isotopic composition (Spies & DesMarais 1983). At an Arctic mud volcano, the proliferation of a single species of nematodes in *Beggiatoa* mats was attributed to the feeding on sulfur bacteria, with a trophic specialization uncommon among meiofaunal organisms (Van Gaever et al. 2006).

Copepods usually represent <1% of the total meiofauna in bacterial sulfide-oxidizing mats (Fenchel & Riedl 1970, Bernard & Fenchel 1995), whereas their dominance is higher in adjacent habitats (Montagna & Spies 1985, Powell et al. 1986, Van Gaever et al. 2006, Sommer et al. 2007). Similarly, copepods presented lower abundances in the present study. This limited abundance of copepods is probably due to a low tolerance to anoxia and sulfide (Levin et al. 1991).

In sulfide seeps, groups of plathylminths, aschelminths and gnathostomulids constitute a high fraction of the meiofaunal community, whereas this contribution is limited in adjacent non-sulfidic sediments (Powell et al. 1986). Abundances of those groups were not evaluated in the present study, but their stable isotope compositions suggested a contribution of *Beggiatoa* to their diets. The high ^{13}C depletion of the

gnathostomulidae *Haplognathia ruberrima* does not appear to be due to endo- or ectosymbioses with sulfur-oxidizing bacteria but to be related to selective ingestion of sulfur-oxidizing bacteria (Pascal et al. in press). *Macrostomum lignano* can easily be cultured with diatoms in experimental conditions (Ladurner et al. 2005), and this turbellarian species is a model organism classically used to investigate hermaphroditic reproduction, developmental biology and ageing (Schärer et al. 2004). *Macrostomum* spp. are not strictly herbivores as, in the present study, they were seen consuming large filamentous sulfur-oxidizing bacteria, and their stable isotope composition suggests this is not an uncommon feeding behavior (P. Y. Pascal pers. obs.).

The ragged sea hare *Bursatella leachii* is a key benthic component of the macrofaunal community of mangrove habitats. They have a daily rhythm and an aggregative distribution in shallow subtidal waters (Ramos et al. 1995). *B. leachii* is a generalist grazer of a wide variety of macroalgae and benthic cyanobacteria (Ramos et al. 1995). In the Manche à Eau system, their abundance was highly variable, with temporal peak densities showing individuals grazing upon *Beggiatoa* mats as well as surficial sediment in adjacent benthic environments (P. Y. Pascal pers. obs.). The very heavy $\delta^{13}\text{C}$ signal of *B. leachii* revealed that this gastropod migrates and feeds outside the mangrove forest, given their more enriched $\delta^{13}\text{C}$ composition (Finlay & Kendall 2007). Sulfur bacteria may represent one of the potential food sources ingested by *B. leachii* but may possibly only be consumed ephemerally when this mollusk is swarming in the mangrove environment.

The variability in infaunal abundance in the studied mangrove mats was higher than in the adjacent sediments. Similarly, high variations in meiofaunal abundances have been revealed in other bacterial mats from seeps in coastal (Montagna & Spies 1985) and deep-sea environments (Van Gaever et al. 2006). Our results supported the idea that sulfide systems are heterogeneous environments characterized by highly variable geochemistry.

Beggiatoa mat sediments were characterized by a higher abundance of diatoms than adjacent sediments. Most studies focusing on free-living sulfur-oxidizing bacteria like *Beggiatoa* were done in deep-sea environments where primary producers are absent. Bacterial mats in a coastal petroleum seep (15 m water depth) also present high chlorophyll *a* concentrations, even if the dominance of phaeophytin *a* indicates stressed and decaying microalgal populations (Montagna & Spies 1985). Similarly, high abun-

dances of diatoms were reported in sulfur-oxidizing bacterial mats in shallow-water (6.5 m water depth) sediments in Denmark (Bernard & Fenchel 1995) and in deeper sediments (60 m water depth) in the Gulf of Mexico (Powell et al. 1986). These high abundances may reflect the tolerance of benthic diatoms to relatively high sulfide concentrations (Admiraal & Peletier 1979) and possibly higher nutrient availability at mat sites given the very high mineralization rates in mangrove sediments (Bouillon et al. 2008).

The transect investigated in this study showed that bacterial biomass was not significantly higher in *Beggiatoa* mat sediments than in those without such mats. The limited contribution of *Beggiatoa* to total bacterial carbon was also suggested by differences in their respective $\delta^{13}\text{C}$ values. Unlike other bacteria, *Beggiatoa* cells only have 2% of their biovolume consisting of active cytoplasm (Schulz & Jørgensen 2001), and, as they are strongly vacuolated, they represent a considerably smaller amount of dry matter than suggested by their volume (Bernard & Fenchel 1995). *Beggiatoa* biomass is consequently negligible compared to the total amount of bacteria found in the surficial sediment.

The concentration of bacteria in mats likely makes them easier for grazers to feed upon. However, none of the studied infaunal species exhibited a significantly higher abundance in *Beggiatoa* mat sediments. This constant abundance could hide changes in community composition, with an increased abundance of species specialized in *Beggiatoa* consumption. However, compartments linked with bacteria remain unchanged, and the general food-web structure is not strongly influenced by the presence of *Beggiatoa*. Similar enrichment levels in the ^{13}C of bacteria and ^{13}C of meiofauna suggest a constant contribution of bacteria to the diet of meiofauna along the transect. Consequently, complementary approaches utilizing natural and enriched stable isotopes suggest that the global bacterial food role is not increased by the presence of *Beggiatoa*. Most studies suggest that deep-sea microbial mats increase standing stocks of micro-, meio- and macrobenthic communities (Levin 2005). Isotopic data have revealed that the contribution of chemosynthetic carbon to the diet of benthic species increases with depth and the absence of photosynthetic primary production (Levin & Michener 2002, Levin 2005). Despite this relationship with depth, sulfur-oxidizing bacteria can constitute an important food source in some shallow continental shelf systems (Powell et al. 1983, Montagna & Spies 1985). Overall, mangrove forests are highly productive, with a large number of organic matter

sources, such as labile leaf detritus and primary producers like diatoms and cyanobacteria. The mangrove system reveals that the additional trophic resource constituted by *Beggiatoa* does not influence infaunal abundances or the contribution of bacteria to their diet.

Infauna can be influenced by the toxicity of bacterial mat environments. *Beggiatoa* can create anoxic conditions as they can consume up to 70% of the total oxygen in the sediment (Fenchel & Bernard 1995). Moreover, *Beggiatoa* are found in sediments rich in sulfide, which is toxic at low concentrations for many aerobic metazoans as it blocks the cytochrome *c* oxidase of their respiratory chain (Bagarinao 1992). This toxicity is particularly high for small-sized grazers where diffusional fluxes of sulfide into body tissue are extremely fast (Jahn et al. 1997). Some meiofaunal species have developed a sulfide detoxification system based on the oxidation in their body wall (Fenchel & Findlay 1995). However, this tolerance is restricted to certain species (Pape et al. 2011), and the diversity observed in bacterial mats is consequently often lower than in the adjacent sediment (Van Gaever et al. 2006). As a result, we suggest that in the studied marine mangrove systems, eukaryotic species able to tolerate this toxicity do not necessarily have a higher contribution of bacteria in their diets than species dwelling in the adjacent sediment.

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