Impact of feeding by Arenicola marina (L.) and ageing of faecal material on fatty acid distribution and bacterial community structure in marine sediments: An experimental approach

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Vincent Grossi [a,*], Philippe Cuny [a], Sarah Caradec [a,b,1], David Nerini [a], Richard Pancost [b], Franck Gilbert [a]

[a] *Laboratoire de Microbiologie, Géochimie et Ecologie Marines — UMR CNRS 6117, Centre d'Océanologie de Marseille (OSU), Université de la Méditerranée, Campus de Luminy — Case 901, 13288 Marseille Cedex 9, France*

[b] *Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, United Kingdom*

Abstract

The fate of ingested eukaryotic photoautotrophic fatty acids during gut transit in the lugworm *Arenicola marina* (L.) and the influence of *A. marina*'s faeces on the evolution of fatty acid distribution and bacterial community structure in superficial sediments were studied under laboratory conditions. Dead phytoplanktonic cells (food portions) were fed to individual *A. marina* and subsequently incubated, or allowed to directly incubate in the presence of fresh egesta or non-ingested sediment. Changes in fatty acid composition and genetic structure of bacterial communities during gut transit and/or incubation were monitored using gas-chromatography/mass-spectrometry and a DNA fingerprint approach (RISA), respectively. Results, supported by principal component analyses, suggest that *A. marina*'s feeding activity can directly and indirectly affect the lipid biomarker composition and the bacterial community structure of inhabited sediments. Faecal casts produced from food portions appeared qualitatively enriched in saturated fatty acids relative to (poly)unsaturated ones due, partly, to an increase of some bacterial fatty acids and to the preferential removal of some polyunsaturated fatty acids (PUFAs). The incubation of food portions in the presence of fresh *A. marina*'s egesta (designed to study the indirect impact of feeding by *A. marina*) induced a significant increase in the concentrations of C_{20} and C_{22} polyunsaturated fatty acids (PUFAs), whereas these compounds almost disappeared following direct feeding and subsequent incubation, indicating that some dietary fatty acids may be more accessible to biodegradation following passage through the gut of *A. marina*. The aforementioned increase in PUFAs was attributed to a bacterial production during incubation, suggesting the presence of PUFA-producing bacteria in the fresh egesta of *A. marina*. Those bacteria were either enteric bacteria that were released with the egesta or ingested bacteria that have survived gut passage, as suggested by the variations of the bacterial community structure (i.e. RISA profiles) during incubation. The results suggest that aged faeces from *A. marina* might be, in some circumstances, of relatively high nutritional value to trophic levels which are unable to synthesize essential PUFAs de novo. The presence of PUFA-producing bacteria in guts of marine lugworms deserves further attention.

Keywords: Arenicola marina; Feeding activity; Fatty acids; PUFA-producing bacteria; Bacterial community structure; RISA; Superficial sediments

* Corresponding author. Present address: Paléoenvironnements et Paléobiosphère — UMR CNRS 5125, Université Claude Bernard Lyon 1, Campus de la Doua — Bâtiment Géode, 69622 Villeurbanne Cedex, France. Tel.: +33 472 445 813; fax: +33 472 431 688.

E-mail address: vincent.grossi@univ-lyon1.fr (V. Grossi).

[1] Present address: Laboratoire de Chimie des Eaux, Service d'analyses et de caractérisation, 16 route de Gray, 25030 Besançon Cedex, France.

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1. Introduction

Benthic macrofauna have a significant impact on the biogeochemical processes in marine sediments, through activities such as particle reworking, irrigation, feeding and bacterial gardening/stimulation (Banta et al., 1999; Aller et al., 2001). To meet their nutritional needs, deposit feeders ingest massive volumes of sediment by bulk feeding, and extract organic matter (OM) from different food sources such as digestible detritus (e.g. phytoplanktonic remains), microbes and benthic microbiota (Lopez and Levinton, 1987; Andresen and Kristensen, 2002). As a result of the ingestion process, marine worms produce faeces which can represent a potential food source for other consumers and be the site of intense geochemical transformation of OM (Wilde and Plante, 2002; Plante and Wilde, 2004).

Feeding by deposit-feeders can significantly affect the qualitative and quantitative distribution of specific components of OM (Bradshaw and Eglinton, 1993 and references therein). Among those compounds, lipids (such as fatty acids) appear particularly important due to their key role in the transfer of carbon and energy through aquatic food webs (Müller-Navarra et al., 2000). Moreover, the specific structure of some lipids allow them to be widely used as biomarkers in geochemical studies for determining the source, the transformation and the fate of OM (Grossi et al., 2003 and references therein).

The differential digestion of bacteria and the release of enteric bacteria by marine deposit-feeders have been demonstrated. This can lead to structural shifts of the bacterial assemblages in faeces compared to the ingested sediment, and affect bacterial population dynamics by cellular destruction (bacteriolytic activity) and/or by stimulating growth during passage through the digestive tract (Dobbs and Guckert, 1988; Wilde and Plante, 2002; Lucas et al., 2003).

It is thus evident that the overall feeding activity of worms (ingestion of sediment/egestion of faecal material) influences the quality and the quantity of the OM, but also its subsequent evolution. However, to the best of our knowledge, there have been few studies at the molecular level on both the direct (i.e. gut transit) and the indirect (i.e. bacterial inoculation and ageing) impact

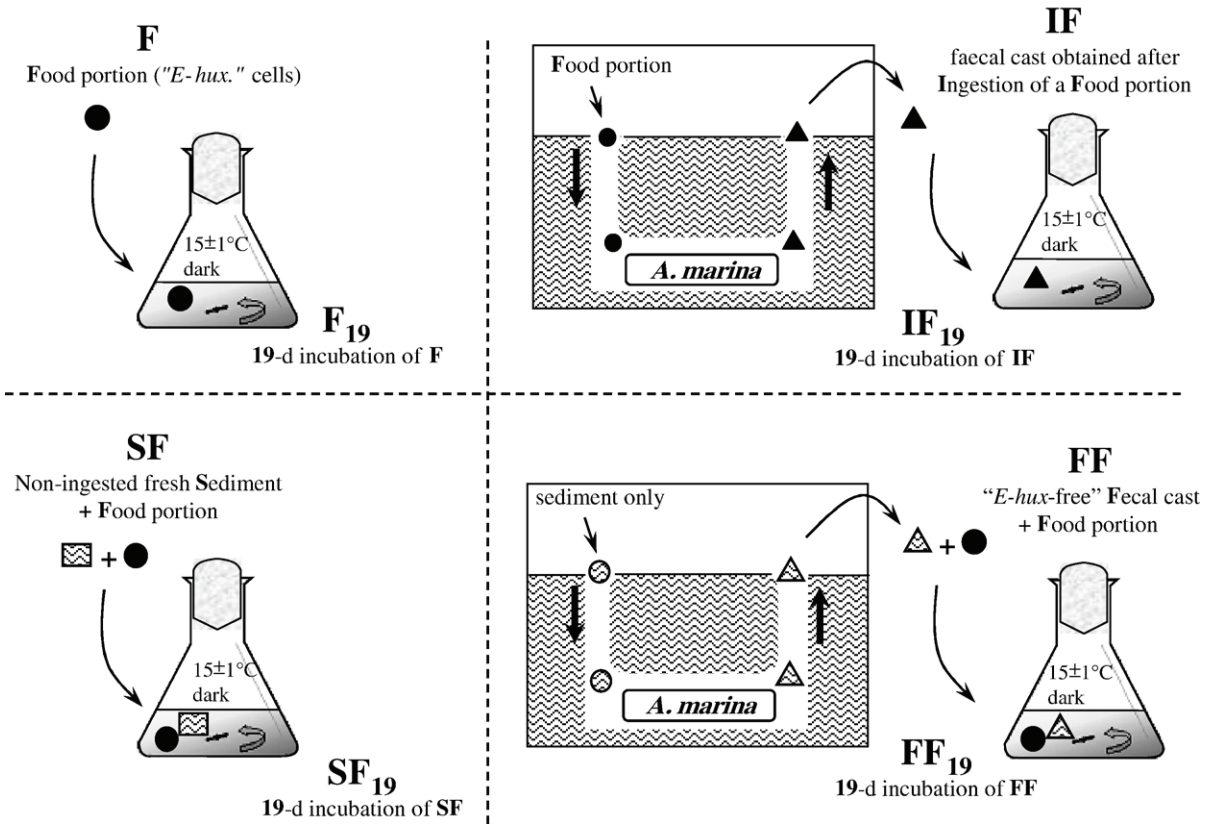

Fig. 1. Outline of the experimental design.

Table 1
Samples description and analyses performed

Samples	Description[a]	Qualitative changes in FA	Quantitative changes in FA	DNA analyses
F	**F**ood portion	X	n.d.[b]	n.d.
F_{19}	**19**d-incubation of **F**	X	n.d.	n.d.
IF	Faecal cast obtained after **I**ngestion of a **F**ood portion	X	n.d.	n.d.
IF_{19}	**19**d-incubation of **IF**	X	n.d.	n.d.
FF	"*E. hux*-free" **F**aecal cast + **F**ood portion	X	X	X
FF_{19}	**19**d-incubation of **FF**	X	X	X
SF	Non-ingested fresh **S**ediment + **F**ood portion	X	X	X
SF_{19}	**19**d-incubation of **SF**	X	X	X

[a] See Experimental design section for the rationale behind each individual treatment.
[b] n.d. = not determined.

of deposit-feeders on the fate of dietary lipids. To address these questions, dead phytoplanktonic cells (food portions) were fed to the lugworm *Arenicola marina* (L.) and subsequently incubated, or directly incubated in the presence of fresh egesta or non-ingested sediment. Changes in fatty acid composition and structure of bacterial communities during gut transit and/or incubation were then monitored using gas-chromatography/mass-spectrometry and a DNA fingerprint approach, respectively. This allowed to specifically determine i) the fate of dietary fatty acids during gut transit of *A. marina* and, ii) the potential influence of *A. marina*'s faeces (i.e. bacterial inoculation and ageing) on the evolution of fatty acid distribution and bacterial community structure in superficial sediments.

2. Material and methods

2.1. Material

Seawater and sediment were collected manually from Carteau beach (Gulf of Fos, Mediterranean Sea). This sediment is occupied by a macrofauna assemblage characteristic of sandy areas with a dominant contribution of polychaetes, from which sampled Arenicolidae individuals (*A. claparedii*) were too small to be used in our experiment. The sediment was maintained in isothermic bags during its transportation to the laboratory where it was sieved (1 mm) to remove all large organisms, homogenised and stored at 15 ± 1 °C before use. The same sediment was used all along the study.

A. marina (L.) was provided by the company "Normandie Appats". *A. marina* was chosen as a model deposit-feeder as it is a dominant macrobenthic species along Western European coasts (Retraubun et al., 1996; Riisgård and Banta, 1998). *A. marina* assimilates OM from different food sources (Grossmann and Reichardt, 1991; Retraubun et al., 1996; Andresen and Kristensen, 2002), and ejects characteristic faecal casts at the surface of the sediment (Riisgård and Banta, 1998). Animals used in the experiment (~4 g wet weight) were collected in the Bay de Somme (Channel, France) at a temperature close to our experimental temperature, one day prior to their introduction in tanks containing sieved sediment. There, animals were allowed to acclimatise for two weeks at 15 ± 1 °C before the beginning of the experiment.

For the experiment, the marine phytoplankton *Emiliania huxleyi* strain CS-57 (CSIRO Collection of Living Microalgae) was used to prepare food portions. *E. huxleyi* is an ubiquitous phototrophic eukaryote which constitutes a high proportion of marine biomass and which can contribute to significant inputs of OM to sediments (Conte et al., 1995). Batch cultures were grown non-axenically to the stationary phase at 15 °C with Si-free f/2 medium. Cells were harvested by centrifugation, freeze-dried, and mixed with sieved sediment (1 g freeze-dried cells/100 g wet sediment) for an accurate splitting into food portions (2.9 ± 0.1 g), and to facilitate feeding of individual *A. marina*. Those food portions were kept frozen until the beginning of the experiment.

2.2. Experimental design

The experimental design is outlined in Fig. 1. At the beginning of the experiment, selected individual *A. marina* were introduced either in U-shaped PVC tubes or in aquaria, each filled with fresh (sieved) sediment. Each worm living in a U-shaped tube was fed with one food portion and the corresponding faecal cast was collected, whereas worms living in aquaria fed on the surrounding sediment and produced the so-called "*E. hux*-free" faecal casts. Then, different incubation experiments (i.e. ageing) were performed under aerobic conditions (19 days, 15 ± 1 °C, dark) with 10 ml of filtered and sterilized seawater.

The different samples considered and the rationale behind each experimental treatment is:

- Individual food portions (samples F; $n=6$) used as a source of dietary fatty acids;
- Faecal casts produced by *A. marina* fed with a food portion (samples IF; $n=4$) which allowed to determine the impact of gut transit on F;
- "*E. hux*-free" faecal casts individually mixed with one food portion (1:4, weight/weight) (samples FF; $n=6$) designed to study the ageing of F after indirect bacterial inoculation by *A. marina* (i.e. indirect impact of feeding);
- Non-ingested (sieved) sediment mixed with one food portion (1:4, weight/weight) (samples SF; $n=6$) designed as a control for the latter treatment.

Half of each sample set was stored for lipid and/or DNA analysis, whereas the other half was incubated for 19 days (to mimic ageing of detritus) as described above, yielding samples F_{19}, IF_{19}, FF_{19} and SF_{19} (Fig. 1 and Table 1).

2.3. Extraction and analysis of lipids

Each sample was extracted 5 times ultrasonically with a mixture of methylene chloride–methanol–water

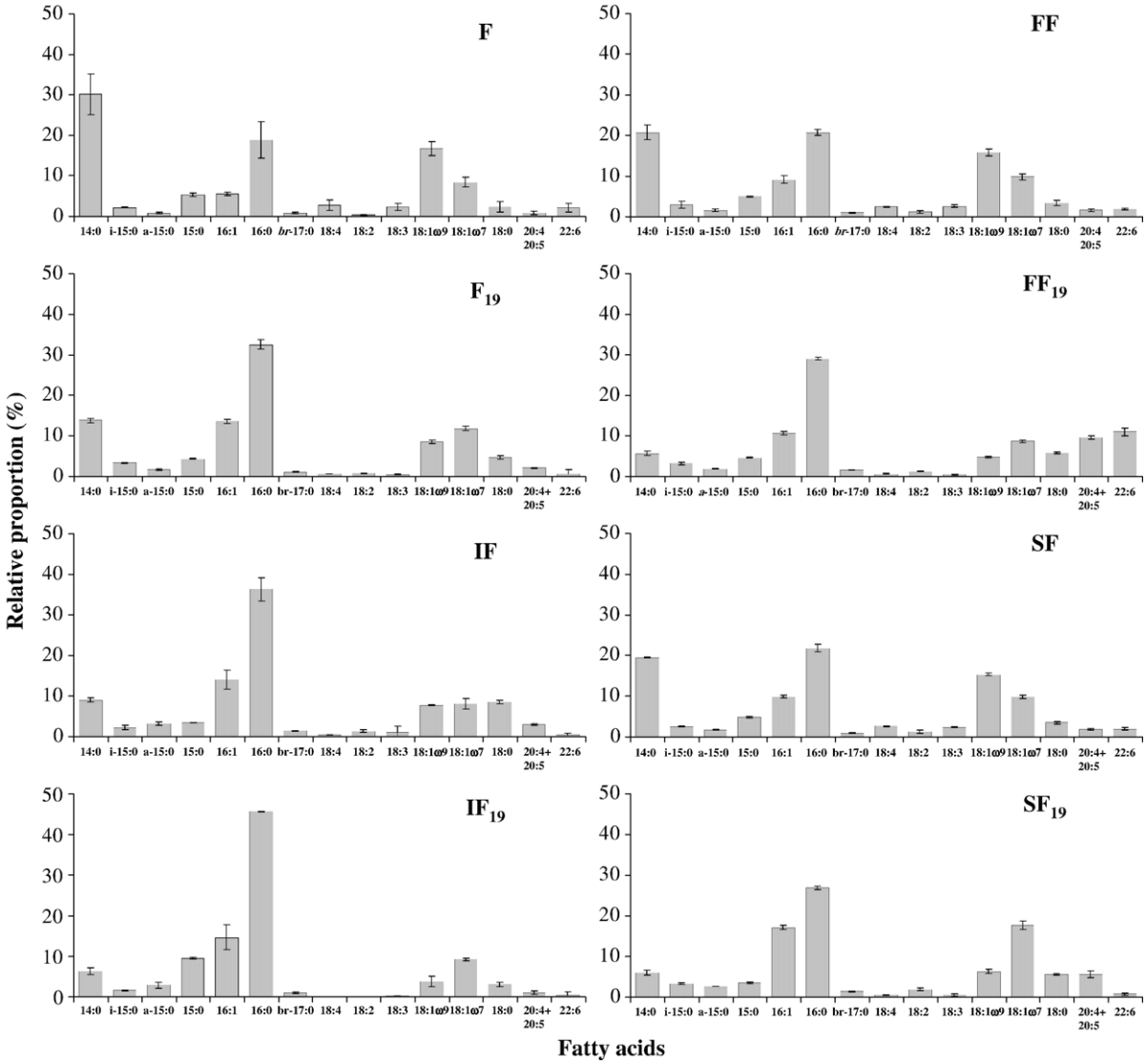

Fig. 2. Relative proportions of individual fatty acids in all non-incubated and incubated samples (mean±S.D.; $n=2$ for IF and IF_{19} and $n=3$ for all other samples). *i*-, *a*- and *br*- refer to *iso*-, *anteiso*- and undefined branched fatty acids, respectively. See Table 1 for samples description.

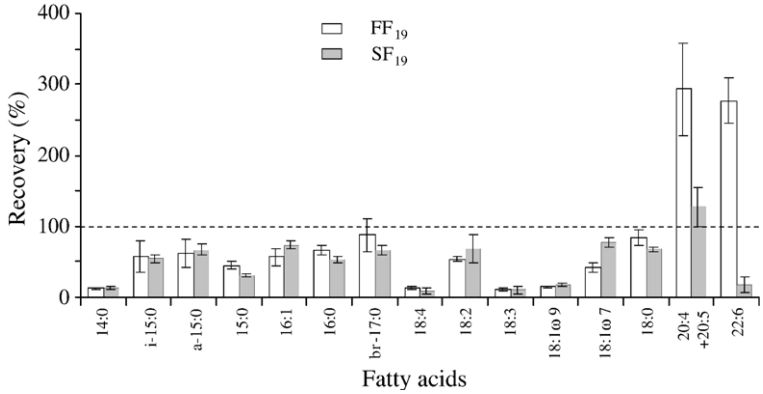

Fig. 3. Recovery percentages of fatty acids after 19d-incubation of FF and SF samples (mean ± S.D.; $n = 3$ for each condition). See Table 1 for samples description.

(DCM–MeOH–H_2O, 20 ml, volume ratio 1:2:0.8). DCM and H_2O were added to the combined extracts to bring the DCM–MeOH–H_2O volume ratio to 1:1:0.9, which allowed two solvent layers to separate. Lipids were recovered in the lower DCM phase and the aqueous phase was extracted again twice with DCM. The combined organic extracts were concentrated by rotary evaporation and saponified with 1 N KOH in MeOH/H_2O (volume ratio 1:1, reflux 2 h). After extraction of the neutral lipids from the basic solution (*n*-hexane, 3 × 20 ml), acids were extracted (DCM, 3 × 20 ml) following addition of HCl (pH = 2).

Fatty acids were converted to trimethylsilyl-derivatives by reaction with bis-trimethylsilyl-trifluoroacetanamide (Supelco) in pyridine (volume ratio 1:1; 30 min at 60 °C). Arachidic acid (Sigma) was used as an internal standard for fatty acid quantification. Individual compounds were identified and quantified using a HP 5890 Series II Plus gas chromatograph coupled to a HP 5972 mass spectrometer, operated at 70 eV with a mass range *m*/*z* 50–700. The gas chromatograph was equipped with an on-column injector and a Solgel-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), and Helium was used as the carrier gas. Samples were injected at 60 °C and the oven temperature was programmed from 60 to 130 °C at 20 °C/min and then at 4 °C/min to 300 °C at which it was held for 10 min.

2.4. Extraction and analysis of DNA

The protocol for DNA extraction was modified from Tsai and Olson (1991). Freeze-dried portions of the FF, FF_{19}, SF and SF_{19} samples were carefully ground, and triplicate subsamples (0.25 g dry weight) of each portion were mixed with 500 μl of TES buffer (100 mM Tris–HCl [pH = 8.0], 100 mM Na_2HPO4 [pH = 8.0], 100 mM Na_2EDTA [pH = 8.0], 1.5 M NaCl). Three cycles of freezing (liquid nitrogen, 2 min) and thawing (water bath 100 °C, 2 min) were conducted to allow the lysis of microbial cells (Roose-Amsaleg et al., 2001). Lysozyme (10 mg, Sigma) and proteinase K (7 μl of a 10 mg ml^{-1} solution, Boehringer Mannheim) were then added to the samples which were incubated for 2 h at 37 °C under agitation (300 rpm). Following the addition of hexadecyltrimethylammonium bromide (10 mg, Sigma), the samples were further incubated for 2 h at 65 °C under agitation (300 rpm) before centrifugation at 12,000 ×*g* (20 °C, 15 min). Supernatants were mixed with an equal volume of chloroform–isoamyl alcohol (volume ratio 24:1). The aqueous phase was recovered by centrifugation (12,000 ×*g*, 20 °C, 15 min) and precipitated with isopropanol overnight at room temperature. The crude DNA pellets were obtained by centrifugation at 15,000 ×*g* (room temperature, 30 min) and were gently washed with 70% ethanol (−20 °C). Ethanol was removed after centrifugation (15,000 ×*g*, 20 °C, 15 min) and air-drying (10–15 min). The pellets were suspended in 50 μl of sterile ultra pure water and stored at −20 °C.

rRNA intergenic spacer analysis (RISA) was used to characterize changes in the genetic structure of bacterial communities (Acinas et al., 1999; Ranjard et al., 2000). Amplification reactions were performed in a final volume of 50 μl containing HighFidelity buffer (5 μl of 10× dilution), $MgCl_2$ (2.5 mM), dNTP (200 μM of each), primers (25 pmol of each), T4 gene 32 protein (1 μg, Boehringer Mannheim), and TripleMaster polymerase (1.75 units, Eppendorf AG). Reactions were performed in an automated DNA thermal cycler (Eppendorf Mastercycler) for 25 cycles. After an initial denaturation step (30 s. at 94 °C), each cycle consisted of a denaturing step (1 min at 94 °C), annealing at 55 °C for 30 s and an extension step of 1 min at 72 °C. A final elongation step at 72 °C for 5 min

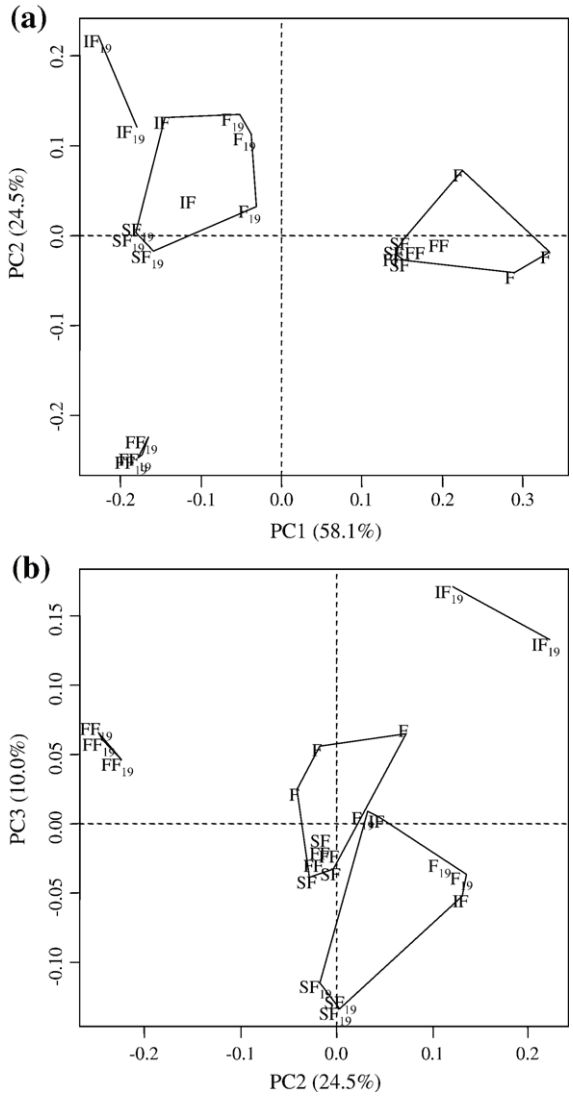

Fig. 4. Plots of the first (PC1) and second (PC2) **(a)** and of the second (PC2) and third (PC3) **(b)** principal components derived from PCA of the relative distribution of individual fatty acids in all non-incubated and incubated samples (see Table 1 for samples description). Line-linked observations correspond to boundaries of K-means clusters. Replicates always belong to the same clusters.

preceded cooling at 4 °C. PCR products (16 µl) were loaded on a 5% non-denaturing acrylamide gel (*N*-acrylamide/*N*-methylenebisacrylamide, 29:1, Bio-Rad) and separated by electrophoresis (DSG200-02, C.B.S. Scientific) for 15 h at 8 mA. Gels were stained with SYBR green I (FMC Bioproducts) according to the manufacturer's instructions. RISA profiles were photographed and analysed using a Quantity One GelDoc2000 system (Bio-Rad) yielding data matrices which were used for principal component analysis (PCA). Those matrices took into account the presence or absence of bands and their normalized intensity. RISA profiles were assumed to be indicative of the main bacterial community changes, and variation between RISA profiles generated from different samples was assumed to reflect variation in the corresponding bacterial communities (Yannarell and Triplett, 2004).

2.5. Principal component analysis and classification

Two Principal component analyses (PCA) were carried out to analyse variations in fatty acid distribution (relative abundances) and bacterial community RISA profiles amongst non-incubated and incubated samples. In both cases, we disposed of a sequence of n observations (fatty acid distributions or RISA profiles) $\{x_i, i=1,\ldots, n\}$, caracterized by p variables (each peak) such that $x_i=(f_{i1},\ldots, f_{ip})$ with:

$$\begin{cases} f_{ik} \geq 0, k = 1, \ldots, p, i = 1, \ldots, n \\ \sum_{k=1}^{p} f_{ik} = 1, i = 1, \ldots, n \end{cases}$$

that were row-combined into a single matrix X.

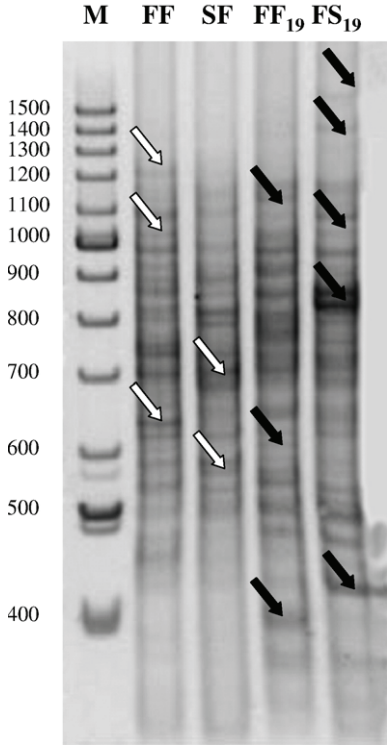

Fig. 5. RISA profiles from the incubated and non-incubated FF and SF samples. Black and white arrows respectively indicate major discriminating bands between incubated and non-incubated FF and SF samples. M: molecular markers (bp).

Once the PCA of X performed (i.e. the eigenvalue decomposition of the covariance matrix of X in order to determine the main directions into the observations space along which the data have the highest variability; Hastie et al., 2001), the initial table X was resumed through a matrix Y of $m<p$ principal components accounting for a given quantity of variability (e.g. 90%). The other components accounting for small proportions of the total variance were discarded. The K-means clustering method was then applied to Y to gather observations lying in the space of the m first principal components (Hastie et al., 2001). This two-steps procedure (PCA+K-means classification) allowed gathering observations that were most similar regarding the squared Euclidean distance between observations. A filtering step via the PCA was simply considered to make the K-means clusters more robust. Results of the classification were displayed in the form of convex envelops into the 2-dimensional plots of the PCA.

3. Results

Table 1 presents the different types of samples considered in this study and the analyses performed. Due to our experimental design, *A. marina* living in the U-shaped tubes likely ingested some of the surrounding sediment in addition to the food portions (Fig. 1). The quantitative impact of *A. marina*'s gut transit on fatty acid distribution (comparison between F and IF samples) was therefore not possible, and only qualitative changes were considered for those samples. However, the influence of egested bacterial communities on the fatty acid distribution of aged faecal casts (FF and FF_{19} samples), could be qualitatively and quantitatively compared with that of non-ingested sediment (SF and SF_{19} samples), owing to the use of similar dilution rates of food portions. For those latter samples (FF, FF_{19}, SF, SF_{19}), the varying fatty acid distribution was simultaneously compared with changes in the genetic structure of the bacterial communities assessed by DNA fingerprinting (Table 1).

3.1. Changes in fatty acids distribution

Fifteen major fatty acids present in the food mixture (F) were considered for this study (Fig. 2). Although some fatty acids were present in the original sediment which was used to prepare the food portions, the mixing with non-axenic *E. huxleyi* cells induced an enrichment ≥95% for each component. The $C_{14:0}$, $C_{16:0}$, $C_{18:1\omega9}$, and $C_{18:1\omega7}$ were the most abundant compounds and contributed to ca. 75% of the total fatty acids. The presence of branched odd-carbon-number fatty acids in food portions suggests the presence of bacteria in the cultured *E. huxleyi*.

Ingestion of a food portion by *A. marina* resulted in a significant increase of the relative proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1\omega7}$ fatty acids, and in the concomitant decrease of $C_{14:0}$ and $C_{18:1\omega9}$ homologues (Fig. 2, F and IF). Gut transit also induced a pronounced decrease in relative abundances of $C_{18:3}$, $C_{18:4}$ and $C_{22:6}$ components, whereas $C_{20:4+20:5}$ fatty acids slightly increased from 2 to ca. 6%. Incubation of a food portion for 19 days induced similar changes in the overall distribution of fatty acids as gut transit (Fig. 2, F_{19} and IF) but also a further decrease in $C_{18:1\omega9}$ fatty acid and the complete removal of C_{18} polyunsaturated fatty acids (PUFAs).

The mixing of food portions with either "*E. hux*-free" faecal casts (FF samples) or fresh sediment (SF samples), induced only small changes in the relative fatty acid distribution (Fig. 2). The most significant change was observed for the $C_{14:0}$ fatty acid, the relative proportion of which decreased by 10% compared to the original food portion. As observed for food portions alone, the proportion of $C_{14:0}$ and $C_{18:1\omega9}$ fatty acids and of most C_{18} PUFAs decreased during incubation of FF and SF mixtures, whereas those of C_{20} PUFAs significantly increased, especially during the incubation of food portions in the presence of "*E. hux*-free" faecal casts (Fig. 2, FF_{19}). This latter condition of incubation also induced a 4 times increase of the proportion of the C_{22} PUFA, which exhibited a decrease during all incubations (Fig. 2, F_{19}, IF_{19} and SF_{19}).

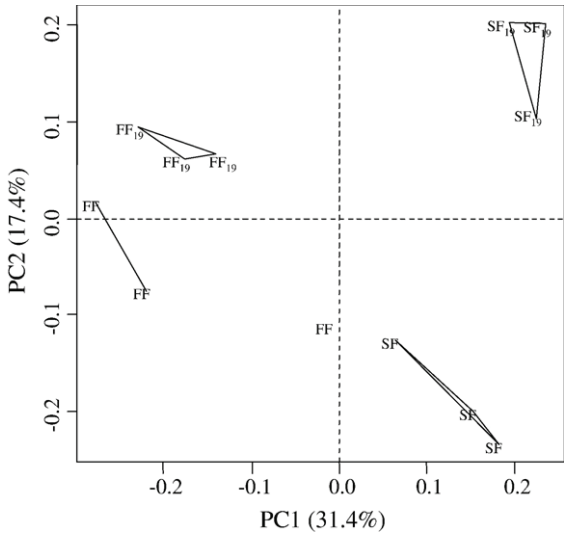

Fig. 6. Comparison of the bacterial communities structure in non-incubated and incubated FF and SF samples (see Table 1 for samples description). Plot of first (PC1) and second (PC2) principal components derived from PCA of RISA profiles. Observations belonging to the same cluster are linked with a black line.

Table 2
Relative proportions of different classes of fatty acids in the different samples (see Table 1 for samples description)

Samples	Σ saturated FA (%)***	Σ branched FA (%)	Σ monounsaturated FA (%)	Σ PUFAs (%)	Σ unsaturated FA (%)****
F*	60	4	31	9	40
F_{19}*	61	6	34	5	39
IF**	64	7	30	6	36
IF_{19}**	70	6	28	2	30
FF*	55	5	35	10	45
FF_{19}*	52	7	24	24	48
SF*	55	5	35	10	45
SF_{19}*	49	7	41	10	51

*average of triplicate samples, **average of duplicate samples, ***including bacterial branched FA, ****sum of monounsaturated+PUFAs.

Pronounced quantitative changes of the fatty acid distribution were also observed in 19d-incubated SF and FF samples (Fig. 3). All but C_{20} and C_{22} fatty acids showed a sharp decrease in concentration upon incubation. The greater losses (>80%) were observed for the $C_{14:0}$ and the (poly)unsaturated $C_{18:4}$, $C_{18:3}$ and $C_{18:1\omega9}$ fatty acids, whereas the amounts of $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:1\omega7}$ and $C_{18:2}$ fatty acids decreased to a lower extent (15–40%). Similar to the qualitative observations, the concentrations of C_{20} and C_{22} PUFAs increased by 3-fold during incubation of food portions in the presence of "*E. hux*-free" faecal casts (Fig. 3, FF_{19}). A lower but significant increase in C_{20} PUFAs concentration (Rec= ~130%) was also observed during incubation in the presence of non-ingested fresh sediment, while only 18% of the initial $C_{22:6}$ FA remained after incubation under these conditions (Fig. 3, SF_{19}).

The qualitative fatty acid distribution in all incubated and non-incubated samples was further analysed through PCA (Fig. 4). Components 1, 2, and 3 accounted for a total of 92% (58, 25 and 10%, respectively) of the variability amongst all samples. The first component distinguished ingested and/or incubated samples (F_{19}, IF, IF_{19}, FF_{19}, SF_{19}) from those that were neither ingested nor incubated (F, FF, SF; Fig. 4a). To better explain the variations in fatty acid distribution observed during the different experimental treatments, PCA was considered for the second and the third components (Fig. 4b). This clearly distinguished all incubation conditions from one another and confirmed that ingestion (IF) or 19d-incubation (F_{19}) of food portions resulted in similar qualitative changes in fatty acids distribution.

3.2. Changes in bacterial communities

RISA profiles obtained before incubation of FF and SF samples already showed some differences in the dominant bacterial communities present in the two types of samples (Fig. 5); this was likely due to the presence of enteric bacteria in the egested sediment and/or to the preferential removal of certain types of bacteria during gut transit. Incubation of those samples during 19 days induced a clear and distinct evolution of the bacterial community structure for both conditions. These changes were determined from the RISA profiles which showed a decrease or an increase of the relative intensity of pre-existing bands, and by the appearance or disappearance of other bands (Fig. 5).

Differences of bacterial community structure in incubated and non-incubated FF and SF samples were further confirmed by PCA analysis of the RISA profiles. Components 1, 2, and 3 explained 61% of the data variance (31, 17 and 13%, respectively). The first principal component clearly discriminated between SF and FF experimental conditions whereas changes during incubation were essentially explained by the second principal component (Fig. 6).

4. Discussion

4.1. Direct influence of feeding

A common effect of marine food webs on dietary fatty acids is the preferential assimilation and/or degradation of (poly)unsaturated fatty acids, resulting in a relative increase of the proportion of saturated homologues in the egested material. However, in the case of benthic invertebrates, the effect appears species dependent (e.g. Bradshaw et al., 1990b; Meziane et al., 2002). Deposit-feeder activity can also result in minor changes of the fatty acid distribution or in an enrichment of the monounsaturated fatty acid content of sediments (Bradshaw and Eglinton, 1993 and references therein). In the present case, faecal casts produced from food portions were slightly enriched in saturated fatty acids relative to (poly)unsaturated ones (Table 2, F and IF),

although we could not determine the quantitative variations of the different classes of compounds during gut transit. This relative increase of saturated fatty acids was partly explained by an increase of branched $C_{15:0}$ and $C_{17:0}$ bacterial fatty acids (Fig. 2 and Table 2). The greater proportions of some individual fatty acids in the egested material could be due to the selective adsorption of other homologues by the worm and/or to a release from gut cells/microbes. It has already been suggested that an increase in branched C_{15} and C_{17} fatty acids in faeces of different marine invertebrates reflects a contribution from enteric bacteria (Harvey et al., 1987; Bradshaw et al., 1990b). Variations in the distribution of bacterial fatty acids (i.e. $C_{18:1\omega7}$ and branched $C_{15:0}$ and $C_{17:0}$) during feeding of *A. marina* may be further explained by differential digestibility of killed bacterial communities present in the original food portions. Plante and Shriver (1998) indeed demonstrated that only 11 of 43 bacterial species isolated from intertidal sediments were digested in gut extracts from *A. marina*. Feeding by *A. marina* did not change the relative proportion of the $C_{18:1\omega7}$ fatty acid but induced a significant decrease of the proportion of the $C_{18:1\omega9}$ isomer (Fig. 2, F and IF). Invertebrate herbivory is generally considered to have the inverse effect on C_{18} monounsaturated fatty acids, as faeces often contain a predominance of the ω9 isomer even when the diet contain the reverse (Bradshaw et al., 1990a, 1991; Bradshaw and Eglinton, 1993; Meziane et al., 2002); this is typically attributed to an animal contribution of the ω9 isomer which is usually the dominant isomer in animal tissues. Interestingly, the polychaete *Nereis diversicolor* was also shown to affect the distribution of $C_{18:1}$ fatty acids in a different way from other invertebrates (Bradshaw et al., 1990b). This suggests that polychaete worms have a distinct dietary impact on the sedimentary fatty acid distribution compared to other invertebrate species.

Although ingestion of food portions by *A. marina* induced significant variations in the relative (and probably quantitative) distribution of individual fatty acids, all major dietary fatty acids were still detected in the produced faecal casts (Fig. 2, F and IF). The further (sometimes complete) removal of (poly)unsaturated fatty acids during 19d-incubation of those faeces (Fig. 2 and Table 2, IF and IF_{19}) could be attributed to microbial degradation. Moreover, the greater removal of (poly)unsaturated fatty acids during incubation of pre-ingested food portions compared to non-ingested ones (Table 2, IF_{19} and F_{19}) suggested that these compounds were more accessible to biodegradation after passage through the gut of *A. marina*.

The differences in the structure of bacterial communities between FF and SF samples reflected the direct impact of *A. marina* on sedimentary bacteria, when the lugworm was allowed to feed on sieved sediment. These results are in good agreement with previous studies which clearly demonstrated, using a variety of approaches, compositional shifts in bacterial assemblages with passage through deposit-feeders gut (Dobbs and Guckert, 1988; Findlay et al., 1990; Grossmann and Reichardt, 1991; Wilde and Plante, 2002), although the degree and nature of the disturbance seems to vary among invertebrate taxa (Plante and Wilde, 2004). In all cases, the modification of the bacterial community can be attributed to the digestion of a subset of ingested bacteria with the concomitant stimulation or inoculation of other groups.

4.2. Indirect influence of feeding (i.e. inoculation and ageing of faeces)

The incubation of food portions in the presence of "*E. hux*-free" egesta (FF samples) yielded a very different qualitative distribution of fatty acids compared to ingested and subsequently incubated food portions (Figs. 2 and 4b, FF_{19} and IF_{19}). The major difference was the significant increase of the proportions of C_{20} and C_{22} PUFAs during incubation in the presence of "*E. hux*-free" faecal casts whereas these compounds almost disappeared following direct feeding (Fig. 2 and Table 2, FF, FF_{19} and IF_{19}). The quantitative analysis of fatty acids in non-incubated and incubated FF samples further demonstrated the important production of C_{20} and C_{22} PUFAs during incubation (Fig. 3).

The analysis of *A. marina*'s gut tissues indicated the presence of minor amounts of C_{20}–C_{22} PUFAs (data not shown), but an input of these compounds from gut cells possibly released in the "*E. hux*-free" faecal casts could not explain an increase in concentration during incubation. On the other hand, a production from autotrophic benthic microalgae that might be present in the original sediment appears very unlikely since incubations were performed under complete darkness. Although we cannot totally discard the presence of mixotrophic organisms, our results likely indicate a bacterial origin of the C_{20} and C_{22} PUFAs produced during incubation of FF samples. C_{20} and C_{22} PUFAs have long time been considered to be characteristic of eukaryotes (Harwood and Russell, 1984), but there is growing evidence that specific bacterial strains can also biosynthesised these compounds in relatively high amount (Yano et al., 1997; Nichols, 2003). In the present case, the formation of PUFAs was obviously due to the presence of PUFA-producing bacteria in the "*E. hux*-free" faecal casts. Those bacteria were either enteric bacteria that were

released with the egesta, or ingested bacteria (present in the sediment used to fill the aquaria) that have survived gut passage. Both explanations are supported by the presence of specific bacterial groups (bands) clearly visible in the RISA profiles of the FF samples (Fig. 5).

C_{20} PUFAs were also produced (although to a much lesser extent) when food portions were incubated in the presence of non-ingested sediment but this was accompanied by a significant decrease in C_{22} PUFA concentration (Fig. 3, SF_{19}). Since the incubation conditions of FF and SF samples were similar, we speculate that the preponderance of C_{22} PUFA in FF_{19} samples was due to the growth of released enteric bacteria. If so, the production of C_{20} PUFAs in FF_{19} and SF_{19} samples involved bacteria different from the enteric C_{22} PUFA-producing population. The greater production of C_{20} PUFAs in FF_{19} compared to SF_{19} may further reflect the presence of (at least two) different C_{20} PUFA-producing bacterial populations (one in the original sediment and one in the gut) or a stimulation (or an enrichment) of sedimentary C_{20} PUFA-producing bacteria during *A. marina* feeding. The fact that no relative increase in (C_{22}) PUFA was observed following ingestion and incubation of food portions (Fig. 2, IF_{19}) does not seem contradictory to the above hypotheses. It is indeed possible that PUFA-producing bacteria were released in IF samples but that their abundance was too low and/or that the conditions were not good enough to allow a significant growth within 19 days.

Although the structure of the bacterial community was clearly different in non-incubated FF and SF samples (Figs. 5 and 6), those contained similar fatty acid distributions (Fig. 2). These fatty acid profiles reflect the preponderance of *E. huxleyi* cells in the samples. Incubation for 19 days of each series of samples induced significant shifts in the structure of the bacterial community (Figs. 5 and 6), in addition to the aforementioned changes in fatty distribution. Classical bacterial fatty acids (i.e. $C_{18:1\omega7}$ and branched $C_{15:0}$ and $C_{17:0}$) had relatively high percentages of recovery compared to other fatty acids (i.e. $C_{18:4}$, $C_{18:3}$, $C_{18:1\omega9}$) originating from dietary phytoplanktonic cells (Fig. 3). This indicates a significant microbial contribution to fatty acid in FF_{19} and SF_{19} and further supports a bacterial origin of the produced PUFAs. The distinct evolution of the bacterial community structure during incubation of FF and SF samples is in agreement with different PUFA-producing bacterial populations (see discussion above).

The strong bacterial production of PUFAs during incubation of faecal casts indicates that these bacteria could potentially contribute a substantial proportion of the C_{20} to C_{22} PUFAs level in sediment. This is of particular importance since bacteria present in egesta are generally considered to be of poor nutritional value as they are more likely to be resistant to digestion (Plante and Wilde, 2001). Colonization of PUFA-synthesizing bacteria on surficial sediment may represent an important source of these vital nutrients to high trophic levels, which are unable to synthesize essential PUFA de novo (Müller-Navarra et al., 2000). As far as we know, the presence of PUFA-producing bacteria in guts of marine lugworms has never been reported.

It should be noted, however, that the environmental relevance of our experimental observation may be limited since disturbances associated with gut passage are a function of numerous variables, including the longevity of faecal materials as distinct habitats. Our experimental design (i.e. incubation in separated flasks and long incubation time) favoured the regrowth of enteric bacteria and/or bacteria that have survived gut passage, and precluded any recolonization of the faecal casts from bacteria in surrounding sediment. By using field manipulative experiments, Plante and co-authors (Plante and Wilde, 2001; Plante and Stinson, 2003) demonstrated that recolonization of naturally incubated faeces from different deposit feeders including the polychates *A. cristata* and *N. succinea*, occurred rapidly and was dominated by immigration from adjacent sediments. These authors recently confirmed the primary role of immigration using molecular techniques (Denaturing Gradient Gel Electrophoresis, DGGE) but, interestingly, also observed notable differences between the genetic fingerprints of naturally incubated egesta and surrounding sediments (Plante and Wilde, 2004). The presence of some unique DGGE bands in aged egesta might represent (bacterial) phylotypes introduced by the animals, which would agree with the different RISA profiles observed in the present study.

5. Conclusions

The present experimental work indicates that passage through the gut of *A. marina* can increase the proportions of (bacterial) saturated fatty acids relative to dietary (poly)unsaturated ones of ingested sediments, make some dietary fatty acids more accessible to subsequent biodegradation and, induce significant compositional shifts in sedimentary bacterial assemblages.

The results further show that *A. marina*'s faeces can contain PUFA-producing bacterial populations which can be of relatively high nutritional value to other trophic levels which are unable to synthesize essential PUFAs de novo. Further work on the presence of PUFA-producing bacteria (isolation, phylogenetical affiliation, etc.) in guts of marine lugworms is undoubtedly needed.

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