Kill●Spill

Integrated solutions for combating marine oil spills

Deliverable D5.2

Report on infaunal changes to oil-polluted sediment in microcosms

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1 About this deliverable

The overall objective of WP5 is to develop novel or improved technologies that accelerate the biodegradation of hydrocarbons in contaminated sediments. Deliverable 5.2 shows the proof of principle that sediment-burrowing infauna can accelerate hydrocarbon degradation by stimulating aerobic hydrocarbon-utilising bacteria. Deliverable 5.2 takes a 'whole microbial ecosystem' approach to characterise the impacts of burrowing infauna on microbial (prokaryote and eukaryote) communities in contaminated sediments.

2 Introduction

Oil pollution remains a major threat to marine ecosystems (Shahidul Islam and Tanaka, 2004), with recent significant oil spills, such as the Prestige oil tanker disaster, highlighting that coastal ecosystems can be particularly sensitive to oil pollution impact (de la Huz et al., 2005). Coastal ecosystems are also exposed to oil pollution from other sources, such as from estuarine outflows and land-based runoff (Shahidul Islam and Tanaka, 2004).

Petroleum oil contains a diverse mixture of hydrocarbon compounds, such as alkanes and polycyclic aromatics hydrocarbons (PAHs), which can be utilised as sources of carbon and energy by some marine bacteria. The obligate hydrocarbonoclastic bacteria (OHCB), including *Alcanivorax*, *Marinobacter* and *Cycloclasticus*, have been shown to be important marine oil degraders (Yakimov et al., 2007). Aerobic degradation of hydrocarbons by OHCB is critical in the remediation of oil-contaminated sediments (Coulon et al., 2012), however, the degradation of oil in marine sediments can be limited by the availability of oxygen (Venosa et al., 2010).

Through activities such as particle reworking and burrow irrigation, bioturbating fauna act as 'ecosystem engineers' by significantly altering the geochemical properties of sediments, and having a resulting impact on microbial communities and subsequent microbially-mediated biogeochemical processes (Kristensen et al., 2012). Within faunal sediment burrows, bacterial abundance can be greater than that in the surrounding unaffected sediment (Papaspyrou et al., 2005; Papaspyrou et al., 2006). Bacterial community structure and diversity in faunal burrows can also be distinct (Papaspyrou et al., 2005; Papaspyrou et al., 2006; Laverock et al., 2010). For example, bacterial communities in the burrows of coastal shrimp are significantly different to bacterial communities in ambient surface and subsurface sediments, with shrimp bioturbation increasing bacterial diversity (Laverock et al., 2010), and stimulating nitrogen fixation by sulfate reducing bacteria (Bertics et al., 2010).

The potential impact of bioturbation on sediment microbial eukaryote communities has received substantially much less attention compared to that of bacterial communities. Molecular assessment using 18S rRNA gene pyrosequencing of DNA isolated from sediment burrows of the lugworm *Arenicola marina* has indicated that distinct protist communities can be present in burrows that are different to those in surrounding sediment and at the sediment-water interface (Engel et al., 2012).

Bioturbating fauna in oil-contaminated sediments could theoretically accelerate the remediation process by stimulating aerobic hydrocarbon degradation by OHCB. Assessment of the effect of the burrowing polychaete *Hediste* (*Nereis*) *diversicolor* on oil-contaminated sediment bacteria community structure has been conducted using ribosomal intergenic spacer analysis (RISA). One RISA sequenced band was affiliated to the OHCB genera *Alcanivorax* (Cuny et al., 2007). More recently using a combination of terminal restriction fragment length polymorphism (TRFLP) and clone libraries, *H. diversicolor* has been shown to cause broad changes in general bacteria community structure in oil-contaminated sediments (Stauffert et al., 2013). Both Cuny et al (2007) and Stauffert

et al (2013) determined bacteria community structure in homogenised sediments samples and did not consider the spatial heterogeneity formed by burrowing fauna. Other studies of the effects of bioturbation on bacteria diversity in non-contaminated sediments have shown that it is vital to consider burrows and un-affected sediments separately because they are geochemically dissimilar and subsequently microbiologically divergent habitats (e.g. Laverock et al 2010).

The potential for bacterial communities to remediate polluted sediments could be decreased by top-down control from meiofauna (Näslund et al., 2010). Using a radiorespirometry-based approach, studies have shown that bacterial degradation of ^{14}C-napthalene in marine sediments is reduced in the presence of bacterivorous meiofauna such as nematodes and ostracods, which decrease mineralisation rates and modify bacterial community structure (Näslund et al., 2010).

3 Aims and Objectives

In this study, we have investigated the effects of the important coastal bioturbating polychaete *H. diversicolor* on oil degradation and microbial community structure in oil-contaminated sediments. In particular, we have focused on diversity within the burrows formed by the polychaete, using high-throughput sequencing of bacterial 16SrRNA and eukaryote 18S rRNA genes and transcripts to describe the community composition of the burrow ecosystem.

4 Material and Methods

4.1 Experimental setup

Surface sediment (top 20cm) was collected from the mouth of the Plym Estuary (50°22’17.22’’N, 04°06’34.45’’W) in the South West of the UK. The sediment was sieved through a 1mm mesh to remove large fauna, stones and other debris. In order to contaminate the sediment with oil, 375g was air dried before 20g of IFO-180 crude oil was dissolved in 100mL hexane and mixed with the dry sediment. The oil-contaminated sediment was incubated overnight with continuous air flow and after the hexane had evaporated was mixed with 1125g of fresh sediment. The contaminated sediment was maintained for 2 months submerged in a continuous flow seawater aquarium at 15°C and in the dark before experimental setup.

Oil-contaminated (450g) sediment was transferred to individual plastic tubes (length 18cm, diameter 6cm) that were sealed at one end. The tubes were maintained submerged in continuous flow seawater aquaria at 15°C and in the dark. After 7 days, 2 adult *H. diversicolor* with the same length and weight were added to 3 experimental cores and 3 cores were used as controls (i.e. no bioturbation). The polychaetes formed gallery burrows within 2-3 days and remained active during the experimental period.

4.2 DNA extraction

DNA was extracted using the DNeasy extraction kit (Qiagen) with minor modifications. Sediment (0.25g) was weighed into tubes containing 0.5g glass beads (100–300 µm, MPBIO) and 600µl lysis buffer (Qiagen) before an additional bead-beating step followed by the manufactures DNA extraction protocol. An additional cleanup step was also included using QIA-shredder columns (Qiagen) according to the manufactures protocol.

4.3 RNA extraction and cDNA synthesis

Sediment (0.25g) was weighed into tubes containing 0.5g glass beads (100–300 µm, MPBIO) and 1mL TRI Reagent® (Ambion) before bead beating. Samples were heated at 60°C for 10 min before 600 µL

of supernatant was transferred to a new tube containing 100µl 1-bromo-3-chloro-propane and vortexed. The tubes were centrifuged to separate the organic and aqueous phases before the aqueous phase was transferred to QIA-shredder columns. The resulting filtrate was precipitated with 2-propanol (equal volume) and sodium acetate (1/10 volume) for 1 hr at -20°C before the RNA pellet was isolated and washed by centrifugation. The RNA pellet was resuspended in 100 µL RNAse-free water and further cleaned using the RNasy kit (Qiagen, U.K) according to the manufacturer's instructions. DNAse treatment was performed using RQ1 RNase-Free DNase (Promega) according to the manufactures instructions. Control PCRs confirmed the presence of only RNA. cDNA generation was performed using an Omniscript RT kit (Qiagen) in accordance with manufacturer's instructions.

4.4 16S rRNA and 18S rRNA transcript quantification

Bacterial 16S rRNA cDNA and eukaryote 18S rRNA cDNA was used as target for quantitative PCR (Q-PCR) following previously published protocols (Suzuki et al., 2000; Zhu et al., 2005). Q-PCR was carried out using the Sensi-FAST SYBR Q-PCR kit (Bioline) and in a Qiagen Rotor-Gene (Qiagen).

4.5 16S rRNA/18S rRNA amplicon sequencing and bioinformatics

Bacterial 16S rRNA and eukaryote 18S rRNA gene amplicon sequencing was carried as previously described (Taylor et al., 2014; Taylor and Cunliffe, 2014). In summary, the V4 variable region of the 16S rRNA gene was amplified using the PCR primers 515F and 806R (Caporaso et al., 2011), and the following PCR conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, and a final elongation step at 72°C for 5 minutes. The V9 variable region of the 18S rRNA gene was amplified using the polymerase chain reaction (PCR) primers 1391F (Lane, 1991) and EukB (Medlin et al., 1988). The same PCR conditions were used except the annealing temperature was changed to 57°C. Sequencing of amplified 16S rRNA and 18S rRNA genes was performed on an Ion Torrent PGM (Life technologies) according to manufactures instructions.

Sequences were analysed using the QIIME software package (Caporaso et al., 2010) as previously described for 16S rRNA (Taylor et al., 2014) using the Greengenes database (DeSantis et al., 2006) and 18S rRNA (Taylor and Cunliffe, 2014) using the SILVA database (Quast et al., 2013) as a reference. In brief, quality filters were used to remove short (<150bp) and low quality reads (average phred score <25). Chimeras were then identified and removed. Operational taxonomic units (OTUs) were defined at 97% similarity and classified against the reference databases.

4.6 Hydrocarbon analysis

After 30 days, the *H. diversicolor* were removed and the sediment containing tubes sacrificed for hydrocarbon analysis. The sediment was homogenised before hydrocarbon analysis using standard protocols. In summary, aliphatic and aromatic hydrocarbons were extracted in hexane/acetone and analysed using GC-FID. PAHs were extracted in hexane/acetone/triethylamine and analysed using GC-MS.

4.7 Statistical analyses

Significant differences in hydrocarbon concentrations between bioturbated and non-bioturbated sediments were determined by t-tests using the SPSS statistics software package (IBM). Permutational Multivariate Analysis of Variance (PERMANOVA) was performed with 999 permutations in QIIME using UniFrac distance matrices and OTU tables as inputs to investigate differences in community composition at the OTU level. In order to establish if there were significant differences between burrows and sediment in the relative abundance of specific eukaryote and

bacteria groups, data were converted from percentages to arcsin values before t-test analysis using SPSS.

5 Results

5.1 Hydrocarbon degradation

After 30 days incubation, there was a significant reduction in the total concentrations of hydrocarbon aliphatics (>C5-C35), aromatics (>EC8-EC44) and EPA16 PAHs in the bioturbated sediment cores compared to the control (non-bioturbated) sediment cores (t-test; $p < 0.05$) (Figure 1). The level of hydrocarbon degradation was related to hydrocarbon molecular mass, with lower mass compounds degraded more than higher mass compounds (Table 1). For example, analysis of individual EPA16 PAHs showed that naphthalene, acenaphthene, fluorine, phenanthrene and anthracene were significantly more degraded in bioturbated sediments compared to control sediments (t-test; $p < 0.05$). However, fluoranthene and larger PAHs remained at similar concentrations in both control and bioturbated sediments (Table 1).

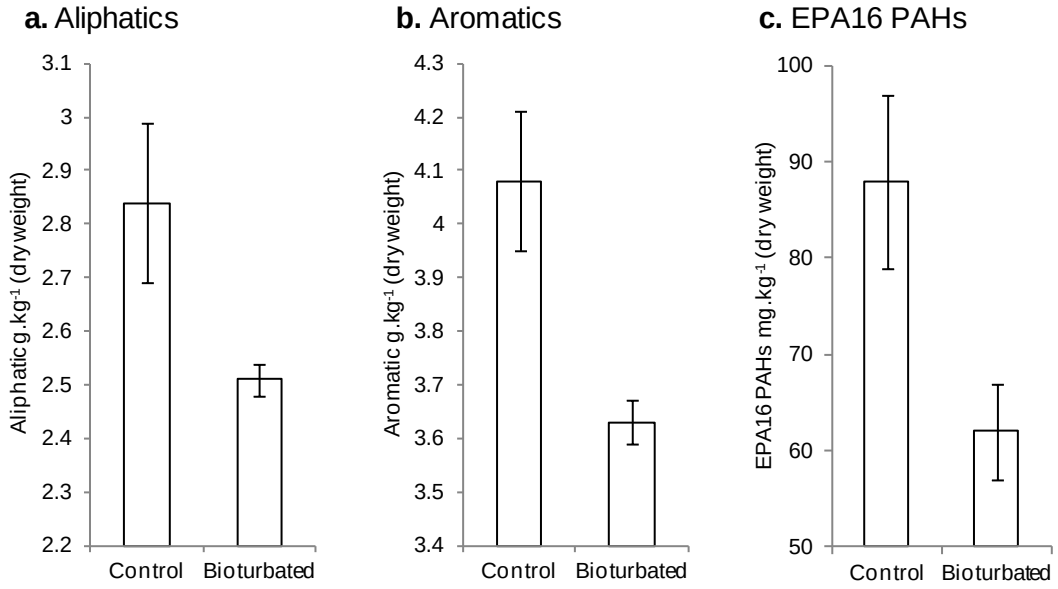

Figure 1 **Concentration of total aliphatic hydrocarbons (a), aromatic hydrocarbons (b) and Environmental Protection Agency 16 polycyclic aromatic hydrocarbons (EPA16 PAHs, c) in control sediments (i.e. no bioturbation) and sediments bioturbated by the burrowing polychaete after 30 days incubation. Values shown are means ± standard deviation (n = 3).**

Table 1 **Concentrations of size-based groupings of aliphatic and aromatic hydrocarbons and individual EPA16 PAHs in control sediments (i.e. no bioturbation) and sediments bioturbated by the burrowing polychaete *Hediste* (*Nereis*) *diversicolor* after 30 days incubation.**

	Control		Bioturbated
	Mean ± STDEV		Mean ± STDEV
Aliphatics (mg.kg^{-1})			
Aliphatics >C5-C6	1.7 ± 0.4		0.9 ± 0.5
Aliphatics >C6-C8	0.1 ± 0.0		0.0 ± 0.0
Aliphatics >C8-C10	3.5 ± 0.0		2.0 ± 1.0
Aliphatics >C10-C12	9.8 ± 0.0		7.5 ± 1.8
Aliphatics >C12-C16	219 ± 0.0		174 ± 12.1
Aliphatics >C16-C21	406 ± 0.0		345 ± 13.2
Aliphatics >C21-C35	2200 ± 0.2		1977 ± 32.1
Aromatics (mg.kg^{-1})			
Aromatics >EC7-EC8	0.0 ± 0.0		0.0 ± 0.0
Aromatics >EC5-EC7	0.0 ± 0.0		0.0 ± 0.0
Aromatics >EC8-EC10	2.9 ± 0.8		1.6 ± 0.8
Aromatics >EC10-EC12	6.5 ± 1.3		5.0 ± 1.2
Aromatics >EC12-EC16	604 ± 36.8		407 ± 27.8
Aromatics >EC16-EC21	696 ± 43.3		581 ± 4.7
Aromatics >EC21-EC35	1857 ± 102.1		1753 ± 64.3
Aromatics >EC35-EC44	636 ± 35.2		616 ± 26.9
Aromatics >EC40-EC44	274 ± 19.1		267 ± 9.8
EPA16 PAHs (mg.kg^{-1})			
Naphthalene	28.1 ± 6.2		15.0 ± 7.5
Acenaphthylene	0.5 ± 0.3		0.3 ± 0.1
Acenaphthene	3.5 ± 0.6		2.5 ± 0.3
Fluorene	4.5 ± 0.6		3.1 ± 0.4
Phenanthrene	16.5 ± 2.5		11.7 ± 1.8
Anthracene	3.7 ± 0.4		2.7 ± 0.3

	Control		Bioturbated
	Mean ± STDEV		Mean ± STDEV
Fluoranthene	2.5 ± 0.5		2.0 ± 0.2
Pyrene	8.3 ± 1.5		6.9 ± 1.2
Benz(a)anthracene	4.6 ± 0.8		3.8 ± 0.9
Chrysene	5.0 ± 1.2		4.7 ± 0.9
Benzo(b)fluoranthene	2.3 ± 0.4		1.8 ± 0.5
Benzo(k)fluoranthene	0.7 ± 0.1		0.6 ± 0.2
Benzo(a)pyrene	3.4 ± 0.6		3.1 ± 1.0
Indeno(1,2,3-cd)pyrene	0.9 ± 0.2		0.8 ± 0.3
Dibenzo(a,h)anthracene	0.7 ± 0.1		0.7 ± 0.2
Benzo(g,h,i)perylene	2.2 ± 0.4		2.0 ± 0.6

Values shown are means ± standard deviation (n = 3). Those data significantly different to each other are underlined (t-test; $p < 0.05$).

5.2 Bacteria activity and community composition

RT-Q-PCR of 16S rRNA transcripts was used to assess the level of bacterial activity in the polychaete burrows compared to sediment without burrows and showed that 16S rRNA transcripts were significantly higher in the burrows (t-test; p 0.04), indicating that the bacterial community was more active (Figure 2a). Comparison of the relative abundance of 16S rRNA gene and transcript operational taxonomic units (OTUs) from t DNA and RNA samples showed that the bacteria communities formed discrete clusters that were significantly different to each other (PERMANOVA; $p < 0.002$) (Figure 3a), indicating that the polychaete burrow communities were distinct from the communities in the ambient sediment. When the presence/absence of OTUs was analysed the communities remained significantly different (PERMANOVA; $p < 0.01$) (Figure 3b).

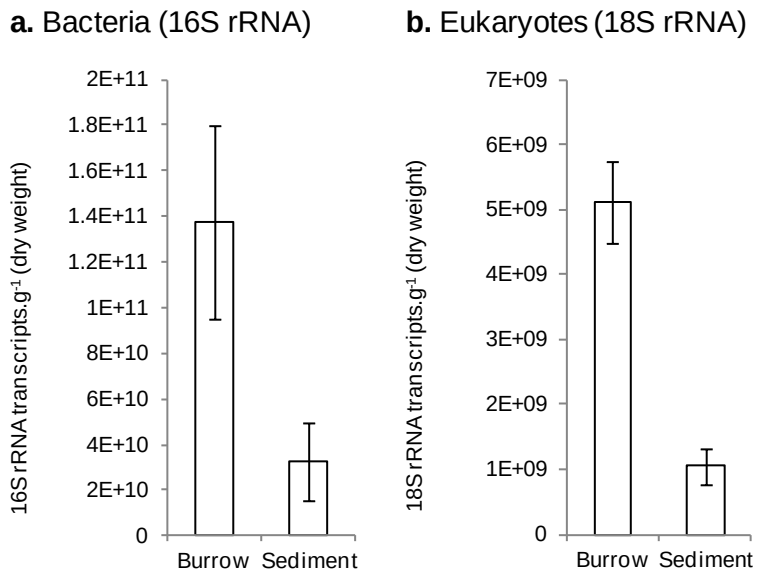

Figure 2 **Abundance of bacteria 16S rRNA (a) and eukaryote 18S rRNA (b) transcripts in *Hediste* (*Nereis*) *diversicolor* burrows and sediment unaffected by bioturbation. Values shown are means ± standard deviation (n = 3).**

There were also significant differences in bacterial community composition between burrows and sediment at the order scale (Figure 4a). In the 16S rRNA gene libraries produced from DNA extracted from the burrows, the relative abundance of the orders *Cytophagales*, *Saprospirales*, ML635J-21 (*Cyanobacteria*), *Nitrosomonadales*, *Alteromonadales*, *Methylococcales*, *Oceanospirillales*, *Thiotrichales* and *Alphaproteobacteria* and *Gammaproteobacteria* not taxonomically assigned at the order level were significantly increased (t-test; $p < 0.05$) compared to the sediment libraries. Conversely, the orders *Acidimicrobiales*, *Bacteroidales*, SHA-20 (*Chloroflexi*), *Bacillales*, *Clostridiales*, MSBL9 (*Planctomycetes*), *Pirellulales*, *Desulfarculales*, *Desulfobacterales*, *Desulfuromonadales*, *Marinicellales* and *Mollicutes* not taxonomically assigned at the order level were significantly more abundant (t-test; $p < 0.05$) in the sediment libraries compared to the burrow libraries.

We subsequently focused on changes in the relative abucance of specific OHCB genera in order to make links with the significant increase in hydrocarbon degradation in the bioturbated sediments compared to the non-bioturbated sediments. *Cycloclasticus* were present in the 16S rRNA gene libraries produced from the sediment DNA (3%) and RNA (1.3%), however increased in relative abundance in the 16S rRNA gene libraries produced from the burrow DNA (7.1%) and RNA (6.3%) (Figure 4b). *Alcanivorax* were also prevalent in the burrow libraries (DNA 1.8%, RNA 1.7%), yet present at only very low levels in the sediment DNA libraries (0.7%) and undetectable in the sediment RNA libraries. *Marinobacter* and *Oleibacter* were only minor components of the burrow communities, with *Oleibacter* undetectable in the sediment libraries and *Marinobacter* at very low levels in the sediment DNA libraries (0.2%).

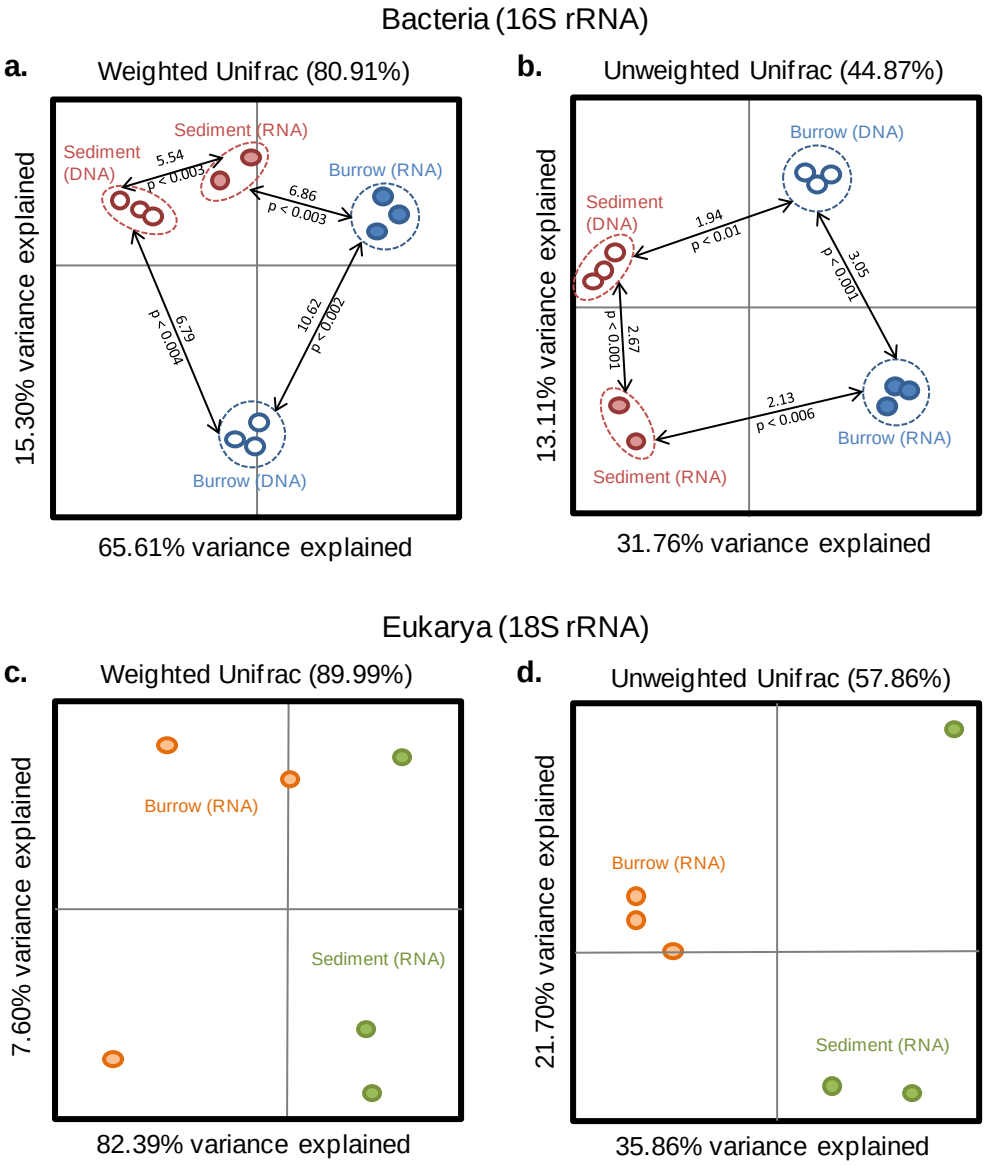

Figure 3 **Principal coordinates analyses plots describing betadiversity of total bacteria communities (a, b) and eukaryote communities (c, d) using UniFrac distance matrices generated from OTU (97% similarity) data. Weighted plots (a, c) are based on the relative abundance of OTUs. Unweighted plots (b, d) are based on the presence/absence of OTUs. The hatched circles joined by arrows highlight bacterial communities that are significantly different to each other determined by PERMANOVA analysis, and include pseudo F and p values.**

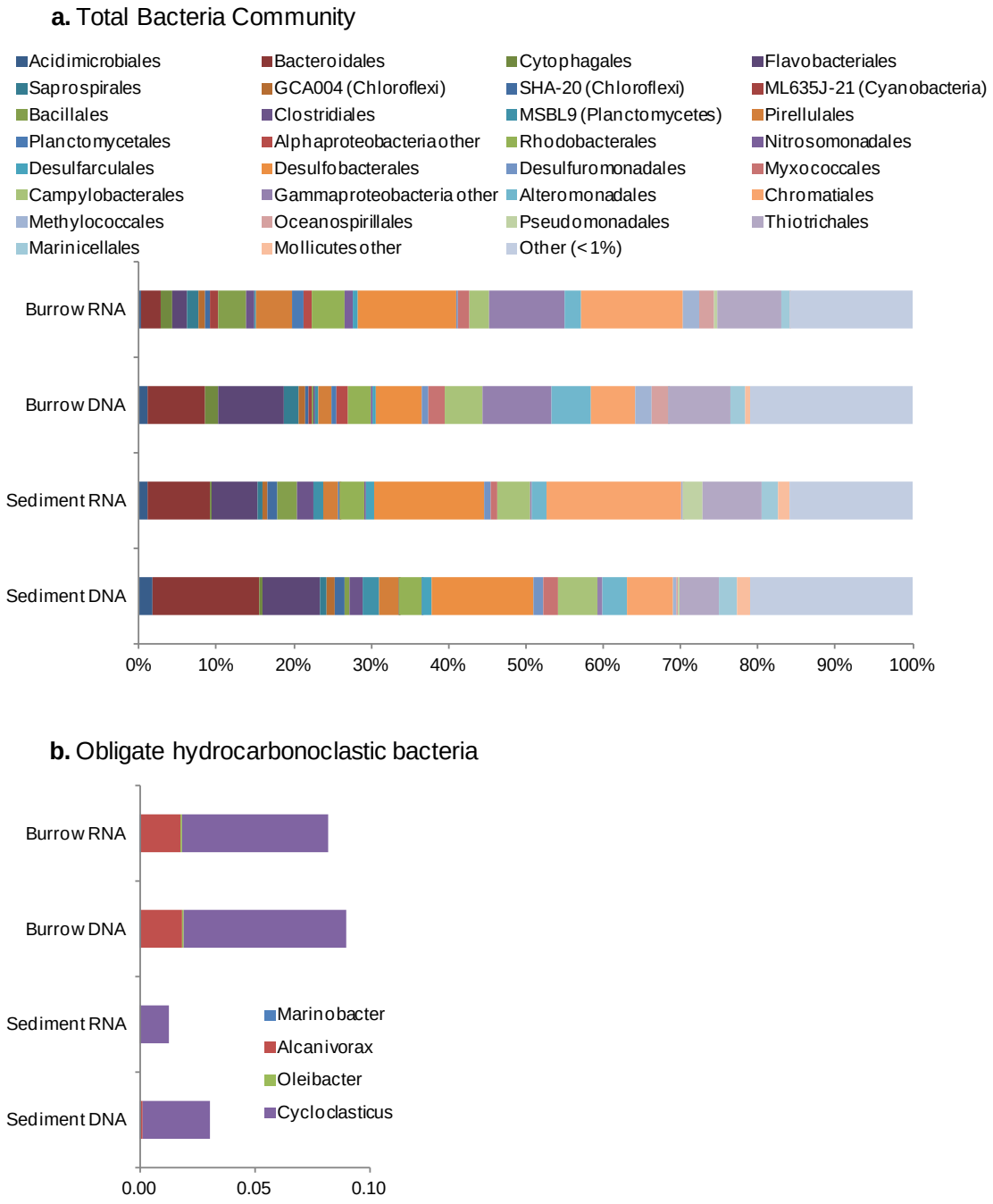

Figure 4 **Bacteria community composition determined as the relative abundance of taxa at the order scale (a). The relative abundance of obligate hydrocarbonoclastic bacteria genera that were detected in the libraries was also calculated (b). Values shown are the means (n = 3 for all data sets except sediment RNA were n = 2).**

5.3 Eukaryote activity and community composition

RT-Q-PCR of 18S rRNA transcripts was used to assess the level of eukaryote activity in the polychaete burrows compared to sediment without burrows. As with bacterial 16S rRNA analysis, 18S rRNA transcripts were significantly higher in the burrows (t-test; p 0.04), indicating that the eukaryote community was also more active (Figure 2b). Comparison of the relative abundance of 18S rRNA transcript OTUs from the RNA samples showed that the eukaryote communities did not form discrete clusters (Figure 3c). When the presence/absence of 18S rRNA transcript OTUs was analysed, the burrow communities formed a distinct cluster, however there was no significant difference between the burrow and sediment eukaryote communities (Figure 3d).

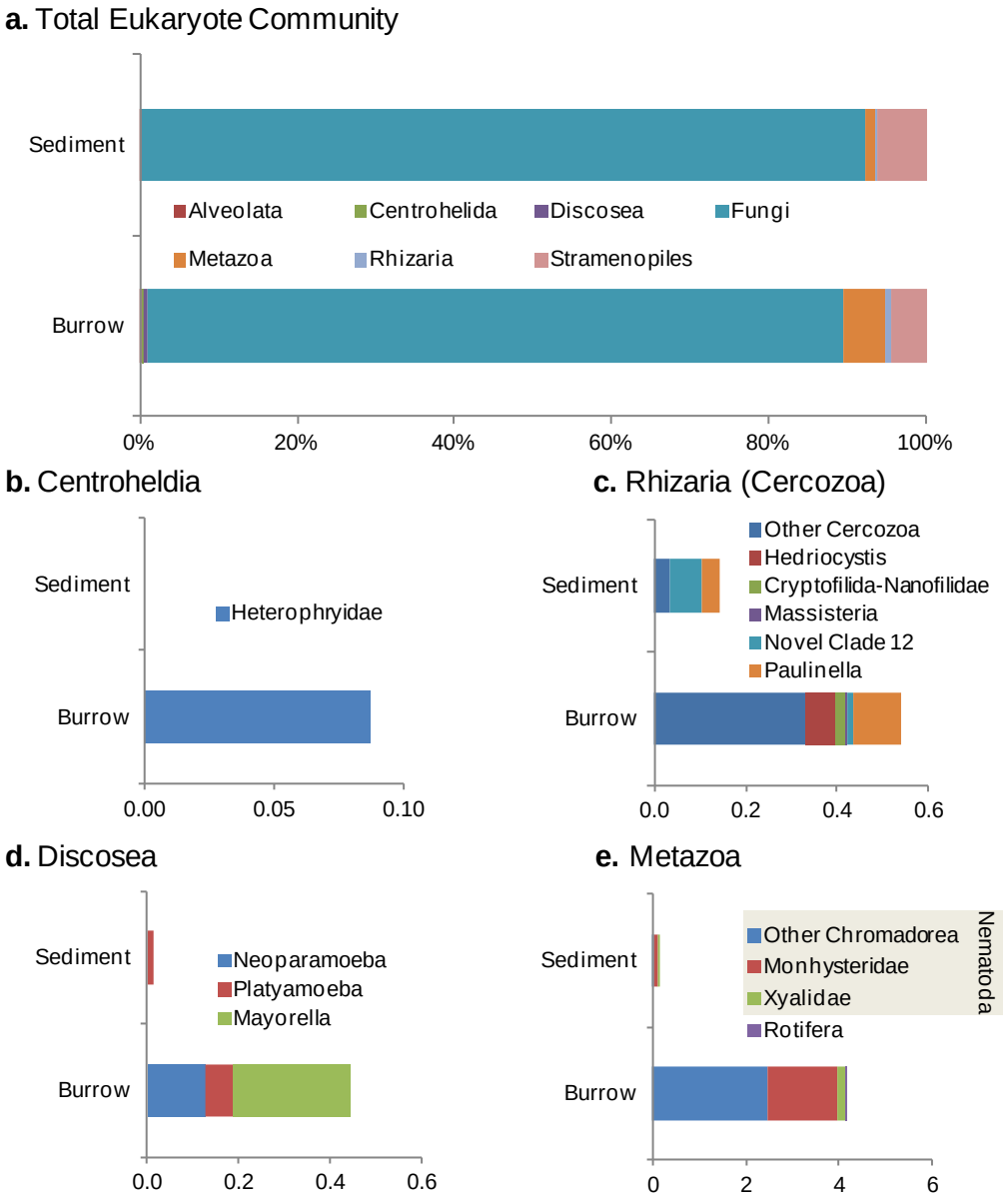

Figure 5 **Eukaryote community composition determined as the relative abundance of major taxa (a). The relative abundance of groups that were significantly more abundant in the burrow communities were studied in more detail (b-e). Values shown are the means (n = 3).**

Fungi dominated both polychaete burrow and control sediment eukaryote communities (Figure 5a), with most of the fungal 18S rRNA sequences associated to the phylum *Basidiomycota* (Figure 6). We focused on major differences in active eukaryote community structure in the polychaete burrows compared to the sediment, and showed that the protists *Centroheldia*, *Rhizaria* and *Discocea* were significantly increased in the burrow communities (t-test; p <0.05) (Figure 5 b-d). The family *Heterophryidae* dominated the *Centroheldia* OTUs enriched in the burrow RNA libraries (Figure 5b). All of the *Rhizaria* in the burrows were *Cercozoa*, and affiliated to several groups including the genera *Paulinella* and *Hedriocystis* (Figure 5c). The *Discosea* OTUs were affiliated with the genera *Neoparamoeba*, *Platyamoeba* and *Mayorella* (Figure 5d). Metazoan 18S rRNA sequences were also significantly increased in relative abundance in the polychaete burrows compared to the sediment, with most sequences affiliated to the nematode class *Chromadorea* (Figure 5e).

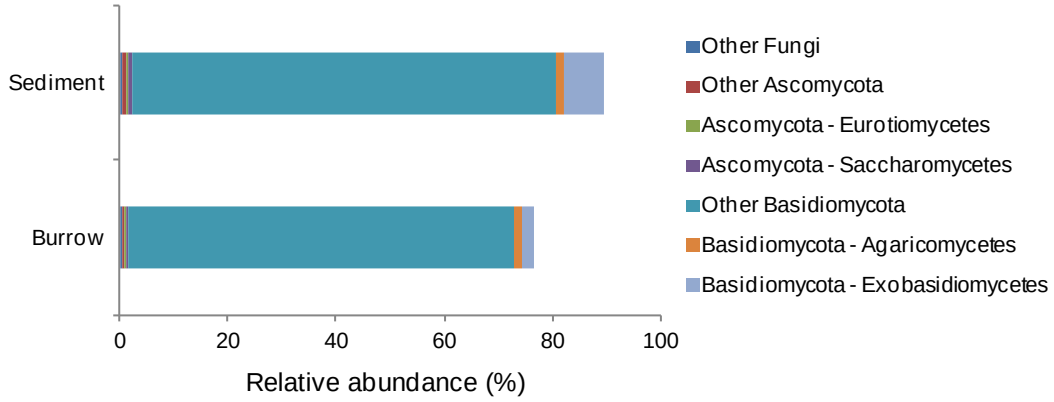

Figure 6 Fungi community composition determined as the relative abundance of major taxa. Values shown are the means (n = 3).

6 Discussion

In this study, we showed that *H. diversicolor* burrows in oil-contaminated sediment harbour distinct bacterial and eukaryote communities compared to un-bioturbated sediment communities. *H. diversicolor* naturally maintain semi permanent burrows from which they can feed directly on detritus and suspension feed, resulting in different geochemical impacts on the sediment, including irrigation with overlying oxygenated water and sediment mixing (Kristensen, 2001). Nereidid polychaetes also line their burrows with proteinaceous mucopolysaccharide secretions, and consequently increase the concentration of microbially-available organic carbon (Papaspyrou et al., 2006). Clustering of DGGE profiles generated from DNA samples collected from *H. diversicolor* burrows in uncontaminated sediments also showed that the communities present are distinct from surrounding ambient sediment (Papaspyrou et al., 2006), however the study did not identify bacterial taxa (i.e. sequence DGGE bands). Laverock et al (2010) using a combination of 16S rRNA gene terminal restriction fragment length polymorphism and clone library analysis, showed that in the distinct bacterial communities present in the burrows of shrimp, *Gammaproteobacteria* are increased in relative abundance and *Planctomycetes* are decreased compared to control ambient sediment. We show a similar pattern in *H. diversicolor* burrows, in that the *Gammaproteobacteria* orders *Alteromonadales*, *Methylococcales*, *Oceanospirillales* and *Thiotrichales* are more abundant, and that the candidate order MSBL9 and *Pirellulales*, both *Planctomycetes*, are less abundant.

Coinciding with the significant decline in sediment hydrocarbon concentrations, we observed the increased relative abundance of the OHCB genera *Cycloclasticus* and *Alcanivorax* in the *H.*

diversicolor burrows. Previous studies have shown that OHCB can respond rapidly to changing conditions, such as oil influx, and increase in abundance (Yakimov et al., 2007). In mesocosm experiments that mimic oil pollution events, both *Cycloclasticus* and *Alcanivorax* rapidly increase in abundance in order to exploit favourable growth conditions (Kasai et al., 2002a; Kasai et al., 2002b).

The *Deltaproteobacteria* orders *Desulfarculales*, *Desulfobacterales*, *Desulfuromonadales* were major components of microbial communities in the anoxic oil-polluted sediments after the Prestige oil spill (Acosta-González et al., 2012). These orders include cultured representatives that are known to anaerobically degrade hydrocarbons coupled with sulfate or iron reduction (Widdel et al., 2010). In the *H. diversicolor* burrows, these groups were less abundant compared to the control sediment. It is therefore possible that oxygenation of the sediment by the polychaetes caused a community switch over from anaerobic to aerobic metabolism that resulted in more rapid hydrocarbon degradation.

In this study, fungi dominated both *H. diversicolor* burrows and sediment communities. Only one previous study has assessed microbial eukaryote diversity in polychaete burrows (Engel et al., 2012). Engel et al (2012) showed that ciliates and diatoms dominate *A. marina* burrows in non-polluted coastal sediment. Assessment of the impact of the Deepwater Horizon oil spill on coastal sediment communities in the Gulf of Mexico also showed using 18S rRNA gene pyrosequencing that post-spill communities fungi become dominant (Bik et al., 2012). Fungi are known to degrade hydrocarbons directly (Cerniglia and Sutherland, 2010), and could also stimulate degradation through interspecies interactions with oil-degrading bacteria (McGenity et al., 2012).

We focused on the significant effects of bioturbation on eukaryote communities, and showed that *Centroheldia*, *Rhizaria*, *Discocea* and nematodes were enriched in the burrows compared to ambient sediment, all of which are bacterivorous. Nematodes can dominate coastal sediment meiofauna communities (Hubas et al., 2010), and have been shown to have a significant top-down impact on bacterial community structure (De Mesel et al., 2004). Previous studies have suggested that nematode predation could have a negative impact on PAH-degrading bacteria that results in reduced PAH degradation rates (Näslund et al., 2010). Other studies of non-polluted sediments suggest that bacterivorous nematodes have a positive impact on bacterial communities by increasing bacteria abundance and production (Hubas et al., 2010).

7 Conclusions

- Bioturbation of oil-contaminated sediment by the model infauna organism *H. diversicolor* accelerates hydrocarbon breakdown.
- Infauna change bacterial community composition, including the enhancement of specific hydrocarbon-degrading bacteria (*Cycloclasticus* and *Alcanivorax*).
- Infauna change eukaryote community composition, including the enhancement of bacterivorous protists and nematodes.
- Infauna could be further developed as a novel technology to accelerate the biodegradation of hydrocarbons in contaminated sediments.

8 References

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