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Temporal and depth-related differences in prokaryotic communities in abyssal sediments associated with particulate organic carbon flux

M.M. Moeseneder [a,b,d,*], K.L. Smith Jr.[c], H.A. Ruhl [d], D.O.B. Jones [d], U. Witte [a,1], J.I. Prosser [b,1]

[a] Oceanlab, University of Aberdeen, Main Street, Newburgh, AB41 6AA, Scotland, UK
[b] Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen, AB24 3UU, Scotland, UK
[c] Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA
[d] National Oceanography Centre, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK

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ABSTRACT

Particulate organic carbon (POC) flux is hypothesized to be the most important parameter influencing activity and biomass of prokaryotic and faunal communities in the abyssal seafloor, but there is little evidence of POC-related changes in community composition of prokaryotes. This hypothesis was tested by 16S rRNA-gene-based analysis of prokaryotic DNA and RNA extracted from abyssal seafloor sediments during periods of low and high POC flux. Fingerprint analysis of prokaryotic communities indicated that approximately 50% of the phylotypes were identical at each sediment horizon, regardless of the temporal variations in POC flux. However, phylotypes were also detected that represented a relatively dynamic component of these communities and were probably strongly influenced by the prevalent POC flux regime. These patterns were also detected in deeper sediment horizons. DNA- and RNA-based community profiles differed, although both approaches had similar community dynamics. Crenarchaeota showed the strongest shift in community composition in response to availability of labile POC, indicating that POC flux may have a more pronounced impact on crenarchaeal communities than on bacterial communities. The high number of phylotypes common to each sample time suggests that both standing stock and active prokaryotic communities are stable.

1. Introduction

Abyssal sediments at depths between 3000 and 6000 m cover > 50% of the earth's surface (Smith et al., 2009), comprising one of its largest biomes. They were traditionally considered to be well buffered from seasonality and changing environmental conditions in the overlying water column (Druffel and Robinson, 1999; Menzies, 1965), and to have low process rates, because of the low temperature and organic nutrient input. Observation of seasonal and inter-annual variation in organic matter supply to the abyssal seafloor, linked to benthic productivity and oxygen consumption (Billett et al., 1983; Smith and Baldwin, 1984), challenged this view, and the close coupling of deep-sea benthic processes to surface water dynamics is now well established. Sedimentation of phytodetrital aggregates and other organic material from the euphotic zone (pelagic–benthic coupling) is the most important energy source for life in the deep-sea (Graf, 1989) and, POC flux to the abyss is hypothesized to be the single most important factor influencing changes in community composition of organisms on the deep seafloor (Danovaro et al., 2000a; Gooday, 2002; Graf, 1989; Smith et al., 2002).

However, while carbon cycling and respiration appear to be dominated by prokaryotes in abyssal sediments (Turley and Lochte, 1990) and both abundance and metabolic activity of prokaryotes vary in response to seasonal nutrient input (Boetius et al., 2000; Rowe et al., 1991; Turley, 2000; Turley and Lochte, 1990), the link between POC flux and prokaryotic community composition is not well established. Prokaryotic cell concentration in sea-floor sediments is greater than in productive open ocean surface waters (10^8 cells per cm^3), in part through the accumulation of material at the sediment surface and limited grazing pressure (Böer et al., 2009; Jørgensen and Boetius, 2007; Schauer et al., 2010), but there is little understanding of the mechanisms leading to and maintaining prokaryotic biomass or diversity.

Community composition of Bacteria in abyssal sediments is highly complex (Li et al., 1999b). Archaeal communities generally show less heterogeneity, with 63% of the clones belonging to Marine Group I (MGI) Crenarchaeota in archaeal clone libraries (Vetriani et al., 1999; Wang et al., 2005). Bacteria have an extensive variety of metabolic traits in these abyssal sediments (Jørgensen and Boetius, 2007), while the role of MGI Crenarchaeota as mediators of

* Corresponding author at: National Oceanography Centre, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK. Tel.: +1 203 432 6737; fax: +1 203 432 6167.
E-mail address: m.moeseneder@noc.ac.uk (M.M. Moeseneder).
[1] Joint last authors.

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biogeochemical cycling of matter is only recently emerging from research on the ocean water column. Vertical profiles from the euphotic zone to abyssal depths have shown that members of MGI Crenarchaeota provide a larger proportion of prokaryotic cells with increasing depth and may contribute significantly to oceanic ammonia oxidation (Agogué et al., 2008; Karner et al., 2001; Wuchter et al., 2006). Comparative genomics recently revealed that MGI Crenarchaeota probably belong to the newly described deep-branching phylum Thaumarchaeota (Brochier-Armanet et al., 2008; Pester et al., 2011). It is therefore likely that a substantial amount of crenarchaeal community composition described here falls within this novel phylum.

Temporal and spatial variability of marine prokaryotic communities has been studied by DNA-targeted analysis of 16S rRNA genes, but RNA-targeted analysis increases sensitivity and is believed to assess active communities (Martinez et al., 2006; Moeseneder et al., 2001), as active prokaryotic cells generally contain greater numbers of ribosomes than inactive or dormant cells (DeLong et al., 1989; Poulsen et al., 1993).

The aim of the present study was to investigate whether the composition of 'total' and active prokaryotic communities in abyssal sediments changes temporally and whether such changes result from temporal variation in POC flux to the abyssal seafloor. Because the POC flux to the abyss is hypothesised to have greatest influence on changes in community composition of organisms on the deep seafloor (Danovaro et al., 2000b; Gooday, 2002; Graf, 1989; Smith et al., 2002), a temporal sampling strategy was employed to determine the impact of POC flux on indigenous prokaryotes in different depth horizons of the abyssal sediment. Research was performed at Station M (Sta. M, 3953 m), a monitoring site in the northeast Pacific for which, more than 20 years of POC flux data to abyssal depths are available (Smith and Druffel, 1998). Vertical POC flux typically increases in early summer at Sta. M, and phytodetritus can completely cover the seafloor by August or September (Baldwin et al., 1998; Smith et al., 1994). POC flux usually decreases sharply in the fall and stays low during winter and early spring. Furthermore, POC utilization, usually assessed as sediment community oxygen consumption (SCOC) is routinely measured in situ with autonomous respiration chambers at Sta. M. Long time series records show a distinct, recurring seasonal signal in SCOC, which is highest in summer and fall and lowest in winter at this site. Prokaryotes and smaller fauna mainly drive these annual patterns in SCOC (Drazen et al., 1998; Ruhl et al., 2008; Smith et al., 1994). Thus, by sampling in June, September and February, respectively, we were able to analyse prokaryotic community composition during phases differing significantly with regard to the vertical POC input and organic matter remineralisation.

2. Materials and methods

2.1. Field sampling

Three replicate push cores each were used to sample sediment at the abyssal long-term study site Sta. M, 200 km off the Californian coast (Fig. 1a) in June 2007, September 2007 and in February 2009 (3953 m depth, temperature constant at 1.49 °C at all sampling events). Samples were taken at the onset of the summer period with elevated vertical POC flux (June), the end of this period, (September) and 17 months later when very low input of POC prevailed (February). At Sta. M, sediment porewater (3953 m depth) oxygen concentration is ~145 μmol l^{-1}, and the oxygen penetration depth is 3 cm (Reimers et al., 1992; Smith, 1992). Silty clay, with a gray-black appearance prevails in sediment horizon depth greater than 3 cm. Sampling was done using the remotely operated vehicles (ROV) *Tiburon* and *Doc Ricketts*. Three replicate cores were taken 2 m apart on each sampling date. Upon arrival of the ROV on deck, the 0–1, 1–2, 2–3, 3–5, 5–10 and 10–15 cm horizons of each core were immediately sliced in a cold room at 4 °C and slices were stored at −80 °C. Sediment horizons from 5–10 to 10–15 cm are not analysed here in this study.

2.2. Measurement of particulate organic carbon (POC)

Sediment traps were moored at 600 and 50 m above bottom (mab), providing 10-day integrated measurements of POC flux to the deep sea as described by (Baldwin et al., 1998). POC flux data for February 2009 are not available and were estimated using an empirical model, which takes into account the preceding climate, upwelling and satellite estimated export of POC from the surface ocean *sensu* (Smith et al., 2006). The quality of the results from the model was tested, and the variability between the in situ measurements and the empirical model are minimal. It uses inputs from the Northern Oscillation Index (Schwing et al., 2002), Bakun Upwelling Index (Bakun, 1973) and satellite estimated export flux (Lutz et al., 2007) all with temporal lag offsets that were determined by cross correlation with between the respective input variable and sediment trap measured POC flux. The variability between the in situ measurements and the empirical model indicate the model can reliably determine if fluxes were high or low.

2.3. Extraction of nucleic acids and cDNA preparation

Nucleic acids were extracted from 0.5 g of sediments (wet weight) using a bead-beating method (Griffiths et al., 2000) and were re-suspended with 30 μl nuclease-free H_2O (Ambion, Life Technologies, Carlsbad, USA) and stored at −80 °C. Nucleic acids were extracted exactly the same way at different times during the temporal sampling. DNA concentration was determined with a Thermo-Scientific Nanodrop 1000 (Labtech, UK). Community cDNA was prepared following (Mahmood et al., 2005) modified by use of random-hexamer primers. Seven microlitre of extracted nucleic acids was incubated at 37 °C for 60 min with 1 μl DNase (2U, Ambion, Life Technologies, Carlsbad, USA) according to the manufacturer's instructions and 1 μl of this reaction was used in a control PCR (using the bacterial primers from the first round PCR), to check for complete DNA digestion. Reverse transcription of RNA to cDNA was done with a SuperScript II Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions.

2.4. Polymerase chain reaction (PCR) amplification

Bacteria and Crenarchaeota were characterized by DNA- and RNA-targeted analysis of 16S rRNA genes, PCR-amplified from extracted DNA and cDNA, respectively. Direct PCR for Denaturing Gradient Gel electrophoresis (DGGE) was not possible, because of low nucleic acid concentration in extracts and/or the presence of inhibitors, and a nested PCR approach was therefore used (Mahmood et al., 2005). First-round amplification was performed using Bacteria-specific primers 27F_B (5′-GGTGTGAGAGCACTTGCC-3′) and pF (5′-ACGAGCTGACGACAGCCATG-3′) or Archaea-specific primers 20F (5′-TTCCGGTTGATCCYGCCRG-3′) and 958R (5′-YCCGGCGTTGAMTC-CAATT-3′) (Edwards et al., 1989; Lane et al., 1991; Massana et al., 1997). The 50-μl PCR reactions contained 0.1 μM of both primers, 16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl (pH 8.8), 5 mM $MgCl_2$, 200 μM of each dNTP, 1% bovine serum albumin (BSA, Ambion, Carlsbad, USA), 1 U of Polymerase, (Bioline, London, UK), and 1 μl of nucleic acid extract. PCR cycling conditions consisted of initial denaturation at 95 °C for 5 min followed by 10 cycles at 94 °C for 0.5 min, annealing at 55 °C for 0.5 min, and extension at 72 °C for 1 min. PCR products were further amplified by an additional 25 cycles of denaturation at 92 °C for 0.5 min, annealing at 55 °C for

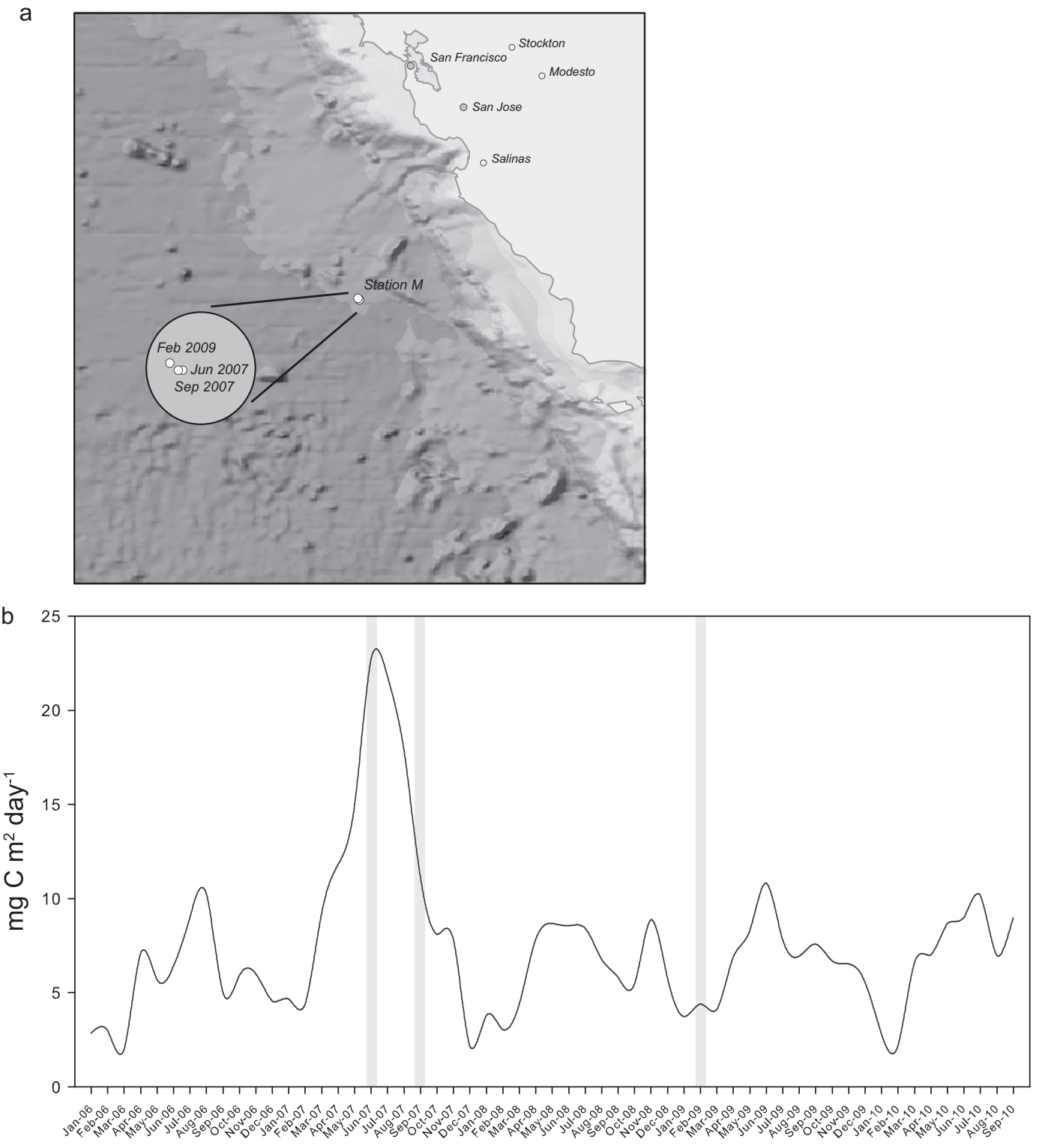

Fig. 1. (a) Sta. M sampling site off the Californian coast. Circle insert represents a detailed view of the location at different sampling times, where the diameter of the circle is ~6 km. (b) Flux of particulate organic matter carbon at Sta. M during 2006 to 2010 from in situ measurements and the empirical model. Gray bars represent the times at which samples were taken. POC flux was 20, 11 and 4 mg C $m^{-2}d^{-1}$ in June–July, September and February, respectively.

0.5 min and extension at 72 °C for 1 min. PCR products were checked for correct size and quality by 1% agarose gel electrophoresis and 1 μl was used as a template for nested PCR with the Bacteria-specific DGGE primers Mf_341F_GC 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) and Mr_534R (5′-ATTACCGCGCTGCTGG-3′) or crenarchaeal DGGE primers CREN_771F (5′-ACGGTGAGGGATGAAAGCT-3′) and CREN_957R_GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCGGCGTTGACTCCAATTG-3′) (Muyzer et al., 1993; Ochsenreiter et al., 2003). Cycling conditions for bacterial and crenarchaeal nested PCR were as

described for first round PCR amplification. Nested PCR products were also checked as described above. For the analysis of rRNA, 1 µl of cDNA reaction was used for first-round PCR, followed by nested PCR amplification as described above.

2.5. Quantification of end point PCR products

First-round PCR products (5 µl) were run on 1% agarose gels and quantified using known concentrations of a Hyperladder (Bioline, London, UK). Agarose gels were imaged using a gel documentation system, and individual bands were quantified with the GelEval software (Frogdance Software, http://www.frogdance.dundee.ac.uk) by comparing pixel intensities of samples to reference bands from the Hyperladder. Lower band intensities were regarded as a rough estimate for lower template concentrations for PCR in the samples (i.e., lower standing stock or active prokaryotes based on DNA and RNA profiles, respectively).

2.6. Denaturing gradient gel electrophoresis (DGGE), shared operational taxonomic units (OTUs), and cluster analysis

Amplification products were analysed using DGGE (as partially shown in Fig. 3) as described previously (Muyzer et **al**., 1993) using 8% polyacrylamide gels, with a denaturant gradient of 35 to 65%, run for 16 h at 75 V and 60 °C. Sensitivity of band detection was increased by silver staining (Mahmood et al., 2005). Bands were considered to represent OTUs and differences in banding patterns indicated differences in OTU composition. Separate binary matrices were constructed for bacterial and crenarchaeal DGGE gels, based on the presence or absence of OTUs and results were used to determine OTUs that were present in all samples from a particular sediment horizon and those shared between DNA- and RNA-targeted analyses in samples taken at all sampling times (June, September and February). The binary matrix was loaded into Primer6 (Primer-E, Lutton, UK) and cluster analysis was used as previously described to evaluate the significance groupings (Bertics and Ziebis, 2009).

2.7. Statistical analysis

Plainstat (Plainstat Software, http://www.plainstat.com) was used to perform Student's *t*-tests of significance. Significance of clustering of DGGE profiles was determined using SIMPROF in the Primer6 package (Bertics and Ziebis, 2009).

3. Results

3.1. Bulk DNA concentrations

DNA concentration of extractable DNA was significantly higher in all samples collected in February than June and September (Fig. 2a). Furthermore, DNA concentration in June and September at sediment horizons 2–5 cm was significantly greater ($p < 0.05$) than in the surface horizon (0–2 cm).

3.2. Temporal and spatial variability of PCR amplifiable prokaryotic nucleic acids during different POC fluxes

End point bacterial PCR concentration was significantly ($p < 0.001$) higher in September (Fig. 2b and c). Variability in DNA concentration between triplicate cores was greater in September samples than in those taken in June and February (Fig. 2b), while variability in end point RNA-based concentration was higher in June than in February and September samples (Fig. 2c). There was no evidence of sediment depth-associated differences in bacterial DNA and RNA end point PCR concentrations, except for significantly higher ($p < 0.01$) bacterial DNA concentration in deeper horizons (2–5 cm) taken in June (Fig. 2b) than in surface samples (0–2 cm).

End point DNA- and RNA-targeted bacterial PCR concentrations were significantly ($p < 0.001$) higher than those of Crenarchaeota (Fig. 2b–e). Crenarchaeal DNA showed no significant temporal differences (Fig. 2d), but crenarchaeal RNA was significantly ($p < 0.01$) higher in September (Fig. 2e), where variability between replicate cores was also higher than in the other months.

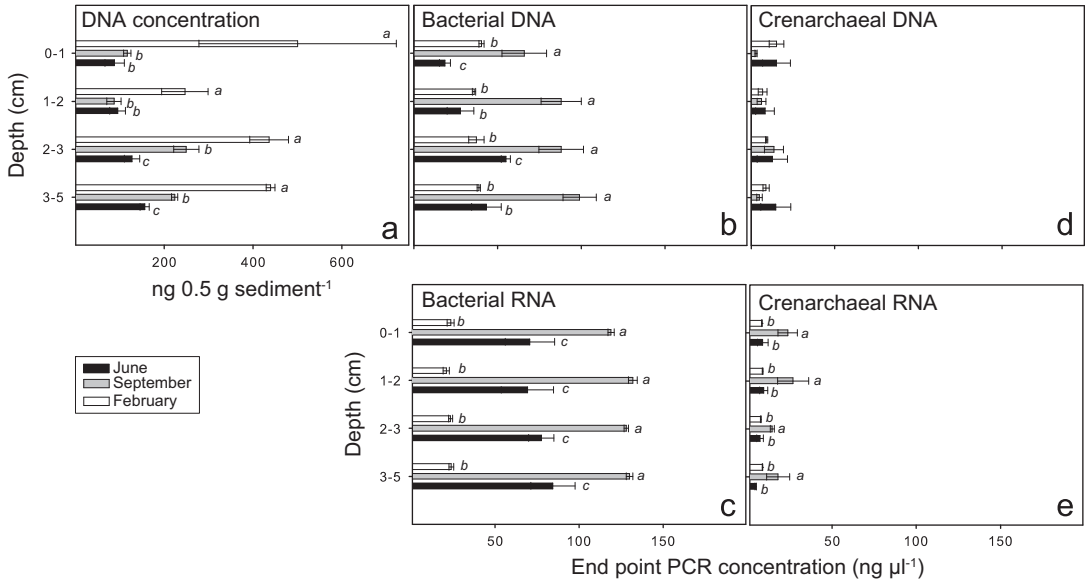

Fig. 2. (a) DNA concentration in 0.5 g abyssal sediment (wet weight) sampled at 4 depths (0–1, 1–2, 2–3, 3–5 cm) in June 2007, September 2007 and February 2009 and concentrations of first-round PCR products amplified from (b) and (d) DNA and (c) and (e) RNA (cDNA) using bacterial and crenarchaeal primers. Error bars represent standard errors of triplicate cores. Values with different letters are significantly different at the $p < 0.05$ level, Students's *t*-test from those at the same sample time.

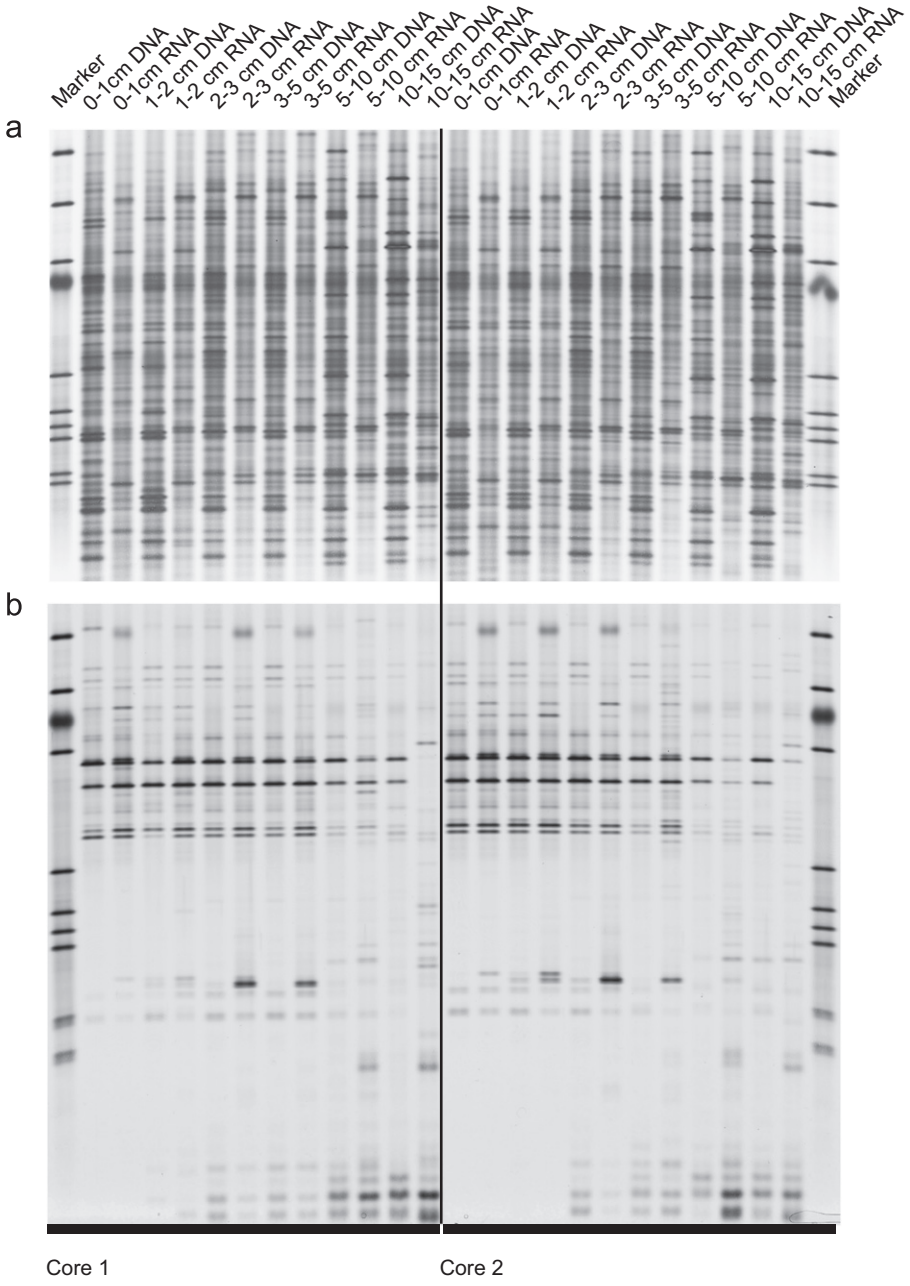

Fig. 3. Representative DNA and RNA DGGE profiles from 2 replicate cores taken in February. Sediment horizons down to 5 cm depth were included in the analysis. (a) Bacterial DGGE profiles and (b) crenarchaeal DGGE profiles. DGGE profiles from replicate cores were reproducible.

3.3. Distributions of OTUs

A large number (44 and 83%) of OTUs were common to all bacterial DNA and RNA DGGE profiles (Fig. 4). Slightly fewer (24 and 79%) were common to all crenarchaeal DNA and RNA profiles. The number of bands shared between bacterial DNA and RNA profiles ranged between 19 and 60%, with lower numbers (12 and 27%) of shared between crenarchaeal DNA and RNA profiles (Fig. 5).

3.4. Comparison of bacterial and crenarchaeal community composition

Representative DGGE profiles indicated a high reproducibility of this fingerprinting approach (Fig. 3). The gels shown are from one sampling, but other samplings (June, September) indicated similar heterogeneity. Although the resolution of the banding patterns in Fig. 3 was beyond the fine scale resolution subsequently used for further analysis, the profiles provide a general overview of the different temporal samplings. Deeper sediment horizons at 5–10 and 10–15 cm were not included in the analysis. Bacterial DNA and RNA profiles formed four major clusters, which were associated with different sediment horizons (Fig. 6a). Surface horizons (0–1 and 1–2 cm) clustered together, indicating greater similarity within bacterial communities at these depths than in deeper horizons (2–5 cm). DNA and RNA profiles fell within distinctive sub-clusters within these depth-related clusters. Profiles from replicate cores taken in June and September generally clustered together, and formed 2 distinct sub-clusters, with communities in February samples being less closely related, but following the same pattern with respect to sediment depth. Cores from the 1–2 cm horizons followed a different temporal pattern, with June and February RNA profiles forming 2 distinct

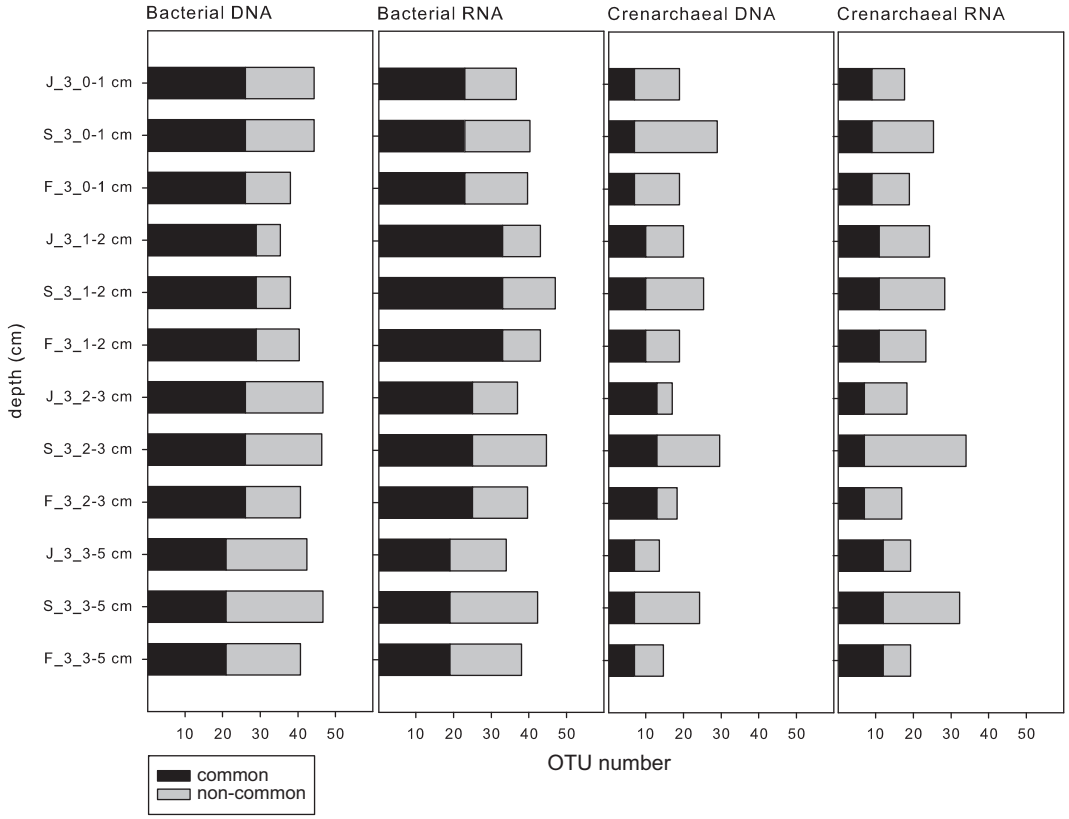

Fig. 4. Common and non-common OTUs in samples taken at different times and sediment depths. Common OTUs are those detected at a certain sediment horizon only (0–1, 1–2, 2–3 or 3–5 cm) but at each sampling time (June, September and February). Non-common OTUs represent the remaining phylotypes detected. Replicate cores are plotted for June (J_), September (S_) and February (F_) samples on the *y*-axis. Data are presented as means of replicate cores. Standard errors were <1 for all data points.

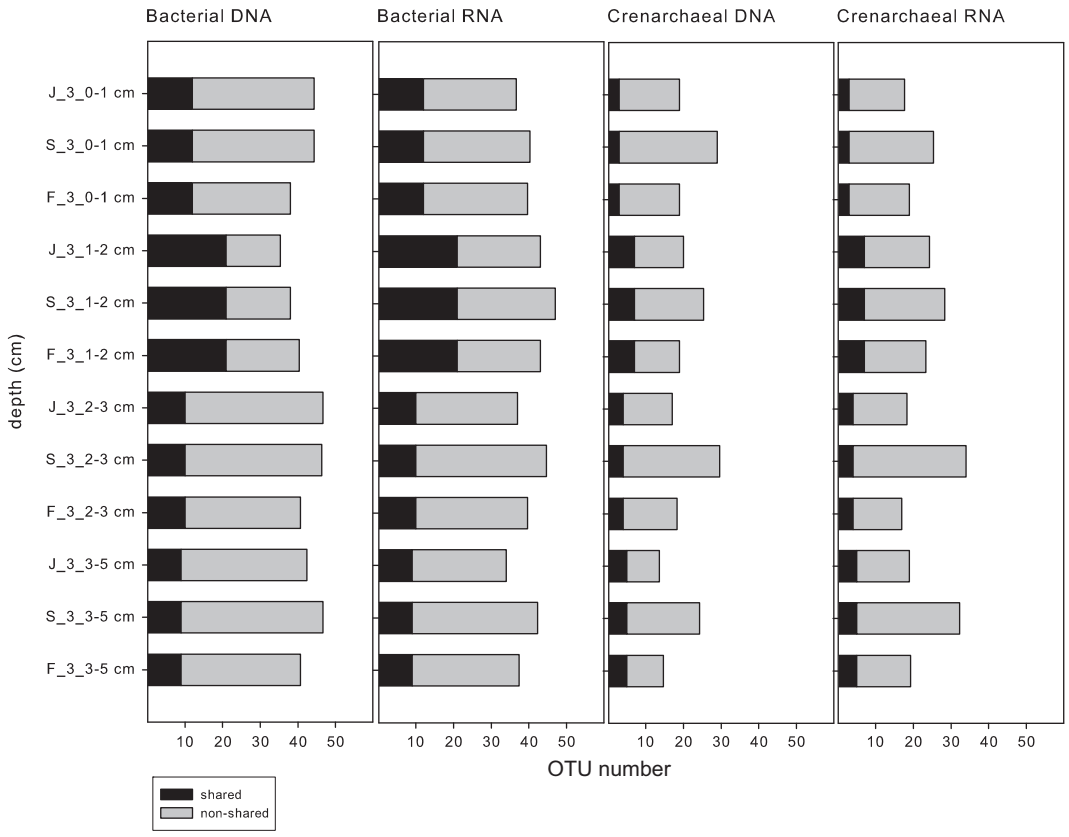

Fig. 5. Shared OTUs in the dataset. Shared OTUs are those identical phylotypes in DNA and RNA based DGGE profiles that were detected at a certain sediment horizon only (0–1, 1–2, 2–3 or 3–5 cm), but at all sampling times (June, September and February). Non-shared OTUs represent the remaining phylotypes detected. Replicate cores are plotted for June (J_), September (S_) and February (F_) samples on the *y*-axis. Data are presented as means of replicate cores. Standard errors were <1 for all data points.

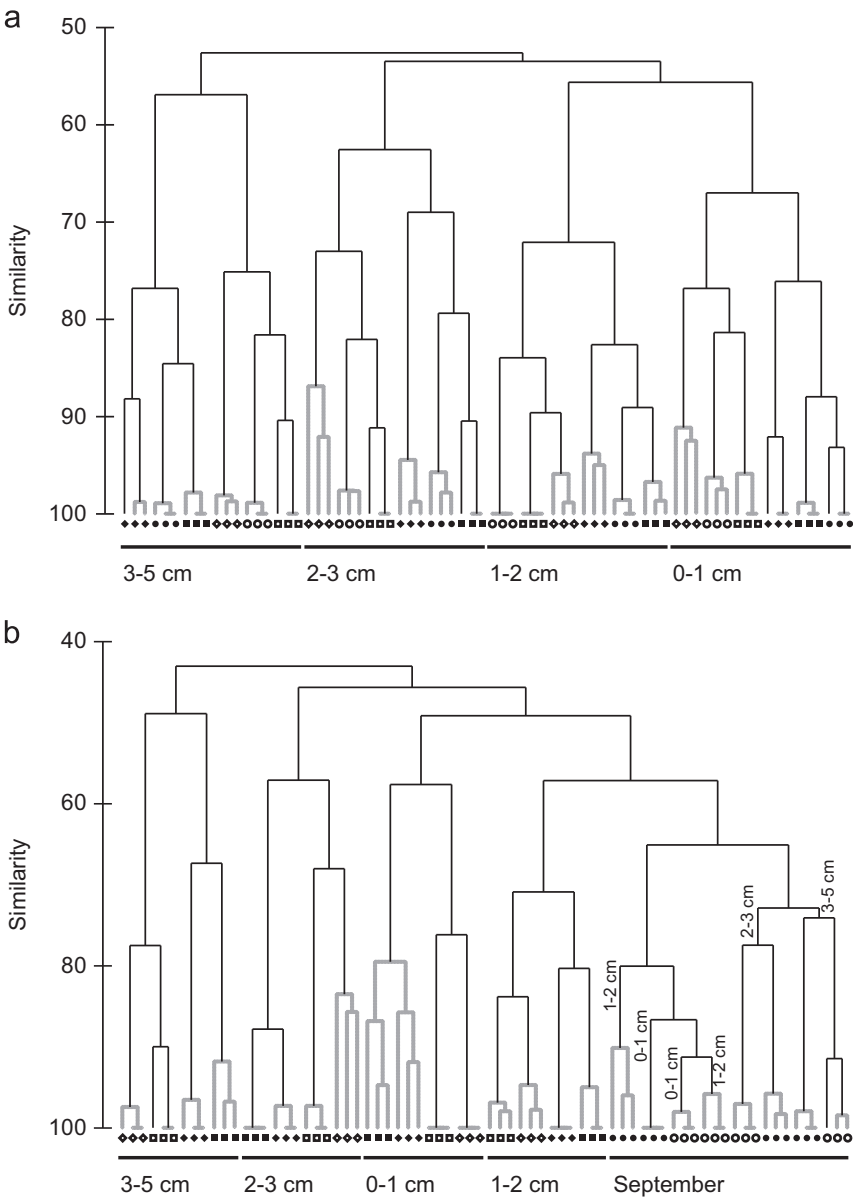

Fig. 6. Cluster analysis of bacterial (a) and crenarchaeal (b) DNA and RNA DGGE profiles in triplicate sediment samples taken in June, September and February at depths of 0–1, 1–2, 2–3 and or 3–5 cm. Similarity values between 50% and 100% for Bacteria and 40% and 100% for Crenarchaeota were considered in the analysis. Clustering of groups with grey branches is not supported statistically. ■ = June DNA, ● = September DNA, ♦ = February DNA, □ = June RNA, ○ = September RNA, ◇ = February RNA.

sub-clusters and September RNA profiles forming a larger cluster for the RNA. Profiles from triplicate cores indicated some spatial variability in bacterial community composition but generally clustered together and spatial differences were generally less than temporal differences.

With the exception of samples taken in September, crenarchaeal DNA and RNA profiles formed separated clusters within all depth-related subclusters, as observed for bacterial profiles (Fig. 6b). However, all September samples formed a major cluster with two sub-clusters comprising profiles from the upper (0–2 cm) and lower (2–5 cm) sediment horizons, with further subclusters discriminating DNA and RNA profiles. Only RNA profiles from the 1–2 cm horizon clustered differently and were more similar to the 0–1 cm RNA community profiles from September. The DNA and RNA crenarchaeal profiles were more closely related to the 1–2 cm sediment depth samples taken in February and June, with increasingly distant relationships to February and June samples at sediment depths of 0–1, 2–3 and 3–5 cm.

4. Discussion

4.1. Bulk DNA concentrations

Since the nucleic acids extraction protocol applied here used a rigorous bead-beating step in the presence of Cetyl Trimethyl Ammonium Bromide (CTAB) to precipitate large amount of humic substances, we are confident that all our extractions yielded DNA and RNA with the same efficiency and are therefore comparable. POC flux at Sta. M could influence prokaryotic and eukaryotic abundance and activity and hence differences in extractable DNA concentrations. Thus, temporal maxima in POM fluxes in late summer might be expressed by the patterns of reoccurring higher standing stocks of organisms (extractable DNA as a proxy) months later. Consistent discrepancies between bulk DNA concentrations and first round PCR concentrations with prokaryotic primers suggested that the elevated extractable DNA in February was not prokaryotic and could therefore originate from eukaryotic

organisms and/or extracellular DNA (Corinaldesi et al., 2005; Guilini et al., 2010; Moodley et al., 2002). Macrofaunal biomass at Sta. M has been shown to peak ~8 month after peaks in POC flux (Ruhl et al., 2008). However, the extraction methods might contain traces of refractory organic matter/humic substances, which could subsequently interfere with DNA measurements to a small extent. DNA concentrations for Bacteria and Crenarchaeota measured in sediments at Sta. M were relatively low in comparison to total nucleic acid and other analyses of abyssal sediments (Dell'Anno and Corinaldesi, 2004; Dell'Anno and Danovaro, 2005), and DNA and RNA PCR amplification yields were also low.

The DNA/RNA extraction protocol used (Griffiths et al., 2000) is believed to be efficient, but it is possible that the proportion of prokaryotic DNA in intact cells may have been small in relation to total extractable DNA. There is evidence for extracellular DNA in marine sediments (Dell'Anno and Corinaldesi, 2004; Dell'Anno and Danovaro, 2005), some of which may be of high molecular weight, although extracellular 16S rRNA genes have not been amplified (Corinaldesi et al., 2005). Because of this uncertainty in the origin of DNA templates, we did not quantify gene abundance by quantitative PCR, but rather quantified amplifiable PCR products in first round PCR reactions, which were subsequently used in nested PCR and DGGE analysis. The quantification of end-point PCR products, as presented here, should be seen as a very rough estimate on concentrations of amplifiable gene products from abyssal sediments, the quantification is prone to identical PCR biases as those found in quantitative PCR (Suzuki and Giovannoni, 1996). Although we did not address the extent of the potential biases, this rough estimate of end-point PCRs gave some conservative estimates on gene dynamics in abyssal sediments. RNA is generally degraded faster than DNA in marine sediments, and extracellular RNA is unlikely to have been amplified (Novitsky, 1986). Since our DNA- and RNA-targeted results showed identical trends, we are confident that there was a negligible impact of extracellular DNA.

Changes in bulk DNA concentration generally occurred in both surface oxygenated sediment layers (~0–3 cm) and in deeper sediment horizons (3–5 cm). This indicates effective sediment mixing by bioturbation, thereby possibly increasing oxygen and POC concentrations in deeper horizons and fuelling microbial growth (Bertics and Ziebis, 2009; Brunnegaard et al., 2004; Laverock et al., 2010; Reichardt, 1988; Ruhl et al., 2008; Smith et al., 2008). These results are contrary to findings of steep decreases in DNA concentration in deeper abyssal sediment horizons, perhaps because sites studied at the northeastern Atlantic Ocean may have had more pronounced and stable anoxic gradients with decreased bioturbation (Dell'Anno et al., 1999).

4.2. Persistence of prokaryotic communities during variable particulate organic carbon flux

A large proportion of all OTUs were common to bacterial and crenarchaeal DGGE profiles in June, September and February, comprising ~50% phylotypes in all samples from a particular sediment horizon. Previous studies indicated similar stability of pelagic and benthic prokaryotic communities over periods ranging from weeks to months to years (Böer et al., 2009; Pereira et al., 2006; Pringault et al., 2008; Stevens et al., 2005). Various studies also showed phylotypes recurring over extensive geographic distances and temporal samplings (Böer et al., 2009; Hewson and Fuhrman, 2006; Pachiadaki et al., 2011, 2010; Polymenakou et al., 2005; Pringault et al., 2008; Schauer et al., 2010). For example, bacterial communities in replicate samples from Sta. M sediment and from deep Eastern Mediterranean sea were found to differ more than those between these two sites (Kouridaki et al., 2010). The same authors also point out the greater role of the quantity and quality of POC fluxes, rather than geographic location, in structuring bacterial communities (Polymenakou et al., 2005). In addition, the same proportions of bacterial phylogenetic classes (e.g., the Gammaproteobacteria) usually occur in abyssal sediments (Arakawa et al., 2006; Bowman and McCuaig, 2003; Kouridaki et al., 2010; Li et al., 1999a,b). This high number of re-occurring phylotypes and classes also point to a compositional persistence of prokaryotic communities in abyssal sediments.

4.3. The impact of variation of POC flux on standing stock and active prokaryotic community composition

Prokaryotic DGGE profiles also indicated temporal differences in OTU composition, suggesting that communities were influenced more by the prevalent physicochemical regime, such as POC flux (Polymenakou et al., 2005). Sediment depth was the primary factor in determining bacterial community composition, with sample time a secondary, but consistent factor. Time-oriented clusters are probably driven by phylotypes that are more influenced by the POC flux. Similar differences were also detected for deeper sediment horizons, although these (here 3–5 cm) are generally regarded as less variable over time (Jørgensen and Boetius, 2007; Llobet-Brossa et al., 1998). One important aspect explaining the depth-related differences in OTU patterns is the transition from oxic (0–5 cm) to anoxic (5–15 cm) redox-regimes in the sediment, thereby changing the metabolic processes of prokaryotes and ultimately the prokaryotic community composition (Jørgensen and Boetius, 2007). Spatial variability between triplicate cores was always less than that between sediment depths or sample times, and triplicates always clustered together. We are therefore confident that our chosen description of prokaryotic community composition provided reliable and reproducible data when triplicate cores from each temporal sampling were compared.

Further, more detailed studies are needed to address temporal changes in prokaryotic community profiles in different sediment horizons, but it seems that deeper sediment horizons potentially host prokaryotic communities that are more dynamic and active than currently expected, possibly fuelled by geoelectric- or geochemical-microniches of the sediments (Allen et al., 2007; Nielsen et al., 2010).

4.4. Standing stock and active prokaryotic communities

All triplicate cores from the 3 temporal samplings formed distinct sub-clusters, for the DNA- and RNA-based approaches, and this trend was found throughout the different sediment horizons. These results indicate significant differences between the 'total' community, or standing stock, and the active community. For bacterial communities on average ~25% of all OTUs were common to both DNA and RNA profiles, while ~12% of crenarchaeal OTUs were shared. Distinct differences between RNA- and DNA-based approaches have been previously reported from other marine habitats (Gentile et al., 2006; Martinez et al., 2006). The concentration of bacterial first round amplification products from DNA was always greatest in September samples, suggesting that high POC flux increased bacterial biomass. These results are consistent with the RNA profiles, which indicated potentially high activity of these communities in September (higher ribosome numbers). However, this might not always be the case. DNA and RNA community profiles may differ temporally, as prokaryotic RNA changes (shifts in activity) are more likely to occur at shorter time scales than DNA changes, which require DNA replication and cell division (Coolen and Shtereva, 2009; Dumont and Murrell, 2005; Manefield et al., 2002; Whiteley et al., 2007). Thus, RNA

profiles probably reflect more accurately prokaryotic OTUs that are involved in biogeochemical cycling of POC.

4.5. Crenarchaeal communities during variable POC flux

Crenarchaeal DGGE profiles in the upper sediment horizons (0–2 cm) differed more than those of Bacteria and all crenarchaeal DNA- and RNA-based profiles from September samples clustered separately. These differences indicate that crenarchaeal communities are influenced most during high POC flux events (here September). Localized heterotrophic decomposition of POC can lead to increases in ammonia (Smith et al., 1983), which may lead to a distinct spatial and compositional distribution of ammonia-oxidizing members of the crenarchaeal community. Although there is only one study indicating that MGI Crenarchaeota are involved in the oxidation of ammonia in abyssal sediments (Roussel et al., 2009), archaeal ammonia oxidation might be a significant process in these organisms (Wuchter et al., 2006), potentially explaining the compositional changes during high POC fluxes to the deep ocean.

The lower initial crenarchaeal nucleic acid concentration, in comparison with Bacteria, suggest that Crenarchaeota are a minor component of the standing stock of prokaryotes in abyssal sediments, but potentially play an important responsive major role in the biogeochemistry of these abyssal sediments. Furthermore, the concentration of first round crenarchaeal PCR products was highest in September for all sediment horizons, indicating enhanced crenarchaeal activity during enhanced POC flux. Although it is not possible to determine whether these increases resulted from crenarchaeal utilisation of organic substrates or ammonia (derived from either the POC flux itself and/or associated microbial activity), the results showed that Crenarchaeota were likely to respond significantly to the increases of POC on the seafloor. These results also suggest a positive correlation between increased POC flux and crenarchaeal activity (here based on RNA), providing the first indication that POC flux to abyssal deep-sea has a greater impact on crenarchaeal than bacterial communities.

5. Conclusions

Standing stock and active prokaryote communities in abyssal sediments appeared to be spatially and temporally stable. Non-common OTUs detectable via DGGE fingerprinting may represent the dynamic component of these communities and may be influenced by the prevalent physicochemical regime, notably POC availability. Total and active communities showed similar temporal trends and crenarchaeal communities were more dynamic than bacterial communities, indicating a greater effect of POC flux to the abyssal deep-sea on Crenarchaeota. Further research on factors controlling abyssal prokaryotic community composition, including phylotypes from the 'rare biosphere', will enable long-term prediction of prokaryotic communities, as recently shown for river bacterioplankton (Crump et al., 2009).

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