



# Spatial patterns of bacterial abundance, activity and community composition in relation to water masses in the eastern Mediterranean Sea

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**ABSTRACT:** To determine the variation of bacterial activity and community composition between and within specific water masses, samples were collected throughout the water column at 5 stations in the eastern Mediterranean Sea corresponding to the regions of the northern Aegean, mid-Aegean, western Cretan, Ionian and southern Aegean Seas. Prokaryotic abundance below 100 m declined with depth at all the stations, while decreasing trends with depth in prokaryotic heterotrophic activity were present only at 2 out of the 5 stations. Bacterial community composition (BCC), determined using both automated rRNA intergenic spacer analysis and terminal-restriction-fragment length polymorphism, was also related to depth although the number of operational taxonomic units was remarkably constant throughout the water column. Overall, the maximums in similarity values of the BCC between water sample pairs decreased with increasing temperature–salinity (T–S) distance of the water samples probably due to distinct biogeochemical characteristics of water masses. However, considerable dissimilarity in the BCC between samples with identical T–S values, and hence within the same water mass, was also observed, possibly reflecting heterogeneity in the organic matter field or in biotic control within a given water body. Thus, we conclude that the richness of bacterial communities is remarkably constant with depth down to bathypelagic waters. The similarity of bacterial communities in water parcels with identical temperature and salinity can range from highly similar to very dissimilar, reflecting variability in substrate supply despite the physical uniformity of water parcels.

**KEY WORDS:** Bacteria · Community composition · Patchiness · Spatial scale · Eastern Mediterranean Sea

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## INTRODUCTION

Prokaryotes play a major role in marine biogeochemical fluxes (Williams 2000). The relation between microbially mediated biogeochemical transformation rates and the prokaryotic community composition is a central research topic in microbial ecology, linked to the function–stability debate (Miki et al. 2008, Fuhrman 2009, Yokokawa & Nagata 2010). Examining distribution patterns of prokaryotes and environmental parameters over space and time is one of the ap-

proaches used to determine how prokaryotes relate to specific biogeochemical processes.

The distribution patterns of prokaryotic abundance and activity throughout the water column of the open ocean have been determined extensively in the Atlantic (Herndl et al. 2005, Reinthaler et al. 2006), in the North Pacific (Nagata et al. 2000), in the Arabian Sea (Hansell & Ducklow 2003) and in the north-western Mediterranean Sea (Tanaka & Rassoulzadegan 2004, Winter et al. 2009). Also, at the 2 open ocean long-term time-series stations, the Bermuda Atlantic

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Time-Series Study and the Hawaii Ocean Time-Series, microbial activity and community dynamics have been related. From these data, the notion emerged that the prokaryotic community composition of the open oceanic water column is highly stratified (Giovannoni et al. 1996, DeLong et al. 2006). Distinct depth profiles of prokaryotes have been found for community composition and specific phylogenetic groups. For example, Crenarchaea are the dominant group in deep waters (Herndl et al. 2005, De Corte et al. 2009). The SAR 202 cluster dominates in waters below 2000 m depth, comprising up to 40% of the bacterial community in the North Atlantic (Varela et al. 2008).

The eastern Mediterranean Sea features several characteristics, which affect prokaryotic activity and probably community composition, such as its oligotrophic nature and the warm deep waters (about 14°C). In the North Aegean Sea, Black Sea Water (BSW) forms the surface waters. Below the BSW, Levantine Intermediate Water (LIW) extends to about 400 m depth, and North Aegean Deep Water (NAeDW) reaches down to the bottom of the North Aegean Trough. In the mid-Aegean Sea, surface waters are comprised by Levantine Surface Water (LSW) followed by LIW, and NAeDW down to the bottom of the Chios Basin. In the South Aegean Sea, the main water masses are the surface Atlantic Water (AW), LIW, Transitional Mediterranean Water mass (TMW) and the Cretan Deep Water (CDW) in the Cretan Basin. In the Ionian Sea, the water masses from surface to bottom waters are AW, LIW, TMW and the East Mediterranean Deep Water mass (EMDW). All the water masses have a specific temperature and salinity signature, although the water masses are composed of waters of different ages (Zervakis et al. 2000). In the layer up to 400 m depth, BSW, LIW, LSW, AW and TMW mix frequently on a seasonal time scale. In contrast, the deep waters (NAeDW, CDW) in the basins of the eastern Mediterranean Sea are fairly isolated and exchange on a decadal scale (Zervakis et al. 2004).

There are only a few studies on the prokaryotic community composition of the water column of the eastern Mediterranean Sea using terminal-restriction-fragment length polymorphism (T-RFLP) (Moeseneder et al. 2001a,b), clone libraries (Moeseneder et al. 2005) and fosmid libraries (Martín-Cuadrado et al. 2007). The differences in the characteristics of the water bodies from north to south in the eastern Mediterranean Sea are reflected by the considerable changes in the BCC (Moeseneder et al. 2001a) and microbial activity (De Corte et al. 2009).

The aim of the present study was to determine the spatial patterns of prokaryotic abundance, activity and BCC in the different water masses of the eastern Mediterranean Sea. Specifically, we assessed spatial

variations of the BCC in relation to the specific water masses identified by their temperature and salinity characteristics.

## MATERIALS AND METHODS

**Study site and sampling.** The POSEIDON cruise was conducted with the RV 'AEGAEON' (Hellenic Center for Marine Research, Greece) in the eastern Mediterranean Sea in May 2007 and sampled 5 stations (Fig. 1). The stations were located in the North Aegean (48° 7.0' N, 24° 32.5' E), mid-Aegean (37° 42.1' N, 25° 26.0' E), western Cretan (36° 13.2' N, 23° 18.3' E), Ionian (36° 15.9' N, 21° 30.1' E) and South Aegean Seas (35° 47.2' N, 24° 54.7' E). Water samples were obtained from surface waters (10 m depth) and the meso- (150 to 1000 m depth) and bathypelagic layers (1000 to 4350 m depth) with 5 l Niskin bottles mounted on a CTD rosette sampler.

**Abundance of prokaryotes.** Prokaryotic abundance was determined in seawater collected from the Niskin bottles and fixed with 0.2 µm filtered formaldehyde (2% final concentration). Thereafter, the samples were frozen in liquid nitrogen for 10 min and kept at -80°C until analysis. Prokaryotic abundance was determined by flow cytometry within 2 mo after collecting the samples. Samples were thawed to room temperature, and 0.5 ml subsamples were stained with SYBR Green I in the dark for 10 min, and, subsequently, 1 µm fluorescent latex beads (Molecular Probes) (~10<sup>5</sup> ml<sup>-1</sup>) were added to the samples as an internal standard. The

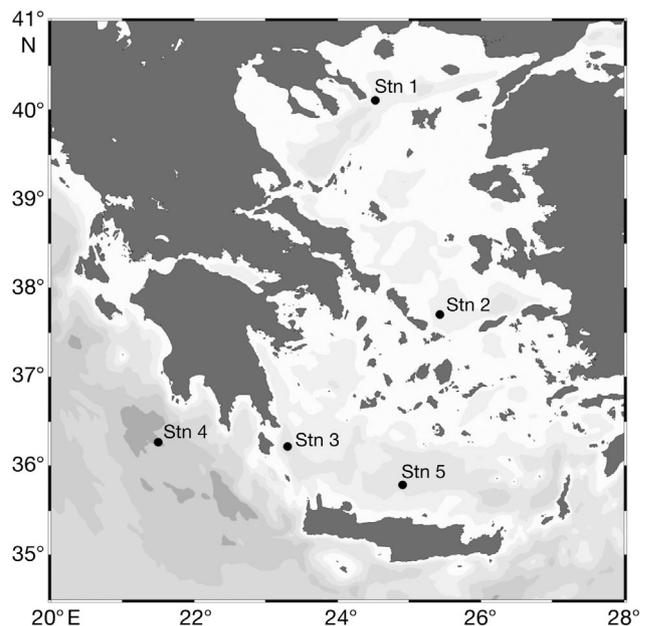


Fig. 1. Sites in the eastern Mediterranean Sea sampled during the POSEIDON cruise in May 2007

prokaryotes were enumerated on a FACScalibur flow cytometer (Becton Dickinson) by their signature in a plot of green fluorescence versus side scatter, and the abundance was calculated based on the ratio of stained cells to the added bead standard.

**Leucine incorporation rate.**  $^3\text{H}$ -leucine incorporation rate was determined as a proxy for prokaryotic production (Kirchman 2001). Subsamples (5 to 40 ml) from each depth were amended with 5 nM  $^3\text{H}$ -leucine (Amersham, specific activity 160 Ci mmol $^{-1}$ ) and incubated at *in situ* temperature ( $\pm 2^\circ\text{C}$ ) in the dark. Incubation time varied depending on depth: 1 h for upper waters (up to 100 m) and 24 h for deep waters (below 100 m). After incubation, samples were filtered onto polycarbonate filters (pore size, 0.2  $\mu\text{m}$ ; Millipore) and rinsed twice with both 5% trichloroacetic acid and 80% ethanol. The samples were radioassayed with a liquid scintillation counter (1211 Rack beta, Wallac) using FilterCount (Packard) as scintillation cocktail. Duplicate samples and 1 trichloroacetic acid-killed control were prepared for each depth. The disintegrations per minute (DPM) of the killed control were subtracted from the mean DPM of the corresponding duplicate samples and converted to leucine incorporation rates.

**Bacterial community composition.** A volume of 10 l of seawater was filtered through a 0.22  $\mu\text{m}$  Sterivex filter cartridge (Millipore). Thereafter, 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris-HCL, 0.75 M sucrose) was added to the filter cartridge and stored at  $-80^\circ\text{C}$ . DNA extraction from Sterivex filter cartridges was performed by Mega Kit extraction (MoBio Laboratories) using the protocol of the manufacturer. DNA extracts were concentrated ( $\sim 10$ -fold) with a Centricon device (Millipore).

**PCR and T-RFLP.** PCR conditions and chemicals were applied as described by Moeseneder et al. (2001a). One microlitre of the DNA extract was used as a template in a 50  $\mu\text{l}$  PCR mixture. The primers used for PCR were the *Bacteria*-specific primer 27F-FAM and the universal primer 1492R-JOE (Lane 1991). Samples were amplified by an initial denaturation step at  $94^\circ\text{C}$  (3 min), followed by 35 cycles of denaturation at  $94^\circ\text{C}$  (1 min), annealing at  $55^\circ\text{C}$  (1 min) and extension at  $72^\circ\text{C}$  (1 min). Cycling was completed by a final extension at  $72^\circ\text{C}$  (7 min). Two negative controls (1  $\mu\text{l}$  of Sigma water and 49  $\mu\text{l}$  of PCR mixture) were included in each PCR run to check for potential contamination and unspecific products. The PCR products were run on 1.0% agarose gels. The gel was stained with a working solution of SYBR Gold (Molecular Probes). The obtained bands were cut, purified by Quick gel extraction kit (Genscript), and quantified using a Nanodrop spectrophotometer. Fluorescently labelled PCR products were digested at  $37^\circ\text{C}$  overnight. Each reaction contained 30 ng of cleaned PCR product, 5 U of tetrameric restriction enzyme

(*HhaI*) and the respective buffer filled up to a final concentration of 50  $\mu\text{l}$  with ultrapure water (Sigma). The restriction enzyme was heat inactivated and precipitated by adding 4.5  $\mu\text{l}$  LPA solution and 100  $\mu\text{l}$  100% isopropanol. The samples were stored at room temperature for 15 min and afterwards were centrifuged at  $15\,000 \times g$  for 15 min. After removing the supernatant, the pellets were rinsed with 100  $\mu\text{l}$  70% isopropanol and precipitated again by centrifugation ( $15\,000 \times g$  for 5 min). Thereafter, the supernatant was removed, and the samples were dried in the cyclor at  $94^\circ\text{C}$  for 1 min and stored at  $-20^\circ\text{C}$ .

The pellet was resuspended in 2  $\mu\text{l}$  of ultraclean water (Sigma), and the product was denatured in 7.8  $\mu\text{l}$  of Hi-Di formamide at  $94^\circ\text{C}$  for 3 min. Each sample contained 0.2  $\mu\text{l}$  GeneTrace 1000 (ROX) marker (Applied Biosystems). Fluorescently labelled fragments were detected and separated with an ABI Prism 310 capillary sequencer (Applied Biosystem) run under GeneScan mode (van der Maarel et al. 1998, Moeseneder et al. 1999). The size of the fluorescently labelled fragments was determined by comparison with the internal GeneTrace 1000 (ROX) size standard. Injection was performed electrokinetically at 15 kV for 15 s (adjustable), and the runs were completed at 15 kV and  $60^\circ\text{C}$  within 35 min.

**Automated rRNA intergenic spacer analysis (ARISA).** ARISA-PCR was performed following the method of Borneman & Triplett (1997) with modifications. One microlitre of the DNA extract was used as a template in a 50  $\mu\text{l}$  PCR mixture. The primers used were ITSf, 5'-GTC GTA ACA AGG TAG GCC GTA-3', and ITSr eub, 5'-GCC AAG GCA TCC ACC-3'. The primer ITSf was end-labelled with the phosphoramidite dye 5-FAM. Samples were amplified by an initial denaturation step at  $94^\circ\text{C}$  for 2 min, followed by 30 cycles of amplification at  $94^\circ\text{C}$  for 15 s,  $55^\circ\text{C}$  for 30 s and  $72^\circ\text{C}$  for 3 min, and a final extension at  $72^\circ\text{C}$  for 9 min. As for T-RFLP, 2 negative controls were included in each PCR run to check for potential contamination and unspecific products. Five microlitres of PCR products were run on a 2.0% agarose gel for quality assessment of the products. The gel was stained with a working solution of ethidium bromide. The PCR products were purified with Quick Clean PCR product purification kit (Genscript) and quantified using a Nanodrop spectrophotometer. Fluorescently labelled fragments (8 ng  $\mu\text{l}^{-1}$  of sample) were separated and detected with an ABI Prism 310 capillary sequencer (Applied Biosystem) run under GeneScan mode. The size of the fluorescently labelled fragment was determined by comparison with the combination of the internal GeneTrace 1000 (ROX), CST 300-1800 and the size standard. Injection was performed electrokinetically at 10 kV for 5 s (adjustable), and the run was completed at  $60^\circ\text{C}$  within 60 min.

The output of T-RFLP and ARISA from the ABI Genescan software was transferred to the Fingerprinting II software (Bio-Rad) to determine peaks and to standardize the peaks with the size marker. The threshold of peaks was set to 0.1% of the maximum peak height per run. Peaks above the 0.1% threshold were defined as operational taxonomic units (OTUs) and were used for further analysis.

**Statistical analyses.** Presence/absence patterns of OTUs of individual samples determined by both T-RFLP and ARISA were further analyzed using the Primer software (Primer-E) to determine the Jaccard similarity. To compare the similarity matrices of prokaryotic communities collected at different sites or depths, the Mantel test was used (Paleontological Statistics [PAST] Ver. 1.75).

## RESULTS

### Environmental parameters

There was little variability in temperature and salinity among the 5 stations (Table 1). The temperature difference between surface and deep waters was relatively small, with a maximum range of 20.35 to 13.36°C. Phosphate and nitrate concentrations exhibited a common depth-related trend, with low concentrations in the top 100 m layer and increasing with depth (Table 1). Oxygen concentrations remained rather constant, with depth exhibiting no pronounced oxygen minimum layer below the euphotic layer.

### Depth distribution patterns of prokaryotic variables

Prokaryotic abundance (PA) ranged from  $2.5 \times 10^5$  to  $5.0 \times 10^5$  cells ml<sup>-1</sup> in the surface layer, and decreased

exponentially with depth to  $0.2 \times 10^5$  cells ml<sup>-1</sup> at 3000 m depth (Fig. 2A). The depth-dependent decrease of PA was described by a log-log linear regression with a slope of  $-0.65 \pm 0.23$  ( $n = 5$ ) (Table 2).

Prokaryotic leucine incorporation (leu incorp.) decreased by 1 order of magnitude from surface waters (97.3 pmol leu incorp. l<sup>-1</sup> h<sup>-1</sup>) to the mesopelagic layer (Fig. 2B). The depth distribution patterns of prokaryotic leu incorp., however, were less pronounced than those for PA and were variable among stations. All the depth profiles of leu incorp. showed, after a decline from the 10 m layer to the 100 m layer, a peak in the mesopelagic layer (200 to 1000 m), varying, however, among stations. Significant depth-dependent decreases (log-log linear regression) were only found at Stn 1 and Stn 4 (Table 2).

Cell-specific activity (prokaryotic leu incorp. divided by PA) exhibited a depth-related trend only in the euphotic layer, but not in upper mesopelagic to bathypelagic waters (Fig. 2C). Maximum cell-specific activity was  $6.3 \times 10^{-3}$  fmol leu cell<sup>-1</sup> d<sup>-1</sup> at 50 m depth at Stn 3 (Fig. 2C). Generally, cell-specific activity was more variable in meso- and bathypelagic layers than in the surface layer. In meso- and bathypelagic layers, the minimum cell-specific activity was  $5.5 \times 10^{-5}$  fmol cell<sup>-1</sup> d<sup>-1</sup> at 750 m depth at Stn 4, and the highest was  $2.4 \times 10^{-3}$  fmol cell<sup>-1</sup> d<sup>-1</sup> at 750 m depth at Stn 5. Overall, the cell-specific activity in the meso- and bathypelagic waters was within 1 order of magnitude, with only a few exceptions (Fig. 2C).

### Bacterial community composition analyzed by T-RFLP and ARISA

The T-RFLP pattern of the bacterial community revealed in total 63 OTUs on the 16S rRNA gene level, ranging from 12 to 22 OTUs sample<sup>-1</sup>, whereas the

Table 1. Physical and chemical characteristics of the stations sampled during the cruise in the eastern Mediterranean Sea. Data for the euphotic layer (<100 m depth) correspond to the values for each sample. Mean  $\pm$  SD are given for meso- and bathypelagic waters

Stn	Region	Bottom depth (m)	Layer (m)	No. of samples	Temperature (°C)	Salinity	Dissolved oxygen (μmol l <sup>-1</sup> )	PO <sub>4</sub> (μmol l <sup>-1</sup> )	NO <sub>3</sub> (μmol l <sup>-1</sup> )	NO <sub>2</sub> (μmol l <sup>-1</sup> )
1	North Aegean	1010	<100	2	14.31, 16.18	36.73, 38.45	129.8, 133.6	0.04, 0.05	0.36, 0.68	0.05, 0.10
			100–950	6	13.84 $\pm$ 0.40	38.99 $\pm$ 0.04	124.2 $\pm$ 4.6	0.12 $\pm$ 0.04	3.10 $\pm$ 1.29	0.05 $\pm$ 0.01
2	Mid-Aegean	800	<100	2	15.54, 16.60	39.16, 40.00	109.6, 141.9	0.02	0.07, 0.13	0.05
			100–750	7	14.38 $\pm$ 0.30	39.01 $\pm$ 0.05	132.6 $\pm$ 3.2	0.07 $\pm$ 0.02	1.76 $\pm$ 0.53	0.05 $\pm$ 0.01
3	Western Cretan	1245	<100	2	15.00, 17.82	38.79, 38.82	137.5	0.03	0.12, 0.41	0.04, 0.08
			100–1200	7	14.45 $\pm$ 0.15	38.94 $\pm$ 0.03	130.1 $\pm$ 2.3	0.10 $\pm$ 0.04	2.56 $\pm$ 1.06	0.05 $\pm$ 0.01
4	Ionian	4322	<100	2	16.85, 19.92	38.67, 38.65	137.3, 147.0	0.02, 0.03	0.03, 0.06	0.04, 0.05
			100–1000	7	14.86 $\pm$ 0.81	38.81 $\pm$ 0.06	129.9 $\pm$ 11.0	0.09 $\pm$ 0.07	1.76 $\pm$ 1.54	0.04 $\pm$ 0.01
			2000–4350	3	14.60 $\pm$ 0.78	38.75 $\pm$ 0.01	123.0 $\pm$ 1.3	0.15 $\pm$ 0.01	3.57 $\pm$ 0.45	0.04 $\pm$ 0.01
5	South Aegean	1420	<100	2	15.63, 20.35	35.98, 39.20	117.6, 152.3	0.03	0.07, 0.33	0.05, 0.08
			100–1350	7	14.68 $\pm$ 0.52	39.00 $\pm$ 0.06	137.2 $\pm$ 4.7	0.10 $\pm$ 0.04	2.52 $\pm$ 1.26	0.07 $\pm$ 0.06

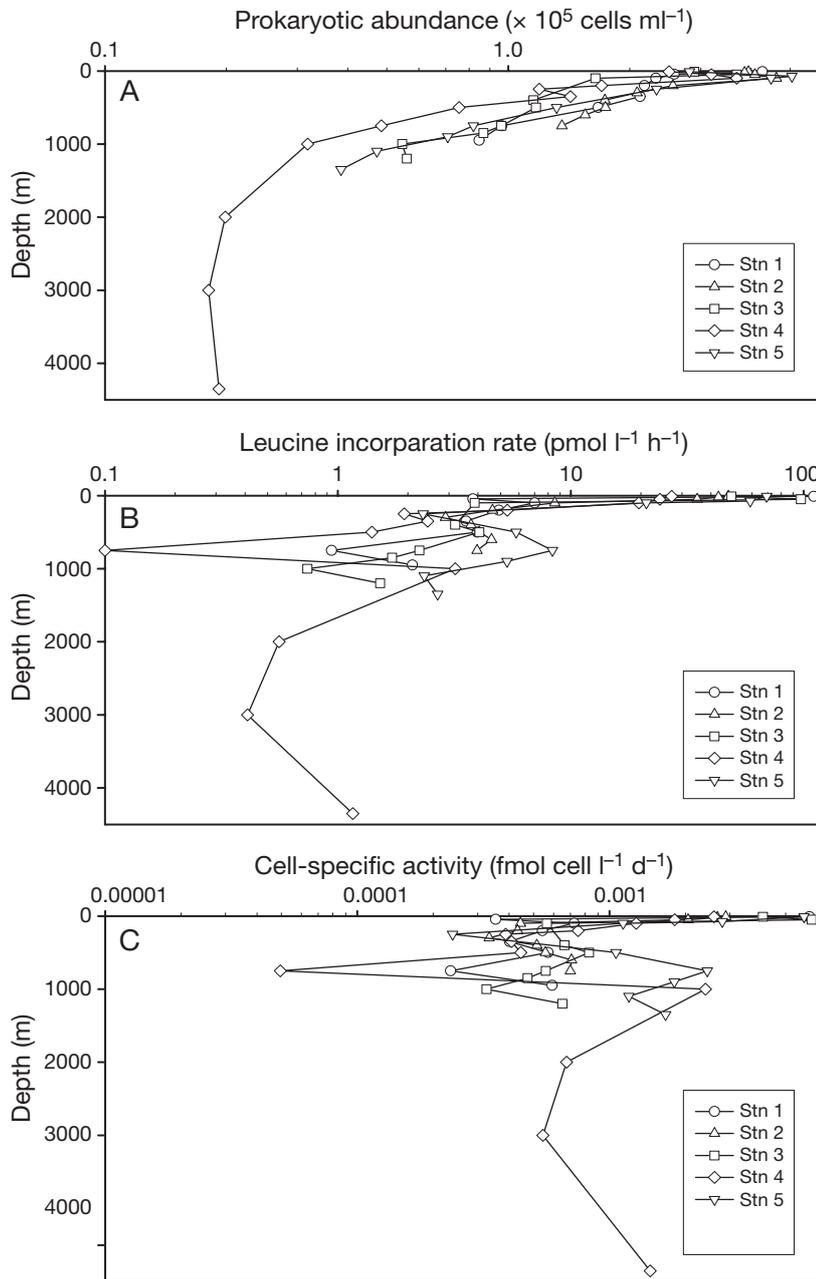


Fig. 2. Depth profiles of (A) prokaryotic abundance, (B) leucine incorporation rate, and (C) specific leucine incorporation rate

ARISA pattern of the bacterial community revealed in total 169 OTUs on the internal transcribed spacer (ITS) level, ranging from 16 to 45 OTUs sample<sup>-1</sup>. Thus, ARISA resulted in an almost 3-fold higher resolution for the bacterial community than T-RFLP and 16S rRNA gene analysis.

Generally, the number of OTUs in both T-RFLP and ARISA were fairly constant throughout the water column (Fig. 3). The mean percentage of OTUs present in a given sample was  $26 \pm 4\%$  (average  $\pm$  SD,  $n = 27$ ) and 16

$\pm 4\%$  ( $n = 28$ ) for T-RFLP and ARISA, respectively, of the total number of OTUs (sum of all OTUs detected). Two OTUs out of 63 determined by T-RFLP analysis were ubiquitously present (at all stations and depths), while only 1 OTU in ARISA analysis was ubiquitously present. The number of unique OTUs was 11 and 39 for T-RFLP and ARISA, respectively. These unique OTUs (i.e. appearing only once in all the samples) were evenly distributed among depths and stations (data not shown).

Although the number of OTUs did not follow a specific depth-related pattern, similarity analysis of the BCC on both the 16S rRNA gene and the ITS level indicated stratification of the BCC with depth. On the 16S rRNA gene level, 3 major clusters were identified (Fig. 4A): a bathypelagic cluster (3000 to 4000 m depth), a mesopelagic cluster (200 to 1400 m depth) and a deep mesopelagic cluster (500 to 945 m depth). At Stn 5, a single cluster was detected for the community between 250 and 1400 m depth (Fig. 4A). On the ITS level, 4 clusters were identified (Fig. 4B). One cluster comprised the surface samples from 45 to 250 m depth, 2 clusters were identified for bathypelagic waters, and 1 was identified for mesopelagic waters. The average percentage of similarity among the samples was  $49 \pm 14$  and  $28 \pm 21\%$  for T-RFLP and ARISA, respectively. The similarity matrices of the BCC based on the 16S rRNA gene level and on the ITS level were significantly correlated (Mantel test,  $r = 0.69$ ,  $p < 0.001$ ).

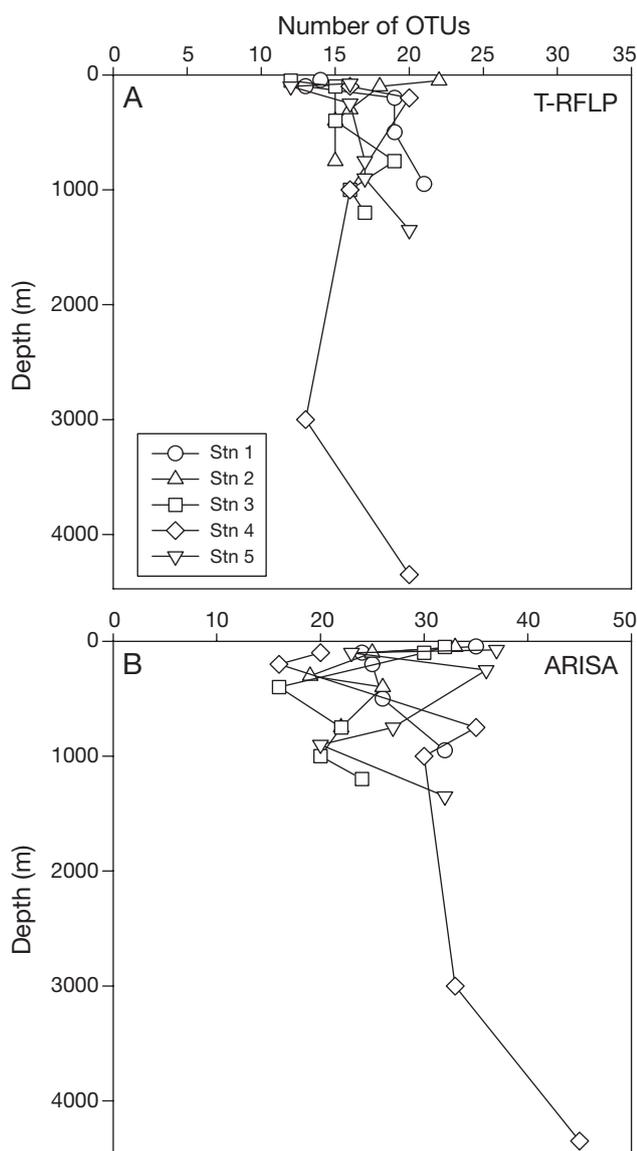
### Bacterial community composition in specific water masses

To determine whether the BCC is water mass specific, we determined the relationship between the similarity of the BCC among several samples with the hydrographic similarity of these water masses. As an indicator of the hydrographic similarity of the different water masses, we calculated the Euclidean distance between 2 water samples on the temperature–salinity (T–S) diagram. We coined this ‘T–S distance’ to be a parameter of water mass similarity. Fig. 5 shows the relationships between the similarity of the BCC and the T–S dis-

Table 2. Values at 100 m and regression results of the decline of prokaryotic abundance (PA) and leucine incorporation rate (leu incorp.) with depth (>100 m depth). The model used was  $N = N_{100} \times (Z/100)^b$ , where  $N$  and  $Z$  are either PA or leu incorp. and depth (m), respectively.  $N_{100}$  is the value of the specific parameter at 100 m depth, and  $b$  is the log-log slope of the depth-dependent decline of  $N$ , which was estimated by the linear regression of  $\log N$  on  $\log Z$ .  $t$ -tests were performed for the datasets of the Atlantic and eastern Mediterranean Sea. A comparison of  $b_{\text{leu incorp.}}$  was not carried out because of the lack of data for  $b_{\text{leu incorp.}}$  in the East Mediterranean Sea. \*: significant difference ( $p < 0.01$ ) between the parameter in the Atlantic and East Mediterranean Sea

	Prokaryotic abundance		Leucine incorporation rate	
	PA <sub>100</sub> ( $\times 10^5$ cells ml <sup>-1</sup> )	$b_{\text{PA}}$	Leu incorp. <sub>100</sub> (pmol l <sup>-1</sup> h <sup>-1</sup> )	$b_{\text{leu incorp.}}$
East Mediterranean Sea (Present study)	3.35 ± 1.32 (n = 5)	-0.65 ± 0.23 (n = 5)	12.03 ± 7.82* (n = 5)	-0.78, -0.70 (n = 2) <sup>a</sup>
Atlantic (G. J. Herndl & T. Reinthaler unpubl. data)	3.03 ± 0.54 (n = 27)	-0.79 ± 0.35 (n = 27)	3.40 ± 2.27* (n = 22)	-1.61 ± 0.38 (n = 22)
NW Mediterranean Sea (Tanaka & Rassoulzadegan 2004)	-	-0.62 ± 0.18 (n = 11)	-	-1.15 ± 0.30 (n = 5)

<sup>a</sup>Two depth profiles (Stn 1 and Stn 4) out of 5 showed a significant ( $p < 0.05$ ) depth-dependent decrease



tance of water masses. The relations between T-S distance and the similarities of the BCC determined by T-RFLP and ARISA showed similar distribution patterns (Fig. 5A,B). Although there was no significant relation between the similarity in the BCC and T-S distance (Mantel test,  $p > 0.05$ ), distinct patterns in the BCC related to T-S distance were noticeable. The similarity in the BCC between samples decreased with increasing T-S distance of the corresponding waters. Low similarity values of the BCC, however, were obtained at all T-S distances, indicating that even in samples with essentially the same T-S signature, the BCC can be very different.

## DISCUSSION

We found 2 overall patterns in the relation between the BCC and water mass variability as indicated by their T-S distance (Fig. 5A,B). While overall, the similarity of the BCC decreased in waters with increasing T-S distance, low similarity in the BCC was also found between samples with essentially identical T-S signatures (Fig. 5A,B). Previous studies showed decreasing similarity in the BCC with spatial distance on a 'hundreds-thousands km scale' (Hewson et al. 2006a,b) and on a 'latitudinal scale' (Fuhrman et al. 2008). Our results reveal that substantial heterogeneity in the BCC can also be found within water masses as indicated by identical T-S signatures.

Fig. 3. Distribution of the number of operational taxonomic units (OTUs) obtained at the stations in the eastern Mediterranean Sea by (A) terminal-restriction-fragment length polymorphism (T-RFLP) and (B) automated rRNA intergenic spacer analysis (ARISA)

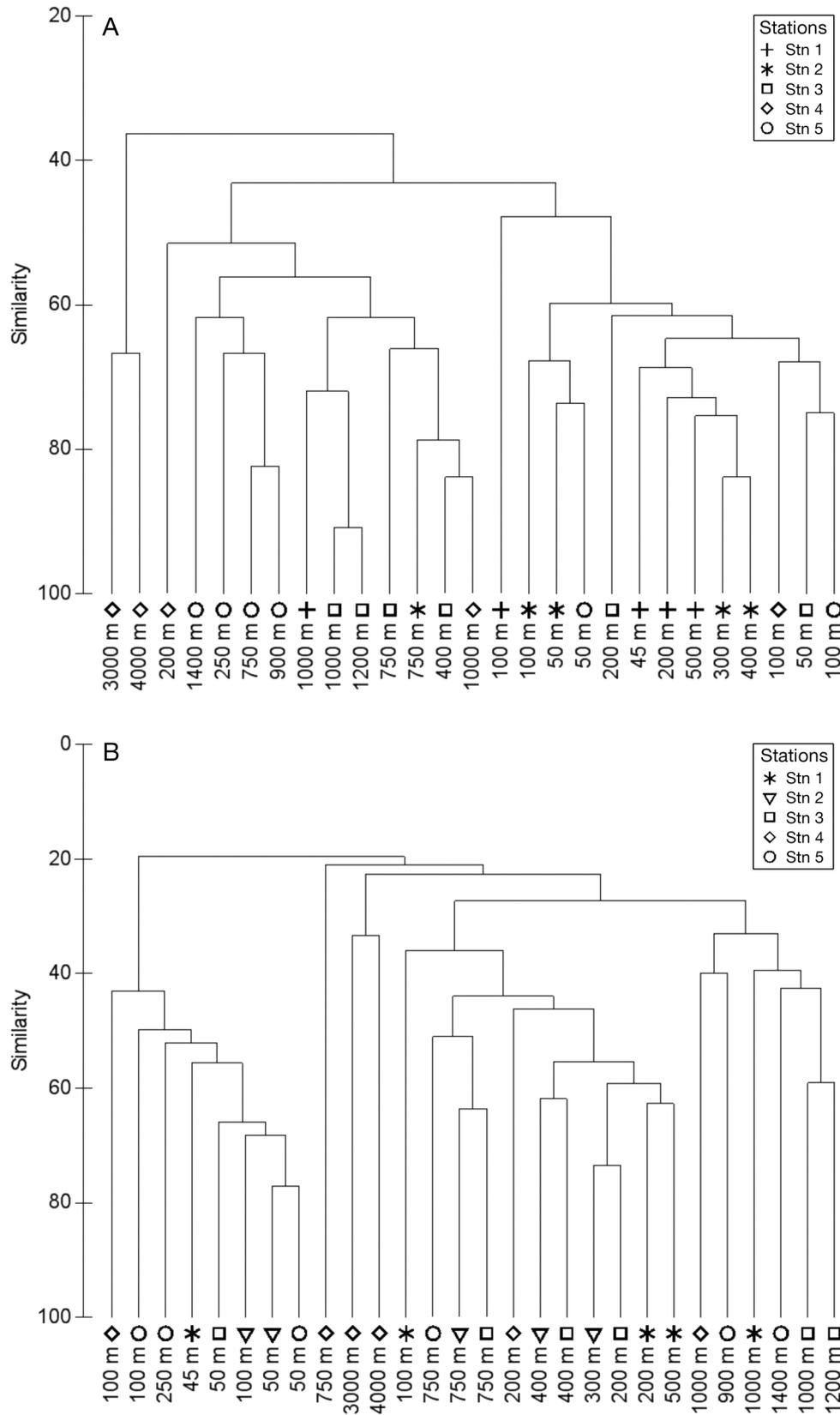


Fig. 4. Similarity matrix for the individual samples determined by (A) terminal-restriction-fragment length polymorphism (T-RFLP) and (B) automated rRNA intergenic spacer analysis (ARISA) in the eastern Mediterranean Sea

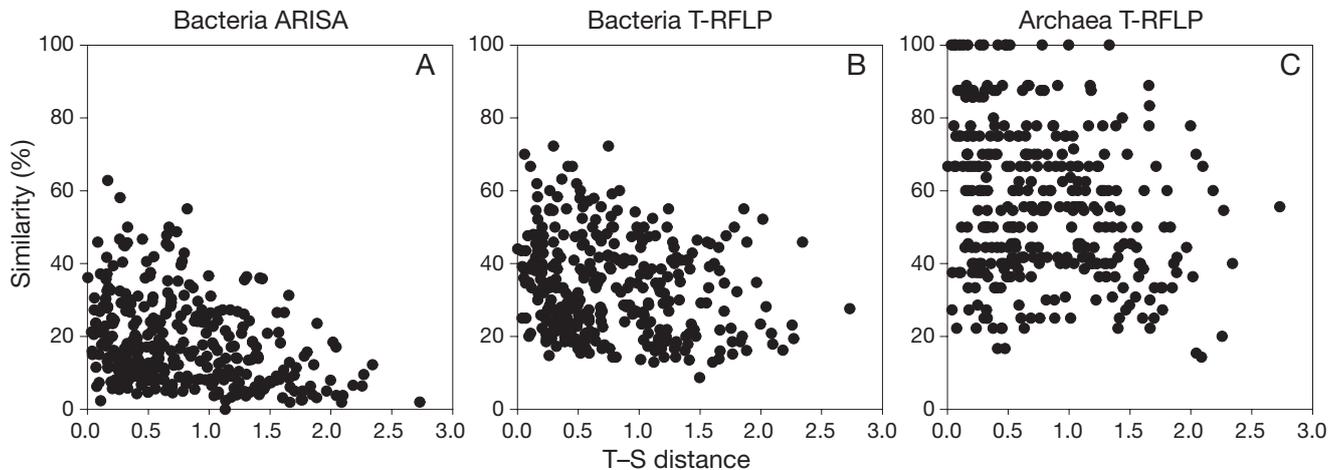


Fig. 5. Relationship between temperature–salinity (T–S) distance of 2 water masses and the similarity of the bacterial community composition in these water masses determined by (A) ARISA and (B) T-RFLP. (C) The similarity of the archaeal community composition determined by T-RFLP is also given for comparison (data from De Corte et al. 2009)

#### Distribution pattern of prokaryotic abundance and activity

The depth profiles of prokaryotic abundance measured in the present study are comparable to those in previous studies of the Atlantic (data from the ARCHIMEDES I cruise; G. J. Herndl & T. Reinthaler unpubl. data) and the northwestern Mediterranean Sea (Tanaka & Rassoulzadegan 2004). There are no significant differences in prokaryotic abundance at 100 m depth between the Atlantic and the eastern Mediterranean Sea and in the depth-dependent decline of abundance (Table 2). Remarkable differences were found, however, in terms of prokaryotic heterotrophic activity between the present study and the Atlantic study. Depth-dependent decreases of prokaryotic activity were only detected in 2 out of the 5 stations in the current study (Fig. 2B), while in the North Atlantic and the northwestern Mediterranean Sea almost all profiles exhibit a depth-dependent decrease (Tanaka & Rassoulzadegan 2004, G. J. Herndl & T. Reinthaler unpubl. data). Cell-specific activity in the mesopelagic layer was similar to that for surface waters (Fig. 2C), suggesting that some populations of prokaryotes are as active in mesopelagic waters as in surface waters. High cell-specific activity in mesopelagic waters seems to be a common feature, also reported in a recent compilation of data from deep waters (Aristegui et al. 2009). These results indicate that the overall decrease in bulk prokaryotic activity with depth is caused by a decrease in the active prokaryotic populations rather than by an overall decrease in cell-specific activity.

Several factors have been reported to limit heterotrophic prokaryotic activity. Studies revealed that

prokaryotic activity reflects availability of usable organic matter (Church 2008), temperature (Kirchman & Rich 1997) and inorganic nutrients (Turley & Stutt 2000). We found that sporadic low prokaryotic activities at 750 m depth of Stn 1, at 1000 m depth of Stn 3 and at 750 m depth of Stn 4 correspond to aged water masses (Zervakis et al. 2004). This distribution pattern of prokaryotic production in the eastern Mediterranean Sea may be partly explained by the patchiness of resources for heterotrophic bacteria because of the isolated deep-water masses in each basin of the eastern Mediterranean Sea. The isolated deep-water masses have their own unique dissolved organic matter signature (Meador et al. in press).

To determine the environmental factors controlling prokaryotes, regression analyses were performed between prokaryotic variables (abundance, activity, cell-specific activity) and environmental variables (temperature, salinity, depth). A significant linear regression was found only between the prokaryotic leu incorp. rate and temperature. Within the small range of water temperature (13.4 to 20.4°C), heterotrophic prokaryotic activity measured via leu incorp. was explained by temperature, as described by the following equation:

$$PLI_{\text{EastMedSea}} = 4.78T - 64.67$$

$$(r^2 = 0.37, p < 0.001, n = 30)$$

where  $PLI_{\text{EastMedSea}}$  and  $T$  are the prokaryotic leu incorp. rate ( $\text{pmol leu l}^{-1} \text{h}^{-1}$ ) and temperature ( $^{\circ}\text{C}$ ), respectively. A temperature-dependent increase in the prokaryotic leu incorp. rate was also found elsewhere, e.g., in the North Atlantic (G. J. Herndl & T. Reinthaler unpubl. data), such that:

$$PLI_{\text{NorthAtlantic}} = 0.18T - 0.94$$

$$(r^2 = 0.51, p < 0.001, n = 130)$$

The relation between temperature and leu incorp. for the eastern Mediterranean Sea, however, might not be a direct effect of temperature on prokaryotic activity, as this temperature dependency is substantially higher than in the North Atlantic (i.e. 26-fold higher than in the Atlantic), but might be explained by some variables co-varying with temperature such as depth. Resource limitation with depth might be more pronounced in the eastern Mediterranean Sea than in other parts of the global ocean, due to the severe oligotrophic nature of this region. Furthermore, to examine both water temperature and depth potentially affecting leu incorp., a forward stepwise regression analysis was conducted. In the North Atlantic (G. J. Herndl & T. Reinthaler unpubl. data) both factors, water temperature and depth, were selected as significant predicting variables ( $p < 0.001$ ), whereas, in the eastern Mediterranean Sea, the forward stepwise regression analysis did not select depth as a significant predicting factor ( $p = 0.27$ ), but did select temperature ( $p < 0.001$ ).

To compare the strength of 'top-down control' in the eastern Mediterranean Sea compared to other oceanic regions, we assumed that a shorter turnover time at a similar prokaryotic abundance as in other oceanic regions indicates top-down control, mediated by grazing or viral infection. To determine the turnover time of prokaryotes, the following equation was used:

$$T = PA / (f \times PHP)$$

where  $T$  is turnover time (days),  $PA$  is prokaryotic abundance ( $\text{cells ml}^{-1}$ ),  $f$  is the conversion factor ( $\text{mol } ^3\text{H-leu incorp. to cells produced: } 1.64 \times 10^{17} \text{ cells mol}^{-1} \text{ leu incorp.}$ ; Chin-Leo & Kirchman 1988),  $PHP$  is prokaryotic heterotrophic production ( $\text{pmol leu l}^{-1} \text{ d}^{-1}$ ). The mean turnover time in the present study was  $11 \pm 18 \text{ d}$  (range: 1.0 to 123,  $n = 49$ ). Prokaryotic turnover time in the eastern Mediterranean Sea is substantially shorter than in the NW Mediterranean Sea ( $81 \pm 32 \text{ d}$ ; Tanaka & Rasoulzadegan 2004), in the North Atlantic ( $18 \pm 70 \text{ d}$ ; Fig. 8 in Reinthaler et al. 2006) and in the Pacific ( $1155 \pm 1803 \text{ d}$ ; Nagata et al. 2000) over the same depth range. Prokaryotic abundance in the eastern Mediterranean Sea, however, is comparable to that in other oceanic regions (Table 2). Thus, there is indication that prokaryotic communities are more 'top-down' controlled in the eastern Mediterranean Sea than in other oceanic regions.

### Variability in bacterial community composition

The total number of OTUs determined by ARISA was higher than with T-RFLP. This is because the ITS

region used for ARISA is more variable than the 16S rRNA gene used for T-RFLP (Brown & Fuhrman 2005). In similar studies, slightly higher total numbers of OTUs have been obtained with ARISA (Hewson et al. 2006a, Fuhrman et al. 2008) and with T-RFLP (Moeseneder et al. 2001b, Winter et al. 2008). Moeseneder et al. (2001b) determined for March 1998 both the attached and free-living bacterial community in the same region as covered in the present study. These authors report numbers of OTUs using T-RFLP ranging between 20 and 65, while we obtained a range of OTUs from 12 to 22 (Fig. 4). Differences in the number of OTUs between our study and others can be explained by seasonal dynamics in OTU numbers (Fuhrman et al. 2006) or, more likely, by the different restriction enzymes used. The number of OTUs did not correlate with any prokaryotic variables ( $p > 0.05$ ). This means that bacterial OTU numbers are not related to prokaryotic abundance and activity, at least not in the range of activity we measured.

T-RFLP analysis revealed that the BCC at the 5 stations displayed a distinct depth-related pattern (Fig. 4A). A parallel analysis based on ARISA also revealed the same trend as the T-RFLP analysis (Fig. 4B). This means that OTUs, which are distinguished on the ITS and 16S rDNA levels, respond in the same manner to environmental variables. Albeit a few ubiquitously present OTUs were detected (data not shown), drastic changes in the BCC were noted with depth. A stratification of the prokaryotic community composition has been reported for the bulk prokaryotic community elsewhere, such as for the deep Pacific (DeLong et al. 2006) and the NW Mediterranean Sea (Winter et al. 2009). This suggests that the factors controlling the BCC are related to depth. Sedimenting particulate organic matter supply and its nutritive value decrease with depth, exerting a structuring effect on the BCC (Moeseneder et al. 2001b). Hydrostatic pressure might also affect the stratification of bacterial communities (Grossart & Gust 2009).

Stratification of community composition was also found in *Archaea* (De Corte et al. 2009) for the same sites as investigated here. Statistical analysis showed that the similarity of the archaeal community composition between sites and depths is related to the similarity in the BCC determined by both T-RFLP (Mantel test,  $r = 0.4671$ ,  $p < 0.001$ ) and ARISA (Mantel test,  $r = 0.5617$ ,  $p < 0.001$ ). This indicates that the archaeal community composition and BCC are structured by similar environmental variables.

Our study also revealed considerable variability in the BCC in waters with identical temperature and salinity. Similarities in the BCC in waters with identical or similar temperature and salinity characteristics (i.e. T-S distance = 0 or close to 0) varied from near 0 to the

highest similarity detected for any of the sample pairs (Fig. 5A,B). This indicates that factors controlling the BCC vary also within individual water masses. Previous studies have revealed that the BCC can vary on a small scale, such as in diatom blooms developing in mesocosms (Riemann et al. 2000). On a micro-scale down to 1  $\mu$ l of water, changes in the BCC have been documented (Long & Azam 2001). This small-scale variability in the BCC or that within waters with identical salinity and temperature characteristics might be generated by heterogeneity of substrate compositions. Hotspots of organic matter (Azam et al. 1994), such as sinking particulate organic carbon plumes (Kiorboe et al. 2001), marine snow (Simon et al. 2002) and release of dissolved organic matter by Protozoa (Ferrier-Pages et al. 1998), or self assemblage of organic compounds to gels (Chin et al. 1998) might generate such within-water mass variability and associated to that, variability in the BCC. Top-down factors might also generate heterogeneity, because the strength of the link between prokaryotes and heterotrophic nanoflagellates varies with depth (Tanaka & Rassoulzadegan 2002, Tanaka et al. 2005). Archeal community composition determined by T-RFLP (data from De Corte et al. 2009), however, did not show any trend with T–S distance of water masses (Fig. 5C). This might indicate that differences in hydrological conditions affected the archaeal community composition to a lesser extend than bacteria.

In the present study we used temperature and salinity, commonly determined to identify water masses, as an index to determine the 'hydrological distance' among samples. This 'T–S distance' is preferable to the simple spatial distance frequently used, as specific water masses are highly variable in their extension, ranging from a few hundred meters in vertical or areal extension to thousands of kilometres in oceanic conveyor belt circulation. Hence, simple spatial distance does not necessarily relate to differences in water column biogeochemistry and biology. Based on the relation between the BCC and T–S distance, the BCC varies within individual water mass conditions as much as between distant water masses. Overall, however, the similarity in the BCC decreased with distance in T–S. Thus, the overall change in similarity of bacterial communities with distance in a given water mass is superimposed over the variability generated apparently by small-scale patchiness in the water column.

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