The following supplement accompanies the article

Seasonal variation in marine-snow-associated and ambient-water prokaryotic communities in the northern Adriatic Sea

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Supplement. Additional data about the sampling site (Fig. S1) with basic physico-chemical environmental data at the time of sampling presented in Table S1. The distribution of 3 main prokaryotic groups at the time of marine snow sample collection, obtained by catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) is shown in Fig. S2, the bacterial richness estimated by terminal restriction fragment length polymorphism (T-RFLP) in Table S2, and the parameters of non-metric multidimensional scaling (nMDS) analysis in Table S3. The rarefaction curves obtained by 16S rRNA and 16S rDNA clone libraries are given in Fig. S3. The last paragraph gives the detailed description of DNA/RNA extraction.

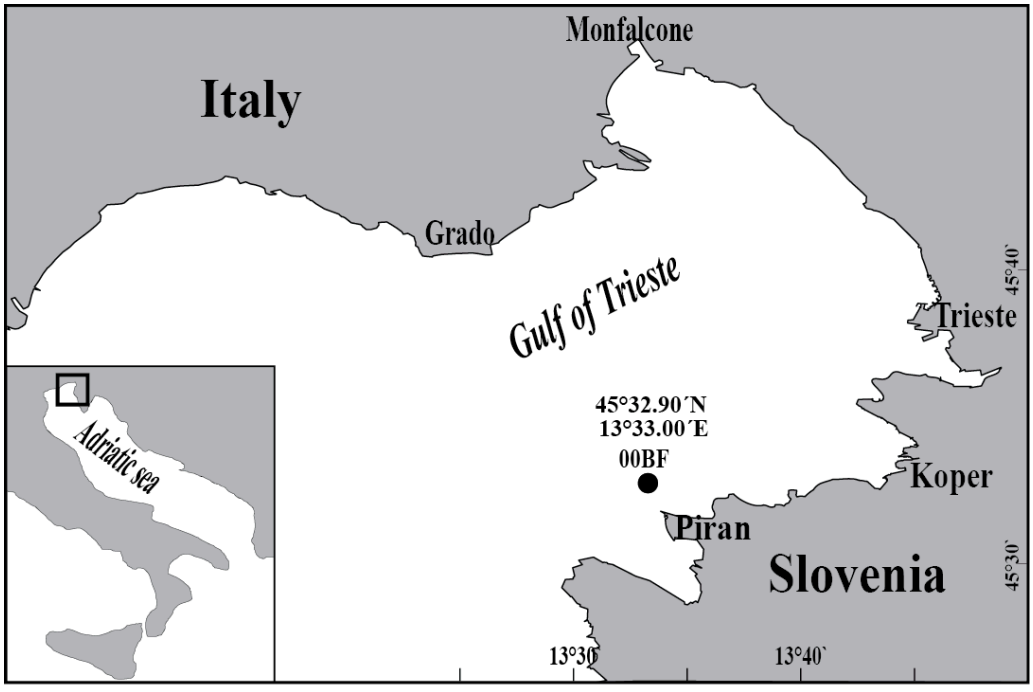

Fig. S1. Study area in the Gulf of Trieste (northern Adriatic Sea). Full circle denotes the sampling site

Table S1. Physico-chemical characteristics of unfiltered seawater at the sampling station in the Gulf of Trieste (northern Adriatic Sea) during the sampling of marine snow and ambient water. On 10 December 2009, the environmental parameters were not measured; therefore, we provide data from monitoring done 1 wk earlier as an indication of the conditions during sampling on 10 December. nd = not determined

Sampling date	Sampling depth (m)	Temperature (°C)	Salinity	Sampling depth: nutrients (m)	PO_4^{3-} (µM)	NO_2^- (µM)	NO_3^- (µM)	NH_4^+ (µM)	SiO_4^{4-} (µM)	Inorganic N:P ratio
03 Dec 09	10	14.6	37.0	10	0.25	1.2	5.36	0.33	6.87	27.6
10 Dec 09	10	13.5, 14.6[a]	36.8[b]	/	nd	nd	nd	nd	nd	/
23 Apr 10	10	14.3	36.4	10	0.06	0.18	3.76	0.3	2.9	70.8
07 Jun 10	13	17.5	35.9	10	0.05	0.1	2.65	0.3	1.3	61.2
				15	0.05	0.11	1.53	1.4	0.5	60.3
15 Jul 10	12	20.4	36	10	0.09	0.05	0.53	0.5	4.5	12.3
				15	0.11	0.05	0.09	0.5	2.5	6
12 Aug 10	13	20.9	36.6	10	0.06	0.03	0.06	0.3	2.9	6.4
				15	0.06	0.06	0.2	0.4	3.9	11.2
				10	0.05	0.04	0.19	0.1	3.3	6.5
26 Aug 10	13	21.2	36.0	15	0.08	0.09	0.18	0.5	4.9	9.6

[a]Temperature measured at 2 and 22 m depth
[b]Salinity measured at 2 m depth

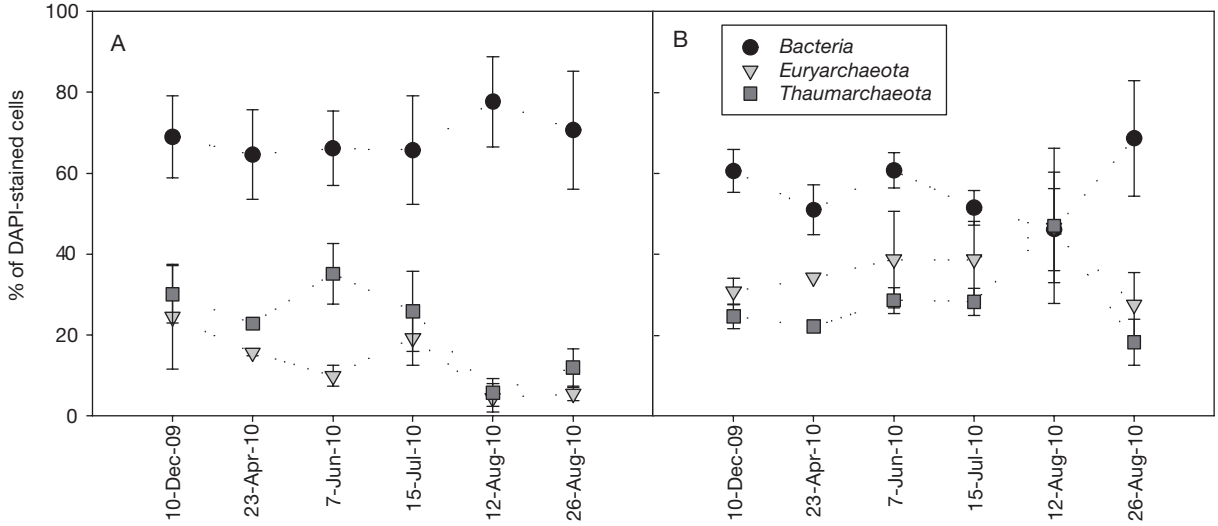

Fig. S2. Relative contribution of *Bacteria*, *Euryarchaeota* and *Thaumarchaeota* to total DAPI-stained cells detected by CARD-FISH in (A) ambient water and (B) marine snow. Error bars are SD

Table S2. Number of operational taxonomic units (OTUs) detected by T-RFLP analysis of bacterial 16S rRNA and 16S rRNA genes (16S rDNA) in ambient water (AW) and marine snow (MS). Numbers of OTUs are presented as a sum of fragments detected for the FAM-labelled forward and VIC-labelled reverse primer. MS: marine snow, AW: ambient water, 16S rDNA: 16S rRNA genes, 16S rRNA: 16S rRNA, nd: not done

Sampling date	Sample type	OTUs 16s rDNA	OTUs 16 rRNA
10 Dec 09	AW	84	49
	MS	59	nd
23 Apr 10	AW	52	11
	MS	22	43
07 Jun 10	AW	70	35
	MS	54	9
15 Jul 10	AW	74	37
	MS	67	35
12 Aug 10	AW	103	45
	MS	50	34
26 Aug 10	AW	66	39
	MS	75	27

Table S3. Final stress, p-value of the Monte Carlo test and r^2 values for the nMDS analysis

Final stress	0.16
Monte Carlo test, p-value[a]	0.01
Axis 1 (non metric r^2)[b]	0.92
Axis 2 (non metric r^2)	0.973

[a]The Monte Carlo p-value is the probability that a similar final stress could have been obtained from a random configuration of points, based on 1000 permutations with randomized data

[b]The r^2 values are the determination coefficient calculated between the distance in the ordination space and distance in the original space (i.e. Bray Curtis dissimilarity)

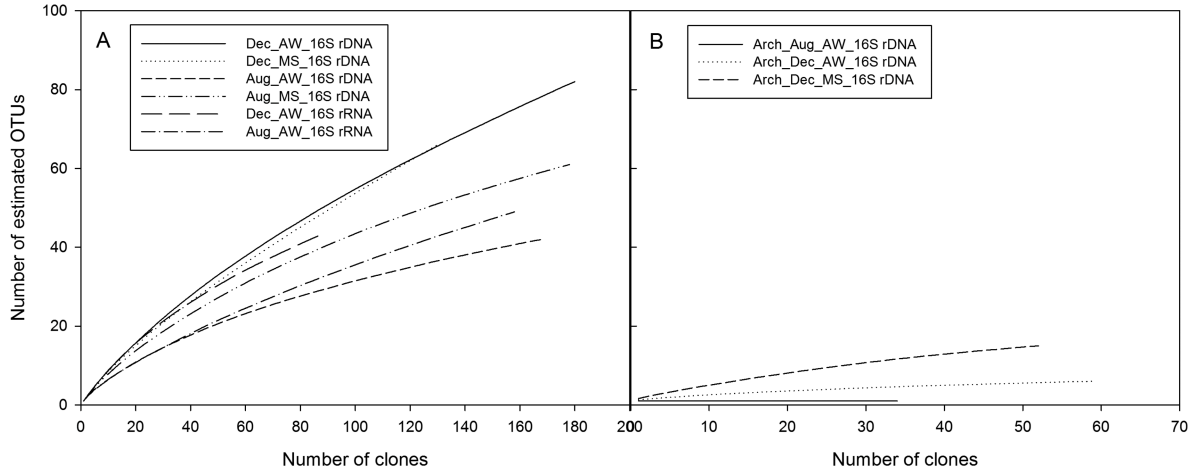

Fig. S3. Rarefaction analysis obtained for (A) bacterial 16S rRNA/rDNA and (B) archaeal 16S rDNA clone libraries in ambient water (AW) and marine snow (MS) collected in Dec 2009 and Aug 2010. 16S rDNA (obtained from DNA extracts), 16S rRNA (obtained from cDNA prepared from the RNA extracts), Arch (Archaeal community)

DNA/RNA detailed extraction method. Filters cut into small pieces were incubated with lysis buffer (10 mM Tris-HCL; pH 8, 25 mM Na_2EDTA; pH 8, 100 mM NaCl) and lysozyme (1 mg ml^{-1}) at 37°C for 30 min. Sodium dodecyl sulphate (final conc. 1% wt/v) and proteinase K (final conc. 100 µg ml^{-1}) were added and samples incubated at 55°C for 1 h. Tubes were vortexed horizontally for 10 min after the addition of 1.5–2 g zirconium-silica beads and incubated at 70°C for 30 min. The lysate was treated with 0.5× volume of phenol (pH 7.9), followed by 0.5× volume of phenol:chloroform:isoamyl alcohol (25:24:1) and 0.5× volume of chloroform. Nucleic acids were precipitated with 2× volume of 100% ethanol and 0.02× volume of 5 M NaCl at –20°C for 1–2 h. The pellet was washed with 70% ethanol, air-dried and re-suspended in 100 µl of RNase/DNase free ultra pure water (Sigma) at 4°C overnight