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Sunlight effects on the DMSP-sulfur and leucine assimilation activities of polar heterotrophic bacterioplankton

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Abstract The influence of solar ultraviolet radiation and photosynthetically active radiation (PAR) on summertime marine bacterial uptake and assimilation of sulfur from radiolabeled dimethylsulfoniopropionate (^{35}S-DMSP) was studied at four Arctic and two Antarctic stations. Incubations with ^{3}H-leucine were also conducted for comparative purposes as a measurement of bacterial activity. Arctic waters were characterized by large numbers of colonial *Phaeocystis pouchetii* and higher DMSP concentrations than in the two diatom-dominated Antarctic samples. Exposure to full sunlight radiation (280–700 nm), and to a lesser extent to PAR + UVA (320–700 nm), generally decreased the bacterial assimilation of ^{3}H-leucine with respect to darkness, and caused variable effects on ^{35}S-DMSP assimilation. By using a single-cell approach involving microautoradiography we found high percentages of sulfur assimilating cells within the bacterial groups *Gammaproteobacteria*, *Bacteroidetes*, SAR11 and *Roseobacter* despite the varying DMSP concentrations between Arctic and Antarctic samples. The dominant SAR11 clade contributed 50–70% of the cells assimilating both substrates in the Arctic stations, whereas either *Gammaproteobacteria* or SAR11 were the largest contributors to ^{3}H-leucine uptake in samples from the two Antarctic stations. Only one station was analyzed for single-cell ^{35}S-DMSP assimilation in Antarctica, and *Gammaproteobacteria* were major contributors to its uptake, providing the first evidence for Antarctic bacteria actively taking up ^{35}S-DMSP. PAR + UVA repeatedly increased the number of SAR11 cells assimilating ^{3}H-leucine. This pattern also occurred with other ^{35}S-DMSP assimilating groups, though not so consistently. Our results support a widespread capability of polar bacteria to assimilate DMSP-sulfur during the season of maximum DMSP concentrations, and show for the first time that all major polar taxa can be highly active at this assimilation under the appropriate circumstances. Our findings further confirm the role of sunlight as a modulator of heterotrophic carbon and sulfur fluxes in the surface ocean.

Keywords Bacteria · DMSP · Leucine · Arctic · Antarctica · Sunlight

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Introduction

Dimethylsulfoniopropionate is a ubiquitous sulfur compound produced by many phytoplankton taxa (Keller et al. 1989) as an intracellular osmolyte (Kirst 1996), although other functions such as cryoprotectant or antioxidant have been also suggested (Karsten et al. 1996; Sunda et al. 2002). Once released into the dissolved pool mainly through grazing, viral lysis or phytoplankton autolysis (Simó 2001), DMSP becomes

a significant carbon source and a major source of reduced sulfur for marine bacteria (Kiene et al. 2000; Vila-Costa et al. 2007; Simó et al. 2009) herbivorous protozoans (Burkill et al. 2002; Saló et al. 2009), and even low-DMSP producing phytoplankton (Vila-Costa et al. 2006).

Algal DMSP production varies both among species (Keller et al. 1989) and within species depending on environmental conditions (Stefels et al. 2007). One of the strongest DMSP producers is the haptophyte *Phaeocystis* spp. (Liss et al. 1994) which is known to form massive blooms causing elevated concentrations of this compound in the particulate and the dissolved forms. In polar waters, summer *Phaeocystis* blooms triggered by ice-melting stratification are accompanied by very high DMSP levels (Curran and Jones 2000; Matrai and Vernet 1997). Within marine bacteria, significant assimilation of DMSP-sulfur has been observed in all major taxonomic groups, although not all cells uniformly assimilate it (Malmstrom et al. 2004a; Vila et al. 2004; Vila-Costa et al. 2007). This widespread use of DMSP is consistent with its suggested role as a source of reduced sulfur for protein synthesis, energetically advantageous with respect to the assimilative sulfate reduction chain (Kiene et al. 1999). It has been suggested, though, that more than the taxonomic composition of the bacterial assemblage, it is the contribution of DMSP to available reduced sulfur in the ecosystem that controls its assimilation (Pinhassi et al. 2005; Vila-Costa et al. 2007). Therefore, given that large DMSP production rates occur in polar waters during summer, it is of interest to examine whether this is reflected in the sulfur assimilation activity and taxonomic composition of the bacterial assemblages. To date, the only study focusing on the identity of polar bacteria taking up DMSP was carried out during the Arctic winter and early spring, where low numbers of active bacteria were found associated with very low-DMSP concentrations (Vila-Costa et al. 2008).

Ultraviolet radiation (UVR, 280–400 nm) is among the environmental factors with a high potential to regulate the availability of DMSP to bacteria and its uptake and assimilation. Exposure to UVR has been found to either increase (Slezak and Herndl 2003; Sunda et al. 2002) or reduce (Sakka et al. 1997) the cellular DMSP content of marine algae. Whether UVR hampers or enhances the DMSP production machinery is, therefore, uncertain, but a recent study with cultures of the haptophyte *Emiliania huxleyi* reported the UVR-enhanced release of DMSP and its cleavage product dimethylsulfide (DMS; Archer et al. 2010). Besides potentially influencing the release of DMSP by phytoplankton, high doses of solar radiation can inhibit the growth and activity of marine bacteria (Aas et al. 1996; Herndl et al. 1993), and there is direct evidence that it can decrease DMSP consumption (Slezak et al. 2001; Slezak et al. 2007).

All this, together with the reported variable sensitivities to UVR within bacterial and phytoplankton species (Arrieta et al. 2000; Hernández et al. 2006; Neale et al. 1998), and the fact that polar summer is characterized by long days of almost continuous light, points to an important role of natural solar radiation in the assimilation of DMSP-sulfur by polar bacterial taxa.

UV radiation studies in the polar regions have attracted increasing attention since ozone depletion (Jones and Shanklin 1995; Müller et al. 1997) and the ongoing thinning of the ice cover in both the Arctic (Rothrock et al. 1999) and some regions of Antarctica (Rignot et al. 2008) are leading to a higher UVR penetration in the water column. However, although some information is available about the impact of UVR on bacterial communities or isolates from the Arctic and Antarctica (e.g., Wickham and Carstens 1998; Davidson and van der Heijden 2000; Hernández et al. 2006), to our knowledge no study has examined the effects of natural solar radiation on the single-cell activities of different polar bacterial groups.

We used a single-cell approach combining CARD-FISH (catalyzed reporter deposition-fluorescence in situ hybridization of RNA) for bacterial identification with microautoradiography for quantification of the active cells. This was applied to natural bacterial assemblages in four Arctic and two Antarctic stations during summer cruises. With the aim of deciphering the bacterial taxa responsible for DMSP-sulfur, radiolabeled ^{35}S-DMSP was used as a substrate. ^{3}H-leucine was used in parallel for comparative purposes since it is considered one of the most universal substrates for marine bacterioplankton and its assimilation is used to estimate protein synthesis (Kirchman et al. 1985). The effect of natural sunlight (and specially UVB, the most harmful fraction within UVR and the one affected by changes in ozone concentration) on substrate assimilation was assessed by exposing samples to different light conditions. Our

starting hypotheses were that (a) the bacterial assemblages in summer polar waters would be adapted to an efficient use of highly available DMSP as a source of reduced sulfur, and (b) that uptake of labile dissolved organic compounds, sulfur assimilation and protein synthesis by bacteria would be dependent on the spectrum of solar radiation, with implications for bacterial activity in situ in response to changes in surface irradiance, mixing, and light penetration.

Methods

Study area, sampling and basic parameters

The study was carried out on board RV Hespérides during the ATOS I (Arctic) and II (Antarctica) cruises. In July 2007, ATOS I visited the Atlantic sector of the Arctic with a transect from Iceland, parallel to the eastern Greenland current, up to the ice cap edge (ca. 81°N) located north/northwest of Svalbard. In February 2009, ATOS II cruised around the Antarctic Peninsula, from the Weddell Sea (65°S) through the Bransfield Strait and into the Bellingshausen Sea (ca. 69°S). Samples were collected at 5 m depth with a rosette of Niskin bottles mounted on a CTD profiler (Seabird SBE 911). Station AN2 was the only one sampled at 20 m depth, where we found a dense bloom of the diatom *Pseudonitzschia* spp. Since this diatom was also present in high numbers in the Arctic samples (Ruiz-González et al. submitted), we chose this sampling depth to examine whether comparable bacterial assemblages would also be associated with this algal group in Antarctic waters. Water characteristics of the sampled stations together with the irradiance measurements and time of incubation during experiments are shown in Table 1.

DMSP analysis

We could not reliably measure dissolved DMSP concentrations because even low volume drip filtration (Kiene and Slezak 2006) seemed to break *Phaeocystis* sp. colonies and enrich the dissolved fraction with intracellular DMSP. Therefore only total DMSP concentrations (DMSPt, Table 1) were measured. Water samples were collected directly from the Niskin bottles into glass vials (120 ml) avoiding bubbling. Subsamples of 3–5 ml were syringe-filtered through GF/F into glass vials, and analyzed for DMS by purging, cryotrapping, and sulfur-specific gas chromatography followed by flame photometry as described by Simó et al. (1996). Aliquots of 40 ml of the original sample were stored in crimp glass vials with two added pellets of NaOH (45 mg each), which hydrolyzed all DMSP into DMS. DMSPt was determined in 0.2–1 ml subsamples the following day as the evolved minus the pre-existing DMS.

Bacterial abundance

In situ bacterial abundances were analyzed by flow cytometry. Samples of 1.2 ml were preserved with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentrations) and kept frozen at −80°C until analysis with a Becton–Dickinson FACSCalibur flow cytometer after staining with SybrGreen I (Molecular Probes, Eugene, Oreg.). Abundances were determined by plotting side scatter (SSC) versus versus FL1 (green fluorescence; Gasol and Del Giorgio 2000).

Experimental design

Six experiments were performed to assess the impact of natural sunlight on the heterotrophic activity of polar bacterial assemblages. The substrates used were chemically synthesized and purified ^{35}S-DMSP, which was kindly donated by Ronald (University of South Alabama, Dauphin Island Sea Laboratory, USA), and ^{3}H-leucine (Amersham).

Briefly, water samples were incubated in 50 ml UV-transparent quartz bottles with the addition of trace concentrations of ^{35}S-DMSP or ^{3}H-leucine under different light conditions. The bottles were exposed either to the full sunlight spectrum (PAR + UVR), the full spectrum without UVB (i.e., PAR + UVA, covered with the plastic foil Mylar-D that excludes only UVB radiation) or kept in the dark (wrapped with aluminum foil and a black plastic bag). The samples were incubated on deck inside a black tank with running seawater to maintain the in situ temperature, which ranged from −0.2 to 3.2°C. To simulate the irradiance level of 5 m, samples were placed 5 cm under the surface below an optically neutral mesh that reduced surface irradiances by 40%, which was approximately the reduction naturally occurring at 5 m. Samples from station AN2 reaching the 20 m sampling depth were covered with a double neutral

Table 1 Characteristics of the sampling stations, total DMSP to chlorophyll *a* ratio, time of exposure and integrated UVB irradiances during deck board incubations

	Stn	Date (day/mo/yr)	Longitude	Latitude	Sampling depth (m)	SW temp (°C)	DMSPt:Chl *a* (nmol μg^{-1})	Incub. time(h)	UVB (kJ m^{-2})
Arctic	AR3	05/07/07	1° 39.82′W	77° 23.23′N	5	3.22	31	12	9.2
	AR4	07/07/07	2°58.49′W	78°43.72′N	5	2.15	42	11.5	4.6
	AR5	12/07/07	10° 11.44′E	80° 13.99′N	5	0.15	167	11.8	7.4
	AR7	19/07/07	13° 14.22′ E	80° 49.57′N	5	0.21	21	9.5	4.5
Antarctica	AN1	03/02/09	55° 45.43′ W	65° 01.17′S	5	-0.17	2	7.6	9.1
	AN2	06/02/09	57° 14.42′ W	62° 10.63′S	20	1.67	17	8	1.2

mesh, in order to mimic the natural irradiance reaching that depth.

Radiation measurements

UVR and PAR irradiances were continuously monitored throughout the incubations with a Biospherical PUV-radiometer 2500 installed inside the incubation tank. Downwelling cosine irradiance was measured in six bands within the UV region (305, 315, 320, 340, 380, 395 nm). UVB (305–320 nm) measured doses during experiments are shown in Table 1.

^{3}H-leucine incorporation rates

Bacterial heterotrophic activities was estimated before and after exposure to sunlight using the ^{3}H-leucine method described by Kirchman et al. (1985) modified as in Smith and Azam (1992). From each quartz bottle, four aliquots (1.2 ml) plus two trichloroacetic acid-killed controls were incubated with ^{3}H-leucine (40 nM final conc., 161 Ci $mmol^{-1}$) for 2 to 3 h in the dark at in situ temperature.

Trace isotope assimilation during incubations

Samples of 50 ml were incubated for 7 to 12 h in quartz bottles with added trace concentrations of ^{35}S-DMSP (845 Ci $mmol^{-1}$, 0.8 pM final conc. for Arctic samples and 120–145 Ci $mmol^{-1}$, 2.5–3 pM final conc. for Antarctic samples) or ^{3}H-leucine (161 Ci $mmol^{-1}$, 0.5 nM final conc.). Killed controls were prepared in 30 ml Teflon flasks by adding paraformaldehyde (PFA, 1% final conc.) before the addition of the radioisotope. After exposure, the incorporation of substrate was stopped by fixing samples overnight with PFA (1% final conc.) at 4°C in the dark. Triplicate aliquots (previously filtered through 5 μm pore-sized filters to exclude the largest organisms) were filtered through 0.2 μm pore-sized filters (GNWP, Millipore) and rinsed with 15 ml of 0.2 μm-filtered seawater. Macromolecules were precipitated by treating filters with 5 ml of cold TCA 5% for 5 min. The filters were then rinsed with milliQ water and their radioactivity counted with a Beckman scintillation counter.

MAR-CARD-FISH (microautoradiography combined with catalyzed reporter deposition-fluorescence in situ hybridization)

Samples of 50 ml were incubated under the different light treatments with added radioactive ^{35}S-DMSP (845 Ci $mmol^{-1}$, 0.04 nM final conc. for Arctic samples and 145 Ci $mmol^{-1}$, 0.03 nM final conc. for Antarctic samples) or ^{3}H-leucine (161 Ci $mmol^{-1}$, 0.5 nM final conc.) for 7 to 12 h. PFA-killed controls were run simultaneously with all live incubations. Microautoradiography of ^{35}S-DMSP samples from station AN2 could not be performed due to insufficient amount of the isotope left for a visible signal.

After sunlight exposure, live samples were fixed overnight with PFA (1% final conc.) at 4°C in the dark. Aliquots of 10–15 ml were first filtered through 5 μm polycarbonate filters (Osmonics, inc.) for identification of the particle-attached bacteria and subsequently filtered through 0.22 μm polycarbonate filters (GTTP, 25 mm diameter, Millipore), rinsed with milliQ, air dried and stored at −20°C until processing. Hybridizations were made following the CARD-FISH protocol (Pernthaler et al. 2002). We used a suite of six horseradish peroxidase (HRP)-probes to characterize the composition of the bacterial community (particle-

attached and free-living bacteria) in the water samples: Eub338-II-III for most *Eubacteria* (Amann et al. 1990; Daims et al. 1999), Gam42a for *Gammaproteobacteria* (Manz et al. 1992), CF319 for many clades belonging to the *Bacteroidetes* group (Manz et al. 1996), Ros537 for the *Roseobacter* clade (Eilers et al. 2001) and SAR11-441R for the SAR11 cluster (Morris et al. 2002). Cells were first permeabilized with lysozyme (37°C, 1 h) and achromopeptidase (37°C, 0.5 h) and hybridizations were carried out at 35°C overnight. Counterstaining of CARD-FISH filters was done with 4,6-diamidino-2-phenylindole (DAPI, 1 μg ml^{-1}) and a minimum of ten fields (500-800 DAPI-stained cells) were manually counted using an Olympus BX61 epifluorescence microscope.

For microautoradiography, we essentially followed the protocol described in Alonso and Pernthaler (2005) modified as in Vila-Costa et al. (2007). Only 0.22 μm filters were used, which were developed after 6 days of exposure for ^{3}H-leucine and 18 days for ^{35}S-DMSP in Arctic samples, or after 5 days for ^{3}H-leucine and 2 months for ^{35}S-DMSP in Antarctic samples. Cells were then stained with DAPI and between 500 and 700 hybridized cells were counted within a minimum of 10 fields.

Results

Phytoplankton biomass in the Arctic stations was generally dominated by *Phaeocystis pouchetti* (Lasternas and Agustí 2010; Ruiz-González et al. submitted), a haptophyte that forms large colonies and produces high concentrations of DMSP. Conversely, at the two Antarctic stations, the highest abundances corresponded to the apparently low-DMSP producing *Thalassiosira* spp. (station AN1) or *Pseudonitzschia* spp. (station AN2; Ruiz-González et al. submitted). As a consequence, higher DMSPt to chlorophyll *a* ratios (DMSPt:Chl *a*, an indicator of the occurrence of DMSP producers within algal assemblages) were found in the Arctic (21–167 nmol μg^{-1}) than in Antarctic samples (2–17 nmol μg^{-1}; Table 1).

Prokaryotic abundances ranged between 0.2×10^6 cells ml^{-1} in Antarctic stations to up to 2×10^6 cells ml^{-1} in station AR4 (Table 2). Instead, the highest bacterial leucine incorporation rate was recorded at station AN1 (~180 pmol leucine l^{-1} h^{-1}) with the other stations presenting lower values (~30 to 90 pmol leucine l^{-1} h^{-1}; Table 3).

Exposure to natural sunlight reduced post-exposure (measured in the dark for 2 to 3 h after light incubation) leucine incorporation (Table 3), but this reduction was only significant (Tukey's test, $p < 0.05$) in comparison to the dark treatment when UVB was included. Only in samples from station AN1 exposure to PAR + UVA caused a significant inhibition. Similarly, the trace ^{3}H-leucine assimilated during incubations by organisms in the 0.22–5 μm fraction (mainly prokaryotes; Fig. 1b) declined by 18% (station AR4) to 85% (station AN1) upon exposure to full sunlight radiation conditions. No significant differences were found between dark and PAR + UVA treatments except in stations AR7 and AN1, where PAR + UVA accounted for most of the observed decrease. Samples from AR3 and AR5 from the Arctic and AN1 from Antarctica received the highest UVB doses during incubations (7–9 kJ m^{-2}, Table 1) and a positive correlation was found between the inhibition of trace ^{3}H-leucine assimilation due to UVB exposure (with respect to PAR + UVA incubation) and the UVB doses measured during each experiment (Spearman's $r = 0.81$, $p < 0.05$, $n = 6$). In contrast, no correlation was apparent between UVB doses and the degree of inhibition of the bacterial leucine incorporation measured after exposure.

The recorded percentages of assimilation of added ^{35}S-DMSP (0.2–2.3%; Fig. 1a) were lower than those of ^{3}H-leucine, and showed a variable behavior with regard to light, with no significant differences among light treatments at three stations (AR4, AR5 and AN2), significant increase by PAR + UVA at station AR7 and significant inhibition by UVB (station AN1) or by both light treatments (station AR7). No significant correlation was found between inhibition and UVB doses.

'Free-living' (0.22–5 μm) and 'particle-attached' (>5 μm) bacterial assemblages were characterized by CARD-FISH. Most prokaryotic cells hybridized with the eubacterial probe EUB338-II-III (92–96% of DAPI counts). Hybridization with specific probes showed that the free-living Arctic bacterial communities were largely dominated by the SAR11 clade (*Alphaproteobacteria*), which accounted for 61–71% of total DAPI counts (Table 2) whereas the rest of the groups showed much lower percentages. Conversely, the two Antarctic stations presented higher numbers of

Table 2 **a** Initial abundances of bacteria (BA) and operationally defined free-living (0.2–5 μm) bacterial assemblage structure described as percentage of hybridized cells with specific probes by CARD-FISH at the beginning of each experiment; **b** particle-attached (>5 μm) bacterial assemblage described by CARD-FISH at the beginning of the experiments

Fraction (%) of total DAPI counts detected with CARD-FISH probe in 0.22 μm filters						
Station	BA (10^6 ml^{-1})	Eub338-II-III	Gam42a	CF319a	Sar11	Ros537
a						
AR3	0.9	96 ± 5	6 ± 3	7 ± 4	68 ± 3	5 ± 4
AR4	2.0	95 ± 7	3 ± 2	10 ± 4	64 ± 10	5 ± 2
AR5	0.8	94 ± 6	6 ± 4	2 ± 2	61 ± 7	3 ± 2
AR7	1.1	92 ± 4	6 ± 3	5 ± 4	71 ± 6	3 ± 2
AN1	0.2	93 ± 3	27 ± 6	42 ± 7	20 ± 4	2 ± 2
AN2	0.2	95 ± 5	19 ± 6	18 ± 4	48 ± 7	4 ± 3

Fraction (%) of total DAPI counts detected with CARD-FISH probe in 5 μm filters					
Station	BA (10^6 ml^{-1})	Gam42a	CF319a	Sar11	Ros537
b					
AR3	0.03	–	94 ± 7	–	–
AR4	0.02	–	95 ± 8	–	–
AR5	0.02	–	94 ± 6	–	–
AR7	0.02	–	95 ± 4	–	–
AN1	0.02	35 ± 12	22 ± 12	2 ± 2	2 ± 3
AN2	0.04	11 ± 6	40 ± 14	3 ± 2	2 ± 2

Eubacteria (EUB338-II-III), *Gammaproteobacteria* (Gam42a), *Bacteroidetes* (CF319a), SAR11 cluster (SAR11-441R), *Roseobacter* (Ros537)

CARD-FISH values represent means ± standard deviations

Table 3 Bulk bacterial activity measured as ^{3}H-leucine incorporation rates before and after exposure to the following radiation conditions: PAR + UVA, PAR + UVR and darkness

	Bacterial heterotrophic activity (pmol ^{3}H-leucine l^{-1} h^{-1})					
	Stn. AR3	Stn. AR4	Stn. AR5	Stn. AR7	Stn. AN1	Stn. AN2
Initial	55 ± 2	77 ± 1	92 ± 2	80 ± 20	182 ± 24	31 ± 1
DARK	93 ± 3^{a}	107 ± 7^{a}	162 ± 16^{a}	160 ± 13^{a}	227 ± 30^{a}	34 ± 7^{a}
PAR + UVA	84 ± 13^{a,b}	93 ± 4^{a}	161 ± 21^{a}	152 ± 10^{ab}	137 ± 18^{b}	26 ± 3^{ab}
PAR + UVR	72 ± 4^{b}	71 ± 4^{b}	155 ± 9^{a}	138 ± 9^{b}	119 ± 11^{b}	23 ± 3^{b}

Values are means ± standard deviations. *Letters* refer to results with a post hoc Tukey's test ($p < 0.05$). *Different letters* indicate significant differences among treatments

Gammaproteobacteria and *Bacteroidetes* and less SAR11 cells, and interestingly, whereas SAR11 dominated the community at station AN2, *Bacteroidetes* was the most abundant group at station AN1, associated with the extremely high chlorophyll concentration (~20 μg l^{-1}) of a dense diatom bloom.

As for the bacteria retained in the 5 μm fraction, they were almost entirely comprised of members of the *Bacteroidetes* cluster in samples from the *Phaeocystis*-dominated Arctic stations (Table 2b). Microscopic examination of integral *Phaeocystis* colonies revealed that *Bacteroidetes* accounted for almost all DAPI counts within the colony mucus (Fig. 2), and very high numbers were also found associated with other particles. The particle-attached bacteria from the two Antarctic stations were less group-specific, but still appeared to be enriched in *Gammaproteobacteria* and *Bacteroidetes* whereas barely any other group was found.

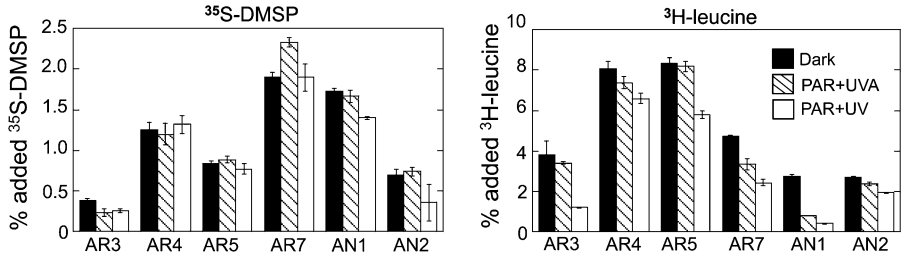

Fig. 1 Percentages of assimilated ^{35}S-DMSP (**a**) and ^{3}H-leucine (**b**) by organisms' 0.2–5 μm during-exposure to the following radiation conditions: PAR + UVA (*dashed bars*), PAR + UV (*white bars*) and darkness (*black bars*). Values are averages ± standard errors

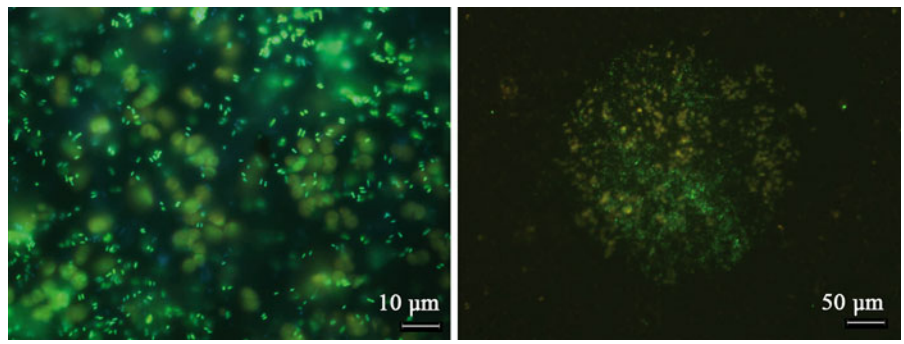

Fig. 2 Epifluorescence images of *Bacteroidetes* cells (hybridized with the CF319a probe) associated to colonies of *Phaeocystis pouchetii* on 5 μm filters. *Left panel*: green *Bacteroidetes* and reddish *Phaeocystis* cells. *Right panel*: view of a whole colony where the green fluorescence of hybridized *Bacteroidetes* is visible. (Color figure online)

The contribution to both trace ^{35}S-DMSP and ^{3}H-leucine assimilation by the different groups of bacteria was further assessed by applying microautoradiography to the hybridized filters. Only the free-living bacterial fraction was subjected to analysis; bacteria retained in the 5 μm filters occurred in aggregates and thus silver grains could not be clearly attributed to individual bacteria. High numbers of active cells were found for both substrates, although more bacteria assimilated ^{3}H-leucine (65 to 83% of Eub, dark treatment) than ^{35}S-DMSP (35 to 45% of Eub, dark treatment) at all locations (Table 4). Negligible numbers of cells were labeled with silver grains in the killed controls.

Only minor differences were found among the total number of active eubacteria exposed to different light conditions. Only bacteria from stations AR5 and AN1 showed significantly smaller percentages of leucine and DMSP-sulfur assimilating cells when exposed to full sunlight radiation compared to PAR + UVA exposure (Tukey's test, $p < 0.05$).

Among the taxonomic groups taking up ^{35}S-DMSP, *Gammaproteobacteria* (Fig. 3a) and *Roseobacter* (Fig. 3d) showed the highest numbers of active cells (60–98% of hybridized cells), followed by the SAR11 clade (Fig. 3c, 24–46%). *Bacteroidetes* (Fig. 3b) were much more variable among stations, with percentages of active cells in ^{35}S-DMSP uptake ranging from 5% (station AN1) to 75% (station AR7).

With respect to ^{3}H-leucine, both *Gammaproteobacteria* and *Roseobacter* presented numbers of active cells as high as those taking up ^{35}S-DMSP (Fig. 4a, d). SAR11 showed up to 83% of cells taking up ^{3}H-leucine (Fig. 4c), which is twice the values for ^{35}S-DMSP samples but in contrast less *Bacteroidetes* ($\sim$10–50%) were active for ^{3}H-leucine (Fig. 4b).

When samples were exposed to different sunlight conditions, some differences were detected within bacterial groups, although the general effects of UVR were small and variable among experiments. *Gammaproteobacteria* seemed to be stimulated in their ^{35}S-DMSP uptake due to dark incubation only at station AR3; no important differences were found for the rest of the stations. Instead, inclusion of UVB led to significantly lower numbers of *Gammaproteobacteria* labeled

Table 4 Percentage of positively hybridized cells with EUB338-II-III probes for *Eubacteria* taking up ^{35}S-DMSP and ^{3}H-leucine (average ± deviation of fields) as measured by MAR-CARD-FISH after exposure to each treatment

	Stn. AR3	Stn. AR4	Stn. AR5	Stn. AR7	Stn. AN1	Stn. AN2
% of hybridized *Eubacteria* active in ^{35}S-DMSP uptake						
DARK	37 ± 8^{a}	35 ± 7^{a}	44 ± 9^{a}	45 ± 8^{a}	44 ± 5^{a}	–
PAR + UVA	30 ± 7^{a}	33 ± 5^{a}	45 ± 5^{a}	44 ± 8^{a}	42 ± 5^{a}	–
PAR + UVR	31 ± 3^{a}	30 ± 4^{a}	33 ± 7^{b}	47 ± 7^{a}	32 ± 7^{b}	–
% of hybridized *Eubacteria* active in ^{3}H-leucine uptake						
DARK	79 ± 5^{a}	77 ± 5^{a}	83 ± 5^{ab}	79 ± 5^{a}	65 ± 7^{a}	65 ± 4^{a}
PAR + UVA	80 ± 3^{a}	71 ± 3^{a}	85 ± 4^{a}	83 ± 4^{a}	59 ± 12^{a}	66 ± 8^{a}
PAR + UVR	82 ± 3^{a}	71 ± 7^{a}	79 ± 4^{b}	79 ± 7^{a}	37 ± 5^{b}	62 ± 6^{a}

Letters refer to results with a post hoc Tukey's test ($p < 0.05$). *Different letters* indicate significant differences among treatments

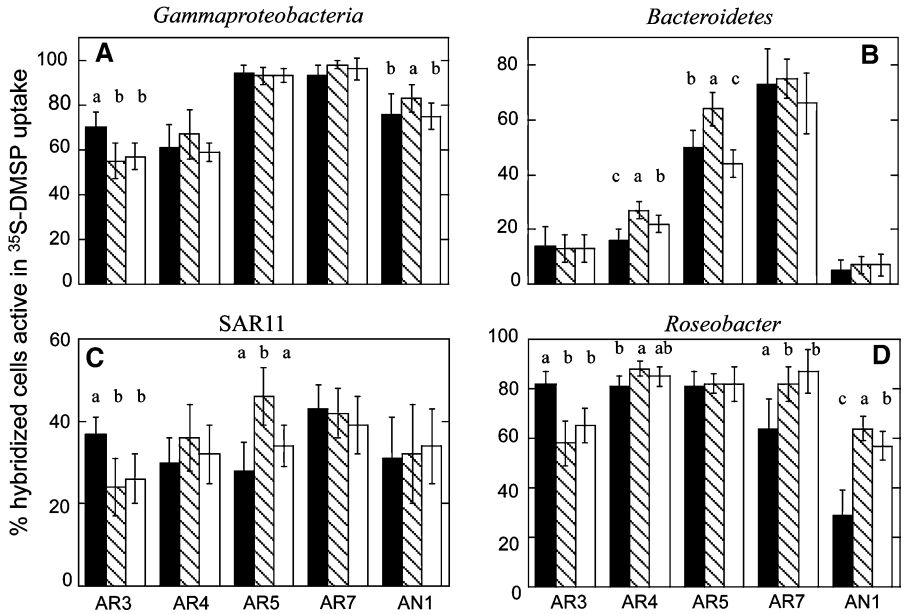

Fig. 3 Percentage of positively hybridized cells with probes for free-living *Gammaproteobacteria* (**a**), *Bacteroidetes* (**b**), SAR11 (**c**), and *Roseobacter* (**d**) taking up ^{35}S-DMSP (average ± standard deviation of fields) as measured by MAR-CARD-FISH after exposure to the following radiation conditions: PAR + UVA (*dashed bars*), PAR + UVR (*white bars*) and darkness (*black bars*). Note that the *Y* axes show different scales. *Letters* refer to results with a post hoc Tukey's test ($p < 0.05$). *Different letters* indicate significant differences among treatments

for ^{3}H-leucine at stations AR5, AR7 and AN1 (11, 15, and 10% reduction in the percentage of active cells compared to the dark treatment, respectively, Fig. 4a) whereas at station AN2 a significant 17% increase was caused by full sunlight exposure compared to dark treatment.

Bacteroidetes showed no significant differences among treatments at most stations, except for stations AR4 and AR5, where the numbers of cells active in ^{35}S-DMSP uptake increased after PAR + UVA exposure compared to the dark treatment and decreased due to UVB (Fig. 3b). ^{3}H-leucine uptake by this group in the Arctic was only affected by light at station AR7, showing a 25% decrease in both light incubations compared to the dark treatment (Fig. 4b). However, the two Antarctic stations showed either a strong light-driven inhibition (72% decrease compared to dark conditions at station AN1) or a significant stimulation of activity (42% increase after PAR + UVA exposure compared to dark conditions at station AN2).

Within *Alphaproteobacteria*, members of the *Roseobacter* cluster were either photoinhibited in their ^{35}S-

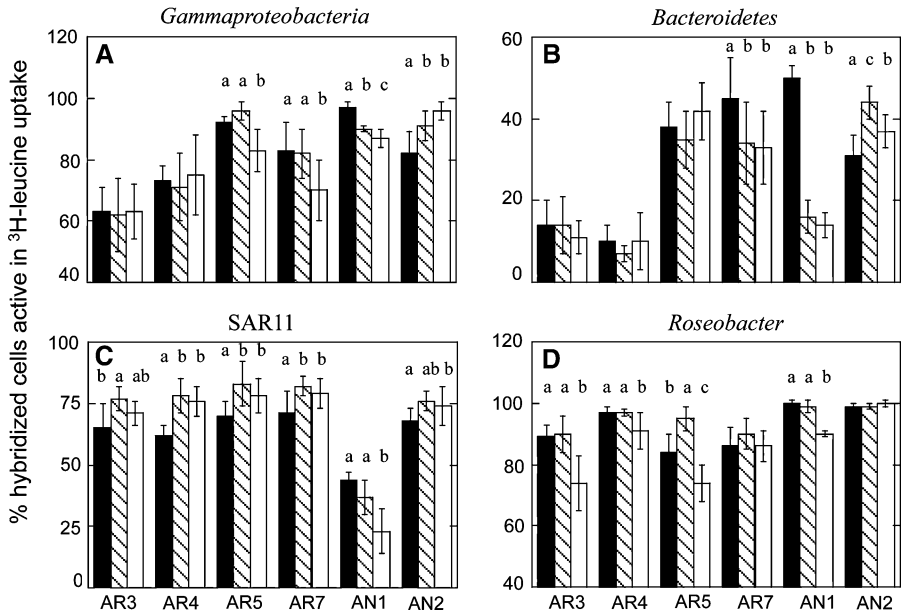

Fig. 4 Percentage of positively hybridized cells with probes for free-living *Gammaproteobacteria* (**a**), *Bacteroidetes* (**b**), SAR11 (**c**), and *Roseobacter* (**d**) taking up ^{3}H-leucine (average ± standard deviation of fields) as measured by MAR-CARD-FISH after exposure to the following radiation conditions: PAR + UVA (*dashed bars*), PAR + UVR (*white bars*) and darkness (*black bars*). Note that the *Y* axes show different scales. *Letters* refer to results with a post hoc Tukey's test ($p < 0.05$). *Different letters* indicate significant differences among treatments

DMSP uptake (station AR3) or significantly stimulated by one or both light types at stations AR4, AR7 and AN1 (i.e. 8, 28, and 124% increase after PAR + UVA exposure, respectively, Fig. 3d). Regarding the uptake of ^{3}H-leucine, significant decreases in active cells caused by UVB were found for stations AR3, AR5, and AN1, the ones with the highest measured doses of UVB (Fig. 4d). Indeed, ^{3}H-leucine-assimilating *Roseobacter* was the only group for which a significant correlation could be found between the reduction in the number of active cells after full sunlight exposure and the UVB doses received (Spearman's $r = 0.81$, $p < 0.01$, and $r = 0.78$, $p < 0.05$, $n = 6$ for percentages of the dark and PAR + UVA treatments, respectively).

Assimilation of ^{35}S from DMSP by SAR11 showed no clear trend, presenting dark stimulation in experiment AR3, PAR + UVA-driven stimulation at station AR5 and no effect in the other 3 experiments (Fig. 3c). Instead, the only repetitive pattern found for this group was in the uptake of ^{3}H-leucine (Fig. 4c). In five out of 6 experiments, the number of active SAR11 was significantly higher after exposure to both light treatments, showing increases ranging from 13 to 25% after PAR + UVA exposure compared to the dark conditions.

To examine the importance of each bacterial group to substrate uptake, we estimated the contribution of each phylogenetic group to the total number of cells assimilating each substrate. This was calculated from the fraction of active cells within one group and its abundance (with respect to total eubacterial cells) relative to the percentage of total eubacteria active at substrate uptake. This relative contribution (average of the three treatments) is shown in Fig. 5. The numbers of cells assimilating ^{35}S-DMSP or ^{3}H-leucine were both largely dominated by the SAR11 group at all Arctic stations (on average 69 and 76% of active bacteria, respectively), whereas at station AN1 *Gammaproteobacteria* was the major contributor to active cells, accounting for 56 and 50% of the ^{35}S-DMSP and the ^{3}H-leucine-assimilating bacteria, respectively. Besides the numeric abundance of *Bacteroidetes* in station AN1, their low numbers of active cells led to a small contribution to total active cells.

Figure 6 shows the percentage of total DAPI-stained prokaryotic cells that hybridized with each probe and incorporated each substrate, pooled from the different treatments and stations. Stations AR3-AR4 and AR5-AR7 were plotted together due to similar behavior of groups (Figs. 6a, b, respectively).

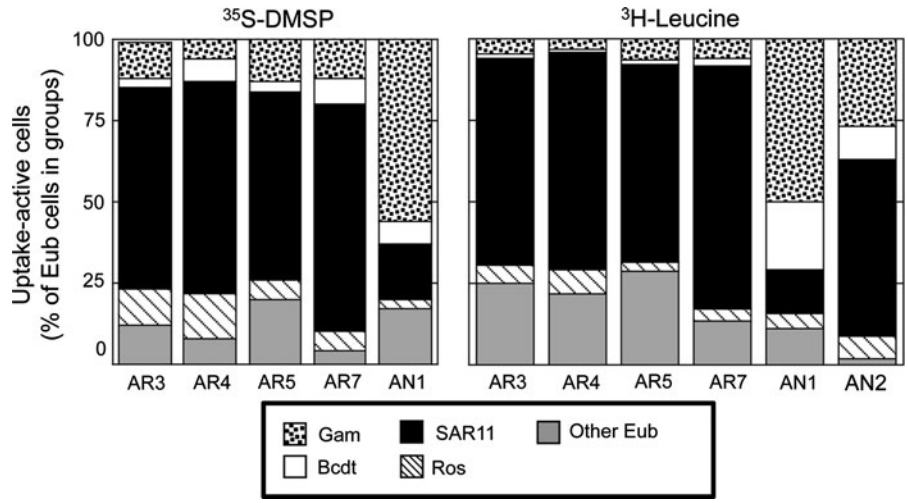

Fig. 5 Relative contribution of each of the analyzed phylogenetic groups (*Gammaproteobacteria* [Gam], *Bacteroidetes* [Bcdt], SAR11, *Roseobacter* [Ros] and other *Eubacteria* [Eub]) to total active bacteria in the uptake of ^{35}S-DMSP (*left panel*) and ^{3}H-leucine (*right panel*) in samples from the stations studied. Percentages were calculated relative to eubacterial cells (probes EUB338-II and -III) as average of the three treatments

In the Arctic, *Roseobacter* and *Gammaproteobacteria* showed similar percentages of ^{35}S-DMSP and ^{3}H-leucine labeled cells (i.e., they appear very close to the 1:1 line), and in stations AR5 and AR7 *Gammaproteobacteria* presented more active cells (Fig. 6b). Conversely, within the *Bacteroidetes* group, the percentage of ^{35}S-DMSP labeled cells was often higher than that of ^{3}H-leucine labeled cells, and, like *Gammaproteobacteria,* much higher percentages of active cells were found at stations AR5 and AR7 (Fig. 6b). at the Antarctic station AN1, all these groups were more active in assimilating ^{3}H-leucine than ^{35}S-DMSP (Fig. 6c). SAR11 behaved distinctly: at the Arctic stations they showed on average 50% fewer ^{35}S-DMSP labeled than ^{3}H-leucine labeled cells (Fig. 6a, b), but at AN1 they fell close to the 1:1 line (Fig. 6c).

The contribution of each taxonomic group to ^{35}S-DMSP and ^{3}H-leucine-assimilating eubacteria was compared with their relative abundance in the bacterial community (Fig. 7). In general, both *Gammaproteobacteria* (Fig. 7a) and *Roseobacter* (Fig. 7d) accounted for a higher fraction of ^{3}H-leucine and ^{35}S-DMSP-assimilating cells than that expected from their relative abundance, and SAR11 fell close to the 1:1 line (Fig. 7c), suggesting that they contributed to the number of cells assimilating both substrates according to their relative abundance. Instead, *Bacteroidetes* were underrepresented among DMSP and leucine-assimilating bacteria, specially in the two studied Antarctic samples (Fig. 7b).

Discussion

Despite the low temperatures and extreme conditions, the Arctic and Antarctica can support high prokaryotic heterotrophic activities due to the spring and summertime development of localized massive phytoplankton blooms when the ice retreats (Fogg 1977; Sakshaug 2004). Our values of heterotrophic bacterial abundance and activity (measured as ^{3}H-leucine incorporation) fell within the ranges previously reported for polar areas (e.g. Rich et al. 1997; Straza et al. 2010) and are similar to those measured in other oceanic regions (Alonso and Pernthaler 2006; Church et al. 2004).

High DMSPt:Chl *a* ratios were observed in the Arctic due to the dominance of the colonial haptophyte *Phaeocystis pouchetii* (Lasternas and Agustí 2010), a well-known DMSP producer (Liss et al. 1994), whereas at the two studied Antarctic stations, where mainly diatoms comprised the phytoplankton assemblage (Ruiz-González et al. submitted), lower ratios were found. The DMSP:Chl *a* ratio is a good indicator of the relative occurrence of DMSP producers among the phytoplankton assemblages (Kiene et al. 2000; Simó et al. 2002), and the values found in the two

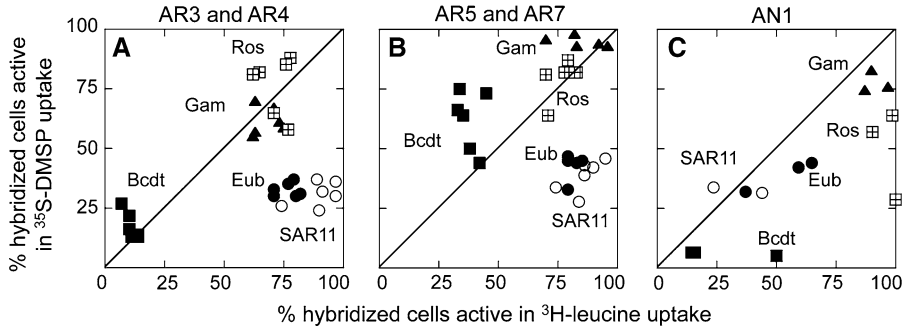

Fig. 6 Comparison of ^{35}S-DMSP versus ^{3}H-leucine labeled cells (as % of total DAPI counts) for each phylogenetic bacterial group from stations AR3 and AR4 (**a**), AR5 and AR7 (**b**) and AN1 (**c**). Each data point represents one single treatment. *Line* indicates a 1:1 relationship (same number of active cells for both substrates). *Gammaproteobacteria* [Gam], *Bacteroidetes* [Bcdt], SAR11, *Roseobacter* [Ros] and total *Eubacteria* [Eub])

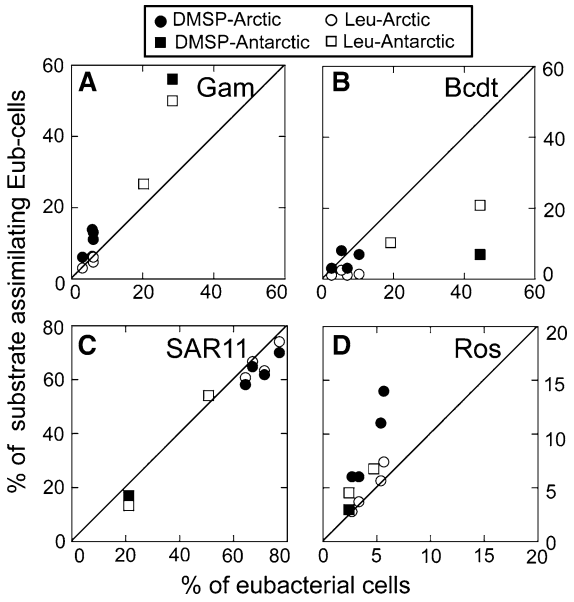

Fig. 7 Contribution of **a** *Gammaproteobacteria* [Gam], **b** *Bacteroidetes* [Bcdt], **c** SAR11 and **d** *Roseobacter* [Ros] from Arctic and Antarctic stations to the number of cells assimilating ^{35}S from DMSP (*solid symbols*) or ^{3}H-leucine (*open symbols*) versus the relative abundance of the group (calculated with respect to *Eubacteria* (EUB) cells). Values are expressed as average of the three treatments and the 1:1 *line is indicated*. Note that the *X* and *Y* axes show different scales

Antarctic stations are typical of low-DMSP producing phytoplankton (Kiene et al. 2000).

Exposure of samples to natural sunlight caused a significant reduction of both the post-exposure ^{3}H-leucine incorporation rates and the percentage of trace ^{3}H-leucine assimilated during incubations (Table 3; Fig. 1b), suggesting that the UVR levels received was actually affecting bacterial heterotrophic activity, with UVB generally accounting for most inhibition compared to dark incubations. This deleterious effect of UVB radiation on leucine uptake by marine bacteria has been reported for many different non-polar systems (Aas et al. 1996; Herndl et al. 1993) yet its effects on the heterotrophic activity of polar free-living bacterial assemblages are still rather understudied (e.g., Pakulski et al. 2008). The large discrepancy between the ^{3}H-leucine incorporation rates and the trace ^{3}H-leucine assimilations measured in station AN1 might be due to the presence of high numbers of large leucine-assimilating diatoms, such as *Thalassiosira* spp., *Eucampia* spp. and *Chaetoceros* spp. (Ruiz-González et al. submitted), which were removed by 5 μm pre-filtration for the trace assimilation measurements but not for the incorporation assays.

Remarkably, a significant correlation between inhibition and UVB doses was found for the trace ^{3}H-leucine assimilation but not for the incorporation measured after exposure. Reasons for this discrepancy can be found in the fact that post-exposure ^{3}H-leucine incorporation was determined by standard 2–3 h dark incubations during which bacteria might have had time to recover from photodamage (Kaiser and Herndl 1997). This further suggests that incubation of samples with added substrates under natural light may give more realistic estimates of the in situ incorporation rates.

The low assimilation percentages of trace ^{35}S-DMSP (Fig. 1a) might be due to the dilution of the radiolabeled tracer within the potentially high (and highly variable) concentrations of dissolved DMSP released by *Phaeocystis* cells. For this reason, comparisons should be done among light treatments within stations but not among stations. No correlation was found between the light-

driven effects on trace DMSP assimilation and UVR doses. Instead, variable responses to the light spectrum were recorded. These results agree with those of Slezak et al. (2001), who observed that the degree of bacterial inhibition due to UVR differed between ^{3}H-leucine incorporation and ^{35}S-DMSP consumption in the Mediterranean Sea. Thus, while bacterial assimilation of leucine in either high or trace concentrations seems to be directly affected by photodamage, trace DMSP-sulfur assimilation seems to follow more complex, yet still unknown, physiological responses to light, or be highly sensitive to light-driven DMSP release by phytoplankton.

The CARD-FISH description of the bacterial communities revealed differences between particle-attached and free-living bacterial assemblages (Table 2), as previously reported (DeLong et al. 1993; Riemann et al. 2000). Nearly all DAPI-stained cells hybridized with the EUB338-II-III probe, suggesting that the summer prokaryotic assemblages from both poles were almost entirely comprised by bacteria, agreeing with the observed low numbers of *Archaea* towards the summer in surface samples from the Arctic (Alonso-Sáez et al. 2008) and Antarctica (Murray et al. 1999).

Whereas the free-living Arctic bacteria were numerically dominated by the SAR11 clade, the particle-attached assemblages were almost entirely comprised of cells assigned to the *Bacteroidetes* cluster, in agreement with some studies that have shown *Alphaproteobacteria* and *Bacteroidetes* groups to be associated to *Phaeocystis* blooms (Brussaard et al. 2005; Simon et al. 1999). At the two studied Antarctic stations, however, members of *Gammaproteobacteria* and *Bacteroidetes* were the dominant in the aggregate fraction, whereas either *Bacteroidetes* (AN1) or SAR11 (AN2) dominated the free-living assemblages. This discrepancy in bacterial composition might be due to the variability between the phytoplankton assemblages from both stations, which were dominated either by the diatoms *Thalassiosira* spp. (AN1) or by *Pseudonitzschia* spp. (AN2; Ruiz-González et al. submitted). This fact, together with the small dataset available for Antarctic samples, precludes making generalizations about bacterial community structure and activities in Antarctic waters.

Relatively few studies have examined the abundance of bacterial groups in polar waters. Within Arctic bacterial assemblages, *Alphaproteobacteria* has often been reported as the most abundant group (15–42% of cell counts), with SAR11, when probed, accounting for the majority of it, particularly in summer (e.g., Alonso-Sáez et al. 2008; Bano and Hollibaugh 2002; Malmstrom et al. 2007). However, none of these authors found such high numbers of SAR11-positive cells. In agreement with our results, Antarctic and Southern Ocean waters have been found to be either dominated by *Bacteroidetes* (Simon et al. 1999; Straza et al. 2010) or *Gammaproteobacteria* (Gentile et al. 2006), and lower numbers or sequences of *Alphaproteobacteria* have been often recovered.

MAR-CARD-FISH was further applied to resolve the substrate utilization activity of single cells belonging to the observed bacterial groups. Very high percentages of active bacteria were found in all stations, in accordance with some previous studies in Arctic waters that showed that prokaryotes are an active component of polar microbial communities (Alonso-Sáez et al. 2008; Elifantz et al. 2007; Straza et al. 2010).

Since leucine is a universal substrate for bacteria that is used to estimate bacterial heterotrophic production (Kirchman et al. 1985), we regarded the number of cells assimilating ^{3}H-leucine (60–85%, Table 4) as a measure of the fraction of active bacteria. Up to 47% of ^{35}S-labeled cells were also recorded, meaning that approximately half of the active bacteria were taking up sulfur from DMSP. The only previous study where bacteria assimilating DMSP and leucine were compared in polar waters is that of Vila-Costa et al. (2008), which was conducted in March and May in a coastal region near the Mackenzie river estuary. They found smaller percentages of ^{3}H-leucine-assimilating cells (47–62%) and much lower proportions of ^{35}S-DMSP labeled cells (4–5%). The authors suggested that these low numbers were due to the low concentrations of labile organic substrates occurring in the ice covered waters prior to the summer phytoplankton bloom which peaked in July (Alonso-Sáez et al. 2008). This was confirmed for DMSP, which showed very low concentrations throughout the study (1–6 nM in total; Vila-Costa et al. 2008). Consistent with that hypothesis, the high percentages of active cells found in our Arctic stations would be explained by the high concentrations of DMSP and other substrates released by the *Phaeocystis* bloom.

At the Antarctic station AN1, where the dense diatom bloom carried a much lower DMSP:Chl *a* ratio, percentages of ^{35}S-active cells were as high as in the

Arctic samples. As a result, no significant correlation was found between DMSP:Chl *a* ratios and the number of ^{35}S-labeled cells across stations. This does not support the hypothesis that the fraction of DMSP-sulfur assimilating bacteria depends on the contribution of DMSP to total reduced sulfur and carbon sources in the ecosystem (Pinhassi et al. 2005; Vila-Costa et al. 2007). However, the fact that just one station was analyzed for single-cell ^{35}S-DMSP assimilation in Antarctica prompts caution in making generalizations; indeed, to our knowledge this is the first time that cell-specific uptake of DMSP is probed for Antarctic bacterial assemblages. In any case, DMSP seemed to be an important source of reduced sulfur for a wide array of polar marine bacteria, consistent with the suggested role of this compound in a methionine synthesis shortcut that fuels the protein factory (Kiene et al. 1999).

High percentages (ca. 20–50%) of SAR11 cells active in the uptake of both substrates were recorded at the Arctic stations (Figs. 3, 4). This high activity, together with their numeric dominance, led to a major contribution of this group to total numbers of active cells in Arctic samples (Fig. 5). This contrasts with the results of previous studies in Arctic and other coastal waters where the very abundant SAR11 were not the major contributors to total substrate uptake (Alonso-Sáez et al. 2008; Elifantz et al. 2005). However, this clade has also shown high activities in oceanic Atlantic waters (Malmstrom et al. 2004a) suggesting a substantial variability in their single-cell activity and/or taxonomic composition changes among oceanic regions. These authors also found that the SAR11 clade dominated the utilization of dissolved DMSP and amino acids due to their high abundances and high percentages of active cells (40–60% and 40–85% of DMSP and amino acid uptake, respectively). Supporting these results, Tripp et al. (2008) showed that SAR11 cells need exogenous sources of reduced sulfur for growth because they lack the genes for sulfate reduction. Therefore, a DMSP-rich environment, such as that we encountered during our cruise through *Phaeocystis*-blooming Arctic waters, might be a suitable environment for these particular SAR11 to grow actively up to high abundances.

Roseobacter and *Gammaproteobacteria* were also highly active in the uptake of both tracers (Figs. 3, 4) in agreement with previous results from other ecosystems such as the NW Mediterranean Sea (Vila-Costa et al. 2007), the North Atlantic and the Gulf of Mexico (Malmstrom et al. 2004b). The capacity to degrade DMSP and assimilate its sulfur is known to be common among *Roseobacter* isolates (González et al. 1999) and their abundances have been positively correlated with DMSP concentrations (González et al. 2000) or DMSP consumption (Zubkov et al. 2002) during blooms of DMSP producers. Similarly, both *Roseobacter* and *Gammaproteobacteria* groups were shown to be favored by the development of a DMSP-rich algal bloom (Pinhassi et al. 2005).

At the Antarctic station AN2, the SAR11 cells were also the major contributors to both abundance and total ^{3}H-leucine-assimilating cells. The relatively abundant *Gammaproteobacteria* contributed more to total ^{3}H-leucine uptake than their Arctic counterparts. A different picture was found at station AN1: the lower abundance of SAR11, perhaps less favored by the bloom of *Thalassiosira* spp. present at this station meant they contributed less than 20% to the total active cells for both substrates (Fig. 6), even though the numbers of ^{35}S-DMSP-active cells were quite similar to those from the Arctic. Despite their dominance, *Bacteroidetes* accounted for only a small proportion of the total active cells. The relatively high abundance of *Gammaproteobacteria* and their high percentages of active cells resulted in a major contribution of this group to both ^{3}H-leucine (50%) and ^{35}S-DMSP (56%) uptake at this Antarctic station. These results add to previous evidence that DMSP-sulfur assimilation is widespread among the major bacterial taxonomic groups in a variety of marine environments, and further provide the first observations in Antarctic waters.

SAR11 was the only group where cells taking up ^{35}S-DMSP were often much fewer than those taking up ^{3}H-leucine (Fig. 6), which suggests that not all active SAR11 were using DMSP-sulfur for protein synthesis. *Gammaproteobacteria* and *Roseobacter*, conversely, presented similar proportions of active bacteria for both substrates. *Bacteroidetes* even showed higher affinity for DMSP-sulfur, yet in this case leucine may not be the best indicator of active cells because *Bacteroidetes* have predilection for large complex carbohydrates and usually present low numbers of amino acid assimilating cells (Cottrell and Kirchman 2000). These results are in accordance with the percentages of active bacteria found for both substrates in coastal temperate waters (Vila et al.

2004; Vila-Costa et al. 2007) but differ considerably from those recorded by Vila-Costa et al. (2008) for winter and springtime Arctic bacterial assemblages, where all groups except *Roseobacter* showed higher affinity for ^{3}H-leucine than for ^{35}S-DMSP.

The contributions of each group to substrate-assimilating cells were compared with their relative abundance in the bacterial community. In general, both *Gammaproteobacteria* and *Roseobacter* accounted for higher fractions of the ^{3}H-leucine and ^{35}S-DMSP-assimilating cells than those expected from their relative abundances (Fig. 7), indicating high activities and a significant role of these groups in the processing of DMSP during the polar summer. Instead, SAR11 fell onto the 1:1 line and *Bacteroidetes* were underrepresented among cells assimilating both substrates. This contrasts with the pattern found by Vila-Costa et al. (2008) with winter and spring Arctic assemblages, where only spring *Bacteroidetes* were overrepresented among cells assimilating ^{35}S-DMSP and the rest of the groups either contributed equally or less to active cells than expected based on abundance.

In general, the effects of solar radiation on the single-cell activity of these broad taxonomic groups were small and variable, showing no clear trends among experiments. Even though there are a few studies on the effects of UVR on polar bacterial assemblages and isolates (e.g. Wickham and Carstens 1998; Davidson and van der Heijden 2000; Hernández et al. 2006), to our knowledge this is the first time that group-specific sensitivities to UVR have been assessed for in dominant polar bacterial taxa in situ.

Apparently, light-driven changes in the number of active cells did not reflect the observed variations in either post-exposure incorporation or during-exposure trace assimilation rates (Table 3; Fig. 1), which could be explained by the fact that single-cell activity was assessed in terms of presence/absence (i.e., each cell is identified as labeled or non-labeled) and not as assimilation per cell which would have required silver grain area measurements (Sintes and Herndl 2006). Hence, higher or lower percentages of active cells do not necessarily equal to higher or lower assimilation rates, since a cell surrounded by silver grains will be counted as labeled regardless of the amount of exposed silver grains.

Nonetheless, some differences could be observed, showing either UVB inhibition or light stimulation of uptake (Figs. 3 and 4). In contrast to the results of Alonso-Sáez et al. (2006) and Ruiz-González et al. (2012), where SAR11 cells from the Mediterranean were the most sensitive to the detrimental effects of solar radiation, we found a slight but significant increase in the number of ^{3}H-leucine-assimilating SAR11 cells upon light exposure in five out of six stations, yet most of those experiments in the Mediterranean were performed under the much higher UVR doses typical of spring and summer. The only group showing a significant correlation between the reduction in the number of active cells due to full sunlight and the UVB doses received was *Roseobacter* in terms of trace ^{3}H-leucine assimilation (Spearman's $r = 0.81$, $p < 0.01$, and $r = 0.78$, $p < 0.05$ for percentages of the dark and PAR + UVA treatments, respectively, $n = 6$). This is also opposite to the results of Alonso-Sáez et al. (2006) and Ruiz-González et al. (2012), who found higher resistance to UVB in *Roseobacter* than in SAR11. The level of resolution of these CARD-FISH probes provides no information about taxonomic variations within the groups, yet these observations point to distinct phylotypes adapted to different light regimes (continuous light vs. diel cycles) and different trophic conditions (eutrophic vs. oligotrophic).

The largest differences among treatments were found at station AN1, where ^{3}H-leucine uptake by all groups appeared to be negatively affected by both UVA + PAR and UVB exposure. Interestingly, the strong photoinhibition of the abundant *Bacteroidetes* in the uptake of ^{3}H-leucine reflects the observed decrease in both post-exposure ^{3}H-leucine incorporation and during-exposure trace ^{3}H-leucine assimilation. On the other hand, uptake of ^{35}S by *Roseobacter* was significantly greater upon light exposure compared to the dark controls, while all other groups showed no significant effects among treatments at this Antarctic station. Such a light-driven enhancement of activity has also been reported for Mediterranean *Roseobacter* by Alonso-Sáez et al. (2006) in both leucine and ATP uptake, and it could be related to the suggested ability of some bacterial groups to derive energy from light with the use of proteorhodospsins (Béjà et al. 2000; Gómez-Consarnau et al. 2007) or bacteriochlorophyll-*a* (Kolber et al. 2000). It seems that having the ability to derive energy from light would be a useful strategy in these areas with such long light periods, although Cottrell and Kirchman (2009) did not find any of these photoheterotrophs to behave as superior competitors during the Arctic summer. The fact that different groups responded

distinctly to light depending on the substrate assimilated points, instead, either to differential regulation or damage of the uptake systems (Herndl et al. 1997) or, as previously suggested, to light-driven variations of the available DMSP released by phytoplankton. In any case, considering that the polar bacterial assemblages are continuously exposed to light during the summer months, incubation under realistic solar irradiances is essential for accurate determinations of bacterial heterotrophic activities and production rates. Unreal dark incubation can lead both to overestimates (as is the case for our leucine assimilation rates) or underestimates (as is the case for our DMSP-sulfur assimilation rates) of the measured activities during the long polar summer.

Notable amounts of UVB may reach 15 m depth and biological effects may be detected at 20 or 30 m in inshore polar waters (Convey and Fogg 2007). In deeply-mixed waters this may not be so important since individual cells are near the surface for short times only and have time for repair in the shade of deeper waters. In these cases, the effects of UVR measured in experimental tanks under near surface conditions may easily be overestimates. It is possible that such an overexposure of samples was partially responsible for the greatest effects found at station AN1, where the mixed layer was ca. 15 m deep and thus our samples were exposed to doses higher than the natural situation. In contrast, in shallower and strongly stratified waters, such as those of the Arctic marginal ice zone, where actively growing phyto- and bacterio-plankton are held in a narrow surface mixing layer (ca. 5 m) in the season of maximum UVR levels, these organisms may be continuously exposed to deleterious light with less chance for repair (Buma et al. 2001). In these cases experimental approaches like ours would provide more realistic estimates.

Overall, our results support a high heterotrophic activity during the polar summer and provide insight into the apparently widespread DMSP-sulfur and leucine assimilation capabilities of different dominant bacterial groups from polar waters, including the first observations of DMSP-sulfur assimilating Antarctic bacteria. Similar percentages of bacteria active in ^{35}S-DMSP uptake were found in the Arctic and in the one Antarctic sample we studied despite the variable DMSP concentrations found across stations, indicating for the first time a widespread role of DMSP as a reduced sulfur source for marine bacteria in polar ecosystems. The present study also documents the substantial impact of UVR on heterotrophic carbon and sulfur fluxes and further suggests that incubations under realistic solar radiation levels and spectrum are necessary in order to obtain meaningful measurements of the contribution of bacterial assemblages and their taxonomic composition to the processing of labile organic compounds.

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