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Vertical zonation of bacterial assemblages attributed to physical stratification during the summer relaxation of the coastal upwelling off Galicia (NW Spain)

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ABSTRACT

We combined flow cytometry, CARD-FISH, and 16S rRNA gene tag pyrosequencing to investigate bacterioplankton dynamics along a transect in shelf waters off A Coruña (Galicia, NW Spain). Over five days (16-20th July 2012) we sampled during the relaxation of a summer upwelling pulse, providing an opportunity to examine the impact of pulses of cold nutrient-rich water into coastal microbial communities. The hydrographic conditions, characterized by intense density stratification of surface waters and the presence of a deep chlorophyll maximum (DCM) at 20–30 m, were relatively maintained over the sampling period. Indeed, bacterial abundance and composition displayed low day to day variation. Alpha diversity analysis suggested that species richness and diversity increased from coastal to shelf stations and from the surface down to the coastal DCM, which could be caused by the mixing of upwelled bacteria with the coastal surface waters. SAR11, SAR86, and *Roseobacter* were the most abundant bacteria detected in the samples by using CARD-FISH. The assemblages observed by pyrosequencing displayed a strong vertical zonation along the transect. Rhodobacteraceae (under class Alphaproteobacteria) and Bacteriodetes dominated the surface waters and decreased during the upwelling pulse, while SAR 86 (under class Gammaproteobacteria), Actinobacteria and SAR11 clade increased their relative abundance at the coastal DCM with upwelling relaxation, particularly at the shelf stations. Bacterial assemblages from surface waters were associated with higher temperature and light conditions, while coastal DCM assemblages were rather associated to salinity, inorganic nutrients and a diatom-bloom leading to high chlorophyll-a. Our findings suggest that the vertical variability in environmental conditions induced by the intense density stratification, the exportation of warmer and less saline surface water from the rias to the adjacent shelf, and the fertilizing effect of recently upwelled water at the deeper layer, determined the composition of distinct bacterial assemblages at the subsurface and DCM layers.

1. Introduction

Ecological and biogeochemical processes in the ocean are dependent on a diverse assemblage of microbes including members of Bacteria (Glöckner et al., 2012). These diverse bacterial assemblages fulfill a wide range of ecological roles in the marine system including the carbon biogeochemical cycle (Falkowski et al., 2008) and the energy transfer to higher trophic levels (Fuhrman et al., 2015). The relatively recent applications of new molecular techniques have revolutionized microbial research and have considerably contributed to an increase in our knowledge of the vast marine microbial diversity. High-Throughput Sequencing (HTS) and bioinformatic analyses offer a sensitive and high resolution approach to study diversity and composition of microbial assemblages. Indeed, these methods have been used in global research surveys (i.e. GOS and Sorcerer cruises, and recently Tara Oceans and Malaspina expedition) aiming at better understanding composition and distribution patterns of microbial communities at the global scale and at detecting significant trends (Sunagawa et al., 2015;

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Salazar et al., 2016). For instance, two recent studies in the global open ocean have illustrated that community composition of phytoplankton (Estrada et al., 2016) but also of bacterioplankton (Sunagawa et al., 2015) in the upper and the lower parts of the euphotic zone contrasted significantly. Previous regional oceanic studies have identified that the vertical stratification of microbes is likely associated to the differences in the physicochemical properties of the water column (i.e. DeLong et al., 2006; Hewson et al., 2006; Treusch et al., 2009, Mapelli et al., 2013). The study of bacterioplankton in coastal zones is also of critical importance, and in particular there is a need for microbial studies in highly dynamic coastal ecosystems (such as upwelling systems). Microorganisms inhabiting these systems have the ability to function under extremely variable conditions, and thus likely play a disproportionately important role in the microbial-mediated cycling of marine nutrients. However, relatively few sequencing studies to study the bacterial community composition have been carried out in upwelling zones (Allen et al., 2012; Bergen et al., 2015; Gregoracci et al., 2015, Zancker et al. 2018, Bachmann et al., 2018), none of them following an upwelling pulse and its relaxation, and the role that bacterioplankton plays in these highly variable zones still not well understood (i.e. Bachmann et al., 2018).

The northwestern Iberian coast, located at the northern limit of the eastern boundary upwelling ecosystem of the Canary current, is a temperate region where alternation between water column stratification and mixing, driven by the seasonal variations in solar irradiance, is modified by wind-driven upwelling-downwelling events (Arístegui et al., 2006). Furthermore, the region is characterized by the influence of freshwater outflows and the presence of the "Rías", coastal inlets that amplify the nutrient inputs provided by the upwelling by the export of regenerated nutrients and organic matter (Álvarez-Salgado et al., 2000). Rapid increases in phytoplankton growth typically occur during early spring and through the summer in this region (Varela et al., 2001, 2005). Notwithstanding the frequent fertilization of surface waters by upwelling, transient situations of stratification occur during periods of upwelling relaxation, leading to the formation of a defined deep chlorophyll maximum (DCM). The persistence and position of this DCM, generally colonized by small diatoms and dinoflagellates, is related with the frequency of upwelling and the renewal of the nutrients consumed in the surface layer (Figueiras and Pazos, 1991; Casas et al., 1997).

Investigations of the role of bacteria in this region have focused on bulk biological measurements of abundance, biomass and single-cell activity (e.g. Valencia et al., 2003; Bode et al., 2006; Teira et al., 2017). In these studies, the variation in bacterioplankton was related to diverse environmental factors, unveiling hydrography and phytoplankton dynamics as the main driving forces. However, the composition of bacterial assemblages has been comparatively less studied and previous analysis of upwelling induced-changes on bacterial composition were limited by low taxonomic resolution (Alonso-Gutiérrez et al., 2009; Teira et al., 2009; Dobal-Amador et al., 2016). The present study represents the first attempt to study the metataxonomy of the bacterial community in the coastal wind-driven upwelling section off A Coruña (NW Iberian Peninsula) within the RADIALES Long-Term Time- Series (http://www.seriestemporales-ieo.net/). Over one week we conducted a survey along a transect in the shelf waters of A Coruña (NW Spain) to investigate the trends in bacterioplankton communities under the influence of upwelling. The aims of this paper is twofold (i) to determineabundance, diversity, community composition and distribution patterns of bacteria population and (ii) to examine the environmental variables that are significantly correlated and possibly drive the observed variations within the bacterial community composition.

2. Material and methods

2.1. Study site and sampling

Seawater samples were collected on board the R/V Lura during HERCULES-0712 cruise (from the 16th to the 20th July 2012, with the exception of the19th) at six shelf stations off A Coruña from the coast in the ria de A Coruña to the adjacent shelf (Fig. 1). At each station, vertical profiles of temperature, salinity, fluorescence and photosynthetically active radiation (PAR) were obtained with a Seabird-25 conductivity temperature depth (CTD) sensor attached to a rosette sampler, and a Licor spherical PAR sensor deployed to a maximum depth of 100m. Seawater samples were collected for the determination of inorganic nutrient concentration, chlorophyll-a concentration (Chl-a) and bacterial abundance at up to 7 discrete depth levels, and for bacterial diversity and community composition at 2 depth layers: surface (at 1m depth) and the deep chlorophyll maximum (DCM, at 20–30m). Seawater samples from six stations (1c, 2, 3a, 3c, 3b and 4, Fig. 1) were sampled for hydrography while microbial analysis (including abundance, activity and bacterial community composition) were performed only on the samples collected from the stations 1C, 2, 3C, and 4.

Aliquots for inorganic nutrient analysis (nitrate±, nitrite, phosphate and silicate) were collected in polyethylene bottles and frozen at −20 °C until analysis by standard colorimetric methods with a Bran-Luebbe segmented flow analyser (Grasshoff et al., 1983). Chl-a was determined from acetonic extracts of plankton retained by GF/F filters and measured by the fluorimetric method (Neveux and Panouse, 1987).

Additionally, hydrographic information at higher spatial resolution was obtained by using a microstructure profiler (MSS, Prandke and Stips, 1998) with microstructure shear and temperature sensors for estimating dissipation of turbulent kinetic energy that was equipped with a high-precision CTD probe. Ten sets of 3–5 profiles each were carried out between stations 2 and 4 on the 17th July, and 16 sets between stations 1c and 4 on the 18th and 20th July. The squared Brunt Väisälä frequency (N^2), a proxy for water column stratification, was computed from the CTD profiles according to the equation:

$$N^2 = -\left(\frac{g}{\rho w}\right)\left(\frac{\partial \rho}{\partial z}\right)(s^{-2})$$

where g is the acceleration due to gravity (9.8 m s^{-2}), ρ_w is seawater density (1025 kg m^{-3}), and $\partial\rho/\partial z$ is the vertical potential density gradient.

Fluorometrically determined Chl-a was used to calibrate the fluorometer attached to the CTD-rosette (Chl a = fluorescence *0.867; $r^2 = 0.961$, n = 16), which was used to plot the chlorophyll *a* distribution on the 16th July, and also to calibrate the fluorometer sensor

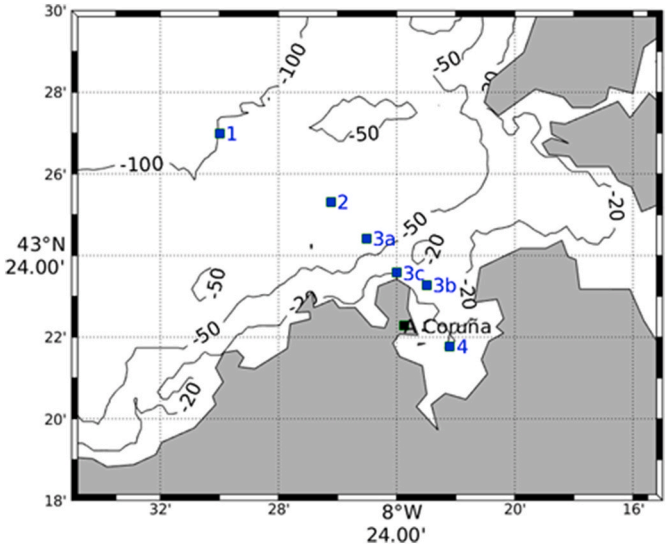

Fig. 1. Map of the study area and location of stations (4, 3b, 3c, 3a, 2, 1) sampled during the oceanographic cruise HERCULES-0712 in the shelf waters in front of A Coruña. Isolines for 20, 50 and 100 m depth are shown.

included in the MSS profiler (Chl a = 2.255 × fluorescence–0.527; $r^2 = 0.859$, n = 134).

Abundance of microphytoplankton was estimated in 50 mL aliquots of a sample collected with a plankton net (30 cm diameter, 40 μm mesh size) deployed between the surface and 50m or 5m near the bottom, according to the bathymetry. The sample was preserved in glutaraldehyde (25% final concentration) and cells were counted using a FlowCAM® system (Fluid Imaging Technologies). Particles in the sample were digitized at x200 to obtain reliable counts of cells a range of 3–50 μm (Álvarez et al., 2012). Prior to the analysis, the samples were screened with a 100 μm nylon mesh to prevent clogging of the FlowCAM cell. Cell identification was made on the digitized images with an emphasis in the identification of major groups (diatoms, dinoflagellates and other small flagellates). Ciliates were counted in Lugol-preserved samples under an inverted microscope. In this study, the abundance of diatoms, dinoflagellates and ciliates are reported as the number of cells per volume of seawater.

The upwelling index (Iw) was estimated by calculating the Ekman transport (QUITAR from surface winds data) computed as estimates every 6h and provided by the Instituto Español de Oceanografía (http://www.indicedeafloramiento.ieo.es) in two cells of 1° x 1° centred at 43.5°N, 9°W, using data from atmospheric pressure at sea level, derived from the WXMAP model (González-Nuevo et al., 2014).

2.2. Microbial abundance by flow cytometry

Microbial abundance was determined in up to 7 depths at each station by flow cytometry following Gasol and Del Giorgio (2000). A volume of 1.8 mL per water sample was preserved with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 10 min and stored at −80 °C. Samples were thawed to room temperature and stained with Syto13 (Life Technologies) in the dark for 10 min. Subsequently, 1 μm fluorescent latex beads (approximately 1×10^5 mL^{-1}) (Molecular Probes, Invitrogen, Carlsbad, CA) were added to all the samples as internal standard. Prokaryotes were counted using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) according to their signature in right angle light scatter and green fluorescence. Cells with high nucleic acid contents (HNA bacterial cells) were discriminated from the group of cells with low nucleic acid contents (LNA cells) (Morán et al., 2007).

2.3. Abundance of the most abundant groups of bacteria by CARD-FISH

CARD-FISH analyses were carried out to quantify the abundance of the specific bacterial groups following the method described by Pernthaler et al. (2002). Immediately after collecting the samples from the Niskin bottles of surface and DCM layers, a volume of 10–20 mL of seawater was fixed with paraformaldehyde (2% final concentration) and stored at 4 °C in the dark. About 12–18 h later, the samples were filtered onto 0.2 μm polycarbonate filters (Millipore GTTP, 25 mm filter diameter) supported by nitrocellulose filters (Millipore, HAWP, 0.45 μm), washed twice with 10 mL Milli-Q water, dried and stored in a microfuge vial at −20 °C until further analysis in the laboratory. Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labelled oligonucleotide probes: Eub338I-III (Bacteria) (Daims et al., 1999); Gam42a (Gammaproteobacteria) (Manz et al., 1992); CF319a (Bacteroidetes) (Manz et al., 1996); Ros537 (Roseobacteria) (Eilers et al., 2001); SAR11-732 (Morris et al., 2002); SAR86-1249 (Eilers et al., 2000) and Non338 (negative control) (Wallner et al., 1993), followed by tyramide-Alexa488 signal amplification. Cells were stained with DAPI-Mix [4′,6-diamidino-2-phenylindole (DAPI, concentration 2 μg mL^{-1}) phosphate buffer (PBS, 0.5 mg mL^{-1}), Vectashield (1 μg mL^{-1}) and Citifluor (5.5 mg mL^{-1})]. Quantification of DAPI-stained cells and cells stained with the specific probes were performed with an epifluorescence microscope (Nikon Eclipse 80i) equipped with a mercury lamp (130 W) and a set of filters appropriate for DAPI and Alexa488 stains. For each sample at least 20 fields (equivalent to ≈500 cells) were counted.

2.4. DNA extraction, PCR amplification and sequencing of the 16S rRNA gene fragments

Samples for DNA pyrosequencing were obtained by filtration of ≈5 L of seawater through sterile Sterivex cartridge 0.22 μm pore size filters (Millipore, USA) and stored at −80 °C until DNA extraction could be performed. DNA extraction was performed using the PowerWater DNA Isolation Kit (Mobio, Carlsbad, CA, USA). DNA extraction and quantification (via Nanodrop, Thermoscientific) was carried out for a total of 17 samples. A subsample of DNA extracted was used for pyrosequencing at the Research and Testing Laboratory (Lubbock, TX, USA: http://www.medicalbiofilm.org/) using 454 GL FLX technology. The primer set 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) (Herlemann et al., 2011) were used to generate amplicons spanning the V3 to V4 regions of the bacterial 16S rRNA gene. The raw sequence data were processed using Quantitative Insights into Microbial Ecology (QIIME) pipeline (https://www.qiime.org) (Caporaso et al., 2010). A quality check was performed to minimize low quality pirotags. These sequences were removed from the subsequent analyses if they were shorter than 100 bp, had an average quality score <25 calculated in sliding windows of 50 bp, and contained primer mismatches. The remaining sequences were run using Denoiser implemented in QIIME to reduce the impact of pyrosequencing errors (Reeder and Knight, 2010). The curated sequences were grouped into operational taxonomic units (OTUs) with 97% similarity threshold. A representative sequence from each phylotype was chosen by selecting the most abundant sequence within that particular phylotype and was used for taxonomic identification. The chimeric sequences were removed using ChimeraSlayer implemented in MOTHUR (Schloss et al., 2009; Haas et al., 2011) based on the alignment file SILVA119 (http://www.arb-silva.de). Blast Classifier (Wang et al., 2007) implemented in QIIME determined the identity of 16S rRNA phylotypes. The chloroplast, mitochondria or archaeal members identified by this method were removed from the dataset. To enable comparisons between samples, the OTU table was downsized to 1552 sequences to ensure an equal number of sequences in each sample. All further analyses, alpha and beta diversity estimations implemented in QIIME, were performed with the subsampled OTU table. Chao 1 is an abundance-based estimator of species richness (it does not consider the numbers of individuals of each species present), while Shannon index is a diversity index providing more inference about the community composition (including the relative abundances of the different species). In addition, the rarefaction curves were plotted to verify that the sequences obtained in each sample showed a tendency of plateauing for most samples of Bacteria (Supplementary Figs. 1A and B). Sequence data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under SRP133560 bioproject number.

2.5. Statistical analysis

One-way ANOVA was performed to verify significant differences between subsets of variables (inorganic nutrients, phytoplankton biomass, microbial abundance, CARD- FISH counts) for the different days, stations and/or depths. These statistical analyses were performed with SPSS software package. Hierarchical cluster analysis based on Bray-Curtis distance matrix, was carried out to explore similarities in the taxonomical composition between samples. Significant differences in bacterioplankton community composition between samples were determined by a permutational analysis of variance (PERMANOVA). Non-metric multidimensional scaling (nMDS) was conducted to illustrate the associations among the relative abundance of OTUs, and environmental or taxonomic group variables by vector overlays. DistLM analysis was built to identify the best factors explaining variation in the

bacterial community structure (temperature, salinity, Chla, nitrate + nitrite, phosphate, silicate, PAR and abundance of diatoms, dinoflagellates and ciliates). Previously all variables were analyzed to test the colinearity between them using a Pearson correlation matrix, eliminating the variables with r2>0.95. In order to assign the contribution of each variable and each set of variables taken alone, an "all specified" selection procedure was carried out using the "r2" as selection criterion. The contribution of each variable was assessed using "marginal test" to assess the statistical significance and percentage contribution of each variable. Finally, all variables were introduced in the model using the "step wise" selection procedure of the DistLM and the "Aikake" information criterion (AIC). Such procedure allowed us to find the best combination of environmental variables that explained the highest proportion of variability found in the bacterioplankton community structure resemblance matrix. A "sequential test" was employed to evaluate the cumulative effect of each variable one the previous variable (s) had been accounted for. Cluster, PERMANOVA, NMDS and DISTLM analysis were performed with PRIMER6 & PERMANOVA+ (Anderson et al., 2008).

3. Results

3.1. Hydrographic conditions

During the five days sampling the situation was of relaxation of upwelling favorable conditions observed during the 16th and 17th July (Fig. 2), which induced an accumulation of diatoms after the pulse (Table 1). An intense vertical density gradient (evident in T and S and quantified by N^2) was observed over the sampling period in the upper layers (Fig. 3, Table 1), with relatively warm (20.20 ± 0.37 °C) and fresher waters (33.95 ± 5.48) at the surface, and cooler (13.79 ± 1.01 °C) and saltier waters (35.77 ± 0.01) below (Fig. 3, Table 1). The base of the pycnocline (20–30m) coincided with the location of a distinct DCM, where Chl-a ranged between 2.03 and 9.86 mg m^{-3} versus 0.48–0.81 mg m^{-3} quantified at surface waters (Fig. 3, Table 1). The intense vertical density gradient acted as a barrier for the transport of nutrients from deep to surface waters (Fig. 4, Table 1). Surface concentrations of nitrate + nitrite, phosphate and silicate at surface waters (1.52 ± 0.17, 0.19 ± 0.01 and 1.30 ± 0.09 µmol L^{-1}, respectively) were lower than those quantified at the DCM (2.60 ± 0.54, 0.31 ± 0.02, and 2.27 ± 0.19 µmol l^{-1}, respectively). (Fig. 4, Table 1). The DCM layer, got thinner vertically forced by the upwelling of deep waters, and got broader again when upwelling relaxed on 18th July and water restratified in the area between stations 2 and 3b.

3.2. Microplanktonic abundance by FlowCAM and light microscopy

A bloom of diatoms occurred during the cruise in response to the upwelling pulse. Diatom concentration in cell per liter increased throughout the week in stations 2, 3c and 4 both at the surface and the DCM in response to the nutrients introduced by upwelling (Table 1). Diatom concentrations were lower in surface waters, where several dinoflagellates (mainly *Lingulodinium polyedrum*) and small unidentified flagellates dominated. In contrast, at the DCM there was a drastic shift to a ciliate- and diatom-dominated community (*Leptocylindrus danicus*, *L. minimus* and *Chaetoceros socialis*) at all stations, which resulted in maximum Chl-a concentrations at a DCM around 20–30 m depth (Table 1).

3.3. Microbial cell abundance by flow cytometry

Microbial cell abundance values were, in general, higher in surface waters ($2.05–6.92 \times 10^5$ cells mL^{-1}) and decreased significantly with depth (ANOVA, $p < 0.05$), showing minimum values at the shelf stations below 60 m ($1.22–3.33 \times 10^5$ cells mL^{-1}) (Fig. 5). Similarly, the contribution of HNA bacteria to total abundance was on average, slightly higher in surface waters ($46 \pm 9.5\%$) than in the DCM ($42 \pm 9.5\%$) and/or below that depth layer (Fig. 5). However, both HNA % and nutrient concentrations were high at the DCM in 3c on the 18th. It is remarkable that microbial abundance at stations 2 and 3c increased throughout the week and was highest on 20th (i.e. in a later stage of the diatom bloom). . Average vertical profiles of the more coastal stations (St. 4 and 3c) consistently showed the highest values of microbial abundance and HNA Bacteria, particularly during the sampling days of the 17th and 18th July, when nutrients concentrations were lowest, and progressively decreased towards the shelf stations (St. 1c and 2) (ANOVA, $p < 0.05$) (Fig. 5). Overall, no significant differences were found from day to day for microbial abundance and for HNA Bacteria considering the whole data set (ANOVA, $p > 0.05$).

3.4. Relative abundance of specific groups of bacteria by CARD –FISH

The relative abundance of Bacteria showed similar values throughout the water column, on average, the recovery was $71 \pm 12\%$ of total DAPI counts (Table 2). Overall, SAR11 (Alphaproteobacteria) and

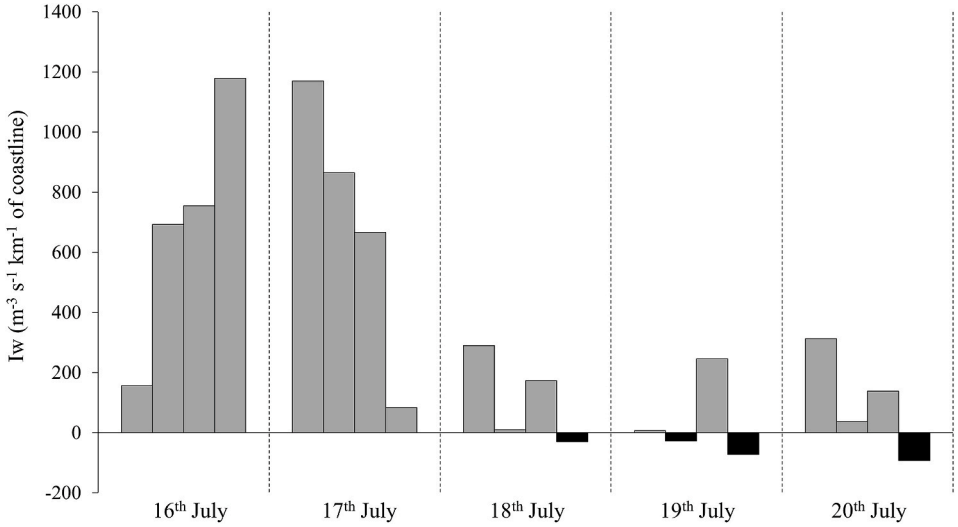

Fig. 2. Distribution of six-hourly (0:00, 06:00, 12:00, and 18:00 GMT) offshore upwelling index (Iw) during the sampling period of the oceanographic cruise HERCULES-0712 in the shelf waters in front of A Coruña.

Table 1
Physical, chemical and biological characteristics of the surface (1m) and the deep chlorophyll maximum (DCM) from different sampling days and stations along a transect in the shelf waters in front of A Coruña during the Hercules III cruise. Depth (m); T: temperature (°C); Sal: salinity; PAR: photosynthetically active radiation (μmol photons m^{-2} s^{-1}); Chl-a: Chlorophyll-a concentration (μg L^{-1}); Diatoms: Diatoms concentration (cells L^{-1}); Dinoflag: dinoflagellate concentration (cells L^{-1}); Cilliates: ciliate concentration (cells L^{-1}); NO_2+NO_3: nitrate + nitrite concentration (μmol L-1); PO_4: phosphate concentration (μmol L-1); SiO_4: silicate concentration (μmol L-1).

Date	Station	Layer	Depth	T	Sal	PAR	Chl-a	Diatoms	Dinoflag	Ciliates	NO_3+NO_2	PO_4	SiO_4
16th July	4	surface	3	18.649	35.580	540.9	0.801	4485	4815	3200	0.71	0.36	1.47
		DCM	12	17.238	35.679	78.4	9.896	5040	11025	2190	0.49	0.33	2.55
	3c	surface	3	18.374	35.448	669.7	0.624	1065	570	740	1.68	0.19	1.11
		DCM	30	15.748	35.730	29.6	2.737	8060	820	260	3.50	0.35	2.39
	2	surface	3	18.221	35.548	659.9	0.480	902	88	420	1.20	0.18	1.13
		DCM	30	15.607	35.733	39.6	2.629	3030	75	890	4.77	0.39	2.96
	1c	surface	3	17.973	35.701	648.8	0.525	550	360	30	2.62	0.17	2.13
		DCM	32	15.244	35.749	34.3	2.579	12405	240	30	2.45	0.32	1.68
17th July	4	surface	3	18.520	35.587	609.1	0.787	1680	520	–	1.25	0.19	1.47
		DCM	18	15.535	35.733	68.6	2.143	7905	1860	–	2.30	0.29	2.43
	3c	surface	3	17.525	35.633	513.6	0.743	–	–	–	1.24	0.17	0.87
		DCM	10	15.996	35.740	184.7	3.206	–	–	–	0.95	0.24	1.67
	2	surface	3	17.801	35.484	187.8	0.608	1770	405	–	0.48	0.21	0.96
		DCM	20	15.434	35.740	26.3	3.406	23955	285	–	2.30	0.29	2.12
	1c	surface	–	–	–	–	–	–	–	–	–	–	–
		DCM	–	–	–	–	–	–	–	–	–	–	–
18th July	4	surface	3	19.424	35.480	334.5	0.805	5235	1035	4140	1.85	0.19	1.41
		DCM	18	16.483	35.711	54.5	2.982	7230	1080	7790	0.59	0.17	1.79
	3c	surface	3	18.179	35.486	611.7	0.748	3795	495	1830	2.63	0.21	1.23
		DCM	20	15.371	35.753	55.6	3.238	18390	240	1050	2.67	0.34	2.21
	2	surface	3	17.975	35.569	289.3	0.604	2820	375	1620	2.92	0.16	0.83
		DCM	20	15.070	35.772	22.8	7.361	17295	195	1460	3.20	0.30	1.95
	1c	surface	3	17.744	35.696	100.5	0.781	3285	165	1340	2.99	0.16	1.80
		DCM	20	15.322	35.761	16.3	6.955	16260	75	6400	1.57	0.21	1.37
20th July	4	surface	3	19.090	35.554	550.9	0.742	5370	600	910	0.90	0.18	1.83
		DCM	18	14.893	35.768	66.2	3.389	36555	495	1730	2.45	0.29	2.72
	3c	surface	3	18.547	35.374	594.7	0.692	13200	690	3270	1.07	0.16	1.22
		DCM	30	14.889	35.748	19.2	3.763	22150	440	800	3.39	0.34	2.99
	2	surface	3	17.990	35.445	365.0	0.611	6870	22.5	960	1.81	0.17	1.16
		DCM	26	14.689	35.772	19.2	6.601	43935	0	1580	2.50	0.30	1.77
	1c	surface	3	17.615	35.707	213.8	0.568	2750	50	1960	1.86	0.17	1.83
		DCM	30	14.477	35.760	15.3	2.029	5775	0	2500	4.25	0.36	3.09

Bacteroidetes phylum showed, on average, the highest relative abundance (28 ± 4% and 31 ± 4% respectively) of total Bacteria (Table 2). By contrast, SAR86 (Gammaproteobacteria) displayed the lowest contribution of the total Bacteria (12%) (Table 2). No significant differences were found from day to day samples for any of the bacterial groups (ANOVA, $p > 0.05$), with the only exception of *Roseobacter*, which was significantly reduced throughout the week (ANOVA, $p < 0.01$). Similarly, no significant differences were found for any of these bacterial groups between stations (ANOVA, $p > 0.05$). Moreover, no significant differences were found between the surface and the DCM waters for any of the groups targeted (ANOVA, $p > 0.05$).

3.5. Diversity and composition of bacterial community by NGS (454 pyrosequencing)

A total number of 69220 pyrosequencing reads of V3–V4 region of the 16S rRNA gene were obtained from 8 to 9 samples of surface water and DCM layer, respectively. Sequences were clustered into a total number of 554 OTUs. Shelf stations (i.e. St.1C and 2) showed in general higher richness values, estimated by the number of OTUs and Chao1 indices, than coastal stations (Table 3). Similarly, the Shannon diversity index indicated that the latter stations had the lowest bacterial diversity, while the highest diversity values were found at the DCM of station 2 on the 17th July (3.77) and on the 20th July (3.36) (Table 3), during upwelling relaxation. The number of OTUs and Chao 1 index values were generally higher in the DCM than in the surface (Table 3), while Shannon diversity values of surface samples were equivalent to those of DCM, ranging from 2.69 to 3.77 (Table 3). No clear trend of day to day variation either in richness or diversity was found (Table 3).

The relative abundance of bacterial OTUs, determined at different phylum and family taxonomic levels, indicated that bacterial assemblages in the water column varied considerably between the surface and the DCM (Fig. 6). Furthermore, some phyla showed slightly differences in their relative contribution to the total Bacteria along with the distance from the coastline and throughout the week. Samples were dominated by Proteobacteria, Bacteroidetes, Actinobacteria, and Deferribacteres phyla (80%, 10%, 7% and 5% of total Bacteria, respectively) (Fig. 6A). Other less abundant groups accounting for ≈1% of Bacteria, included members of SAR202 (Chloroflexi), Acidobacteria, Verrumicrobia, Planctomycetes, and Cyanobacteria (Fig. 6A). Among Proteobacteria, Alphaproteobacteria and Gammaproteobacteria were the most abundant classes accounting for 35% and 30% of total abundance of Proteobacteria, respectively (Fig. 6B). The most abundant bacterial groups, as SAR11 and Rhodobacteraceae belonging to Alphaproteobacteria, showed an opposite distribution pattern with depth and distance from the coastline. The SAR11 clade was relatively more abundant in the DCM layer near the coast, while Rhodobacteraceae dominated in surface waters at the shelf St. 2. Gammaproteobacteria was largely represented by Oceanospirillales, mainly SAR86 clade (25%), JL-ETNP-Y6 (≈5%) and Oceanospirillaceae (<5%), while Alteromonadales mainly consisted of Alteromonadaceae (5%) (Fig. 6B). The SAR86 clade showed high abundance at the DCM of the shelf stations, while Oceanospirillaceae and Alteromonadaceae prevailed in surface waters of the coastal stations. Flavobacteriaceae and Cryomorphaceae (phyla Bacteroidetes) were more abundant at the surface (≈15%) than at the DCM (≈5% of total Bacteria), while OCS-155 (phyla Actinobacteria) showed a relatively higher abundance in the DCM (10%) than in surface waters (<5%). SAR406 (phyla Deferribacteres) accounted for ≈5% of total

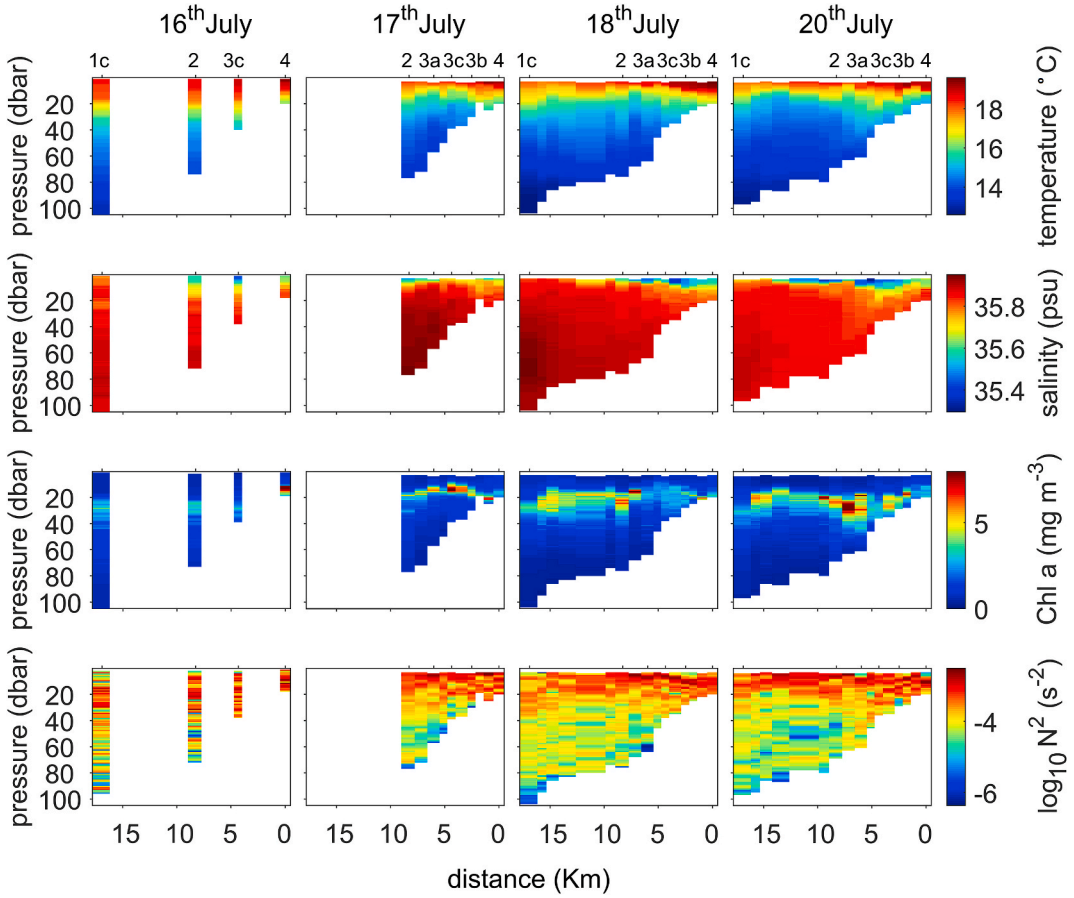

Fig. 3. Daily evolution of physical and biological variables (temperature, salinity, chlorophyll-a and water column stratification, N^2)sampled during the oceanographic cruise HERCULES-0712 in the shelf waters in front of A Coruña.

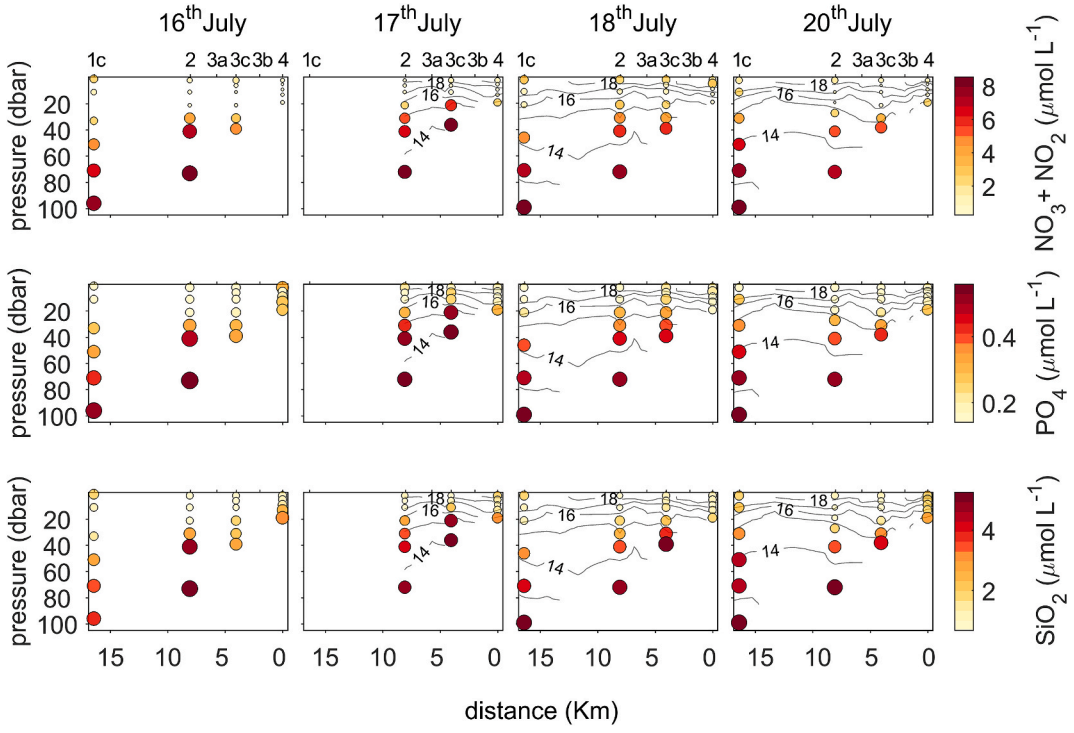

Fig. 4. Inorganic nutrients during the oceanographic cruise HERCULES-0712 in the shelf waters in front of A Coruña. $NO_2 + NO_3$: nitrate + nitrite concentration; PO_4: phosphate concentration; SO_4: silicate concentration.

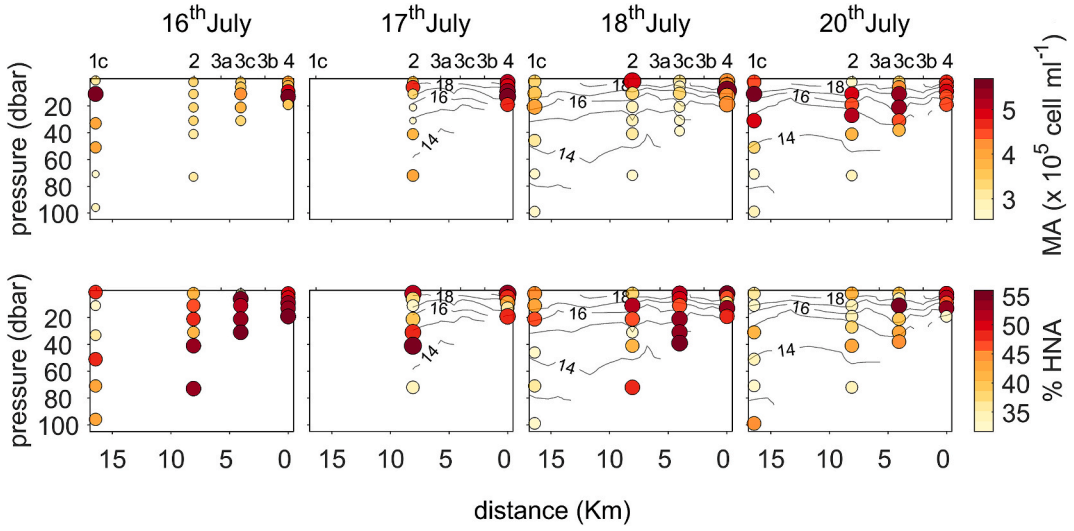

Fig. 5. (A) Microbial abundance (MA, cells mL^{-1}) and high nucleic acid content bacteria (% HNA) in the shelf waters in front of A Coruña.

Table 2
Percentage of relative abundance (mean ± standard deviation) of the main bacterial groups determined by CARD-FISH along a transect in the shelf waters in front of A Coruña during the HERCULES-0712 cruise. DCM: deep chlorophyll maximum.

EUBACTERIA			PROTEOBACTERIA				BACTEROIDETES
		(%DAPI)	*Alphaproteobacteria*		*Gammaproteobacteria*		
			SAR-11	*Roseobacter*	Total	SAR-86	
			(%Eub)	(%Eub)	(%Eub)	(%Eub)	(%Eub)
4	surface	72 ± 2	29 ± 3	17 ± 2	23 ± 3	13 ± 2	34 ± 3
	DCM	75 ± 3	31 ± 4	14 ± 4	21 ± 4	13 ± 3	29 ± 3
3c	surface	72 ± 1	29 ± 3	13 ± 2	22 ± 3	9 ± 2	27 ± 3
	DCM	68 ± 3	21 ± 4	12 ± 4	23 ± 4	9 ± 3	27 ± 3
2	surface	69 ± 1	31 ± 3	15 ± 2	29 ± 3	13 ± 2	34 ± 3
	DCM	67 ± 3	31 ± 4	22 ± 4	29 ± 4	14 ± 3	35 ± 3
1c	surface	73 ± 1	24 ± 3	18 ± 2	26 ± 3	11 ± 2	30 ± 3
	DCM	69 ± 3	26 ± 4	15 ± 4	19 ± 4	9 ± 3	28 ± 3

*% DAPI: The relative contribution of Bacteria to the total prokaryotic community.
*% Eub: The relative contribution of each phylogenetic group (SAR11, Roseobacter, Gammaproteobacteria, SAR-86, Bacteriodetes) to the total bacterial community.

Table 3
Number of OTUs, richness and diversity estimated by 454-pyrosequencing of Bacterial 16S rRNA gene. The samples marked with an asterisk **(*)** are outlier. DCM: deep chlorophyll maximum.

DATE		17th July			18th July			20 th July		
Station	Layer	No. OTUs	Chao 1 value	Shannon index	No. OTUs	Chao 1 value	Shannon index	No. OTUs	Chao 1 value	Shannon index
4	surface	95	120	3.30	98	136	2.77	88	102	3.12
	DCM	20*	20*	2.43*	113	153	3.48	91	139	2.81
3 c	surface	–	–		79	135	2.65	83	98	2.94
	DCM	–	–		128	190	3.21	89	109	2.69
2	surface	114	161	3.26	–	–		89	108	2.51
	DCM	201	308	3.77	19*	19*	1.64*	120	145	3.36
1 c	surface	–	–		–	–		111	154	2.89
	DCM	–	–		–	–		99	128	2.91

Bacteria and showed a patchy distribution (Fig. 6B).

The clustering of samples showed that the main bacterial assemblages were those associated with the two different water layers considered in this study (Fig. 6C). At a similarity level of ≈60%, one cluster grouped the samples belonging to the surface, and a second cluster included the DCM samples (Fig. 6C; PERMANOVA, $p < 0.05$). Surface and DCM assemblages had a large overlap, with most of the shared-OTUs belonging to the more abundant bacterial phyla, which included Rhodobacteraceae, Cryomorphaceae, Flavobacteraceae, Alteromonadaceae, SAR86 and SAR 116. Overall, the number of non-shared OTUs was higher in DCM than in the surface layer. Several bacterial phylotypes such as *Ulvibacter* and *Litoricola* were exclusive of the surface layer, while SAR202 clade and SAR 324 were some of the non shared- OTUs belonging to the DCM. As for the environmental variables, the variability in bacterial community composition was not significantly different from day to day for any of the stations (Fig. 6C,

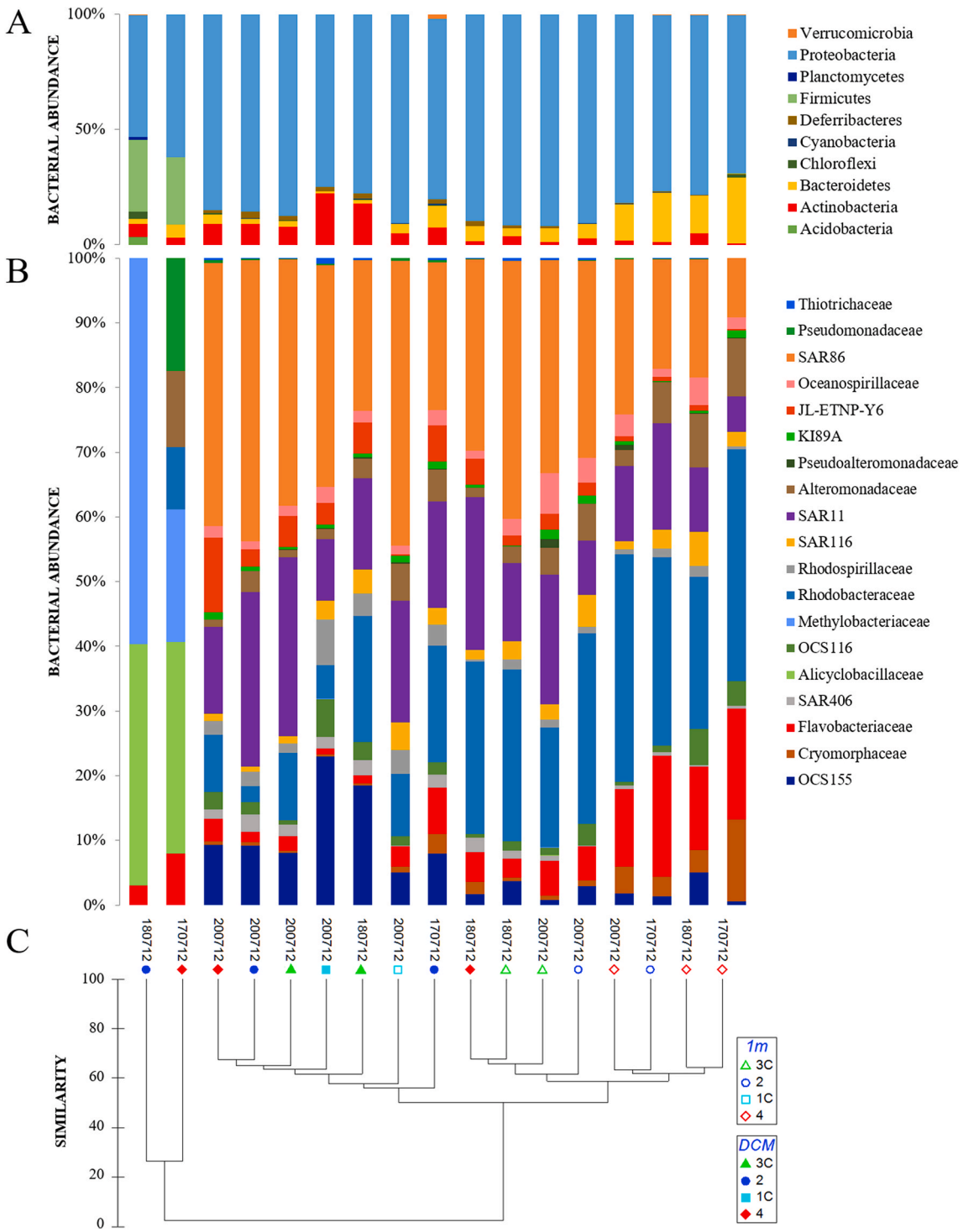

Fig. 6. Comparison of bacterial diversity determined by 454-pyrosequencing among different samples collected in the shelf waters in front of A Coruña (A) Stacked column graph representing the relative distribution of the dominant phyla (B) Stacked column graph representing the taxonomic composition at the order and family level (C) Cluster analysis of bacterial diversity. Sample codes consist of the sampling date (day followed by the month and year), station and depth of sampling (1m: surface samples, DCM: depth chlorophyll maximum samples). Diamond symbols: station 4. Triangle symbols: station 3C; Circle symbols: station 2; Square symbols: Station 1C. Open and close symbols correspond to Surface and DCM horizons, respectively. Samples of 17th July and 18th July of the DCM of station 4 and 2, respectively, are outliers.

PERMANOVA, $p > 0.05$). However, our results showed that DCM samples in the inner most station 4 changed from the surface cluster on 18th to the DCM cluster on 20th in response to the influence of upwelled nutrient-rich waters in deeper layers of station 4 introduced by the upwelling pulse.

3.6. *Relationship between bacterial community composition and environmental variables*

Surface samples were characterized by higher temperature and PAR values (Fig. 7A) and higher number of OTUs of Rhodobacteraceae, Flavobacteriaceae and Alteromonadaceae than those recorded in DCM

samples (Fig. 7B). By contrast, the high nutrient concentrations observed in the DCM layer, particularly at shelf stations (i.e. St. 2 and 1c), along with the high Chl-a and high salinity (Fig. 7A), were associated to the occurrence of SAR86, SAR11, OCS155 and JL-ETNP-Y6 (Fig. 7B). A distance linear-based model (DistLM) showed the better combination of environmental variables explaining the variability in bacterioplankton community structure. According to the results of the marginal test on each set of variables of the DistLM model, physico-chemical and biological variables explain 63.5% and 38.4%, respectively, of the bacterial community structure variability (Supplementary Table 1). Only temperature account for 31% of the variability. Other main contributors include inorganic nutrients as phosphate (25%), PAR (23%), salinity (23%) or diatom abundance (19%). The same analysis was performing by choosing the best fitted model. As a result, only five

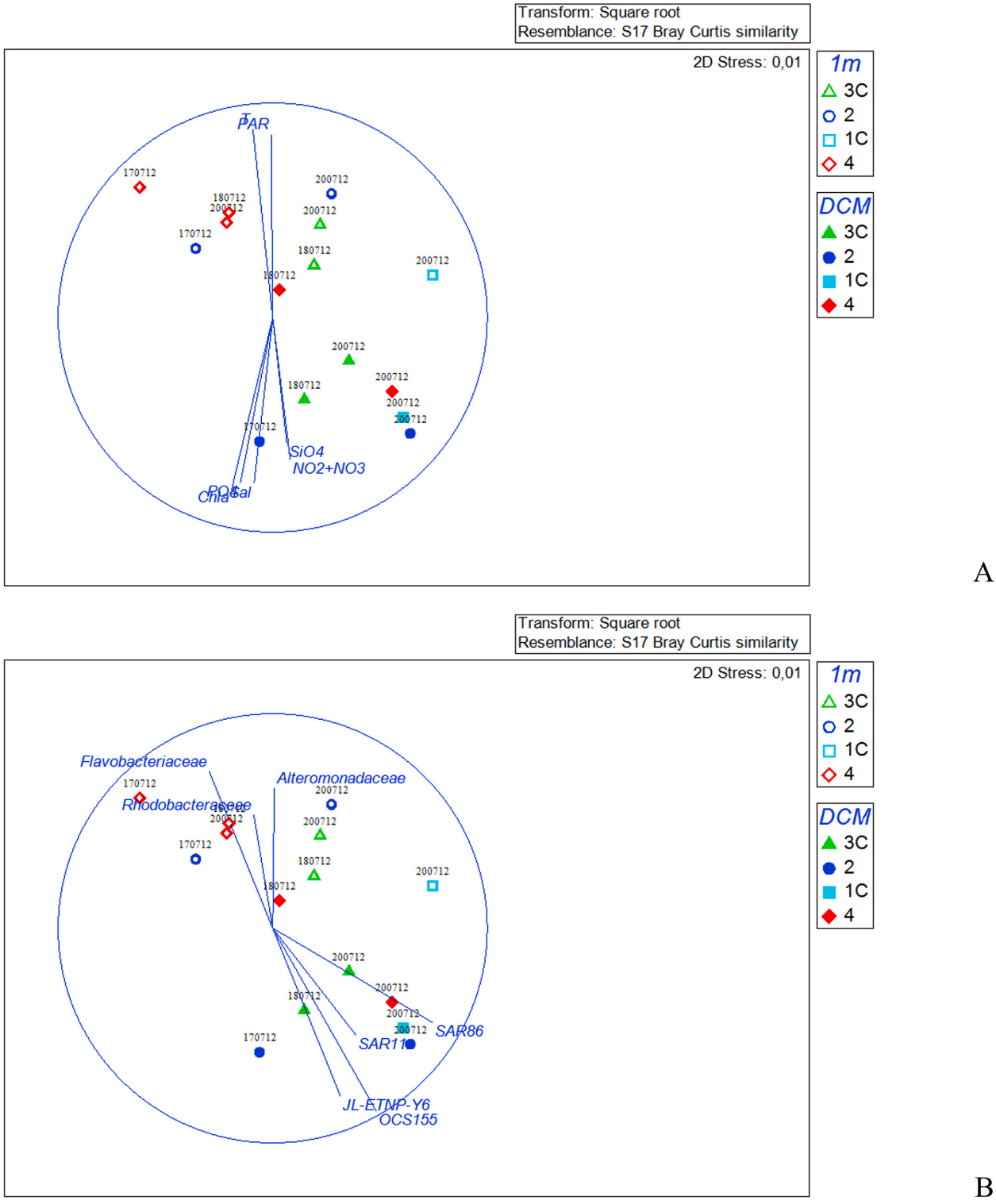

Fig. 7. Non-metric multidimensional scaling (NMDS) analysis showing the correlation between (A) different environmental variables and (B) specific taxonomic phyla and the relative abundance of bacterial OTUs determined by 454-pyrosequencing in the different samples in the shelf waters in front of A Coruña. Sampling points that are close together are more similar in their bacterial composition than those that are far apart. For sample labels see Fig. 6.

variables (temperature, diatom abundance, nitrate + nitrite, phosphate and PAR) were retained in the model and remain significant in the sequence test of the best model. These five variables account for 53.1% of the variability found in the bacterial community resemblance matrix (Supplementary Table 2).

4. Discussion

In this investigation we have generated baseline information on the taxonomic diversity of bacteria in an exploratory survey off A Coruña (NW Spain), as part of a multidisciplinary research examinating short-term hydrodynamic variability and marine plankton in the Galician shelf during upwelling events in the Artabro gulf (Otero-Ferrer et al., 2018). The interest and peculiarity of this study is that it is one the few examples investigating how bacterial community composition evolves during an episode of relaxation of an upwelling, along with hydrography, nutrient concentration and microbial abundance. The different approaches for characterizing the microbial assemblages combined in this study allow usto investigate the abundance and diversity of microbial communities following the upwelling pulse and its relaxation, with important biogeochemical implications for other ecosystems affected by upwelling (Bachmann et al., 2018). Flow cytometry provided total microbial abundance and a very general characterization of two physiological groups of organisms (HNA and LNA populations), while CARD-FISH add more detail by identifying the dominant groups of bacteria and finally 454-pyrosequencing (a more powerful sequencing technique for taxonomic studies) allowed us to further investigate the bacterial diversity including also those bacterial phylotypes that had very low abundance. The latter technique was particularly suited for revealing spatial and temporal changes in diversity that could be related to changes in the environmental conditions.

The distribution pattern of bacterial assemblages showed a marked difference between surface and DCM layers, but it was remarkably uniform across stations and throughout the week, in spite of the variability in hydrographical conditions. Our sampling occurred during the relaxation of a summer upwelling pulse on the 16th and 17th (Fig. 3). The pulse introduced cold, saltier and nutrient rich water in deep layers and exported warmer and less saline surface waters from the rias to the adjacent shelf. This situation is typical during the summer in the Galician coast, where periods of upwelling alternate with situations of stratification when the wind relaxes (Casas et al., 1997; Álvarez-Salgado et al., 2000). Because of the presence of continental water near the coast and the thermal stratification caused by warming of the surface, the upwelled waters only reach the surface when upwelling pulses last for several days, which was not the case in our study. However, the upwelled water with relatively high concentration of nutrients influenced deep layers of the water column (DCM and below). For instance, the characteristics of the water below 50 m at the shelf stations (temperature <14 °C, salinity >35.8, nitrate + nitrite>7 μmol L^{-1}) suggest the presence of the Eastern North Atlantic Central Water, which is modified by warming and remineralization on the shelf (Álvarez-Salgado et al., 2000).

Two distinct clusters of bacterial assemblages along the transect were present, possibly associated with these two distinctive layers. Furthermore, this vertical zonation of bacterioplankton could also be linked to the abundance and quality of phytoplankton, since dissolved organic carbon originating from different phytoplankton species differentially stimulated heterotrophic prokaryotes (Sarmento and Gasol, 2012). Interestingly, there were also marked changes in the composition of microplankton in the two layers, with a marked dominance of ciliate and diatoms in the DCM and an increase of diatoms throughout the week. Diatoms are well adapted to the DCM, generally associated to the nutricline, because of their ability to capture nutrients from the deep layer (Latasa et al., 2017). For instance, upwelling pulses can provide nitrate that can be stored by diatoms and other microphytoplankton groups for later use in biosynthesis (Bode et al., 1997; Lomas and Glibert, 2000). Daily vertical displacements of phytoplankton, notably from dinoflagellates but also from diatoms, can influence the quantity and quality of the organic matter synthesized, as illustrated by the excess of carbohydrates observed in the surface layer during periods of upwelling relaxation reported for the Ria de Vigo, and which contrasts with the more balanced composition of the organic matter in the layers below the DCM where nutrients were abundant (Fraga et al., 1999).

4.1. Abundance of major bacterial groups: CARD–FISH versus 454 pyrosequencing

The diversity of microplanktonic assemblages, including phytoplankton and ciliates has been well studied by conventional sampling methods and microscope techniques in the shelf waters off A Coruña (Varela et al., 2003; Bode et al., 2004; Bode et al., 2006; Varela et al., 2017). However, Flow CAM and microscopy only detect a minor part of the microbial diversity (Rodríguez-Ramos et al., 2014), and do not allow to study of the taxonomic diversity of bacteria. In contrast, the approach employed in the present study is based on a combination of two molecular techniques (i.e. Korlević et al., 2015; Guerrero-Feijóo et al., 2017): the CARD-FISH (accounting for the dominant microbial groups) and the high resolution 454 pyrosequencing (for specifically addressing the identification of both abundant and rare bacterial taxa). A direct correlation between the results obtained by CARD-FISH and pyrosequencing analysis could not be performed because these different techniques target different regions of the 16S rRNA gene. However, in general, this investigation has found a good correspondence between the relative abundance of the major bacterial groups by both methodologies. For instance, pyrosequencing sequences for Alphaproteobacteria or Gammaproteobacteria yielded a percentage of contribution similar to that obtained by CARD-FISH counts (see Table 2), indicating that this cluster was similarly recovered by both methodologies. By contrast, the relative abundance of SAR11 and Bacteroidetes was higher in CARD-FISH counts (see Table 2) than in pyrosequencing. It is well known that the use of different primers complicates the comparison of microbial community composition and diversity between different studies. In this regard, we conducted our study prior to the report of Apprill et al. (2015) and it is possible that the primers we have used here underrepresented the SAR11 clade. In contrast, SAR86 cluster contributed more to the total bacterial abundance in pyrosequencing than in CARD-FISH counts (see Table 2). These different patterns could be due to the different number of samples analyzed, and also to the PCR bias associated with the pyrosequencing approach compared to CARD-FISH, where the probes target directly the 16S rRNA.

4.2. Bacterial diversity and composition by 454 pyrosequencing

Alpha diversity analysis suggested that species richness and diversity tends to increase from coast to shelf stations and from the surface down to the DCM, which could be associated to the mixing of upwelled bacteria with the coastal surface waters and probably also reflecting the marked functional diversity encompassed by bacteria. Consistent with previous studies of the relative richness of dominant phyla, the variability in bacterioplankton community composition in the shelf waters of the Galician coast showed some characteristics in common with oceans globally (i.e. GOS, Yooseph et al., 2007 and/or Tara Oceans expedition, Sunagawa et al., 2015). It is well established that Proteobacteria and Bacteroidetes, and to a lesser extent Actinobacteria are the dominant Bacteria phyla in seawater (DeLong et al., 2006; Agogué et al., 2011; Walsh et al., 2016; Guerrero-Feijóo et al., 2017) and, consequently, they displayed the highest relative abundance in our study. However, the distribution of the bacterial assemblages at different taxonomic levels was driven by the differences induced by upwelling and its relaxation in the area.

The main sequences within Alphaproteobacteria comprised SAR11

and Rhodobacteria, and their distribution varied in surface and DCM layers along the sampling days of the cruise. As expected, a considerable portion of 16S rRNA amplicons belong to SAR 11 (under class Alphaproteobacteria), an ubiquitous clade at the world ocean. We found a significant positive correlation between the abundance of the SAR11 clade and Chl-a, supporting previous findings describing that the abundance of SAR11 is significantly higher in epipelagic waters and correlated with dissolved organic matter produced by phytoplankton (Carlson et al., 2009; Morris et al., 2012; Eiler et al., 2009) and thus suggesting that this clade actively responds to organic matter released by the phytoplanktonic bloom found at the DCM. This is illustrated by the observed increase during the diatom bloom (from the 18th to 20th) of the contribution of SAR11 at the DCM in St. 3c, where the increase of microbial abundance in time was also apparent. In contrast to the SAR11 distribution, the Rhodobacteraceae family (also under class Alphaproteobacteria) was observed to be relatively abundant in the surface layer shelf. Members of the Rhodobacteraceae family have been described to prevail in areas of relatively low nutrient concentrations (Gilbert et al., 2012), which may explain its relative dominance at the surface layer in our study. One of the dominating genera found in this study within the Rhodobacteraceae family, *Roseobacter*, a strong competitor thriving under low-nutrient conditions (Pinhassi and Berman, 2003), which in our case appears to be at the surface. Overall, Rhodobacteraceae was reduced throughout the week at the DCM in stations 4 and 3c, in response to the nutrient enrichment at these stations with the upwelling pulse and the further evolution with relaxation. However, Rhodobacteraceae contribution was high at the DCM in 3c on the 18th, where nutrients and also HNA% were high. The presence of Gammaproteobacteria in upwelling systems has been reported in several studies (Bergen et al., 2015; Zhou et al., 2018). The main sequences under the Gammaproteobacteria class comprised SAR86 and Alteromonadaceae. In our study, SAR86 dominated in the DCM at the shelf stations, suggesting that this group could be stimulated by macronutrients such as nitrate of upwelled water since it has been identified as a typical taxa of open ocean waters in other regions (Treusch et al., 2009; Beman et al., 2011; Walsh et al., 2015). The efficiency of SAR86 in exploiting nutrient pulses is suggested by the increase of its contribution throughout the week (being highest on 20th) at all stations and depths, with the exception of the surface layer of St. 3c. Furthermore, this group is often defined as opportunistic and has a broad substrate spectrum (Skerratt et al., 2002), thus suggesting substrate utilization of algal derived organic matter (Teeling et al., 2012; Buchan et al., 2014). Hence, SAR86 may have been linked to the DCM bloom of *Leptocylindrus* species, which could produce and release compounds that serve as chemoatractants for these bacterial groups. Conversely, Alteromonadaceae (another Gammaproteobacteria family) was relatively more abundant in surface waters, contrasting with the dominance of SAR11 at the DCM, which might be attributed to competition for nutrients and/or to a response to the contrasted environmental conditions of surface versus DCM layers found during our study.

The Bacteroidetes consisted mostly of members of the Flavobacteria class, including Cryomorphaceae and Flavobacteriaceae families. Flavobacteria has been reported to be an important component of the microbial loop in coastal phytoplankton blooms (Williams et al., 2013; Tully et al., 2014). In this study, Flavobacteria were relatively more abundant at the surface waters of the coastal stations, especially at the innermost coastal station 4 (under the influence of water from continental origin). Such abundance may reflect the availability and quality of organic substrates, since several Flavobacteria have been identified as powerful degraders of complex organic matter (Arnosti et al., 2014; Goméz Pereira et al., 2012). In particular, sequences from the Flavobacteriaceae (mainly belonging to genera *Formosa*, *Polaribacter*, *Croceitalea* and to the uncultured marine groups NS3, NS4, NS5) and Cryomorphaceae families (mainly genus *Owenweeksia*) were found at all stations of the transect but exhibited pronounced abundance fluctuations, maybe related with the fact they are highly diverse, displaying distinct niches and different life strategies (Gómez-Pereira et al., 2010; Abell and Bowman, 2005). In this respect, the abundance of Flavobacteria at st. 4 both at the DCM and the subsurface layer along withits considerable reduction throughout the week suggest that before the pulse that introduced nutrients in this station, the Flavobacteria community was adapted to nutrient depleted waters. Some Flavobacteriaceae sequences, such as *Formosa* and *Polaribacter* and NS5, were more abundant at st. 4 before the pulse, thus providing additional support to their adaptation to low nutrient waters. Conversely, NS4 increased during the diatom bloom), while *Croceitalea* and NS3 abundances remained relatively constant throughout the week. We must note that st. 4 is periodically fertilized by upwelled waters during pulses, and during relaxation the bacterial community might depend on the recycling of organic matter, that most likely would increase the variability in its taxonomic composition.

Another widespread and commonly found class is Actinobacteria, which in our study mainly belonged to OCS155 sequences. Recent studies found unique sequences of OCS155 associated to the upwelling in the South Pacific Ocean (West et al., 2016) and Actinobacteria clones were also recovered from the DCM in the South Atlantic gyre (Morris et al., 2012). Indeed, the presence of Actinobacteria in the shelf waters off A Coruña (relatively more abundant at the DCM than at the surface) could be associated with the phytoplankton biomass accumulated at the DCM. Actinobacteria helps to decompose organic matter (i.e. senescent algae) for uptake by phytoplankton, and several investigations have correlated Actinobacteria with phytoplankton blooms (Brown et al., 2005; Eckert et al., 2012; Bunse et al., 2016). Deferribacteres, dominated by the SAR406 clade, showed a patchy distribution, although generally being more abundant in the DCM layer at the shelf stations. In the marine environment SAR406 clade is ubiquitously distributed, and it is also known that is positively correlated with Chl-a (Gordon and Giovannoni, 1996; Cram et al., 2014). SAR 406 tags were more abundant on the 18th and 20th at the DCM of shelf stations 2 and 1c, where Chl-a reached the highest values of the study.

4.3. Environmental factors regulating microbial structure

Past studies of plankton community in the area identified that bacterial abundance and production was highest in periods of upwelling relaxation with a time-lag with the phytoplankton bloom fueled by nutrient-rich upwelled waters (Valencia et al., 2003; Barquero et al., 1998). In our study we have described the variability of hydrography and nutrients in a section from the coast to the mid-shelf during an upwelling-induced diatom bloom and its relaxation concomitant to the characterization of bacterial abundance and community composition. The correlation analysis showed that temperature, Chl-a and inorganic nutrient concentrations had a significant impact on bacterial distribution, especially on its vertical zonation, as illustrated in the two clusters of the nMDS. The surface cluster comprised samples in warm and less saline water (located at the mouth of the ria and advected to the adjacent coastal zone), low Chl-a, dominance of dinoflagellates and high PAR. At this surface layer we found high relative abundance of OTUs affiliated with Rhodobacteraceae, Flavobacteriaceae, and Alteromoadaceae, which have been reported to be abundant in upwelling areas (Baltar et al., 2007). The DCM cluster was characterized by blooming phytoplankton at ≈20–30 m with high abundance of diatoms and dominance of ciliates, high concentration of inorganic nutrients, and low PAR. The richness and phylogenetic diversity of the bacterial community was a higher at the DCM than at the surface. SAR86 and SAR-11 were present at the DCM and increased their contribution with relaxation of upwelling and senescence of diatom bloom. The coexistence of these two assemblages, suggests that they could be specialized in utilizing different DOM compounds released during the DCM phytoplankton bloom and thus avoid direct competition for resources. This is further supported by the presence of specific taxa, represented by the OCS155 group and JL-ETNP-Y6 *Oceanospirilla* sequences that displayed high

abundances in the DCM layer.

In summary, this study provides the first attempt to study the bacterial diversity and community composition during upwelling relaxation using 16S rRNA gene sequencing. This technique was more sensitive than CARD-FISH and flow cytometry for the detection of changes in community composition during the upwelling-relaxation transition. Sampling of surface and DCM layers allowed us to show that while the bacterial communities in each depth layer showed a high day to day similarity across 14 km section, the surface and DCM communities were significantly different. The separation of these communities can be due to dispersal limitation due to differential water densities acting as a physical barrier, but may also be due to the selection of communities adapted to the contrasted environmental conditions. Temperature, phytoplankton composition and nutrient concentrations might have acted as the main drivers of the observed vertical zonation of marine Bacteria, with enhanced diversity and presence of specific taxa in the DCM compared to the surface waters. Further analysis on microbial diversity are currently being carried in the reference station 2 from long term RADIALES time series during a seasonal cycle (monthly sampling), including a more detailed description of community composition at the level of amplicon sequencing variants (ASVs) (PhD in progress). Also, high resolution time-series (monthly sampling) preliminary results reveals short-term variability in bacterioplankton assemblages determined by flow cytometry (unpublished results).

CRediT authorship contribution statement

Tania Montes: Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Elisa Guerrero-Feijóo:** Data curation, Formal analysis, Methodology, Software, Writing - review & editing. **Víctor Moreira-Coello:** Data curation, Formal analysis, Visualization, Writing - review & editing. **Antonio Bode:** Data curation, Funding acquisition, Investigation, Visualization, Writing - review & editing. **Manuel Ruiz-Villarreal:** Funding acquisition, Investigation, Visualization, Writing - review & editing. **Beatriz Mouriño-Carballido:** Investigation, Writing - review & editing. **Marta M. Varela:** Conceptualization, Funding acquisition, Investigation, Resources, Data curation, Methodology, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2020.106791.

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