Niche partitioning and plastisphere core microbiomes in the two most plastic polluted zones of the world ocean

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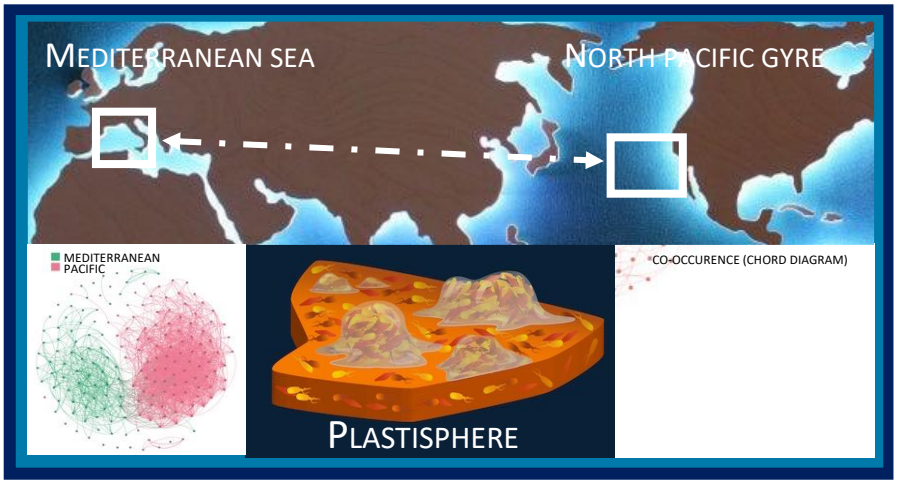
Abstract:

Plastics are offering a new niche for microorganisms colonizing their surface, the so-called 'plastisphere', in which diversity and community structure remain to be characterized and compared across ocean pelagic regions. Here we compared the bacterial diversity of microorganisms living on plastic marine debris (PMD) and the surrounding free-living (FL) and organic particle-attached (PA) lifestyles sampled during the *Tara* expeditions in two of the most plastic polluted zones in the world Ocean, i.e. the North Pacific gyre and the Mediterranean Sea. The 16S rRNA gene sequencing analysis confirmed that PMD are a new anthropogenic ocean habitat for marine microbes at the ocean-basin-scale, with clear niche partitioning compared to FL and PA lifestyles. At an Ocean-basin-scale, the composition of the plastisphere communities was mainly driven by environmental selection, rather than polymer types or dispersal effect. A plastisphere 'core microbiome' could be identified, mainly dominated by *Rhodobacteraceae* and *Cyanobacteria*. Predicted functions indicated the dominance of carbon, nitrogen and sulfur metabolisms on PMD that open new questions on the role of the plastisphere in a large number of important ecological processes in the marine ecosystem.

Highlights:

- Niche partitioning on plastic marine debris (PMD) at the ocean-basin-scale
- Environmental selection rather than plastic dispersion drives plastisphere bacterial diversity
- A plastisphere 'core microbiome' dominated by *Rhodobacteraceae* and *Cyanobacteria*
- Dominance of carbon, nitrogen and sulfur metabolisms on PMD

Graphical abstract



1. Introduction

Plastics are part of the emerging pollutants and they are now regarded as a key geological indicator of the Anthropocene because of their abundance and widespread distribution on Earth (Zalasiewicz et al. 2016). The ocean represents the final destination of mismanaged terrestrial plastic waste, with an estimated 500,000 tons of plastic entering the sea each year (Kaandorp et al. 2023). Plastic stocks at the world's upper oceans amount from 82 to 578 of kilotons (Isobe et al. 2021), mainly dominated by <5mm in size microplastics coming from the fragmentation of larger items (ter Halle et al. 2016). Vast accumulation zones of buoyant plastic were described in the five main oceanic gyres, including the most famous 'Great Pacific garbage patch' in the North Pacific Ocean where up to the order of kilograms (or millions of pieces) per km^2 was found in the center of the gyre (Lebreton et al. 2018). The Mediterranean Sea has been proposed as the sixth great accumulation zone for marine litter, with some of the highest concentrations of floating plastics in the world (Cozar et al. 2014).

Plastics represent physical support for microbial life, the so-called 'plastisphere' (Zettler et al. 2013). A range of 1 to 15 kilotons of microbial biomass has been estimated harbored on the plastic marine debris (PMD), which is in the same range as the estimated microbial carbon mass in the global rivers or both polar regions (Zhao et al. 2021). The plastisphere has several independent or cumulative effects on the fate and impacts of plastic in the marine environment. The biofouling may increase the sedimentation of the plastic particles by decreasing its buoyancy (Kooi et al. 2017; Kane et al. 2020). It is disguising the plastic pieces, thus creating particles more similar to food items for grazers (Vroom et al. 2017). Biofilm growing on plastics has been recently shown to alter the biogeochemical cycles of elements by consuming and releasing nutrients (Chen et al. 2020) and favor the colonization by algae (Nava and Leoni 2021) or metazoan larvae (Ghiglione and Laudet 2020), thus helping indirectly the spread of non-indigenous species over large geographic distances by plastic dispersion (Miralles et al. 2018). The detection of potentially pathogenic *Vibrio* species (such as *V. aestuarianus* and *V. splendidus*) classically found in the plastisphere also raised the question of the diffusion of infectious diseases by plastics (Pedrotti et al. 2022). Finally, the plastic biofilms were shown to host hydrocarbonoclastic bacteria that are thought to be involved in their biodegradation processes (Jacquin et al. 2019).

Bacteria living on plastics have demonstrated distinct niche partitioning in comparison to their counterparts living in organic-particle attached and free-living lifestyles (Dussud et al. 2018a), as well as compared to microorganisms residing on other substrates like wood, cellulose, or glass (Kirstein et al. 2018; Oberbeckmann et al. 2018; Ogonowski et al. 2018).

The reasons for this preferential attachment on plastic particles are still enigmatic. The early stages of colonization may be influenced significantly by factors such as hydrophobicity, topography, roughness, crystallinity, and surface charge (Rummel et al. 2017). However, bacterial communities composing a mature biofilm can undergo alterations, as demonstrated by the occurrence of a diatom bloom on plastic surfaces due to environmental changes (Cheng et al. 2020). The majority of research conducted on the plastisphere was based on controlled incubation experiments using plastics with known compositions, and only a few investigated plastic debris collected at sea, and even fewer were conducted specifically in the open ocean (Amaral-Zettler et al. 2020). This lack of knowledge explains that no consensus has been reached so far to explain the preferential attachment of some bacteria on plastics. Although numerous recent studies have made efforts to characterize the plastisphere niche partitioning, it is still not clear whether a core microbiome (microbial taxa shared by all plastic substrates) can contribute to the description of the plastisphere in the marine environment. Few studies have explored the core plastisphere microbiome in naturally occurring plastic litters from distinct marine geographical areas (Aguilla-Torres et al. 2022, Basili et al. 2020), but these have been carried out on a regional scale and never in distinct geographically distant oceanic regions.

Here, we explored the biogeography of the plastisphere from samples collected during two *Tara* expeditions the most plastic polluted oceanic regions on Earth, i.e., the North Pacific gyre and the Mediterranean Sea. We hypothesized that the preferential attachment of some bacterial species on plastics, as compared to the surrounding free-living and organic particle-attached communities, may be conserved within and between the two distinct environmentally and geographically distant oceanic regions. We also hypothesized that a plastisphere core microbiome may be identified across both regions, which may help to identify and understand critical factors and functions driving the specificity of microbial communities living on plastic debris.

2. Materials and Methods

2.1. Sampling plastic marine debris and the surrounding sea water

Samples were taken using the same protocol during two cruises aboard the research vessel *Tara* in the Western Mediterranean Sea (Tara Mediterranean expedition from April to November 2014) and in the North Pacific gyre (Tara Pacific expedition from June to July 2018) (**Fig. 1**). Plastic marine debris (PMD) floating in surface waters were gathered by utilizing a manta net with a 330 μm mesh attached to a frame and trawled behind the boat for 30 minutes to an hour at a speed of 3 knots. At each of the 39 sampling stations, a subset of the plastic items

of various sizes (ranging from a few mm^2 to a few cm^2), colors and textures (**Fig. 1**) were sorted under a dissecting microscope using sterilized forceps and immediately frozen in liquid nitrogen until further analysis. Prior to deploying each manta net, a conductivity-temperature-fluorescence-depth profiler (CTD – SeaBird SBE 19, in the first 100 m below the surface) and a Niskin bottle for collecting surface seawater (in the first meter below the surface) were deployed at each station. A total of 2 L of seawater was sequentially filtered through 3 µm-pore size polycarbonate filters (referred to as the particle-attached fraction, PA) and 0.2 µm-pore size polycarbonate filters (referred to as the free-living fraction, FL) using a peristaltic pump (pressure <100 mbar). Filters were immediately frozen in liquid nitrogen until DNA extraction.

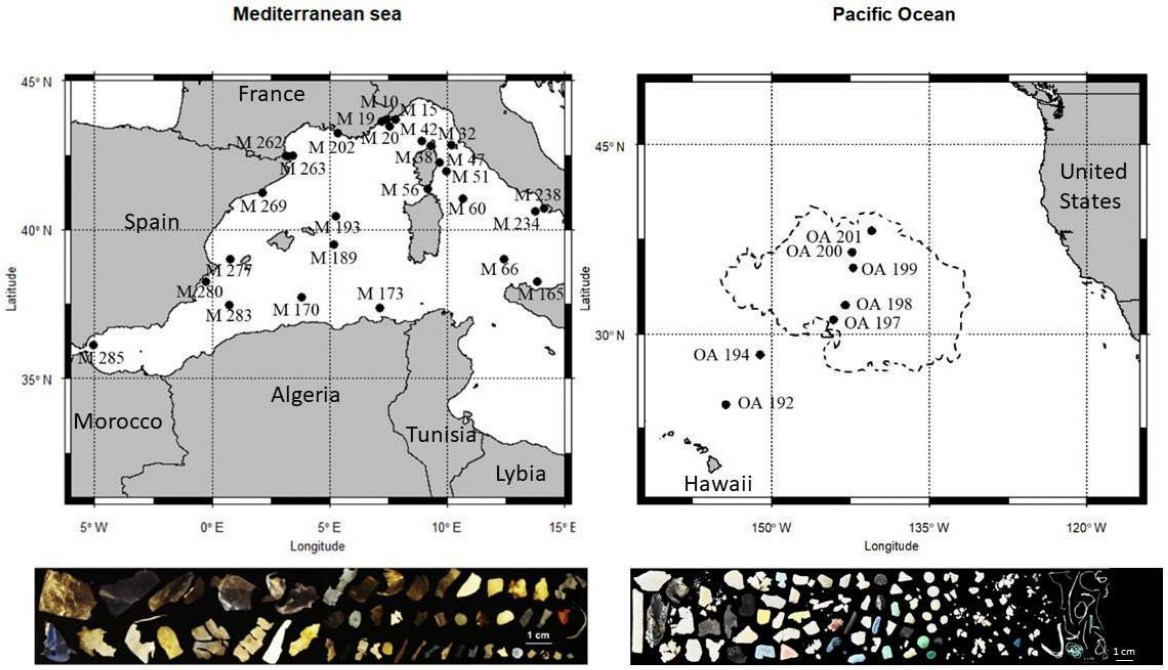


Figure 1: *Station locations in the Western Mediterranean Sea (left part, n=32) and in the North Pacific Ocean (right part, n=7). The corresponding light-micrograph of the 218 PMD collected at each oceanic region is presented below each map, respectively (114 particles collected in the western Mediterranean Sea and 104 in the North Pacific gyre). The mapping of the North Pacific gyre (dotted line) is indicated according to Lebreton et al. (2018).*

2.2. DNA extraction, PCR and sequencing

A first set of 218 DNA extractions was done using the individual PMD sampled in the North Pacific gyre (n=104, between 3 and 38 plastic pieces analyzed per station) and in the NW Mediterranean Sea (n=114, between 2 and 4 plastic pieces analyzed per station). A second set of 39 DNA extracts were obtained from all 3 µm- (particle-attached, PA) and 0.2 µm-pore size

filters (free-living, FL) from the surrounding seawater (n=7 stations in the North pacific gyre and n=32 stations in the Western Mediterranean Sea). The DNA extraction process utilized a standard phenol-chloroform-based protocol (Anderson et al. 1983) with the addition of preliminary steps for plastics, as previously described (Dussud et al. 2018b). Briefly, cell detachment using a sonication pre-treatment (3 x 5 s with 30% amplitude, Branson SLPe) was performed for the disruption of the biofilm in order to improve the further enzymatic and chemical cell lysis of the biofilm (1 mg mL-1 lysozyme at 37°C for 45 min followed by 0.2 mg mL-1 proteinase K and 1% SDS at 50°C for 1 h).

Sequencing was performed using Illumina MiSeq at the Genoscope (Paris, France) after PCR amplification of the 16S rRNA V4–V5 region using 515-FY and 926-R primers (Parada et al. 2016). Raw FASTA files were deposited at NCBI (accession numbers PRJEB58218 and PRJEB52329). The 16S rRNA gene sequences were analyzed using the FROGS pipeline host in the Galaxy platform (Escudié et al. 2018), as previously described (Odobel et al. 2021). Briefly, the merge the paired-ends raw reads were clustered using the SWARM algorithm (Mahé et al. 2014) after quality filtering and primers trimming with cutadapt (Martin, 2011). The removal of chimera was performed after detection by de novo UCHIME method with the VSEARCH algorithm (Edgar et al., 2011; Rognes et al., 2016). Operational Taxonomic Units (OTUs) were defined after clustering (0.03 distance threshold) and further assignment with the Silva128 16S rRNA database (Quast et al., 2012). Chloroplast and mitochondrial sequences as well as clusters that did not belong to Bacteria kingdom were removed.

2.3. Characterization of each PMD

After the cell lysis step of DNA extraction, each individual PMD was processed using the ImageJ software in order to determine the one-dimensional surface area, major length, and circularity. Additionally, the chemical analysis of each PMD was conducted using the Attenuated Total Reflection Fourier Transform Infrared spectrometer (ATR-FTIR Spectrum 100, Perkin-Elmer) and the polymer composition was determined using the POSEIDON software (Kedzierski et al. 2019).

2.4. Statistical analysis and diversity assessment

The confidence interval (*IC*) for the proportion of polymer composition in the sampled plastics at each station and the *IC* for the carbonyl index (CI) and hydroxyl index (HI) of the PE-based PMD were calculated following the method described by Kedzierski et al. (2019).

It is to be expected that the count of unique sequences (and hence OTUs) detected in a

sample will rise with the total number of sequences reads, which may fluctuate according to the sequencing efficiency for each sample (Gilbert et al. 2009). To ensure accurate data comparisons, the bacterial 16S rRNA sequences in each sample were randomly subsampled to match the number of sequences in the sample with the fewest sequences (N=9,115). We decided to remove four samples from the global dataset (with less than 9,115 sequences per sample, and thus not taken into account for further processing), and all the remaining samples stayed in the stationary phase of the rarefaction curve after resampling. All the subsequent results were calculated using this standardized dataset with 9,115 sequences per sample (Ghiglione and Murray 2012). The non-parametric Chao1 species richness estimator (SPADE software; Bandinelli et al. 1993), along with the Shannon diversity and Pielou evenness indices (PRIMER-E; Clarke and Warwick 2001), were used to estimate alpha-diversity. Differences between FL, PA and PMD bacterial richness, evenness and diversity indices were tested using non-parametric Kruskal-Wallis test (package 'stats' version 3.3.1, RStudio software version 1.0.143). For beta-diversity analysis, a non-metric multidimensional scaling (NMDS) on two dimensions was generated based on Bray-Curtis dissimilarity. Statistical analysis of similarities between groups (ANOSIM, PRIMER-E) was used for a combination of natural and Plastic related "niche" lifestyles (FL/PA or PMD) with ocean provenance (Mediterranean Sea vs. Pacific Ocean). ANOSIM is a nonparametric technique designed to allow statistical comparisons for multivariate data sets in a manner similar to univariate techniques (ANOVA) (Clarke and Warwick, 2001). Both NMDS and ANOSIM were performed using the vegan R package (Oksanen et al. 2015).

We utilized the direct gradient approach canonical correspondence analysis (CCA, CANOCO 4.5) to examine the relationships between bacterial community structure and various environmental parameters (pressure, temperature, conductivity, salinity, oxygen and photosynthetically available radiation from CTD - SeaBird SBE 911 data), geographical distance between samples and PMD characteristics (polymer composition and oxidation level from FTIR data, as well as surface and major length of the individual PMD from ImageJ software), as previously described (Ghiglione et al. 2008). The significance of the environmental variables was determined through Spearman rank pairwise correlations.

2.5. Core microbiomes identification, co-occurrence networks inference and predicted metagenomes

Venn diagrams were obtained by identifying the set of OTUs present in each of the Mediterranean and the Pacific fractions using R software (R 5.6.3) and a representation of the

histogram was made using Excel software. The core microbiome was identified by the common OTUs in the two fractions characterized by the overlapping circles.

A co-occurrence network for PMD samples was inferred using the FlashWeave package (Tackmann et al. 2019). To check effects due to the imbalance between Mediterranean and Pacific samples, we randomly selected 75 out of 104 Pacific samples and built co-occurrence networks using the same parameters. Furthermore, the network was decomposed into modules using the leading eigenvectors algorithm (Clauset et al. 2004). Briefly, this algorithm finds communities that maximize modularity, a network property that accounts for the number of edges falling within groups minus the expected number in an equivalent network with edges placed at random (Newman 2006). As a result, modules detected by the leading eigenvector algorithm maximize the number of edges within modules and minimize the connections between modules.

Metagenome prediction was performed using the PICRUSt2 software (Douglas et al. 2020). To detect enriched pathways, we compared the relative abundance of each pathway in each sample. Next, after CLR transformation, we used a Kruskal-Wallis test to detect differences in means (FDR adjustment, q-value < 1e-3), followed by a Dunn post-hoc test to attribute which pathway is enriched in which sample group (ocean-niche).

3. Results

3.1. Chemical characterization showed dominance of PE-based plastics in both oceanic regions

ATR-FTIR analyses showed that polyethylene (PE) dominated the composition of the sorted PMD in both the Western Mediterranean Sea and in the North Pacific Gyre, with 66.1±10.3% and 71.1±8.7%, respectively. In the Mediterranean Sea, PE was followed by propylene (PP; 21.2±6.2%) and polystyrene (PS; 4.2±3.1%), with other polymers representing 3.6±2.8% and undetermined were 4.9±3.3%. Similar proportions were found in the North Pacific Gyre with PP (16.0±7.0%) followed by PS (5.3±4.3%) and other polymers (5.3±5.1%), with undetermined at 2.3±2.9%. The PP/PE ratio is higher in the Mediterranean Sea (0.32) than in the North Pacific Gyre (0.23). Based on PE spectra, no significant differences were found between the carbonyl index (CI) in the Mediterranean Sea and Pacific gyre (CI = 0.78±0.16 and 0.78±0.11, respectively), whereas significant differences were found for the hydroxyl index HI (Mediterranean Sea: 5.7±1.1; Pacific gyre: 2.5±0.5) and the fouling index FI (Mediterranean Sea: 1.0±0.24; Pacific gyre: 0.29±0.04).

3.2. Comparison of α-diversity indexes between the FL, PA and PMD lifestyles in the Mediterranean and Pacific regions

The Chao1 index indicated that the bacterial richness was significantly higher in the North Pacific gyre compared to the Mediterranean Sea for all FL, PA and PMD lifestyles (Man-Withney $p<2.2.10^{-16}$) (**Fig. 2**). Significant differences were found between the three groups within each oceanic region (Mann Withney $p<0.05$), but with different trends depending on the geographic region. The PMD (170.1 and 262.3) presented always intermediate Chao1 estimated richness values compared to FL (174.4 and 251.0) and PA (153.8 and 323.0).

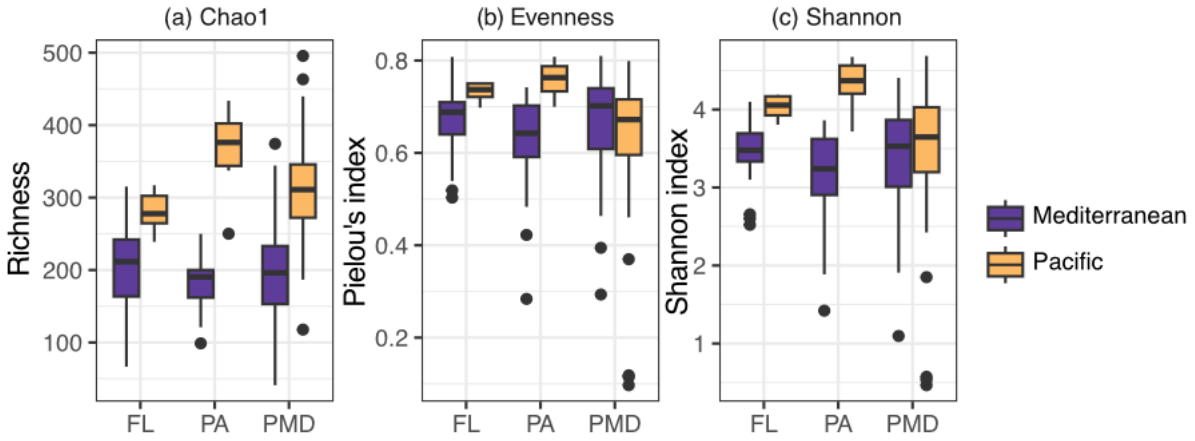


Figure 2: *Comparison of α-diversity indices: (a) Chao1 richness, (b) Pielou evenness and (c) Shannon diversity indexes between the FL, PA and PMD lifestyles according to the sampling location (Western Mediterranean Sea and North Pacific gyre). The median values are marked with a vertical line, and whiskers represent the minimum and maximum values. Outliers are indicated as filled circles.*

The Pielou evenness index was similar for the PMD in the Mediterranean and the Pacific regions (Man-Whitney, p=0.4), whereas significant differences were found for PA and FL lifestyles between the two oceanic regions ($p=4.1\ 10^{-4}$ and $p=2.5\ 10^{-2}$, respectively). No significant differences were observed between PMD, PA and FL in the Mediterranean Sea (Kruskal and Wallis, p=0.24; mean values of 0.67, 0.62 and 0.68, respectively), whereas significant differences were found in the Pacific (Kruskal and Wallis, $p=7.2\ 10^{-5}$; mean values of 0.64, 0.76 and 0.73 on PMD, PA and FL, respectively).

The Shannon index confirmed a significantly higher microbial diversity in the North Pacific gyre compared to the Western Mediterranean samples (Kruskal and Wallis, $p=7.6\ 10^{-5}$) for the FL (Shannon index average values of 4.03 and 3.42, respectively) and PA (4.33 and

3.14, respectively), but not for the PMD (Shannon index average values of 3.52 and 3.41, respectively; Mann-Withney p=0.13).

3.3. Bacterial community structure (β-diversity) with high dissimilarities between PMD compared to FL and PA lifestyles

Clustering according to the three sample lifestyles clearly emerged with higher differences between PMD compared to FL and PA lifestyles (**Fig. 3**). Each group could be divided into two subgroups according to their geographical origin, i.e., Mediterranean Sea or North Pacific gyre (NMDS2 axis). Remarkably, PMD communities were not structured by plastic type (**Fig. 3**). In order to confirm the visual cluster separation from Figure 3, we run a nonparametric ANOSIM test. The analysis showed a significant separation between groups (ANOSIM R=0.67, p=0.001) when using lifestyle/ocean grouping (i.e., FL/PA.Mediterranean, PMD.Mediterranean, FL/PA.Pacific, PMD.Pacific). Furthermore, a detailed ANOSIM pairwise comparison between groups (**Suppl. Table 1**) indicated less difference in FL/PA vs. PMD in the Mediterranean Sea *vs.* the Pacific Ocean samples.

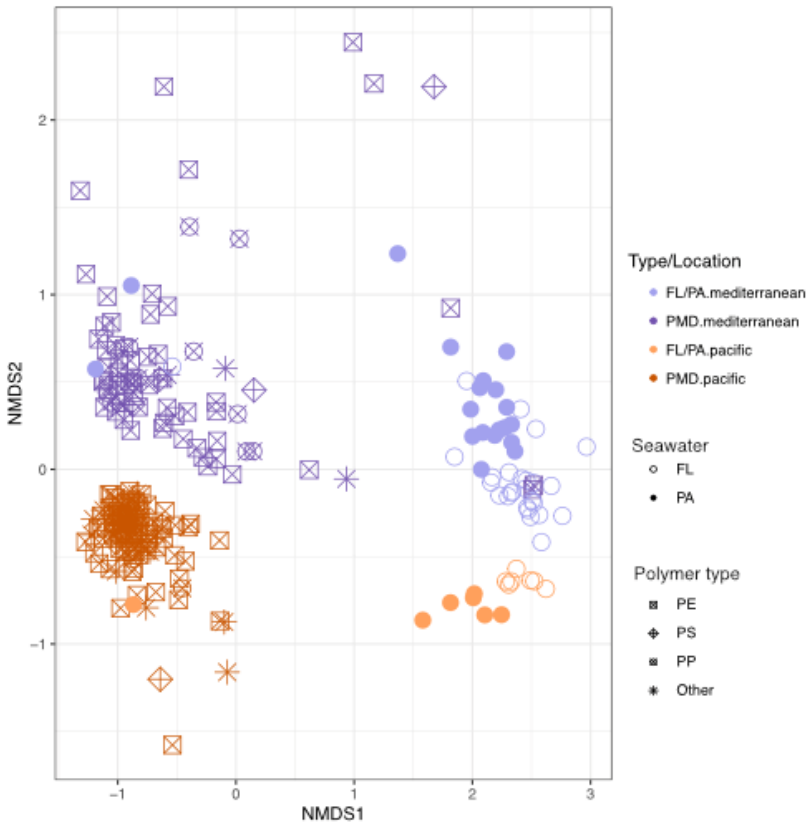


Figure 3. *Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis similarities of bacterial communities (stress = 0.10) of PMD (hollow shapes in non-muted colors, together with information of the polymer type, i.e. PE, PP, PS and others), FL and PA (solid squares or triangles with*

muted colors, respectively). Ocean provenance is depicted in violet or orange for the Western Mediterranean Sea and the North Pacific Ocean, respectively.

3.4. Taxonomic affiliation and similarity analysis of FL, PA and PMD lifestyles between the Mediterranean and Pacific regions

The FL lifestyle was dominated by *Alphaproteobacteria* that represented 45% in the Mediterranean Sea and 53.24% in the North Pacific gyre, with *SAR11* as the dominant group (14.63% and 25.5% of the total relative abundance in the two oceanic regions, respectively) together with *Rhodobacteraceae* (6.0% in Pacific and 6.84% in Mediterranean) (**Fig. 4**). Cyanobacteria represented a higher proportion of the community in the FL Mediterranean Sea (21.6%) than in the North Pacific gyre (8.0%), with *Prochlorococcus* sp. as the dominant group (1.43% and 6.86% of the total relative abundance in the two oceanic regions, respectively). *Bacteroidia* were also very abundant in the FL (16.9% in the Pacific and 26.5% in the Mediterranean) with the dominance of *Flavobacteriaceae* (11.6% in the Pacific and 9.36% in the Mediterranean). Inversely, *Bacteroidia* (mainly *Flavobateriaceae*) and *Gammaproteobacteria* were more abundant in the North Pacific gyre (16.0% and 13.2%, respectively) than in the Mediterranean Sea (12.8% and 12.2%, respectively). Similarity percentage analysis (SIMPER) showed that *Cyanobiaceae* explained 14.44% of the difference between the two oceanic regions (higher abundance in the Mediterranean Sea), whereas *Prochlorococcus* sp. and *Rhosospirillales* explained 5.2 and 4.4%, respectively (higher abundance in the Pacific).

The PA lifestyle was also dominated by the same four taxonomic groups (*Alphaproteobacteria*, *Gammaproteobacteria*, *Cyanobacteria* and *Bacteroidia*), but with different proportions between locations and compared to FL (**Fig. 4**). The proportion of *Bacteroidia* increased in the PA compared to FL, and became even dominant in the North Pacific gyre with 31.7% of the total affiliated reads (mainly *Formosa* sp. and *Fluviicola* sp.), while it represented only 20% in the Mediterranean Sea (mainly *Lewinella* sp.). The proportion of *Alphaproteobacteria* was lower in the North Pacific gyre (19.2%, mainly composed of *Rhodobacteraceae* family) as compared to the Western Mediterranean Sea (27.6%, mainly composed of *Sphingomonadaceae* family), whereas it was similar in both oceanic regions for *Gammaproteobacteria* (25% and 27%, respectively, mainly *Alteromonas* sp.). *Cyanobacteria* represented 9.18% in the North Pacific gyre (mainly *Phormidesmiaceae*) and 14.62% in the Western Mediterranean Sea (mainly *Cyanobiaceae*). The SIMPER analysis showed that *Sphingomonadaceae*, *Alteromonadaceae* and *Lewinella* sp. explained 5.5%, 5.24% and 4.36%

of the dissimilarity in PA between the two oceanic regions of both regions, respectively (higher abundance in the Mediterranean Sea). The North Pacific PA lifestyle is distinguished from the Mediterranean PA lifestyle by a high abundance of the genus *Formosa* sp. (1.4%), *Alteromonas* sp. (1.1%) and *Fluviicola* sp. (1.29%) (not shown on the bubble plot, percentage dissimilarity < 5%).

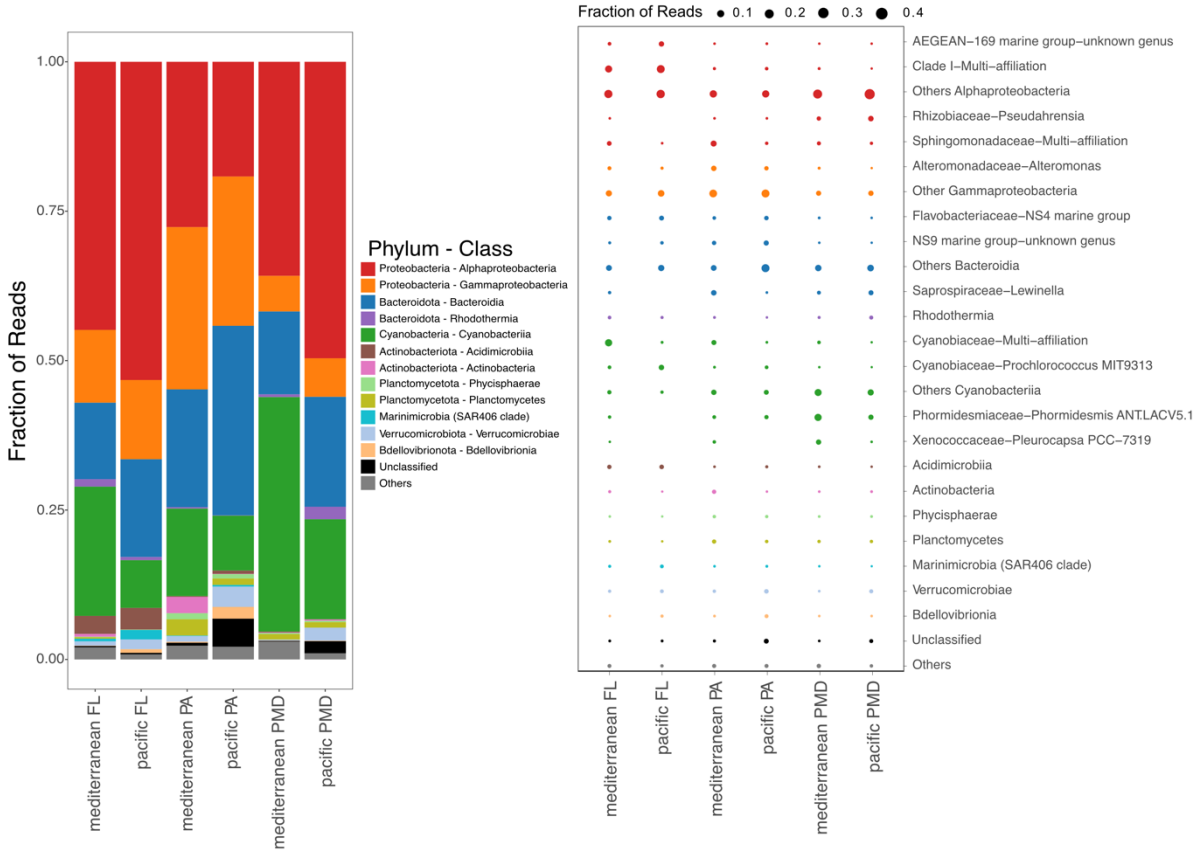


Figure 4. *Bacterial OTUs associated with the FL, PA and PMA lifestyles in the Mediterranean Sea and in the North Pacific gyre. Left panel: relative abundance of the major groups at the Phylum-Class level. Right panel: Main Family-Genus levels explaining differences (assed by SIMPER). Others: 1.88% of total reads, Unclassified: 1.11% of total reads.*

The PMD lifestyle contained a drastically lower proportion of *Gammaproteobacteria* compared to PA and FL, which represented only 5.94% and 6.43% of the average abundance across PMD samples in the Mediterranean Sea and in the North Pacific gyre, respectively (**Fig. 4**). *Cyanobacteria* were much more abundant on PMD in the Mediterranean Sea (39.2%) as compared to the North Pacific gyre (15.0%), where others were present in both regions, such as *Phormidesmis sp.* (16.4% and 6.1%, respectively), *Rivularia sp.* (9.3% and 9.0%, respectively) and *Pleurocaspa sp.* (6.2% and 0.08%, respectively). Inversely, *Alphaproteobacteria* were much less abundant in the Mediterranean Sea (35.8%) compared to

the North Pacific gyre (54.5%), where several members of the *Rhodobacteraceae* were abundant in both oceanic regions (19.8 and 30.5%, respectively), including *Pseudahrensia sp.* (2.9% and 7.3%, respectively), *Tateyamaria sp.* (2.4% and 4.1%, respectively) and *Erythrobacter sp.* (3.3% and 1.4%, respectively). Finally, *Bacteroidetes* were found in similar proportion in the Mediterranean Sea (13.9%) as compared to the North Pacific gyre (15.0%), dominated by *Flavobacteriaceae* (9.8% and 8.7%, respectively), *Saprospiraceae* (2.84% and 7.5%, respectively) and *Rhodothermaceae* (0.4% and 1.84%, respectively), including *Muricauda* sp. (1.2% and 1.7%, respectively) and *Lewinella* sp. (1.9% and 5.4%, respectively). SIMPER analysis confirmed that the OTUs explaining 35 % of the difference between the PMD communities living in both oceanic regions were *Phormidesmis sp., Pleurocapsa sp., Erythrobacter sp., Acrophormium sp., Dokdonia sp.* and *Sagittula sp.* that were more abundant in the Mediterranean Sea (explaining 20.3% of the variability) and *Pseudahrensia* sp., *Lewinella* sp., *Psychrobacter* sp., *Tenacibaculum* sp., *Roseovarius* sp. and *Cobetia* sp. in the North Pacific gyre (explaining 14.7% of the variability). The SIMPER analysis also revealed that *Cyanobiaceae, Phormidesmis* sp., *SAR11* and *Rivularia* sp. explained together 31.5% of the dissimilarity between PMD and FL in the Mediterranean Sea, and *SAR11, Rivularia* sp. and *Pseudahrensia* sp. explained 20.5% in the North Pacific gyre. Distinction between PMD and PA were explained at 30.0% by *Phormidesmis* sp., *Rivularia* sp. Alteromonadaceae, Sphigomonoadaceae and *Lewinella* sp. in the Mediterranean Sea, whereas *Rivularia* sp, *Phormidesmis* sp. and *Pseudahrensia* sp. explained 13.1% of the dissimilarity in the North Pacific gyre.

3.5. Temperature and geographical distance are the main factors driving FL, PA and PMD bacterial community structure in both oceanic regions

Canonical correspondence analysis (CCA) revealed that a combination of multiple regression on matrix analysis using environmental factors and geographical distance accounted for 26.7% (FL lifestyle), 17.6% (PA lifestyle), and 16.8% (PMD lifestyle) of the overall variation in the Bray-Curtis dissimilarity matrix (**Table 1**). The effect of environmental variables on bacterial community structure was statistically significant ($p<0.05$) for the FL (14.3% of the variance), PA (7.6% of the variance) and PMD lifestyles (6.5% of the variance). Temperature was the main environmental driver in all the lifestyles (9.9%, 4.8% and 4.4% of the variance in FL, PA and PMD, respectively). Geographical distance between samples was also significant and explained a high proportion of the variance for all lifestyles (12.4%, 10.0% and 10.3% of the variance in FL, PA and PMD, respectively; $p<0,05$).

	FL	PA	PMD
Physico-chemical (seawater)	14.3	7.6	6.5
Temperature only	9.9	4.8	4.4
Geographical distance	12.4	10.0	10.3
Total variance Env	26.7	17.6	16.8
PMD characteristics	ND	ND	3.6

Table 1. *Percentage of variance explained by CCA analysis of the bacterial community structure data when constrained by physico-chemical variables (temperature, salinity, oxygen and PAR), geographical distance and/or PMD characteristics (polymer composition, oxidation level, surface and major length). All values were significant ($p<0.05$) when using a Monte Carlo permutation full model test (199 permutations). ND: non-determined.*

3.6. Identifying core microbiomes on FL, PA and PMD across oceanic regions

With the objective to identify and describe core microbiomes in FL, PA and PMD lifestyles, we first analyzed OTUs shared among bacterial consortia living in both the oceanic regions, represented by UpSet plots (**Fig. 5**). A core microbiome was identified for the FL samples, representing 303 OTUs (45% of all OTUs) composed of *Alphaproteobacteria* (51%), mainly represented by the order *SAR11* (27.45%), *SAR116* (6.5%), *Rhodobacteraceae* (6.26%) and *Sphingomonadaceae* (2.5%). *Gammaproteobacteria* represented nearly 17.7% of the FL core microbiome, mainly represented by the order *SAR86* (4.22%) and the *Alteromonadaceae* family (2.86%), of which the genus *Alteromonas* sp. is very dominant (1.5%). The *Cyanobacteria* represented 14.5%, with *Cyanobiaceae* and the genus *Prochlorococcus* sp. (4.5%). *Bacteroidia* were found at 13.1% of the entire core microbiome, mainly represented by the *Flavobacteriaceae* (10%) including the *Formosa* sp. (0.85%) and *Fluviicola* sp. (0.34%).

The core microbiome of 412 OTUs in PA samples (49% of all OTUs) was dominated by *Gammaproteobacteria* (30.5%), mainly *Alteromonadaceae* (18.02%) including *Alteromonas* sp. (4.5%) and *Aesturiibacter* sp. (2.26%). *Alphaproteobacteria* represented 24.5% of the PA core microbiome, represented by the *Rhodobacteraceae* (8.7%, including *Parococcus* sp. and *Ruegeria* sp.), *Sphingomonadaceae* (7.7%, mainly *Sphingobium* sp.), and *Rhizobiaceae* (1.4%, mainly *Fulvimarina* sp.). *Bacteroidia* represent 28.05% of the PA core microbiome, mainly composed of *Saprospiraceae* (4.9%, mainly *Lewinella* sp.), *Flavobacteriaceae* (12.7%, including *Formosa* sp. and *Fluvicola* sp.). Finally, *Cyanobacteria* represents only 8.8% of the PA core microbiome, mainly represented by the *Cyanobiaceae* (4.8%, mainly *Prochlorococcus* sp. and *Phormidesmiaceae*).

In the PMD lifestyle, 665 OTUs was shared between both oceanic regions, a higher percentage (69%) compared to FL and PA lifestyles. This core microbiome within the PMD samples was dominated by *Alphaproteobacteria* (45.35%), followed by *Cyanobacteria* (28.8%), *Bacteroidia* (14.9%) and *Gammaproteobacteria* (6.25%). *Alphaproteobacteria* was mainly represented by *Rhodobacteraceae* (27%, mainly *Tateyamaria* sp., *Yoonia-Loktanella* sp. and *Sagitula* sp.), *Rhizobiaceae* (9.34%, mainly *Pseudahrensia* sp.) and *Sphingomonadaceae* (4.44%, mainly *Erythrobacter* sp. and *Sphingobium* sp.). *Cyanobacteria* were dominated by Phormidesmiaceae (13.13%, mainly *Phormidesmisis* sp.), Rivulariaceae (10.55%, mainly *Calothrix parasitica* and *Calothrix* sp.) and *Xenococcaceae* (3.06%, mainly *Pleurocapsa* sp.). *Bacteroidia* were dominated by *Flavobacteriaceae* (8.7%, mainly *Muricauda* sp., *Dohdonia* sp. and *Winogradskyella* sp.) and *Saprospiraceae* (5%, mainly *Lewinella* sp.). *Gammaproteobacteria* were dominated by the *Moraxellaceae* (2.28%, mainly *Acinetobacter* sp. and *Psychrobacter* sp.) and *Halomonadaceae* (0.92%, mainly *Cobetia* sp. and *Halomonas* sp.). Other families were also present such as the *Nostocaceae* (belonging to *Cyanobacteria* 30.2%), as well as the *Rhizobiaceae* (13%), *Sphingomonadaceae* (8.3% of the core microbiome) and *Hyphomonadaceae* (3.5%). The most represented genus in the PMD core microbiome was *Rivularia* sp. (belonging to the *Nostocaceae* family), followed by *Pseudahrensia* sp. (13%; belonging to the *Pseudahrensia* sp. family. In the *Rhodobacteraceae* family, the genus *Tateyamaria* sp. and *Roseovarius* sp. represented 14% of the PMD core microbiome. The *Sphingomonadaceae* family was represented by the *Erythrobacter* sp., which represents 5.4% of the PMD core microbiome. Interestingly, we could not identify any persistent OTUs that was consistently detected in 100% of the PMD samples. When arbitrarily decreasing the cut-off to 90%, we found only 17 OTUs belonging mainly to the *Rhodobacteraceae* family, representing 45% of the abundance of the core microbiome families.

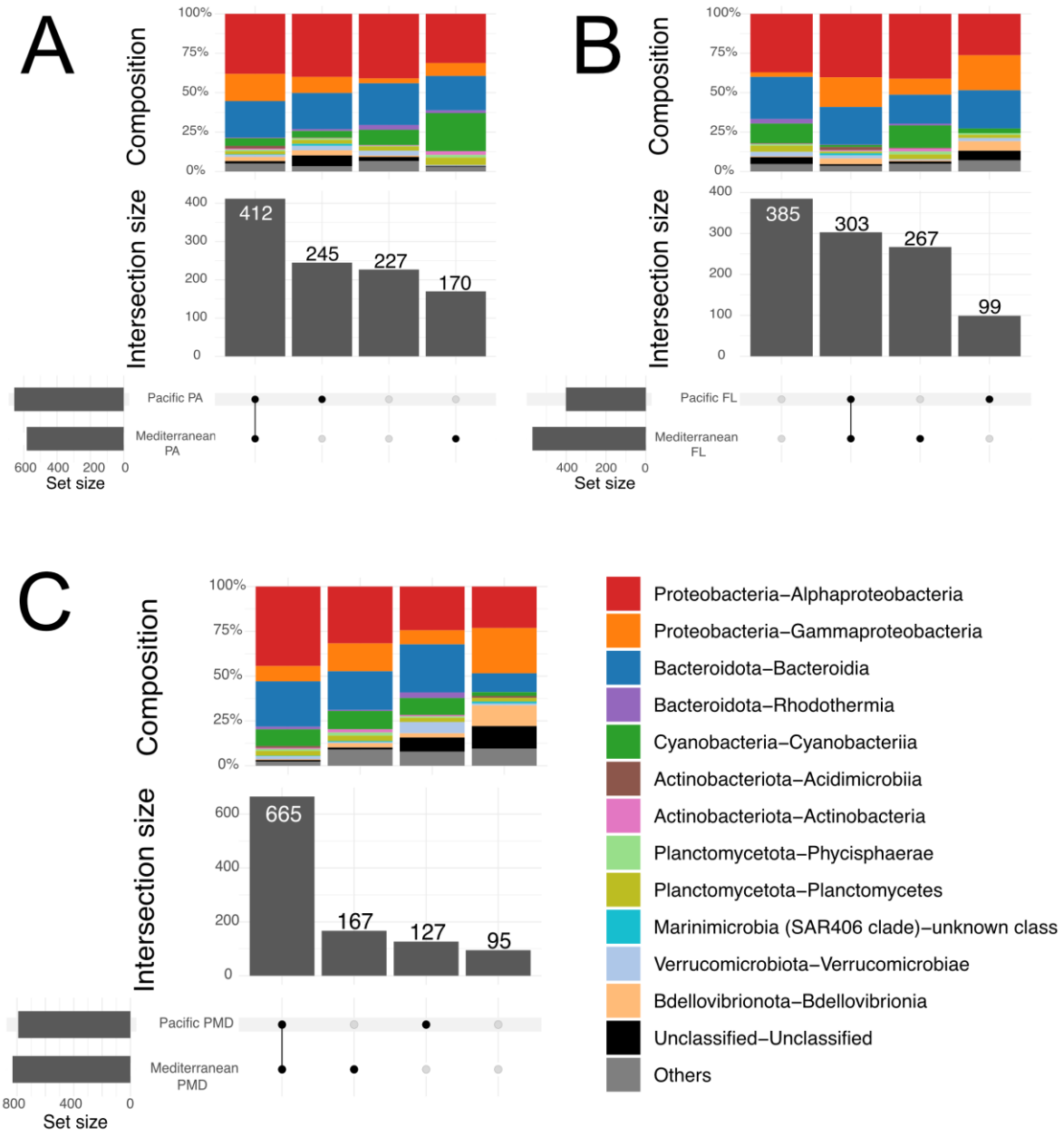


Figure 5: *UpSet Plots from PA (A), FL (B) and PMD lifestyles found in the Mediterranean Sea and North Pacific gyre. Black dots represents the set of OTUs in either North Pacific gyre (upper black dot), the Mediterranean Sea (low black dot), their intersection (connected black dots) or not in the corresponding lifestyle (disconnected grey points). Grey bars show the number of OTUs per set and colored bars their composition in Phylum – Class categories.*

3.7. A global PMD co-occurrence network for the Mediterranean and Pacific regions

A co-occurrence network was inferred using all PMD samples across both oceanic regions with the goal to identify and delineate potentially shared community network structures. Although there was a small imbalance in sampling between the Mediterranean Sea (n=114) and the Pacific Ocean (n=104), the inferring a global PMD network using all PMD samples allowed

us to gather more information without affecting ecological interpretations (see supplementary text). Thus, this global network derived using the entire PMD dataset including both ocean regions was used in the following analyses. We decomposed the PMD co-occurrence network into modules using the leading eigenvector algorithm. We identified seven modules (**Fig. 6A**), each representing a distinct community of highly connected OTUs. We considered modules 1 to 6 for further analysis, as the module 7 was composed of only 3 OTUs and was thus not relevant in terms of relative abundance.

Specific modules presented distinct abundance patterns within Mediterranean and Pacific regions (**Fig. 6B**). Modules 1 and 3 were detected significantly more abundant in the Pacific Ocean, while modules 2 and 6 were more abundant in the Mediterranean Sea. In contrast, modules 4 and 5 did not appear to be driven by geographical provenance. The geographic specificity of these modules highlighted the presence of conserved plastisphere communities in both ocean regions. The module 1 was clearly more related to co-occurring OTUs in PMD samples from the Pacific, while module 2 was more related to co-occurring OTUs in PMD samples from the Mediterranean Sea. The module 3 appeared present in both Pacific and Mediterranean regions, but was significantly enriched in abundance in Pacific samples.

The taxonomic distribution of delineated modules (**Fig. 6C**) highlighted a higher prevalence of *Bacteroidota* in module 1 (enriched in Pacific samples), while *Cyanobacteria* were more prevalent in module 2 (enriched in Mediterranean samples). Module 2 (enriched in Mediterranean) and 3 (enriched in Pacific) showed a similar composition and network structure as shown by a detailed analysis of associations between modules (**Suppl. Fig. 2**), indicating a relatively high number of shared co-occurrences between OTUs of both modules. Module composition showed that biogeographic distinct communities associated with PMD articulate around the primary producers *Cyanobacteria* (Module 2 for the Mediterranean Sea and 3 for the Pacific Ocean). Less abundant and less distinct modules showed more diverse taxonomic composition and stronger in-group correlation (**Fig. 6C**). Noteworthy, modules 4-6 showed a contrasting taxonomic structuring, characterized by a low diversity in *Cyanobacteria* OTUs and an increased richness in *Bacteroidota* and *Gammaproteobacteria*.

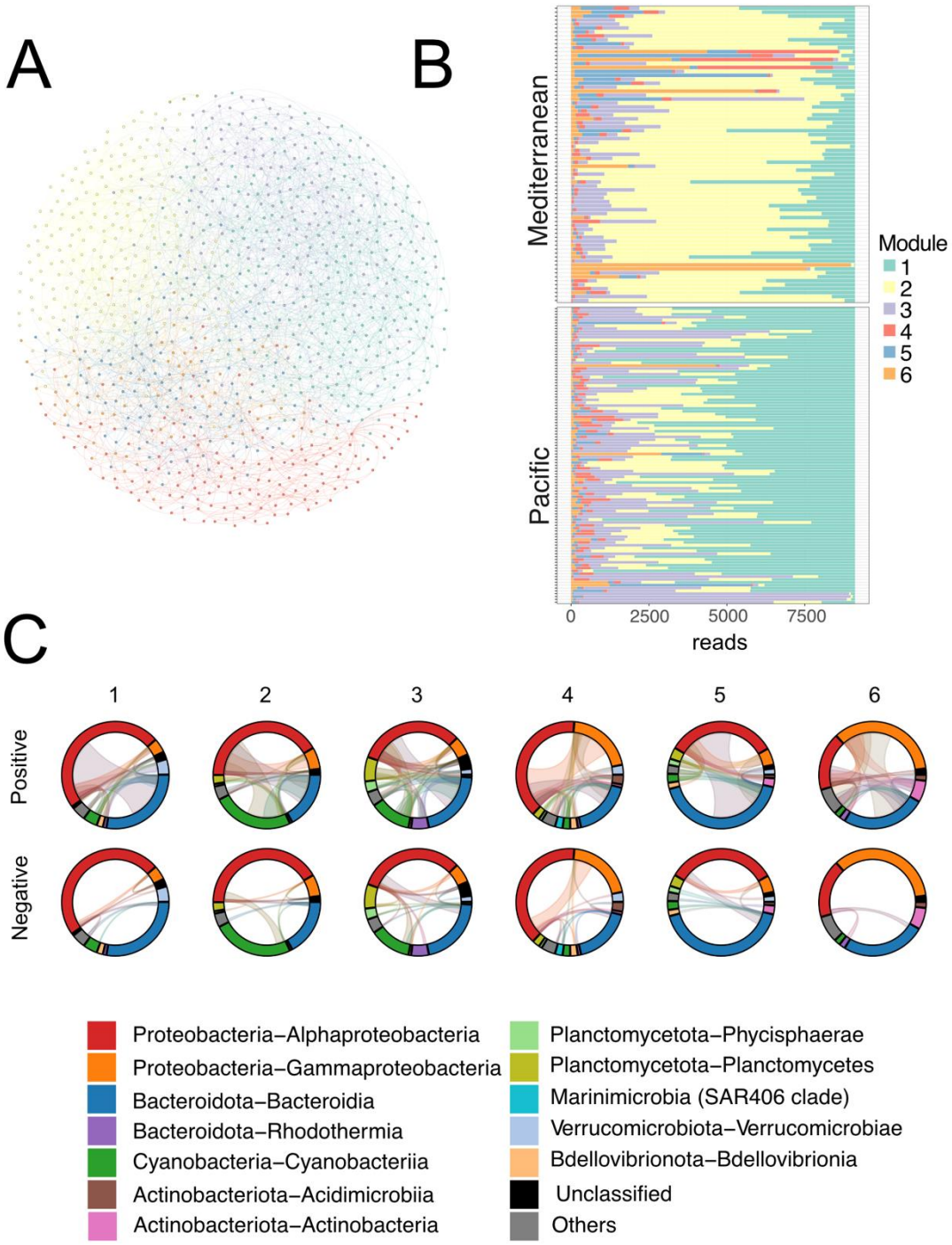


Figure 6. *Co-occurrence network in PMD originated from Mediterranean and Pacific regions with (A) module decomposition, (B) distribution of modules per oceanic region and (C) chord diagrams.*

3.8. Metagenome prediction to evaluate the functional redundancy in FL, PA and PMD

Analysis of the predicted metabolic capacities encoded by the bacterial communities associated with FL, PA and PMD lifestyles revealed a number of systematic differences (**Fig. 7**). Here, we first identified predicted functions that were prevalent in a combination of ocean-niche samples (**Fig. 7A**) to guide the interpretation. For instance, L2 categories "Protein

Families: signalin and cellular process" and "Biosynthesis of other secondary metabolites" were enriched in both PA and PMD, but not in FL (therefore grouped in the PA+PMD subpanel). "Membrane transport" and "Cellular Community - prokaryotes" functions were abundant in both Mediterranean-PMD and Pacific-PMD samples, marked as enriched in PMD context.

Next, we analyzed the relative contribution of each module to the total predicted metagenome by ocean-niche, pointing out enriched functions that were driven by the relative abundance of previously detected community modules in each ocean (**Fig. 7B**). Despite the different taxonomic composition of modules, predicted L2 functions were equally enriched in most cases, thus showing functional redundancy between niche-specific distinct communities.

To dig further in specific categories in FL, PA and PMD systems, we calculated the relative abundance of L3 paths within the selected L2 pathways of Figure 7 (**Suppl. Fig. 3**).

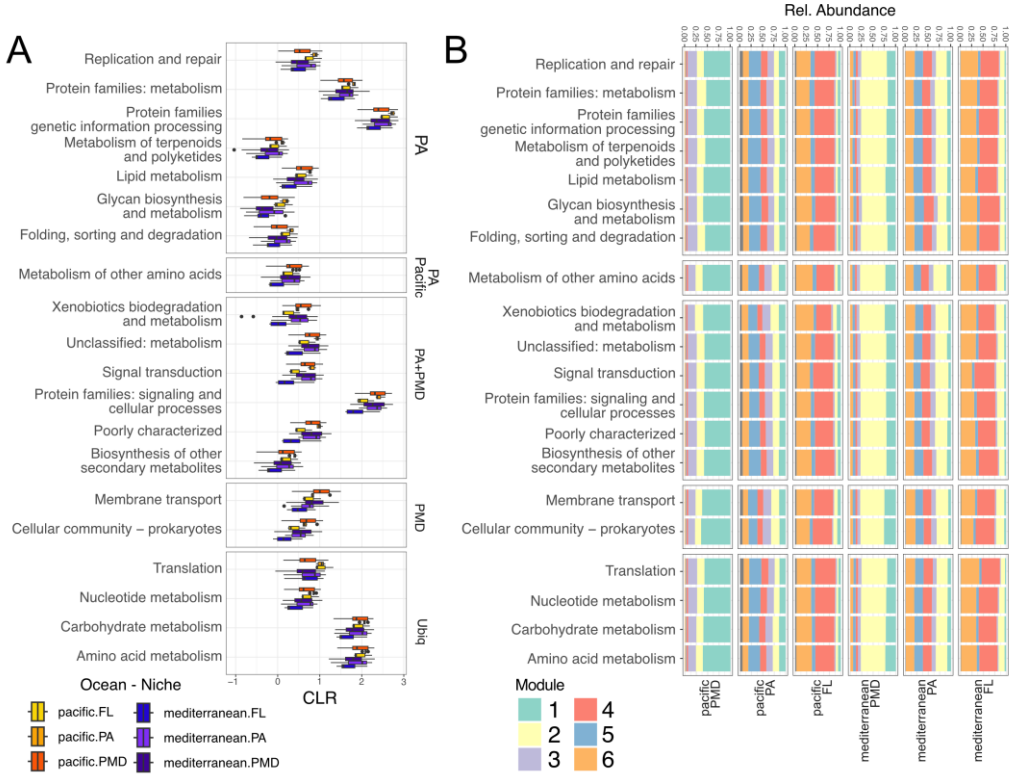


Figure 7. Metagenome prediction. *(A) CLR transformed abundances for enriched functions per ocean-niche. Subpanels show combinations of ocean-niche samples where functions show a significantly higher CLR mean. Subpanel codes: PA= Particle Attached, PA_Pacific = Particle Attached in the Pacific, PA+PMD = Particle Attached and PMD, PMD = PMD, and Ubiq = Ubiquitous. (B) Relative abundance of function by module per niche and ocean-niche.*

4. Discussion

4.1. Niche partitioning between FL, PA and PMD lifestyles

The results presented here demonstrated a clear niche partitioning in seasurface prokaryotes at the ocean-basin-scale as reflected in significant differences in the composition of FL, PA and PMD bacterial communities. FL and PA marine microbial communities have repeatedly been shown to differ in their diversity (α-diversity) and composition (β-diversity) in different locations worldwide (Crump et al. 1999; Ghiglione et al. 2005, 2007; Grossart 2010; Ortega-Retuerta 2013). More recently, another type of marine bacterial communities living on PMD was shown to differ from FL (Zettler et al. 2013; Debroas et al. 2017) and also from PA (Dussud et al. 2018a; Oberbeckmann et al. 2018). In this study, we observed that the spatially isolated Western Mediterranean and North Pacific Gyre hosted FL, PA and PMD communities that consistently differed in β-diversity. This result is in accordance with previous studies showing that differences between FL and PA lifestyles exceeded the global-scale geographical variation (Acinas et al. 1997; Salazar et al. 2015). Here, we show that it is not only the case for FL and PA lifestyles, but also for the PMD, which indicates that the strong niche partitioning already observed in different locations (Oberbeckmann et al. 2018; Xu et al. 2019) is confirmed at the ocean-basin-scale.

4.2. Ocean-basin-scale comparison revealed a clear influence of environmental parameters on FL, PA and PMD lifestyles

Our ocean-basin-scale comparison showed that the bacterial community structure (β-diversity) within the FL, PA and PMD lifestyles were clearly influenced by their geographical origin, i.e. Mediterranean vs. Pacific regions. This was also visible for α-diversity, with higher Shannon diversity index on FL, PA, and PMD lifestyles in the North Pacific gyre compared to the Western Mediterranean Sea. In particular, we observed a higher selection of given species on PMD in the Pacific (higher richness and lower evenness) compared to the Mediterranean Sea. Our results are in accordance with previous data revealing clear links between environmental variables and global bacterial biogeography of both FL, PA and PMD lifestyles (Ghiglione and Murray 2012; Salazar et al. 2015; Amaral-Zettler et al. 2015).

Such biogeographical patterns can result from two ecological processes: (a) the ‘environmental selection’, build on the principle that the existence of environmental difference between basins exert different pressures on each group (FL, PA or PMD) or (b) the ‘dispersal effect’, where the reduced dispersal capabilities of microbes between basins are considered by

each lifestyle (Hanson et al. 2012). We estimated the relative contribution of both processes by relating community composition to a set of environmental variables, and to the geographical distance between sampling locations, using multiple regression. First, we found that temperature was the main environmental driver of bacterial communities in all lifestyles, which was in accordance with another study based on *Tara* expeditions on global planktonic ocean microbiome in the epipelagic zone (Sunagawa et al. 2015). On another hand, we observed that the effect of geographical distance was also significant between both ocean basins for the three lifestyles ($p<0.05$), but not within the same basin. One of the potentially effects of marine debris is that it offers opportunities for the dispersal, or 'hitchhiking' of species around the world (Debroas et al. 2017). This may be true on a regional scale, but not at the global Ocean-basin-scale, as shown in our study. We observed a clear distance-decay in all bacterial lifestyles (PMD, FL and PA) that remained relatively close in the same basin (Mediterranean Sea or the North Pacific gyre), but significantly differed at the global Ocean-basin-scale. As a consequence, our results indicate that environmental selection rather than dispersal effects appear to shape the biogeography of the FL, PA and PMD lifestyles at the Ocean-basin-scale, although spurious distance effects may arise as a result of unmeasured environmental variables (Hanson et al. 2012).

4.3. Relation between the polymer type and its corresponding plastisphere

In the specific case of PMD, we also evaluated the influence of the polymer composition, morphology, size and age in shaping this specific community. As in many other places in the world surface oceans (Erni-Cassola et al. 2019), PE was the most abundant polymer in both oceanic regions (> 70%), followed by PP, PS together or not with PET in the North Pacific gyre or in the western Mediterranean Sea, respectively. After comparing a total of 179 plastic pieces of different polymer composition, we could not find any significant relation between the bacterial community structure and the polymer type, morphology, size or age. Our study gives another proof that the environmental conditions rather than the polymer characteristics are driving the plastisphere composition at both global (Amaral-Zettler et al. 2015) and regional scales (Dussud et al. 2018a; Basili et al. 2020). This result may appear in contradiction with other studies showing distinct biofilm community structure according to different polymer types (Oberbeckmann et al. 2018; Dussud et al. 2018b; Pinto et al. 2019). Such discrepancy may be explained by the fact that these latter studies were focused on the formation of the biofilm when pristine new plastic were immersed for several weeks or months in seawater, which is different from sampling microplastics directly in the environment that likely stayed

longer in seawater. PMD are long-lived substrates that can transport microbial communities across ocean basins. Our results, together with other global comparisons between the North Pacific and North Atlantic gyres (Amaral-Zettler et al. 2015), are indicating that the environmental selection is even more important in shaping the specific community growing on PMD.

4.4. Conservative patterns in the FL, PA and PMD in both oceanic regions

Previous studies highlighted the importance of identifying core microbiomes to unravel the ecology of microbial consortia. It has been proposed that these commonly occurring organisms that appear in all assemblages associated with a particular habitat are likely critical to the function of that type of community (Hamad and Knight 2009). Our dataset using ocean-basin-scale comparison was particularly well suited to describe core microbiomes since distinct habitats could be distinguished in both geographical regions according to biological niche spaces, i.e., seawater, organic particles and plastics (FL, PA and PMD lifestyles, respectively).

First, we found a classical trend of the FL and PA core microbiomes in the Mediterranean and Pacific regions. The FL lifestyle was dominated by the genus *SAR11* in both oceanic regions, already identified as a highly prevalent group in the world oceans (Dussud et al. 2018b; Basili et al. 2020). Shared presence of *Cyanobacteria* in the two oceanic regions were also identified in the FL core microbiome, being 2.7 times more important in the Mediterranean than in the Pacific. *Cyanobacteria* are generally influenced by water temperature, excess of nitrogen and by the N:P ratio (Dolman et al. 2012). Differences between the two regions resulted in the dominance of *Synechococcus* sp. in the Mediterranean and *Prochlorococcus* sp. in the Pacific, two of the most widespread phototrophic bacterial genera in the oceans (Suzuki et al. 2017; Sunagawa et al. 2020). As depicted elsewhere, the core microbiome in PA samples was characterized by the dominance of *Gammaproteobacteria* and *Alteromondaceae*. It is well known that *Alteromonas* bacteria have generally higher nutrient requirements than the free-living *SAR11*, and attach to particles to avoid the nutrient-depleted conditions in the surrounding waters (Crespo et al. 2013).

Second, we demonstrated that conservative patterns found in PMD communities originated from the two distinct environmentally and geographically distant oceanic regions. We identified a higher number of OTUs shared between both oceanic regions in the PMD (69% of the total OTUs) compared to FL (45%) and PA (49%). Similar results were found in other studies exploring the biogeography of FL marine bacteria worldwide (Salazar et al. 2016, Villarino et al. 2022). Our results confirm again the specificity of the distinct and uniquely PMD

habitat at global scale, and suggests that the relative homogeneity of PMD characteristics (composition, morphology, size and age) across the two oceanic regions is a conducive environment for consistent components across the complex plastisphere assemblages. The PMD core microbiome was dominated by *Alphaproteobacteria* (45% of the shared PMD OTUs across the two oceanic regions), *Cyanobacteria* (29%) and *Bacteroidia* (15%). *Rhodobacteraceae* were particularly well represented (25% of the PMD community on average in the two regions) with *Tateyamaria* sp. already isolated in the plastisphere of several regions of the world, such as the North Atlantic, the North Pacific, the North Sea, in estuaries or in the Baltic Sea (Rogers et al. 2020). The *Rhodobacteraceae* has been described as a key taxonomic group in marine biofilm formation, as they were classically found as primary colonizers and the extracellular polymeric substances (EPS) they produce promotes the further attachment of other microorganisms (Dang et al. 2013, Elifantz et al. 2013). It has previously been suggested that these specific bacteria colonizing a virgin plastic surface might have a distinct advantage over other microbial lineages over a given period of time (Zettler et al. 2020). Despite to other taxonomic groups of primo-colonizers that generally do not remain during the different phases of the mature biofilm formation (Odobel et al. 2021), *Rhodobacteraceae* have been repeatedly found in the marine plastisphere (Zettler et al. 2020), thus suggesting that this taxonomic group may remain for longer period of time than other primo-colonizers. It is probably due to the genome plasticity of this taxonomic group that might explain its adaptability and very versatile physiology to dwell in a wide variety of plastic biofilms (Simon et al. 2017). Marine *Rhodobacteraceae* are one of the major subdivisions of *Alphaproteobacteria* and they are highly abundant in the pelagic zone and in algae-associated biofilms, thus playing a crucial role in the cycling of biogeochemical elements (Elifantz et al. 2013). Members of the family *Rhodobacteraceae* are also known to break down hydrocarbons in environmental settings, such as post Deepwater Horizon (Jin et al. 2012). This is not the first study to identify hydrocarbonoclastic bacteria in the plastisphere, a finding which suggests that such group may be involved in the biodegradation of petroleum-based plastics (Zettler et al. 2013). Further studies are needed to evaluate if plastic biodegradation capabilities may be part of the selection processes and community dynamics that influence the fate of plastic-specific bacteria in marine ecosystems.

The PMD core microbiome was also dominated by *Cyanobacteria* (13% of the shared PMD OTUs across the two oceanic regions). *Phormidium* and *Rivularia* (mainly *Calothrix* sp.) dominated this photoautotrophic group, as previously found in other plastisphere communities (Zettler et al. 2013; Bryant et al. 2016; Dussud et al. 2018a). Such finding is of great interest

according to the recent demonstration of high autrotrophic activity on plastics, which can support the bacterial carbon demand needed for heterotrophic activities (net autotrophy) (Bryant et al. 2016). These results confirm the potential role played by the plastisphere in the biogeochemical cycles in the context of an increasing number of plastic particles in the marine environment (Conan et al. 2022). Interestingly, we also found almost 17% of potentially hydrocarbonoclastic bacteria in the PMD core microbiome, including well-known linear or branched hydrocarbon degraders such as *Alcalinovorax* sp. and *Marinobacter* sp. (Gutierrez 2019). However, similar percentages were also found in FL and PA core microbiome, underlining their ubiquitous presence in our samples. This was also the case for putative pathogens, even if OTUs belong to *Vibrio* sp. (including *V. parahaemolyticus, V. anguillarum, V. harveyi, V. pectinicida, V. xiamenensis*) were 3 to 32 times higher on PMD compared to FL and PA in both regions. Mechanism for the persistence and spread of potential pathogens such as *Vibrio* bacteria remain unknown (Amaral-Zettler et al. 2015). Because the metabarcoding approach is not an appropriate method for describing bacterial virulence, more in-depth studies are needed to assess the pathogenicity of the OTUs identified in this study.

4.5. Ocean-specific PMD communities identified through co-occurrence network analysis

With the goal to identify PMD-specific communities, we inferred a cross-ocean co-occurrence network of OTUs from PMD samples. The analysis of this network using a community detection algorithm revealed communities specifically associated to plastic particles in both the Pacific Ocean and the Mediterranean Sea. While a specific community was identified as enriched in abundance in the Pacific samples (Module 1), distinct from the community enriched Mediterranean samples (Module 2), both these communities (or modules) were detected in all PMD samples and harbored similar functional potential with specific enriched predicted functions (as discussed below). A specific signature of Module 1 (Pacific enriched) was the higher prevalence of associations between *Alphaproteobacteria* and *Bacteroida*, while Module 2 (Mediterranean enriched) had a distinctive signature with a specific enrichment of associations between *Cyanobacteria* and *Alphaproteobacteria* as well as *Bacteroida*. These results confirm previous observation of the importance of *Cyanobacteria* in the plastisphere bacterial communities classically found in the Mediterranean Sea (Dussud et al. 2018, Zettler et al. 2021). Nitrogen-fixing cyanobacteria are playing key roles in the primary production of organic matter, especially in an oligotrophic environment like the Mediterranean Sea (Le Moal and Biegala 2009; Uysal and Koksalan 2006). A recent study underlined the strong relationships between microbial activities in the Mediterranean plastisphere by primary

producers and heterotrophic bacteria (Conan et al. 2022). This type of relationship might explain the co-occurrence of both autotrophic *Cyanobacteria* and heterotrophic *Alphaproteobacteria* and *Bacteroida* found in the Module 1. Causal effect between *Cyanobacteria* and eukaryotic dinoflagellates was also previously demonstrated in the plastisphere from the Mediterranean Sea, a pairing that may be related to nitrogen-fixing capacity in the cyanobacterium in oligotrophic environment (Zettler et al. 2021). Overall, this cross-ocean co-occurrence network analysis thus revealed the relative contribution of specific PMD-specific communities across samples, but also likely underlined the need for further studies to better understand the importance of co-occurrence associations in the role of the plastisphere in global biogeochemical cycles.

4.6. Functional potential and redundancy in PMD compared to FL, PA lifestyles in both oceanic regions

Finally, we explore the functional redundancy of the plastisphere in comparison to FL and PA lifestyles in the two oceanic regions. Functional redundancy has been approached by prediction of microbial metagenomes in FL or PA against PMD lifestyles and across the two oceanic regions. PICRUST analysis highlighted the dominance of a set of putative functions on PMD compared to FL and PA lifestyles. Although these comparisons were based purely on predictions made from 16S rRNA gene sequences (Langille et al. 2013; Louca et al. 2016), we found that several important function in the carbon biogeochemical cycle, biofilm formation, microbial turnover and nutrient efficiency were overrepresented functional categories on PMD compared to FL and PA. The dominance of photosynthesis and heterotrophic metabolism (carbohydrate metabolism, amino acid, vitamin metabolism) on PMD are consistent with the role played by the plastisphere in the biogeochemical cycles in the context of an increasing number of plastic particles in the marine environment (Bryant et al. 2016). Another prediction of overrepresented functional categories on PMD compared to FL and PA was also plausible: the membrane transport functions and the ABC transporters, as well as the cellular processes and signal transduction were previously described as essential functions in biofilm formation and maturation (Lasa and Penadés 2006; Porter et al. 2011; He and Bauer 2014). Other metabolic pathways, including nitrogen and sulfur metabolism were also enhanced in PMD, which is in accordance with recent findings showing that microbial turnover and nutrient efficiency was enhanced in the plastisphere (Zhou et al. 2021). These all suggested that plastisphere communities could participate in a large number of important ecological processes

in the marine ecosystem (Bhagwat et al. 2021). Interestingly, our study does not put into evidence the enhanced metabolic pathways belonging to xenobiotics biodegradation in the plastisphere, as compared to FL and PA lifestyles. Previous studies underlined the presence of various contaminants sorbed on PMD surface, including persistent organic pollutants (POPs) and heavy metals (Alimba and Faggio 2019). We also observed the presence of putative xenobiotic degradation genes on PMD, as well as the conservative presence of well-known linear or branched hydrocarbon degraders such as *Alcalinovorax* sp. and *Marinobacter* sp. (Sekiguchi et al. 2011). However, our study suggests that the plastisphere does not suffer from more environmental stress compared to the surrounding seawater.

5. Conclusion

The clear niche partitioning together with the description of a core microbiome that could be characterized at the ocean-basin-scale have important implications for understanding microbial interactions with plastic on a global scale. Autotrophic and heterotrophic organisms were always found on PMD in the two oceanic regions, thus reinforcing the importance of future studies exploring the relative importance of the plastisphere on the carbon sink (absorbing carbon dioxide from the atmosphere) or source in the Ocean. Our functional predictive analysis also revealed the importance of nitrogen and sulfur metabolisms on microplastics that open new questions on the role of the plastisphere in a large number of important ecological processes in the marine ecosystem. Finally, our finding that Pacific and Mediterranean plastisphere communities are distinct from each other allowed us to statistically prove the importance of environmental selection, rather than polymer type or dispersal effects, in driving changes in plastisphere communities at the Ocean-basin-scale. It opens new routes for further studies on our ability to predict the plastisphere community responses to environmental changes.

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Declarations

-Ethical Approval: This article follows the Committee on Publication Ethics (COPE) guidelines. including the ethical responsibilities of the authors. The authors declare that they obtained study-specific approval from the appropriate ethics committee for the research content of this article.

-Consent to Participate: All the authors agreed to participate in coauthorship. The authors have no competing interests to declare that they are relevant to the content of this article.

-Consent to Publish: All the coauthors agreed with the content of this article, and they all provided explicit consent for submission. The authors obtained consent from the responsible authorities at the institute/organization where the work was carried out before the work was submitted.

-Author Contributions (CRediT taxonomy): Justine Jacquin: Formal analysis, Investigation, Methodology, Visualization, Writing - Original Draft, **Marko Budinich:** Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing, **Samuel Chaffron:** Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing, **Valérie Barbe**: Formal analysis, Visualization, Writing - review & editing, **Fabien Lombard:** Conceptualization, Funding acquisition, Investigation, Writing - review & editing, **Pedrotti Maria-Luiza:** Conceptualization, Funding acquisition, Project administration, Investigation, Writing - review & editing, **Gabriel Gorsky:** Conceptualization, Funding acquisition, Project administration, Investigation, Writing - review & editing, **Alexandra ter Halle:** Visualization, Writing - review & editing, **Stephane Bruzaud:** Visualization, Writing - review & editing, **Mikaël Kedzierski:** Formal analysis, Methodology, Visualization, Writing - review & editing, **Jean-François Ghiglione:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - original draft, review & editing.

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in the current study are available upon reasonable request.

Supplementary Text, Tables and Figures

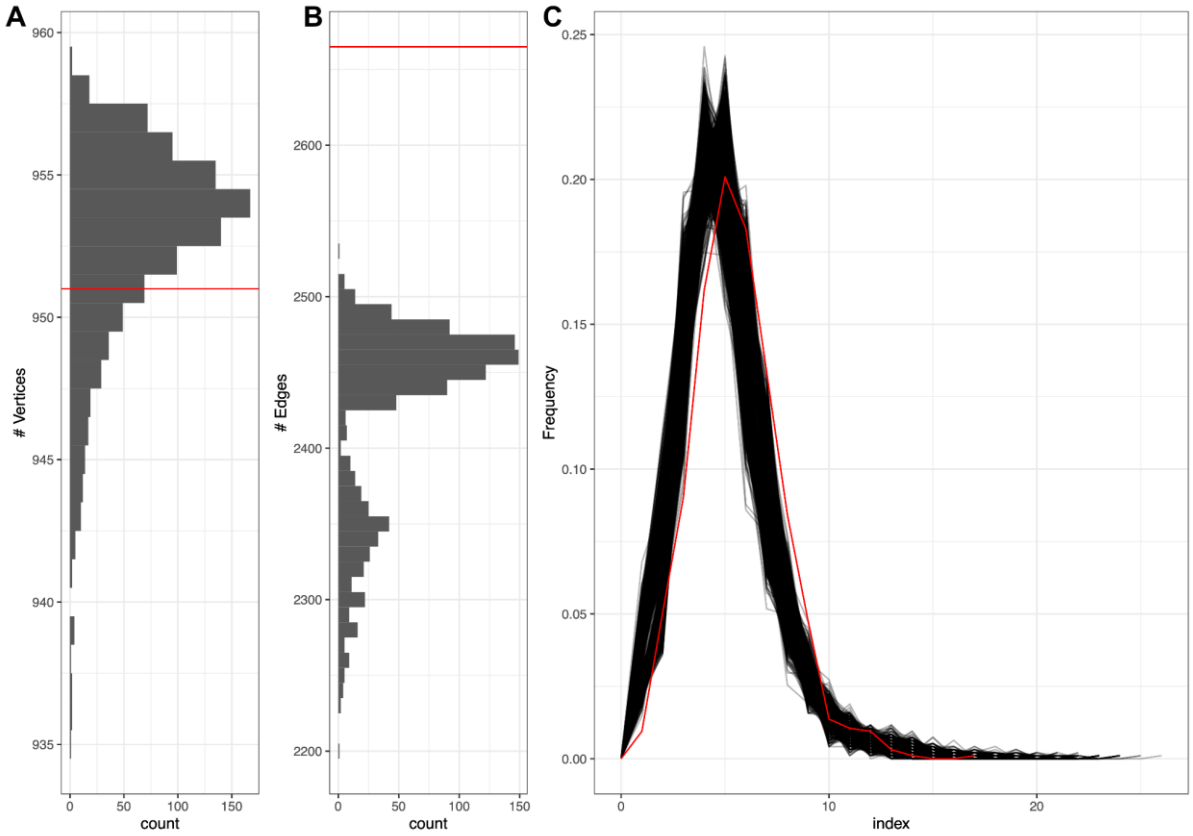
1. ***Supplementary Table 1.*** *Analysis of similarities test (ANOSIM, R and p-values) to evaluate if differences of bacterial community structure were significantly related to different locations (Pacific vs. Mediterranean) and type (FL, PA, PMD).*

Location_1	Type_1	Location_2	Type_2	ANOSIM	p-value
Pacific	FL	Mediterranean	FL	0.4066	0.004
Pacific	FL	Pacific	PA	0.7191	0.001
Pacific	FL	Mediterranean	PA	0.6211	0.001
Pacific	FL	Pacific	PMD	0.9933	0.001
Pacific	FL	Mediterranean	PMD	0.8165	0.001
Mediterranean	FL	Pacific	PA	0.7427	0.001
Mediterranean	FL	Mediterranean	PA	0.5333	0.001
Mediterranean	FL	Pacific	PMD	0.9544	0.001
Mediterranean	FL	Mediterranean	PMD	0.7136	0.001
Pacific	PA	Mediterranean	PA	0.4253	0.001
Pacific	PA	Pacific	PMD	0.8711	0.001
Pacific	PA	Mediterranean	PMD	0.6720	0.001
Mediterranean	PA	Pacific	PMD	0.9178	0.001
Mediterranean	PA	Mediterranean	PMD	0.6204	0.001
Pacific	PMD	Mediterranean	PMD	0.4761	0.001

2. Supplementary text and Supplementary Figure 1

Biases due to sample imbalance in Network Construction

To check biases due to imbalanced sampling between the Mediterranean Sea (n=114) and the Pacific Ocean (n=104), we reconstructed 1000 networks by randomly selecting 75 Pacific samples and compared them with the network obtained using the entire PMD dataset (**Figure 1**). We observed that using the entire dataset inferred approximately the same number of nodes (**Figure 1 A**) while ~10% more associations (**Figure 1 B**). These extra associations did not impact the network structure measured by its degree distribution (**Figure 1 C**), so we concluded that using the entire PMD dataset network allowed us to gather more information without affecting ecological interpretations. Therefore, the network derived using the entire dataset was used in the analyses.

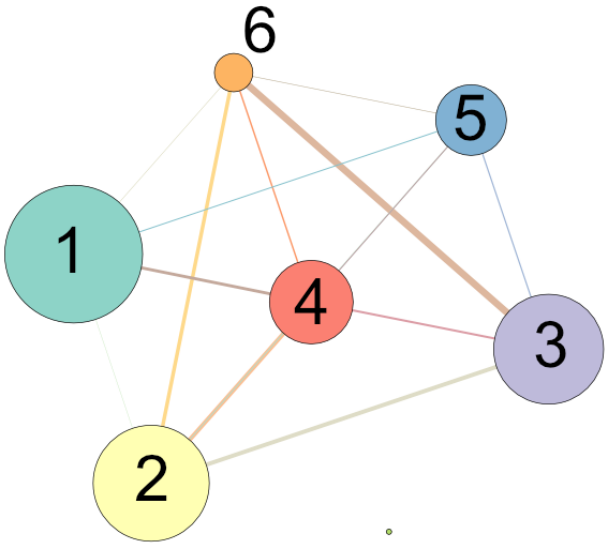


Supplemental Figure 1. *Simulation analysis for network reconstruction. The red line in panels A, B and C shows the value obtained for the network containing all the samples. A) Number of vertices (i.e., nodes) in the 1000 network dataset. B) Number of edges (i.e., associations) in the 1000 network dataset and C) Degree distribution in the 1000 networks*

2. Relative abundance of modules in samples and co-occurrences between modules

Supplementary Table 1. *Wilcoxon tests on Mediterranean / Pacific prevalence based on CLR values on PMD Samples. Q.value correction using FDR.*

module	p.value	q.value
1	4.44E-17	3.11E-16
2	7.03E-15	2.46E-14
3	2.71E-04	6.33E-04
4	9.69E-01	9.69E-01
5	7.60E-01	8.87E-01
6	9.80E-03	1.71E-02
7	2.44E-01	3.41E-01



Supplemental Figure 2. Links between modules. The entire network was contracted into modules and represented as a graph. Node size is proportional to the number of OTUs in each module, whereas edge size between nodes is proportional to the number of co-occurrences between OUTs in each module

3. Predictive metagenomic analysis

Supplemental Figure 3: *Relative abundance of L3 pathways within L2 and ocean-niche groups.*