RESEARCH ARTICLE

Vibrio spp and other potential pathogenic bacteria associated to microfibers in the North-Western Mediterranean Sea

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Abstract

Microfibers, whether synthetic or natural, have increased dramatically in the environment, becoming the most common type of particles in the ocean, and exposing aquatic organisms to multiple negative impacts. Using an approach combining morphology (scanning electron microscopy-SEM) and molecular taxonomy (High-Throughput DNA Sequencing- HTS), we investigated the bacterial composition from floating microfibers (MFs) collected in the north-western Mediterranean Sea. The average number of bacteria in 100 μm^2 on the surface of a fiber is 8 ± 5.9 cells; by extrapolating it to a whole fiber, this represents 2663 ± 1981 bacteria/fiber. Attached bacterial communities were dominated by Alteromonadales, Rhodobacterales, and Vibrionales, including the potentially human/animal pathogen *Vibrio parahaemolyticus*. This study reveals a high rate of bacterial colonization on MFs, and shows that these particles can host numerous bacterial species, including putative pathogens. Even if we cannot confirm its pathogenicity based only on the taxonomy, this is the first description of such pathogenic *Vibrio* living attached to MFs in the Mediterranean Sea. The identification of MFs colonizers is valuable in assessing health risks, as their presence can be a threat to bathing and seafood consumption. Considering that MFs can serve as vector for potentially pathogenic microorganisms and other pollutants throughout the ocean, this type of pollution can have both ecological and economic consequences.

1. Introduction

Plastics are synthetic organic polymers whose mass production began in the 1950s, and has grown from 1.5 million tons/year to 359 million tons in 2018 [1], with cumulative global production expected to triple by 2050 to 33 billion tons [2]. Due to their versatility, low production cost, and resistance to degradation, plastic became a key product in our society; however, its low degradation rate has become an environmental threat [3, 4]. Plastics are the most abundant contributors to marine litter (60–90%), and in their majority they consist of microplastics

($<$ 5 mm in size) [5, 6]. It is currently estimated that 4.8 to 12.7 Mt of plastic enter the ocean each year [7], with floating plastic concentrations between 1–5 millimeters accounting for 24.4 trillion items, weighing between 82,000 and 578,000 tons [8]. Despite current strategies to reduce plastic pollution, the projected growth in plastic production, and thus plastic waste, exceeds efforts to mitigate plastic pollution [9].

The textile industry is of great economic value where more than half of the world's production is based on synthetic fibers, with polyester, polyamide, acrylic and polyolefin being the most common types, the rest of the production comes from natural fibers with cotton being the most important [10]. Synthetic microfibers are a ubiquitous class of microplastics that can have several origins: they come from household laundry textiles that enter the oceans via urban wastewater treatment plants (WWTPs) or rivers that carry plastic waste from inland [11, 12], through atmospheric deposition or aerosols [13–15], as well as derived from fishing activities [16].

Microfiber pollution is widespread in coastal and offshore surface waters in all ocean basins; synthetic fibers account for up to 50% of fibrous items; the remainder are natural fibers such as cotton and wool [17–19]. Whether synthetic or natural polymers, their release into the environment has become an emerging pollution concern because organisms are exposed to this mixture on a daily basis, and little is known about the degradation of MFs in the marine environment and how it can pose a potential long-term risk to ecosystems and human health [20, 21]. In addition, synthetic microfibers are now the most common type of anthropogenic particles in the oceans, in some cases accounting for 80–90% of the number of microplastics, with higher concentrations than granules or fragments [22]. Their release into the environment poses a threat to marine ecosystems, as they are the most common type of ingested microplastics [23, 24]. They may be potentially harmful physically and chemically for aquatic environments or the food chain through the release of additives and dyes, with unknown consequences, including for humans [25–27].

Once at the sea, like other microplastics, the fibrous material can be rapidly colonized by microorganisms, such as bacteria or benthic microalgae [28]. The establishment of a biofilm on microplastics, which can appear and smell like food [29] may enhance their ingestion. Several studies have shown that bacterial communities inhabiting plastic on the surface of the oceans differ significantly from bacterial communities in the surrounding water [30–33]. Plastics can also become vectors for potentially dangerous or pathogenic microorganisms, and their impact on marine environments and human health is the subject of numerous studies [34–37].

The Mediterranean is a semi-enclosed sea with a densely populated coastline and high economic activity, tourism, and maritime traffic that result in a significant land-based plastic pollution, which represents over 80% of the marine litter [6, 38]. This sea, although it represents only 0.8% of the world's marine waters, was predicted by global models to account for nearly 7% of microplastic pollution in the global ocean [6]. The Mediterranean also hosts a high level of biodiversity with 17,000 marine species, 28% of which are endemic [39], that are likely to be affected by the presence of plastic debris in all marine compartments [40].

Here we examined the interaction between MFs and the microbial community of the Mediterranean Sea, with focus on Vibrionales. We also discussed the potential role of these fibrous particles, now ubiquitous in the marine environment, as vectors for the spread of potential pathogens such as *Vibrio parahaemolyticus*. This study is part of a larger survey where general information on the abundance of MFs, as well as their chemical composition in the Mediterranean Sea is published in Pedrotti and colleagues [19]; they found, in the same samples we used to study the organisms attached to MFs, that 14–50% of MFs are synthetic fibers. The term MFs used in this study therefore refers to all types of fibers (synthetic or natural).

2. Material and methods

2.1 Sampling collection

Samples were collected aboard the research vessel The Alchemy, an 11 m long sailing ship, in the northwestern Mediterranean Sea, Liguria Sea, during the ECOSEASTEM cruise (February—October 2014) [19]. For this experiment, a total of seven sites were sampled in spring (6–8 May) and summer (5–8 August). Six sites were located along the coastal zone from the Var River, from in front the city of Nice (where the Haliotis waste water treatment plant—WWTP —is located) to the entrance of the bay of Villefranche-sur-mer (Point B). Another sampling station, characterized by low anthropogenic contamination, was located 70 km off the coast (DYFAMED station). Samples were also collected from the urban and treated water of the Nice Haliotis WWTP (Fig 1).

For *in situ* samples, fifty liters of surface seawater were collected with a stainless-steel bucket in each location and filtered through a 20 μm pore sized stainless-steel sieve. Samples were then resuspended in 200 mL of 0.2 μm filtered seawater. A quarter of each sample was used to investigate the microbial diversity associated to MFs by DNA analysis, and another quarter
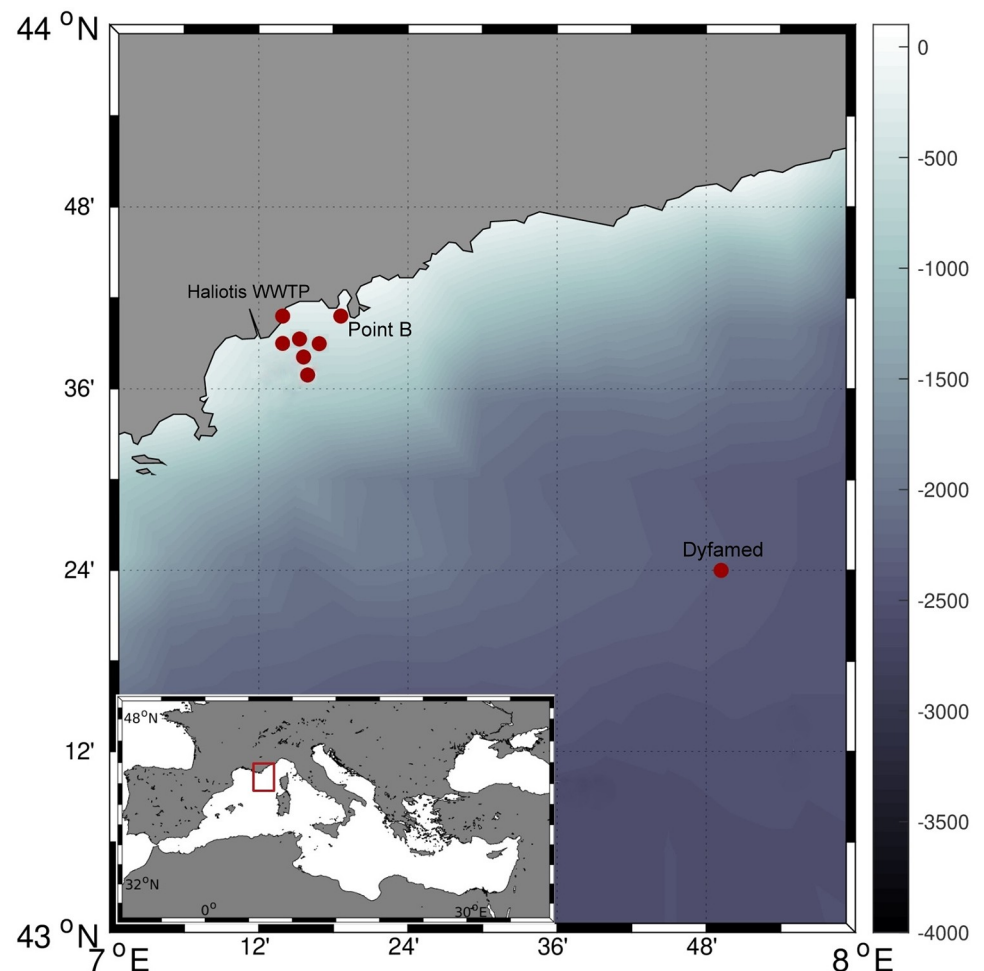

Fig 1. Map of the northwestern Mediterranean Sea where seasonal sampling of MFs and seawater was carried out in the coastal area between Nice and Monaco, as well as in the offshore Dyfamed station, and the Haliotis waste water treatment plant. This map was created at MATLAB software by using the *M_Map* package.

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was destined for image analysis of MFs-attached biota. The remaining subsamples were dedicated to the quantification and characterization of MFs by Fourier transform infrared spectroscopy (ATR-FTIR) in a larger experiment, provided in Pedrotti and colleagues [19].

In parallel, 2 L of surface seawater were sampled to analyze the free-living microbial community. For sampling in the WWTP, three replicates of 2 L of urban and treated wastewater were also collected during the two seasons. All water samples were collected in amber bottles and fixed in 2% formaldehyde.

To minimize contamination, a stainless-steel bucket, held by sheathed steel wire, was used for collecting surface water and, at each sampling point, it was rinsed three times with distilled water. Sampling was carried by placing the bucket on the surface of the water, so that only the top 10 cm was sampled. To avoid cross-contamination, in both in situ sampling and laboratorial manipulation, all material was previously washed with alkaline and acidic detergents, and rinsed three times before each handling with 0.2 μm milli-Q microfiltered water.

2.2 Scanning electron microscopy (SEM) & image analysis

SEM images were obtained to quantify and evaluate the morphology of microbial organisms on MFs. Seawater was filtered through 0.2μm-pore-size Anodisc membrane (aluminumoxide, 47mm). The filters were dehydrated with alcohol (1 rinsing for 10 min at 70%, rinsing 10 min at 96% and 3 rinses 10 minutes with absolute ethanol) and fixed under a chemical hood with Hexamethyldislazan (HDMS). Analysis was performed with a JEOL6700F field emission gun SEM equipped with a cryo-fracture platinum Gryan Alto 2500 and a YAG Autrata backscattered electron detector (X25-X20 000). Prior the analyzes, the fiber length was measured using a stereomicroscope (Zeiss Discovery V12 SZX10) and ImageJ v.1.5 software, and benchmarks were sheared on the filter to first identify the MFs to allow their identification under a scanning microscope. The number of bacteria attached to the fibers was calculated based on the SEM images. For this, bacteria were counted in several fields of 30 MFs using ImageJ v.1.5 software. In order to report the bacterial abundance on the surface of a fiber, we considered it as a cylinder without the spherical ends. Considering the median length (601 μm) and the median diameter (18μm) of the fibers analyzed, this represented an average scanned area of 33986 μm^2.

2.3 Samples processing prior DNA extraction

In the laboratory, the resuspended sieved sample represented the community associated with the MFs (MF fraction). To study the microbial diversity of free-living community, the seawater was filtered through 0.2μm-pore-size filters (47 mm diameter, polycarbonate, Nuclepore). For wastewater, 100 ml of inlet samples and 300 ml of outlet samples were resuspended through the 20μm sieve, and filtered through 0.2μm-pore-size filters (47 mm diameter, polycarbonate, Nuclepore). Filters were stored at -80˚C until DNA extraction.

2.4 DNA extraction, PCR and high-throughput sequencing

DNA extraction was performed using a thermo-saline lysis protocol [41]. The partial 16S rRNA gene was amplified by PCR using primers 357F and 907R, which amplify the V3-V5 hypervariable regions. The molecular size and the purity of the DNA extracts were analyzed by agarose gel electrophoresis (1%). PCRs were carried out in 50μl reactions using 0.5–5 μl of DNA template, 2 mM MgCl$_2$, 0.25 μM of each primer (forward and reverse), 0.25 mM dNTP and 1.25 U Taq polymerase, completed with ultrapure water. Negative controls containing ultrapure water instead of DNA template were performed at all PCR steps. Amplifications

were confirmed by agarose gel electrophoresis run. DNA sequencing was carried out with the Illumina MiSeq by Research and Testing Laboratory (Lubbock, Texas).

2.5 Sequence data and diversity analysis

Paired-ends raw reads (2 x 250) were merged, quality-filtered and assigned to taxa after primers trimming, sequence clustering and chimera checking using the Mothur pipelines [42]. Clusters were assigned with the Silva 128 16S rRNA database [43] and clusters that did not belong to Bacteria kingdom were removed, as well as chloroplast and mitochondrial sequences. Operational Taxonomic Units (OTUs) were defined as clusters sharing 97% of sequence identity. The taxonomy assignments were completed using the SILVA v.128 database (https://www.arb-silva.de/documentation/release-128/). Bacterial sequences were randomly resampled in the OTU file to enable comparison between samples, by normalizing the number of sequences between samples to the sample with the fewest sequences (n = 468) using Mac-Qiime 1.9.0 (single_rarefaction.py). All further analyses were performed on the randomly resampled OTU table.

OTUs richness was estimated by a non-parametric estimator of Chao1. The Jaccard dissimilarity matrix was used to visualize patterns in the community composition [44] by producing a Principal Coordinates Analysis (PcoA) plot among all samples [45]. Statistical analyses were done with the *vegan* package [46] in *R studio* 1.1.456 (R Development Core Team). The *ggplot2* package [47] was used in *R studio* to build boxplots with number of OTUs between MFs and seawater samples, as well as the PCoA plots.

3. Result and discussion

3.1 Morphological analysis of microfibers and their associated organisms

Optical microscopy of MFs showed a variety of MFs in all samples, in different sizes, thicknesses and colors, with an average length of 939 ± 1011 (size range from 52 to 6018 µm (Figs 2A and 2B, S1). SEM analysis showed that many MFs presented signs of degradation, including cracks and pitting. Bacterial cells were the most commonly associated organisms with the MFs. Although we also have observed many diatoms, it was not possible to estimate their abundance on MFs with the magnifications we used to capture the images, due the large size range of this group. Extracellular Polymeric Substances (EPS) were regularly observed (Fig 2C–2F). The average number of bacteria on the surface of a fiber was 8 ± 5.9 cells 100µm^{-2}; extrapolating it to an entire fiber, this represents 2663 ± 1981 bacteria per fiber, revealing a strong bacterial presence on MFs. To date, only a few studies have quantified the surface area colonized by bacteria on microplastics in the marine environment [48, 49]. Our counts, although on different types of MFs and not only microplastics, yielded results in the same range as the above-mentioned studies in the Mediterranean, respectively 0.5 and 4.4 cells 100µm^{-2}.

3.2 Description of microfibers-associated bacteria

We found a total of 195 bacterial OTUs belonging to twelve phyla and one unclassified bacterium. PCoA analysis showed clear distinctions between the bacterial community structure attached to MFs in comparison to seawater and the WWTP samples (Fig 3). WWTP samples were dominated by *Cloacibacterium normanense* (Bacteroidetes; Flavobacteriia) and *Arcobacter cryaerophilus* (Proteobacteria; Epsilonproteobacteria), both representing less than 0.01% of bacteria we found attached to MFs or seawater samples. Previous studies showed significant differences between bacterial assemblages on microplastics, borosilicate spheres, and bacterial
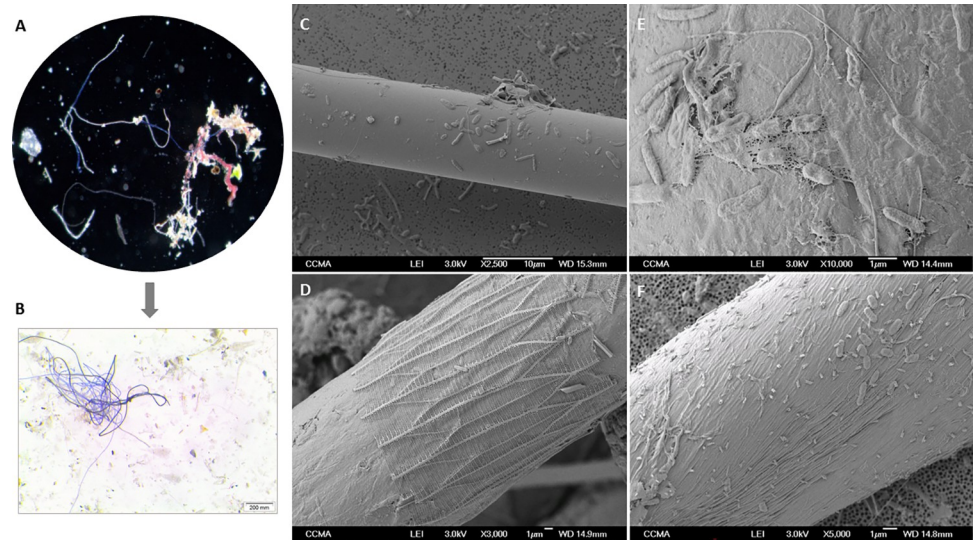
Fig 2. Optical microscopy of floating fibers sampled at the Mediterranean Sea (A, B), and Scanning Electron Microscopy (SEM) images of their attached bacteria, with elongated and rounded cells, as well as Extracellular Polymeric Substances (EPS) (C-F).
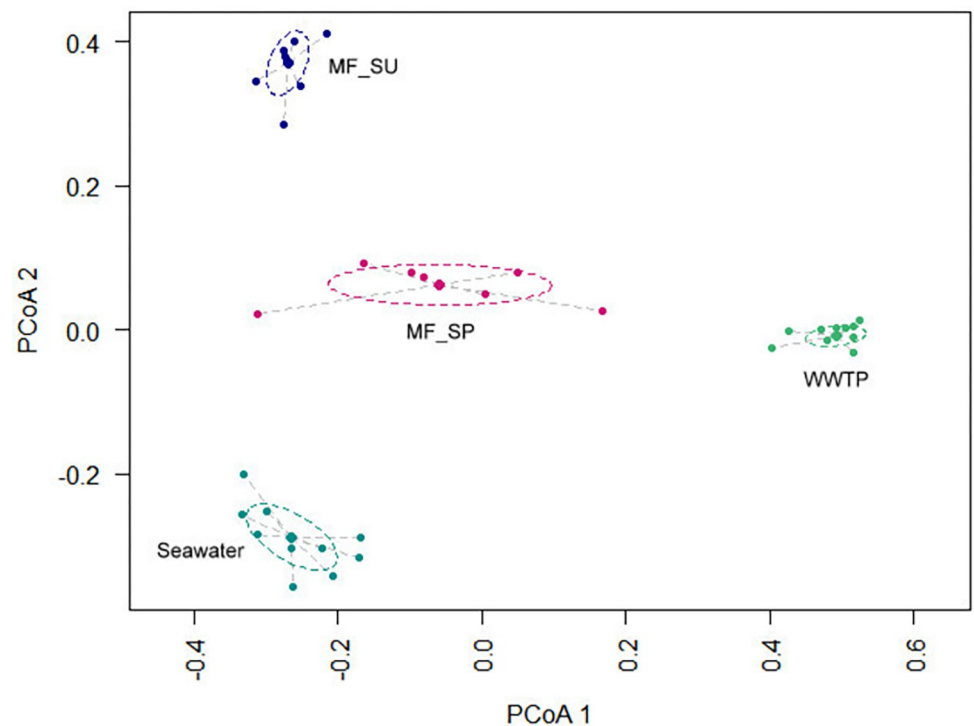
Fig 3. Principal coordinate analysis (PCoA) plot showing dissimilarities among the bacterial community composition attached to microfibers from summer (MF_SU) and spring (MF_SP) seasons, the free-living bacteria from the Seawater and from the Haliotis Waste Water Treatment Plant (WWTP). Samples were grouped based on Jaccard distance matrix.
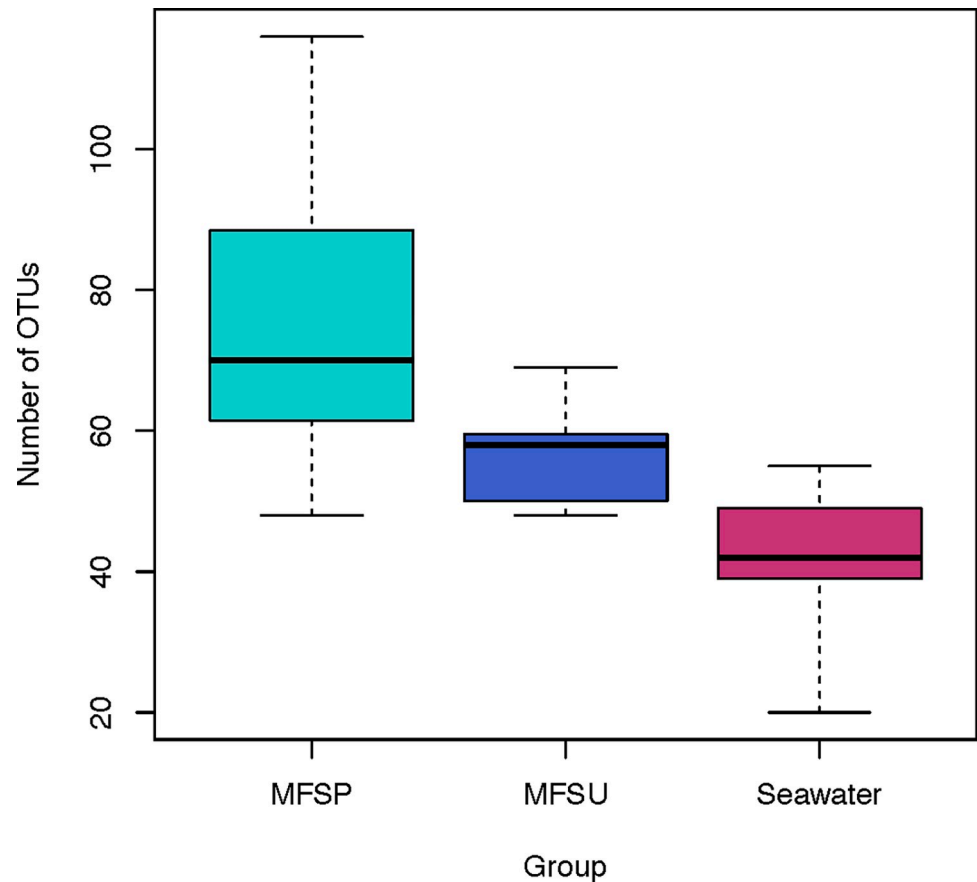
Fig 4. Number of observed OTUs per group of microfibers from spring (MFSP) and summer (MFSU) seasons, as well as from the Seawater, obtained from 16S amplicon sequence library, with significant difference between microfibers and seawater samples (Kruskal-Wallis, p < .01).

communities in WWTP effluent water [50]. The presence of freshwater in WWTP, in opposition of the salt water found in coastal regions, could be the main factor distinguishing these populations. For that reason, we choose to exclude WWTP samples from further analysis as the description of WWTP bacteria was not the main goal of the present study.

The OTUs richness among MFs and seawater samples was significantly different (Kruskal-Wallis, p < 0.01), being higher on MFs (Fig 4). Free-living bacterial community was dominated by (Alpha) Proteobacteria, Bacteroidetes, and unclassified Cyanobacteria, divided into eight OTUs that together represented 84% of free-living prokaryotes, all of them being frequent in all seawater samples. *Candidatus Pelagibacter* was the most abundant species (relative abundance of 28%). This is a ubiquitous bacteria member of the SAR11 clade, and indeed is one of the most abundant microbial plankton cells [51].

MFs-attached bacteria showed a different community composition, dominated by eight (Gamma or Alpha) Proteobacteria OTUs that represented 80% of the prokaryotes associated to MFs. *Alteromonas* sp. (Gammaproteobacteria) was the most abundant OTU (48% of reads), frequent in all MFs samples. This taxon is commonly found as the most abundant OTU in the marine plastisphere worldwide [45]. In addition, marine *Alteromonas spp* exhibit algicidal activity, which may attack cells and kill or lyse nearby microalgal cells [51]. In our study area, the putative pathogen *Vibrio parahaemolyticus* was the second most abundant OTU (7%)
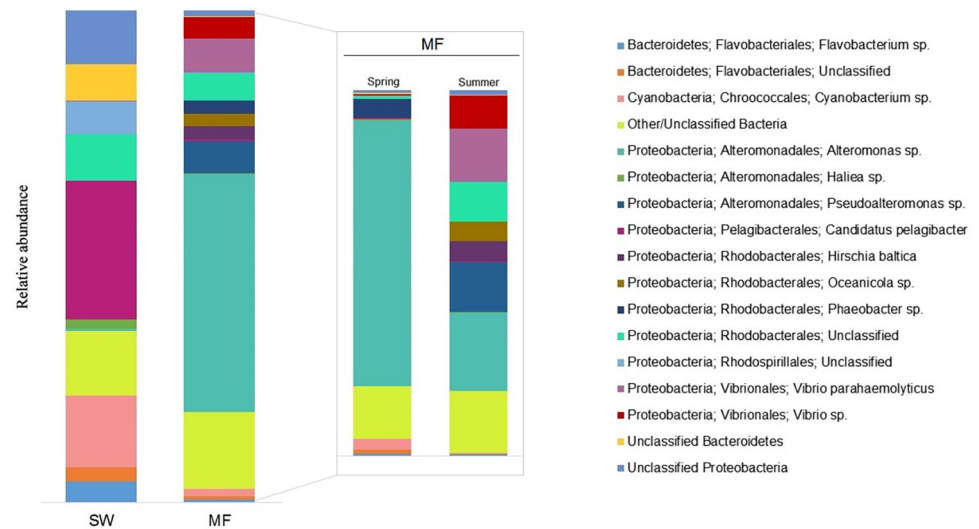
Fig 5. Bar chart showing taxonomic composition and dominance of a relatively small number of abundant OTUs, for seawater (SW) and floating microfibers (MF) in the Mediterranean Sea. Similarities between microfibers collected during spring and summer seasons are detailed. For more clarity, OTUs representing less than 1% or unclassified at the class level are grouped in the Other/Unknown series.

associated to MFs, with frequency of occurrence (FO) of 71% in MFs samples. Another *Vibrio* sp. was also highly frequent (FO 86%), even with low abundance (4.4%).

Comparison between summer and spring seasons (Fig 5) showed that four individual OTUs contributed to 68% of the dissimilarity among the MFs samples, with *Alteromonas* sp. dominating in the spring, while during the summer *Pseudoalteromonas* sp. and *Vibrio parahaemolyticus* were more representative. Considering the relevance of this taxon, we discuss the *Vibrio* spp. in a separate section hereafter. *Alteromonas* sp. was previously identified as an organic particle-attached group [52], whereas *Pseudoalteromonas* sp. is frequently associated to marine algae [53]. These associations support the fact that the plastisphere may be a self-sufficient ecosystem [54], with many ecological relations among its members that include symbionts, saprotrophs and parasites [55]. The communities we have identified living on MFs in the Mediterranean Sea form distinct groups in relation to the sampling seasons (summer and spring) (Fig 4). This is in accordance to what has been demonstrated in other regions [56–58] to plastisphere organisms, as they are highly dependent on environmental factors [28].

Other groups of bacteria found attached to MFs belong to the genera *Marinobacter* (FO 71%), *Pseudomonas* (FO 50%), *Acidovorax* and *Clostridium* (FO 43% each), *Acinetobacter* and *Comamonas* (FO 29% each), some of them described as able of biodegrading various types of polymers [59]. For example, a *Pseudomonas* strain has been shown to have enzymes such as monooxygenase that play a central role in polyethylene degradation [60]. The richness of OTUs estimated by Chao 1 shows that a very high diversity of bacteria exists on MFs, highlighting a vast and largely unknown functional potential. Although our data indicate the presence of these taxa associated to MFs in the Mediterranean, they do not allow us to assess the functionality of their genes. The identification of mechanisms involved on pathogenicity and biodegradation of synthetic polymers is therefore a priority issue [61].

Many bacteria found associated to MFs in the Mediterranean Sea were previously identified as potential animal and/or human pathogens, such as *Tenacibaculum*, *Vibrio* and *Pseudomonas* species [62]. These groups were also described as part of the marine plastisphere in the North/Baltic Seas [33, 63, 64], South Atlantic [65] and North Pacific [31] oceans. As

mentioned above, the presence of *Vibrio* has been confirmed in various sampled fibers recovered in your sampling, which included both synthetic and natural fibers. Although our results are not exclusively related to synthetic fibers, they do reinforce the role of plastics, including synthetic microfibers, in harboring potentially harmful organisms.

3.3 Vibrio spp. associated to microfibers in the Mediterranean Sea

To date, only a few studies have reported bacteria that could be a putative human pathogen on MFs or other types of microplastics in Mediterranean waters [49, 54]. This study is the first to describe V. *parahaemolyticus* on floating MFs at the region. Members of the genus *Vibrio* were also found on plastic debris at a Mediterranean beach (Calvi, Corsica) with the pathogenic *V. splendidus* accounting for up to 70% of the total reads [54]. Other studies detected *V. parahaemolyticus* attached to microplastics, one in the South Atlantic Ocean [65], and another in the North Sea/Baltic Sea [35], including polyethylene fibers, polyethylene films and polypropylene fragments. Kirstein and colleagues [35] also identified this species in the surrounding water where microplastics were sampled, thus suggesting that the seawater could be a potential reservoir of *Vibrio* species. Recently, Kesy et al. [64] showed that the colonization of plastics by *Vibrio* sp. was observed within the first hour of exposure of these materials *in situ*, highlighting that *Vibrio* sp. are amongst the very first colonizers on plastics.

In our survey, this putative pathogen represented around 6% of the total community in the MFs fraction, mostly present during the summer, and it counted for 28% of reads in a single sample from a coastal station; this taxon was not found in the seawater samples from the offshore Dyfamed station, and it was found in a very low abundance attached to the MFs (0.01%) from Dyfamed, emphasizing that the risk of contamination may be higher in areas of high anthropogenic impact. In the Baltic Sea, a positive correlation was found between *Vibrio* abundances and the presence of cities with more than 100,000 inhabitants [64]. *Vibrio parahaemolyticus* can potentially infect humans when ingesting raw or partially cooked shellfish [66] This is noteworthy since *Vibrio* spp are rarely found in concentrations that can account for more than 1% of the community attached to plastics [67].

Other potentially pathogenic *Vibrio* species not related to human diseases were observed on microplastics in the Mediterranean Sea, such as *V. anguillarum*, *V. harveyi*, *V. pectinicida*, *V. xiamenensis* but again as a low percentage of the community (< 0.1%) [48]. In our samples, *Enterovibrio calviensis* (first described in the Mediterranean as *Vibrio* sp.) was frequent in all MFs samples, in low abundances, but this is a species resistant to antibiotics (lincomycin, oxacillin and spectinomycin), and a facultative anaerobe, able to reduce nitrate to nitrite [68]. Vibrionales are generally known to express antagonistic activities, being the most prolific producers of inhibiting materials but also the most resistant to them [69]. In addition, some *Vibrio* species are capable of degrading toxic polycyclic aromatic hydrocarbons (PAHs] in polluted marine sediments [70]. The evaluation of the potential virulence of environmental strains of *Vibrio parahaemolyticus* has shown that they have the ability to regulate newly acquired virulence factors, e.g. in response to the temperature, but the ability to adapt to a human host environment has not yet been demonstrated [71]. Although abundant in the Mediterranean Sea and occurring in many areas in the Baltic Sea, metaproteomic analyses have shown that, in the Vibrionaceae family, proteins related to virulence processes are not very active [54, 72].

Furthermore, while it is now established that there is a specific microbiome growing on plastics that differs from the seawater (free-living and associated with organic particles), there is less evidence regarding the differences between natural and synthetic substrates [67]. A study of three marine ecosystems under various environmental conditions found that a large proportion of the OTUs present on plastics were absent in non-plastic particles and in the

seawater [45]. Regarding *Vibrio* sp, they are known to be associated with various natural substrates such as wood, cellulose or glass, and their abundance appears to be low on plastic debris compared to natural ones [31, 67]. Nevertheless, in our study, we were able to confirm the presence of *Vibrio* in the MFs, although it cannot be established directly on synthetic or natural fibers. A recent meta-analysis of several environments shows that the variety of potentially pathogenic species found on microplastics is comparable to natural particles [37]. Many questions remain open as to whether, rather than a selection of distinct microbial colonizers, persistent plastic debris could lead to sustain selection of biofilms, thereby increasing the risk of pathogen transport and disease occurrence [37].

3.4 Impacts of microfibers and their attached bacteria in the Mediterranean Sea

In a global fiber study covering surface waters of six ocean basins, the Mediterranean Sea had the highest concentrations (median of 4.2 fibers L^{-1}) [18]. The median concentration of synthetic microfibers in the western basin was even higher (10.7 fibers L^{-1}), as the characterization by ATR-FT-IR revealed that they represent 14–50% of the raw materials [19]. This high concentration of fibers could be related to the higher density of Mediterranean waters (generally >1.026 g cm^{-3}), which allows fibers such as polyamides, commonly used in the textile industry and fishing (density between 1.02 and 1.15 g cm^{-3}), to remain in the upper layers in higher proportions [15], when compared to other seas [73, 74]. Another explanation is related to their shape, as vertical advection velocities are lower for fibrous microplastics than for sheets and other shapes [75], which may contribute to a longer residence time for these fibrous materials, favoring their transport as potential vectors for organisms, including putative pathogens.

Studies have shown that temperature and salinity affect *Vibrio* species, in particular temperature has a significant correlation with the increase of *Vibrio* spp. [33, 76, 77]. In the Baltic Sea, gradual warming of the water favored the appearance of potentially pathogenic *Vibrio* and the emergence of infections [78]. Higher temperature was also positively correlated with the density of *V. parahaemolyticus* in oysters [66]. A recent study showed that the increase of seawater temperature has an important influence on the adhesion properties of free-living *V. parahaemolyticus* to plastic, with all analyzed factors being transiently expressed in 27˚C and even more upregulated at 31˚C, emphasizing the role of climate change in the spread of this pathogenic bacteria [79]. In the Thau Lagoon (Gulf of Lyon), episodes of massive oyster mortality coinciding with single or double infections involving mainly OsHV-I and *Vibrio splendidus* have been observed when seawater temperature is above 24˚C [80].

Indeed, the temperature during the summer cruise ranged from 25.2 to 26.5˚C. During this season, although low runoff may decrease plastic transport from freshwater systems [81], the population double in this area as this is the touristic season. The western coasts of the Mediterranean are among the most densely populated areas, with population size expected to increase nearly twofold over the next decade [38]. Much of the world's plastic waste enters the oceans at heavily populated coastal sites and near wastewater drainage systems. While the increased spread of potentially pathogenic bacteria on floating plastics is a real threat to low-income countries [82], the presence of putative pathogenic *Vibrio* in a highly persistent (or ubiquitous) anthropogenic particle (that can be transported long distances) in the Mediterranean Sea is also becoming a major environmental concern.

4. Final remarks

Our study shows clear distinctions between bacterial communities on MFs compared to the free-living bacteria inhabiting surface waters in the Mediterranean Sea. It highlights a strong

colonization of MFs by microorganisms and reports the first occurrence of the pathogenic *Vibrio parahaemolyticus* attached to floating MFs, including synthetic ones, in surface waters of Northwestern Mediterranean Sea, especially during the summer. We have shown that microfibers, both natural and synthetic, have the ability to host bacterial species, including potential pathogens, although the comparison is not sufficient to draw conclusions on the enrichment of certain bacterial species on microplastics.

This study raises the question of whether the increasing amount of persistent plastic waste in the environment may influence the dynamics of various hitchhikers offering greater transport opportunities, thus leading to an increased risk of contamination compared to other short-lived natural particles, such as wood or sediments. Considering that synthetic fibers can serve as a vector for potentially pathogenic microorganisms and other pollutants in the ocean, due to their longevity, this type of pollution may have ecological and economic consequences. These results on the characteristics of microbial assemblages are valuable for future assessments of the health risks associated with plastic pollution, as their presence may pose a threat to swimming and seafood consumption.

The Mediterranean is under constant anthropogenic pressure regarding pollution, as well as the consequences of climate change exceeding global trends for most variables, with waters warming faster than the rest of the ocean, especially during the summer months [83]. These changing conditions can lead to shifts in marine microbial community structure, including particle colonizers in areas with high levels of plastic pollution. Further studies are needed to target genes associated with virulence to prevent the spread of diseases. Human discharges of chemicals and plastics into continents and oceans have reached a critical threshold, and plastic pollution meets the criteria for planetary boundary threats [84].

Supporting information

S1 File.
(DOCX)

Acknowledgments

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Supervision: Maria Luiza Pedrotti.

Writing – original draft: Maria Luiza Pedrotti, Ana Luzia de Figueiredo Lacerda.

Writing – review & editing: Stephanie Petit, Jean François Ghiglione, Gabriel Gorsky.

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