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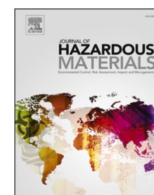


The invisible threats of sunscreen as a plastic co-pollutant: Impact of a common organic UV filter on biofilm formation and metabolic function in the nascent marine plastisphere

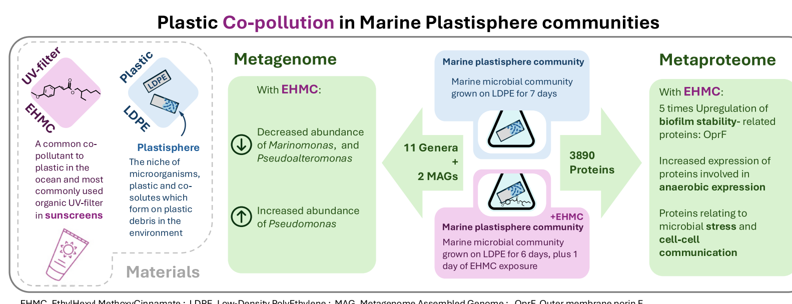
Charlotte E. Lee [a], Lauren F. Messer [a], Ruddy Wattiez [b], Sabine Matallana-Surget [a,*]

[a] Division of Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, Scotland FK9 4LA, UK
[b] Laboratory of Proteomics and Microbiology, Research Institute for Biosciences, University of Mons, Place du Parc 20, Mons 7000, Belgium

HIGHLIGHTS

- First study on how plastic and EHMC sunscreen co-pollution alters biofilm functioning.
- EHMC favours biofilm-forming bacteria and generalists over aerobic hydrocarbonoclastic degraders.
- EHMC upregulates proteins involved in biofilm formation and enhances bacterial anaerobic respiration.
- Biofilm formation may support EHMC resistance and stress adaptation.
- UV-filters may inhibit photodegradation and biodegradation of plastics in the ocean.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic debris in marine environments serves as a substrate for microbial colonisation, forming biofilms known as 'plastispheres'. Also accumulated on plastic debris are co-pollutants including UV-protective organic UV-filters from sunscreens, which likely interact with this niche through their lipophilicity. Despite their widespread use and environmental accumulation, the influence of UV-filters on plastisphere composition and function has never been investigated. This study therefore investigates, for the first time, how co-pollution – specifically by an organic UV-filter – impacts the composition and function of marine plastisphere communities. To achieve this, low-density polyethylene (LDPE) was incubated with marine microbial communities for six days to cultivate a nascent plastisphere, which was then exposed to 5 mg/L of EthylHexyl MethoxyCinnamate (EHMC); the most used organic UV-filter in sunscreens, and a prevalent marine pollutant. Metagenomic analyses revealed that EHMC favoured the growth of bacterial generalists *Pseudomonas* and *Psychromonas* while reducing pollutant-degrading genera like *Marinomonas*. Analysis of 3070 proteins revealed a consistent upregulation of proteins used for biofilm maintenance by *Pseudomonas* with EHMC exposure, including the considerable upregulation of outer membrane porin F (OprF) which regulates exopolymeric substance (EPS) production. Additionally, proteins thought to indicate a shift from aerobic to anaerobic respiration were frequently expressed after exposure to EHMC. This may have selected against the obligate aerobes *Marinomonas* and *Pseudoalteromonas*, contributing to the observed shift in community composition. These findings underscore the importance of considering chemical

* Correspondence to: Division of Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK
E-mail address: sabine.matallanasurget@stir.ac.uk (S. Matallana-Surget).

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co-pollutants in plastisphere research as we now begin to discover how ecologically significant, and potentially harmful microbial genera are affected by this interaction.

1. Introduction

An estimated 19–23 million tonnes of plastic pollution enters the marine ecosystem each year [14]. This ongoing pollution has incentivised thousands of global initiatives aimed at mitigating plastic waste in the ocean [92]. However, as plastic continues to persist in the marine ecosystem, it impacts all domains of marine life, leading to widespread disruption [40]. A notable consequence of this pollution is the formation of the 'plastisphere', the microbial community that colonises plastic surfaces in the ocean [108]. Research has shown that the marine plastisphere modifies microbial community structures, impacting ecology, and produces a number of hydrocarbonoclastic microorganisms which may now be investigated for their bioremediative potential [58]. Studies have also begun to address how the mature plastisphere functions at different locations, on different plastics, and now at different time-points [31,74,75,81]. However, the early-stage or 'nascent' plastisphere presents a unique opportunity for investigation, as its relatively immature biofilm community allows for greater direct interaction between microorganisms and the plastic substrate [59]. This enhanced interaction is hypothesised to increase the likelihood of biodegradation processes compared to mature plastispheres with thicker and more complex biofilm layers [10,36]. Interestingly, compared to biofilms formed on natural substrates, plastisphere communities also closely reflect the broader biofilm-forming microorganisms in the surrounding marine environment [3,81]. These studies therefore provide valuable insight into the behaviour of marine biofilms as a whole, as well as their response to plastic, as a novel marine substrate. However, this niche is also susceptible to further contamination from other pollutants [58].

As of 2024, persistent organic pollutants (POPs) have been recorded at concentrations of up to 820,000 ppb in oceans worldwide [58]. Among these pollutants are organic UV-filters, chemicals commonly found in personal care products (PCPs) from toothpastes, to moisturisers, to sunscreens as preservatives and agents used to protect skin from the sun [22]. With growing awareness of the harmful effects of UV exposure [103,33], demand for products containing UV protection has grown exponentially [83]. However, the frequent use of these PCPs, especially during activities like swimming, directly exposes marine life to these pollutants at the coast, while its predicted content in municipal runoff further pollutes the ocean in these areas [51,63]. Indeed, wastewater effluent loadings of organic UV filters are even recorded to reach levels as high as 800 ng/day/1000 population [55], highlighting the continuous environmental input of these compounds. This pollutant load is also expected to increase during the summer months, where beaches can host up to 800,000 visitors per day (Busan, South Korea), and oil slicks from sunscreen use become visible to the naked eye [11,48]. Here, the UV-filter pollution from beach visitors alone would likely reach or exceed 640 μg/day based on the UV-filter emission rate calculated by Langford et al. [55]. Concerningly, the coast also accumulates the highest recorded levels of plastic pollution due to littering, proximity to municipal sources, and additional inputs from oceanic currents [82]. Organic UV-filters are therefore an emerging pollutant, contributing to ocean contamination in areas of significant concurrent plastic pollution [78].

Many of these UV-filters are semi-persistent ($\leq$ 2 years), lipophilic pollutants, meaning they tend to form surface films and/or adhere to floating marine plastic debris rather than dissolving in seawater [105,109,48,58,101]. In addition to this physical persistence, many UV-filters exhibit strong bioaccumulation potential in marine organisms, including in microbial biofilms that colonise the surface of plastic debris [12]. This can lead to the accumulation of lipophilic pollutants on plastics in the ocean, resulting in concentrations on plastic surfaces ($>$ 10 μg/g) which exceed those found in the surrounding water column (av. $\approx$ 1 μg/g) [43,58]. By accumulating on plastics, UV-filters could then increase plastic persistence in the ocean by shielding the plastics from UV degradation [66]. This UV protection can also create microhabitats that favour certain bacterial strains, potentially facilitating the persistence of microorganisms which do not benefit the wider microbial community [63,9,100]. The most used organic UV-filter in sunscreens, EHMC (EthylHexyl MethoxyCinnamate) has been shown to be the most potent of five studied UV-filters in reducing the growth of five marine microorganisms [22,63]. EHMC is also associated with coral bleaching, reduced fecundity, and impaired development of many higher trophic organisms in the ocean due to its reported interaction with endocrine receptors [57]. Additionally, recent research has now revealed EHMC to be the most concentrated UV-filter in three distinct marine regions – North Atlantic Ocean, South Carolina coastline, and Tunisian coastline [17,37,5] –, designating EHMC as a prevalent (4.3 ng/L to 4.043 μg/L) pollutant of concern [13,99]. Previous study has also shown that organic UV-filters, as co-solutes to microplastics have a deleterious effect on the growth of the microalga *Chlorella vulgaris* [45]. However, co-pollutants, including organic UV-filters, have not been investigated for their role in marine plastisphere structuring, or for their potential impact on key functions.

In this study, a seven-day-old model marine plastisphere – enriched for hydrocarbonoclastic microorganisms using a minimal medium (*Section 2.2*), and previously shown to be functionally distinct from earlier stages [59] – was compared to an equivalent plastisphere with added co-pollution. This plastisphere was dosed with a high concentration (5 mg/L) of EHMC for a short duration (17 hrs) to simulate acute, localised pollution that may occur near popular bathing areas during peak visitation [32,48,55,58]. The duration of exposure was chosen to reflect short-term, high-impact exposure scenarios rather than chronic background contamination. Following exposure, metaproteomics was used to examine the plastisphere's key processes and how these may be facilitated or inhibited by co-pollutant exposure. Low-density polyethylene (LDPE), one of the most widely produced plastics globally (45.7 Mt annually) [86], was selected as the test substrate to ensure ecological relevance. Furthermore, prior research indicates that the plastisphere is most susceptible to change during early development [36,56,60]. Therefore, the study focused on plastispheres cultivated over six days before EHMC exposure to allow for optimal biomass accumulation while still reflecting a 'nascent' plastisphere. This represents the first investigation of the molecular effects of EHMC – one of the most widely used UV-filters in sunscreens and a prevalent pollutant in coastal marine environments – on marine microbial activity [54,58]. Critically, it also marks the first study to examine the combined influence of plastic, co-pollutants, and microorganisms within the marine plastisphere, revealing how such co-pollution may impact microbial functioning.

2. Materials and methods

2.1. Recovery of plastisphere stock communities

EthylHexyl MethoxyCinnamate (EHMC; CAS-No. 5466–77–3) was purchased from Sigma-Aldrich (Steinheim, Germany). Plastic debris was collected from Oban Bay beach (56° 24' 50.4'' N, 5° 28' 19.2'' W) August 6th 2022. After collection, this plastic was washed using sterile artificial seawater supplemented with 3 mM d-glucose, vitamins, and trace elements to remove debris (ASW-G) [34], then followed by preservation at −20℃. To pre-enrich and recover the plastisphere [59], the collected transparent plastic was incubated in glass containers containing 300 ml of ASW-G at 15℃ under constant shaking (65 rpm) for three days, simulating the peak seawater temperature at Oban Bay.

Following incubation, excess ASW-G was removed, leaving behind any medium which would have disturbed the newly formed biofilms with removal. The plastics were then vortexed in the remaining medium before rinsing with additional ASW-G. The total remaining medium was then removed, and centrifuged (14℃, 5000 g, 7 mins), then the pellet was resuspended with more ASW media, cryopreserved with 30 % glycerol, and stored at −80℃ for subsequent analysis.

2.2. Plastisphere growth

Five folded pieces of low-density polyethylene (LDPE; 2 ×10 cm; ET31-FM-000101; Goodfellow, England) per replicate were placed in 250 ml glass Erlenmeyer flasks containing 150 ml of the minimal medium Bushnell Haas Broth supplemented with vitamins and trace metals (BHB-v; Supplementary file S1) [19]. This was then inoculated with 50 µl of plastisphere stock, following which all replicates were incubated at 15°C with gentle shaking (65 rpm). The experiment consisted of eight replicates, four of which received 5 mg/L EHMC under sterile conditions after six days incubation. All cultures were incubated for an additional 17 h following EHMC addition before collection, simulating an acute pollution event.

2.3. Protein extraction

Proteins were extracted from the plastisphere biofilm following the protocol outlined by Messer *et al.* (2024). The plastics were retrieved, air-dried under laminar flow, and then bead beaten with 1 mm glass beads in a 2 % sodium dodecyl sulphate (SDS) solution three times, for 10 min each. The cell suspension was then sonicated (1 s pulse, 1 s gap, 40 % Amp., 2 times), and centrifuged (8000 g, 10 min) twice. After the second centrifugation, a fraction of the supernatant was stored at −80℃ for metagenomic analyses (*Section 2.4*), while the remainder (5–6 ml) was concentrated using 3 K centricons (7000 g) until the sample volume was reduced to ≤ 250 µl. Protein precipitation was performed using acetone at a 4:1 acetone-to-sample ratio overnight at −80°C. The resulting protein pellet was resuspended in 6 M guanidine hydrochloride in dipotassium phosphate buffer, sonicated (1 s pulse, 1 s gap, 40 % Amp.), and centrifuged (13,000 g, 15 min). The supernatant was then diluted with LC-MS grade water (1:1) and stored at −80°C. Protein yields averaged 6.68 mg (±4.76) and 7.08 mg (±2.58) for the control and EHMC-exposed groups, respectively.

For digestion, 100 mg of protein per sample was reduced with 10 mM 1,4-Dithioerythritol, alkylated using 25 mM iodoacetamide, and subjected to acetone precipitation (4:1 acetone-to-sample ratio) before incubation with 20 µL of sequencing-grade trypsin (EC 232–650–8) overnight at 37°C [74].

Protein analysis was conducted using an ultra-high-performance liquid chromatography–high-resolution tandem mass spectroscopy (UHPLC-HRMS/MS) system (Eksigent nanoLC 400 and AB Sciex TriplеTOF 6600). Two micrograms of peptides were analysed using a 75-minute LC separation with data-dependent acquisition (DDA) mode, following established parameters [30].

2.4. Shotgun metagenomics

Metagenomic sequencing was performed using the Oxford Nanopore Technologies (ONT) NEBNext companion module for MinION. DNA co-precipitation was performed by adding protein precipitation solution (PPS; Promega, A7951) to the previously preserved plastisphere sample (*Section 2.3*) in a 2:1 sample-to-PPS ratio, and centrifuging (20,000 g, 15 mins). The supernatant was then supplemented with filtered 3 M sodium acetate (300 µL) and cold ethanol (>95 %) at a 1:2 sample-to-ethanol ratio, then incubated at −20°C overnight. The DNA pellet was retrieved by centrifugation (20,000 g, 15 min), washed twice with 70 % ethanol, centrifuged again (16,000 g, 15 min), and air-dried. Samples were resuspended in UltraPure water (20µl), briefly heated to (55°C, 5 mins), vortexed, and stored at −80 °C. DNA quantification revealed average concentrations of 197.1 ng/µL (±168.4) and 256.9 ng/µL (±188.1) for the control and EHMC-exposed groups, respectively.

Library preparation was performed using the Nanopore Native Barcoding Kit 24 (SQK-NBD112.24) with 800 ng of pooled sample per condition. Sequencing was carried out on a MinION (Mk1c) device with a FLO-MIN106 flow cell for 24 h. Reads shorter than 200 bp or with a quality score below 8 were filtered out during real-time basecalling in MinKnow (v22.10.5). A total of 6.72 Gb of sequence data (5.66 million reads) was obtained, processed with BBDuk and BBNorm for quality trimming (minimum quality score 7) and normalisation, taxonomically annotated using Kaiju [73], assembled (2955 total contigs) using MetaFlye [53], and functionally annotated with DRAM, leveraging KBase [74].

2.5. Shotgun metaproteomics

Protein searches were performed using ProteinPilot (v5.0.3.1029, 9521aa4603a; Paragon Algorithm: 5.0.3.1029, 1029; AB SCIEX), and the AB SCIEX OneOmics software package, with databases created from our metagenomic data (*Section 2.4*). The genus-level taxonomic assignments from Kaiju (.txt), and functional annotations from DRAM (.faa) (*Section 2.4*) were utilised in the mPies (v1.0) [102] database creation workflow (https://github.com/johanneswerner/mPies), which uses NCBI taxon IDs to retrieve available proteomes from the UniProt database prior to dereplicating at 100 % sequence similarity. This non-redundant database was then used to identify protein groups in spectral (DDA) data within ProteinPilot including a global false discovery rate (FDR) of 1 %, using parameters described in Messer *et al.,* (2024) for protein grouping. The identified protein groups were annotated using mPies, whereby the Last Common Ancestor approach of MEGAN [6] was used to assign taxonomy, and DIAMOND BLAST [18] alignments against UniProt and NCBI databases were used to assign function [102]. All taxonomic data was subsequently filtered according to score (≥80 %), and proteins with < 2 associated peptides were removed. Before annotation, relative quantification between the conditions (EHMC: Control) was performed in MarkerView. An adjusted P-value of < 0.05 and Log2 fold change of 0.5 or −0.5 was considered significant. Fold change values displayed in-text are untransformed, and exceptions to P-value cutoffs are reported. Missing values resulting from protein inference issues were manually annotated using BLAST (https://www.uniprot.org/blast) if the protein was repeatedly matched (>50 % matches), had a highest-rated peptide score of ≥ 100, had a species level match, or met a combination of these criteria. Images of gels, bacterial pellets, raw data files, MarkerView exports for relative quantification, and manually annotated files are provided in Supplementary files S1: S6.

Data visualization and statistical analyses were conducted in RStudio (v. 4.2.2).

3. Results

With the use of our consensus approach (*Sections 2.5), 73.3*% (control) and 79.6 % (+EHMC) proteins were annotated automatically within mPies, leaving 26.7 % (control) and 20.4 % (+EHMC) proteins to be annotated manually. Within the control, a total of 1886 proteins were identified across all replicates, and 2004 were identified for the '+EHMC' group, both equalling a spectral coverage of 28.1 %. Of this, 1449, and 1621 proteins were classified as 'non-redundant' (*Section 2.5*) for the control and '+EHMC' conditions respectively.

The studied metaproteomes were taxonomically annotated (score ≥80); 98.1 % (±0.4 %) to the phylum level, 96.9 % (±0.3 %) to the class level, 92.65 % (±0.25 %) to the order level, 91.3 % (±0.3 %) to the family level, 84.3 % (±0.2 %) to the genus level, and 21.15 % (±2.15 %) to the species level. In terms of quantification, of 1385 total proteins, 748 non-redundant (>1 peptide) proteins were compared successfully,

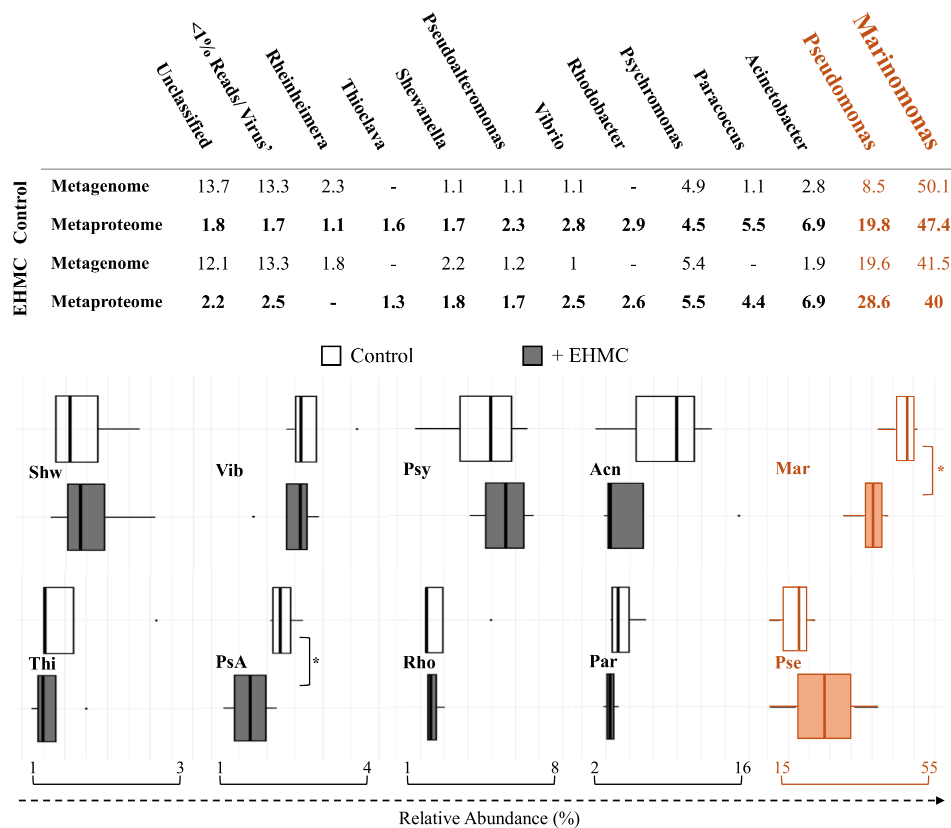
returning 288 upregulated proteins in the control, and 460 upregulated proteins with EHMC. Acronyms of proteins are provided in the figure legends.

3.1. EHMC favours Pseudomonas in the nascent marine plastisphere

Interestingly, metagenomic analyses of all genes assigned to an organism within these data revealed a 99.93 % sequence return rate for bacterial taxa (score >80; Supplementary file S2). The active bacterial classes represented within the metaproteome belonged primarily to the Proteobacteria phylum – namely Gammaproteobacteria (86.5 % ±1.15), and Alphaproteobacteria (13 % ±1.3) –, followed by Bacteroidetes (0.45 % ±0.05), then Firmicutes, for which one protein was found in the '+EHMC' condition. With EHMC, the relative abundance of Gammaproteobacteria increased from 85.3 % to 87.6 %, and decreased from 14.3 % to 11.7 % for Alphaproteobacteria, and the colour of the bacterial pellet changed from a dark brown to light beige (Supplementary file S1).

At the level of genera, 11 taxa were identified with > 1 % reads (Fig. 1). To calculate relative abundance, the number of proteins associated with each genus relative to all the proteins found in that condition (Metagenome), or to all proteins found in each replicate of that condition (Metaproteome) were used. Averaged values were then calculated and statistical tests performed (*Kruskal-Wallis <0.1) for significant differences between genus abundances (Fig. 1). The most active and abundant bacteria according to current annotations were *Marinomonas, Pseudomonas, Acinetobacter, Paracoccus*, and *Psychromonas* (Fig. 1). The least active bacteria were *Rheinheimera, Thioclava, Shewanella, Pseudoalteromonas, Vibrio*, and *Rhodobacter*. With the exception of *Rheinheimera* (ˇ53.9 % ±1.7) and *Psychromonas,* all genera exhibited greater abundance in the metaproteome compared to the metagenome (Fig. 1). The greatest increases in relative abundance from the metagenome to the metaproteome were observed in *Paracoccus* (ˆ394.5 % ±5.5), *Rhodobacter* (ˆ223.5 % ±1.5), and *Acinetobacter* (ˆ204.5 % ±58.5). In contrast, the relative abundance of unclassified and low-abundance taxa (<1 % Reads) decreased by 84.4 % (±2.6) and 84.3 % (±2.9) respectively in their representation of the metaproteome compared to the metagenome.

With the addition of EHMC, the relative abundances of the *Pseudomonas, Psychromonas*, and *Shewanella* trended upwards, whereas the majority of the other genera trended downwards (Fig. 1). Of the nine identified taxa whose expression of proteins were significantly differentially regulated by the addition of EHMC ($P < 0.05$), *Pseudomonas* was the most differentially regulated (42 proteins), followed by *Marinomonas* (35 proteins), *Acinetobacter* (6 proteins) and the six other genera (Fig. 2). In total, 95 proteins were significantly upregulated, while 13 proteins were downregulated with EHMC treatment. This included 26 upregulated and 9 downregulated proteins for *Marinomonas*, and 40 upregulated and 2 downregulated proteins for *Pseudomonas* (Supplementary files S3, S6). Additionally, in the set of proteins unique to the control condition, *Pseudomonas, Marinomonas, Acinetobacter*, and other genera represented 20.6 %, 44.5 %, 7.6 %, and 27.3 % of the total metaproteome. While in the set of proteins only expressed with EHMC exposure, *Pseudomonas* represented 77.5 %, *Marinomonas* represented 6.1 %, *Acinetobacter* represented 3.8 % and all other genera represented 12.6 % of the metaproteome. A significant increase for *Pseudomonas*, and decrease for the remaining genera. Additionally, a significant decrease in relative abundance (Kruskal-Wallis, P 0.08) was found for *Marinomonas* and *Pseudoalteromonas* with the addition of EHMC (Fig. 1). The increased and decreased abundance of respective genera is made apparent through their relative expression of cell cycle proteins. Within the 'cell cycle control, cell division, chromosome partitioning' function,



		Unclassified	<1% Reads/ Virus'	Rheinheimera	Thioclava	Shewanella	Pseudoalteromonas	Vibrio	Rhodobacter	Psychromonas	Paracoccus	Acinetobacter	Pseudomonas	Marinomonas
Control	Metagenome	13.7	13.3	2.3	-	1.1	1.1	1.1	-	4.9	1.1	2.8	8.5	50.1
	Metaproteome	**1.8**	**1.7**	**1.1**	**1.6**	**1.7**	**2.3**	**2.8**	**2.9**	**4.5**	**5.5**	**6.9**	**19.8**	**47.4**
EHMC	Metagenome	12.1	13.3	1.8	-	2.2	1.2	1	-	5.4	-	1.9	19.6	41.5
	Metaproteome	**2.2**	**2.5**	**-**	**1.3**	**1.8**	**1.7**	**2.5**	**2.6**	**5.5**	**4.4**	**6.9**	**28.6**	**40**

Fig. 1. Relative abundance of microbial genera found within the metaproteome of a marine plastisphere with (+ EHMC) and without (control) exposure to EHMC. The two most abundant genera are highlighted in orange. Acronyms: Mar, Marinomonas; Pse, Pseudomonas; Acn, Acinetobacter; Par, Paracoccus; Psy, Psychromonas; Rho, Rhodobacter; Vib, Vibrio; PsA, Pseudoalteromonas; Shw, Shewanella; Thi, Thioclava.

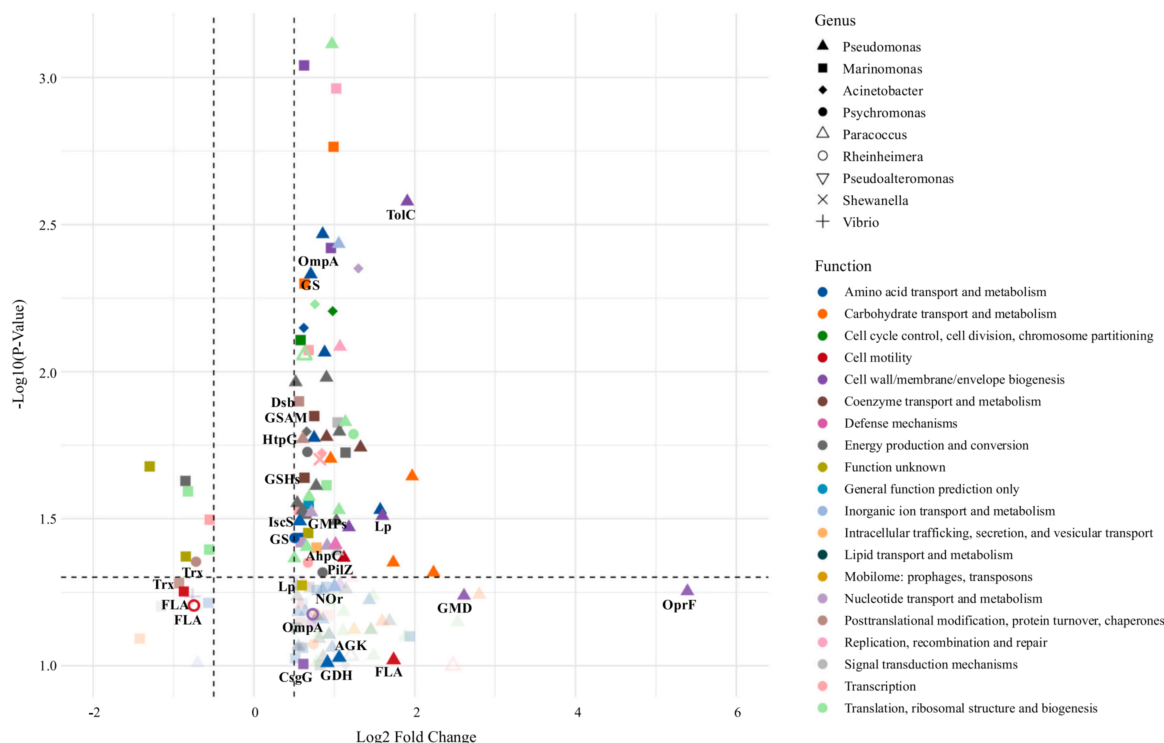


Fig. 2. Differential regulation of proteins between the control, and EHMC-exposed plastispheres. Horizontal dashed line: p-value 0.05; Vertical dashed lines: Log2 Fold change −0.5 and 0.5 (Positive fold change: >EHMC; Negative fold change: >Control). Saturated points represent differentially regulated proteins with a p-value of < 0.05 and proteins of interest, greyed out points represent differentially regulated proteins with a p-value of > 0.05. Acronyms: **Adhesion/ Motility**: CsgG, curli assembly protein; FLA, flagellin; GMD, GDP-mannose 6-dehydrogenase; LP, lipoprotein; OprF, outer membrane porin F. **Cell-cell communication**: OmpA, outer membrane protein A; PilZ, pilus assembly protein. **Glutamine/ Arginine cycling**: AGK, acetylglutamate kinase; GDH, glutamate dehydrogenase; GMPs, GMP synthase; GS, glutamine synthetase; GSAM, glutamate-1-semialdehyde 2,1-aminomutase. **Nitrogen respiration**: NOr, nitrous-oxide reductase. **Stress**: AhpC, alkyl hydroperoxide reductase C; Dsb, thiol:disulfide interchange protein; GSHs, glutathione synthase; HtpG, chaperone HtpG; IscS, cysteine desulferase; Trx, thioredoxin. (Protein of interest= protein related to function(s) discussed in the manuscript).

three proteins were upregulated with EHMC (Fig. 2). Of these, two were cell-shape determining proteins (*Mar*: FC 1.49; *Pse*: P 0.08, FC, 2.74), and one was a septum-site determining protein (*Acn*: FC 1.97) directly involved in cell division. Of a total 14 proteins associated with this function, three *Marinomonas* proteins were unique to the control, and three *Pseudomonas* proteins were unique to the '+EHMC' test condition (Supplementary file S6).

3.2. Lipophilic co-pollutants induce biofilm formation in a nascent marine plastisphere

In this study, a total of 51 proteins associated with biofilm formation and maintenance were identified. With a broader search of differentially regulated proteins ($P < 0.1$), the biofilm stability associated proteins OprF (*Pse:* P 0.06, FC 42.1), and GMD (*Pse:* P 0.06, FC 6.1) were > 6 times more expressed with EHMC, proving to be the most upregulated proteins in this dataset (Fig. 2). In an additional search for proteins relating to biofilm stability and arrangement, six proteins involved in glutamine cycling were found to be upregulated by *Marinomonas, Pseudomonas*, and *Psychromonas* with EHMC (Fig. 2). Of the 10 total active genera (>1 % reads), *Pseudomonas* expressed the most proteins (n = 23) associated with biofilm formation and maintenance (Fig. 3c). Of these, eight were upregulated (Fig. 2), and nine were uniquely expressed in response to EHMC (AidA, ADI, AGPR, AlgF, ALS, CK, CP, gaABC, OTC). Six of these were then involved in the recycling of amino acids glutamate and arginine, aligning with the observed rise in amino acid metabolism protein expression from 9.99 % to 10.99 % relative to the control and '+EHMC' metaproteomes respectively (Fig. 2).

A downregulation of motility proteins and an upregulation of substrate adhesion and cell-cell interaction process was also observed. FLA was downregulated by the identified *Marinomonas* (P 0.06, FC 0.55) and *Rheinheimera* (P 0.06, FC 0.6), and upregulated by *Pseudomonas* (P 0.1, FC 3.34) in the '+EHMC' treatment group (Fig. 2). Flagellin may have been used for motility or adhesion [15], though the CsgG protein, upregulated by *Marinomonas* (P 0.1, FC 1.54), and LP protein, upregulated by both *Marinomonas* (FC 1.6) and *Pseudomonas* (FC 3.03), are primarily associated with substrate adhesion. In contrast to the downregulation of FLA, *Marinomonas* and *Rheinheimera*, concurrently upregulated OmpA (*Mar*: FC 1.94; *Rhe*: P 0.07, FC 1.66), which is a cell-cell communication protein. Another cell-cell communication protein, PilZ was also upregulated by *Pseudomonas* with EHMC (FC 2.17; Fig. 2). Besides FLA and PilZ, no proteins associated with cell motility were differentially regulated (Fig. 2), and only two of the 25 motility proteins expressed were unique to each respective condition (Supplementary file S6). However, cell-cell communication proteins – also found regularly in the established biofilm – were upregulated by the most abundant genera within this nascent plastisphere when exposed to EHMC (Fig. 3b). In addition, the proteins Agg and TolB expressed by *Pseudomonas*, and proteins DNase and TVISS expressed by *Acinetobacter* were only found expressed after the addition of EHMC.

In contrast with the previously stated results, expression of proteins involved in the TCA cycle by *Pseudomonas*– a process which may be associated with early biofilm formation – decreased from 14.23 % in the control to 8.95 % with EHMC exposure. A trend observed despite the upregulation of enzymes aconitate hydratase B (AHB; FC 2.21), isocitrate dehydrogenase (P 0.06, FC 1.45), pyruvate kinase (PK; P 0.06, FC 6.97), and succinate dehydrogenase (FC 1.43) by *Pseudomonas* once exposed to EHMC. The similar upregulation of AHB (*Acn*: P 0.09, FC 1.44), PK (*Mar*: FC 1.99), and oxaloacetate decarboxylase (*Mar*: FC 1.55) was also observed for *Marinomonas* and *Acinetobacter* despite a

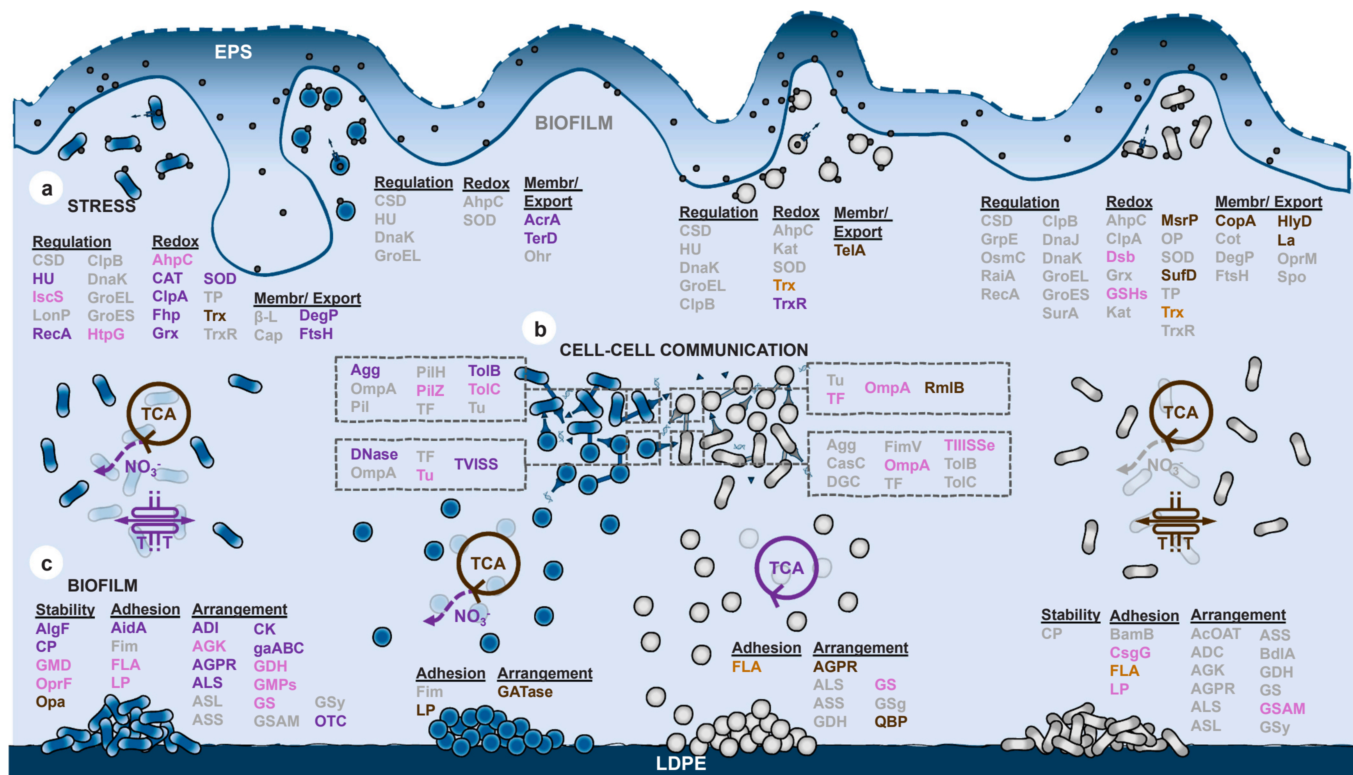


Fig. 3. Graphic representation of the plastisphere's proteomic response to EHMC exposure. Responses include stress (a), cell-cell communication (b), biofilm maintenance (c), the tricarboxylic acid (TCA) cycle, the use of nitrate (NO_3-) for nitrogen respiration, and transmembrane transport (TT). Ubiquitous = expressed evenly across treatment groups, control = only expressed in the control, Log2 FC < -0.5 = downregulated with the addition of EHMC, EHMC = only expressed with EHMC, Log2 FC > 0.5 = upregulated with the addition of EHMC. Acronyms: **General**: EPS, exopolymeric substances; LDPE, low-density polyethylene; ROS, reactive oxygen species. **Stress**: AcrA, acriflavine resistance protein A; AhpC, alkyl hydroperoxide reductase C; b-L, beta-lactamase; Cap, capsular biosynthesis protein; CAT, catalase; ClpA, ATP-dependent Clp protease ATP-binding subunit; CopA, copper resistance protein A; Cot, spore coat protein; CSD, cold-shock DNA-binding protein; DegP, periplasmic serine endoprotease; Dsb, thiol:disulfide interchange protein; Fhp, flavohemoprotein; FtsH, ATP-dependent zinc metalloprotease; GrpE, protein GrpE; Grx, glutaredoxin; GSHs, glutathione synthase; HlyD, HlyD family efflux transporter; HU, transcriptional regulator HU; IscS, cysteine desulferase; Kat, catalase peroxidase; La, endopeptidase La; LonP, Lon protease; MsrP, methionine sulfoxide reductase; Ohr, organic hydroperoxide resistance protein; OP, 5-oxoprolinase; OprM, outer membrane efflux protein; OsmC, osmotically-inducible protein; RaiA, ribosome-associated translation inhibitor; RecA, SOS-response protein; SOD, superoxide dismutase; Spo, sporulation protein; SufD, FeS cluster assembly protein; TelA, toxic anion resistance protein; TerD, tellurium resistance protein; TP, thiol peroxidase; Trx, thioredoxin; TrxR, thioredoxin reductase; Chaperones/ chaperonins- ClpB, DnaJ, DnaK, GroEL, GroES, HtpG, SurA. **Cell-cell communication**: Agg, agglutination protein; CasC, CRISPR system cascade subunit CasC; DGC, diguanylate cyclase; DNase, type I site-specific deoxyribonuclease; OmpA, outer membrane protein A; Pil, pilin; RmlB, dTDP-glucose 4,6-dehydratase; TF, trigger factor; TIIISS, type III secretion system protein; TIIISSe, TIIISS PLP-dependent enzyme; Tu, elongation factor Tu; TVISS, Hcp1 family type VI secretion system protein; Pilus proteins- (Pil) FimV, PilH, PilZ. **Biofilm maintenance**: AcOAT, acetylornithine aminotransferase; ADC, biosynthetic arginine decarboxylase; ADI, arginine deiminase; AGK, acetylglutamate kinase; AGPR, N-acetyl-gamma-glutamyl-phosphate reductase; AidA, adhesin; AlgF, alginate biosynthesis protein; ALS, acetolactate synthase; ASL, arginosuccinate lyase; ASS, arginosuccinate synthase; BamB, outer membrane protein assembly factor; BdlA, biofilm dispersion protein; CK, carbamate kinase; CP, cytosol aminopeptidase; CsgG, curli assembly protein; Fim, fimbrial protein; FLA, flagellin; gaABC, glutamate/aspartate ABC transporter; GATase, glutamine amidotransferase; GDH, glutamate dehydrogenase; GMD, GDP-mannose 6-dehydrogenase; GMPs, GMP synthase; GS, glutamine synthetase; GSAM, glutamate-1-semialdehyde 2,1-aminomutase; GSy, glutamate synthase; LP, lipoprotein; OmpF, outer membrane porin F; Opa, opacity protein; OTC, ornithine carbamoyltransferase; QBP, L-glutamine-binding protein.

respective drop in TCA cycle protein expression by 39.01 % and 24.22 % with EHMC. Conversely, when examined together, all other genera expressed 11.5 % more TCA cycle-related proteins with EHMC compared to the control (Supplementary file S6) with *Psychromonas* notably upregulating two such proteins in response to EHMC (PK: P 0.08, FC 1.67; oxoglutarate dehydrogenase: FC 1.81). EHMC also induced a 18.5 % drop in the expression of transcription-related proteins, while increasing the expression of translation proteins by 17.4 % within the plastisphere metaproteome (Supplementary file S6). In addition, *Acinetobacter* only expressed LP and GATase in the control, indicating a downregulation of the associated substrate adhesion properties of these proteins for this genus (Fig. 3c).

3.3. The plastisphere's regulation of oxidative stress in response to EHMC

Proteins related to stress, including redox regulation, competitive interactions, and cellular repair mechanisms represented 9.43 % (±0.22 %) of proteins identified for the nascent plastisphere (Fig. 3a). This was therefore the fifth most prevalent metabolic process within these plastispheres behind translation (14.97 % ±1.43), amino acid metabolism (10.49 % ±0.5), transmembrane transport (10.07 % ±0.17), and the TCA cycle (9.71 % ±0.55). Of the limited number of proteins downregulated by EHMC exposure, the oxidative stress regulatory protein thioredoxin (Trx), was downregulated by both *Marinomonas* (FC 0.53) and *Psychromonas* (FC 0.61; Fig. 2). Further proteins involved in the regulation of oxidative stress were upregulated by *Marinomonas* (Dsb: FC 1.5; GSHs: FC 1.55), and *Pseudomonas* (AhpC: FC 2.01; HtpG: FC 1.52; IscS: FC 1.48; NOr: FC 2.01; Fig. 2).

Pseudomonas had the greatest stress response of all nascent plastisphere organisms exposed to EHMC with the expression of two regulation proteins (HU, RecA), five redox response proteins (CAT, ClpA, Fhp, Grx, SOD), and two proteins associated with membrane maintenance in stress conditions (DegP, FtsH) only with EHMC (Fig. 3a). *Acinetobacter* also exclusively expressed the proteins AcrA and TerD in the EHMC-

exposed plastisphere; transmembrane transport (TT) proteins often expressed in direct response to anthropogenic stress. Interestingly, seven other TT proteins were upregulated by *Marinomonas* (translocase subunit SecA: FC 1.71), and *Pseudomonas* (acetyl CoA synthetase: P 0.07, FC 1.72; amino acid ABC transporter substrate-binding protein: P 0.09, FC 1.96; branched-chain amino acid ABC transporter: P 0.09, FC 1.47; OprD family porin: FC 2.1; porin: FC 2.27; spermidine/ putrescine-binding periplasmic protein: P 0.06, FC 2.7) with the introduction of EHMC (Fig. 2), making this one of the most upregulated functions of this nascent plastisphere. A total of 15 and 14 TT proteins were also unique to the control and '+EHMC' conditions respectively (Supplementary file S6). Bacterioferritin (*Mar*: P 0.06, FC 1.52) and sulfite reductase (*Pse*: FC 2.08), were also upregulated with EHMC, and two ion and metal transport proteins expressed by *Marinomonas* were unique to the control (molybdopterin oxidoreductase and Trk system potassium uptake protein).

3.4. EHMC-induced metabolism of nitrogen and complex carbons

One of the most affected metabolic processes in the EHMC-exposed plastisphere was nitrogen cycling and respiration (Fig. 3). Of a total 22 proteins found associated with this function, six proteins were unique to the '+EHMC' treatment group, compared to one in the control (Supplementary file S6). The addition of EHMC induced an upregulation of the P-II family nitrogen regulator (*Mar*: P 0.06, FC 1.72), and nitrogen regulatory protein P-II (*Pse*: P 0.07, FC 1.56). Furthermore, six proteins (*Mar, Pse*: nitrate reductase; *Pse*: NOr, FC 2.01; *Acn* : nitrite reductase; *Pse, Shw*: periplasmic nitrate reductase) expressed or upregulated after the addition of EHMC, and three proteins (*Mar, Pse, Lelliottia*: nitrate reductase) expressed in the control, were related to the use of nitrate (NO_3^-) for nitrogen respiration (Supplementary files S3, S6). Similarly, one (*Mar*: aryl-alcohol dehydrogenase-like predicted oxidoreductase) and three (*Acn* , *Bacillus*: aldehyde dehydrogenase; *Pse*: alcohol dehydrogenase) of a total 11 proteins associated with the alkane degradation pathway – and consequentially potential LDPE biodegradation – were unique to the control and '+EHMC' conditions respectively. One such protein – Aldedh domain-containing protein – was also upregulated by *Pseudomonas* (FC 1.71) with EHMC. Three of 10 proteins found associated with aromatic hydrocarbon (AH) degradation (*Pse*: acetyl-CoA acetyltransferase, enoyl-CoA hydratase; *Rho*: acetyl-CoA dehydrogenase domain-containing protein) were also unique to the '+EHMC' condition, though no proteins were differentially regulated for this function (Supplementary files S3, S6).

4. Discussion

This study provides the first molecular-level investigation into how a widely used organic UV-filter prevalent in coastal environments affects microbial communities in the nascent marine plastisphere. Using plastispheres enriched from marine plastic debris, we compared communities on pristine LDPE exposed to an acute dose (5 mg/L) of EHMC with unexposed controls. EHMC exposure led to the formation of a distinct plastisphere community relative to the control, with altered microbial composition and function. Furthermore, metaproteomic analysis revealed key stress responses, enhanced biofilm formation, and respiratory inhibition, indicating a disruption of core microbial processes. Together, these results underscore the influence of concentrated copollutants in shaping plastisphere dynamics, and their impact on environmentally and anthropogenically relevant processes.

4.1. Bacterial response to EHMC is taxa-dependent

In this study, fewer than 12 total genera were characterised with > 1 % reads. These were therefore simple biofilms compared to other plastisphere communities [58], likely due to culturing conditions, and use of frozen stock, which can induce selective pressures [19,98]. However, this reduced community complexity enabled deeper insight into the functional contributions of each genus and facilitated direct comparisons between metagenomic and metaproteomic data. The two most abundant genera characterised in this study, *Marinomonas*, and *Pseudomonas*, are well-known for biofilm-formation and bioremediation in marine environments [68,72,77]. Importantly, the use of a minimal medium in this study with plastic as the sole carbon source (*Section 2.2*) facilitated these hydrocarbonoclastic taxa, as previously reported [75]. Among these, *Pseudomonas* showed increased abundance and activity, consistent with its known role as a plastic-degrading and hydrocarbonoclastic genus [46,93]. Protein abundance decreased for *Pseudoalteromonas* but increased for *Psychromonas* upon EHMC exposure. The expression of cell division proteins by *Pseudomonas* and *Marinomonas* mirrored their respective responses to EHMC, suggesting that EHMC may influence microbial proliferation and cell division in a taxa-specific manner.

Such varied responses to pollutant exposure have been linked to the structural similarity between some pollutants and dissolved organic carbon (DOC), as well as the known toxicity of such lipophilic compounds [38,9]. Indeed, some organisms may utilise the pollutant as a source of carbon, while others are negatively impacted by its toxicity. By analysing the metaproteomic data presented here, we aim to elucidate the mechanisms behind these species-specific growth responses to co-pollutants like EHMC and the multi-dimensional factors that govern them.

4.2. EHMC induces biofilm formation in Pseudomonas

Pseudomonas and *Marinomonas* are biofilm-forming bacteria [68,77], demonstrated here through the expression of adhesive proteins [35,52,80,94], cell-cell communication proteins [26,67], and the glutamine and arginine cycling proteins associated with biofilm arrangement [79,85]. Interestingly, all but one of the differentially regulated or exclusively expressed proteins mediating biofilm formation were upregulated or exclusively expressed with EHMC. The sole exception to this was an opacity protein, which may have facilitated the adhesion of *Pseudomonas* to the membrane of other cells in the control [29].

The most upregulated protein within the EHMC treatment, OprF, plays a critical role in biofilm stability and defence. For instance, OprF regulates biofilm formation in *Pseudomonas* through retaining extracellular DNA in the matrix [21,58]. Another significantly upregulated protein, GMD, is a key enzyme in alginate biosynthesis [49]. The co-expression of GMD with another alginate biosynthesis protein, AlgF, suggests that alginate was synthesized by *Pseudomonas* in response to EHMC exposure. This may have facilitated membrane synthesis, biofilm formation, xenobiotic resistance, or all three [27]. Similarly, the OmpA protein upregulated by *Marinomonas* and *Rheinheimera* is homologous to OprF [24], and may have served a similar function in biofilm stability. The increased expression of cell-cell communication proteins (i.e., PilZ, TIIISSe, TolB) often involved in horizontal gene transfer (HGT) by *Pseudomonas* and *Marinomonas* also indicates a level of cooperation, particularly with EHMC exposure [26,44,58]. However, biofilm-related proteins in other genera, like *Marinomonas*, were not as strongly upregulated in response to EHMC. Notably, flagellin, a protein used for substrate binding, was downregulated by *Marinomonas*. This downregulation of flagellin, despite the upregulation of other adhesion mechanisms, suggests that *Marinomonas* may shift its strategy from motility to biofilm formation under EHMC exposure [80]. Overall, these findings suggest that, while *Pseudomonas* and *Marinomonas* both actively contributed to biofilm stability under EHMC exposure, they may do so through different mechanisms.

The upregulation of biofilm stabilisation proteins, also known to confer xenobiotic resistance [79,85], alongside stress-response proteins (*Section 4.3*) suggests that biofilm formation may function as an extension of the stress response to EHMC. Indeed, biofilms are well-known for providing protection against environmental stressors and predation [79,

85]. However, the availability of key nutrients within a conditioning film that forms rapidly on marine surfaces, attracts early microbial colonisers to the plastic surface [61]. Following the initial stages of biofilm formation (< 1 day), nutrients will also accumulate within the plastisphere through processes such as cell lysis [50,62]. This accumulation would provide a nutrient-rich environment, increasing the advantage of biofilm formation compared to remaining in a planktonic state [61]. The upregulation of amino acid transport and metabolism proteins with EHMC indicates that these biomolecules could have been utilised within the nascent plastisphere community through cross-feeding [42,7]. EHMC may have therefore accelerated biofilm formation in *Pseudomonas* by inducing the microbial stress-response in other genera, and causing cell lysis (*Section 4.3*), resulting in an accumulation of biomolecules.

4.3. The toxicity of EHMC within a nascent marine plastisphere

In our prior study investigating the impact of an organic UV-filter on planktonic microbial metabolism, toxicity was found to be induced through oxidative stress, subsequent DNA damage, membrane damage, and downregulation of protein synthesis [62]. In the present study, antioxidants, chaperones, transcription regulators, amino acid metabolism proteins, and protein- and DNA- repair proteins were differentially expressed after EHMC exposure. Crucially, nineteen stress-related proteins were upregulated or dysregulated with EHMC exposure compared to the control, primarily involving the regulation of oxidative stress, potentially induced by EHMC [8]. To counteract reactive oxygen species (ROS), antioxidants such as AhpC and SOD were expressed to neutralise oxidative protein and DNA damage [20]. Despite displaying enrichment in response to EHMC, *Pseudomonas* expressed the highest levels of antioxidants compared to other taxa, and also increased expression of the membrane repair protein ATP-dependent zinc metalloprotease (FtsH). This indicates that EHMC caused membrane damage in *Pseudomonas*, either directly or potentially via ROS [106]. Additionally, the expression of RecA, involved in the SOS response, and the protein repair enzyme IscS, is indicative of more widespread cellular damage induced by EHMC [20,70]. This suggests that antioxidant production was insufficient to protect *Pseudomonas* from EHMC toxicity.

Conversely, EHMC did not induce the same antioxidant response in other genera, and in some cases, even reduced antioxidant expression. For example, *Marinomonas* showed downregulation of thioredoxin (Trx), a redox enzyme that reduces ROS and repairs damaged proteins [64,95]. Critically, the dysregulation or deletion of Trx is lethal to many organisms, including bacteria [64]. *Marinomonas*'s downregulation of Trx was potentially caused by an excess of ROS with EHMC [95], and this may have contributed to its reduced growth. In addition, the expression of the AcrA and TerD proteins by *Alteromonas* indicates that EHMC, its metabolites, or other xenobiotic stressors may have been internalised by these organisms before inducing stress, suggesting a potential pathway for xenobiotic response [69]. The general upregulation of TT proteins is also predicted to confer an increased resistance of marine bacteria to organic UV-filters through xenobiotics exocytosis [62]. However, in this study, the majority of TT proteins upregulated were porins used to endocytose hydrophilic compounds, rather than lipophilic compounds such as EHMC [8]. This upregulation of TT may therefore have a different function in marine biofilms compared to planktonic bacteria [62,63].

These findings suggest that EHMC induces widespread damage in the nascent marine plastisphere. However, the increased expression of stress-related proteins may also reflect a general increase in protein expression. Notably, the increased expression of stress proteins in *Pseudomonas* with EHMC exposure does not correspond with reduced growth, as would typically occur in response to stress [20,38]. Instead, the enhanced growth of *Pseudomonas* through other means may have led to better detection of proteins, including those related to stress, compared to the control where fewer active *Pseudomonas* were present [74]. It is also important to note that the concentration of EHMC used in this study (5 mg/L) exceeds what plastisphere communities are likely to encounter in the natural marine environment [58]. Importantly, this study was designed to represent an acute exposure scenario – a high, short-term dose of EHMC – such as would occur through direct contact with sunscreen pollution [32]. In contrast, chronic exposure to lower concentrations over longer periods may elicit fundamentally different microbial responses, including adaptation, tolerance, or the gradual selection of detoxification pathways [62,9]. Further study of chronic, low-dose scenarios may therefore also reveal a more diverse range of toxicity responses to this marine pollutant than those first explored here.

4.4. EHMC-induced biofilm formation increases anaerobic respiration and competition in the plastisphere

Interestingly, there is evidence to support that *Pseudomonas, Marinomonas, Acinetobacter*, and *Shewanella* switched from aerobic to anaerobic respiration with EHMC exposure, indicating a decreased availability of oxygen. Most notably, nitrogen-respiration proteins (i.e., nitrate reductase, nitrite reductase, NOr) were upregulated, indicating that in this nascent plastisphere, nitrate, instead of oxygen, started being used as the final electron acceptor in respiration [107,88]. This decline in aerobic respiration is often indicative of oxygen-depleted conditions which may have been caused by increased respiration due to stress (*Section 4.3*). Alternatively, the increased biofilm density which results from accelerated biofilm formation may have led to oxygen depletion. Organic UV-filters are also expected to directly inhibit aerobic respiration pathways [62], meaning EHMC could have also directly inhibited aerobic respiration.

This inhibition of aerobic respiration may have driven the decline of the plastisphere's aerobic bacteria, *Marinomonas,* and *Pseudoalteromonas,* which utilise cytochromes (e.g., cytochrome C) and pigments (e.g., melanin) to perform aerobic respiration [16,90,91]. Contrastingly, the facultative anaerobes *Psychromonas* and *Pseudomonas* were more active with EHMC [76,84]. The inhibition of aerobic respiration may have therefore been one of the most significant drivers of community shifts within the nascent plastisphere. Such a reduction of aerobic processes would also justify the reduced pigmentation observed in the extracted bacterial cell pellet in response to EHMC (Supplementary file S1). The addition of EHMC also induced an increased expression of a Type VI secretion system (TVISS) protein, often associated with bacterial virulence, in *Acinetobacter* [26]. In a competitive environment, the TVISS can be used to deliver toxins into neighbouring cells, thereby inhibiting competing processes, or causing cell death [1]. The expression of TVISS indicates that the addition of EHMC could have increased competition within the nascent plastisphere, likely through the described biofilm formation. Consequently, the expression of a TVISS may have facilitated *Acinetobacter's* activity after the nascent plastisphere's exposure to EHMC.

4.5. Biocatalytic processes within the nascent marine plastisphere

Due to the potential implications that pollutant bioremediation has for the future of marine ecology, pollutant-degrading enzymes have been searched for in every study of the marine plastisphere metaproteome to-date [31,74,75,81]. Indeed, the experimental conditions used in this study – growth in minimal (BHB-v) medium with plastic as the sole carbon source – were designed to favour the proliferation of plastic-associated microbial taxa. While not specifically intended to enrich for hydrocarbonoclastic microorganisms, this setup naturally selects for taxa capable of utilising complex hydrocarbons. Marine plastisphere communities are frequently reported to be enriched in such hydrocarbon-degrading organisms [59,74,75], many of which are known to express key enzymes involved in alkane degradation (e.g., alkane monooxygenases such as AlkB), aromatic ring-cleaving dioxygenases, and esterases or lipases that could potentially contribute to the

breakdown of plastic-associated compounds.. In addition to the 17 enzymes found in these studies, this study identified another enzyme – Enoyl-CoA hydratase – which may be associated with this function, and confirms the presence of eight previously reported proteins [59]. Enoyl-CoA hydratase catalyses the second step of fatty acid beta-oxidation by hydrating trans-2-enoyl-CoA to L-3-hydroxyacyl-CoA; a key reaction in the degradation of aliphatic hydrocarbons [96]. This function has been implicated in xenobiotic degradation pathways involving structural analogous to fatty acids – such as linear alkanes and polycyclic hydrocarbons, where beta-oxidation-like mechanisms facilitate degradation [2,74,96]. Notably, this enzyme was only expressed following exposure to EHMC, while five additional associated proteins were also exclusively expressed under EHMC exposure conditions. Three of these enzymes were expressed by *Pseudomonas*, which may have contributed to its growth, and the species-specific response discussed above. Within the '+EHMC' metaproteome, 5.4 % of proteins also remained uncharacterised after manual annotation (*Section 2.5*), equalling a significant pool of unknown proteins which may have contributed to xenobiotic biodegradation. These uncharacterised proteins likely reflect limitations in current genome annotations, underscoring the need for improved functional characterisation, which may be achieved through the use of proteogenomic tools [25,4,71]. Meanwhile, within the present pool of characterised proteins, the organisms identified as *Acinetobacter, Bacillus*, and *Rhodobacter* were found to exclusively express enzymes related to xenobiotic biodegradation with EHMC. However, unlike *Pseudomonas*, none of these genera were found to be enriched in the presence of EHMC. *Marinomonas*, another genus closely associated with xenobiotic biodegradation [72,77], also showed significantly reduced activity and abundance with EHMC exposure despite expressing five related proteins. This suggests that EHMC was not a significant source of carbon for *Acinetobacter, Bacillus*, *Marinomonas*, or *Rhodobacter*. Instead, the discussed enzymes were likely used for their housekeeping functions, rather than xenobiotic degradation [38,9]. Moreover, the observed lack of correlation between enzyme expression and microbial growth indicates that *Pseudomonas'* increased growth and activity may be unrelated to the expression of these proteins or EHMC biodegradation.

4.6. Organic UV-filter co-pollution increases plastic persistence and potential virulence

Plastispheres are unique niches, though also contain marine bacteria representative of the ocean's most abundant and most influential organisms [39]. The observed community shift—marked by increased activity of *Pseudomonas* and *Psychromonas,* and a decline in *Marinomonas* and *Pseudoalteromonas*—raises important ecological questions. While some *Pseudomonas* species are known opportunistic pathogens [84,87], the genus is also well recognised for its remarkable metabolic plasticity and pollutant degradation capacity. For example, despite its harmful effects on human health, *P. aeruginosa* has also been widely studied for the remediation of hydrocarbons and organic pollutants, aided by biosurfactants like rhamnolipids that enhance solubility and uptake of hydrophobic compounds [93,47].

This dual identity—as both a potential health concern and a key degrader—highlights the need for caution in interpreting shifts in microbial communities. The enrichment of *Pseudomonas* may reflect adaptive advantages under EHMC exposure, but further investigation is needed using *in situ* communities and more complex sunscreen formulations, which may elicit different ecological responses and microbial dynamics.

In contrast, the decline in *Pseudoalteromonas*—a ubiquitous marine bacterium known for its role in regulating pathogenic populations [89] —is ecologically significant and likely taxa-specific. Similarly, the reduced abundance of *Marinomonas* suggests that EHMC may suppress certain microbial lineages associated with plastic degradation [39,77]. These observations point to targeted impacts of EHMC on particular taxa rather than a general inhibition of microbial activity. A shift towards a biofilm increasingly dominated by potentially pathogenic genera, accompanied by a decline in ecologically important groups, could destabilise marine microbial community structure and function. By shielding plastics from UV degradation [66], and interfering with biodegradation processes, acute plastic co-pollution by EHMC and other organic UV-filters may therefore significantly increase the persistence of plastic in the ocean.

This acute exposure to EHMC (5 mg/L) was designed to simulate a high-intensity plastisphere contamination scenario, such as direct contact with freshly applied sunscreen and its subsequent accumulation on plastic surfaces (*Section 1 ; 4.3*) [32,58]. Though even at lower, environmentally recorded concentrations of EHMC (4.3 ng/L to 4.043 μg/L), co-exposure to complex pollutant mixtures in the ocean is still likely to drive plastisphere restructuring due to the recorded cumulative (<3 mg/L), and potentially interactive effects of multiple contaminants [104,109,41]. The nature of this restructuring may differ from what was observed herein due to possible synergistic or antagonistic interactions among pollutants [28], and species-specific responses of marine microorganisms [63]. Nonetheless, EHMC's increased detection in coastal waters [37], and elevated concentrations near densely populated areas [55], indicates that EHMC and other organic UV-filters, are becoming a prominent and widespread marine pollutants. Our results suggest that in regions heavily affected by both plastic and UV-filter pollution, plastic persistence may be increased, and the activity of taxa associated with polymer degradation may be decreased. Importantly, this interaction remains poorly understood, which is particularly concerning given the proximity of densely populated regions, as well as some of the world's most biodiverse marine ecosystems to this pollution [82,97]. This study therefore highlights the urgent need for targeted research to discover the biogeochemical, ecological, and health-related impacts of the ocean's most combined pollutants on prevalent marine microbial communities, including the plastisphere.

5. Conclusion

This study investigated the molecular response of a nascent marine plastisphere community to 5 mg/L of the plastic co-pollutant EHMC, revealing how organic UV-filters may interact with plastic in highly polluted marine ecosystems. Interestingly, the plastisphere community responded to UV-filter co-pollution in a largely taxa-specific manner, with increased activity of *Pseudomonas* and a decline in other genera such as *Marinomonas* and *Pseudoalteromonas*. Notably, *Pseudomonas* upregulated many biofilm stability and maintenance proteins including outer membrane porin F, upregulated 42.1 times, and GDP-mannose 6-dehydrogenase, upregulated 6.1 times; both of which are believed to have roles in xenobiotic resistance. The addition of EHMC also led to a marked increase in the expression of six anaerobic respiration proteins by generalist bacteria, suggesting a potential inhibitory effect on aerobic respiration within the nascent plastisphere. This may have contributed to the decline in the relative abundance of the obligate aerobes *Marinomonas* and *Pseudoalteromonas* when exposed to EHMC. Our findings indicate that acute EHMC exposure imposes selective pressure on early-stage plastisphere communities, favouring stress-tolerant generalists—including *Pseudomonas*, a genus known for both its metabolic versatility and, in some cases, opportunistic pathogenicity—while suppressing key hydrocarbonoclastic genera associated with plastic degradation. Moreover, the UV-shielding properties of EHMC, combined with its apparent inhibitory effect on aerobic degraders, may slow down both photodegradation and biodegradation of plastics, potentially enhancing their persistence in marine environments. These shifts in microbial composition and function could have wider ecological implications, particularly in coastal regions where plastic and UV-filter pollution co-occur with tourism and wastewater inputs—potentially affecting both ecosystem stability and human health. Further research under environmentally relevant conditions—using native microbial communities

without artificial enrichment—is essential to better understand the broader ecological implications of co-pollutant exposure, particularly when using more complex, commercially available sunscreen formulations.

Environmental implications

The co-pollution of plastics by organic UV-filters, such as the commonly used EHMC, significantly alters the structure and function of marine biofilm communities. This promotes the growth of biofilm-forming generalist bacteria while reducing the abundance of hydrocarbonoclastic taxa, impacting plastic degradation processes and increasing plastic persistence in marine environments. Additionally, the favoured biofilm-forming taxa and the upregulation of biofilm-stability proteins, like OprF, may enhance biofilm stability and confer resistance to environmental stressors, making the plastisphere more resilient to chemical pollutants. These findings underscore the need to account for chemical co-pollutants in marine ecosystem management and plastic pollution mitigation strategies.

Data statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org/) via the MassIVE partner repository [23,65] with the dataset identifier PXD061450. Currently made accessible to editors and reviewers in MassIVE using the password: 'EHMCPlasisph3re'.

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CRediT authorship contribution statement

Sabine Matallana-Surget: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Data Curation, Funding acquisition, Conceptualization. **Charlotte E. Lee:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lauren F. Messer:** Writing – review & editing, Methodology, Data curation. **Ruddy Wattiez:** Resources, Methodology, Data curation, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author C.E.L used ChatGPT in order to improve the readability and language of the manuscript. After using this service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sabine Matallana-Surget reports financial support was provided by University of Stirling. Charlotte Lee reports financial support was provided by University of Stirling. Lauren Messer reports financial support was provided by University of Stirling. Ruddy Wattiez reports financial support was provided by University of Mons. Sabine Matallana-Surget reports a relationship with University of Stirling that includes: funding grants. Charlotte Lee reports a relationship with University of Stirling that includes: funding grants. Lauren Messer reports a relationship with University of Stirling that includes: funding grants. Ruddy Wattiez reports a relationship with University of Mons that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.139103.

Data availability

Data will be made available on request.

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