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Early biofilm colonization on traditional and biodegradable plastics in the Baltic Sea using a mesocosm approach

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ABSTRACT

Bioplastics are promising alternatives to conventional plastics, but their potential entry into marine ecosystems highlights the need for a better understanding of their interactions with microbial communities, including their role in the plastisphere. Here, we characterized the early biofilm formation on traditional plastics and bioplastics using a mesocosm approach. We tested the hypothesis that distinct bacterial communities selectively colonize traditional and biodegradable plastics in the marine environment. Specifically, fragments of the petroleum-based plastic polypropylene (PP) and the bioplastics Poly(3-hydroxybutyrate)- hydroxyvalerate (PHBv) and polylactic acid (PLA) were submerged in Baltic Sea mesocosms for three weeks. Biofilm colonization, prokaryotic abundance, and community composition were assessed through scanning electronic microscopy analysis, epifluorescence microscopy and 16S rRNA gene metabarcoding, respectively. Biofilm development increased over time on both traditional and bioplastics, with photosynthetic organisms appearing after 3 weeks. However, prokaryotic abundance decreased over time except on PLA surfaces. Prokaryotic communities' composition differed among biofilms formed on the different polymers. The microbial community associated with conventional plastic PP was more similar to that of the seawater in the control treatment, while biofilms on PLA and PHBv shared a higher degree of similarity with each other. These findings suggest that microbial communities selectively colonize different plastic types, with bioplastics supporting distinct and specific bacterial biofilm assemblages over three-week exposure. The great diversity observed in bioplastics, particularly PLA, suggests they may support more complex and potentially active plastisphere communities after only three weeks of exposure to the Baltic Sea.

1. Introduction

Plastic is currently used in wide applications within our daily life owing to its good processability, excellent mechanical properties and low cost (Pan et al., 2020; Fredi and Dorigato, 2021). In 2021, global plastic production reached nearly 390 million tons (Plastics Europe, 2022) and its productivity is expected to increase worldwide, reaching 1600 million tons by 2050 (Ali et al., 2021). Because of its persistency and mismanaged waste disposal, plastic enters the aquatic ecosystems through sea- and land-borne sources (i.e. fishing activity, runoff water, sewage, industrial activities), resulting in an environmental issue we are facing today. To mitigate this problem, which increasingly threatens both environmental and human health, alternatives to replace traditional not degradable plastics are urgently needed. A promising alternative to conventional plastics, such as petroleum-based polymers (i.e. polypropylene, PP), is represented by "bioplastics", innovative biopolymers that are considered sustainable and environmentally friendly (Ali et al., 2023). Bioplastics are either bio-based or biodegradable or have both properties (European Bioplastics, 2024). Bio-based plastics derive from primary and secondary feedstock (i.e. plant-based biomass, cooking oil wastes and petroleum-based feed; Ali et al., 2023), while biodegradable ones can work for a limited time before being degrading

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while leaving no toxic residues behind (i.e. polymers that can return to nature; Peng et al., 2021). Bioplastics have a wide range of applications (i.e. medicinal implants, packaging; Zhong et al., 2020); thus, their global production is expected to increase up to 2.87 million tonnes by 2025 (Hernández-García et al., 2022). Some of the main drivers of this increase are innovative biopolymers such as Polylactic acid (PLA) and polyhydroxyalkanaotaes (PHAs), characterized by bio-based and biodegradable and compostable properties (Jogi and Bhat, 2020; European Bioplastics, 2024).

PLA is an aliphatic polyester thermoplastic polymer (Saini, 2017), synthesized from renewable materials (i.e. wheat, potato starch, rice bran corn; Shah and Vasava, 2019), readily available and low in cost. Due to the rigidity, transparency and processability (Hernández-García et al., 2022), it is currently used in several applications, including packaging, where it has been proposed to substitute traditional polymers (i.e. polystyrene, PP, etc., Ali et al., 2023). PHAs are biopolyesters naturally produced by various bacteria species as intracellular granules for carbon storage (Fernandes et al., 2020). While conventional carbon sources for PHA production commonly include glucose or glycerol (Koller et al., 2017), these can be substituted with agricultural residues, such as purple sweet potato and rice waste (Brojanigo et al., 2020), or other feedstock waste materials, contributing to a circular economy and enhancing sustainability (Koller et al., 2005, 2017; Zhou et al., 2023). Together, PHAs and polylactic acid (PLA) account for approximately 60 % of the global bioplastics production, which exceeds 1.2 million tonnes (Naser et al., 2021). Moreover, their production is projected to continue expanding in the next future (European Bioplastics, 2024). Polyhydroxybutyrate (PHB) is the most extensively PHA investigated biopolymer, that can be biodegraded depending on PHB depolymerase produced by microorganisms (Zhang et al., 2022). Poly (3-hydroxybutyrate)- hydroxyvalerate (PHBv) is a polymer, that has increased recent attention for biomedical uses (i.e. in drug deliver), due to its enhanced flexibility and mechanical robustness (Pachekoski et al., 2009; Wang et al., 2013; Ali and Jamil, 2016; Ferreira et al., 2017).

Any surface - including plastics - can be colonized by a wide variety of marine microorganisms that form biofilms, structured communities of surface-associated microbial cells embedded within a self-secreted extracellular polymeric matrix (Lobelle and Cunliffe, 2011; Fabra et al., 2021). Biofilm formation leads to biofouling, firstly developed by dissolved organic molecules' absorption and bacteria attachment, and then enriched by eukaryotes, larvae and spores (Dobretsov, 2010). Thus, microbial biofilm can trigger the settlement of specific invertebrates and algae, thereby increasing the extent of biofouling (Zardus et al., 2008). The biofilm associated with plastic, termed "plastisphere", may act as a vector of pollutants (i.e. metals, phthalates, antibiotics; Basili et al., 2020; Bowley et al., 2021) and a promoter of the spread of pathogenic microorganisms (Wang et al., 2021). For this reason, microbial colonization of plastic surfaces is considered an emerging environmental issue, and understanding the dynamics between microbial communities and surface biofilm development is important for evaluating plastic behaviour in natural ecosystems.

In the last years, biofilm formation on plastic surface have received attention (De Tender et al., 2017; Trotter et al., 2019; Miao et al., 2020; Bowley et al., 2021; Ganesan et al., 2022). Biofilm on different plastic polymers may differ in microbial composition (Ribba et al., 2022; Ali et al., 2023; Barbe et al., 2024), suggesting that colonization could be substrate-specific (Hansen et al., 2021). However, limited information is available so far on biofilm growth on biodegradable plastics, since their use is minimal if compared to traditional plastics (Ribba et al., 2022). Polymers of traditional plastics (i.e. polyethylene (PE), PP, polystyrene, PS) affect the microbial community's assembly and biofilm differs from the free-living fraction of sea water (De Tender et al., 2017). Biofilm of traditional plastics have been widely studied under both laboratory and in field conditions (Lobelle and Cunliffe, 2011; Oberbeckmann et al., 2016a, 2016b; Wright et al., 2021a; Candlen et al., 2024), conversely to bioplastic biofilm growth in the natural environments (Marín et al., 2023). In addition, few studies investigate and compare biofilm formation on bioplastics and traditional plastics in the field (Saygin and Baysal, 2020; Marín et al., 2025). Increasing knowledge on bioplastics-associated plastisphere in marine environments is useful to select materials that are more ecofriendly and prone to environmental degradation (Sabatino et al., 2024), besides understanding biopolymers' degradation behaviour under natural conditions (Dilkes-Hoffman et al., 2019). To fill this gap, we investigated early biofilm formation on traditional plastics and bioplastics using a mesocosm experiment with natural microbial communities from the Baltic Sea over a three-week period. We aim**ed** to evaluate whether specific microbial community may select and colonize traditional and biodegradable plastics - PP, PHBv, PLA - in the marine environment. To achieve this goal, a multiple analytical approach based on macroscopic observations, chemical characterization and metabarcoding analysis for microbial community assessment was used.

2. Materials and methods

2.1. Experimental set up

Traditional and biodegradable plastics were obtained in pellet form. PP pellets were purchased from Sigma-Aldrich (reference 428116, CAS Number: 9003-07-0), polylactic acid (PLA850) pellets from Sakata 3D were sourced through Garhem 3D (Spain), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) pellets were obtained from Heliam Polymers (China). Plastics were then thermoformed to sheets with a temperature-controlled press. Sheets of 300–500 μm thickness were then cut into scraps of 20 × 20 mm surface area; each one was used for a different analysis, as described below. The samples were then assembled in a frame holder (110 × 110 mm), where 9 samples of each plastic polymer were attached by using a fishing line. A total of 27 boards and 243 samples were sent to UMEA facility (Sweden) to be further submerged in mesocosms.

The experiment was conducted at the Umeå Marine Sciences Centre, located in the Gulf of Bothnia (Baltic Sea) in May–June 2023, in the mesocosm facility. We used 12 mesocosms, each one constituted by 2 m^3 tank filled with natural seawater, sediment and biota. The surface seawater (salinity ~3.5 PSU) to fill the mesocosms was sourced from an inlet located 800 m offshore at a depth of 2 m (coordinates: 63°34′ N, 19°50′ E). The biota in the mesocosms includes microbial and planktonic organisms and small benthic bivalves (Baltic clam *Macoma balthica*). The 12 tanks were divided into 4 groups to have 3 replicates for each polymer and a negative control (blank). Micronized plastics were added in each mesocosm to reach a concentration of 0.2 g/L, except for the blank tanks. The experimental set up (mean water temperature 7,8 °C, mean salinity 3,45 PSU, mean oxygen concentration 353,04 μM, mean chlorophyll-a 4617 μg/L, 12:12 h light-dark cycle) lasted 3 weeks (Fig. 1). Nutrient concentrations—including nitrate (NO_3^--N), phosphate (PO_4^{3-}-P), ammonia (NH_4^+-N), and silicate (SiO_2-Si)—were analysed following Grasshoff et al. (1983) using a continuous segmented flow analyzer (QuAAtro, SEAL Analytical). Nitrate and phosphate were occasionally supplemented during the experiment to restore nutrient levels and avoid depletion, thereby preventing the collapse of the autotrophic community. Nutrient concentrations remained consistent across treatments (Supplementary Table S1). Water temperature was regulated at three different depths to create a thermocline, ranging from 8,1 °C at the surface to 7,9 °C at the bottom, with complete convective mixing occurring every 6 h (Båmstedt and Larsson, 2018). An air bubbling system was also installed to stir the surface water, using PVC tubing that released 1–2 bubbles per second at a depth of 10 cm. Each mesocosm was equipped with an individual light source (Valoya R-258) set to an intensity of approximately 170 μE m^2/s under a 12:12 h light-dark cycle.

Each week, 3 boards of the same polymer type were added to each mesocosm, secured to the tank and suspended 0.5 m below the water's

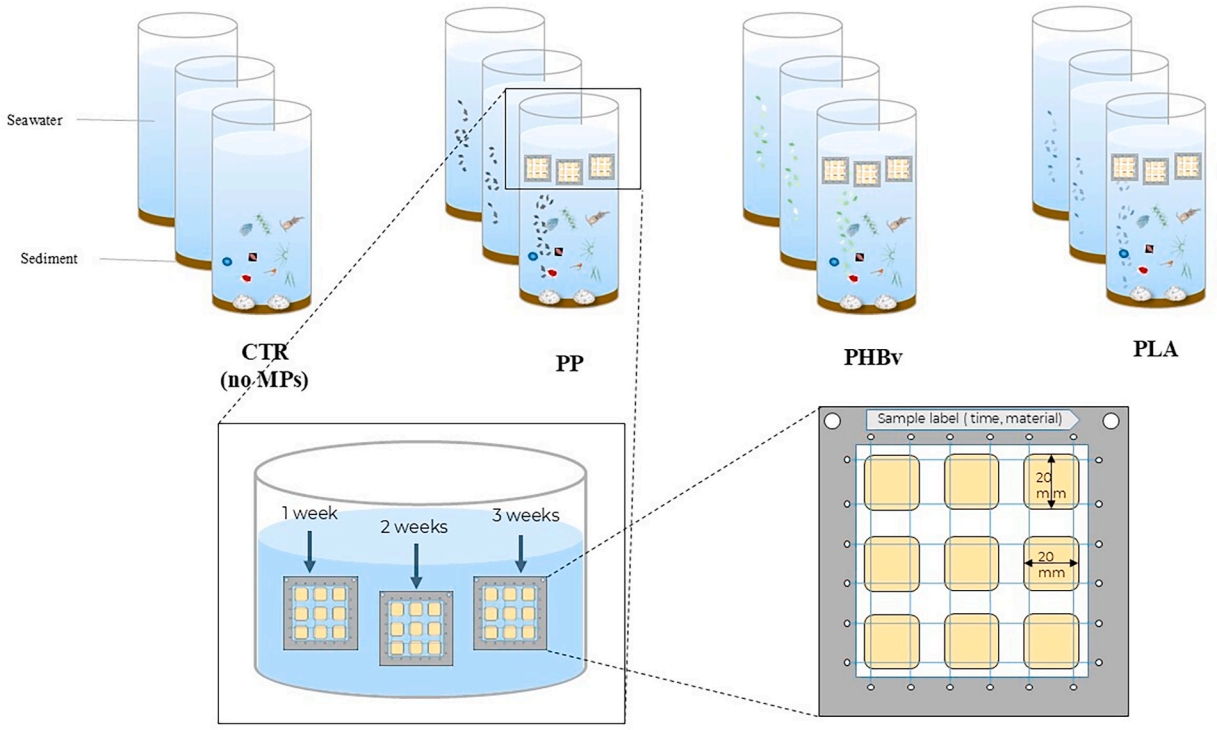

Fig. 1. Schematic diagram of the experimental set up used in the present study at the UMEA mesocosm facility (Sweden). Twelve tanks filled with seawater, sediment and biota were divided into four groups, namely control (no plastics, CTR), conventional plastic (polypropylene, PP) and bioplastics, including polylactic acid (PLA) and poly(3-hydroxybutyrate)- hydroxyvalerate (PHBv). Three boards for each plastic typology composed by 9 sheet samples (20 mm × 20 mm) were inserted in each tank every week. At the end of the exposure boards - corresponding to 1, 2 and 3 weeks-were removed and analysed.

surface. At the end of the experiment, boards were removed and used for analyses corresponding to 1, 2 and 3 weeks of exposure. For each board, three replicates were used for SEM characterization, histological characterization and metabarcoding analyses.

2.2. Scanning Electron Microscope (SEM) imaging

A subset of plastic fragments for each exposure time and polymer typology was used for biofilm observation by Scanning Electron Microscop**ye** (SEM). Briefly, plastic fragments were dried in air for 72 h prior to loading in the SEM. Samples were not coated with conductor film, since the SEM used in this study allows imaging of non-conductive samples in medium and low vacuum. Images were then obtained with TM4000Plus SEM (Hitachi, Tokyo, Japan) at an accelerating voltage of 15.00 kV in medium vacuum with the back scattered electron detector.

2.3. Prokaryotic abundance

Total prokaryotic abundance (TPA) on plastic samples was assessed using epifluorescence microscopy with acridine orange staining, following Luna et al. (2002). For each plastic polymer and exposure time, three replicate samples were used for TPA. Each replicate was transferred into a sterile tube and fixed with 10 mL of pre-filtered, 2 % formalin solution, previously buffered with $Na_2B_4O_7$ x 10 H_2O, ensuring a complete immersion of the samples. The samples were stored overnight at 4 °C and then stained using acridine orange at a final concentration of 0.025 %. Samples were then observed under epifluorescence microscopy (ZEISS Axioscope 5). For each slide, ten randomly selected microscope fields were analysed by manually counting microbial cells. The average number of prokaryotic cells per field was calculated for each replicate to determine cell abundance.

2.4. Diversity and community composition of plastisphere

Microbial DNA was extracted exclusively from plastic fragments (2 × 2 cm each) collected after 3 weeks of exposure, and from 1 L of seawater for the control (CTR), which was filtered through a 0.22 μm membrane using a vacuum pump. Extractions were performed according the DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's protocol with some modifications described by Basili et al. (2020). DNA concentrations were quantified using a Qubit fluorometer (Thermo Fisher Scientific). HTS libraries were prepared following the Illumina Nextera protocol, targeting the V3–V4 hypervariable regions of the 16S rRNA gene. Sequencing was carried out on the Illumina MiSeq platform (2 x 300 bp, V3 chemistry) by IGAtech (Italy). Amplification was conducted using the universal bacterial primers 341F (5′-CCTACGGGNBGCASCAG-3′) and 805R (5′-GACTACNVGGGTATC-TAATCC-3'; Eiler et al., 2012). Primer and adapter sequences were removed from raw sequences using Cutadapt (Martin, 2011). The paired-end reads were processed in RStudio (version 4.4.0; RStudio Team, 2020) with the *dada2* package Callahan et al. (2016). Reads were quality checked and trimmed following the package instructions (max estimated error >2 and 2 per 100 bp for forward and reverse reads, respectively) and merged in Amplicon Sequence Variants (ASVs). Chimeric sequences were identified and removed from the dataset. Taxonomic classification was performed using the SILVA database (version 138; https://www.arb-silva.de/documentation/release-138/) via the implemented native Bayesian classifier. Sequences identified as chloroplast or eukaryotes were removed and analysed separately. For the most abundant ASVs identified as chloroplasts or mitochondria, the representative sequence was identified using BLASTN (Altschul et al., 1990).

2.5. Statistical analysis

All data are expressed as mean ± standard error of the three

replicates. The normality of the data and homogeneity of variance between polymers and exposure times were tested through Shapiro-Wilk and Levene's tests. Since no normality or homogeneity of variances were found, permutational ANOVA (PERMANOVA) was carried out. "Polymer type" (PLA, PHBv and PP) factor and "time" (1 week, 2 week and 3 week) factor were considered. In detail, PERMANOVA analysis was based on Euclidean distance; maximum of 9999 permutations were used to obtain the p values ($\alpha < 0.05$) in each dataset, applying Monte Carlo correction. Analyses were performed using the R statistical software (R version 4.0.2) and a PRIMER 6 software implemented with Permanova + routine were used.

Regarding sequencing, samples with low numbers of reads were excluded from the ASV table prior to analysis. The ASV table was rarefied to the lowest number of reads observed among samples (n = 4998) with the *vegan* package (Oksanen et al., 2017). Significant differences in microbial community composition among the different polymers were evaluated using the analysis of similarity (*anosim*), based on a Bray–Curtis similarity matrix. Alpha-diversity indices (i.e., richness and Shannon index) were calculated in *vegan*, and any significant differences were tested using ANOVA analysis followed by Tukey's *post-hoc* test, Shapiro-Wilk and Levene's tests were used for homogeneity of the values. To identify potential biomarkers of each group, Linear Discriminant Analysis Effect Size (LEfSe; Segata et al., 2011) was conducted using the *microeco* package in R studio (Liu et al., 2021) and the results were visualized through the *ggplot2* package (Wickham, 2016).

3. Results

3.1. Biofilm formation and characterization

Visible biofilm formation was observed on the plastic surfaces after 2 weeks of submersion, with an increasing coverage throughout the duration of the experiment (Fig. 2). SEM images revealed smooth surfaces without visible differences in all three polymers (PP, PLA, PHBv) after 1- and 2-weeks exposure in mesocosms. Biofilm formation on the submerged conventional and bioplastics increased after 3 weeks (Fig. 3). At this exposure time, differences among biofilm colonization were observed in the three different plastic types (PP, PLA, PHBv), characterized by an increase of eukaryotes (Fig. 4).

3.2. Prokaryotic abundance

Epifluorescence microscopy counts showed that conventional and bioplastics were colonized by prokaryotes (Fig. 5). Overall, prokaryotic density ranged from 1.18 to 3.60 $\times$ 10^6 cell/cm^2. Prokaryotic community decreased according to the time exposure (3 weeks), although significant differences among time were only observed for PHBv and PP.

Overall, the prokaryotic density associated with the different

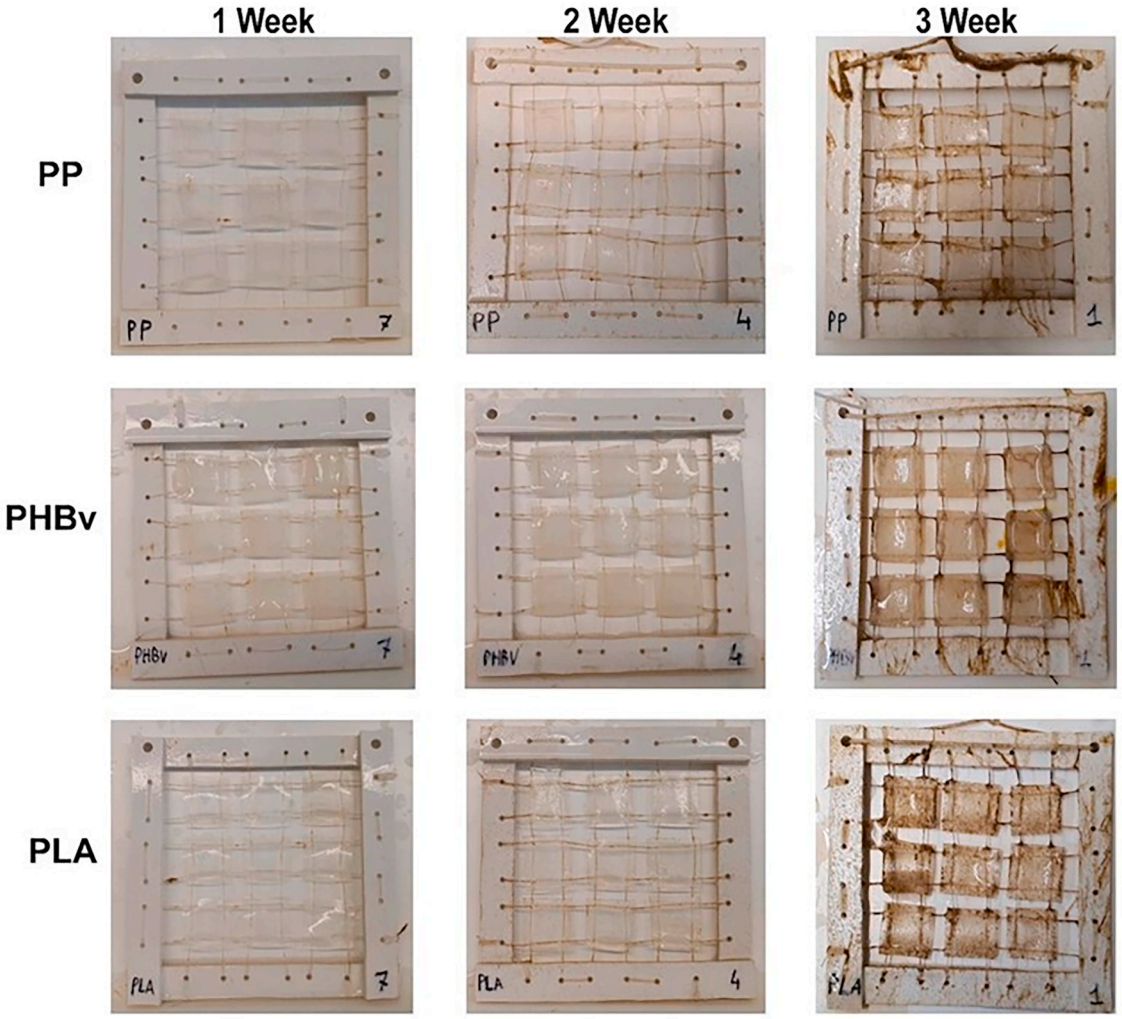

Fig. 2. Photographs of polypropylene (PP), Poly 3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBv) and polylactic acid (PLA) samples after 1, 2 and 3 weeks of submersion at 0.5 m depth in the mesocosms at UMEA facility (Sweden).

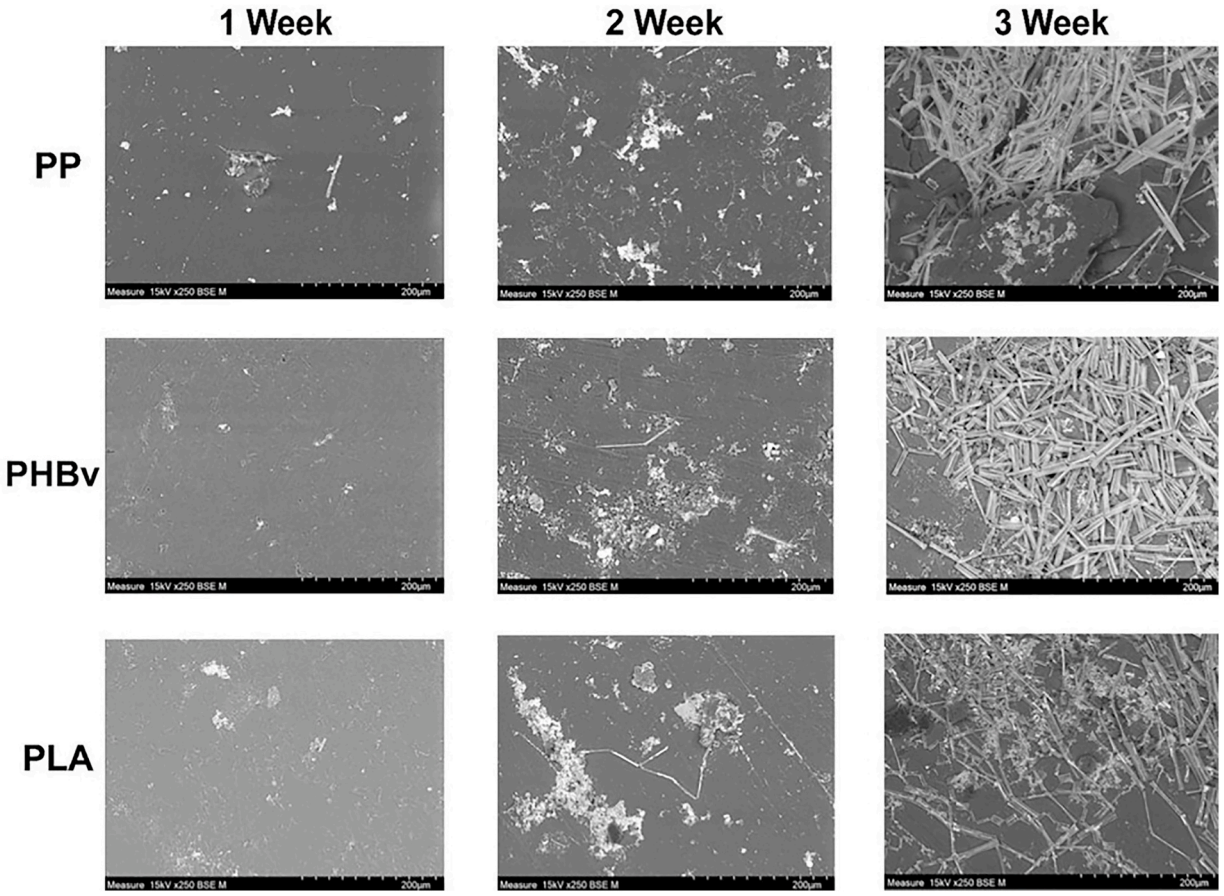

Fig. 3. Scanning Electron Microscope (SEM) images of biofilm colonization in PP, PHBv and PLA samples after 1, 2 and 3 weeks of submersion at 0.5 m depth in the mesocosms at UMEA facility (Sweden). Bars equal 200 µm.

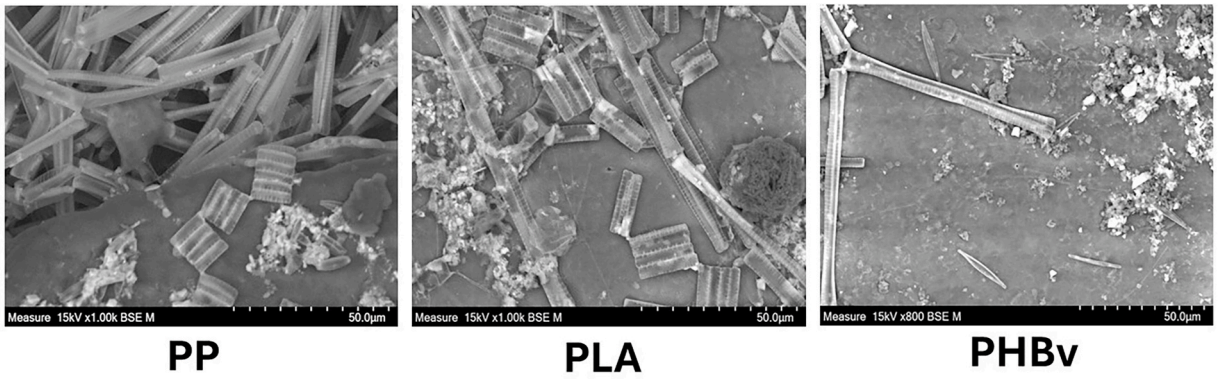

Fig. 4. Scanning Electron Microscope (SEM) images of biofilm colonization in PP, PHBv and PLA samples after 3 weeks of submersion in the mesocosms at UMEA facility (Sweden). Bars equal 50 µm.

substrates statistically differed according to polymer type and time of exposure (Table S1; $p < 0.001$). After one week exposure, PLA and PHBv showed a significant difference if compared to PP ($p = 0.042$, $p < 0.001$ for PLA and PHBv, respectively). Although no differences were observed during the second week, the third week was characterized by a significant prokaryotic community decrease for PHBv and PP compared to PLA ($p < 0.001$ for both PHBv and PP; Supplementary Tables S2–S4).

3.3. Microbial communities associated with (bio)plastics and seawater

The analysis of HTS 16S rRNA gene data showed that both prokaryotic (n. of ASVs = 516) and eukaryotic (n. of ASVs = 64) sequences were found on the analysed fragments. The number of reads assigned to chloroplasts and mitochondria were found to be generally higher in CTR (i.e., seawater) than on plastic and bioplastics, with almost all eukaryotic reads identified as microalgae, and, in more detail, as (putative) *Diatoma tenuis* and *Chaetoceros tenuissimus* (Supplementary Figure). These sequences were not considered for further analyses.

From the analysis of the prokaryotic communities associated with the different types of plastic and bioplastics, we found that each type of polymer displayed a specific associated prokaryotic community (*anosim* R = 0.92, significance = 0.001). In more detail, PLA- and PHB- associated communities clustered separately from PP and CTR, highlighting a higher similarity of communities living on plastic (i.e., PP) to those in

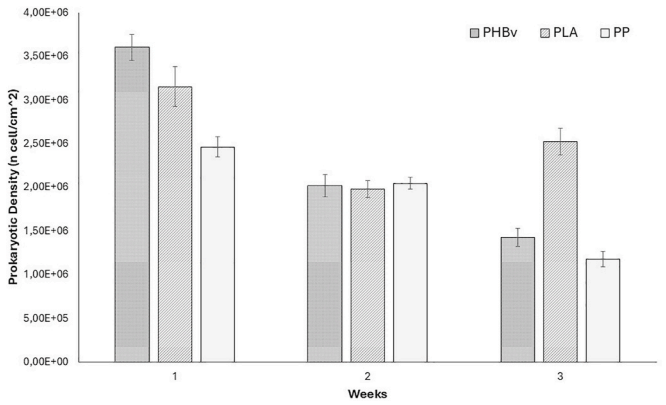

Fig. 5. Prokaryotic cell density (cells/cm^2) in the biofilms formed on PP, PLA and PHBv fragments estimated by epifluorescence microscopy.

seawater than on bioplastics (i.e., PLA and PHBv) (Fig. 6A). At the phylum level, Proteobacteria (avg. 80.62 ± 5.82 %), particularly Gammaproteobacteria (avg 76.57 ± 6.08 %) and Alphaproteobacteria (avg. 4.04 ± 1.32 %) and Bacteroidota (avg 12.49 ± 3.81 %) were the most abundant phyla across the entire dataset (Fig. 6A), with Verrucomicrobiota (avg. 1.90 ± 1.42 %) and Planctomycetota (avg. 1.23 ± 0.71 %) found on all plastic types and in seawater. Despite an apparently similar community composition, microbial communities associated with the different substrates differed statistically in the relative abundance of some taxa. More in detail, PHBv showed higher abundances of Gammaproteobacteria than CTR and PLA (*anova*, $p < 0.01$), but not PP; Bacteroidota were also significantly lower in PHBv than CTR, PP and PLA (*anova*, $p < 0.01$), whereas Desulfobacterota were lower in PHBv than PLA (*anova*, $p < 0.01$) but not PP. On the other hand, Bdellovibrionota had higher abundances in PLA (*anova* $p < 0.05$ with CTR; $p < 0.01$ with PP and PHBv).

Alpha diversity analyses (ASV richness and Shannon index; Fig. 6B) indicated an overall higher diversity of (bio)plastic-associated biofilm communities than those associated with seawater. PLA biofilm hosted the most diverse communities of the entire dataset. Significant differences were found between the number of ASVs (i.e., ASV richness) harbored by seawater samples and the (bio)plastic biofilms. Shannon index values significantly differed between PLA compared with water and PP biofilms.

LEfSe analysis (Fig. 6C) showed that Rhodocyclaceae and Rhodobacteraceae were identified as key discriminant taxa for PP (respectively, avg. 39.43 ± 5.84 % and 2.15 ± 1.06, in contrast to avg. 10.06 ± 14.47 % and 0.69 ± 0.65 % in bioplastics). In contrast, Alteromonadaceae and Flavobacteriaceae characterized PLA-associated communities (avg. 29.41 ± 3.92 % and avg. 6.83 ± 0.73 %, respectively); Comamonadaceae and Oxalobacteraceae (avg. 16.08 ± 12.92 % and avg. 38.38 ± 5.29 %, respectively) were identified as discriminant families for PHBv. ASVs identified as belonging to the *Dechloromonas* genus represented the main components of the Rhodocyclaceae, accounting for more than 40 % of the microbial community of the PP biofilm. ASVs identified as *Dechloromonas* were also observed as major components of the seawater (i.e., control) samples, whereas they were scarcely represented in bioplastic samples. Among the discriminant genera identified in bioplastics, we observed that the most abundant genus for PLA was represented by *Paraglaciecola*, and, for PHBv, by *Caenimonas*.

4. Discussion

We report here, for the first time, short-term biofilm colonization on both traditional and biodegradable plastics by natural microbial communities from the Baltic Sea, one of the world's largest brackish ecosystems (Kettner et al., 2019). This study was conducted during summer, when plastics-attached biofilms have been previously shown to have a faster growth rate than in other seasons, likely thanks to several environmental parameters (i.e. temperature, nutrients) that promote biofilm formation either in marine or freshwater environment (Zhang et al., 2021; Klun et al., 2025). For instance, high nutrients can lead to a more rapid succession of biofilm communities as it has been reported on traditional plastics submerged in the Baltic Sea (Oberbeckmann et al., 2016a). To date, short-term biofilm colonization in field communities has been investigated only for traditional plastics (i.e. polyethylene; Lobelle and Cunliffe, 2011; Eich et al., 2015), thus making this study the first reporting on biofilm colonization and early development in bioplastics in a brackish ecosystem.

Both prokaryotic and eukaryotic cells are widely reported as prevalent in biofilms on aquatic (micro) plastics, including marine, freshwater and brackish waters (Pinto et al., 2019; Basili et al., 2020; Nguyen et al., 2021, 2023; Reisoglu et al., 2024; Ventura et al., 2024; and references therein). In the present study, biofilm growth changed along time in all plastic types when considering both prokaryotic and eukaryotic organisms. Specifically, eukaryotic organisms, mainly represented by microalgae, increased after three weeks, conversely to prokaryotic cells. Bacterial abundances have been shown to increase on bioplastics over long-term experiments (Marín et al., 2023; 2025); however, it must be pointed out that these results were obtained by culture-based quantification of microbial cells, which are widely acknowledged to create biases due to their ability in quantifying only cultivable strains (Marín et al., 2023). In addition, prokaryotic community abundance may have been partially covered by eukaryotic organisms (both visible by SEM and detected through 16S RNA gene sequencing). Finally, the density of bacteria growing on all plastics was one order of magnitude higher than that reported in literature (10^6 cells/ml this study *versus* 10^4 -10^5 cells/ml in Lobelle and Cunliffe, 2011). This could be ascribed to the environmental parameters used in the two studies - conducted in different geographic areas - that are known to influence bacteria growth and therefore abundance.

Sequencing data showed a higher relative abundance of eukaryotic reads in seawater than in plastics, including both traditional ones and bioplastics. Regarding plastics, a similar diatom community abundance was found in traditional and bioplastics (i.e. mater-Bi) submerged in the marine and brackish ecosystems (Eich et al., 2015; Kettner et al., 2019) for two weeks, while differences occurred after a long period (i.e. one month; Eich et al., 2015). These findings confirm our results, since eukaryotic reads identified as microalgae were found between PP and bioplastics.

Plastics represent a habitat that may select the bacterial communities that are attached (Martínez-Campos et al., 2022). Generally, the initial biofilm colonization phase on plastics is characterized by Proteobacteria (Oberbeckmann et al., 2015; De Tender et al., 2017), while the second phase is due to a rapid succession of growing bacteria, including Bacteroidetes, Actinobacteria and Planctomycetes (Kirstein et al., 2018; Bech et al., 2017; Marín et al., 2025). According to previous studies on brackish ecosystems (Weig et al., 2021; Jiao et al., 2024), the dominant prokaryotic phyla in both conventional and bioplastics were represented by Proteobacteria and Bacteroidota; conversely, Planctomycetota were not identified here among the most abundant phyla. Proteobacteria were already reported in PP, PHBv and PLA biofilms in aquatic environments (Jacquin et al., 2021; Weig et al., 2021; Guo et al., 2022; Marín et al., 2023; 2025); similarly to our findings, among them, Gamma- and Alphaproteobacteria were the most abundant proteobacterial classes, since they colonize biofilm mainly during the early development stages (Dussud et al., 2018; Pinto et al., 2019). Proteobacteria and Bacteroidota are pioneer colonizers on floating plastics in the aquatic ecosystem (Oberbeckmann et al., 2015; De Tender et al., 2017; Basili et al., 2020), from freshwater (Morohoshi et al., 2018) to the marine environment. Besides them, we found the phylum Verrucomicrobiota and Planctomycetota in all plastic type and seawater, in line with previous literature

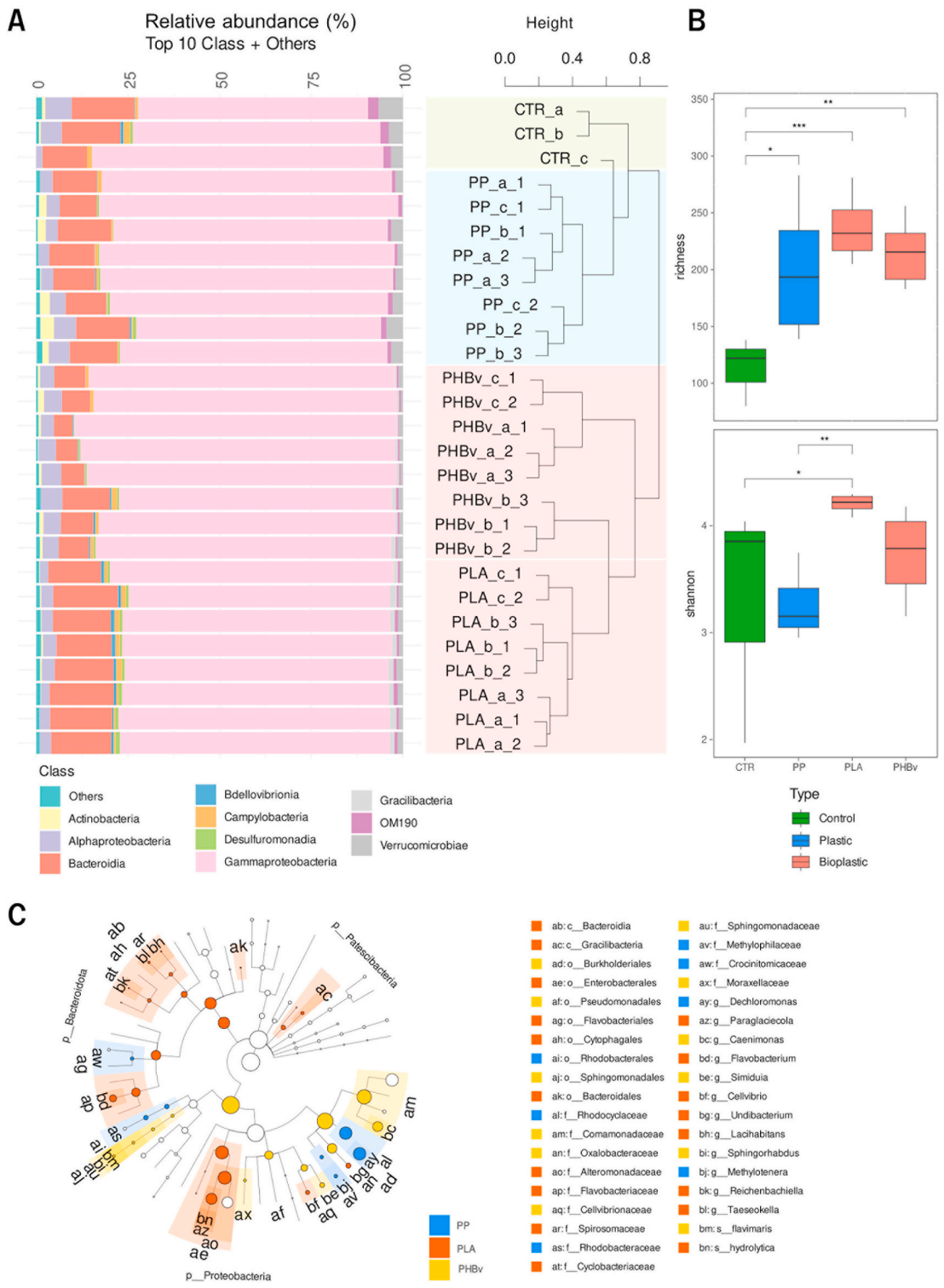

Fig. 6. **A**: Bar plot showing the prokaryotic community composition (as relative abundance) at the Class level. Taxa with an average relative abundance across all samples <1 % were aggregated as "Others". In the middle, a cluster analysis of community composition based on Bray–Curtis dissimilarity matrix is shown; the letter and number on the sample names correspond to the replicates (1-2-3) and the different tanks (a-b-c) used). **B**: ASV richness values calculated for the different types of samples (i.e., green for the seawater, blue for the PP, and red for the bio-plastic samples); asterisks indicate the occurrence of significant differences as calculated by the ANOVA test ($p < 0.05$); non-significant comparisons are not reported in the plot. **C**: Taxonomic cladogram comparing biofilm microbiota using LEfSe analysis. Significantly discriminant taxon nodes are colored in blue for PP, in red for PLA and in yellow for PHBv. In addition, branch areas are shaded according to the highest ranked group for that taxon. Not significantly discriminant taxa are represented in white. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

data on the same polymers (Pinto et al., 2022; Marín et al., 2025). Members of these phyla have been detected in the Baltic Sea (Laas et al., 2014; Kesy et al., 2019) and in plastics submerged in the brackish waters of this geographic area (Eronen-Rasimus et al., 2022). Furthermore, a high abundance of these phyla has been reported in traditional polymers – including PP - in freshwater and estuarine waters (Laverty et al., 2020; Cheng et al., 2021; Weig et al., 2021; Vaksma et al., 2022), thus confirming their importance in the plastisphere community of the aquatic ecosystems (Wright et al., 2021b).

Each polymer showed a specific associated prokaryotic community, although we observed that PP's plastisphere was more similar to that in seawater (i.e., control) than to that of bioplastics; similarly, microbial communities appeared to be more similar between PLA and PHBV with respect to PP and control samples. Our results agree with those described in a 6-months experiment conducted in the Mediterranean Sea by Marín et al. (2023), indicating significant differences between PHBv and PLA biofilm's communities. Interestingly, Marín et al. (2025) showed that, over a long-term experiment (i.e. 12 months), differences in bacterial community composition between PHBv, PLA and PP varied more significantly based on exposure time rather than on polymer type. Taken together, this information suggests that biofilm community composition on different (bio)plastic substrates might evolve over time, with a more polymer-specific setting characterizing the early biofilm formation stages, as in our study, whereas a more uniform microbial composition is likely observable in medium-to-later stages (Marín et al., 2023; 2025; Jacquin et al., 2021). These differences may be ascribed to specific substrates or organic chemicals (additives) released from the surface of plastics during the first days of exposure to the marine environment (Suhrhoff and Scholz-Böttcher, 2016). Over time, these compounds are depleted, likely making the weathered plastic surfaces less suitable for certain microbial communities.

Our findings on alpha-diversity indicate that plastic and bioplastic substrates harboured more diverse microbial communities compared to the surrounding water, consistent with previous studies confirming the role of polymer surfaces as novel microbial habitats in estuarine waters (Laverty et al., 2020). In addition, the higher diversity of PLA samples compared to PHBv (and PP) is a finding in agreement with recent studies in the marine and freshwater environment (Nguyen et al., 2023; Marín et al., 2023; 2025), while, at the best of our knowledge, no literature is available so far on brackish waters. The observed increase in ASV richness on bioplastics suggests selective colonization after a short period, and microbial succession facilitated by surface properties and polymer composition (Kirstein et al., 2018; Marín et al., 2025). Notably, the significantly higher Shannon diversity on PLA supports the hypothesis that biodegradable plastics may promote more complex community structures, potentially due to their enhanced degradability and nutrient release (Dussud et al., 2018). Since bacteria can utilize plastic additives as carbon sources, the higher diversity of organic additives presents in biopolymers such as PLA and PHBv, compared to PP (Laranjeiro et al., 2024), may promote greater prokaryotic biodiversity in biopolymer-associated biofilms as observed in the present study.

Our results confirms that polymer type can influence bacterial community in the biofilms (Jiang et al., 2018; Guo et al., 2020), suggesting that colonization could be substrate-specific (Hansen et al., 2021). Here, in line with previous data on PP and other conventional plastics (i.e. PS, PP, PET, PVC), the key discriminant taxa for PP were represented by Rhodobacteriaceae and Rhodocyclaceae (Xie et al., 2021; Liu et al., 2023). Rhodobacteraceae are core members of the PE, PET and PP biofilms (De Tender et al., 2017; Oberbeckmann et al., 2016a; Zettler et al., 2013; Kesy et al., 2019) and commonly reported both as early (Dang and Lovell, 2000; Dang et al., 2008) and late colonizers of plastics (Tu et al., 2020). In addition, they have been recently detected on PLA film submerged in brackish northern Baltic Sea (Eronen-Rasimus et al., 2022). As widely reported in association with conventional plastics (e.g., PP), members of this family are able of breaking down specific parts of plastic materials (Pinto et al., 2022; Marín et al., 2025). Gammaproteobacteria dominated the PHBv biofilm, in accordance with Eronen-Rasimus and colleagues (2022), which reported a predominance of this taxon in the same polymer along a one-year incubation in the brackish waters of the Baltic Sea. On the other hand, this finding differs from those reported by Marín et al. (2023), which indicated a higher abundance of this taxon in PLA rather than in PHBv after a 6-months exposure in the Mediterranean Sea. We hypothesize that such differences may be linked to different environmental conditions characterizing the two geographical areas (i.e., Mediterranean *vs*. Baltic seas), supporting the greater effect on biofilm formation and plastisphere composition of environmental factors rather than material composition, as suggested by Harrison et al. (2018).

Other differences between bioplastics were observed in Bacteroidota abundance. The latter was higher in PLA than in PHBv, in accordance with previous literature on the same polymers (Marín et al., 2023). This difference may be due to the presence of Flavobacteriaceae, one of the dominant discriminant family in PLA, able to degrade complex carbon substrates and specialized in estuarine and marine surface-associated or biofilm/microbial mat lifestyles (López-Pérez et al., 2012; Oberbeckmann et al., 2016a; Nguyen et al., 2021). In addition, Flavobacterium may proliferate at optimal oxygen and salinity levels, which can be found in freshwater or estuarine waters (Nagaraj et al., 2017; Laverty et al., 2020). Considering that the present study was carried out in brackish water mesocosms in the Baltic Sea, we hypothesize that this may explain the high abundance of this family found in PLA.

Besides Proteobacteria and Bacteroidota, we reported for the first time significant differences between PLA and PHBv in the relative abundances of Desulfobacterota and Bdellovibrionota. Specifically, the relative abundance of these phyla was higher in PLA than in PHBv. Bdellovibrionota and Desulfobacteria are groups of bacteria already reported as potential plastic degraders, also in the brackish waters of the Baltic Sea (Korneeva et al., 2015), due to the predicted presence of PHA depolymerases genes in their genome (Viljakainen and Hug, 2021), and documented as plastisphere members in PLA (Sosa and Chen, 2022; Sun et al., 2022); however, to our knowledge, this is the first study reporting a significant difference in these taxa between PLA and PHBv.

Other discriminant taxa for PLA plastisphere were represented by Alteromonadaceae and *Paraglaciecola*. *Paraglaciecola* spp. are widely reported in studies on biodegradable plastic polymers associated communities, and they have been indicated as potential seaweed-derived polysaccharides degraders (Bech et al., 2017; Schultz-Johansen et al., 2018; Di Gregorio et al., 2024). Similarly, Alteromonadales (which includes the Alteromonadaceae family) have been consistently found among the most abundant taxa on plastics across multiple studies and environments (Wright et al., 2021a). Comamonadaceae represented, together with Oxalobacteraceae, one of the discriminant taxa for PHBv. Comamonadaceae have been detected on marine and freshwater microplastics (Nguyen et al., 2021 and reference therein); interestingly, the genus *Ideonella*, from this family has raised scientific attention due to its ability to degrade PET (Yoshida et al., 2016).

In conclusion, this study provides new insights into short-term biofilm colonization on conventional and biodegradable plastics in the Baltic Sea over a three-week exposure period. Our results show that diverse microbial communities establish on different polymers over the analysed period, indicating that substrate properties may influence early colonization. The greater diversity observed on biodegradable plastics, particularly PLA, suggests they may support more complex and potentially active plastisphere communities; however, further research is needed to confirm this hypothesis. Comparisons with previous studies on long term colonization of the same polymers suggest that biofilm communities likely evolve over time, shifting from substrate-specific assemblages to more convergent profiles, highlighting the dynamic nature of plastisphere succession, also on biodegradable polymers.

CRediT authorship contribution statement

Chiara Gambardella: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Basili:** Writing – original draft, Software, Formal analysis, Data curation. **Filippo Castelli:** Writing – original draft, Methodology, Investigation, Formal analysis. **Roberta Miroglio:** Writing – original draft, Methodology, Investigation, Formal analysis. **Elena Manini:** Writing – review & editing, Writing – original draft, Supervision, Software, Data curation. **Grazia Marina Quero:** Writing – review & editing, Writing – original draft, Supervision, Software, Formal analysis, Data curation. **Rodrigo Almeda:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition. **Francesco Regoli:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **Marco Faimali:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization. **Francesca Garaventa:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107592.

Data availability

Data will be made available on request.

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