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Site-specific response of sediment microbial community to supplementation of polyhydroxyalkanoates as biostimulants for PCB reductive dechlorination

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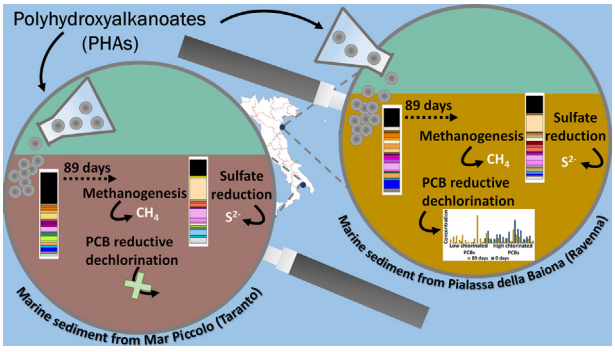
HIGHLIGHTS

- Efficacy of PHA biostimulation of PCB dechlorination depends on sediment's features.
- Biodegradable polymers entering marine sediments impact the resident microbiome.
- PHA as carbon source selects for abundant microbial primary degraders.
- Site-specific signatures persist in subdominant sediment microbiome members.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of biodegradable plastics is constantly raising, increasing the likeliness for these polymers to end up in the environment. Environmental applications foreseeing the intentional release of biodegradable plastics have been also recently proposed, e.g., for polyhydroxyalkanoates (PHAs) acting as slow hydrogen releasing compounds to stimulate microbial reductive dehalogenation processes. However, the effects of their release into the environment on the ecosystems still need to be thoroughly explored. In this work, the use of PHAs to enhance the microbial reductive dechlorination of polychlorobiphenyls (PCBs) and their impact on the metabolic and compositional features of the resident microbial community have been investigated in laboratory microcosms of a polluted marine sediment from Mar Piccolo (Taranto, Italy), and compared with recent findings on a different contaminated marine sediment from Pialassa della Baiona (Ravenna, Italy). A decreased biostimulation efficiency of PHAs on PCBs reductive dechlorination was observed in the sediment from Mar Piccolo, with respect to the sediment from Pialassa della Baiona, suggesting that the sediments' physical-chemical characteristics and/or the biodiversity and composition of its microbial community might play a key role in determining the outcome of this biostimulation strategy. Regardless of the sediment origin, PHAs were found to have a specific and pervasive effect on the sediment microbial community, reducing its biodiversity, defining a newly arranged microbial core of primary degraders and consequently affecting, in a site-specific way, the abundance of subdominant bacteria, possibly cross-feeders. Such potential to dramatically change the structure of autochthonous microbial communities should be carefully considered, since it might have secondary effects, e.g., on the natural biogeochemical cycles.

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1. Introduction

Biodegradable plastics have been developed and are being increasingly used to help facing the devastating effects of plastic pollution on terrestrial and aquatic environments (Directorate-General for Research And Innovation of the European Commission, 2020). Biobased biodegradable polymers are considered to be environmentally friendly, due to their biodegradability and to the possibility to produce them from renewable resources, representing an alternative to fossil-based plastics (Polman et al., 2021; Samer et al., 2022). As the global market and utilization of biodegradable plastics is foreseen to consistently rise in the future (European bioplastics, 2022), the likeliness for these polymers to end up in the environment (i.e. soil, freshwater, and marine environments) is expected to increase parallelly. The ecological impact of biodegradable polymers and monomers/oligomers generated by their progressive degradation in the natural environment is still to be clarified (Awasthi et al., 2022; Cruz et al., 2022), but such lack of information has not stopped their applications, which find everyday usage in different industrial, agricultural and biomedical fields (Alaswad et al., 2022).

Polyhydroxyalkanoates (PHAs) are a family of microbial biopolyesters that can be produced with pure and mixed cultures using a wide range of organic waste streams as feedstock and which are degraded in the environment, aerobically and anaerobically, by microbes that use them as a carbon source (Van Roijen and Miller, 2022; Yukesh Kannah et al., 2022). PHAs have been widely employed in biomedical applications and devices, food packaging, biosensors, cosmetics, drug delivery among other interesting uses (Fernandez-Bunster and Pavez, 2022). Recently, the use of PHAs as carbon source for post-denitrification and micropollutant co-metabolism has been proposed, as well as their use as slow releasing source of electron donors for microbial reductive dechlorination processes (Baric et al., 2014; Pierro et al., 2017; Santorio et al., 2019).

The use of PHAs for stimulating reductive dehalogenation is of particular interest since halogenated organic compounds have been extensively utilized in both industry and agriculture fields and a large number of organohalides are included in both the priority pollutants list of the United States Environmental Protection Agency and in the list of persistent organic pollutants (POPs) of the Stockholm Convention (He et al., 2021). Among organohalides, polychlorinated biphenyls (PCBs) are widespread pollutants, in spite of their ban from production in the late 70s, for which the development of sustainable bioremediation approaches still presents a considerable challenge (Šrédlová and Cajthaml, 2022). This is particularly true for marine sediments, which represent both a sink of many organic pollutants and source for marine food web contamination (Omar and Mahmoud, 2017). Indeed, within this anaerobic environment, the bacteria responsible for reductive dechlorination (i.e. organohalide respiring bacteria, OHRB) compete against abundant sulfate-reducing (SRB) and methanogenic (MB) bacteria for the available hydrogen (Dolfing, 2003; Wiegel and Wu, 2000; Zanaroli et al., 2012b). From a kinetic point of view, OHRB should be favored by their higher affinity for hydrogen (Dolfing, 2003). Yet, in the presence of high concentrations of other electron acceptors (i.e., SO_4^{2-}), large fractions of reducing equivalents can prime other anaerobic species, to the detriment of OHRB (Dolfing, 2003; Fennell and Gossett, 2003). In this context, the use of biodegradable bioplastics as biostimulating agents has been proposed, given the possibility to slowly release the reducing equivalents, resulting in lower hydrogen concentration available (Aulenta et al., 2006). Although this approach showed to be promising in groundwater and freshwater, little is known on the effect of PHAs on microbial reductive dechlorination processes in marine environments, where the biodegradation of PHAs has been reported to be faster than in freshwater ecosystems (Kasuya et al., 1998; Mergaert et al., 1994) thus providing electron donors at higher concentrations to OHRB and their competitors. Recently, a first glance on the possibility to exploit PHAs to promote PCBs reductive dechlorination in anaerobic marine sediments was provided (Botti et al., 2023). Briefly, two PHAs with different composition (ratio of 3-hydroxybutyrate to 3-hydroxyvalerate 75:25 and 88:12 mol%, respectively) and their monomer were added to lab-scale microcosms of a marine sediment from a salt marsh (Pialassa della Baiona, Ravenna, Italy) inoculated with a marine PCB-dechlorinating culture and maintained under in situ like biogeochemical conditions. PHAs increased the initial rate of the reductive dehalogenation process and were fermented more slowly compared to the monomer, reducing the stimulation of anaerobic competitors such as MB. The same study highlighted that the other side of the coin of this specific bioplastics' application could be a pervasive modification of the microbial community of the surrounding environment, as the hyperproliferation of specific bacterial groups was observed.

This study aimed at deepening the existing knowledge both on the effectiveness of PHAs as biostimulating agent in PCBs-contaminated marine sediments and on the effects of their sinking in the sediment on microbial communities, by extending the previous study (Botti et al., 2023) to a different contaminated marine sediment from another anthropized area (Mar Piccolo, Taranto, Italy). Both locations are shallow basins within the Mediterranean Sea, characterized by high anthropization and bordered by several urban areas and large industrial sites. Pialassa della Baiona (Ravenna) is influenced by inputs from the Po river (Ponti and Airoldi, 2009) which drains a heavily anthropized and extensive cultivated inland. Mar Piccolo is a semi enclosed lagoon in Taranto with scarce water circulation that encourages organic matter sedimentation and accumulation of pollutants, i.e. organic compounds and heavy metals deriving from the high urbanization and the massive industrialization of the surrounding area (Cardellicchio et al., 2016). Sediments from the two sites have been reported to differ in terms of granulometry, with Mar Piccolo sediments having higher percentages of clay and silt (Guerra, 2012; Guerra et al., 2014; Todaro et al., 2020; Mali et al., 2020) and higher total organic carbon content (Borghesi et al., 2016; Sfriso et al., 2020; Todaro et al., 2020; Mali et al., 2020). The proposed comparative approach allows to gain insights into the impact of the sediments' features not only on the efficacy of proposed biostimulation strategy for bioremediation, but also on the effects of PHAs environmental release on the sediment microbial community.

2. Material and methods

2.1. Microcosms preparation, sampling and maintenance

Sediments were collected in the Mar Piccolo (MP) (Taranto, Italy, 40.29090 N 17.15040 E). Main chemical-physical parameters of the sediments from Mar Piccolo, as well as of the reference sediments collected in Pialassa della Baiona (PB) (Ravenna, Italy, 44.2938 N 11.2034 E) (Botti et al., 2023) are reported in Supplementary Tables S1-S2, as collection of the recent data available in the literature (from 2000 on). Microcosms were prepared and maintained as described in Botti et al. (2023). Briefly, a sediment slurry was prepared in 100 mL glass serum bottles under anaerobic conditions, inoculated (5 % v/v) with a previously obtained marine culture enriched in OHRB able to reductively dechlorinate PCBs (Nuzzo et al., 2017) and spiked with Aroclor 1254 to a final PCBs concentration of 100 $mg \cdot kg^{-1}_{dry\ sediment}$. To study the influence of the biopolymers on the metabolic activities and on the microbiota, the following compounds were monthly supplied to the microcosms at a final concentration of 20 mM in the main constituent monomer: poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate having a 3-hydroxybutyrate:3-hydroxyvalerate molar ratio 75:25 (PHBHV75) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate having a 3-hydroxybutyrate:3-hydroxyvalerate molar ratio 88:12 (PHBHV88); 3-hydroxybutyric acid (3HB), employed as a reference readily fermentable organic molecule with short life-span. PHAs were both added as suspension of 100–1000 μm particles, coherently with Aulenta et al. (2008) and Amanat et al. (2022), whereas 3HB was supplied from a 2.3 M stock solution in sterile distilled water. Microcosms with no added compounds were set up as negative controls to estimate the extent of the anaerobic metabolic activities supported by the indigenous organic matter (CTR). Microcosms were incubated statically in the dark at 30 °C for 89 days. Periodic sampling (after 0, 30, 61, 75 and 89 days of incubation) was performed to analyze the methane production, the concentration of SO_4^{2-} in the water phase and the concentration of PCBs in the sediment.

Consumed SO_4^{2-} were replenished periodically to maintain microcosms under actual site biogeochemical conditions, avoiding the alteration of the natural competition for electron donors between different terminal-electron accepting processes.

2.2. Extraction and analysis of PCBs

PCBs in the sediment were extracted following a modified method from Rosato et al. (2020), as described by Botti et al. (2023). Quantitative analysis of the PCBs and their possible dechlorination products was performed by using GC-ECD. PCBs concentrations were expressed as μmol of PCBs·$kg^{-1}_{dry\ sediment}$. The chlorination degree was calculated as average number of chlorines per biphenyl molecule, as follows,

$$Chlorination\ degree = \frac{\mu mol\ of\ organic\ chlorine}{\mu mol\ of\ total\ PCBs} = \frac{\sum C_i \times n_i}{\sum C_i} \quad (1)$$

where C_i is the molar concentration of each detected PCB congener (μmol·$kg^{-1}_{dry\ sediment}$) and n_i is the number of its Cl substituents. The percentage reduction of the chlorination degree was used to express the extent of the reductive dechlorination. The $\log_{10}$ fold change (i.e., the $\log_{10}$ of the ratio between the percentage reduction of the chlorination degree at the end of the experiment in the microcosms of MP and PB sediments) was used to compare the extent of the reductive dechlorination in the sediments of the two studied sites.

2.3. Analysis of sulfates and head-space gas

Gas production in the microcosms was measured with an airtight syringe while its CH_4, content was analyzed with a μGC (model 3000 A – Agilent Technologies, Milano, Italy) under the following conditions: injector temperature 90 °C; column temperature 60 °C; sampling time 20 s; injection time 50 ms; column pressure 25 psi; run time was 45 s and the carrier gas was nitrogen. The concentration of SO_4^{2-} in the water phase of the sediment slurry was determined using a Dionex ICS-1000 ion chromatograph equipped with an IonPac AS14 4 mm × 250 mm column, a conductivity detector combined to an AERS-500 suppressor system (Dionex, Sunnyvale, CA, USA). Quantitative analysis were performed by using the conductivity detector response factor obtained from a five-points calibration curve (0.5–50 mg·L^{-1}) of Na_2SO_4. The $\log_{10}$ fold change was used to compare the extent of methanogenic and sulfate-reducing metabolisms in the two sites. It was calculated as $\log_{10}$ of the ratio between the metabolic activities (i.e. cumulative methane production and sulfate depletion rate) measured in microcosms of MP and PB sediments. Since at the end of the first and of the second month of incubation sulfates were completely depleted, hampering the calculation of the actual sulfate-reduction rate, the $\log_{10}$ for sulfate depletion rate was calculated considering the data collected during the third month of incubation, when an additional sampling point was taken on day 75.

2.4. Chemicals

Aroclor 1242, Aroclor 1254 and octachloronaphtalene were provided by Ultra-Scientific. Inorganic ions for IC analysis, 3-hydroxybutyric acid and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with 25 mol% 3-hydroxyvalerate (3HV) units (PHBHV75, powder, custom grade) and with 12 mol% 3HV units (PHBHV88, powder, custom grade) were supplied by SigmaAldrich. Acetone and hexane (both for analysis in capillarycolumn GC systems) as well as the ultra-resi analyzed water for ionchromatography were supplied by Mallinckrodt-Baker.

2.5. Bacterial DNA extraction, 16S rRNA gene amplification and sequencing

Total genomic DNA was extracted from approximately 300 mg of sediment samples taken from the inoculated sediment at the beginning of the experiment (0 day) and from all the microcosms at the end of the experiment (89 days) using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA samples were quantified using Qubit 3.0 fluorimeter (Invitrogen, Waltham, MA, USA) and stored at −20 °C until further processing. The V3-V4 hypervariable region of the 16S rRNA gene was PCR amplified as already described in Botti et al. (2023). PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, United States). Indexed libraries were prepared by limited-cycle PCR with Nextera technology and cleaned-up with the same magnetic beads protocol. Libraries were then normalized to 4 nM and pooled, prior to denaturation with 0.2 N NaOH. Sequencing was performed on Illumina MiSeq platform using a 2 × 250 bp paired-end protocol, following the manufacturer's instructions (Illumina, San Diego, CA, United States).

2.6. Bioinformatics and statistics

Paired-end sequenced reads from this study were analyzed together with those from Botti et al. (2023) (NCBI SRA; BioProject ID PRJ PRJNA884891). Subsamples of 30.000 sequencing reads per sample obtained using Seqtk tool (https://github.com/lh3/seqtk) were merged using the VSEARCH algorithm (v2.15.2) (Rognes et al., 2016) and analyzed using QIIME2 (version 2022.8) (Bolyen et al., 2019). DADA2 (Divisive Amplicon Denoising Algorithm 2) (Hall and Beiko, 2018) plugin was used to remove noise, chimeras, and to generate Amplicon Sequence Variants (ASVs). Taxonomy attribution of ASVs was performed using the feature classifier VSEARCH with SILVA reference database version 138 (Yilmaz et al., 2014). Normalization by rarefaction to the number of sequences in the sample with the least coverage was performed. Microbial community relative abundance profiles at different phylogenetic level were obtained. Statistical analysis was performed using the R statistical software (www.r-project.org), v. 4.2.2 and the libraries vegan, made4, randomForest, and rfPermute. Two different metrics were used to evaluate alpha diversity: Faith's Phylogenetic Diversity (PD) (Haard et al., 1975) and the Simpson diversity index. Weighted and unweighted UniFrac distances were computed to explore samples beta-diversity and plotted as Principal Coordinates Analyses (PCoA). Data separation on PCoA plots was tested using a permutation test with pseudo-F ratios (function “adonis2” in the vegan package). *t*-test was used to assess significance of differences in bacterial relative abundance values among groups of samples. The impact of different variables (sampling site and type of amendments) on the microbiota phylogenetic structure (family and genus level relative abundance profiles) was estimated using the Random Forest machine learning algorithm (Breiman, 2001). Sequence reads from the present study (samples from Mar Piccolo, Taranto) were deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA; BioProject ID PRJNA926484).

3. Results

3.1. Influence of the biodegradable polymers on the microbial anaerobic metabolisms in sediments from Mar Piccolo (Taranto)

In order to evaluate the dynamics of microbial anaerobic metabolisms of interest in microcosms, methane production, sulfate reduction and PCBs reductive dechlorination were monitored (Supplementary Fig. S1) and compared to the results obtained on PB sediments (Botti et al., 2023). To express the differences between the metabolic activities of the studied sediments, $\log_{10}$ fold changes were calculated (Fig. 1, Supplementary Table S3). An overall methane production of 0.29 ± 0.14 mM was observed in MP unamended microcosms (Supplementary Fig. S1A), showing negligible differences with respect to PB sediments ($\log_{10}$ fold changes proximal to 0, Fig. 1). Conversely, at the end of the incubation, methane concentrations were 45.8 ± 11.6 mM, 6.4 ± 1.1 mM, and 13.0 ± 3.6 mM in the presence of 3HB, PHBHV88 and PHBHV75, respectively (Supplementary Fig. S1A), with a $\log_{10}$ fold change with respect to the corresponding PB microcosms ranging from −0.1 to −0.34. Concerning sulfate reducing activity, in unamended MP sediments negligible sulfate

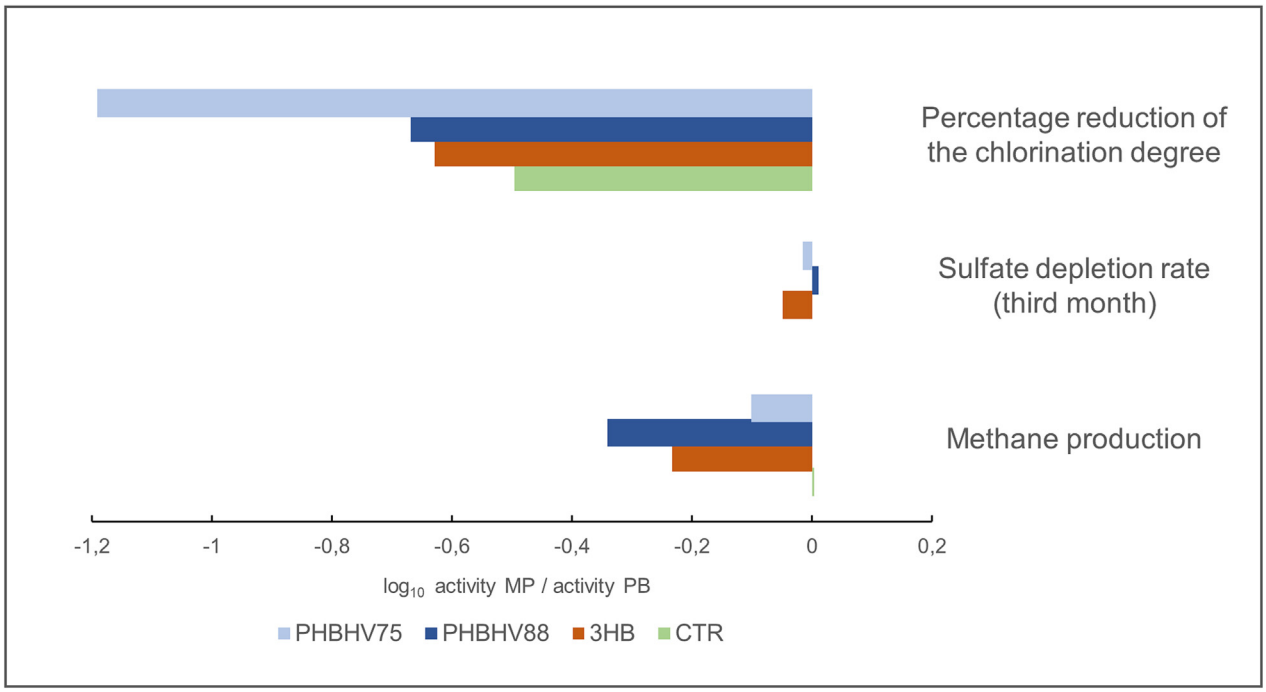

Fig. 1. Differences in the anaerobic metabolic activities measured in the Mar Piccolo, Taranto (MP) sediments in relation to the ones from Pialassa della Baiona, Ravenna (PB). Bar plot representing the average values of $\log_{10}$ ratio of different metabolic activities in the two sediments' site, MP and PB, and the different amendment strategies: PHBHV75 (light blue), PHBHV88 (blue), 3HB (orange), and no amendment (green). Log_{10} fold change of the methane production (mM) and of the reductive dechlorination were calculated at the end (89 days) of the experiment, while sulfate reduction was calculated considering only the data of the third month of incubation. Values of $\log_{10}$ fold change of reductive dechlorination were expressed as the percentage reduction of the chlorination degree of the PCBs mixture, whereas sulfate reduction was measured following the sulfate depletion rate ($g{\cdot}L^{-1}{\cdot}d^{-1}$).

reduction was detected during the third month of incubation, whereas sulfate depletion rates measured were 0.1 ± 0.1 $g{\cdot}L^{-1}{\cdot}d^{-1}$, 0.13 ± 0.01 $g{\cdot}L^{-1}{\cdot}d^{-1}$ and 0.10 ± 0.08 $g{\cdot}L^{-1}{\cdot}d^{-1}$ when adding 3HB, PHBHV88 and PHBHV75, respectively (Supplementary Fig. S1B). These results were coherent with what previously observed on PB sediments ($\log_{10}$ fold changes below 0.05, Fig. 1). Differently from PB sediments, where both the tested PHAs stimulated constantly sulfate-reduction over one month of incubation (Botti et al., 2023), in MP sediments the polymer PHBHV75 stimulated a higher sulfate reduction rate during the first 15 days of the month (0.17 $g{\cdot}L^{-1}{\cdot}d^{-1}$ on days 61–75 vs 0.03 $g{\cdot}L^{-1}{\cdot}d^{-1}$ on days 75–89), suggesting this polymer might be degraded faster in MP sediments compared to PB sediments (Supplementary Fig. S1C). The major differences in terms of metabolic activities between the two sites were identified for reductive dechlorination. In MP sediments, PCBs dehalogenation started from month 2, progressively leading to a 4.6 ± 1.8 % reduction of the chlorination degree of the PCBs mixture at the end of the incubation in the unamended microcosms (Supplementary Fig. S1D). The monomer and PHAs inhibited the dehalogenation process, since after 2 months no or remarkably lower reduction of the chlorination degree was observed in microcosms treated with these amendments. At the end of the experiment, the chlorination degree was reduced of 2.8 ± 3.7 %, 0.9 ± 1.4 % and 2.7 ± 0.3 % in presence of 3HB, PHBHV75 and PHBHV88, respectively (Supplementary Fig. S1D). In comparison to what observed using PB sediments, the extent of PCBs dehalogenation observed in MP microcosms under all the studied conditions was decreased, showing $\log_{10}$ fold changes ranging between −0.5 and -1.2 (Fig. 1). The congeners that accumulated in higher abundance were 2,2',4,5-CB and 2,4,4',6-CB, possibly formed via reduction of 2,2',3,4,4',6-CB, 2,2',4,4',5,5'-CB, 2,2',3,4,5,6-CB, 2,2',4,5,5'-CB, 2,2',4,4',6,6'-CB, 2,2',4,5,6,-CB, which decreased most remarkably after 89 days (Supplementary Fig. S2), through removal of chlorine mainly from flanked para and meta positions. No differences were observed in the dechlorination patterns taking place in the control and in the amended microcosms (Supplementary Fig. S2), as the same highly chlorinated congeners were depleted and the same medium and low chlorinated one accumulated.

3.2. Microbial community characterization

16S rRNA gene amplicon reads from the work of Botti et al. (2023) and from the present paper were analyzed together and beta diversity was explored using multivariate statistical approaches, in order to explore the differences in microbial communities of MP and PB sediments that might have contributed to the differences in the amendment effect on anaerobic metabolisms.

Fig. 2. Microbial community characterization. (A-B) PCoA based on weighted (A) and unweighted (B) UniFrac distances among sediments microbiota profiles of samples collected at the beginning (0 days) and at the end (89 days) of the experiment. Sediments' geographical origins, Pialassa della Baiona, Ravenna (PB) and Mar Piccolo, Taranto (MP), are displayed as circles and triangles, respectively. Color legend for samples representation is reported (right). First and second coordination axes (MDS1 and MDS2) are plotted for each analysis. Percentages of variance in the dataset accounted for MDS1 and MDS2 are reported. (C) Phylogenetic profiles at the family level of sediments from Ravenna (PB) and Taranto (MP) at the beginning of the experiment (0 days) and of the amended and control sediments at 89 days. Color band (left) according to the previous PCoA color legend (A-B) are displayed to clearly identify groups of samples. Bacterial families having relative abundance >2 % in at least 3 sample are depicted in the barplots, for which color legend is reported in the right panel. Black color is used to indicate the percentage of "Other" reads, including unassigned sequences and families with a relative abundance which did not pass the mentioned threshold. (D-E) Boxplot representation of alpha diversity calculated using Simpson (D) and Faith's Phylogenetic Diversity (PD) (E) indices of amended (3HB, PHBVH88, and PHBHV75 at 89 days) and unamended (at 0 and 89 days) sediments. Biodiversity significantly decreases in MP-sediments amended with PHBHV88 and PHBHV75 compared to those at the beginning (MP 0 days) (p-values < 0.05).

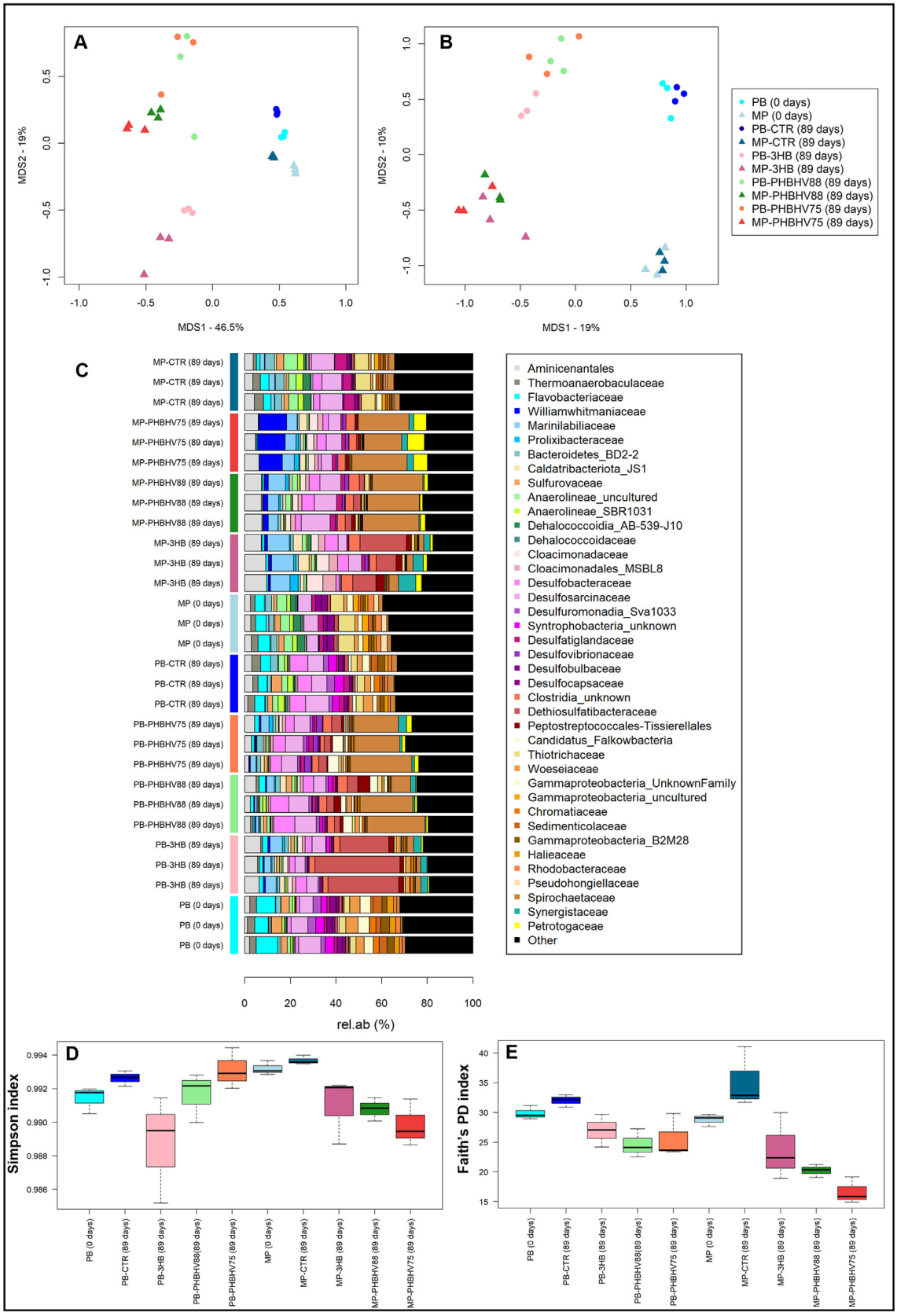
A
B
MDS2 - 19%
MDS1 - 46.5%
MDS2 - 10%
MDS1 - 19%
PB (0 days)
MP (0 days)
PB-CTR (89 days)
MP-CTR (89 days)
PB-3HB (89 days)
MP-3HB (89 days)
PB-PHBHV88 (89 days)
MP-PHBHV88 (89 days)
PB-PHBHV75 (89 days)
MP-PHBHV75 (89 days)
C
MP-CTR (89 days)
MP-PHBHV75 (89 days)
MP-PHBHV88 (89 days)
MP-3HB (89 days)
MP (0 days)
PB-CTR (89 days)
PB-PHBHV75 (89 days)
PB-PHBHV88 (89 days)
PB-3HB (89 days)
PB (0 days)
Aminicenantales
Thermoanaerobaculaceae
Flavobacteriaceae
Williamwhitmaniaceae
Marinilabiliaceae
Prolixibacteraceae
Bacteroidetes_BD2-2
Caldatribacteriota_JS1
Sulfurovaceae
Anaerolineae_uncultured
Anaerolineae_SBR1031
Dehalococcoidia_AB-539-J10
Dehalococcoidaceae
Cloacimonadaceae
Cloacimonadales_MSBL8
Desulfobacteraceae
Desulfosarcinaceae
Desulfuromonadia_Sva1033
Syntrophobacteria_unknown
Desulfatiglandaceae
Desulfovibrionaceae
Desulfobulbaceae
Desulfocapsaceae
Clostridia_unknown
Dethiosulfatibacteraceae
Peptostreptococcales-Tissierellales
Candidatus_Falkowbacteria
Thiotrichaceae
Woeseiaceae
Gammaproteobacteria_UnknownFamily
Gammaproteobacteria_uncultured
Chromatiaceae
Sedimenticolaceae
Gammaproteobacteria_B2M28
Halieaceae
Rhodobacteraceae
Pseudohongiellaceae
Spirochaetaceae
Synergistaceae
Petrotogaceae
Other
rel.ab (%)
D
Simpson index
E
Faith's PD index

Table 1
Random forest misclassification rates of sediments geographical origins (PB vs. MP), expressed as "out-of-bag" (OOB) error rate, based on the microbiota phylogenetic structure (at family and genus level).

Random Forest variable	PB vs. MP[a]					
Taxonomy level	Family			Genus		
OOB error rate (%)	0			4.2		
		Relative abundance (%, mean ± standard deviation)			Relative abundance (%, mean ± standard deviation)	
		PB	MP		PB	MP
Top10 predictors[b]	*Desulfuromonadia* Sva1033	1.4 ± 0.8	0.02 ± 0.05	*Desulfuromonadia* Sva1033	1.4 ± 0.8	0.02 ± 0.05
	Pseudohongiellaceae	0.004 ± 0.011	0.3 ± 0.2	*Woeseia*	1.9 ± 1.0	0.2 ± 0.4
	Candidatus *Falkowbacteria*	2.0 ± 1.8	0.04 ± 0.09	Candidatus *Falkowbacteria*	2.0 ± 1.8	0.04 ± 0.09
	Halieaceae	0.6 ± 0.5	0.03 ± 0.07	*Pseudohongiella*	0.004 ± 0.011	0.3 ± 0.2
	Woeseiaceae	1.9 ± 1.0	0.2 ± 0.4	Syntrophobacterales uncultured	1.5 ± 1.3	0.1 ± 0.2
	Syntrophobacteria unknown	1.5 ± 1.3	0.1 ± 0.2	Gammaproteobacteria uncultured	0.04 ± 0.09	0.6 ± 0.6
	Gammaproteobacteria uncultured	0.04 ± 0.09	0.6 ± 0.6	*Williamwhitmaniaceae* Blvii28_wastewater-sludge_group	0.5 ± 0.3	3.7 ± 4.8
	Dethiosulfatibacteraceae	9.6 ± 12.8	4.3 ± 6.0	Candidatus *Cloacimonas*	0.9 ± 0.8	2.8 ± 1.9
	Williamwhitmaniaceae	0.5 ± 0.3	3.7 ± 4.8	*Marinilabiliaceae* uncultured	1.8 ± 1.7	5.3 ± 2.7
	Cloacimonadaceae	0.9 ± 0.8	2.8 ± 1.9	Dehaloccoccoidia SGCG_AB-539-J10	0.1 ± 0.2	0.8 ± 1.2

[a] Controls and amended samples at 89 days were considered.
[b] 10 bacterial taxa at family or genus level showing the highest mean decrease in Gini value, being identified as most reliable and relevant predictors to perform classification of sediments.

The Principal Coordinates Analysis (PCoA) based on both weighted and unweighted UniFrac distances (Fig. 2A and B) showed that, after 89 days, amended sediments clustered separately from the unamended ones and from the original sediments (day 0) (Adonis test, p value = 0.001) along the first (horizontal) coordination axis, which accounted for the majority of the variance in the dataset (46.5 % for weighted UniFrac based PCoA, 19 % for Unweighted metric). Whilst such separation along the first axis occurred regardless of the sediments' geographical origins, we can observe that PB and MP sediments separates along the second axis when unweighted UniFrac distance is used (Fig. 2B, Adonis test, p value = 0.001), both for amended and unamended microcosms. Conversely, weighted UniFrac distances-based PCoA (Fig. 2A) allowed to highlight separation, along the second ordination axis, between samples treated with the monomer, using both MP and PB sediments, and with the polymeric amendments (PHBHV75 and PHBHV88). This observation was in line with what was predicted using the Random Forest machine learning algorithm for samples classification based on sequences data (Roguet et al., 2018). Indeed, Random Forest classification highlighted that both geographical origins of the sediments (MP vs. PB, Table 1) and monomer (3HB vs. all other samples, Table 2) or polymer-(PHBHV vs. all other samples, Table 3) based amendments were well discriminated based on the compositional profile of the sediments microbiota at day 89, at both the family and genus levels ("out-of-bag" (OOB) error rates between 0 and 8.3 %). The different variables (sediments geographical origin, monomer and polymer-based amendment) fed to the Random Forest algorithm resulted in different and scarcely overlapping sets of taxa able to predict samples classification ("top 10 predictors") (Tables 1–3). Largely subdominant bacterial groups discriminated between microcosms of MP and PB sediments (relative abundances of top 10 predictors ranging between 9.6 ± 12.8 % and 0.004 ± 0.011 % at family level, and between 5.3 ± 2.7 % and 0.004 ± 0.011 % at genus level; Table 1), confirming the separation obtained only using unweighted metrics. On the contrary, among the top 10 predictors for classifying the monomer-amended (Table 2) and polymers-amended (Table 3) microcosms, bacterial groups that became dominant at the end of the experiments in both MP and PB sediments were listed (e.g. *Dethiosulfatibacteraceae* for 3HB and *Spirochaetaceae* for PHBHV88 and PHBHV75) (Fig. 2C). Indeed, comparing the microbial communities after 89 days of incubation with the one at day 0, monomer and

Table 2
Random forest misclassification rates of sediments amendment based on monomer-based amendment (3HB vs. all other samples), expressed as "out-of-bag" (OOB) error rate, based on the microbiota phylogenetic structure (at family and genus level).

Random Forest variable	3HB vs. all others[a]					
taxonomy level	Family			Genus		
OOB error rate (%)	5.5			0		
		Relative abundance (%, mean ± standard deviation)			Relative abundance (%, mean ± standard deviation)	
		3HB	Others		3HB	Others
Top10 predictors[b]	*Dethiosulfatibacteraceae*	21.5 ± 11.2	2.1 ± 1.4	*Dethiosulfatibacter*	21.5 ± 11.2	2.1 ± 1.4
	Desulfatiglandaceae	0.6 ± 0.5	2.1 ± 1.3	Peptostreptococcales-Tissierellales	2.2 ± 0.8	0.6 ± 0.8
	Synergistaceae	4.0 ± 1.9	1.0 ± 1.1	*Desulfatiglans*	0.6 ± 0.5	2.1 ± 1.3
	Peptostreptococcales-Tissierellales	2.4 ± 0.8	1.0 ± 1.3	*Thermovirga*	2.7 ± 1.2	0.8 ± 0.9
	Desulfosarcinaceae	4.2 ± 2.0	8.1 ± 2.1	Aminicenantales	7.5 ± 1.6	4.3 ± 1.9
	Spirochaetaceae	2.7 ± 0.8	15.1 ± 9.9	*Spirochaetaceae* uncultured	0.3 ± 0.3	12.1 ± 9.6
	Marinilabiliaceae	6.8 ± 2.9	2.7 ± 2.2	*Marinilabiliaceae* uncultured	6.3 ± 2.9	2.6 ± 2.1
	Aminicenantales	7.5 ± 1.6	4.3 ± 1.9	Cloacimonadales MSBL8	2.6 ± 1.9	1.0 ± 0.7
	Cloacimonadaceae	3.6 ± 2.1	1.3 ± 1.1	Candidatus *Cloacimonas*	3.6 ± 2.1	1.3 ± 1.1
	Gammaproteobacteria unknown family	0.1 ± 0.2	1.0 ± 0.9	Gammaproteobacteria unknown family	0.1 ± 0.2	1.0 ± 0.9

[a] Controls and amended samples at 89 days were considered.
[b] 10 bacterial taxa at family or genus level showing the highest mean decrease in Gini value, being identified as most reliable and relevant predictors to perform classification of sediments.

Table 3
Random forest misclassification rates of sediments amendment based on polymer-based (PHBHV vs. all other samples), expressed as "out-of-bag" (OOB) error rate, based on the microbiota phylogenetic structure (at family and genus level).

Random Forest variables	PHBHV vs. all others[a]					
taxonomy level	Family			Genus		
OOB error rate (%)	8.3			8.3		
		relative abundance (%, mean ± standard deviation)			relative abundance (%, mean ± standard deviation)	
		PHBHV	Others		PHB	Others
Top10 predictors[b]	*Spirochaetaceae*	21.3 ± 4.9	2.6 ± 0.6	*Spirochaetaceae* uncultured	18.0 ± 5.6	0.4 ± 0.3
	Desulfovibrionaceae	1.3 ± 0.9	0.1 ± 0.4	*Desulfovibrio*	1.3 ± 0.8	0.1 ± 0.2
	Desulfobulbaceae	0.2 ± 0.3	0.9 ± 0.6	Clostridia unknown	3.4 ± 0.7	1.8 ± 1.9
	Petrotogaceae	2.7 ± 2.3	0.6 ± 0.9	*Desulfosarcinaceae*-Sva0081 sediment group	0.6 ± 0.5	2.3 ± 1.6
	Clostridia unknown	3.4 ± 0.7	1.8 ± 1.9	*Sediminispirochaeta*	1.7 ± 0.7	0.7 ± 0.8
	Rhodobacteraceae	0.2 ± 0.2	0.7 ± 0.5	*Petrotogaceae*-SC103	2.7 ± 2.3	0.6 ± 0.9
	Flavobacteriaceae	0.9 ± 0.9	2.5 ± 1.4	*Desulfosarcina*	2.1 ± 1.7	0.6 ± 0.5
	Dethiosulfatibacteraceae	2.5 ± 1.4	11.3 ± 13.1	*Desulfobulbaceae*-uncultured	0.2 ± 0.3	0.8 ± 0.6
	Desulfatiglandaceae	1.6 ± 0.5	1.9 ± 1.8	*Dethiosulfatibacter*	2.5 ± 1.4	11.3 ± 13.1
	Candidatus *Falkowbacteria*	1.7 ± 2.0	0.4 ± 0.5	*Desulfosarcinaceae* uncultured	1.6 ± 0.8	0.8 ± 0.6

[a] Controls and amended samples at 89 days were considered.
[b] 10 bacterial taxa at family or genus level showing the highest mean decrease in Gini value, being identified as most reliable and relevant predictors to perform classification of sediments.

polymer-based amendments caused a transition from a diverse microbial community to ecosystems showing noticeable dominances (i.e. single taxa accounting for approximately 20 % of the microbial community profile in average) and consequently lower phylogenetic diversity (Fig. 2C and E). The effect of amending was particularly evident in MP sediments, where a significant decrease of alpha diversity was observed, especially for what concern PHBHV88 (*t*-test, p value = 0.01 and 0.0006 for Simpson index and Faith's PD index, respectively) and PHBHV75 (p value = 0.04 and 0.004), with respect to sediments at day 0. Differently, both the control microcosms (MP and PB) after three months of incubation (day 89) maintained a family-level community structure very similar to the one reported for the sediment at day 0 (Fig. 2A-2B), without noticeable compositional dominances (Fig. 2C) and even an increased alpha diversity (Fig. 2D-E).

In sediments amended with 3HB, besides the noticeable *Dethiosulfatibacteraceae* dominance, an increase in *Synergistaceae* was detected with respect to all other samples (Table 2), in particular of the genus *Thermovirga* that reached relative abundances of 3.2 ± 1.8 % and 2.3 ± 0.2 % in MP and PB microcosms, respectively. Another 3HB related feature was represented by the family *Marinilabiliaceae* (all sequences assigned to uncultured bacteria), within the phylum Bacteroidetes, which was enriched in all microcosms amended with 3HB (reaching 9.2 ± 0.5 % and 4.3 ± 1.3 % in MP and PB microcosms, respectively) (Fig. 2C and Table 2). Moreover, 3HB amended microcosms were the only ones in which sequences assigned to the methanogenic Archaea genus *Methanosaeta* were detectable (0.2 ± 0.3 % and 0.7 ± 0.6 % in MP and PB-sediments).

Concerning the amendments with PHAs (PHBHV75 and PHBHV88), besides the dominance of the *Spirochaetaceae* family, samples were characterized by an enrichment in *Petrotogaceae*, a group among the Random Forest top 10 predictors (Table 3) that enriched especially in PHBHV75 amended sediments (6 ± 0.9 % and 1.8 ± 0.3 % in MP and PB sediments, respectively), with respect to PHBHV88 (1.7 ± 0.4 % and 0.8 ± 0.2 %). This family was also observed at smaller relative abundances in 3HB amended microcosms, whereas no sequences were detected in control samples at 89 days. Of particular interest, within the phylum Bacteroidetes, the family *Williamwhitmaniaceae* (all sequences assigned to "Blvii28_wastewater-sludge_group") was strongly stimulated in MP sediments by PHBHV75, reaching approximately the 11.5 ± 1.1 % of the whole community (Fig. 2C) and ending up among the top 10 predictors for classifying the geographical origin of the samples (Table 1).

Finally, it is possible to appreciate that in all observed conditions, a relevant portion of the sediment microbial community (25 ± 8 % in average) was related to putative sulfate reducing bacteria (i.e. members of Desulfobacterota phylum and few families from the phylum Firmicutes (*Desulfitobacteriaceae, Desulfotomaculales*) as previously reported (Waite et al., 2020; Zhou et al., 2011), depicted mainly in purple shades in Fig. 2C). The highest abundances were reached in microcosms amended with 3HB (41.8 ± 6.1 % and 23 ± 9.6 % in PB-3HB and MP-3HB, respectively), mainly due to the previously highlighted significant increase in *Dethiosulfatibacteraceae*; the difference between PB and MP microcosms in this case reflects the slightly increased sulfate consumption in PB-3HB microcosms with respect to MP-3HB. No significant differences were found in the cumulative percentage of sulfate reducers between other conditions.

For what concern putative dechlorinating bacteria (i.e. members of Dehalococcoidia class) (Kalogerakis et al., 2015; Zanaroli et al., 2012a), no correlations between metabolic output (i.e. chlorination degree) and cumulative Dehalococcoidia abundance was found. However, MP sediments at day 0 were characterized by a larger cumulative fraction of bacterial groups belonging to the phylum Chloroflexi (2.95 ± 1.04 %, depicted in shades of green in Fig. 2C), including uncultured Anaerolineae members, Anaerolineae group SBR1031 group and Dehalococcoidia group AB-539-J10, with respect to PB sediments at day 0 (0.8 ± 0.5 %). Such larger abundance of Chloroflexi is maintained in MP control microcosms at day 89 (3.2 ± 1.4 %), whereas it noticeably shrinks in amended microcosms due to the pervasive modifications induced by the amendments. It is interesting to point out that Dehalococcoidia AB-539-J10 also emerged among the Random Forest best predictors of sediments' geographical origin (Table 1).

4. Discussion

In the experiments described here (i.e. the one reported in Botti et al. (2023) and the present study) sediments from Mar Piccolo (Taranto) and Pialassa della Baiona (Ravenna) coastal regions (Italy) were both biostimulated via addition of different formulations of PHAs, as supposedly "environmentally friendly" slow fermenting polymers of industrial and ecological interest (Awasthi et al., 2022). While the anaerobic metabolisms of OHRB's most relevant competitors (i.e., sulfate reduction and methanogenesis) were similarly stimulated in both the studied sediments, reductive dechlorination proceeded to a different extent in sediments from PB compared to the ones from MP, supporting the onset of reductive dehalogenation in the first case (Botti et al., 2023) while inhibiting it in the second. This was particularly evident in the case of the polymer PHBHV75, which instead performed better than PHBHV88 in PB sediments (Botti et al., 2023). Conflicting results were previously obtained when comparing degradation rates of PHA heteropolymers with different

hydroxybutyrate and hydroxyvalerate content (Amanat et al., 2022; Kaplan et al., 1994; Kasuya et al., 1998; Mergaert et al., 1994). Thus, our results confirm that the sediments physical-chemical characteristics and/or the biodiversity and composition of the indigenous microbial community might have played a key role in determining the different fates of the added fermentable carbon sources, and consequently, the stimulation of the reductive dechlorination. For instance, PB sediments differ from those from MP for the granulometry, with the former notoriously being richer in sand content compared to the latter ones that have a higher fraction of mud (Di Leo et al., 2016; Guerra, 2012; Guerra et al., 2014; Mali et al., 2020, 2017; Ponti et al., 2011; Quero et al., 2015; Todaro et al., 2020; Vitone et al., 2016). Sandy sediments are characterized by a higher permeability (Huettel et al., 2014), which favors mass transport possibly resulting in a more efficient usage of the organic carbon available by the microbial community (Woulds et al., 2016). As for muddy sediments, they are usually more anoxic, richer in organic matter, and consequently able to sustain higher biodiversity (Boey et al., 2022). Indeed, the available literature showed that MP sediments can reach higher values of total organic carbon (TOC) (Borghesi et al., 2016; Di Leo et al., 2016; Guerra, 2012; Guerra et al., 2022, 2014, 2013; Mali et al., 2020, 2017; Ponti et al., 2011; Quero et al., 2015; Sfriso et al., 2020; Sollecito et al., 2019; Todaro et al., 2020, 2019). Thus, concerning the differences in the extent of reductive dehalogenation, the higher sand content of PB sediments might have supported the bacterial activities and the fermentation of organic matter delivering the required hydrogen to OHRB. The sandier feature might then help explaining the differences observed in the extent of PCBs reductive dechlorination when treating diverse aquatic sediment sites (Kjellerup et al., 2008). As for the inhibition of the reductive dehalogenation occurring in MP sediments amended with PHAs, it was interesting to find that MP samples were characterized by higher percentage of indigenous Dehalococcoidia assigned to the "AB-539-J10" group. This particular group of bacteria was reported as lacking dehalogenases in their genome, according to the available literature (Wasmund et al., 2014), in spite of belonging to the most commonly reported OHRB taxonomic group (Kalogerakis et al., 2015). The presence of more abundant Chloroflexi (Anaerolineae and Dehalococcoidia) in MP sediments might be related to the higher Pb contamination reported for this site with respect to PB (Bellucci et al., 2016; Borghesi et al., 2016; Mali et al., 2020, 2017; Quero et al., 2015; Todaro et al., 2020, 2019), as a positive correlation between this lineage and Pb concentration was previously reported (Li et al., 2020). Finally, sandy sediments have been previously associated to indigenous microbial communities able to respond more rapidly to changing environmental conditions with respect to muddier ones (Boey et al., 2022). It is tempting to hypothesize that, along the relatively short timeframe of the microcosm experiment, the microbial community from the sandier location (PB) might have been able to promptly reestablish a functioning microbial metabolic network in which even low-abundant members, such as OHRB, fulfill their ecological role.

On the contrary, from other anaerobic metabolisms point of view, accordance between the sediments from the two locations was reported, as sulfate-reduction was the main metabolism stimulated by the amendments and sulfate-reducing taxa represented an important fraction of the microbial community under all tested conditions, in both sediments.

Two levels of complexity are appreciable in data obtained from the microbial community characterization, since both site-specific (MP vs. PB) and amendment-specific (PHBHV vs. 3HB vs. controls) features were detected in the dataset. Site-specific microbial features resided within the subdominant fraction of the sediments' microbial community, as site-wise clustering in amended microbial communities (after three months of microcosm experiment) occurred when using statistics that do not take into account the abundance of the single species (i.e. unweighted UniFrac metric). Conversely, abundant members of the microbial communities responded to the use of either polymers or the monomer as amendment, determining the noticeable separation of the two types of samples when weighted statistical analyses (i.e. weighted UniFrac metric) were performed. Concerning site-specific features, coherently with the previously reported highest percentage of silt and clay in MP sediments (Di Leo et al., 2016; Mali et al., 2020, 2017; Quero et al., 2015; Todaro et al., 2020; Vitone et al., 2016), MP samples were initially characterized by higher alpha diversity compared to PB sediments. MP samples showed compositional features previously associated to muddy sediments, such as higher abundances of Anaerolineae (Aldeguer-Riquelme et al., 2022) and decreased *Flavobacteriaceae* (Chen et al., 2022). These features, as well as the levels of alpha diversity, were maintained during the three months incubation in microcosms in the absence of biostimulation (control experiments), whereas the different types of amendments led to the selection of specific microbial communities with decreased alpha diversity. Indeed, the addition of monomer and polymers led to the enrichment of specific fermenting bacteria in both sediments, with the instauration of a dominance in the microbial community profile. As expected, the monomer-based amendment enriched fermenting and syntrophic bacteria, such as those belonging to the family *Dethiosulfatibacteraceae,* both in MP and PB sediments. This bacterial lineage belongs to the Firmicutes phylum, and it is known to ferment hydroxyl-fatty acids (An et al., 2017; Matturro et al., 2017), explaining the preferential enrichment with the monomer addition. Also, this group is able to reduce thiosulfate and elemental sulfur to sulfide (Takii et al., 2007), with thiosulfate being an inorganic intermediate of sulfur cycle that is only accumulated in sediments with particularly high organic load and sulfide production, such as salt marsh beds (Zopfi et al., 2004). Moreover, the genus *Dethiosulfatibacter* have been reported to potentially thrive in presence of a large number of aliphatic and aromatic hydrocarbons, since it is equipped with genes for their anaerobic degradation (Vigneron et al., 2021). It is tempting to link the more striking dominance of *Dethiosulfatibacteraceae* obtained in the PB sediments with the higher polycyclic aromatic hydrocarbon (PAH) contamination reported for this location (Guerra, 2012). Moreover, in spite of being classified as syntrophic bacteria able to oxidize acetate and sustain the growth of hydrogenotrophic methanogens, which were not detected in the studied sediments, the genus *Thermovirga* has been found in cooperative association with *Methanosaeta*, an acetoclastic methanogen, in anaerobic reactors (Ito et al., 2011; Xu et al., 2018). This sustains the possibility that a functioning and cooperative microbial community based on fermentative processes establishes when amending with readily fermentable monomers. Conversely, the amended polymers led to a proliferation of *Spirochaetaceae* in both sediments, representing a marked amendment-specific feature. This family was previously reported to enrich when amending microbial communities with PHAs (Matturro et al., 2018; Pinnell and Turner, 2019; Yang et al., 2020) and have been reported among lineages encoding PHAs depolymerases (Viljakainen and Hug, 2021). *Spirochaetes* were also addressed as possible acetogens in an enriched microbial community able to reductively dechlorinate trichloroethene (TCE) (Ziv-El et al., 2011). Thus, they might have possibly acted as first degraders of the added complex organic matter, subsequently leading to the stimulation of other anaerobic species as sulfate reducing bacteria, as previously speculated by Pinnell and Turner (2019).

Trying to combine the two levels of complexity ("site effect" and "amendment specificity") is harder task. For instance, in both MP and PB sediments, the amendments (both polymers and monomer) caused a shrinkage of the relative abundance of members of *Flavobacteraceae* (phylum Bacteroidetes), which was not detected in control microcosms. However, only in amended MP sediments the Bacteroidetes population was replenished by a proliferation of other families within the same phylum, namely *Marinilabiliaceae* and, especially in the case of PHBHV75 amendment, *Williamwhitmaniaceae*. The available literature does not offer clear suggestions neither about the ecological role of such bacterial families in marine sediments, nor on possible links with biogeochemical features of the MP sediments. Interestingly, members of the *Marinilabiliaceae* family have been reported as lipolytic bacteria (Shalley et al., 2013) and the lipolysis process is strictly linked to the metabolism of PHAs, not only in PHAs producing bacteria but also in degraders-only (Bashiri et al., 2022). The bacterial group "Blvii28_wastewater-sludge_group", to which all sequences attached to the family *Williamwhitmaniaceae* were assigned, was reported as able to perform β/ω-oxidation (de Melo Pirete et al., 2022), a metabolic

pathway involved in the degradation of oligomers and monomers generated by PHAs hydrolysis (Altaee et al., 2016; Prieto et al., 2016).

From a community ecology standpoint, the changes in the microbial community profile of the amended microcosms likely mirrored rearrangements in the metabolic network. Indeed, *Flavobacteriaceae* are known primary degraders in marine ecosystems, often keystone species of modules controlled by - and specialized in the degradation of - the most abundant and available carbon source (Enke et al., 2019; Gralka et al., 2020). The decrease or disappearance of such group in amended microcosms was a direct consequence of the change in the main available macromolecular carbon source, because of which other types of primary degraders took over and became dominant (i.e. fermenters in the case of microcosms provided with the monomer, macromolecules-degrading *Spirochateaceae* in the case of polymers). Marine communities are also equipped with secondary modules of bacteria independent from the nature and abundance of the major complex substrate, whose dynamics are less predictable and determined by inter-species metabolic interactions, e.g. cross-feeding. Members of these secondary modules lack hydrolytic capability and rely on other community members for provision of metabolic intermediates: organic acids, saccharides, nucleotides, amino acids, as well as other secondary metabolites such as signaling molecules and antimicrobial compounds, which emerge from the interaction of the microbial community members and specific biogeochemical features of the environment (Enke et al., 2019; Gralka et al., 2020). The addition of a specific amendment to the sediment created the opportunity for the sedimentary microbial community to re-assemble, with a structure centered on a common module of primary degraders selected by the exogenous primary carbon source (monomer or polymers), but with decentralized modules of subdominant, secondary consumers which are idiosyncratic to the two different sediments (Gralka et al., 2020). In the present model, the Bacteroidetes families with unclear ecological role (*Marinilabialiaceae* and *Williamwhitmaniaceae*) that only enriched in amended MP microcosms might represent an example of those secondary functional clusters. In fact, they were selected starting from the plethora of subdominant microorganisms provided by the original sediment, with its peculiar biogeochemical context, and thrived in the specific novel environment resulting from the shift in the primary carbon source degradation and the consequent changes in released metabolites.

5. Conclusions

The present study broadens the current knowledge about the use of PHAs as potential biostimulants for microbial reductive dechlorination processes in marine sediments contaminated by organohalides. The comparative approach using sedimentary materials from two highly anthropized coastal sites in the Mediterranean basin revealed opposite effects of PHAs on PCBs reductive dechlorination. This remarks the need to deepen the comprehension on how different factors, i.e. the composition of resident microbial community and the chemical-physical parameters of the starting environment, interact among each other and with the biostimulating agent to determine the outcome. Such interactions, and our lack of understanding of it, can determine the failing of bioremediation strategies that showed promising elsewhere.

Parallelly, even if with all the due limitations of microcosm experiments, the study contributed in meeting the recently highlighted need to explore the potential impact of the introduction of biodegradable plastics into the marine environment, either intentional (i.e., as biostimulants) or incidental (i.e., as in the context of future large-scale use of disposable items), on the ecosystem balance (Chen, 2022). A specific and pervasive effect of PHAs on the sedimentary microbial community was reported, reducing the biodiversity, defining a newly arranged microbial core of polymer degraders and shaping the composition of subdominant fractions of the microbial community. This represents a first glance on the secondary effects of PHAs biodegradation on the surrounding ecosystem. In a perspective scenario in which biodegradable plastic materials take over traditional poorly degradable plastics, it is reasonable to picture that an increasing load of such polymers will reach the natural environment. Once dispersed, PHAs will become an abundant source of carbon and will consequently have the potential to change the structure of autochthonous microbial communities with possible secondary effects on the biogeochemical cycles.

CRediT authorship contribution statement

Botti Alberto: Investigation, Data curation, Formal analysis, Writing – original draft. **Musmeci Eliana:** Investigation, Data curation, Formal analysis, Writing – original draft. **Negroni Andrea:** Resources, Writing – review & editing. **Capuozzo Rosaria:** Investigation, Writing – review & editing. **Fava Fabio:** Funding acquisition, Writing – review & editing. **Biagi Elena:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **Zanaroli Giulio:** Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

Data availability

Data have been deposited on NCBI-SRA (Bioproject ID: PRJNA926484)

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.scitotenv.2023.165485.

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