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Exploring the biodegradation of PET in mangrove soil and its intermediates by enriched bacterial consortia

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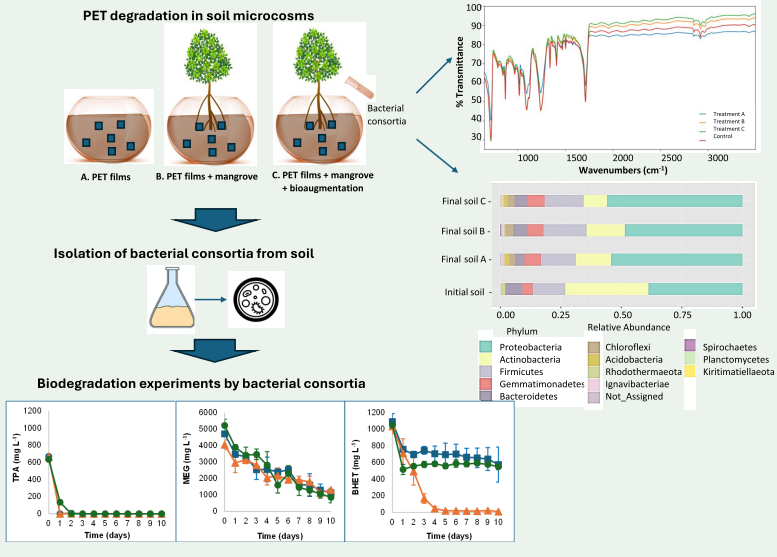
ABSTRACT

The biodegradation of Polyethylene terephthalate (PET) is important due to the environmental impact of plastic waste. This study investigates the degradation of PET films in soil microcosms, with and without mangrove plants, and with mangrove plants bioaugmented with a bacterial consortium (*Bacillus* sp.- GPB12 and *Enterococcus* sp.- WTP31B-5) while following the evolution of soil microcosm microbiome. The ability of bacterial consortia retrieved from soil microcosms of each tested condition to degrade PET intermediates - bis(2-hydroxyethyl) terephthalate (BHET), terephthalic acid (TPA), and monoethylene glycol (MEG) was also assessed. In the microcosms' assays with mangrove plants, variations in functional groups and surface morphology detected by FTIR and SEM analysis indicated PET degradation. Soil microcosms microbiome evolved differently according to the conditions imposed, with dominance of phylum Proteobacteria in all final microcosms. After 270 days, bacterial consortia retrieved from all soil microcosms revealed to be able to completely degrade TPA within three days. MEG degradation reached ca. 84% using the consortium retrieved from the microcosm with bioaugmented mangrove plants. BHETdegradation was ca. 96% with the consortium obtained from the microcosm with non-bioaugmented mangrove plants. These intermediates are key molecules in PET degradation pathways; thus, their degradation is an indicator of biodegradation potential. To the best of authors' knowledge, this is the first report on biodegradation of PET, BHET, TPA, and MEG by microbial community from mangrove soil, providing insights into key taxa involved in PET degradation. These findings can pave a way to develop bioremediation strategies and more efficient waste management solutions.



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1. Introduction

The mangrove ecosystem is a unique coastal wetland and the sole aquatic forest ecosystem, which acts as a vital carbon reservoir with high productivity [1]. Mangroves play a crucial role in coastal protection and ecological purification of water resources [2]. In addition, the well-developed root system enables mangrove plants to thrive against dynamic environmental stress and act as traps for marine litter [3, 4]. Mangroves are primarily found in coastal wetland areas, where they are exposed to various pollutants, including plastics. While exposed to contaminants, mangroves also serve as natural filters and retention points for continental waste entering the ocean [5]. This ecosystem experiences large-scale accumulation of organic, inorganic and plastic wastes due to its typical vegetation, dense root systems and reduced tidal flow. Rhizosphere microorganisms are highly adaptable to dynamic environmental conditions, such as variations in salinity, oxygen levels, and nutrient availability. This adaptability makes them effective in facilitating biodegradation processes within dynamic ecosystems like mangroves. In these ecosystems, rhizosphere microorganisms play a vital role by decomposing organic matter and recycling nutrients like nitrogen and phosphorus, which are essential for mangrove plant growth. This study hypothesizes that mangrove ecosystems, with their unique microbial communities and plant-microbe interactions, can facilitate the biodegradation of PET and its key intermediates, as these microorganisms are likely to have been chronically exposed to plastic pollution and may be more adapted to degrade such compounds.

Phytoremediation is a sustainable remediation process that harnesses the ability of plants and rhizosphere microorganisms to reduce or eliminate harmful contaminant levels in diverse environments [6]. In fact, rhizosphere microorganisms can potentially enhance the biodegradation of polylactide (PLA) and conventional polyethylene terephthalate (PET) films, a global environmental threat [7]. Recently, Dhaka et al. [8] investigated the biodegradation of PET sheet by three rhizosphere bacterial isolates, namely *Priestia aryabhattai* VT 3.12, *Bacillus pseudomycoides* VT 3.15, and *Bacillus pumilus* VT 3.16 achieving degradation efficiencies of 40, 36, and 32% respectively, in 28 days. The study emphasizes the importance of the rhizosphere as a source of microorganisms with PET-degrading capabilities, supporting the concept of rhizoremediation. However, the experiments were conducted under controlled laboratory conditions, which may not fully represent the complexities of natural environments. In what concerns mangroves environments, previous studies demonstrated the potential of bacteria isolated from mangrove sediments to degrade microplastics [9]. Moreover, another study pointed out mangrove rhizosphere bacteria as potential candidates for plastic degradation [10]. Evidence of polymer biodegradation was observed on the surface of polyethylene, polyamide 6 and polyvinyl chloride microplastics after 3-months exposure in mangrove ecosystems. Moreover, the study showed that microplastics with different chemical structures would attract different microbes to colonize on their surfaces.

The degradation of PET primarily involves a catalytic reaction mediated by PET-degrading enzymes to depolymerize PET macromolecules. The process begins with the adherence of microorganisms to the polymer surface, followed by the secretion of extracellular enzymes that bind to PET and initiate its biodegradation [11]. So far, researchers have isolated and identified a variety of enzymes with PET-degrading ability, mainly including esterases, lipases, hydrolases, and cutinases [12]. A PET-degrading specific enzyme, PETase, isolated from *Ideonella sakaiensis* 201-F6, and capable of hydrolysing PET, was first reported by Yoshida et al. [13]. In fact, *I. sakaiensis* produces two enzymes capable of hydrolyzing PET and the reaction intermediate, mono(2-hydroxyethyl) terephthalic acid (MHET). The combined action of both enzymes allows for the efficient enzymatic conversion of PET in its monomers, terephthalic acid (TPA) and monoethylene glycol (MEG). Then, these small water-soluble molecules enter the cells and are further metabolized to produce protocatechuic acid, which is finally completely mineralized into small molecules such as CO_2, H_2O, and other small molecules through tricarboxylic acid cycle metabolism [12]. In recent years, a range of PET-degrading enzymes, that mainly target PET ester bonds, able to break down PET into smaller molecules such as TPA, MEG, MHET, and BHET, has been reported. The latter is a commercial product that shares structural similarity with the core structure of PET and has been widely used as a model compound for studying PET biodegradation [14]. BHET, MHET, and TPA are reported as the major degradation products of PET during the enzymatic hydrolysis and chemical recycling process [15, 16]. BHET has also been studied as a model molecule for PET recycling [17]. Currently, there are no reports on the toxicity and pollution levels of BHET [18], while TPA has been reported to cause bladder stones and bladder cancer, as well as impairment of liver and testicular functions [19, 20]. As for MEG, the parent compound (ethylene) is considered nontoxic. However, prolonged exposure to MEG can cause varying toxicity levels in rats [21]. The study of the biodegradation of these intermediates

is essential in the context of bioremediation, providing insights into the efficacy of microbial degradation of PET and its degradation products, although knowledge about the specific enzymes or microbial species involved in such degrading processes remains limited. *Enterobacter* sp. HY1, isolated from a plastic waste treatment station, was capable of degrading BHET, through the conversion of BHET to MHET and then to TPA [18]. Li et al. [22] has reported the metabolism of MEG by *Pseudomonas putida* KT2440, which resulted in different oxidation products such as glycolaldehyde, glyoxal, glycolate, and glyoxylate. Developing effective remediation strategies for plastic pollution is essential, and obtaining bacterial consortia capable of degrading PET and its monomers is a crucial step towards this goal. The present study aimed to evaluate PET films biodegradation in soil microcosms, both with and without mangrove plants, and to investigate the potential of bioaugmentation, a technique that consists in the introduction of specific bacterial strains in contaminated environments to improve the degradation of recalcitrant compounds. In addition, the potential of bacterial consortia retrieved from these soil microcosms to biodegrade PET monomers, specifically TPA, MEG, and BHET was assessed, with the aim of harnessing these naturally adapted microbial communities for future biotechnological applications.

2. Materials and methods

2.1. Chemicals and materials

PET films (thickness 0.1 mm, crystalline) obtained from Goodfellow (Cambridge, England) were cut into 1 cm^2 sizes, washed with 75% alcohol and dried overnight at 60 °C. After drying, the weight of the PET films was determined. Garden soil (decomposed leaves humus) was purchased from DAISO (Japan). MEG, BHET, and TPA (purity >98%) were purchased from Sigma (Saint Louis, USA).

2.2. Plants and microorganisms

Mangrove plants (*Kandelia* spp.) were collected from the Plants Nursing Centre Helen Garden Coloane, Macao SAR, and 12 saplings were selected for the experiment.

A bacterial consortium consisting of two bacterial isolates namely, *Bacillus* sp. GPB12 (isolated from city Green Park soil samples – an old landfill, Macao SAR, China) and *Enterococcus* sp. WTP31B-5 (isolated from wastewater sludge samples, Macao SAR, China) was assembled and used to bioaugment the soil microcosms. The bacterial strains used were previously isolated from plastic contaminated sites and selected due to their ability to form biofilm on PET granules (details on supplementary material). The bacterial isolates (*Bacillus* sp. GPB12 and *Enterococcus* sp. WTP31B-5) were subcultured in nutrient broth and incubated for 24 h. A consortium was prepared by mixing an equal proportion of each culture at a cell density of 2.3×109 CFU ml^{-1}, following the protocol of Dąbrowska et al. [23] and 1.43 ml/kg was inoculated in the soil for the bioaugmented treatment.

2.3. Degradation of PET films in soil microcosms

Each experimental glass and round bottom pot (20 cm height, 24 cm diameter) was half-filled with soil (3.5 kg). In each pot, six pieces of pre-weight PET films were buried at 4 cm depth and 4 cm apart from each other. Three different microcosms conditions were assembled: A- pots containing soil and PET films; B- pots containing soil, PET films, and one mangroves plant (*Kandelia* spp.); C- pots containing soil, PET films, one mangroves plant, and the bacterial consortium (Figure S1). These conditions were selected to evaluate the effect of mangrove plants and their rhizosphere microorganisms on PET degradation (B), as well as the impact of bioaugmentation with promising strains to further improve the process (C). The A-pots which contain neither mangrove plants nor the bacterial consortium, served as the experimental control. Each type of microcosm condition was performed quadruplicate. The experimental pots were evenly arranged (Figure S1) in the open university garden for 270 days, on a levelled ground, under a roof to prevent overflow from raining. The experiment was run under weather conditions: average temperature 23 °C and humidity 75%. The soil microcosms were watered every two days to keep the soil moist and submerged. These conditions were representative of the natural mangrove environment.

2.4. Determination of dry weight of the PET films

After the soil microcosms assays, the PET films were manually retrieved from the soil and washed with 1% of aqueous sodium dodecyl sulphate (SDS) solution for 4 h, to remove any surface contaminants, followed by rinsing with distilled water and 75% alcohol, to ensure cleanliness and prevent microbial contamination [24]. The use of SDS was chosen for its effectiveness in removing organic residues, while ethanol was selected due to its ability to dissolve and remove any remaining impurities without damaging the PET structure. The washed PET films were placed on a glass petri dish and dried

overnight at 60 °C to prevent moisture interference with the weighing process. After drying, the final weight of the PET films was determined. This protocol was followed to ensure accurate weight measurements by minimizing any potential errors from surface contaminants or moisture retention. The weight loss expressed as a percentage (%) was the difference in weight of the PET films at the beginning and end of the microcosm experiments, using the formula below:

$$\{\text{PET degradation (\%)} = \frac{\text{Initial film weight} - \text{Final film weight}}{\text{Initial film weight}} \times 100$$

2.5. FTIR

FTIR spectra were obtained using a Nicolet IS50 spectrometer (Thermo Scientific, USA), coupled with an Attenuated Total Reflectance (ATR) sampling accessory. FTIR identifies changes in functional groups and chemical bonds, critical for tracking PET degradation. It is non-destructive, surface-sensitive (ATR-FTIR), and quantifiable via indices like the carbonyl index. Four repeated scans were performed within the wavenumber range of 500–4000 cm^{-1}. The carbonyl index was calculated from the ratio of the absorbance of the carbonyl peak (C = O), around 1712 cm^{-1}, to a reference peak, often from methylene (CH_2) groups, around 1450 cm^{-1} [25].

2.6. SEM

SEM was used to visualize physical changes on PET surfaces, such as cracks, pits, or roughness, caused by degradation. The recovered PET films were washed with 1% aqueous SDS solution for 4 h, then rinsed with distilled water and 75% alcohol, and subsequently dried at 60 °C. The PET films were coated with gold particles and analysed using SEM imaging on a TESCAN VEGA3 (Czech Republic).

2.7. Molecular characterization of soil bacterial community

Soil samples were collected from all the microcosm conditions at the experiment's beginning and end. The soil samples were taken at 4 cm depth. Approximately 1 g of soil was collected to a sterile plastic bag and stored at −20 °C for further processing.

Genomic DNA extraction was performed for each soil sample using Power soil DNA isolation kit (Qiagen, USA) as per the manufacturer's protocol. The DNA extracted was quantified using a Qubit fluorometer (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol. The extracted DNA was kept at - 20 °C until further analysis. Next-generation sequencing (NGS) was performed on genomic DNA extracted, in triplicate, by Eurofins Genomics (Konstanz, Germany). The workflow included DNA amplification, preparation of library sequencing, and bioinformatic analysis. Paired-end sequencing was carried out on the Illumina MiSeq platform to ensure high-quality reads for microbial community analysis. The V3-V4 hypervariable region of the 16S rRNA gene was targeted using two specific primers: 357F – TACGGGAGGCAGCAG and 800R – CCAGGGTATCTAATCC . Raw sequencing data were processed by Eurofins Genomics using their in-house pipeline, which included demultiplexing, primer sequence clipping, read merging, quality filtering, and microbiome profiling. For the microbiome analysis, reads with ambiguous bases were removed, and chimeric reads were identified and removed based on the de-novo algorithm of UCHIME [26] as implemented in the VSEARCH package [27]. High-quality reads were then clustered into OTUs using Minimum Entropy Decomposition (MED) [28,29]. Taxonomic assignments were made using DC-MEGABLAST alignments to a reference database, requiring a minimum of 70% sequence identity across at least 80% of the representative sequence. Further processing of OTUs and taxonomic assignments was performed using the QIIME software package (version 1.9.1, http://qiime.org/). Abundances of bacterial taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates [30]. The raw sequence data were deposited in Sequence Read Archive (SRA) from NCBI database, associated to the BioProject, under the accession number BioProject PRJNA1063621.

2.8. Screening for TPA, MEG, and BHET cultivable degrading bacteria

Soil samples collected at the end of the PET film biodegradation microcosm experiment were used to enrich for bacterial consortia able to degrade BHET, MEG, and TPA. A soil sample (1 g) was collected from each replicate and inoculated into a 250 ml flask containing 90 mL of sterilized minimal salts medium [31] at pH 7 and supplemented with TPA, MEG or BHET, at a concentration of 1000 mg L^{-1}. The flasks were incubated at 30 °C and 200 rpm for five days. After this period, the cultures were plated on a minimal salts' agar medium containing the respective compound at the same concentration and further incubated for five days at 30 °C. After incubation, all colonies observed for each treatment were aseptically pooled together and used for further biodegradation assays.

2.9. Biodegradation of TPA, MEG, and BHET by bacterial consortia

The obtained bacterial consortia were sub-cultured and used for biodegradation assays of TPA, MEG, and BHET. The biodegradation assay was conducted in sterilized minimal salts medium [31], and each target compound was individually supplied, at an initial measured concentration of 700 mg L^{-1} for TPA, 1000 mg L^{-1} BHET and of 4500 mg L^{-1} for MEG as the sole carbon source. The bacterial consortia were inoculated at an initial density of 8.0×10^7 cells mL^{-1}. Each condition was conducted in quadruplicate. Culture flasks were incubated at 30 °C with continuous shaking at 200 rpm for 10 days. Three control sets were also included: minimal media and each target compound (TPA, BHET or MEG) without bacterial inoculation; minimal media inoculated with the bacterial consortia but without the target compounds; minimal media, the target compounds, and heat-inactivated consortia to evaluate adsorption.

Aliquot samples were aseptically collected daily to assess the growth and degradation of each target compound. Bacterial growth was monitored by spectrophotometry at 600_{nm} (Biowave II, UK).

2.10. Analytical methods

2.10.1. BHET, MEG and TPA quantification

Over the batch degradation assays, aliquot samples were collected and centrifuged at 13,500 g for 10 min to remove the biomass. The concentrations of BHET and TPA were analysed by high performance liquid chromatography (HPLC). The HPLC analyses were performed on a System Gold 126 (Beckman Coulter, Fullerton, USA) using a reversed phase 250–4 HPLC Cartridge LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany), operated in isocratic mode at room temperature, with a flow rate of 0.8 mL min^{-1} and an injection volume of 20 µL. Acetonitrile/water acidified to pH 2 with trifluoroacetic acid (60:40, V/V) was used as the mobile phase.

MEG concentration was analysed by gas chromatography (GC) using a gas chromatograph Varian CP-3800 (Agilent Technologies, California, USA) and a CP-Wax 52 CP capillary column (Chrompack International B.V., Middelburg, The Netherlands), using a temperature programme starting at 80 °C for 2 min, increasing to 180 °C at a rate of 10 °C min^{-1} with 5 min hold. Injector and detector temperatures were set to 250 °C.

Degradation rate constants were calculated assuming first-order kinetics. With this model, the concentration changes with time (t) were determined according to the following relationship: $C = C_{0e}\text{-}kt$, where C_0 is the initial concentration and k is the degradation rate constant. The half-life of biodegradation ($t_{1/2}$) was estimated from k using $t_{1/2} = \ln 2/k$.

2.10.2. Total organic carbon (TOC)

TOC content was evaluated with a Vario TOC cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The combustion tube with the sheath tube, ash crucible, quartz chips (15 mm), Pt-Kat (25 mm), quartz chips (85 mm), and quartz wool (5 mm) was set up from top to bottom. A working standard solution (500 mg L^{-1}) of KHP and Na_2CO_3 in Milli-Q water was prepared and further diluted with Milli-Q for the standard curve measurements. Synthetic air (around 1000 mbar, purity 99.995%) was used as the operating gas with a gas flow of 200 mL min^{-1} and the combustion tube temperature set to 680 °C.

2.11. Statistical analysis

A paired t-test (2-tailed) was conducted to compare the overall initial and final weight of PET films. A general linear model with the repeated measured test was performed using SPSS (IBM SPSS Statistics 28.0.0.0) to compare the degradation significance of TPA, MEG, and BHET within the time points and between the different conditions tested. The general linear model is a good choice for multiple independent variables, especially in this study with three distinct experimental conditions and accommodates categorical predictors and continuous covariates, enabling simultaneous analysis of their effects on degradation rates.

Microbiome comparisons and diversity analyses were performed on the MicrobiomeAnalyst web-based platform (https://www.microbiomeanalyst.ca/). Alpha diversity was calculated using Richness (total number of observed OTUs), Shannon (Shannon-Wiener- H') and Evenness (Pielou's evenness index – J') indexes. A $p < 0.05$ was considered significant. To visualize patterns in beta diversity, Principal Coordinates Analysis (PCoA) was conducted. Community dissimilarities were calculated at the genus level using the Bray–Curtis distance metric. Statistical significance of differences between groups was assessed using Permutational Multivariate Analysis of Variance (PERMANOVA). Additionally, a linear discriminant effect size (LEfSe) analysis was used to identify differentially abundant bacterial genus taxa across soil samples. A False discovery rate (FDR)-adjusted cut-off, a threshold on the logarithmic LDA score for discriminative features of 2.0 and a p-value of 0.05 was set. Univariate Statistical Comparisons package included in MicrobiomeAnalyst was used at feature-level using t-test with a p-value cutoff adjusted to 0.05.

3. Results

3.1. PET film degradation in soil microcosms

Weight loss of the PET films was not observed in any of the conditions tested during the experimental period of 270 days. However, microbial activity can induce significant chemical and morphological changes in PET without resulting in a measurable reduction in mass, especially over the time frame. In this study, PET film degradation was evidenced by the FTIR and SEM analysis. Figure 1 shows the FTIR spectra of PET films collected from the different microcosms' treatments (A, B or C) after 270 days, and of the control untreated PET film. It is possible to notice that all FTIR spectra for the PET films at the end of each treatment presented shifts in characteristic peaks compared to the control PET film (not buried in the soil). Notably, an OH group (hydroxyl) is indicated by a band at 3432 cm^{-1}. The symmetrical stretch of CH_2 appears at 3054 cm^{-1}, while the C–H symmetrical stretching is evident at 2969 and 2908 cm^{-1}. Additionally, an axial symmetrical deformation of CO_2 is observed around 2350 cm^{-1}. The stretching of the C = O bond in carboxylic acid groups is noted at 1730 cm^{-1}. Vibrations associated with the aromatic skeleton, including stretching of C = C, are identified at 1577 and 1504 cm^{-1}. The deformation of the C–O group and bending modes of the ethylene glycol segment manifest at 1453, 1410, and 1342 cm^{-1}.

Furthermore, the terephthalate group ($OOCC_6H_4$-COO) is characterized by bands at 1240 and 1124 cm^{-1}, while the methylene group and vibrations of the ester C–O bond are observed at 1096 and 1050 cm^{-1}. Aromatic rings show absorption at 972, 872, and 848 cm^{-1}, along with vibrations of adjacent aromatic hydrogen in p-substituted compounds in 1960 and 795 cm^{-1}. A band indicates the interaction of polar ester groups with benzene rings at 712 cm^{-1}. Peaks at 746 cm^{-1} – assigned to C–H bending, shifted to 744 cm^{-1} in treatment A and to 745 cm^{-1} in treatments B and C; peaks at 1429 cm^{-1} – assigned to O-H bending (carbonyl group) shifted to 1421 cm^{-1} in all treatments; 1491 cm^{-1} shifted to 1490 cm^{-1} in treatment A, 1480 cm^{-1} in treatments B and C; 1519 cm^{-1} shifted to 1511 cm^{-1} – assigned to N-O stretching in treatments B and C. The carbonyl index for all PET films exposed to the different treatment was higher than that of the initial PET film, with treatment C having the highest carbonyl index, indicating increased oxidative degradation of this PET film (Table 1). A reduction in the C = O peak intensity at 1712 cm^{-1}indicates hydrolysis of PET's ester bonds, an ester bond cleavage a hallmark of degradation. Shifts in peaks associated with crystalline (1341 cm^{-1}) and amorphous (1410 cm^{-1}) PET regions reflect structural reorganization during degradation, indicating crystallinity changes. FTIR results showed that PET films were altered chemically.

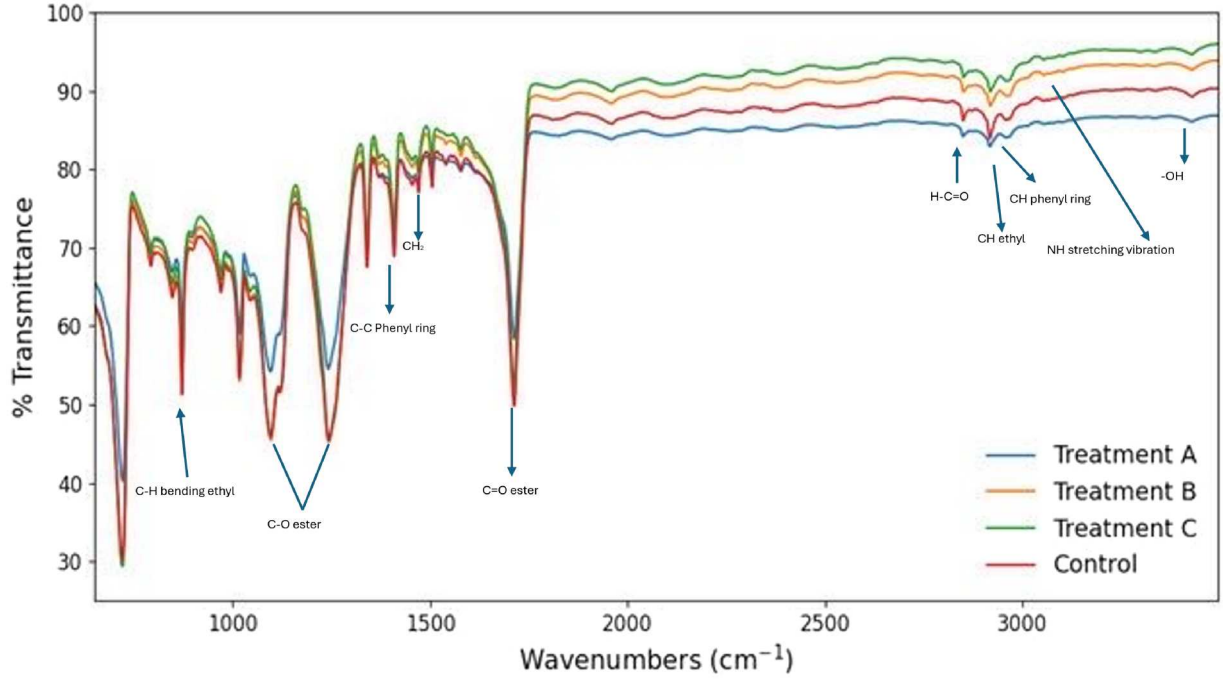

Figure 1. FTIR spectra of PET films collected from the different microcosms' treatments (A, B or C) after 270 days, and of the control untreated PET film.

Table 1. The absorbance values at the relevant wavelengths and carbonyl indices.

Treatments	C = O Absorbance (1712 cm^{-1})	CH_2 Absorbance (1450 cm^{-1})	Carbonyl index (CI)
Control	0.10 ± 0.02	0.90 ± 0.01	0.111 ± 0.02
A	0.15 ± 0.03	0.85 ± 0.02	0.176 ± 0.04
B	0.20 ± 0.04	0.80 ± 0.02	0.250 ± 0.05
C	0.25 ± 0.05	0.75 ± 0.03	0.333 ± 0.07

Results are expressed as mean ± SD.

In relation to the morphology captured by SEM, it was possible to observe that the surface of PET films in treatments with mangroves (Figure 2b,c) showed roughness and abnormal drape, indicating disruption as result of degradation. In comparison, no substantial damage was observed on the PET films from treatment A, which showed no differences in morphology in relation to beginning of the assay (untreated PET film) (Figure 2a, d). The presence of microcracks, pits, or roughness on PET surfaces suggests surface erosion, indicating the occurrence of physical degradation.

3.2. Microbial community of soil

Soil samples from the beginning and end of microcosms experiments were sequenced. The microbial community in the initial soil diverged from that in final soil samples in all treatments. In the initial soil, Proteobacteria (39.1 ± 0.3%) and Actinobacteria (34.4 ± 1.5%) were the most abundant phyla, together representing on average 73.5 ± 1.6%, followed by Firmicutes as the third most abundant phylum (13.0 ± 0.5%), Bacteroidetes (7.1 ± 0.6%) and Gemmatimonadetes (4.3 ± 0.5%). In final soil,

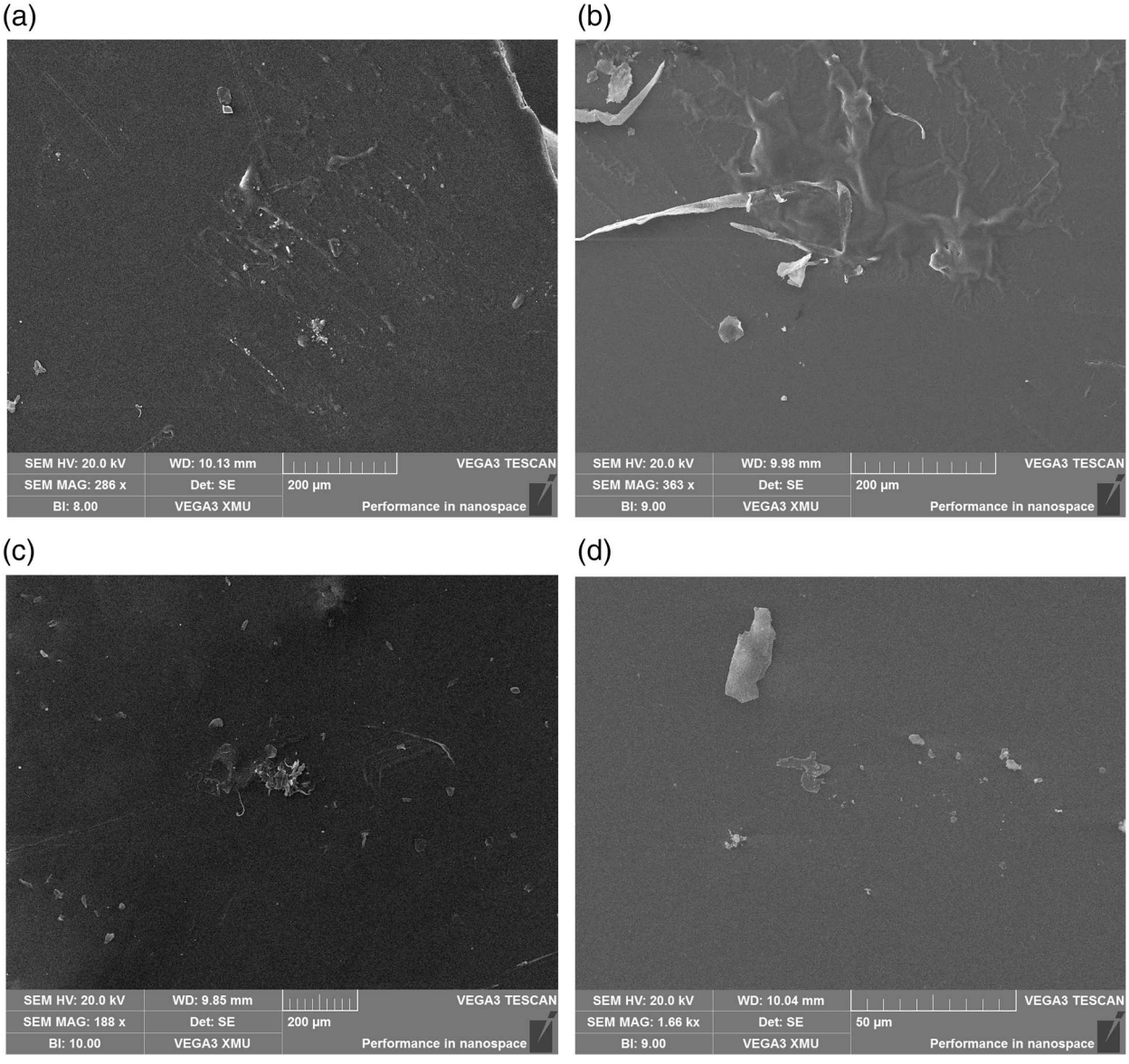

Figure 2. SEM images of PET films collected from the different microcosms' treatments (A, B or C) after 270 days, and of the control untreated PET film.

independently of the treatment that they were subjected to, the abundance of Proteobacteria increased to 53.1 ± 4.5% while that of Actinobacteria decreased to 13.4 ± 3.5% and Firmicutes became the second most abundant phylum (15.9 ± 1.8%). The abundance of the Gemmatimonadetes (6.7 ± 0.1%) and Bacteroidetes(5.0 ± 1.0%) phyla remained similar to that in the initial soil (Figure 3a).

At class level, Actinobacteria was the most abundant in initial soil (28.8 ± 0.6%), followed by Alphaproteobacteria (15.2 ± 0.7%). Conversely, this latter class was the most abundant in final soil samples (18.8 ± 1.7%), followed by Gammaproteobacteria (13.2 ± 2.4%), Deltaproteobacteria (11.4 ± 2.3%) and Betaproteobacteria (9.9 ± 4.0%), while Actinobacteria decreased to 4.9 ± 0.7%. The classes Anaerolineae, Bacteroidia, Coriobacteriia, Ignavibacteria and Saprospiria were not detected in the soil microbiome at the beggining of the experiment but were present in all final soil microbiomes (Figure 3b).

The relative abundance of microorganisms, at class level, in soil microcosms at the beginning and end of the different treatments was clustered (Figure 4). In the microbiome of the initial soil there were eight dominant taxa – Thermomicrobia, Sphingobacteriia, Limnochordia, Flavobacteria, Actinobacteria, Rhodothermia, Cytophagia and Thermoleophilia. These classes were notably less abundant in the microbiomes of the final soil samples. The dominant taxa were quite different in the soil microbiome subject to the different treatments, suggesting that the different conditions imposed lead to the prevalence of different taxa in the microbiome. No single taxon was dominant across all samples. However, three classes were more abundant in relation to initial soil, namely Longimicrobia, Anaerolineae and Ignavibacteria.

Alpha and beta diversity analysis of the bacterial microbiome of the soil revealed that community diversity (Shannon index) and richness were slightly higher in the final soils (Table 2). On the other hand, evenness (Simpson index) did not change from the beginning to the end of the experiment (Table 2). The PCoA ordination plot revealed noticeable changes, at genus level, in the bacterial community structure from the initial to the final and between final samples from different treatments, whereas soil samples from the same treatment clustered tightly together (Figure 5a). A more comprehensive analysis at the genus level revealed that some genera found in the initial soil were no longer identified in soils microcosms by the end of the experiment (e.g. *Aequorivita*, *Alcaligenes*, *Arenibacter*, *Devosia*, *Glaciibacter*, *Luteimonas*, *Methylotenera*, *Nocardioides*, *Pusillimonas* and *Rhodanobacter*) (Figure 5b). Conversely, new genera were only identified in the microbiome of soil microcosm at the end of the experiment (e.g. *Desulfuromonas*, *Ilumatobacter*, *Lewinella*, *Longilinea*, *Methylocaldum*, *Phenylobacterium*, *Piscinibacterium*, *Syntrophus*, *Sulfuritortus*, *Tangfeifania* and *Thiobacillus*). Nevertheless, there are some genera consistently detected across all soil samples (e.g. *Aciditerrimonas*, *Alkalilimnicola*, *Aquihabitans*, *Bacillus*, *Chelativorans*, *Clostridium*, *Conexibacter*, *Desulfonatronum*, *Hyphomicrobium*, *Longimicrobium*, *Mesorhizobium*, *Pseudomonas*, *Racemicystis*, *Streptomyces*, *Symbiobacterium*, *Tuberibacillus*, *Ureibacillus*, *Vulgatibacter*) although in some cases their relative abundances vary substantially.

Additionally, a linear discriminant analysis effect size (LEfSE) was performed to investigate the statistically significant differences in the soil bacterial community (at genus level) among the different soils' samples. The LEfSE allowed to identify the bacterial fingerprint of each microcosm (Figure 5c). For instance, the genera *Methylotenera*, *Mesorhizobium*, *Rhodanobacter* and *Glaciibacter* were significantly abundant in the microbiome of the initial soil and thus, considered the most prominent biomarkers of this soil sample. In contrast, post-treatment soil microcosms showed distinct shifts in

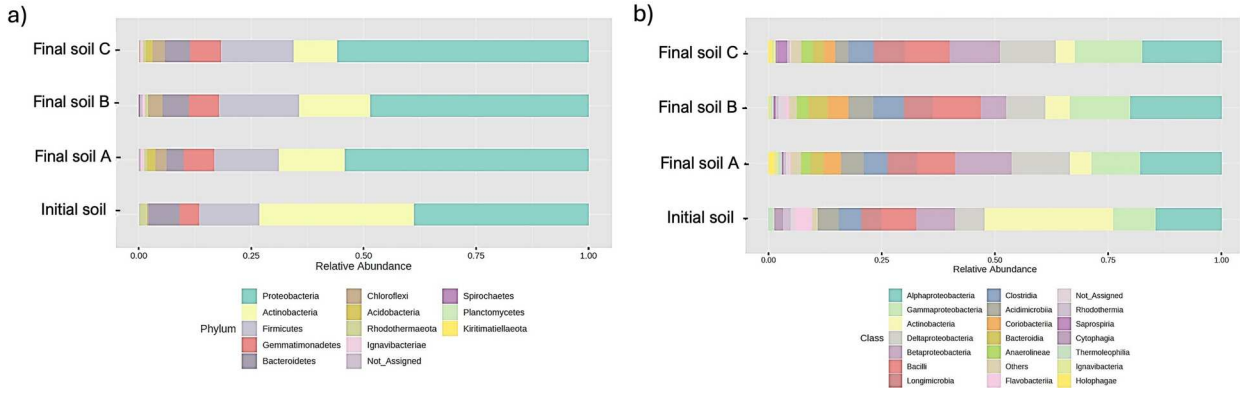

Figure 3. Relative abundance of (a) soil bacterial taxa classified at phylum level and (b) the most abundant classes, with each bar representing the mean of three biological replicates.

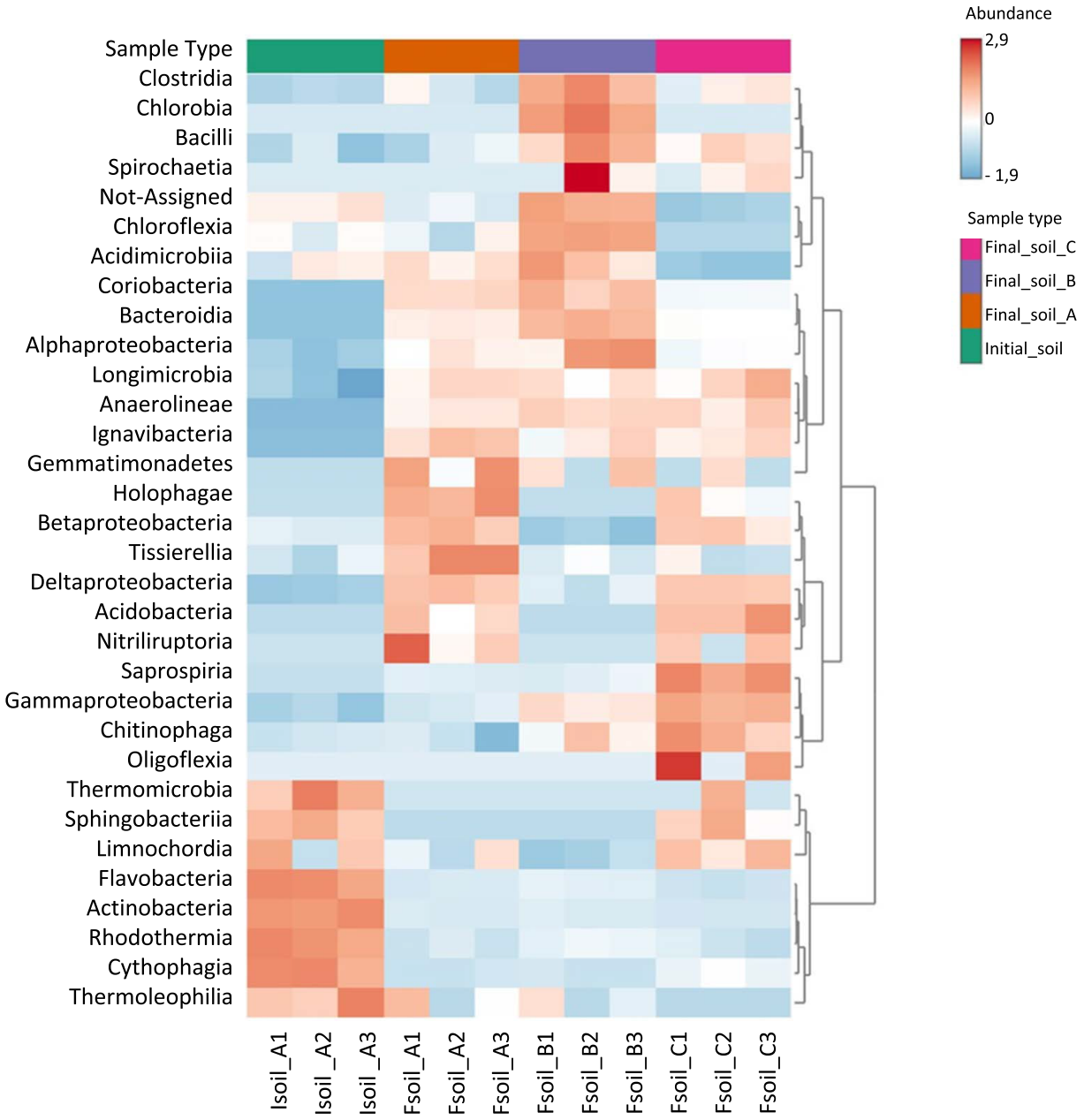

Figure 4. Hierarchical clustering heatmap at the class level of the different soil samples. The Euclidean distance measure and Ward clustering method were used.

microbial composition. Final Soil A was characterized by a significant enrichment of *Thiobacillus*, *Desulfuromonas*, and *Holophaga* genera while soil microcosm in treatment B exhibited a higher abundance of bacteria belonging to the *Raoultibacter*, and *Hyphomicrobium* genera and soil microcosm in treatment C was characterized by a high enrichment of *Methylocaldum*, *Pseudomonas*, *Lewinella*, *Phenylobacterium* and *Piscinibacterium* genera.

Table 2. Bacterial richness and α-diversity of soil microcosms.

	Shannon	Simpson	Richness
Initial Soil	3.97 ± 0.02	0.97 ± 0.00	93.7 ± 2.5
Final Soil A	4.08 ± 0.03	0.97 ± 0.00	102.7 ± 1.5
Final Soil B	4.12 ± 0.03	0.98 ± 0.00	97.3 ± 5.5
Final Soil C	4.11 ± 0.02	0.98 ± 0.00	106.0 ± 3.5

Results are expressed as mean ± SD

3.3. Biodegradation of TPA, MEG, and BHET by bacterial consortia

The three consortia composed of cultivable bacterial isolates retrieved from each soil microcosm by the end of the PET degradation assays were evaluated for their ability to degrade TPA, MEG, and BHET. Figure 6 shows

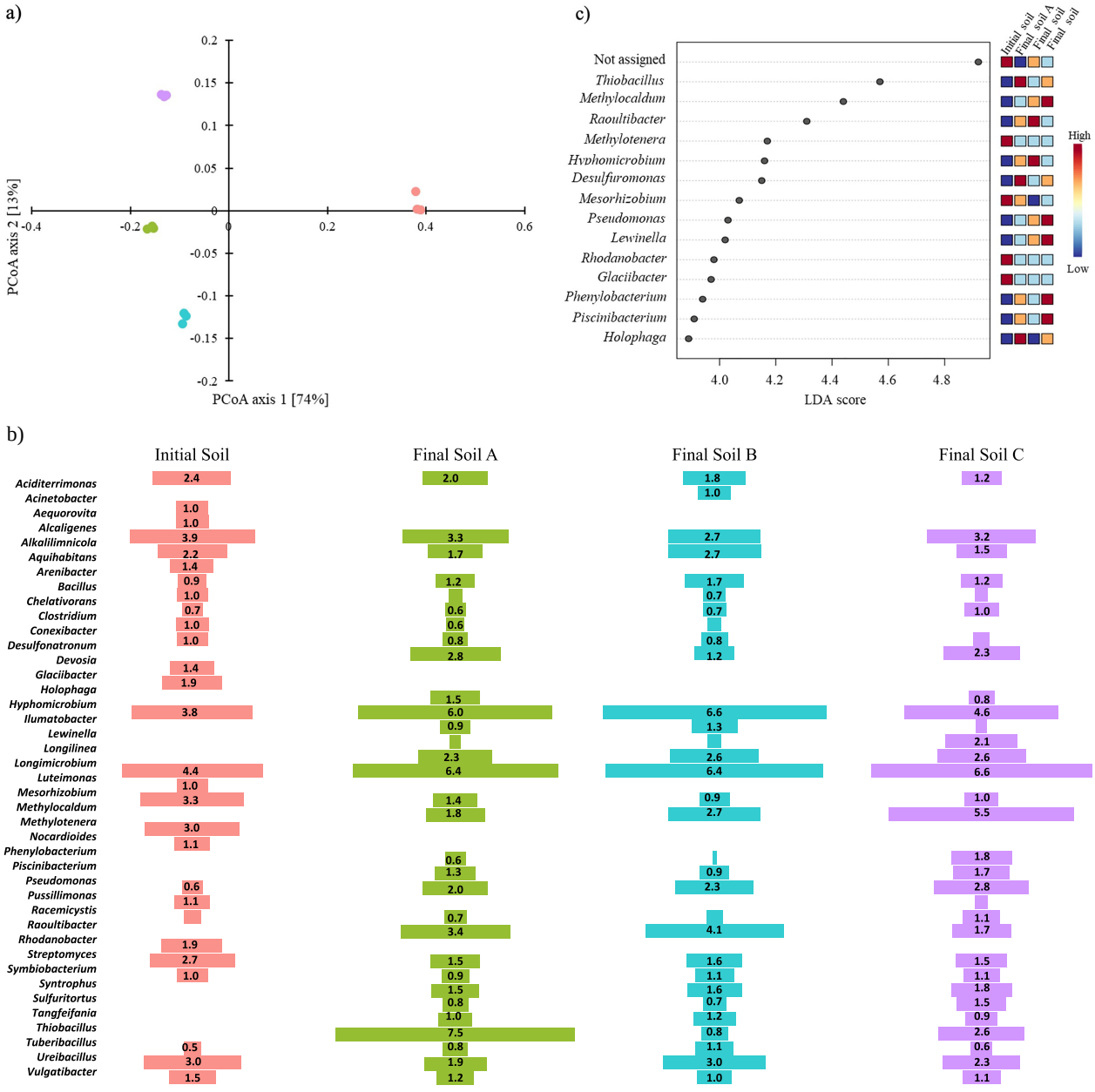

Figure 5. (a) Two dimensional PCoA ordination with a stress value of 0.01 of microcosms bacterial genera beta-diversity based on Bray-Curtis index for dissimilarity. (b) Microcosms' relative abundances at genus level. (c) LEfSE of the microcoms' microbiome at the genus level, presenting a LDA score > 2.0 and a $p < 0.05$.

that the three bacterial consortia grew in the presence of each target compound, which indicates that they can use the PET monomers and intermediate as carbon sources. All bacterial consortia showed faster growth in TPA (Figure 6b), with maximum OD achieved in the first two days, and gradually declining. Although in MEG (Figure 6d), the maximum OD achieved was slightly higher than that on TPA, it was achieved later. In fact, bacterial consortia from all the treatments maintained steady growth in MEG for the 10 days. In BHET, consortia A and C showed an exponential growth within the first 24 h and then a bit of decline, while the growth of consortia B occurred slowly and stabilized only after day 4 (Figure 6f). Bacterial growth was not observed in the control assays without the target compounds (data not shown) which demonstrated the use of the target compounds as carbon source.

Complete TPA degradation was observed on day 2, irrespective of the consortia employed (Figure 6a). This coincided with the maximum OD achieved (Figure 6b), suggesting the use of TPA as carbon source for bacterial growth. In relation to MEG, complete degradation was not observed for any of the consortia in the time course of the experiment (Figure 6c). Consortia A and

Table 3. First-order rate constant (k) and half-life ($t_{1/2}$) for TPA, MEG, and BHET degradation by bacterial consortia.

	Consortium		
	A	B	C
TPA			
Degradation (%)	100	100	100
k (d^{-1})	NA	NA	NA
$t_{1/2}$ (d)	NA	NA	NA
TOC removal (%)	93.8	91.9	89.1
MEG			
Degradation (%)	75.9	75.7	83.7
k (d^{-1})	0.148 ± 0.033	0.099 ± 0.008	0.194 ± 0.029
$t_{1/2}$ (d)	2.636 ± 0.241	3.008 ± 0.077	2.339 ± 0.154
TOC removal (%)	40.80	62.30	57.10
BHET			
Degradation (%)	47.2	98.9	47.5
k (d^{-1})	NA	0.466 ± 0.030	NA
$t_{1/2}$ (d)	NA	1.457 ± 0.065	NA
TOC removal (%)	55.5	95.3	53.2

Results are expressed as mean ± SD.
NA: Not applicable.
TOC: Total organic carbon.

B showed around 75% of MEG degradation, while consortium C showed a slightly higher degradation efficiency (84%). The MEG degradation rate constants were well-fitted to the first-order kinetics (the value of $R^2 = 0.92–0.98$). The rate constant k values followed the trend C > A > B, with the corresponding half-life t1/2 values increasing in the same order (Table 3).

A different pattern was observed for BHET degradation. For consortia A and C, there was a decrease in the concentration of the target compound in the first day but then the degradation stopped or proceeded very slowly (Figure 6e). In other hand, for consortium B, almost complete degradation was achieved after the first five days of the experiment (Figure 6e). The BHET degradation rate of consortium B assay followed first-order kinetics (the value of $R^2 = 0.96–0.99$) (Table 3). No decrease in the concentration of target compounds on controls was observed, indicating that no abiotic losses nor adsorption occurred during the experiment. The most evident difference observed between consortia is on the ability of consortium B for BHET degradation, which suggests a positive role of mangrove rhizosphere in the enrichment of bacteria with ability to degrade this compound.

Over the batch degradation assays, TOC content showed a decrease by the end the experiments following the same trend as the compound removal across all assays (Table 3). The results suggest complete mineralization of the amount of compound degraded. The reduction in TOC for TPA experiment with consortia A, B, and C were 93.8%, 91.9%, and 89.1%, respectively. The decrease in TOC for MEG assay with the consortia A, B, and C were 40.8%, 62.3%, and 57.2%, respectively. Regarding the TOC of the BHET assay, consortium B had the maximum reduction of 95.3%, compared to consortia A and C, with TOC reductions of 55.5% and 53.2%, respectively. As for the control experiments of the heat-inactivated consortia, TOC decrease was up to 11.51%, 22.43%, and 0.83% in TPA, BHET, and MEG respectively.

4. Discussion

4.1. PET degradation overview

This study presents evidence for crystalline PET film degradation by soil microorganisms, which includes changes in the chemical structures revealed by ATR-FTIR and surface modification determined by SEM. These changes were greater in samples from microcosms with mangrove plants. Moreover, bacterial consortia retrieved from PET degradation microcosms were able to degrade PET monomers and intermediate. While extensive degradation of TPA and MEG was observed for all the consortia, significantly higher degradation of BHET was observed for the consortium retrieved from the microcosm with mangrove plants. PET is not readily degraded in the environment, the aromatic groups of PET make it resistant to hydrolysis and prevent its microbial degradation under natural conditions [32, 33]. Therefore, strategies must be implemented to assist and enhance its degradation. Mangroves are known to harbour a high diversity of microbes capable of breaking down organic and some inorganic compounds, which makes them a promising option for biodegrading recalcitrant plastics [34]. The mangrove genus *Kandelia* was chosen because of its resistance to harsh environmental conditions and growth characteristics. In this study, a measurable weight loss was not directly observed. This might be due to several factors, as microbial and enzymatic attacks often begin at the surface of the PET films, leading to localized changes such as pitting, etching, or the formation of microcracks, as evidenced by FTIR and SEM analyses. These alterations may not be sufficient to cause a detectable change in the overall weight of the film, particularly if degradation is limited to the outermost layers. Additionally, degradation products generated at the film surface may remain loosely attached or embedded within the PET matrix, rather than being completely removed during the washing and drying process. This can result in an underestimation of actual material loss. Moreover, the mass of PET degraded may be below the detection limit of the analytical scale, especially when working with small film samples or when the degradation rate is relatively slow. Also, minor losses in mass can be masked by experimental variability or residual moisture content after drying. Lastly, the PET films used were of crystalline

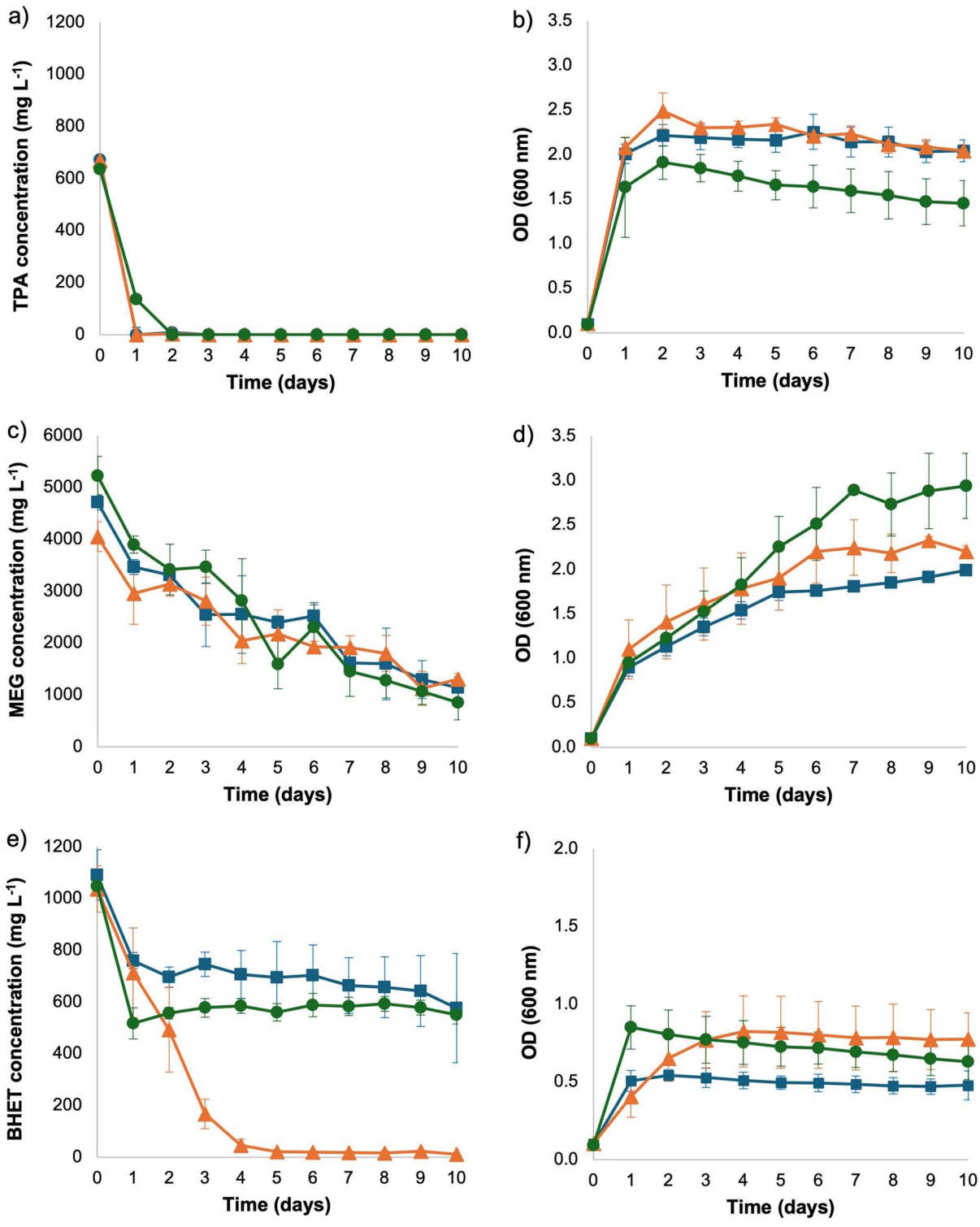

Figure 6. Biodegradation of the PET monomers and intermediate by bacterial consortia A (■), B (▲) and C (●). Variations in (a) TPA, (c) MEG and (e) BHET concentration, and respective bacterial growth (b, d and f) are shown. Error bars represent the standard error among independent biological replicates.

type, which is known to be more resistant to microbial and enzymatic attack than amorphous PET. This structural characteristic can further limit the extent of measurable weight loss over the experimental period. Given PET's inherent resistance to biodegradation, especially under non-controlled environmental conditions, the 270 day incubation may not be sufficient to observe substantial mass loss, even if chemical and

morphological changes are evident. Our results are in accordance with those from an earlier study by Janczak et al. [7], in which no significant weight loss of PET was observed for a period of 6 months in compost soil. Low degradation was observed by Taghavi et al. [35], which after 100 days of incubation, achieved a PET weight loss between 0.1 and 0.6% with microbial strains isolated from various inocula. A similar result has been reported by Beltrán-Sanahuja et al. [36, 37] while conducting plastics degradation experiments. In separate studies, the authors reported about 0.5% and 2% of PET weight loss after a period of 365 days.

Despite the weight reduction was not substantially, the changes in the chemical structures of the PET films revealed by the FTIR spectroscopic analysis confirms their alteration in composition. Changes in transmittance in PET films under different treatments reflect the material degradation. The control (red line) has a steady transmittance, which suggests that PET that has not been subject to any treatment absorbs and scatters more light, with bands that correspond to PET vibrational modes showing almost no change. Treatment A (blue line) exhibits significant variations between 1700 and 2900 cm^{-1}. The drop at 1700 cm^{-1}, caused by the stretching of C = O bonds in esters, forming ketone groups, shows that the material is being degraded, which may be due to physical or biological factors. Previous studies have reported that the appearance of ketone or aldehyde groups in PET could be due to the oxidation or degradation of the polymer [33, 34]. The variation in the absorption peaks of the functional groups reveals the conformational change in PET from all the treatments and of most from treatment with mangrove plants [35]. The disappearance or appearance of functional groups is a strong indication of PET degradation [29, 36–38]. On the other hand, the small rise at 2900 cm^{-1}, which is caused by the stretching of C–H bonds, shows microbial interactions causing structural changes. The adherence of microorganisms to PET plastics promotes their alteration through oxidation reactions [39]. As observed in this study, the formation of oxidation products, such as carbonyls, hydroxyls, esters, aromatics, and alcohols, and the observed peak shifts in the PET films reflect changes in their chemical structure [29]. Treatment B (orange line) exhibits significant changes at 1600 cm^{-1}. The rise at 1600 cm^{-1} is probably caused by C = C stretching or aromatic ring vibrations. It suggests that root interactions are making the breakdown process faster, which may release compounds that help breaking down the PET film. Enhanced transmittance near 2800 cm^{-1} reveals additional structural alterations due to biological factors. There is a peak area between 2800 to 4000 cm^{-1} wavelength representing primary amines, secondary amines and carboxylic acids [40]. In Treatment C (green line: PET film under soil with *Kandelia* sp. and bacterial consortium), considerable changes occur at 1500, 1700, and 2900 cm^{-1}. The rise at 1500 cm^{-1}, maybe due to C = C stretching or $–CH_2$ bending, suggests substantial alteration due to degradation, and the band at 1700 cm^{-1} confirms the breakdown of ester bonds, while the maximum transmittance at 2900 cm^{-1} indicates molecular changes during PET degradation. The effects of the different treatments on PET film transmittance range from 1000 to 3000 cm^{-1}, with Treatment C showing the most significant deterioration.

The appearance of new infrared bands at 3992 cm^{-1} (alcohol group), observed in the treatment only with soil and at 808 cm^{-1} (aromatic ring), observed in the bioaugmented assay, can be related to the formation of oxidation products at different frequencies [33]. The ester carbonyl group (C = O) stretch (1712cm^{-1}) is a defining feature of PET's polymer backbone, linking TPA and MEG units. A reduction in the intensity of this peak indicates hydrolytic cleavage of ester bonds, which is the primary enzymatic attack site for PET hydrolases such as cutinases, esterases, and PETase. The emergence or intensification of peaks in the range stretch 1600–1680 cm^{-1} corresponds to the formation of carboxylic acid groups (COO^-), primarily from TPA generated by enzymatic hydrolysis, indicative of polymer breakdown. The bands C–O stretch (1240–1100 cm^{-1}) are associated with the ester C–O bonds in PET, and their decrease further supports the cleavage of ester linkages during degradation. The FTIR shifts, together with SEM observations, suggest that degradation starts at the polymer surface, where enzymes access and cleave ester bonds. The chemical changes reflect progressive polymer chain scission. The vibrational changes in the different treatments indicate the change in the carbonyl group in PET. Focusing on the carbonyl index (CI) as an additional key indicator of oxidative degradation, the results demonstrated a clear increase in the carbonyl index across all treatment groups compared to the untreated control, suggesting an oxidative degradation of PET. In this study, the untreated control exhibited a carbonyl index of 0.111. In contrast, treatment A, B, and C showed average carbonyl indices of 0.176, 0.250, and 0.333, respectively. The progressive increase in carbonyl index values across these treatments further highlights PET degradation, with microcosm treatment C demonstrating the highest degradation potential. This finding aligns with previous studies that have reported biodegradation of PET due to the action of microbial consortia. For instance, studies by Torena et al. [33] on biodegradation

of PET microplastics by bacterial communities from activated sludge have shown that increase in carbonyl index validate microbial interaction with PET, which points to alterations in chemical structure. The presence of diverse microbial populations may facilitate synergistic interactions that enhance enzymatic activity against PET, resulting in increased carbonyl formation as a byproduct of oxidative degradation [41].

Therefore, the FTIR results indicated that the interaction between the microorganisms and PET films could have occurred as the PET films composition is different from that of the PET film at the beginning.

The SEM analysis of PET films showed modification of the PET morphology in the treatments with mangrove plants. Changes in the surface structure were observed in the form of bumps, dulling, or abnormal drapes. In comparison, the most significant roughness was observed in the film from the treatment C with mangrove and bioaugmented with the bacterial consortium. Similar changes on PET surfaces have been reported by Torena et al. [33] in a study on PET biodegradation by bacterial communities from activated sludge for 168 days. Our finding is also in line with a study that investigated the degradation of PET bottles in the marine environment and found that the surface of older PETs was highly cracked and uneven, while the surface of newer PETs was smooth [38]. Another study investigated the biodegradation of PET by two insect gut symbionts and found that the degradation of PET resulted in surface morphological changes such as roughness and scratches after six weeks [42]. PET surface roughness and cracks are considered evidence of biodegradation [43, 44].

FTIR and SEM are valuable tools for detecting chemical and morphological changes during PET degradation, but each has notable limitations when used as standalone evidence. FTIR primarily provides information about surface or near-surface chemical bond changes, such as the cleavage of ester linkages, but cannot quantify the overall extent of degradation nor confirm complete mineralization. In addition, the observed spectral shifts may also result from superficial modifications, adsorption of metabolites, or abiotic processes rather than true polymer breakdown. Similarly, SEM reveals surface features like pitting or erosion, yet these changes can arise from sample preparation artefacts or physical weathering and do not necessarily correlate with significant mass loss or conversion of PET to monomers. Both methods assume that observed changes are due to biodegradation, but without complementary quantitative data (such as weight loss, TOC reduction, or identification of soluble degradation products), there remains uncertainty about the depth, completeness, and biological specificity of PET degradation.

It is important to notice that in the present study were employed crystalline PET films for the biodegradation experiment. Crystallinity is one of the vital polymer characteristics that can affect microbial attacks on PET polymers [11, 45]. The degree of crystallinity of a polymer refers to the proportion of the polymer in a crystalline state as opposed to an amorphous state. Crystalline regions are structured and ordered with polymer chains aligned in a repeating pattern. In contrast, the amorphous regions are disorganized and lack a regular structure. Crystallinity reduces the movement of the backbone, therefore limiting the availability of the polymer chains for enzymatic attacks [46]. It is well known that PET biodegradation depends on the polymer's crystallinity, purity, and orientation of polymer chains [47]. Although reported PET-degrading enzymes can degrade amorphous PET films or low-crystallinity PET to varying extents, their degradation efficiency is significantly constrained as the crystallinity increases [12, 48]. Therefore, the degradation of highly crystalline regions of PET remains a challenge. A recent study on enzymatic PET hydrolysis with an industrially relevant PET-degrading enzyme, revealed a pronounced lag phase for crystalline PET [49]. The high degree of crystallinity is one of the major reasons why PET is not readily biodegradable [50, 51]. Hence, the degradation obtained may have been hampered by crystallinity of the PET film used. Some plastics pretreatments can be employed to increase the susceptibility to biodegradation. Among these, ultraviolet (UV) irradiation is widely used to induce surface oxidation, which facilitates the formation of cracks on the plastic surface, contributing to increase the susceptibility of plastic to enzymatic degradation. Mechanical forces can also facilitate enzymatic plastic degradation by making the surface of the polymer more available for enzymatic action [46]. Furthermore, chemical pretreatments on plastics have also shown promising results. In one study, the hydrolysable bonds in PET were made more accessible through alkaline treatment with NaOH, reducing the crystallinity, which in turn increased enzymatic degradation [52]. These pretreatments may be used in future experiments to try to improve the PET degradation in soil microcosms.

Mangrove forests possess a significant diversity of microorganisms, which play essential roles in numerous environmental processes and applications [53], harbouring microorganisms capable of degrading plastic polymers [29]. The development of microbial populations in the mangrove environment is favoured by high temperature, salinity, pH, organic matter content and low aeration and moisture levels [54]. In a study to assess the impact of plastics on mangroves, Van Bijsterveldt

et al. [55] pointed out that although plastic was abundant, covering up to 50% of the mangrove forest floor, with 27 plastic items per m^2 (on average), microorganisms in the mangrove environment could evolve and survive. Mangrove plants and their associated environments enhance PET degradation more effectively than many other ecosystems due to several unique microbiological and chemical characteristics. Mangrove soils are biodiversity hotspots that harbour exceptionally diverse and metabolically versatile microbial communities, including bacteria with plastic-degrading enzymes [3]. These communities are shaped by the constant influx of organic matter from plant roots (rhizodeposition), tidal mixing, and frequent exposure to both saline and freshwater inputs. Such conditions select for microbial taxa with robust stress tolerance and adaptive metabolic pathways, which are well-suited to attack recalcitrant polymers like PET. Recent research has demonstrated that mangrove soils contain bacterial consortia with unique or previously uncharacterized PET-hydrolyzing enzymes, such as monohydroxyethyl terephthalate hydrolases, which are crucial for breaking down PET by-products. Notably, the novel genus *Mangrovimarina plasticivorans* was identified as carrying genes encoding these hydrolases, highlighting the evolutionary adaptation of mangrove microbiomes to persistent plastic contamination. Additionally, the chemical environment of mangrove soils, rich in organic acids, phenolics, and other root exudates, can stimulate microbial metabolism and promote the expression of plastic-degrading genes. Beyond microbial action, mangrove habitats also support a wide array of metazoans and macrofauna that can physically bioerode plastics, increasing their surface area and making them more accessible to microbial attack [61]. This synergy between physical and biochemical degradation pathways further accelerates PET breakdown in mangrove systems compared to less dynamic terrestrial or aquatic environments. Collectively, these factors make mangrove ecosystems particularly effective natural laboratories for the discovery and activity of PET-degrading microorganisms and enzymes, offering promising solutions for plastic pollution remediation. In this study, the presence of mangrove plants and their rhizosphere caused structural and chemical changes on the PET surface, more pronounced than in soil without plants.

The bacterial consortia used for bioaugmentation comprised two bacterial strains, *Enterococcus* sp. WTP31B-5 and *Bacillus* sp. GPB12 isolated from wastewater treatment plant sludge and an old landfill, respectively. Bacteria from activated sludge have been reported to degrade up to 17% of PET [33]. A landfill is also an environment with diverse bacterial potential to degrade plastics [56–58]. In particular, *Bacillus* sp. has demonstrated PET degradation potential [29, 59, 60]. Dąbrowska et al. [59] observed intense PET film degradation in the presence of *Bacillus* sp. and plants. Further, they concluded that the *Bacillus* strain combined with miscanthus plantings may be a promising method for accelerating PET degradation in compost soil [59]. In the current study, it is difficult to quantify the PET film degradation extent in the various treatments and the contribution of each factor. Still, it seemed that both the presence of mangrove plants and rhizosphere microorganisms had a significant impact in the degradation of PET in soil microcosms.

4.2. Microbial community dynamics

The soil bacteriome changed during the microcosms experiment. In fact, the bacterial community in the microcosms at the end of the experiment are quite different from the initial community while the bacterial community in soil microcosms by the end of the experiment, irrespective of the conditions applied, are quite similar although in each treatment the dominat taxa were different. Therefore, it seems that the main changes in bacteriomes were mainly due to the long-term of the experiment (270 days). Ng et al [61] reported that the bacterial community of forest soil significantly changed in PET treated soils. The PCoA ordination highlighted the distinctness of the bacterial communities. Contrary to other studies, in which PET enrichment resulted in a significant decrease in community richness and species diversity [61, 62], in the present study, it was oberved a slighty increase in richness and Shannon indices. The phylum Proteobacteria became overwhelmingly dominant in the microbiomes of final soil samples, followed by the phyla Firmicutes and Actinobacteria. An increase in the relative abundance of Proteobacteria and a decrease in the Actinobacteria was also reported in the plastisphere of biodegradable plastics in alpine soils compared to bulk soil [63]. Dominance of members of the Proteobacteria phylum was also observed in PET-degrading consortia [62] and plastisphere community [64]. A shift in the microbial community structure due to PET exposure with dominance of Proteobacteria and Firmicutes was also observed in marine environment [65, 66]. The increase in the abundance of Proteobacteria and Firmicutes is very significant since both phyla have emerged as the most important and common phyla responsible for the degradation of plastics [67, 68]. Reviews on the degradation of microplastics in soil have been indicating Proteobacteria as the phylum with highest relevant abundance [68, 69]. The incubation of diverse polymeric materials with

samples from distinct environments (landfill soil, sewage sludge, and river water) aiming at the enrichment and isolation of plastic-degrading strains revealed that the majority of bacteria with polymer-degrading potential belonged to Proteobacteria [70]. In a study that used targeted community enrichment protocols to identify microorganisms involved in plastic degradation, Proteobacteria emerged as the predominant phylogenetic group on degrading consortia [71]. The trait for PET degradation appears to be limited to a few bacterial phyla. Metagenomic studies suggested that putative PET hydrolases, enzymes involved in PET degradation, are mainly detected in taxa belonging to the Actinobacteria and Proteobacteria phyla, in terrestrial metagenomes. Within the Proteobacteria, the Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria were assigned as the main hosts [72]. In another study to explore the global potential of microorganisms to degrade plastics, in which were compiled a data set of all known plastic-degrading enzymes, further analysis of metagenome-assembled genomes from the ocean revealed a significant enrichment of plastic-degrading enzymes within members of the classes Alphaproteobacteria and Gammaproteobacteria [73]. In the present study, the most abundant class in final soil microbiomeswas Alphaproteobacteria across all treatments although this taxon was more prevalent on the soil microbiome of treatment B. Gamma-, Delta- and Betaproteobacteria were also present in the microbiome of soils at the end of the experiment though at different relative abundancies: Delta- and Betaproteobacteria were more abundant in treatments A and C, while Gammaproteobacteria was more abundante in treatment C. The dominance of Gammaproteobacteria within the phylum Proteobacteria was also observed following PET enrichment of microbial communities both from marine plastic for six weeks [74] and from deep-sea sediment for 2 years [62]. For PET submerged *in situ* on the sediment and in the water column in the Mediterranean Sea during 82 days, it was reported dominance of Alpha- and Gammaproteobacteria by Delacuvellerie et al. [64]. Anaerolineae, a class that was enriched in all final soil microbiomes in relation to initial soil in the present study, and Alphaproteobacteria were both previously reported as the main microbial taxa, at the class level, that were enriched in biofilms colonizing microplastics [75] Bacteria belonging to the Anaerolineae class were also abundant in microplastics surface in an anoxic salt marsh sediment [66]. Other classes not detected in the initial soil but enriched in final soil microbiomes are Bacteroidia, Coriobacteriia, Ignavibacteria and Saprospiria. Bacteroidia was detected in bacterial communities of PET-degrading consortia enriched from deep-sea sediments [62], and together with Alphaproteobacteria and Gammaproteobacteria, this class was present in plastiphere in marine environment [65]. In the black soil, nanoplastics induced abundance increases of Coriobacteriia [76]. Members of the *Ignavibacteria* class were reported to decompose complex polysaccharides (e.g. cellulose and hemicellulose) [83].

An analysis of the core microbiome across the microcosms revealed several genera consistently detected in all soil microbiomes, suggesting their potential role as putative keystone taxa. These include *Aciditerrimonas*, *Alkalilimnicola*, *Aquihabitans*, *Bacillus*, *Chelativorans*, *Clostridium*, *Conexibacter*, *Desulfonatronum*, *Hyphomicrobium*, *Longimicrobium*, *Mesorhizobium*, *Pseudomonas*, *Racemicystis*, *Streptomyces*, *Symbiobacterium*, *Tuberibacillus*, *Ureibacillus*, and *Vulgatibacter*. Although these genera were present across all soil microbiomes, their relative abundances varied substantially, reflecting differences in the evolution of the microcosm microbiome. For instance, at the end of the experiment, microcosm microbiomes were enriched in taxa belonging to the *Pseudomonas* and *Hyphomicrobium* genera, which is relevant since *Pseudomonas* genus has been pointed out as PET degrading microbes, with the ability to colonize and use plastic as carbon source [30]. The importance of *Pseudomonas* in the context of plastic degradation was reviewed [39, 77]. *Hyphomicrobium* were significantly enriched in soil with 7% (w/w) low-density polyethylene microplastics [78] and in the plastisphere of polyethylene mulching film [79]. Additionally, the genera *Methylocaldum*, *Thiobacillus* and *Synthrophus* were not detected in the initial soil but were present in all final microcosms microbiomes, and all of these genera were previously linked to plastic degradation [80–82]. Other genera related with plastic degradation were not enriched in all final microcosms. For example, *Acinetobacter* was only enriched in microcosm B, despite other authors have reported this genus as significantly discriminative of PET communities [83] and identified as plastic degraders [84]. On the other hand, the *Lewinella* genus, identified as part of microbial communities attached to PET drinking bottles submerged in the North Sea [83], was only enriched in microbiomes of microcosms C. *Phenylobacterium*, a genus described as microplastics colonizer and polymeric substances degrader [85, 86], was enriched in microcosms A and C. Similarly, the *Ilumatobacter* genus, also enriched in microcosms A and C, was detected in the microbial community composition of the biofilm formed on the surface of aromatic-aliphatic copolyester plastic [87]. To further evidence the differences in the microbial community composition among different microcosms, LEfSe analysis

was performed, highlighting that the enriched genera serving as biomarkers of final microcosms C namely, *Methylocaldum*, *Pseudomonas*, *Lewinella* and *Phenylobacterium*, are all genera reported as plastic-degrading taxa.

4.3. Biodegradation of PET intermediates

Complete biodegradation of PET is imperative to mitigating the dreaded impacts of plastic waste. There are various methods for degrading PET and its monomers and intermediates, among which biodegradation is more environmentally friendly and technologically suitable. For successful biodegradation, it is important to have not only microorganisms with the ability to degrade the PET itself but also microorganisms with the capacity to biodegrade and assimilate the resulting monomers and intermediate. Therefore, TPA, MEG, and BHET were used as model substrates in biodegradation assays with bacterial consortia retrieved from PET degradation microcosms. All bacterial consortia possessed the ability to biodegrade and grew up in media containing TPA, MEG, and BHET as sole carbon sources, demonstrating the potential of these bacteria for the degradation of PET monomers and intermediate. The complete degradation of TPA by all the consortia is greater than the results reported in other studies. *Rhodococcus* sp. SSM1 achieved 100% of TPA degradation when supplied at 5000 mg L^{-1} after 96 h [88] and *Rhodococcus biphenylivorans* N2 degraded 99.6% of TPA supplied at 1000 mg L^{-1} in 5 days [89]. While these studies used single bacterial strains, in the present study were employed microbial consortia, which may provide synergistic effects, allowing for more efficient biodegradation of PET intermediates. Another study has demonstrated the total removal of 100 mg L^{-1} of TPA by a concortium composed by a few bacterial strains, namely *Pseudomonas* sp., *Chryseobacterium* sp., *Burkholderia* sp., and *Arthrobacter* sp., within 24 h [90]. Furthermore, in the present study, the TOC analysis results supported evidence of degradation by all the bacterial consortia, regardless of the treatment from where they were retrieved. The correspondence between compounds degradation and TOC removal suggested that the amount of degraded compounds underwent mineralization. Consortium C obtained from bioaugmented mangrove soil showed slightly higher MEG degradation (83.7%), with higher degradation rate constant and the lower half-life (2.339 ± 0.154), which may be attributed to the presence of mangrove plants and the consortium in this treatment. A similar degradation extent of MEG at varying concentrations (0.25-1.0% (v/v)) has been reported by Ghogare and Gupta [91] when working with isolates *Oliptrichum macrosporum, Bacillus niacin, Streptomyces* sp-1, *Aspergillus terreus, and Aspergillus faecalis*, individually and in consortium [91]. After seven days, the microbial consortium achieved 75.49% of MEG degradation, which is approximately 5% higher that the degradation obtained by the best degrader - *O. macrosporum* - individually [91]. Despite the degradation obtained with microbial isolates, this report highlights the advantages of mixed microbial communities for MEG degradation. In addition, MEG (3103 mg L^{-1}) oxidation using *Pseudomonas putida* strains KT2440 and JM37 has also been reported with both converting MEG into glycolic acid and glyoxylic acid [92]. The engineered *Pseudomonas putida* KT2440 has demonstrated the ability to completely degrade MEG, enabling its conversion to medium-chain-length polyhydroxyalkanoates [93]. *Acenetobacterium woodii* has been reported to convert MEG into ethanol and acetyl coenzyme A (acetyl-CoA), which was further converted to acetate [94]. The decrease in the TOC corroborated the degradation evidence by all the bacterial consortia as mineralization was not observed in MEG. However, it is important to notice that degradation was still occurring when the experiment finished and probably it was not complete due to the high concentration of MEG used in these assays. There is no evidence of toxicity to the microorganisms nor degradation inhibition.

In relation to BHET, the bacterial consortium retrieved from the treatment B with mangrove plants showed much higher degradation (96%) compared with the consortium retrieved from treatment A without mangroves and, surprisingly, from treatment C with mangrove plants plus bioaugmentation. In these experiments, the degradation stopped after the first day and did not follow a first-order kinetic as expected in case of successful degradation, suggesting some inhibition on the degradation process, such as accumulation of an inhibitory intermediary metabolites or the lack of a degrading microorganism or enzyme for further degradation. Comparing with results for PET degradation, promising in treatment C, the results for BHET degradation suggest that probably key degrading microorganisms were lost during the enrichment procedure to obtain a culturable microbial consortia for intermediates degradation. On the other hand, BHET degradation by bacterial consortium B was higher than what has been reported by individual bacterial strains in literature. For instance, Qiu et al. [18] reported 80.8% of removal of an initial BHET concentration of 1000 mg L^{-1} within 120 h by *Enterobacter* sp. HY1. The analysis of the metabolites produced revealed that BHET was hydrolysed to MHET and then to TPA. The

esterase EstB cloned from *Enterobacter* sp. HY1, specifically hydrolyses BHET to MHET, revealing enzymatic specificity. These findings can establish a foundation for using *Enterobacter* sp. HY1 or its esterase EstB as a biocatalyst for BHET and PET intermediates degradation. On the other hand, the present study demonstrates that complex microbial consortia, especially those associated with mangrove environments, can also achieve high BHET degradation, highlighting ecological and applied bioremediation potential. Other microorganisms have been reported in previous studies as BHET-degrading strains including *Ideonella sakaiensis* [13], *Humicola insolens* [95], and *Bacillus subtilis* [96] although, the actual degradation rate of BHET was not reported as BHET removal occurred during the process of PET degradation. The significant decrease in the TOC, similar to the percentage of compound degraded, corroborated the degradation evidence by all the bacterial consortia, especially of the consortium B which showed an indication of mineralization and, TOC decrease was up to 95.25%. The evidence of mineralization combined with the degradation rate constant, which corresponds to a fast degradation process with a half-life of 1.457 ± 0.065 days, reveals the potential of the consortium retrieved from mangrove soils for biodegradation of BEHT. In fact, looking for the degradation rates and mineralization degree from the three target compounds, this consortium revealed to be the most promising to apply for remediation of contaminated sites.

In the present study, the biodegradation of BHET was limited to the consortium B, while for TPA and MEG, the degradation was effective for the three consortia. These results are aligned with what is known about the degradation of these compounds. While TPA and MEG can be used by different microorganisms and be further metabolized into the tricarboxylic acid cycle (TCA cycle) [47, 97], BHET degradation requires specific BHET-degrading enzyme. This enzyme converts BHET into MHET, which is further degraded by the action of a MHET-degrading enzyme into TPA and MEG, small water-soluble molecules [98]. The high accumulation of BHET during PET degradation experiments with *Thioclava* sp. BHET1 supports the specificity and limiting-step nature of BHET degradation [74]. On the other hand, oxygenase enzymes, which are usually involved in the degradation of most aromatic hydrocarbons, such as TPA, as well as of further intermediates, protocatechuate and catechol, tend to be widely distributed in the environment [74]. Also, the ability to grow with MEG as a sole source of carbon and energy has been demonstrated for various microorganisms, some of which initially oxidize it to glyoxylic acid via glycolic acid [47].

The findings of this study expand the functional understanding of PET-degrading microorganisms by highlighting the importance of microbial communities composed of microorganism possessing primary PET hydrolases and downstream esterases to achieve efficient PET degradation. Our findings suggest that the microbial communities likely express not only PETase-like enzymes for primary chain scission but also carboxylesterases or MHETases that efficiently process BHET and MHET. Despite the enzymes involved in the degradation process were not identified, our data suggesting mineralization of the degraded intermediates, demonstrate that the degradation process does not end with these intermediates; rather, efficient mineralization requires further enzymatic steps specifically, the hydrolysis of BHET and MHET into TPA and MEG by specialized esterases. These downstream enzymes are crucial for relieving product inhibition and enabling the complete conversion of PET-derived oligomers into assimilable monomers. The biodegradation process in soil microcosms likely involved PETase or cutinase-like hydrolases initiating PET depolymerization by cleaving ester bonds within the polymer, producing BHET and MHET. Esterases or MHETases were putatively present in the enriched consortia to further hydrolyse BHET to MHET, and MHET to TPA and MEG, as well as the metabolic pathways to assimilate these compounds.

Our data, therefore, reinforce the emerging view that efficient PET biodegradation in natural or engineered systems is a multi-microorganism, multi-enzyme, multi-step process. It requires the coordinated action of PET hydrolases and downstream esterases/MHETases, and possibly benefits from cell-surface display or biofilm formation to enhance substrate accessibility and catalytic turnover. This comprehensive enzymatic toolkit is essential for overcoming the recalcitrance of PET and achieving meaningful rates of depolymerization and mineralization in environmental or industrial contexts.

5. Conclusions and future research directions

In conclusion, although no significant weight reduction of PET was observed in the soil microcosms experiments, evidence of crystalline PET films degradation was demonstrated through surface and chemical modifications, which were more pronounced in the presence of mangrove plants. These findings strengthen our hypothesizes that mangrove ecosystems can facilitate the biodegradation of PET. Additionally, the soil bacteriome changed during the PET film degradation experiments, with the phylum Proteobacteria, which have emerged as the most important and common phyla responsible for the degradation of plastics, becoming

dominant in the final soil microbiomes. Moreover, some enriched genera at the final microcosms, are genera reported as plastic-degrading taxa. The bacterial consortia retrieved from the soil microcosms assays demonstrated ability to biodegrade TPA, MEG, and BHET. The successful biodegradation of BHET is of particular relevance since it has been widely used for studying PET biodegradation, as it is a monomer similar to the core structure of PET. Our findings demonstrate that the microbiome of soil microcosms evolved for PET degradation, since not only PET film degradation was observed, but the microbial consortia retrieved from those microcosms showed ability to degrade and mineralize PET intermediates.

This study demonstrated the potential of the rhizosphere of mangrove plants to promote a microbial community in soil with the ability to degrade PET and their intermediates. These microorganisms may have unique potential due to the environmental conditions, which differ from other ecosystems and offer opportunities to obtain unique enzymes for plastic waste recycling. Moreover, the retrieved bacterial consortia, especially the consortium B can be used to develop bioaugmentation strategies to improve PET degradation in contaminated environments, namely mangrove ecosystems, and to develop recycling and waste management technologies. For example, composting plants enriched with microbial consortia to improve PET-degrading potential could be implemented.

Building on our findings, future research should focus on unravelling the specific microbial interactions within each consortium that drive efficient PET and intermediates degradation. Detailed metagenomic and metatranscriptomic analyses could identify key functional genes and metabolic pathways, clarifying how synergistic relationships, such as cross-feeding or cooperative enzyme production enhance the overall biodegradation. Additionally, isolating and characterizing dominant microbial strains from each consortium will be crucial to pinpoint species with high PET-degrading potential and to elucidate their enzymatic mechanisms. Refining bioaugmentation strategies by combining these well-characterized isolates into optimized synthetic consortia could further improve degradation rates under environmental conditions. Moreover, exploring the effects of environmental factors, such as nutrient availability, salinity, and pH, on microbial community dynamics and enzyme expression will help tailor bioremediation approaches for diverse habitats, especially mangrove ecosystems. Ultimately, integrating microbial ecology with enzyme engineering and process optimization may advance the development of robust, scalable PET bioremediation technologies.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The 16S rRNA gene sequence of bacterial isolates used for bioaugmentation of microcosmos experiments were deposited in the GenBank database. The raw sequence data of 16S rRNA gene (conducted on Illumina MiSeq platform) of the microbial community from soil was deposited in Sequence Read Archive (SRA) from NCBI database.

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References

[1] Alongi DM. Carbon cycling and storage in mangrove forests. Ann Rev Mar Sci. 2014;6:195–219. doi:10.1146/ANNUREV-MARINE-010213-135020
[2] Barbier EB. The protective service of mangrove ecosystems: A review of valuation methods. Mar Pollut Bull. 2016;109(2):676–681. doi:10.1016/J.MARPOLBUL.2016.01.033
[3] Martin C, Almahasheer H, Duarte CM. Mangrove forests as traps for marine litter. Environ Pollut. 2019;247:499–508. doi:10.1016/J.ENVPOL.2019.01.067
[4] Srikanth S, Lum SKY, Chen Z. Mangrove root: adaptations and ecological importance. Trees. 2015;30(2):451–465. doi:10.1007/S00468-015-1233-0
[5] Canepa L, Ramón J, Olivier S, et al. Transformation of plastic debris to microplastics: An approximate analysis of mangrove environments. Urban Resil Sustain. 2024;2(December):348–364. doi:10.3934/urs.2024018
[6] Jha S. Bioremediation pollutants. Elsevier; 2020. p. 57–123. doi:10.1016/B978-0-12-819025-8.00004-1
[7] Janczak K, Hrynkiewicz K, Znajewska Z, et al. Use of rhizosphere microorganisms in the biodegradation of PLA and PET polymers in compost soil. Int Biodeter Biodegrad. 2018;130(November 2017):65–75. doi:10.1016/j.ibiod.2018.03.017

[8] Dhaka V, Singh S, Ramamurthy PC, et al. Biological degradation of polyethylene terephthalate by rhizobacteria. Environ Sci Pollut Res. 2022;30:116488–116497. doi:10.1007/s11356-022-20324-9

[9] Auta HS, Emenike CU, Fauziah SH. Screening of Bacillus strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. Environ Pollut. 2017;231:1552–1559. doi:10.1016/j.envpol.2017.09.043

[10] Xie H, Chen J, Feng L, et al. Chemotaxis-selective colonization of mangrove rhizosphere microbes on nine different microplastics. Sci Total Environ. 2021;752:142223. doi:10.1016/j.scitotenv.2020.142223

[11] Kawai F, Kawabata T, Oda M. Current knowledge on enzymatic PET degradation and its possible application to waste stream management and other fields. Appl Microbiol Biotechnol. 2019,: June 4;103:4253–4268. doi:10.1007/s00253-019-09717-y

[12] Qiu J, Chen Y, Zhang L, et al. A comprehensive review on enzymatic biodegradation of polyethylene terephthalate. Environ Res. 2024;240:117427–117441. doi:10.1016/J.ENVRES.2023.117427

[13] Yoshida S, Hiraga K, Takehana T, et al. A bacterium that degrades and assimilates poly(ethylene terephthalate). Science (New York, N.Y.). 2016;351(6278):1196–1199. doi:10.1126/science.aad6359

[14] Joo S, Cho IJ, Seo H, et al. Structural insight into molecular mechanism of poly(ethylene terephthalate) degradation. Nat Commun. 2018;9(1):382–394. doi:10.1038/S41467-018-02881-1

[15] Vertommen MAME, Nierstrasz VA, Veer MVD, et al. Enzymatic surface modification of poly(ethylene terephthalate). J Biotechnol. 2005;120(4):376–386. doi:10.1016/J.JBIOTEC.2005.06.015

[16] Salvador M, Abdulmutalib U, Gonzalez J, et al. Microbial genes for a circular and sustainable Bio-PET economy. Genes (Basel). 2019;10(5):373. doi:10.3390/GENES10050373

[17] Ion S, Voicea S, Sora C, et al. Sequential biocatalytic decomposition of BHET as valuable intermediator of PET recycling strategy. Catal Today. 2021;366:177–184. doi:10.1016/J.CATTOD.2020.08.008

[18] Qiu L, Yin X, Liu T, et al. Biodegradation of bis(2-hydroxyethyl) terephthalate by a newly isolated Enterobacter sp. HY1 and characterization of its esterase properties. J Basic Microbiol. 2020;60(8):699–711. doi:10.1002/jobm.202000053

[19] Zhang Z, Ma L, Zhang XX, … Cheng S. Genomic expression profiles in liver of mice exposed to purified terephthalic acid manufacturing wastewater. J Hazard Mater. 2010;181(1–3):1121–1126. doi:10.1016/J.JHAZMAT.2010.05.131

[20] Ordaz-Cortés A, Thalasso F, Salgado-Manjarrez E, et al. Treatment of wastewater containing high concentrations of terephthalic acid by Comamonas sp. and Rhodococcus sp.: kinetic and stoichiometric characterization. Water Environ J. 2014;28(3):393–400. doi:10.1111/WEJ.12048

[21] Snellings WM, Corley RA, McMartin KE, et al. Oral reference dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect. Regul Toxicol Pharmacol. 2013;65(2):229–241. doi:10.1016/J.YRTPH.2012.12.005

[22] Li WJ, Jayakody LN, Franden MA, et al. Laboratory evolution reveals the metabolic and regulatory basis of ethylene glycol metabolism by Pseudomonas putida KT2440. Environ Microbiol. 2019;21(10):3669–3682. doi:10.1111/1462-2920.14703

[23] Dabrowska G, Baum C, Trejgell A, et al. Impact of arbuscular mycorrhizal fungi on the growth and expression of gene encoding stress protein - metallothionein BnMT2 in the non-host crop brassica napus L. J Plant Nutr Soil Sci. 2014;177(3):459–467. doi:10.1002/JPLN.201300115

[24] Ambika K, Ratnasri PV, Lakshmi BKM, et al. Isolation of polythene degrading bacteria from marine waters of Viskhapatnam, India. Int J Current Microbiol Appl Sci. 2014;3(10):269–283.

[25] Janczak K, Dąbrowska G, Znajewska Z, et al. Wpływ szczepienia bakteryjnego na wzrost miskanta i liczebność bakterii i grzybów w glebie zawierającej polimery, Cz. 2. Polimery niebiodegradowalne. Przemysł Chemiczny. 2014;93(12):2222–2225.

[26] Edgar RC, Haas BJ, Clemente JC, et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194–2200. doi:10.1093/bioinformatics/btr381

[27] Rognes T, Flouri T, Nichols B, et al. VSEARCH: A versatile open source tool for metagenomics. PeerJ. 2016;18:1–22. doi:10.7717/peerj.2584

[28] Eren AM, Maignien L, Sul WJ, et al. Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene data. Methods Ecol Evol. 2013;4(12):1111–1119. doi:10.1111/2041-210X.12114

[29] Eren AM, Morrison HG, Lescault PJ, et al. Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J. 2015;9:968–979. doi:10.1038/ismej.2014.195

[30] Angly FE, Dennis PG, Skarshewski A, et al. CopyRighter: A rapid tool for improving the accuracy of microbial community profiles through lineage-specific gene copy number correction. Microbiome. 2014;2(11):1–13. doi:10.1186/2049-2618-2-11

[31] Moreira IS, Amorim CL, Carvalho MF, et al. Effect of the metals iron,: copper and silver on fluorobenzene biodegradation by labrys portucalensis. Biodegradation. 2013;24(2):245–255. doi:10.1007/s10532-012-9581-6

[32] Webb HK, Arnott J, Crawford RJ, et al. Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate). Polymers (Basel). 2013;5(1):1–18. doi:10.3390/polym5010001

[33] Xia XL, Liu WT, Tang XY, et al. Degradation behaviors,: thermostability and mechanical properties of poly (ethylene terephthalate)/polylactic acid blends. J Central South Univ. 2014;21(5):1725–1732. doi:10.1007/s11771-014-2116-z

[34] Helen AS, Uche EC, Hamid FS. Screening for polypropylene degradation potential of bacteria isolated from mangrove ecosystems in peninsular Malaysia. Int J Biosci Biochem Bioinformat. 2017;7(4):245–251. doi:10.17706/ijbbb.2017.7.4.245-251

[35] Taghavi N, Singhal N, Zhuang WQ, et al. Degradation of plastic waste using stimulated and naturally occurring

microbial strains. Chemosphere. 2021;263:127975–127989. doi:10.1016/J.CHEMOSPHERE.2020.127975

[36] Beltrán-Sanahuja A, Benito-Kaesbach A, Sánchez-García N, et al. Degradation of conventional and biobased plastics in soil under contrasting environmental conditions. Sci Total Environ. 2021;787:147678. doi:10.1016/j.scitotenv.2021.147678

[37] Beltrán-Sanahuja A, Casado-Coy N, Simó-Cabrera L, et al. Monitoring polymer degradation under different conditions in the marine environment. Environ Pollut. 2020;259:113836. doi:10.1016/j.envpol.2019.113836

[38] Torena P, Alvarez-Cuenca M, Reza M. Biodegradation of polyethylene terephthalate microplastics by bacterial communities from activated sludge. Can J Chem Eng. 2021;99(S1):S69–S82. doi:10.1002/cjce.24015

[39] Panowicz R, Konarzewski M, Durejko T, et al. Properties of Polyethylene Terephthalate (PET) after thermo-oxidative aging. Materials (Basel). 2021;14(14):3833.

[40] Djebara M, Stoquert JP, Abdesselam M, et al. FTIR analysis of polyethylene terephthalate irradiated by MeV He +. Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms. 2012;274:70–77. doi:10.1016/j.nimb.2011.11.022

[41] Naz I, Batool SAU, Ali N, et al. Monitoring of growth and physiological activities of biofilm during succession on polystyrene from activated sludge under aerobic and anaerobic conditions. Environ Monit Assess. 2013;185(8):6881–6892. doi:10.1007/S10661-013-3072-Z

[42] Skariyachan S, Setlur AS, Naik SY, et al. Enhanced biodegradation of low and high-density polyethylene by novel bacterial consortia formulated from plastic-contaminated cow dung under thermophilic conditions. Environ Sci Pollut Res Int. 2017;24(9):8443–8457. doi:10.1007/S11356-017-8537-0

[43] Ioakeimidis C, Fotopoulou KN, Karapanagioti HK, et al. The degradation potential of PET bottles in the marine environment: An ATR-FTIR based approach. Sci Rep. 2016;6(March):1–8. doi:10.1038/srep23501

[44] Wilkes RA, Aristilde L. Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. J Appl Microbiol. 2017;123(3):582–593. doi:10.1111/JAM.13472

[45] Falah W, Chen FJ, Zeb BS, et al. Polyethylene terephthalate degradation by Microalga Chlorella vulgaris along with pretreatment. Materiale Plastice. 2020;57(3):260–270. doi:10.37358/MP.20.3.5398

[46] Jaiswal S, Sharma B, Shukla P. Integrated approaches in microbial degradation of plastics. Environmental Technology and Innovation. 2020;17:100567. doi:10.1016/j.eti.2019.100567

[47] Kim J, Lee S, Lee B, et al.. Biodegradation Potential of Polyethylene Terephthalate by the TwoInsect Gut Symbionts Xanthomonas sp. HY-74 and Bacillus sp. HY-75. Polymers. 2023;15:3546–3557. doi:10.3390/polym15173546

[48] Sarkhel R, Sengupta S, Das P, et al. Comparative biodegradation study of polymer from plastic bottle waste using novel isolated bacteria and fungi from marine source. J Polym Res. 2020;27(1):1–8. doi:10.1007/s10965-019-1973-4

[49] Yan ZF, Wang L, Xia W, et al. Synergistic biodegradation of poly(ethylene terephthalate) using Microbacterium oleivorans and thermobifida fusca cutinase. Appl Microbiol Biotechnol. 2021;105(11):4551–4560. doi:10.1007/S00253-020-11067-Z

[50] Wei R, Zimmermann W. Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. Microb Biotechnol. 2017;10(6):1302–1307. doi:10.1111/1751-7915.12714

[51] Dhali SL, Parida D, Kumar B, et al. Recent trends in microbial and enzymatic plastic degradation: a solution for plastic pollution predicaments. Biotechnol Sustain Mater. 2024;1(1):1–23. doi:10.1186/s44316-024-00011-0

[52] Mohanan N, Montazer Z, Sharma PK, et al. Microbial and Enzymatic Degradation of Synthetic Plastics. Front Microbiol. 2020;11:1–22. doi:10.3389/fmicb.2020.580709

[53] Kushwaha A, Goswami L, Singhvi M, et al. Biodegradation of poly(ethylene terephthalate): mechanistic insights, advances, and future innovative strategies. Chem Eng J. 2023;457(October 2022):141230. doi:10.1016/j.cej.2022.141230

[54] Thomsen TB, Hunt CJ, Meyer AS. Influence of substrate crystallinity and glass transition temperature on enzymatic degradation of polyethylene terephthalate (PET). New Biotechnol. 2022;69(March):28–35. doi:10.1016/j.nbt.2022.02.006

[55] Gong J, Kong T, Li Y, et al. Biodegradation of microplastic derived from poly(ethylene terephthalate) with bacterial whole-cell biocatalysts. Polymers (Basel). 2018;10(12):1–13. doi:10.3390/polym10121326

[56] Maurya A, Bhattacharya A, Khare SK. Enzymatic remediation of polyethylene terephthalate (PET)–based polymers for effective management of plastic wastes: An overview. Front Bioeng Biotechnol. 2020;8(November):1–13. doi:10.3389/fbioe.2020.602325

[57] Giraldo-Narcizo S, Guenani N, Sánchez-Pérez AM, et al. Accelerated polyethylene terephthalate (PET) enzymatic degradation by room temperature alkali Pre-treatment for reduced polymer crystallinity. ChemBioChem. 2023;24(1):1–6. doi:10.1002/CBIC.202200503

[58] Thatoi H, Behera BC, Mishra RR, et al. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann Microbiol. 2012;63(1):1–19. doi:10.1007/S13213-012-0442-7

[59] Michelato Ghizelini A, Cristina Santana Mendonça-Hagler L, & Macrae A. Microbial diversity in Brazilian mangrove sediments - a mini review. Braz J Microbiol [publication of the Brazilian Society for Microbiology]. 2012;43(4):1242–1254. doi:10.1590/S1517-83822012000400002

[60] van Bijsterveldt CEJ, van Wesenbeeck BK, Ramadhani S, et al. Does plastic waste kill mangroves? A field experiment to assess the impact of macro plastics on mangrove growth, stress response and survival. Sci Total Environ. 2021;756:143826–143837. doi:10.1016/J.SCITOTENV.2020.143826

[61] Obonaga LD, Ortiz A, Wilke T, et al. Plastic litter is rapidly bioeroded in mangrove forests. Mar Environ Res. 2025;207:107027–107042. doi:10.1016/j.marenvres.2025.107027

[62] Montazer Z, Habibi-Najafi MB, Mohebbi M, et al. Microbial degradation of UV-pretreated Low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil. J Polym

Environ. 2018;26(9):3613–3625. doi:10.1007/s10924-018-1245-0

[63] Haedar N, Clara T, Fahrudin AA, et al. Selection of plastic degradation indigenous bacteria isolated from tamangapa landfill macassar city. J Phys: Conf Ser. 2019;1341(2):1–8. doi:10.1088/1742-6596/1341/2/022023

[64] Park SY, Kim CG. Biodegradation of micro-polyethylene particles by bacterial colonization of a mixed microbial consortium isolated from a landfill site. Chemosphere. 2019;222:527–533. doi:10.1016/j.chemosphere.2019.01.159

[65] Dąbrowska GB, Janczak K, Richert A. Combined use of *Bacillus* strains and Miscanthus for accelerating biodegradation of poly(lactic acid) and poly(ethylene terephthalate). 2021. doi:10.7717/peerj.10957

[66] Roberts C, Edwards S, Vague M, et al. Environmental consortium containing pseudomonas and bacillus species synergistically degrades polyethylene terephthalate plastic. MSphere. 2020;5(6):1–20. doi:10.1128/msphere.01151-20

[67] Ng EL, Lin SY, Dungan AM, et al. Microplastic pollution alters forest soil microbiome. J Hazard Mater. 2021;409(August 2020):124606. doi:10.1016/j.jhazmat.2020.124606

[68] Zhao S, Liu R, Wang J, et al. Biodegradation of polyethylene terephthalate (PET) by diverse marine bacteria in deep-sea sediments. Environ Microbiol. 2023;25(12):2719–2731. doi:10.1111/1462-2920.16460

[69] Rüthi J, Rast BM, Qi W, et al. The plastisphere microbiome in alpine soils alters the microbial genetic potential for plastic degradation and biogeochemical cycling. J Hazard Mater. 2023;441(July 2022):129941–129955. doi:10.1016/j.jhazmat.2022.129941

[70] Delacuvellerie A, Benali S, Cyriaque V, et al. Microbial biofilm composition and polymer degradation of compostable and non-compostable plastics immersed in the marine environment. J Hazard Mater. 2021;419(January):126526–126536. doi:10.1016/j.jhazmat.2021.126526

[71] Abelouah MR, Idbella M, Nouj N, et al. Marine plastic exposure triggers rapid recruitment of plastic-degrading bacteria and accelerates polymer-specific transformations. J Hazard Mater. 2025;490(February):137724–137737. doi:10.1016/j.jhazmat.2025.137724

[72] Rosato A, Barone M, Negroni A, et al. Bacterial colonization dynamics of different microplastic types in an anoxic salt marsh sediment and impact of adsorbed polychlorinated biphenyls on the plastisphere. Environ Pollut. 2022;315(October):120411. doi:10.1016/j.envpol.2022.120411

[73] Malik N, Lakhawat SS, Kumar V, et al. Recent advances in the omics-based assessment of microbial consortia in the plastisphere environment: deciphering the dynamic role of hidden players. Process Saf Environ Prot. 2023;176(June):207–225. doi:10.1016/j.psep.2023.06.013

[74] Zhang X, Li Y, Ouyang D, et al. Systematical review of interactions between microplastics and microorganisms in the soil environment. J Hazard Mater. 2021;418(March):126288. doi:10.1016/j.jhazmat.2021.126288

[75] You X, Wang S, Li G, et al. Microplastics in the soil: A review of distribution, anthropogenic impact, and interaction with soil microorganisms based on meta-analysis. Sci Total Environ. 2022;832(March):154975. doi:10.1016/j.scitotenv.2022.154975

[76] Wróbel M, Deja-Sikora E, Hrynkiewicz K, et al. Microbial allies in plastic degradation: specific bacterial genera as universal plastic-degraders in various environments. Chemosphere. 2024;363(March):142933–142947. doi:10.1016/j.chemosphere.2024.142933

[77] Roman EKB, Ramos MA, Tomazetto G, et al. Plastic-degrading microbial communities reveal novel microorganisms,: pathways, and biocatalysts for polymer degradation and bioplastic production. Sci Total Environ. 2024;949(May):174876–174890. doi:10.1016/j.scitotenv.2024.174876

[78] Danso D, Chow J, Zimmermann W, et al. New insights into the function and global distribution of polyethylene terephthalate (PET)-degrading bacteria and enzymes in marine and terrestrial metagenomes. Appl Environ Microbiol. 2018;84(February):1–13.

[79] Zrimec J, Kokina M, Jonasson S, et al. Plastic-Degrading potential across the global microbiome correlates with recent pollution trends. MBio. 2021;12(5):1–15. doi:10.1128/mBio.02155-21

[80] Wright RJ, Bosch R, Langille MGI, et al. A multi-OMIC characterisation of biodegradation and microbial community succession within the PET plastisphere. Microbiome. 2021;9(1):1–22. doi:10.1186/s40168-021-01120-y

[81] Wang L, Tong J, Li Y, et al. Bacterial and fungal assemblages and functions associated with biofilms differ between diverse types of plastic debris in a freshwater system. Environ Res. 2021;196(September 2020). doi:10.1016/j.envres.2020.110371

[82] He Z, Hou Y, Li Y, et al. Increased methane production associated with community shifts towards methanocella in paddy soils with the presence of nanoplastics. Microbiome. 2024;12(1):259–274. doi:10.1186/s40168-024-01974-y

[83] Bei Q, Peng J, Liesack W. Shedding light on the functional role of the Ignavibacteria in Italian rice field soil: A meta-genomic/transcriptomic analysis. Soil Biol Biochem. 2021;163:108444–108455. doi:10.1016/j.soilbio.2021.108444

[84] Lv S, Li Y, Zhao S, et al. Biodegradation of typical plastics: from microbial diversity to metabolic mechanisms. Int J Mol Sci. 2024;25(1):593–618. doi:10.3390/ijms25010593

[85] Rong L, Zhao L, Zhao L, et al. LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci Total Environ. 2021;773:145640–145651.doi:10.1016/j.scitotenv.2021.145640

[86] Wang P, Liu J, Han S, et al. Polyethylene mulching film degrading bacteria within the plastisphere: Co-culture of plastic degrading strains screened by bacterial community succession. J Hazard Mater. 2023;442(September 2022):130045–130057. doi:10.1016/j.jhazmat.2022.130045

[87] Kumar A, Lakhawat SS, Singh K, et al. Metagenomic analysis of soil from landfill site reveals a diverse microbial community involved in plastic degradation. J Hazard Mater. 2024;480(April):135804–135820. doi:10.1016/j.jhazmat.2024.135804

[88] Islami AN, Tazkiaturrizki T, Rinanti A. The effect of pH-temperature on plastic allowance for Low-density

polyethylene (LDPE) by thiobacillus sp. and clostridium sp. J Phys: Conf Ser. 2019;1402:3–6. doi:10.1088/1742-6596/1402/3/033003

[89] Morris BEL, Henneberger R, Huber H, et al. Microbial syntrophy: interaction for the common good. FEMS Microbiol Rev. 2013;37(3):384–406. doi:10.1111/1574-6976.12019

[90] Oberbeckmann S, Osborn AM, Duhaime MB. Microbes on a bottle: substrate,: season and geography influence community composition of microbes colonizing marine plastic debris. PLoS One. 2016;11(8):1–24. doi:10.1371/journal.pone.0159289

[91] Thapliyal C, Priya A, Singh SB, et al. Potential strategies for bioremediation of microplastic contaminated soil. Environmental Chemistry and Ecotoxicology. 2024;6(January):117–131. doi:10.1016/j.enceco.2024.05.001

[92] Kublik S, Gschwendtner S, Magritsch T, et al. Microplastics in soil induce a new microbial habitat,: with consequences for bulk soil microbiomes. Frontiers in Environmental Science. 2022;10(August):1–10. doi:10.3389/fenvs.2022.989267

[93] Luo S, Wang S, Zhang H, et al. Plastic film mulching reduces microbial interactions in black soil of northeastern China. Appl Soil Ecol. 2022;169(August 2021):104187–104197. doi:10.1016/j.apsoil.2021.104187

[94] Meyer-Cifuentes IE, Werner J, Jehmlich N, et al. Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. Nat Commun. 2020;11(1):5790–5803. doi:10.1038/s41467-020-19583-2

[95] Kumar V, Maitra SS, Singh R, et al. Acclimatization of a newly isolated bacteria in monomer tere-phthalic acid (TPA) may enable it to attack the polymer poly-ethylene terephthalate(PET). J Environ Chem Eng. 2020;8(4):103977–103984. doi:10.1016/j.jece.2020.103977

[96] Suwanawat N, Parakulsuksatid P, Nitayapat N, et al. Biodegradation of terephthalic acid by rhodococcus biphenylivorans isolated from soil. Int J Environ Sci Develop. 2019;10(1):30–33. doi:10.18178/ijesd.2019.10.1.1141

[97] Aksu D, Vural C, Karabey B, et al. Biodegradation of terephthalic acid by isolated active sludge microorganisms and monitoring of bacteria in a continuous stirred tank reactor. Braz Arch Biol Technol. 2021;64:1–10. doi:10.1590/1678-4324-2021200002

[98] Ghogare PD, Gupta SG. Degradation of mono ethylene glycol by few selected microorganisms and developed microbial consortium director,: government institute of forensic science, Aurangabad, Maharashtra, India. International Journal of Microbiological Res. 2012;3(2):93–98. doi:10.5829/idosi.ijmr.2012.3.2.5380

[99] Mückschel B, Simon O, Klebensberger J, et al. Ethylene glycol metabolism by pseudomonas putida. Appl Environ Microbiol. 2012;78(24):8531–8539. doi:10.1128/AEM.02062-12

[100] Franden MA, Jayakody LN, Li WJ, et al. Engineering pseudomonas putida KT2440 for efficient ethylene glycol utilization. Metab Eng. 2018;48:197–207. doi:10.1016/J.YMBEN.2018.06.003

[101] Trifunović D, Schuchmann K, Müller V. Ethylene glycol metabolism in the acetogen acetobacterium woodii. J Bacteriol. 2016;198:1058–1065. doi:10.1128/JB.00942-15

[102] Carniel A, Valoni É, Nicomedes J, et al. Lipase from Candida antarctica (CALB) and cutinase from humicola insolens act synergistically for PET hydrolysis to terephthalic acid. Process Biochem. 2017;59:84–90. doi:10.1016/J.PROCBIO.2016.07.023

[103] Ribitsch D, Heumann S, Trotscha E, et al. Hydrolysis of polyethyleneterephthalate by p-nitrobenzylesterase from bacillus subtilis. Biotechnol Prog. 2011;27(4):951–960. doi:10.1002/BTPR.610

[104] Qi X, Yan W, Cao Z, et al. Current advances in the biodegradation and bioconversion of polyethylene terephthalate. Microorganisms. 2022;10(1):1–25. doi:10.3390/microorganisms10010039

[105] Lee GH, Kim DW, Jin YH, et al. Biotechnological plastic degradation and valorization using systems metabolic engineering. Int J Mol Sci. 2023;24(20):15181–15206. doi:10.3390/ijms242015181