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. BHET degradation 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.

Keywords: biodegradation, polyethylene terephthalate, bis(2-hydroxyethyl) terephthalate, monoethylene glycol, terephthalic acid.

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 (Alongi, 2014). Mangroves play a crucial role in coastal protection and ecological purification of water resources (Barbier, 2016). In addition, the well-developed root system enables mangrove plants to thrive against dynamic environmental stress and act as traps for marine litter (Martin et al., 2019; Srikanth et al., 2015). 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 (Canepa et al., 2024). 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 hypothesises 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 (Jha, 2020). In fact, rhizosphere microorganisms can potentially enhance the biodegradation of polylactide (PLA) and conventional polyethylene terephthalate (PET) films, a global environmental threat (Janczak et al., 2018). Recently, Dhaka et al. (Dhaka et al., 2022) 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 emphasises 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

(Auta et al., 2017). Moreover, a study on microplastic degradation pointed out mangrove rhizosphere bacteria as potential candidates for plastic degradation (Xie et al., 2021). 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 (Kawai et al., 2019). So far, researchers have isolated and identified a variety of enzymes with PET-degrading ability, mainly including esterases, lipases, hydrolases, and cutinases (Qiu et al., 2024). A PET-degrading specific enzyme, PETase, isolated from *Ideonella sakaiensis* 201-F6, and capable of hydrolysing PET, was first reported by Yoshida et al., (2016). 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 CO2, H2O, and other small molecules through tricarboxylic acid cycle metabolism (Qiu et al., 2024). In recent years, a range of PET-degrading enzymes, that mainly target PET ester bonds, breaking 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 (Joo et al., 2018). BHET, MHET, and TPA are reported as the major degradation products of PET during the enzymatic hydrolysis and chemical recycling process (Vertommen et al., 2005; Salvador et al., 2019). BHET has also been studied as a model molecule for PET recycling (Ion et al., 2021). Currently, there are no reports on the toxicity and pollution levels of BHET (Qiu et al., 2020), while TPA has been reported to cause bladder stones and bladder cancer, as well as impairment of liver and testicular functions (Zhang et al., 2010; Ordaz-Cortés et al., 2014). As for MEG, the parent compound (ethylene) is considered nontoxic. However, prolonged exposure to MEG can cause varying toxicity levels in rats (Snellings et al., 2013). 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 (Qiu et al., 2020). Li et al., (2019) 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 toward 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 0.1mm, crystalline) obtained from Goodfellow (Cambridge, England) were cut into 1 cm^2 sizes, washed with 75% alcohol and dried overnight at 60 ºC. 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: *Bacillus* sp. GPB12 (isolated from city Green Park soil samples – an old landfill, Macao SAR, China) and *Enterococcus* sp. WTP31B-5 (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 x 109 CFU ml^{-1}, following the protocol of Dąbrowska et al. (2014) 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 23oC 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 (Ambika et al., 2014). 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 ^{o}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:

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

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} (Janczak et al., 2014).

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 ºC. The PET films were coated with gold particles and analysed using SEM imaging on a TESCAN VEGA3 (Czech Republic).

2.8. Molecular characterisation 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 in a sterile plastic bag and stored at -20 ºC 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 ºC 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 (Edgar et al. 2011) as implemented in the VSEARCH package (Rognes et al., 2016). High-quality reads were then clustered into OTUs using Minimum Entropy Decomposition (MED) (Eren AM 2013 and 2015). 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 (Angly FE, 2014). The raw sequence data were deposited in Sequence Read Archive (SRA) from NCBI database, associated to the BioProject, under the accession number BioProject PRJNA1063621.

2.9. 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 sterilised minimal salts medium (Moreira et al., 2013) 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 ºC. After incubation, all colonies observed for each treatment were aseptically pooled together and used for further biodegradation assays.

2.10. 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 sterilised minimal salts medium (Moreira et al., 2013), 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 x 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 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).

2.11. Analytical methods

2.11.1. BHET, MEG and TPA quantification

Over the batch degradation assays, aliquot samples were collected and centrifuged at 13500 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 program starting at 80 ºC for 2 min, increasing to 180 ºC at a rate of 10 ºC min^{-1} with 5 min hold. Injector and detector temperatures were set to 250 ºC.

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: $C = C_{0e}-kt$, 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 $t_{1/2} = \ln 2/k$.

2.11.2. 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 ºC.

2.2. 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 analysis 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 characterised 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.

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 and 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 and 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. TIn 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, 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%). This 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, eight taxa – Thermomicrobia, Sphingobacteriia, Limnochordia, Flavobacteria, Actinobacteria, Rhodothermia, Cytophagia and Thermoleophilia - were the dominant taxa. 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 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.

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 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 ten days. In BHET, consortia A and C showed an exponential growth within the first 24 hours 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 B showed around 75 % 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 2).

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 2). No decrease in the concentration of tested compounds on controls was observed, indicating that no abiotic losses or 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 measurements showed a decrease by the end the experiments following the same trend as the compound removal across all assays (Table 2). The results suggest complete mineralisation of the amount of compound degraded. The reduction in TOC for TPA experiment with consortia A, B, and C was 93.8 %, 91.9 %, and 89.1 %, respectively. The decrease in TOC for MEG assay with the consortia A, B, and C are 40.8 %, 62.3 %, and 57.2 %, respectively. Regarding the TOC of the BHET assay, consortium B was observed to have 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 (Webb et al., 2013; Xia et al., 2014). 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 (Helen et al., 2017). 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 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., 2018), 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. (2021), 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., (2021; 2020) 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 cm^{-1} 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 (Torena et al., 2021; Panowicz et al., 2021). The variation in the absorption peaks of the functional groups reveals the conformational change in PET from all the treatments and of most from treatments with mangrove plants (Djebara et al., 2012). The disappearance or appearance of functional groups is a strong indication of PET degradation (Helen et al., 2017; Naz et al., 2013;

Skariyachan et al., 2017; Ioakeimidis et al., 2016). 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 (Wilkes and Aristilde, 2017). 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 (Helen et al., 2017). 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 break 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 (Falah et al., 2020). 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 1000–3000 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 (Torena et al., 2021). The ester carbonyl group (C=O) Stretch (1712 cm^{-1}) is a defining feature of PET's polymer backbone, linking TPA and EG 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. (2021) 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 (Jaiswal et al., 2020).

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 with mangrove and bioaugmented with the bacterial consortium. Similar changes on PET surfaces have been reported by Torena et al. (2021) 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 degradationof 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 (Ioakeimidis et al., 2016). 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 (Kim et al., 2023). PET surface roughness and cracks are considered evidence of biodegradation (Sarkhel et al., 2020; Yan et al., 2021).

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 or 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 (Wei and Zimmermann, 2017; Kawai et al., 2019). 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 (Dhali et al., 2024). It is well known that PET biodegradation depends on the polymer's crystallinity, purity, and orientation of polymer chains (Mohanan et al., 2020). 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 (Qiu et al., 2024); Kushwaha et al., 2023). Therefore, the degradation of highly crystalline regions of PET remains a challenge. A recent study on enzymatic PET hydrolysis showed how an industrially relevant PET-degrading enzyme, revealed a pronounced lag phase for crystalline PET (Thomsen et al., 2022). The high degree of crystallinity is one of the major reasons why PET is not readily biodegradable (Gong et al., 2018; Maurya et al., 2020). 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 (Dhali et al., 2024). 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 (Giraldo-Narcizo et al., 2023). 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 (Thatoi et al., 2012), harbouring microorganisms capable of degrading plastic polymers (Helen et al., 2017). 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 (Ghizelini et al., 2012). In a study to assess the impact of plastics on mangroves, Van Bijsterveldt et al. (2021) 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 novel plastic-degrading enzymes (Martin et al., 2019). 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-hydrolysing 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 (Obonaga et al., 2024). 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 the plants.

The bacterial consortia used for bioaugmentation comprised two bacterial strains, *Enterococcus* sp. WTP31B-5 and *Bacillus* sp. GPB12 isolated from the activated wastewater treatment plant sludge and an old landfill, respectively. Bacteria from activated sludge samples have been reported to degrade up to 17 % of PET microplastics (Torena et al., 2021). A landfill is also an environment with diverse bacterial potential to degrade plastics (Montazer et al., 2018; Haedar et al., 2019; Park & Kim, 2019). In particular, *Bacillus* sp. has demonstrated PET degradation potential (Dąbrowska et al., 2021; Roberts et al., 2020; Helen et al., 2017). Dąbrowska et al. (2021) 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 (Dąbrowska et al., 2021). 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 communty 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 (2021) 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 (Zhao et al., 2023;Ng et al., 2021), 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 plastisphere of biodegradable plastics in alpine soils compared to bulk soil (Rüthi et al., 2023). Dominance of

members of the Proteobacteria phylum was also observed in PET-degrading consortia (Zhao et al., 2023) and plastiphere community (Delacuvellerie et al., 2021). A shift in the microbial community structure due to PET exposure with dominance of *Proteobacteria* and *Firmicutes* was also observed in marine environment (Abelouah et al., 2025; Rosato et al., 2022). 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 (Malik et al., 2023; Zhang et al., 2021). Reviews on the degradation of microplastics in soil have been indicating *Proteobacteria* as the phylum with highest relevant abundance (Zhang et al., 2021; You et al., 2022). 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 (Wróbel et al., 2024). 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 (Roman et al., 2024). 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 (Danso, Chow, Zimmermann, & Wei, 2018). 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* (Zrimec et al., 2021). In the present study, the most abundant class in final soil samples was 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. Dominance of Gammaproteobacteria within the phylum Proteobacteria was also observed after enrichment with PET of a microbial community from marine plastic for six weeks (Wright et al., 2021) and deep-sea sediment for 2 years (Zhao et al., 2023). For PET submerged in situ on the sediment and in the water column in the Mediterranean Sea during 82 days was reported dominance of Alpha-

and Gammaproteobacteria by Delacuvellerie et al. (2021). *Anaerolineae*, a class that was enriched in all final soil samples 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 (Wang et al., 2021) Bacteria belonging to *Anaerolineae* were also abundant in microplastics surface in an anoxic salt marsh sediment (Rosato et al., 2022). 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 (Zhao et al., 2023), and together with *Alphaproteobacteria* and *Gammaproteobacteria*, this class was present in plastiphere in marine environment (Abelouah et al., 2025). In the black soil, nanoplastics induced abundance increases of *Coriobacteriia* (He et al., 2024). Members of the *Ignavibacteria* class were reported to decompose complex polysaccharides (e.g. cellulose and hemicellulose) (Bei et al., 2021).

An analysis of the core microbiome across the microcosms revealed several genera consistently detected in all soil samples, 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 samples, 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 a PET degrading microbe, with the ability to colonize and use plastic as carbon source (Taghavi et al., 2021). The importance of *Pseudomonas* in the context of plastic degradation was reviewed (Wilkes & Aristilde, 2017; Lv et al., 2024). *Hyphomicrobium* were significantly enriched in soil with 7% (w/w) low-density polyethylene microplastics (Rong et al., 2021) and in the plastisphere of polyethylene mulching film (Wang et al., 2023). 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 (Kumar et al., 2024;Islami et al., 2019; Morris et al., 2013). 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 (Oberbeckmann et al., 2016) and identified as plastic degraders

(Thapliyal et al., 2024). On the other hand, the *Lewinella* genus, identified as part of microbial communities attached to PET drinking bottles submerged in the North Sea (Oberbeckmann et al., 2016), was only enriched in microcosms C. *Phenylobacterium*, a genus described as microplastics colonizer and polymeric substances degrader (Kublik et al., 2022; Luo et al., 2022), 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 (Meyer-Cifuentes et al., 2020). 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, *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 relting 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 degradation of PET monomers and intermediate. The complete degradation of TPA by the consortia is greater than the results reported in other studies. *Rhodococcus* sp. SSM1 achieved 100 % TPA degradation of 5000 mg L^{-1} at 96 h (Kumar et al., 2020) and *Rhodococcus biphenylivorans* N2 degraded 99.6 % 1000 mg L^{-1} in 5 days (Suwanawat et al., 2019). While in these reports were 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 few bacterial strains, namely *Pseudomonas* sp., *Chryseobacterium* sp., *Burkholderia* sp., and *Arthrobacter* sp., within 24 hours (Aksu et al., 2021). Furthermore, the TOC analysis results supported evidence of degradation by all the bacterial consortia, regardless of the treatment from where they were retrieved. The

742 correspondence between compounds degradation and TOC removal suggested that the amount of
743 degraded compounds underwent mineralization. Consortium C obtained from bioaugmented
744 mangrove soil showed slightly higher MEG degradation (83.7 %), with higher degradation rate
745 constant and the lower half-life (2.339±0.154), which may be attributed to the presence of
746 mangrove plants and the consortium in this treatment. A similar degradation extent of MEG at
747 varying concentrations (0.25-1.0 % (v/v)) has been reported by Ghogare and Gupta (2012) when
748 working with isolates *Oliptrichum macrosporum, Bacillus niacin, Streptomyces* sp-1*, Aspergillus*
749 *terreus, and Aspergillus faecalis*, individually and in consortium (Ghogare and Gupta, 2012). After
750 seven days, the microbial consortium achieved 75.49 % of MEG degradation, which is
751 approximately 5% higher that the degradation obtained by the best degrader - *O. macrosporum* -
752 individually (Ghogare and Gupta, 2012). Despite the degradation obtained with microbial isolates,
753 this report highlights the advantages of mixed microbial communities for MEG degradation. In
754 addition, MEG (3103 mg L^{-1}) oxidation using *Pseudomonas putida* strains KT2440 and JM37 has
755 also been reported with both converting MEG into glycolic acid and glyoxylic acid (Mückschel et
756 al., 2012). The engineered *Pseudomonas putida* KT2440 has demonstrated the ability to completely
757 degrade MEG, enabling its conversion to medium-chain-length polyhydroxyalkanoates (Franden
758 et al., 2018). *Acenetobacterium woodii* has been reported to convert MEG into ethanol and acetyl
759 coenzyme A (acetyl-CoA), which was further converted to acetate (Trifunović et al., 2016). The
760 decrease in the TOC corroborated the degradation evidence by all the bacterial consortia as
761 mineralisation was not observed in MEG. However, it is important to notice that degradation was
762 still occurring when the experiment finished and probably it was not complete due to the high
763 concentration of MEG used in these assays. There is no evidence of toxicity to the microorganisms
764 or degradation inhibition.
765 In relation to BHET, the bacterial consortium from the treatment with mangrove plants showed
766 much higher degradation (96 %) compared with the consortium retrieved from treatment without
767 mangroves and, surprisingly, from treatment with mangrove plants plus bioaugmentation. In these
768 experiments, the degradation stopped after the first day and did not follow a first-order kinetic as
769 expected in case of successful degradation, suggesting some inhibition on the degradation process,
770 such as accumulation of an inhibitory intermediary metabolite or the lack of a degrading
771 microorganism or enzyme for further degradation. Comparing with results for PET degradation,
772 promising in treatment C, the results for BHET degradation suggests that probably key degrading
773 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., (2020) reported 80.8 % of removal of initial BHET concentration of 1000 mg L^{-1} within 120 h by *Enterobacter* sp. HY1. The analysis of the metabolites produced revealed that BHET is hydrolysed to MHET and then to TPA. The esterase EstB cloned from Enterobacter sp. HY1 specifically hydrolyses BHET to MHET, revealing enzymatic specificity. While 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* (Yoshida et al., 2016), *Humicola insolens* (Carniel et al., 2017), and *Bacillus subtilis* (Ribitsch et al., 2011) 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 mineralisation 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 consortium from mangrove soils for biodegradation of BEHT. In fact, looking for the degradation rates and mineralization degree from the three tested 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) (Qi et al., 2022; Mohanan et al., 2020). BHET degradation requires specific BHET-degrading enzyme that converts BHET into MHET, which is further degraded by the action of an MHET-degrading enzyme into TPA and MEG, small water-soluble molecules (Lee et al., 2023). The high accumulation of BHET during PET degradation observed in experiments with Thioclava sp. BHET1 supports the specificity and limiting-step nature of BHET degradation (Wright et al., 2021). On the other hand, oxygenase enzymes, which are usually involved in the degradation of most

aromatic hydrocarbons, such as TPA, as well as a potential further intermediates, protocatechuate and catechol, tend to be widely distributed in the environment (Wright et al., 2021). 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 (Mohanan et al., 2020).

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 hypothesises 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 the 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, since it is a monomer similar to the core structure of PET. Our findings demonstrate that the microbiome of soil microcosms evolved for complete PET degradation, since not only PET 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 and stability 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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Declaration of Competing Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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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Figure captions:

Fig. 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

Fig. 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

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

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

Fig. 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 $p < 0.05$.

Fig. 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

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

Table 2. Bacterial richness and α-diversity of soil

	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

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

	Consortium		
TPA	**A**	**B**	**C**
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	**A**	**B**	**C**
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	**A**	**B**	**C**
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

Figure 1

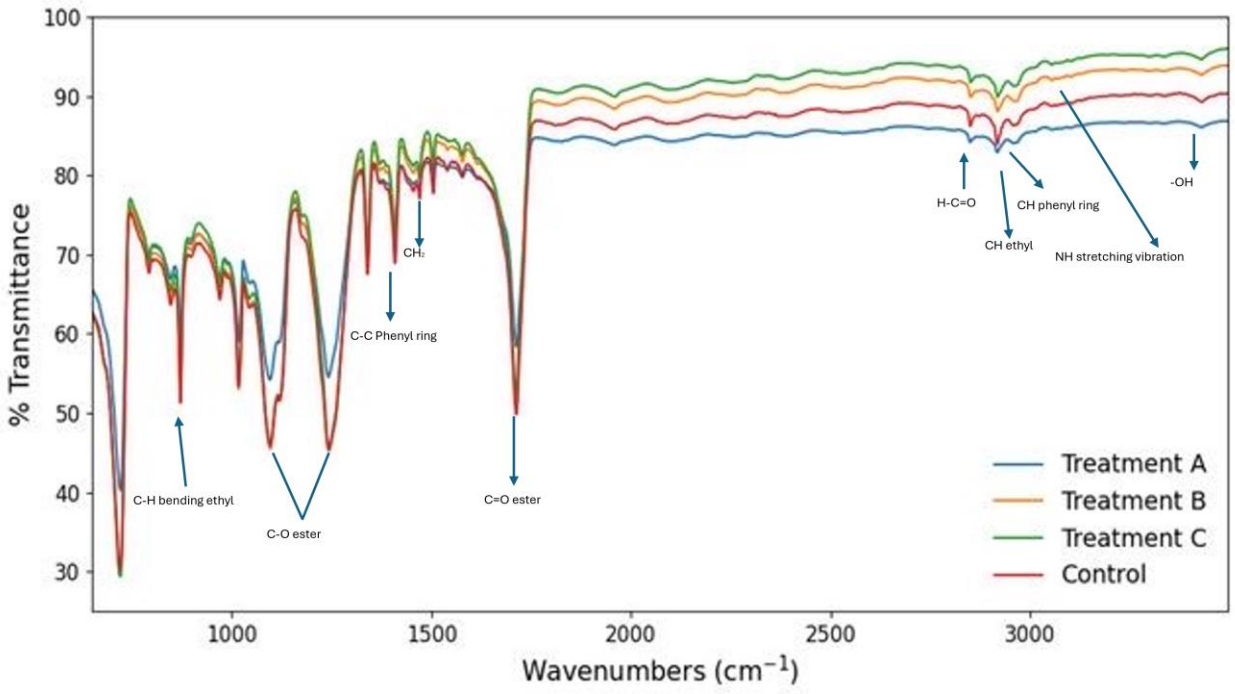

% Transmittance
Wavenumbers (cm^{-1})
C-H bending ethyl
C-O ester
C-C Phenyl ring
CH₂
C=O ester
H-C=O
CH ethyl
CH phenyl ring
NH stretching vibration
-OH
Treatment A
Treatment B
Treatment C
Control

Figure 2

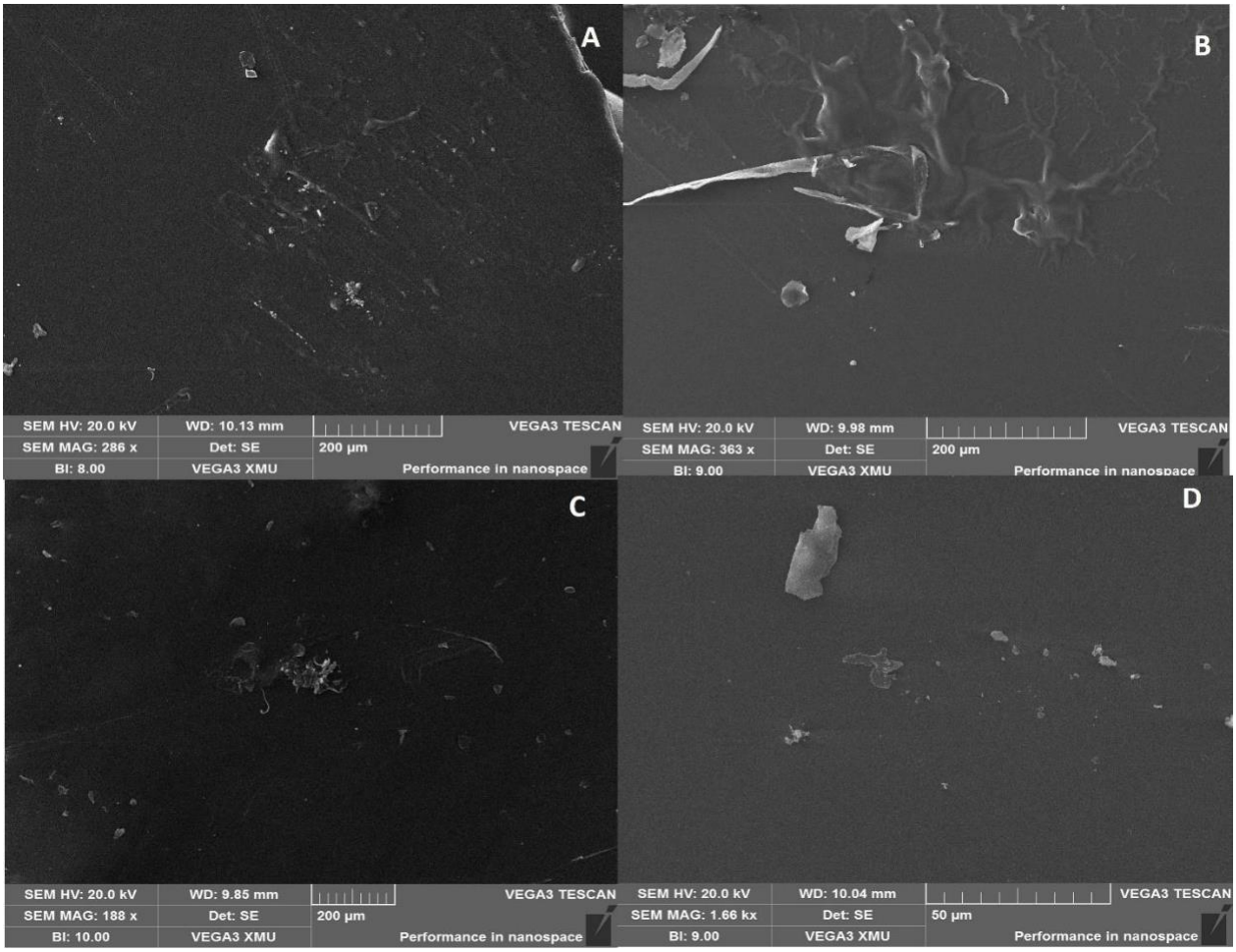
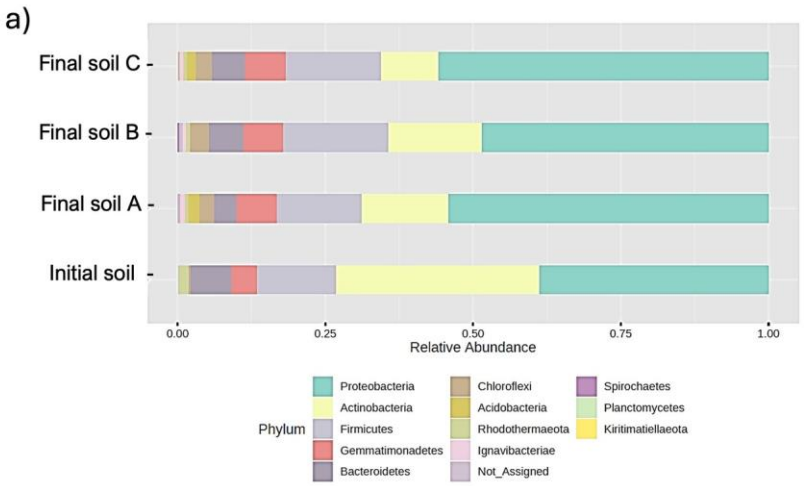

A
SEM HV: 20.0 kV
WD: 10.13 mm
VEGA3 TESCAN
SEM MAG: 286 x
Det: SE
200 µm
BI: 8.00
VEGA3 XMU
Performance in nanospace
B
SEM HV: 20.0 kV
WD: 9.98 mm
VEGA3 TESCAN
SEM MAG: 363 x
Det: SE
200 µm
BI: 9.00
VEGA3 XMU
Performance in nanospace
C
SEM HV: 20.0 kV
WD: 9.85 mm
VEGA3 TESCAN
SEM MAG: 188 x
Det: SE
200 µm
BI: 10.00
VEGA3 XMU
Performance in nanospace
D
SEM HV: 20.0 kV
WD: 10.04 mm
VEGA3 TESCAN
SEM MAG: 1.66 kx
Det: SE
50 µm
BI: 9.00
VEGA3 XMU
Performance in nanospace

Figure 3


a)
Final soil C
Final soil B
Final soil A
Initial soil
0.00
0.25
0.50
0.75
1.00
Relative Abundance
Phylum
Proteobacteria
Actinobacteria
Firmicutes
Gemmatimonadetes
Bacteroidetes
Chloroflexi
Acidobacteria
Rhodothermaeota
Ignavibacteriae
Not_Assigned
Spirochaetes
Planctomycetes
Kiritimatiellaeota

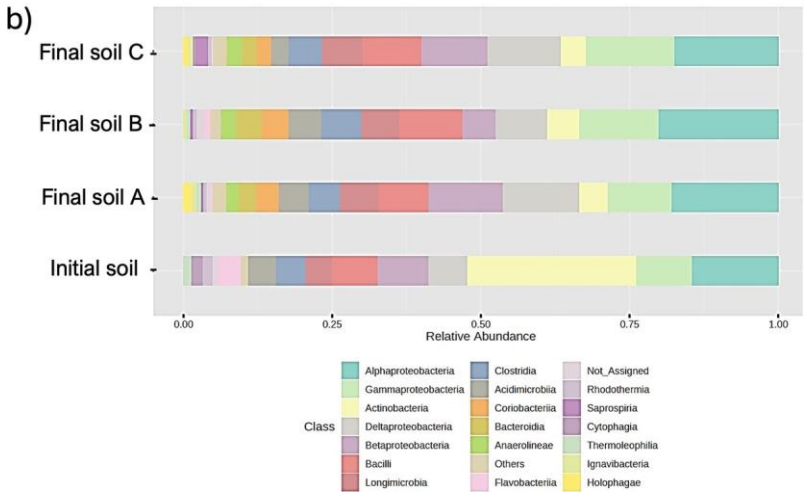
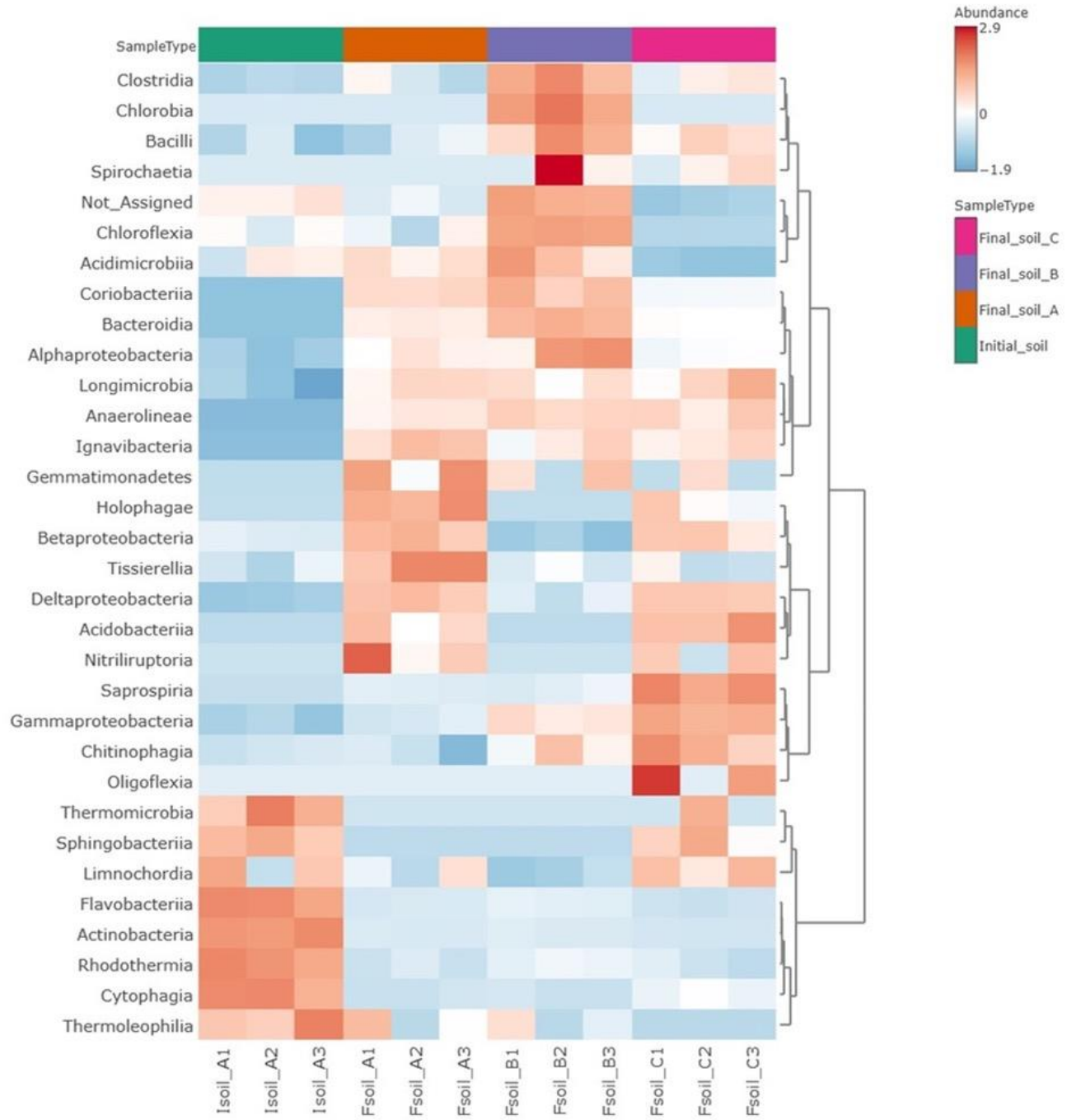

b)
Final soil C
Final soil B
Final soil A
Initial soil
0.00
0.25
0.50
0.75
1.00
Relative Abundance
Class
Alphaproteobacteria
Gammaproteobacteria
Actinobacteria
Deltaproteobacteria
Betaproteobacteria
Bacilli
Longimicrobia
Clostridia
Acidimicrobiia
Coriobacteriia
Bacteroidia
Anaerolineae
Others
Flavobacteriia
Not_Assigned
Rhodothermia
Saprospiria
Cytophagia
Thermoleophilia
Ignavibacteria
Holophagae

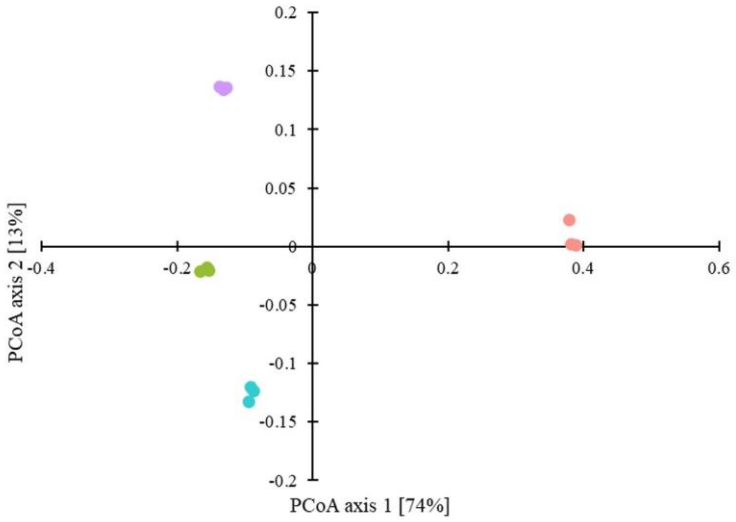
Figure 4



1334

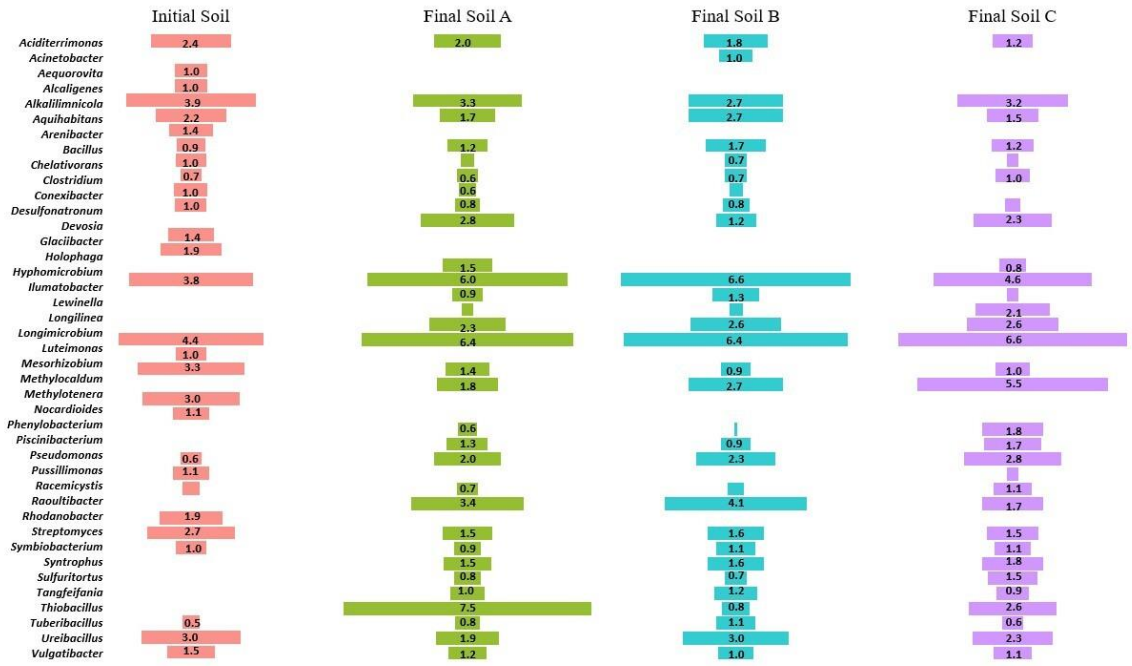
1335 Figure 5

a)



1336

1337 **b)**



1338

1339

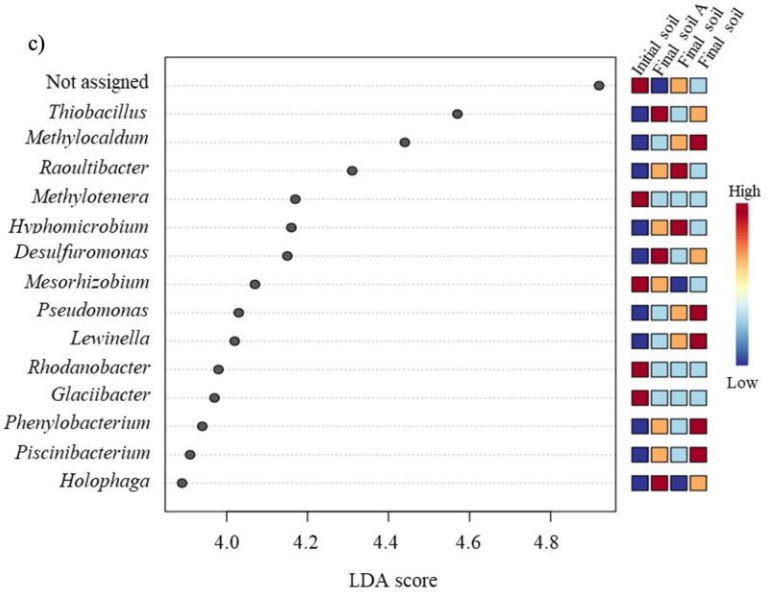
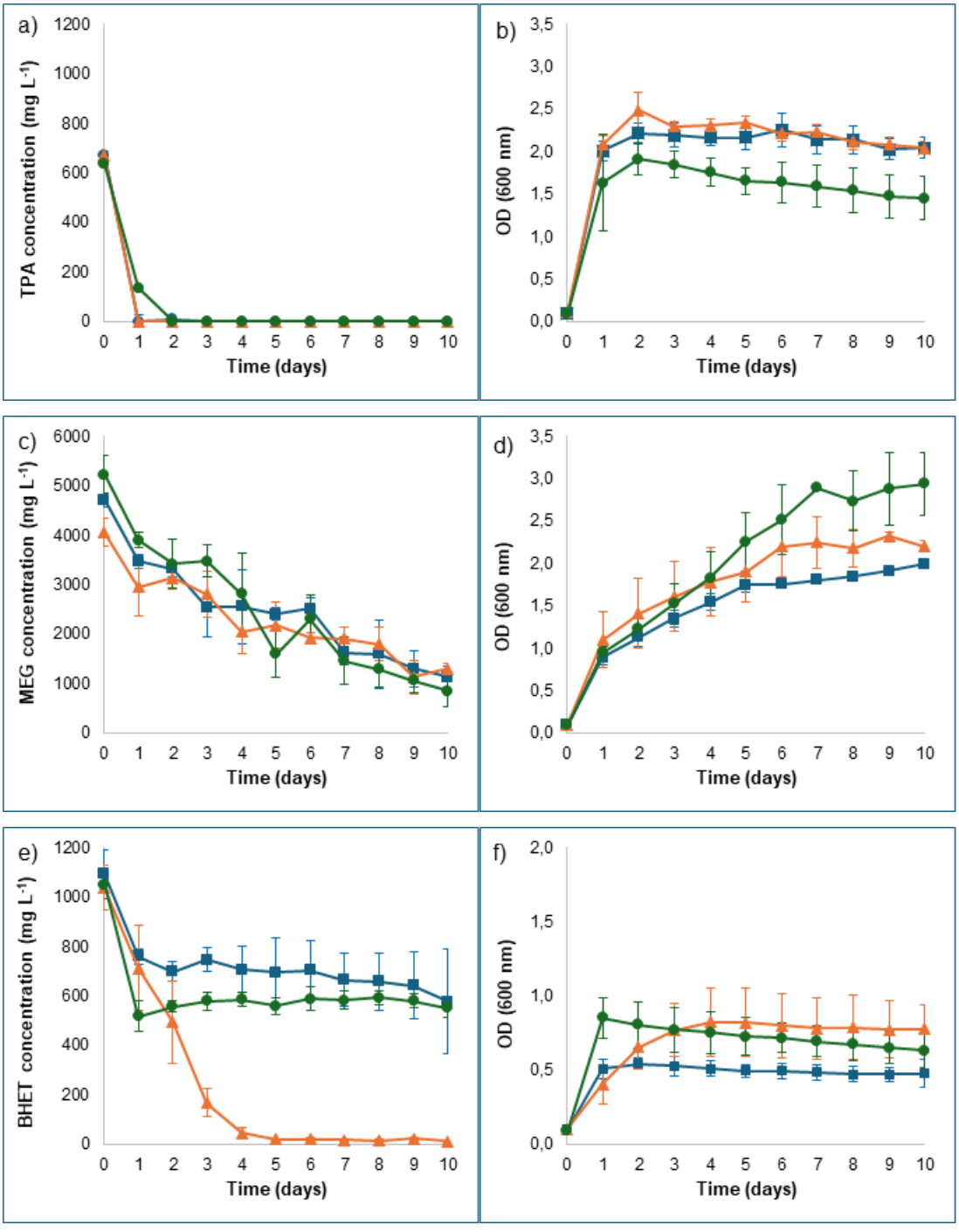

c)
Not assigned
Thiobacillus
Methylocaldum
Raoultibacter
Methylotenera
Hyphomicrobium
Desulfuromonas
Mesorhizobium
Pseudomonas
Lewinella
Rhodanobacter
Glaciibacter
Phenylobacterium
Piscinibacterium
Holophaga
4.0
4.2
4.4
4.6
4.8
LDA score
Initial soil
Final soil A
Final soil
Final soil
High
Low

Figure 6


a)
TPA concentration (mg L-1)
Time (days)
b)
OD (600 nm)
Time (days)
c)
MEG concentration (mg L-1)
Time (days)
d)
OD (600 nm)
Time (days)
e)
BHET concentration (mg L-1)
Time (days)
f)
OD (600 nm)
Time (days)