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# A community of marine bacteria with potential to biodegrade petroleum-based and biobased microplastics

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ABSTRACT

The biodegradability conditions for both, petroleum-based plastics and bioplastics needs to be evaluated under environmentally realistic conditions. We assessed the biodegradability of low-density polyethylene and biobased polyethylene terephthalate microplastic films by a consortium of marine bacteria during 45 days. Bacterial growth and pH were higher in the samples inoculated with bacteria, compared to the controls. Fourier Infrared spectroscopy-Attenuated Total Reflectance and scanning electron microscopy indicated changes in the chemical functional groups, and the presence of fractures and biofilms in the surface of both plastics exposed to the bacterial community, respectively. The chemical oxygen demand further indicated signs of biodegradation of both polymers. Specific groups of bacteria showed preference for each type of microplastic. Overall, our results show signs of biodegradation, or at least biodeterioration and biofragmentation, of both types of plastics, when subjected to the selected bacterial community. Biobased PET was no more prone to biodegradation than conventional, petroleum-based LDPE.

## 1. Introduction

Plastics have become very popular and attractive materials to the global market as they are lightweight, cheap, durable, and corrosion-resistant, among other properties (Gago et al., 2018). This has led to an exponential increase in their manufacturing since the 1980s, reaching a global production of 367 million tonnes in 2020 (Plastics Europe, 2021). The properties that make plastics a good market product, however, also make them inert and ubiquitous in the natural environment. Low-density polyethylene (LDPE) and polyethylene terephthalate (PET) are among the most demanded plastics worldwide (Gewert et al., 2015; Plastics Europe, 2021), widely used in the production of daily products such as trays and containers or food packaging film in the case of LDPE, and bottles or bags in the case of both polymers. Together, they accounted for approximately 26 % of the total plastic demand in the European Union, Norway, Switzerland, and the United Kingdom in 2020 – 17.4 % LDPE and 8.4 % PET (Plastics Europe, 2021).

In Europe, 150,000 to 500,000 t of plastic waste enter the oceans every year (Jambeck et al., 2015), the equivalent weight of up to 111 % the global population of blue whales. These plastics undergo slow fragmentation (up to centuries; Ojeda, 2013) into small particles until reaching a size smaller than 5 mm, what has been defined as microplastics (MPs) (Arthur et al., 2009). MPs are the most reported type of plastic pollution in all environments, accounting for 92.4 % of the 5.25 trillion particles present in the ocean surface (Eriksen et al., 2014). Because of their small size, MPs can enter the aquatic food chain from the lowest trophic level (i.e., phytoplankton) and be transported through the food chain, posing a risk to both protected species and species relevant for human consumption, such as oysters, shrimp, mussels and fish (Hwang et al., 2019; Rochman et al., 2015).

The current situation regarding the COVID-19 pandemic is also environmentally-wise alarming as it has driven a global surge in the use of plastics – especially of single use products – since it started in January 2020, leading to a follow-up waste entering the environment (Klemes et al., 2020; Pinto Da Costa et al., 2020). For instance, Bondaroff and Cooke (2020) have reported an estimation of 1.56 million face masks alone entering the oceans in 2020. It is therefore very urgent to implement policies to reduce plastic pollution, and to find cheap and environmentally safe solutions to remove the plastic that is already in the environment.

Among the possible solutions to eliminate plastics once they reach the environment, their biodegradation is considered the most acceptable

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one in terms of costs and because it is considered environmentally-friendly (ICPE, 2006). In general, the chemical structure and the additives present in LDPE and PET make them resistant to biodegradation (Andrady, 2017; Gewert et al., 2015; Ojeda, 2013). Previous studies have reported some microbial strains with the potential to biodegrade these polymers (e.g., Auta et al., 2017a, 2017b; Skariyachan et al., 2018; Yoshida et al., 2016). However, very few studies have reported the biodegradation of MPs by bacterial communities present in the marine environment (e.g., Syranidou et al., 2019). This is important mainly to know if and at which rate MPs may be degraded in natural waters, but also to evaluate which changes microbial communities may undergo after the exposition to MPs, and what will be the ultimately impact at ecological and chemical level.

As a partial solution to the problem of plastic pollution, the production of bioplastics, which encompass biobased – materials (partly) derived from biomass – and/or biodegradable – the material can be transformed in $CO_2$, water and new biomass by the action of microorganisms, under aerobic conditions (European Bioplastics, 2018) – plastics, has gained a lot of attention. Nonetheless, the biodegradability of these bioplastics is usually limited to specific environmental conditions (Emadian et al., 2017). More particularly, the biodegradability of biobased PET, which accounts for ca. 10 % of total global annual bioplastic production (European Bioplastics, 2019), remains particularly understudied (Mecozzi and Nisini, 2019).

The aim of this study is to assess the potential of a consortium of marine bacteria to biodegrade conventional (low density polyethylene) and biobased (polyethylene terephthalate) microplastics, referred as LDPE and BPET hereafter. Since the PET bag used for the experiment was labelled as "biodegradable", it was initially presumed that this plastic polymer could experience a higher biodegradation as compared to LDPE. However, after revising it, what was labelled as "biodegradable" PET is actually biobased PET, meaning that it has an origin in plants, but the chemical structure is the same as that of conventional, petroleum-based, PET (European Bioplastics, 2018). Hence, in principle, its biodegradability should be the same as of conventional PET. However, biobased or oxo-degradable PET may contain additives that accelerate the oxidation process (prodegradants) (Kubowicz and Booth, 2017). Therefore, we hypothesized that the biodegradation of both polymers would be limited, but higher changes (at a given period of time) were expected in the case of BPET.

## 2. Material and methods

### 2.1. Sample collection and preparation of inoculum

In July 2020, 6 samples from marine organisms were collected from two marine caves, close to the city of Sagres (37°00′31″N; 8°55′36″ W), in southern Portugal. Three samples were collected from the Catedral cave, and three from the Queijo Suiço cave (Fig. 1). To collect the samples, a team of scientific divers scraped and collected around 5 g of the surface of organisms belonging to the phylum Annelida, Cnidaria, Hydrozoa, Porifera and Tunicata. Bacterial communities were then recovered from these samples by adding 0.5 g of the sample to 4.5 ml (1:10 dilution) of an artificial, sterilized, marine broth (PanReac). The samples were left in the dark, at 25 ± 1 °C and under orbital agitation (150 rpm), for 24 h. They were subsequently stored in glycerol, at −20 °C, until further analysis. Before starting the experiment, the bacterial inoculum was prepared by adding one aliquot of these samples to a flask containing sterile marine broth (1:10 dilution). The samples were then incubated under the same conditions as described previously. After 48 h of incubation, bacteria samples were centrifuged (4000 *g*; 10 min), the supernatant discarded, and the pellet washed and resuspended in new marine broth. This washing procedure was repeated two times, and the final pellet was resuspended in the sterile marine broth and used as inoculum in the experiment.

### 2.2. Microplastics of study

Plastic polymers used for the experiment came from commercially available plastic bags, which chemical composition was determined by means of Fourier Transform Infrared spectroscopy – Attenuated Total Reflectance (FTIR-ATR). We used a plastic bag from a supermarket made

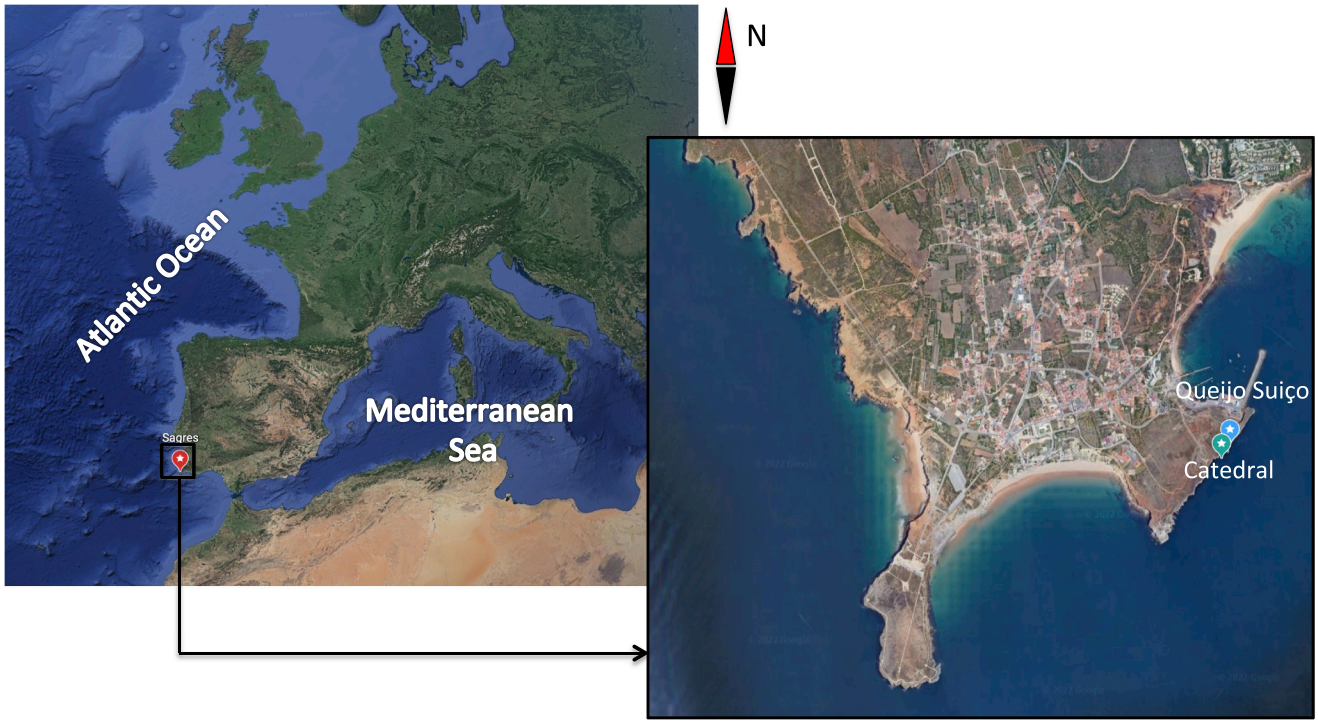


**Fig. 1.** Geographical location of the marine caves (Catedral and Queijo Suiço), in Sagres (southern Portugal), from where bacterial communities were recovered from inhabiting marine invertebrates. Maps retrieved from Google Earth.

of petroleum-based, low-density polyethylene (referred to as "LDPE" hereafter) and a plastic bag from a local shop labelled as "biodegradable", made of polyethylene terephthalate (referred to as "BPET" hereafter). According to the Plastics Europe classification (European Bioplastics, 2018), there is currently no PET classified as "biodegradable", and therefore we understand that this bag was instead made of "biobased" PET, which presents the same chemical structure than conventional PET. Biobased plastics may contain additives that accelerate the oxidation process (prodegradants) (Kubowicz and Booth, 2017), so they can still be more prone to biodegradation in the natural environment than their conventional counterparts.

Both plastic bags were manually cut, with a bistoury and tweezers, into microplastic (MP) films of an approximate area of 2 $mm^2$, under sterile conditions. The MP particles were further sterilized under UV-radiation (253, 7 nm) for 30 min, as this was the sterilization technique proven to be the most effective and easy to implement in a previous test (see Supp. Material, Figs. S1 and S2). This UV-radiation step was performed with a second purpose, since photodegradation is a phenomenon that can naturally enhance the oxidation of plastic polymers in surface waters (Auta et al., 2017b; Gewert et al., 2015; Syranidou et al., 2019).

### 2.3. Experimental setup

The 6 marine bacterial consortia were used in a preliminary screening, to test their potential to biodegrade the MPs of interest. The screening consisted of 5 different assays, including two experimental treatments and two types of controls. The negative control consisted of the marine broth in which either LDPE or BPET MP films were added, and no inoculum. The positive control only contained the inoculum diluted in the marine broth (10 % v/v), without MPs. Bacterial inocula were prepared as detailed in Section 2.1, and added to the samples in concentration 10 % v/v. All treatments were performed in duplicates. Samples were kept in 30 ml glass flasks, in the dark (25 ± 1 °C; 150 rpm), for up to 75 days. Biodeterioration and biofragmentation of the MP particles was determined by means of FTIR-ATR. Based on this test, we selected the inoculum number 18, recovered from the tunicate *Didemnum* sp. from the Catedral cave, as the bacterial community with the most potential to biodegrade both types of MPs (results not shown).

The setup of the subsequent biodegradation experiment was similar to that of the screening, including negative controls (10 particles of LDPE or BPET immersed in the marine broth, with no bacterial inoculum), the positive control (inoculum 18 added to the marine broth in concentration 10 % *v*/v) and the experimental treatments, consisting of 10 LDPE or BPET MPs enriched with the inoculum 18 (Fig. 2). The final concentration in the experimental bioreactors was 1000 MPs $L^{-1}$. This concentration is higher than the values usually reported in marine waters worldwide (e.g. Auta et al., 2017a, and references therein), but even higher concentrations of MPs have been reported in places such as the North Sea (up to 1770 MPs $L^{-1}$; Dubaish and Liebezeit, 2013). With the expected increase of plastic pollution in marine waters in the near future, the concentration of MPs used here can be considered as realistic. All assays were performed in triplicates. Samples were kept in 30 ml glass flasks, in the dark (25 ± 1 °C; 150 rpm), for 45 days. The time of the experiment was reduced as compared to that of the screening because in the latter, no further changes were observed in the FTIR after this time. Sampling was performed at four different times, after 7, 14, 31 and 45 days since the inoculation. An extra replicate was used at the last sampling day to perform Scanning Electron Microscopy (SEM) analysis, with a total of 65 samples.

Each sampling time, we determined the bacterial growth, the pH, and changes in the polymer functional groups by means of FTIR-ATR in each treatment. In addition, at the end of the experiment (after 45 days), we analysed the Chemical Oxygen Demand (COD) of the MP particles, the composition of the bacterial community in the particles and the surrounding broth, and MP particles from each treatment were subjected to SEM analysis.

### 2.4. Physico-chemical analysis

#### 2.4.1. Optical density and pH analysis

Each sampling time, bacterial growth and pH were analysed in each of the three replicates from each treatment. Bacterial growth was assessed by measuring the absorbance of each replicate at 600 nm, using a Hach-Lange™ DR 2800 spectrophotometer (Sköndal, Sweden). Marine broth with no inoculum, neither MPs, was used as zero value. The pH was measured using a pH-meter (CRISON, GLP21, Spain). This analysis was performed at each sampling time, except at the second sampling time (that is, after 14 days), due to technical issues with the instrument.

#### 2.4.2. FTIR-ATR spectroscopy analysis

The functional groups on the surface of the polymer were detected by means of Fourier transform infrared spectroscopy coupled with Attenuated Total Reflection (FTIR-ATR). For this, each sample was filtered onto 25 μm-pore size cellulose filters (Whatman), and 2–3 particles of each replicate were analysed. In order to remove possible bacterial aggregates, which could interfere in the FTIR-ATR analysis, the filters were previously cleaned with ethanol (70 % *v*/v) and left to dry in the stove (50 °C) for 15 min (Skariyachan et al., 2018). MP particles were analysed in a Nicolet iN10MX micro-FTIR (Thermo Fisher Scientific; USA), by means of ATR, using a germanium tip. A Mercury Cadmium Telluride (MCT) detector cooled with liquid nitrogen was used. Spectra were collected in the middle infrared region (from 4000 to 675 $cm^{-1}$), recording 16 scans at 16 $cm^{-1}$ spectral resolution.

#### 2.4.3. Chemical oxygen demand (COD)

The COD was analysed to determine the amount of oxygen that is required to chemically oxidize MPs into their metabolic products (Shah

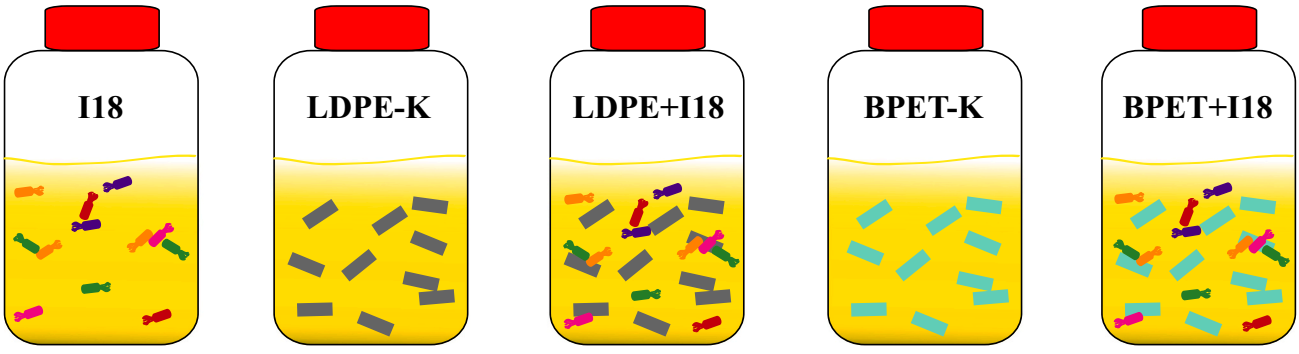


**Fig. 2.** Scheme of the experimental setup. 10 particles of either LDPE or BPET MPs were added to 10 ml of marine broth (1000 MPs $L^{-1}$). The experimental treatment was inoculated with 10 % of inoculum 18. Positive (I18) and negative (K) controls including either the inoculum or the MP films, respectively, were used.

et al., 2008). For this, we introduced 5 MP particles from each polymer into COD cuvette tests for photometric determination (HACH; Germany). These cuvettes contain a mixture of oxidizing reagents (mercury sulphate, silver sulphate and sulphuric acid), and the amount of oxygen equivalent to the mass of potassium dichromate that reacts with the oxidisable substances released by the MP particles is measured, following the ISO 15705:2002 protocol. The theoretical oxygen demand needed to degrade five particles of either LDPE or PET was previously calculated according to Van Haandel and Van Der Lubbe (2007). High absorbance values determined by the test indicate high oxygen demand needed to degrade the particles, and therefore that low biodegradation has occurred, and vice versa.

#### 2.4.4. Scanning electron microscopy (SEM)

At the end of the experiment, we had an extra replicate that was used for Scanning Electron Microscopy (SEM) analysis. This analysis was performed to examine the changes on the polymer surface on the samples containing microplastics (all but the positive controls). Previous to their observation in a high-resolution SEM (Hitachi S3700, Bruker, USA), MP particles from each treatment were washed with 2 % (v/v) aqueous sodium dodecyl sulphate (SDS) during 4 h, and then rinsed with distilled water and ethanol (70 % v/v), following the protocol reported by Skariyachan et al. (2018). Since plastics are non-conductive, MP films were coated with gold nanoparticles before mounted on the microscope, and then visualized under varying magnifications to observe the distinctive attributes of the particles.

### 2.5. Bacterial community composition

To identify the bacterial community developed in the samples after 45 days of incubation, DNA was extracted from the samples (3 replicates from each treatment, including the positive and negative controls) using the DNeasy® PowerSoil Pro Kit (QIAGEN, Germany) following the protocol recommended by the manufacturer. This kit has been reported to adequately extract bacterial DNA (Debeljak et al., 2017, and references therein). In the treatments containing MP particles (negative controls and samples inoculated with the marine bacterial consortium), DNA was extracted from both the marine broth (referred to "free" bacteria hereafter) and from the MP particles themselves (5 particles; referred to as "attached" bacteria or biofilm hereafter), to observe possible differences in the bacterial community found attached to the particles compared to free bacteria developed in the surrounding broth. The quality and concentration of eluted DNA was determined by measuring the absorbance at 260 and 280 nm wavelengths using a NanoDrop spectrophotometer (NanoDrop One C, Thermo Scientific, United States). Extracted DNA was then sent to the Integrated Microbiome Resource laboratory (Halifax, Nova Scotia, Canada) for PCR amplification and sequencing of the full 16S gene (PacBio Sequel). The primers 27F (AGRGTTYGATYMTGGCTCAG) and 1492R (RGYTACCTTGTTACGACTT) (Paliy et al., 2009) were used for PCR amplification, following the conditions detailed in Comeau et al. (2017). Raw sequences were treated for quality control through the Microbiome Helper pipeline (https://github.com/LangilleLab/microbiome_helper/wiki; Comeau et al., 2017), based on QIIME2. Briefly, after demultiplexing and a first quality control, sequences were $>$ 400 bp long and showed a quality score $\geq$ 30. Cleaned reads were clustered into operational taxonomic units (OTUs) at a sequence identity level of 97 %. To remove spurious OTUs that are a result of unfiltered chimeras or "bleed-through" between sequencing runs, a dynamic cutoff was employed to filter out OTUs having $<$0.1 % of the total number of sequences. Sequence data can be found at the GenBank database under accession numbers 25684492 to 25684505. The OTU table was then normalized per sample by subsampling (or rarefying) to a minimal number of reads for further analysis.

### 2.6. Statistical analysis

Variables under study were subjected to the Shapiro-Wilk test to evaluate whether data came from a normal distribution. Since this was not the case for most of the data ($p < 0.05$), the effect of the addition of the selected bacterial consortium on microplastic degradation was evaluated by means of non-parametric tests. The Wilcoxon signed-rank test was performed to look for differences between the samples containing the bacterial inoculum and the negative controls ($N = 24$ for either LDPE and BPET). Similarly, we looked for statistical differences through the experiment (i.e., between the different sampling times). The test was performed for optical density measurements, pH and the absorbance peaks of LDPE and BPET detected through the experiment by FTIR-ATR. In addition, a principal component analysis (PCA) was performed for LDPE and BPET samples to visualize how they distribute across the bi-dimensional space, and to look for possible correlations between the samples and the variables under study. For this, data were previously log-transformed to accomplish with the normality criteria. Wilcoxon test were performed with JMP (SAS) version 16. R studio (version 1.0.143) was used to perform Shapiro-Wilk test and compute the PCAs, using packages 'dplyr' (Wickham et al., 2022; https://cran.r-project.org/web/packages/dplyr/index.html) and 'ggfortify' (Horikoshi et al., 2022; https://cran.r-project.org/web/packages/ggfortify/ggfortify.pdf), respectively. Significance was considered for an alpha cut-off value of 0.05. For bacterial community composition, we evaluated the differences in read counts among the different treatments (positive controls and LDPE and BPET samples containing the I18, since the negative controls showed no reads). In LDPE and BPET samples inoculated with marine bacteria, we assessed the differences in read counts for bacteria found attached to the MP particles, as compared to those in the surrounding broth ("free" bacteria). For this, we used the DESeq2 package (Love et al., 2014) in R studio. Analyses were done at Genus taxonomic level. *P*-adjusted values according to the false discovery rate (Benjamini and Hochberg, 1995) were calculated from a negative binomial distribution and significant differences were considered when *p*-adjusted was below an alpha cut-off value of 0.1.

## 3. Results

### 3.1. Physico-chemical analysis

#### 3.1.1. Optical density and pH

Results from optical density at 600 nm, as indicative of bacterial growth, showed significant higher values in the MP samples containing bacteria inoculum compared to the negative controls ($p < 0.001$ and $p < 0.01$, for LDPE and BPET, respectively; Tables 1 and 2) (Fig. 3) – as expected. The exception was for the BPET control after 7 days of incubation, where suspected contamination may have influenced the results (Fig. 3). In the case of LDPE, pH values in the samples with the bacterial consortium were significantly higher compared to the controls ($p < 0.01$; Table 1), with values of $8.5 \pm 0.19$ and $7.6 \pm 0.2$, respectively (Fig. 4). In the case of BPET, pH values did not differ significantly among treatments according to the Wilcoxon-test (Table 2). For both polymers, pH values remained similar throughout the incubation time (Fig. 4, Tables 1 and 2). Overall, these analyses showed higher bacterial growth and pH in the samples incubated with I18 for both polymers compared to the controls, which is clearly visualized in the PCA bi-plot in the case of LDPE (Fig. 5A). The PCA shows a clear distribution of the samples, with those containing the marine inoculum located at the right side of the first component (PC1), and the negative controls distributed across its left side. While the pattern is less clear in the case of BPET, samples inoculated with I18 tend to show negative values of the second component (PC2), closer to higher values of OD and pH (Fig. 5B).

#### 3.1.2. FTIR-ATR spectroscopy

FTIR-ATR analysis showed changes in the functional groups of both

**Table 1**
Results of non-parametric Wilcoxon-test for the different variables measured in the LDPE samples (K and samples containing the bacterial inoculum #18; $N = 24$) at the 95 % level of statistical significance. T1–T4 correspond to the different sampling times, that is, after 7, 14, 31 and 45 days, respectively.

| Measured variable | Assay (K vs. I18) | Incubation time (T1–T4) |
|---|---|---|
| Optical density | (I18 > K)*** | n.s. |
| pH | (I18 > K)** | n.s. |
| 3728–3733 $cm^{-1}$ | n.s. | (T2 < T1)** |
| | | (T3 < T1)** |
| 2915–2917 $cm^{-1}$ | (I18 < K)** | n.s. |
| 2849–2851 $cm^{-1}$ | (I18 < K)** | n.s. |
| 1648–1650 $cm^{-1}$ | (I18 > K)* | n.s. |
| 1459–1470 $cm^{-1}$ | n.s. | (T2 < T1)* |
| | | (T3 < T1)* |
| 1078–1081 cm-1 | n.s. | n.s. |
| 870–871 $cm^{-1}$ | (I18 > K)** | n.s. |
| 730 $cm^{-1}$ | n.s. | n.s. |
| 717–718 $cm^{-1}$ | n.s. | (T2 < T1)** |
| | | (T3 < T1)** |
| | | (T4 > T3)* |

n.s.: non-significant ($p > 0.05$).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

**Table 2**
Results of non-parametric Wilcoxon-test for the different variables measured in the BPET samples (K and samples containing the bacterial inoculum #18; N = 24) at the 95 % level of statistical significance. T1–T4 correspond to the different sampling times, that is, after 7, 14, 31 and 45 days, respectively.

| Measured variable | Assay (K vs. I18) | Incubation time (T1–T4) |
|---|---|---|
| Optical density | (I18 > K)** | (T2 < T1)* |
| | | (T3 < T1)* |
| | | (T4 < T1)* |
| pH | n.s. | n.s. |
| 3728–3733 $cm^{-1}$ | n.s. | (T3 < T1)** |
| | | (T4 > T3)** |
| 2921–2924 $cm^{-1}$ | n.s. | (T4 > T1)** |
| | | (T4 > T2)** |
| 1715 $cm^{-1}$ | n.s. | (T2 < T1)* |
| 1648–1655 $cm^{-1}$ | n.s. | (T2 < T1)* |
| | | (T3 < T1)* |
| 1458 $cm^{-1}$ | n.s. | (T2 < T1)** |
| | | (T3 > T2)* |
| | | (T4 > T2)** |
| 1410 $cm^{-1}$ | (I18 > K)** | n.s. |
| 1270 $cm^{-1}$ | n.s. | n.s. |
| 1120 $cm^{-1}$ | n.s. | (T2 < T1)* |
| 1100–1104 $cm^{-1}$ | n.s. | n.s. |
| 1019–1020 $cm^{-1}$ | (I18 < K)* | (T2 < T1)* |
| | | (T3 < T1)* |
| 938 $cm^{-1}$ | n.s. | (T2 < T1)* |
| 871–874 $cm^{-1}$ | (I18 > K)* | n.s. |
| 728–730 $cm^{-1}$ | (I18 > K)* | n.s. |

n.s.: non-significant ($p > 0.05$).
* $p < 0.05$.
** $p < 0.01$.

polymers through the incubation time. Figs. 6 and 7 show examples of spectra of LDPE and BPET MP films measured at the end of the experiment (after 45 days of incubation) in the negative controls (LDPE/BPET-K) and inoculated samples (LDPE/BPET+I18), compared to the spectrum of each polymer before the incubation time (0 days). These examples are used to illustrate the main changes that occurred in the polymers by the end of the experiment. Results of the most characteristic peaks of the spectra measured at all sampling times were included in the statistical analysis, which revealed significant differences at certain peak positions among treatments and sampling times (Tables 1 and 2).

In the case of LDPE, on the one hand, new absorption peaks were observed in the inoculated samples with respect to the spectra at the beginning of the experiment (0 days) and the negative controls (Fig. 6). New peaks were detected at positions ~3729, ~3629, ~3296 $cm^{-1}$ of the spectrum after 45 days of incubation with the marine bacteria, attributed to hydroxyl or amino groups (Brandon et al., 2016; Coates, 2006; Syranidou et al., 2019) (Table 3). A new absorption peak at ~1650 $cm^{-1}$, indicative of the C=C bond of the vinyl group (Fotopoulou and Karapanagioti, 2015; Syranidou et al., 2019), was also observed. Other authors attributed this peak to the formation of a carbonyl group (Skariyachan et al., 2018). The absorbance of this peak through the incubation time was significantly higher in the inoculated samples than in the negative controls (Table 1). We also observed the appearance of peaks at positions 1081 and 871 $cm^{-1}$ (Fig. 6), which correspond to carbon-oxygen bonds (Brandon et al., 2016) and trans bending of oxymethylene groups (Mecozzi and Nisini, 2019), respectively (Table 3). The peak at 871 $cm^{-1}$ was significantly higher in the inoculated samples than in the negative controls (Table 1). In addition, Fig. 6 shows a broad band between ~1300 and ~1500 $cm^{-1}$, which has been attributed to end methyl groups of polyethylene (Syranidou et al., 2019). The PCA showed a positive and tight correlation between the peaks at positions 871 $cm^{-1}$, 1081 $cm^{-1}$ and 1650 $cm^{-1}$, located at the right side of the PC1, where most of the LDPE+18 samples where distributed. Instead, negative controls were mostly distributed across the left side of the plot, where peaks at positions ~2916 and ~2851 $cm^{-1}$, the principal absorption peaks characterizing LDPE, were located. Indeed, these two peaks showed significantly lower values in the samples exposed to I18 with respect to the controls (Table 1). Following a similar reasoning than Brandon et al. (2016), we calculated the ratio between the peak at 1650 $cm^{-1}$ and the peaks at ~2916 and ~ 2851 $cm^{-1}$, what could be called the "vinyl:methylene index" (as similarly calculated by Fotopoulou & Karapanagioti for PET). We observed an increase of these indices with time in the samples inoculated with the bacterial consortium, while in the controls it was 0 during the whole incubation time (no peak at 1650 $cm^{-1}$ was observed in these samples) (Table 4).

For BPET, same as for LDPE, the spectrum of the negative control samples remained similar to that of particles not incubated (T0), but for a peak at the region ~3310 $cm^{-1}$ that decreased and moved to a higher position of the spectrum (3327 $cm^{-1}$; Fig. 7). In the case of samples inoculated with marine bacteria, changes were observed in the bands at the footprint region (that is, from the absorbance bands from ~1715 to ~729 $cm^{-1}$) after 45 days incubation time (Fig. 7). Compared to the characteristic absorption peaks of PET, the spectrum illustrated the appearance of several new peaks. Likewise in LDPE, the peak at 1648–1650 $cm^{-1}$ was observed in the inoculated samples, although in this case non-significant differences were observed with the negative controls (Table 2). Same as with LDPE, we calculated the vinyl:methylene index, using the peak at 2921 $cm^{-1}$ as indicative of $CH_2$ asymmetric stretch in this case (Table 5), but we did not find a clear pattern with time (results not shown). Indeed, considering all the samples, the peak was significantly higher after 7 days of incubation than after 15 or 30 days (Table 2). We also calculated the vinyl bond index, after Fotopoulou and Karapanagioti (2015), using the peak at 1458 $cm^{-1}$ in the denominator, as also indicative of methylene bands (Table 5), but no clear pattern was observed either. A new absorption peak at 1410 $cm^{-1}$, related to in-plane vibrations of the benzene ring (Cole et al., 2002; Denaro et al., 2020), was observed, which was significantly higher in the inoculated samples, same as the peak at 871 $cm^{-1}$ (trans bending oxymethylene group) (Tables 2 and 5). These two peaks showed a high positive correlation among them (Fig. 5B). New peaks were also detected at positions ~3729 $cm^{-1}$, and ~3629 $cm^{-1}$, similar to those observed in LDPE spectra, but these peaks were also observed in the controls (Fig. 7) and thus can not be attributed to biodegradation by the marine bacteria. To a lesser extent, the peaks at 871 and 1410 $cm^{-1}$ were also correlated with the peaks at positions 1458 $cm^{-1}$, 1648 $cm^{-1}$ and 3729 $cm^{-1}$, all of them situated at the negative side of PC2, showing in general more correlation with the samples inoculated with I18 (Fig. 5B). On the contrary, the peak at position 1020 $cm^{-1}$, assigned to crystalline

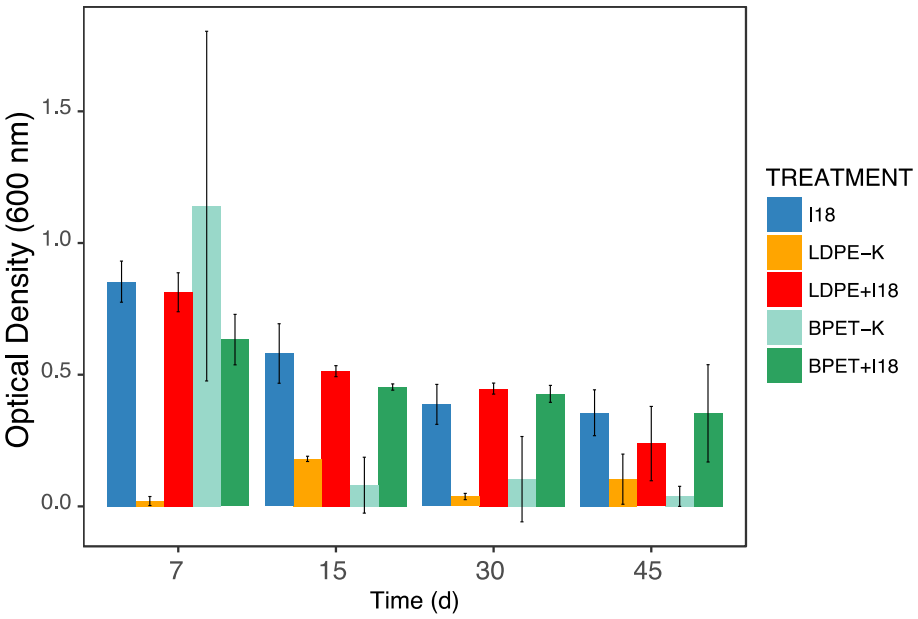


**Fig. 3.** Results of the measurement of the optical density (at 600 nm) – as indicative of bacterial growth – throughout the incubation time in the different treatments. I18 and K stand for the positive (bacterial inoculum) and negative (microplastic particles, without bacteria) controls, respectively. Error bars represent the standard error from three replicates. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

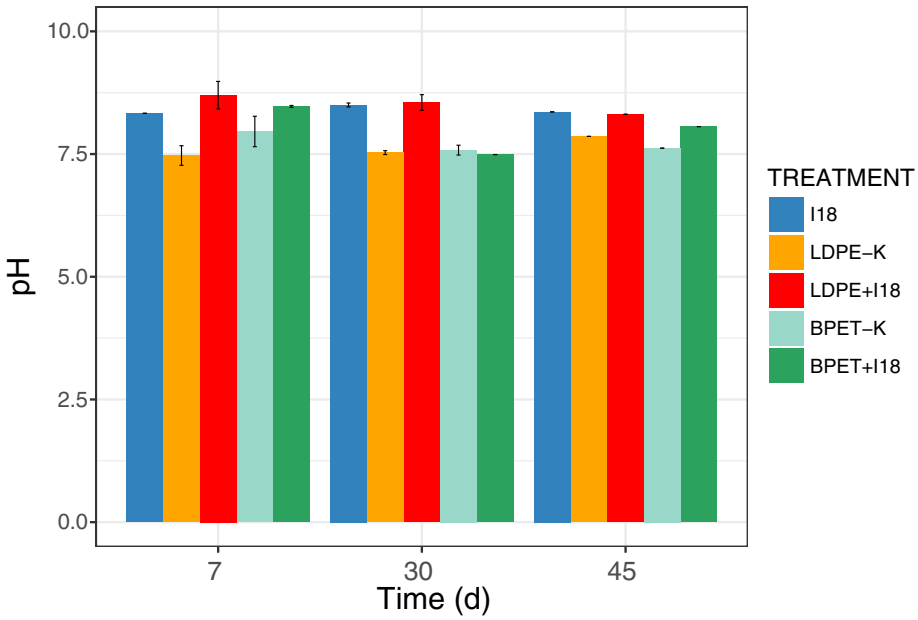


**Fig. 4.** pH measured in each treatment throughout the incubation time. I18 and K stand for the positive (bacterial inoculum) and negative (microplastic particles, without bacteria) controls, respectively. Error bars represent the standard error from three replicates. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

regions (Table 5), was significantly lower in the inoculated samples (Table 2). This peak, and those at positions 728, 938, 1100, 1120, 1270, 1715 and 2924 $cm^{-1}$, all of them characteristic of PET, are located at the right side of the PC1 (Fig. 5B). No clear correlation between these peaks and any of the samples is observed.

### 3.1.3. Chemical oxygen demand analysis

COD results showed that more oxygen was required to degrade the MP particles of each polymer in the negative controls than in the experimental samples inoculated with bacteria (Fig. 8). Interestingly, LDPE microplastics containing the inoculum showed lower COD values than their BPET counterparts, even though the theoretical COD calculated previously was higher for LDPE (250 and 500 mg COD $L^{-1}$ for 5 particles of PET and LDPE, respectively) (see Section 2.4.3). These results suggest that LDPE MPs may require less oxygen to be degraded than biobased PET MPs.

### 3.1.4. Scanning electron microscopy

SEM micrographs illustrated morphological changes in the surface features of LDPE and BPET MP particles exposed to bacterial inoculum 18 (LDPE+I18 and BPET+I18) for 45 days, in comparison to the negative controls (LDPE-K and BPET-K). These features include the presence of fractures and holes in the surface of test samples (Fig. 9B and D), whereas the MP particles from the controls showed smoother surfaces

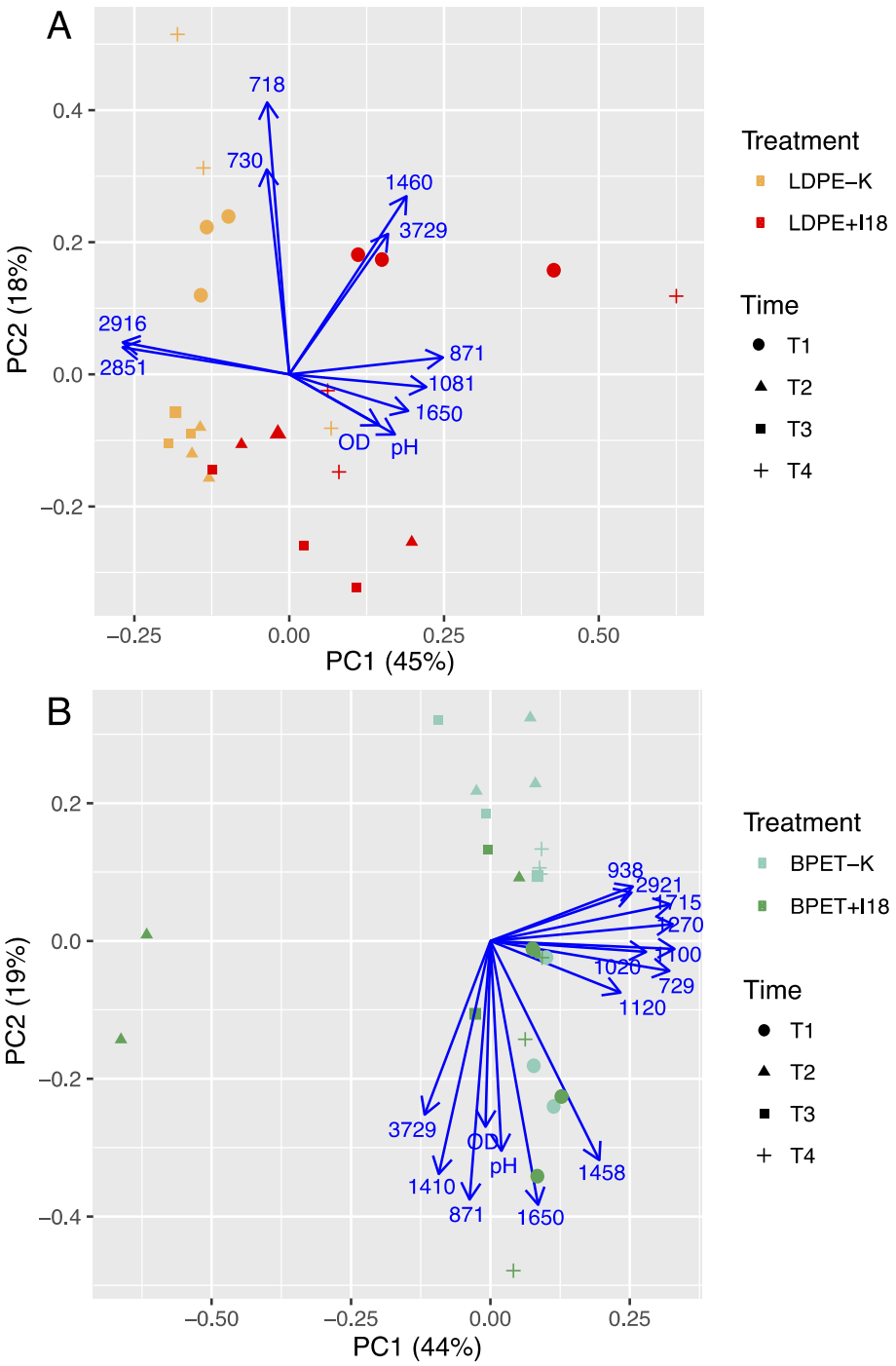


**Fig. 5.** PCA biplot showing the distribution of LDPE (N = 24) (A) and BPET (*N* = 24) (B) samples according to the treatment and incubation time. Arrows show the different FTIR peaks, optical density (OD) and pH values measured through the incubation time. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

(Fig. 9A and C). Additionally, unlike in the controls, the inoculated samples showed scattered bacteria on their surface (Fig. 9B and D) and the formation of bacterial biofilms (Fig. 9E), even after having washed the particles with SDS, distilled water and ethanol (see Section 2.4.4).

### 3.2. Bacterial communities developed in the samples

The concentration of DNA extracted from the bacterial community adhered to the MP particles ("attached" bacteria) after 45 days of incubation ranged from 1,69 to 5,85 ng/μl. In the marine broth ("free" bacteria), slightly higher DNA concentrations were obtained, reaching values up to 15 ng/μl in some samples. PCR amplification was weak or failed in the negative controls, and thus we are certain that no bacterial contamination occurred in the samples. The PCR for the third replicate of the MP particles for LDPE samples inoculated with I18 was also weak and thus not considered in further analyses. The remaining samples (*N* = 14) showed a minimum of 1095 reads across 138 OTUs that belonged to 13 bacteria genus (17 genus before subsampling), as showed in Fig. 10. Some significant differences in the abundance of these groups, as assessed by DESeq2 analysis, were found between treatments. Considering the free bacteria community developed in the two treatments (LDPE and BPET samples from the marine broth) and the positive

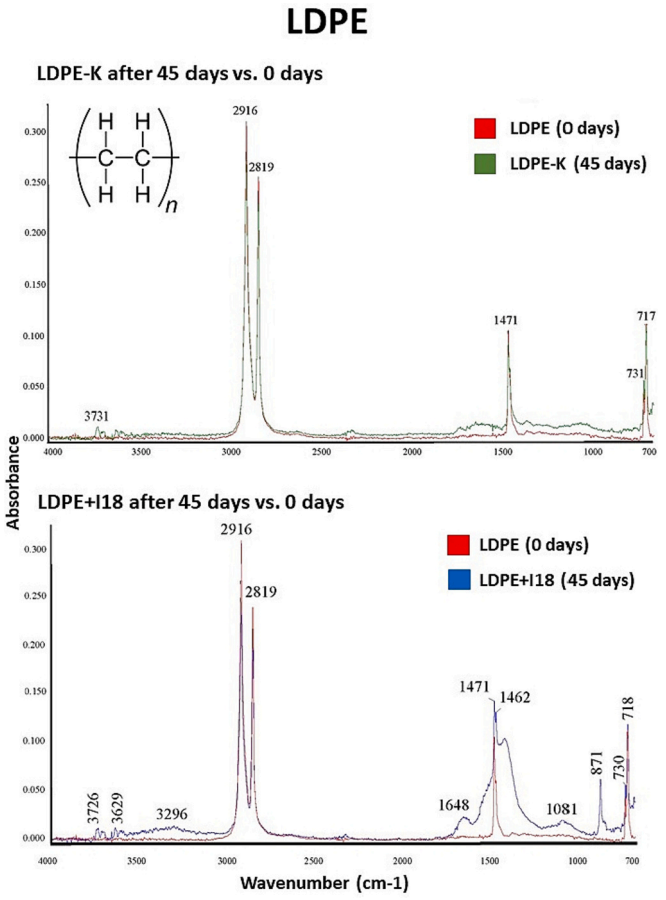


**Fig. 6.** Spectral profile of LDPE, as determined by FTIR-ATR. The plot shows the results of the spectrum of the negative control (LDPE-K, on the top, in green) and the sample with the bacterial inoculate (LDPE+I18, on the bottom, in blue) at the end of the experiment (after 45 days of incubation), superimposed to the spectrum of a LDPE MP measured at the beginning of the experiment (0 days; in red). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

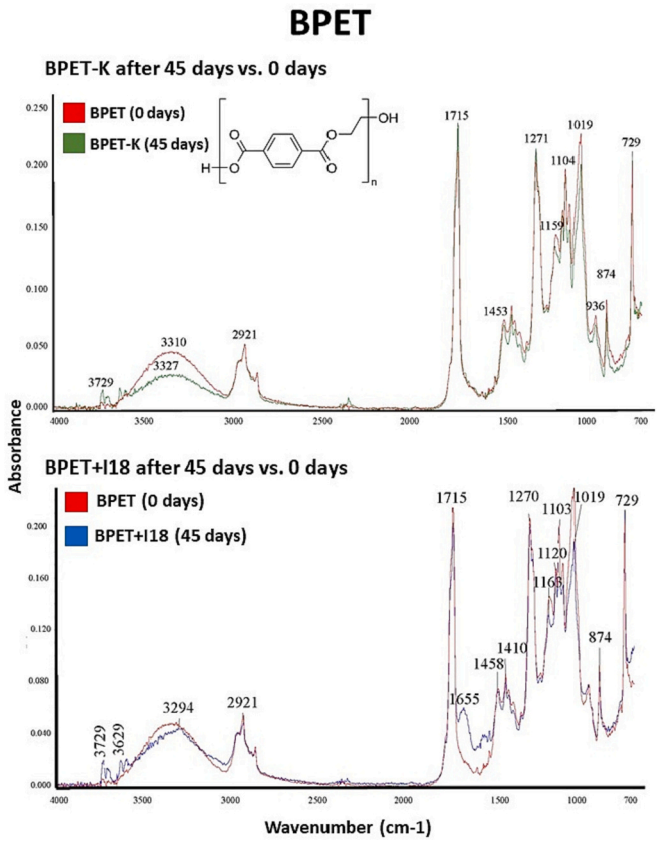


**Fig. 7.** Spectral profile of BPET, as determined by FTIR-ATR. The plot shows the results of the spectrum of the control (BPET-K, on the top, in green) and the sample with the bacterial inoculate (BPET+I18, on the bottom, in blue) after 45 days of incubation, superimposed to the spectrum of a BPET MP measured at the beginning of the experiment (0 days; in red). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

control (I18), the genus *Vibrio* showed a higher abundance in the LDPE samples, while the genera *Marinobacter, Ruegeria* and *Shewanella* were more abundant in the control and the BPET samples (Fig. S3). Comparing attached and free bacteria for each type of microplastic, we found that *Cobetia*, *Pseudoalteromonas* and *Ruegeria* were more abundant in the LDPE particles than in the surrounding broth, while *Arcobacter* and *Vibrio* were more common in the broth (Figs. 10 and S3). In the case of BPET samples, *Arcobater* was also more abundant as free bacteria in the broth, and so was *Marinobacter*. Instead, the genera *Halodesulfovibrio*, *Pseudoalteromonas*, *Pseudomonas* and *Tepidibacter* were more abundant attached to the BPET microplastics (Figs. 10 and S3). The Shannon index showed that the diversity of attached bacteria was higher than that of free bacteria (Fig. S4), but results were statistically non-significant.

## 4. Discussion

### *4.1. Potential of marine bacteria to biodegrade conventional and biobased plastics*

The purpose of this study was to evaluate the potential of a marine bacterial community to biodegrade conventional, petroleum-based (i.e., LDPE), and biobased (i.e., PET) microplastics under simulated natural conditions. To our knowledge, only the studies of Denaro et al. (2020) and Syranidou et al. (2019) have focused on the potential of marine

**Table 3**
Principal spectral absorption peak positions ($cm^{-1}$) of the functional groups characterizing LDPE, according to the current literature, including the new peaks observed after the incubation with the marine bacterial consortium.

| Peak band ($cm^{-1}$) | Functional group | Reference |
|---|---|---|
| 3728–3733 | Hydroxyl or amino groups | Brandon et al. (2016); Coates (2006); Syranidou et al. (2019) |
| 2915–2917 | $CH_2$ asymmetric C—H stretching | Coates (2006); Jung et al. (2018); Rajandas et al. (2012) |
| 2849–2851 | $CH_2$ symmetric C—H stretching | Coates (2006); Jung et al. (2018); Rajandas et al. (2012) |
| 1648–1650 | C=C bond of the vinyl group/ C=O bond of the carbonyl group | Fotopoulou and Karapanagioti, 2015; Syranidou et al., 2019/ Brandon et al., 2016; Denaro et al., 2020; Skariyachan et al., 2018 |
| 1459–1470 | Bending formation; $CH_2$ deformation split when PE is crystalline | Coates (2006); Jung et al. (2018); Rajandas et al. (2012) |
| 1078–1081 | Carbon-oxygen bonds | Brandon et al. (2016) |
| 870–871 | Trans bending oxy-methylene group | Mecozzi and Nisini (2019) |
| 730 | $CH_2$ rocking deformation | Coates (2006); Jung et al. (2018) |
| 717–718 | Rocking deformation; $CH_2$ deformation split when PE is crystalline | Coates (2006); Jung et al. (2018); Rajandas et al. (2012) |

**Table 4**
Vinyl:methylene indices (mean ± standard deviation) in LDPE samples through the incubation time. T1–T4 correspond to the different sampling times, that is, after 7, 14, 31 and 45 days, respectively. T0 correspond to LDPE particles not incubated.

| Sample | 1650/2916 | 1650/2851 |
|---|---|---|
| LDPE-T0 | 0 | 0 |
| LDPE-K-T1 | 0 | 0 |
| LDPE+I18-T1 | 0.017 ± 0.018 | 0.020 ± 0.022 |
| LDPE-K-T2 | 0 | 0 |
| LDPE+I18-T2 | 0 | 0 |
| LDPE-K-T3 | 0 | 0 |
| LDPE+I18-T3 | 0.031 ± 0.031 | 0.036 ± 0.047 |
| LDPE-K-T4 | 0 | 0 |
| LDPE+I18-T4 | 0.034 ± 0.051 | 0.041 ± 0.064 |

**Table 5**
Principal spectral absorption peak positions ($cm^{-1}$) of the functional groups characterizing PET, according to the current literature, including the new peaks observed after the incubation with the marine bacterial consortium.

| Peak range | Functional group | Reference |
|---|---|---|
| 3728–3733 | Hydroxyl or amino groups | Chen et al. (2012); Coates (2006) |
| 2921 | $CH_2$ asymmetric stretch | Chen et al. (2012); Coates (2006); Jung et al. (2018) |
| 1715 | C═O stretch (ketones) | Chen et al. (2012); Coates (2006); Jung et al. (2018); Fotopoulou and Karapanagioti (2015); Mecozzi and Nisini (2019) |
| 1648–1655 | C═C bond of the vinyl group/C═O bond of the carbonyl group | Fotopoulou and Karapanagioti, 2015; Syranidou et al., 2019/Brandon et al., 2016; Denaro et al., 2020; Skariyachan et al., 2018 |
| 1458 | Bending $CH_2$ | Skariyachan et al., 2018 Coates (2006); Jung et al. (2018); Mecozzi and Nisini (2019) |
| 1410 | In-plane vibrations of the benzene ring | Cole et al. (2002); Denaro et al. (2020) |
| 1270 | Stretching of the ester (O═C—O—) bond | Cole et al. (2002); Denaro et al. (2020); Mecozzi and Nisini (2019) |
| 1100–1103 | C—O—C stretch | Chen et al. (2012); Coates (2006); Cole et al. (2002); Jung et al. (2018); Mecozzi and Nisini (2019) |
| 1019–1020 | Aromatic ring in-plane C—H bend | Chen et al. (2012); Coates (2006); Cole et al. (2002) |
| 938 | Gauche bending oxy-methylene group | Mecozzi and Nisini (2019) |
| 871–874 | Trans bending oxy-methylene group | Mecozzi and Nisini (2019) |
| 728–730 | Out-of-plane C—H deformation of the aromatic ring | Denaro et al. (2020); Ioakeimidis et al. (2016) |

bacterial consortia to degrade plastics, but they used plastics of a bigger size (≥1 $cm^2$; i.e., mesoplastics), compared to the size of our particles (~2 $mm^2$; i.e., microplastics). With the increase in plastic surge after the COVID-19 pandemic (Bondaroff and Cooke, 2020; Klemes et al., 2020; Pinto Da Costa et al., 2020), it is becoming now more urgent than ever to look for environmentally-friendly solutions to the global problem of plastic pollution. Especially so for the case of microplastics, which are the final product of plastics fragmentation, and they cannot be removed easily from the environment. It is also important to assess the effect of microplastics not only in macrofauna but also on microbial communities inhabiting marine waters, since any change in these communities may yield a change in the biochemistry of the receiving waters.

More subtle techniques other than e.g. measurement of the weight loss are needed to determine changes in the polymers in the case of microplastics. In this study, we used a range of analytical techniques to assess the biodeterioration (FTIR, SEM) and biofragmentation (FTIR) of LDPE and PET MP films. We also estimated indirectly the remineralization of these polymers by means of the COD. In addition, we analysed bacterial growth in the different samples by means of optical density, and further characterized the specificity of the different bacterial groups by any of the polymers, and as compared to the surrounding marine water, by next-generation sequencing. As expected, bacteria grew in the samples inoculated with the selected consortium significantly more than in the negative controls. We observed slightly higher (but significant in the case of LDPE) pH values in the inoculated samples, as compared with the controls, which are likely indicative of bacteria requiring certain alkalinity in the medium to biodegrade plastic polymers (Auta et al., 2017b; Dilkes-Hoffman et al., 2019). FTIR-ATR and COD analyses further proved that there were chemical changes taking place in the treatment samples.

FTIR-ATR spectroscopy is becoming a promising, quick and precise tool to assess and quantify polymer degradation (Rajandas et al., 2012). It allows for the straight identification of absorption bands at given positions (wavenumbers) of the spectrum. In fact, the formation of new peaks and/or the increase/decrease of different peaks in the FTIR-ATR spectrum has previously been reported as signs of biodegradation of the polymers under study (e.g., Jung et al., 2018; Mecozzi and Nisini, 2019; Rajandas et al., 2012). Tables 3 and 5 show the most common peaks of the FTIR spectrum reported for LDPE and PET by the literature currently available. The most remarkable change observed by FTIR-ATR in both polymers was the appearance of a peak at ~1650 $cm^{-1}$, which has been either attributed to a vinyl bond or a carbonyl group. This could be interpreted as the oxidation of the polymer and substitution of a C═C bond by a C═O bond. According to Skariyachan et al. (2018), the carbonyl absorption band found in this peak in LDPE is due to the formation of ketone or aldehyde groups by the activity of microorganisms on the surface of plastics. The production of carbonyl groups is indicative of an enhancement of bacterial adherence and/or biodegradation of the polymer (Rajandas et al., 2012; Skariyachan et al., 2018). According to Esmaeili et al. (2013), microorganisms consume carbonyl groups facilitating the formation of double bonds and the consequent breakdown of the polymer chain. In the marine environment (simulated in this experiment using an artificial marine broth), PET is susceptible to hydrolytic cleavage (Gewert et al., 2015), which eventually could trigger the biodegradation of smaller compounds. In this case, the observed peak at ~1650 $cm^{-1}$ could be the result of the hydrolysis of the ester group, resulting in the formation of carboxyl or carboxylate terminal groups (Denaro et al., 2020). In the case of LDPE, there was also a significant decrease in the characteristic methylene groups at ~2916 and ~ 2851 $cm^{-1}$. According to Rajandas et al. (2012), this is a consequence of the polymer being oxidized, breaking the double bond of the methylene group (═$CH_2$). As a consequence of these changes, in LDPE samples inoculated with marine bacteria, we observed an increase in what we have called the "vinyl:methylene index" over time, which has been reported as indicative of biotic degradation (Fotopoulou and Karapanagioti, 2015; these authors calculated the vinyl index for PET, using other peak bands). In both polymers subjected to the marine bacteria, we also found a significant increase in the peak band at ~871 $cm^{-1}$, which has been attributed to trans bending oxy-methylene group in the case of PET (Mecozzi and Nisini, 2019), and could also be applied to LDPE. This is indicative of polymer oxidation. In the case of PET, a significant decrease in the peak at positions ~1020 $cm^{-1}$ was observed in the inoculated samples, which is related to crystalline regions of the polymer (Mecozzi and Nisini, 2019). A decrease in crystalline or increase in amorphous regions can trigger biodegradability, since enzymes mainly attack the amorphous domains of a polymer (Andrady, 2017; Tokiwa et al., 2009). Ioakeimidis et al. (2016) studied the degradation of PET bottles recovered from the marine environment and found that samples < than 15 years still preserved the peak at ~1020 $cm^{-1}$, while this was not present in the older samples. Umamaheswari et al., 2013 assesed the biodegradation of PET in soil and observed the disappearance of the band at ~1020 $cm^{-1}$ after the exposition of the polymer to *Penicillium* sp. for 4 weeks. In any case, there is a lack of information regarding the changes in the functional groups of PET due to microbial

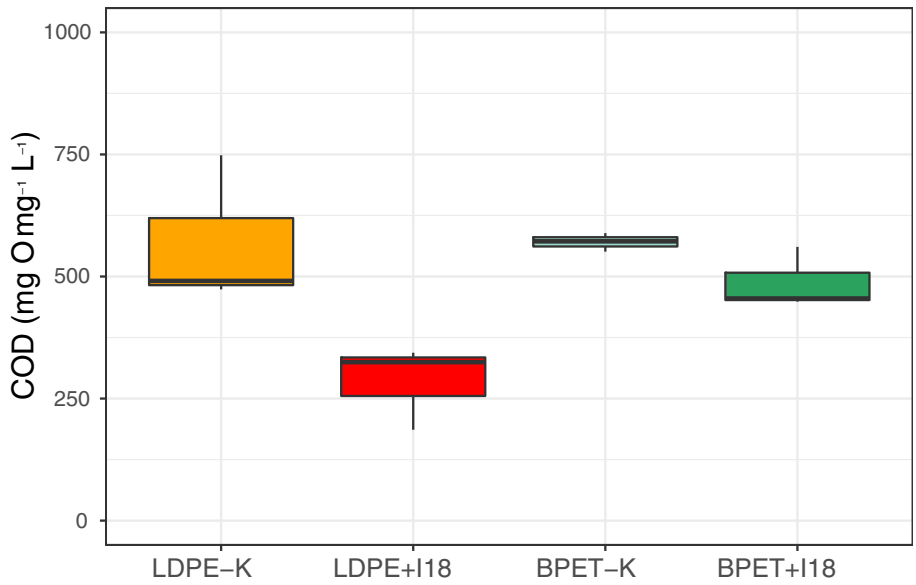


**Fig. 8.** COD measured at the end of the experiment (after 45 days of incubation) for LDPE and BPET particles. I18 and K stand for the positive (bacterial inoculum) and negative (microplastic particles, without bacteria) controls, respectively. The boxes indicate median and quartile values. The whiskers extend to 1.5 times the interquartile range. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

biodegradation, especially for "biobased" PET, which is the polymer of interest in this study. Our results can in this sense pave the way for further interpretation of changes in the functional groups of PET due to biodegradation.

While results of FTIR-ATR analysis are indicative of LDPE and BPET biodeterioration or biofragmentation, oxygen consumption or carbon dioxide release are the ultimate proof of biodegradation under aerobic conditions (Lucas et al., 2008). As the artificial marine broth used in the experiments contained yeast (1 g $L^{-1}$) as a carbon source, measuring the $O_2$ consumption or $CO_2$ release may have not yielded observable changes among treatments, since most $O_2$ consumed or $CO_2$ released could be attributed to the biodegradation of yeast. Therefore, we decided to measure the COD from the MP particles themselves, following the normative ISO 15705:2002. This technique was easy to conduct and provided an insight on whether the selected bacterial consortium was actually consuming oxygen to biodegrade the microplastics. The COD of the MP particles after 45 days of incubation indicated that certain biodegradation had occurred, as the COD values in the controls were higher than in the samples exposed to I18 for both polymers. More interestingly, LDPE MPs, especially particles inoculated with marine bacteria, showed lower COD values than their BPET counterparts. An explanation to these results could be that the BPET particles may have not been completely dissolved once the COD test had finished, in comparison to the LDPE particles. Otherwise, biobased PET MPs were actually less biodegradable than conventional LDPE ones, contrary to what expected. The methodology used to determine changes in the COD for MP particles has been proven effective and could substitute other, more cost-effective, techniques such as the carbon dioxide evolution test (ISO 9439:1999).

FTIR and COD results were further corroborated with the images obtained through SEM in which micrographs of LDPE and BPET films exposed to I18 showed changes in the polymer's surface, such as the presence of fractures and holes. Furthermore, we observed the presence of bacteria biofilms on both types of MPs, the first step of biodegradation. Non-polar polymers show usually limitations in biodegradation because of their hydrophobic surface properties, and the formation of biofilms overcomes this barrier (Pathak and Navneet, 2017). Similar features on the surface of LDPE have been reported by other authors, after their exposition to marine bacterial isolates for longer periods of time of 90 (Li et al., 2020) or 140 days (Skariyachan et al., 2018). Denaro et al. (2020) also observed by SEM analysis uneven surfaces and the presence of small cracks and furrows in PET films exposed to a community of hydrocarbon-degrading bacteria for 45 days, while Umamaheswari et al., 2013 observed fungal colonization on the surface of PET particles after being exposed to *Penicillium* sp. for 1 month. Results from 16S sequencing further proved that the negative controls contained no bacteria. In the samples enriched with the selected inoculum, we found that bacterial groups showed different affinity for either LDPE or BPET particles. This was not unexpected, since polymer characteristics act as substrate that can discriminate the associated planktonic community (Syranidou et al., 2019, and references therein).

Contrary to what expected from a pollutant, we found higher bacterial diversity on LDPE and BPET MP particles than in the surrounding marine broth. Bacteria from the genus *Cobetia*, *Pseudoalteromonas* and *Ruegeria* showed a higher affinity to LDPE particles, compared to the surrounding broth, while the groups *Arcobacter* and *Vibrio* were more abundant in the broth. *Pseudoalteromonas*, *Ruegeria* and *Vibrio* have been reported as common taxa found in polyethylene particles from the open ocean, what has been called "the plastisphere" (Amaral-Zettler et al., 2020). More interestingly, the genus *Cobetia*, belonging to the order Oceanospirillales, has been described as bacteria with potential to degrade oil spills (Dombrowski et al., 2016). This means that these bacteria could also play a role in the biodegradation of petroleum-based plastics, such as LDPE. In the case of BPET, we also found different bacterial groups in the MP particles as compared to the surrounding marine broth. Bacteria from genus *Halodesulfovibrio*, *Pseudoalteromonas*, *Pseudomonas* and *Tepidibacter* were more abundant in BPET microplastics. *Pseudomonas* have also been reported as common taxa found in polyethylene and polypropylene particles from the open ocean, being part of the "plastisphere" (Amaral-Zettler et al., 2020), while, to our knowledge, there are no data on bacterial assemblages on PET particles from open waters. *Tepidibacter* is a moderately thermophilic bacterial genus that has been isolated from deep-sea hydrothermal vents (Slobodkin et al., 2003). The results from 16S sequencing did not allow for the identification of the species, but the only species described so far is anaerobic (*Tepidibacter thalassicus*). Therefore, the species reported here may be a new one with potential to biodegrade plastics. Denaro et al. (2020) have reported the presence of *Halodesulfovibrio* within a community of bacteria enriched with naphthalene-phenanthrene, growing on a compostable shopping bag. Some species of this genus have been

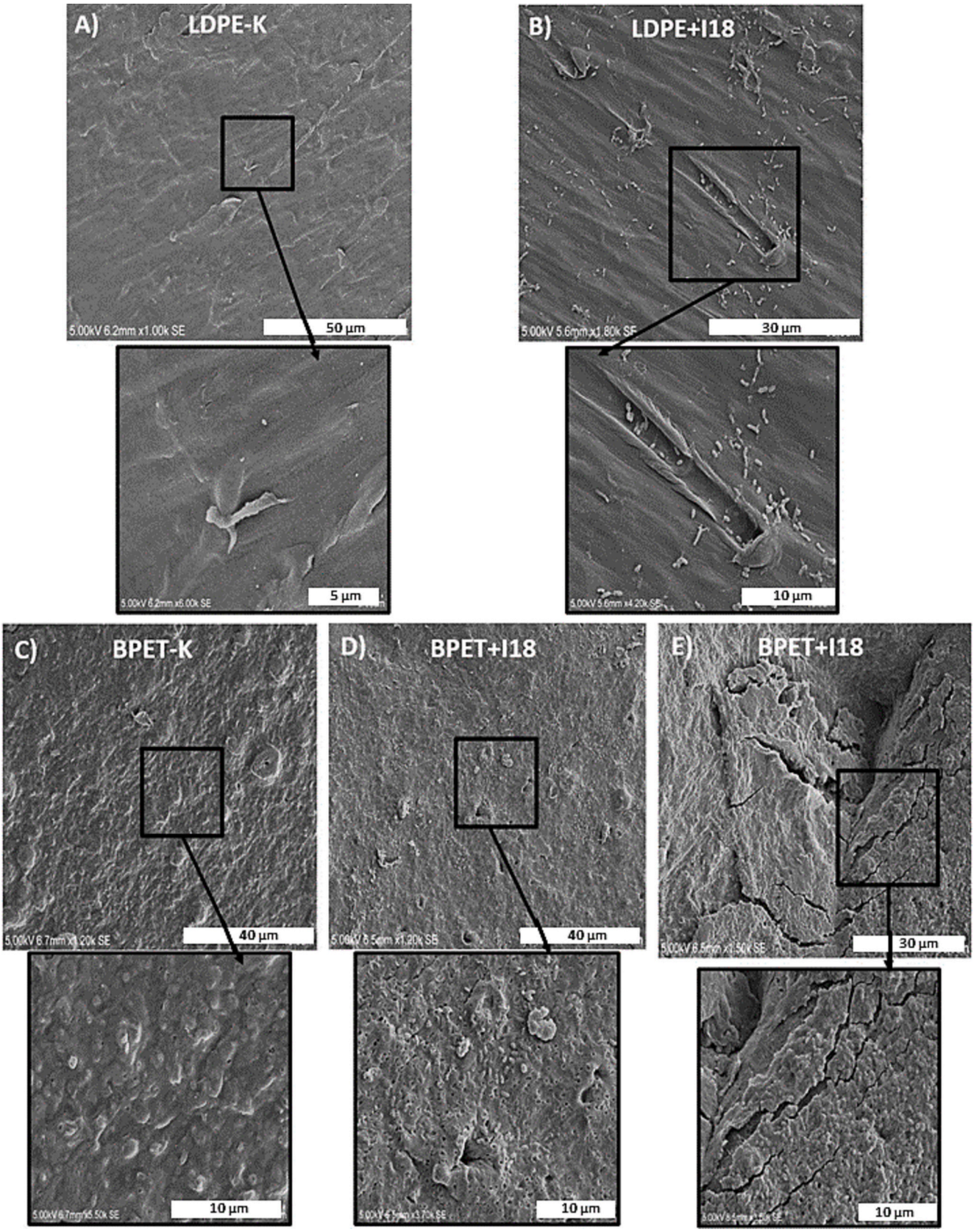


**Fig. 9.** SEM micrographs of LDPE and BPET MP films after 45 days of incubation with the bacterial community (labelled as I18), compared to the negative controls (labelled as K). A) Surface of a LDPE control particle showing a smooth surface; B) Surface of a LDPE particle incubated with I18, showing the presence of bacteria into present fractures; C) Surface of a BPET control particle; D) Surface of a BPET particle after incubation with I18 showing the formation of holes and scattered bacteria; E) Surface of a BPET particle displaying a bacterial biofilm. Note the differences in scale.

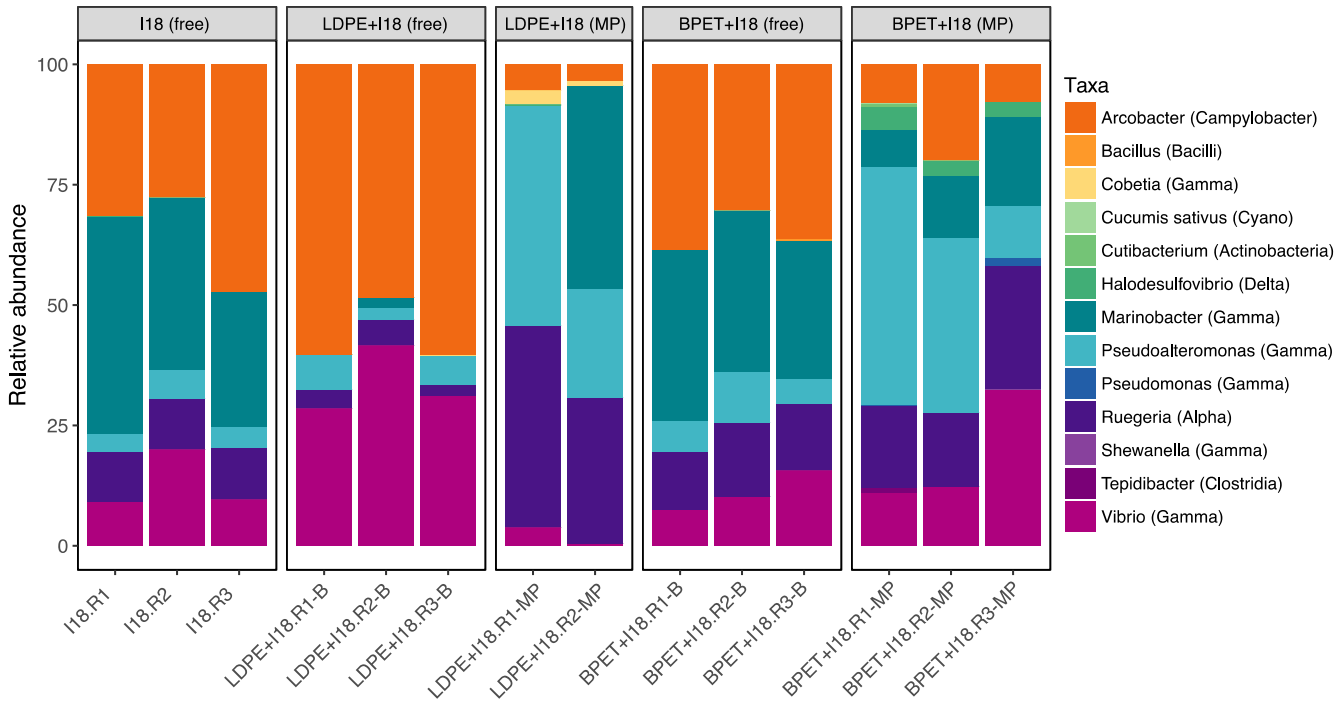


**Fig. 10.** Relative abundance (%) of bacterial taxa at genus level (class within parenthesis) developed in the positive controls (I18) and microplastic samples inoculated with I18 after 45 days of incubation. MP refers to the bacteria found attached to the microplastic particles, as compared to free bacteria found in the surrounding marine broth (B). Alpha = Alphaproteobacteria; Cyano = Cyanobacteria; Delta = Deltaproteobacteria; Gamma = Gammaproteobacteria. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

described as sulfate-reducing bacterium (Shivani et al., 2017). However, their role on the degradation of conventional and biobased plastics is unclear. Future research aimed on the degradation of (biobased) PET could explore different strains of *Halodesulfovibrio* and *Tepidibacter*.

Several strains of *Pseudomonas* have shown potential to biodegrade both conventional aromatic plastics (Shah et al., 2008; Lee et al., 2020), and biobased plastics such as PLA and PCL (Emadian et al., 2017; Sekiguchi et al., 2011). Thus, some strains of *Pseudomonas* may be able to biodegrade both, biobased and conventional PET. *Marinobacter* were more abundant in the marine broth than in the BPET microplastics. Still, it is noteworthy to mention that several species of *Marinobacter* have been reported to be involved in the biodegradation of marine oil spills (Gutierrez et al., 2013; Dombrowski et al., 2016), some even described as "obligate hydrocarbonoclastic bacteria" (Yakimov et al., 2007). They are better known to degrade aliphatic (Yakimov et al., 2007; Gutierrez et al., 2013), rather than aromatic compounds such as PET. However, the most common species found in our samples, *Marinobacter litoralis*, has not been reported as one of those, and could eventually be a species with a potential to biodegrade aromatic hydrocarbons.

Previous studies also reported that the taxonomic composition of microbial biofilms formed around plastic particles was different from that on the surrounding water (McCormick et al., 2016; Syranidou et al., 2019) or to that on natural substrates (Miao et al., 2019). Similar to what observed in our study, Syranidou et al. (2019) also found that bacteria from the phylum Proteobacteria dominated the plastic associated communities, *Alpha-* and *Gammaproteobacteria* exhibiting higher abundances in the acclimated biofilm communities. Miao et al. (2019) observed that *Gammaproteobacteria* were more enriched on PE MPs as well. This is not surprising, since marine bacteria from the class *Gammaproteobacteria* are well-known to respond quickly to organic matter inputs coming from anthropogenic sources such as oil-spills (e.g. Dombrowski et al., 2016) or anthropogenic aerosols (e.g. Marín-Beltrán et al., 2019). According to Miao et al. (2019), the introduction of MPs in the environment is likely to alter the microbial communities and genetic exchange in natural water, and consequently affect the ecological function of the microbial communities. This is something that needs to be further explored. In this work, we observed that microplastics, coming from either petroleum-based (LDPE) or biobased (PET) plastics, had a potential to modify the structure of the marine bacteria community under study. Similarly, Denaro et al. (2020) observed that the structure of a biofilm of hydrocarbon-degrading bacteria was affected by the typology of conventional and compostable plastics. It must be pointed that our community was not original from marine waters, but from the tunicate *Didemnum* sp. Microbial communities associated with marine organisms may differ from the community present in the surrounding water, and therefore it would also be important to observe the degradation of MPs in the marine environment itself. Still, species of the ascidian *Didemnum* are widely distributed across the seas (e.g. Bullard et al., 2007). Therefore, bacterial groups from this organism that showed an affinity for each polymer can be easily recovered and isolated, and have a potential to be further explored for bioremediation purposes.

### 4.2. Limitations of the study and future work

Some authors have pointed that plastics used for toxicological experiments are not realistic (e.g. Arthur et al., 2009). It is important to use realistic concentrations of MPs in laboratory experiments to understand what is truly happening in the natural environment. In this study, we used a MP concentration of 1000 MPs $L^{-1}$, which is higher than values normally reported in the marine environment (see e.g. Auta et al., 2017a, and references therein). A lower concentration may have had a different impact on marine bacterial assemblages. Still, higher concentrations have been reported in places such as the North Sea (Dubaish and Liebezeit, 2013). Additionally, MP pollution is expected to increase in the near future, and the COVID pandemic has only worsen the problem. Therefore, the concentration used is likely representative of what we will see in our oceans in the near future. On the other hand, virgin pellets have been commonly used in previous experiments (e.g. Auta et al., 2017b; Emadian et al., 2017). In this study, we used commercially available plastic products and cut them into smaller pieces, simulating

well the entrance of secondary MPs from land to marine waters, which are likely the main source of MPs (Andrady, 2017). Ideally, we would have assessed the biodegradation of two polymers with a different chemical structure (i.e. LDPE and PET) and with a different origin (petroleum-based and biobased), but logistic did not allow for such a big experiment. Therefore, we decided to select microplastics with a different composition and origin, but in the future it will be interesting to do the comparison of one type of plastic with a different origin (e.g. petroleum-based versus biobased LDPE/PET).

Initial breakdown of polymer chains by microorganisms is a very complex and time-consuming step in their degradation (Ojha et al., 2017). Thus, ideally, experiments aimed to observe the biodegradation of plastics under natural conditions should last several months. Experiments that run for <3 months have exhibited minor to moderate changes on plastic biodegradation (e.g. Auta et al., 2017b; Denaro et al., 2020; Lee et al., 2020), similar to those observed in the present study. In principle, the longer the incubation times, the higher production of carbonyl groups (Abraham et al., 2016), which increase the hydrophilicity of the polymer (Gewert et al., 2015), promoting microbial attachment and ultimately biodegradation. However, studies where experiments were run for longer periods of time (e.g. Skariyachan et al., 2018, up to 140 days; Syranidou et al., 2019, 5 months) observed similar changes in terms of biodeterioration and biofragmentation of plastics than those reported here. This means that relatively short experiments of 1 to 3 months (45 days in our case) can be a good compromise between time and effort devoted and the information acquired regarding the potential of certain microbial species or communities to biodegrade different plastics.

The selected bacterial consortium has shown potential to biodegrade LDPE and biobased PET, and could be applied for bioremediation strategies. The greater potential of microbial consortium over monocultures in several bioprocesses have been recently acknowledged (e.g. Ghosh et al., 2016; Jiménez et al., 2019). Consortia are easier to recover from the environment, being more cost-effective, easy to maintain and, in principle, less dependent from changes in the environmental conditions. Also, positive interactions among the microbial members of a community can favour cooperative work, ensuring the industrial application and long-term stability of the product outcome (Ghosh et al., 2016). Nonetheless, future work could include the use of both isolates and mixed-cultures (mostly those that were found attached to the microplastic particles) from the selected bacterial community, to study the interactions between the different members, and their role on the biodegradation of microplastics.

Although all the techniques used in combination allow detecting early signs of biodegradation (Auta et al., 2017b; Ioakeimidis et al., 2016; Skariyachan et al., 2018), studies on biodegradation of (micro) plastics may benefit from further microbiological and chemical techniques. For example, the use of metatranscriptomics could unveil the metabolic pathways taking place during the biodegradation process (e.g. Miao et al., 2019; Yoshida et al., 2016). Gas or liquid chromatography-mass spectrometry (GC/LC-MS) analysis may also be of interest to identify the products of polymer biodeterioration and biofragmentation, and have been used in other similar studies (Abraham et al., 2016; Skariyachan et al., 2018). This would bring additional advantages such as understanding whether the products from plastic biodegradation can be toxic for marine fauna as well.

## 5. Conclusions

The current study aimed to assess the potential of a marine bacterial community to biodegrade conventional (LDPE) and biobased (PET) microplastics from commercially available products, simulating natural conditions in the marine environment. Overall, our results showed signs of biodegradation – or at least biodeterioration and biofragmentation –, as determined chemically (FTIR-ATR, COD) and physically (SEM), of both types of polymers under study, when subjected to the selected marine bacterial community. The results suggest that biobased PET was no more prone to biodegradation than conventional, petroleum-based LDPE. Distinct bacterial taxa showed affinity for the two types of microplastics, as compared to free bacteria characterized from the surrounding broth. Some of the bacterial groups found attached to the microplastics have been previously reported to have potential to degrade different types of plastics or oil spills (*Cobetia*, *Pseudomonas*). The functionality of other taxa (*Halodesulfovibrio*, *Pseudoalteromonas*, *Ruegeria*, *Tepidibacter*) as plastic-degraders is still unknown. Metabolic pathways of these attached bacteria can be further studied to assess their potential for bioremediation purposes.

## CRediT authorship contribution statement

Nuria Fernández: Laboratory analysis, Data curation, Writing - original draft preparation. Maria Clara Costa: Conceptualization, Funding acquisition, Supervision, Writing - review & editing, Isabel Marín-Beltrán: Conceptualization, Methodology, Laboratory analysis, Data curation, Supervision, Writing - original draft preparation, Writing - review & editing.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114251.

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