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Long-term immersion of compostable plastics in marine aquarium: Microbial biofilm evolution and polymer degradation

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ABSTRACT

The best-selling compostable plastics, polylactic acid (PLA) and polybutylene adipate-co-terephthalate (PBAT), can accidentally end up in the marine environment due to plastic waste mismanagement. Their degradation and colonization by microbial communities are poorly documented in marine conditions. To better understand their degradation, as well as the dynamics of bacterial colonization after a long immersion time (99, 160, and 260 days), PBAT, semicrystalline, and amorphous PLA films were immersed in a marine aquarium. Sequencing and chemical analyses were used in parallel to characterize these samples. Despite the variation in the chemical intrinsic parameters of these plastics, their degradation remains very slow. Microbial community structure varied according to the immersion time with a high proportion of Archaea. Moreover, the plastisphere structure of PBAT was specific. A better understanding of compostable plastic degradability is crucial to evaluate their impact on ecosystems and to eco-design new recyclable plastics with optimal degradation properties.

1. Introduction

Due to their large panel of properties and light weight, petroleum-based plastics are vastly implemented in diverse applications with an annual growth rate of 4 % (Plastics, 2020). Even if they facilitate our daily lives, plastic materials are increasingly becoming controversial, particularly when they are accidentally or purposely disposed of in our environment. It results in the exponential accumulation of plastic waste in the natural environment (Geyer et al., 2017), provoking a global ecological impact due to waste ingestion by fauna and ultimately leading to death (Rochman et al., 2013; Wilcox et al., 2015).

In this respect, biodegradable plastics, particularly polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT), have attracted particular attention to address this pollution issue. Due to its excellent mechanical properties, PLA represents a good alternative for non-compostable rigid plastics, i.e., polystyrene (PS) and polypropylene (PP), while PBAT is used as a flexible equivalent of low-density polyethylene (LDPE) (European Bioplastics, 2018). The biodegradation process is driven by two important factors: (1) abiotic degradation, i.e., the action of the physicochemical parameters from the surrounding environment, and (2) biotic degradation, i.e., microbial activity (Vaksmaa et al., 2021a). To be considered compostable plastic, these polymers must follow industry norms referring to a specific time frame and conditions, e.g., degradation of up to 90 % into CO_2 in controlled compost at 70 °C after 180 days (ISO 14855). However, when the degradation of these biodegradable polymers is conducted in marine conditions, i.e., at low temperature and in an aqueous medium, they are less prone to degradation (Wang et al., 2021).

For instance, recent studies have shown that PLA is not biodegradable in home-composting or reactors mimicking marine environments (Narancic et al., 2018). A set of studies highlighted the fact that PLA was not even degradable in the marine environment (Karamanlioglu et al., 2017; Bagheri et al., 2017; Tsuji and Suzuyoshi, 2002; Deroiné et al., 2014; Beltran-Sanahuja et al., 2020). Only three research groups have focused on PBAT degradation in seawater. They investigated degradation after 1 month, 6 months, and 1 year (Nakayama et al., 2019; Kedzierski et al., 2018; De Monte et al., 2022), showing surface erosion (approximately 20 to 40 μm of thickness was lost after 1 year (Kedzierski

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et al., 2018)) and low weight loss (approximately 8 % after 6 weeks of immersion (Nakayama et al., 2019)). These studies were focused only on the physicochemical parameters of the polymers and not on microbial colonization. Moreover, two recent studies described the microbial communities developed on several biodegradable and nonbiodegradable polymers in seawater (Jacquin et al., 2021; Odobel et al., 2021). The first study analyzed successive biofilm steps (i.e., initial, growth and mature phases (Odobel et al., 2021)) on PLA between 3 and 206 days of immersion, while the second analyzed the microbial community structures on PLA and PBAT after 40 days of immersion in natural seawater (Jacquin et al., 2021). Unfortunately, the quantification of plastic degradation by physicochemical parameters was not monitored. In addition, the sample position in the water column was reported to strongly influence abiotic degradation and the bacterial community structure developed on the polymer (Delacuvellerie et al., 2021). PBAT, PLA, and noncompostable plastics were immersed in situ in the water column and on sediment in the Mediterranean Sea after 82 days (Delacuvellerie et al., 2021). The microbial structure of compostable plastic biofilms on PBAT has not yet been studied after long-term exposure.

The plastisphere, i.e., microbial communities developing on plastic surfaces, is a microecosystem composed of diatoms, fungi, archaea and bacteria (Zettler et al., 2013; Delacuvellerie et al., 2022). The structure of these microbial biofilms evolves gradually according to the immersion duration of the polymer. For instance, *Gamma-* and *Alphaproteobacteria* were the primary colonizers, and *Bacteroidetes* were the secondary colonizers (De Tender et al., 2017a; De Tender et al., 2017b). After 80 days of immersion in situ, the bacterial community structure colonizing compostable and noncompostable polymers was similar (Delacuvellerie et al., 2021). Bacteria are unspecific for plastic degradation, and plastic surfaces are used by microbial organisms as a growth support rather than a carbon source (Delacuvellerie et al., 2021).

As mentioned above, the biodegradability studies of compostable plastics collecting analyses of both physicochemical parameters of polymers and bacterial community structures after long immersion times in the marine environment are poorly documented. In this context, we studied compostable plastic degradation by physiochemical analysis in parallel with the dynamics of the microbial colonization of these polymer surfaces by 16S rRNA amplicon sequencing after 99, 160, and 260 days of immersion in a marine aquarium mimicking a natural environment. A better understanding of biodegradation and microbial colonization of PLA and PBAT, the best-selling compostable plastics, is essential to evaluate their impact on the natural environment and to ecodesign environmentally friendly new recyclable polymers with optimal degradation behaviors in all environments (e.g., compost, freshwater, and seawater).

2. Materials and methods

2.1. Plastic films

Three compostable plastics (PBAT, amorphous, and semicrystalline PLA) and one noncompostable plastic used as a control for conventional thermoplastic (PS) were used during this study. PS (ST311125/5) was supplied by Goodfellow © (England). The density and thickness of the amorphous polystyrene were 1.05 g/cm^3 and 125 μm, respectively (technical data provided by the supplier), and its glass transition temperature was 106 °C (Table 1). Pellets of PBAT were provided by BASF company and distributed by B-Plast 2000 (product code: Ecoflex F Blend C 1200). Pellets of the semicrystalline (PLA 4032D, Ingeo™ 4032D) and amorphous PLA (PLA 4060D, Ingeo™ 4060D) were provided by Nature Works (USA).

Pellets were dried overnight and films of 200 μm tickness and 5 cm diameter were pressed using a Carver 3851–0 hot-press machine (Wabash, USA). The temperature settings of the plate were 200 °C and 150 °C for PLA and PBAT, respectively. PLA and PBAT were melted for 5 min and 3 min, respectively, followed by 2 degassing steps. Finally, plastic films were pressed for 5 min at 250 bar and cooled at room temperature for 5 min using water circulation within the pressure plates (Delacuvellerie et al., 2021).

Table 1
Characteristics of compostable plastic pellets (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS).

Plastic type	Density[1]	Glass transition temperature	Melting temperature	Crystallinity
PBAT	1.26 g/cm^3	−29 °C	120 °C	13 %
PLA (4060D)	1.24 g/cm^3	58 °C	/	Amorphous
PLA (4032D)	1.24 g/cm^3	62 °C	166 °C	4 %

2.2. Experimental design

Plastic films were sterilized in 70 % ethanol (v/v) and placed in an aquarium of 97 cm × 196 cm with a depth of 50 cm in cold marine water (temperature of 14–22 °C, salinity of 38 g/l, and pH of 8; Fig. S1) in the museum aquarium of Liège. We immersed polymers in an aquarium with a water circulation system connected to other basins containing marine organisms and marine flora. Four replicates of each polymer were fixed on a plastic framework of 30 cm × 30 cm to avoid metal contamination and deposited on the bottom of the aquarium (Fig. S1). A total of three frameworks were set (one for each sampling time). Plastics of different chemical compositions were alternately fixed on the frames and immersed on 03 March 2020. The samplings took place on the (1) 10th of June 2020 (t_{99}, 99 days of immersion); (2) on the 11th of August 2020 (t_{160}, 160 days), and on the 18th of November 2020 (t_{260}, 260 days). The water temperature was monitored with a HOBO ® during immersion (Fig. S2).

In parallel, to highlight the abiotic degradation, semicrystalline and amorphous PLA, PBAT and PS were also immersed (each film was cut into 1 cm × 1 cm squares in three replicates per sample) in artificial sea water (sea salt, 38 g/l; pH: 8) at 20 °C for 69 and 153 days. These plastics were used for chemical characterization.

2.3. Plastic sample processing

Polymer films were rinsed using sterile salt water to remove microorganisms that were not well attached to the biofilm. Biofilm was detached using a sterile inoculation loop, and the obtained biomass was used for DNA extraction, while polymer films were cleaned with 70 % ethanol (v/v) and deionized water to remove organic coatings, and dried at 30 °C before performing their chemical characterizations.

2.4. Physicochemical characterization

2.4.1. Weight loss method

After cleaning and drying, polymer films were weighed and the percentage of weight loss was determined as follows (Roy et al., 2008):

$$\text{Weight loss } (\%) = \frac{(m_i - m_f)}{m_i} \times 100$$

where m_i is the weight of the plastic at the initial time and m_f is the weight after the immersion time.

2.4.2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry analyses were carried out with a DSC Q2000 from TA Instruments®, New Castle, USA. Enthalpy and temperature calibrations were performed using an indium standard. Approximately 5–10 mg of samples were sealed in an aluminum standard pan and, heated from −20 °C to 200 °C for amorphous and

semicrystalline PLA and − 80 °C to 200 °C for PBAT and PS with a heating ramp of 10 °C/min under a nitrogen atmosphere. The parameters of interest (i.e., melting temperature (T_m), and glass transition temperature (T_g)) were obtained at the second heating scan. The equation for determining the crystallinity percentage was as follows (Benali et al., 2015):

$$\chi_c\ (\%) = \left[\frac{\Delta H_{m(t)} - \Delta H_{c(t)}}{\Delta H_m^0}\right] \times 100$$

where $\Delta H_{m(t)}$ and $\Delta H_{c(t)}$ are the melting and cold crystallization enthalpies at the same time t (after or before immersion in the marine aquarium); $\Delta H_m^0$is the melting enthalpy of the 100 % crystalline polymer: ΔH_m^0 is 93.0 Jg^{-1} for PLA (Benali et al., 2015) and 114.0 Jg^{-1} for PBAT (Bastarrachea et al., 2010).

2.4.3. Size exclusion chromatography (SEC)

Polymer solutions were prepared at 2 mg polymer/ml of $CHCl_3$. The SEC procedure used was the same as described previously (Paul et al., 2003). Agilent liquid chromatography was used for the analysis of molecular weight such as the number average molecular weight and the dispersity (Mn and ĐM, respectively). A total of 100 μl of the samples was injected with an Agilent autosampler at a flow rate of 1 ml/min. The calibration was performed with a PS standard for the separation of Mw (PS) ranging from 200 to 4 × 10^5 g/mol.

2.4.4. ATR-FTIR spectroscopy

Spectra of the plastic surface before and after incubation (after biofilm removal) were obtained using Fourier transform infrared spectroscopy (FTIR) using the attenuated total reflectance (ATR) technique (Bruker, Tensor 27) with OPUS 6.5 software. The spectra were acquired over the wavenumber range of 4000–600 cm^{-1} with 64 spectral scans (Mahoney et al., 2013).

2.5. Microbial taxonomic profile

2.5.1. DNA extraction and PCR

The Power soil ® DNA kit (Power soil ® DNA kit, QIAGEN) was used to extract the DNA of biofilms following the manufacturer's instructions. A 460 pb sequence of the hypervariable V3-V4 region of the 16S rRNA of bacteria and archaea was amplified using the following primers: 806R (5′-GGACTACNNGGGTATCTAAT-3′) and 341F (5′-CCTAYGGGRBGCASCAG-3′) (Nunes et al., 2016). To allow compatibility with Illumina index and sequencing adapters, overhang adapter sequences are added to the primer pair sequences.

2.5.2. 16S rRNA amplicon sequencing and generation of the OTU contingency table

The 2 × 300 bp paired-end high-throughput sequencing was performed on the Illumina® MiSeq ® platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions using high-throughput sequencing by GIGA, Liège, Belgium. Amplicon sequencing analysis was carried out on the qiime2 pipeline (https://qiime2.org/; (Bolyen et al., 2019)). The diversity spacers were trimmed using Cutadapt (Martin, 2011). Pair-end joining, sequence filtering, dereplication, clustering, verification, and exclusion of chimeric sequences were performed with *Vsearch*. The sequences with a minimum read length of 200 bp were preserved, and paired-end matings were performed according to a minimum quality of 50 and a minimum overlap of 15 (Jacquiod et al., 2018). Regarding the clustering, 97 % identity was chosen. Finally, a contingency table was exported using the *Greengenes* database (DeSantis et al., 2006) with the *feature-classifier classify-consensus vsearch* followed by the collapse feature table on taxonomy. Chloroplast sequences were removed as well as sequences that did not belong to the bacterial or archaeal kingdoms. 16S rRNA amplicon sequences were deposited in the SRA (Sequence Read Archive) of NCBI under accession number PRJNA788865 (http://www.ncbi.nlm.nih.gov/bioproject/788865).

2.5.3. Analysis of the diversity index

PAST software was used to verify the sequencing quality using rarefaction curves (Hammer et al., 2001) (Fig.S3). The richness and the equitability indices (alpha-diversity) were calculated on the rarefied data (10,935 read counts, *Limma* RGui package). Constrained analysis of principal coordinates (CAP) was obtained from the 16S sequencing data using the pairwise community dissimilarity (Bray-Curtis) distance and variance-adjusted weighted UniFrac with the RGui packages *ape*, *picante,* and *GUniFrac* (Paradis and Claude, 2004; Kembel et al., 2010; Chen et al., 2012). The evolution of phylogenetic relatedness in our samples (after 99, 160, and 260 days of immersion for PBAT, semicrystalline and amorphous PLA, and PS) was assessed using the phylogenetic tree obtained from qiime (qiime phylogeny align-to-tree-mafft-fasttree). The weighted net relatedness index (NRI) and the nearest taxon index (NTI) were calculated with the RGui package *picante* (Kembel et al., 2010; Cyriaque et al., 2020). Briefly, mean pairwise distance (MPD) quantifies the mean relatedness in a group of OTUs and is calculated by considering all possible pairs of OTUs, while mean nearest taxon distance (MNTD) quantifies the mean relatedness by considering only the nearest phylogenetic neighbors. NRI and NTRI assess the number of standard deviations in comparison with the MPD or MNTD, respectively, calculated on the corresponding null distribution with 1000 randomizations (95 % confidence interval, p-value <0.05) for each immersion time (Webb et al., 2008; Stegen et al., 2015). The coexisting taxa are significantly close when NTI or NRI values are higher than 2, while NTI or NRI values lower than 2 indicate significant phylogenetic overdispersion (Stegen et al., 2012).

2.5.4. Identification and validation of response groups (RGs)

A heatmap was generated with 63 OTUs significantly affected by plastic chemical compositions (PLA-4032D, PLA-4060D, PBAT, and PS) and by the immersion times (99 days, 160 days, and 260 days) identified with a nbGLM (negative binomial distribution and generalized linear model) revised by 1000 resampling iterations of the residual variance using the *mvabund* Rgui package (Dixon, 2003). Three RGs were drawn from the cluster dendrogram using the Euclidean distance, and average clustering was obtained with the *vegan* RGui package. RGs were validated with a Monte- Carlo simulation (Fig. S4), comparing the RG clustering with a null model containing all the OTUs to reinforce the power of randomization (Jacquiod et al., 2018).

3. Results and discussion

3.1. Physicochemical properties of the polymers

Compostable plastics, i.e., PBAT, amorphous and semicrystalline PLA, and polystyrene (as a control for conventional thermoplastic), were submerged in a marine aquarium for 99, 160, and 260 days. Two grades of PLA were used to study the impact of crystallinity on microbial colonization and biodegradation processes. The immersed plastics have different chain structures: (i) compostable plastics, having an unstable matrix degradable by hydrolysis, and (ii) PS, which is a commodity plastic with hardly degradable chains. Macroscopic observations of biofilm development reveal a similar biofilm structure based on the polymer chemical compositions (PBAT, PLA-4032D, PLA-4060D, and PS) and on the immersion time (Fig. S5). We first investigated the physicochemical alterations of these polymers as follows:

3.1.1. Polybutylene adipate-co-terephthalate films

The weight loss of PBAT reached 3.0 ± 0.5 % after 260 days and this loss was significantly different from those of the other plastics (Fig. 1). Regarding the SEC parameters, M_n decreased over time (51,000 to 46,000 g/mol) and the dispersity slightly increased (2.2 to 2.4; Fig. 2).

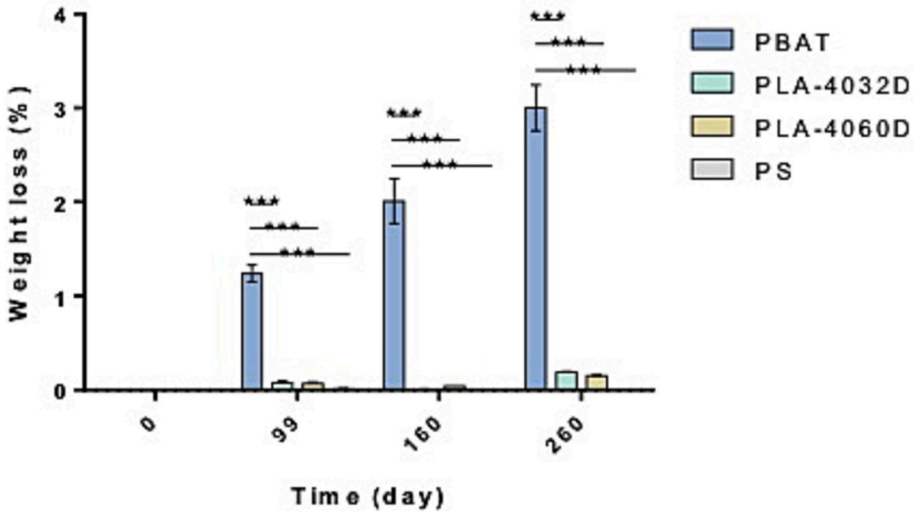

Fig. 1. Plastic weight loss after 99, 160 and 260 days in the marine aquarium (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS)), ANOVA with Tukey's post- hoc test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

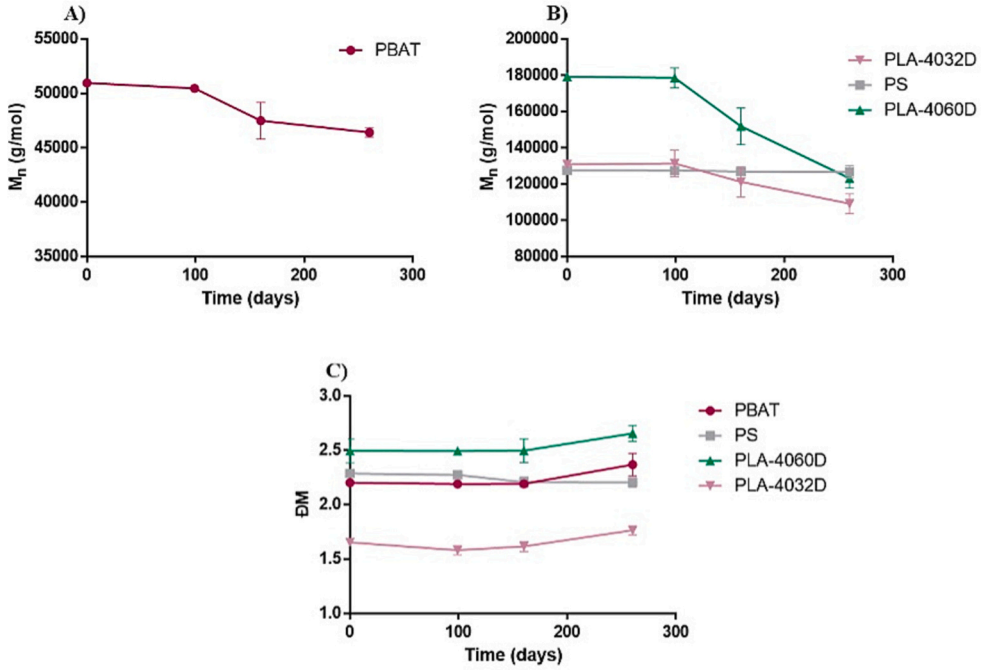

Fig. 2. Number average molecular weight (M_n) (A & B) and dispersity (ÐM) evolution (C) of the plastic films (semicrystalline PLA (4032D); amorphous PLA (4060D); PBAT and PS) before and after the different immersion times (99, 160 and 260 days) in the marine aquarium (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS)).

The shape of the SEC trace remained monomodal, indicating that degradation occurred homogeneously across the polymer (Fig. S6). The DSC parameters were stable over time (Table 2). These results are in accordance with a previous study of PBAT in seawater under controlled conditions after 10 weeks of immersion (Nakayama et al., 2019). Finally, a wide peak appeared in the ATR-FTIR spectra at 3650–3100 cm^{-1} after 160 and 260 days of immersion, as well as a peak at 1635–1535 cm^{-1} from 260 days of immersion (Fig. S7). The 3500–3100 cm^{-1} band corresponds to OH bond stretching due to polymer hydration by water molecules. OH formation resulted from the hydrolysis of the ester functional group, forming a carboxylic acid and an alcohol functional group. Moreover, the NH group of amides can be assigned to 3400–3100 cm^{-1} peaks, corresponding to the residues of proteins from the bacterial biofilm (Arrieta et al., 2014). Finally, the formation of carboxylate groups can be attributed to the 1635 cm^{-1} peak and 1535 cm^{-1} is assigned to proteinic material (Arrieta et al., 2014; Bonhomme et al., 2003). After 260 days of immersion, the weight (Fig. 1), DSC parameters (Table 2), SEC analysis (Fig. 2 & Fig. S6), and ATR-FTIR spectra remained similar (Fig. S7).

These results showed that PBAT degradation started with a very low rate of hydrolysis and are in accordance with our first study in which different polymers were immersed for 80 days in situ in the Mediterranean Sea in the water column and on the sediment (Delacuvellerie et al., 2021). Indeed, PBAT lost 1 % of its weight (Delacuvellerie et al., 2021). Moreover, the weight loss of our samples was proportional to the immersion time (Fig. S8A). As far as the SEC parameters are concerned, the

Table 2
Differential scanning calorimetry (DSC) parameters from the second heating for the polymer at the initial time (t_i: before the immersion), and after 99 days (t_{99}), 160 days (t_{160}), and 260 days (t_{260}) of immersion in the marine aquarium (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS), melting temperature (T_m), glass transition temperature (T_g), crystallinity χ_c).

Polymer	Sample name	T_g (°C)	T_m (°C)	χ_c (%)[a]
PLA (4032D)	t_i	62	166	4
	t_{99}	62	166	5
	t_{160}	62	166	5
	t_{260}	62	167	4
PLA (4060D)	t_i	58	/	Amorphous
	t_{99}	58	/	Amorphous
	t_{160}	58	/	Amorphous
	t_{260}	58	/	Amorphous
PBAT	t_i	−29	121	13
	t_{99}	−29	121	13
	t_{160}	−29	122	13
	t_{260}	−29	122	14
PS	t_i	106	/	Amorphous
	t_{99}	105	/	Amorphous
	t_{160}	105	/	Amorphous
	t_{260}	105	/	Amorphous

[a] $\chi_c\ (\%) = \left[\frac{\Delta H_{m(t)} - \Delta H_{c(t)}}{\Delta H_m^0}\right] \times 100$

M_n started to decrease from 160 days of immersion, with an increased dispersity from 260 days. Only a few studies have been out on PBAT biodegradability in marine water. For instance, PBAT lost <2.5 % of its weight over 56 weeks of immersion in tanks with natural seawater at room temperature (Wang et al., 2019), and the formation of microplastic fragments was observed after 10 weeks of immersion at 23 °C (Wei et al., 2021). The composting degradation of the same PBAT was tested in two phases: (i) thermophilic conditions for 20 days at 58 °C and (ii) mesophilic conditions for 40 days at 37 °C (Ruggero et al., 2021). After only 60 days in the compost, approximately 10 % weight loss was observed with fragmentation of the polymers, showing that the composting conditions are far from the marine environment, relating to a low temperature and a reduced concentration of microorganisms. Even if a slight degradation of the PBAT is observed, this remains too slow.

3.1.2. Amorphous and semicrystalline polylactic acid films

After 260 days, the semicrystalline PLA (4032D) and the amorphous PLA (4060D) presented weight losses of 0.19 ± 0.02 % and 0.15 ± 0.01 %, respectively, without a significant difference with respect to PS (the control of conventional thermoplastic), as shown in Fig. 1. The DSC parameters before and after the immersion times remained similar (Table 2). The SEC parameters highlighted decreases in M_n for amorphous PLA (180,000 to 120,000 g/mol) and the semi-crystalline PLA (130,000 to 120,000 g/mol) (Fig. 2B). The physical structure of PLA affects its hydrolysis, as the cleavage of the chains occurs preferentially in the amorphous zone (Tsuji and Ikada, 2000). Increases in the dispersity were also observed after 260 days of immersion from 1.7 to 1.8 and 2.5 to 2.7 for semicrystalline and amorphous PLA, respectively (Fig. 2C), showing that some slight hydrolysis occurred in the polymer matrix. Regarding the profile of the SEC curves, the trace remained monomodal, but a slight shift to a lower elution volume was observed, indicating the release of oligomers (Fig. S6). Finally, ATR-FTIR spectra were similar before and after the immersion times (Fig. S9 & S10).

Even though the PLA degraded poorly after 260 days, the SEC analysis highlighted modifications, showing that the PLA had undergone some changes in the molecular chains. Previous studies showed no significant degradation after 82 days of immersion in situ in the Mediterranean Sea (Delacuvellerie et al., 2021), after 206 days of immersion in natural seawater (Odobel et al., 2021), or in a marine environment in a bioreactor at 30 °C (Narancic et al., 2018). However, PLA is biodegradable in industrial composting (Narancic et al., 2018). For instance, the biodegradation of semicrystalline PLA was tested in compost (20 days at 58 °C and 40 days at 37 °C), and <10 % was degraded (Ruggero et al., 2021). The marine environment parameters were therefore not appropriate for the efficient biodegradation of PLA. All these results highlighted the fact that plastics considered biodegradable can have the same impact on the environment as nonbiodegradable polymers. A change in the macromolecular design of the PLA can be a solution. For example, a research group realized a new PLA concept inspired by RNA, accelerating its degradation in seawater (Rheinberger et al., 2021).

3.2. Temporal dynamics of the microbial community structure

A better understanding of the microbial community structure on compostable plastics after a long immersion time (99 days, 160 days, and 260 days) is necessary to better understand their impacts on the marine environment. This study was performed in a marine aquarium to be as close as possible to conditions found in natural marine environment and to avoid the loss of samples as observed in previous in situ (Delacuvellerie et al., 2021). The experimental design of the present study type was applied previously in others studies to analyze the structure plastisphere and follow plastic degradation (Jacquin et al., 2021; Odobel et al., 2021). In this type of study, the complexity of the marine environment is decreased, to avoid uncontrolled environmental variations (e.g., temperature, UV radiations).

Regarding the alpha-diversity, the richness indices of PBAT were smaller than those of the other polymers (Fig. S11A). Those of PS, amorphous, and semicrystalline PLA were similar over time. The equitability of PBAT was similar to those of the three other plastics after t_{99} and decreased after 160 days of immersion (Fig. S11B). The CAPscale analyses using both Bray-Curtis (p-value = 0.000999) and UniFrac (p-value = 0.000999) distances (Fig. 3A & B) showed a different assembly process on PBAT in comparison with the other three polymers (PLA-4032D, PLA-4060D, and PS). Moreover, plastisphere communities also diverged with incubation time (t_{99}, t_{160}, and t_{260}) on all types of plastic (UniFrac associated p-value = 0.000999), revealing a dynamic assembly process over time. The microbial community structure was significantly impacted by the polymer chemical composition. The phylogenetic diversity (Fig. S12; Table S2) revealed that the community assembly initially selected for closely related OTUs (NRI and NTI >2 (Stegen et al., 2012)) highlighting a strong selection process when plastic colonization began. The NTI index remained stable over time and between the polymer types showing no phylogenetic shift between close relatives during the community assembly process. However, the NRI index remained stable for PS and significantly decreased with immersion time for the compostable polymers (PBAT and both PLAs), corresponding to the diversification of the bacterial communities. This could be explained by a strong selection process during the early colonization of the different plastics as it requires specific abilities such as efficient biofilm formation on the polymer structure, or nutrient capture from the plastic, or the surrounding water. Nevertheless, once set up, there are easier ways to carry out these processes, e.g., symbiosis and nutrient sharing, leading to a mature biofilm (Flemming et al., 2016; Donlan, 2002; Prakash et al., 2003), losing the selective pressure and giving room to a broader range of microorganisms. The diversification of the PS microbial communities was slower than that on compostable plastics. Compostable plastics being hydrolyzable, more bioavailable molecules could be used by the bacterial communities on compostable plastic surfaces.

The plastisphere was mainly represented by *Proteobacteria,* including *Alpha-* and *Gammaproteobacteria* from Bacteria and *Crenarchaeota* from Archaea, with slight variations in proportions over time (Fig. 4). The relative abundance of *Crenarchaeta*, mainly represented by the *Cenarchaeaceae* family (Fig. S13), significantly increased according to the immersion time. This family was mainly present on the amorphous and semicrystalline PLAs (Table S3), where *Crenarchaeta* represented up to

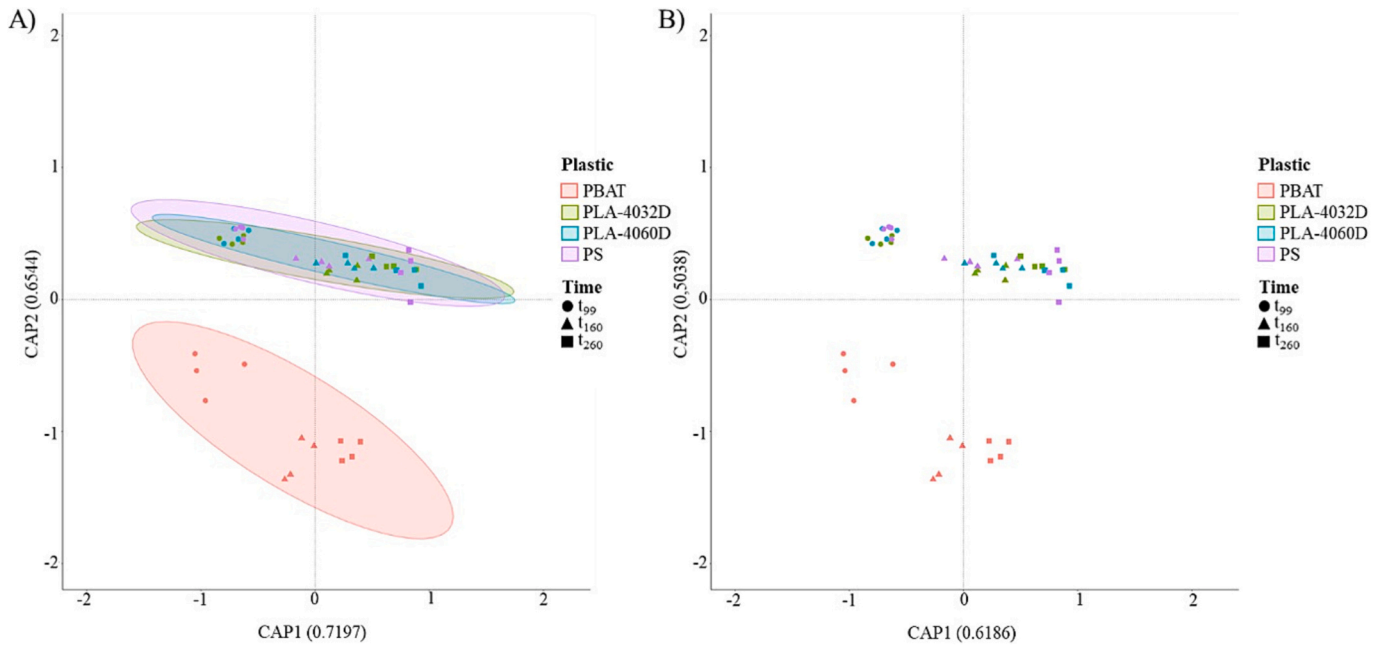

Fig. 3. (A) Constrained analysis of principal coordinates (CAP) scaling analysis profile of the pairwise community dissimilarity (Bray-Curtis) index of the 16S sequencing data of plastics after 99 days (t_{99}), 160 days (t_{160}), and 260 days (t_{260}) of immersion in the marine aquarium; (B) CAP scale analysis using a variance adjusted weighted UniFrac (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS)). ANOVA with 1000 permutations indicated that the plastic chemical composition and the immersion time significantly influenced the bacterial communities (p-value = 0.000999 and 0.000999, respectively).

55 % of the microbial structure. Several studies have focused on the bacterial and archaeal structures in the plastisphere (Debroas et al., 2017; Woodall et al., 2018), but very little information is available regarding the role of archaea in these environments. *Gamma-* and *Alphaproteobacteria* are known to be plastic first colonizers and *Bacteroidetes* as second colonizers (De Tender et al., 2015; Elifantz et al., 2013). The abundances of *Alphaproteobacteria* and *Bacteroidetes* were stable over time, while *Gammaproteobacteria* slightly decreased after 260 days (Table S3). In our study, the shift from primary to secondary colonizers was not well discernible because our first sampling was carried out after 99 days of immersion, when the biofilm was already well established. The shift between *Bacteroidetes* and *Proteobacteria* (*Alpha-* and *Gammaproteobacteria*) generally occurs between the initial and 2-week-old biofilm (Elifantz et al., 2013), while from 30 days, stabilization of the bacterial abundance is reached, corresponding to the maturation phase (Cheng et al., 2021). Even though the biofilm was in the mature phase, the community structure continued to evolve over time, while the selective pressure exerted by the substrate and the marine environment decreased over time, as demonstrated by decreasing phylogenetic relatedness (NRI). Interestingly, several microorganisms were selected according to the plastic chemical composition, i.e., *Alphaproteobacteria* were mainly represented by *Rhodobacteraceae* and *Hyphomicrobiaceae* on PBAT (Fig. S13), where their relative abundances were higher than those of the other polymers. In accordance with these results, Jacquin's group showed that PBAT communities were dominated by *Alphaproteobacteria* (mainly represented by *Rhodobacteraceae*) and *Gammaproteobacteria* after 40 days of immersion in natural seawater (Jacquin et al., 2021). The previous biofilm succession stage experiment was carried out over a shorter period for the PBAT polymer, making the comparison difficult.

In contrast with previous studies (Delacuvellerie et al., 2021; Debroas et al., 2017; Wang et al., 2018), the *Vibrionaceae* family was poorly represented in our samples, with <0.5 % of microbial communities. The study of the same compostable plastic-associated communities in the Mediterranean Sea contained abundant families similar to those in our research, e.g., *Rhodobacteraceae*, *Oceanospirillaceae*, *Saprospiraceae,* and *Flavobacteraceae* (Delacuvellerie et al., 2021). These results highlight the fact that even if our analyses were performed in an aquarium (with a water circulation system connected to other basins containing marine organisms and flora), biofilms contained the most represented families developed on plastic in the natural environment. Therefore, our bacterial community observation in the aquarium seemed to be close to our in situ observations.

Fig. S14 highlights the most abundant genera present on the different polymers (relative abundance >5 %). The *Nitrosopumilus* genus, belonging to Archaea, was the most represented genus, reaching up to 50 % of the microbial communities on PLA after 260 days of immersion. *Nitrosopumilus*, an ammonia-oxidizing archaea, was found in a previous study to be associated with PS (Vaksmaa et al., 2021b). However, too little information about Archaea was available to well understand their role in the plastisphere and, in our case, on the compostable PLA. Further investigations targeting Archaea should be carried out. Our heatmap displays 63 genera statistically significantly affected by the polymer chemical composition and the immersion time, identified using nbGLM analysis, forming three RGs (Fig. 5). RG1 represented genera mainly present after 99 days of immersion on all plastics, e.g., *Marinicella*, and unclassified genera from *Hyphomonadaceae.* The *Hyphomonadaceae* family, known for its ability to form long holdfast filaments, is commonly found on plastics (Zettler et al., 2013). Interestingly, members of this family, e.g., the *Hyphomonas* genus, are methylotrophic and able to degrade hydrocarbons (Coulon et al., 2007; Wang et al., 2016a; Kappell et al., 2014). *Marinicella* is a genus containing species living in seawater or marine sediment (Romanenko et al., 2010; Wang et al., 2016b; Wang et al., 2018). RG2 represented genera present on all polymers after 160 and 260 days of immersion, e.g., *Loktanella* and unclassified from *Oceanospirillaceae*. The *Loktanella* genus has already been found on plastic biofilms (Pinto et al., 2019; Basili et al., 2020). Finally, RG3 contained genera, such as unclassified genera from *Rhodospirillaceae,* HTCC2089, BD7-4, JdFBGBact, Congregibacter and *Bdellovibrio*, specifically present on PBAT. *Congregibacter* is a photosynthetic *Gammaproteobacteria (*Fuchs et al., *2007)*, and HTCC2089 is a marine *Gammaproteobacteria* and an obligate oligotroph. JdFBGBact corresponded to an *Acidobacteria* attached to the basal blade of an algae, *Sargassum muticum*, while *Bdellovibrio* is a pathogen of other bacteria.

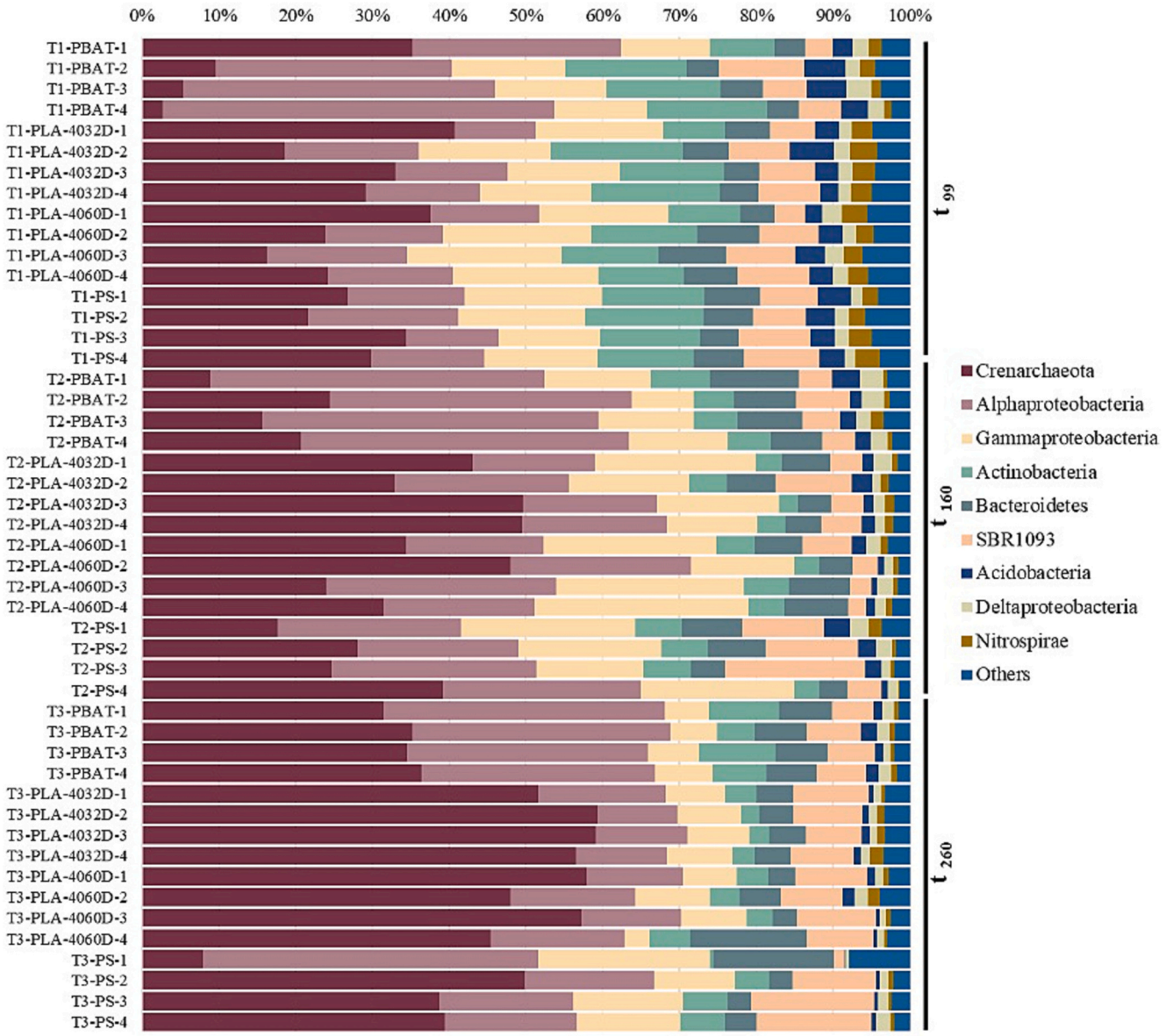

Fig. 4. Phylogenetic profiles based on 16S rRNA amplicon sequencing for plastic films (PBAT, amorphous PLA (4060D), semicrystalline PLA (4032D), and PS) after 99, 160, and 260 days (t_{99}, t_{160} and t_{260}, respectively) of immersion in the marine aquarium (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS)).

The specificity of these bacteria on PBAT seemed to be dependent on the specificity of other organisms, i.e., JdFBGBact might be present on the PBAT surface due to the presence of algae, and *Bdellovibrio* was a pathogen of specific bacteria present mainly on PBAT. *Rhodospirillaceae* contains a genus, e.g., *Thalassospira*, involved in polycyclic aromatic hydrocarbon bacteria (Wang et al., 2021). Interestingly, BD7-4, corresponding to the *Sedimentaribacter* genus, contained species such as *Sedimentibacter hydrobenzoicus* or *Sedimentibacter* sp., which are able to carboxylate phenol or degrade toluene (Tischer et al., 2013), respectively. These compounds contain aromatic cycles such as PBAT. The selection of microorganisms specific to PBAT can be explained by several hypotheses. (I) These bacteria could be involved in the biotic degradation of PBAT, i.e., the enzymatic degradation of PBAT by microbial communities. (II) A second hypothesis would be that PBAT has been hydrolyzed by abiotic degradation, e.g., water action, forming two monomers: adipic acid and bis(4-hydroxybutyl) terephthalate (Muthuraj et al., 2015), and the specific bacterial communities could have been involved in monomer uptake. (III) Finally, the physicochemical properties of the plastic could influence the bacterial community structure, e.g., preferential adsorption of microorganisms depending on plastic hydrophobicity. A hydrolysis test degradation of PBAT was carried out, i.e., immersion of PBAT films in artificial seawater without microorganisms at 20 °C to follow the PBAT weight loss and highlight the microorganism's role in polymer degradation (Fig. S8B). The weight loss of PBAT in the aquarium in the presence of microorganisms was not significantly higher than that in artificial seawater after 153 days of immersion. Therefore, these bacteria were not involved in the biotic degradation of PBAT. The plastic hydrophobicity was also measured by contact angle (Fig. S15). Interestingly, PBAT was the most hydrophilic plastic, followed by both PLAs and PS. The most likely hypothesis is then that these microorganisms preferentially adsorb on PBAT due to its chemical surface properties, i.e., it is the most hydrophilic plastic, or were involved in PBAT monomer degradation. In our previous study on compostable plastics immersed in situ in the Mediterranean Sea, no difference in the bacterial community was observed between the polymers after 82 days of immersion

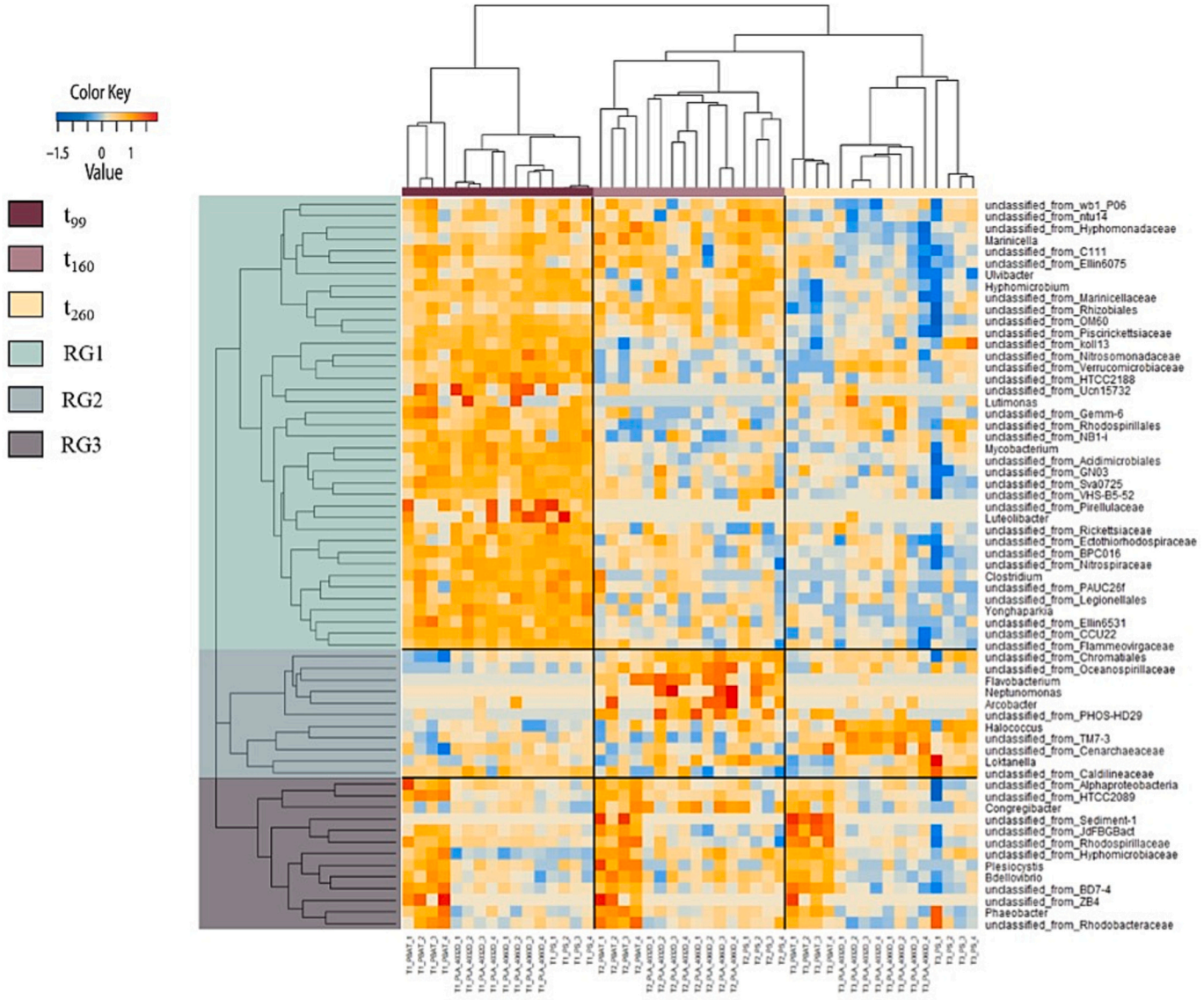

Fig. 5. Heatmap of the 63 genera significantly affected by the polymer chemical composition, (polylactic acid (PLA), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS)) and by the immersion time (99 days (t_{99});160 days (t_{160}) and 260 days (t_{260})). Three response groups (RGs) were defined with hierarchical clustering based on center-scaling abundance.

(Delacuvellerie et al., 2021). The immersion time in the present study was longer, which would explain this difference.

Hydrocarbonoclastic bacteria (HCB) are commonly found in plastisphere communities (Zettler et al., 2013; Delacuvellerie et al., 2019). In the natural marine environment, HCB are present in low abundance, and their growth is stimulated by the presence of hydrocarbons. In general, HCB were poorly represented in the marine plastisphere (Delacuvellerie et al., 2021; Delacuvellerie et al., 2019) and were significantly selected after enrichment culture (i.e., in poor medium containing the plastic as the main carbon source) (Delacuvellerie et al., 2019).It seems that these bacteria could be involved in the degradation of carbon-carbon structures such as plastics (Zettler et al., 2013; Delacuvellerie et al., 2019). The presence of 24 genera considered HCB was identified in our analysis (Table 3). HCB bacteria were poorly represented in the plastisphere communities, reaching up to 2.5 % of the total community for the amorphous PLA after 160 days of immersion. Previous studies carried out on PLA and PBAT after 40 days of immersion contained 22.7 % and 21 % HCB, respectively (Jacquin et al., 2021). These differences could be explained by the difference in immersion time.

Our results showed a gradual community assembly process during the period of immersion and plastic-specific microbial selection. Despite the differences observed in the different kinds of plastic, a microbial core of plastic samples was defined (Table S4). In total, 29 core genera were identified on all polymer chemical compositions after the three immersion times and could be divided into several groups depending on the peak abundance timing: (1) genera with a higher abundance after the first sampling time, e.g., *Mycobacterium*; (2) genera with a higher relative abundance in the intermediate immersion time, e.g., unclassified *Chromatiales*; (3) genera with a higher abundance after 260 days of

Table 3
Percentage of selected genera containing putative hydrocarbonoclastic bacteria on PBAT, semicrystalline PLA (4032D), amorphous PLA (4060D) and PS after 99, 160 and 260 days of immersion.

Immersion time	99 days (% ± SD)	160 days (% ± SD)	260 days (% ± SD)
PBAT	0.24 ± 0.06	0.61 ± 0.32	1.44 ± 0.61
PLA 4032D	0.43 ± 0.35	0.88 ± 0.57	0.43 ± 0.15
PLA 4060D	0.92 ± 0.58	2.45 ± 2.01	0.66 ± 0.59
PS	0.38 ± 0.23	0.63 ± 0.10	0.45 ± 0.23

immersion, e.g., *Nitrosopumilus* and finally (4) genera with a similar relative abundance over time, e.g., unclassified from *Rhodospirillaceae*. Strikingly, together, these microorganisms composing this microbial core of plastic represented approximately 80 % of the total number of OTUs, showing that the majority of these microbes were common in all the samples. Although our plastics from one immersion time came from an independent construction sunk in the marine aquarium, the pieces of plastic showed high reproducibility for the taxonomic compositions. A large number of genera were shared between all the plastic samples, confirming the precedent observation that a "core" of bacteria was shared among all the polymers as a more general biofilm (Basili et al., 2020; Kirstein et al., 2018).

4. Conclusion

This study provided valuable insights into the degradation behavior and bacterial colonization of compostable plastic in a simulated marine environment. In conclusion, compostable plastics are poorly degraded in the marine environment which can cause environmental hazards similar to noncompostable plastics. The temporal dynamics of the bacterial communities colonizing the compostable polymers showed a strong selection process during the early colonization of the different plastics, leading to a mature biofilm, and this selective pressure decreased over time. The microbial community structure varied across time and was unique for PBAT. For the first time, a high proportion of archaea was observed on the plastic surface, increasing with immersion time. Supplementary studies focusing on the role of Archaea in the plastisphere should be carried out to better understand their role in the plastisphere. Moreover, the eco-design of new recyclable biodegradable plastics with optimal degradability properties in not only one (e.g., industrial composting) but all environmental compartments (e.g., marine water, freshwater, soil, home composting) must be developed, and plastic use should be minimized. These new polymers should be recyclable with greener methods, e.g., enzymatic or chemical pathways, and biodegradable to limit the environmental impact.

CRediT authorship contribution statement

Alice Delacuvellerie: Methodology, Formal analysis, Investigation, Writing- original draft preparation, Conceptualization. **Axelle Brusselman**: Investigation, Writing- Review & Editing. **Valentine Cyriaque:** Formal analysis, Writing- Review, Critical thinking. **Samira Benali**: Resources, Writing- Review. **Sébastien Moins**: Resources. **Jean-Marie Raquez**: Resources, Writing- Review & Editing. **Sylvie Gobert**: Methodology, Resources, Writing- Review & Editing. **Ruddy Wattiez**: Supervision, Conceptualization, Methodology, Resources, Writing- Review & Editing.

Declaration of competing interest

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

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.2023.114711.

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