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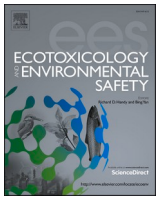

Evolution of prokaryotic colonisation of greenhouse plastics discarded into the environment

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ABSTRACT

Current knowledge on the capacity of plastics as vectors of microorganisms and their ability to transfer microorganisms between different habitats (i.e. air, soil and river) is limited. The objective of this study was to characterise the evolution of the bacterial community adhered to environmental plastics [low-density polyethylene (LDPE)] across different environments from their point of use to their receiving environment destination in the sea. The study took place in a typical Mediterranean intermittent river basin in Larnaka, Cyprus, characterised by a large greenhouse area whose plastic debris may end up in the sea due to mismanagement. Five locations were selected to represent the environmental fate of greenhouse plastics from their use, through their abandonment in soil and subsequent transport to the river and the sea, taking samples of plastics and the surrounding environments (soil and water). The bacterial community associated with each sample was studied by 16S rRNA metabarcoding; also, the main physicochemical parameters in each environmental compartment were analysed to understand these changes. The identification and chemical changes in greenhouse plastics were tracked using Attenuated Total Reflection Fourier Transform Infra-red spectroscopy (ATR-FTIR). Scanning Electron Microscope (SEM) analysis demonstrated an evolution of the biofilm at each sampling location. β-diversity studies showed that the bacterial community adhered to plastics was significantly different from that of the surrounding environment only in samples taken from aqueous environments (freshwater and sea) (*p-value* p-value > 0.05). The environmental parameters (pH, salinity, total nitrogen and total phosphorus) explained the differences observed at each location to a limited extent. Furthermore, bacterial community differences among samples were lower in plastics collected from the soil than in plastics taken from rivers and seawater. Six genera *(Flavobacterium, Altererythrobacter, Acinetobacter, Pleurocapsa, Georgfuchsia* and *Rhodococcus)* were detected in the plastic, irrespective of the sampling location, confirming that greenhouse plastics can act as possible vectors of microorganisms between different environments: from their point of use, through a river system to the final coastal receiving environment. In conclusion, this study confirms the ability of greenhouse plastics to transport bacteria, including pathogens, between different environments. Future studies should evaluate these risks by performing complete sequencing metagenomics to decipher the functions of the plastisphere.

1. Introduction

The rapid development of synthetic polymers, the main constituent of plastics, caused revolutionary progress in the past century (Andrady and Neal, 2009). Plastics vary in chemical structure and can be manufactured in various shapes to meet the demand of multiple uses, including packaging, building, automotive, electronic, household and agriculture. A total amount of 368 million tonnes of plastics were

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produced in the world in 2019 to cover this demand, 9 million more than in 2018 (PlasticsEurope, 2020). The widespread use of plastic and improper post-consumer management disseminates plastic debris into the environment. Plastic debris acts as a persistent pollutant in receiving environments (Pazienza and De Lucia, 2020), such as terrestrial (Rillig and Lehmann, 2020; Baho et al., 2021), freshwater (Li et al., 2021d; Zhang et al., 2017) and marine ecosystems (Pattiaratchi et al., 2021; Lebreton et al., 2018). Low-density polyethylene (LDPE) is particularly interesting among thermoplastics since it is widely used for agricultural purposes. The European demand for LDPE is estimated at 8.85 million tonnes, the second most used plastic after polypropylene (PlasticsEurope, 2020). LDPE is the primary material used for protected cultivation in greenhouse plastics since it has relatively good mechanical and optical properties, extended useful life and a low price (Briassoulis, 2005). Greenhouse plastics are widely used in the Mediterranean, facilitating the all-year cultivation of vegetables (Saltuk, 2018). They fragment during in-service conditions making them functional for 1–4 seasons (Dehbi et al., 2017; Dilara and Briassoulis, 1998). The improper management of end of use greenhouse plastics generates high volumes of waste that usually get disposed of in fields, near water bodies or simply burnt. The problem arises when greenhouses are dismantled, producing a vast amount of plastic waste, estimated to have reached more than 850 million metric tons in 2019 globally (Afxentiou et al., 2021; Scarascia-Mugnozza et al., 2012). Ultimately, discarded greenhouse plastic debris finds its way to riverbeds and is eventually dragged into the sea.

The plastic interactions with receiving environments and when moving between environmental compartments are not fully understood yet. In this context, Bank and Hansson (2019) use the terms “biogeochemical cycle” and “plastic cycle” to describe the processes occurring when plastics move between compartments of the receiving environment. Understanding the “plastic cycle” is pivotal to identifying potential risks posed to the ecosystems from the trophic transfer of plastics (Cox et al., 2019; Latchere et al., 2021). Plastics are hydrophobic and are known to adsorb and then transport toxic chemicals such as PCBs, PBDEs, PAHs and DDTs (Wang et al., 2018). By modifying their structure, plastics retain nutrients and microorganisms adhered to them, leading to an increasing C:N ratio in the long term due to their decomposition in the soil (Rillig et al., 2019). Plastics create a new type of habitat for the biota, mainly microorganisms, also known as the plastisphere (Zettler et al., 2013), which is prone to changes when moving between environmental compartments (Li et al., 2021a). Some studies examined the colonisation of plastics in soil (Puglisi et al., 2019); however, very limited information about the plastisphere continuum exists (Latchere et al., 2021).

This study aims to describe the bacterial greenhouse plastisphere during its lifecycle by characterising the evolution of the community from the time the greenhouse plastic is in use, discarded and transferred between soil, river and sea environment. Specifically, the bacterial community of LDPE is compared via microscopy and metabarcoding to the one of surrounding environments at (1) the point of use; (2) soils; (3) a riverbed at various distances from the point of use (both dry and water-covered riverbed) and (4) a sea site. To investigate whether plastics act as vectors of bacteria between environmental compartments, we hypothesise that the plastisphere differs from the bacterial communities of the receiving environments at each sampling location. To the authors’ knowledge, no previous study investigates how the plastic-associated bacterial community changes during its lifecycle from its initial point of use towards receiving environments. This is the first study confirming that greenhouse plastics act as a vector for certain bacteria, thereby allowing the transfer of microorganisms between different environmental compartments.

2. Material and methods

2.1. Study area and sampling strategy

An intensive agricultural region representative of Mediterranean agricultural locations in the Larnaka district, Cyprus, was selected. It is located between Maroni and Zygi villages, with extensive greenhouse plastic use. Agriculture accounts for 2% of gross domestic product and 13.5% of national exports in 2019, an important part of the country’s economy (Adamides, 2020). Cyprus has a typical Mediterranean climate, so farming methods are adapted to the high summer temperatures and limited water supply favouring its location by rivers and the prevalence of small and fragmented farm holdings, which promotes the development of small greenhouse exploitations primarily used for early horticultural crops (Adamides, 2020). In particular, the area selected (shown in Fig. S1a in Supplementary Material 1) has an estimated cultivating area of 78.3 ha in 2016 and an estimated greenhouse plastic use of approximately 250 tonnes (Afxentiou et al., 2021). Sampling was carried out in the Maroni river basin, a typical intermittent Mediterranean river, during the dry phase of the river.

Five sampling locations were selected to assess the changes of the bacterial communities during the greenhouse plastics transfer to the sea. G1 is a sampling location where greenhouse plastic is in use. CG2 is located 20 m from the greenhouse and 30 m from the river, where many greenhouse plastics were discarded. The R3 sampling location is 1.5 km downstream of CG2 in the dry riverbed. The R4 sampling location is 400 m downstream from R3 in the river delta next to the sea, where water was still available. Finally, the S5 sampling location is in the sea, 50 m from R4. More information about the location, type of sample collection and images of each sampling location is given in Table S1 in Supplementary Material 1 and Fig. S1 in Supplementary Material 1.

Large fragments of greenhouse plastics were present at the sampling locations. Three fragments (denoted as replicates) were randomly selected in each location and collected using sterile gloves. They were cut into smaller pieces using sterile scissors and stored in four sterile tubes. Plastics collected from soil (G1, CG2, and R3 plastics) were rinsed to remove soil particles using sterile Milli-Q water.

To evaluate the differences between the microbial communities of greenhouse plastics and the surrounding environment, samples were taken according to the following procedure: At G1, CG2, and R3, approximately 100 g of soil adjacent to plastics were taken and placed in sterile tubes for the metabarcoding analyses. For the rest of the analyses, 1 kg of soil was sampled and stored in a sterile plastic bag for further processing in the laboratory. At R4 and S5, 3 L of water were collected in sterilised glass bottles and kept in the dark. All the samples were collected on the same day (July 15th, 2019).

Immediately after sampling, all samples were transported to the laboratory at 4 °C using cooling boxes. 1 L water was filtered through 2.7 μm glass Millipore filters to retain the particulate material in suspension. Subsequently, 250 mL of the filtered water was further filtered by 0.22 μm sterile membrane Millipore filters to collect the free-living microbial community. The process was repeated three times to obtain three replicates. Two tubes containing plastics were kept frozen at −20 °C until performing DNA extraction, along with soil and filter samples. The two tubes were stored at 4 °C to be used for further analyses, as explained below.

2.2. Nutrients and physicochemical analysis

In water samples (R4 and S5), the pH, temperature and conductivity were measured *in situ* using an ExStik II multiparameter probe (pH/conductivity EC500, Extech Instruments, USA). Dissolved oxygen was measured using a Hanna HI98193 oximeter (Hanna Instruments, USA). Water from R4 and S5 was analysed for nutrients. Nitrate (NO_3^-), nitrite (NO_2^-), ammonium ions (NH_4^+) and orthophosphate ions (PO_4^{3-}) concentrations were measured using Spectroquant Tests (Merk Millipore,

USA) following the instructions indicated by the manufacturer with a Spectroquant Pharo 100 spectrophotometer (Merck Millipore, USA). Total inorganic nitrogen of water samples (TIN) was calculated by summing NO_3^-, $NO_2^-$and NH_4^+ values. From G1, CG2 and R3 soils, pH, conductivity, bulk density, Total Organic Carbon (TOC), the total nitrogen (TN) and total phosphorus (TP) concentrations, and soil texture (which includes % sand, % silt, and % clay) were determined. The bulk density was measured *in situ*, collecting the sample in an aluminium tube of a given volume and measuring its weight. Soil samples of 1 kg were passed through a 2 mm sieve to remove large particles. In addition, 10 g of soil dispersed into 25 mL of Milli-Q water were used for pH measurements. A similar procedure was followed for conductivity, evaluating a soil/water suspension, but the ratio between soil and water was 1:4. The soil texture was assessed using particle size analysis based on the hydrometer method (Bouyoucos, 1962); the total organic carbon (TOC) was calculated using the loss on ignition method (Heiri et al., 2001); the total nitrogen was measured by the Kjeldahl method (Bremner, 1960), and the phosphorus concentration was determined using the sulfomolybdo-phosphate method (Tan, 1996).

2.3. Identification and assessment of weathering of greenhouse plastics using ATR-FTIR

The chemical composition of plastics was assessed using ATR-FTIR to ensure that the plastics collected at the different sampling locations were LDPE from greenhouses. The organic matter covering plastic specimens was removed by digestion with H_2O_2 (33% w/v) and heating at 60 °C for 24 h. ATR-FTIR spectra were obtained using a ThermoScientific Nicolet iS10 apparatus with a Smart iTR-Diamond ATR module. Spectra were taken in the 4000–650 cm^{-1} range with a resolution of 4 cm^{-1} (data spacing of 0.483 cm^{-1}). A minimum of five spectra were taken per specimen at five different points. The spectra were compared with the library provided by the OMNIC Spectra software v 9.1.26 using Pearson's correlation (Aldrich and Goodfellow library, Thermo Fisher Scientific Inc., USA). The minimum matching for identification was set to 80% (Rios Mendoza et al., 2018).

Three indexes were used to estimate the possible weather-related change in plastics between sampling locations according to previous research (Brandon et al., 2016): carbonyl, carbon-oxygen and hydroxyl index. These indices were calculated as the quotient of the peak height of carbonyl groups (1550–1810 cm^{-1}), carbon-oxygen (1000–1200 cm^{-1}) and hydroxyl groups (region of 3300–3400 cm^{-1}) to a reference peak (2920 cm^{-1}), which corresponds to the C-H asymmetric stretching vibration (Brandon et al., 2016). Before calculating indexes, the spectral baselines were corrected (OMNIC Spectra software v 9.1.26).

2.4. Scanning electron microscopy analysis

The qualitative assessment of the biofilm structure and cellular integrity on greenhouse plastics was performed using SEM. Briefly, one randomly selected piece of plastic of 3 cm^2 (from the sterile tubes described in Section 2.1) was cut into smaller pieces and immersed in 4% paraformaldehyde solution for 30 min to fix the biofilm. Afterwards, the supernatant was removed and washed three times with 1X phosphate-buffered saline. Three replicates per sample were dried at room temperature overnight. The samples were gold-sputtered (32 nm thick films) using an SC7640 Sputter coater (Quorum Technologies, UK) and evaluated using a Quanta 200 microscope (FEI, USA).

2.5. Microbial diversity analysis

2.5.1. DNA extraction

Plastics of 10 cm^2 and water filters were cut into smaller pieces and transferred to 2-mL tubes. DNA extraction was performed based on a phenol:chloroform extraction method followed by absolute ethanol precipitation as previously described by Martínez-Campos et al. (2021). Briefly, 400 μL of Tris 10 mM – EDTA 0.1 mM (7.5 pH), 0.010 g of silica beads, 20 μL of 10% SDS and 250 μL hot ultrapure phenol (pH 8, 65 °C) were added in each tube. The samples were then vortexed for 1 min and heated to 65 °C for 1 min in three repeating cycles. 250 μL chloroform were added, and samples were vortexed and frozen 6 times. Finally, samples were centrifuged at 13,000 min^{-1} at 4 °C for 20 min. The supernatant was transferred to a new Eppendorf 1 mL hot phenol (pH 8, 65 °C) was added, and the tubes were centrifuged at 13,000 min^{-1} at 4 °C for 5 min. This step was repeated once. Next, the supernatant was placed in a new Eppendorf, and 1 mL chloroform was added. The sample was mixed by shaking 10 times and centrifuged at 13,000 min^{-1} at 4 °C for 5 min. Finally, the supernatant of the Eppendorf tubes that belonged to the same sample were mixed, and ethanol was added (double volume of ethanol than supernatant). The sample was then mixed and frozen at − 20 °C overnight to precipitate the DNA. The following day, the samples were centrifuged at 13,000 min^{-1} at 4 °C for 20 min. Samples were dried, and 40 μL of Milli-Q water was added to resuspend the DNA, the concentration of which was measured spectrophotometrically (NanoDrop™ 1000 Spectrophotometer, Thermo-Scientific, USA).

2.5.2. Metabarcoding

Twenty-three samples were used for DNA metabarcoding, including 15 greenhouse plastics (3 samples from each sampling location) and 8 samples from surrounding environments (soil, freshwater, and seawater). Library preparation was performed as instructed by the Illumina workflow at AVVA Pharmaceuticals (de Muinck et al., 2017). Briefly, two consecutive PCR reactions were performed using KAPA HiFi HotStart (KAPA Biosystems, USA). During PCR1, PCR amplicon was produced using 12.5 ng of DNA template and the following primers, including adaptor sequences: 16 S Amplicon PCR Forward Primer (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAGCCTACGGGNGGCWGCAG) and 16 S Amplicon PCR Reverse Primer (5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-CAGGACTACHVGGGTATCTAATCC) to amplify the 16 S V3 and V4 regions, respectively. PCR2 was performed by attaching dual indices and Illumina sequencing adaptors using the Nextera XT Index Kit. PCR clean-up was performed between PCR reactions using AMPure XP beads (Beckman Coulter, UK) according to the manufacturer's instructions. The final pool was sequenced on an Illumina MiSeq paired-end 2x250bp V3 sequencing programme.

2.5.3. Bioinformatics and data analysis

The analysis of the Illumina MiSeq results was performed using the DADA2 pipeline, which uses the amplicon sequence variants (ASV) (Callahan et al., 2017, 2016) using R v 3.6.2 (Rstudio, 2020). Briefly, quality profiles of the reads were evaluated using the plotQualityProfile function. Quality filtering, denoising, merging and removing chimeric sequences were applied to the dataset. Taxonomic assignment was performed using the Silva 132 99% OTU Database with a bootstrap threshold of 75% (Callahan, 2018).

α-diversity analysis, including the Gini Index (Gini, 1912) and Shannon Diversity Index (Shannon, 1948), was performed via alphaDiversity function from the otuSummary package (Yang, 2018). The Gini coefficient is a ratio between 1 and 0, measuring the inequality, whereas the Shannon index calculates species uniformity. The differences found between samples were estimated using the Kruskal-Wallis statistic method, and results were plotted using ggplot2 v 3.3.2 function of the tidyverse package (Wickham et al., 2019).

For β-diversity analysis, two methods were employed to compare the similarity of bacterial communities among samples. First, a hierarchical treemap based on the Bray-Curtis similarity matrix (Beals, 1984) was combined with a heatmap based on ASV abundance using the hclust function from the stats package (Team, 2013) to identify the most similar samples. The significant differences between samples (confidence interval 95%) were assessed using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), considering 999

permutations.

Distance-based redundancy analysis (db-RDA) was performed to establish the correlation between environmental parameters and the bacterial community attached to each substrate using the dbrda function of the vegan package (Oksanen et al., 2013). The analysis was performed based on the Bray- Curtis similarity matrix. The environmental factors considered were nitrogen, phosphorus, salinity and pH. To perform a linear regression analysis, the function envfit of the vegan package was used. Envfit shows the maximum correlations between environmental variables and the ordination configuration. The length of the vectors represents the strength of the correlations (Oksanen et al., 2013). The "anova.cca" function of the vegan package (Oksanen et al., 2013) with 999 permutations was used to perform the significance test of db-RDA. The linear discriminant analysis effect size method (LEfSe) (Segata et al., 2011) was used to determine the differentially more abundant taxa (up to genus level) in sampled plastics and their surrounding environments. This analysis was performed with the LEfSe online tool available in the Galaxy framework, using default settings for data formatting. LDA (Linear discriminant analysis) effect size was performed using the strategy for multi-class analysis one-against-all.

Venn diagrams mine the plastics' common and unique bacterial genera at different sampling locations. The same method was applied to evaluate bacterial communities changes between plastic substrates and their surrounding environments at each sampling location using the "Bioinformatics & Evolutionary Genomics" tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.5.4. Accession number

The sequences data obtained in this study were submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) under the Bioproject accession number: PRJNA747817.

3. Results

3.1. Spectroscopic analyses

Except for plastics taken directly from the greenhouse at G1, the selection of plastics was carried out *in situ* by visual inspection. The identification of the plastics was confirmed using ATR-FTIR spectroscopy. All spectra (Fig. 1) showed characteristic absorption bands at 2915 cm^{-1} and 2848 cm^{-1} (CH_2 asymmetrical and symmetrical stretching), at~1460 cm^{-1} (CH_2 bending), a small absorption band at 1370 cm^{-1} (bending of -CH_3 terminal groups that only appeared in LDPE) and a double band in the region of ~ 728^{-1} cm and ~ 718 cm^{-1} (corresponding to CH_2 rocking deformation in the amorphous phase and crystalline phase respectively). These are the native bonds present in LDPE (Rajandas et al., 2012). Pearson correlations (Table S2 in Supplementary Material 1) confirmed this result with a matching of over 80% in all samples. Some small peaks between ~ 1550–1810 cm^{-1} correspond to carbonyl stretching vibration. A broad absorption band of vibrations at 1037–1012 cm^{-1} indicates a C-O stretching vibration. Both result from the oxidation of the backbone of LDPE.

The weathering indices (carbonyl groups, carbon-oxygen, hydroxyl, shown in Table S2 in Supplementary Material 1) indicate major photo-oxidation of CG2 plastics (summation of three indices: 1.97) followed by R4 and R3 plastics (0.79 and 0.66, respectively). S5 and G1 plastics were the least photo-oxidated (0.56 and 0.5, respectively). However, the hydroxyl index does not indicate a substantial plastic degradation, in contrast with the carbonyl groups and carbon-oxygen ratios.

3.2. Visualisation of bacterial communities onto greenhouse plastics

A detailed examination using Scanning Electron Microscopy (Fig. 2) confirmed the presence of microbial communities and intact microorganisms on the surface of the plastics. Collected plastics showed fouling covering the surface (Fig. S3 in Supplementary Material 1). The fouling

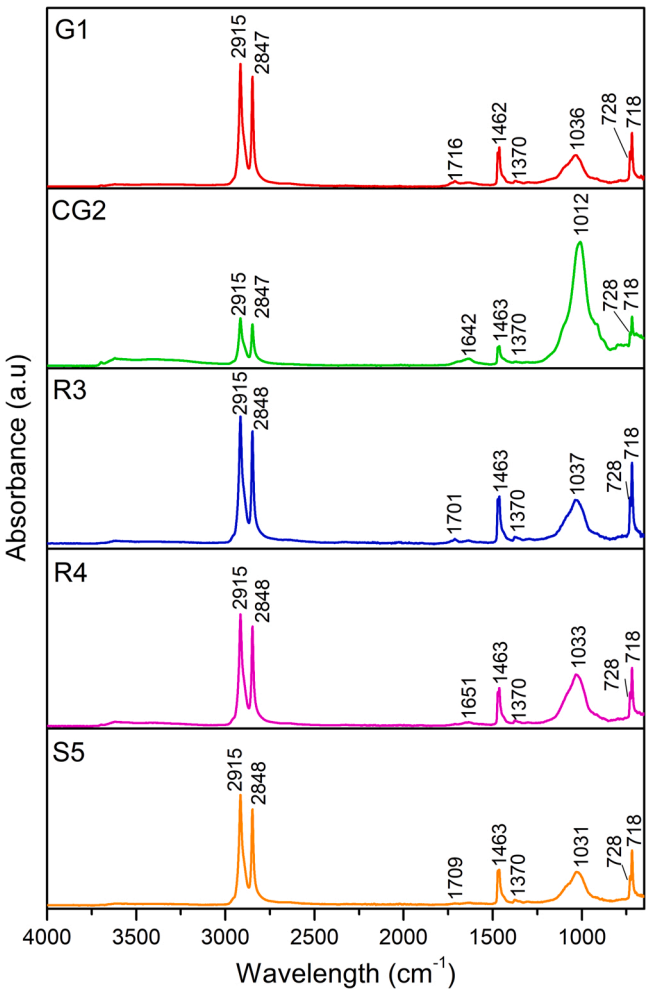

Fig. 1. ATR—FTIR spectra of plastics collected in each sampling location: G1: greenhouse sampling location; CG2: sampling location close to the greenhouses; R3: dry riverbed; R4: end of the river near the sea; S5: sea, near the shoreline.

on plastics increased as the distance from G1 increased, from G1 plastics to the plastic sample in the sea (S5 plastics). At the same time, the abundance of microbes and their distribution on the plastics changed substantially between sampling locations. In addition, a smooth surface primarily characterised G1 plastics with the scattered presence of crystalline structures and diatoms. A true biofilm was not observed, but coccoid- and rod-shaped bacteria embedded in extracellular polymeric substances (EPS) could be seen in hollows around the crystals. Moreover, CG2 plastics had a higher number of crystals, and a clear biofilm spread over the entire surface of the plastic. The biofilm density prevented the clear visualisation of microorganisms embedded in it. R3 plastics had thick inorganic fouling covering all surfaces. The presence of biofilm was limited to the cracks and holes generated in this inorganic fouling. A very dense microbial community was present on the surface of submerged R4 and S5 plastics. Rod-shaped bacteria, diatoms and fungal hyphae dominated R4 plastics. The inorganic fouling forming small crystals was more significant over the biofilm. S5 plastics had a mature biofilm, with a major dominance of Vibrio-shaped bacteria embedded in EPS with a relatively rough surface. The overall biofilm extent was more significant in the greenhouse plastics submerged in water (R4 and S5 plastics) compared to plastics collected from soil (riverbed, CG2 and R3 plastics) or in use (G1 plastics).

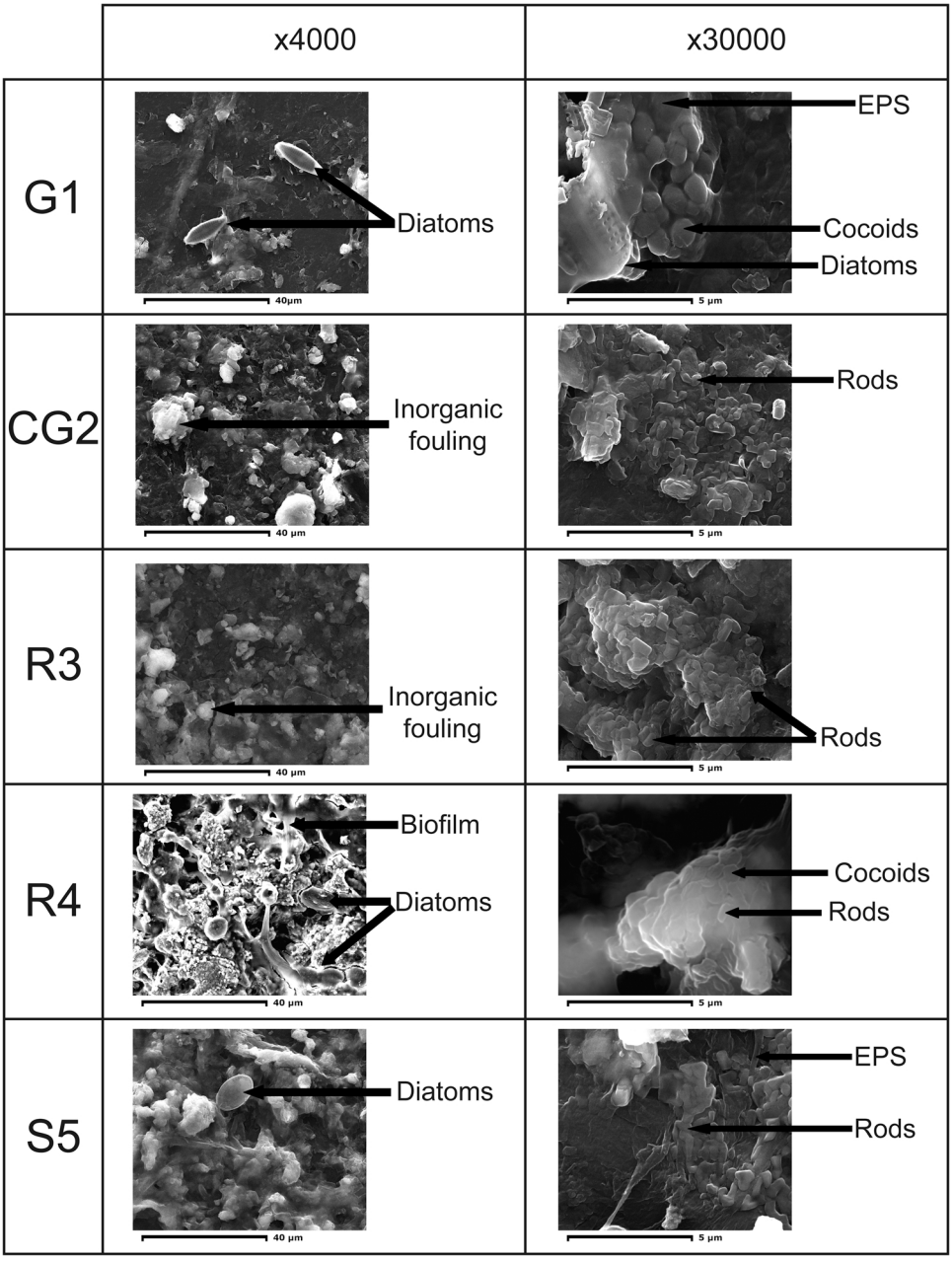

Fig. 2. Scanning electron microscope images of plastics collected in each sampling location. The first column shows lower magnification to appreciate the development of the biofilm. The second column showed the presence of microorganisms in larger magnification. Legend of sampling locations: G1: greenhouse sampling location; CG2: sampling location close to the greenhouse; R3: dry riverbed; R4: end of the river near of the sea; S5: sea near the shoreline. Abbreviations meaning: EPS, extracellular polymeric substances.

3.3. Metabarcoding of greenhouse plastic bacterial communities and surrounding environments

3.3.1. Sequencing data pre-processing and ASVs assignment

Each sample produced at least 65,023 reads after Illumina sequencing, with a total of 3409,254 reads for all samples. After removing the adaptors, filtration of the sequences, merging, and removal of possible chimeras, 1,148,609 high-quality reads remained. The remaining sequences were associated with 8829 ASVs.

3.3.2. α-diversity analysis

Bacteria diversity was initially assessed using the Gini coefficient and Shannon index (Fig. 3). Gini coefficient results were higher than 0.95 (the lower value corresponding to freshwater with 0.96 ± 0.01), indicating that specific taxa dominated the bacterial community. Global ANOVA indicated significant differences between samples (*p-value* < 0.05) but the pairwise comparison only demonstrated significant differences (ANOVA *p-value* < 0.05; Table S3 in Supplementary Material 1) between R4 plastics and freshwater. This suggests a lower bacterial community diversity associated with plastics than the surrounding freshwater environment. The values obtained by the Shannon index were, in general, slightly lower for plastics (average value of 4.28 ± 0.53) compared to the surrounding environment (average value of 4.78 ± 0.68). Despite that, no significant differences were found in the global ANOVA (*p-value* > 0.05) or the pairwise ANOVA tests (Table S4 in Supplementary Material 1

3.3.3. Bacterial community composition

All obtained ASVs were compared with SILVA 132 database to obtain its taxonomy classification. Forty-two bacterial phyla classified in 85 classes were identified in the whole sample set. The complete taxonomical assignation can be found in Supplementary Material 2.

Bacterial taxonomy distribution at the phylum level in all samples is presented in Fig. 4. The most abundant phyla, independent of the type of sample (plastic, soil, or water) or sampling location, were Proteobacteria (43.6%) followed by Bacteroidetes (16.3%), Cyanobacteria (13.6%),

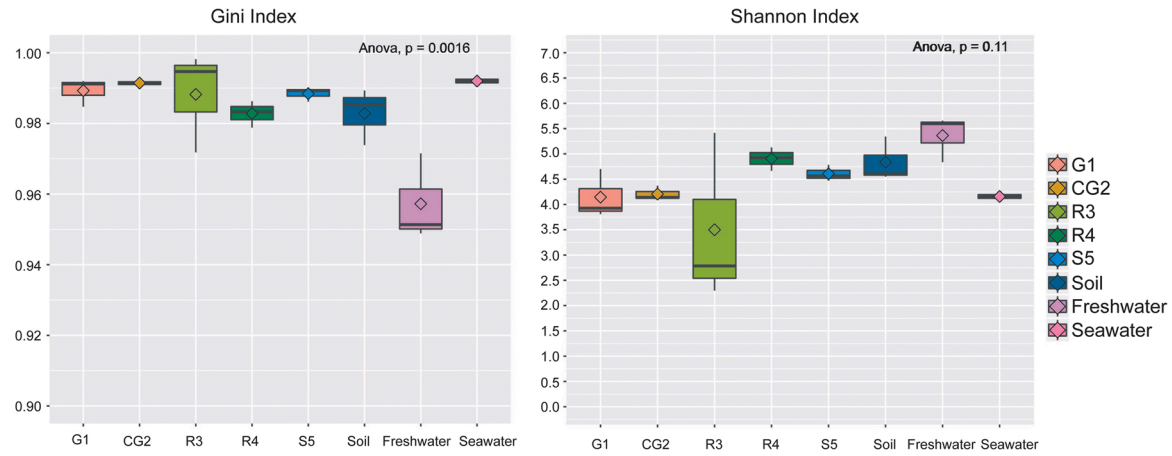

Fig. 3. Results of the α-diversity analysis using Gini Index and Shannon Index in the plastics collected from each sampling location (G1, CG2, R3, R4, and S5) in comparison with the surrounding environment of each sampling location: soil (from G1, CG2, and R3), river freshwater (R4) and seawater (S5).

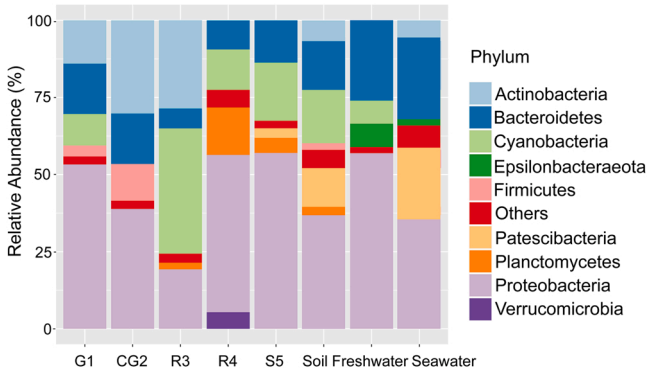

Fig. 4. Relative abundance of prokaryotic community based on 16S rRNA metabarcoding at the phylum level.

and Actinobacteria (10.9%). Although Proteobacteria were dominant in most collected plastics (G1, CG2, R4, and S5 plastics), Cyanobacteria predominated on R3 plastic (40.6%). Regarding surrounding environmental samples, the soil had Proteobacteria (36.87%), Cyanobacteria (17.3%), and Bacteroidetes (15.8%). The phyla Proteobacteria (35.49%), Bacteroidetes (26.5%), and Pastecibacteria (23.22%) were more abundant in freshwater. The most abundant phyla in seawater were Proteobacteria (56.92%), Bacteroidetes (26%), and Epsilonbacteraeota (7.6%).

The most abundant classes detected in plastics, independently of the sampling location, were Alphaproteobacteria (39%), Oxyphotobacteria (16.7%), Actinobacteria (14.6%), and Bacteroidia (11.33%). Alphaproteobacteria (19.1%), Oxyphotobacteria (17.2%), Bacteroidia (15%), and Gammaproteobacteria (14.4%) were dominant in the soil samples. Bacteroidia (26.3%), Gammaproteobacteria (18.6%), Alphaproteobacteria (15.45%) and Parcubacteria (15.45%) were dominant in freshwater. In seawater, the classes with the highest abundance were Alphaproteobacteria (34%), Bacteroidia (25.5%), Gammaproteobacteria (22.8%), and Campylobacteria (7.6%).

At the order- and the family- levels, the bacterial distribution differed between the plastics and the surrounding environments at each location. G1 plastics were colonised by the orders Rhizobiales (28.4%; represented mainly by the family Beijerinckiaceae with a 26.9% abundance), Sphingomonadales (14.5%; family Sphingomonadaceae), and Cytophagales (13.4%) represented by the families Hymenobacteraceae (11.81%) and Spirosomaceae (1.6%). On CG2 plastics, the orders with the highest abundance were Cytophagales (15.6%; family Hymenobacteraceae represented 15.5% of total abundance), Micrococcaceae (14.8%; Micrococcaceae represented 13.0%), and Sphingomonadales (12.8%) represented primarily by Sphingomonadaceae (12.8%). The orders Kineosporiales (17.0%; represented by family Kineosporiaceae), Frankiales (5.3%; family Geodermatophilaceae: 4.8%), Sphingomonadales (5.1%; totally represented by the family Sphingomonadaceae) had a higher relative abundance on R3 plastics. The surrounding soil environment was dominated by Saccharimonadales (7.7%), Rhodobacterales (6.9%; represented by the family Rhodobacteraceae: 6.8%), and Flavobacteriales (6.8%; represented by Flavobacteriaceae: 5.1%). On R4 plastics, a substantial change could be observed in the taxonomical distribution at the order level, with the higher abundances of Rhodobacterales (25.2%; family Rhodobacteraceae: 25.24%), Sphingomonadales (13.3%; family Sphingomonadaceae: 13.3%), Pirellulales (12.0%; family Pirellulaceae: 11.9%). In freshwater, the orders with the highest presence were Flavobacteriales (19.5%; family Cryomorphaceae: 12.8%), Rhodobacterales (10.36%; Rhodobacteraceae: 10.4%), and Betaproteobacteriales (9.7%). The bacterial community of S5 plastics was represented by Rhodobacterales (36.8%; family Rhodobacteraceae: 36.8%), Rhizobiales (10.1%; family Rhizobiaceae: 8.8%), and Phormidesmiales (9.8%; family Phormidesmiaceae: 9.8%). In seawater, the orders with significant abundance were Flavobacteriales (25.1%; family Flavobacteriaceae: 18.9%), Vibrionales (13.9%; family Vibrionaceae: 13.9%), and Rhodospirillales (11%).

3.3.4. *β-diversity analysis*

The differential bacterial taxonomy distribution suggests significant variations between the plastics at each location and between the plastics and their surrounding environments (soil, freshwater, or seawater). The distribution of the samples is presented in the db-RDA ordination plot (Fig. 5). In general, the plastics were more distant between different sampling locations than their surrounding environment, showing a consistent pattern depending on where the plastics were collected (soil, freshwater, or seawater). Global PERMANOVA analysis (Table S5 in Supplementary Material 1) confirmed significant differences between all samples (*p-value* < 0.05). Furthermore, the PERMANOVA test comparison between the plastics and the surrounding environments (PERMANOVA *p-value* < 0.05) also confirmed significant differences. Plastics collected from soil showed minor differences, distributed along the second axis (8.9%). Replicates from G1 and CG2 plastics were ordinated together, constituting the same cluster without significant differences between them (pairwise PERMANOVA test *p-value*: 0.07), but significantly different from R3 plastic (pairwise PERMANOVA test *p-value* < 0.05). R4 and S5 plastics were distributed along the first axis (15.3%), denoting a major difference in comparison with the greenhouse plastics collected from soil (pairwise PERMANOVA test *p-value* < 0.05) and significantly different between them (pairwise PERMANOVA test *p-value*

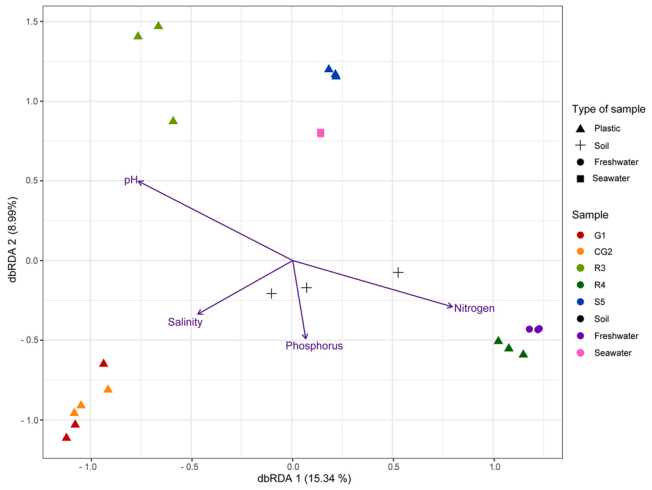

Fig. 5. Distance-based redundancy analysis (dbRDA) ordination plot based on Bray-Curtis dissimilarity of 16S rRNA metabarcoding and environmental variables between the different environments selected in this study (soil and water). Each point in the ordination plot represents the community in a given sample.

< 0.05). Furthermore, pairwise PERMANOVA comparison between plastic and their corresponding environment indicated that G1, CG2, and R3 plastic bacterial communities were not significantly different from soil (pairwise PERMANOVA *p-value* > 0.05). In contrast, R4 and S5 plastics had significantly different bacterial communities than their surrounding environment (*p-value* < 0.05). The hierarchical clustering tree based on the Bray-Curtis matrix (Fig. S4 in Supplementary Material 1) confirmed these results. To explain the possible influence of environmental parameters in the evolution of the bacterial community adhered to greenhouse plastics, the environmental variables measured in the soil (Table S6 in Supplementary Material 1) and the water (Table S7 in Supplementary Material 1): pH, salinity, total nitrogen, and total phosphorus were used in the db-RDA analysis. In general, locations in dry conditions were characterised by higher values of salinity and pH (G1, CG2, and R3), in contrast with the R4 and S5 with higher values of nutrients (total nitrogen and phosphorus). The high nitrogen values at R4 explained the eutrophication observed during the sampling day. The analysis (Table 1) confirmed a significant influence of the pH, TN, salinity, and TP (*p-value* < 0.05). The environmental parameter with the most significant influence was pH (7.9% of explained variation), followed by TN (3.75%), salinity (3.6%), and TP (1.8%). The model only explained 19.6% of the variation, suggesting a low correlation between samples (summation of the explained variables had comparable values).

LEfSe analysis of the plastic bacterial communities at each sampling location (Table S8 in Supplementary Material 1) revealed significant differences in the abundance of some genera. G1 plastics were dominated by *Methylobacterium*, *Sphingomonas*, *Frigobacterium*, *Pantoea*, *Weisella*, *Corynebacterium*, *Bacillus*, *Turicibacter*, *Curtobacterium*, *Jeotgalicoccus*, and *Clostrium_sensu_stricto_1*. The genera *Hymenobacter*, *Arthrobacter*, *Massilia*, *Kocuria*, *Paracoccus*, *Planomicrobium*, *Modestobacter*, *Kineococcus*, *Kineosporia* and *Rhizorhapis* were more abundant on the CG2 plastic. On R3 plastic, the genera with higher abundance were *Geodermatophilus*, *Nocardiopsis, Marmoricola, Quadrisphaera*, *Roseomonas*, *Blastococcus*, *Skermanella*, *Tepidisphaera*, *Pseudomonas* and *Actinomycetospora*. Plastics collected from the freshwater aquatic environment (R4 plastics) had a higher abundance of specific taxa, including *Porphyrobacter*, *Rhodopirellula*, *Tabrizicola*, *Rubribacterium*, *Ketogulonicigenium*, *Luteolibacter*, *Sandaracinobacter, Sandarakinorhabdus, Germmobacter, Terrimicrobium, Rhodobacter, Legionella* and *Runella*. This result coincides with that obtained in the beta diversity analysis, which shows a greater difference in the bacterial community R4 plastic than the other plastic samples. In contrast, on S5 plastics, the genera *Rubrivirga*, *Maribius, Loktanella, Lewinella, Pseudahrensia, Parvularcula, Erythrobacter, Algimonas, Truepera* and *Granulosicoccus* were dominant.

Furthermore, LEfSe analysis was used to determine differentially abundant genera between each plastic and its surrounding environment. The bacterial community attached to G1 plastic (Table S9 in Supplementary Material 1) was characterised by *Rhizorhapis, Jeotgalicoccus, Fructobacillus, Romboutsia, Aureimonas, Turicibacter, Emticicia,* and *Rhodococcus*. Genera *Hymenobacter, Arthrobacter, Methylobacterium, Planococcus, Sphingomonas, Planomicrobium, Roseomonas, Modestobacter, Kineococcus, Geodermatophilus* and *Marmoricola* were enriched in biofilms colonised on CG2 plastic (Table S10 in Supplementary Material 1). On R3 plastic (Table S11 in Supplementary Material 1), the characteristic genera were *Geodermatophilus, Methylobacterium, Nocardiopsis, Marmoricola, Hymenobacter, Roseomonas, Fiedmanniela, Arthrobacter, Aquipuribacter, Blastococcus* and *Rhodococcus*. In soil locations, the genera *Acinetobacter*, *Micrococcus*, *Delftia* and *Acidibacter* were more abundant (Tables S9–S11 in Supplementary Material 1).

The plastics collected in the aquatic environments showed a significant number of specific genera constituting the plastisphere than the soil plastic. Genera *Porphyrobacter, Rhodopirellula, Tabrizicola, Rubribacterium, Algoriphagus, Ketogulonicigenium, Luteolibacter, Sandaracinobacter* and *Roseomonas* were significantly more abundant on the R4 plastic (Table S12 in Supplementary Material 1). In contrast, the characteristic taxa in freshwater were *Fluviicola, Sediminibacterium, Limnobacter, Hydrogenophaga, Rheinheimera, Arcobacter, Perlucidibaca*, *Vogesella, Flavobacterium, Marivivens* and *Vibrio*. Bacterial communities in S5 seawater (Table S13 in Supplementary Material 1) were dominated by *Vibrio, Arcobacter, Formosa, Catenococcus, Nereida, Shimia, Phaedactylibacter, Marinomonas, Reichenbachiella* and *Fluviicola*. In contrast, S5 plastics were dominated by *Rubrivirga, Maribius, Loktanella, Lewinella, Perudahrensia, Parvularcula, Erythrobacter, Aquimarina, Algimonas and Nonlabens.*

3.3.5. Plastic-associated bacterial genera

The Venn diagram presents the number of specific genera and those shared among the greenhouse plastics (Fig. 6). The results demonstrate that many genera are unique to a single plastic, indicating that the bacterial community attached to the plastic in each sampling location was different. Additionally, some genera were shared between the different sampling locations. Specifically, 29 genera (Table S14 in Supplementary Material 1) were common between the dry stations (G1-CG2-R3), highlighting the presence of *Kineococcus, Fibrella, Blastocatella, Novosphingobium, Rhodocytophaga, Dyadobacter, Aureimonas, Solirubrobacter, Rathayibacter, Pseudoclavibacter, Pantoea, Streptococcus, Friedmanniella, Staphylococcus, Stenotrophomonas, Bacillus, Aeromicrobium, Rhizorhapis, Variovorax, Lactobacillus* and *Salana* as dominant.

Thirty-one genera were shared between G1, CG2, R3, and R4 plastics, including Brevundimonas, Pedomicrobium, Lamia, Chryseobacterium, Nocardiopsis, Roseomonas, Pseudomonas, Blastococcus, Spirosoma, Luteimonas, Sphingomonas, Geodermatophilus, Deinococcus, Kocuria, Paracoccus, Modestobacter, Pedobacter, Microvirga,

Table 1
Adjusted percentage of proportion variation explained by each variable in separate db-RDA analysis (gross effects). The total consideration in a single db-RDA model includes all variables (pure effects). The significance of explained variation was tested using the Monte Carlo test with 999 permutations.

Environmental factor	Df	Sum. of squares	F	*p*-value	The proportion of explained variation adjusted (%)
Salinity	1	0.78	2.18	0.009	3.57
pH	1	1.12	3.10	0.001	7.93
Phosphorus	1	0.58	1.61	0.05	1.76
Nitrogen	1	0.99	2.48	0.001	7.50
Residual	18	6.51			
Total	22				19.61

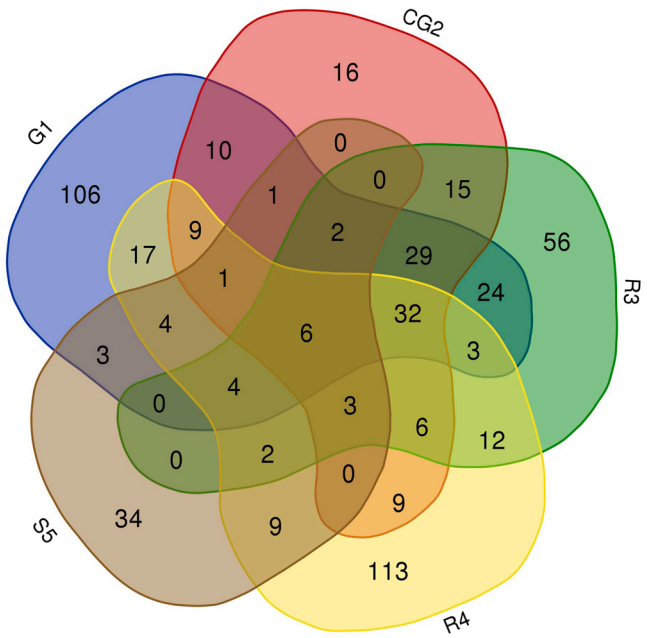

Fig. 6. Venn diagram obtained using the taxonomy assignment at the genus level. The figure is the analysis chart of the five plastics in the different sampling locations. Legend of sampling locations: G1: greenhouse sampling location; CG2: sampling location close to the greenhouse; R3: dry riverbed; R4: end of the river near the sea; S5: sea, near the shoreline.

Massilia, Arthrobacter, Rubellimicrobium, Pseudorhodobacter, Skermanella, Hymenobacter and Devosia. The S5 plastics had a lower number of shared genera. Only six genera were identified in all the plastics samples, constituting the plastic core bacteriome between the sampling locations. These genera were Flavobacterium, Georgfuchsia, Acinetobacter, Pleurocapsa_PCC-7319, Altererythrobacter and Rhodococcus.

Furthermore, the Venn diagrams detect the common genera between plastics with their surrounding environment (Figs. S4–S8 in Supplementary Material 1). There was a high percentage of common genera between the plastics and their surrounding environments at G1, CG2, R3, and R4 except for S5 plastics with only 19 genera in common with seawater Table S15 in Supplementary Material 1).

4. Discussion

Our study provides novel information about the evolution of the bacterial assemblages on greenhouse plastics along their life cycle: from the time of use to the time the plastic ends up in the sea. Previously, a meta-study investigated the bacterial assemblages present on microplastics in different environments, collecting the data from previous experiments (Wright et al., 2021). As suggested by the authors, the evident problem was that the experimental process, such as DNA extraction, selection of primers and the time of plastic incubation in the environment, can impact the final reported results, making comparisons very challenging. Furthermore, the studies that include different habitats where incubation experiments are performed (Martínez-Campos et al., 2021; De Tender et al., 2017; Puglisi et al., 2019) use artificially aged plastics (Dussud et al., 2018) or the origin of the plastic is unknown (Wu et al., 2020; Puglisi et al., 2019).

The weathering of plastics was assessed using the degradation indices defined above. The appearance of a broad band centred at 1030 cm^{-1} relates to the oxidation reactions under natural weathering facilitated by the loss of polymer stabilisers (Scoponi et al., 2000). The degradation indexes implied higher degradation for CG2 plastic. This is consistent with the loss of stabilisers in LDPE discarded after its useful life. The fact that specimens collected along the riverbed and in the shoreline displayed lower degradation bands may be explained because, once in the environment, the plastic loses superficial layers. This assumption is supported by data showing that microorganisms from soil can biodegrade the superficial layers of plastics (Chamas et al., 2020; Li et al., 2021a) and the backbone photochemical oxidation induced by the ultraviolet radiation from sunlight can favour the biodegradation of plastics (Tribedi and Dey, 2017).

The capacity of ATR-FTIR to penetrate the samples is typically 0.5–2.0 μm (Mirabella, 1992). Accordingly, the loss of superficial layers can expose less weathered plastic explaining the lower indices for samples exposed to the environment for a longer time. In their journey to the sea, as SEM images detected, the plastics may be covered with soil and later by water. For this reason, the degradation increased between sampling locations, although it did not yield a higher value than that of CG2 plastic. SEM images detected a significant inorganic layer covering the plastic surface.

Recent studies suggest that the photosynthetic degradation of LDPE in aquatic environments releases microplastics and other chemical compounds, dependent on the possible additives associated with the plastics with a substantial toxicity effect on the environment (Walsh et al., 2021). Our findings indicate a significant degradation of LDPE in terrestrial ecosystems. This process, combined with the fact that plastics are transported to rivers and later to the sea, can act synergistically, contributing significantly to the release of harmful substances to aquatic ecosystems. Additionally, our study confirms that biofilm development slows the degradation produced by sunlight, confirming the hypothesis proposed by Walsh et al. (2021).

Our study provides evidence that plastics represent a habitat that selects, to a certain extent, the bacteria that are attached to them. We found a slightly lower diversity in the plastics compared to their surrounding environments, although this was not statistically significant. In agreement with our observations, different studies have shown that when compared to a different environment such as landfill or aquatic ecosystems, lower α-diversity is observed on plastics (McCormick et al., 2014; Puglisi et al., 2019).

β-diversity analysis shows that the bacterial community attached to plastics evolves as the greenhouse plastics move towards the sea, except for G1 and CG2 plastics because these locations are similar biotopes. This confirms the findings of previous studies that location is the most important factor affecting the variation of plastic-associated bacterial communities. (Amaral-Zettler et al., 2020; Wright et al., 2021; Martínez-Campos et al., 2021). In this study, we also address the influence of environmental factors in modifying the microbial community adhered onto plastic, resulting in pH being the most relevant factor. pH was described previously as an important factor in the bacterial community developed in plastics located in soil (Li et al., 2021c). This can explain the difference between G1 and CG2 plastics and R3 plastics. Furthermore, nitrogen concentration was the second environmental factor with a key impact on the changes in the bacterial community diversity. The concentration of nitrogen-related ions was confirmed previously as an environmental factor that significantly affects the plastisphere in freshwater and seawater ecosystems (Li et al., 2021b). Still, this study confirms its influence on dry environments. The high concentration of nitrates in R4 freshwater can explain the main difference of this plastic compared to the rest. The high concentration of nitrate in the R4 may be due to the intense fertiliser application in all the peripheral crops. Moreover, the river water was largely stagnant in the dry season, without any water renewal. The effect of fertilisers in increasing nitrogen concentration in rivers has been previously demonstrated (Lassaletta et al., 2009). Phosphorus was considered another important factor affecting the community attached to the plastisphere (Amaral-Zettler et al., 2020), and this study corroborates this hypothesis. Furthermore, our study confirmed the potential role of salinity in affecting the plastisphere's community composition, as previous studies denoted in different marine ecosystems (Oberbeckmann and Labrenz, 2020). Our

study, which includes dry sampling locations (G1, CG2, R3) and freshwater (R4), denotes that the effect of this factor was not only limited to marine ecosystems but also influenced terrestrial and freshwater environments.

The significantly more abundant genera identified by Lefse in the microbial communities of the plastics at each location played different roles in the maturation of the biofilm. They adapt to their environment, confirming an evolution of the LDPE-associated microbial community as greenhouse plastic are transferred between sampling locations.

On G1 plastics, where the biofilm is in the first stage, more abundant genera were associated with this process. The high abundance genus *Methylobacterium*, usually implicated in the phyllosphere (Green and Ardley, 2018), can be explained because it was found as the primary coloniser in the plastisphere in aquatic ecosystems (Purohit et al., 2020). Furthermore, the presence of the genera *Sphingomonas* (Bereschenko et al., 2010; Martínez-Campos et al., 2018) and *Jeotgalicoccus* (Arti et al., 2020) are associated with the first stages of the formation of the biofilm under high salinity conditions, producing the EPS, which facilitates the adhesion and colonisation of other microorganisms over the plastic. The presence of primary producers, such as *Calothrix_KVSF5* and *Chamaesiphon_PCC_7430*, can stimulate biofilm growth and develop complex bacterial communities (Yokota et al., 2017). Also, the genus *Calothrix* produces microcystin (Shardlow, 2021), which could be toxic when the plastic arrives in the aquatic environment. Lastly, the presence of *Corynebacterium* can indicate the initiation of LDPE biodegradation as previous studies suggested the potential of this genus to biodegrade the polymer in marine conditions (Sudhakar et al., 2008).

On the CG2 plastics, the high abundance of *Hymenobacter,* previously detected in biodegradable plastic mulching (Bandopadhyay et al., 2020), can implicate the importance of this genus in the formation of the biofilm attached to plastics in soil ecosystems (Bandopadhyay et al., 2020). The elimination of the superficial layers on plastic in this sampling location can be explained by the significant abundance of the genera *Arthrobacter* and *Kocuria*, microorganisms with the capacity to biodegrade the LDPE in natural conditions (Bolo et al., 2015; Han et al., 2020). The high abundance of *Modestobacter*, involved in the nitrate reduction (Song et al., 2018), indicates major function activities in the microbial community attached to the plastic. R3 plastics had a significant layer of biofilm (detected using SEM). For this reason, the high abundance of the genus *Pseudomonas* is not a surprise since this genus is known for its importance in the development of biofilms (Chien et al., 2013) and its potential to degrade polymers (Abdullah et al., 2021; Sivan et al., 2006). Other genera that could be involved in the biodegradation of the LDPE are *Rhodococcus*, which have some species that only used LDPE as a carbon source (Gilan and Sivan, 2013), *Devosia*, found previously in marine plastic debris (Zettler et al., 2013) and known by its capacity of biodegrading a high number of substrates, including hydrocarbons compounds (Talwar et al., 2020) and *Nocardiopsis*, that can biodegrade LDPE and may favour the biodegradation for the rest of the microorganisms producing biosurfactant (Priyadarshini et al., 2018). Also, the high abundance of the genus *Crinalium*, a cyanobacterium common in terrestrial sandy areas with a high desiccation-resistance (Wickham et al., 2019), indicates the importance of the primary producers in the community attached to the plastic. Furthermore, the high abundance of genera that can be opportunist pathogens, such as *Roseomonas* (Rihs et al., 1993), indicates the plastic's potential to carry pathogens, even on the ground.

On R4 plastics, some of the more abundant genera were previously associated with biofilms that grow in different freshwater ecosystems, such as *Porphyrobacter* (Di Pippo et al., 2020), *Tabrizicola* (Murphy et al., 2020), *Gemmobacter* (Nguyen et al., 2021), and *Pseudorhodobacter* (Di Pippo et al., 2020). Specifically, *Porphyrobacter* is an aerobic bacterium that participates in biogeochemical cycles in aquatic environments (Liu et al., 2017); *Rhodopirellula* and *Rubribacterium* have been reported as hydrocarbon-degrading bacteria (de Araujo et al., 2021; Urbance et al., 2001); *Algoriphagus* has been associated with polypropylene in a freshwater lake, whose development indicates significant algae growth on plastic (Szabó et al., 2021). *Ketogulonicigenium* is a facultatively anaerobic chemoheterotroph (Urbance et al., 2001) although its role in the plastisphere has not been defined and *Sandaracinobacter* is mainly found in freshwater environments (Lee et al., 2020). *Sandarakinorhabdus*, *Nodosilinea,* and *Rhodobacter* are primary producers playing a role in biofilm formation as previously documented (Yokota et al., 2017). Furthermore, some species of *Roseomonas*, are known to be opportunistic bacteria for humans (Rihs et al., 1993); and *Legionella*, a well-known biofilm participant is usually denoted as a pathogen as well (Edelstein and Lück, 2015). The specific eutrophic conditions in this location, along with the presence of R4 weathered plastics, providing an extra carbon source and a surface in which nutrients can adhere are probably the main drivers of the significant increase of genera and their respective abundances in R4 plastics.

On S5 plastics, the plastic-associated communities agreed with the genera found in other studies in marine habitats such as *Lewinella* (Roager and Sonnenschein, 2019), *Dokdonia* (Basili et al., 2020), *Loktanella* (Delacuvellerie et al., 2019, Pinto et al., 2019), *Pseudahrensia* (Zhang et al., 2021), *Erythrobacter* (Kirstein et al., 2019) and *Parvularcula* (Kirstein et al., 2019). The repetitive detection of these genera suggested having an essential role in the marine plastic biofilm, suggesting that future studies can discover their function in these communities. Furthermore, the abundance of autotrophs at all sampling locations and their changes according to the different sampling locations (on S5 plastics, the more abundant genera were *Pleurocapsa* and *Schizothrix*) shows their importance in the plastisphere, independently of the environment. Most of the studies that address the plastisphere are based mainly on heterotrophic bacteria (Yokota et al., 2017), but determining the presence of photosynthetic bacteria can contribute information about the different relationships established in the bacterial communities associated with the plastisphere.

Many studies suggest that plastics and their smaller fractions (microplastics and even nano plastics) can be vectors of microorganisms between different habitats (Meng et al., 2021; Shen et al., 2019). The negative effect on ecosystems is not entirely clear, but some studies propose that plastics can introduce invasive species (Carter et al., 2010), pathogens (Goldstein et al., 2014; Kirstein et al., 2016), or increase the gene exchange between attached biofilm communities and the surrounding environments (Arias-Andres et al., 2018). Other studies indicate the potential of these microorganisms to use plastics as a carbon source (Bornscheuer, 2016). Our study confirms that greenhouse plastics can effectively function as vectors of bacteria, showing six genera (i.e., *Flavobacterium, Georgfuchsia, Acinetobacter, Pleurocapsa, Altererythrobacter* and *Rhodococcus*) preserved on the plastics independently of the sampling location and their surrounding environment (soil, freshwater or seawater). The genus *Flavobacterium* can be found generally in soil and freshwater (Bernardet and Bowman, 2006); it is a potential pathogen for some fish species (Bernardet and Bowman, 2006; Nematollahi et al., 2003). *Georgfuchsia* has been described previously as capable of biodegrading aromatic hydrocarbons (Staats et al., 2011). The genus *Acinetobacter* was reported for its implication in some human infections (Joly-Guillou, 2005) and its capacity for its resistance to multiple antibiotics (Manchanda et al., 2010), as it also happens with the genus *Pleurocapsa* (Li et al., 2021a). Some species of the genus *Altererythrobacter* were reported as PHA/PHB degraders in previous studies (Vannini et al., 2021), and lastly, the genus *Rhodococcus*, can degrade LDPE under laboratory conditions (Abdullah et al., 2021; Sivan et al., 2006). Nonetheless, the highest number of these common taxa were detected in G1, CG2, R3, and R4 plastics suggesting that the arrival of the plastics to the sea may limit the capacity of the plastics to act as a vector of microorganisms. The presence of bacteria involved in the development of biofilms such as *Sphingomonas* (Bereschenko et, 2010; Martínez-Campos et al., 2018); others capable of biodegrading plastics such as *Pseudomonas* (Kyaw et al., 2012) and *Arthrobacter* (Han et al., 2020); potential pathogens such as *Brevundimonas* (Ryan and Pembroke,

2018) and *Roseomonas* (Rihs et al., 1993) urges not to underestimate the impact that plastics and associated plastisphere can have in each environment along their life cycle.

5. Conclusions

This study analyses for the first time the evolution of the bacterial community adhered to plastics across different environments from their point of use to their final destination in the sea. Greenhouse plastics were chosen for this study because their mismanagement facilitates their debris reaching the ocean. Bacterial communities detected on greenhouse plastics change with increasing distance from the point of use. Additionally, changes were caused by their surrounding environments, especially for plastics arriving in freshwater and the sea. Furthermore, the statistical analysis revealed that the pH, salinity, and concentration of nutrients (nitrogen and phosphorus) had an essential role in the successive changes produced in the bacterial community attached to the plastics.

The presence of six common genera independently of the sampling location (*Flavobacterium, Altererythrobacter, Acinetobacter, Pleurocapsa, Georgfuchsia and Rhodococcus*) confirmed that plastics could act as vectors of microorganisms between different environments along their life cycle. The potential of these bacteria to act as human and animal pathogens, invasive species, or to carry antibiotic resistance genes could be an important concern for human health and the environment. Nevertheless, the demonstrated implication of these genera in the degradation of different types of plastics provides insights into the possible future elimination of these plastics in the environment. Future studies should perform complete sequencing metagenomics to evaluate the real impact of the plastisphere on the ecosystems of the planet. Lastly, studies should focus on verifying which of these microorganisms may pose a real risk to the environment or the importance of isolating degrading microorganisms to discover better mechanisms for eliminating plastic waste. This work provides information about the way by which greenhouse plastics act as vectors of microorganisms posing an added risk to receiving environments. Finally, appropriate waste management techniques such as centralised collection systems and targeted waste management education seminars can be proposed.

CRediT authorship contribution statement

Sergio Martínez-Campos: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing – original draft, Visualization. **Panayiota Pissaridou:** Conceptualization, Methodology, Software, Investigation, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **Miguel González-Pleiter:** Conceptualization, Methodology. **Francisca Fernandez-Piñas:** Conceptualization, Methodology, Writing – review & editing. **Francisco Leganes:** Conceptualization, Methodology, Writing – review & editing. **Roberto Rosal:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Katerina Drakou:** Conceptualization, Methodology, Investigation. **Christos Shammas:** Methodology, Resources. **Kostas Andreou:** Methodology, Investigation, Resources. **Michalis Koutinas:** Methodology, Resources, Validation. **Konstantinos Kapnisis:** Methodology, Software, Resources. **Marlen Ines Vasquez:** Conceptualization, Methodology, Validation, Resources, Funding, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113213.

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