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ORIGINAL PAPER

Potential of sediment bacterial communities from Manila Bay (Philippines) to degrade low-density polyethylene (LDPE)

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Abstract

The persistence of plastics and its effects in different environments where they accumulate, particularly in coastal areas, is of serious concern. These plastics exhibit signs of degradation, possibly mediated by microorganisms. In this study, we investigated the potential of sediment microbial communities from Manila Bay, Philippines, which has a severe plastics problem, to degrade low-density polyethylene (LDPE). Plastics in selected sites were quantified and sediment samples from sites with the lowest and highest plastic accumulation were collected. These sediments were then introduced and incubated with LDPE in vitro for a period of 91 days. Fourier transform infrared spectroscopy detected the appearance of carbonyl and vinyl products on the plastic surface, indicating structural surface modifications attributed to polymer degradation. Communities attached to the plastics were profiled using high-throughput sequencing of the V4-V5 region of the 16S rRNA gene. Members of the phylum Proteobacteria dominated the plastic surface throughout the experiment. Several bacterial taxa associated with hydrocarbon degradation were also enriched, with some taxa positively correlating with the biodegradation indices, suggesting potential active roles in the partial biodegradation of plastics. Other taxa were also present, which might be consuming by-products or providing nourishment for other groups, indicating synergy in utilizing the plastic as the main carbon source and creation of a microenvironment within the plastics biofilm. This study showed that sediment microbes from Manila Bay may have naturally occurring microbial groups potentially capable of partially degrading plastics, supporting previous studies that the biodegradation potential for plastics is ubiquitously present in marine microbial assemblages.

Keywords Biodegradation · Microbial communities · Coastal marine environment · Plastic accumulation · Sediments · Manila Bay · Hydrocarbonoclasts

Introduction

Plastics are synthetic hydrocarbon polymers used by humans for their durability and wide range of applications (Thompson et al. 2009). Waste mismanagement, however, resulted in their accumulation in landfills or in natural environments (Geyer et al. 2017), with more than 8 million metric tons leaking into the ocean (Jambeck et al. 2015). Among the plastic types, polyethylene (PE) or the common material used as plastic bags (low-density polyethylene—LDPE) or single-use packaging, has become one of the most abundantly found material in coastal areas and surveys in other habitats (Erni-Cassola et al. 2019; Cyvin et al. 2021). PE comprises a significant portion of plastic pollution that has now become an emerging global threat due to its several negative implications (Caruso 2015) affecting the smallest microorganisms (Tetu et al. 2019; Sánchez-Fortún et al. 2021) to the largest megafauna (Besseling et al. 2015). Because of their persistence and long life, their effects in the environment where they accumulate have been of serious concern.

Increasing evidence showed that aged plastics deposited in different marine habitats exhibited signs of deterioration and even decomposition, which have been suggested to be mediated by microorganisms. An indication of this is the formation of pits conforming to the shape of microbial cells

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or colonies that are hydrolyzing the hydrocarbon polymer as shown by scanning electron micrographs (Zettler et al. 2013; Harshvardhan and Jha 2013). Once plastics are released into the environment, they are rapidly colonized by a unique microbial community different from its surrounding environment, now known as the 'plastisphere' (Zettler et al. 2013). Many of the bacteria found and enriched in the plastisphere are putative hydrocarbonoclastic species with xenobiotic biodegradation genes (Dashti et al. 2015; Bryant et al. 2016; Quero and Luna 2017; Dussud et al. 2018a, b). These taxa are widely found on surfaces of plastics accumulating in the open ocean (Zettler et al. 2013; Bryant et al. 2016), coastal waters (Dussud et al. 2018b), and sediments (Harrison et al. 2014), also indicating that genes possibly involved in plastics degradation are ubiquitous in the marine environment and can be enriched once the material has been introduced. Several incubation experiments of plastics with microbes from different marine environments either in situ or in vitro also showed the formation of new functional groups such as carbonyl and double bonds as detected by Fourier transform infrared (FTIR) spectroscopy (Montazer et al. 2020; Harshvardhan and Jha 2013). Molecular studies further showed a correlation of some microbial groups with the observed biodegradation indices, suggesting that they mediate possible deterioration and eventual degradation of the plastics (Harshvardhan and Jha 2013; Delacuvellerie et al. 2019).

Among marine habitats, coastal areas or beaches have been suggested as one of the most vulnerable since the majority of plastics tend to accumulate on the coasts aided by wave motion or wind influence (Harrison et al. 2014; De Tender et al. 2015; Lebreton et al. 2019; Cruz and Shimozono 2021), leaving these ecosystems and sediments at risk to plastic pollution (Barnes et al. 2009; Pinnell and Turner 2019). An example of such a scenario are the coastal areas of Manila Bay, which is an embayment in the southwestern part of Luzon Island in the Philippines (Jacinto et al. 2006). It serves as the drainage basin of four of the top ten largest emitting rivers of plastics in the world, namely Pasig, Tullahan, Meycauayan, and Pampanga rivers (Meijer et al. 2021), accounting for approximately 10% of the global riverine plastics (Ritchie 2021). A waste audit conducted by environmental groups in 2014 revealed that around 62% of the marine litter collected in the bay were classified as plastic wastes, with plastic bags commonly made from polyethylene (PE) were found to be the most abundant, followed by plastic wrappers, miscellaneous discards such as cigarette butts, clothes, rags, and sponges (Ranada, 2014). Due to the high abundance of plastics as a potential substrate for marine microorganisms, it is expected that naturally occurring taxa capable of degrading the plastics could possibly be enriched from the sediment microbial communities of Manila Bay.

In this study, the potential of the sediment microbes to biodegrade the plastics accumulating on the coast of Manila Bay was investigated. To do this, we first quantified the macroplastics (> 2.5 cm) concentration in different coastal environments around Manila Bay to confirm the presence and assess the extent of plastics pollution in the area and quantify the abundance of the different plastic types. We then collected sediments from select sites representing varying degrees of plastics accumulation. Based on the survey results, unused or unexposed LDPE sheets, hereafter referred to as pristine LDPE, were used and pre-exposed to natural weathering before co-incubating with collected sediments. The changes occurring in both the substrate and communities over time in vitro were monitored. Changes in the substrate were detected using FTIR spectroscopy through the formation of functional groups as biodegradation indices, which can be attributed to polymer degradation. Since plastics are new substrates with different physico-chemical properties, selective enrichment of taxa that can grow and possibly utilize the material is expected. To investigate this, we profiled changes in the community using high throughput sequencing of the 16S rRNA gene to determine responses and activity coupled with high-resolution confocal laser scanning microscopy. After which we correlated biological patterns with the biodegradation indices to infer the taxa potentially responsible for the observed changes. Understanding the ecological response of the sediment microbes to the accumulation of plastics could provide insights into its implications for the ecological processes in coastal environments in the presence of plastics pollution. Further down the line, this could provide information on how to manage plastics by exploring genes with potential biotechnological applications, as a natural approach to addressing this problem (Caruso 2015).

Materials and methods

Sampling sites

To determine the extent of potential plastics accumulation in Manila Bay, five (5) coastal sites on the southern coast of the embayment (Fig. 1) were selected for their sandy beach shorelines, accessibility, and length (> 100 m) as recommended by Lippiatt et al. (2013). The different sites were characterized by varied coastal activities and settings, and thus, possible varied accumulation of plastics. The site in Ternate (Site 1: 14.28523°E, 120.70441°N) was a stretch of shoreline adjacent to a beach resort, Naic (Site 2: 14.324969°E, 120.754345°N) was near a fishing community, Noveleta (Site 3: 14.442292°E, 120.875152°N) was an abandoned stretch of beach in-between two resorts, Las Piñas-Parañaque Critical Habitat and Ecotourism Area (LPPCHEA Site 4: 14.49744°E,120.98185°N) site was in an area with nearby mangrove forests, and the site in Baseco

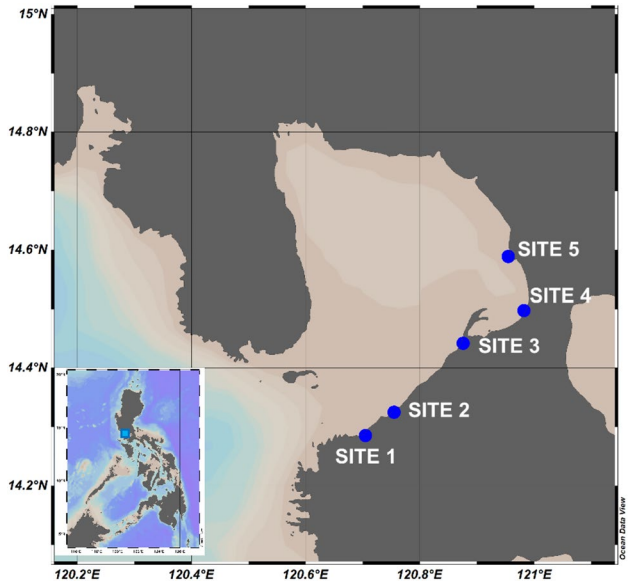

Fig. 1 Map of the sampling sites in coastal environments around the southern and eastern sections of Manila Bay, Philippines. Inset: Map of the Philippines with Manila Bay indicated in a blue square. Site 1 (Ternate), Site 2 (Naic), Site 3 (Noveleta), Site 4 (Las Piñas-Parañaque Critical Habitat and Ecotourism Area), and Site 5 (Baseco)

(Site 5: 14.588967°E, 120.954459°N) was a stretch of beach where people frequent for recreational activities. Baseco was also the site nearest to a dense coastal community. These sites also represent geographic areas along the southern coast of Manila Bay where coastal communities tend to be the densest (Philippine Statistics Authority 2015) and where major contributing river deltas (van Emmerik et al. 2020) are located. Sample collection was done only once during the inter-monsoon period from May to early June 2019, characterized by less water movement, thus, allowing plastics to accumulate on the coasts as well as their associated microbial communities to develop.

Plastics survey and sample collection

Percent cover and standing stock surveys were carried out to quantify the abundance of macroplastics (> 2.5 cm) per site. Percent cover is the proportion of the beach covered in plastics represented by the number of squares (grids) with the presence of plastics in a quadrat with 100 squares. Results of which were then used in selecting sites where samples were taken for the biodegradation assays (see succeeding section). Proper permits for field work and sample collection were coordinated with the local government units and relevant agencies.

Surveys were done along a 100-m long area of a beach in each site following the protocols by the National Oceanic and Atmospheric Administration (NOAA; Lippiatt et al. 2013; Whitmire and Van Bloem 2017; Supplementary Fig. 1A). However, only plastic materials were accounted for during the surveys as they were the main substrates being investigated. Four (4) random 5-m transects were selected for both surveys. For the percent cover assessment, five (5) 1 × 1-m quadrats with 0.1-m grids were laid along the wrack line (Supplementary Fig. 1B). Percent cover was quantified by counting the number of squares with plastics, each square representing 1% cover. For standing stock surveys, the standardized protocol by NOAA was followed (Lippiatt et al. 2013). In brief, during low tide, plastics greater than 2.5 cm in diameter within the 5-m transects were surveyed from the edge of the water to the back of the shoreline (where the primary substrate changes or at the first barrier such as vegetation), to quantify the number of plastics in a defined area (Supplementary Fig. 1C).

To determine the most abundant plastic types, the materials observed were classified into six categories. These include polyethylene terephthalate (PET; e.g., beverage bottles), high-density polyethylene (HDPE; e.g., other jugs or containers), low-density polyethylene (LDPE; e.g., plastic bags), polypropylene (PP; e.g., plastic cups and straws), polystyrene (PS; e.g., styrofoam), and miscellaneous (e.g., cigarette lighters, ropes, and net pieces). Raw data were submitted to the PlastiCount Pilipinas portal (plasticount.ph) - a repository for archiving plastics pollution survey data in the Philippines.

After the standing stock surveys, sediment samples used as inocula for the enrichment and biodegradation assays were collected using a seagrass corer, only collecting the upper 5 cm of the sediment in the submerged part lower than the low tide line. However, for this purpose, we only compared the two sites with the highest and lowest plastic accumulation based on the conducted surveys, which were identified to be Sites 1 and 4 (Fig. 2, see Results).

Samples for bacterial community characterization and incubation experiments were stored in sterile conical tubes but potential microplastics in the sediment samples were not further considered in this study. The overlying seawater was also collected and then used as the medium for the cultures. All samples were stored in coolers containing ice during transport until further processing. Upon arrival in the laboratory, sediment samples for biodegradation assays were stored at 4 °C until inoculation and seawater were filtered through a 0.22 µm membrane filter (Whatman®) and autoclaved.

Incubation of sediment microbes with plastics

Results of the survey revealed low-density polyethylene (LDPE) to be the most abundant type of single-use plastics accumulating in Manila Bay (see Results), which was then used as the model substrate to investigate the possible

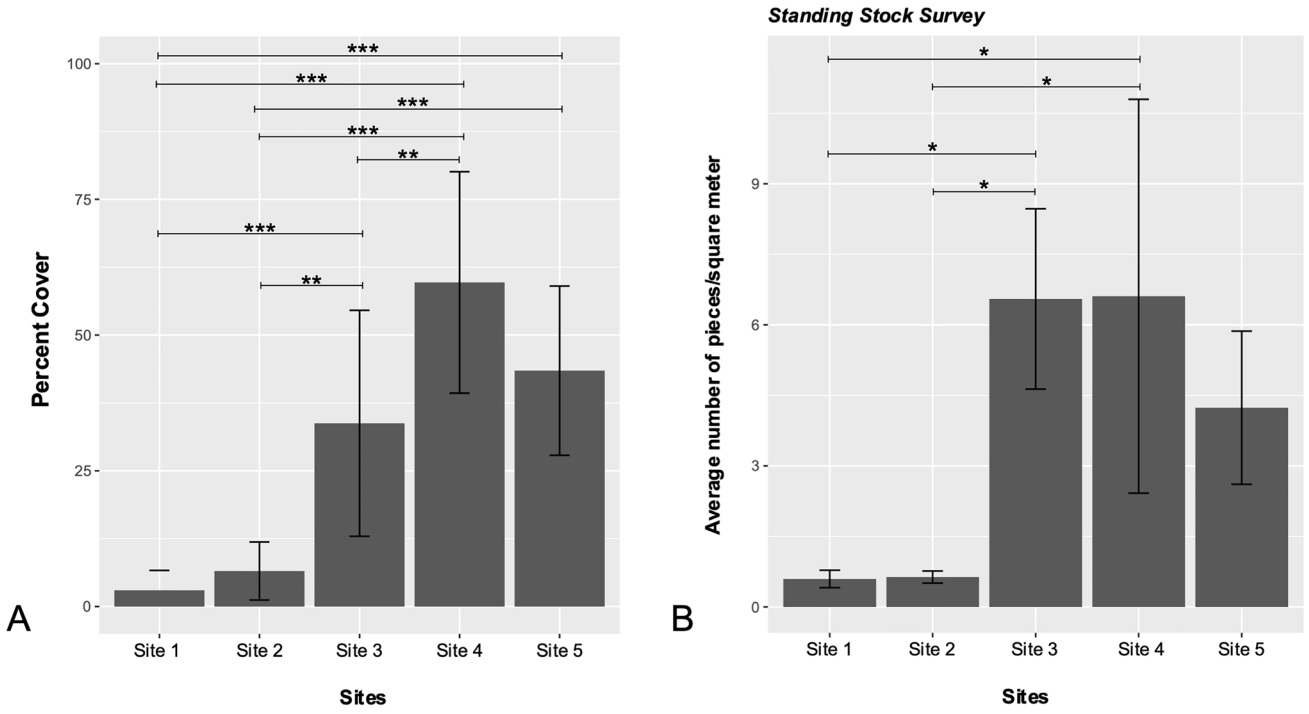

Fig. 2 Percent cover (**A**) and the number of plastic pieces per square meter (**B**) of macroplastic per site. The site with the highest plastic accumulation was in Site 4, and the least was in Site 1. Significance codes: '***' for 0, '**' for 0.001, '*' for 0.01, and '.' for 0.05

biodegradation potential of sediment microbial communities. Specifically, a popular LPDE plastic bag was purchased from a local supermarket (Whitehorse®). The sheets were cut open sterilized in 70% ethanol for five minutes, and air-dried inside the biosafety cabinet before use. The pre-sterilized sheets were hanged on a grid to be exposed to direct sunlight for two weeks to simulate the disposal of plastics in the environment before going to the ocean prior to being used in the enrichment experiment.

The exposed LDPE sheets were cut into 1.5×1.5 cm squares to simulate fragmentation and were sterilized in 70% ethanol for one minute as oversoaking would lead to discoloration. Microbial communities from the sediment samples of stations with the highest (Site 4) and lowest (Site 1) plastic deposition, hereafter referred to as the High and Low treatments, respectively, were then tested for their possible responses to LDPE introduction by looking at changes in the attaching community through time. These squares were collected periodically to look at changes in the attached microbial communities. Partial biodegradation of plastics as a possible response was also monitored following the combined methods of Harshvardhan and Jha (2013) and Dela Torre et al. (2018). In brief, approximately 35 g of the sediment samples were individually cultivated in a 500-mL Erlenmeyer Flask containing 320 mL of filtered and sterilized seawater from each site, and 30 mL of Bushnell-Haas medium (0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3, and 0.05 g $FeCl_3$) with 20 strips of pre-weighed LDPE. The flasks were incubated for 91 days at 30 °C with a 12:12 light–dark cycle, shaken at 140 rpm for the first 42 days to allow the bacteria in the sediments to attach to the plastic. Stirring was then stopped on Day 42 to simulate the settlement of the plastic squares into the sediments and allow further enrichment. Each set-up had three replicates while a set-up with no added sediment served as the control.

Monitoring change in plastic properties

To monitor the changes in the structure of the plastics, which could indicate partial biodegradation through time, both quantitative and qualitative methods were used. These include the quantitative loss in dry weight and Fourier Transform infrared spectroscopy (FTIR) analyses, and qualitative and semi-quantitative confocal laser scanning (CLS) microscopy techniques. For these, separate plastic samples were collected from each flask on Days 0, 7, 42, and 91.

Loss in dry weight

To determine the possible loss in dry weight due to the solubilization of plastic polymers, the dry weight of the residual polymer was determined following the methods of Harshvardhan and Jha (2013). One LDPE square per sample per

set-up was collected, and were incubated in 2% aqueous sodium dodecyl sulfate (w/v) solution for 4 h at 50 °C. After incubation, the squares were swabbed to ensure the removal of the remaining biofilm, washed with warm distilled water and 70% ethanol to remove cells and other debris. The washed LDPE squares were placed on filter paper and dried overnight at 60 °C before weighing. Weight loss was calculated using the following equation (Konduri et al. 2010):

$$\%\text{weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Fourier transform infrared spectroscopy (FTIR) using Attenuated total reflection (ATR)

To check for the formation of new functional groups, which can be attributed to polymer degradation, Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR; Bruker, Ettingen, Germany) was done following the methods of Dela Torre et al. (2018) and (Jung et al. 2018). The LDPE squares collected at different time points in all treatments were first washed with 2% SDS, followed by sterile distilled water, stored in petri dishes lined with filter paper, and then dried overnight at 60 °C before being used for FTIR analysis. The instrument collected spectra from the plastic samples from 4000 to 450 cm^{-1} with a data interval of 1 cm^{-1}, with the resolution set to 2 cm^{-1}. The ATR diamond crystal was cleaned with 70% 2-propanol and background scans were performed between samples. Each sample was compressed against the diamond with the minimum force recommended by the manufacturer to ensure good contact between the sample and the ATR crystal. Each sample was read ten times and the intensity of the absorption bands was averaged. The absorption bands were identified using a peak height algorithm within the equipment's software (OPUS 8.5, Bruker Corporation). The relative absorbance intensities of the ester carbonyl bond at 1740 cm^{-1}, the keto carbonyl bond at 1715 cm^{-1}, the terminal double bond (vinyl) bond at 1650 $cm^{-1}$and the internal double bond at 908 cm^{-1} to that of the methylene bond at 1465 cm^{-1} were recorded and evaluated using the formulas by (Albertsson et al. 1987):

$$\text{Keto Carbonyl Bond Index (KCBI)} = \frac{I_{1715}}{I_{1465}}$$

$$\text{Ester Carbonyl Bond Index (ECBI)} = \frac{I_{1740}}{I_{1465}}$$

$$\text{Vinyl Bond Index (VBI)} = \frac{I_{1650}}{I_{1465}}$$

$$\text{Internal Double Bond Index (IDBI)} = \frac{I_{908}}{I_{1465}}$$

The crystallinity percentage of the LDPE squares was also measured as described by (Zerbi et al. 1989). A reduction in crystallinity would mean the conversion of the structured polymer into an amorphous structure (Dela Torre et al. 2018), a change in structure would mean possible biodegradation. The formula:

$$\%\text{Crystallinity} = 100 - \left[\left\{1 - \frac{\frac{Ia}{1.233Ib}}{1} + \frac{Ia}{Ib}\right\} \times 100\right]$$

where Ia is the absorbance at 1473 cm^{-1} and Ib is the absorbance at 1463 cm^{-1}.

Confocal laser scanning microscopy

To visualize bacterial colonization and biofilm structure for subsequent partial biodegradation of the LDPE squares in all set-ups, confocal laser scanning microscopy (CLSM) was used. The LDPE squares were collected and preserved in 1% glutaraldehyde, gently washed by 1% phosphate buffer saline by immersion to remove excess aldehyde, stained as described in Onda et al. (2013, 2015), and observed under the confocal laser scanning microscope (CLSM 710, Carl Zeiss, Germany). Cellular components were visualized using different fluorescent stains. Calcofluor White M2R and SYBR Green DNA Gel stain were used to visualize the beta-polysaccharides and nucleic acids, respectively, as described in Baird et al. (2012) and Onda et al. (2014). The following wavelengths were used to capture the emissions of the fluorescent stains: beta-polysaccharides at 360–443 nm, nucleic acids at 485–498 nm, extracellular polymeric substances (EPS) polysaccharide at 555–580 nm, and EPS protein at 610 nm. All auto-fluorescent structures possibly pertaining to lipids were detected at 650 nm. Average fluorescence intensity of the different cellular structures and components per micrograph was measured using Zeiss Zen blue v 3.3 software and calculated as the mean of three different z-stacks (depths) per field.

DNA extraction, amplicon sequencing, and post-sequencing analysis

Separate plastic squares were collected at different time points from all set-ups as described in the previous

sections to determine possible changes and enrichment in the attaching community. Samples for DNA extraction on Day 0 were directly taken from the sediment samples collected from the field, representing the in situ or starting microbial inocula before enrichment. From the same set-up in the incubation experiments, samples were collected on Days 7, 42, and 91 for the microbial communities that had grown on the LDPE squares. Day 7 represents the primo-colonization stage of bacterial species on the plastic surface (Dussud et al. 2018a), while Day 42 is almost the half-way between Days 0 and 91 when substantial partial biodegradation was observed as reported by Dela Torre et al. (2018). DNA from LDPE squares from each sampling day and set-up were extracted using PowerSoil DNA Kit (MoBio Qiagen, Hilden, Germany) following the manufacturer's protocol. Extracts were visualized on 1% agarose gel using SYBR™ Safe DNA Gel Stain and concentration was quantified using a Nanodrop spectrophotometer. The eluted DNA was stored at – 20 °C until downstream processing.

Before sequencing, DNA samples were subjected to PCR amplification as a quality control step to check for the integrity of the target gene. The V4-V5 region of the 16S rRNA gene was amplified using the 515F (Parada) forward (GTGYCAGCMGCCGCGGTAA) and 926R (Quince) reverse (CCGYCAATTYMTTTRAGTTT) primers (Quince et al. 2011; Parada et al. 2016; Walters et al. 2016). Amplification was done with the following conditions: initial denaturation at 94 °C for 8 min, followed by 28 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 2 min, and final extension at 72 °C for 10 min. The quality of the amplicons was visualized using 1% agarose gel stained with SybrGreen. Then, the separate DNA extracts (genomic DNA) from each plastic square (replicate) per treatment at every time point were combined in equimolar concentrations into one tube per sample to ensure equal representation of microbial communities living on the surfaces of the plastics and account for possible variabilities associated with various factors (e.g., non-uniform colonization, extraction biases, handling, etc.; Feinstein et al. 2009). Multiplex sequencing using the MiSeq Illumina platform was performed in the Integrated Microbiome Resource (IMR) of Dalhousie University (Canada) using the same primer pairs. The raw reads have been deposited in the NCBI Short Reads Archive under Accession No. PRJNA752037.

Post-sequence processing was mainly done in Quantitative Insights into Microbial Ecology v. 1.9.1 (QIIME: Caporaso et al. 2010). In brief, USEARCH was used to filter sequence noise, check for chimeras, and pick operational taxonomic units (OTUs). The primers were trimmed using 'mothur' (Schloss et al. 2009) and OTU clustering using USEARCH was done with 97% similarity (Caron et al. 2009). Taxonomic assignments were carried out using mothur against the Silva reference database v.138.1. The OTU tables were rarefied based on the sample with the lowest number of sequences (Low Day 91, $d = 5209$) after quality filtering and removal of OTUs present in the negative control for diversity comparisons. OTUs present in the negative control set-up (medium only) and in experimental treatments were removed to account for the possible contaminant taxa, which could have adhered to the plastic during pre-exposure.

Statistical and ecological analyses

Results of the plastic survey were expressed as means ± standard deviation of four replicates (four five-meter transects in the 100-m long area of the beach) and were tested for the significant difference against all mean values by ANOVA followed by F-statistic at $\alpha = 0.05$ and post-hoc analysis using Tukey's Test. Partial biodegradation indices were expressed as means ± standard deviation of three independent replicates and were tested for significant differences against the control using a *t* test with a significance value of $p \leq 0.05$. Statistical analyses were done in Past 4.0 (Hammer et al. 2001) and R (version 4.0.2; R Core Team 2021).

For diversity analyses, alpha diversity indices Chao1, Shannon, ACE, observed OTUs, and inverse Simpson and beta diversity Bray–Curtis dissimilarity were obtained through QIIME using the 'vegan R' package (Dixon 2003). OTUs were clustered using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Bray–Curtis similarity derived from the OTU table which was computed in PAST v4.0 (Hammer et al. 2001). For the construction of the heatmap for bacterial families, R packages 'pheatmap', 'dplyr', and 'RColorBrewer' were used (Neuwirth 2014; Kolde 2019; Wickham 2016). For the construction of the correlation table, only OTUs with a read sum of ≥ 50 were used in the analysis. Correlations between partial biodegradation indices and OTUs were determined using Spearman's rank correlation using the 'corrplot' package v. 0.84 also in R (Wei and Simko 2017), corrected using "BH" or its alias "fdr", with $\alpha = 0.01$, and r values greater than or equal to 0.5. The correlogram was plotted using the R package 'ggplot2' (Wickham 2016).

Results

Plastic accumulation in select coastal environments of Manila Bay

Among the sites surveyed, Site 4 (LPPCHEA) had the highest percent cover (Fig. 2a, 59.7 ± 11.8%), followed by Site 5 (Baseco; 43.5 ± 9.1%), then Site 3 (Noveleta; 33.8 ± 12.1%), Site 2 (Naic; 6.6 ± 2.6%), and Site 1 (Ternate) with the least at 3 ± 1.1%.

Similarly, in terms of standing stock (Fig. 2b), Site 4 also had the highest plastic count per square meter at 6.6 ± 4.2 plastic pieces, followed by Site 3 with 6.5 ± 2 plastic pieces, then Site 5 with 4.2 ± 1.6 plastic pieces, Site 2 with 0.63 ± 0.1 plastic pieces, and Site 1 with the least at 0.6 ± 0.2 plastic pieces. Again, this included all plastic pieces with varying sizes from ≥ 2.5 cm. Increasing plastic accumulation was observed towards the inner parts of the bay including Sites 3, 4, and 5 with comparable standing stock results (a value > 0.05, $p = 0.44$). Meanwhile, Sites 1 and 2, which were part of the outer bay, had lower standing stocks. LDPE (red bar) was the most abundant plastic-type found in three out of five sites (Sites 1, 2, and 5), while PP (blue bar; Site 3) and PS (yellow bar; Site 4) were found to be the most abundant in the other two sites (Fig. 3). Notably, a high standard deviation was observed in some of the counts, implying that plastic types were not equally distributed within the sites.

Changes in the surface properties of the plastic

Loss in percent dry weight has been used as the simplest way to measure structural changes in the plastic polymer by the attached microbes. Studies attribute this change in weight to the solubilization of the plastic polymer components (Harshvardhan and Jha 2013). However, results in this study yielded a broad range of standard deviation (Supplementary Table 1) and may not be an accurate measure for partial biodegradation, as weight loss can result from the solubilization of volatile and soluble impurities and low molecular mass PE materials (i.e., accessible side chains) and not from the fragmentation of the backbone polymer chains (Lucas et al. 2008; Montazer et al. 2020).

Structural changes of the LDPE due to natural weathering were observed by examining unexposed and 2-week UV-exposed squares under an FTIR. Specific bonds had unique chemical signatures that can be seen in the IR spectrum (Fig. 4). For the unexposed LDPE square (Fig. 6, solid

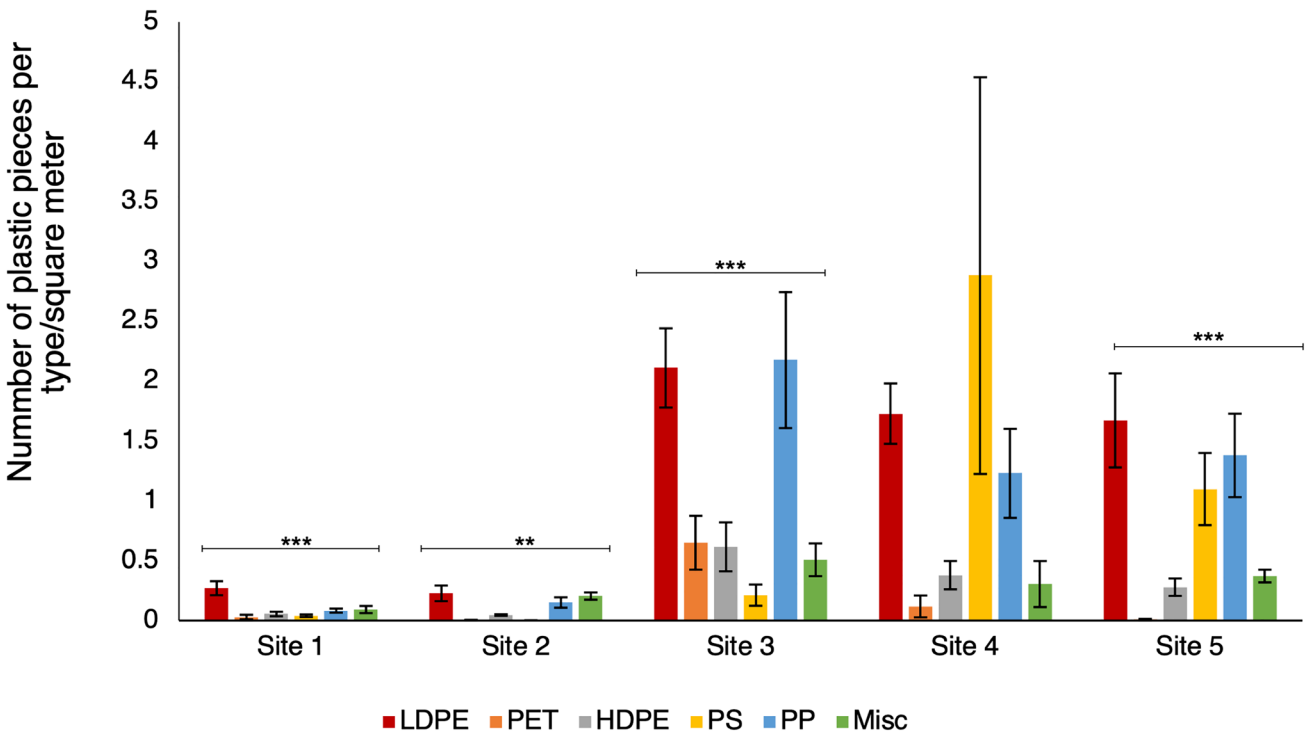

Fig. 3 Most abundant plastic type in terms of the total number of pieces per type per site. Blue—polyethylene terephthalate (PET); orange—high-density polyethylene (HDPE); gray—low-density polyethylene (LDPE); yellow—polypropylene (PE); and green—miscellaneous (Misc). LDPE was found to be the most abundant in three out of five sites (Sites 1, 2, and 5), polypropylene was found to be the most abundant in one site (Site 3), as well as polystyrene (Site 4). Significance codes: '***' for 0, '**' for 0.001, '*' for 0.01, and '.' for 0.05 (colour figure online)

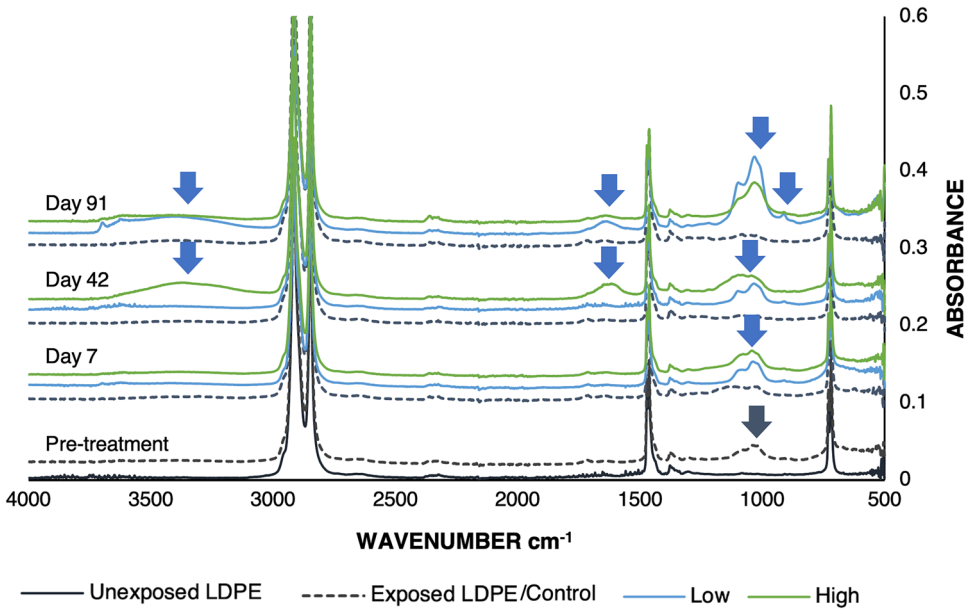

Fig. 4 Overlay of the IR spectra of the unexposed (solid line), UV-exposed LDPE/control (dotted line), low (blue), and high (green) squares. Unexposed LDPE shows characteristic doublet peaks at 2915 and 2848 cm^{-1} for CH_2 asymmetric and symmetric stretching, at 1472 and 1462 cm^{-1} for bending deformation and at 720–730 cm^{-1} for rocking deformation (Gulmine at el. 2002). UV-exposed LDPE showed peaks from 950 to 1150 cm^{-1} and at 1710 cm^{-1} (dark gray arrow) possibly correlated to the formation of carbonyl products. Low and high samples showed peaks at 905–915 cm^{-1}, 950–1150 cm^{-1}, 1550–1750 cm^{-1}, and 2900–3700 cm^{-1} (blue arrows) relative to the control (colour figure online)

line), strong bands or a doublet was observed at 2915 and 2848 cm^{-1} for CH_2 asymmetric and symmetric stretching; medium peaks (another doublet) at 1472 and 1462 cm^{-1} were for bending deformation, and medium peaks (another doublet) at 720–730 cm^{-1} were for rocking deformation (Gulmine et al. 2002).

Sampled LDPE squares from different set-ups were also subjected to FTIR spectroscopy to track possible changes in the structures over time. The overlay of the IR spectra of these samples is shown in Fig. 4. Through time, peaks (blue arrows) have appeared at 905–915 cm^{-1}, 950–1150 cm^{-1}, 1550–1750 cm^{-1}, and 2900–3700 cm^{-1} in the experimental samples, which consistently appeared in all samples regardless of the sediment used (low: blue, high: green) compared to the control.

Indices of unexposed and UV-exposed LDPE squares, and LDPE squares in incubation experiments with sediment samples, and the control set-up were also calculated (Fig. 5) by using the formulas provided by Albertsson et al. (1987). Results of these indices were tested for significance against the control sample. A significant increase in the internal double bond index ($p=0.009$) was seen early as Day 7 in the low treatment, which further increased significantly through Day 42 ($p=0.013$) and Day 91 ($p=0.029$). On the other hand, keto carbonyl ($p=0.023$), ester carbonyl ($p=0.010$), and vinyl bond ($p=0.023$) indices were also observed to increase but only on Day 42. Only the VBI (vinyl bond index) increased significantly until Day 91 ($p=0.046$), while ECBI (ester carbonyl bond index) and KCBI (keto carbonyl bond index) decreased, making the index values comparable with the control set-up. For the high treatment, a significant increase in IDBI (internal double bond index, $p=0.018$) was only seen on Day 42. Malfunctioning of the shaker which led to the breaking of some flasks did not allow us to conduct significance tests due to the loss of some of the replicates. However, based on the graph (Fig. 5), ECBI and KCBI also decreased on Day 91, while VBI and IDBI increased relative to the control.

Percent crystalline content of the PE samples, which indicates the ratio of the crystalline part of the PE polymer to its amorphous part (Hamzah et al. 2018), were also observed to have changed through time (Supplementary Fig. 2). The control set-up showed a decreasing percent crystalline value, − 0.57% difference from Day 7 to Day 91. Low set-up showed a 1.21% increase, while the High showed a 5.81% increase. However, these changes were not found to be statistically significant (p values >0.05).

Attachment of microbial communities to plastics

Based on the results of the survey, only the sediment samples from sites with the lowest (Site 1) and highest (Site 4) percent cover ($p<0.001$) and plastics count per square meter ($p=0.01$, Tukey's post hoc, Supplementary Fig. 3) were

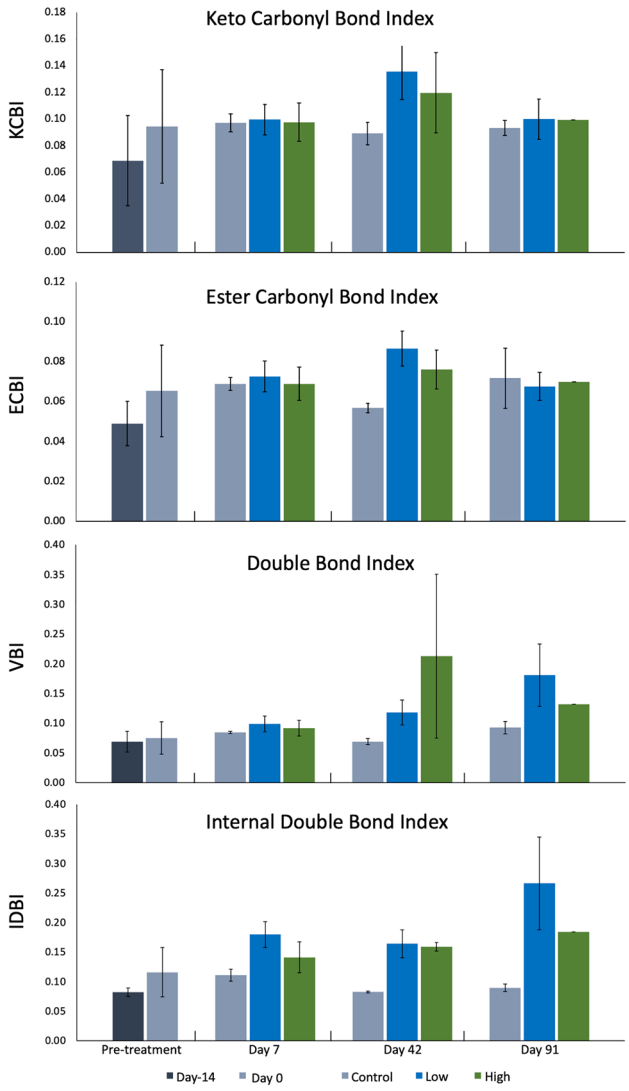

Fig. 5 Keto carbonyl, ester carbonyl, vinyl bond, and internal double indices (y-axes) of unexposed LDPE (dark gray), UV-exposed LDPE (light–dark gray), control (light to dark gray bars), low (light to dark blue bars), and high (light to dark green bars). Shades of each color signify sampling points per set-up, lighter shades represent Day 7, medium shades represent Day 42, and darker shades (colour figure online)

used in the bioassay. Beta diversity, which is the measure of community-level change (Supplementary Fig. 4), showed differences between time points in all treatments, indicating a substantial change in community composition through time at the OTU level. UPGMA clustering of the OTUs further showed strong similarity by time point, where communities from low (Site 1) and high (Site 4) plastic treatments clustered together on Day 0 (inocula), with around 25% of OTUs being shared. Similarly, communities from Day 7, also clustered together but started to differentiate from Days 42 and 91 (Fig. 6). Further, Day 0 samples directly extracted from the sediments had the highest species richness and evenness based on OTUs (Supplementary Fig. 5). In contrast, alpha diversity metrics derived from attached communities decreased through time. The attachment of these microbes to the LDPE squares was also observed using CLSM (Supplementary Fig. 6). Figure Decreasing red auto-fluorescent intensities through time, which was a proxy for attachment to surfaces of cells and EPS materials (Supplementary Fig. 7).

Generally, sediment samples (Day 0) from both high and low plastics environments were commonly dominated by Bacteroidota (40–60%), Proteobacteria (15–22%), and Acidobacteria (5–9%) (Fig. 6). Interestingly, on Day 7, attached communities in both plastic treatments became dominated by Proteobacteria (81–85%), with Bacteroidota still present but now in lower abundance (10–11%). On High Day 42, Proteobacteria (77%) was still the dominant phyla with Bacteroidota (9%) and Acidobacteriota (9%). For High Day 91, the dominant taxa shifted to Bacteroidota (60%) with Firmicutes (15%) and Proteobacteria (11%). In contrast, on Day 91, the microbial community in the Low treatment sample remained dominated by Proteobacteria (80%) with Bacteroidota (16%).

Diverse sets of microbial families were found in the samples with Muribaculaceae (17–55%) and Other Families (21–37%) dominating in both (Fig. 7) set-ups for Day 0. Alcanivoraceae (29–45%) was the dominant microbial family in both treatments, along with Marinobacteraceae (6–28%) and Methylophagaceae (8–15%) on Day 7. On High Day 42, a shift to a Rhodanobacteraceae-dominated (64%) was observed. For High Day 91, the dominant taxa shifted to Muribaculaceae (65%) being the most abundant family. In contrast, bacterial families from Methylophagaceae (50%), Flavobacteriaceae (15%), Rhodobacteraceae (11%), Alteromonadaceae (3.7%), and Sphingomonadaceae (2.5%) were further enriched (Fig. 5) in Low Day 91.

Correlation with biodegradation indices

Despite the presence of several OTUs associated with known hydrocarbon degraders or hydrocarbonoclastic groups, correlation based on stringent measures only showed a total of eighteen (18) OTUs that were positively and significantly correlated ($\alpha = 0.01$, $r \geq 0.5$) with the partial degradation indices (Fig. 8). These 18 OTUs represent only less than 1% of the total number of OTUs across all samples but are 18% of the total number of reads.

Among these, OTUs from *Methylotenera, Bdellovibrio, Winogradskyella*, and BD7-11 were strongly, positively correlated ($r > 0.4$) with three indices—IDBI, KCBI, and VBI. Three OTUs from Rhodanobacteraceae were also correlated ($r > 0.6$) with three indices, but with ECBI, KCBI, and VBI. In addition, three OTUs from the same family were correlated with ECBI and VBI. *Alcanivorax*, *Kangiella*, and two OTUs from *Methylophaga* were correlated with IDBI

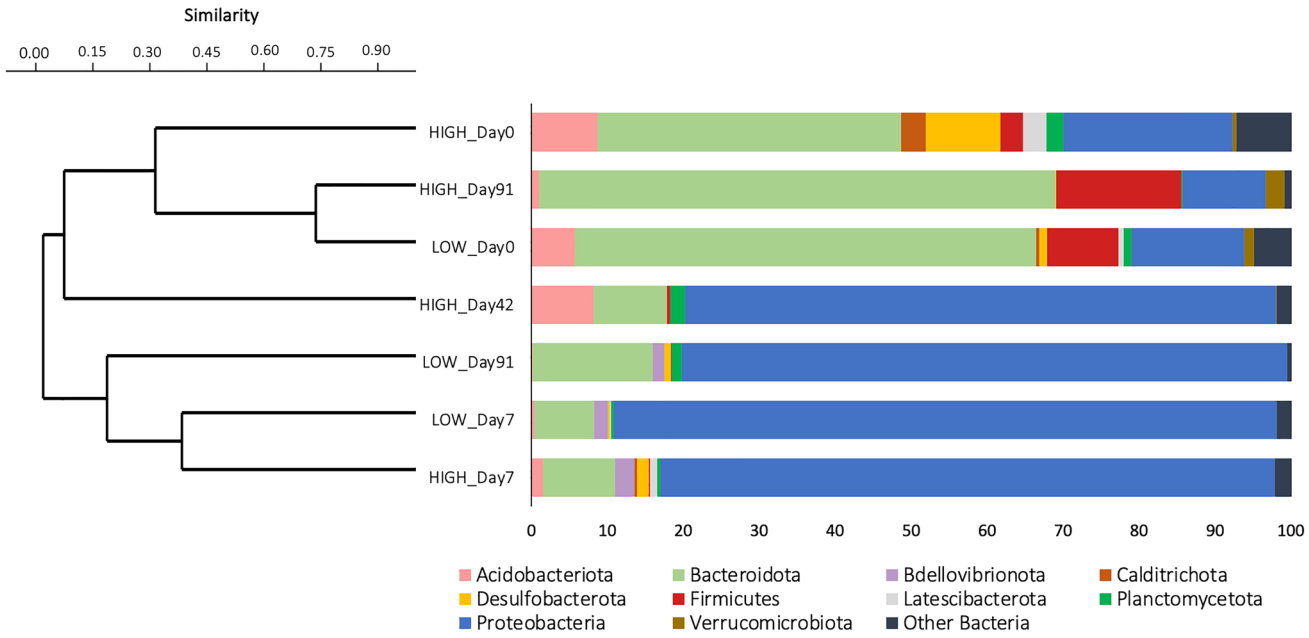

Fig. 6 Phyla of microbial communities in sediment samples (Day 0) and on surfaces of plastics (Days 7, 42, and 91) paired using UPGMA method with Bray–Curtis similarity index. Bateroidota dominated Day 0 samples, while Proteobacteria dominated plastic samples

and KCBI ($r > 0.4$). While two OTUs from *Methylophaga*, *Aestuariibacter*, and *Alteromonas* were only strongly correlated with IDBI.

Discussions

Plastics accumulation in Manila Bay

In this study, we observed high accumulation (ca. 1–7 plastic pieces/m^2) of plastics in the different coastal areas of Manila Bay, with increasing patterns toward the known point-sources in the inner part (e.g., Pasig River; van Emmerik et al. 2020). Specifically, Sites 3, 4, and 5 all had high and comparable plastic concentrations in terms of plastics per sq. m, being closest to the point-sources, while Sites 1 and 2 had lower and comparable concentrations of plastics being on the outer part of the bay. These results coincide with the particle tracking model simulation for floating litter done by Cruz and Shimozono (2021), where they identified five accumulation hotspots in the southeastern to the northeastern portions of the bay. Two of the hotspot areas include Sites 4 and 5. The surrounding thick vegetation covering 36 hectares of mangrove swamp (Mayor-Gordove and Aguinaldo 2013) in Site 4 could have also contributed to the retention, accumulation, and deposition of marine litter for long periods of time. Site 3 may not have been identified in the model but because of its location in the Cavite spit, particles from coastal areas near the mouth of the bay may have the tendency to pass by this area (Cruz and Shimozono 2021), thereby also making it an accumulation point. Site 5 was located near the mouth of the Pasig River, one of the major river systems situated in the most densely populated region in the Philippines and is estimated to discharge around 63,000 tons of plastic waste per year (Lebreton et al. 2017; Meijer et al. 2021).

PE, PP, and PS were the most abundantly observed plastic types in the surveyed areas, as these are mainly used in packaging and have a very short "in-use" lifetime, hence the dominant generators of plastic wastes (Geyer et al. 2017; Ritchie and Roser 2018). These three plastic types also have relatively higher buoyancy compared to the other plastic types such as PET, HDPE, and polyvinyl chloride (PVC), allowing a wider range of movement around and outside the bay, and higher particle travel distance (Cruz and Shimozono 2021).

Changes in plastic properties

PE is known to be highly recalcitrant and inert, however, exposure to direct sunlight (natural weathering) may cause the formation of carbonyl products (Albertsson et al. 1987; Ghatge et al. 2020). Here, we observed the presence of peaks in the FTIR spectrum of the LDPE squares after exposure to natural weathering for two weeks as compared to the unexposed LDPE squares. Peaks from 950 to 1150 cm^{-1} correspond to the –C–O stretch of alcohols, carboxylic acids,

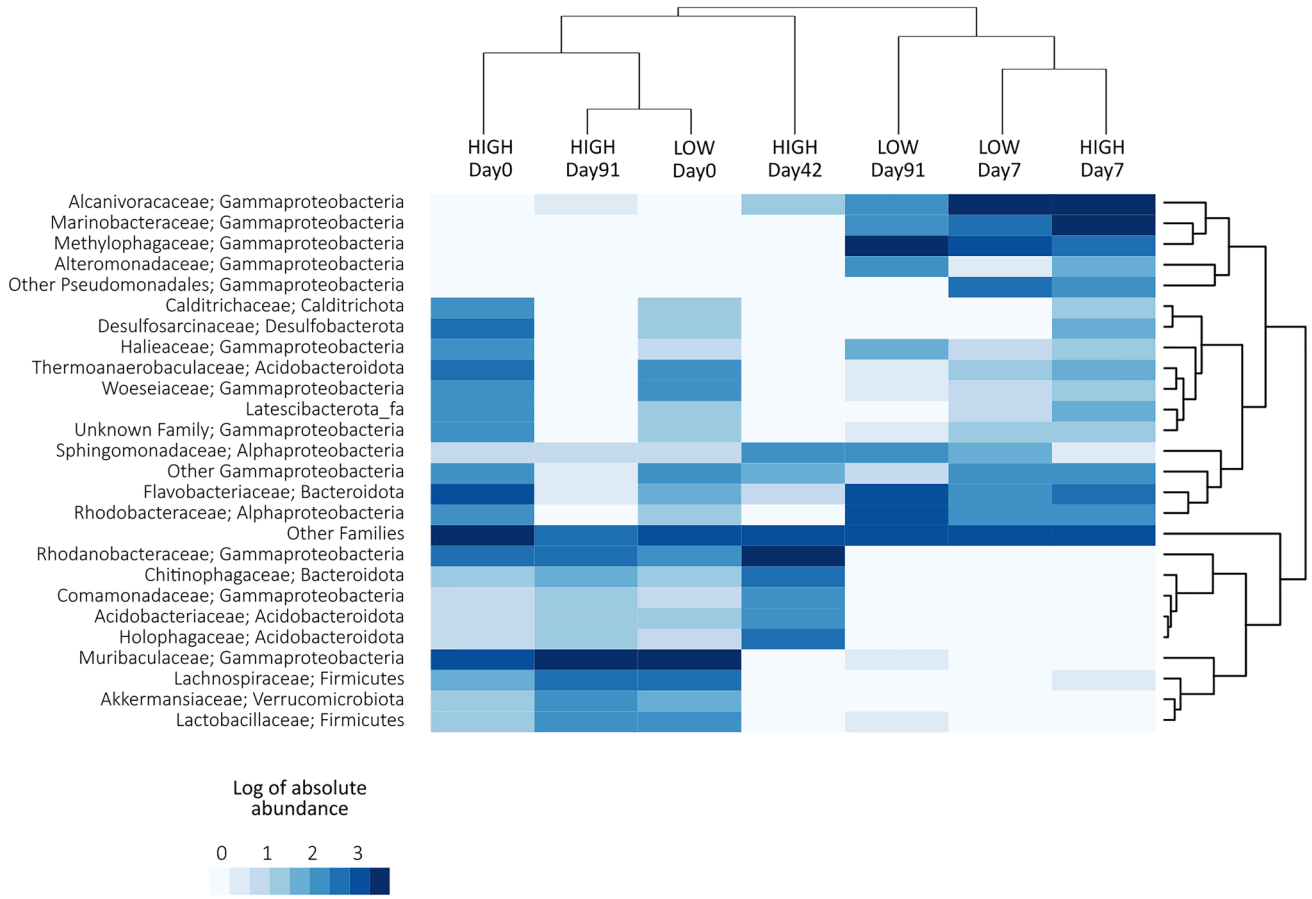

Fig. 7 Heat map showing absolute abundances (log transformed) of families of microbial communities in sediment samples (Day 0) and on surfaces of plastics (Days 7, 42, and 91) paired using the UPGMA method with Bray–Curtis similarity index. A shift in the microbial family composition was observed over time. Muribaculaceae dominated Day 0 samples. Alcanivoracaceae dominated Day 7 samples with the presence of Marinobacteraceae and Methylophagaceae. Day 42 shifted to a Rhodanobacteraceae-dominated community. Day 91 was dominated by bacterial families from Methylophagaceae, Flavobacteriaceae, Rhodobacteraceae, Alteromonadaceae, and Sphingomonadaceae

esters, and ethers, and a weak peak at 1710 cm^{-1} corresponds to the –C = O stretch of ketones and aldehydes (Montazer et al. 2020). This process is usually accompanied by thermal oxidation; thus, the absorption of oxygen led to the formation of hydroperoxides (Montazer et al. 2020), producing carbonyl-containing compounds (Albertsson et al. 1987; Hamzah et al. 2018; Montazer et al. 2020).

Changes in the structure of the plastic co-incubated with microbial communities from the sediments were tracked over time. Compared to the control, we observed the presence of peaks unique to samples on Day 7 at 950–1150 cm^{-1} (Fig. 4) for the experimental set-ups, which can be attributed to the –C–O stretch of the alcohols, carboxylic acids, esters, and ethers (Montazer et al. 2020). This same peak was still seen on Day 42 in the experimental set-ups with the appearance at 1550–1750 cm^{-1} and 2900–3700 cm^{-1}. These peaks may be attributed to the C = O stretch of aldehydes and ketones as products of the oxidation of the plastic polymer and possibly microbial activities on the surfaces of the plastic samples (Vimala and Mathew 2016), and the introduction –O–H (hydroxyl) groups formed validated the product formation of some carbonyl groups (Zbyszewski and Corcoran 2011). Aside from peak appearances, the disappearance of peaks was also observed, specifically for peaks at 1550–1750 cm^{-1} and 2900–3700 cm^{-1}, which disappeared in one of the experimental set-ups on Day 91. A new peak at 905–915 cm^{-1} may correspond to the formation of double bonds from the Norrish Type II reaction of the oxidized carbonyl compounds (Albertsson et al. 1987; Albertsson and Karlsson 1990; Artham et al. 2009). The presence of these peaks in the plastics co-incubated with microbes and the absence of the same peaks in the control showed that possible surface modifications by

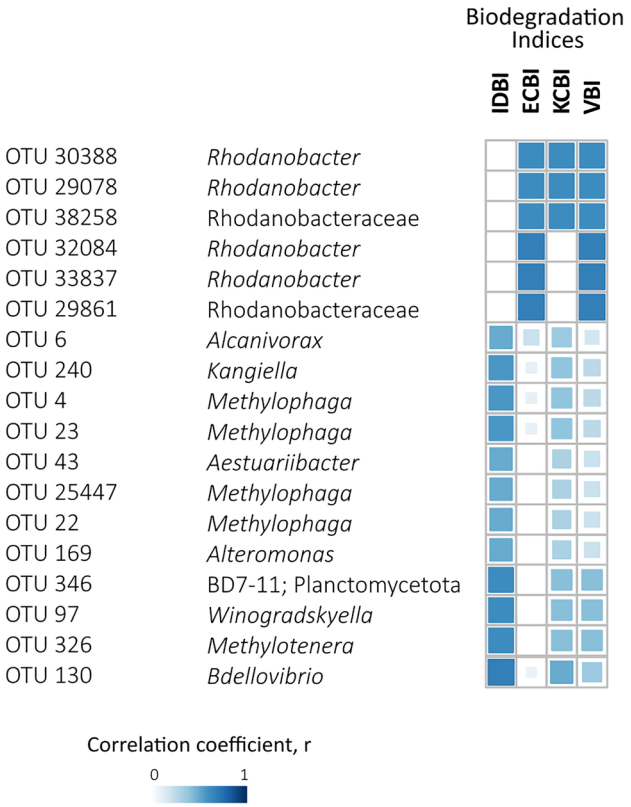

Fig. 8 Correlation plot of positively correlated OTUs ($r \geq 0.5$) with biodegradation indices (*VBI* vinyl bond index, *KCBI* keto carbonyl bond index, *ECBI* ester carbonyl bond index, *IDBI* internal double bond index)

microorganisms had occurred, indicating microbial degradation or biodegradation.

Microbial attachment and enrichment on a plastic surface

Changes in the structure of the plastics after the experiment were accompanied by significant shifts in the composition of the attached bacteria on LDPE surfaces. Here, the bacteria present in collected sediments rapidly colonized the LDPE in vitro, consistent with previous reports (Harrison et al. 2014). The sediments found in both sites (Site 1 and Site 4) were dominated by OTUs belonging to Bacteroidetes and Proteobacteria, which mainly make up the core microbiome of the seafloor and sub-surface plastisphere (Jacquin et al. 2019). Members of the phylum Bacteroidetes have been associated with significant contributions to ocean carbon and nutrient cycling (Zheng et al. 2020), while Proteobacteria are involved in organic degradation and nitrogen and sulfur metabolism in sediments (Huang et al. 2017; Chen et al. 2020). These two phyla also dominated in sediment samples on Day 0, with Bacteroidetes being the more dominant.

Upon introduction of plastics, however, plastics-associated bacteria became more Proteobacteria-dominated (Day 7), which again was consistent with previous reports describing these taxa to be primo-colonizers of plastic surfaces in the marine environment, with Bacteroidetes being the secondary colonizers (De Tender et al. 2015; Delacuvellerie et al. 2019). As seen on Day 7 (Fig. 6), the communities among the plastics in the different treatments clustered together despite the starting community coming from separate geographic locations within the bay. Alcanivoraceae, Marinobacteraceae, and Methylophagaceae were the dominant families in both treatments. Interestingly, members from the families Alcanivoraceae and Marinobacteraceae are known as hydrocarbon degraders (Mishamandani et al. 2016; Radwan et al. 2019) or hydrocarbonoclasts, while members from the family Methylophagaceae are known as putative oil-degraders (Mishamandani et al. 2016). Hydrocarbonoclastic bacteria can metabolize aliphatic and aromatic hydrocarbons (Teramoto et al. 2013) and are involved in the mineralization or removal of hydrocarbons in the environment (Dashti et al. 2015; Berry and Gutierrez 2017). These bacteria were also found to be dominant in floating plastic debris isolated from the Mediterranean Sea and the Beaufort Inlet in Western Australia, suggesting the possibility of them having metabolic potentials to degrade plastics but needs more assessment (Mishamandani et al. 2014; Dussud et al. 2018a; Delacuvellerie et al. 2019; Vaksmaa et al. 2021).

Days 42 to 91 were characterized by continued differentiation in the community with the appearance of taxa capable of degrading by-products of hydrocarbon degradation, although the abundance of the hydrocarbonoclastic taxa was still significant. On High Day 42 for example, the community shifted to hydrocarbon-degrading related group Rhodanobacteraceae, along with species from polyaromatic hydrocarbons (PAH)-degrading Chitinophagaceae, and members from the known plastic colonizer Acidobacteriaceae. The EPS-producing *Granulicella* was notably the predominant OTU from Acidobacteriaceae, which possibly played a role in biofilm formation by improving the adhesion and enhancing habitability of the plastic, and a methoxylated aromatic compound-degrader *Holophaga* from Holophagaceae (Liesack et al. 1994; Rummel et al. 2017; Gutierrez 2017; Blanco-Enríquez et al. 2018; Pinto et al. 2019; Pankratov and Dedysh 2010). This continued to Day 91 where an enriched bacterial community composed mainly of hydrocarbonoclasts from Flavobacteriaceae, Rhodobacteraceae, Alteromonadaceae, and Sphingomondaceae, and the putative oil-degrader Methylophagaceae were still observed (Abed et al. 2014; Mishamandani et al. 2014; Al-Mailem et al. 2017; Berry and Gutierrez 2017; Prince et al. 2018; Neethu et al. 2019). Similarly, OTUs from Muribaculaceae

(formerly S24-7), Lachnospiraceae and an unknown bacterium, which are known to inhabit animal guts (Ormerod et al. 2016; Jiang et al. 2018; Vacca et al. 2020), along with Rhodanobacteraceae were found to compose the bacterial community of the LDPE sheet in High Day 91. It is also possible that some of the taxa attached to the plastic may also be occurring in the bulk liquid, however, this was not investigated. This shift may have also been caused by the lack of stirring in the set-up after 42 days to simulate the settlement of plastics in the sediments, resulting in the establishment of microenvironments and causing differentiation of the communities.

The decreasing α-diversity metrics from the original sediment microbial community (Day 0) towards the end of the enrichment assays could suggest habitat selection (Ogonowski et al. 2018), as previous studies showed significant differences between those attached to plastics and the free-living communities (e.g., Dussud et al. 2018b). Moreover, the inverse Simpson metric also suggests that from a large, more stable, and complex community on Day 0, the communities have shifted to a smaller, less table, and simpler environment on Day 91 (Franco 2018). As the community in the biofilm matures, resources could also become more limited compared to the sediments or the surrounding environment, creating specific niches and variability in resources. These then result in a trophic cascade and the establishment of a microecosystem with decreasing diversity.

Potential interaction and role of microbes in plastics biodegradation

Biodegradation of plastics is a stepwise process involving several key processes as outlined by Lucas et al. (2008). First is biodeterioration, followed by bio-fragmentation or depolymerization, then assimilation, and finally remineralization which is the complete degradation of the incorporated molecules resulting in the release of metabolites (such as CO_2, CH_4, and H_2O) (Mueller 2006). Biodeterioration is classified as superficial degradation as the result of microorganisms growing on the plastic material (Lucas et al. 2008). This occurs during the early stages of colonization, microbes release EPS to facilitate attachment and biofilm formation (Rummel et al. 2017). Consequently, both experimental treatments showed biofilm formation on plastic surfaces (Day 7) noted by red auto-fluorescent lipids and green-stained genetic material (Supplementary Fig. 7), indicating prior EPS secretion.

In bio-fragmentation or depolymerization, enzymes breakdown the polymer into small, soluble molecules (Lucas et al. 2008; Dussud and Ghiglione 2014) through a series of oxidation, reduction, hydrolysis, and esterification reactions, and sometimes molecular inner conversion (Harshvardhan and Jha 2013; Kopeček and Rejmanova 2019), creating new functional groups on the surface of the plastics. In this study, a significant increase in the keto and ester carbonyl indices for Low Day 42 was observed, consistent with previous studies on biodegradation (Nowak et al. 2011; Harshvardhan and Jha 2013; Dela Torre et al. 2018). These can be possibly attributed to the product formation of oxidoreductases secreted by microbes (Harshvardhan and Jha 2013). However, other studies revealed the opposite where a decrease in the carbonyl indices was observed after incubation with microorganisms (Albertsson et al. 1987; Manzur et al. 2004; Montazer et al. 2018) possibly due to the oxidation of carbonyl groups through enzymatic hydrolysis by microorganisms (i.e., bacteria and fungi). Our results also showed a significant increase in the vinyl and internal double bonds or indices in the experimental set-ups as compared to the control, similar to previous studies (Manzur et al. 2004; Balasubramanian et al. 2010; Nowak et al. 2011; Harshvardhan and Jha 2013; Dela Torre et al. 2018; Syranidou et al. 2019). This may be attributed to the Norrish Type II reactions of the formed carbonyl products, which led to the formation of double bonds (Syranidou et al. 2019). The trend of carbonyl and double bond indices upon incubation with microbial organisms may not be universal, but these studies show that different microbial communities have different ways to degrade polyethylene (Restrepo-Flórez et al. 2014). What is definite is that incubation of polyethylene with possible biodegrading microorganisms resulted in the changes in surface concentrations of functional groups on the plastic surface, which then potentially confirm degradation (Harshvardhan and Jha 2013; Restrepo-Flórez et al. 2014).

Many studies suggested that bio-fragmentation of the intact polymer can only be determined by measuring the molecular weight of the polymer (Montazer et al. 2020). While this is true, we believe that surface modifications observed using FTIR should also be another way to measure this second stage of biodegradation, as enzymes are biocatalysts responsible for breaking down a section of the polymer, converting the polymeric backbone into smaller molecules for the microbes to assimilate in their biomass or use as energy. Although many OTUs were found to belong to hydrocarbonoclastic groups, only a few OTUs showed a strong positive correlation with the biodegradation indices, which could indicate their roles in the formation of functional groups. The low number of OTUs (< 1% of OTUs) strongly correlated with the biodegradation indices suggest that only a few taxa could be driving the degradation, but their high abundance (18% of all reads) on the other hand indicates their significant contribution to the community. One OTU from *Methylotenera* correlated with three indices. Species from this genus are methylotrophs that can utilize single carbon compounds as their carbon and energy source, with one species *M. mobilis* found to be an obligate methylamine utilizer and may be a putative

hydrocarbon degrader (Kalyuzhnaya et al. 2006; Thompson et al. 2017). Members of the Rhodanobacteraceae family, which correlated with three indices—ECBI, KBI, and VBI, are categorized as generalist hydrocarbon-degraders, as they can utilize carbon as their sole energy source (Gutierrez 2017). The identified genus of this family correlating to the indices was *Rhodanobacter*, which was previously found to have stimulated responses to the presence of petroleum hydrocarbons (van Dorst et al. 2016). Interestingly, species of *Rhodanobacter* did not grow in the presence of simple hydrocarbons nor aromatic hydrocarbons in pure cultures but grew in hydrocarbon-mineralizing consortia, which may mean that this genus utilizes the metabolites or by-products produced by other members (Kanaly et al. 2002; Bacosa et al. 2012). Meanwhile, OTUs belonging to *Alcanivorax* and two OTUs from *Methylophaga* correlated with IDBI and KCBI. *Alcanivorax* species are found in different marine environments and utilize hydrocarbons as the sole carbon source (Schneiker et al. 2006; Cappello and Yakimov 2010). Recently, this genus was identified as a key player in LDPE degradation (Delacuvellerie et al. 2019). A strain of *Methylophaga* was found to degrade and utilize hydrocarbons as the sole carbon and energy source (Mishamandani et al. 2014; Thompson et al. 2017). *Aestuariibacter*, two OTUs from *Methylophaga*, and *Alteromonas* were strongly positively correlated with IDBI. Members from the genus *Aestuariibacter* are known to produce biosurfactants and EPS, with oil-degrading capabilities (Wang et al. 2014; Bacosa et al. 2012, 2018), and was reported to select for weathered PE in the Bay of Palma (Erni-Cassola et al. 2019). *Alteromonas* has been conventionally found to be the key agent of PAH biodegradation in crude oil-contaminated coastal sediments (Jin et al. 2012).

The underlying mechanisms of how these microbes (represented by OTUs) correlated with the biodegradation indices are beyond the scope of this study. However, there seems to be an association of hydrocarbon-degraders with plastic surfaces and biodegradation indices, suggesting that some of the members of the plastic biofilm or plastisphere might have the ability to degrade plastics in our oceans (Pinto et al. 2020), as plastic surfaces are composed mainly of hydrocarbons. Some microbes that were known to be hydrocarbon-degrading were also found to have plastic-degrading capabilities. *Rhodococcus ruber,* an aliphatic-degrading bacterium, was found to produce laccase involved in the biodegradation of PE (Orr et al. 2004; Zhukov 2017; Sivan 2011). The diverse strains of the genus *Pseudomonas* have not been only found to degrade crude oil, but PE and other types of plastics as well (Yoon et al. 2012; Pasumarthi et al. 2013; Wilkes and Aristilde 2017). Recently, a microbial consortium of hydrocarbon-degrading bacteria composed of *Alcanivorax* and *Cycloclasticus* have been found to alter the surface chemistry and morphology of polyethylene terephthalate (PET) films (Denaro et al. 2020). *Alcanivorax borkumensis* was observed to have high affinity and significantly degraded LDPE after 80 days of incubation (Delacuvellerie et al. 2019). A strain of this bacterium was reported to have genomic traits that can metabolize oil-derived hydrocarbons (Schneiker et al. 2006; Oberbeckmann et al. 2016). *Alcanivorax* species have alkane monooxygenases *AlkB1* and *AlkB2*, which can degrade C5 to C12 hydrocarbons (HCs) and C8 to C16 HCs (Schneiker et al. 2006; Miri et al. 2010) and three cytochrome P450 alkane hydrolases (Liu et al. 2011; Yakimov et al. 2019).

It may be possible that only the initial communities or those found in the innermost part of the biofilm may be directly involved in the decomposition of the plastic polymer because of their proximity and access to the substrate. However, the development of the biofilm as a microenvironment also created more niches and provide a platform for different non-plastic biodegraders to interact and benefit from the products leaching out of the biofilm. Such interactions may influence the recycling and exchange of major nutrients, resulting in more efficient utilization of the plastic polymer as a carbon source and its associated compounds or their by-products (De Tender et al. 2015; Delacuvellerie et al. 2019). For example, some members from the family Saprospiraceae, known as biofilm formers with their ability to produce exopolysaccharides and scavenge resources from the biofilm for energy, was found present with the other hydrocarbonoclasts in Day 7. This suggests their possible role in the initial colonization by biofilm formation, which then allowed other organisms to attach (Caruso 2020). On Day 42, hydrocarbonoclasts co-occurred with OTUs from Rhodanobacteraceae and *Rhodanobacter*, which can metabolize metabolic products produced by hydrocarbon degraders (Kanaly et al. 2002; Bacosa et al. 2012). The presence of intermediate-metabolizing microbes may further enhance biodegradation by consuming the by-products produced in the process which may be toxic to the hydrocarbon degraders (Park et al. 2004; Bacosa et al. 2012). Day 91 had the greatest number of positively correlated OTUs with biodegradation indices but along with these was the high abundance of co-occurring predatory bacterial species *Peredibacter*, *Bdellovibrio*, and an OTU from Bradymonadales, which may indicate a selective force in the microbial community (Davidov and Jurkevitch 2004; Iebba et al. 2014; Mu et al. 2020). Also observed co-occurring were the uncultured Cellvibrionaceae and *Thalassococcus*, which may have roles in nitrogen fixation and conversion of nitrate to nitrite that can be used by the other members of the community (Pujalte et al. 2014; Suarez et al. 2014). *Robiginitalea* are *Pyruvatibacter* bacterial heterotrophs that were also found to co-occur in the same cluster, indicating the presence of

organic compounds on the surface which may have been produced through microbial action on the plastic polymer (Oh et al. 2009; Rong et al. 2021). All these coincided with the decreasing fluorescence intensity of the biofilm captured by CLSM, possibly indicating consumption of the organic material through time.

Summary and implications

This study showed that the anthropogenic input of plastics in the marine environment triggers some of the members of the naturally occurring taxa in the sediments to occupy the surfaces of plastics, using it as an alternate substrate. Through time, the substrate may select a specific set of taxa adapted to the conditions in the plastisphere, and here, some were found to be positively and strongly correlated with biodegradation indices. These suggest that some of the hydrocarbonoclasts may be playing active roles or functions in the partial biodegradation of plastics. Further, our results support the notion that biodegradation potential for plastics could be ubiquitously present in marine microbial assemblages (Quero and Luna 2017) since microbial communities from all sediments collected from Manila Bay exhibited biodegradation activity. Notably, hydrocarbonoclasts also co-occurred with other bacteria that have varied functions, demonstrating that the colonization of plastic surfaces by microorganisms is not limited to biodegradation, but also to the production and consumption of the by-products of biofilm formation and degradation. Most biodegradation studies have focused on pure strains to degrade plastics; however, microbes exist with other microbial populations as communities in nature, allowing them to perform ecosystem functions (Friedrich 2011). The biodegradation of LDPE in the environment is a process possibly involving many different microbial taxa, with the different members directly or indirectly contributing to the partial degradation of plastics in the environment.

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Author contributions NCFG: conducted field surveys and sample collection, performed laboratory experiments, did data analysis, wrote the manuscript, and prepared the figures and tables. DFLO: reviewed and edited the manuscript, figures, and tables, as well as provided advice and guidance throughout the study. All authors conceptualized the project idea, designed the experiment and data analyses, and finalized the manuscript.

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Data availability All data from this study were included in this article.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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