FEMS Microbiology Ecology, 96, 2020, fiaa230

doi: 10.1093/femsec/fiaa230
Advance Access Publication Date: 12 November 2020
Research Article

RESEARCH ARTICLE

Temporal changes in water temperature and salinity drive the formation of a reversible plastic-specific microbial community

Lee J. Pinnell† and Jeffrey W. Turner*

Department of Life Sciences, Texas A&M University – Corpus Christi, 3600 Ocean Drive, Corpus Christi, Texas, 78412, USA

*Corresponding author: Department of Life Sciences, Texas A&M University - Corpus Christi, 3600 Ocean Drive, Corpus Christi, Texas, 78412, USA. E-mail: jeffrey.turner@tamucc.edu

One sentence summary: Temperature and salinity drive the formation of a reversible plastic-specific community.

Editor: Tillmann Lueders
†Lee J. Pinnell, http://orcid.org/0000-0002-6238-3313

ABSTRACT

Plastic is a ubiquitous pollutant in the marine environment. Here, we investigated how temporal changes in environmental factors affect the microbial communities formed on plastic (polyethylene terephthalate; PET) versus a ceramic substrate. *In situ* mesocosms ($N = 90$ replicates) were deployed at the sediment–water interface of a coastal lagoon and sampled every 4 weeks for 424 days. Sequencing data (16S rRNA) was parsed based on variation in temperature with the exposure starting in fall 2016 and remaining *in situ* through the next four seasons (winter, spring, summer and fall 2017). PET biofilms were distinct during the summer when salinity and temperature were highest. In particular, a significant shift in the relative abundance of Ignavibacteriales and Cytophagales was observed during the summer, but PET and ceramic communities were again indistinguishable the following fall. Water temperature, salinity and pH were significant drivers of PET biofilm diversity as well as the relative abundance of plastic-discriminant taxa. This study illustrates the temporal and successional dynamics of PET biofilms and clearly demonstrates that increased water temperature, salinity, pH and exposure length play a role in the formation of a plastic-specific microbial community, but this specificity can be lost with a change in environmental conditions.

Keywords: plastisphere; plastic; marine debris; microbial community; sediment, succession

INTRODUCTION

Beginning with the mass production of plastic in the 1940s, the Plastic Age has transformed modern society (Thompson *et al.* 2009). Today, plastic pollution is widely regarded as an environmental crisis. It was estimated that 5.5 billion metric tons of plastic waste has entered our landfills and natural environments (Geyer, Jambeck and Law 2017). The oceans in particular are sinks for plastic debris and it was estimated that between 4.8 and 12.7 million metric tons of plastic debris entered the oceans in 2010 alone (Jambeck *et al.* 2015).

Aquatic microorganisms rapidly form biofilms on plastic debris (Lobelle and Cunliffe 2011; Fazey and Ryan 2016; Tu *et al.* 2020) and plastic-associated microbial communities are distinct in comparison to free-living and sediment-associated microbial communities (Zettler, Mincer and Amaral-Zettler 2013; Harrison *et al.* 2014; Bryant *et al.* 2016; Oberbeckmann, Osborn and Duhaime 2016; De Tender *et al.* 2017; Kettner *et al.* 2017; Dussud *et al.* 2018; Ogonowski *et al.* 2018; Muthukrishnan Al Khaburi and Abed 2019; Pinnell and Turner 2019; Pinto *et al.* 2019; Dudek *et al.* 2020). Microbial fouling then contributes to a decrease in

Received: 1 July 2020; **Accepted:** 10 November 2020

buoyancy, sinking and seafloor deposition (Lobelle and Cunliffe 2011; Fazey and Ryan 2016). The plastisphere is a diverse community of prokaryotic and eukaryotic microorganisms associated with plastic debris (Zettler, Mincer and Amaral-Zettler 2013).

Many have postulated that plastic is colonized by a distinct microbial community, and Amaral-Zettler, Zettler and Mincer (2020) recently described the plastic-specific community hypothesis as a topic of intense investigation. Indeed, *ex situ* (laboratory-based) and *in situ* (field-based) studies have tested this hypothesis by including a biofilm reference substrate in their experimental design. The *ex situ* studies indicate that plastic-attached communities are distinct in comparison to glass and cellulose substrates (Kirstein *et al.* 2018; Ogonowski *et al.* 2018; Kirstein *et al.* 2019). However, the findings of *in situ* studies are less clear, and differences in the selection of reference substrate and exposure environment have resulted in contrasting results. For example, Oberbeckmann, Osborn and Duhaime (2016) used glass as a reference substrate during different seasons and concluded that polyethylene terephthalate (PET) was not colonized by a distinct community although PET-associated communities differed between seasons. Pinnell and Turner (2019) used ceramic as a reference substrate during the fall and also concluded that PET was not colonized by a distinct community. Pinto *et al.* (2019) used glass as a reference substrate during the winter and observed that polyvinyl chloride (PVC) was initially colonized by a distinct community whereas high-density polyethylene (HDPE), low-density polyethylene (LDPE) and polypropylene (PP) were not. By contrast, when Oberbeckmann, Kreikemeyer and Labrenz (2018) used wood as a reference substrate, a distinct PET community was observed at study sites under nutrient limitation and higher salinity. Similarly, Kettner *et al.* (2017) as well as Muthukrishnan, Al Khaburi and Abed (2019) observed that polyethylene (PE), polystyrene (PS) and PET were colonized by distinct communities in comparison to a wood substrate. These mixed findings suggest that *in situ* plastic colonization may be governed by conventional marine biofilm processes and subject to seasonal fluctuations in physiochemical conditions (Oberbeckmann, Osborn and Duhaime 2016; Pinto *et al.* 2019; Dudek *et al.* 2020).

This study utilized 16S small subunit ribosomal RNA (16S rRNA) sequencing to monitor microbial succession during the colonization of PET nurdles in coastal marine sediment over a 424-day period. It was designed to test how temporal changes in environmental factors affect the succession and specificity of the plastic-attached community. Our study was highly replicated, randomized, continuous and included ceramic nurdles as the reference substrate. We hypothesized that microbial communities colonizing PET would be distinct in comparison to the ceramic substrate. Further, we hypothesized that the composition of the plastic-attached microbial community would be modulated by temporal changes in temperature, salinity, pH, dissolved oxygen and exposure length.

MATERIALS AND METHODS

Site description

The Laguna Madre, a bar-built coastal lagoon, is the largest estuarine system along the Texas coast of the United States. The lagoon is divided into two subunits, the Upper Laguna Madre (ULM) and the Lower Laguna Madre. The mesocosms described below were deployed in the ULM adjacent to a dredge material island located at 27°32′39.0‘N and 97°17′07.7’W (Figure S1, Supporting Information).

Experimental design

Mesocosms were designed as described previously (Pinnell and Turner 2019). Briefly, 3.0 g of PET nurdles (M&G Chemicals, Ettelbruck, Luxembourg) and 6.0 g of ceramic nurdles (Lyman Products, Middletown, CT) were deployed in custom-made capsules at the sediment–water interface. A total of 90 capsules (n = 45 ceramic, n = 45 PET) were deployed on October 18, 2016. Triplicates of each sample type were collected at approximately 4-week intervals for a total of 424 days (final collection date December 15, 2017). Additionally, triplicate 1 L seawater samples from the sediment–water interface were collected. All samples were stored on ice, transported to the laboratory and processed within 2 h of collection. Environmental parameters (i.e. temperature, salinity, dissolved oxygen and pH) were measured using a 6920 V2–2 Multi-Parameter Water Quality Sonde (YSI, Yellow Springs, OH; Table S1, Supporting Information) at the deployment date and each of the 15 collection timepoints.

DNA isolation

Genomic DNA was isolated from triplicates for each sample type (seawater, ceramic and PET). Nurdles were washed three times with 25 mL of 0.22 μm filter-sterilized, site-specific seawater to remove any organisms that were not part of the biofilm. Seawater was pre-filtered (315 μm Nitex mesh) and the free-living microorganisms were collected by vacuum filtration on a 0.22 μm polycarbonate filter (MilliporeSigma, Burlington, MA). DNA was isolated from the nurdles and filters using a modified version of a high-salt and sodium dodecyl sulfate-based method (Zhou, Bruns and Tiedje 1996) as described previously (Pinnell and Turner 2019). DNA was quantified (ng/L) and assayed for quality (A_{260}/A_{280} and A_{260}/A_{230}) using a BioPhotometer D30 (Eppendorf, Hamburg, Germany). DNA concentrations were verified using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA) and stored in the dark at −20°C prior to sequencing.

16S rRNA PCR Amplification and Sequencing

The V4 region of the 16S rRNA gene was amplified with an iTaq DNA Polymerase Kit (Bio-Rad, Hercules, CA) using the improved 515f (5′—GTG YCA GCM GCC GCG GTA A–3′) and 806r (5′—GGA CTA CNV GGG TWT CTA AT–3′) primers (Walters *et al.* 2016) and 40 ng of DNA as template. Amplification conditions were 95°C for 3 min followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 72°C for 1 min. Final elongation occurred at 72°C for 5 min. Amplification was confirmed visually using gel electrophoresis. Excess primers and unincorporated nucleotides were removed using an EXOSAP-IT Express PCR Cleanup Kit (Thermo Fisher Scientific, Waltham, MA). The cleaned amplicons were pooled in equal proportions based on their molecular weight and DNA concentrations. Pooled amplicons were purified using calibrated Ampure XP beads (Beckman Coulter, Indianapolis, IN) and the resulting pooled library was sequenced on an Illumina MiSeq instrument using paired-end chemistry (2 × 250 bp) at Molecular Research LP (Shallowater, TX).

Community structure

Raw sequence reads were processed using a combination of QIIME v1.9 and QIIME2 version 2018.11 (Caporaso *et al.* 2010). Barcodes were extracted from the paired-end reads using the 'extract_barcodes.py' tool in QIIME v1.9. Reads were then imported into QIIME2 where they were demultiplexed and denoized with DADA2 (Callahan *et al.* 2016) to generate amplicon sequence variants (ASVs). DADA2 was also used to filter the reads for quality, remove chimeric sequences and merge overlapping paired-end reads. Trim lengths of 242 and 233 bp were used on the forward and reverse reads, respectively. Taxonomy was assigned using a Naïve Bayes classifier trained on the SILVA release 132 99% OTU database (Quast *et al.* 2013), where sequences had been trimmed to include only the 250 bases from the V4 region bound by the 515F/806R primer pair. Reads that mapped to chloroplast and mitochondrial sequences were filtered from the sequence variants table using the 'filter_taxa' function, and a phylogenetic tree was then generated using the 'q2-phylogeny' pipeline with default settings, which was used to calculate phylogeny-based diversity metrics. Data was then imported into phyloseq (McMurdie and Holmes 2013) using the 'import_biom' and 'import_qiime_sample_data' functions and merged into a phyloseq object. Samples with read depth of less than 4086 reads were discarded and Faith's Phylogenetic Diversity (FPD) was calculated for remaining samples ($n = 44$ seawater, $n = 37$ ceramic, $n = 44$ PET) using the 'estimate_pd' function within the package btools. Samples were then proportionally transformed to a read depth of 6048. Beta-diversity was analyzed using generalized UniFrac (Lozupone *et al.* 2011; Chen *et al.* 2012) distances calculated using phyloseq and GUniFrac. From these distances, a principal coordinates analysis (PCoA) was calculated and plotted, and a permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences between communities using the 'vegan' (Oksanen *et al.* 2019) and 'pairwiseAdonis' (Arbizu 2017) packages in R version 3.5.2 (R Core Team 2017). To ensure differences in microbial communities were not due to unequal dispersion of variability among groups, permutational analyses of dispersion (PERMDISP) were conducted for all significant PERMANOVA outcomes with the 'vegan' package in R. Hierarchal clustering was performed using Ward's agglomeration clustering (Murtagh and Legendre 2014) method of generalized UniFrac distances and the 'hclust' function. Further, the relative abundances of ASVs within each sample were calculated and plotted using phyloseq.

Temporal dynamics

To facilitate the observation of temporal patterns, seasons were determined based on daily temperatures from the nearby Naval Air Station Corpus Christi collected by the National Oceanic and Atmospheric Administration. Data was accessed from the National Centers for Environmental Information database. To determine seasonal trends in temperature, daily maximum temperatures were plotted for a time period encompassing the entirety of the experiment (Figure S2, Supporting Information). Seasons were defined as follows: fall: September 21–December 14, winter: December 15–March 6, spring: March 7–June 2, summer: June 3–September 20 and fall: September 21–final sample collection (December 15; Figure S2, Supporting Information). Alpha and beta diversity analyses within seasons were compared between sample types.

Plastic-discriminant taxa

In the summer, when PET- and ceramic-associated communities were significantly different from each other, linear discriminant analysis effect size (LEfSe; Segata *et al.* 2011) was used to identify taxa that discriminated the plastic-associated community from the ceramic-associated community. This was done using the online LEfSe tool on the Galaxy server (https://huttenhower.sph.harvard.edu/galaxy), using default settings with the exception of a more stringent alpha of 0.01, due to the small sample size. Genera with a mean relative abundance $> 0.1\%$ across all plastic and ceramic summer samples were considered, and the factor 'substrate type' was set as class, and an 'all-against-all' strategy was applied. The relative abundances of the three taxonomic lineages considered discriminant of plastic-specific communities in the summer, were then visualized and compared between all three sample types in each of the five sampled seasons.

Statistical analyses

Unless specified otherwise, R version 3.6.1 (R Core Team 2017) was used for statistical analysis of data. Pairwise Wilcoxon rank-sum tests were performed with a Bonferroni (PW+B) correction for multiple comparisons. Differences in beta diversity were tested using pairwise PERMANOVA with a Bonferroni correction for multiple comparisons and 9999 permutations. Additionally, pairwise PERMDISPs were carried out for all PERMANOVAs using 9999 permutations to test for differences in dispersions. To determine drivers of substrate-specific alpha diversity, a linear model was fitted using all combinations of collected environmental parameters (temperature, salinity, pH, dissolved oxygen and exposure length) and the best model was chosen based on corrected Akaike Information Criterion (AICc). Due to the non-linear relationship between some environmental parameters and the relative abundance of plastic-discriminant taxa, a generalized additive mixed-model (gamm) was used to fit a model using the 'mgcv' package. Individual parameters were fit to relative abundances and the proportion of variance each individual parameter explained on its own was reported. Shapiro–Wilk tests and quantile–quantile plots were used to test data for normality, and Breusch–Pagan tests were used to test for homoskedasticity with alphas of 0.05. If needed, heteroskedasticity was accounted for with an exponential variance function using the package 'nlme'.

Data availability

All sequence reads were made available through the BioProject PRJNA551219 at the NCBI's Sequence Read Archive.

RESULTS

Temporal diversity

The diversity of biofilm communities (i.e. ceramic and PET) was significantly higher than that of the surrounding seawater community across all five seasons (Fig. 1; Pairwise Wilcoxon rank-sum + Bonferroni correction or PW+B, $P < 0.05$). The only significant difference between ceramic and PET diversity occurred in the spring when ceramic communities were significantly more diverse (Fig. 1; PW+B, $P < 0.05$). In general, diversity exhibited a temporal pattern in that FPD increased with exposure time and

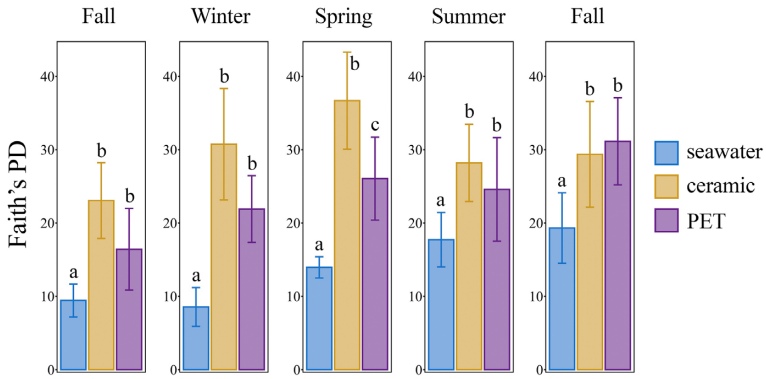

Figure 1. Barplot displaying Faith's Phylogenetic Distance (FPD) for each sample type (seawater, ceramic and PET) during each season. Error bars display the standard error of the mean. Significant differences in FPD between sample types within each season are illustrated by different letters (PW+B, $n = 6$–12, $P < 0.05$).

slight peaks occurred during spring and fall (Fig. 1). The diversity of PET-associated biofilms was best explained by a combination of exposure time (i.e. maturity of the biofilm), water temperature and salinity (Table 1; $R^2 = 0.406$, overall model $P < 0.0001$). Contrastingly, no combination of recorded environmental parameters (see Table S1, Supporting Information, for temperature, salinity, conductivity, dissolved oxygen and pH measurements) significantly impacted the FPD in ceramic biofilm communities (results of the non-significant model not shown).

Temporal community structure

The effect of sample type (seawater, ceramic and PET) on temporal community structure was analyzed using PCoA, hierarchal clustering and PERMANOVA. The PCoA illustrated that particle-attached communities (i.e. ceramic and PET biofilms) were clearly distinct from the free-living seawater communities during all seasons (Fig. 2), and that difference was statistically significant (Table S2, Supporting Information; pairwise PERMANOVA, $P < 0.05$). Interestingly, the specificity of plastic-associated communities differed between seasons (Fig. 2). In the fall, winter and spring, the microbial biofilms attached to PET and ceramic exhibited similar community structure; however, during the summer, they differed significantly (Fig. 2; Table S2, Supporting Information; pairwise PERMANOVA, $P < 0.05$).

To further investigate the summer specificity of the PET biofilm community structure, hierarchal clustering was performed on all summer samples. Clustering revealed that each sample type (i.e. seawater, ceramic and PET) was most similar to itself with the formation of three sample type-specific clades (Fig. 3). Importantly, the formation of a distinct PET-specific clade confirmed the development of a plastic-specific community during the summer. Additionally, the clustering of PET and ceramic samples the following fall confirmed the reversion of plastic-specificity with PET and ceramic biofilms intermixing evenly in a clade that remained distinct from the free-living, seawater communities (Figure S3, Supporting Information). The dichotomy between free-living and biofilm communities was primarily the result of large differences in the abundances of α-proteobacteria (~46% seawater and ~5% biofilms), δ-proteobacteria (~5% seawater and ~30% biofilms) and Actinobacteria (~9% seawater and <1% biofilms; Fig. 3; Table S3, Supporting Information). Differences between PET and ceramic biofilms were more subtle and are detailed in the following section.

Plastic-discriminant taxa

Three lineages of microbial taxa were found to be differentially abundant within PET biofilms versus ceramic biofilms during the summer: the Melioribacteraceae family (order Ignavibateriales, class Ignavibacteria), an uncultured genus of Cyclobacteriaceae (order Cytophagales) and the *Candidatus Electrothrix* genus (family Desulfobulbaceae, phylum Proteobacteria; Fig. 4). The differential abundance of the later lineage, however, was primarily the result of two PET samples having very high abundances (~20%) of *Candidatus Electrothrix* while the majority had less than 0.5% (Table S4, Supporting Information), and therefore, this lineage was excluded from subsequent analyses.

Temporal drivers of plastic-discriminant taxa

Temporal changes in the relative abundance of plastic-discriminant taxa were visualized, and generalized additive mixed models (gamms) were used to identify the environmental conditions driving the differential abundance between plastic and ceramic biofilms. Melioribacteraceae was significantly more abundant within PET biofilms during the summer (Fig. 5; PW+B, $P < 0.05$). Further, gamms revealed that water temperature, salinity, pH, dissolved oxygen and exposure length were drivers of increased Melioribacteraceae abundance within PET biofilms (Table 2), while none of the collected parameters impacted its abundance in ceramic biofilms. The gamms for dissolved oxygen and exposure length failed normality testing and therefore do not fully explain the data. This trend of increasing Melioribacteraceae abundance with increasing temperature, salinity, pH and dissolved oxygen is depicted in Fig. 6A. The significant impact of exposure length is misleading, as the abundance followed a seasonal pattern with highest abundances occurring in summer (~day 300 in mid-August) opposed to strictly increasing with exposure length (Fig. 6A).

While the uncultured Cyclobacteriaceae genus was also more abundant within PET biofilms during the summer (Fig. 5; PW+B, $P < 0.05$), it was a result of a decrease in ceramic biofilms opposed to an increase in PET communities. Gamms revealed that water temperature, salinity and exposure length were drivers of decreased Cyclobacteriaceae abundance within ceramic biofilms, while none of the collected parameters impacted its abundance in PET biofilms (Table 2). This trend of decreasing abundance of the uncultured Cyclobacteriaceae genus with increasing temperature, salinity and exposure length in ceramic biofilms is depicted in Fig. 6B. Again, the significant impact of exposure length is misleading, as the abundance

Table 1. Summary of multiple linear regression on environmental variables that predict the diversity (Faith's phylogenetic distance) of PET biofilms.

	Estimate	Std. Err.	t-value	P
Intercept	−0.012	0.559	−2.089	0.043
Salinity	4.153	1.578	2.632	0.012
Water temperature	3.729	1.908	1.954	0.058
Exposure length	0.0164	0.008	1.937	0.060
Salinity:water temperature	−0.119	0.052	−2.278	0.028
F-statistic: 10.24 on 4 and 39 d.f.; adjusted R^2: 0.462; overall model $p = 9.118^{-6}$				

*abbreviations: Std. Err. Standard error; d.f. degrees of freedo

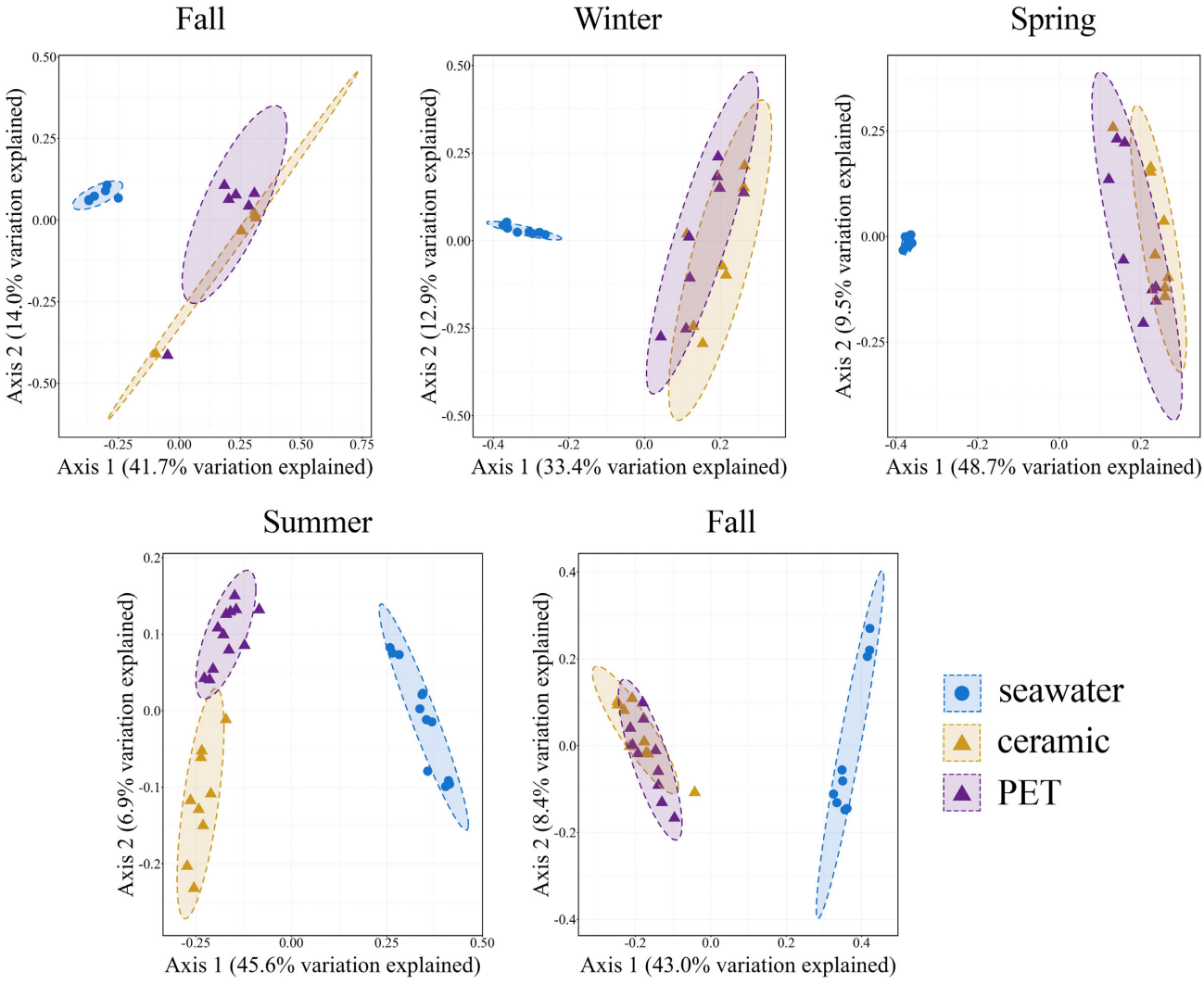

Figure 2. Principal coordinates analysis (PCoA) of generalized UniFrac distances illustrating the variation in microbial community structure within each season. The PCoA demonstrates clustering of 16S rRNA gene sequences from seawater, ceramic and PET microbial communities. Dashed lines and shaded areas represent 95% confidence ellipses for each community type.

of the uncultured Cyclobacteriaceae genus follows a seasonal pattern with its lowest abundances occurring in summer (~ day 300 in mid-August) opposed to increasing over time (Fig. 6B).

DISCUSSION

Numerous studies have investigated the microorganisms that colonize the plastisphere; however, a recent review article highlighted important unresolved questions about plastic-associated microbial communities (Amaral-Zettler, Zettler and Mincer 2020). Among these, how does the plastisphere microbial community change over time, and is the plastisphere colonized by a plastic-specific microbial community? To address these two questions, we conducted a long-term, continuous *in situ* study to assess the temporal dynamics and specificity of the plastisphere community in marine sediments.

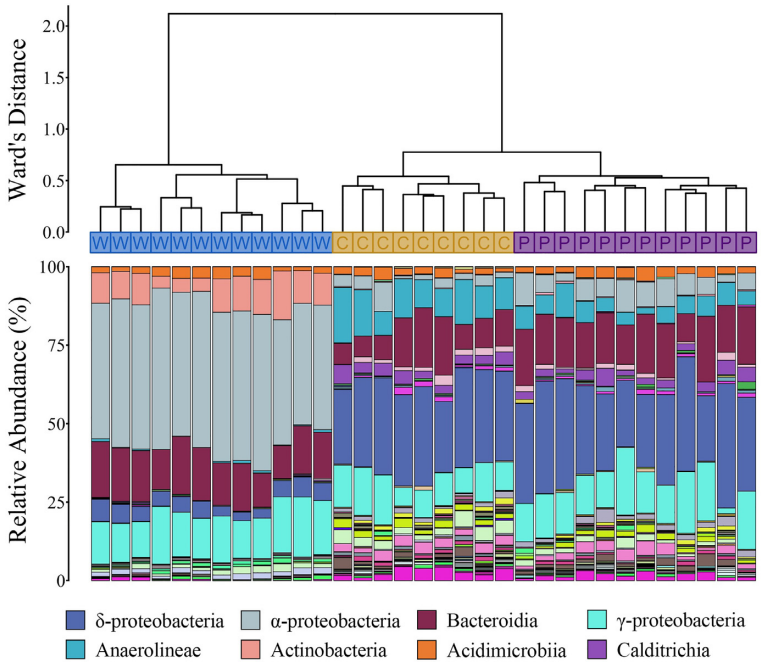

Figure 3. The relatedness of seawater, ceramic and PET microbial communities based on normalized ASVs from samples collected during the summer. Hierarchal clustering was performed on generalized UniFrac distances. Blue boxes (W) represent seawater communities, gold boxes (C) represent ceramic communities, and purple boxes (P) represent PET communities. The barplot shows the relative abundance of microbial classes within each sample. Abundances were normalized to the total number of sequences and the top eight most abundance classes across all samples are displayed in the legend.

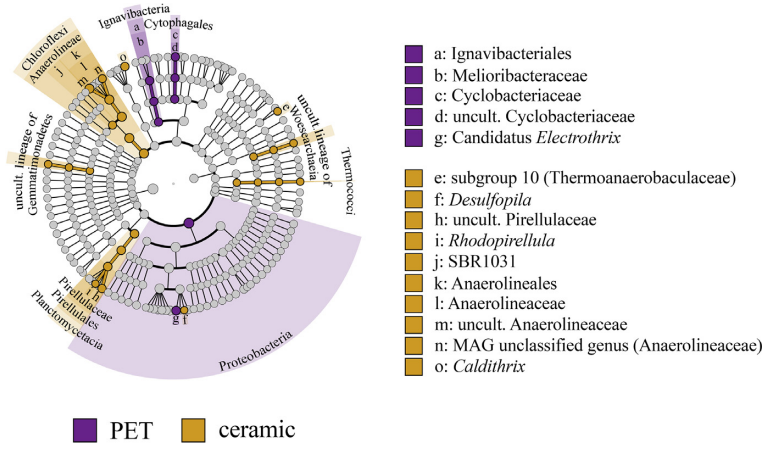

Figure 4. Cladogram demonstrating the microbial taxa with relative abundances of greater than 1% of the total community across PET and ceramic biofilm communities. Taxa discriminant of ceramic communities are highlighted in gold, and lineages discriminant of PET communities are highlighted in purple (LEfSe, $n = 9–12$, $P < 0.01$).

Differences in the diversity and community structure between substrate-attached and free-living communities is a well-established dichotomy in microbial ecology (DeLong, Franks and Alldredge 1993; Crump, Armbrust and Baross 1999; Murrell *et al.* 1999; Hollibaugh, Wong and Murrell 2000; Kellogg and Deming 2014). Likewise, numerous studies have reported phylogenetic and metabolic differences between plastic-associated and free-living communities (Zettler, Mincer and Amaral-Zettler 2013; Oberbeckmann, Osborn and Duhaime 2016; De Tender *et al.* 2017; Muthukrishnan, Al Khaburi and Abed 2019). In this study, the diversity (determined by FPD) and community structure (determined by generalized UniFrac values) of substrate-attached communities (i.e. PET and ceramic biofilms) was always significantly different compared to free-living communities. Further, this study showed that plastic-attached communities were significantly more diverse than free-living communities, in agreement with previous studies (Zettler, Mincer and Amaral-Zettler 2013; De Tender *et al.* 2017; Tu *et al.* 2020). The higher diversity of the plastic-attached communities may be the product of a biofilm lifestyle, which provides access to a more stable, more nutrient rich microenvironment (Mestre *et al.* 2017). We acknowledge however that a biofilm lifestyle may not always favor higher diversity. Case in point, contrasting studies have reported that communities attached to natural particles are sometimes less diverse than free-living communities (Hollibaugh, Wong and Murrell 2000; Moeseneder, Winter and Herndl 2001).

Mature aquatic biofilm communities are typically more diverse than early biofilm communities (Jackson, Churchill and Roden 2001; Chung *et al.* 2010), and exposure time has been

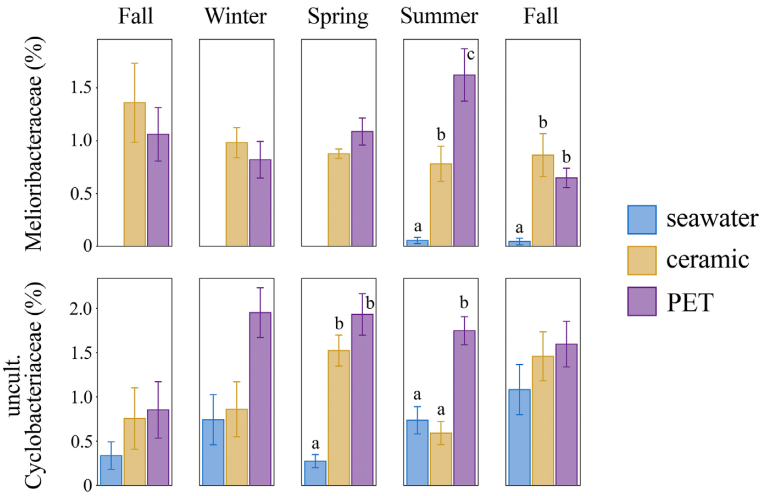

Figure 5. Barplot showing the relative abundances of plastic-discriminant taxa by season throughout the course of the study. Error bars display the standard error of the mean of the total relative abundance of Melioribacteraceae or uncult. Cyclobacteriaceae in seawater, ceramic and PET communities within each season. Significant differences between relative abundances are illustrated by different letters (PW+B, $n = 9–12$, $P < 0.05$).

Table 2. Coefficients of determination and p-values from linear regression based on generalized additive mixed models between the relative abundance of plastic-discriminant taxa and measured environmental parameters. Significant predictors of a taxa's relative abundance are bolded.

		PET biofilms					Ceramic biofilms				
		W.T. (°C)	Salinity	pH	D.O (%)	E.L.	W.T (°C)	Salinity	pH	D.O (%)	E.L.
Ignavibacteria	r^2	0.184	0.167	0.187	0.022	0.079	0.021	0.014	0.008	0.009	−0.002
	p	0.001	0.004	0.012	0.162	0.128	0.451	0.416	0.569	0.252	0.333
Ignavibacteriales	r^2	0.220	0.237	0.220*	0.063	0.216*	0.046	0.000	0.015	0.011	−0.008
	p	0.001	0.011	0.000*	0.052	0.013*	0.296	0.518	0.513	0.237	0.643
Melioribacteraceae	r^2	0.294	0.292	0.203	0.085*	0.247*	0.087	0.049	−0.013	−0.014	0.061
	p	0.000	0.006	0.001	0.029*	0.011*	0.141	0.262	0.778	0.473	0.108
Cytophagales	r^2	−0.014	0.024	−0.010	−0.009	0.009	0.165	0.375	0.015	0.019	0.231
	p	0.524	0.394	0.451	0.580	0.546	0.006	0.000	0.410	0.197	0.028
Cyclobacteriaceae	r^2	0.006	0.060	−0.013	−0.014	0.066	0.251	0.347	0.002	0.013	0.295
	p	0.263	0.190	0.502	0.517	0.177	0.015	0.000	0.461	0.226	0.013
uncult. Cyclobacteriaceae	r^2	0.000	0.045	−0.013	−0.020	0.063	0.222	0.313	−0.025	−0.007	0.260
	p	0.317	0.256	0.503	0.691	0.183	0.028	0.000	0.711	0.394	0.035

*model failed normality testing
Abbreviations: W.T., water temperature; D.O., dissolved oxygen; E.L., exposure length; PET, polyethylene terephthalate

correlated with plastisphere biofilm thickness and diversity (Tu *et al.* 2020). In this study, temperature and salinity, in addition to exposure time, were correlated with FPD diversity, and slight peaks in biofilm diversity were observed during spring and fall. Temperature and salinity are known to strongly affect marine biofilm biomass and diversity (Chiu *et al.* 2005; Moss *et al.* 2006). Freshwater inflows into the subtropical Laguna Madre are highest in the spring and fall (Tunnell 2002), and corresponding peaks in diversity may reflect temporal changes in additional factors, such as dissolved organic matter (DOM) concentration and primary productivity, which are also established regulators of bacterial community structure (Murrell *et al.* 1999; Langenheder and Ragnarsson 2007). However, these spring and fall peaks were not significant and additional factors not recorded in this study (e.g. light intensity, nutrients, mixing and water turbidity and the abundance of fungal taxa or bactivorous grazers) could be modulators of temporal biofilm diversity in this system.

Previous studies have suggested that environmental factors play a role in the structuring of plastic-specific communities (De Tender *et al.* 2015; Oberbeckmann, Osborn and Duhaime 2016; Oberbeckmann, Kreikemeyer and Labrenz 2018). For example, environmental factors such as nutrient limitation and salinity have been proposed as drivers of a plastic-specific community (Oberbeckmann, Kreikemeyer and Labrenz 2018). In this study, the presence of a plastic-specific community was evident during the summer based on PCoA and hierarchal clustering analyses. This result should not however be confused with the results of previous studies that conducted short-term exposure experiments during different seasons (De Tender *et al.* 2015; Oberbeckmann, Osborn and Duhaime 2016; Oberbeckmann, Kreikemeyer and Labrenz 2018). By contrast, this continuous study clearly demonstrates that a mature non-specific plastisphere community was succeeded by a specific plastisphere community during summer conditions (e.g. warmer water and higher salinity). Further, we show for the first time that plastic-specific community structure can be lost with a subsequent seasonal change in environmental conditions.

While subtle shifts in a large number of taxa played a role in the formation of a plastic-specific microbial community in the summer, two lineages were differentially abundant. The

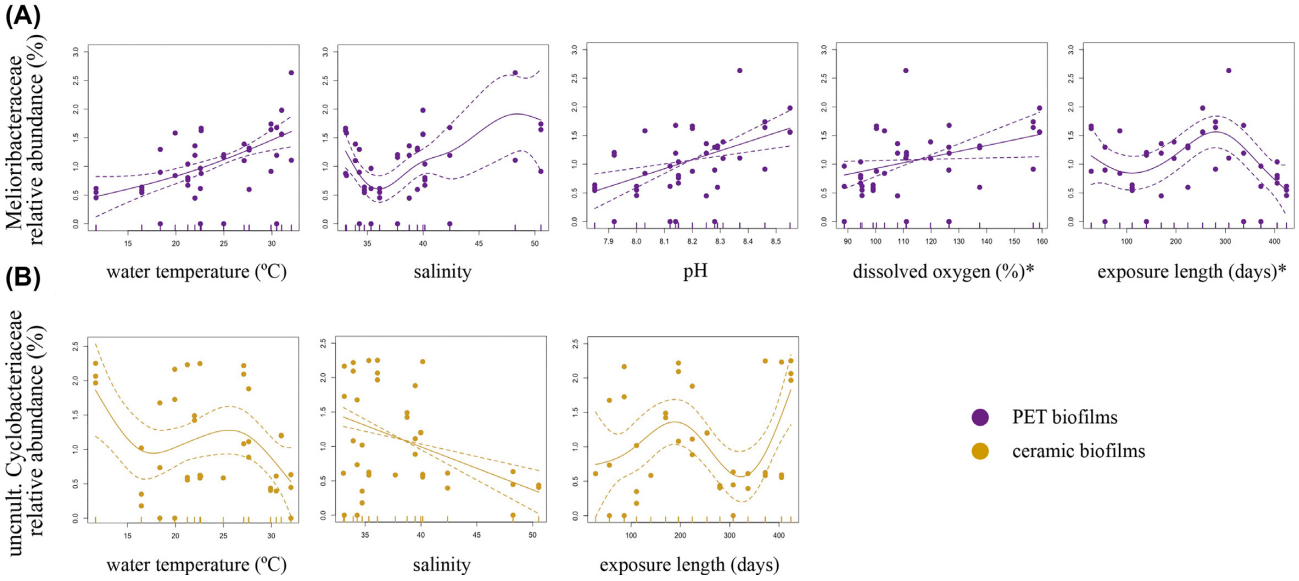

Figure 6. Plot of the gamms demonstrating the relationship between significant environmental predictors and the relative abundance of the plastic-discriminant taxa Melioribacteraceae **(A)** and uncult. Cyclobacteriaceae **(B)**. * model failed normality testing.

moderately thermophilic and halotolerant Melioribacteraceae family was preferentially associated with PET during summer conditions. An uncultured genus of Cyclobacteriaceae was also differentially abundant during the summer. However, in contrast to Melioribacteraceae, the differential abundance of uncultured Cyclobacteriaceae was the result of a decreased affinity for ceramic under summer conditions as opposed to an increased affinity for PET.

Melioribacteraceae is a largely uncharacterized family, containing only a single defined genus (*Melioribacter*) with only one cultivated isolate that is a moderately thermophilic, halotolerant, facultative anaerobe (Podosokorskaya *et al.* 2013; Kublanov and Podosokorskaya 2020). By contrast, Cyclobacteriaceae are morphologically and physiologically diverse, tolerate a wide range of physiochemical conditions and are broadly distributed in marine environments including sediment (Pinnaka and Tanuku 2014). Importantly, Cyclobacteriaceae have previously been reported as more abundant on microplastic substrates compared to natural substrates (Miao *et al.* 2019).

Previous studies have linked temperature and salinity with the formation of plastic-specific microbial assemblages (De Tender *et al.* 2015; Oberbeckmann, Kreikemeyer and Labrenz 2018), but to our knowledge, this is the first study to link pH with plastic-specificity. This effect could be direct or indirect although pH is a known regulator of community structure in terrestrial soils (Lauber *et al.* 2009; Zhalnina *et al.* 2015) and streams (Besemer 2015). Additionally, while the length of exposure (i.e. biofilm maturity) affected the diversity and community structure of the plastic-specific communities, a summer increase in temperature and salinity appeared to attenuate the importance of biofilm maturity. Further characterizing the effect of temporal change could reveal that plastisphere communities vary considerably with latitude and magnitude of seasonal change.

On the whole, these findings advance basic knowledge of the *in situ* temporal dynamics of plastisphere microbial communities, which is a critical endeavor given that plastic is a ubiquitous pollutant in marine environments worldwide. In particular, this study demonstrates that temporal changes in environmental parameters, namely temperature, salinity and pH, play an important role in shaping the diversity and structure of the plastisphere community. The formation of a plastic-specific community was clear during summer months when temperature and salinity were highest. This plastic-specificity appears to be largely driven by environmental conditions seeing that a subsequent decrease in temperature and salinity was correlated with the dissolution of specificity. However, it is important to state the effects of temperature and salinity could be direct or indirect. To rule out indirect effects, future studies should consider measuring additional factors such as light intensity, nutrients, turbidity and the abundance of bactivorous predators (e.g. *Bdellovibrios* and *Bacterioroax*) that can also impacted by temporal changes in temperature and salinity (Kelley *et al.* 1997, Williams, Turng and Kelley 2009).

ACKNOWLEDGMENTS

This study would not have been possible without the support of our friends and colleagues. Specifically, we thank Robert 'Bobby' Duke and the entire Center for Coastal Studies staff for assistance in the field. This study was funded by the Texas General Land Office's Coastal Management Plan (GLO-CMP), the Texas Research and Development Fund (TRDF), Texas Sea Grant (TSG) and the National Sciences and Engineering Research Council of Canada (NSERC). The majority of computational data analysis was performed on TAMU-CC's high-performance computing cluster, which is funded in part by the National Science Foundation's CNS MRI Grant (Number 1429518).

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

Conflict of interest. We declare no conflict of interest.

REFERENCES

Amaral-Zettler LA, Zettler ER, Mincer TJ. Ecology of the plastisphere. *Nat Rev Microbiol* 2020;**18**:139–51.

Arbizu PM. pairwiseAdonis: pairwise multilevel comparison using adonis: R package version 0.0.1 Edition. https://github.com/pmartinezarbizu/pairwiseAdonis, 2017.

Besemer K. Biodiversity, community structure and function of biofilms in stream ecosystems. *Res Microbiol* 2015;**166**:774–81.

Bryant JA, Clemente TM, Viviani DA *et al*. Diversity and activity of communities inhabiting plastic debris in the North Pacific gyre. *mSystems* 2016;**1**:e00024–16.

Callahan BJ, McMurdie PJ, Rosen MJ *et al*. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Caporaso JG, Kuczynski J, Stombaugh J *et al*. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**:335–6.

Chen J, Bittinger K, Charlson ES *et al*. Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* 2012;**28**:2106–13.

Chiu J, Thiyagarajan V, Tsoi M *et al*. Qualitative and quantitative changes in marine biofilms as a function of temperature and salinity in summer and winter. *Biofilms* 2005;**2**:183–95.

Chung HC, Lee OO, Huang Y-L *et al*. Bacterial community succession and chemical profiles of subtidal biofilms in relation to larval settlement of the polychaete *Hydroides elegans*. *ISME J* 2010;**4**:817.

Crump BC, Armbrust EV, Baross JA. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. *Appl Environ Microbiol* 1999;**65**:3192–204.

DeLong EF, Franks DG, Alldredge AL. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol Oceanogr* 1993;**38**:924–34.

De Tender C, Schlundt C, Devriese LI *et al*. A review of microscopy and comparative molecular-based methods to characterize "Plastisphere" communities. *Anal Methods* 2017;**9**:2132–43.

De Tender CA, Devriese LI, Haegeman A *et al*. Bacterial community profiling of plastic litter in the Belgian part of the North Sea. *Environ Sci Technol* 2015;**49**:9629–38.

Dudek KL, Cruz BN, Polidoro B *et al*. Microbial colonization of microplastics in the Caribbean Sea. *Limnol Oceanogr Lett* 2020;**5**:5–17.

Dussud C, Meistertzheim AL, Conan P *et al*. Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. *Environ Pollut* 2018;**236**:807–16.

Fazey FMC, Ryan PG. Biofouling on buoyant marine plastics: an experimental study into the effect of size on surface longevity. *Environ Pollut* 2016;**210**:354–60.

Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. *Sci Adv* 2017;**3**:e1700782.

Harrison JP, Schratzberger M, Sapp M *et al*. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. *BMC Microbiol* 2014;**14**:1–15.

Hollibaugh JT, Wong PS, Murrell MC. Similarity of particle-associated and free-living bacterial communities in northern San Francisco Bay, California. *Aquat Microb Ecol* 2000;**21**:103–14.

Jackson CR, Churchill PF, Roden EE. Successional changes in bacterial assemblage structure during epilithic biofilm development. *Ecology* 2001;**82**:555–66.

Jambeck JR, Geyer R, Wilcox C *et al*. Plastic waste inputs from land into the ocean. *Science* 2015;**347**:768–71.

Kelley JI, Turng B, Williams HN *et al*. Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic bdellovibrios. *Appl Environ Microbiol* 1997;**63**:84.

Kellogg CTE, Deming JW. Particle-associated extracellular enzyme activity and bacterial community composition across the Canadian Arctic Ocean. *FEMS Microbiol Ecol* 2014;**89**:360–75.

Kettner MT, Rojas-Jimenez K, Oberbeckmann S *et al*. Microplastics alter composition of fungal communities in aquatic ecosystems. *Environ Microbiol* 2017;**19**:4447–59.

Kirstein IV, Wichels A, Gullans E *et al*. The plastisphere – uncovering tightly attached plastic "specific" microorganisms. *PLoS One* 2019;**14**:e0215859.

Kirstein IV, Wichels A, Krohne G *et al*. Mature biofilm communities on synthetic polymers in seawater - Specific or general? *Mar Environ Res* 2018;**142**:147–54.

Kublanov IV, Podosokorskaya OA. Melioribacter. In: Whitman W.B., Rainey F., Kämpfer P. *et al*. (eds). *Bergey's Manual of Systematics of Archaea and Bacteria*, 2020, 1–6. DOI: 10.1002/9781118960608.gbm01506.

Langenheder S, Ragnarsson H. The role of environmental and spatial factors for the composition of aquatic bacterial communities. *Ecology* 2007;**88**:2154–61.

Lauber CL, Hamady M, Knight R *et al*. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 2009;**75**:5111.

Lobelle D, Cunliffe M. Early microbial biofilm formation on marine plastic debris. *Mar Pollut Bull* 2011;**62**:197–200.

Lozupone C, Lladser ME, Knights D *et al*. UniFrac: an effective distance metric for microbial community comparison. *ISME J* 2011;**5**:169–72.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;**8**:e61217.

Mestre M, Borrull E, Sala MM *et al*. Patterns of bacterial diversity in the marine planktonic particulate matter continuum. *ISME J* 2017;**11**:999–1010.

Miao L, Wang P, Hou J *et al*. Distinct community structure and microbial functions of biofilms colonizing microplastics. *Sci Total Environ* 2019;**650**:2395–402.

Moeseneder MM, Winter C, Herndl GJ. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. *Limnol Oceanogr* 2001;**46**:95–107.

Moss J, Nocker A, Lepo J *et al*. Stability and change in estuarine biofilm bacterial community diversity. *Appl Environ Microbiol* 2006;**72**:5679–88.

Murrell M, Hollibaugh J, Silver M *et al*. Bacterioplankton dynamics in northern San Francisco Bay: Role of particle association and seasonal freshwater flow. *Limnol Oceanogr* 1999;**44**:295–308.

Murtagh F, Legendre P. Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? *J Classif* 2014;**31**: 274–95.

Muthukrishnan T, Al Khaburi M, Abed RMM. Fouling microbial communities on plastics compared with wood and steel: are they substrate- or location-specific? *Microb Ecol* 2019;**78**:361–74.

Oberbeckmann S, Kreikemeyer B, Labrenz M. Environmental factors support the formation of specific bacterial assemblages on microplastics. *Front Microbiol* 2018;**8**.

Oberbeckmann S, Osborn AM, Duhaime MB. Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *PLoS One* 2016;**11**:e0159289.

Ogonowski M, Motiei A, Ininbergs K *et al*. Evidence for selective bacterial community structuring on microplastics. *Environ Microbiol* 2018;**20**:2796–808.

Oksanen J, Blanchet FG, Friendly M *et al*. vegan: community ecology package: R package version 2.5-5 Edition. https://CRAN.R-project.org/package=vegan, 2019.

Pinnaka AK, Tanuku NRS. The family Cyclobacteriaceae. In: Rosenberg E, DeLong EF, Lory S *et al*. (eds). *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*, Berlin, Heidelberg: Springer, 2014, 551–75.

Pinnell LJ, Turner JW. Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. *Front Microbiol* 2019;**10**. DOI: 10.3389/fmicb.2019.01252.

Pinto M, Langer TM, Hüffer T *et al*. The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. *PLoS One* 2019;**14**:e0217165.

Podosokorskaya OA, Kadnikov VV, Gavrilov SN *et al*. Characterization of *Melioribacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class Ignavibacteria, and a proposal of a novel bacterial phylum Igavibacteriae. *Environ Microbiol* 2013;**15**:1759–71.

Quast C, Pruesse E, Yilmaz P *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–D6.

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2017.

Segata N, Izard J, Waldron L *et al*. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60.

Thompson RC, Moore CJ, vom Saal FS *et al*. Plastics, the environment and human health: current consensus and future trends. *Philos Trans R Soc Lond B Biol Sci* 2009;**364**:2153–66.

Tu C, Chen T, Zhou Q *et al*. Biofilm formation and its influences on the properties of microplastics as affected by exposure time and depth in the seawater. *Sci Total Environ* 2020;**734**:139237.

Tunnell JW. Geography, climate, and hydrography. In: Tunnell JW, Judd FW (eds). *The Laguna Madre of Texas and Tamaulipas*. College Station, TX: Texas A&M University Press, 2002, 7–27.

Walters W, Hyde ER, Berg-Lyons D *et al*. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 2016;**1**:e00009–15.

Williams HN, Turng B-F, Kelley JI. Survival response of bacteriovorax in surface biofilm versus suspension when stressed by extremes in environmental conditions. *Microb Ecol* 2009;**58**:474–84.

Zettler ER, Mincer TJ, Amaral-Zettler LA. Life in the "plastisphere": microbial communities on plastic marine debris. *Environ Sci Technol* 2013;**47**:7137–46.

Zhalnina K, Dias R, de Quadros PD *et al*. Soil pH determines microbial diversity and composition in the park grass experiment. *Microb Ecol* 2015;**69**:395–406.

Zhou J, Bruns MA, Tiedje JM. DNA recovery from soils of diverse composition. *Appl Environ Microbiol* 1996;**62**:316–22.