SUPPLEMENTARY MATERIAL

Early colonization of weathered polyethylene by distinct bacteria in marine coastal seawater

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This file contains 11 pages (cover page included), containing 4 figures, 4 tables and on page S9 specific 16S rRNA gene sequences used in BLAST searches:

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Figure S1: Principal coordinate analysis (PCoA; Bray-Curtis distance) plot of the full 16S rRNA gene sequencing data.

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Table S2: Statistical summary of PERMANOVA tests on UniFrac ordinations of 16S rRNA data from polyethylene communities.

Table S3: Contrast summaries of generalized linear model results for Shannon diversity of bacterial communities (16S rRNA gene) colonizing PE and glass at two different time points.

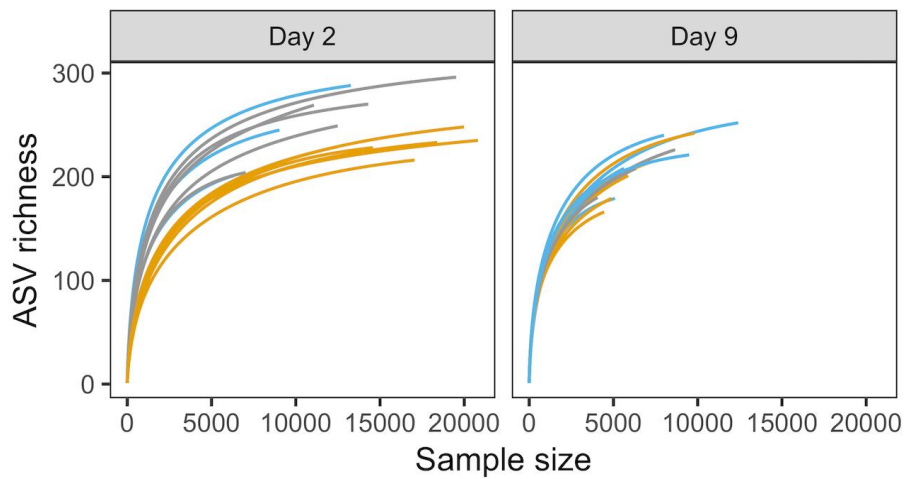**Table S4:** Details for most abundant ASVs from figure 4.

Table S1. 16S rRNA gene primer pairs tested for OHCB coverage.

Primer name	Sequence	Reference
515F-Y(*) 926R(*)	GTGYCAGCMGCCGCGGTAA CCGYCAATTYMTTTRAGTTT	Parada *et al.* [1] Quince *et al.* [2]
515F 806R	GTGCCAGCMGCCGCGGTAA GGACTACHVGGGTWTCTAAT	Oberbeckmann *et al.* [3]
341F 805R	CCTACGGGNGGCWGCAG GACTACHVGGGTATCTAATCC	De Tender *et al.* [4]
518F 1046R	CCAGCAGCYGCGGTAAN CGACAGCCATGCANCACCT	Zettler *et al.* [5]
341F 785R	CCTACGGGNGGCWGCAG GACTACHVGGGTATCTAATCC	Kirstein *et al.* [6]
343F 908R	TACGGRAGGCAGCAG CGTCAATTCMTTTGAGTT	Berry and Gutierrez [7]

*Note: (*) indicates primer pair used in this study.*
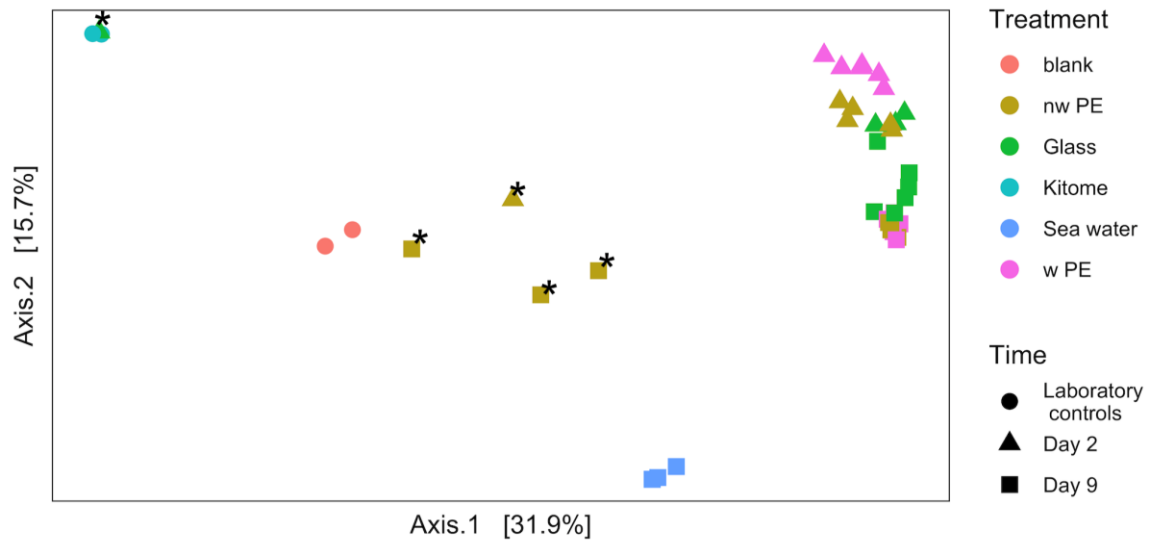
Figure S1. Principal coordinate analysis (PCoA; Bray-Curtis distance) plot of the full 16S rRNA gene sequencing data. Note that controls (Kitome, blank), as well as Sea water samples cluster separately from bulk experimental samples (nw PE, w PE and Glass). (*): experimental samples that were excluded from downstream analysis due to clustering with controls.

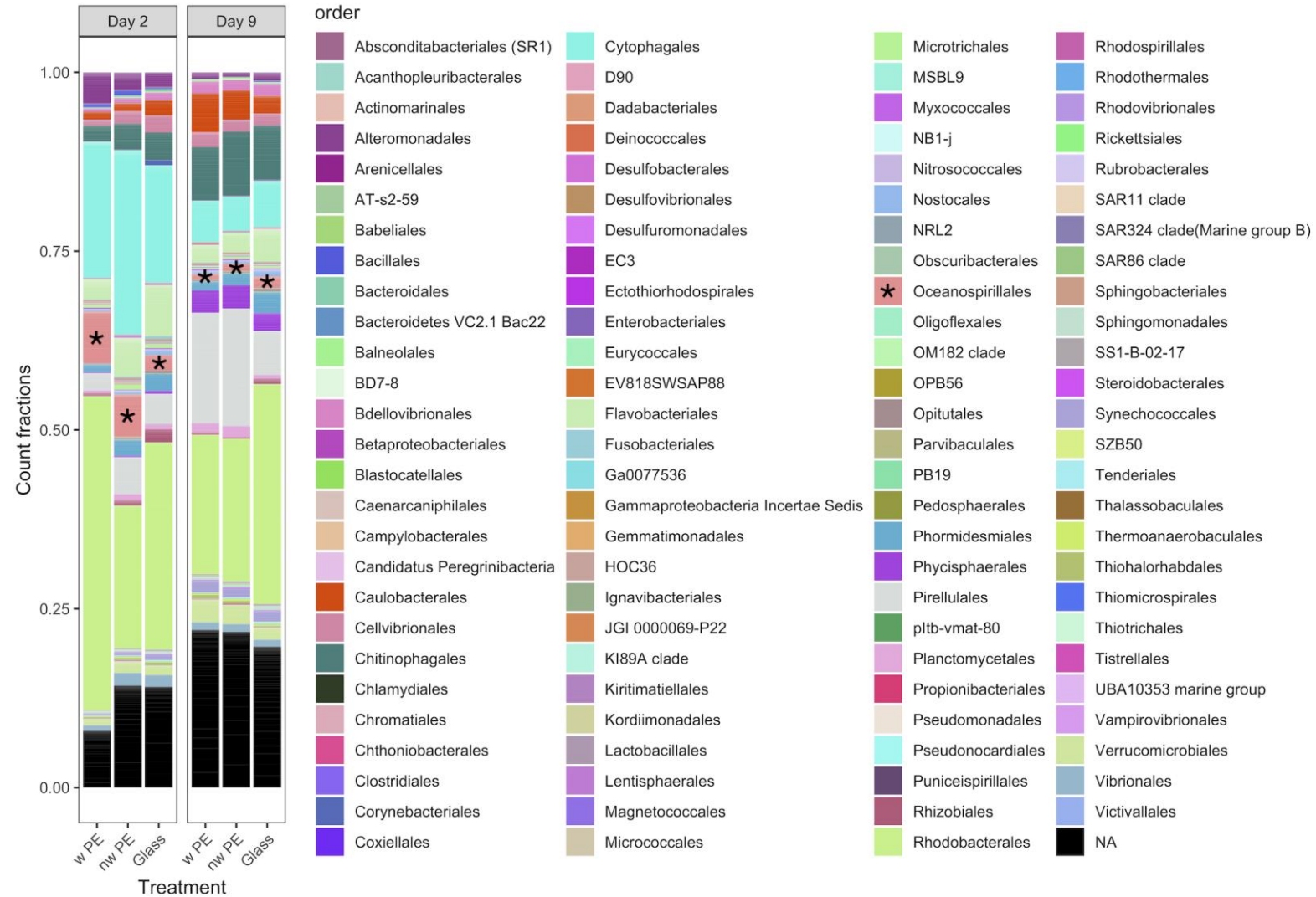**Figure S2.** Rarefaction curves for bacterial communities (16S rRNA genes) colonizing weathered polyethylene (w PE), non-weathered PE (nw PE), and glass after 2 and 9 days of incubation in coastal Mediterranean seawater.
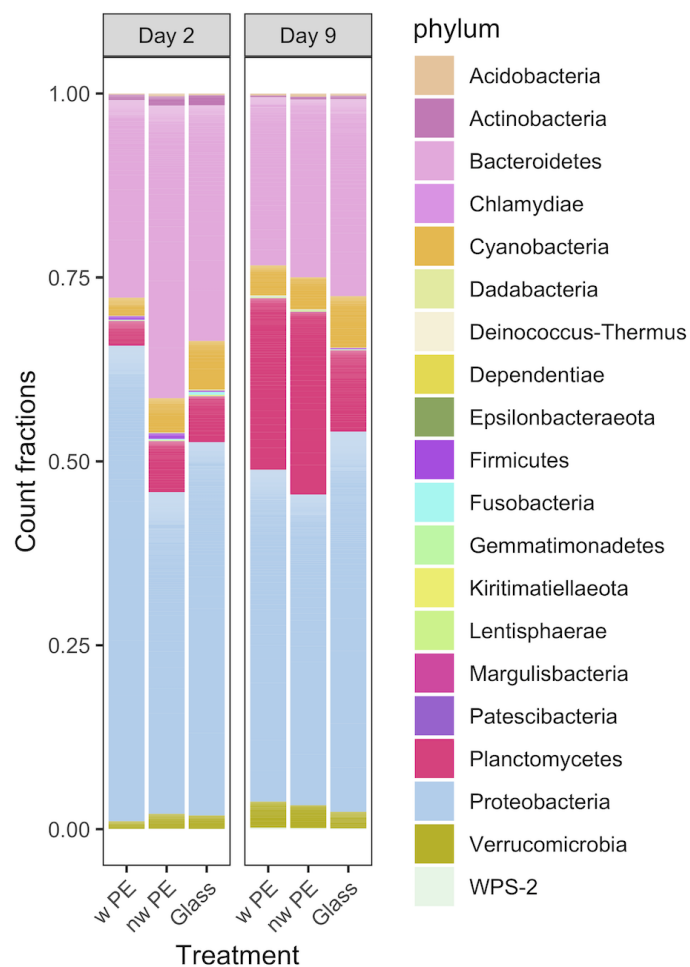
Figure S3. Bar chart showing dominant bacterial phyla (16S rRNA genes) in microbial communities colonizing weathered polyethylene (w PE), non-weathered PE (nw PE), and glass after 2 and 9 days of incubation in coastal Mediterranean seawater.

Figure S4. Bar chart showing dominant bacterial orders (16S rRNA gene) in microbial communities colonizing weathered polyethylene (w PE), non-weathered PE (nw PE), and glass after 2 and 9 days of incubation in coastal Mediterranean seawater. (*) denotes genus *Oceanospirillales*

Table S2. Statistical summary of PERMANOVA tests on UniFrac ordinations of 16S rRNA gene data from polyethylene communities.

16S rRNA gene						
UniFrac: weighted	**Df**	**Sums of Sqs**	**Mean Sqs**	**F Model**	**R^2**	**Pr(>F)**
treatment	2	1.450	0.725	5.916	0.147	**0.001**
timepoint	1	4.588	4.588	37.453	0.466	**0.001**
treatment:timepoint	2	1.108	0.554	4.522	0.113	**0.003**
Residuals	22	2.695	0.123		0.274	
UniFrac: unweighted						
treatment	2	0.153	0.077	1.304	0.084	0.077
timepoint	1	0.239	0.239	4.074	0.131	**0.001**
treatment:timepoint	2	0.147	0.074	1.255	0.080	0.098
Residuals	22	1.291	0.059		0.705	

Note: significant p-values are denoted in bold.

Table S3. Contrast summaries of generalized linear model results for Shannon diversity of bacterial communities (16S rRNA gene) colonizing PE and glass at two different time points.

Compared conditions	Estimate	Std. Error	z value	Pr(>|z|)
glass, day 2 – non-weathered PE, day 2	-0.005	0.014	-0.354	0.999
weathered PE, day 2 – non-weathered PE, day 2	0.094	0.014	6.861	**<0.001**
non-weathered PE, day 9 – non-weathered PE, day 2	-0.023	0.014	-1.649	0.563
glass, day 9 – non-weathered PE, day 2	-0.001	0.012	-0.089	1.000
weathered PE, day 9 – non-weathered PE, day 2	-0.022	0.012	-1.811	0.455
weathered PE, day 2 – glass, day 2	0.100	0.015	6.467	**<0.001**
non-weathered PE, day 9 – glass, day 2	-0.018	0.015	-1.145	0.861
glass, day 9 – glass, day 2	0.004	0.014	0.289	1.000
weathered PE, day 9 – glass, day 2	-0.017	0.014	-1.207	0.832
non-weathered PE, day 9 – weathered PE, day 2	-0.117	0.015	-7.852	**<0.001**
glass, day 9 – weathered PE, day 2	-0.096	0.013	-7.196	**<0.001**
weathered PE, day 9 – weathered PE, day 2	-0.116	0.013	-8.692	**<0.001**
glass, day 9 – non-weathered PE, day 9	0.022	0.013	1.631	0.575
weathered PE, day 9 – non-weathered PE, day 9	0.001	0.013	0.064	1.000
weathered PE, day 9 –glass, day 9	-0.021	0.011	-1.809	0.457

Note: significant p-values are denoted in bold.

Table S4. Details for most abundant ASVs from figure 4.

ASV	Abundance on wPE at day2 [%]	Closest relative	Identity score
ASV3 [1]	26.9	*Thalassococcus halodurans*	99.73%
ASV10 [2]	6.1	*Marivivens niveibacter*	98.66%
ASV23 [3]	0.6	*Boseongicola aestuarii*	97.31%
ASV28 [4]	2.1	*Oleiphilus mesinensis*	94.41%
ASV34 [5]	0.8	*Oleiphilus mesinensis*	95.48%
ASV47 [6]	1.3	*Oceanospirillum nioense*	94.39%
ASV52 [7]	0.9	*Aestuariibacter aggregatus*	97.33%
ASV123 [8]	0.4	*Aestuariibacter aggregatus*	97.33%
ASV2019 [9]	0.01	*Aestuariibacter aggregatus*	98.40%

[1]

TACGGAGGGGGTTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGATTGGAAAGTTGGGGGTGAAATCCCGGGGCTCAACCCCGGAAC
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AGTCGTCAGCAAGCATGCTTGTTGGTGACACACCTAACGGATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGATTA

[2]

TACGGAGGGGGTTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGACTGGAAAGTTAGAGGTGAAATCCCGGGGCTCAACCCCGGAAC
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AGTCGTCGGGTTGCTTGCAACTCGGTGACACACCTAACGGATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGATTA

[3]

TACGGAGGGGGTTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATTATTAAGTGAGAGGTGAAATCCCAGGGCTCAACCCTGGAAC
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AGACGTCGGGCAGTATACTGTTCGGTGTCACACCTAACGGATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGATTA

[4]

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[5]

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AGCCGTTGGGGAACTTGATTCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTA

[6]

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AGCCGTTGGGGAACTTGATTCTTTAGTGGCGCAGCTAACGCACTAAGTAGACCGCCTGGGGAGTACGGTCGCAAGATTA

[7]

TACGGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGATTGTTAAGCTAGAGGTGAAAGCCCCGCGCTCAACGTGGGAAT
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[8]

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[9]

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AGCTGTTTGTGTATTTAATACGTGAGTAGCGAAGCTAACGCGCTAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTA

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