

Year-Long Microbial Succession on Microplastics in Wastewater: Chaotic Dynamics Outweigh Preferential Growth

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Experimental Section

Microplastic Information

The microplastic types studied were PE (Goodfellow Ltd, Cambridge, UK), PP (Shawingigan Ltd, London, UK),^[1] PVC (Cellomer Associates inc., New York, USA),^[2] nylon-6 (Koch-Light Laboratories Ltd, Colnbrook, UK) and trimmed PET fragments obtained from a commercial mineral water bottle (Costcutter Montgomery Q4 Natural Water, Uxbridge, UK). With the exception of PET, all microplastics were ~1.

Microplastic Extraction from Microcosm Tubes

Larger microplastics (e.g. PET) and large glass beads were removed at each sampling interval using a small spatula. Smaller microplastics (150–250 µm) were extracted by multiple pipetting and rinsing with sterilised ultra-high quality [UHQ] water. This was repeated 3–6 times until no more biogenic organic matter (BOM) could be observed. In a final step all microplastics were drawn into a 250-µL pipette tip and the remaining liquid was absorbed using sterilised tissue paper (VWR, Germany). The outside of the tip was cleaned with 80% (v/v) ethanol (VWR, Germany) and the retained microplastics were stored in 1 ml of UHQ water at 4 °C until all samples were ready for DNA extraction. All microplastic extraction work was undertaken in a laminar flow cabinet to avoid contamination from the air.

For microplastics which would form a pellet by centrifugation, samples were centrifuged (up to 2038× g) for 2 minutes before being extracted from storage suspension. The sample was placed on clean, water resistant laboratory film (Parafilm MTM, Sigma Aldrich, USA) and the remaining suspension liquid was removed using a fine 2 µL pipette tip (see Appendix B; Figure B1). For PE and PP, which could not be centrifuged, a 1 µL inoculation loop was used to transfer microplastics from storage suspension directly to the extraction tube. For the extraction of larger size microplastics like PET and large glass beads (~1–2 mm) sterile fine tweezers were used for transfer. Glass microbeads were transferred to extraction tubes using a 20 µL pipette tip without ejecting any of the suspension fluid.

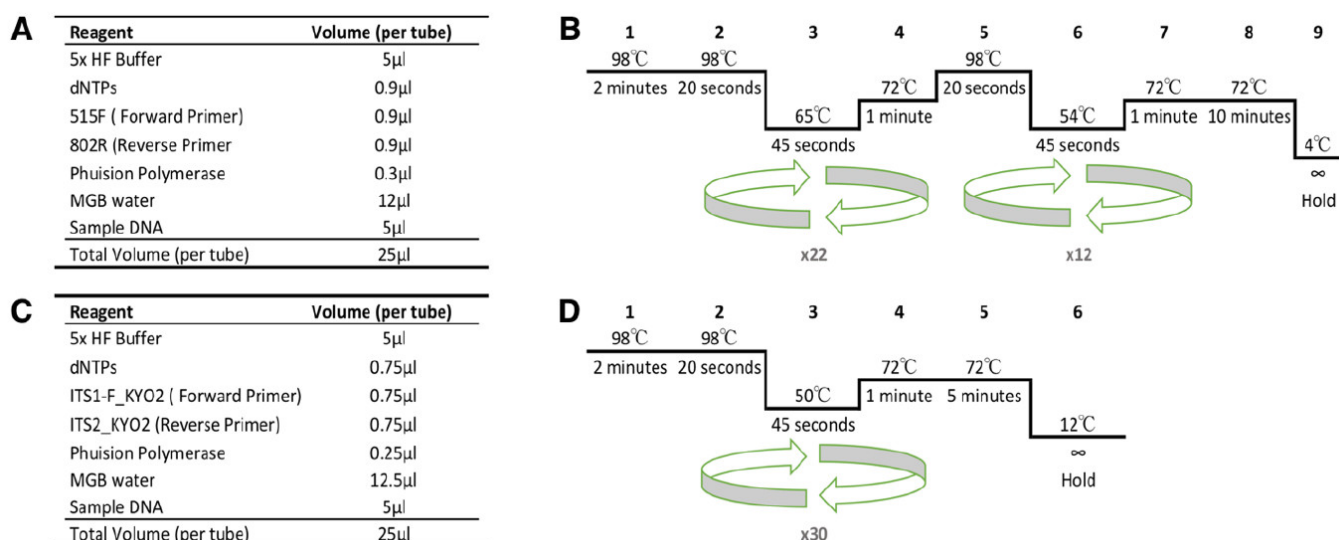


Figure S1. Experimental details of amplicon PCRs. (A): Amplicon PCR mix for 16S. (B): Amplicon PCR run protocol for 16S. (C): Amplicon PCR mix for ITS. (D): Amplicon PCR run protocol for ITS.

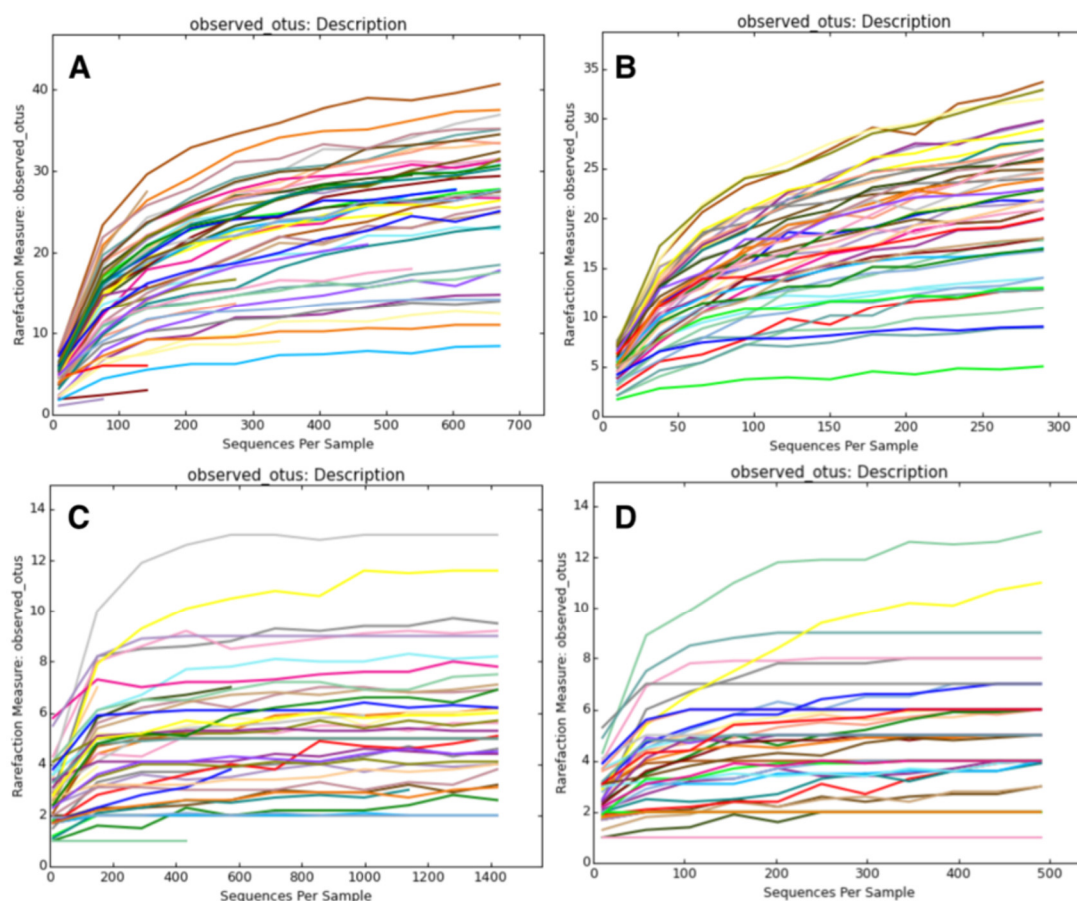


Figure S2. Rarefaction curves based on OTU richness. A: 16S before rarefaction; B: 16S after rarefaction at 297 sequences per sample; C: ITS before rarefaction; D: ITS after rarefaction at 499 sequences per sample.

Table S1. National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) best matches for 16S OTUs. See main text for full references.

OTU ID	UTAX Taxonomy	BLAST “Best Hit” Taxonomy	BLAST Score	BLAST Similarity (%)	“Best Hit” Reference (If Available)	“Best Hit” Genbank ID
OTU 3	<i>Pseudomonas</i> sp.	<i>Pseudomonas</i> sp.	468	100	Hamm et al., 2017 [78]	KX928315
OTU 7	<i>Rhodanobacter</i> A	<i>Rhodanobacter thiooxydans</i>	468	100	Thijs et al., 2014 [79]	HG794331
OTU 8	<i>Armatimonas</i> Gp1	Uncultured bacterium	470	100	Unpublished	KU113498
OTU 10	<i>Sphingomonas</i>	<i>Sphingomonas</i> sp.	468	100	Colin et al., 2017 [80]	KX419227
OTU 11	<i>Corynebacterineae</i>	<i>Rhodococcus erythropolis</i>	468	100	Mikolasch et al., 2016 [81]	KU663058
OTU 12	<i>Mucilaginibacter</i> A	<i>Mucilaginibacter</i> sp.	468	100	Unpublished	EU423305
OTU 14	<i>Geothrix</i>	Uncultured bacterium	468	100	Rodrigues et al., 2010 [82]	GU169059
OTU 15	<i>Dokdonella</i>	Uncultured bacterium	468	100	Unpublished	KU316012
OTU 16	<i>Thiobacillus</i>	Uncultured <i>Thiobacillus</i>	468	100	Suzuki et al., 2016 [83]	LC070284
OTU 17	<i>Mucilaginibacter</i> B	Uncultured <i>Bacteroidetes</i>	468	100	Zinger et al., 2009 [84]	FJ569393
OTU 18	<i>Rhodanobacter</i> B	Uncultured bacterium	468	100	Zeglin et al., 2016 [85]	KP909173
OTU 19	<i>Corynebacterineae</i>	<i>Mycobacterium fortuitum</i>	468	100	Azadi et al., 2016 [70]	KX954379
OTU 20	<i>Rhodanobacter</i> C	Uncultured <i>Rhodanobacter</i> sp.	468	100	Unpublished	KY445711
OTU 21	Unidentified <i>Edaphobacter</i> (Order)	Uncultured bacterium	468	100	Zeglin et al., 2016 [85]	KP911732
OTU 22	<i>Micrococcineae</i>	<i>Lysinimonas</i> sp.	468	100	Liu et al., 2002 [86]	KT803390
OTU 23	<i>Poवालibacter</i>	Uncultured bacterium	468	100	Peipoch et al., 2015 [87]	KT908136
OTU 24	Unidentified (Family) <i>Rhizomicrobium</i>	Uncultured bacterium	468	100	Zeglin et al., 2016 [85]	KP911577
OTU 25	<i>Devosia</i>	<i>Devosia</i> sp.	468	100	Colin et al., 2017 [80]	KX418822
OTU 26	<i>Acidocella</i>	Uncultured <i>Acidoella</i> sp.	468	100	Unpublished	EF016441
OTU 28	<i>Chitinophaga</i>	Uncultured <i>Bacteroidetes</i>	468	100	Zinger et al., 2009 [84]	FJ569783
OTU 29	Unidentified <i>Terriglobus</i> (Order)	Uncultured <i>Granulicella</i> sp.	468	100	Unpublished	KR831665
OTU 30	<i>Afipia</i>	<i>Nitrobacter</i> sp.	468	100	Unpublished	KY117526
OTU 32	<i>Flavisolibacter</i>	Uncultured bacterium	468	100	Fonseca-García et al., 2016 [88]	KU539980
OTU 33	<i>Ferruginibacter</i>	Uncultured <i>Sphingobacteriales</i> bacterium	462	99	Sun et al., 2012 [89]	JN541173
OTU 35	<i>Aminobacter</i>	<i>Mesorhizobium ciceri</i>	468	100	Haskett et al., 2016 [71]	KX226352
OTU 36	<i>Burkholderia</i>	<i>Burkholderia</i> sp.	468	100	Colin et al., 2017 [80]	KX419240
OTU 38	<i>Pseudonocardineae</i>	<i>Pseudonocardia xishanensis</i>	470	100	Zhao et al., 2012 [90]	NR_108411
OTU 39	<i>Mesorhizobium</i>	<i>Mesorhizobium waimense</i>	468	100	De Meyer et al., 2015 [91]	NR_137372
OTU 40	Unidentified <i>Aridibacter</i> (Order)	Uncultured bacterium	462	99	Yin et al., 2015 [92]	KP784910
OTU 41	<i>Brevundimonas</i>	Uncultured <i>alpha proteobacterium</i>	468	100	Yergeau et al., 2007 [93]	EF219826
OTU 42	<i>Nevskia</i>	Uncultured bacterium	468	100	Zeglin et al., 2016 [85]	KP912254
OTU 43	Unidentified (Class) <i>Verrucomicrobia</i> Sub3	Uncultured bacterium	468	100	Dunbar et al., 2012 [94]	JQ366564
OTU 45	<i>Oleigrimonas</i>	Uncultured archaeon	468	100	Rastogi et al., 2009 [95]	FJ184749
OTU 46	<i>Beijerinckia</i>	Uncultured <i>Rhizobiales</i>	468	99	Hodkinson et al., 2012 [96]	JF814502
OTU 47	<i>Rhodopseudomonas</i> A	Uncultured bacterium	468	100	Kelly et al., 2010 [97]	GU219805
OTU 52	<i>Flaviumibacter</i>	Uncultured <i>Bacteroidetes</i>	468	100	Zinger et al., 2009 [84]	FJ569311
OTU 53	<i>Romboutsia</i>	<i>Romboutsia timonensis</i>	466	100	Ricaboni et al., 2016 [98]	NR_1447
OTU 54	<i>Phenylobacterium</i>	Uncultured bacterium	462	99	Zeglin et al., 2016 [85]	KP904793
OTU 55	<i>Nitrobacter</i>	Uncultured bacterium	468	100	Faltesek and Cepicka, 2012 [99]	JQ217878
OTU 56	Unidentified (Family) <i>Rhizomicrobium</i>	Uncultured <i>alpha proteobacterium</i>	468	100	Chen et al., 2014 [100]	KF182818
OTU 61	<i>Flavisolibacter</i>	<i>Flavitalea</i> sp.	468	100	Unpublished	KX146487
OTU 62	WPS-1 “ <i>Genera incertae sedis</i> ” (Class)	Uncultured bacterium	446	98	Unpublished	JQ711725
OTU 63	Unidentified (Class) <i>Acidobacteria</i> Gp1	Uncultured bacterium	451	99	Zeglin et al., 2016 [85]	KP913651
OTU 68	<i>Parvibaculum</i>	Sediment bacterium	468	100	Hilyard et al., 2008 [101]	EU167984
OTU 69	<i>Rhodopseudomonas</i> B	<i>Rhodopseudomonas</i> sp.	468	100	Ganzert et al., 2014 [102]	KF974286
OTU 73	<i>Acidicoccus</i>	Uncultured bacterium	462	99	Zeglin et al., 2016 [85]	KP929003
OTU 74	<i>Aquisphaera</i> B	Uncultured bacterium	464	99	Fonseca-Garcia et al., 2016 [88]	KU542490
OTU 76	<i>Micrococcineae</i>	<i>Leifsonia lichenia</i>	468	100	Al-Sadi et al., 2016 [103]	KU220847
OTU 77	Unidentified (Class) <i>Acidobacteria</i> Gp3	Uncultured <i>Acidobacteria</i>	468	99	Unpublished	KR844218
OTU 78	<i>Ferruginibacter</i>	Uncultured bacterium	468	100	Zeglin et al., 2016 [85]	KP906926
OTU 82	<i>Dyadobacter</i>	<i>Dyadobacter</i> sp.	468	100	Colin et al., 2017 [80]	KX418930
OTU 84	<i>Vampirovibrio</i> A	Uncultured <i>Vampirovibrio</i> sp.	468	100	Unpublished	KR839383
OTU 85	<i>Pectobacterium</i>	<i>Pectobacterium carotovorum</i>	468	100	Al-Kharousi et al., 2016 [104]	KR265429

OTU 89	<i>Lacibacterium</i>	Uncultured <i>Rhodospirillaceae</i>	468	100	Lesaulnier et al., 2008 [105]	EF018478
OTU 92	<i>Nitrospira</i>	Uncultured <i>Nitrospira</i> sp.	468	100	Zhao et al., 2015 [106]	KP890815
OTU 98	<i>Staphylococcus</i>	<i>Staphylococcus epidermidis</i>	468	100	Sepulveda et al., 2016 [107]	KX108950
OTU 108	<i>Vampirovibrio B</i>	Uncultured <i>Vampirovibrio</i> sp.	451	99	Unpublished	KR836560
OTU 113	<i>Haliangiaceae</i>	Uncultured soil bacterium	468	100	Lueders et al., 2006 [108]	DQ643693
OTU 116	<i>Pseudolabrys</i>	Uncultured alpha proteobacterium	468	100	Sun et al., 2012 [89]	JN541152
OTU 129	<i>Aquisphaera A</i>	Uncultured bacterium	464	99	Unpublished	KY256905
OTU 136	<i>Propionibacterineae</i>	<i>Marmoricola</i> sp.	468	100	Unpublished	LN833253
OTU 138	Unidentified <i>Acidipila</i> (Order)	Uncultured <i>Acidobacterium</i>	468	100	Sanchez-Andrea et al., 2011 [109]	HQ730658
OTU 139	<i>Candidimonas</i>	<i>Parapusillimonas granuli</i>	462	99	Auffret et al., 2015 [110]	KM047480
OTU 140	<i>Ewingella</i>	<i>Ewingella</i> sp.	468	100	Xie et al., 2017 [111]	KX378962
OTU 276	<i>Herbaspirillum</i>	<i>Herbaspirillum hiltneri</i>	451	99	Poosakkannu et al., 2015 [112]	KJ529095
OTU 373	<i>Sediminibacterium</i>	Uncultured bacterium	457	99	Egert et al., 2010 [113]	FN401290
OTU 380	<i>Mucilaginibacter C</i>	Uncultured <i>Bacteroidetes</i>	451	99	Zinger et al., 2009 [84]	FJ569664
OTU 801	<i>Mizugakiibacter</i>	Uncultured <i>Xanthomonadaceae</i>	440	98	Ceja-Navarro et al., 2010 [114]	FJ889338

Table S2. National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) best matches for ITS OTUs. See main text for full references.

OTU ID	UTAX Taxonomy	BLAST "Best Hit" Taxonomy	BLAST score	BLAST Similarity (%)	BLAST "Best Hit" Reference (If Available)	"Best Hit" Genbank ID
OTU 1	Unidentified <i>Tremellomycetes</i> (Class)	<i>Trichosporon porosum</i>	416	99	Jimenez et al., 2014 [115]	KF285994
OTU 2	<i>Orpinomyces</i>	Uncultured fungus	512	98	Unpublished	KU000514
OTU 3	<i>Exophiala</i>	<i>Exophiala equina</i>	555	99	Unpublished	JF747078
OTU 5	<i>Trichoderma</i>	<i>Trichoderma lixii</i>	573	99	Anees et al., 2010 [116]	HM176572
OTU 6	<i>Candida</i>	<i>Candida sake</i>	366	99	Unpublished	KY106736
OTU 7	<i>Tyromyces</i>	<i>Trypanosoma evansi</i> -Tyropanosome	124	99	Wen et al., 2016 [117]	KU552351
OTU 10	Unidentified <i>Nectriaceae</i> (Family)	<i>Fusarium oxysporum</i>	477	100	Unpublished	KT241034
OTU 11	Unidentified <i>Sordariomycetes</i> (Class)	Uncultured fungus	488	100	Unpublished	JX364633
OTU 13	Unidentified <i>Fungi</i> (Kingdom)	Uncultured eukaryote	154	98	Unpublished	KY690346
OTU 14	<i>Serendipita</i>	Uncultured fungus	124	99	Taylor et al., 2008 [118]	EU292563
OTU 15	Unidentified <i>Basidiomycota</i> (Phylum)	Uncultured fungus	311	91	Unpublished	KU000525
OTU 16	Unidentified <i>Fungi</i> (Kingdom)	<i>Fungal</i> ^{sp.} NLEndoHerit_025_2008N1-27-3H	488	99	Lamit et al., 2014 [119]	JX978254
OTU 17	<i>Penicillium</i>	<i>Penicillium corylophilum</i>	520	99	Bukovska et al., 2010 [120]	GU566277
OTU 19	<i>Devriesia</i>	Uncultured fungus	455	100	Adams et al., 2013 [121]	KF221844
OTU 20	<i>Pulchromyces</i>	<i>Metschnikowia matae</i> var. <i>matae</i>	122	96	De Oliveira Santos et al., 2015 [122]	KR779914
OTU 21	<i>Pestalotiopsis</i>	<i>Pestalotiopsis maculans</i>	462	99	Unpublished	KX610327
OTU 22	Unidentified <i>Helotiales</i> (Order)	Uncultured fungus	479	100	Cordier et al., 2012 [123]	JN906781
OTU 23	Unidentified <i>Nectriaceae</i> (Family)	Uncultured <i>Ascomycota</i>	475	99	Kartzinel et al., 2013 [124]	JX998704
OTU 24	<i>Malassezia</i>	Uncultured <i>Malassezia</i>	671	99	Unpublished	KY430579
OTU 25	Unidentified <i>Fungi</i> (Kingdom)	Uncultured <i>Basidiomycota</i>	128	99	Unpublished	KU000462
OTU 26	<i>Guehomyces</i>	<i>Tausonia pullulans</i>	483	99	Unpublished	KY105583
OTU 27	Unidentified <i>Pleosporales</i> (Order)	<i>Alternaria tenuissima</i>	503	99	Unpublished	KX674653
OTU 28	<i>Aspergillus</i>	<i>Aspergillus pseudoglaucus</i>	462	99	Unpublished	KX610153
OTU 30	Unidentified <i>Sporidiobolales</i> (Family Incertae sedis)	<i>Sporidiobolales</i> sp. LM531	479	99	Unpublished	EF060822
OTU 32	Unidentified <i>Sporidiobolales</i> (Order)	Uncultured <i>Sporobolomyces</i>	479	99	Unpublished	KT334689
OTU 34	<i>Malassezia</i>	<i>Malassezia restricta</i>	588	99	Alaei et al., 2009 [125]	EU400587
OTU 35	Unidentified <i>Fungi</i> (Kingdom)	Uncultured fungus	451	89	Scoble et al., 2014 [126]	KF577829
OTU 37	<i>Sporobolomyces</i>	Uncultured fungus	424	88	Waldrop et al., 2006 [127]	DQ420716
OTU 63	<i>Monographella</i>	<i>Microdochium</i> sp. 00019	460	99	Carter et al., 1999 [131]	AJ246155
OTU 131	Unidentified <i>Fungi</i> (Kingdom)	Uncultured fungus	327	96	Unpublished	KU000475
OTU 226	Unidentified <i>Tremellomycetes</i> (Class)	<i>Trichosporon porosum</i> strain 2t1F	407	99	Jiménez et al., 2012 [115]	KF285994

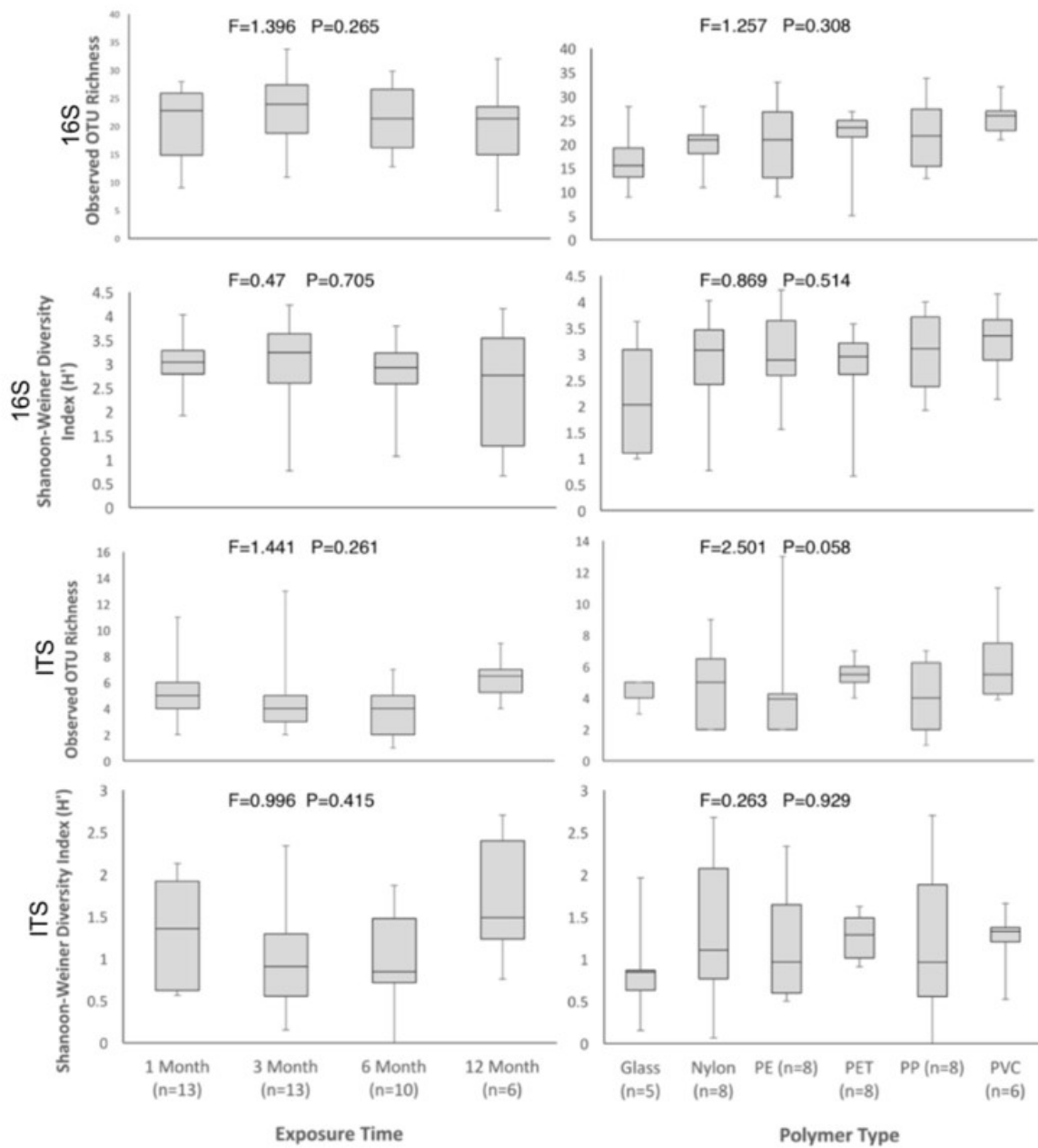


Figure S3. Box plots of bacterial or fungal OTU richness and Shannon-Wiener diversity for both polymer type and time. ANOVA F and *p* values show no significant differences within each tested box plot data (randomly subsampled to highest possible equal level of *n*). In the case of graph F where the *p* value indicates near-significance, post-hoc Tukey's HSC test proved no significant differences between any variables.

Results

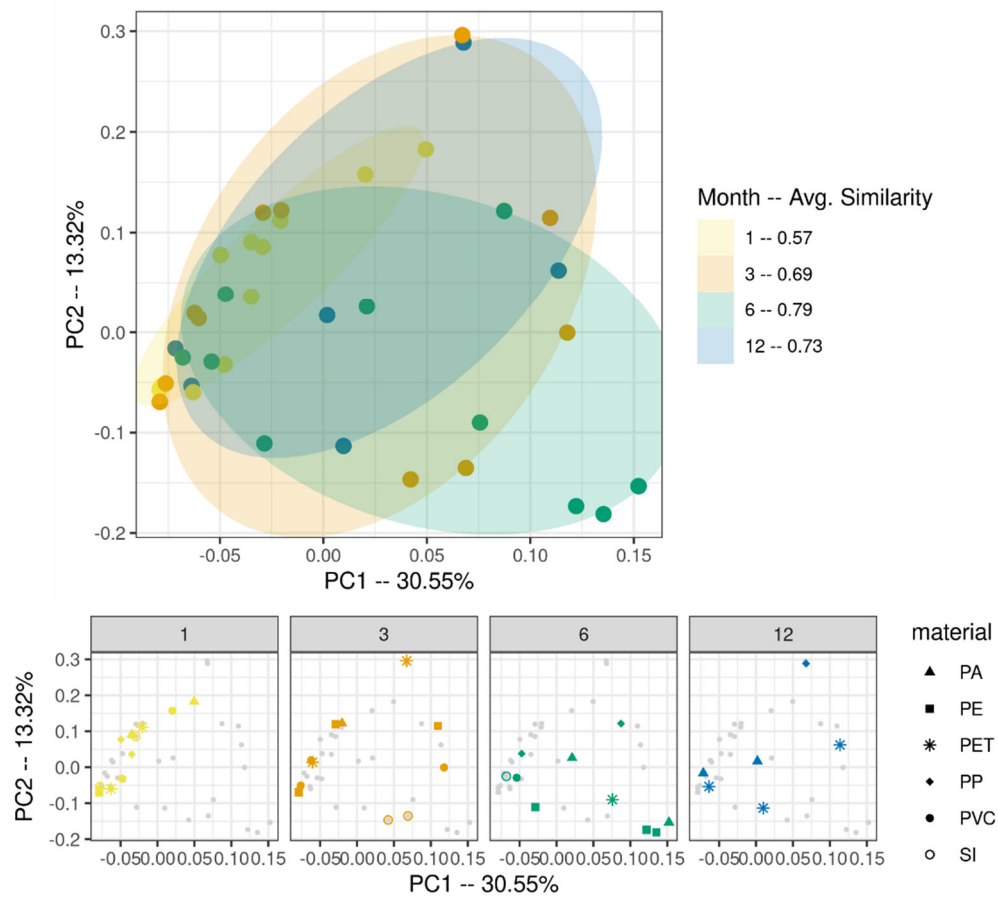


Figure S4. Principle coordinate analyses of ITS data based on Bray-Curtis dissimilarity matrix. The top plot shows the overall dataset while the bottom plots give details on polymer types.