

Fungal communities differ with microplastic types in deep sea sediment enrichments of the Eastern Pacific

Shuai Yang^{a,1}, Wei Xu^{a,1}, Kai Zhang^{a,1}, Jiege Hu^a, Yuanhao Gao^a, Guojie Cui^{a,d}, Hans-Peter Grossart^{e,f,**}, Zhuhua Luo^{a,b,c,*}

^a Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, 178 Daxue Road, Xiamen, 361005, PR China

^b Co-Innovation Center of Jiangsu Marine Bioindustry Technology, Jiangsu Ocean University, Lianyungang, 222005, PR China

^c School of Marine Sciences, Nanjing University of Information Science & Technology, Nanjing, 210044, PR China

^d Shenzhen Key Laboratory of Marine Microbiome Engineering, Institute for Advanced Study, Shenzhen University, Shenzhen, 518060, PR China

^e Department of Experimental Limnology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, 16775, Germany

^f Institute of Biochemistry and Biology, Potsdam University, Potsdam, 14469, Germany

ARTICLE INFO

Keywords:

Deep sea fungi
Fungal diversity
Community compositions
Microplastics

ABSTRACT

In this study, we used three plastic powders (polystyrene (PS), polyethylene terephthalate (PET) and polyurethane (PU)) to conduct micro-enrichments with deep-sea sediments from the Eastern Pacific. High-throughput sequencing of the ITS marker gene was performed during the enrichment process. The results showed that in comparison to culture time, plastic type significantly influenced the richness and diversity of the associated fungal community. The fungal community structures in PS and PET enrichments were similar, and there was no significant difference in fungal β diversity. Culture time, however, did not have any significant impact on the fungal community. On the other hand, based on FUNGuild analysis, we revealed that the fungal community compositions in PS and PET samples were highly similar, whereas PU enrichment was very different. The OTU network indicated more interactions between the different OTUs in the PU-enriched samples, demonstrating a highly complex interaction network. Fungal abundance, however, was not significantly affected by plastic type or culture time. In conclusion, compared to the original deep-sea sediments, addition of microplastics results in changes of the fungal community structure. Furthermore, different plastic types lead to different fungal communities, and compared with PS and PET, the enrichment effect of PU was stronger. Finally, rather than culture time, plastic type has a significant impact on fungal diversity and community composition.

1. Introduction

Microplastics generally refer to plastic particles smaller than five mm and larger than 0.1 μ m in size, and nowadays occur in almost all aquatic ecosystems worldwide, such as lakes, rivers, coastal and marine waters (Galloway et al., 2017; Gigault et al., 2018; Gomiero et al., 2019; Zhang et al., 2021). Marine microplastics pollution originates from a variety of sources, including land-based, sea-based, and air-based sources. The main sources of marine plastics are land-based and about 80% of all plastics in the ocean originate from terrestrial environments (Kane et al., 2020). Microplastics pose multiple harmful effects on

marine organisms. For instance, polystyrene microplastic particles can decrease the chlorophyll content, esterase activity, cell growth and photosynthetic efficiency of diatoms (González-Fernández et al., 2019). On the other hand, microplastics also adsorb various pollutants, which may combine to further and largely unknown health risks for the marine environment and humans. Le Bihan et al. (2020) investigated the influence of organic pollutants adsorbed to microplastics on the development of early life stages of marine medaka fish. These authors showed that perfluorooctanesulfonic acid (MP-PFOS) could cause a decrease in embryo survival rate and inhibit hatching, and benzo(a)pyrene (MP-BaP) as well as benzophenone-3 (MP-BP3) could cause abnormal

* Corresponding author. Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, 178 Daxue Road, Xiamen, 361005, PR China.

** Corresponding author. Department of Experimental Limnology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, 16775, Germany.

E-mail addresses: hgrossart@igb-berlin.de (H.-P. Grossart), luozhuhua@tio.org.cn (Z. Luo).

¹ These authors have contributed equally to this work.

larval development and behavior.

Although microplastics can lead to many adverse effects on organisms, they can also provide a substrate for microbial growth, namely the plastisphere (Zettler et al., 2013; Kettner et al., 2019). Bacterial communities colonizing on the surface of marine microplastics are significantly different from those in the surrounding waters or other more natural types of particles (Oberbeckmann et al., 2017; Debroas et al., 2017; Kettner et al., 2019). Interspecies interactions, such as competition, viral infection, and horizontal gene transfer, can affect the structure, stability, and functionality of biofilms, and may affect microbial plastics colonization and biodegradation (Arias-Andres et al., 2018; Pollet et al., 2018; Oberbeckmann and Labrenz 2020). Li et al. (2020) reported that exposure of plastics to marine environment leads to the occurrences of peaks in the micro-Fourier transformed infrared spectra - corresponding to the oxidation group of polyethylene (PE) and polyethylene terephthalate (PET). Increased crack intensities and microbial growth was observed on PE and PET particle surfaces, suggesting microplastics are actively degraded by microorganisms. Furthermore, a few recent studies reveal that the type of microplastics affects the metabolic performance of the colonizing microbial communities (Wang et al., 2021). Research on microbial microplastics colonization is a hot topic, yet, most studies focus on bacteria and largely neglect eukaryotic microorganisms such as fungi.

Fungi, like bacteria, represent ubiquitous microorganisms, which can grow in almost all environments including microplastics. A recent study revealed that members of the Ascomycota, Chytridiomycota, and Cryptomycota dominate fungal assemblages on polyethylene and polystyrene substrates in fresh to brackish surface waters of North-East Germany (Kettner et al., 2017). Moreover, 8 fungal phyla and 64 orders were detected on plastics in surface waters of the Western South Atlantic and the Antarctic Peninsula, whereby *Aspergillus*, *Cladosporium*, and *Wallemia* constituted the dominant fungal genera (Lacerda et al., 2020). Among various plastic types, polyurethane can be best degraded by fungi, e.g. by members of *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, and *Cladosporium* (Alvarez-Barragan et al., 2016; Kemonia and Piotrowska, 2016; Khan et al., 2017; Beneš et al., 2020).

Recent microcosmos studies illustrate the relationship between microplastics and its microbial colonization from another perspective. Seeley et al. (2020) conducted a microcosm experiment using salt marsh sediments amended with polyethylene (PE), polyvinyl chloride (PVC), polyurethane foam (PUF), or polylactic acid (PLA) plastics. They reported that the presence of plastics greatly altered sediment microbial community composition, whereby significant differences in the relative abundance of microbial families occurred on different plastic types. A variety of researches focused on identifying and quantifying microplastics in the ocean, showing that plastic debris found in the marine environment consists of a variety of plastic types: mainly polyurethane (PU), polystyrene (PS), foamed polystyrene, high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), etc. (Balasubramanian et al., 2010; Auta et al., 2017). Furthermore, several studies have addressed microbial biofilm formation on plastics in marine sediments (Harrison et al., 2014; Nauendorf et al., 2016; Pinnell and Turner, 2019; Kettner et al., 2019). Yet, to the best of our knowledge, incubation studies of plastics with deep-sea sediments have not been carried out, which greatly limits our understanding on ecological and biochemical effects of plastics in the deep ocean, a primary site of final plastics accumulation (Woodall et al., 2014; Kane et al., 2020; Pabortsava and Lampitt, 2020).

Therefore, in this study, we investigated the effect of three common plastic types (PS, PET, and PU) on fungal community when incubated in mineral salt medium with deep sea sediments of the Eastern Pacific Ocean. We addressed three major questions related to deep sea fungi: 1) Does the presence of microplastics impact fungal community structure? 2) Do different microplastic types result in different fungal community composition and interaction networks? and 3) Does plastic type or incubation time have a greater impact on fungal community structure?

Thus, the present study aims to investigate the response of deep sea fungal communities to the amendment of microplastics under laboratory conditions.

2. Materials and methods

2.1. Study sites, sampling and incubation experiment

Four sediment samples were collected with a box sampler in the Eastern Pacific Ocean during DY 125–50 cruises of R/V “Xiangyanghong 03” in September 2018. Details of the collected sediment samples are described in Table S1 and Table S2.

In the present study, we used a mineral salt medium implemented with three different microplastic powders (ca. 0.075 mm grain size) of PU, PET and PS. The mineral salt medium refers to Mathur's experimental method with some modification (Mathur and Prasad, 2012). Briefly, incubations were initiated by inoculating 1.0 g of the respective deep sea sediment samples in 100 ml of mineral salt medium. Samples were incubated at 25 °C on a rotary shaker at 120 rpm in the dark. The remaining sediments were stored at –80 °C before DNA extraction. After 3 months of incubation, 1 ml aliquots were transferred to 100 ml of fresh culture medium, which was incubated at the same conditions for another 3 months (in total 6 months).

2.2. DNA extraction, PCR amplification, and high-throughput sequencing

Total DNA was extracted from 0.5 g sediment using the FastDNA™ SPIN Kit for Soil (MP Biomedicals LLC, USA), following the manufacturer's instructions. Primers ITS3 (GATGAAGAACGYAGYRAA) and ITS4 (TCCTCCGCTTATTGATATGC) were used to amplify the ITS2 regions of the ITS rRNA gene (Toju et al., 2012). Polymerase chain reaction (PCR) amplification conditions were set to an initial denaturation at 95 °C for 10 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 7 min. Amplicons were extracted from 2% agarose gels and purified using the AMPure XP Beads (Beckman Agencourt, USA) according to the manufacturer's instructions, and quantified using the ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). Purified amplicons were pooled and paired-end sequenced (PE250) on an Illumina HiSeq2500 platform according to the standard protocols by Genedenovo Biotechnology Co., Ltd. in Guangzhou. For incubation cultures, 0.5 ml of samples was used for DNA extraction with the FastDNA™ SPIN Kit. PCR amplification and sequencing steps were the same as given above.

2.3. Sequence processing, OTU clustering and taxonomy annotation

Raw reads were filtered using FASTP (version 0.18.0) (Chen et al., 2018): 1) removal of reads containing more than 10% of unknown nucleotides (N); 2) removal of reads containing <50% of bases with Q-value of >20. Paired end clean reads were merged as raw tags using FLSAH (version 1.2.11) (Magoc and Salzberg, 2011) with a minimum overlap of 10 bp and mismatch error rates of 20%. The raw tags were filtered by QIIME (Caporaso et al., 2010) (version 1.9.1) to obtain high-quality clean tags (Bokulich et al., 2013). UPARSE (version 9.2.64) was used to cluster effective tags into operational taxonomic units (OTUs) with a similarity ≥97% (Edgar, 2013). All chimeric tags were removed using the UCHIME algorithm resulting in effective tags for further analysis (Edgar et al., 2011). The tag sequence with the highest abundance was selected as a representative sequence within each cluster. The representative OTU sequences were classified into organisms by a naive Bayesian model using RDP classifier (version 2.2) (Wang et al., 2007) based on the UNITE database (version 8.0) (Koljalg et al., 2013) with a confidence threshold value of 0.8. Before analysis, the non-fungal ITS sequences were removed and the number of sequences in each sample was standardized to 29,000. The stacked bar plot of the community composition was visualized using the R project ggplot2 package (version 2.2.1) (Wickham, 2011).

2.4. Statistical analysis

Statistical analyses were performed on the obtained OTU table and α -diversity indices including the Sob, Chao1, ACE, Shannon index, and Simpson index were determined. In addition, phylogenetic diversity of the whole tree (PD whole tree) was calculated. The QIIME software was used to reveal alpha diversity indices (Caporaso et al., 2010), and the PD-whole tree index was calculated using the R package picante (version 1.8.2) (Kembel et al., 2010). Alpha indices were determined and compared among samples by using Welch's *t*-test in the R project Vegan package (version 2.5.3) (Oksanen et al., 2015). Non-parametric test analysis of independent samples was used to reveal the impact of culture time and types of plastics on OTU richness and PD whole tree with the SPSS software (version 24.0) according to Wang et al. (2021).

The NMDS (Non-Metric Multidimensional Scaling) analysis based on Bray-Curtis dissimilarity was generated by using the vegan package and visualized via the R ggplot2 package (version 2.2.1) (Wickham, 2011). Statistical analysis via Welch's *t*-test, Adonis, and Anosim tests of all items were performed via the Vegan package.

The FUNGuild tool was used to assign each OTU to a functional guild (for example, parasitic, phytopathogenic, and saprophytic fungi) (Nguyen et al., 2016). FUNGuild is currently the largest database used to assign the fungal genus to one of several functional guilds based on a community annotation database of fungal taxa with known or unclear ecological functions. In this study, only probable and highly probable confidence score guild assignments were used for further analysis. Analysis of functional differences between groups was determined by PERMANOVA using the Vegan package. Based on the method of Qiao et al. (2019), Pearson coefficients were calculated to represent the correlation of the selected main functional groups. Gephi v0.9.2 was used to construct the OTU network diagram.

2.5. Quantification of fungal abundance

ITS gene copy number of fungi in each sample was determined by quantitative PCR (qPCR) using a Light Cycler 480II Real-Time PCR system (Roche Life Science, Swiss). Primer pair ITS5 (GGAAGTAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGAT ATGC) (White et al., 1990) was used to specifically detect the fungal ITS gene. Following reagents were used for the fungal qPCR: 1 μ l of DNA template (10-fold-diluted to avoid interference of humic acids), 10 μ l of Premix (Fast Start Universal SYBR Green Master, Roche), and 0.4 μ l of forward and reverse primers (each 10 μ M). Real-time PCR was run with the following program: 94 °C for 3 min, followed by amplification (45 cycles of 30 s at 95 °C and 30 s at 55 °C) and a melting curve (5 s at 95 °C, 60 s at 65 °C, and increasing temperature from 65 °C to 97 °C at 0.11 °C s⁻¹ with 5 fluorescence acquisitions s⁻¹). One positive ligated plasmid of pGEM-T easy vector with gene fragments from previously prepared PCR products (*Meyerozyma*) was used to prepare the successive 10-fold dilution series for generating standard curves for fungal qPCR. Copy numbers of standard plasmid dilution were calculated by measuring the DNA concentration in a Qubit system applying it in the following equation: abundance of gene copy number/ μ l = (amount μ l⁻¹ \times 6.022 $\times 10^{23}$)/(length $\times 10^{-9} \times 660$).

2.6. Nucleotide sequence accession numbers

Raw sequences of all 28 subsamples were deposited in the Sequence Read Archives of the NCBI (Table S3).

3. Results

3.1. Effects of incubation time and plastic types on fungal α -diversity

OTU number declined after 3 months of enrichment on all three types of microplastics compared to the original sediment samples (Table S4). After 6 months (second round of enrichment), the number of

OTUs again increased (Table S4). The highest number of fungal OTUs, Chao1 and ACE indices were found in the PU2 samples, in which fungal Shannon and Simpson indices were lowest. During the incubation process, Shannon and Simpson indices increased for PS- and PET-enriched samples, but decreased for the PU-enriched samples. On the other hand, fungal richness increased in all microplastics-enriched samples independent of plastic type.

We compared the richness and diversity of the fungal community via Chao1 and Shannon indices (Zeng et al., 2019), respectively. The results revealed that richness and diversity of fungi in the PS- and PET-enriched samples increased when incubation time changed from 3 to 6 months, while fungal diversity in the PU-enriched samples decreased. Our results demonstrate that microplastic types affect deep-sea sedimentary fungal communities (Fig. S1).

Impacts of culture time and microplastic types on α -diversity of the obtained fungal communities were compared quantitatively using two-way ANOVA analysis. These data illustrate that microplastic types influenced all α -diversity indices, whereas incubation time (3 vs. 6 months) only showed significant effects on ACE and Shannon indices (Table 1).

OTU Venn diagrams enabled the comparison of fungal communities between different sets of samples. Thereafter, the number of shared OTUs between the original sediment sample and the first incubation (after 3 months) was greater than those between the first and second incubation (i.e. after 3 vs. 6 months). In addition, during the first incubation (after 3 months), the numbers of OTUs shared between PS and PET were higher than between PU and PET or PS. The second incubation (after 6 months) revealed a similar pattern indicating that fungal communities on PET and PS are similar, but different from PU (Fig. 1). In summary, the tested three microplastic types exerted significant influences on richness and diversity of fungi retrieved from deep sea sediments, yet, effects of PS and PET were very similar.

3.2. Composition and β -diversity of the obtained fungal communities

The NMDS plot based on Bray Curtis dissimilarity reveal differences in fungal communities among the different samples (Fig. 2). At the same time, distinct patterns in fungal community structure between the initial sample vs. the first and second round of enrichment incubations were observed (based on Bray-Curtis dissimilarity or weighted UniFrac dissimilarity; Table 2). All three microplastic types significantly changed the fungal community obtained from the different deep sea sediment samples. Whereas no significant differences were found between the fungal community structures enriched by PET and PS ($p > 0.05$), significant differences were observed in the PU-enriched incubations when compared to PET or PS, respectively ($p < 0.05$).

We examined the effects of microplastic types and incubation times on fungal β -diversity and the results showed that microplastic types exhibited stronger effects (PERMANOVA, Bray-Curtis dissimilarity: $F = 6.636$, $P = 0.002$; weighted UniFrac dissimilarity: $F = 3.617$, $P = 0.031$, Table 3) on the fungal community than that of incubation time (PERMANOVA, Bray-Curtis dissimilarity: $F = 0.947$, $P = 0.332$; weighted UniFrac dissimilarity: $F = 0.596$, $P = 0.442$, Table 3).

3.3. Fungal community structure of the different incubations

Ascomycota dominated the fungal assemblages in all samples (including the initial deep sea sediments) at the phylum level (Fig. S2). We detected representatives of the generally common classes: Eurotiomycetes, Sordariomycetes, Saccharomycetes, Dothideomycetes, Microbotryomycetes (belonging to Basidiomycota), which showed moderate relative abundances in the original deep sea sediment samples. Eurotiomycetes, Sordariomycetes and Saccharomycetes, however, exhibited the most abundant taxa on the PS1, PET1, PS2, and PET2 incubations. In addition, PU1 incubations were dominated by the classes Eurotiomycetes and Sordariomycetes. Different to all other samples, on

Table 1

Two-way ANOVA showing the effects of microplastics and culture time on the alpha diversity of fungal community.

	sobs			Chao			ACE			Shannon			Simpson			PD whole tree		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Microplastics	2	3.694	0.026	2	3.479	0.032	2	3.36	0.035	2	7.539	0.001	2	5.963	0.003	2	8.345	0.001
Culture time	2	2.199	0.132	2	3.142	0.061	2	3.547	0.044	2	6.018	0.007	2	1.803	0.186	2	1.744	0.195

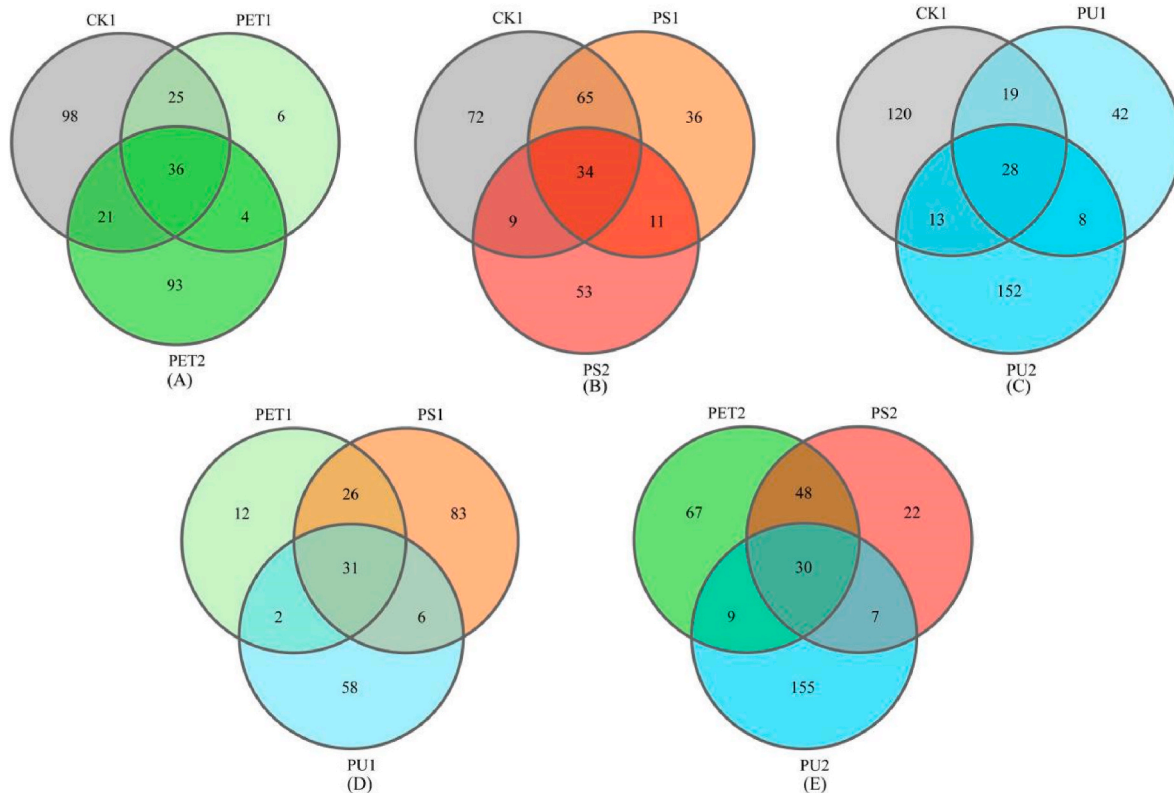


Fig. 1. Venn diagram showing fungal OTU overlap for different groups. CK1 represent Control, PET1 & PET2 represent the first round and second round of PET enrichment incubation, PS1 & PS2 represent the first round and second round of PS enrichment incubation, PU1 & PU2 represent the first round and second round of PU enrichment incubation.

PU2, Eurotiomycetes had a relative abundance of >80% (Fig. S3).

Lower similarities between fungal community composition at genus level existed between the original sediment samples vs. all microplastic enrichments (Fig. S4). *Penicillium* was the predominant fungi in the original sediment samples, which were set as the control group. On PS and PET, however, relative abundance of *Penicillium* was very low. Compared to the original sediment samples, *Aspergillus*, *Exophiala*, *Meyerozyma* and *Fusarium* were enriched effectively in PET1 (after 3 months of incubation). After 6 months (second round of enrichment incubation), the relative abundance of *Penicillium* and *Purpureocillium* in PET2 increased, whereas *Aspergillus* and *Exophiala* decreased. *Aspergillus*, *Exophiala*, *Meyerozyma* and *Fusarium* were also enriched in PS1 and the fungal community in PS2 was dominated by the genera *Penicillium*, *Aspergillus*, *Exophiala* and *Purpureocillium*.

The most dominant genera in PU1 were *Penicillium* and *Purpureocillium* (*Exophiala* only appeared in PU1-4) and thus very different from PS1 and PET1. Extension of the enrichment incubation resulted in a drastic change of the fungal community structure in PU2. For instance, *Aspergillus* and *Exophiala* were dominant in PU2-1 and PU2-4 with a relative abundance of more than 80%, respectively. In PU2-6 and PU2-13, *Penicillium* was the predominant fungal genus (relative abundance >90%). Moreover, several fungal strains, including *Aspergillus versicolor* (Genbank accession No. MF476045), *Cladosporium halotolerans* (LN834365), *Fusarium oxysporum* (MN018399), *Penicillium chrysogenum*

(MK801311), and *Exophiala jeanselmei* (KY292527) were isolated from PU2 enrichment cultures which had the ability to growth on the medium with Impranil PU as sole carbon source, demonstrating the potential of PU degradation.

We used ANOVA to analyze differences of the top 10 fungal taxa among all three plastic enrichment cultures (Fig. S5, Table S5). The analysis revealed distinct fungal community composition in incubations with different microplastic types. For instance, when compared to PS or PET, the relative abundance of *Penicillium* in the PU incubation was significantly higher, and thus closest to the original sediment communities. Additionally, PS and PET enhanced the relative abundance of *Meyerozyma* compared to PU additions ($p < 0.05$). The fungal genus *Purpureocillium* was most dominant in PU1, PET2 and PS2, whereas *Fusarium* showed a similar distribution as *Penicillium*, i.e. without any significant difference between PS and PET. Changes of *Aspergillus* in PS and PET were similar during the prolonged incubation time (from 3 to 6 months), but its relative abundance decreased significantly. In contrast, *Aspergillus* showed the opposite trend on PU.

3.4. Fungal abundance differed between treatment groups

The fungal qPCR results revealed that fungi were most abundant across all four sites in the PU2 incubations with abundances varying from $5.05 \times 10^7 \pm 7.7 \times 10^7$ copies/ml, which is significantly higher

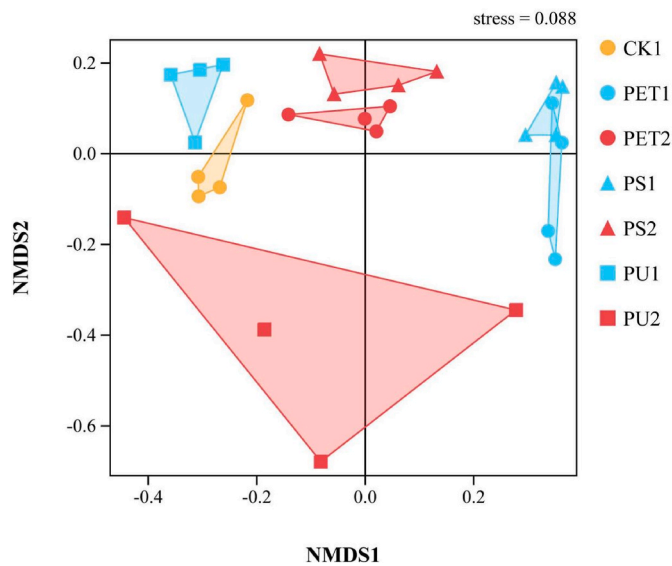


Fig. 2. NMDS analysis of each sample based on the Bray-Curtis dissimilarity. CK1 represent Control, PET1 & PET2 represent the first round and second round of PET enrichment incubation, PS1 & PS2 represent the first round and second round of PS enrichment incubation, PU1 & PU2 represent the first round and second round of PU enrichment incubation.

Table 2
Significant tests of fungal community composition between different compartments.

Distance matrix	Multiple comparisons	PERMANOVA		ANOSIM	
		R ²	p	R	p
Bray-Curtis dissimilarity	PET1-vs-PET2	0.44	0.03	0.69	0.03
	PS1-vs-PS2	0.58	0.03	0.81	0.03
	PU1-vs-PU2	0.35	0.03	0.59	0.03
	PS1-vs-PET1	0.23	0.25	0.13	0.19
	PS1-vs-PU1	0.8	0.02	1	0.03
	PET1-vs-PU1	0.63	0.03	1	0.03
	PS2-vs-PET2	0.15	0.39	0.1	0.24
	PS2-vs-PU2	0.38	0.04	0.5	0.03
	PET2-vs-PU2	0.33	0.03	0.41	0.03
	CK1-vs-PET1	0.58	0.03	1	0.03
	CK1-vs-PS1	0.76	0.03	1	0.02
	CK1-vs-PU1	0.59	0.03	1	0.03
	PET1-vs-PET2	0.29	0.17	0.26	0.15
	PS1-vs-PS2	0.47	0.05	0.5	0.06
Weighted UniFrac dissimilarity	PU1-vs-PU2	0.39	0.02	0.53	0.03
	PS1-vs-PET1	0.29	0.19	0.19	0.21
	PS1-vs-PU1	0.79	0.03	1	0.04
	PET1-vs-PU1	0.54	0.03	0.84	0.04
	PS2-vs-PET2	0.11	0.54	0.05	0.35
	PS2-vs-PU2	0.48	0.04	0.65	0.02
	PET2-vs-PU2	0.44	0.03	0.61	0.02
	CK1-vs-PET1	0.4	0.03	0.52	0.03
	CK1-vs-PS1	0.76	0.03	1	0.03
	CK1-vs-PU1	0.61	0.03	0.99	0.03

Table 3

Permutational multivariate analysis of variance (PERMANOVA) based on Bray – Curtis dissimilarity and weighted UniFrac dissimilarity in fungal community of different microplastics and culture time.

	Bray-Curtis dissimilarity				Weighted UniFrac dissimilarity			
	df	R ²	F	P	df	R ²	F	P
Microplastics	2	0.380	6.636	0.002	2	0.205	3.617	0.031
Culture time	1	0.080	0.947	0.332	1	0.043	0.596	0.442

than those in the initial CK1 ($1.1 \times 10^5 \pm 1.6 \times 10^4$ copies/ml) (Kruskal Wallis test, $p < 0.05$), followed by PET1 ($5.78 \times 10^6 \pm 2.2 \times 10^6$ copies/ml) and PS1 ($2.48 \times 10^6 \pm 1.9 \times 10^6$ copies/ml). This suggests that PU effectively increases the abundance of fungi after 6 months of incubation (Fig. 3).

3.5. Comparison and co-occurrence patterns of fungal functional groups

Trophic modes and functional groups of fungal communities were determined by FUNGuild. Saprotrophic fungi, within the Ascomycota, were the most frequently detected fungal taxa throughout the incubations. In general, PU and PS/PET samples reveal a significant difference in the function and composition of fungal communities (Fig. 4).

Fungi affiliated with “pathotrophs” had significantly more sequences in PU1 ($46.07 \pm 13.22\%$) than in other samples, but did not show significant difference in PS2 ($17.36 \pm 3.97\%$) vs. PET2 ($26.41 \pm 10.88\%$) samples. Furthermore, fungi, belonging to “pathotrophs-saprotrophs-symbiotrophs” in PU1 ($1.29 \pm 0.27\%$) and PU2 ($0.33 \pm 0.3\%$) samples were significantly less than other functional groups. Yet, the other five trophic modes did not show any significant differences among all groups. Fungi that were classified into “unidentified” trophic modes had a significantly higher proportion in the original deep sea sample CK1 ($17.91 \pm 12.21\%$), followed by PET2 ($5.06 \pm 5.38\%$) and PS2 ($3.23 \pm 5.74\%$) (Table 4).

In order to discern the relationships between OTUs during the incubations, co-occurrence network analysis was performed by separating according to microplastic types (PU, PS and PET) and incubation times (after 3 and 6 months, respectively). Compared to PS2 and PET2, PU2 was found to have a much more complex OTU interaction network. Thereby, OTU 7 (*Fusarium*) showed positive correlations to 17 other OTUs, followed by OTU18 (*Cladosporium cladosporioides*) having

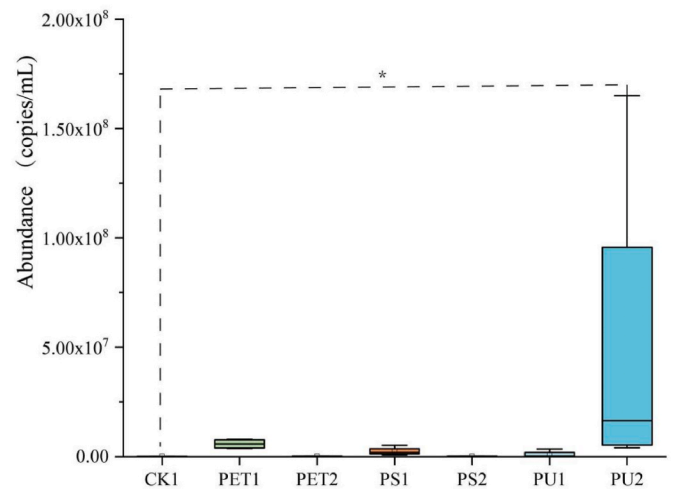


Fig. 3. Fungal abundance in different samples determined by qPCR. Asterisk shows significant differences, $p < 0.05$. CK1 represent Control, PET1 & PET2 represent the first round and second round of PET enrichment incubation, PS1 & PS2 represent the first round and second round of PS enrichment incubation, PU1 & PU2 represent the first round and second round of PU enrichment incubation.

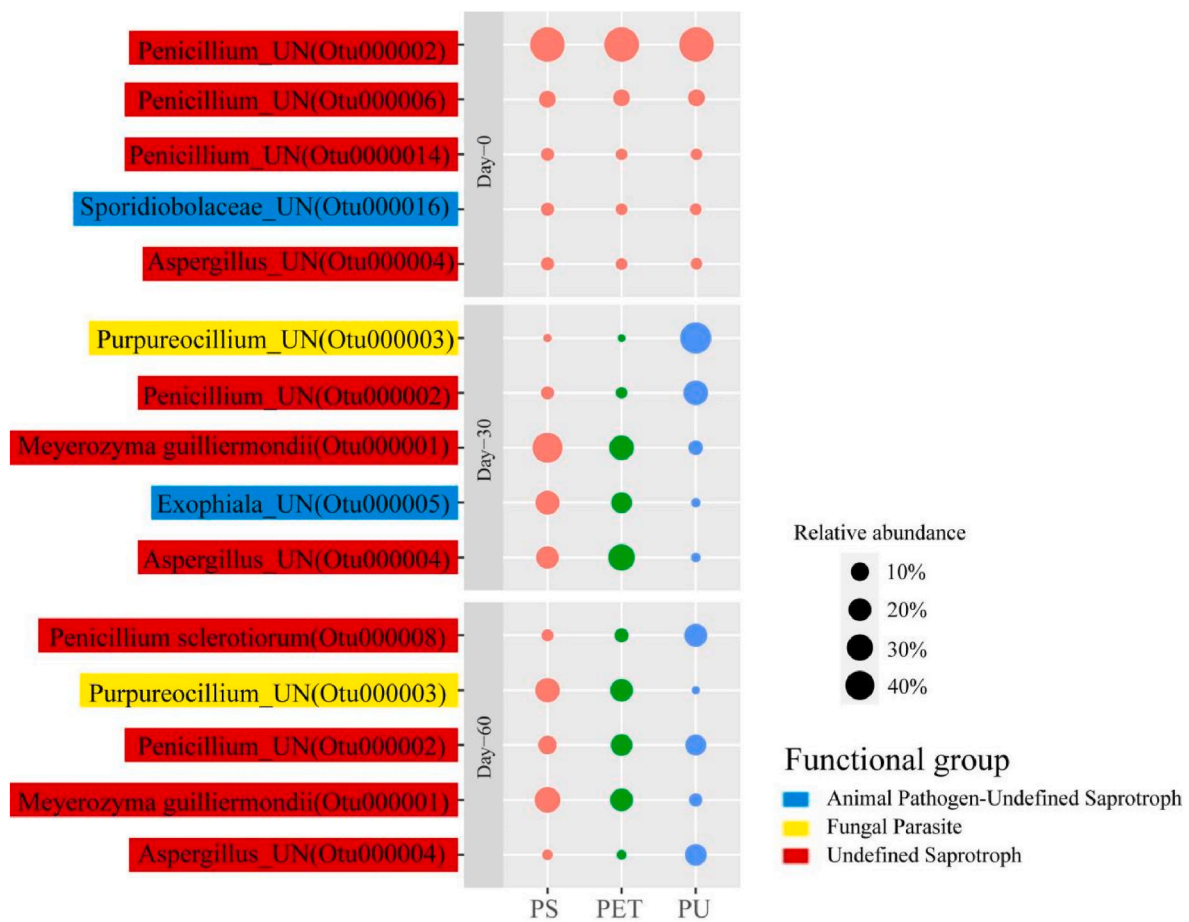


Fig. 4. Bubble chart displaying the five most commonly observed fungal OTUs in original sediments (0 day), first enrichment (30 days) and second enrichment (60 days). Guild assignments for the top 5 fungal species using the FUNGuild database.

Table 4

Relative abundance of fungal sequences classified by the corresponding fungal trophic mode in original sediments and microplastic enriched samples inferred by FUNGuild.

Trophic mode	CK1	PET1	PS1	PU1	PET2	PS2	PU2	p
Pathotroph	3.05 ± 3.75 ^a	0.14 ± 0.07 ^a	0.11 ± 0.17 ^a	46.07 ± 13.22 ^b	17.36 ± 3.97 ^c	26.41 ± 10.88 ^c	0.79 ± 0.98 ^a	7.86E-09
Pathotroph-Saprotroph	6.42 ± 2.54	27.99 ± 20.02	19.56 ± 20.89	12.81 ± 23.76	6.02 ± 4.88	13.6 ± 9.01	20.79 ± 39.95	0.745
Pathotroph-Saprotroph-Symbiotroph	6.37 ± 4.87 ^a	7.7 ± 4.42 ^a	8.7 ± 5.9 ^a	1.29 ± 0.27 ^b	7.69 ± 4.98 ^a	7.86 ± 4.08 ^a	0.33 ± 0.3 ^b	0.039
Pathotroph-Symbiotroph	2.54 ± 3.01	0.17 ± 0.11	0.18 ± 0.11	0.52 ± 0.19	0.95 ± 0.5	1.48 ± 0.67	0.5 ± 0.41	0.103
Saprotroph	63.71 ± 12.27	62.39 ± 15.05	71.09 ± 23.79	37.44 ± 9.84	62.69 ± 8.01	45.9 ± 15.43	75.85 ± 43.13	0.192
Saprotroph-Symbiotroph	0	0.01 ± 0.02	0	0.65 ± 0.68	0.23 ± 0.24	1.52 ± 2.74	0.01	0.377
Symbiotroph	0	0	0	0	0	0	0.02 ± 0.02	0.108
Unassigned	17.91 ± 12.21 ^a	1.6 ± 1.2 ^b	0.36 ± 0.23 ^b	1.22 ± 1.22 ^b	5.06 ± 5.38 ^b	3.23 ± 5.74 ^b	1.71 ± 1.47 ^b	0.003

Note: different letters in the upper right corner of the numbers represent significant difference.

positive correlations to 13 other OTUs. OTU 20 (*Cladosporium cladosporioides*) had positive correlations to 12 other OTUs. OTU 22 (*Fusarium*) and OTU 52 (*Cladosporium cladosporioides*) were positively correlated to 11 other OTUs (Fig. S6).

Microbial network complexity increased strongly with prolonged incubation times, exhibiting higher OTU numbers and more fungal ecotypes in microcosms supplemented with different microplastic types. This result indicates that enrichment with microplastics increases the functional diversity of fungi. In the first incubation round, samples contained a sediment inoculum and saprophytic fungi dominated the fungal community. In the second incubation round, microplastics were the major particle type and carbon source, shifting the functional diversity of the fungal community, yet, saprophytic fungi remained

dominant.

4. Discussion

In recent years, microplastics research has developed into a hot topic in marine sciences, yet, there is little knowledge about microorganisms colonizing and potentially degrading microplastics in deep-sea environments, especially fungi. In this study, we investigated the impact of three different types of microplastics on fungal diversity and community composition obtained after lab incubations of deep sea sediments of the Eastern Pacific. Fungi from the deep sea sediments showed distinct responses to different microplastic types, i.e. α and β diversity of PS and PET samples were relatively similar, but significantly different when

compared to PU samples.

4.1. Microplastics addition resulted in significant shifts of the fungal community from deep sea sediments

Results of high-throughput sequencing revealed that the dominant fungal taxa in the original deep sea sediments were Ascomycota. This is consistent with previous studies, indicating that Ascomycota dominate deep sea sediments (Zhang et al., 2016; Nagano and Nagahama, 2012; Xu et al., 2018; Rojas-Jimenez et al., 2020). Not only in the deep sea, but also in surface waters, fungal communities on PET bottles in the North Sea were comprised of 24 fungal OTUs assigned to Ascomycota, Basidiomycota, and Chytridiomycota (Oberbeckmann et al., 2016). Similarly, fungi on microplastics in the Baltic Sea were mainly dominated by Ascomycota, Chytridiomycota and Cryptomycota (Kettner et al., 2017). Previous studies further demonstrated that fungi belonging to Ascomycota had the potential to effectively degrade PS and PET (Motta et al., 2009; Rodrigo et al., 2021). This indicates that marine fungi can colonize microplastics and use it as a carbon source for growth.

Based on our qPCR results, fungal abundance increased in the incubations where microplastics have been added and reached consistently higher values than that of the original deep sea sediments. The pronounced dominance of Ascomycota on microplastics incubation cultures indicates a strong enrichment of these potentially microplastics degrading fungi. This notion is supported by an earlier study of Kettner et al. (2017) who reported that fungal richness and Shannon diversity on PS and PE microplastic particles were lower than in the surrounding water and wood of the River Warnow. This indicates a strong selection of specialist fungi, which use microplastics either as substrate for colonization or growth (carbon source). Thus it is not surprising that our FUNGuild analysis also revealed pronounced changes in functional groups of fungi upon addition of microplastics (Fig. 4). Yet, the main ecological type of fungi remained “saprophytes”, which is consistent with previous findings of microplastic fungi in surface waters of the Southern Atlantic and the Antarctic Peninsula (Lacerda et al., 2020).

4.2. PS, PET and PU addition significantly altered fungal communities from deep sea sediments in the lab incubations

In the same batch of incubations, there was no obvious difference in fungal community structure on PS and PET, which is most likely related to their high resistance to fungal degradation (Fotopoulou and Karapanagioti, 2019). In contrast, PU incubations showed significant differences with both PS and PET incubations. PU has been reported to be degradable by a large number of fungi including many Ascomycota (Zafar et al., 2013; Kemonia and Piotrowska, 2016; Khan et al., 2017; Khan et al., 2017). Furthermore, PU is more likely to be colonized by fungi than PE and PLA (Seeley et al., 2020).

In particular in PU2 incubations (after 60 days), *Penicillium* was significantly enriched (Fig. S4). This is in accordance to findings that many *Penicillium* strains can utilize PU for growth (Howard, 2012; Kemonia and Piotrowska, 2016; Alvarez-Barragan et al., 2016). And even in PS and PET incubations *Penicillium* increased after 60 days of incubation (second round), but less than in PU2. Some studies indicate that *Penicillium* can also metabolize PS and PET (Taghavi et al., 2021). Rodrigo et al. (2021) reported that *Penicillium* spp. had a much higher degradation efficiency in PU than in both PS and PE. Moreover, PS can be only colonized by *Curvularia* after microplastic pretreatments via chemical oxidation (Motta et al., 2009), which supports that PS is extremely difficult to be degraded by fungi (Krueger et al., 2017). Furthermore, Umamaheswari and Margandan (2015) isolated a *Penicillium* fungus from PET waste, and observed by scanning electron microscopy (SEM) that *Penicillium* can successfully colonizes the surface of PET flakes and exhibits morphological changes including the formation of hyphae, surface corrosion, and a multilayer dense network of crystals. Consequently, it is obvious that *Penicillium* can

invade or degrade PU or PET materials, but without chemical or physical pretreatment the fungus can hardly degrade PS. These findings confirm our results, which showed the lowest relative abundance of *Penicillium* on PS and the highest on PU. The following fungal strains were recovered from PU enrichment cultures after 60 days of incubation including *Aspergillus versicolor*, *Cladosporium halotolerans*, *Fusarium oxysporum*, *Penicillium chrysogenum*, and *Exophiala jeanselmei*, which also exhibited high relative abundances in the same PU enrichment cultures when using high-throughput sequencing. In addition, subsequent inoculation of these fungal strains on the mineral salt medium with Impranil PU as the sole carbon source revealed growth of all fungi, indicating that these fungi may be capable of degrading PU.

4.3. Type of microplastics has a greater impact on fungal community composition than incubation time

ANOVA analysis was used to test for the influence of microplastic type vs. incubation time on fungal α - and β -diversity, abundance and functional composition in the different incubations. Microplastic type significantly affected all calculated α -diversity parameters, whereas incubation time affected Shannon and ACE indices. In particular, microplastic type affected fungal community composition and interaction networks. Seeley et al. (2020) used PUF, PLA, PVC and PE as plastic substrates for their micro-culture enrichment study. They found big differences in the α -diversity of the fungal community of different plastic enrichment cultures, especially fungal communities in the PVC enrichment samples were significantly different from other plastic types. Several other researchers showed that fungal communities on microplastics substantially changed with different types of plastics, indicating a highly selective effect of microplastics on fungal communities (McCormick et al., 2014; Kettner et al., 2017; Ren et al., 2020). Similarly, in our study, fungal communities significantly differed between PU and PS or PE ($p < 0.05$). A reasonable explanation for these significant differences is that physical as well as chemical properties of the different microplastic types are greatly different (Lambert et al., 2017), causing the observed, pronounced changes between the respective fungal communities (Seeley et al., 2020).

In a two-month community study of bacteria on plastic surfaces in the coastal Northern Adriatic Sea, Pinto et al. (2019) found that bacterial communities significantly differed with plastic types and also incubation duration. In summary, our results indicate that microplastic types significantly affect deep sea fungal community composition as well as α -diversity in the different incubations.

It was reported that esterases secreted by fungi can degrade PU (Oceguera-Cervantes et al., 2007) and PS (Tahir et al., 2013) individually via attacking the ester bonds, while PET may require more extracellular enzymes for biodegradation of its aromatic ring structure (Wei and Zimmermann, 2017). The degradation intermediate of microplastics are usually toxic but may serve as new carbon source for specific bacterial and fungal utilization or co-metabolism. Breakdown products of PET are terephthalic acid (TPA) and ethylene glycol, which are toxic to cells (Franden et al., 2018) and could be subsequently biodegraded by specific fungal species such as *Aspergillus* and *Penicillium* (Soni et al., 2009). Polyols and alkanes were reported to be biodegradation intermediate of PU by *Cladosporium*.sp. (Alvarez-Barragan et al., 2016) and only a few fungal species were reported to use alkanes as carbon sources (Singh et al., 2011). Styrene was the biodegradation intermediate of PS (Ho et al., 2018), the degradation pathway of this toxic compound was only reported for some fungal species, such as white-rot fungi (Braun-Lülleman et al., 1997). Additionally, only a few fungal species were shown grow on styrene as sole carbon and energy sources, such as *Cladosporium sphaerospermum* (Qi et al., 2002). In summary, different microbial biodegradation intermediates released from different types of microplastics can shape fungal community composition and diversity.

5. Conclusions

We studied the effects of three kinds of plastics on the fungal community of deep-sea sediments. The results showed that PU has a greater impact on the fungal community composition than PS and PET, suggesting that deep-sea sediments may harbor a large number of potential PU degrading fungi. At the same time, addition of PU in our incubations resulted in more complex and diverse fungal networks than PS and PET. Most of the PU-associated fungi represent saprophytic fungi, highlighting a high potential for polymer and presumably plastic degradation. Diverse fungal species were isolated from PU enrichment cultures and could utilize aqueous polyester PU as a sole carbon source. In future, we will explore in greater detail the degradation characteristics of these fungal strains to better understand their potential for plastic degradation.

Declaration of competing interest

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

Acknowledgments

This research work was financially supported by the Natural Science Foundation of Fujian Province of China (2022J02011), China Postdoctoral Science Foundation (2021M693781), Xiamen Ocean Research and Development Institute Project, China (KFY202201), China Ocean Mineral Resources R&D Association (COMRA) Program (DY135-B-09 and DY135-B-01), National Natural Science Foundation of China (41776170 and 41606145), Scientific Research Foundation of Third Institute of Oceanography, Ministry of Natural Resources, China (2016039), and European Union Horizon 2020 Research and Innovation 772 programme under Grant Agreement number 965367 (PlasticsFatE). We would like to thank the crew and scientific team of R/V Xiang yang hong 03, the pilots and the supporting team in 50th Da yang Cruise for the sampling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ibiod.2022.105461>.

References

- Alvarez-Barragan, J., Dominguez-Malfavon, L., Vargas-Suarez, M., Gonzalez-Hernandez, R., Aguilar-Osorio, G., Loza-Tavera, H., 2016. Biodegradative activities of selected environmental fungi on a polyester polyurethane varnish and polyether polyurethane foams. *Appl. Environ. Microbiol.* 82, 5225–5235.
- Arias-Andres, M., Klumper, U., Rojas-Jimenez, K., Grossart, H.P., 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. *Environ. Pollut.* 237, 253–261.
- Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. *Environ. Int.* 102, 165–176.
- Balasubramanian, V., Natarajan, K., Hemambika, B., Ramesh, N., Sumathi, C.S., Kottaimuthu, R., Rajesh Kannan, V., 2010. High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar, India. *Lett. Appl. Microbiol.* 51, 205–211.
- Beneš, H., Vlčková, V., Paruzel, A., Trhlíková, O., Chalupa, J., Kanizsová, L., Skleničková, K., Halecký, M., 2020. Multifunctional and fully aliphatic biodegradable polyurethane foam as porous biomass carrier for biofiltration. *Polym. Degrad. Stabil.* 176, 109156.
- Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D. A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* 10, 57–59.
- Braun-Lüthmann, A., Majcherczyk, A., Hüttermann, A., 1997. Degradation of styrene by white-rot fungi. *Appl. Microbiol. Biotechnol.* 47, 150–155.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336.
- Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890.
- Debroas, D., Mone, A., Ter Halle, A., 2017. Plastics in the North Atlantic garbage patch: a boat-microbe for hitchhikers and plastic degraders. *Sci. Total Environ.* 599–600, 1222–1232.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200.
- Fotopoulou, K.N., Karapanagioti, H.K., 2019. Degradation of various plastics in the environment. In: Takada, H., Karapanagioti, H.K. (Eds.), *Hazardous Chemicals Associated with Plastics in the Marine Environment*. Springer, pp. 71–92.
- Franden, M.A., Jayakody, L.N., Li, W.-J., Wagner, N.J., Cleveland, N.S., Michener, W.E., Hauer, B., Blank, L.M., Wierckx, N., Klebensberger, J., Beckham, G.T., 2018. Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization. *Metab. Eng.* 48, 197–207.
- Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* 1, 116.
- Gigault, J., Halle, A., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: what is a nanoplastic? *Environ. Pollut.* 235, 1030–1034.
- Gomiero, A., Oysa, K.B., Agustsson, T., van Hoytema, N., van Thiel, T., Grati, F., 2019. First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. *Chemosphere* 227, 705–714.
- González-Fernández, C., Toullec, J., Lambert, C., Le Goïc, N., Seoane, M., Moriceau, B., Huvet, A., Berchel, M., Vincent, D., Courcot, L., Soudant, P., Paul-Pont, I., 2019. Do transparent exopolymeric particles (TEP) affect the toxicity of nanoplastics on *Chaetoceros neogracile*? *Environ. Pollut.* 250, 873–882.
- Harrison, J.P., Schratzberger, M., Sapp, M., Osborn, A.M., 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. *BMC Microbiol.* 14, 232.
- Ho, B.T., Roberts, T.K., Lucas, S., 2018. An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach. *Crit. Rev. Biotechnol.* 38, 308–320.
- Howard, G.T., 2012. Polyurethane biodegradation. In: Singh, S.N. (Ed.), *Microbial Degradation of Xenobiotics*. Springer, pp. 371–394.
- Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., Pohl, F., 2020. Seafloor microplastic hotspots controlled by deep-sea circulation. *Science* 368, 1140–1145.
- Kemmel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464.
- Kemona, A., Piotrowska, M., 2016. Microorganisms Potentially Useful in the Management of Polyurethane Foam Waste. *Infrastruktura i Ekologia Terenów Wiejskich*, pp. 1297–1308.
- Kettner, M.T., Oberbeckmann, S., Labrenz, M., Grossart, H.-P., 2019. The eukaryotic life on microplastics in brackish ecosystems. *Front. Microbiol.* 10, 538.
- Kettner, M.T., Rojas-Jimenez, K., Oberbeckmann, S., Labrenz, M., Grossart, H.P., 2017. Microplastics alter composition of fungal communities in aquatic ecosystems. *Environ. Microbiol.* 19, 4447–4459.
- Khan, S., Nadir, S., Shah, Z.U., Shah, A.A., Karunaratna, S.C., Xu, J., Khan, A., Munir, S., Hasan, F., 2017. Biodegradation of polyester polyurethane by *Aspergillus tubingensis*. *Environ. Pollut.* 225, 469–480.
- Koljal, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martin, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Pöldmaa, K., Saag, L., Saar, I., Schüssler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 22, 5271–5277.
- Krueger, M.C., Seiwert, B., Prager, A., Zhang, S., Abel, B., Harms, H., Schlosser, D., 2017. Degradation of polystyrene and selected analogues by biological Fenton chemistry approaches: opportunities and limitations. *Chemosphere* 173, 520–528.
- Lacerda, A., Proietti, M.C., Secchi, E.R., Taylor, J.D., 2020. Diverse groups of fungi are associated with plastics in the surface waters of the western South Atlantic and the Antarctic Peninsula. *Mol. Ecol.* 29, 1903–1918.
- Lambert, S., Scherer, C., Wagner, M., 2017. Ecotoxicity testing of microplastics: considering the heterogeneity of physicochemical properties. *Integrated Environ. Assess. Manag.* 13, 470–475.
- Le Bihan, F., Clerdeau, C., Cormier, B., Crebassa, J.C., Keiter, S.H., Beiras, R., Morin, B., Begout, M.L., Cousin, X., Cachot, J., 2020. Organic contaminants sorbed to microplastics affect marine medaka fish early life stages development. *Mar. Pollut. Bull.* 154, 111059.
- Li, J., Huang, W., Jiang, R., Han, X., Zhang, D., Zhang, C., 2020. Are bacterial communities associated with microplastics influenced by marine habitats? *Sci. Total Environ.* 733, 139400.
- Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963.
- Mathur, G., Prasad, R., 2012. Degradation of polyurethane by *Aspergillus flavus* (ITCC 6051) isolated from soil. *Appl. Biochem. Biotechnol.* 167, 1595–1602.

- McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. *Environ. Sci. Technol.* 48, 11863–11871.
- Motta, O., Proto, A., De Carlo, F., De Caro, F., Santoro, E., Brunetti, L., Capunzo, M., 2009. Utilization of chemically oxidized polystyrene as co-substrate by filamentous fungi. *Int. J. Hyg Environ. Health* 212, 61–66.
- Nagano, Y., Nagahama, T., 2012. Fungal diversity in deep-sea extreme environments. *Fungal Ecol.* 5, 463–471.
- Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M., Wahl, M., Treude, T., 2016. Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. *Mar. Pollut. Bull.* 103, 168–178.
- Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J.R., Schilling, J.S., Kennedy, P.G.J.F.E., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* 20, 241–248.
- Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2017. Environmental factors support the formation of specific bacterial assemblages on microplastics. *Front. Microbiol.* 8, 2709.
- Oberbeckmann, S., Labrenz, M., 2020. Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. *Ann. Rev. Mar. Sci.* 12, 209–232.
- Oberbeckmann, S., Osborn, A.M., Duhaime, M.B., 2016. Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *PLoS One* 11, e0159289.
- Oceguera-Cervantes, A., Carrillo-García, A., Lopez, N., Bolanos-Nunez, S., Cruz-Gomez, M.J., Wachter, C., Loza-Tavera, H., 2007. Characterization of the polyurethanolytic activity of two alicyclophilus sp. strains able to degrade polyurethane and N-methylpyrrolidone. *Appl. Environ. Microbiol.* 73, 6214–6223.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B., Simpson, G., Solymos, P., Stevens, H., Wagner, H., 2015. *Vegan: community ecology package. R Package. Version 2.2-1.* <http://CRAN.R-project.org/package=vegan>.
- Pabortsava, K., Lampitt, R.S., 2020. High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. *Nat. Commun.* 11, 4073.
- Pinnell, L.J., Turner, J.W., 2019. Shotgun metagenomics reveals the benthic microbial community response to plastic and bioplastic in a coastal marine environment. *Front. Microbiol.* 10, 1252.
- Pinto, M., Langer, T.M., Huffer, T., Hofmann, T., Herndl, G.J., 2019. The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. *PLoS One* 14, e0217165.
- Pollet, T., Berdjeb, L., Garnier, C., Durrieu, G., Le Poupon, C., Misson, B., Jean-Francois, B., 2018. Prokaryotic community successions and interactions in marine biofilms: the key role of flavobacteria. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 94, 83.
- Qi, B., Moe, W.M., Kinney, K.A., 2002. Biodegradation of volatile organic compounds by five fungal species. *Appl. Microbiol. Biotechnol.* 58, 684–689.
- Qiao, C., Ryan Penton, C., Liu, C., Shen, Z., Ou, Y., Liu, Z., Xu, X., Li, R., Shen, Q., 2019. Key extracellular enzymes triggered high-efficiency composting associated with bacterial community succession. *Bioresour. Technol.* 288, 121576.
- Ren, X., Tang, J., Liu, X., Liu, Q., 2020. Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil. *Environ. Pollut.* 256, 113347.
- Rodrigo, O.-A., Daynet, S., Jaime, N.-M.n., Nora, F., JosÁ, Á.I.-B., Alejandro, A.n., Villafuerte, J., Milton, B.-A., 2021. Analysis of the degradation of polyethylene, polystyrene and polyurethane mediated by three filamentous fungi isolated from the Antarctica. *Afr. J. Biotechnol.* 20, 66–76.
- Rojas-Jimenez, K., Grossart, H.-P., Cordes, E., Cortes, J., 2020. Fungal communities in sediments along a depth gradient in the Eastern Tropical Pacific. *Front. Microbiol.* 11, 575205.
- Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. *Nat. Commun.* 11, 2372.
- Singh, S., Kumari, B., Mishra, S., 2011. Microbial degradation of alkanes. In: Singh, S.N. (Ed.), *Microbial Degradation of Xenobiotics*. Springer, pp. 439–469.
- Soni, R.K., Soam, S., Dutt, K., 2009. Studies on biodegradability of copolymers of lactic acid, terephthalic acid and ethylene glycol. *Polym. Degrad. Stabil.* 94, 432–437.
- Taghavi, N., Singhal, N., Zhuang, W.-Q., Baroutian, S., 2021. Degradation of plastic waste using stimulated and naturally occurring microbial strains. *Chemosphere* 263, 127975.
- Tahir, L., Ali, M., Zia, M., Atiq, N., Hasan, F., Ahmed, S., 2013. Production and characterization of esterase in lantinus tigrinus for degradation of polystyrene. *Pol. J. Microbiol.* 62, 101–108.
- Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS One* 7, e40863.
- Umamaheswari, S., Margandan, M.M., 2015. Potential of soil fungi (*Penicillium* sp.) to form biofilm on polyethylene terephthalate surface. *J. Pure Appl. Microbiol.* 9, 1583–1586.
- Wang, L., Tong, J., Li, Y., Zhu, J., Zhang, W., Niu, L., Zhang, H., 2021. Bacterial and fungal assemblages and functions associated with biofilms differ between diverse types of plastic debris in a freshwater system. *Environ. Res.* 196, 110371.
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267.
- Wei, R., Zimmermann, W., 2017. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? *Microb. Biotechnol.* 10, 1308–1322.
- White, T., Bruns, T., Lee, S., Taylor, J., Innis, M., Gelfand, D., Sninsky, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, pp. 315–322.
- Wickham, H., 2011. ggplot2. *Wiley Interdiscipl. Rev.: Comput. Stat.* 3, 180–185.
- Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The Deep Sea Is a Major Sink for Microplastic Debris, vol. 1. *Royal Society Open Science*, 140317.
- Xu, W., Gong, L.F., Pang, K.L., Luo, Z.H., 2018. Fungal diversity in deep-sea sediments of a hydrothermal vent system in the Southwest Indian Ridge. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* 131, 16–26.
- Zafar, U., Houlden, A., Robson, G.D., 2013. Fungal communities associated with the biodegradation of polyester polyurethane buried under compost at different temperatures. *Appl. Environ. Microbiol.* 79, 7313–7324.
- Zeng, J., Lin, Y., Zhao, D., Huang, R., Xu, H., Jiao, C., 2019. Seasonality overwhelms aquacultural activity in determining the composition and assembly of the bacterial community in Lake Taihu, China. *Sci. Total Environ.* 683, 427–435.
- Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "plastisphere": microbial communities on plastic marine debris. *Environ. Sci. Technol.* 47, 7137–7146.
- Zhang, L., Kang, M., Huang, Y., Yang, L., 2016. Fungal communities from the calcareous deep-sea sediments in the Southwest India Ridge revealed by Illumina sequencing technology. *World J. Microbiol. Biotechnol.* 32, 78.
- Zhang, L., Xie, Y., Zhong, S., Liu, J., Qin, Y., Gao, P., 2021. Microplastics in freshwater and wild fishes from Iijiang river in Guangxi, Southwest China. *Sci. Total Environ.* 755, 142428.