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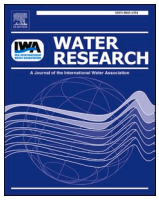

Dynamics and functions of microbial communities in the plastisphere in temperate coastal environments

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ABSTRACT

Microbial attachment and biofilm formation on microplastics (MPs <5 mm in size) in the environment have received growing attention. However, there is limited knowledge of microbial function and their effect on the properties and behavior of MPs in the environment. In this study, microbial communities in the plastisphere were explored to understand microbial ecology as well as their impact on aquatic ecosystems. Using the amplicon sequencing of 16S and internal transcribed spacer (ITS) genes, we uncovered the composition and diversity of bacterial and fungal communities in samples of MPs (fiber, film, foam, and fragment), surface water, bottom sediment, and coastal sand in two contrasting coastal areas of Japan. Differences in microbial diversity and taxonomic composition were detected depending on sample type (MPs, water, sediment, and sand) and the research site. Although relatively higher bacterial and fungal gene counts were determined in MP fragments and foams from the research sites, there were no significant differences in microbial community composition depending on the morphotypes of MPs. Given the colonization by hydrocarbon-degrading communities and the presence of pathogens on MPs, the complex processes of microbial taxa influence the characteristics of MP-associated biofilms, and thus, the properties of MPs. This study highlights the metabolic functions of microbes in MP-associated biofilms, which could be key to uncovering the true impact of plastic debris on the global ecosystem.

1. Introduction

Microplastics (MPs <5 mm in size) in the environments have received growing attention because of their harmful effects on the ecosystem. An alarming amount of plastic debris, especially MPs, has been discovered within environmental compartments, such as water, sediment, sand, and biota, ranging from the most remote islands to highly populated regions worldwide (Jambeck et al., 2015; Nunes et al., 2023). Land-based sources of plastic debris account for approximately 80 % of marine debris with the dominant input being river transport (Nakayama and Osako, 2023). Because of plastic emissions from the land into the ocean by rivers, coastal ecosystems have received considerable pressure from plastic pollution. Given that differences in hydrological regimes are influenced by climate conditions, population density, and levels of urbanization and industrialization, several orders of magnitude of MP concentrations have been reported in coastal systems (Atwood et al., 2019; Tsang et al., 2017). Although interactions between MPs and organisms (de Smit et al., 2021) and inorganic and organic pollutants (León et al., 2019; Turner and Lau, 2016) in coastal environments were reported, few studies have considered the colonization of microbial communities and the effect of their composition and diversity on MPs.

In the environment, MPs are immediately colonized by unique microbial assemblages and act as a long-lasting surface for a wide variety of heterotrophic microorganisms. Generally, bacterial, archaeal, and eukaryotic communities attach to MPs and produce extracellular polymeric substances to protect their colonies from environmental alterations. Extracellular polymeric substances mainly consist of polysaccharides, proteins, lipids, and nucleic acids (Battulga et al., 2022). The complex mixture of these biopolymers forms a biofilm—microbial cells embedded in the extracellular polymeric matrix—on the plastic surface. Based on water retention, nutrient

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storage, and high extracellular enzymatic activity in biofilms (Jiang et al., 2020), microbes in the biofilm receive many benefits. In a biofilm, microbes communicate through quorum sensing and synchronically regulate their actions for drastic changes in the ambient environment. Thus, to examine microbial taxa on MPs and distinguish them from free-living communities in the environment, Zettler et al. (2013) described the concept of "plastisphere." The plastisphere represents a thin layer of microbial life on plastics. Moreover, biofouling, the mechanism of microbial attachment and accumulation on biotic and abiotic surfaces, leads to hotspots of the heterotrophic activities on plastic debris, resulting in the potential alteration of aquatic biogeochemical processes by the presence of plastics (McCormick et al., 2014; Shen et al., 2020). Hence, understanding the composition and diversity of microbial communities on MPs and their impact on the ecosystem is of great importance.

A broad spectrum of microbial colonies on MPs as well as distinct species from free-living organisms in the ambient environment have been explored in previous studies (Amaral-Zettler et al., 2020; Wu et al., 2019). Miao et al. (2019) investigated the community structure and function of microbes growing on natural and MP substrates by incubation under controlled conditions and observed distinct community structures between the substrates. Similar results that emphasized taxonomically distinct and less diverse bacterial and fungal communities on MPs compared with surrounding water, sediment, and organic matter, such as plant litter and seston, have been discussed in some published literature (Kettner et al., 2017; McCormick et al., 2014, 2016). Microbial colonization on MPs alerts the environmental risk and threatens human health by increasing the abundance of pathogens (harmful microorganisms causing disease) and vectoring the non-indigenous species into new environments (Ma et al., 2020). Sardi et al. (2014) reviewed the formation of pathogenic biofilms by fungal species and emphasized their role in infection. Considering that many animal and plant pathogens and parasites are fungi, particular interest in the accumulation of fungal pathogens in the plastisphere has increased in the plastic study (Xue et al., 2021). Although the occurrence of pathogenic species in marine plastic debris was reported in the previous studies, there is still a lack of information about the plastic-specific pathogens and the species vectored by plastic particles from terrestrial to marine environments.

Fungi, such as *Aspergillus* and *Fusarium*, were recognized to degrade synthetic plastics (Paço et al., 2017; Zhang et al., 2021). Furthermore, bacteria such as *Pseudomonas* and *Bacillus* strains have the potential to utilize hydrocarbons in plastic fragments contributing to the biodegradation of synthetic particles (Roager and Sonnenschein, 2019; Zettler et al., 2013). Generally, the biodegradation mechanism relies on the hydrolyzation of macromolecules into small molecules by microbial cells as the sole carbon source based on specific enzymatic activities (Sonawane et al., 2022; Sooriyakumar et al., 2022). Given the microorganisms' ability to degrade plastics, biodegradation is a promising strategy for cleaning plastic debris with an environmentally friendly approach. Although an increasing number of studies have revealed plastic-degrading microorganisms, limited studies pointed out the key enzymes involved in biodegradation. Furthermore, members of the plastisphere play a significant role in the behavior of MPs in the ecosystem, as yet data on the microbial functions of MP-associated biofilms in the coastal environment are limited. Therefore, it is crucial to reveal microorganisms including potentially pathogenic taxa in the plastisphere and improve our understanding of microbial function including how microorganisms metabolize the plastics as well as their potential effects on the ecosystem.

The main goal of the current study is to uncover the bacterial and fungal communities on MP from coastal ecosystems. The objectives of this study were to i. reveal the diversity and abundance of bacterial and fungal communities on MPs and environmental compartments (water, sediment, and sand) from two contrasting coastal sites in Japan; ii. uncover hydrocarbon-degrading and potential pathogenic taxa on MPs; iii. explore the enzymes that play a key role in microbial functions of developing biofilm and utilizing synthetic plastics; and iv. investigate the potential influences of microorganisms in the plastisphere on coastal ecosystems. We have monitored the abundance and characteristics of MPs in coastal environments of Japan using analytical and thermogravimetric approaches (Battulga et al., 2023) and the properties of plastic-associated biofilms using isotope tracer techniques (Battulga et al. (2024) submitted). As an important part of monitoring, this study addresses microbial ecology in the plastisphere in two contrasting coastal areas of Japan. The novelty of the current study is to provide critical discussion in terms of pathogens and hydrocarbon-degrading taxa on plastics besides emphasizing the microbial community composition and diversity in the plastisphere.

2. Materials and methods

2.1. Sample collection

MP, surface water, bottom sediment, and coastal sand samples were collected four times from the shores of the Shin and Shinano Rivers during 2021–2022 in Japan (Fig. S1A–B). In previous studies, various sampling strategies have been utilized to collect MPs and investigate colonized communities (Li et al., 2019). In this study, MPs were randomly collected four times from the shores of the Shin (May, September, December, and March) and Shinano (July, October, January, and April) Rivers (Table S1) to avoid the influence of random effects in the coastal environments. Although samples were collected at different months throughout the year, the seasonal effect on the diversity and structure of microbial assemblages was beyond the scope of the current study.

The research sites were selected as representative study locations for typical estuarine and coastal environments. In addition, this study addressed the river mouth area since plastic particles can be trapped due to the bidirectional movement of the surface water. The site at Shin River (catchment area: 30.1 km^2) was located at Tokai village (population density: 999 inhabitants per km^2) in Ibaraki Prefecture facing the Pacific Ocean. The site at Shinano River (catchment area: 11,900 km^2) was located at Niigata city (population density: 1069 inhabitants per km^2) in Niigata Prefecture facing the Sea of Japan. The catchment of the Shinano River is occupied by agricultural and highly urbanized areas with industrial and aquaculture activities, while the Shin River catchment is surrounded by mainly agricultural areas. Given the dissimilarities in outflow and catchment areas of the rivers, the research sites were characterized by their contrasting geographic and hydrological conditions as well as differences in their land-use patterns.

MP samples were collected by visual sorting using a tweezer from the shores of the two rivers. The total number of collected MPs ranged from 476 to 4079 particles (Table S1). Bottom sediment samples (up to 5 cm depth) and coastal high tide line (HTL) and low tide line (LTL) sand samples (within 40 × 40 cm^2 quadrat areas with up to 5 cm depth) were collected using a scoop. At the research sites, we measured the physicochemical parameters (pH, electrical conductivity, and temperature) of surface water using a portable pH/EC meter (HI98129, Hanna Inc., USA) (Table S1). Surface water samples (>1 L) were collected and transported to the laboratory in a cooler box with field-collected MP, sediment, and sand samples. Details of sample preparation were provided in Supplementary Chapter 1.1.

Water samples were filtered with a 0.45-μm pore-sized membrane filter (Merck Millipore, Tullagreen, Ireland), and total organic carbon (TOC) content was measured with a TOC analyzer (TOC-L CPH/CPH, Shimadzu, Japan) (Table S1). The distribution of morphotypes (Fig. S1B) and colors (Fig. S2A–B) of field-collected MPs was recorded. Scanning electron microscopy (JSM-IT100, JEOL, Japan) was used to visualize microbial colonization on MPs (Fig. S2D). In addition, the surface property of MPs was observed using scanning electron microscopy (Fig. S2D). We identified the polymer types of MPs (N = 100,

randomly selected) using a Laser Raman spectrometer (NRS-5100, Jasco, Japan); the most common MP polymers were polyethylene (42.7 %), polypropylene (29.4 %), and polystyrene (18.5 %) MPs (Fig. S2C). The remaining 9.4 % was mainly occupied by polyurethane, polyvinyl, polyethylene terephthalate, polyvinyl chloride, and polyester plastics in this study.

2.2. DNA extraction and sequencing

Microbial DNA was extracted from up to 250 mg samples of MP ($N = 31$), water ($N = 8$), sediment ($N = 8$), and sand ($N = 16$) using DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. For MP samples, we applied particles based on morphotypes (fiber, film, foam, and fragment; Fig. S1C) to DNA extraction to identify differences in microbial community composition among the morphotypes. However, fiber-MP from Shin River (sample from Dec. 2021) was unable to apply DNA extraction due to the relatively small amount collected from the research site. To check whether that extraction was successful, nucleic acid content was determined in the DNA extracts at 230 nm using a NanoDrop Spectrophotometer (ND-1000; Thermo Fisher Scientific, Massachusetts, USA) (Table S2).

We performed a two-step tailed Polymerase chain reaction (PCR) using Thermal Cycler Dice Touch (Takara Bio Inc., Japan). For the first PCR, primer pairs 515F (Parada et al., 2016)/806R (Apprill et al., 2015) and ITS1F_KYO2/ITS2_KYO2 (Toju et al., 2012) with the hanging region were used to amplify prokaryotic 16S rRNA and fungal ITS, respectively. In the second PCR, Illumina sequencing adaptors were added using fusion primers. Reaction mixture composition and thermal cycling conditions of the first and second PCRs are shown in Tables S3 and S4, respectively. High-throughput sequencing of 16S rRNA and ITS genes was conducted using the Illumina MiSeq platform (Hokkaido System Science Co., Ltd., Tokyo, Japan). Detailed experimental steps for PCR and data analysis in sequencing are explained in Supplementary Chapter 1.2. Real-time quantitative PCR was conducted using CFX 96 (Bio-Rad Laboratories, Inc., CA, USA) to quantify the abundance of bacterial and fungal gene copies in the DNA extracts (Supplementary Chapter 1.3). Because the sample number was large, each DNA sample was quantified once each for bacteria and fungi.

Considering the potential biodegradation of synthetic plastics by microorganisms, we selected and examined 15 bacterial and 11 fungal genera that can degrade hydrocarbons based on taxonomic classification (Tables S5 and S6). The relative read counts of the selected hydrocarbon-degrading microbial genera were evaluated. We reviewed the presence of opportunistic bacterial pathogens, reported in previous studies, on MPs (Table S7).

In this study, we employed the PICRUSt2 pipeline (ver. 2.5.1) (Gavin et al., 2020) using the Kyoto Encyclopedia of Gene and Genomes catalog to investigate microbial (especially bacterial) functions of MP-associated biofilms. Functional prediction generated the copy number of enzymes based on the EC number. In total, 2337 enzyme commission (EC) numbers were assigned from the functional prediction. Key enzymes including histidine kinase (EC2.7.13.3), diguanylate cyclase (EC2.7.7.65), aldose 1-epimerase (EC5.1.3.3), l-iditol 2-dehydrogenase (EC1.1.1.14), and cellulose synthase (EC2.4.1.12) were selected, and their read counts in the samples were addressed to explore the metabolic processes, especially those related to biofilm formation on MPs.

Several key enzymes were also selected in this study based on their importance in the biodegradation of hydrocarbons in synthetic plastics according to previous reports (Jacquin et al., 2019; Ji et al., 2023; Zhang et al., 2015). This included protocatechuate 4,5-dioxygenase (EC1.13.11.8), alkane 1-monooxygenase (EC1.14.15.3), haloalkane dehalogenase (EC3.8.1.5), medium-chain acyl-CoA dehydrogenase (EC1.3.8.7), enoyl-CoA hydratase (EC4.2.1.17), 3-hydroxyacyl-CoA dehydrogenase (EC1.1.1.35), and 3-oxoadipyl-CoA thiolase (EC2.3.1.174).

For the fungal community, we predicted fungal ecological guilds using the FUNGuild database (Nguyen et al., 2016). Three main groups of trophic modes (pathotroph, symbiotroph, and symbiotroph) were classified mainly based on the FUNGuild database. We further addressed the fungal pathotroph group to identify harmful taxa (pathogen and parasite) on MPs and the coastal ecosystem.

2.3. Statistical analysis

All statistical analyses were performed using R (ver. 4.3.1) ("R Core Team" 2023). Shannon diversity index (H) was calculated using operational taxonomic units (OTUs) data to evaluate the alpha (α) diversity of bacterial and fungal communities. We used a two-way analysis of variance (ANOVA) to test differences in diversity indices among sample types (MP, water, sediment, and sand) and research sites (Shin and Shinano Rivers). The significant differences in morphotypes (fiber, film, foam, and fragment) of MP particles were also addressed to explore the specific taxa and diversities depending on morphotypes using two-way ANOVA. One-way ANOVA followed by Tukey's honestly significant difference (HSD) was additionally conducted to examine significant differences based on sample type and research site.

Nonmetric multidimensional scaling (NMDS) was performed to visualize the beta (β)-diversity of microbial communities based on the Bray–Curtis dissimilarity matrix. Differences in microbial community structure and dispersion among sample types and research sites were analyzed using two-way permutational ANOVA [PERMANOVA] using adonis2 function in the "Vegan" package (ver. 2.6–4) (Oksanen et al., 2022) and permutational multivariate analysis of dispersion (PERMDISP). We additionally conducted multiple comparison for the microbial community structures using the permanova_pairwise function with 999 permutations in package ecole (Robert, 2019).

2.4. Data availability

The sequence data was uploaded to the DDBJ Sequence Read Archive under the accession number PRJDB17697 (BioProject).

3. Results

3.1. Bacterial and fungal diversities

In this study, we performed amplicon sequencing to identify the microbial communities on MPs and in surrounding environmental media (water, sediment, and sand). The α and β diversity were computed based on detected OTUs to identify variations in the diversity of bacterial and fungal communities. The α-diversity which expresses the richness of communities on a local scale is determined using the observed OTUs number and the Shannon diversity (H) index. The α-diversity indices observed in the samples from Shin and Shinano Rivers are shown in Fig. 1. Although the interaction effect between sample types and research sites was significant on observed values of OTUs based on two-way ANOVA (Table S8), one-way ANOVA revealed clear differences among sample types at both research sites (Shin River: $F = 14.5$, $p < 0.001$ and Shinano River: $F = 5.6$, $p < 0.01$). According to the statistical multiple comparison test, bacterial communities in MPs had significantly lower values of α-diversity indices than in sediment and sand samples (Fig. 1). For the fungal community, Shannon diversity indices were statistically distinct depending on the research site, while the effects of sample types on the index were marginally significant based on two-way ANOVA (Table S8).

For β-diversity (overall community diversity between different environments), NMDS demonstrated differences in microbial diversity between samples from Shin and Shinano Rivers (Fig. 2). Although there were no significant differences between the research sites, bacterial (one-way PERMANOVA: $F = 5.1$, $p < 0.001$ for Shin River and $F = 3.4$, $p < 0.001$ for Shinano River) and fungal (one-way PERMANOVA; $F = 1.8$, $p < 0.001$ for Shin River and $F = 1.4$, $p < 0.05$ for Shinano River) communities differed between sample types (MPs, water, sand, and

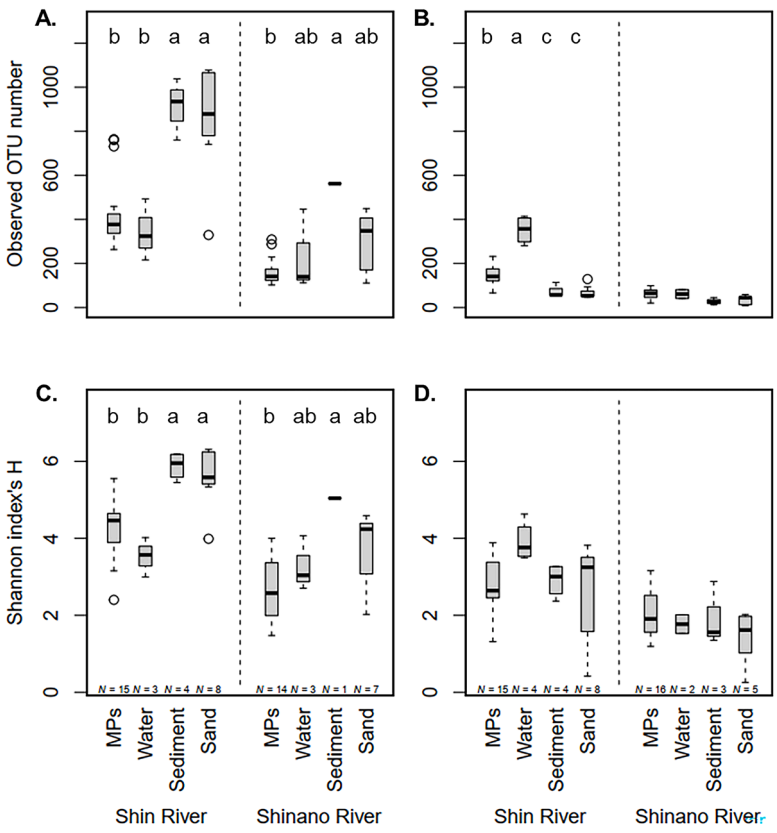

Fig. 1. Alpha-diversity of microbial community composition based on observed operational taxonomic units (OTUs) number (A and B) and Shannon diversity index (C and D) of bacterial (A and C) and fungal (B and D) communities in samples from Shin and Shinano Rivers. Different letters represent significant differences among sample types (Tukey's HSD test; $p < 0.05$).

sediment). Based on multiple comparison analysis, bacterial communities on MPs in Shin River were significantly different from other sample types (Bonferroni-adjusted $p < 0.05$; Fig. 2A). In Shinano River, bacterial communities on MPs were distinct from that on water and sand (Bonferroni-adjusted $p < 0.05$), while the differences between MPs and sediments were unclear (Bonferroni-adjusted $p = 0.300$; Fig. 2B). The fungal community on MPs in Shin River were significantly different from other sample types (Bonferroni-adjusted $p < 0.05$; Fig; 2C), while that in Shinano River did not significantly differ among sample types (Bonferroni-adjusted $p > 0.05$; Fig. 2D). The PERMDISP test showed significant dispersion differences in bacterial ($F = 3.9$, $p < 0.05$ for Shin River and $F = 5.6$, $p < 0.01$ for Shinano River; Fig. 2A–B) and fungal ($F = 3.5$, $p < 0.05$ for Shin River and $F = 6.1$, $p < 0.01$ for Shinano River; Fig. 2C–D) diversities. Regarding the PERMDISP results, fungi seem to have less variation in MPs than other samples demonstrating the high number of fungal OTUs that are specific to MPs.

We evaluated the similarity of bacterial and fungal communities using Venn diagrams (Fig. S3A–D) to identify shared communities among the sample types. Plastic-specific bacterial assemblages (approximately 60 % of the total OTU values) were predominant in MPs, whereas only 5.2 % were occupied by common species. Similarity analysis exclusively addressed communities in MPs based on morphotypes (Fig. 3). MPs from Shin River were characterized by the dominance of MP-specific species, species belonging to MP–sediment–sand, and common species in all sample types (Fig. 3A and C). However, samples from Shinano River represented the dominant contribution of species dispersed in MP–sand and MP–water–sand. Our results demonstrated that a higher OTU value in MP-specific communities did not always represent a higher proportion of the relative read count. Phylogenetic tree analysis of the bacterial communities observed in MP samples from rivers (Fig. S4A–B) was performed to obtain a comprehensive picture of the colonizing communities on MPs in the environment. The results showed that MP-specific bacterial assemblages were phylogenetically diverse, especially in Shin River. However, the phylogenetic tree showed phylogenetically less diverse MP-specific communities in Shinano River.

Venn diagram revealed that approximately 70 % of the total OTU values were assigned to plastic-specific fungal communities on MPs (Fig. S3C–D). Therefore, 2.7 % were identified as common species observed in all sample types. Based on site specificity, the interaction between MPs and water (shared species: 15.6 %) seemed peculiar in Shin River. Given the 9.6 % shared species between MPs and sand, their interaction is more common than interactions between MPs and other environmental media (water and sediment) in Shinano River. Consistently, similarity analysis revealed clear differences in fungal communities (Fig. 3B and D). In Shin River, species in MPs were dominated by common fungal species that were also observed in water, sediment, and sand samples (Fig. 3D). However, MPs from Shinano River were characterized by the dominance of shared communities with sediment and sand and MP-specific communities (Fig. 3D).

3.2. *Microbial community composition and abundance*

The most abundant bacterial and fungal phyla in the analyzed samples from Shin and Shinano Rivers are shown in Fig. 4. Based on mean relative read counts, Proteobacteria (55.8 % ± 2.1 %; average percentage ± standard error), Actinobacteriota (15.8 % ± 2.0 %), Bacteroidota (9.5 % ± 1.0 %), Firmicutes (7.7 % ± 1.4 %), and Acidobacteriota (2.1 % ± 0.4 %) were the predominant bacterial phyla in MP, water, sediment, and sand samples (Fig. 4A and B). Ascomycota (53.8 % ± 4.3 %), Basidiomycota (16.2 % ± 2.7 %), and Chytridiomycota (4.7 % ± 2.0 %) were the most commonly detected fungal

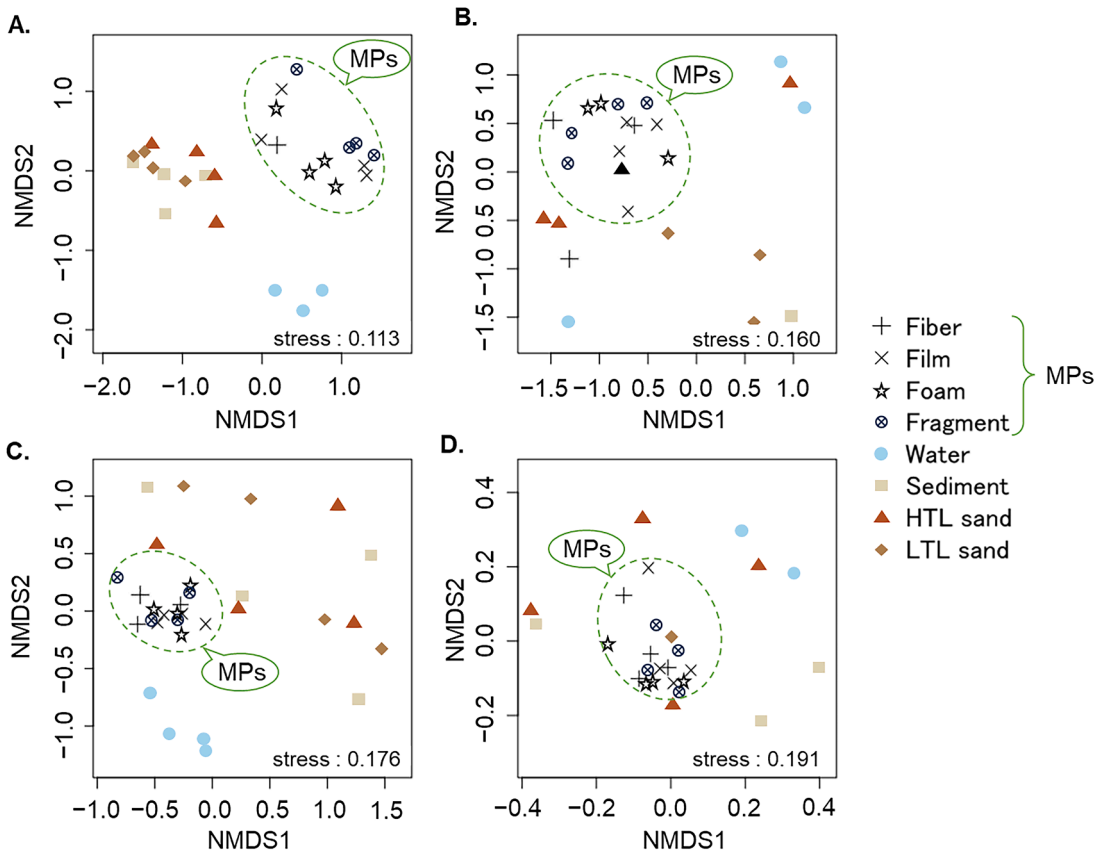

Fig. 2. Nonmetric multidimensional scaling (NMDS) of high-throughput sequencing data of bacterial (A and B) and fungal (C and D) communities detected in the samples (MP, water, sediment, and sand) from Shin River (A and C) and Shinano River (B and D). MP samples are provided with their morphotypes (fiber, film, foam, and fragment). The sand was obtained with high tide line (HTL) and low tide line (LTL) samples. Dashed ellipses highlight the MP samples.

phyla in the analyzed samples, in addition to unidentified phyla (24.5 % ± 4.0 %) (Fig. 4C and D). Interestingly, relatively low fungal read counts (8.8 % ± 2.3 %) were assigned to unidentified fungal phyla in MP samples compared with those in water (64.6 % ± 13.8 %), sediment (46.0 % ± 12.2 %), and sand (31.8 % ± 9.0 %) samples. Given the detected bacterial and fungal phyla in the samples, niche-specific functions may affect the properties of MPs and the coastal ecosystem. For example, the species belonging to the Proteobacteria and Ascomycota have various metabolic functions and ecological roles such as driving the carbon and nitrogen cycling in the environments (Challacombe et al., 2019; Zhou et al., 2020). In addition, some species belonging to the Ascomycota and Basidiomycota fungi can degrade synthetic plastics (Zhou et al., 2022). Furthermore, qPCR results demonstrated variable abundance of bacterial and fungal communities on MP, water, sediment, and sand samples (Table 1). Interestingly, MPs, especially foam and fragments, from either site represented convenient surfaces for bacterial and fungal colonization providing the highest quantities of gene copies. However, there were no significant differences among the bacterial and fungal community compositions depending on morphotypes of MPs.

3.3. Hydrocarbon-degrading microorganisms

Given the capability of the bacterial and fungal communities to degrade and metabolize synthetic plastic polymers through specific functions, we selected 15 bacterial and 11 fungal genera and evaluated their relative abundance on MPs and environmental compartments (Tables S5 and S6). The selected hydrocarbon-degrading bacterial genera accounted for 10.9 % (mean value; Fig. 5A and B) of the total read counts of prokaryotes on MPs. Differences in the relative read counts of selected bacterial genera can be observed from the taxonomy (Fig. S5A–B). Polyethylene, polystyrene, polyurethane, polyethylene terephthalate, and polyvinyl chloride degrading *Bacillus* (Sooriyakumar et al., 2022), *Pseudomonas* (Mughini-gras et al., 2021), and *Streptomyces* (Huang et al., 2019) species were detected in this study with different contributions from bacterial genera. Some species such as *Nakamurella, Paenibacillus, Rhodococcus*, and *Streptomyces* were dominantly identified in MPs, especially samples from Shin River, while higher contributions of *Exiguobacterium* and *Paracoccus* were observed in MPs from Shinano River (Fig. 5A and B and Tables S5).

Hydrocarbon-degrading fungal genera on MPs accounted for 18.8 % (mean value; Fig. 5C and D) of the total fungal read count. *Acremonium* and *Cladosporium* have reported their capability to degrade polyethylene (Ekanayaka et al., 2022; Gao et al., 2022), which explains their larger proportion in relative read counts of fungal genera (Fig. 5C and D). The predominant occurrence of *Cladosporium* was detected in samples from Shin River rather than samples from Shinano River. Further attention should be directed to *Exophiala* and *Plectosphaerella* based on their dominant contribution and potential to degrade polyurethane (Owen et al., 1996; Srikanth et al., 2022). Although *Exophiala* was detected with higher relative read counts in MPs from Shinano River, *Plectosphaerella* showed peculiar read counts in not only MPs but also water, sediment, and sand samples from Shin River (Tables S6). Differences in the relative abundance of the hydrocarbon-degrading fungal genera depending on the research site were evident in the *Aspergillus, Fusarium, Plectosphaerella*, and *Trichoderma* suggesting influences from site-specificity on fungal taxonomy.

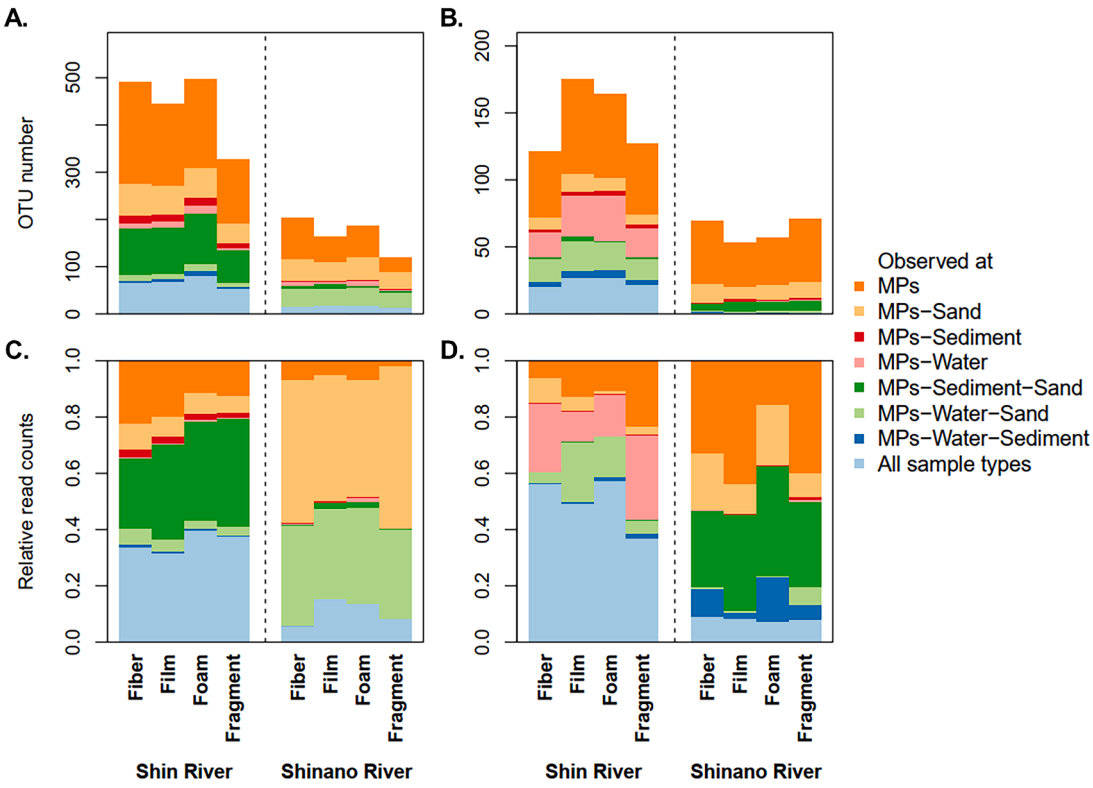

Fig. 3. Shared operational taxonomic units (OTUs) of bacterial (A) and fungal (B) communities from MPs based on differences in morphotypes. Contributions of shared OTUs were identified by examining relative read counts of bacteria (C) and fungi (D). The color composition indicates MP-specific taxa, taxa shared between MPs and environmental compartments (water, sediment, and sand), and taxa observed in all sample types.

3.4. Microbial functions

Considering that microbial communities in biofilms perform crucial functions through their functional genes, we examined several essential enzymes encoded by these genes (especially in bacteria) in this study (Table 2A). The enzymes EC2.7.13.3: histidine kinase, EC2.7.7.65: diguanylate cyclase, EC5.1.3.3: aldose 1-epimerase, EC1.1.1.14: l-iditol 2-dehydrogenase, and EC2.4.1.12: cellulose synthase (Table 2A), classified according to EC number, play important roles in providing energy, motility, and robustness in biofilm formation (Sambanthamoorthy et al., 2012; Wang et al., 2023; Wei et al., 2023). The histidine kinase was detected with relatively high abundances in all samples, especially in MPs (Table 2A). As a result, two-way ANOVA revealed significant differences among sample types ($F = 2.9, p < 0.05$). We detected 403–1328 and 196–1537 counts of diguanylate cyclase in MPs from Shin and Shinano Rivers, respectively. Higher counts of aldose 1-epimerase, l-iditol 2-dehydrogenase, and cellulose synthase were observed for MPs from Shin River than Shinano River (Table 2A). To highlight the characteristics of biofilm formation on MPs based on morphotypes, two-way ANOVA was conducted for MP samples. The results showed no differences in the selected enzymes depending on the morphotype of MP particles. However, statistically significant differences were observed in aldose 1-epimerase ($F = 9.4, p < 0.01$), l-iditol 2-dehydrogenase ($F = 16.8, p < 0.001$), and cellulose synthase ($F = 7.0, p < 0.05$) between the research sites.

Given the occurrence of hydrocarbon-degrading bacterial and fungal genera in the samples, we examined enzymes that have the potential to mineralize plastic polymers (Table 2B). In samples of MP, water, sediment, and sand, we detected 171 ± 30, 1546 ± 482, 327 ± 98, and 341 ± 94 counts (average count ± standard error) of protocatechuate 4,5-dioxygenase (EC1.13.11.8), respectively. The enzyme is important for aromatic hydrocarbon catabolism in the bacterial degradation pathway and reported to degrade polyethylene terephthalate (Wright et al., 2021). Although long-chain alkane monooxygenase (EC1.14.14.28) and alkane 1-monooxygenase (EC1.14.15.3) were reported to have capabilities to degrade the polyethylene plastics (Jacquin et al., 2019; Ji et al., 2023), we only detected alkane 1-monooxygenase (772 ± 102 in Shin River and 565 ± 89 in Shinano River) in MPs. Medium-chain acyl-CoA dehydrogenase (EC1.3.8.7), which is one of the key enzymes in the oxidation of hydrocarbons, was detected in MPs with average counts of 6339 ± 681 and 2282 ± 358 from Shin and Shinano Rivers, respectively. In MPs, enoyl-CoA hydratase (EC4.2.1.17), 3-hydroxyacyl-CoA dehydrogenase (EC1.1.1.35), and 3-oxoadipyl-CoA thiolase (EC2.3.1.174) were detected with average read counts of 7301 ± 442, 4016 ± 139, and 84 ± 10, respectively (Table 2B). The enzymes were reported for their significant role in the degradation mechanisms of polystyrene plastics. Haloalkane dehalogenase (EC3.8.1.5), showed significant differences between sample types ($F = 9.1, p < 0.001$) and research sites ($F = 6.8, p < 0.05$) in two-way ANOVA. Given that various halogenated alkanes and aromatic compounds can be broken down by haloalkane dehalogenase originates from bacteria and eukaryotes (Ji et al., 2023), occurrence in haloalkane dehalogenase may encourage the biodegradation of MPs in the environment.

3.5. Pathogens on MPs

In terms of fungal ecology, we classified the fungal communities based on trophic modes (pathotroph, saprotroph, and symbiotroph) (Fig. 6A). Although a large proportion of communities was unidentified (44.2 ± 4.0 %), saprotrophs (fungi that acquire nutrients from dead organic matter through extracellular digestion (Xue et al., 2021)) were dominantly detected with 18.8 ± 3.0 % (mean value ± standard error). Given the diverse fungal communities with their multiple ecological guilds, we only examined pathotrophs to identify harmful taxa on MPs. Moreover, pathotrophic fungi could influence the structural characteristics of MPs through their potential metabolic function to utilize the

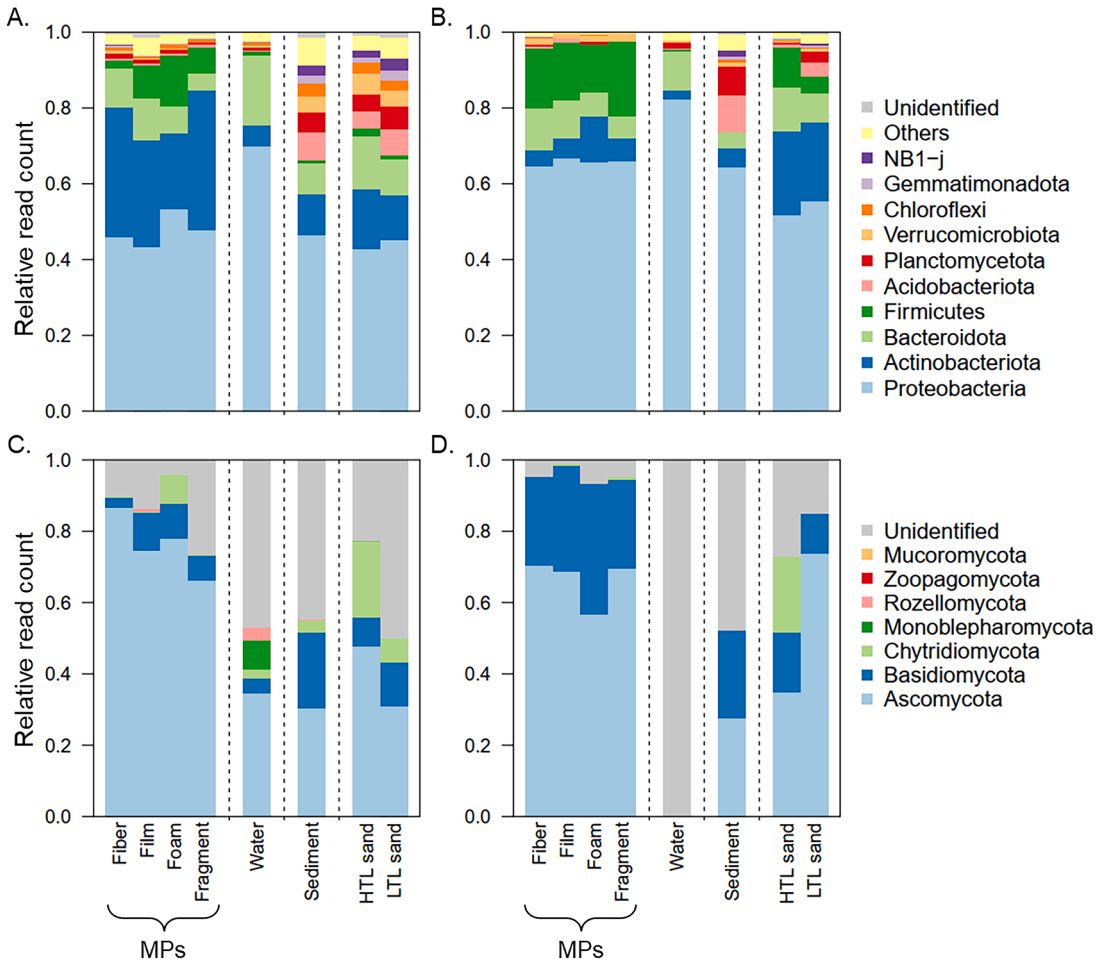

Fig. 4. Relative mean abundance of the most abundant bacterial (A and B) and fungal (C and D) phyla based on read counts of sequencing data from MP, water, sediment, and high tide line (HTL) sand and low tide line (LTL) sand samples collected from Shin River (A and C) and Shinano River (B and D).

Table 1
Absolute abundance (average ± standard error) of bacterial and fungal communities in the samples.

	Shin River	Shinano River
Bacteria		
Water ($\times 10^8$ copy L^{-1})	10.9 ± 3.7	1.6 ± 1.0
Sediment ($\times 10^8$ copy g^{-1} (dw))	0.11 ± 0.08	0.02 ± 0.01
Sand ($\times 10^8$ copy g^{-1} (dw))	0.2 ± 0.1	1.5 ± 1.5
MPs ($\times 10^8$ copy g^{-1} (dw))	16.6 ± 13.9	13.2 ± 12.7
Fiber	373.1 ± 352.8	67.5 ± 40.1
Film	171.2 ± 150.4	26.3 ± 9.1
Foam	5709 ± 5108	80.6 ± 36.6
Fragment	45.8 ± 37.2	5119 ± 5104
Fungi		
Water ($\times 10^3$ copy L^{-1})	1.4 ± 0.7	0.7 ± 0.5
Sediment ($\times 10^3$ copy g^{-1} (dw))	0.02 ± 0.02	0.4 ± 0.3
Sand ($\times 10^3$ copy g^{-1} (dw))	0.07 ± 0.04	0.4 ± 0.4
MPs ($\times 10^3$ copy g^{-1} (dw))	100 ± 61	19.6 ± 14.9
Fiber	30.1 ± 28.3	5.4 ± 3.4
Film	10.1 ± 6.9	6.9 ± 3.9
Foam	338 ± 194	65.2 ± 59.2
Fragment	4.3 ± 1.7	0.9 ± 0.3

hydrocarbons in aged plastics (Ekanayaka et al., 2022; Srikanth et al., 2022).

In the pathotroph group, animal pathogens and fungal parasites were dominantly occupied in the samples based on the mean relative read counts of fungal taxonomy (Fig. 6B and C). Interestingly, in Shinano River, relative mean read counts of animal pathogens and fungal parasites were higher in MPs than in surrounding environmental media (water, sediment, and sand) (Fig. 6C). In contrast, distinctive sinks for fungal parasites and animal pathogens in Shin River were observed in sediment and high tide line (HTL) sand samples, respectively (Fig. 6B). In terms of fungal genera, our results demonstrate the higher enrichment of the fungal parasite *Cystobasidium* (0.44 % in Shin River and 5.24 % in Shinano River) in MPs. Although only two fungal genera (*Cystobasidium* and *Purpureocillium*) were detected as fungal parasites, a higher prevalence on MPs from Shinano River was observed than Shin River. Of the 49 plant pathogenic genera observed from the FUNGuild database, several fungal genera including *Devriesia* (0.11 %), *Protomyces* (1.20 %), *Strelitziana* (0.58 %), and *Triodiomyces* (0.22 %) occupied relatively higher read counts in MP samples from Shinano River. However, *Devriesia* (1.16 % in MPs), *Diatrype* (1.93 % in HTL sand), *Lasiodiplodia* (1.34 % in HTL sand), *Septoria* (2.78 % in water), and *Strelitziana* (2.44 % in HTL sand) were peculiar based on predominant read counts in the Shin River.

Furthermore, we selected 12 pathogenic bacterial genera and observed their abundances among the analyzed samples based on mean relative read counts (Table S7). Although *Vibrio* is one of the most common pathogens on plastic debris in marine and coastal environments (Bhagwat et al., 2021), almost all the MP samples (except MP-film in Shinano River) were not colonized by *Vibrio*. Several selected bacterial pathogens were dominantly observed in the surface water (i.e., *Acinetobacter, Bacteroides, Shewanella*), and sand (i.e., *Aeromonas, Vibrio*) samples. However, the mean relative read counts of *Bacillus* (0.58 %),

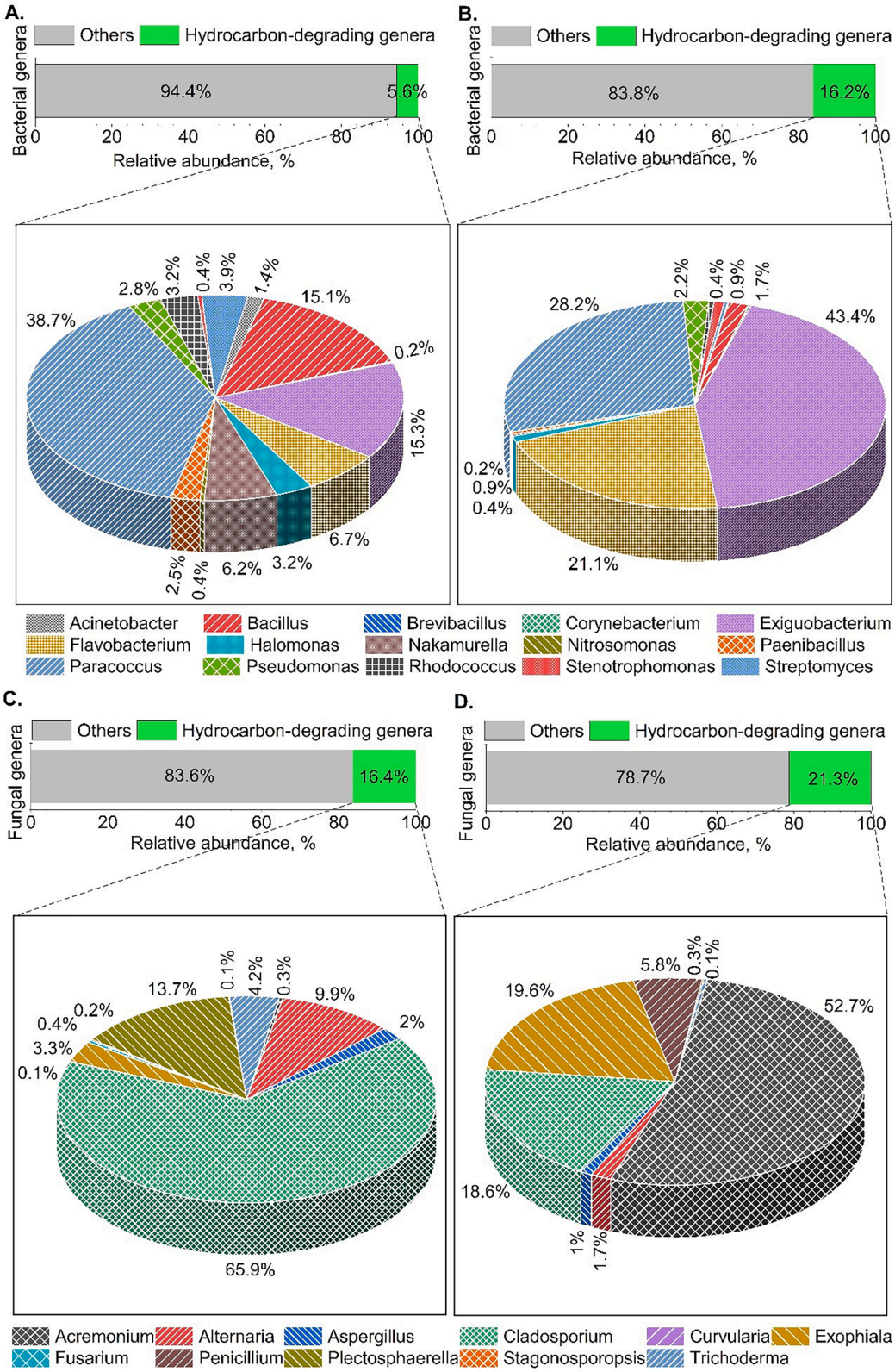

Fig. 5. Relative abundance (%) of selected hydrocarbon-degrading bacterial (A–B) and fungal (C–D) genera in MPs from Shin River (A and C) and Shinano River (B and D) based on detected read counts.

Mycobacterium (0.41 %), *Psychrobacter* (23.7 %), and *Stenotrophomonas* (0.07 %) were relatively higher in MPs than that in the surrounding environmental compartments. We detected *Pseudomonas*, which is common pathogenic taxa in the aquatic environment along with their potential to degrade hydrocarbons, in MPs (0.15 % in Shin River and 0.35 % in Shinano River). However, higher read counts of *Pseudomonas* were accounted in water and sand samples from Shin and Shinano Rivers, respectively.

Table 2
Average predicted counts of enzymes with standard error that are specific to biofilm formation (A) and hydrocarbon-degrading (B) genes in the detected samples obtained from Shin River and Shinano River. Key enzyme commission (EC) numbers are provided for the selected enzymes (EC2.7.13.3: histidine kinase, EC2.7.7.65: diguanylate cyclase, EC5.1.3.3: aldose 1-epimerase, EC1.1.1.14: l-iditol 2-dehydrogenase, EC2.4.1.12: cellulose synthase, EC1.13.11.8: protocatechuate 4,5-dioxygenase, EC1.14.15.3: alkane 1-monooxygenase, EC3.8.1.5: haloalkane dehalogenase, EC1.3.8.7: medium-chain acyl-CoA dehydrogenase, EC4.2.1.17: enoyl-CoA hydratase, EC1.1.1.35: 3-hydroxyacyl-CoA dehydrogenase, and EC2.3.1.174: 3-oxoadipyl-CoA thiolase) based on predicted microbial metabolic function.

EC	Shin River				Shinano River			
	MPs	Water	Sediment	Sand	MPs	Water	Sediment[†]	Sand
A. Enzymes that specific to biofilm formation genes								
EC2.7.13.3	22,673 ± 465	21,168 ± 1012	21,065 ± 373	20,554 ± 522	23,055 ± 830	19,726 ± 2193	21,535	21,898 ± 1054
EC2.7.7.65	23,055 ± 69	385 ± 18	548 ± 134	601 ± 75	964 ± 121	681 ± 72	398	816 ± 134
EC5.1.3.3	2331 ± 222	2043 ± 451	1236 ± 316	1583 ± 270	1439 ± 176	2005 ± 348	500	2425 ± 537
EC1.1.1.14	957 ± 104	244 ± 62	849 ± 64	886 ± 70	425 ± 70	422 ± 232	558	712± 215
EC2.4.1.12	771 ± 87	1026 ± 128	266 ± 65	257 ± 44	438 ± 79	301 ± 268	44	571 ± 137
B. Enzymes that specific to hydrocarbon degrader genes								
EC1.13.11.8	271 ± 40	2470 ± 254	404 ± 77	464 ± 155	63 ± 18	599 ± 473	17	201 ± 80
EC1.14.15.3	772 ± 102	250 ± 64	410 ± 168	555 ± 113	565 ± 89	1263 ± 111	655	738 ± 141
EC3.8.1.5	672 ± 108	169 ± 30	426 ± 64	457 ± 50	1220 ± 163	221 ± 24	294	436 ± 88
EC1.3.8.7	6338 ± 681	7154 ± 969	3828 ± 406	4225 ± 269	2282 ± 358	4299 ± 1266	2300	4621 ± 964
EC4.2.1.17	8929 ± 570	6820 ± 479	6358 ± 344	7128 ± 242	5557 ± 214	5648 ± 1016	5149	7434 ± 907
EC1.1.1.35	3563 ± 113	4607 ± 155	3047 ± 279	3249 ± 244	4501 ± 191	4344 ± 255	2853	3497 ± 143
EC2.3.1.174	66 ± 11	46 ± 11	42 ± 16	78 ± 16	103 ± 17	39 ± 25	5	56 ± 19

[†] Replicate sample was removed after rarefaction.

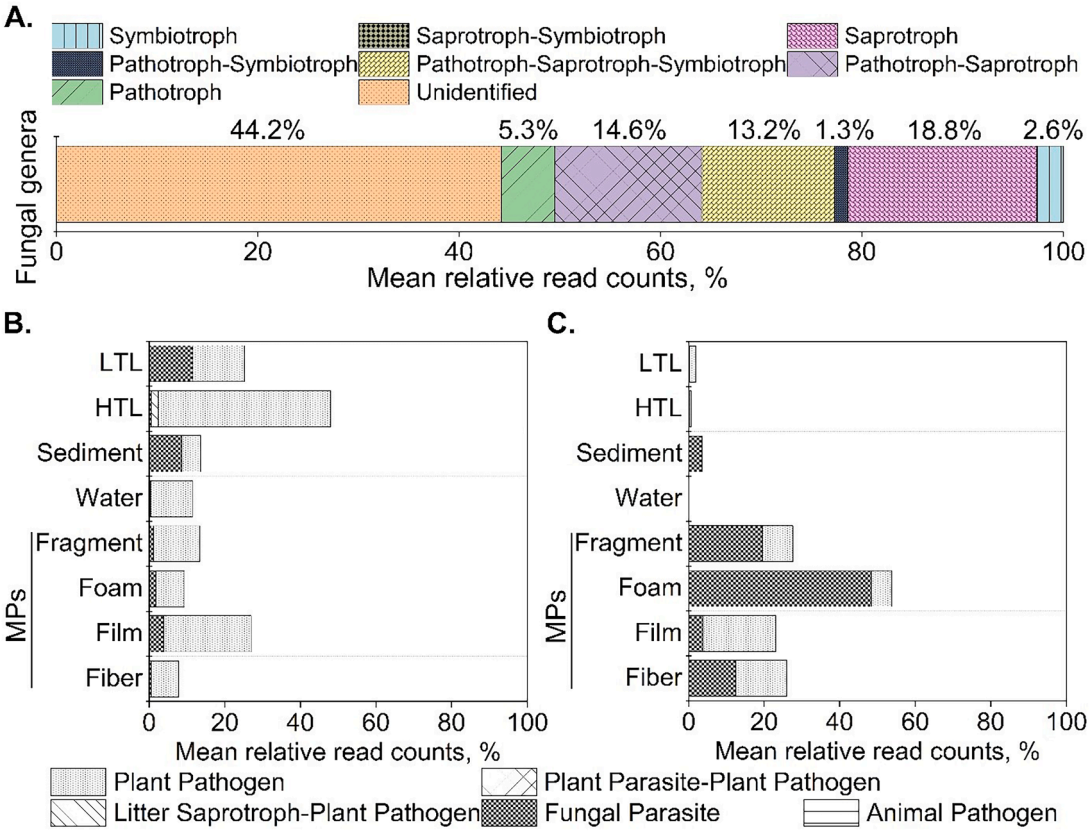

Fig. 6. Total trophic modes of fungal communities in the samples based on mean relative read counts (A). The sole pathotroph is shown with its contribution of fungal ecological guilds in Shin River (B) and Shinano River (C).

4. Discussion

Our results demonstrate significantly distinct bacterial and fungal communities on MPs compared with those in the ambient environment based on diversity indices (observed OTU number and Shannon index) and NMDS (Figs. 1 and 2). The differentiation of microbial taxa depending on plastic debris of the coastal systems was explored from the shared taxa among samples (Fig. 3) and community composition (Fig. 4). Given the large variety of MPs in the selected research sites (Figs. S1 and S2), MPs can be colonized by microbial assemblages based on size, shape, and surface properties (extended surface area due to weathering and/or aging in the environment) (Reisser et al., 2014). Although our results reveal a lower diversity of microbial communities on MPs, considering differences in diversity indices among the samples (Fig. 1 and Table S8), relatively higher 16S rRNA and ITS gene quantities were observed in MPs especially foams and fragments than in water, sediment, and sand samples (Table 1). This may be a result of the degraded (aged) and hydrophobic surfaces of MPs (Fig. S2D), which

provide a favorable surface for microbial colonization. However, no significant differences were observed in microbial colonization and biofilm formation depending on the morphotypes of MPs. Most MPs have low density (~1 g cm^{-3}) (Song et al., 2017) with relatively rough surfaces that provide shelter to microbial cells and allow them to interact with air and sunlight while floating in rivers and coastal oceans. The availability of the required biotic factors encourages the metabolic activities of the microbes on MPs resulting in active cellular processes via enzymatic reactions of the functional genes to grow and divide. This might be the reason for the higher quantities of bacterial and fungal colonies in MPs than in the ambient environment (Table 1).

Microbial community composition and diversity can be affected by physicochemical parameters (pH, temperature, and electrical conductivity) and nutrient availability in the ambient environment (Ge et al., 2021; Mughini-gras et al., 2021), which encourage biofilm formation for a stable habitat on MPs (Yan et al., 2021). Therefore, microbial survival, metabolic processes, and ecological functions can be differentiated depending on site specificity. This is evidenced by the observed differences in the detected bacterial and fungal communities among the sample types and research sites (Fig. 4). Moreover, higher occurrence of hydrocarbon-degrading and pathogenic communities was detected on MPs from Shinano River than on MPs from Shin River (Figs. 5 and 6). Since higher salinity and lower total carbon (TOC) contents were recorded in surface water from Shinano River than Shin River (Table S1), the environmental factors may influence the composition and diversity of the microbial taxa differentiating their metabolic activities. In addition, bacterial and fungal taxa are likely to form biofilms on MPs to survive and adapt to any environment. The adaptation and survival strategy of microorganisms could be driving their habitation in the plastisphere. The evolutionary patterns of bacterial communities in the two contrasting research sites (Fig. S4) provide evidence for the adaptation mechanisms of the communities on MPs. Phylogenetic tree analysis showed that although cyanobacteria and diatoms were present early in the succession of biofilms on MPs, Proteobacteria are primary colonizers, revealing their resistance to changing environmental conditions, especially in the mixing zone of fresh and salt waters in the coastal area. Furthermore, histidine kinase promotes microbial response to environmental stress (Table 2A) through biofilm formation and signal transduction. Signal transduction, which is related to quorum sensing, is important for antibiotic resistance, plasmid transfer, motility, etc., and promotes cooperative adaptation to environmental change (Sonawane et al., 2022). On the other hand, the formation of a conditioning film or "eco-corona" on MPs through instant interaction with dissolved organic matter (Rummel et al., 2017), before microbial colonization, is notable. Organic nutrients in the conditioning film accompanied by weathering (aging) of MP surfaces, immediately attract a variety of microorganisms to the MPs from the surrounding environment. This suggests that the colonization of microbial communities is not only dependent on the MP surface but also on the conditioning film on MPs (Oberbeckmann and Labrenz, 2020). Thus, the nutrient content in the ambient environment is possibly a limiting factor for the attachment of microbial communities and biofilm formation on MPs.

Microbial colonization offers a variety of metabolic functions. The main components of the plastisphere included Gammaproteobacteria (33.8 % ± 4.3 %) and Alphaproteobacteria (22.6 % ± 2.6 %) for bacterial communities and Dothideomycetes (47.3 % ± 4.3 %) and Sordariomycetes (12.7 % ± 2.9 %) for fungal communities; their relative abundance differed from that of environmental media (Fig. S6). To explore the dynamics and functions of microbial communities in the plastisphere, we examined biofilm formation by microorganisms (Table 2A). Although examination of biofilm formation and development on MPs was beyond the scope of this study, the prediction of metabolic pathways enabled us to understand microbial processing in MP-associated biofilms. Generally, the development and impairment of the biofilm are directly related to key enzymes of functional genes. Our results suggest the development of stable biofilms on MPs based on a variety of occurrences of the selected key enzymes on MPs (Table 2A). However, the presence of key enzymes in water, sediment, and sand samples may reveal the potential of free-living communities to form biofilms and/or detached biofilms from any hard (e.g., MPs, stone) surface in the ambient environment. Although it is challenging to clarify the formation and development of biofilms in the analyzed samples, the statistically significant differences between sample types reveal the presence of distinct enzymatic processes in the microbes on MPs compared with free-living microbes. Considering the degraded MPs in the coastal environment, microbial metabolism in biofilm on aged surfaces may induce the degradation of MPs. Furthermore, previous studies mentioned the tendency of polymer degradation where the carbon source is represented, via microbial communities (Miao et al., 2020). Therefore, synthetic MP substrate may encourage specific enzymatic activities by microbial taxa.

We examined hydrocarbon-degrading communities and their enzymatic activities to explore microbial functions of biodegradation of synthetic plastic (Fig. 5 and Table 2B). For example, the observed genera *Bacillus, Exiguobacterium, and Pseudomonas* reported their potential to degrade synthetic plastics and produce dioxygenase, dehydrogenase, and other enzymes for the biodegradation process (Bai et al., 2017; Bhatt et al., 2021; Sooriyakumar et al., 2022). Considering that the chemical structure of MPs experiences stress from solar radiation, temperature change, salinity fluctuation, and wet and dry cycles in a coastal system, degraded plastic particles (Fig. S2D) might be easier to utilize by hydrocarbon-degrading taxa. After polymers are broken down into smaller molecules, oxidation (also known as beta-oxidation) converts oxidized molecules to acetyl/succinyl coenzyme A (CoA) through several enzymatic reactions. Then, the acetyl/succinyl CoA enters the tricarboxylic acid (also known as Krebs) cycle, resulting in the formation of carbon dioxide and water. The cycle is a part of cellular respiration and plays a central role in producing energy through a series of enzymatic reactions. Based on the occurrence of hydrocarbon-degrading microbial genera (Fig. 5) and the relative read counts of the selected enzymes (Table 5B), the microbial degradation of MPs in coastal ecosystems can be predicted. In addition, the developed biofilm provides a beneficial microenvironment for microbes, allowing genetic exchange and metabolic cooperation in the plastisphere (Galgani et al., 2019). Hence, cooperated metabolic functions by microbial interactions potentially promote the degradation of hydrocarbons (Wilkes and Aristilde, 2017). For example, Gu (2003) reported the enhanced degradation of polyethylene glycol by *Flavobacterium* when co-cultured with *Pseudomonas*. Although the selected hydrocarbon-degrading bacterial and fungal taxa accounted for >10 % of the total read counts of microbial communities, diverse functions and functional genes of the selected microbial taxa should be noted in addition to hydrocarbon-degrading genes. On the other hand, some genera such as *Acinetobacter* and *Flavobacterium* presented relatively higher relative read counts in water samples than MPs (Table S5) revealing the other potential sources of hydrocarbons in surface water via various land use patterns in the coastal areas of both regions. Although our results suggest the biodegradation of synthetic plastics in coastal ecosystems, the degradability and degradation rate of plastics must be investigated further to provide accurate information on the biodegradation of plastics in the environment. The research findings would be beneficial for the isolation of plastic-degrading microbial strains and for developing carbon recycling technology utilizing microorganisms.

Furthermore, we examined the presence of pathogenic bacteria and fungi on MPs (Fig. 6 and Table S7) to identify the harmful effects of microbes in the plastisphere. In addition to the alarming number of MPs in the environment, harmful taxa, including potential pathogenic organisms, that cooperate with MPs, can cause serious concern in the aquatic ecosystem. Predominant contributions of animal pathogens and fungal parasites into the plastisphere were observed in this study (Fig. 6), which is in agreement with previous reports (Gkoutselis et al., 2021; Yu et al., 2021). Moreover, pathogenic communities have the

potential to form biofilms, resulting in tolerance to antibiotics, leading to infections in humans and aquatic wildlife. Although our results provide evidence that invasive species, including pathogens, such as *Bacillus, Pseudomonas*, and *Stenotrophomonas*, hitchhike on MPs in coastal ecosystems, there is a need to assess their impact on aquatic wildlife and humans through the food chain.

5. Conclusions

This study demonstrated the bacterial and fungal community composition and diversity in the plastisphere and surrounding environmental media to uncover the microbial ecology and its effect on the coastal environment. Aged property of MPs in the coastal environment encourages bacterial and fungal colonization and biofilm formation on the surface of plastic particles allowing various metabolic functions by microbes. The microbial community composition in MPs can vary depending on the site, surrounding environmental conditions, and probably the history and pathways of MP transport, which result in the distinct ecological functions in coastal ecosystems. Statistical analyses support the site-specificity of microbial dynamics in the plastisphere. Therefore, the characteristics of MPs, nutrient availability in the ambient environment, etc., can be considerations for microbial colonization and biofilm formation on MPs. Conversely, microbial metabolic function and biofilm development can modify the properties of MPs, and thus, the impacts of plastic debris on ecosystems. The occurrence of hydrocarbon-degrading taxa and specific enzymes suggests the potential biodegradation of MPs in the coastal environment. The enrichment of pathogens in MPs compared to surrounding environmental media (water, sediment, and sand) provides useful implications regarding the ecotoxicological effect of MPs on the ecosystem. Patterns in microbiome composition and selective enrichment of specific taxa on MPs observed in this study can probably be found in other coastal environments, suggesting that plastic debris can alter the global ecosystem. Given the variety of metabolic functions and enzymatic reactions of microbial communities, investigation on plastisphere merits further consideration.

CRediT authorship contribution statement

Batdulam Battulga: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Masataka Nakayama:** Visualization, Methodology, Formal analysis, Data curation. **Shunsuke Matsuoka:** Investigation, Formal analysis. **Toshiaki Kondo:** Investigation, Formal analysis. **Mariko Atarashi-Andoh:** Investigation, Formal analysis. **Jun Koarashi:** Writing – review & editing, Supervision, Investigation.

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.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122207.

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