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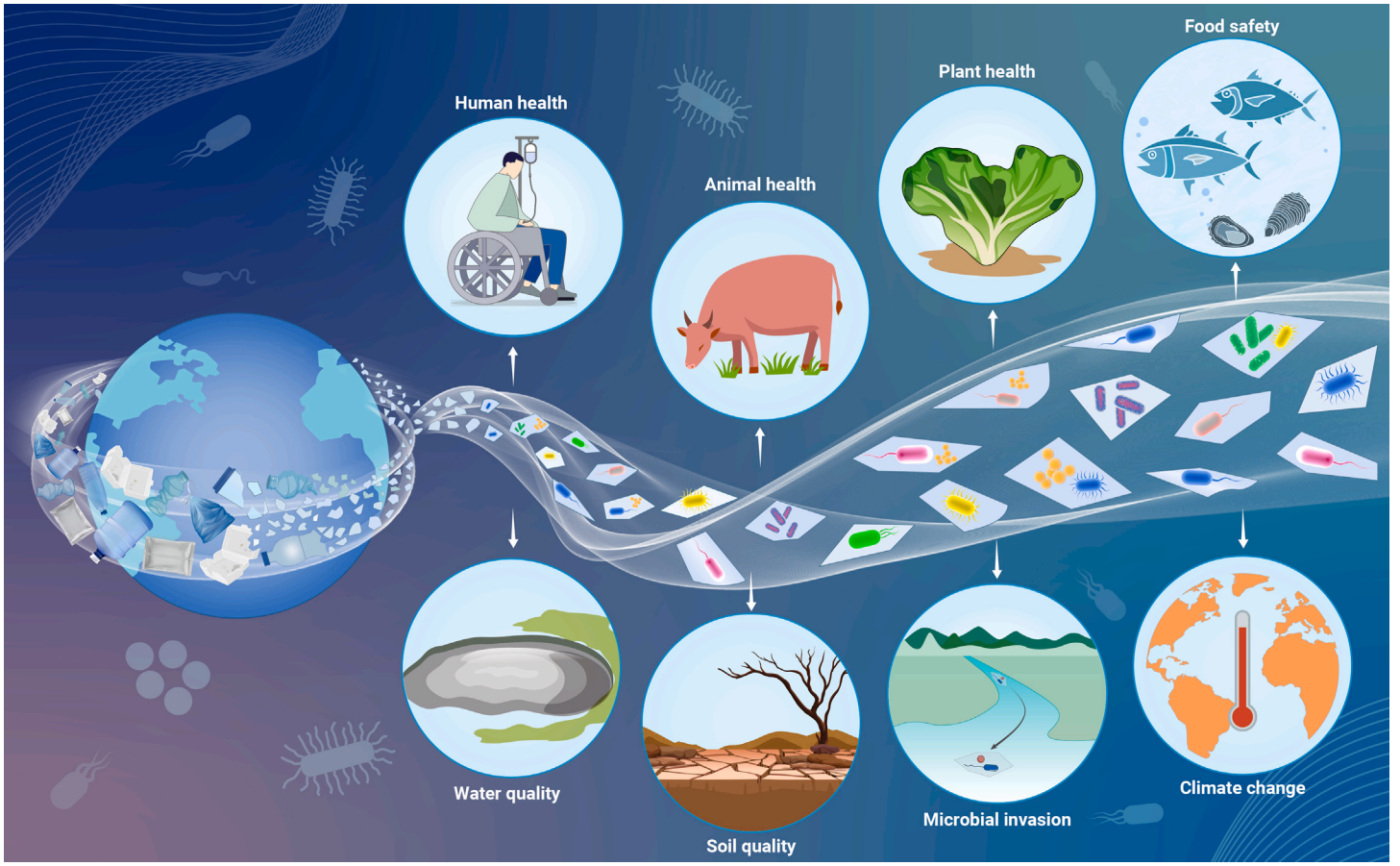
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- The plastisphere selectively assembles a microbiome distinct from that of natural habitats.
- New microbial coexistence patterns are yielded in the plastisphere.
- Altered microbial functions in the plastisphere threaten natural ecosystem functioning.
- Enrichment of pathogens in the plastisphere poses a critical challenge to “One Health”.

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Plastic offers a new niche for microorganisms, the plastisphere. The ever-increasing emission of plastic waste makes it critical to understand the microbial ecology of the plastisphere and associated effects. Here, we present a global fingerprint of the plastisphere, analyzing samples collected from freshwater, seawater, and terrestrial ecosystems. The plastisphere assembles a distinct microbial community that has a clearly higher heterogeneity and a more deterministically dominated assembly compared to natural habitats. New coexistence patterns—loose and fragile networks with mostly specialist linkages among microorganisms that are rarely found in natural habitats—are seen in the plastisphere. Plastisphere microbiomes generally have a great potential to metabolize organic compounds, which could accelerate carbon turnover. Microorganisms involved in the nitrogen cycle are also altered in the plastisphere, especially in freshwater plastispheres, where a high abundance of denitrifiers may increase the release of nitrite (aquatic toxicant) and nitrous oxide (greenhouse gas). Enrichment of animal, plant, and human pathogens means that the plastisphere could become an increasingly mobile reservoir of harmful microorganisms. Our findings highlight that if the trajectory of plastic emissions is not reversed, the expanding plastisphere could pose critical planetary health challenges.

INTRODUCTION

Plastic is a ubiquitous aspect of human life and is a marker of the present, new geological era—the Anthropocene.[1–5] The large-scale production, use, and disposal of plastics mean that plastic pollution has become one of the most problematic global environmental issues.[2–4] Global plastic production has climbed from 1.5 million tons in 1950 to more than 390 million tons in 2021.[6] Plastic products generated approximately 6,300 million tons of waste between 1950 and 2015.[7] Only a minority of plastic waste can be recycled or incinerated, whereas the vast majority (approximately 80%) ends up in landfills or the natural environment.[7] For example, the accumulation of plastic in the Pacific Ocean has created the infamous Great Pacific Garbage Patch, which is three times the size of France and is still expanding.[8] However, the size of individual plastic particles can be small enough to reenter the food chain, as shown by the presence of microplastics in plants,[9–11] animals,[12,13] human feces,[14,15] and even human placentas.[16] The amount of plastic waste in the environment will continue to increase due to the unabated production of plastic and its poor degradability.[3,7] As estimated, emissions of plastic waste may reach 12,000 million tons by 2050 if disposal is not effectively controlled.[7] By that time, the weight of plastic waste in the seas will far exceed the collective weight of fish,[17] and 99% of seabird species will be tainted with plastic.[18]

Plastics are a persistent, inert, hydrophobic, organic, and long-distance transportable substrate that can be colonized by microorganisms.[19–21] The resultant ecological system, characterized by diverse microorganisms thriving within the plastic matrix, is commonly known as the "plastisphere."[19,22] The magnitude of plastic waste means that it can harbor significant microbial biomass.[19,23] Using marine plastic debris as an example, the biomass on 1 g of marine plastic debris can be nearly an order of magnitude higher than the microbial biomass in 1,000 L of open-ocean seawater.[23] It has been estimated that marine plastic debris harbors between 0.01% and 0.2% of the total microbial biomass in the open ocean.[19,23] Because we can only account for approximately 1% of the plastic waste released into the ocean, the microbial biomass harbored by the plastisphere could be orders of magnitude larger.[19,24]

The increasing emission and intractability of plastic waste will lead to a continuous expansion of the plastisphere and a consequent increase in the microbial biomass that it harbors. Microorganisms control many elemental cycles and can affect the health of environments, animals, and humans.[25–27] Due to the increasing area of the plastisphere,[7,19] its significant microbial biomass,[19,23] and mobility in the environment,[28] it is imperative to explore the microbial ecology of this new habitat and its effects on the functioning of ecosystems. Furthermore, increased microbial exposure, via entry of plastic debris into the food chain,[10,28] poses a threat to biological safety and human health. Therefore, elucidating the microbial ecology in the plastisphere is central to predicting and managing the risks posed by plastic pollution, contributing to achieving the "One Health" goal.[29]

However, individual research programs may not be sufficient to generate a synoptic view of microbial ecology in the plastisphere. A more generalized understanding is required to determine how this newly expanding habitat assembles characteristic microbiomes, and the associated functional implications for ecosystem services, biosecurity, and human health. By combining our field-collected samples with publicly available raw sequences, we constructed a global dataset of plastisphere communities, covering freshwater, seawater, and terrestrial ecosystems. We analyzed the distinctiveness of the plastisphere microbiome in terms of community structure, assembly mechanisms, coexistence patterns, ecologically relevant functions, and potential pathogenic risks, and revealed the resulting ecological threats. Using our macrogenomic data, we validated the results of global sample-based microbial function prediction. Plastic is one representative of man-made surfaces, and plastic pollution is one of the most important ways by which humans exert an impact on planetary health.[3] Therefore, an effort was made in this study to reveal the ecology of the plastisphere and the associated effects inherent in plastic pollution, broadening our understanding of human effects on the natural world.

RESULTS

The plastisphere harbors distinct microbial assemblages

After strict data screening, we obtained a final total of 1,013 microbial samples collected from the plastisphere and its associated natural environment (water or soil) and used these to investigate plastisphere ecology in freshwater, seawater, and terrestrial ecosystems (Figure 1A; Tables S1–S3). Rarefaction analyses (Figure S1) showed that the number of samples

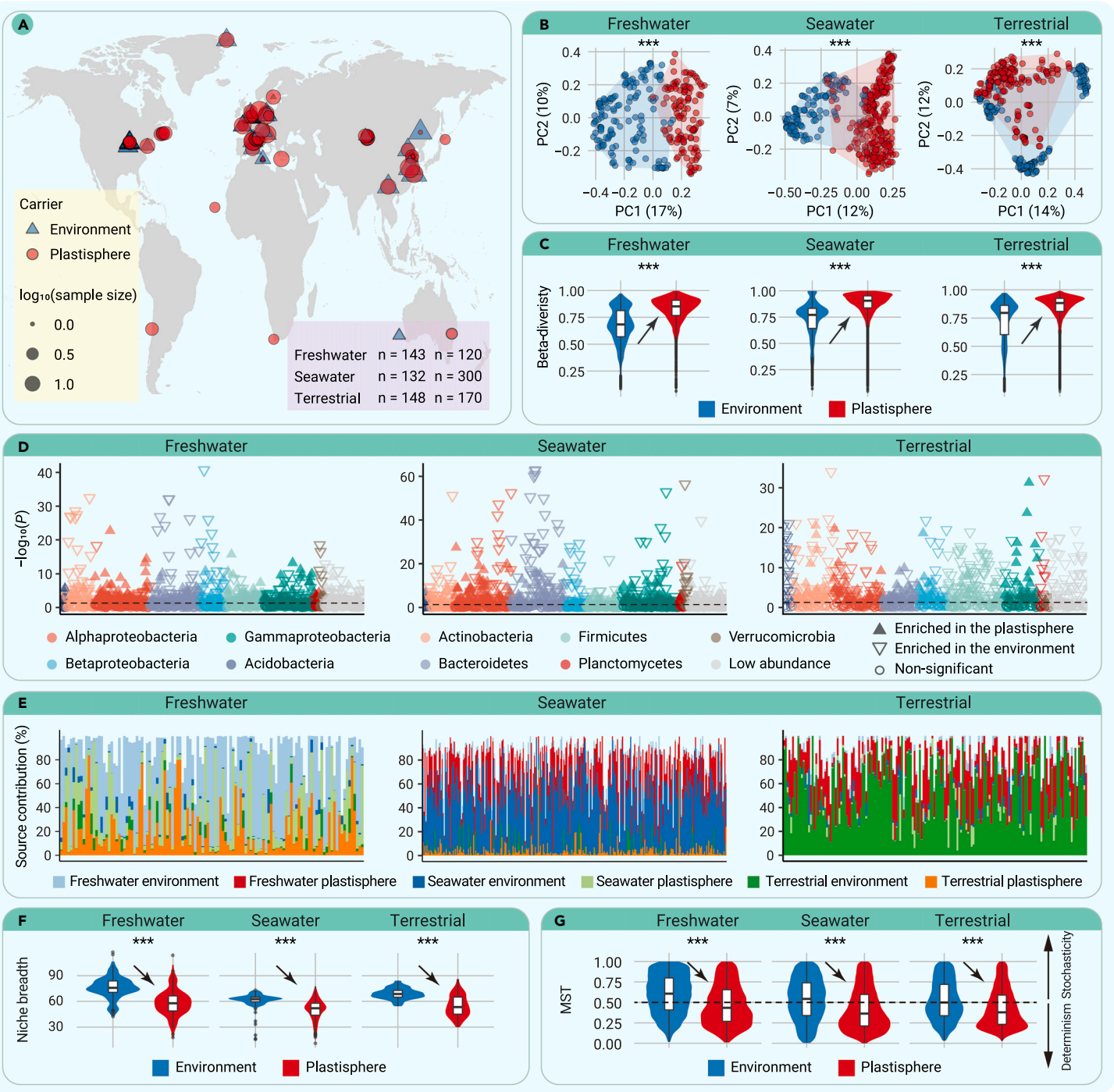

Figure 1. A distinct community assembles in the plastisphere from natural environments (A) Sources of the plastisphere and natural environment samples from freshwater, seawater, and terrestrial ecosystems that were analyzed in this study. (B) Unconstrained PCoA with PERMANOVA showing that the plastisphere has a distinct microbial community from that of the natural environment (PERMANOVA: ***$p < 0.001$). (C) Comparisons of β-diversity between the community in the plastisphere and that of the natural environment (***$p < 0.001$; Wilcoxon rank-sum test). (D) Manhattan plots showing genera with significant differences between the plastisphere and the natural environment in freshwater, seawater, and terrestrial ecosystems. Each circle or triangle represents a single genus. An upward and filled triangle represent a genus significantly enriched in the plastisphere, a downward and empty triangle represents a genus significantly enriched in the natural environment, and a circle represents a genus with nonsignificant difference between the plastisphere and the natural environment ($p < 0.05$; Wilcoxon rank-sum test). (E) A source analysis of microorganisms in the plastisphere based on the FEAST tool revealing that the corresponding natural environment contributes the largest part, but only a subset, of the sources of microorganisms in the plastisphere. (F) Comparison of habitat niche breadths between the plastisphere and the natural environment in each ecosystem (***$p < 0.001$; Wilcoxon rank-sum test). (G) The MST of the plastisphere and the natural environment in each ecosystem (***$p < 0.001$; Wilcoxon rank-sum test). A higher MST value represents a more stochastic assembly, with 0.5 defining the boundary between a deterministic (MST <0.5)- and stochastic (MST >0.5)-dominated assembly.

in our study was sufficient to capture the majority of microorganisms in the plastisphere and in the corresponding natural environment of each ecosystem. Canonical correspondence analysis was carried out to identify the important drivers of the structure of the global microbial meta-community, and we found that the ecosystem identity was the strongest driver, followed by the carrier identity (i.e., the plastisphere or natural environments), and then latitude and study identification (representing study-specific factors such as different methods applied in different studies) (Figure S2). The unconstrained principal-coordinate analysis (PCoA) with permutational multivariate analysis of variance (PERMANOVA) further showed that the plastisphere community was significantly distinct from the natural environmental microbial community (Figures 1B and S3), but the differences in community structure caused by different ecosystems were greater than those caused by the heterogeneity between the plastisphere and the

natural environment (Figure S3). The above results suggest that understanding the microbial ecology in the plastisphere should be studied specifically in its corresponding ecosystem. More importantly, although samples in different studies may be collected from different geographical locations in different seasons, and using different research methods, plastisphere samples and environmental samples still tend to form two separate clusters, demonstrating the fundamental differences between the plastisphere and natural habitats. Although the aim of our study was to understand the uniqueness and the associated risks of the plastisphere as a newly expanding habitat by analyzing its difference with natural habitats, we also tested the compositional difference between the plastisphere microbiome and other natural or unnatural biofilms (e.g., glass, natural seston, plant leaves) to further illustrate the distinctiveness of the plastisphere. Results showed that significant compositional differences existed between the plastisphere microbiome and other biofilms, indicating that the plastisphere was indeed a unique ecological niche for microorganisms (see supplemental information for details: Result S1; Figure S4; and Tables S4 and S5).

Similarities in community composition decreased significantly with increasing geographic distance (Figure S5A), indicating that the microbial community in the plastisphere followed a distance-decay pattern. Compared to the natural environments, the microbial communities in the plastisphere had significantly higher β-diversity (i.e., significantly lower similarity; Figures 1C and S5B).

Members of *Gammaproteobacteria*, *Betaproteobacteria*, and *Alphaproteobacteria* were prevalent within the freshwater plastisphere; members of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes* were highly abundant in the seawater plastisphere; and members of *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* comprised the majority of the terrestrial plastisphere community (Figures 1D, S6, and S7; Table S6). Most of the taxa exhibited significant differences in relative abundance between the plastisphere and the natural environment (Figures 1D and S8). In the freshwater ecosystem, compared to the natural environment, the plastisphere had a significantly higher abundance of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes*, and a lower abundance of *Bacteroidetes*, *Actinobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* (Figures 1D and S8A; Table S6). In the seawater ecosystem, the abundance of *Gammaproteobacteria*, *Firmicutes*, *Acidobacteria*, and *Planctomycetes* was higher, whereas that of *Bacteroidetes*, *Actinobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* was lower in the plastisphere than in the natural environment (Figures 1D and S8B; Table S6). Moreover, in the terrestrial ecosystem, the abundance of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Verrucomicrobia* was higher, whereas that of *Firmicutes*, *Acidobacteria*, and *Planctomycetes* was lower in the plastisphere (Figures 1D and S8C; Table S6). There were 478 genera commonly present in the plastisphere across the 3 ecosystems, and the number of unique genera in the plastisphere of each ecosystem ranged from 86 to 195 (Figure S9). However, only 26 genera were commonly enriched in the plastisphere across the 3 ecosystems, whereas 134–194 genera were enriched specifically in the plastisphere of each ecosystem (Figure S10), indicating that the plastisphere may play a different role in different ecosystems.

Using a random-forest classification model, we identified a group of biomarker taxa in the plastisphere in each ecosystem (see supplemental information for details: Result S2; Figures S11–S14; and Tables S7–S9). These biomarker taxa represented the most distinct differences in the taxonomic structure between the plastisphere and their corresponding natural habitats. Using these biomarker taxa could distinguish the plastisphere from the natural environment with high accuracy, which once again illustrates a fundamental difference between the plastisphere and natural habitats.

An analysis using the fast expectation maximization for microbial source tracking (FEAST) tool[30] was carried out to quantify the effects of environmental and plastisphere environments on the plastisphere community. The results showed that for all three studied ecosystems, the surrounding environment was the most important source of microorganisms, but it contributed only a subset of the residents of the plastisphere (Figures 1E and S15; Table S10), indicating the sheltering effect of the plastisphere and its ability to raft microorganisms in long-distance, cross-ecosystem transport.

The community-level niche breadth and the modified stochasticity ratio (MST) were calculated to reveal the underlying mechanism for community assembly in the plastisphere. The ecological niche breadth was significantly lower in the plastisphere compared to that of the natural environment in all three ecosystems (Figure 1F; Table S11), indicating that microorganisms in the plastisphere were subject to more environmental filtering. The MST model further revealed that the assembly process of the plastisphere community was dominated by determinism (MST <0.5), and stochastic factors played a much less important role in plastisphere community assembly than did microbial community assembly in the corresponding natural environment across all three ecosystems (Figure 1G; Table S12).

The plastisphere yields new patterns of coexistence

To explore the dominant factors driving global microbial cooccurrence patterns, we constructed a global ecological meta-network and found that, consistent with the findings in the structure of the global microbial meta-community, the cooccurrence pattern of the global microbial meta-community was also dominated by ecosystem identity (see supplemental information for details: Result S3; Figures S16 and S17; Tables S13–S15).

Therefore, we further constructed ecological subnetworks in each ecosystem to compare microbial cooccurrence patterns between the plastisphere and the natural environment (Figure 2A; Tables S16 and S17). All of the networks presented nonrandom and scale-free features (R^2 of the power law ranging from 0.854 to 0.982; Figure S18). Indexes characterizing the complexity of ecological networks, the number of links, the connectance, the average degree, and the natural connectivity showed a clearly lower level in the plastisphere than in natural environments (Figure 2B; Table S18). By randomly removing a percentage of the nodes, simulating species extinction, we tested the stability of the networks and found that the microbial networks in the plastisphere were consistently less robust than those in the natural environment (Figure 2C; Table S19).

In addition to the topological properties, we found that the nodes supporting the networks in the plastisphere were largely different from those supporting the networks in the natural environment (Figure 2D). Furthermore, more than 80% of the links between microorganisms in the plastisphere were specialist links (links occurring in the plastisphere but absent from the corresponding natural environment) in each ecosystem (Figure S19). In the freshwater ecosystem, the plastisphere specialist links consisted mainly of associations between members of *Alphaproteobacteria*, *Bacteroidetes*, and *Gammaproteobacteria* (Figure 2E). The plastisphere specialist links in the seawater ecosystem derived mainly from members of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes* (Figure 2E). Connections between members of *Firmicutes* and *Betaproteobacteria* contributed most of the plastisphere specialist links in the terrestrial ecosystem (Figure 2E).

Altered ecologically relevant functional profile in the plastisphere

Ecologically relevant functional signatures in the plastisphere and the natural environment were annotated with the Functional Annotation of Prokaryotic Taxa (FAPROTAX) tool.[31] Unconstrained PCoA with PERMANOVA revealed that significantly distinct functional features existed between the plastisphere and the natural environment in all three ecosystems (Figures S20 and S21; Table S20). Notably, the plastisphere in freshwater ecosystems exhibited significantly higher functional potentials related to denitrification, respiration of nitrogen and nitrogen oxides, and nitrate reduction, while having a lower functional potential for nitrification (Figure 3; Table S21). Functions related to the degradation or decomposition of organic compounds, including ligninolysis, oil bioremediation, hydrocarbon degradation, and aromatic hydrocarbon degradation, showed generally higher potentials in the plastisphere (Figure 3; Table S21). Based on databases PlasticDB[32] and Microbial Biodegradation of Persistent Organic Pollutants (POPs) Database (mibPOPdb),[33] we showed that the potential for plastic and POP degradation was remarkably higher in the plastisphere of all of the studied ecosystems (Figure 3; Table S21).

To validate the robustness of the global sample-based functional evaluations, we further analyzed functional genes related to nitrogen metabolism and organic compound metabolism between the plastisphere and the natural environment, using our own metagenomic samples collected from regional freshwater, seawater, and terrestrial ecosystems. The metagenomic data-based results also showed that the plastisphere in freshwater ecosystems exhibited a significantly higher abundance of genes encoding

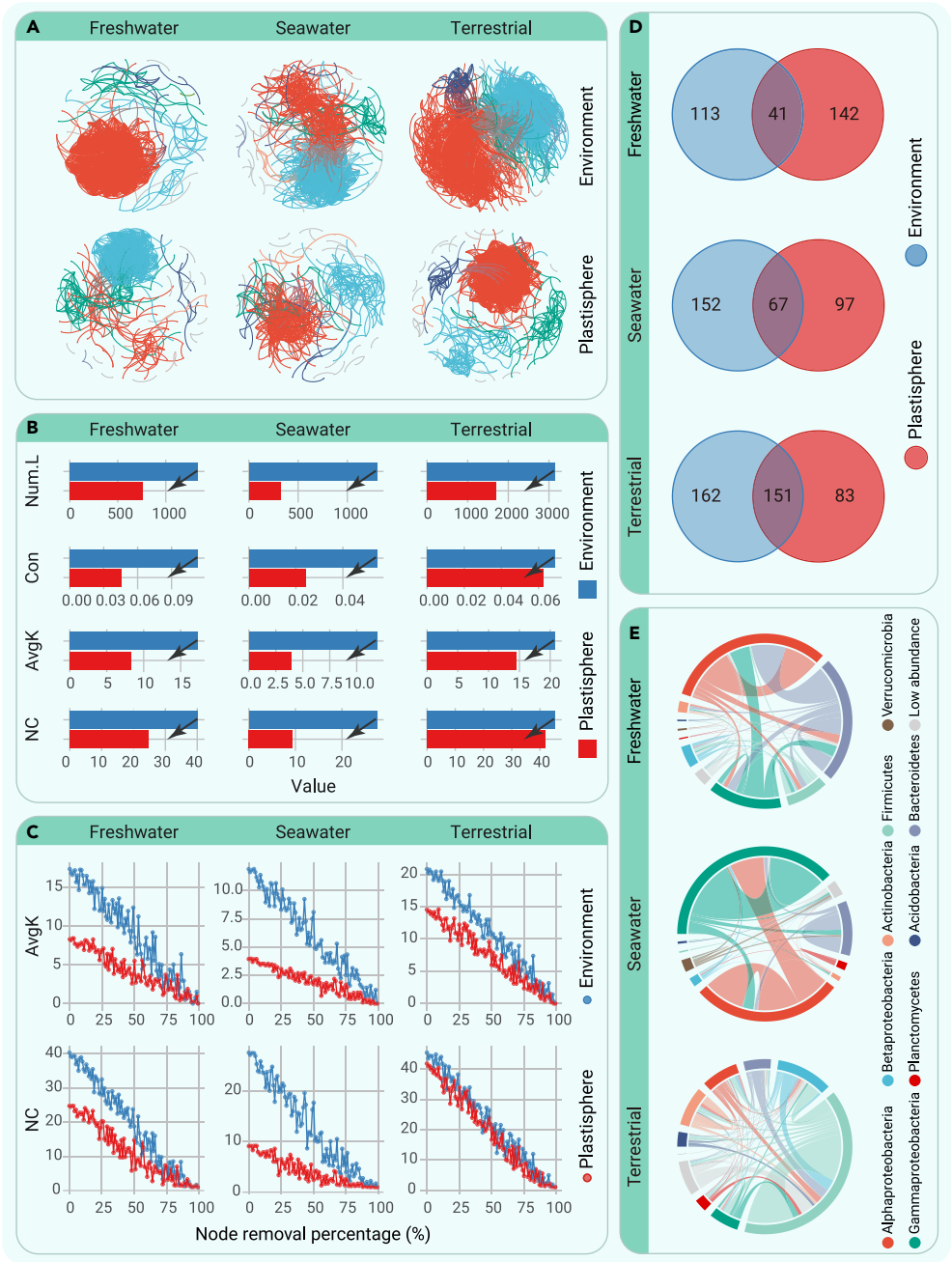

Figure 2. Microbial ecological networks in the plastisphere and the natural environment (A) An overview of the ecological networks. Each node represents a unique microbial genus. Each connection between the 2 nodes represents a strong cooccurrence relationship (Spearman's $\rho > 0.6$ and $p < 0.05$). Different colors indicate different modules. (B) The number of links (Num.L), connectance (Con), average degree (AvgK), and natural connectivity (NC) of the ecological networks in the plastisphere and the natural environment in each ecosystem. (C) The robustness of the ecological networks in the plastisphere and the natural environment in each ecosystem based on node removal to simulate species extinction. (D) Venn diagrams showing differences in the composition of the ecological network community between the plastisphere and the natural environment in each ecosystem. (E) Circos plots showing the composition of the specialist links (only present in the plastisphere) in the ecological network of the plastisphere in each ecosystem.

for denitrification functions (Figure S22; Table S22), whereas functional genes related to organic compound metabolism, such as carbohydrate metabolism (Figure S23; Table S22), plastic degradation (Figure S24; Table S22), and POP degradation (Figure S25; Table S22), showed generally high levels in the plastisphere in all ecosystems.

Increased pathogenic risks in the plastisphere

Using the multiple bacterial pathogen detection (MBPD) tool,[34] we evaluated pathogenic risks to animals, plants, and humans from the plastisphere. The MBPD database, designed specifically under the One Health vision, contains 72,685 full-length 16S gene sequences from 1,986 reported bacterial causes of plant, animal, and human diseases.[34] By aligning our samples to the MBPD database, a total of 642 pathogenic species (462 animal, 91 plant, and 89 zoonotic) were detected from all of the samples, of which 589 species (418 animal, 83 plant, and 88 zoonotic) were detected in the freshwater ecosystem, 594 species (422 animal, 87 plant, and 85 zoonotic) were detected in the seawater ecosystem, and 529 species (369 animal, 78 plant, and 82 zoonotic) were detected in the terrestrial ecosystem (Table S23). Notably, total abundances of plant pathogens, animal pathogens, and zoonotic pathogens showed a significant increase in the plastisphere (Figure 4; Table S24). A wide range of pathogens underwent upregulation in the plastisphere: 230 of 589 detected pathogens were enriched in the plastisphere in the freshwater ecosystem, 96 of 594 detected pathogens were enriched in the plastisphere in the seawater ecosystem, and 232 of 529 detected pathogens were enriched in the plastisphere in the terrestrial ecosystem (Figure 4; Table S23). In each ecosystem, a significant portion of pathogenic species not occurring in the associated natural environment was detected in the plastisphere (Figure 4; Table S23), emphasizing that the potential of the plastisphere to harbor pathogens for long-distance, cross-ecosystem transport.

We also analyzed clinical pathogens in the samples specifically using the 16S Pathogenic Identification Process (16SPIP) pipeline[35] to further evaluate the human disease risk posed by the plastisphere. The 16SPIP is an effective tool for rapid pathogen detection in clinical samples and also widely applied in environmental samples.[36–38] A detailed description of the result is presented in Result S4. Briefly, higher clinically pathogenic risks were observed in the plastisphere; the total abundance of clinical pathogens generally showed a higher level in the plastisphere. In each ecosystem, all of the pathogens detected in the natural environment also occurred in the plastisphere, but the plastisphere harbored additional pathogenic species that were not detected in the corresponding natural environment (Figures S26 and S27; Tables S25–S28).

Because aquatic animals, compared to organisms in other ecosystems, are more likely to accidentally ingest plastic debris via filter feeding, we further specifically identified potential fish pathogens in the samples based on the Fish Pathogen Database[39] to reveal the threat from the plastisphere to fish health. Results showed that, in all three ecosystems, the total abundance of fish pathogens always demonstrated significantly higher abundance in the plastisphere rather than in the natural environment (Figure S28; Table S29).

Using our metagenomic samples obtained from regional freshwater, seawater, and terrestrial ecosystems, we analyzed genes encoding virulence factors in the plastisphere and the natural environment to illustrate the robustness of our global sample–based pathogenic potential assessment results. We found generally higher levels of genes encoding virulence factors in the plastisphere rather than the natural environment in all of the studied ecosystems (Figure S29; Table S22).

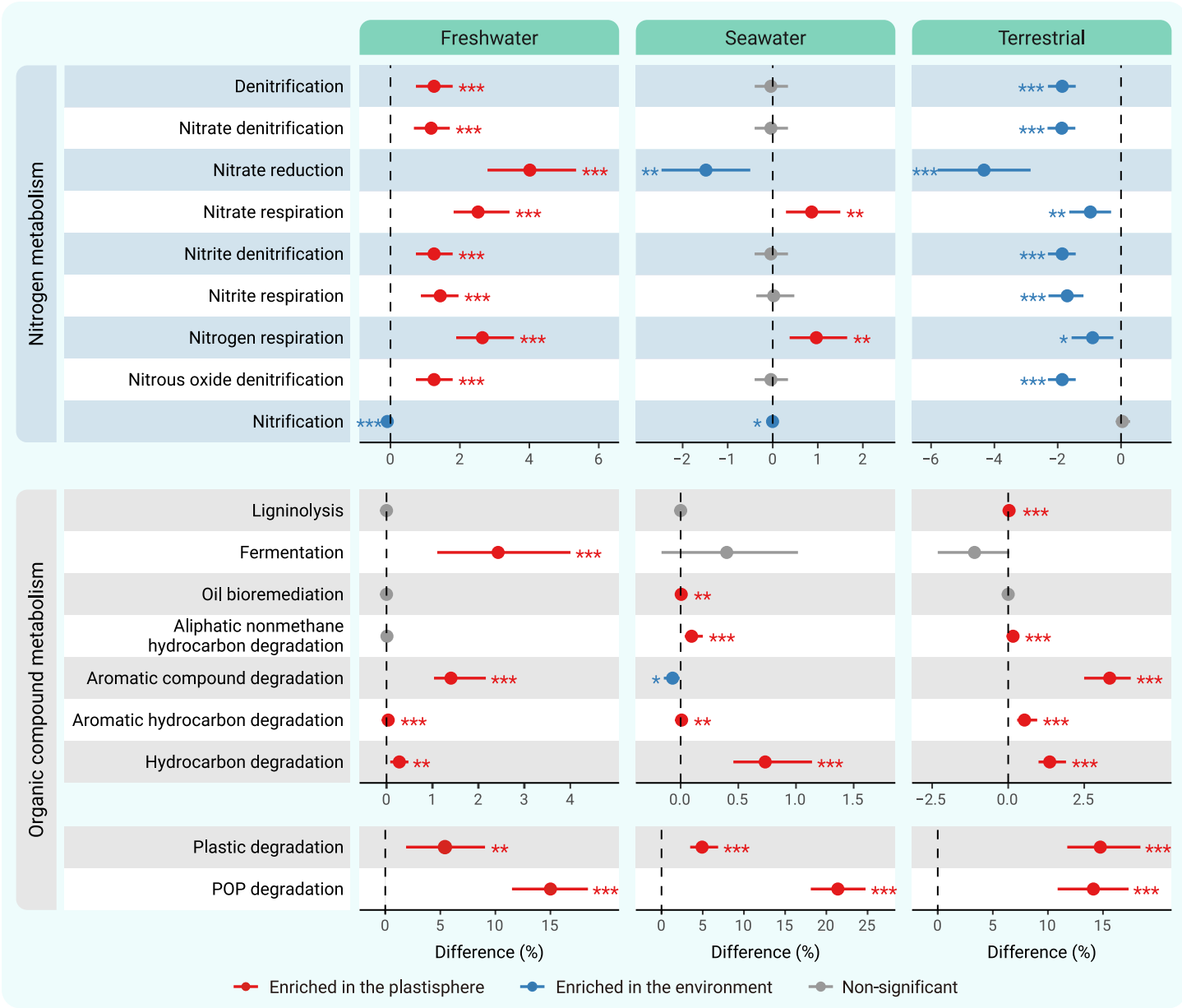

Figure 3. Differences in ecologically relevant functions between the plastisphere and the natural environment Plastic degradation potential is revealed based on the PlasticDB database, POP degradation potential is estimated with the mibPOPdb, and other functional potentials are predicted based on the FAPROTAX platform. A dot represents an estimate of the difference in functional potentials between the plastisphere and the natural environment, and the corresponding bar represents a confidence interval. A red dot with the corresponding bar indicates a significantly higher functional potential in the plastisphere, a blue dot indicates a significantly higher functional potential in the natural environment, and a gray dot indicates a nonsignificant difference in functional potential (*p < 0.05, **p < 0.01, ***p < 0.001; Wilcoxon rank-sum test).

DISCUSSION

Here, we constructed a global catalog of microbial communities from the plastisphere, covering samples from freshwater, seawater, and terrestrial ecosystems. We explored the ecological processes and mechanisms of microbial assembly in the plastisphere. This analysis shows that the human production of plastics is altering the natural microbial world, potentially influencing ecological processes, biosecurity, and human health (Figure 5).

The unique habitat selectively recruits a distinct microbiome with new coexistence patterns

As a habitat, the plastisphere has unique characteristics. First, the substrate of the plastisphere is organic, hydrophobic, buoyant, and persistent.[19,20,28] Plastic is composed of organic carbon compounds[40,41] that also tend to adsorb organic matter from the environment.[28] This provides nutrients for some microorganisms.[42] Harmful compounds can be released as plastic degrades—for example, the phthalate plasticizers, bisphenol A, and metal additives such as zinc, copper, and nickel.[43] These have potential effects on photosynthesis and community composition.[44] Hazardous hydrophobic pollutants such as polychlorinated biphenyls[45] and polycyclic aromatic hydrocarbons[46] are adsorbed from the ambient environment by the surface of plastic polymers. These exert strong selection pressure on microorganisms.

Consequently, the plastisphere selectively enriches microorganisms.[20,47] Microorganisms that can adapt to the unique environment are promoted and those that cannot adapt are inhibited, resulting in the formation of distinct microbial communities in the plastisphere (Figure 1B). The ability of plastics to travel through different environmental media (also referred to as the "plastic cycle"[48,49]) and the protection afforded to the microbial community by the plastisphere[20] allows plastics to carry microorganisms for long distances and between ecosystems.[28,50] This unique characteristic is verified in the present study by the source analysis of plastisphere microorganisms, which shows that plastispheres from different ecosystems have common sources and that plastisphere microorganisms can partially originate from the natural environment of other ecosystems (Figure 1E). Based on our field-collected samples, we found that, compared to the ambient microbial community, the plastisphere microbial community was

Figure 4. Animal, plant, and zoonotic pathogens in the plastisphere and the natural environment Potential pathogens were annotated with the MBPD database. Dot plots in the center of the figure show that the total relative abundance of animal, plant, and zoonotic pathogens exhibit significantly high levels in the plastisphere in all of the studied ecosystems (*p < 0.05, ***p < 0.001; Wilcoxon rank-sum test). A dot represents a mean value, and the length of a bar represents the corresponding standard deviation. The circular diagram characterizes the distribution of the monitored pathogens between the plastisphere and the natural environment in each ecosystem. Except for the outermost ring annotating the classification of the pathogens, the diagram has 9 layers, and each of the 3 layers from the inside to the outside characterizes the pathogen distribution between the plastisphere and the natural environment in one ecosystem. From the 3 layers of each ecosystem, the innermost layer characterizes the relative proportion of the mean abundance for each pathogen between the plastisphere and the natural environment, with a red bar representing a higher mean abundance in the plastisphere and a blue bar representing a higher mean abundance in the natural environment. The center layer characterizes the difference test result of the relative abundance of each pathogen between the plastisphere and the natural environment, filling with red means that the pathogen is enriched in the plastisphere, filling with blue means that the pathogen is enriched in the natural environment, and the rest means no significant differences. The third layer characterizes the occurrence of the pathogens unique to the plastisphere, with a gray dot indicating that the pathogen occurs only in the plastisphere but not in the natural environment.

less affected by environmental physicochemical properties (see supplemental information for details: Result S5; Figure S30). Therefore, the plastisphere can act as a vector for transporting microorganisms through different ecosystems. This may be an important driver of differences between the structure of the microbial community, in particular, plastispheres and their corresponding natural environment. This property also makes it possible for the plastisphere to transport invasive species into new environments, which may disturb the stability of natural ecosystems.[19,28,51]

The second unique characteristic of the plastisphere is its great heterogeneity, which is caused by the complexity and diversity of the plastic pollutants, including complex polymers, additives, and aging time.[51,52] The third characteristic of the plastisphere habitat is its high degree of fragmentation and isolation. The combination of these three characteristics explains the significantly increased importance of deterministic processes in the assembly of plastisphere microbial communities (Figure 1G). The unique microenvironmental conditions exert vast selection pressures on microorganisms, the high heterogeneity increases heterogeneous selection, and fragmentation and isolation make it more difficult for microorganisms to disperse among these microhabitats. It is well documented that deterministic and stochastic processes jointly lead to distance-decay patterns in biotic communities, because increases in geographic distance can increase the difficulty of dispersal and are associated with differences in environmental conditions.[53,54] The environmental conditions of the plastisphere are shaped by both the plastic substrate[55,56] and the physicochemical properties of its ambient environment.[20] Different types of plastic can recruit different microbial communities.[55,56] Our previous study revealed that the similarity of plastic-type composition also decreased significantly with increasing geographic distance,[52] and this finding has been confirmed by subsequent studies.[57,58] Therefore, strong dispersal limitation, high environmental selection pressure resulting from the heterogeneity of the substrate, and bulk physicochemical variation combine to generate a significant distance-decay pattern and a high β-diversity of the plastisphere microbiome (Figure 1C).

Associations among microorganisms shape microbial diversity and functions.[59–62] The complexity and stability of microbial networks in the plastisphere are lower than those in the natural environments in all three ecosystems (Figures 2A–2C). Food and resource availability are usually important drivers of network structures.[62,63] An adequate supply of resources facilitates the formation of complex networks.[62,63] In contrast, harsh and underresourced environments can limit interactions among microorganisms, leading to loose networks.[62–65] In addition to the effects of the above environmental selections, dispersal limitation also mediates microbial coexistence.[53,66,67] The strong dispersal limitation of the plastisphere can reduce the chances of species association. Supporting the core ecological theory that complexity begets stability,[67] the low complexity of plastisphere networks leads to their low robustness (Figure 2C). Notably, this study reveals that the composition of the nodes supporting the ecological networks is largely different between the plastisphere and the natural environments, and that most of the microbial associations in the plastisphere are specialist links (Figures 2D and 2E). These results demonstrate the fundamental difference between the plastisphere and the natural environment, and once again illustrate the unique environmental properties of the plastisphere, leading to new patterns of coexistence among microorganisms.

The distinct biotope threatens ecosystem functioning and One Health

The plastisphere has significantly distinct functional potentials compared to the natural environment in which it is embedded (Figure 3). Arguably, this affects the normal functioning of the whole ecosystem, especially because the microbial biomass of the plastisphere is often higher than that of the surrounding medium.[19] Due to the nature of its organic substrate, the plastisphere domesticates or selects microorganisms with functions related to the decomposition or degradation of organic compounds (Figure 3). For example, higher degradation potentials for hydrocarbons, aromatic hydrocarbons, plastics, and POPs prevail in the plastisphere of all of the ecosystems, a higher ligninolysis potential presents in the plastisphere from the terrestrial ecosystem, which is the most lignin-rich ecosystem, and a higher oil remediation potential exists

Figure 5. Schematic diagram showing potential plastisphere threats The plastisphere, which harbors microorganisms moving across ecosystems, may cause microbial invasions and disturb the stability of ecosystems. The general recruitment of organic-metabolizing bacteria and the altered nitrogen-metabolizing bacteria indicate that the plastisphere has potential effects on ecological processes, environmental quality, and climate change. The enrichment of human pathogens, plant pathogens, and animal pathogens means that the plastisphere poses critical challenges for One Health.

in the plastisphere in seawater ecosystems, which is the most oil-affected ecosystem (Figure 3).

These results indicate that plastic pollution has the potential to accelerate organic compound metabolism, which is detrimental to the sequestration of organic matter. Our findings provide an in-depth explanation of the observations in previous global investigations that high plastic biodegradation potential occurs in areas with high plastic contamination[68] and the accumulation of plastic residues negatively affects soil organic matter in croplands.[69,70] Furthermore, the higher decomposition and degradation potentials in the plastisphere also may increase the release of greenhouse gases such as CO_2 and CH_4, which are the end products of the decomposition and degradation. The functions of denitrification, respiration of nitrogen and nitrogen oxides, as well as nitrate reduction exhibit higher potential in the plastisphere from the freshwater ecosystem (Figure 3), which increases the chances of producing N_2O and NO_2^-.[71] N_2O is also a strong greenhouse gas with a global warming potential of 298 times that of CO_2 on a 100-year timescale,[72] whereas NO_2^- is toxic to aquatic organisms.[73] Metabolites from the plastisphere are released directly into the surrounding environment, consequentially disturbing the normal nutrient cycles of the natural ecosystem, as supported by microcosm studies.[74,75] The high potential for the metabolism of organic compounds to be altered and for there to be distinct functional signatures related to the nitrogen cycle means that the plastisphere could alter normal biogeochemical flows and help drive changes in climate.[20,56,76]

In addition to the above-mentioned effects on ecological processes, our findings that potential animal, plant, and human pathogens are enriched in the plastisphere indicate that the plastisphere poses a critical threat to biosecurity and, potentially, human health (Figure 4). Plastic debris–carrying pathogens could directly enter animals, especially aquatic animals via filter feeding,[13] which could negatively affect the growth, behavior, and feeding of animals.[70,77] There is evidence that even micron-sized plastics can be absorbed and accumulated by terrestrial plants.[10,11] The increased exposure of plants to the contaminating pathogens from the plastisphere may be an important cause of reports that plastic residues impair crop yields (an average yield reduction of 3% for every additional 100 kg/ha of plastic film residue)[69] and impede the normal growth of plants (e.g., reduced biomass).[69,70] The high abundance of human pathogens in the plastisphere means that plastic pollution increases the risk of human exposure to pathogens; moreover, plastic debris absorbed by animals and plants causes an increase in disease risk for humans indirectly.

The discovery that unique pathogens are present in the plastisphere but absent in the corresponding natural environment applied to all of the studied ecosystems (Figure 4) further confirms that plastics can act as vectors, harboring microorganisms for cross-ecosystem transport, in line with a previous *in situ* sequential incubation study.[50] This unique property of plastics poses an invasion risk from pathogens and other microorganisms, greatly increasing the disturbance to natural ecosystems and the uncertainty of infection in animals and humans.[19,27,28,50] Therefore, in line with the core notion of One Health,[78] the plastisphere can be said to pose a challenge to the health of environments, plants, animals, and humans.

The plastisphere can cause different ecological effects in different ecosystems

Our findings that the plastisphere differentially enriches microbial taxa, elemental metabolic functions, and conditional pathogenic taxa in different ecosystems (Figures 1D, 3, and 4) indicate that the plastisphere could cause different ecological effects under different scenarios. Our previous study[52] revealed that the composition of plastic debris varies significantly in different ecosystems due to the great differences in the physical and chemical properties of the environmental substrates and the highly different pollution sources; therefore, the different substrate composition of the plastisphere may be an important cause of the different ecological effects of the plastisphere in different ecosystems.[20] In addition, due to the largely different physicochemical properties of different ecosystems, the plastisphere can play different roles for microorganisms in different ecosystems.[19,20] For example, in terrestrial ecosystems, soils are usually rich in nutrients for microorganisms, and although the plastisphere can effectively adsorb organic matter, the availability of nutrients may not be an important reason for the enrichment of microorganisms by the plastisphere. However, in aquatic ecosystems, especially in the harsh and nutrient-poor environment of seawater ecosystems, the plastisphere, which is inert organic carbon itself and also can effectively adsorb ambient organic matter,[28,40,41] could serve as "nutrient islands" within these nutrient deserts.[19] Therefore, the different roles of the plastisphere under different environmental conditions could be another important cause of differentiated microbial taxa or functions enriched by the plastisphere in different ecosystems. Given these findings, plastic pollution is a global problem that requires local pollution control management. Therefore, further efforts are needed to reveal the specific effects of the plastisphere in representative human-influenced areas and to identify areas where plastic pollution should be prioritized for control.

In conclusion, the plastisphere distinguishes itself from natural habitats by selectively recruiting microbial communities and generating new coexistence patterns in which emerging microbial associations occur in loose and fragile networks. The functional implications of such a unique plastisphere assemblage are reflected in its distinct metabolic potential for nitrogen cycling and organic compounds, and great enrichment of animal, plant, and human pathogens, which may perturb the functioning of ecosystems and critically challenge the achievement of One Health. Our results provide a theoretical basis for quantifying the effects of the plastisphere on a number of planetary health issues, such as carbon turnover, greenhouse gas emission, pathogen-related food safety, and biological health, in relationship to the surface area of the plastisphere under the projected trajectory of its production and release. With the plastisphere as an example, understanding how the expansion of man-made surfaces introduced by human civilization is altering the natural microbial world contributes to informed global actions on the consequence of evolving microbiology in the Anthropocene. In the future, quantifying the extent of ecological processes, climate changes, and health events driven by plastic pollution will be necessary to fully assess plastic pollution risks. Given that our findings demonstrate the ability of the plastisphere to foster microbial communities with a heightened capacity for organic compound degradation, if we can establish microbial technologies and products that could effectively degrade plastics via the screening of microorganisms sourced from the plastisphere, it would make a substantial contribution to the solution of global plastic pollution.

MATERIALS AND METHODS

See supplemental information for details.

DATA AND CODE AVAILABILITY

The raw sequencing data from the field-collected samples have been deposited in the NCBI under accession identification numbers PRJNA717904 (for the amplicon sequencing data) and PRJNA984432 (for the metagenomic sequencing data). The sources of publicly available data are provided in Table S1. All of the data used in the analysis of this study are provided in Tables S2–S29. R scripts for key analyses in this study are available at https://github.com/Changchao-Li/global_plastisphere_ecology.

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AUTHOR CONTRIBUTIONS

C.L., L. Jin, and J. Liu designed the study; C.L. conducted the field sampling, performed the laboratory work, and collected publicly available data, with D.Z. and J.W. contributing a part of the metagenomic samples; C.L. analyzed the data under the guidance of L. Jin and J. Liu; C.L. L. Jin, and J. Liu prepared the original draft of the manuscript; and the following authors contributed significantly to reviewing and editing: M.R.G., C.Z., Q.C., D.Z., J.W., K.Z., Q.X., P.L., and X.D.L. All of the authors read and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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LEAD CONTACT WEBSITE

https://www.polyu.edu.hk/cee/people/academic-staff/dr-ling-jin/.

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Supplemental Information

Ecology and risks of the global plastisphere as a newly expanding microbial habitat

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This PDF file includes:

Other Supplemental Materials for this manuscript include the following:

Tables S1 to S29 are in a separate excel file (captions listed below).

Table S1 Metadata source.

Table S2 Sample information.

Table S3 Relative abundance of genera.

Table S4 Design of studies investigating microbial information of both the plastisphere and biofilms from other substrates.

Table S5 Genus relative abundance of the plastisphere and biofilms from other substrates.

Table S6 Taxonomic composition.

Table S7 Relative abundance of families.

Table S8 The importance of each microbial family on the accuracy of the random forest model in each ecosystem.

Table S9 Relative abundance of the plastisphere biomarkers in each ecosystem.

Table S10 Source analysis of microorganisms in the plastisphere based on the fast expectation-maximization for microbial source tracking (FEAST).

Table S11 Habitat niche breadth.

Table S12 The modified stochasticity radio (MST).

Table S13 Meta-network links.

Table S14 Meta-network nodes.

Table S15 Relative abundance of nodes in the meta-network.

Table S16 Sub-network links.

Table S17 Sub-network nodes.

Table S18 Sub-network indexes.

Table S19 Robustness of sub-networks in the plastisphere and the natural environment in each ecosystem.

Table S20 Relative abundance of ecologically relevant functions.

Table S21 Comparison of ecologically relevant functions between the plastisphere and the natural environment.

Table S22 Relative abundance of functional genes based on the metagenomic data (logTPM).

Table S23 Detected animal, plant and zoonotic pathogens in samples based on the MBPD database.

Table S24 Total abundance of animal, plant and zoonotic pathogens in each sample.

Table S25 Relative abundance of clinical pathogens detected based on the 16SPIP tool.

Table S26 Total abundance of clinical pathogens in each sample detected based on the 16SPIP tool.

MATERIALS AND METHODS

Field sampling strategy and processing of samples

We collected plastic samples and associated water samples from three freshwater bodies (the Wulong River, the Moshui River, and the Dagu River) and three seawater areas (Dingzi Bay, Southwest of Jiaozhou Bay, and Northeast of Jiaozhou Bay) in Qingdao and Yantai of Shandong province, China, during the month of September 2020. A Manta trawl (333 μm) was used to capture the plastic debris. A plastic sample was obtained in every 30 minutes of capture. Plastic debris trapped in the trawl were placed in a 50-mL centrifuge tube. Simultaneously, 2 L of surface water were collected in a sterile glass bottle. All of the samples were immediately placed on dry ice. A total of 36 plastic samples and 36 bulk water samples were obtained during the field sampling. The aim of this study was to reveal microbial ecological patterns and associated risks in the plastisphere, a huge and expanding man-made ecosystem, generated by environmental plastics. And it is generally accepted that the size of plastic debris does not significantly affect the structure of its residents as long as it is not so small as to affect the colonization of microorganisms.[1-4] Furthermore, if the plastic is too small to form a biofilm, *e.g.*, nanoplastics, it cannot form a plastisphere but instead forms an eco-corona,[5] which is beyond the aim and scope of our study. Therefore, the plastic debris included in this study include microplastics, mesoplastics, and macroplastics, and our findings and conclusions are generalizable for plastisphere research.

Each water sample was vacuum filtered successively through an 80–120 μm quantitative filter (to remove interfering substances) and a 0.22 μm membrane filter (to collect microorganisms). DNA was extracted from cells retained on the 0.22 μm filters and from plastic debris using a cetyltrimethylammonium bromide (CTAB) method.[6,7] A portion of the 16S rRNA gene was amplified with primer pairs of 515F and 806R, and subsequently sequenced to obtain 2 × 250 bp paired-end reads using an Illumina Novaseq 6000 platform.

Metadata collection and data preprocessing

To expand our view of the ecological patterns and threats associated with the plastisphere, we made an extensive effort to collect data from publications and bioprojects that used high-throughput sequencing to examine bacterial populations in the plastisphere. We first reorganized the dataset created by Wright et al. [8] in January 2020, which included 35 studies from the Web of Science Core Collection and Science Direct. Then, in October 2021 we obtained another 25 studies by searching NCBI using the search term “plastisphere”. To filter the metadata, the following criteria were applied: (i) The plastisphere had been collected or incubated in freshwater, seawater, or terrestrial ecosystems; (ii) the raw sequence data were available; and (iii) the sample information was clear, or could be obtained from the corresponding authors. Sequences satisfying all of these criteria were downloaded.

Paired-end sequences were joined, primer-cut, and quality-filtered in each project (with our own field-collected samples also treated as a project) using USEARCH [9] and VSEARCH [10]. Then, the sequences of all of the projects were combined into one file for subsequent analysis. Since the dataset was composed of thousands of samples with complex sources, it was more appropriate in this study to cluster the sequences as OTUs with a 97% similarity threshold, in order to avoid overestimations of diversity. OTUs were mapped to the RDP database to remove sequences generated from chimera, mitochondria, and chloroplasts. Then, an OTU table was generated using USEARCH. The taxonomic identity of representative sequences was annotated with the RDP classifier [11].

We first obtained 2,035 microbial samples, including plastisphere samples, the associated environmental samples, and biofilm samples on other substrates, from these plastisphere studies performed around the globe. To minimize the effect of different sequencing depths, samples with < 2,000 reads were removed, and all subsequent analyses were performed based on relative abundance. To address the effect of different sequencing regions in different studies, data at the genus level were used for all potentially affected analyses. To avoid data bias, 80 samples were randomly selected if the number of plastisphere or environmental samples in one study was greater than 80. Further, the number of seawater plastisphere samples was large compared with other subgroups, so 300 seawater plastisphere samples were randomly selected to avoid data bias. After carrying out the above data-trimming processes, we finally obtained a total of 1,192 samples from 35 bioprojects, including 143 freshwater-environment samples, 120 freshwater-plastisphere samples, 132 seawater-environment samples, 300 seawater-plastisphere samples, 148 terrestrial-environment samples, 170 terrestrial-plastisphere samples, and 179 biofilm samples from other substrates such as glass, natural seston, and plant leaves. The starting point of this study was that the plastisphere was a new microbial habitat with a vast and expanding area. This study aimed to clarify the differences in the microbial ecology between this new habitat and natural habitats, and to reveal the associated ecological threats. Therefore, we focused on the analysis of the microbial communities from the plastisphere and the natural environment (see Tables S1 and S2 for sample sources, Figure 1A for sample distributions, and Table S3 for the abundance of genera). The comparison of the plastisphere with biofilms from other substrates is presented in Result S1, and the result shows that the plastisphere is indeed a unique ecological niche that differ from other substrates significantly (Result S1, Figure S4, and Tables S4 and S5).

Microbial community structure analysis

Factors such as different sample handling, different primers, and different sequencing platforms potentially influence the microbial information of samples. To demonstrate the robustness of our findings and the fundamental difference between the plastisphere and natural environments, CCA was carried out and the relative importance of potential drivers of compositional variations in the global meta-community were quantified. These potential drivers included the ecosystem identity (*i.e.*, the freshwater ecosystem, the seawater ecosystem, and the terrestrial ecosystem), the carrier identity (*i.e.*, the plastisphere and the natural environment), the location latitude, and the study ID. Consistent with the approach applied in a Earth Microbiome Project study for revealing multi-scale microbial diversity on Earth,[12] we used the study ID as a proxy for a wide range of other potential drivers because the explanation of the variation in the meta-community composition by the study ID covered the explanation by factors like different research methods in different studies. The three categorical variables, the ecosystem identity, the carrier identity, and the study ID, needed to be converted to dummy variables for CCA and relative importance calculations. Since the carrier identity contained only two categories (the plastisphere and the environment), we replaced them directly with 0 and 1. The ecosystem identity contained three categories (the freshwater ecosystem, the seawater ecosystem, and the terrestrial ecosystem), and three different distance relationships might occur between the three groups, which we used “1,2,3”, “1,3,2”, and “2,1,3” to replace the three ecosystem IDs, respectively. For the study ID, we performed random permutations of the study IDs and then replaced the IDs with numerical values and repeated the process for 99 times. Then, with the rdacca.hp package,[13] we performed CCA analysis using the replaced variables and computed the explanation of each potential driver to the meta-community structural variation for a total of 3 × 99 = 297 times. Finally, we obtained the importance ranking of the drivers using the scores of each driver derived from the 297 calculations.

An unconstrained PCoA based on Bray-Curtis distance was carried out to analyze differences in microbial community structure between the plastisphere and the natural environment, and between different ecosystems. A PERMANOVA was used to test the statistical significance of the difference. A linear regression model between community similarity (1 – Bray-Curtis dissimilarity) and geographic distance was implemented to explore the distance-decay pattern of microbial communities in the plastisphere and the natural environment. A Wilcoxon rank sum test was used to compare the similarity in communities between the plastisphere and the natural environment. The FEAST tool [14] was used to quantify the impact of the natural environment as well as the traits of the plastisphere itself on the structure of the plastisphere microbial community. To avoid data bias due to sample size, 100 samples were randomly selected from each potential source for a FEAST analysis.

Community assembly mechanism

To reveal the community assembly mechanisms underlying microbial ecological patterns, including community structure and diversity, we computed the ecological niche breadth and the modified stochasticity ratio (MST). Habitat niche breadth is a key feature that influences species sorting and dispersal limitation in community assembly processes.[15] Microbiota with wider niches are usually more metabolically flexible at the community level, implying less influence from environmental filtering.[16] Using Levins' niche breadth index,[17] we estimated the habitat niche breadth of each genus in a metacommunity and then evaluated the community-level niche breadth by calculating the average habitat niche breadth of all taxa present in the community. The MST based on a null model is usually used to quantify the relative importance of stochasticity and determinism in the community assembly process. The MST model reflects the community assembly process by relative difference, rather than by the significance of the difference between the observed situation and the null expectation, and therefore provides a better quantitative measure of the stochasticity in community assembly.[18,19] The values of MST range from 0 to 1, with MST = 0 representing completely deterministic assembly and MST = 1 representing completely stochastic assembly, with 0.5 as the boundary defining deterministic (MST < 0.5) or stochastic (MST > 0.5) dominated assembly processes.

Ecological network construction and analysis

A meta-network was constructed to explore co-occurrence patterns of the global microbial meta-community. Genera with a relative abundance of > 0.001% and occurring in more than 60 samples were selected for a correlation calculation. Spearman's rank correlations were computed using the Benjamini-Hochberg method for multiple-testing-correction. Links with Spearman's $\rho \leq 0.4$ or P-value ≥ 0.05 were discarded. Further, we constructed ecological sub-networks of the plastisphere and the natural environment in each ecosystem to reveal the difference in the co-occurrence pattern between the plastisphere and the natural environment. To avoid data bias caused by sample size, 100 samples in the plastisphere or the natural environment subgroup in each ecosystem were randomly selected, and genera occurring in more than 10 samples were selected to construct the sub-networks based on the Spearman's correlation with the Benjamini-Hochberg correction method. Since the number of samples in the sub-datasets was much smaller than that in the meta-dataset, more stringent criteria were used when selecting the links used to build the sub-networks. Only links with Spearman's $\rho \geq 0.6$ and P-value ≤ 0.05 were chosen for the further construction of sub-networks. Node properties, module partition, and topological characteristics were analyzed using the igraph package. The small-world property of the network was tested using the power-law model with a good fit representing a scale-free and non-random network. To compare the robustness of the ecological networks in the plastisphere and the natural

environment, we further calculated the average degree and the natural connectivity after nodes were randomly removed to simulate species extinction.

Ecologically functional signatures

The FAPROTAX platform v.1.2.3 [20] was used to extrapolate the functional potential of the plastisphere. FAPROTAX is a tool that maps prokaryotic taxa to their corresponding metabolically or ecologically relevant functions based on current literature on cultured strains.[20] Unconstrained PCoA with PERMANOVA was carried out to test the difference in the overall functional signatures between the plastisphere and the natural environments. We extracted ecologically important functions involved in nitrogen metabolism and organic compound metabolism and examined the difference in potentials of these functions between the plastisphere and natural environments using the Wilcoxon rank sum test with false discovery rate (FDR) correction. Furthermore, using the usearch_global command in VSEARCH, we mapped our sequences to PlasticDB [21] and mibPOPdb [22] databases to evaluate the functional potential of plastic biodegradation and POP biodegradation in the plastisphere and the natural environment.

Pathogenic risks

The MBPD database is a newly established, specialized, large, and curated database for the monitoring of animal, plant, and zoonotic pathogens in biological and environmental samples under the “One Health” vision.[23] We annotated potential animal, plant, and zoonotic pathogens in our samples by aligning our sequences to the MBPD database with the usearch_global command. The 16SPIP pipeline is a comprehensive tool for rapid pathogen detection in clinical samples and also widely applied in environmental samples.[24-27] Using the 16SPIP pipeline, we further explored potential human pathogens in the plastisphere and in the natural environment. Moreover, we mapped our sequences to the Fish Pathogen Database [28] to specifically identify potential fish pathogens in the samples and reveal the threat from the plastisphere to fish health. The abundance of identified potential pathogens in the plastisphere and the natural environment was compared using the Wilcoxon rank sum test with FDR correction.

Metagenomic sample collection and analysis

To validate the robustness of the functional potential evaluations based on global bacterial communities, we conducted metagenomic-based analyses on our 38 paired-, field-collected plastisphere and natural environmental samples. These samples were also obtained from freshwater, seawater, and terrestrial ecosystems. Seawater plastic debris and the bulk water samples were pair-collected from two sites (120.315° E, 36.255° N and 120.3° E, 36.071° N) in coastal areas across Qingdao, China, in August 2021. At each site, no less than three sample pairs were collected. The sampling method was consistent with that used to collected samples for amplicon sequencing as described before, the Manta-trawl method. A total of seven pairs of plastic and water samples were obtained in the seawater ecosystem. Each water sample was vacuum filtered successively through an 80–120 μm quantitative filter (to remove interfering substances) and a 0.22 μm membrane filter (to collect microorganisms). Total genomic DNA from the collected plastic debris and filters was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer’s protocol. The extrated DNA from plastic and water samples were shotgun-metagenomic sequenced on the MGISEQ-2000 platform using a pair-end (2 × 150 bp) sequencing strategy. Freshwater plastic debris and the bulk water samples were pair-collected from nine sampling sites along the Huangpu River in Shanghai, China, in October 2021. At each site, the plastic debris for metagenomic sequencing was collected by passing 5 L

of water through a 50 μm mesh sieve, and additional 5 L of water was collected for the detection of the bulk water genome. The detailed methods for sample treatment, DNA extraction and sequencing can be found in our previous publication (to separate samples collected from the freshwater ecosystem and from the seawater ecosystem, one pair of samples collected from the estuary of the Huangpu River included in the previous paper was excluded from this study).[29] For the metagenomic-based investigation of the plastisphere in the terrestrial ecosystem, we employed an *in-situ* incubation strategy in Harbin, China. The microplastics were purchased from Youngling Electromechanical Technology Co. (Shanghai, China). Before incubation, these microplastics were soaked in 1% sodium hypochlorite for 30 min and then washed with sterile water five times to remove the microorganisms inherent in the microplastics. The microplastics were transferred into a nylon mesh bag and then buried in the soil. After eight weeks of incubation, the microplastic samples and the surrounding soil samples were collected. The detailed methods for sample treatment, DNA extraction and sequencing can be found in our previous publication.[30] In our previous study, we performed metagenomic sequencing for ten microplastic samples and three soil samples.[30] Since the plastic and environment samples for metagenomic sequencing in freshwater and seawater ecosystems were obtained using a paired-sampling strategy, three samples were randomly selected from the ten terrestrial plastic samples to balance the sample sizes of the two groups (the plastisphere and the environment). Finally, we obtained a total of 38 metagenomic samples (including nine freshwater-plastisphere samples, nine freshwater-environment samples, seven seawater-plastisphere samples, seven seawater-environment samples, three terrestrial-plastisphere samples, and three terrestrial-environment samples) for the characterization of functional genomes in the plastisphere and the natural environment to support our global sample-based findings on the ecological risks posed by the plastisphere.

Metagenomic raw sequences of each sample were quality-filtered to remove adapters and low-quality sequences with fastp v0.23.2 [31] with default parameters. The filtered sequences were assembled using MEGAHIT v1.2.9 [32]. Assembled contigs with length >500 bp were selected for further analysis. Using Prodigal v2.6.3,[33] open reading frames (ORFs) were predicted from the assembled contigs. All the predicted ORFs were further clustered to generate a non-redundant gene set by employing CD-HIT v4.8.1 [34] at 95% sequence identity with >90% coverage. The filtered reads were mapped to the non-redundant gene set to quantify the relative abundance (transcripts per million, TPM) of each gene in each sample with Salmon v1.10.1 [35]. Specialized functional gene databases including NCycDB,[36] CAZy,[37] PlasticDB,[21] mibPOPdb,[22] and VFDB [38] were employed to identify and quantify the genes encoding for nitrogen cycle-related functions, carbohydrate-active enzymes (CAZymes), plastic biodegradation functions, POP biodegradation functions and bacterial virulence factors, respectively. Non-redundant genes were translated into protein sequences with Seqkit v2.4.0 [39], and then the protein sequences were aligned to the above target functional gene datasets using DIAMOND v 2.1.6 (For the CAZymes annotation, the recommended *e*-value threshold of 1e–102 was adopted, and for other databases, the *e*-value threshold was set as 1e–5).[40]

SUPPLEMENTAL RESULTS

S1 Plastisphere microbial community distinct from other biofilms

Although the aim of this study was to reveal the microbial ecology in a new microbial habitat with a huge and expanding area – the plastisphere, its difference with the natural habitats, and the accompanying ecological threats of the plastisphere, we still tested the compositional difference between the plastisphere microbial community and other natural or unnatural biofilms to further illustrate the distinctiveness of the plastisphere as a microbial habitat. We screened 16 studies [1,30,41-54] from the metadata set that investigated microbial information of both the plastisphere and other biofilms, and obtained 289 plastisphere samples and 179 biofilms samples from other substrates including glass, natural seston, plant leaves, plant litters, tile, aluminium, cardboard, cellulose, and rock (see Tables S4 and S5 for the sample design and compositional information). The unconstrained principal coordinate analysis (PCoA) with the permutational multivariate analysis of variance (PERMANOVA) showed that significant differences existed in the microbial community composition between the plastisphere and other biofilms, both overall ($P < 0.001$) and specifically in each ecosystem ($P < 0.001$; Figure S4), indicating that the plastisphere was indeed a unique ecological niche for microorganisms. The underlying mechanism is that plastics are a persistent, inert, hydrophobic, buoyant, organic, and long-distance transportable substrate, which is distinguished from other natural or unnatural substrates. In addition, the whole area of plastics is huge and expanding with an unabated momentum in the near future, but the size of individual plastics can be small enough to enter into plants, animals, and even humans, which is the starting point of this study to decipher the microbial ecology of the plastisphere.

S2 Plastisphere biomarkers in each ecosystem

To identify a set of microbial features, which could best distinguish the plastisphere from the natural environment in each ecosystem, among numerous microbial taxa with significant difference between the plastisphere and the natural environment, we carried out a random-forest machine-learning model.[55-57] The model was established based on relative abundances of microbial families in the plastisphere and the natural environment in each ecosystem (Table S7) using the randomForest package [58] in R.

In each ecosystem, the model explained >97% of the variation in microbial communities between the plastisphere and the natural environment, showing the reliability of the models and the fundamental difference between the plastisphere and the natural habitats. Ten-fold cross-validation with five repeats was carried out in each ecosystem to evaluate the importance of each microbial feature. The error-rate curves stabilized before the 20 most relevant microbial features were used by the model, so we uniformly selected the top 20 microbial features that were most important for the accuracy of the models to discriminate between the plastisphere and the natural environment as biomarkers of the plastisphere in each ecosystem (Figure S11 and Table S8).

The plastisphere biomarker taxa in the freshwater ecosystem were from 5 phyla (Figure S12A), of which 9 taxa were enriched (namely, *Enterobacteriaceae*, *Rhizobiaceae*, *Burkholderiales incertae sedis*, *Erythrobacteraceae*, *Bacillaceae-1*, *Sphingomonadaceae*, *Bacillales Incertae Sedis XII*, *Halomonadaceae*, and *Xanthomonadaceae*) while 11 taxa were depleted (namely, *Streptomycetaceae*, *Cryomorphaceae*, *Microbacteriaceae*, *Burkholderiaceae*, *Demequinaceae*, *Flammeovirgaceae*, *Sutterellaceae*, *Puniceicoccaceae*, *Chitinophagaceae*, *Flavobacteriaceae*, and *Cyclobacteriaceae*) in the plastisphere (Figure S12B, C and Table S9).

The plastisphere biomarker taxa in the seawater ecosystem were from 4 phyla (Figure S13A), of which 9 taxa were enriched (namely, *Erythrobacteraceae*, *Saprospiraceae*, *Arenicellaceae*, *Rhizobiaceae*, *Hyphomonadaceae*, *Alteromonadaceae*, *Burkholderiaceae*, *Phyllobacteriaceae*, and *Hyphomicrobiaceae*) while 11 taxa were depleted (namely, *SAR11*, *Methylophilaceae*, *Euzebyaceae*, *Cryomorphaceae*, *Pseudomonadaceae*, *Verrucomicrobiaceae*, *Puniceicoccaceae*, *Rhodospirillaceae*, *Flavobacteriaceae*, *Chitinophagaceae*, and *Oceanospirillales incertae sedis*) in the plastisphere (Figure S13B, C and Table S9).

The plastisphere biomarker taxa in the terrestrial ecosystem were from 6 phyla (Figure S14A), of which 10 taxa were enriched (namely, *Pseudomonadaceae*, *Nocardiaceae*, *Burkholderiaceae*, *Moraxellaceae*, *Caulobacteraceae*, *Chromatiaceae*, *Peptococcaceae-2*, *Phyllobacteriaceae*, *Enterobacteriaceae*, and *Nocardioidaceae*) while 10 taxa were depleted (namely, *Gaiellaceae*, *Actinomycetaceae*, *Conexibacteraceae*, *Thermomonosporaceae*, *Hyphomicrobiaceae*, *Solirubrobacteraceae*, *Ktedonobacteraceae*, *Rhodocyclaceae*, *Planctomycetaceae*, and *Gemmatimonadaceae*) in the plastisphere (Figure S14B, C and Table S9).

S3 Ecosystem identity controls the microbial coexistence pattern

Based on the Spearman's rank correlations corrected by the Benjamini-Hochberg method, we constructed a global ecological meta-network to explore the dominate factor of the global microbial co-occurrence pattern (Figure S16 and Tables S13 and S14). The degree of the meta-network followed a power-law distribution (R^2 = 0.858; Figure S17), displaying non-random and scale-free features. The meta-network contained 660 nodes that formed 11,752 significant associations (Figure S16A and Tables S13 and S14). The top three large modules formed in the meta-network encompassed more than 96% of the nodes (Figure S16A, B and Table S15). By analyzing the relative abundance of the nodes in each sample, we found that each of the three modules reflected a corresponding ecosystem. Module 1 consisted mainly of members of the *Alphaproteobacteria*, *Gamaproteobacteria*, and *Bacteroidetes*, and was prevalent mainly in seawater ecosystems (Figure S16B, C and Table S15). Module 2 was comprised mainly of members of the *Firmicutes*, *Actinobacteria*, and *Alphaproteobacteria*, and reflected terrestrial ecosystems (Figure S16B, C and Table S15). Module 3 was mainly formed by members of the *Betaproteobacteria*, *Bacteroidetes*, and *Gammaproteobacteria*, and represented freshwater ecosystems (Figure S16B, C and Table S15). These phenomena indicated that the ecosystem identity was a more important driver of the co-occurrence pattern of the global microbiome than differences between the plastisphere and the natural environments.

S4 Increased risk from clinical pathogens in the plastisphere

We explored the potential for clinical pathogens to be present in the plastisphere based on the 16SPIP (16S Pathogenic Identification Process),[27] a comprehensive tool for rapid pathogen detection in clinical samples and also widely applied in environmental samples.[24-26] A total of 40 pathogenic species were observed in our dataset after matching with >99% similarity in sequence (Table S25). Overall, the plastisphere exhibited a significantly higher pathogenic potential compared to the natural environment (Figure S26 and Table S26). In the freshwater ecosystem, pathogens accounted for 10.4% of the plastisphere community, which was more than four times the proportion in the natural environment. In the terrestrial ecosystem, pathogens accounted for 9.3% of the plastisphere community, 5.7 times that of the community in the natural environment (Table S26).

Notably, in each ecosystem, all pathogens detected in the natural environment also occurred in the plastisphere, but the plastisphere harbored additional pathogens that were not detected in the

corresponding natural environment (Figure S26). By comparing the plastisphere and the natural environment in terms of the abundance of each pathogen, we found that a significant proportion of pathogenic species showed higher abundance in the plastisphere in all studied ecosystems (Figure S26 and Table S27). This suggests that the plastisphere promotes the growth of diverse pathogens. For example, 25 of 40 pathogens were enriched in the plastisphere in the freshwater ecosystem (Figure S26 and Table S27). In particular, the relative abundance of four pathogenic species (*Erysipelothrix rhusiopathiae*, *Proteus vulgaris*, *Citrobacter freundii*, and *Morganella morganii*) in the freshwater plastisphere was two to three orders of magnitude higher than that in the natural environment (Table S28). Similarly, a total of 17 out of 39 pathogens were plastisphere-enriched in the terrestrial ecosystem (Figure S26 and Table S27). Of these, the relative abundance of *Escherichia coli*, *Acinetobacter lwoffii*, *Citrobacter freundii*, *Acinetobacter baumannii*, and *Nocardia asteroides* in the plastisphere was again two to three orders of magnitude higher than that in the natural environment (Table S28). Pathogens unique to the plastisphere and plastisphere-enriched pathogens were different between ecosystems (Figures S26 and S27), showing that the plastisphere could pose different health threats in these different ecosystems.

S5 The plastisphere shelters its residents from external disturbances

Using our own field-collected samples, we explored the driving effect of the physicochemical properties of the surrounding medium on the plastisphere microbiome. The measurement methods for environmental physicochemical parameters were as described in our previous study.[57] Procrustes analysis and Mantel test showed that significant correlation existed between variation in the physicochemical properties of the surrounding medium and variation in the structure of the plastisphere community (Procrustes: $r = 0.563$, $P < 0.001$; Mantel: $r = 0.252$, $P < 0.001$; Figure S30A). Among the measured physicochemical factors, oxidation-reduction potential, concentrations of nutrients (dissolved organic carbon, NO_3^-, and NH_4^+), and salinity, explained more of the variation in the plastisphere microbial community and may be significant environmental drivers of the plastisphere microbial community (Figure S30B). Compared to the ambient microbial community (Procrustes: $r = 0.582$, $P < 0.001$; Mantel: $r = 0.366$, $P < 0.001$), the microbial community in the plastisphere were less driven by environmental physicochemical factors (Figure S30 A and C), demonstrating the sheltering effect of the plastisphere on its residents. In addition, while there was a significant association between the ambient microbial community and the plastisphere microbial community, changes in the ambient microbial community explained only a small fraction of the changes in the plastisphere microbial community (Procrustes: $r = 0.428$, $P < 0.01$; Mantel: $r = 0.119$, $P < 0.05$; Figure S30D), suggesting the selective assembly of the plastisphere with its preferred microorganisms, the sheltering effect of the plastisphere on its residents, and the potential of the plastisphere to raft its residents for long-distance transport.

SUPPLEMENTAL FIGURES

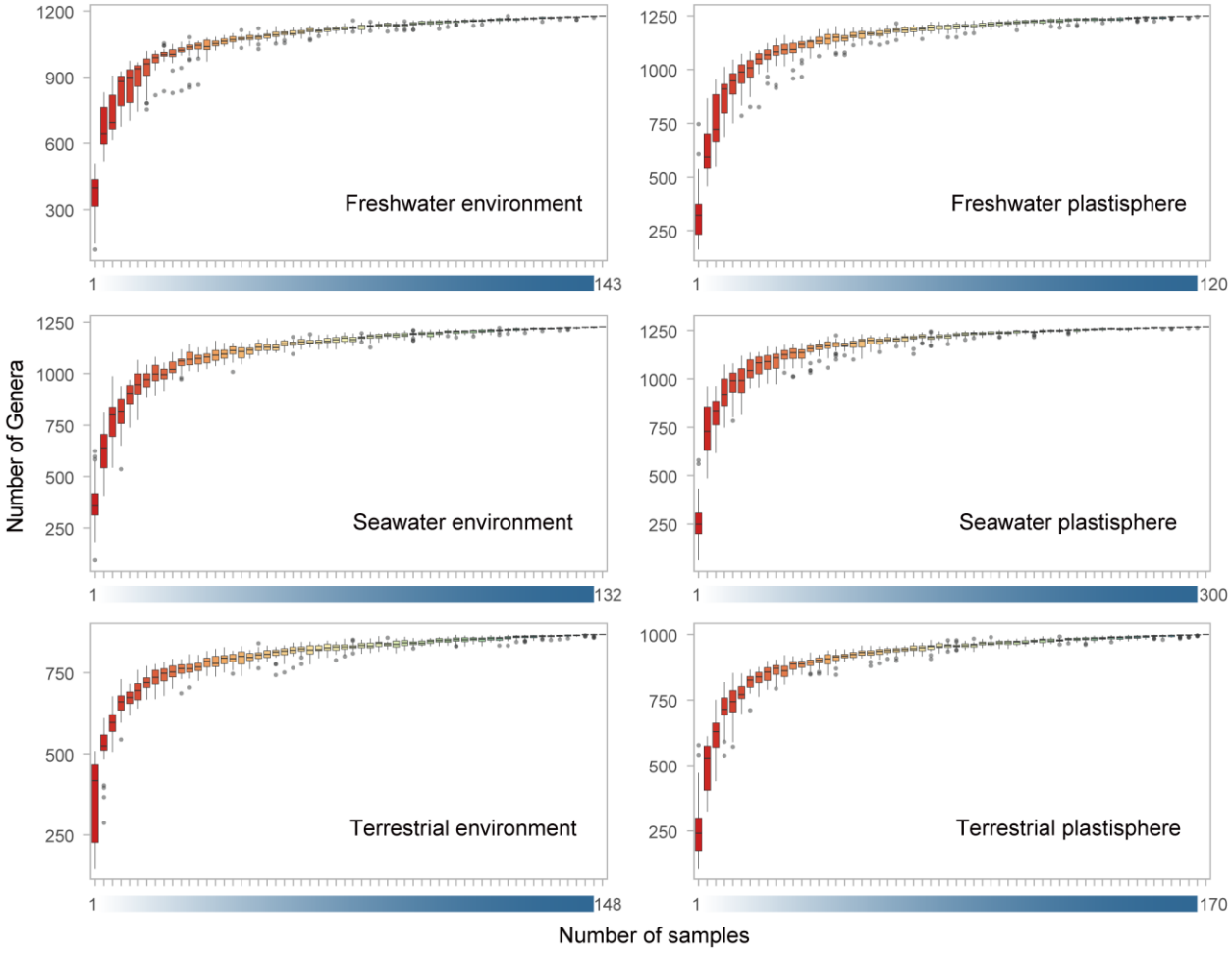

Figure S1 Rarefaction curves.

Rarefaction curves of the number of genera in the plastisphere and the natural environment in freshwater, seawater, and terrestrial ecosystems reach the saturation stage with increasing numbers of samples, indicating that the number of samples in our study is sufficient to capture most microorganisms from the plastisphere and the natural environment in each ecosystem.

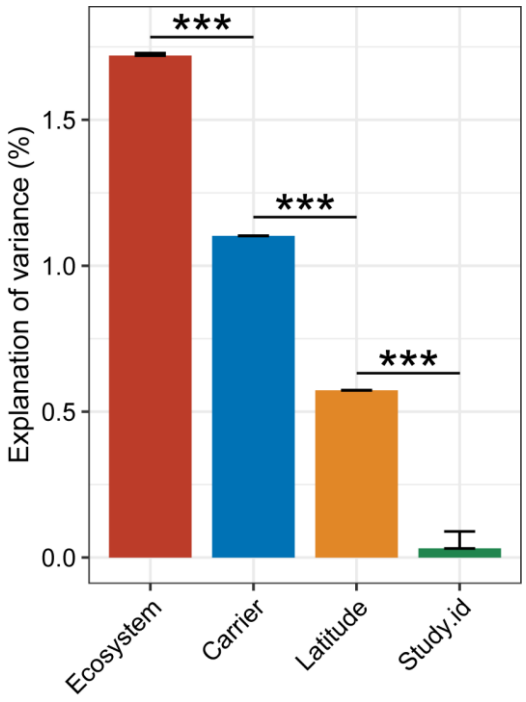

Figure S2 Explanations of the meta-community variation by different potential drivers.

The result was obtained based on the canonical correspondence analysis, and shows that, except for the ecosystem identity, the carrier identity, *i.e.*, the difference between the plastisphere and the natural environment, is the most important factor driving the variation in the meta-community structure.

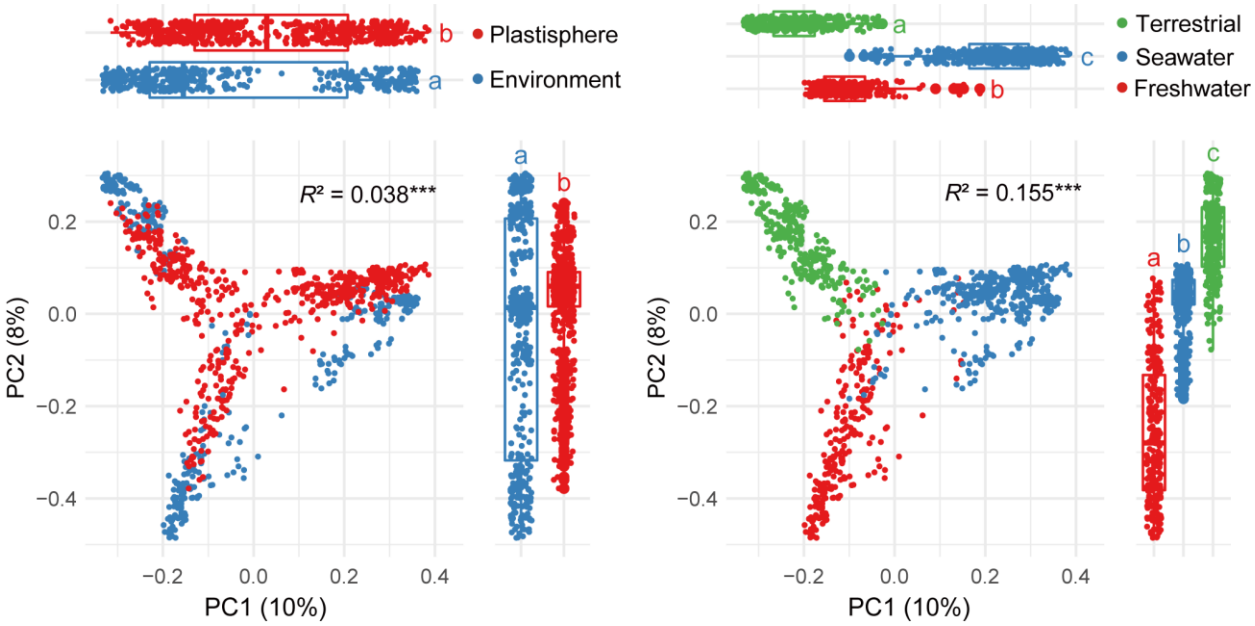

Figure S3 Differences in microbial community structure between the plastisphere and the natural environment and among different ecosystems.

Unconstrained principal coordinate analysis (PCoA) with permutational multivariate analysis of variance (PERMANOVA) showing that the plastisphere has a distinct microbial community from that of the natural environment (R^2 = 0.038, ***P < 0.001), but that the structure of the community is more dependent on the ecosystem (R^2 = 0.155, ***P < 0.001).

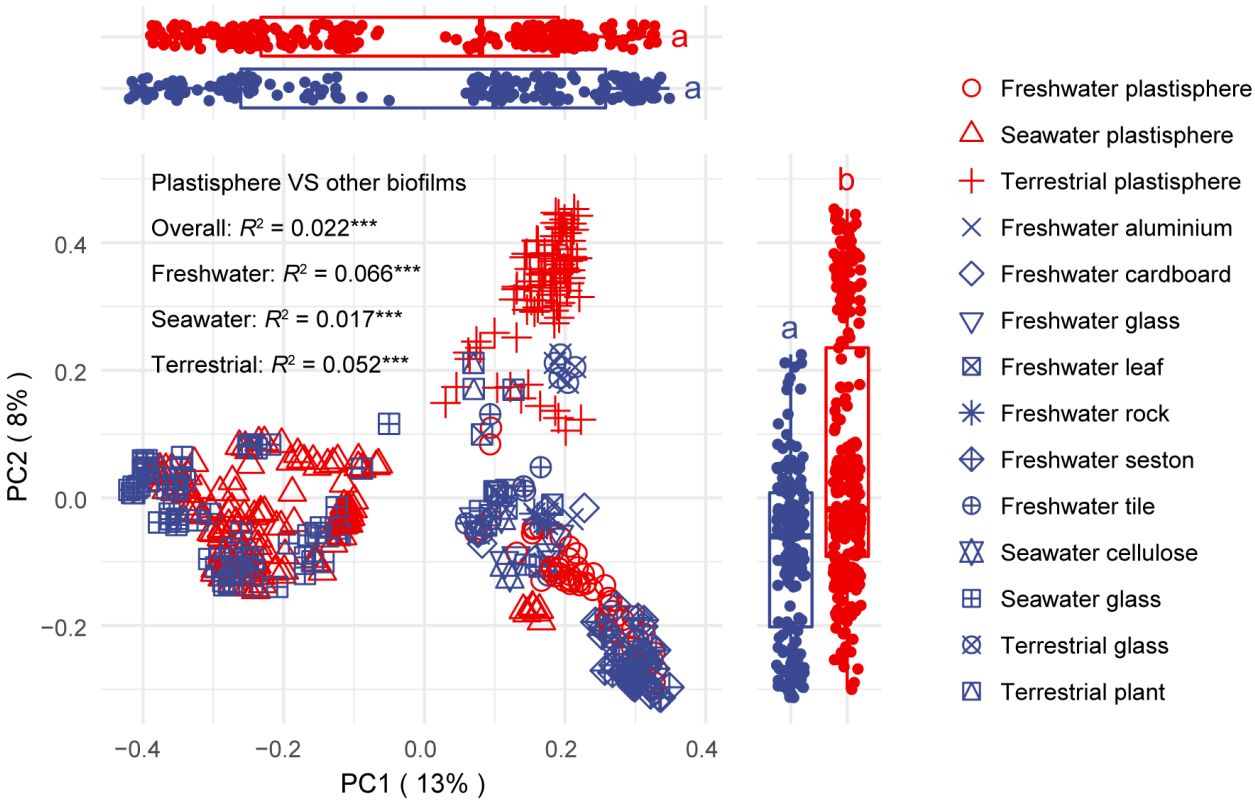

Figure S4 Significant differences between plastisphere microbial communities and other biofilms.

Unconstrained principal coordinate analysis (PCoA) with permutational multivariate analysis of variance (PERMANOVA) showing that the plastisphere has a distinct microbial community from other biofilms, both overall (R^2 = 0.022, ***P < 0.001) and in each ecosystem specifically (in the freshwater ecosystem: R^2 = 0.066, ***P < 0.001; in the seawater ecosystem: R^2 = 0.017, ***P < 0.001; in the terrestrial ecosystem: R^2 = 0.052, ***P < 0.001).

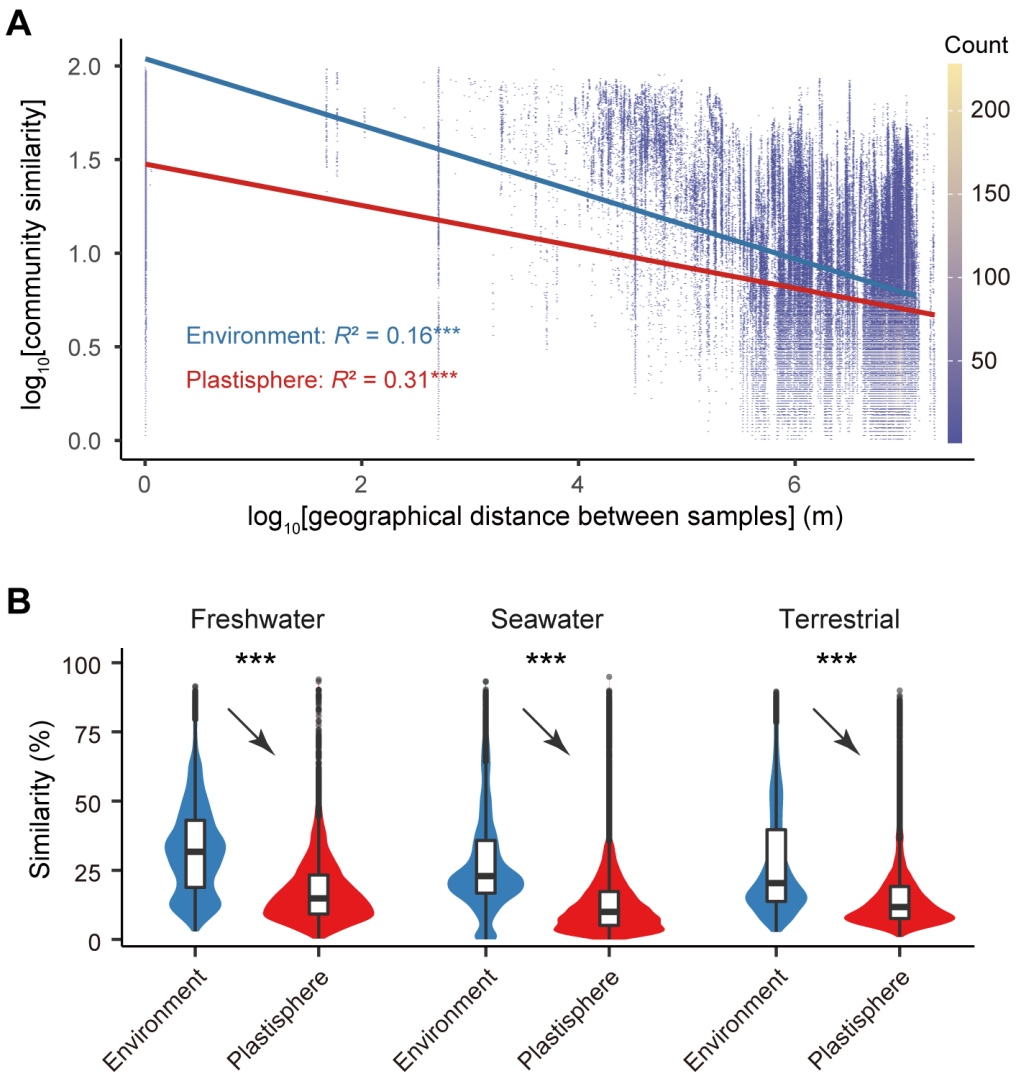

Figure S5 Between-sample compositional similarity.

(**A**) Significant distance-decay patterns in the plastisphere and the natural environment (***P < 0.001; linear regressions). (**B**) Comparisons of compositional similarity between the community in the plastisphere and that of the natural environment (***P < 0.001; Wilcoxon rank sum test), and the numbers of replicated samples are as follows: freshwater plastisphere (n = 120), freshwater environment (n = 143), seawater plastisphere (n = 300), seawater environment (n = 132), terrestrial plastisphere (n = 170), terrestrial environment (n = 148).

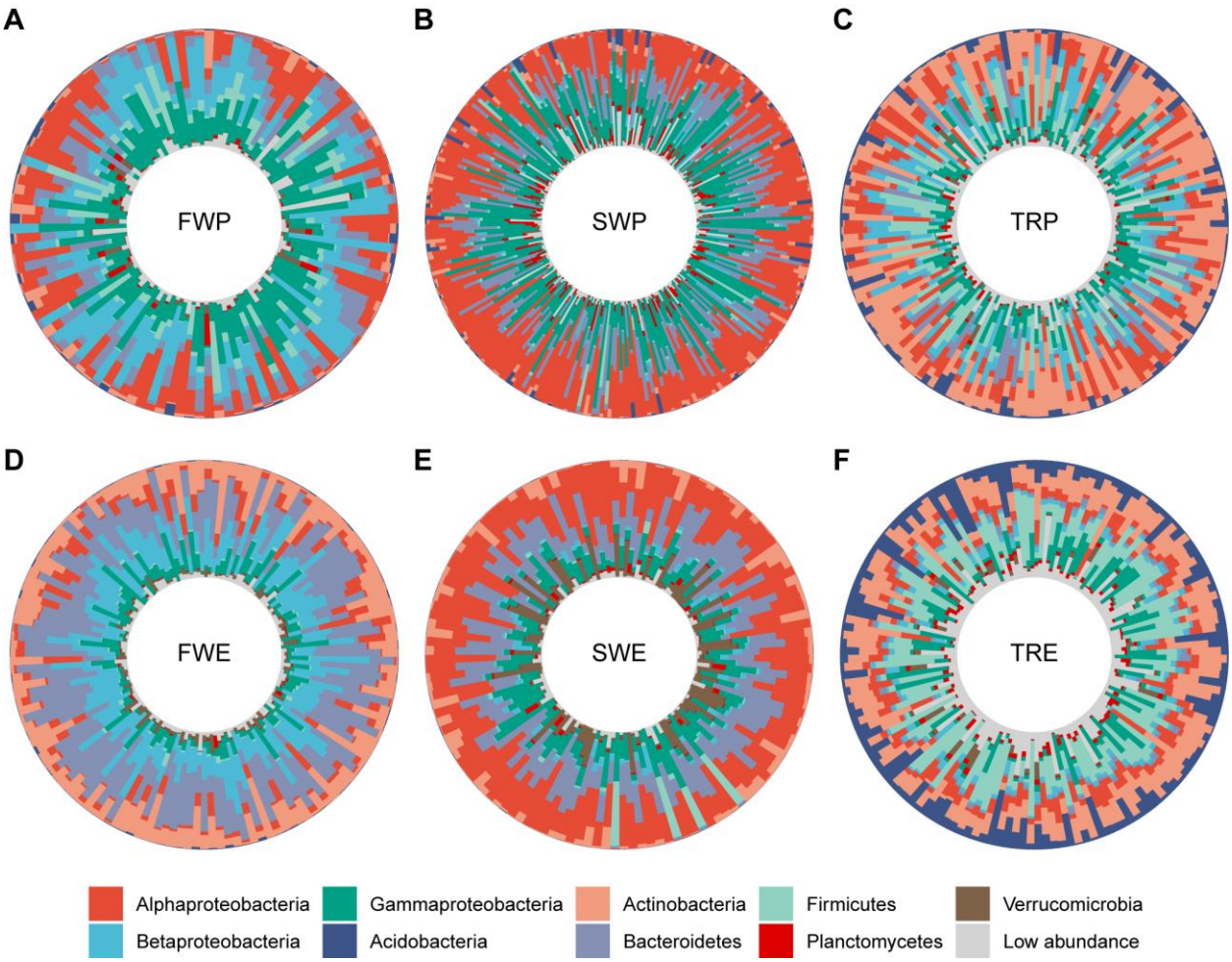

Figure S6 Taxonomic composition of microbial communities in the plastisphere and the natural environment.

(**A**-**C**) Phylum-level (with Proteobacteria being shown at the class level) composition of microbial communities in the plastisphere in freshwater (**A**), seawater (**B**) and terrestrial (**C**) ecosystems. (**D**-**F**) Phylum-level (with *Proteobacteria* being shown at the class level) composition of microbial communities in the natural environment in freshwater (**D**), seawater (**E**) and terrestrial (**F**) ecosystems. FWP = freshwater plastisphere (n = 120); FWE = freshwater environment (n = 143); SWP = seawater plastisphere (n = 300); SWE = seawater environment (n = 132); TRP = terrestrial plastisphere (n = 170); TRE = terrestrial environment (n = 148).

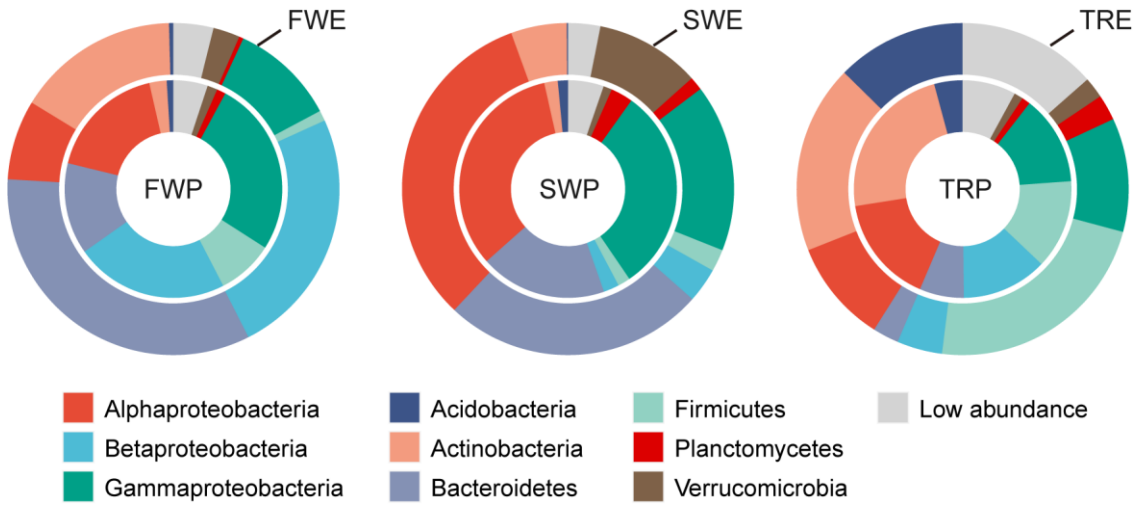

Figure S7 Taxonomic composition of microbial communities in the plastisphere (inner circles) and the natural environment (outer circles).

The numbers of replicated samples are as follows: freshwater plastisphere (*n* = 120), freshwater environment (*n* = 143), seawater plastisphere (*n* = 300), seawater environment (*n* = 132), terrestrial plastisphere (*n* = 170), terrestrial environment (*n* = 148).

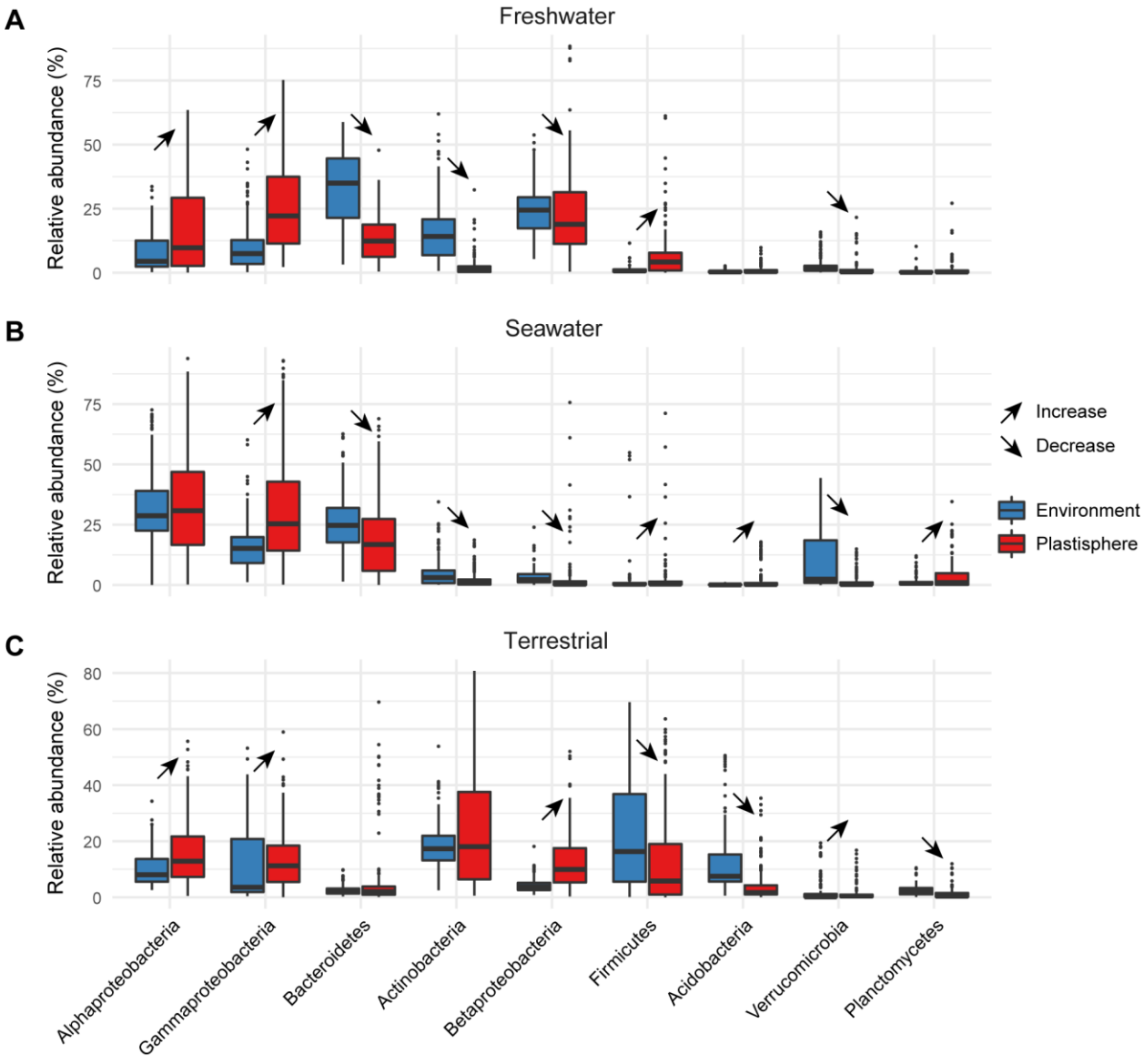

Figure S8 Differences in the relative abundance of microbial taxa between the plastisphere and the natural environment.

Wilcoxon rank sum tests for the relative abundance of the top nine most abundant microbial taxa between the plastisphere and the natural environment in freshwater (A), seawater (B), and terrestrial (C) ecosystems showing that most microbial taxa are significantly altered in the plastisphere ($P < 0.05$). An upward arrow represents that the relative abundance of the microbial taxon is significantly higher in the plastisphere than in the natural environment, while a downward arrow represents that the relative abundance of the taxon is significantly lower in the plastisphere than in the natural environment ($P < 0.05$; Wilcoxon rank sum test). The numbers of replicated samples are as follows: freshwater plastisphere ($n = 120$), freshwater environment ($n = 143$), seawater plastisphere ($n = 300$), seawater environment ($n = 132$), terrestrial plastisphere ($n = 170$), terrestrial environment ($n = 148$).

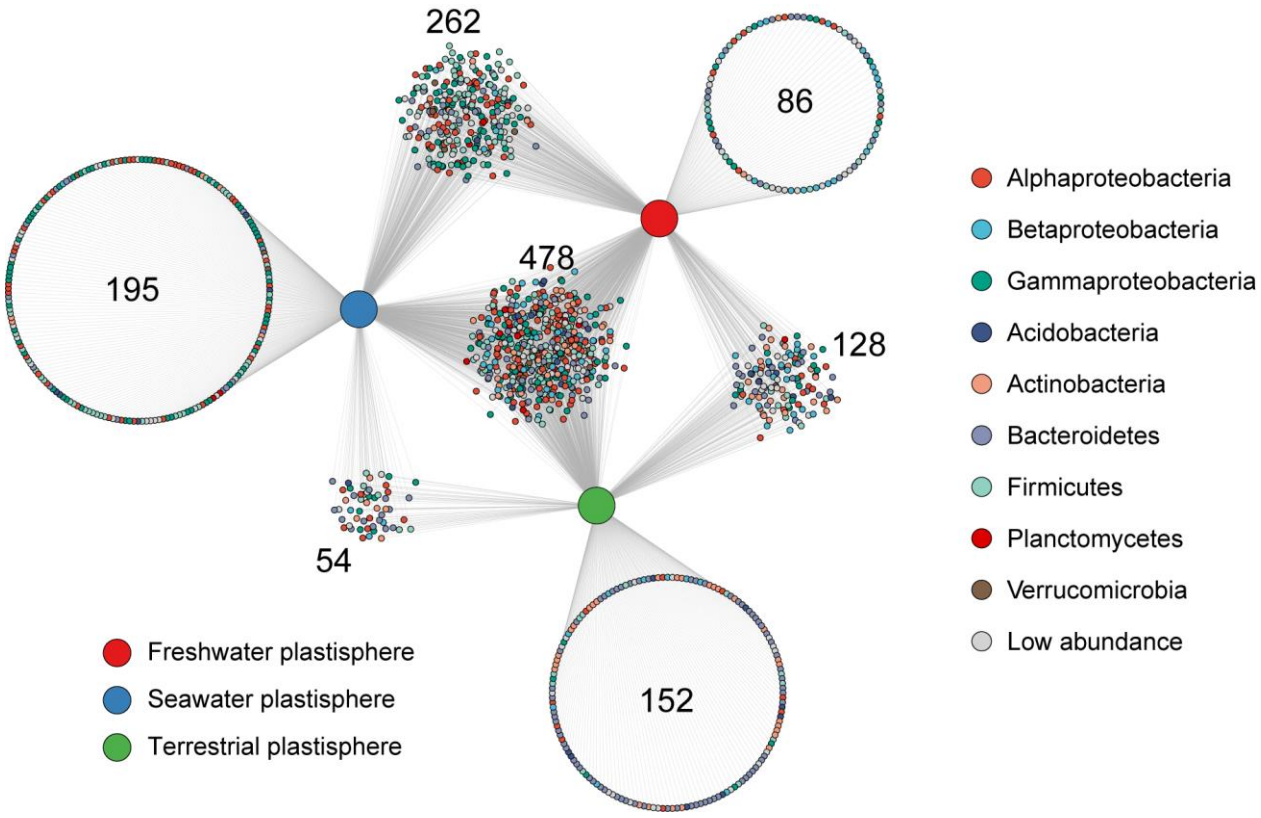

Figure S9 Shared and unique taxa between the plastisphere in freshwater, seawater, and terrestrial ecosystems.

Each small dot represents a microbial genus, and its color represents the taxonomic information. Each large dot represents a group (the freshwater plastisphere, the seawater plastisphere, and the terrestrial plastisphere). A line between a small dot and a large dot represents the presence of this taxon in the corresponding plastisphere.

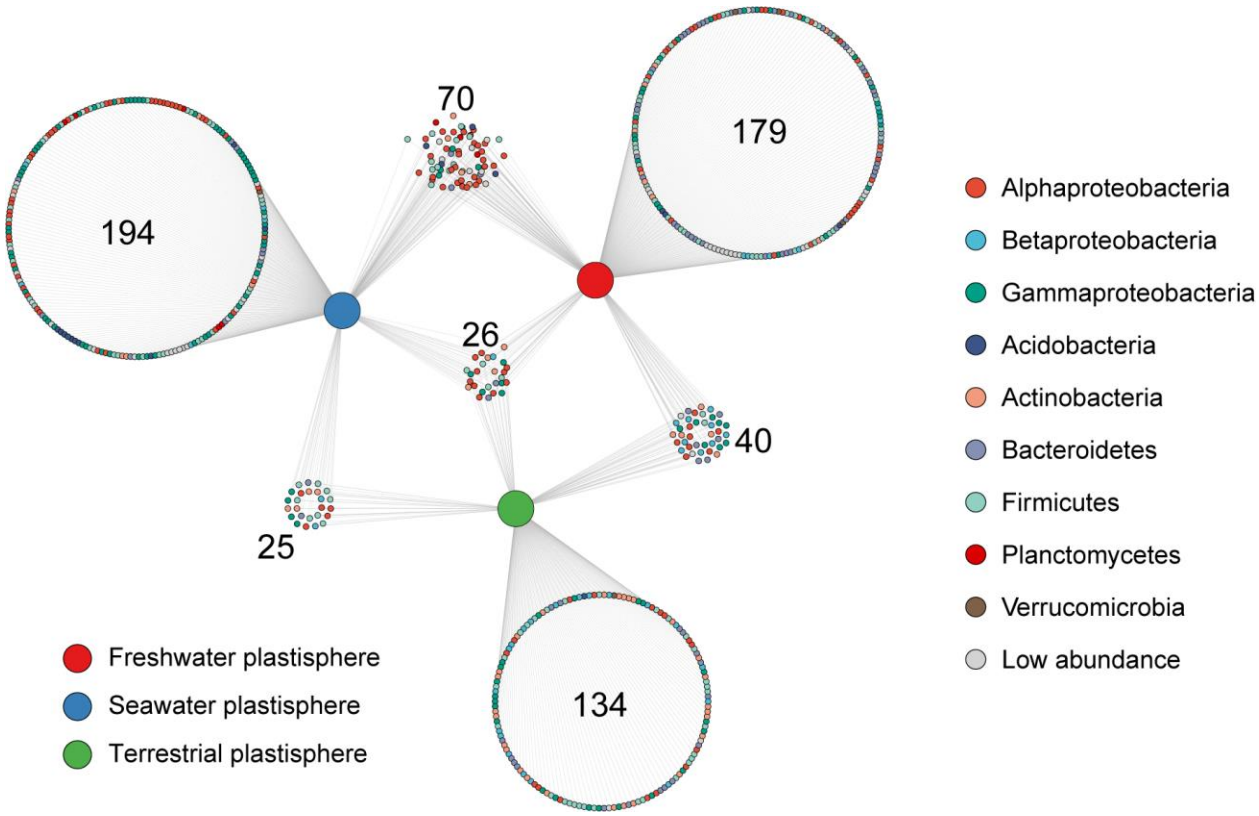

Figure S10 Commonly and uniquely enriched taxa between the plastisphere in freshwater, seawater, and terrestrial ecosystems.

Each small dot represents a microbial genus, and its color represents the taxonomic information. Each large dot represents a group (the freshwater plastisphere, the seawater plastisphere, and the terrestrial plastisphere). A line between a small dot and a large dot represents the presence of this taxon in the corresponding plastisphere.

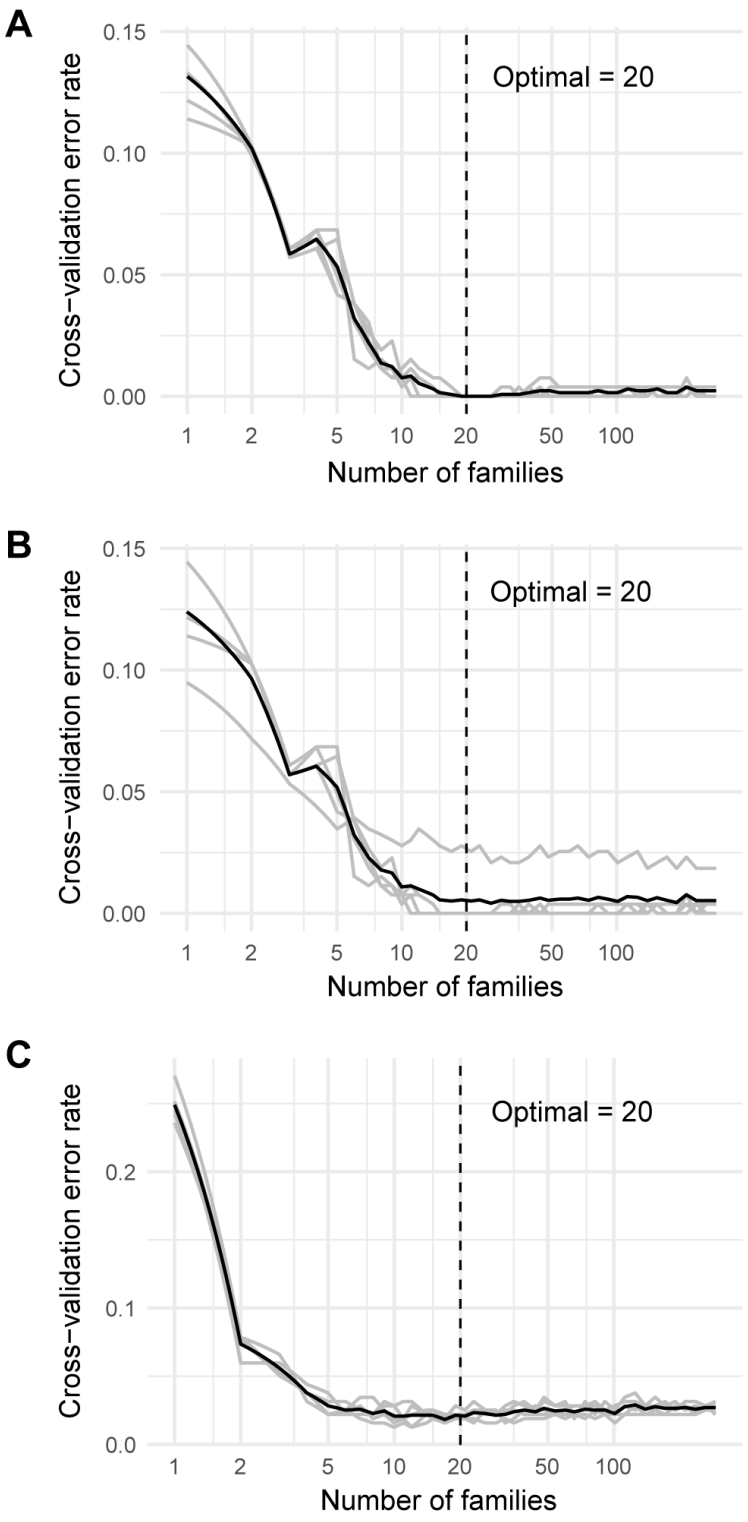

Figure S11 Identification of the number of plastisphere biomarkers.

Ten-fold cross-validation with five repeats revealing that cross-validation error curves have stabilized when 20 microbial families are included with error rates having reduced to a low level in freshwater (**A**), seawater (**B**), and terrestrial (**C**) ecosystems.

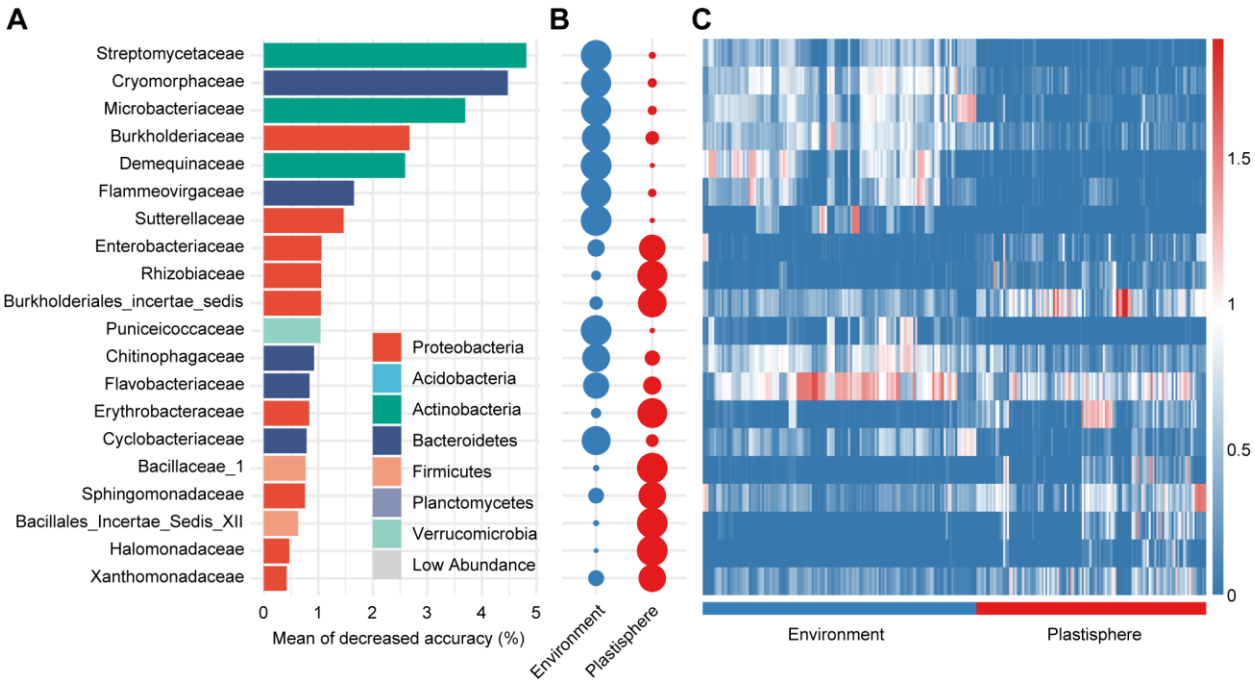

Figure S12 Plastisphere biomarkers in the freshwater ecosystem identified based on a random-forest model.

(**A**) The top 20 microbial families most important to the accuracy of the random-forest classification model for distinguishing the plastisphere from the natural environment were identified as plastisphere biomarkers in the freshwater ecosystem. The biomarker taxa are listed in descending order of importance to the accuracy of the model. (**B**) Relative proportions of mean abundance of the biomarker taxa in the plastisphere and the natural environment. (**C**) Relative abundance profiles for the biomarker taxa in each sample of the plastisphere and the natural environment. Relative abundances are log-transformed for a clear presentation in the heatmap. The numbers of replicated samples used in the model are as follows: the plastisphere (n = 120), the natural environment (n = 143).

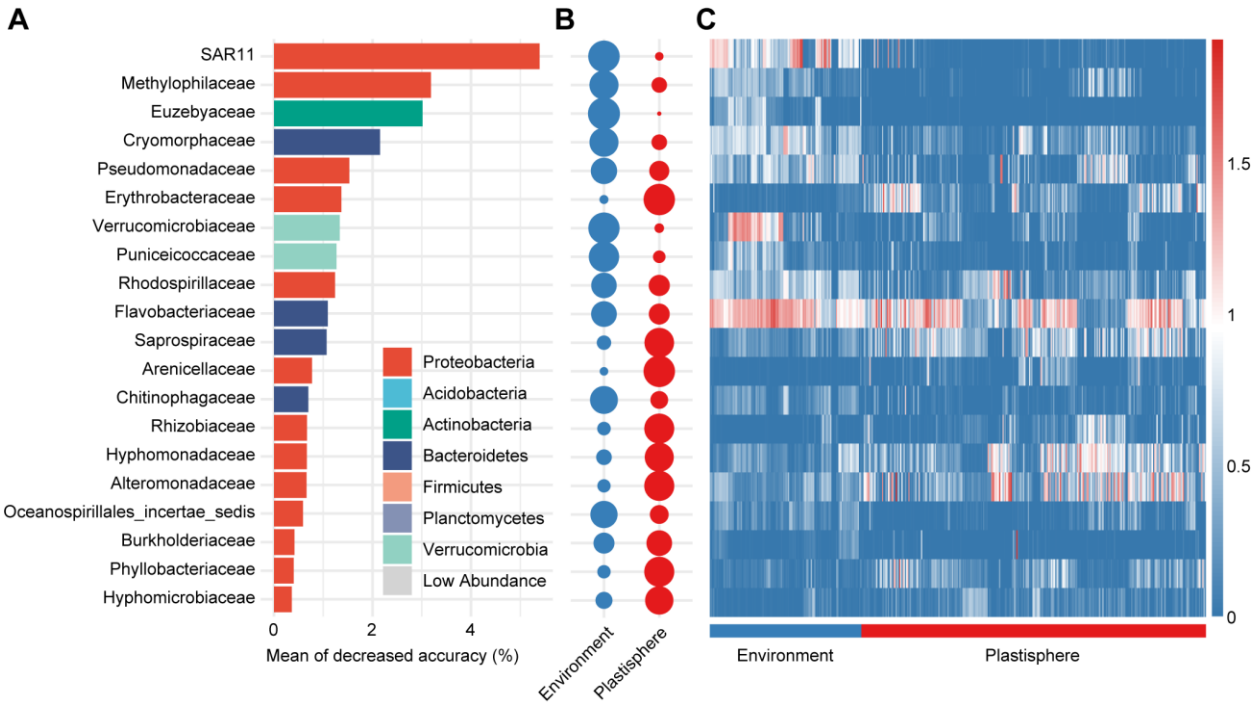

Figure S13 Plastisphere biomarkers in the seawater ecosystem identified based on a random-forest model.

(**A**) The top 20 microbial families most important to the accuracy of the random-forest classification model for distinguishing the plastisphere from the natural environment were identified as plastisphere biomarkers in the seawater ecosystem. The biomarker taxa are listed in descending order of importance to the accuracy of the model. (**B**) Relative proportions of mean abundance of the biomarker taxa in the plastisphere and the natural environment. (**C**) Relative abundance profiles for the biomarker taxa in each sample of the plastisphere and the natural environment. Relative abundances are log-transformed for a clear presentation in the heatmap. The numbers of replicated samples used in the model are as follows: the plastisphere (n = 300), the natural environment (n = 132).

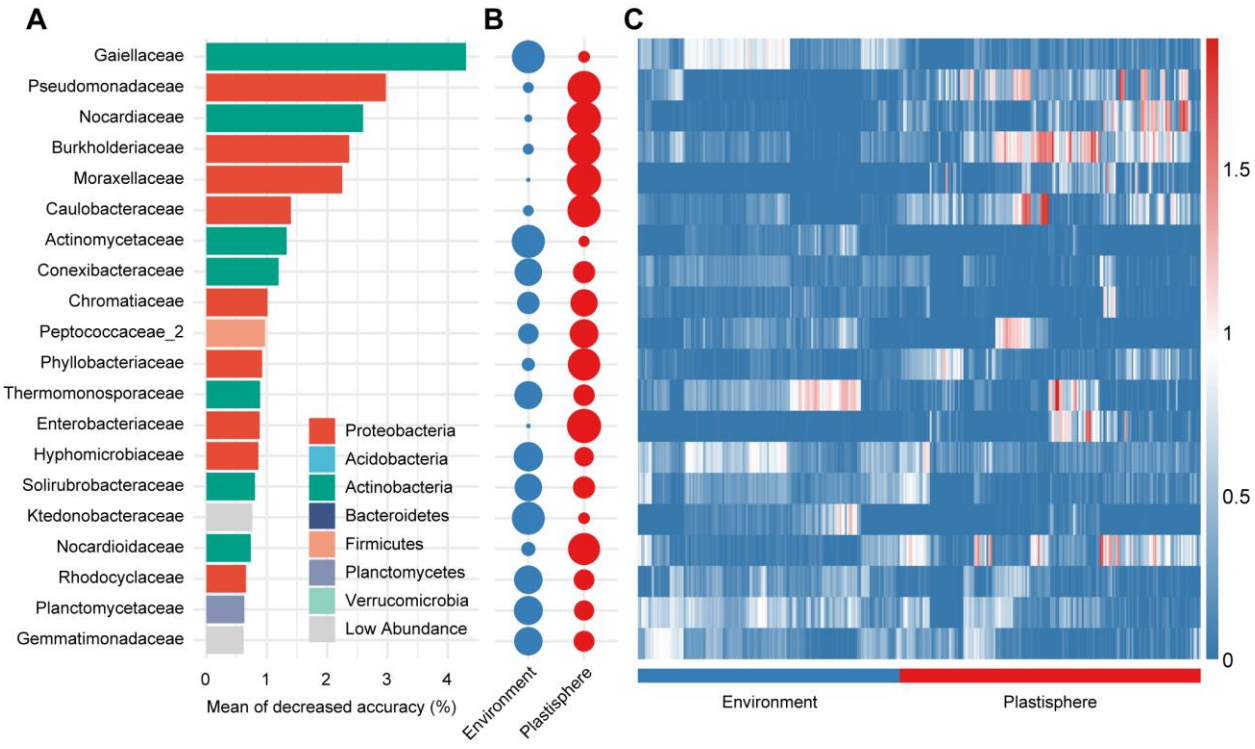

Figure S14 Plastisphere biomarkers in the terrestrial ecosystem identified based on a random-forest model.

(**A**) The top 20 microbial families most important to the accuracy of the random-forest classification model for distinguishing the plastisphere from the natural environment were identified as plastisphere biomarkers in the terrestrial ecosystem. The biomarker taxa are listed in descending order of importance to the accuracy of the model. (**B**) Relative proportions of mean abundance of the biomarker taxa in the plastisphere and the natural environment. (**C**) Relative abundance profiles for the biomarker taxa in each sample of the plastisphere and the natural environment. Relative abundances are log-transformed for a clear presentation in the heatmap. The numbers of replicated samples used in the model are as follows: the plastisphere (n = 170), the natural environment (n = 148).

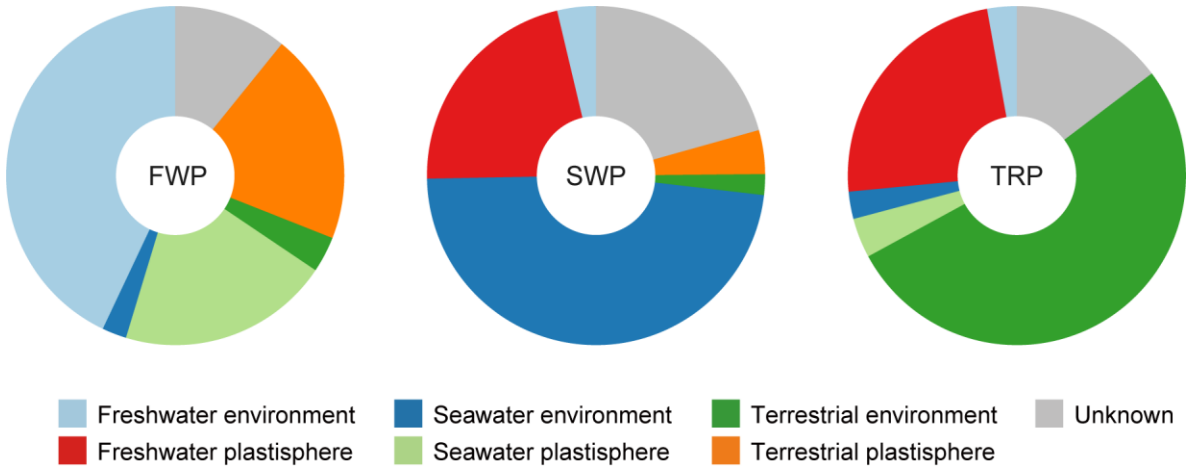

Figure S15 Source analysis.

The fast expectation-maximization for microbial source tracking (FEAST) analysis showing that the corresponding natural environment contributes the largest part, but only a subset, of the sources of microorganisms in the plastisphere.

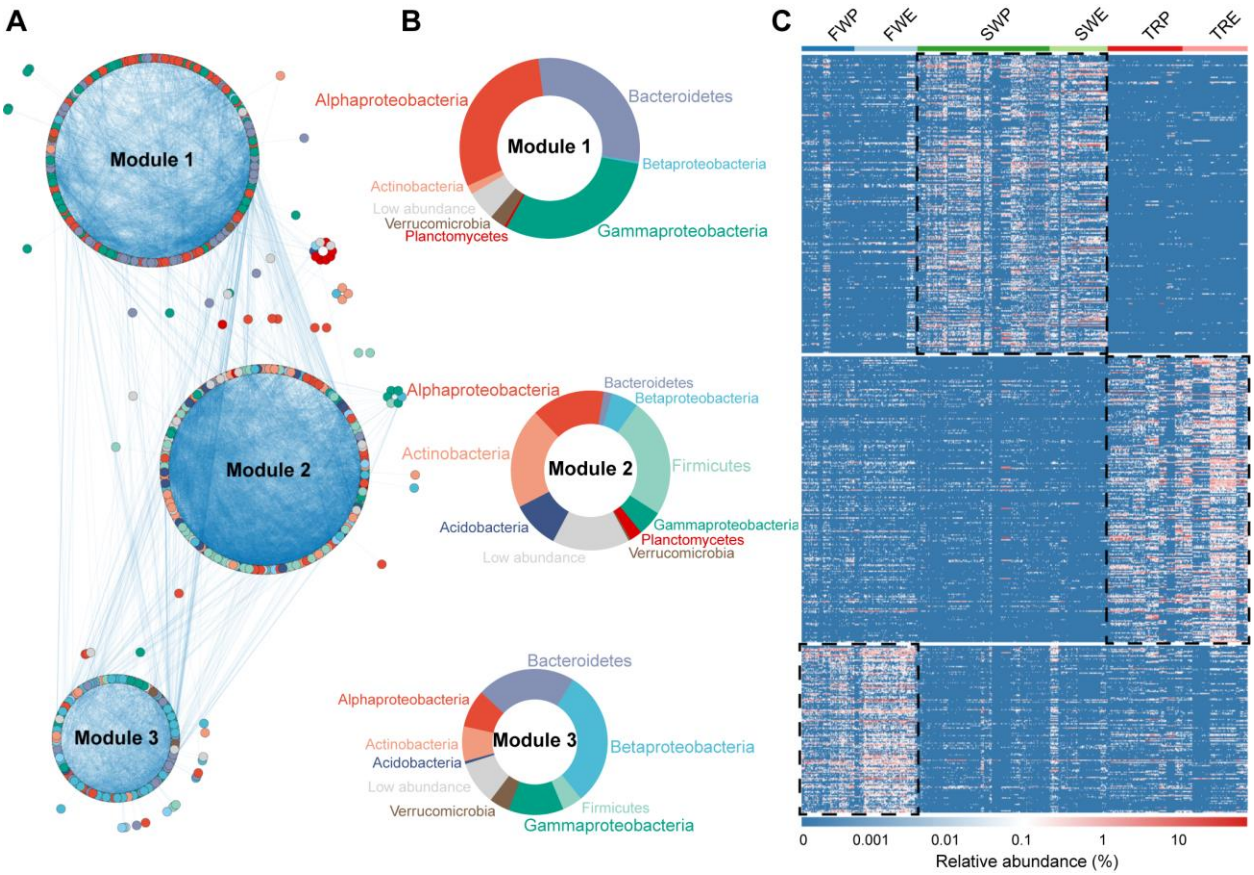

Figure S16 Global ecological meta-network.

(**A**) An overview of the meta-network. Each node represents a unique genus. Each connection between the two nodes represents a significant co-occurrence relationship (Spearman's $\rho > 0.4$ and $P < 0.05$). The size of each module indicates the number of nodes that it contains. The colors of the nodes indicate taxonomic identity. (**B**) Taxonomic composition of the top three largest modules, containing more than 96% of the total nodes in the meta-network. (**C**) Patterns of relative abundance of the nodes in different ecosystems. FWP = freshwater plastisphere; FWE = freshwater environment; SWP = seawater plastisphere; SWE = seawater environment; TRP = terrestrial plastisphere; TRE = terrestrial environment.

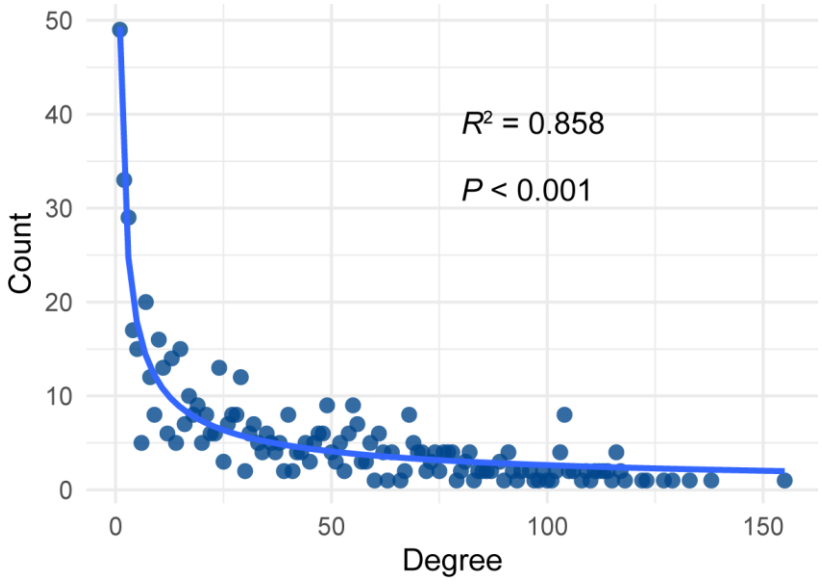

Figure S17 Degree distributions of the microbial ecological meta-network.

R^2 represents the goodness of fit of a power-law model.

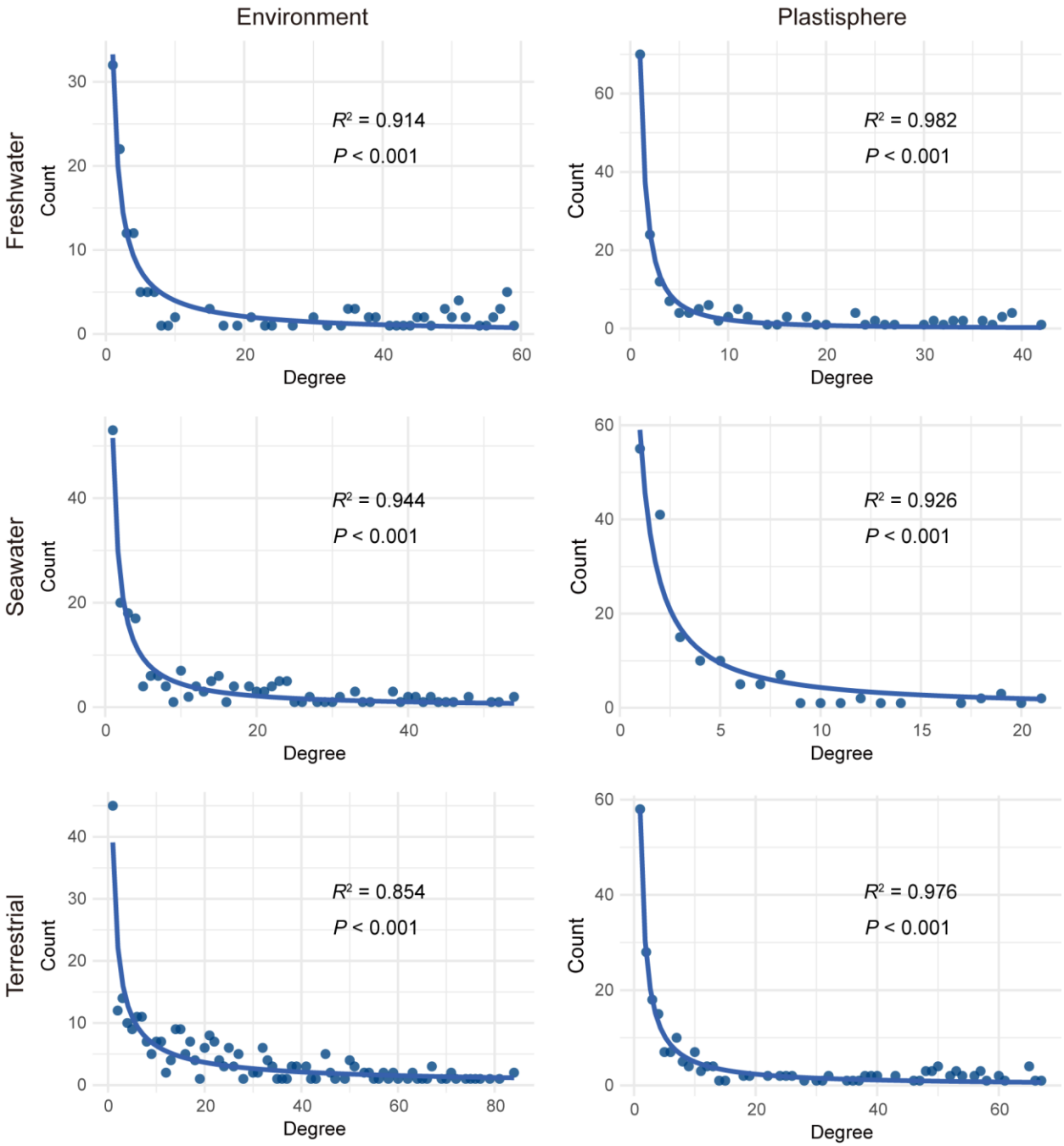

Figure S18 Degree distributions of the microbial ecological sub-networks.

R^2 represents the goodness of fit of a power-law model.

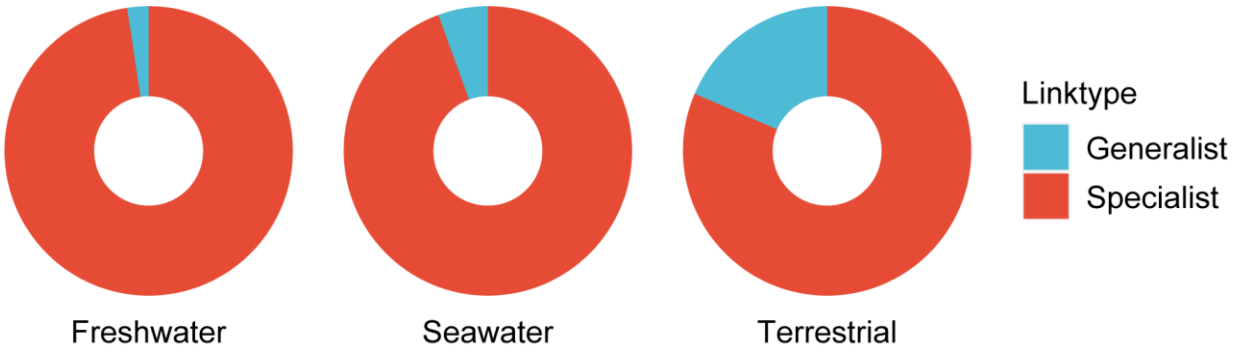

Figure S19 Proportions of specialist links in the plastisphere sub-networks.

The specialist link means that the microbial association occurs only in the plastisphere and not in the corresponding natural environment of that ecosystem. The generalist link means that the microbial association occurs in both the plastisphere and the natural environment.

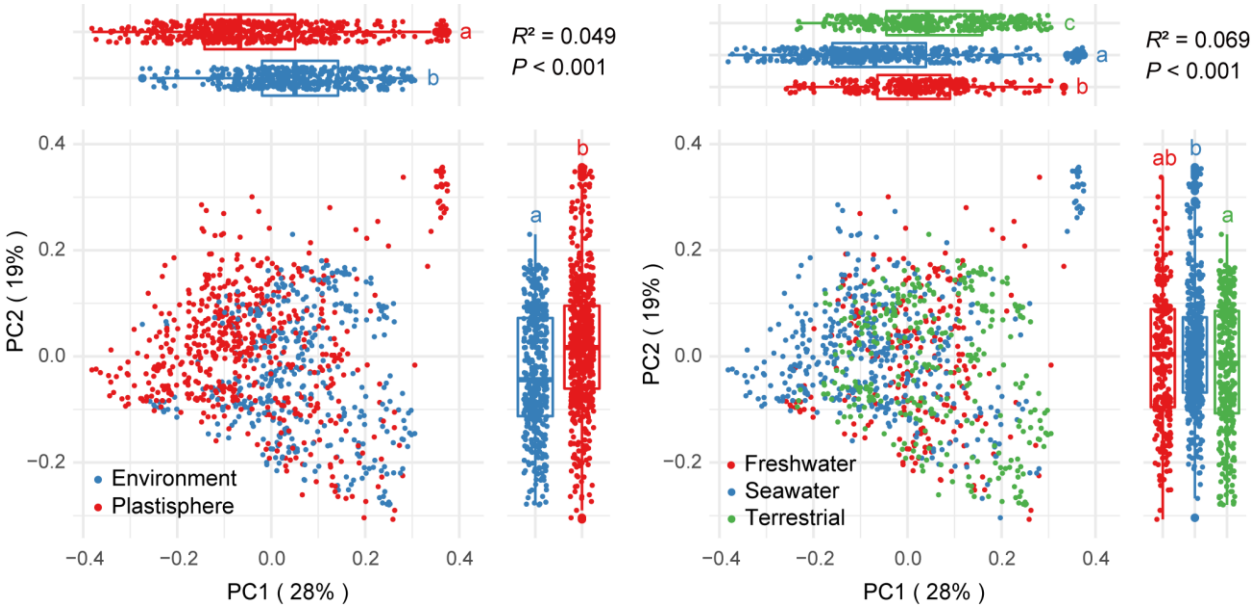

Figure S20 Differences in functional composition between the plastisphere and the natural environment among different ecosystems.

Unconstrained principal coordinates analysis (PCoA) showing the difference in ecologically functional composition between the plastisphere and the natural environment and among different ecosystems, and permutational multivariate analysis of variance (PERMANOVA) showing the statistical significance of the differences ($P < 0.001$).

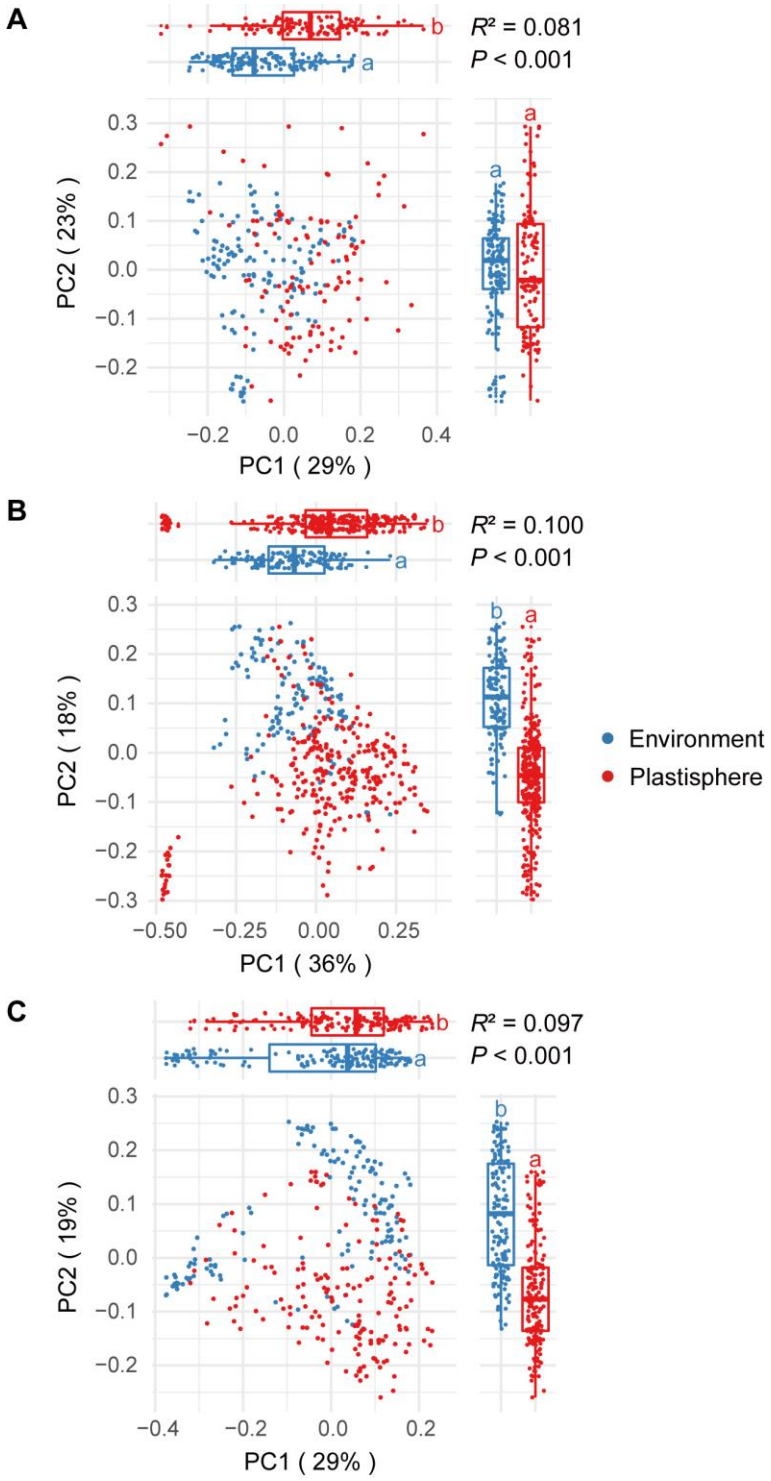

Figure S21 Differences in functional composition between the plastisphere and the natural environment in each ecosystem.

Unconstrained principal coordinates analysis (PCoA) showing the difference in ecologically functional composition between the plastisphere and the natural environment in freshwater (**A**), seawater (**B**), and terrestrial (**C**) ecosystems, and permutational multivariate analysis of variance (PERMANOVA) showing the statistical significance of the differences ($P < 0.001$).

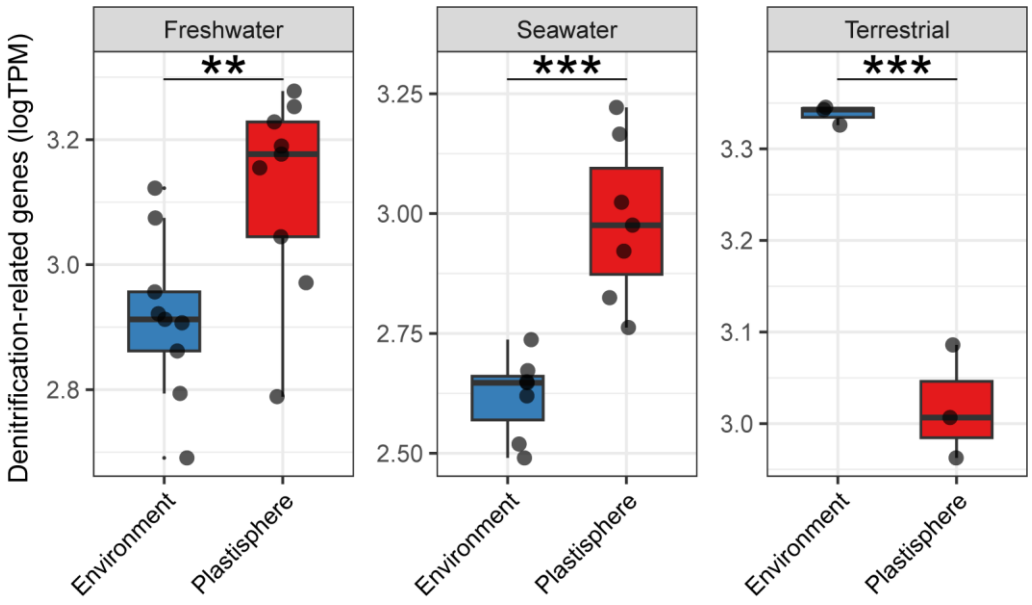

Figure S22 Comparison of the abundance of genes encoding for the denitrification function between the plastisphere and the natural environment.

TPM = transcripts per million. ***P* < 0.01, ****P* < 0.001; *t*-test. The numbers of replicated samples are as follows: freshwater plastisphere (*n* = 9), freshwater environment (*n* = 9), seawater plastisphere (*n* = 7), seawater environment (*n* = 7), terrestrial plastisphere (*n* = 3), terrestrial environment (*n* = 3).

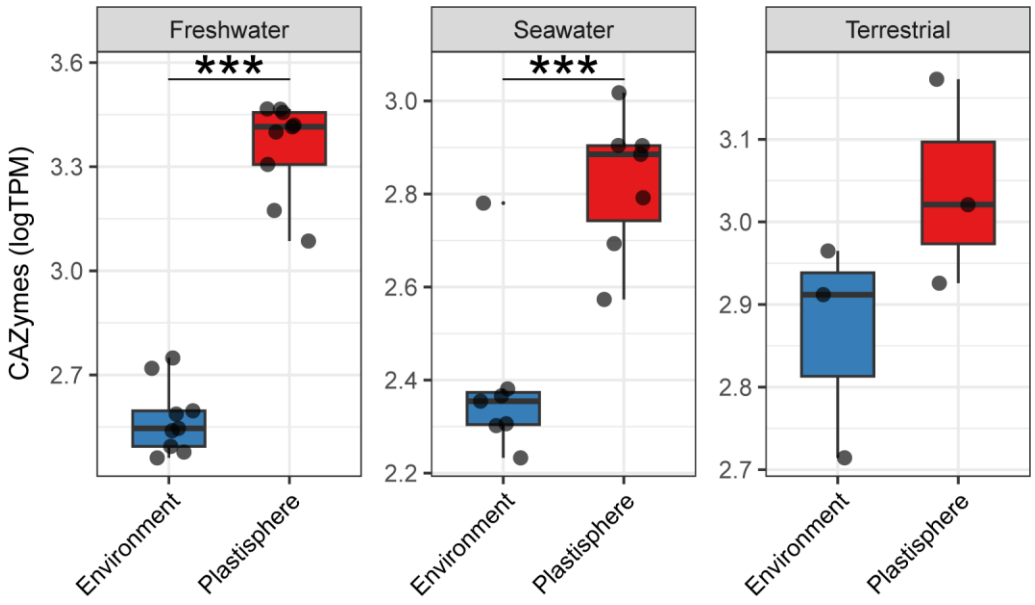

Figure S23 Comparison of the abundance of genes encoding for carbohydrate-active enzymes (CAZymes) between the plastisphere and the natural environment.

TPM = transcripts per million. ***P < 0.001; *t*-test. The numbers of replicated samples are as follows: freshwater plastisphere (n = 9), freshwater environment (n = 9), seawater plastisphere (n = 7), seawater environment (n = 7), terrestrial plastisphere (n = 3), terrestrial environment (n = 3).

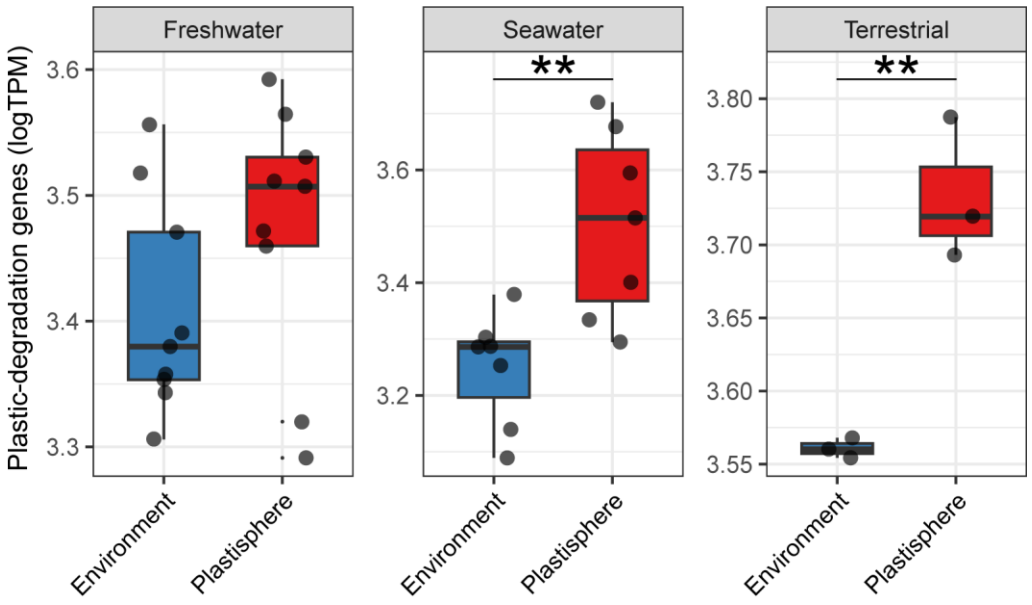

Figure S24 Comparison of the abundance of genes encoding for plastic degradation between the plastisphere and the natural environment.

TPM = transcripts per million. $^{**}P < 0.01$, *t*-test. The numbers of replicated samples are as follows: freshwater plastisphere (n = 9), freshwater environment (n = 9), seawater plastisphere (n = 7), seawater environment (n = 7), terrestrial plastisphere (n = 3), terrestrial environment (n = 3).

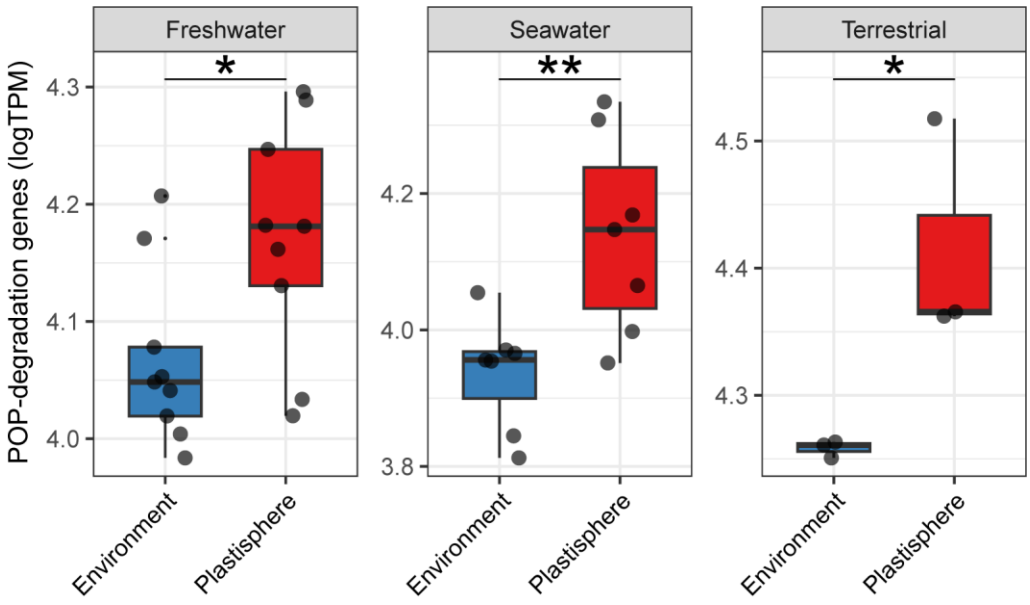

Figure S25 Comparison of the abundance of genes encoding for persistent organic pollutant (POP) degradation between the plastisphere and the natural environment.

TPM = transcripts per million. *P < 0.05, **P < 0.01; t-test. The numbers of replicated samples are as follows: freshwater plastisphere (n = 9), freshwater environment (n = 9), seawater plastisphere (n = 7), seawater environment (n = 7), terrestrial plastisphere (n = 3), terrestrial environment (n = 3).

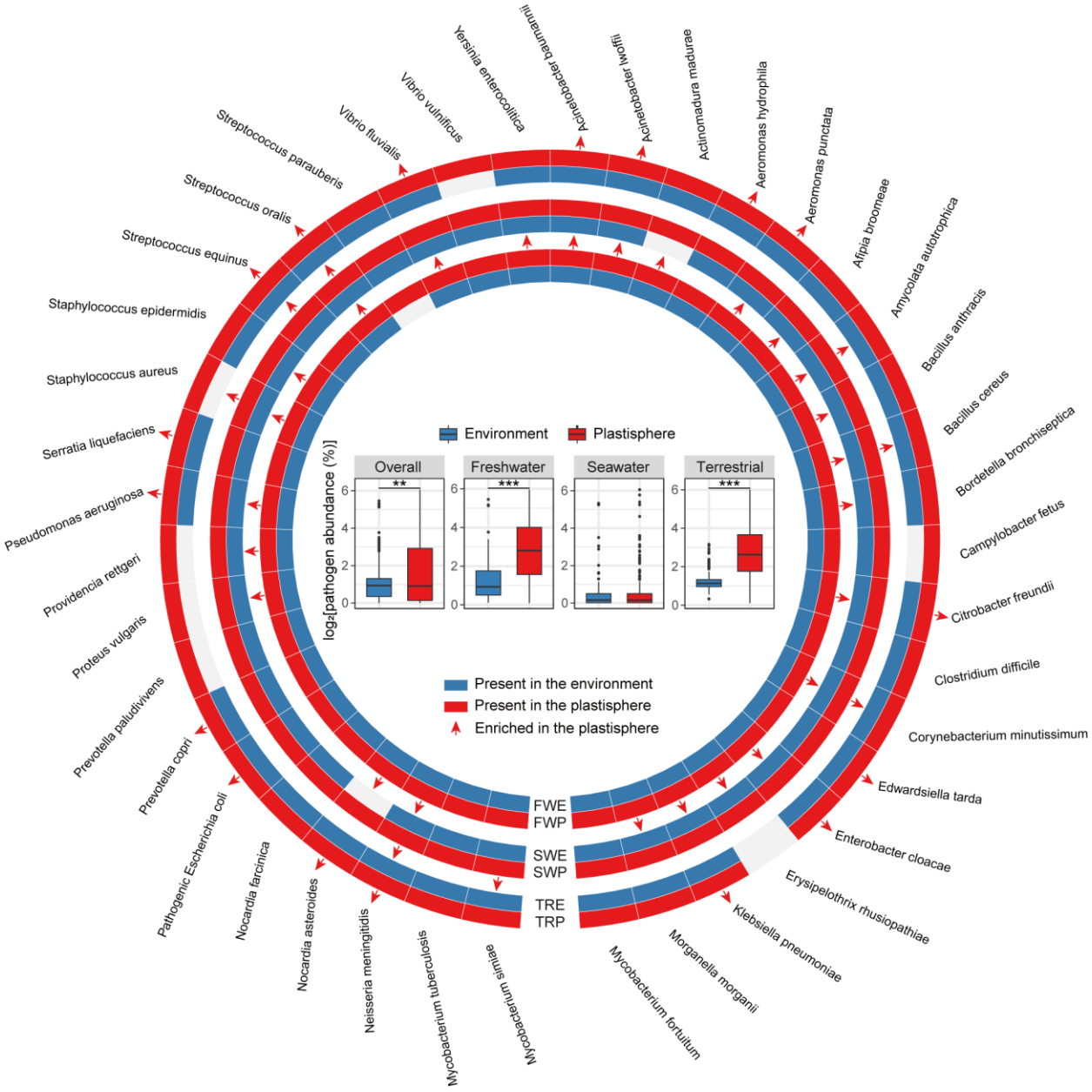

Figure S26 Clinically pathogenic threat of the plastisphere.

Potentially clinical pathogens are identified based on the 16S Pathogenic Identification Process (16SPIP). Box plots showing the difference in the total abundance of the identified pathogens between the plastisphere and the natural environment in each ecosystem (**P < 0.01, ***P < 0.001; Wilcoxon rank sum test). Circle diagram showing the species and number of pathogens that are present in the plastisphere and the natural environment in each ecosystem, and that are enriched in the plastisphere in each ecosystem (P < 0.05; ns = non-significant; Wilcoxon rank sum test). FWP = freshwater plastisphere (n = 120); FWE = freshwater environment (n = 143); SWP = seawater plastisphere (n = 300); SWE = seawater environment (n = 132); TRP = terrestrial plastisphere (n = 170); TRE = terrestrial environment (n = 148).

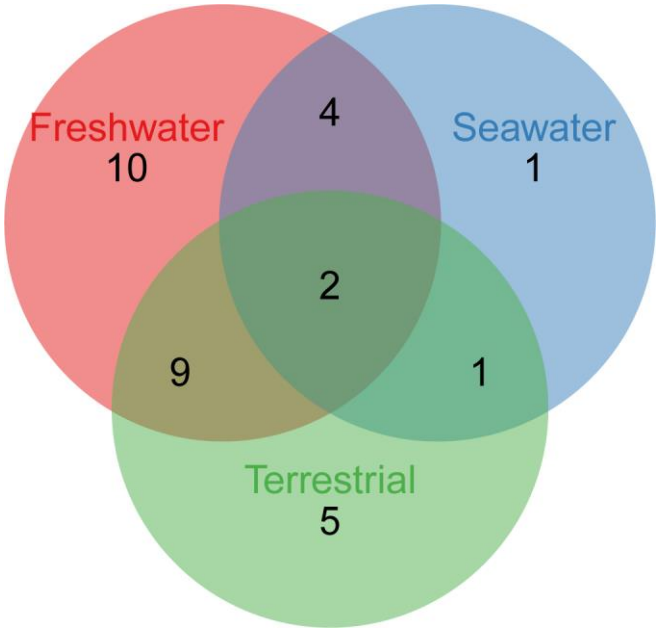

Figure S27 Venn diagram showing that the plastisphere-enriched clinically pathogenic species vary greatly among different ecosystems.

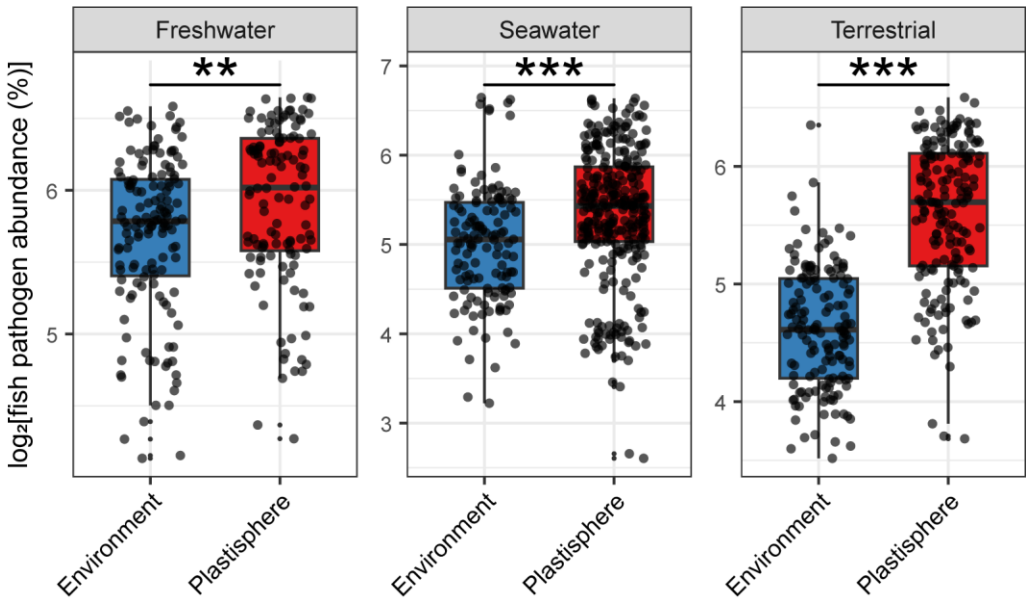

Figure S28 Comparison of the abundance of fish pathogens between the plastisphere and the natural environment.

***P* < 0.01, ****P* < 0.001; Wilcoxon rank sum test. The numbers of replicated samples are as follows: freshwater plastisphere (*n* = 120), freshwater environment (*n* = 143), seawater plastisphere (*n* = 300), seawater environment (*n* = 132), terrestrial plastisphere (*n* = 170), terrestrial environment (*n* = 148).

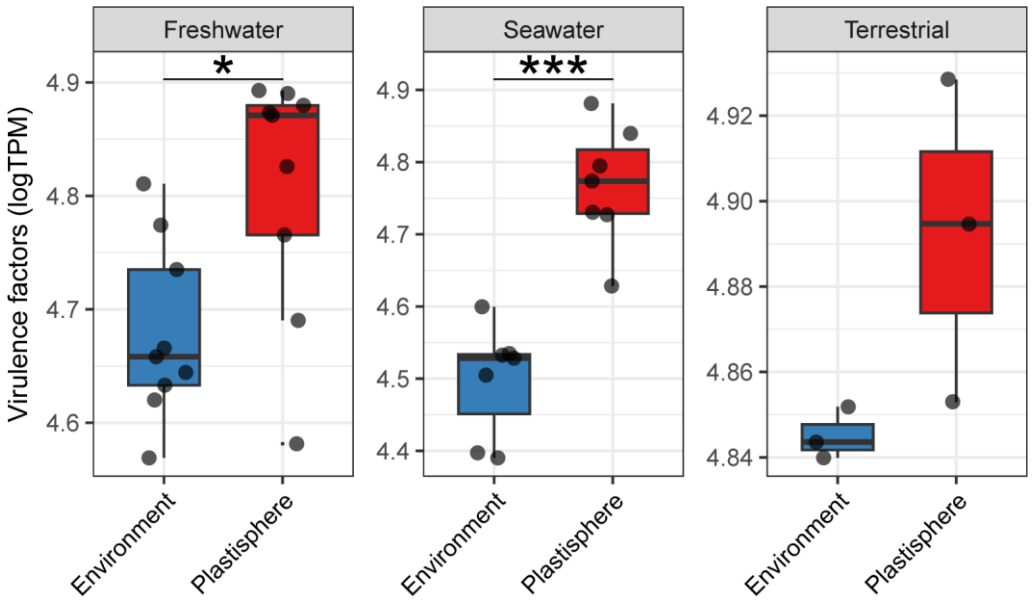

Figure S29 Comparison of the abundance of genes encoding for virulence factors between the plastisphere and the natural environment.

TPM = transcripts per million. *$P < 0.05$, ***$P < 0.001$; *t*-test. The numbers of replicated samples are as follows: freshwater plastisphere (n = 9), freshwater environment (n = 9), seawater plastisphere (n = 7), seawater environment (n = 7), terrestrial plastisphere (n = 3), terrestrial environment (n = 3).

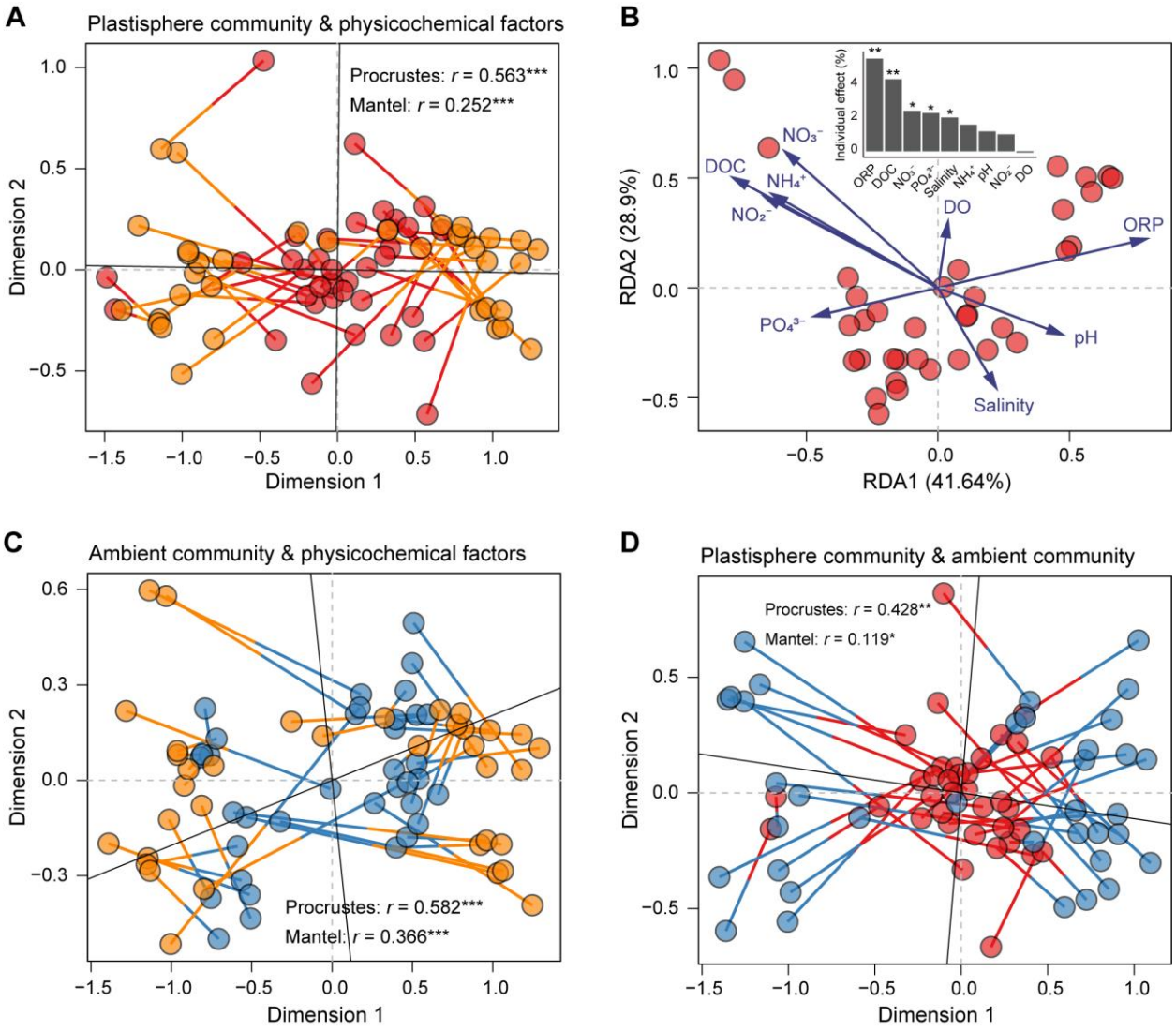

Figure S30 Driving factors of the plastisphere microbiome.

(**A**) Correlations between the plastisphere community and environmental physicochemical properties revealed by Procrustes analysis and Mantel test. (**B**) Potential environmental drivers of the plastisphere microbiome revealed by distance-based redundancy analysis (db-RDA). Correlations between the ambient community and environmental physicochemical properties (**C**), and between the plastisphere community and the ambient community (**D**) revealed by Procrustes analysis and Mantel test. ORP = oxidation-reduction potential, DOC = dissolved organic carbon, DO = dissolved oxygen. *P < 0.05, **P < 0.01, ***P < 0.001.

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