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Plastics in the marine environment are reservoirs for antibiotic and metal resistance genes

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ABSTRACT

Plastics have been accumulated offshore and in the deep oceans at an unprecedented scale. Microbial communities have colonized the plastisphere, which has become a reservoir for both antibiotic and metal resistance genes (ARGs and MRGs). This is the first analysis of the diversity, abundance, and co-occurrence of ARGs and MRGs, and their relationships within the microbial community, using metagenomic data of plastic particles observed in the North Pacific Gyre obtained from the National Centre for Biotechnology Information Sequence Read Archive database. The abundance of ARGs and MRGs in microbial communities on the plastics were in the ranges 7.07×10^{-4}–1.21×10^{-2} and 5.51×10^{-3}–4.82×10^{-2} copies per 16S rRNA, respectively. Both the Shannon-Wiener indices and richness of ARGs and MRGs in plastics microbiota were significantly greater than those of ARGs and MRGs in seawater microbiota in the North Pacific Gyre via one-way analysis of variance. Multidrug resistance genes and multi-metal resistance genes were the main classes of genes detected in plastic microbiota. There were no significant differences in the abundance or diversity of ARGs and MRGs between macroplastics biota and microplastics biota, indicating that particle size had no effect on resistance genes. Procrustes analysis suggested that microbial community composition was the determining factor of the ARG profile but not for MRG. Some ARGs and MRGs had a higher incidence of non-random co-occurrence, suggesting that the co-effects of selection for antibiotic or metal resistance are important factors influencing the resistome of the microbiota on the plastic particles.

1. Introduction

Plastic pollution is of great concern in aquatic environments and is well-documented in seawater ecosystems which include the Mediterranean Sea, the southwestern Indian Ocean (Woodall et al. 2014), the Bohai Sea (Zhang et al. 2017), the Baltic Sea (Tamminga et al. 2018), and the North Atlantic Ocean (Courtene-Jones et al. 2017). Able to resist degradation, plastics are persistent and ubiquitous in the environment (Gewert et al. 2015; Shah et al. 2008). Over 250,000 tons, or > 5 trillion pieces of plastic were estimated to float in the oceans in 2014 (Eriksen et al. 2014). There are three categories of environmental plastic pollution, classified by size: nanoplastics (< 100 nm), microplastics (100 nm–5 mm), and macroplastics (> 5 mm) (Anderson et al. 2016; Galloway et al. 2017). Furthermore, environmental plastics can be ingested by and accumulate in many marine animals throughout the food web (Wright et al. 2013).

Plastics are vectors for inorganic and organic pollutants in water. Concentrations of copper and zinc have been observed to increase significantly on both virgin polystyrene beads and aged polyvinyl chloride fragments in seawater (Brennecke et al. 2016). Various organic pollutants, such as polycyclic aromatic hydrocarbons (Rochman et al. 2013), polychlorinated biphenyls (Velzeboer et al. 2014), and antibiotics (Li et al. 2018a), can be adsorbed onto plastics. Moreover, plastics can also be colonized by different microbial communities, thus becoming vectors for the selection and spread of bacteria and algae (Keswani et al. 2016; Zalasiewicz et al. 2016). The environmental niches for microbial life on plastics are collectively known as the *plastisphere* (Zettler et al. 2013). Several studies have investigated microbial community structures on the plastics in the marine environment (Bryant et al. 2016; Oberbeckmann et al. 2014). Microplastics have recently been found to influence the evolution of microbial communities and to increase the exchange of genes, including antibiotic

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resistance genes (ARGs), between different bacterial taxa in laboratory experiments (Arias-Andres et al. 2018). To date, there has been no published research concerning the abundance and diversity of antibiotic resistance genes in bacterial taxa on plastics in marine environment, although seawater has been identified as a global reservoir of ARGs (Hatosy and Martiny 2015). We suggest that plastics are a more important sink for metal resistance genes (MRGs) and ARGs in marine environment than seawater. In this study, the metagenomic data, available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database, for microbial communities found on the microplastics, macroplastics and seawater of the North Pacific Gyre were used to investigate: (1) the difference in the abundance and diversity of ARGs and MRGs between the plastics microbiota and seawater microbiota; (2) the diversity, abundance, and co-occurrence patterns of ARGs and MRGs in bacterial communities on microplastics and macroplastics in the marine environment; (3) the effect of the category size of plastics (microplastics or macroplastics) on the abundance and diversity of ARGs and MRGs; and (4) the relationship between the species composition of the microbial community and resistance genes (ARGs and MRGs). This study is the first to investigate the distribution of resistance genes in microbe colonies on microplastics and macroplastics, and it will importantly increase our understanding of the marine ecological influence of plastics.

2. Methods and materials

2.1. Data collection and information

The metagenomic data for microbial communities on plastics (microplastics and macroplastics) and in seawater in the North Pacific Gyre were deposited by Bryant et al. (2016) in the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra). The seawater samples were collected in the North Pacific Gyre but not at sites where plastics samples were collected were used as controls for study of resistance genes. Bryant et al. (2016) used the same seawater metagenomic data as controls to investigate the bacterial community inhabiting the plastics. Metagenomic data with the accession numbers SRS1401924 – SRS1401935 (plastics, Table S1), SRX 556050, and SRX 556052 – SRX 556067 (seawater, Table S2) were obtained from the database. The distributions of plastics and the diversity and activity of microbes inhabiting the plastics were comprehensively investigated by Bryant et al. (2016). We used microplastics (2 mm–5 mm) and macroplastics (> 5 mm) from six sampling sites for metagenomic analysis (Table S1 and Fig. S1, Supplementary materials). We downloaded the raw data and processed them as follows Li et al. (2018b): (1) data quality was checked and filtered using PRINSEQ (prinseq-lite.pl with parameter settings mean quality score ≥ 20 and number of ambiguous ≤1); (2) only sequences with length ≥ 100 bp were included, to eliminate inconsistencies in sequences, and then all sequences were trimmed to 100 bp. In the final datasets, the average number of sequences across all samples of plastics was 33,060,796 with a minimum of 967,606 and a maximum of 91,520,464 sequences. The average number of sequences across all seawater samples was 447,292 with a minimum of 162,880 and a maximum of 752,284 sequences.

2.2. Analysis of antibiotic resistance genes

Reference ARGs for analysis of the metagenomic data of plastics (microplastics and macroplastics microbiota) and seawater from the North Pacific Gyre were retrieved through the ARGs-OAP online pipeline (http://smile.hku.hk/SARGs) following the procedures in previous publications (Li et al. 2018b; Yin et al. 2018). Briefly, the UBLAST algorithm, using Perl script supplied by the platform, was run to prescreen ARG-like and 16S rRNA gene sequences. The candidate ARG sequences were matched against the SARG database using BLASTX. The sequences found that met the BLASTX criteria (alignment length 25 aa, similarity 80%, and e-value $1e^{-5}$) were classified according to the SARG hierarchy (type–subtype–sequence). ARG abundance (copies of ARG per copy of 16S rRNA) in the metagenomic data was calculated and recorded.

2.3. Analysis of metal resistance genes

MRGs in the metagenomic data were identified using the protocol followed by Gupta et al. (2018). Briefly, experimentally confirmed MRGs were downloaded from the BacMet database (Version 2.0) as reference (Pal et al. 2014). Clean reads of MRGs were matched against the BacMet reference dataset using BLASTX with the following search criteria: e-value 10^{-5}, amino acid identity ≥90%, and alignment length > 25 amino acids (Buchfink et al. 2014; Kristiansson et al. 2011). The abundance of MRGs was calculated and recorded.

2.4. Statistical analysis

Differences in the abundance and diversity of ARGs and MRGs between microplastics, macroplastics and seawater microbiota were analyzed using one-way analysis of variance (ANOVA). Heatmaps of ARGs and MRGs were generated using the open-source software R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria) with pheatmap (Kolde 2013) and heatmap.2 within the gplots package (Warnes et al. 2015). The Shannon-Wiener indices and the richness of ARGs and MRGs were found using the R 3.4.1 vegan package (Oksanen et al. 2013).The co-occurrence of ARGs and MRGs in microplastics and macroplastics biota was analyzed by Spearman rank correlation using R software. Data for the co-occurrence of resistance genes and the composition of the microbial communities on the plastics was obtained from Bryant et al. (2016), who analyzed the same metagenomic data using R for Spearman analysis. The correlation network was visualized using open-source Gephi software (Bastian et al. 2009). In the network, the observed (O%) and random incidences (R%) of co-occurrence or correlation of ARG and MRG were calculated and statistically analyzed using the methods in previous publications (Ju et al. 2016; Ju and Zhang 2015). The ratio of O% to R% (O/R ratio) was used as a threshold to determine non-random co-occurrence patterns (Ju et al. 2016; Ju and Zhang 2015). Pairwise concordance between resistance gene (ARG and MRG) profile and microbial community composition on the plastics was investigated using non-metric multidimensional scaling (NMDS) Procrustes analysis with 999 permutations using data of the microbial community and the resistance gene profiles. The statistical test was performed by the protest function in the R package Vegan (Oksanen et al. 2013). A p value of the Procrustes analysis < 0.05 indicated a significant correlation between the resistance gene (ARG or MRG) and the composition of the microbial community on the plastics.

3. Results

3.1. Antibiotic resistance genes on microplastics and macroplastics

ARGs were found in all the plastic samples, but only in 4 of the 17 seawater samples. There was no significant difference in the relative abundance of total ARGs between macroplastics and microplastics biota ($p = 0.441$), but the relative abundance of total ARGs in both macroplastics and microplastics was significantly greater than for seawater (one-way ANOVA, $p < 0.05$) (Fig. 1a). The Shannon-Wiener indices and the richness of ARGs in both macroplastics and microplastics were also significantly higher than for seawater ($p < 0.05$) (Fig. S2, Supplementary materials). The average relative abundance of total ARGs in macroplastics (6.55×10^{-3} copies per 16S rRNA) and microplastics biota (5.15×10^{-3} copies per 16S rRNA) were 7.23 and 5.69 times higher than those in seawater (9.06×10^{-4} copies per 16S rRNA). These results indicated that ARGs on plastics in the marine environment were more abundant and more diverse than in seawater. Highest

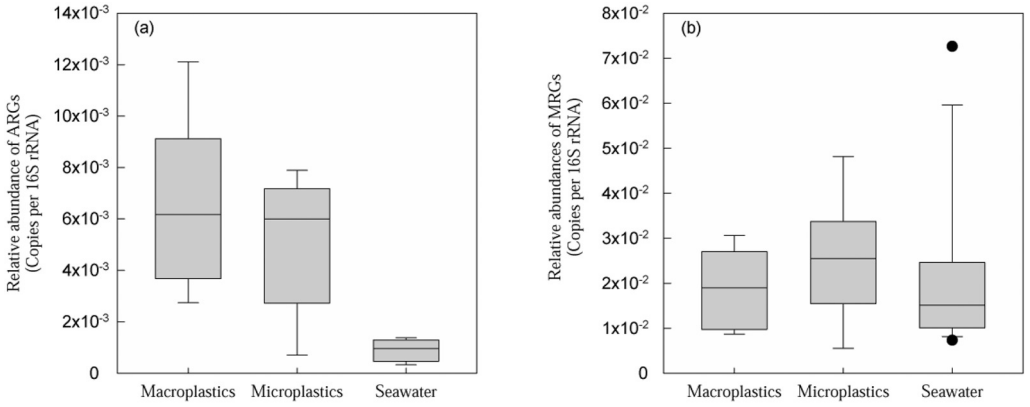

Fig. 1. Boxplots of the relative abundance of (a) antibiotic resistance genes and (b) metal resistance genes in macroplastics, microplastics and seawater samples.

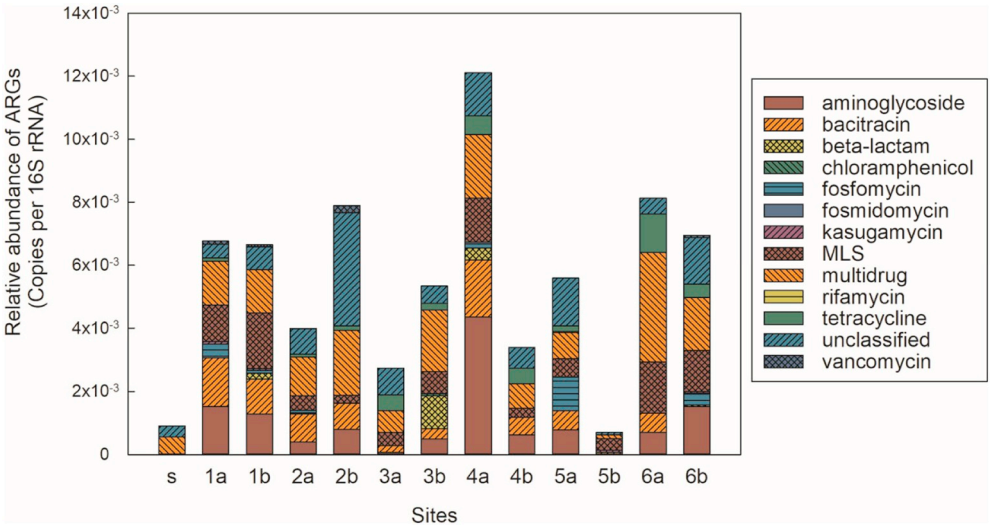

Fig. 2. The relative abundance of antibiotic resistance genes in (s) seawater, (a) macroplastics microbiota and (b) microplastics microbiota at different sampling sites (MLS: macrolide–lincosamide–streptogramin).

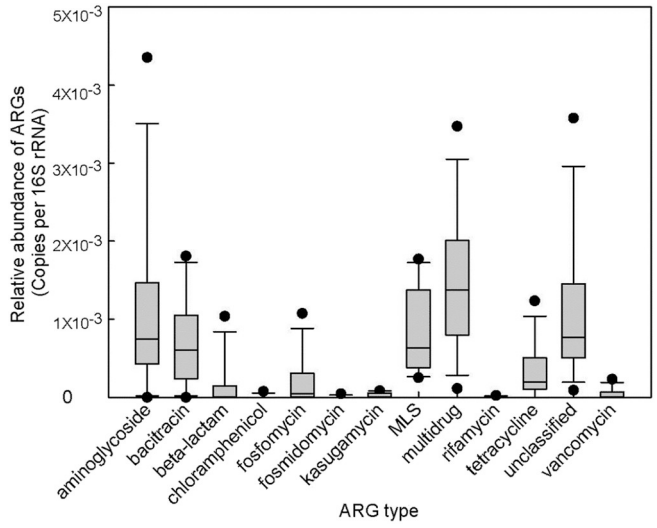

Fig. 3. Comparison of different ARGs in both macroplastics microbiota and microplastics microbiota combined (MLS: macrolide–lincosamide–streptogramin).

relative abundance of total ARGs was observed in macroplastics biota at site 4 with values of 1.21×10^{-2} copies per 16S rRNA, followed by macroplastics biota at site 6 (8.13×10^{-3} copies per 16S rRNA) and microplastics biota at site 2 (8.13×10^{-3} copies per 16S rRNA) (Fig. 2). Thirteen ARG types that provide resistance to various antibiotics were found in the microbiota on the plastics, while only two ARG types (multidrug resistance genes and unclassified ARGs) were found in the seawater samples. Multidrug-resistance genes showed the highest average relative abundance in the plastics microbiota (1.47×10^{-3} copies per 16S rRNA), followed by aminoglycoside-resistance genes and unclassified ARGs (Fig. 3). ARGs that provide resistance to chloramphenicol, fosmidomycin, kasugamycin, rifamycin or vancomycin were detected at 10^{-6}–10^{-5} copies per 16S rRNA, which are lower values than for other ARGs ($p < 0.05$).

We found 64 ARG subtypes that provide resistance to 13 kinds of antibiotics in the macroplastics and microplastics biota (Fig. S3 and Table S3, Supplementary materials), while only 6 ARG subtypes were found in the seawater samples (Table S4, Supplementary materials). It can be seen that most ARGs were detected at levels $< 10^{-4}$ copies per 16S rRNA. An aminoglycoside resistance gene (*aac*(3) − I), an unclassified resistance gene (transcriptional regulatory protein CpxR cpxR), a bacitracin resistance gene (*bac*A), an MLS (macrolide-lincosamide-streptogramin) resistance gene (*mac*B), and multidrug resistance genes (*mex*F and multidrug ABC transporters) had average relative abundance $> 4 \times 10^{-4}$ copies per 16S rRNA, higher than for other ARG subtypes detected in this study. ARGs at different sites could

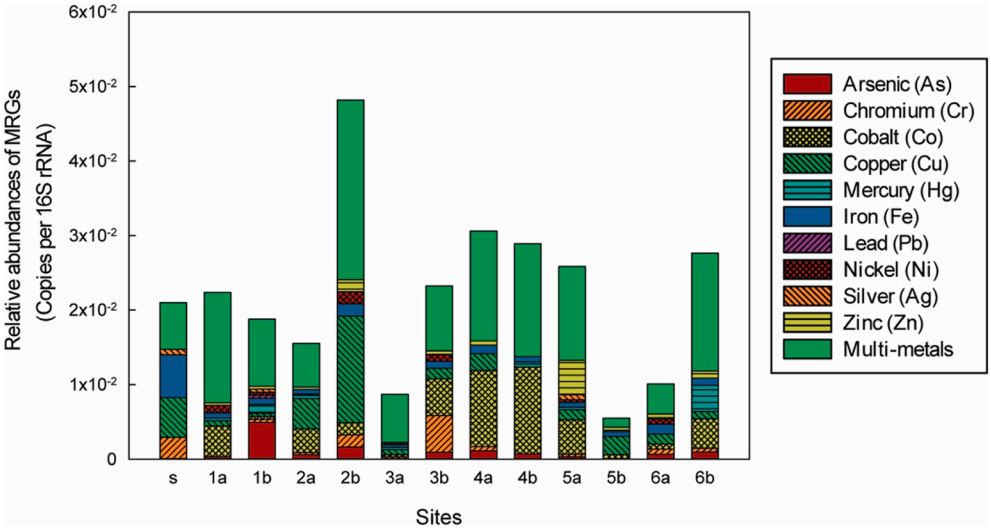

Fig. 4. The relative abundance of metal resistance genes in (s) seawater, (a) macroplastics microbiota and (b) microplastics microbiota at different sampling sites.

not be characterized according to the plastic size (microplastic or microplastic; Figs. S3 and S4, Supplementary materials). Thus, plastics size did not influence ARG abundance or diversity.

3.2. Metal resistance genes on microplastics and macroplastics

MRGs were found in all the microplastics and macroplastics biota, but only in 13 of the 17 seawater samples. There was no significant difference in the relative abundance of total MRGs between the microplastics, macroplastics and seawater samples (one-way ANOVA, $p < 0.05$) (Fig. 1b). However, both the Shannon-Wiener indices and the richness of MRGs in macroplastics and in microplastics were significantly greater than for seawater ($p < 0.05$) (Fig. S5, Supplementary materials). These results indicated that the plastics had more varieties of MRGs than the surrounding seawater. Highest relative abundance of total MRGs was found in microplastics biota at site 2 (4.82×10^{-2} copies per 16S rRNA), then in microbial communities on both microplastics (4.82×10^{-2} copies per 16S rRNA) and macroplastics (2.89×10^{-2} copies per 16S rRNA) at site 4 (Fig. 4). The relative abundance of MRGs in plastics was 10^{-3}–10^{-2} copies per 16S rRNA, which is significantly higher than the relative abundance of ARGs ($p < 0.05$). Multi-metal resistance genes were detected with highest relative abundance in the microbial communities of both plastics combined (average value of 1.10×10^{-2} copies per 16S rRNA), followed by cobalt resistance genes (3.86×10^{-3} copies per 16S rRNA) and copper resistance genes (2.49×10^{-3} copies per 16S rRNA) (Fig. 5). The lowest relative abundances were found for lead and silver resistance genes, which were detected at 10^{-5} copies per 16S rRNA level.

Forty-seven MRG subtypes, which provided resistance to eleven metals, were detected in microbial communities on both plastics combined (Fig. 5 and Table S5, Supplementary materials). However, 15 MRG subtypes, providing resistance to five metals, were found in the seawater samples (Table S6, Supplementary materials). We divided the forty-seven MRG subtypes into three groups (Fig. S6, Supplementary materials). Group I included the arsenic resistance gene *arsH* and the cobalt resistance gene *corT/coaT*. These two genes were found at a high relative abundance in microbiota on all the sampled plastics except *corT/coaT* on macroplastics biota at site 4. Group II included copper resistance genes *copA* and *actP* and the arsenic resistance gene *arsB*. These three genes had high relative abundance in microplastics biota at site 2. The remaining 42 MRG subtypes constituted the third group (Group III), which were detected with low frequency and low relative abundance. MRG abundance in the microbial communities on the

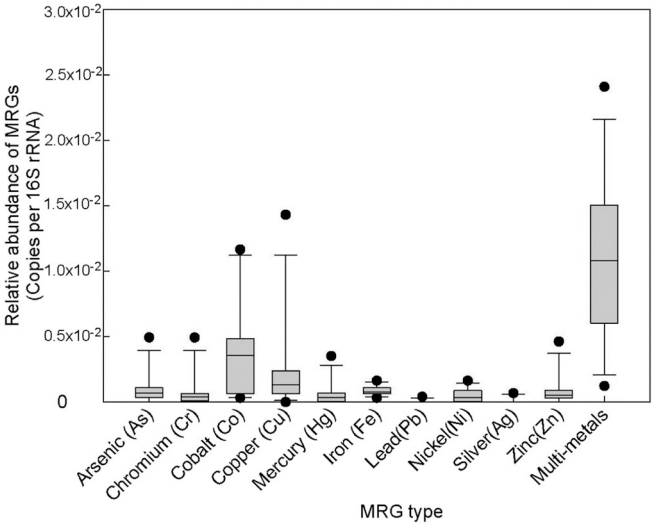

Fig. 5. Comparison of different MRGs in macroplastics microbiota and microplastics microbiota combined.

plastics at different sampling sites could not be clustered by plastic size (microplastics or macroplastics; Figs. S6 and S7, Supplementary materials). Thus, plastic size had no effect on the relative abundance and diversity of MRGs.

3.3. Relationship between ARGs and MRGs

There was no correlation between the relative abundance of total ARGs and MRGs on the plastics via Pearson analysis ($p > 0.05$) (Fig. 6). Spearman correlation analysis of the co-occurrence of ARG and MRG subtypes was shown in Fig. 7. Analysis of the network showed 12 modules. Module 1 had the most complex correlations between the resistance genes. The gene at the hub of module1, *tro*B, which encodes resistance to Zinc (Zn), Manganese (Mn), and Iron (Fe), had significant Spearman correlations with one MLS resistance gene (*vat*D), one beta-lactam resistance gene (bla_{VEB-6}), four multidrug resistance genes (*mdt*C, *mdt*F, *mdt*B, and *amr*B), and one mercury resistance gene (*mer*R). A non-random co-occurrence pattern of resistance genes was found within or across ARGs and MRGs (Fig. 7 and Table S7). Only genes conferring resistance to tetracycline (7 subtypes) and beta-lactam (8 subtypes) tended to co-occur (O% = 5.55%) more than would be

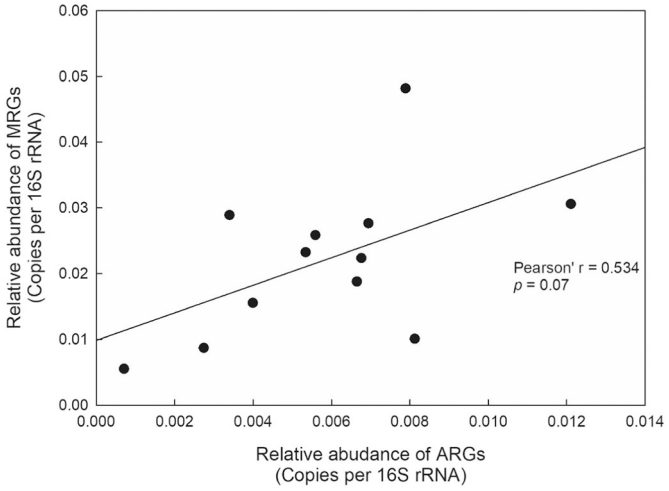

Fig. 6. Correlation between the relative abundance of ARGs and MRGs in microbial communities for all the plastic samples combined.

expected under random association (R% = 1.63%) within the same type. Higher non-random co-occurrences were found for different ARG and MRG subtypes. Multidrug resistance genes and multimetal resistance genes tended to co-occur nonrandomly, with highest O % = 10.56% and O/*R* = 2.2. Higher co-occurrences than would be expected by chance were also observed between the ARG subtypes conferring resistance to multidrugs and ARG/MRG subtypes conferring resistance to beta-lactam (O% = 5.56%, *R* = 2.09%), MLS (O % = 4.44%, *R* = 1.05%), mercury (O% = 3.33%, *R* = 0.78%), silver (O % = 2.78%, *R* = 0.52%), nickel (O% = 2.22%, R = 1.05%), aminoglycoside (O% = 1.11%, R = 0.52%), and chloramphenicol (O % = 1.11%, *R* = 0.26%). Similar co-occurrence patterns were also observed between genes conferring resistance to multi-metals and genes conferring resistance to MLS (O% = 2.78%, R = 1.05%), mercury (O % = 1.67%, R = 0.78%), silver (O% = 1.67%, R = 0.52%), zinc (O % = 1.67%, R = 0.26%), and vancomycin (O% = 1.11%, R = 0.52%).

3.4. Relationship between microbial diversity and resistance genes

Procrustes analysis provides an overall correlation between resistance genes and the bacterial community. The *p* value of Procrustes analysis between the ARG profile and the bacterial community was

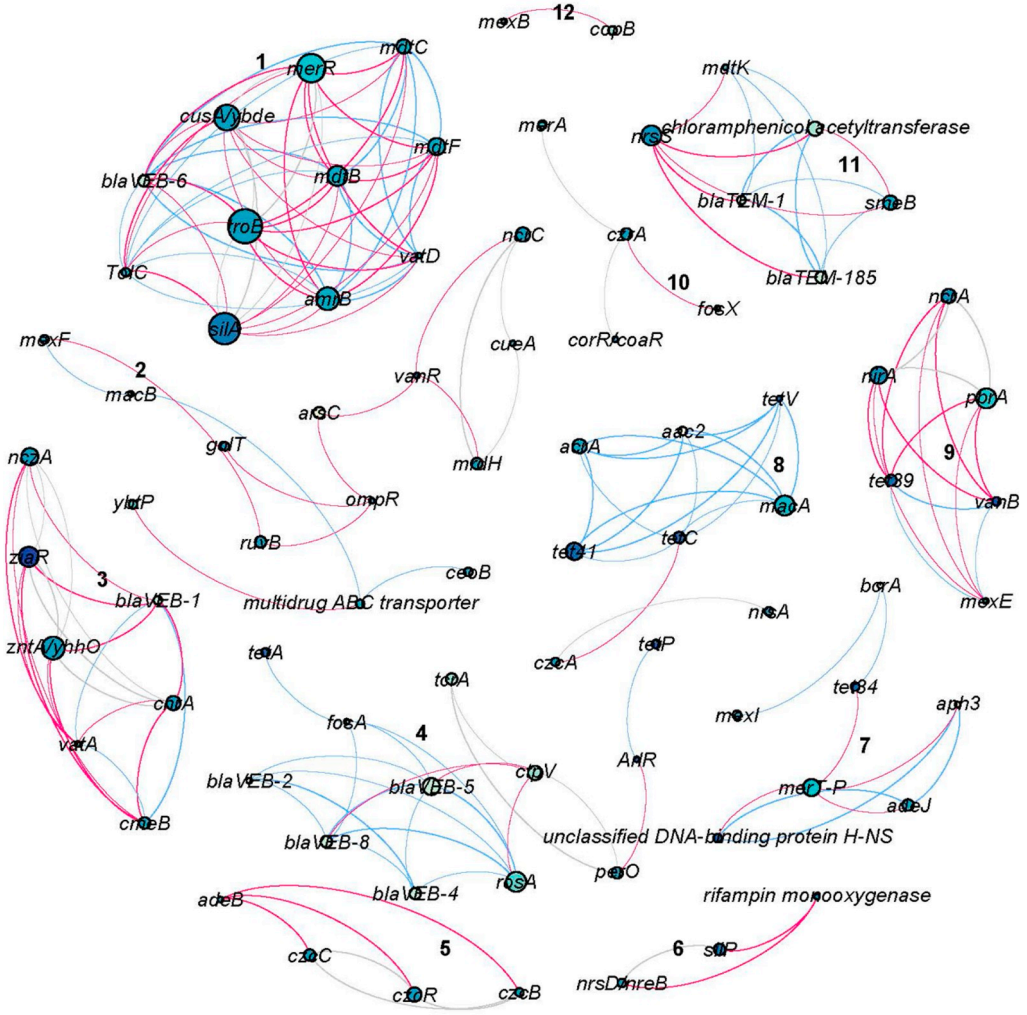

Fig. 7. Co-occurrence patterns among ARG and MRG subtypes shown by network analysis. The numbers in the figure indicate different modules. A connection represents a strong correlation based on Spearman analysis ($r > 0.7$ and $p < 0.01$). Red indicates connections between ARG and MRG subtypes; gray indicates connections between different MRG subtypes; and blue indicates connections between different ARG types. The size of each node is proportional to the number of connections. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

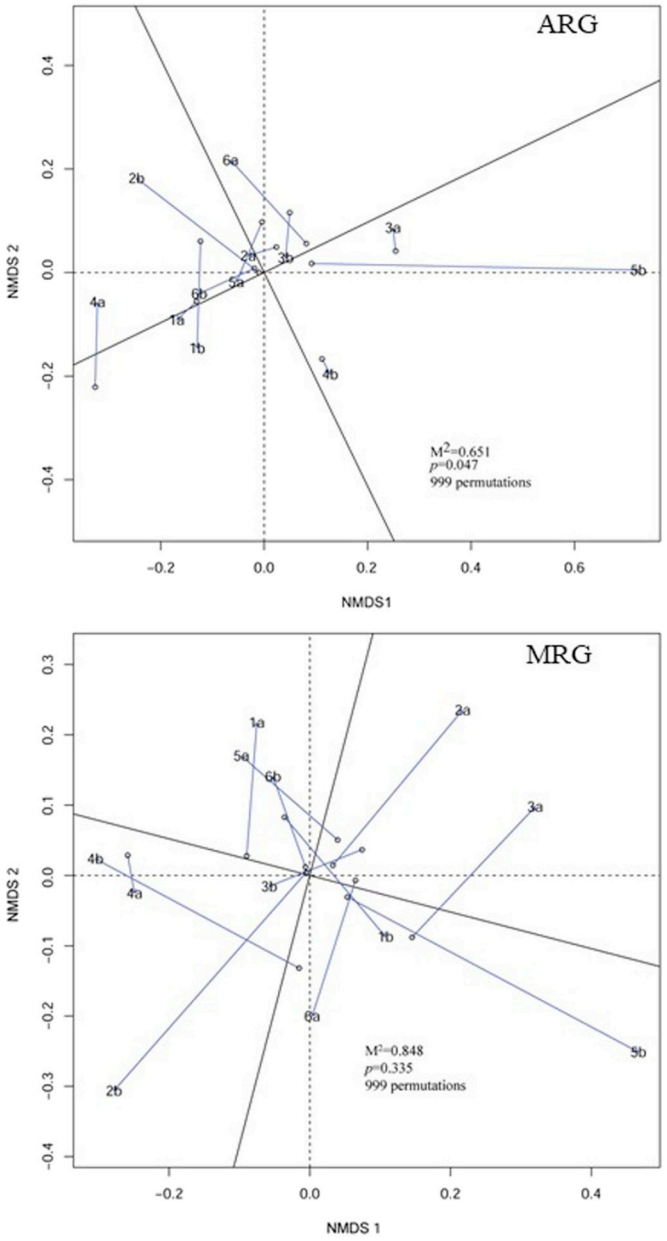

Fig. 8. Procrustes analysis of the correlations between resistance genes (ARGs and MRGs) and microbial community based on the NMDS (Bray-Curtis) results of resistance gene data and microbial community data.

0.047, while the *p* value of Procrustes analysis between the MRG profile and the bacterial community was 0.34 (Fig. 8). These results indicate that there was a significant congruence between bacterial community and the ARG profile, but no significant congruence between the bacterial community and the MRG profile. The co-occurrence pattern of ARG and the bacterial community is shown in Fig. S8 (Supplementary materials). 11 subtype ARGs confer resistance to five kinds of antibiotics (aminoglycoside, multidrugs, bacitracin, tetracycline, and MLS) were proposed to be contained by the following groups of organisms: *Flavobacteriaceae*; *Muricauda*, Cyanobacteria subsection III family I, *Sneathiellaceae*; *Sneathiella* and *Flammeovirgaceae*; *Tunicatimonas* and *Flammeovirgaceae*; and *Reichenbachiella* (Fig. S8). Three subtype MRGs (the copper resistance gene *ctp*V and the multimetal resistance genes *cor*R/*coa*R and *czc*A) may be contained in Cyanobacteria, *Flammeovirgaceae*; *Tunicatimonas* and *Flavobacteriaceae*; *Muricauda* (Fig. S9, Supplementary materials). Thus, bacteria of the family *Flavobacteriaceae* may be important hosts for both ARGs and MRGs.

4. Discussion

To our knowledge, this is the first study to use metagenomic analysis to investigate the abundance and diversity of ARGs and MRGs in microbial communities on plastic particles in a marine environment. The Shannon-Weiner indices and richness of ARGs and MRGs in plastics microbiota were all greater than those of ARGs and MRGs obtained from seawater microbiota. These results indicate that plastics are an important reservoir for ARGs and MRGs in the marine environment when compared to seawater. The particle size of the plastics strongly influences sorption capacity and the time taken for traditional pollutant loads to reach equilibrium (Goedecke et al. 2017). Although the greatest relative abundances of ARGs and MRGs were observed in macroplastics microbiota at site 4 and microplastics microbiota at site 2, we found in this study that their diversity and abundance in the plastic debris did not depend on particle size (microplastic or macroplastic). Bryant et al. (2016) also confirmed that the nature of the bacterial communities on the plastics in the North Pacific Gyre does not depend on particle size. Procrustes analysis showed that the distribution of ARGs in the plastics was largely shaped by the bacterial community, but this was not the case for the MRG distribution. The bacterial community as a driver of resistome composition has been discussed in various environments (Yang et al. 2018), such as lakes (Bengtsson-Palme et al. 2014; Chu et al. 2018) and rivers (Zheng et al. 2017; Zhou et al. 2017). We suppose that pollutants adsorbed on the plastics might be an important factor influencing the composition of ARGs and MRGs on the plastics at different sites. Metals are absorbed by and accumulated in the microplastics, with concentrations that can reach > 600 times those found in seawater (Brennecke et al. 2016). Microplastics are also vectors for organic pollutants, such as antibiotics (Li et al. 2018a) and polycyclic aromatic hydrocarbons (Rochman et al. 2013). However, more study is needed to understand the relationship between the pollutants and resistance genes in marine plastics in both field studies and microcosm research.

In this study, 64 ARG subtypes of 11 ARG types and 47 MRG subtypes were found in microbes on plastic particles in the North Pacific Gyre. In other studies, 526 ARG subtypes of 21 ARG types were identified in landfill leachate by metagenomic analysis (Zhao et al. 2018); 20 ARG types were found in urban sewage across China (Su et al. 2017); and 192 MRG subtypes conferring resistance to 21 metals were found in the influent and effluent of a wastewater treatment plant (Gupta et al. 2018). Our results indicate that, compared to environments high in direct anthropogenic activity (e.g., sewage sludge and landfill leachate), fewer ARGs and MRGs were detected in microbiota on the plastic particles in marine environment. ARG abundance in ten typical environments (sewage, swine wastewater samples, treated wastewater, river water, drinking water, soils, sediments, wastewater biofilm, sludge samples, and fecal samples) were found to be in the range 3.2×10^{-3}–3.1×10^{0} copies per 16S rRNA gene by metagenomic analysis (Li et al. 2015). ARG abundance in a pristine Swedish lake and a polluted Indian lake were found to be 4×10^{-3} and 28.4 copies per 16S rRNA gene by metagenomic analysis (Bengtsson-Palme et al. 2014). We found that ARG abundance in microbial communities on plastic particles in a deep ocean environment ranged from 7.7×10^{-4} to 1.2×10^{-2} copies per 16S rRNA, less ARG abundance than in studies for other environments. Sulfonamide resistance genes are prevalent in typical pollution sources of ARGs. They have been investigated in many different ecosystems, such as inland lake waters and sediment (Yang et al. 2016; Yang et al. 2017) and Arctic marine sediment (Tan et al. 2018). In this study, we did not find any sulfonamide resistance genes in plastics microbial communities in the marine environment. Similar results to ours have been found for deep ocean sediment (Chen et al. 2013) and lake sediment in Tibet (Chen et al. 2016), which are relatively pristine sites subject to little anthropogenic influence. Multidrug resistance and multi-metal resistance genes were most abundant among the resistance genes in plastics microbiota detected in this study.

Similar results have been found for the influent and effluent of a wastewater treatment plant (Gupta et al. 2018). Vancomycin resistance genes, which were identified in this study, have been observed to be widely distributed in various environments (Li et al. 2015), showing that antibiotic resistance is a natural and not new phenomenon (D'Costa et al. 2011).

The co-occurrence patterns among ARG and MRG subtypes show that more non-random co-occurrences of ARGs were found within the same types and across different types than random co-occurrences. More non-random co-occurrences were found among different MRGs than random co-occurrences. Similar co-occurrence patterns for ARGs have also been found in municipal sewage sludge digesters (Ju et al. 2016) and landfill leachate (Zhao et al. 2018). Higher numbers of non-random co-occurrences (O%) were found between different ARGs and classes of MRGs than for the same ARGs and classes of MRGs, showing that selection for the co-effects of antibiotic resistance and metal resistance was important in determining the composition of resistome in the plastics microbiota. MRGs and ARGs are co-selected because the factors that regulate efflux pump gene expression can also regulate resistance gene expression (Eckert et al. 2018; Perron et al. 2004). ARGs and MRGs can be linked on the same mobile genetic elements, particularly on plasmids (Baker-Austin et al. 2006). Class 1 integron-integrase gene (*intI1*), one of the important mobile genetic elements, is also linked to ARGs and MRGs (Gillings et al. 2015). Furthermore, ARGs and MRGs can be co-transferred via the same mobile gene elements, which have been found in various environments, including sediment (Rosewarne et al. 2010) and soil (Johnson et al. 2016).

Bacteria of the family *Flavobacteriaceae* are supposed to be important hosts for ARGs and MRGs in the plastics via co-occurrence network analysis. The relationship between microbial community composition and ARGs in an ocean microbiome is exemplified in *Flavobacterium* spp., which was a hub node in a species–species co-occurrence network in the ocean microbiome (Hao et al. 2018). Bacteria in the family *Flavobacteriaceae* were resistant to many antibiotics and heavy metals and constitute an environmental pool of ARGs and MRGs (Chang et al. 1997; De Souza et al. 2006). As Zhu et al. (2017) suggested, the identification of potential host microorganisms of ARGs and MRGs by co-occurrence analysis of the plastics still needs to be validated by mobilome sequencing or by the isolation of antibiotic or metal resistant strains.

5. Conclusion

Plastics in the marine environment are of great concern because of their increasing quantity and the risk they pose to aquatic animals via uptake. This is the first study to examine the abundance, diversity, and co-occurrence patterns of ARGs and MRGs in microbial communities that have colonized plastics in the marine environment. We found no significant difference between microplastics and macroplastics in the abundance of microbial ARGs and MRGs. MRG abundance was greater than ARG abundance in the microbiota of the plastics. Bacterial multidrug resistance genes had the greatest average relative abundance, followed by aminoglycoside resistance genes and unclassified ARGs. The relative abundance of MRGs was detected at the level of 10^{-3}–10^{-2} copies per 16S rRNA for all the plastics samples. More research is needed to better understand the ecological risks due to ARGs and MRGs in microbial communities in the aquatic plastisphere.

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Appendix A. Supplementary data

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