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Original Research Article

Nanoplastics promote the dissemination of antibiotic resistance genes and diversify their bacterial hosts in soil

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ABSTRACT

The wide application of plastics has led to the ubiquitous presence of nanoplastics and microplastics in terrestrial environments. However, few studies have focused on the mechanism underlying the effects of plastic particles on soil microbiomes and resistomes, especially the differences between nanoplastics and microplastics. This study investigated the microbiome and resistome in soil exposed to polystyrene microplastics (mPS) or nanoplastics (nPS) through 16S rRNA and shotgun metagenomic sequencing. Distinct microbial communities were observed between mPS and nPS exposure groups, and nPS exposure significantly changed the bacterial composition even at the lowest amended rate (0.01%, w/w). The abundance of antibiotic resistance genes (ARGs) in nPS exposure (1%) was 0.26 copies per cell, significantly higher than that in control (0.21 copies per cell) and mPS exposure groups (0.21 copies per cell). It was observed that nanoplastics, bacterial community, and mobile genetic elements (MGEs) directly affected the ARG abundance in nPS exposure groups, while in mPS exposure groups, only MGEs directly induced the change of ARGs. *Streptomyces* was the predominant host for multidrug in the control and mPS exposure, whereas the primary host was changed to *Bacillus* in nPS exposure. Additionally, exposure to nPS induced several bacterial hosts to exhibit possible multi-antibiotic resistance characteristics. Our results indicated that the effects of plastic particles on the soil microbial community were size-dependent, and nano-sized plastic particles exhibited more substantial impacts. Both microplastics and nanoplastics promoted ARG transfer and diversified their bacterial hosts. These findings bear implications for the regulation of plastic waste and ARGs.

1. Introduction

Due to the high production, inadequate disposal, and slow degradation of plastics, the ubiquitous and long-lasting presence of microplastics (diameter < 5 mm) in multiple environments has received worldwide attention [1–4]. The sources of microplastics can be divided into two categories, that is, primary ones unintentionally released from the raw industrial materials and secondary ones due to the physical breakdown of larger debris [5]. A number of investigating studies have well documented the accumulation of microplastics in a variety of environmental media, including waters (e.g., rivers, lakes, glaciers, and oceans), soils (e.g., farmlands, industrial soils, natural reserve areas, and even polar regions), and the air (including both urban and remote areas) [6–11]. Consequently, the adverse ecological effects of microplastics have become one of the greatest scientific and policy concerns [12–14].

The occurrence and potential impacts of microplastics on the soil ecosystems are recently more recognized since the amount of microplastics entering the land can be 4–23 times greater than the ocean [15]. Previous work has shown that microplastics can change the structure and succession of soil microbial communities, and the effects were closely associated with the microplastic properties. For instance, Fei et al. observed that the exposure of polyvinyl chloride (diameter 18 μm) and polyethylene (PE, diameter 678 μm) microplastics at 1% can lead to decline in the richness and diversity of soil bacterial community [16], while Wang et al. reported that PE micro-fragments (2 × 2 mm) did not change the diversity of soil communities but significantly increased the bacterial community turnover rate in soil [17]. Additionally, in aquatic environments, microplastic exposure has been shown to influence the evolution of microbial communities and to increase horizontal gene transfer (HGT), which may potentially facilitate the flow of antibiotic

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resistance genes (ARGs) between microorganisms [18–21]. Given the fact that soils are the most important reservoirs for both environmental antibiotic resistance and microplastics in terrestrial ecosystems, there is a growing concern about whether microplastics can promote the dissemination of ARGs in soils [22–24]. ARGs, associated with a variety of soil ecosystem functions, including antibiotic defense, signal transduction, and intermediate detoxification [25,26], circulate among the microbiomes of humans, animals, plants, and the environments, forming a critical "One Health" issue [27]. Therefore, an adequate evaluation of the patterns of soil ARG succession with microplastics is essential for understanding the spread and evolution of antibiotic resistance. This effort would significantly contribute to the "One Health" approach aimed at combating this global risk.

One particular concern of microplastic pollution is related to the issues regarding nanoplastics (<100 nm), which are the smaller nanoscale fractions of the microplastics [28,29]. Although the occurrence of nanoplastics in the environment remains largely unexplored, potentially due to the unsystematic sampling surveys and analytical limitations, the formation of nanoplastics through the weathering of microplastics has been evidenced in laboratory-based studies [30,31]. Thus, it is estimated that the abundance of nanoplastics is 10^{14} times greater than that of microplastics on the basis of mass conservation principles [32,33]. Despite their similar composition and origin, the nano-specific properties (e.g., transport properties, bioavailability, interactions with natural colloids, and potential toxicity) of nanoplastics distinguish them from microplastics. With increasing recognition of their potentially huge environmental load and unique characteristics, an empirical understanding of the specific hazards of nanoplastics is mandatory. Although there were several studies evaluating the potential effects of nanoplastics on ARG propagation, very few studies have comprehensively investigated the changes in ARG profiles and their potential bacterial hosts.

Therefore, the current study attempted to characterize the influence of microplastics and nanoplastics on the soil microbiome and antibiotic resistomes. Two sizes of polystyrene (PS) particles, including microplastics (mPS, 150 μm in diameter) and nanoplastics (nPS, 50 nm in diameter), were selected as the representative model plastics, as PS is one of the most widely used polymers [34]. The main aims of this study were (1) to detect the impact of mPS and nPS on the soil microbial communities by sequencing the bacterial 16S rRNA gene, (2) to investigate the abundance and diversity of ARGs in soil exposed to mPS and nPS via shotgun metagenomic sequencing, and (3) to estimate the changes in ARG hosts via the contig assembling and binning methods. We hypothesized that nanoplastics may exhibit a higher level of impact on soil microbiome and resistomes, and the ARG characteristics were affected by different mechanisms in mPS and nPS-treated soils.

2. Materials and methods

2.1. Plastics and soil

The PS microplastic particles (150 μm) were purchased from Aladdin Industrial Corporation (Hangzhou, China), and the PS nanoplastic particles (50 nm) were obtained from Tianjin Baseline Chromtech Research Center (Tianjin, China) (Fig. S1). The soil used in this study was collected from the surface soil (0–10 cm) in a greenhouse field in September 2019 in Daxing, Beijing, China (39°33′N, 116°6′E). Organic fertilizers were commonly used in the greenhouse. The soil was air-dried and sieved through a 2-mm mesh before use. The soil chemical properties were as follows: pH 6.34, total carbon 40.9 g/kg, total nitrogen 3.35 g/kg, clay 18.4%, silt 28.4%, and sand 53.2%.

2.2. Experimental design

Each microcosm experiment was conducted in a sterilized glass jar with 50 g of soil. To estimate the effects of plastic concentration on soil microbiome and antibiotic resistance, the plastic particles were added to the soil to reach a mass fraction of 0.01%, 0.1%, and 1%, followed by vigorous homogenization on a rolling mixer. Even though limited field studies have estimated the micro/nanoplastic concentrations in the land, previous studies indicated the microplastic weight level could be up to 7% in heavily contaminated area [35]. In the soil samples from natural reserve areas, almost without human activities, the baseline of microplastics can be up to 0.002% [36]. Thus, the concentrations in our study can be considered to simulate the low, medium, and heavy plastic contamination in soil. A total of seven treatments with three replicates were included in the current study: control soil (CK), soil + 1% mPS (mPS1), soil + 0.1% mPS (mPS2), soil + 0.01% mPS (mPS3), soil + 1% nPS (nPS1), soil + 0.1% nPS (nPS2), and soil + 0.01% nPS (nPS3). Sterilized water was added to microcosms to maintain the soil moisture content at approximately 20%, and all microcosms were incubated at 25 °C in the dark. After 30 days, the soils were collected and stored at −80 °C before DNA extraction.

2.3. DNA extraction and sequencing

The total soil genomic DNA was extracted from approximately 0.5 g of soil using the Qiagen PowerSoil® DNA isolation kit (Qiagen, Shanghai, China) following the manufacturer's instructions, and 50 μL of DNA was obtained for each sample. A NanoDrop Spectrophotometer (ND-2000, Thermo Scientific, Wilmington, DE, US) was used to assess the DNA quality, and the DNA concentration of each sample ranged 21–49 ng/μL. Each DNA sample was adjusted to a concentration of 10 ng/μL. One microliter template DNA was used for the amplicon sequencing. The 16S rRNA gene encompassing the V3–V4 regions was targeted using the forward 338F (ACTCCTACGGGAGGCAGCA) and reverse 806R (GGACTACHVGGGTWTCTAAT) primers. Afterward, the amplicons were purified and further sequenced on the Illumina Miseq platform in a paired-end format in Majorbio BioPharm Technology Co. Ltd (Shanghai, China). The same DNA extractions (25 μL) were used for shotgun metagenomic sequencing with the Illumina Novaseq 6000 platform, generating approximately 12 Gbp of 150-bp paired-end reads per sample.

2.4. Amplicon sequence processing and community analysis

The Quantitative Insights Into Microbial Ecology 2 (QIIME 2, version 2020.02) was used to analyze the amplicon sequencing data [37]. After trimming, the reads were denoised via DADA2 and clustered into amplicon sequence variants (ASVs) [38]. The taxonomy of each ASV was assigned according to the Silva reference database (version 132). The alpha diversity indices based on ASV level, including Chao 1 richness, Pielou's evenness, Shannon diversity, and Faith's Phylogenetic Diversity, were estimated using QIIME2. Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance was used to visualize the beta diversity, and the significant difference was further examined by the Adonis test with the "vegen" package [39]. Statistical analysis of differentially abundant genera was performed using the "edgeR" package by fitting a negative binomial generalized linear model to the genera [40].

2.5. Analysis of ARGs and mobile genetic elements

After quality control of the shotgun metagenomic reads by fastp (version 0.12.1) with default filtering parameters, the potential ARG reads were extracted via the ARG-analysis pipeline ARG-OAP (version 2.2) [41,42]. The parameters were set at the cutoff of E value of 10^{-7}, sequence identification of 90%, and alignment length of more than 25 amino acids. For the quantification of mobile genetic elements (MGEs), the pipeline of ARG-OAP was also used, and the reference database was replaced by an MGE signature sequence database [43]. The quantification of ARGs or MGEs was estimated by normalizing their abundance to the cell number (copies per cell) [44].

PCoA on the basis of Bray-Curtis distance was used to identify the differences between ARG and MGE profiles among the treatments. To estimate the correlation between bacterial community, ARG, and MGE profiles, Procrustes analysis and Mantel test based on the Bray-Curtis dissimilarity metrics were performed. Partial redundancy analysis (pRDA) was used to delineate the effects of explanatory variables, including plastic properties (plastic types and concentrations), microbial communities (alpha diversities and community distances), and MGE characteristics (abundances and Bray-Curtis dissimilarity) on the ARG profiles. The partial least squares-path modeling (PLS-PM) was employed to explore the linkages between plastic concentration, microbial community, MGE abundance, and ARGs using the "PLS-PM" package [45]. The fit model was examined by the goodness-of-fit index (*GoF*), with a higher value indicating better prediction performance.

2.6. Identification of ARG bacterial hosts

Clean reads in the three replicates for CK, mPS1, and nPS1 treatments were de novo assembled using Megahit (version 1.2.9) with a minimum contig length of 1000 bp [46]. Kraken2 (version 2.1.2) was applied on contigs for taxonomic annotation against Genome Taxonomy Database (GTDB) [47,48]. The open reading frames (ORFs) within the assembled contigs were predicted using Prodigal (version 2.6.3) [49]. The predicted ORFs were then identified for ARGs using BLASTX against the ARG database of the ARG-OAP pipeline with parameters: E values $\leq 10^{-10}$, similarity $\geq$ 80%, and coverage $\geq$ 70%. The coverage (times per Giga base, /Gb) of ARG-like ORFs were defined as:

$$\text{Coverage} = \sum_{1}^{n} \frac{N \times 150/L}{S}$$

where N is the number of the reads mapped to ARG-like ORF, L is the sequence length of the corresponding target ARG-like ORFs, 150 is the length of our Illumina sequencing reads, S is the sequencing data size (Gb), and n is the number of ARG-like ORFs [50].

Sequence composition-independent binning was performed by MetaWRAP (version 1.3) to obtain the metagenome-assembled genomes (MAGs) [51]. After assembly, binning was performed using three different method tools, i.e., MaxBin2 (version 2.2.6), Metabat2 (version 2.13), and CONCOCT (version 1.1.0), with default options, followed by bin-refinement in MetaWRAP with parameters: completeness $\geq$ 50% and contamination $<$ 10%. For each treatment, the amino acid identity (AAI) between genomes was estimated using CompareM (version 0.1.2) with default parameters, and MAGs with AAI $\geq$ 99.5% were considered as belonging to the same species. The abundance of each MAG was estimated using MetaWRAP in terms of fragments per kilobase of gene sequence per million reads mapped. The taxonomic classification of MAGs was conducted using GTDB-Tk (version 1.5.1) against GTDB r202 [52]. The ARGs carried by MAGs were identified using BLASTX against the Structured Antibiotic Resistance Gene (SARG) database with an e-value cutoff of 10^{-10}, 80% similarity, and 70% query coverage [42].

3. Results and discussion

3.1. Effects of exposure to mPS and nPS on soil microbial community

Illumine sequencing of the 21 samples yielded a total of 654,252 high-quality sequences, which were clustered into 6,388 ASVs. The rarefaction curves of all samples reached the plateau phase, and all Good's coverage scores were higher than 99.9%, suggesting sufficient depth of sequencing (Figs. S2 and S3).

The phyla Firmicutes (27.7%), Proteobacteria (20.2%), Actinobacteria (12.3%), Chloroflexi (12.8%), and Bacteroidetes (5.1%) were the predominant taxonomic phyla in the control soils (Fig. S4). There were no significant compositional shifts of the phyla Firmicutes, Proteobacteria, and Actinobacteria in the soil exposed to lower concentrations of (0.01% and 0.1%) mPS and nPS. As shown in Fig. 1, a higher concentration of mPS (1%) significantly decreased the relative abundance of Chloroflexi, whereas the value in the soil amended with 1% nPS was (15.8%) statistically higher than that in CK (10.1%). The increase of the phylum Chloroflexi within the nanoplastic amendment was also observed in other studies. For instance, Zhou et al. evaluated the effects of the differentially charged PS nanoplastics on soil microbial community structure and found that Chloroflexi showed significantly higher relative abundance in the nanoplastic-treated groups [53]. The phylum Chloroflexi was reported to be oligotrophic bacteria, which would thrive in environments with limited nutrients. The results may suggest that nanoplastics could influence the nutrient supply in the soil. However, Chloroflexi in soil have not been thoroughly studied, and further studies on the effects of nanoplastics on these soil inhabitants are still needed. Additionally, the amendment of 1% nPS significantly decreased the relative abundances of Bacteroidetes (2.9%) and Patescibacteria (0.9%) compared with the control group (5.2% and 3.6%, respectively, for Bacteroidetes and Patescibacteria). To better illustrate the compositional differences between the control, mPS1, and nPS1 treatments, the differential genera were analyzed. Compared with the control, only one genus, *Sporichthya*, was significantly enriched in the soil amended with 1% mPS, whereas the microbiota in soil amended with 1% nPS enriched 50 genera and depleted 48 genera, respectively (Fig. 1). The depleted genera mainly belonged to the phyla Bacteroidetes and Patescibacteria, which was consistent with the decreased relative abundance of these two phyla. The enriched genera mainly belonged to the phylum Proteobacteria, such as *Ramlibacter*, *Pseudomonas*, *Azoarcus*, *Legionella*, *Phenylobacterium*, *Hahella*, and *Panacagrimonas*.

Exposure to different concentrations of mPS had no effects on soil bacterial alpha diversity (Fig. S5). Compared to mPS, nPS exposure at the highest dose (1%) significantly decreased all bacterial alpha diversity indices. For instance, the values of richness and phylogenetic diversity in the soil amended with 1% nPS were 1,461 and 143, respectively, which were significantly lower than those in the control (1,669 and 161, respectively). To compare the similarities of soil bacterial community after exposure to mPS and nPS, we conducted PCoA analysis on the base of Bray-Curtis distance (Fig. 2). All mPS-treated samples clustered together and were not distinguishable from the control, while a clearly significant separation (R^2 = 0.5259, p = 0.001, Adonis) between the control and nPS treatments along the primary principal coordinate (26.43%) was observed. Additionally, the microbiomes in the soil exposed to 0.01% and 0.1% of nPS clustered together and separated from 1% nPS treatment along the secondary principal coordinate, suggesting exposure to the highest concentration of nPS exerted more drastic effects on soil bacterial compositions. The community distances between the control and mPS/nPS-treated samples further indicated that nPS can lead to more distinct soil bacterial communities even at the lowest amended rate (0.01%).

The nPS exhibited greater effects on soil microbiota in comparison with mPS. As inferred in Fig. S4, exposure to 1% nPS significantly decreased the richness, evenness, and diversity of soil microbiota, whereas no statistical difference was observed between the control and the mPS exposure groups. Additionally, the microbial community structure was notably changed in nPS-treated groups, embodied in the greater community distances and the variations in the relative abundance of biomarker taxa (Figs. 1 and 2). Although very few studies have investigated the effects of nano-scale plastics on soil microbial communities, previous studies indicated that the influences of plastic particles were size-dependent. For instance, Shi et al. reported that exposure to 200–500 nm PS particles induced greater dissimilarity in microbial composition in municipal landfill leachate than 9.0–9.9 μm PS particles [54]. Additionally, Zhou et al. observed that PS nanoplastics (100 nm) decreased the abundance of nitrifiers and denitrifiers in activated sludge and suppressed the nitrification and denitrification genes, while PS microplastics (100 μm) showed no significant effects [55]. Concerning the effects on soil microbial communities, previous studies

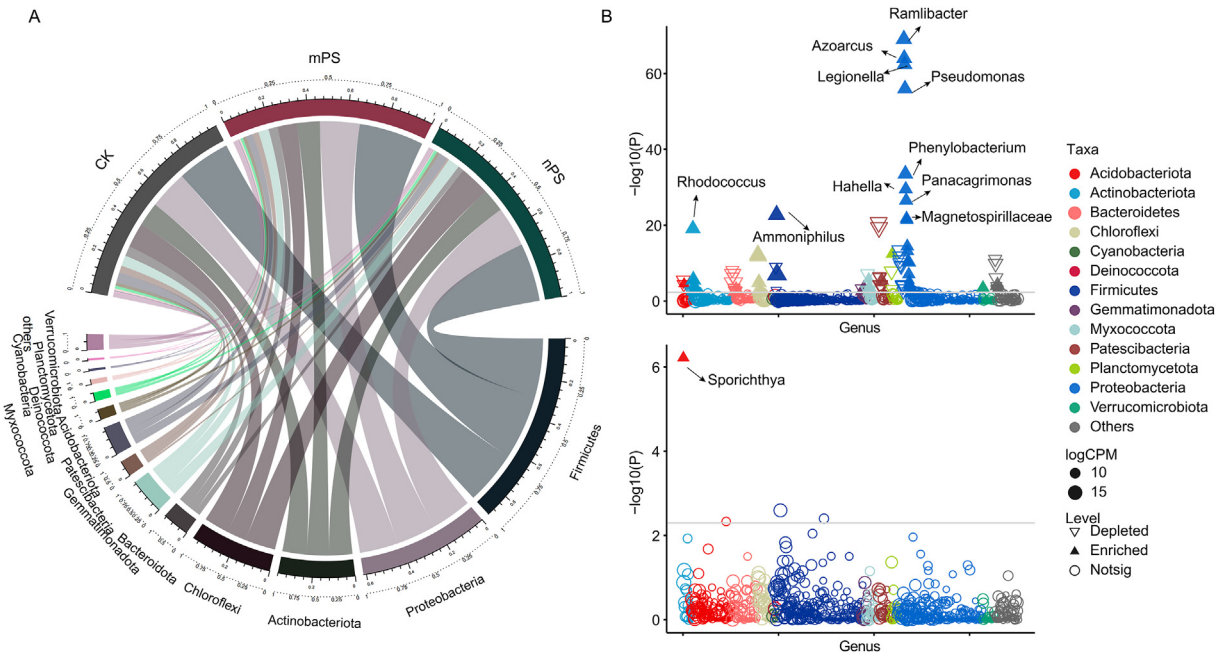

Fig. 1. (**A**) The bacterial composition at the phylum level in the control and mPS/nPS exposures. (**B**) The biomarker genera in nPS and mPS exposure compared to the control.

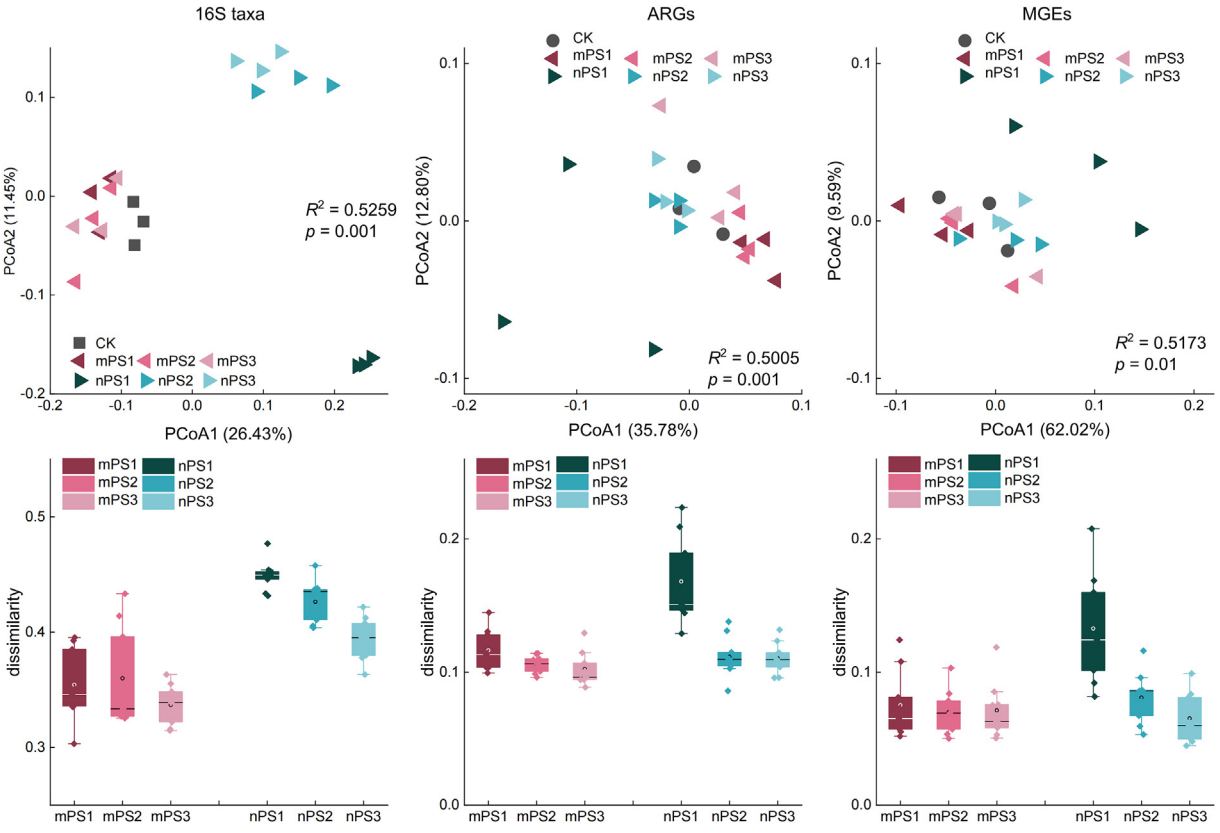

Fig. 2. Principal coordinate analysis (PCoA) plots and community distances showing the differences in bacterial community, ARGs, and MGEs. ARG, antibiotic resistance gene; MGE, mobile genetic element.

predominantly investigated micro-scale plastic particles, and their results were always inconspicuous [56–58]. For example, low-density PE microplastics showed significant changes in soil microbial community structure only when the amendment rate was higher than 3% [59]. By contrast, even though the concentration of nanoplastics in this study was set as low as 0.01%, the exposed bacterial communities were clearly separated from the control, suggesting nanoplastics may cause more pressure on soil microbiota.

In this study, compared to the control, the relative abundance of keystone taxa (several genera) was clearly altered in the nPS and mPS

exposure groups. In particular, several opportunistic pathogenic genera, such as *Legionella*, *Pseudomonas*, *Hahella*, and *Rhodococcus*, were significantly enriched in the soil exposed to 1% nPS. *Legionella* has been reported to be an opportunistic pathogen causing legionellosis, which refers to two clinical syndromes: Legionnaires' disease and Pontiac fever [60]. Also, several *Pseudomonas* species, such as *Pseudomonas aeruginosa*, can cause infections and diseases in both plants and animals, including human hospital-acquired infections [61]. One *Rhodococcus* species, *Rhodococcus equi*, is ubiquitous in soil and can cause lung infections via fecal-oral cycling [62]. These findings suggest that nanoplastics in terrestrial environments may potentially facilitate the spread and propagation of opportunistic pathogens.

3.2. Effects of mPS and nPS on ARGs and MGEs

Exposure to mPS was found to decrease the total abundance of ARGs in the soil without significant differences (Fig. 3). In contrast to mPS, amendment with 1% nPS resulted in a significant increase in the abundance of ARGs. For instance, the total abundance of ARGs in the control was 0.21 copies per cell, significantly lower than the value in soil amended with 1% nPS (0.26 copies per cell). Especially, the genes for resistance to multidrug (0.062 copies per cell), macrolide-lincosamide-streptogramin (0.021 copies per cell), bacitracin (0.023 copies per cell), and beta-lactam (0.0037 copies per cell) were significantly ($p < 0.05$) more abundant in the soil amended with 1% nPS than those in control (0.053, 0.017, 0.012, and 0.0021 copies per cell, respectively). The major resistance mechanisms in all treatments were antibiotic efflux, inactivation, and target alteration (Fig. S6). Exposure to 1% mPS decreased the percentage of antibiotic inactivation from 14.13% to 12.00%, while the value increased after exposure to 1% nPS (15.23%). Among the extensive array of 288 detected ARG subtypes, 85 genes spanning across 18 types were present in all samples, with 26 belonging to multidrug, 12 belonging to MLS, and 10 belonging to aminoglycoside (Fig. S7). The vancomycin resistance gene *vanR*, the multidrug resistance gene *ABC transporter*, and the bacitracin resistance gene *bacA* were the three most abundant ARG subtypes. Linear discriminant analysis effect size was used to identify the most differentially ARG subtypes between the control and exposed groups. Compared with the control, there were 11 and 18 ARG subtypes enriched in the soil amended with 1% mPS and 1% nPS, respectively (Fig. S8). PCoA at the subtype level was further conducted based on the Bray-Curtis distances to illustrate the shite of ARG profiles (Fig. 2). A significant separation ($R^2 = 0.5005$, $p = 0.001$, Adonis) between the 1% PS-treated samples and the other samples along the primary principal coordinate (35.78%) was observed. The community distances also confirmed that exposure to 1% nPS significantly changed the ARG profile in the soil.

Nanoplastics were found to increase the abundance of MGEs in the soil (Fig. 3). For instance, the total abundance of MGEs in the control was

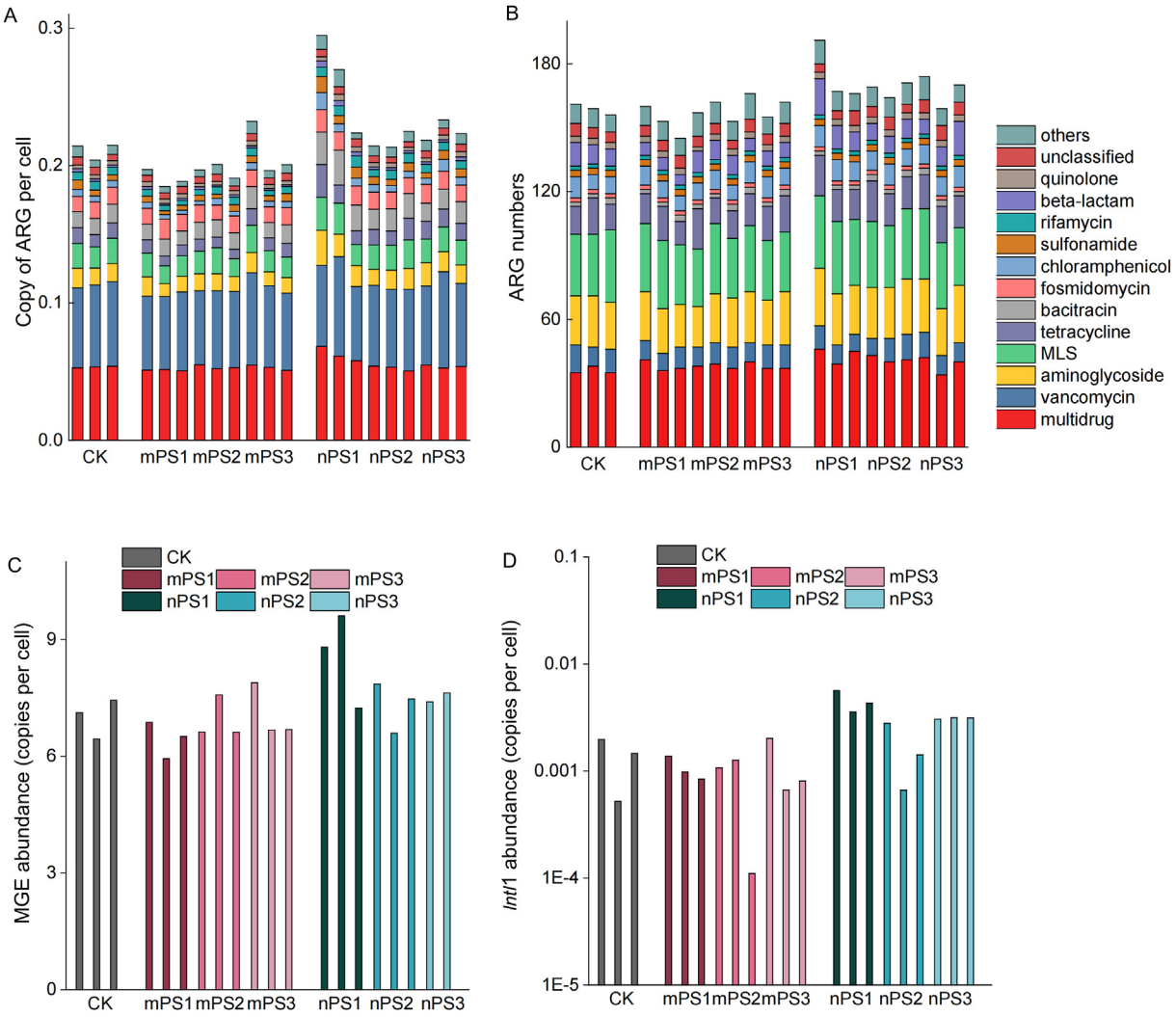

Fig. 3. (**A**) The abundance of ARG types in all samples. (**B**) The ARG numbers in all samples. (**C**) The abundance of MGEs in all samples. (**D**) The abundance of intI1 in all samples.

6.89 copies per cell, significantly lower than the value (8.55 copies per cell) in the soil amended with 1% nPS. The Bray-Curtis distances and Adonis test further indicated that the MGE profile was significantly affected by 1% nPS (Fig. 2). Additionally, the abundances of class 1 integron–integrase gene (*intI*1), a typical integron strongly associated with HGT of ARGs, were increased in the soil amended with nPS but decreased in mPS exposure. Additionally, a significant positive correlation ($p < 0.001$) was observed between the abundance of ARGs and *intI*1 in all samples (Fig. S9).

To date, only a limited number of studies have estimated the effects of plastics on ARGs. For instance, by comparing the effects of PS microplastics (2 μm), both along and in combination with sulfamethoxazole, on the gut microbiota of collembolan (*Folsomia candida*), Xiang et al. reported an enrichment of multidrug efflux pump genes in the guts exposed to PS microplastics [63]. Similarly, Xu and Yu observed that 100 mg/kg of 10 μm PS microplastics significantly changed the profile of ARGs in earthworm guts [64]. Additionally, Shi et al. found that 200–500 nm PS particles increased the ARG abundances, including resistance genes for sulfonamide, aminoglycoside, macrolide, and beta-lactam [54]. The results potentially suggest that plastic particles with small sizes (e.g., <10 μm) may increase the abundance of ARGs in various environments.

To investigate potential correlations among the bacterial community, MGEs, and ARGs, Procrustes analysis and Mantel test based on Bray-Curtis dissimilarity metrics were performed (Fig. S10). The Procrustes analysis revealed significant correlations ($M^2 = 0.2033$, $p = 0.001$) between the bacterial community and ARG profiles across different samples, as well as significant correlations ($M^2 = 0.3740$, $p = 0.001$) between bacterial community and MGE profiles, suggesting that both ARGs and MGEs were strongly associated with the bacterial community. Additionally, the ARG characteristics were also significantly correlated with the MGE profiles ($M^2 = 0.2268$, $p = 0.001$). The Mantel test further confirmed the significant correlations among the bacterial community, MGEs, and ARGs ($R > 0.4242$, $p < 0.001$). pRDA was used to better understand the effects of plastic properties, bacterial community, and MGE characteristics on ARG profiles (Fig. 4). The results showed that over 60% of the observed variation in ARGs could be explained by selected factors, with the bacterial community being the most important contributor (45.18%) to the variance of ARGs, followed by MGEs (35.11%). After excluding their interactive effects, the plastic properties, bacterial community, and MGE characteristics can explain 10.60%, 14.18%, and 8.08% of the variance of ARGs, respectively. To understand how plastic mediates alterations in soil ARGs, we conducted PLS-PM for both mPS and nPS treatments (Fig. 4), respectively. The models fitted the data well, explaining 89.2% and 82.3% of the variance in ARG abundance for mPS and nPS treatments, respectively. In mPS treatments, only MGEs showed a significantly positive effect on ARG abundance, indicating that MGEs abundance strongly explained the ARG abundance in mPS treatments. By contrast, for nPS treatments, nanoplastic concentration, MGEs, and bacterial alpha diversity all displayed significantly positive effects on ARG abundance, suggesting the importance of direct effects of the three aspects in nPS treatments.

The mechanisms underlying the impact of microplastics and nanoplastics on the ARG propagation may be different. In this study, the diameter of microplastic particles was approximately 150 μm, which can provide a habitat for biofilm development. In such biofilms, frequent contact between surface-attached bacteria may favor the HGT, potentially influencing the spread of ARGs. This can be demonstrated by the strong positive relationship between MGEs and ARGs in mPS treatments. In contrast, the nanoplastics (50 nm) employed in this study may increase the permeability of the bacterial membrane. Qiu et al. reported that nano-alumina can damage bacterial cell membranes, promote the conjugative transfer of RP4 plasmid, and facilitate the horizontal transfer of multi-resistance genes between bacteria [65]. Similarly, nano-scale plastic particles were shown to elevate the intracellular oxidative levels and increase membrane permeability [54]. The nanoplastics could increase the production of reactive oxygen species, damaging the bacterial cell, and consequently increasing the membrane permeability, potentially causing more bacteria to become ARG receptors via the

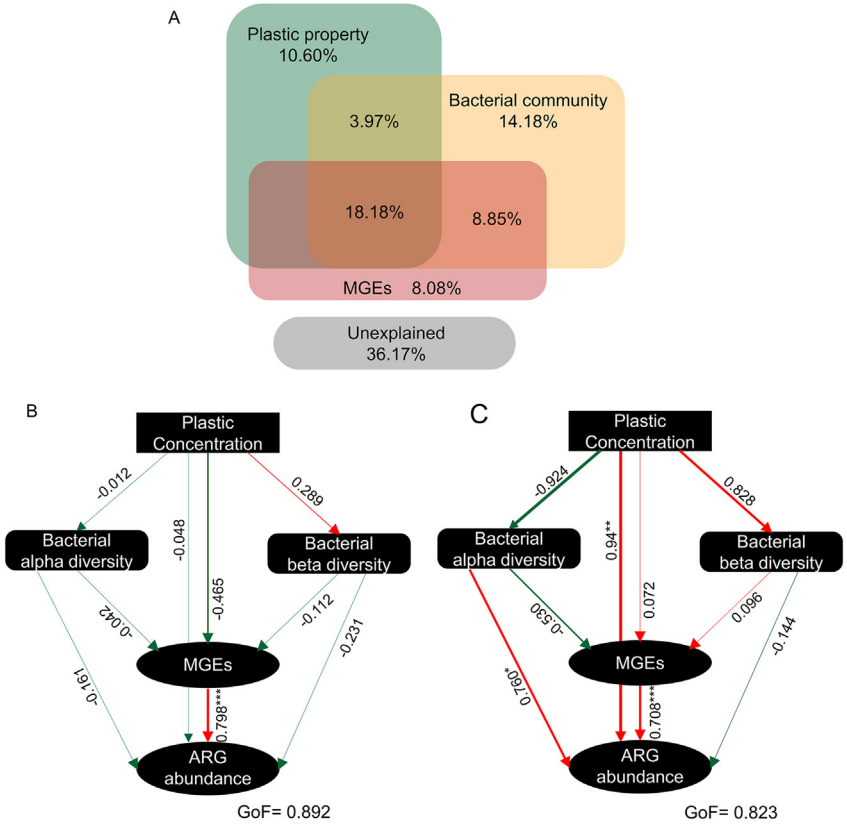

Fig. 4. (**A**) Partial redundancy analysis (pRDA) differentiating the effects of plastic property, bacterial community, and MGEs on ARG profiles. (**B**) Partial least squares path model (PLS-PM) showing the connection of plastic concentration, bacterial community, MGEs, and the ARG abundance in mPS treatments. (**C**) PLS-PM showing the connection of plastic concentration, bacterial community, MGEs, and the ARG abundance in nPS treatments. Numbers labeling the lines are indicative of the path coefficients. The asterisks indicate that the path coefficients are significant: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

intra-bacterial community transfer of MGEs [66,67]. Therefore, nanoplastics in our study can directly participate in the regulation of ARG spread or indirectly show influences via impacting bacterial community and MGEs.

3.3. The effects of mPS and nPS on the ARG bacterial host

After assembly, we obtained a total of 1,762,493 contigs containing 4,352,154 ORFs, where 1,088 ORFs were annotated as ARG-like ORFs. For the taxonomic annotation, the taxa of approximately 75% of contigs carrying ARGs were predicted at their genera level (Fig. S11). The genes resistant to multidrug and vancomycin were found to be the most abundant across all samples. Exposure to mPS and nPS decreased the abundance of multidrug but increased the abundance of vancomycin, aminoglycoside, bacitracin, rifamycin, macrolide-lincosamide-streptogramin (MLS), and chloramphenicol. The total coverage of ARGs in the assembled contigs was lower in the mPS exposed samples (79.2 ×/Gb) than the control (98.7 ×/Gb), while the coverage of ARGs was the highest in the nPS exposure (127.9 ×/Gb). With the exception of unclassified genera, *Streptomyces* was the predominant bacterial host in the control (50.2%) and harbored diverse ARGs, including the genes resistant to multidrug, MLS, bacitracin, aminoglycoside, vancomycin, tetracycline, quinolone, and sulfonamide (Fig. 5). For mPS exposure, the genes resistant to multidrug, vancomycin, quinolone, and aminoglycoside were mainly harbored in *Streptomyces*. However, the predominant bacterial host for sulfonamide, tetracycline, and MLS changed to *Planifilum*, *Mycolicibacterium*, and *Ruminiclostridium*. Notably, more discriminative hosts of ARGs were observed for nPS exposure. For instance, the major host of sulfonamide-resistant genes was *Escherichia*, and the resistant genes to multidrug, quinolone, tetracycline, and aminoglycoside were predominantly harbored in *Bacillus*, *Micromonospora*, *Mycolicibacterium*, and *Oceanobacillus*, respectively. The results clearly indicated that both mPS and nPS facilitated the spread of ARGs across different bacteria, suggesting that more diverse bacteria may show resistance to antibiotics after exposure to mPS or nPS. Additionally, exposure to nPS also induced some bacteria to exhibit possible multi-antibiotic resistance characteristics. For instance, the genus *Bacillus* did not harbor any resistant gene in the control samples (Fig. S12). After exposure to nPS, the resistant genes to bacitracin, fosfomycin, MLS, multidrug, quinolone, and vancomycin were observed in the contigs annotated to *Bacillus*. The diverse hosts of ARGs and the trigger of multiple-antibiotic resistance under nPS pressures potential indicated that nanoplastics may play a critical role in the acquisition and spread of antibiotic resistance in the environment and introduce great uncertainty into the administration strategy of treating resistant pathogens. For example, the species *Enterococcus faecium* only harbored chloramphenicol-resistant genes in the control soil (Table S1), allowing for treatment of *Enterococcus* infection using effective antimicrobials except for chloramphenicol. However, after exposure to nPS, the genes resistant to aminoglycoside and sulfonamide were found in *E. faecium*, necessitating the adjustments of specific targeted administration.

The metagenomic binning was further performed, generating a total of 293 high- (completeness ≥ 90% and contamination < 5%) and medium-quality (completeness ≥ 50% and contamination < 10%) MAGs (Fig. S13). Among these recovered MAGs, 84 MAGs were identified as carrying ARGs, which were assigned to 12 phyla. The hosts of ARGs mostly belonged to the phylum Proteobacteria (30 MAGs), followed by Chloroflexi (17 MAGs) and Actinobacteria (15 MAGs) (Table S2). In addition, 79 MAGs were classified at the family level (44 families), among which 48 MAGs were classified at the genus level (30 genera) (Table S2). The relative abundance of ARG-carrying MAGs in nPS treatments (31.8%) was higher than those in CK (27.5%) and nPS (25.2%) treatments (Table S3). Furthermore, the results also indicated that the application of microplastics/nanoplastics changed the ARG-carrying MAGs in the control (Fig. 6). For instance, two MAGs affiliated to the genus *WHUA01* and *Nocardioides* showed the greatest abundances (12.1% and 8.9%, respectively) in the CK treatments, which were identified as the hosts of dfrA1 and multidrug ARB transporter. However, the hosts with the greatest abundances changed to the genus *SCGC-AG-212-J23* and *Sphingomicrobium* in the nPS treatments and the genus *WHUA01*

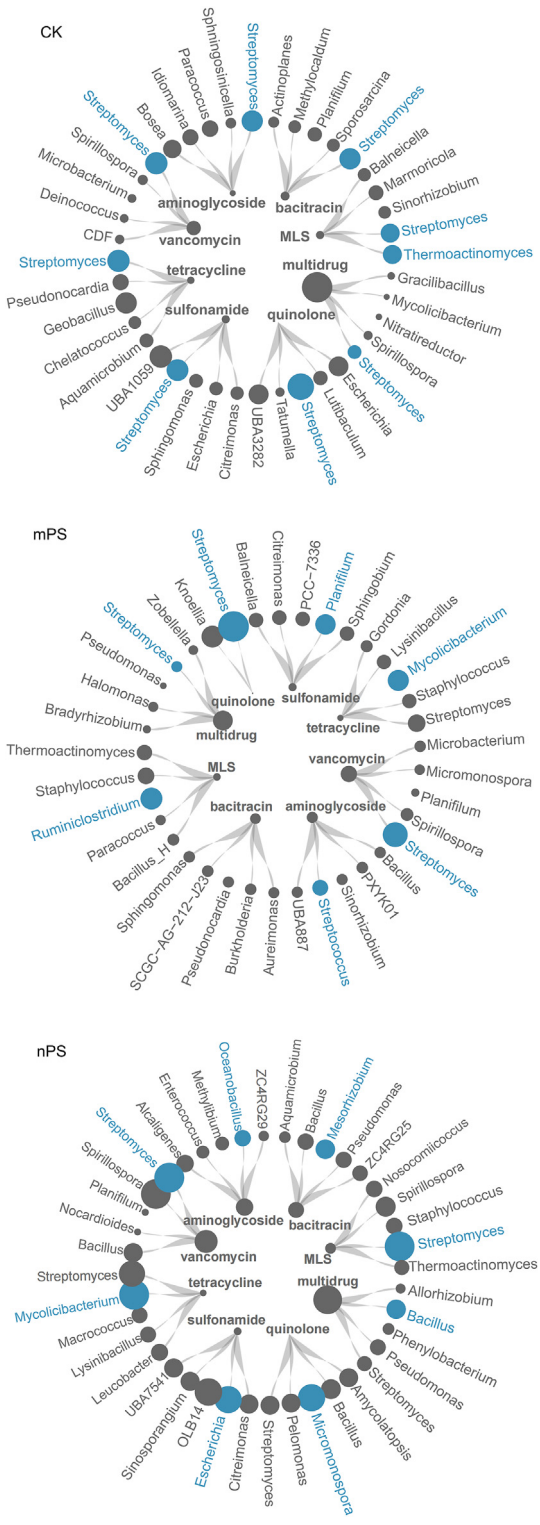

Fig. 5. Variations of the predominant ARGs carried by bacterial hosts in different treatments with node size corresponding to percentage. The percentage of a single ARG type was calculated using the coverage of the ARG divided by the sum of the coverage of all ARGs in one sample.

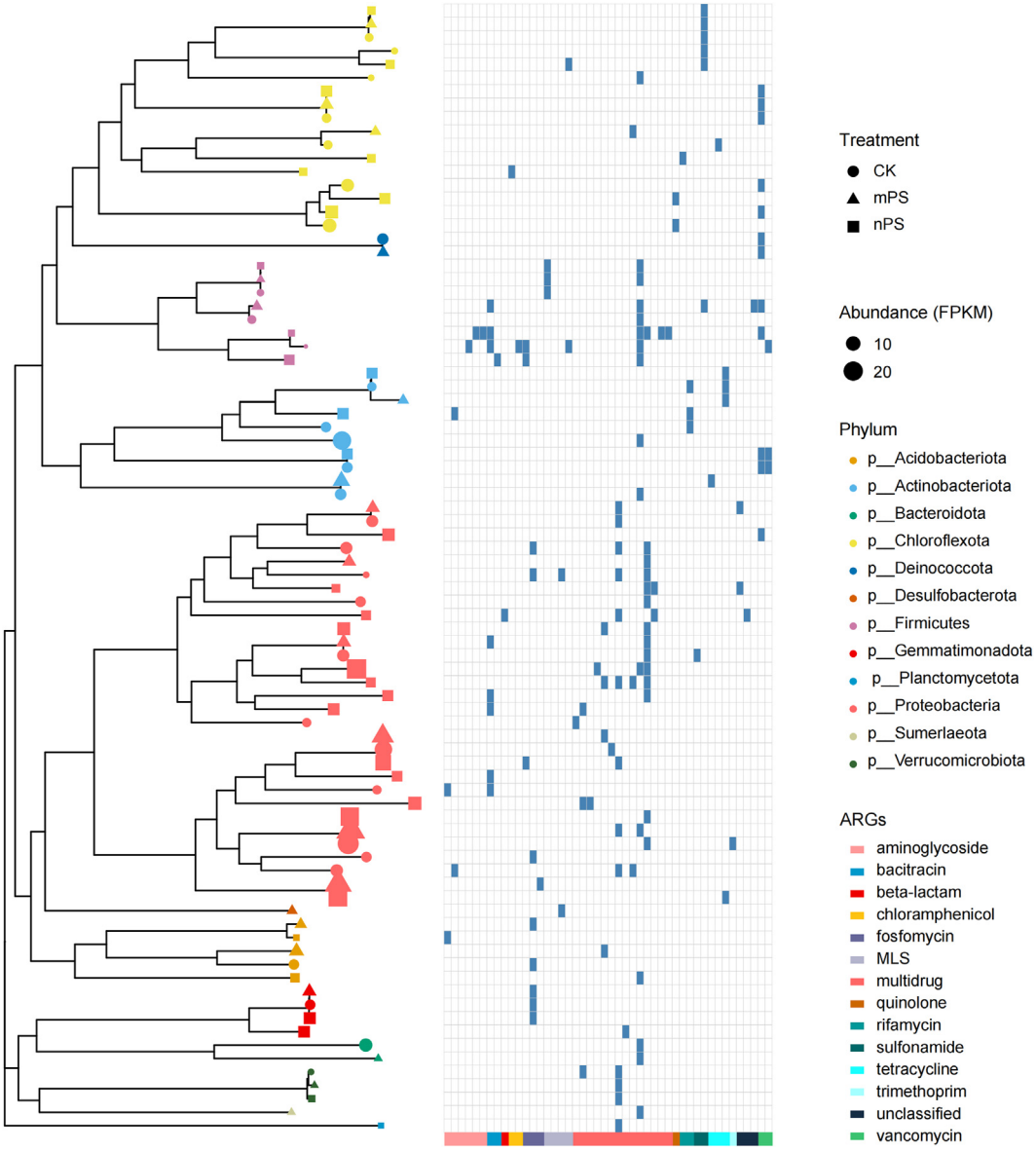

Fig. 6. Phylogenetic tree of ARG-carrying MAGs and their carrying ARGs in all treatments.

and *Sphingomicrobium* in the mPS treatments (Tables S2 and S3). Several bacteria also exhibited possible multi-antibiotic resistance characteristics. For example, three MAGs assigned to the class Bacilli in the nPS treatments were resistant to the antibiotic types of aminoglycoside, bacitracin, fosfomycin, multidrug, MLS, and vancomycin (Fig. 6). Generally, the binning results further confirm that the application of microplastics/nanoplastics facilitate the horizontal acquirement of ARGs.

In conclusion, this study conducted an integrated analysis of changes in microbiome structure and variations of ARG contents to estimate the effects of microplastics and nanoplastics on soil microbiomes and resistomes. We found that microplastics showed limited impacts on soil microbiomes, while nanoplastics significantly changed their community structure even at low amended rate (0.01%). The effects of plastic particles on the soil resistome were closely related to the changes in microbial communities and MGEs. Additionally, the shifts in HGT on microplastics significantly impacted the ARGs in mPS exposure, whereas nPS can directly shape ARG contents via changing bacterial membrane permeability. The risks that microplastics and nanoplastics may promote the spread of ARGs should not be neglected. This comprehensive study enhances our understanding of the pressures of plastic particles on soil health and provides valuable insights into the risk assessment of plastic waste.

Author contributions

L.J.L.: data curation, investigation, visualization. Y.Z.S.: writing–original draft, visualization, software. S.T.D.: resources, writing–review and editing. Y.M.L.: resources, writing–review and editing. J.W.: conceptualization, supervision, writing–review and editing.

Declaration of competing interests

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eehl.2023.09.005.

References

[1] A.D. Vethaak, J. Legler, Microplastics and human health, Science 371 (6530) (2021) 672–674, https://doi.org/10.1126/science.abe5041.
[2] M.C. Rillig, A. Lehmann, Microplastic in terrestrial ecosystems, Science 368 (6498) (2020) 1430–1431, https://doi.org/10.1126/science.abb5979.
[3] D.M. Mitrano, W. Wohlleben, Microplastic regulation should be more precise to incentivize both innovation and environmental safety, Nat. Commun. 11 (1) (2020) 5324, https://doi.org/10.1038/s41467-020-19069-1.
[4] L. Zinke, Moving marine microplastics, Nat. Rev. Earth Environ. 1 (4) (2020) 186, https://doi.org/10.1038/s43017-020-0047-9, 186.
[5] R. Geyer, J.R. Jambeck, K.L. Law, Production, use, and fate of all plastics ever made, Sci. Adv. 3 (7) (2017) e1700782, https://doi.org/10.1126/sciadv.1700782.
[6] Q. Zhang, E.G. Xu, J. Li, Q. Chen, L. Ma, E.Y. Zeng, H. Shi, A review of microplastics in table salt, drinking water, and air: direct human exposure, Environ. Sci. Technol. 54 (7) (2020) 3740–3751, https://doi.org/10.1021/acs.est.9b04535.
[7] S. Klein, E. Worch, T.P. Knepper, Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany, Environ. Sci. Technol. 49 (10) (2015) 6070–6076, https://doi.org/10.1021/acs.est.5b00492.
[8] L.T. Yonkos, E.A. Friedel, A.C. Perez-Reyes, S. Ghosal, C.D. Arthur, Microplastics in four estuarine rivers in the Chesapeake Bay, U.S.A, Environ. Sci. Technol. 48 (24) (2014) 14195–14202, https://doi.org/10.1021/es5036317.
[9] R. Ambrosini, R.S. Azzoni, F. Pittino, G. Diolaiuti, A. Franzetti, M. Parolini, First evidence of microplastic contamination in the Supraglacial Debris of an Alpine Glacier, Environ. Pollut. 253 (2019) 297–301, https://doi.org/10.1016/j.envpol.2019.07.005.
[10] J. Brahney, N. Mahowald, M. Prank, G. Cornwell, Z. Klimont, H. Matsui, K.A. Prather, Constraining the atmospheric limb of the plastic cycle, Proc. Natl. Acad. Sci. USA 118 (16) (2021), https://doi.org/10.1073/pnas.2020719118.
[11] M. Bergmann, S. Mützel, S. Primpke, M.B. Tekman, J. Trachsel, G. Gerdts, White and wonderful? Microplastics prevail in snow from the Alps to the Arctic, Sci. Adv. 5 (8) (2019) eaax1157, https://doi.org/10.1126/sciadv.aax1157.
[12] A.A. de S. Machado, W. Kloas, C. Zarfl, S. Hempel, M.C. Rillig, Microplastics as an emerging threat to terrestrial ecosystems, Global Change Biol. 24 (4) (2018) 1405–1416, https://doi.org/10.1111/gcb.14020.
[13] R.C. Hale, Are the risks from microplastics truly trivial? Environ. Sci. Technol. 52 (3) (2018) 931, https://doi.org/10.1021/acs.est.7b06615, 931.
[14] B. Worm, Silent Spring in the Ocean, Proc. Natl. Acad. Sci. USA 112 (38) (2015) 11752–11753.
[15] A.A. Horton, A. Walton, D.J. Spurgeon, E. Lahive, C. Svendsen, Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities, Sci. Total Environ. 586 (2017) 127–141.
[16] Y. Fei, S. Huang, H. Zhang, Y. Tong, D. Wen, X. Xia, H. Wang, Y. Luo, et al., Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil, Sci. Total Environ. 707 (2020) 135634, https://doi.org/10.1016/j.scitotenv.2019.135634.
[17] J. Wang, M. Huang, Q. Wang, Y. Sun, Y. Zhao, Y. Huang, LDPE Microplastics significantly alter the temporal turnover of soil microbial communities, Sci. Total Environ. 726 (2020) 138682, https://doi.org/10.1016/j.scitotenv.2020.138682.
[18] M. Arias-Andres, U. Klümper, K. Rojas-Jimenez, H.-P. Grossart, Microplastic pollution increases gene exchange in aquatic ecosystems, Environ. Pollut. 237 (2018) 253–261, https://doi.org/10.1016/j.envpol.2018.02.058.
[19] J. Wang, X. Qin, J. Guo, W. Jia, Q. Wang, M. Zhang, Y. Huang, Evidence of selective enrichment of bacterial assemblages and antibiotic resistant genes by microplastics in urban rivers, Water Res. 183 (2020) 116113, https://doi.org/10.1016/j.watres.2020.116113.
[20] X. Wu, J. Pan, M. Li, Y. Li, M. Bartlam, Y. Wang, Selective enrichment of bacterial pathogens by microplastic biofilm, Water Res. 165 (2019) 114979, https://doi.org/10.1016/j.watres.2019.114979.
[21] Y. Liu, W. Liu, X. Yang, J. Wang, H. Lin, Y. Yang, Microplastics are a hotspot for antibiotic resistance genes: Progress and perspective, Sci. Total Environ. 773 (2021) 145643, https://doi.org/10.1016/j.scitotenv.2021.145643.
[22] V.M. D'Costa, C.E. King, L. Kalan, M. Morar, W.W.L. Sung, C. Schwarz, D. Froese, G. Zazula, et al., Antibiotic Resistance Is Ancient, Nature 477 (7365) (2011) 457–461, https://doi.org/10.1038/nature10388.
[23] J.L. Martínez, Antibiotics and antibiotic resistance genes in natural environments, Science 321 (5887) (2008) 365–367, https://doi.org/10.1126/science.1159483.
[24] H.K. Allen, J. Donato, H.H. Wang, K.A. Cloud-Hansen, J. Davies, J. Handelsman, Call of the wild: Antibiotic resistance genes in natural environments, Nat. Rev. Microbiol. 8 (4) (2010) 251–259, https://doi.org/10.1038/nrmicro2312.
[25] Z.-Z. Yan, Q.-L. Chen, C.-Y. Li, B.-A. Thi Nguyen, Y.-G. Zhu, J.-Z. He, H.-W. Hu, Biotic and abiotic factors distinctly drive contrasting biogeographic patterns between phyllosphere and soil resistomes in natural ecosystems, ISME COMMUN 1 (1) (2021) 1–9, https://doi.org/10.1038/s43705-021-00012-4.
[26] K. Faust, J. Raes, Microbial interactions: From networks to nodels, Nat. Rev. Microbiol. 10 (8) (2012) 538–550, https://doi.org/10.1038/nrmicro2832.
[27] L. Jin, A. Pruden, A.B. Boehm, P.J.J. Alvarez, L. Raskin, T. Kphm, X.D. Li, Integrating environmental dimensions of "One Health" to combat antimicrobial resistance: Essential research needs, Environ. Sci. Technol. 56 (21) (2022) 14871–14874.
[28] D.M. Mitrano, P. Wick, B. Nowack, Placing nanoplastics in the context of global plastic pollution, Nat. Nanotechnol. 16 (5) (2021) 491–500, https://doi.org/10.1038/s41565-021-00888-2.
[29] J. Gigault, H. El Hadri, B. Nguyen, B. Grassl, L. Rowenczyk, N. Tufenkji, S. Feng, M. Wiesner, Nanoplastics are neither microplastics nor engineered nanoparticles, Nat. Nanotechnol. 16 (5) (2021) 501–507, https://doi.org/10.1038/s41565-021-00886-4.
[30] L.M. Hernandez, E.G. Xu, H.C.E. Larsson, R. Tahara, V.B. Maisuria, N. Tufenkji, Plastic teabags release billions of microparticles and nanoparticles into tea, Environ. Sci. Technol. 53 (21) (2019) 12300–12310, https://doi.org/10.1021/acs.est.9b02540.
[31] M. González-Pleiter, M. Tamayo-Belda, G. Pulido-Reyes, G. Amariei, F. Leganés, R. Rosal, F. Fernández-Piñas, Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments, Environ. Sci.: Nano 6 (5) (2019) 1382–1392, https://doi.org/10.1039/C8EN01427B.
[32] S.M. Mintenig, P.S. Bäuerlein, A.A. Koelmans, S.C. Dekker, A.P. van. Wezel, Closing the gap between small and smaller: Towards a framework to analyse nano- and microplastics in aqueous environmental samples, Environ. Sci.: Nano 5 (7) (2018) 1640–1649, https://doi.org/10.1039/C8EN00186C.
[33] O.S. Alimi, J. Farner Budarz, L.M. Hernandez, N. Tufenkji, Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport, Environ. Sci. Technol. 52 (4) (2018) 1704–1724, https://doi.org/10.1021/acs.est.7b05559.
[34] E. Schmaltz, E.C. Melvin, Z. Diana, E.F. Gunady, D. Rittschof, J.A. Somarelli, J. Virdin, M.M. Dunphy-Daly, Plastic pollution solutions: Emerging technologies to prevent and collect marine plastic pollution, Environ. Int. 144 (2020) 106067, https://doi.org/10.1016/j.envint.2020.106067.
[35] S. Fuller, A. Gautam, A procedure for measuring microplastics using pressurized fluid extraction, Environ. Sci. Technol. 50 (11) (2016) 5774–5780, https://doi.org/10.1021/acs.est.6b00816.
[36] M. Scheurer, M. Bigalke, Microplastics in Swiss floodplain soils, Environ. Sci. Technol. 52 (6) (2018) 3591–3598, https://doi.org/10.1021/acs.est.7b06003.
[37] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, H. Alexander, E.J. Alm, et al., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, Nat. Biotechnol. 37 (8) (2019) 852–857, https://doi.org/10.1038/s41587-019-0209-9.
[38] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: High-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (7) (2016) 581–583, https://doi.org/10.1038/nmeth.3869.
[39] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, et al., Vegan: Community Ecology Package, 2020.
[40] M.D. Robinson, D.J. McCarthy, G.K. Smyth, EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data, Bioinformatics 26 (1) (2010) 139–140, https://doi.org/10.1093/bioinformatics/btp616.
[41] S. Chen, Y. Zhou, Y. Chen, J. Gu, Fastp: An ultra-fast all-in-one FASTQ preprocessor, Bioinformatics 34 (17) (2018) i884–i890, https://doi.org/10.1093/bioinformatics/bty560.
[42] X. Yin, X.-T. Jiang, B. Chai, L. Li, Y. Yang, J.R. Cole, J.M. Tiedje, T. Zhang, ARGs-OAP v2.0 with an expanded SARG database and Hidden Markov Models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes, Bioinformatics 34 (13) (2018) 2263–2270, https://doi.org/10.1093/bioinformatics/bty053.
[43] G.A. Arango-Argoty, D. Dai, A. Pruden, P. Vikesland, L.S. Heath, L. Zhang, NanoARG: A web service for detecting and contextualizing antimicrobial resistance genes from nanopore-derived metagenomes, Microbiome 7 (1) (2019) 88, https://doi.org/10.1186/s40168-019-0703-9.
[44] Y.G. Zhu, Y. Zhao, B. Li, C.L. Huang, S.Y. Zhang, S. Yu, Y.S. Chen, T. Zhang, et al., Continental-scale pollution of estuaries with antibiotic resistance genes, Nat Microbiol 2 (4) (2017) 1–7, https://doi.org/10.1038/nmicrobiol.2016.270.
[45] Y. Lavaan Rosseel, An R package for structural equation modeling, J. Stat. Software 48 (1) (2012) 1–36, https://doi.org/10.18637/jss.v048.i02.
[46] D. Li, C.-M. Liu, R. Luo, K. Sadakane, T.-W. Lam, MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph, Bioinformatics 31 (10) (2015) 1674–1676, https://doi.org/10.1093/bioinformatics/btv033.
[47] D.E. Wood, J. Lu, B. Langmead, Improved metagenomic analysis with Kraken 2, Genome Biol. 20 (1) (2019) 257, https://doi.org/10.1186/s13059-019-1891-0.
[48] D.H. Parks, M. Chuvochina, P.-A. Chaumeil, C. Rinke, A.J. Mussig, P. Hugenholtz, A complete domain-to-species taxonomy for Bacteria and Archaea, Nat. Biotechnol. 38 (9) (2020) 1079–1086, https://doi.org/10.1038/s41587-020-0501-8.
[49] D. Hyatt, G.-L. Chen, P.F. LoCascio, M.L. Land, F.W. Larimer, L. Hauser, J. Prodigal, Prokaryotic gene recognition and translation initiation site identification, BMC Bioinf. 11 (1) (2010) 119, https://doi.org/10.1186/1471-2105-11-119.
[50] L. Ma, Y. Xia, B. Li, Y. Yang, L.-G. Li, J.M. Tiedje, T. Zhang, Metagenomic assembly reveals hosts of antibiotic resistance genes and the shared resistome in pig, chicken, and human feces, Environ. Sci. Technol. 50 (1) (2016) 420–427, https://doi.org/10.1021/acs.est.5b03522.
[51] G.V. Uritskiy, J. DiRuggiero, J. Taylor, MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis, Microbiome 6 (1) (2018) 158, https://doi.org/10.1186/s40168-018-0541-1.

[52] P.-A. Chaumeil, A.J. Mussig, P. Hugenholtz, D.H. Parks, GTDB-Tk: A toolkit to classify genomes with the Genome Taxonomy Database, Bioinformatics 36 (6) (2020) 1925–1927, https://doi.org/10.1093/bioinformatics/btz848.
[53] Y. Zhou, G. He, G. Bhagwat, T. Palanisami, Y. Yang, W. Liu, Q. Zhang, Nanoplastics alter ecosystem multifunctionality and may increase global warming potential, Global Change Biol. 29 (2023) 3895–3909, https://doi.org/10.1111/gcb.16734.
[54] J. Shi, D. Wu, Y. Su, B. Xie, Nano)microplastics promote the propagation of antibiotic resistance genes in landfill leachate, Environ. Sci.: Nano 7 (11) (2020) 3536–3546, https://doi.org/10.1039/D0EN00511H.
[55] K. Tallec, A. Huvet, C. Di Poi, C. González-Fernández, C. Lambert, B. Petton, N. Le Goïc, M. Berchel, et al., Nanoplastics impaired oyster free living stages, gametes and embryos, Environ. Pollut. 242 (2018) 1226–1235, https://doi.org/10.1016/j.envpol.2018.08.020.
[56] L. Rong, L. Zhao, L. Zhao, Z. Cheng, Y. Yao, C. Yuan, L. Wang, H. Sun, LDPE microplastics affect soil microbial communities and nitrogen cycling, Sci. Total Environ. 773 (2021) 145640, https://doi.org/10.1016/j.scitotenv.2021.145640.
[57] H.-Z. Li, D. Zhu, J.H. Lindhardt, S.-M. Lin, X. Ke, L. Cui, Long-term fertilization history alters effects of microplastics on soil properties, microbial communities, and functions in diverse farmland ecosystem, Environ. Sci. Technol. 55 (8) (2021) 4658–4668, https://doi.org/10.1021/acs.est.0c04849.
[58] M. Xu, W. Du, F. Ai, F. Xu, J. Zhu, Y. Yin, R. Ji, H. Guo, Polystyrene microplastics alleviate the effects of sulfamethazine on soil microbial communities at different CO_2 concentrations, J. Hazard Mater. 413 (2021) 125286, https://doi.org/10.1016/j.jhazmat.2021.125286.
[59] B. Gao, H. Yao, Y. Li, Y. Zhu, Microplastic addition alters the microbial community structure and stimulates soil carbon dioxide emissions in vegetable-growing soil, Environ. Toxicol. Chem. 40 (2) (2021) 352–365, https://doi.org/10.1002/etc.4916.
[60] A.L. Benin, R.F. Benson, K.E. Arnold, A.E. Fiore, P.G. Cook, L.K. Williams, B. Fields, R.E. Besser, An outbreak of travel-associated Legionnaires disease and Pontiac fever: The need for enhanced surveillance of travel-associated legionellosis in the United States, J. Infect. Dis. 185 (2) (2002) 237–243, https://doi.org/10.1086/338060.
[61] M.W. Azam, A.U. Khan, Updates on the pathogenicity status of *Pseudomonas aeruginosa*, Drug Discov. Today 24 (1) (2019) 350–359, https://doi.org/10.1016/j.drudis.2018.07.003.
[62] J.F. Prescott, Rhodococcus Equi: An animal and human pathogen, Clin. Microbiol. Rev. 4 (1) (1991) 20–34, https://doi.org/10.1128/CMR.4.1.20.
[63] Q. Xiang, D. Zhu, Q.-L. Chen, P. O'Connor, X.-R. Yang, M. Qiao, Y.-G. Zhu, Adsorbed sulfamethoxazole exacerbates the effects of polystyrene (~2 μm) on gut microbiota and the antibiotic resistome of a soil collembolan, Environ. Sci. Technol. 53 (21) (2019) 12823–12834, https://doi.org/10.1021/acs.est.9b04795.
[64] G. Xu, Y. Yu, Polystyrene microplastics impact the occurrence of antibiotic resistance genes in earthworms by size-dependent toxic effects, J. Hazard Mater. 416 (2021) 125847, https://doi.org/10.1016/j.jhazmat.2021.125847.
[65] Z. Qiu, Y. Yu, Z. Chen, M. Jin, D. Yang, Z. Zhao, J. Wang, Z. Shen, et al., Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera, Proc. Natl. Acad. Sci. USA 109 (13) (2012) 4944–4949.
[66] S. Zhang, Y. Wang, J. Lu, Z.G. Yu, H.L. Song, P.L. Bond, J.H. Guo, Chlorine disinfection facilitates natural transformation through ROS-mediated oxidative stress, ISME J. 15 (2021) 2969–2985, https://doi.org/10.1038/s41396-021-00980-4.
[67] S. Mohammad Mirsoleimani Azizi, B.S. Zakaris, N. Haffiez, B.R. Dhar, Sludge thermal hydrolysis for mitigating oxidative stress of polystyrene nanoplastics in anaerobic digestion: Significance of the solids content, ACS Sustainable Chem. Eng. 11 (2023) 7253–7262, https://doi.org/10.1021/acssuschemeng.3c01349.