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# Exposure to heavy metal and antibiotic enriches antibiotic resistant genes on the tire particles in soil

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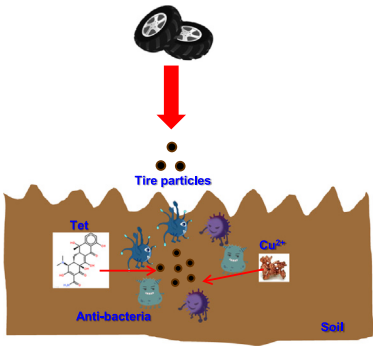
## HIGHLIGHTS

- This is the first report on response of microbe and ARGs on TPs to soil pollutants.
- Bacterial community on TPs is different from their surrounding soil.
- Cu and Tet exposure in soil shifts the bacterial community composition on TPs.
- ARGs on TPs are less diverse than soil, but strongly impacted by soil pollutants.
- There is a close connection between bacterial taxa and ARGs on TPs.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The widespread occurrence of tire particles (TPs) in soils has attracted considerable attention due to their potential threats. The assemblage of bacteria and associated antibiotic resistant genes (ARGs) on TPs is yet largely unknown, especially under the stress of soil pollutants. In the present study, TPs were incubated in soils with or without the stress of heavy metal ($Cu^{2+}$) or/and antibiotic (tetracycline), and bacterial community and ARG profile on TPs and in soils were explored using high-throughput sequencing and high-throughput quantitative PCR. Results indicated that bacterial community structure on TPs was significantly different from the surrounding soils, with a lower diversity, and significantly shifted by heavy metal and antibiotic exposure. Additionally, a diverse set of ARGs were detected on TPs, and their abundances were significantly increased under the stress of heavy metal and antibiotic, revealing a strong synergistic effect. Moreover, a good fit was observed for the correlation between bacterial community and ARG profile on TPs. Taken together, this study, for the first time, demonstrates that TPs can provide a novel niche for soil bacteria and soil resistome, and heavy metal and antibiotic exposure may potentially increase the abundance of ARGs on TPs, threatening soil ecosystems and human health.


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## 1. Introduction

The global plastics production has approached 350 million tonnes in 2017 and has continued to increase (Brahney et al., 2020). The increasing production has led to the global pollution of microplastics (MPs, <5 mm), attracting public and regulatory attention (Rochman and Hoellein, 2020). Research focus has recently started to turn to terrestrial systems, after having concentrated on aquatic ecosystems for at least a decade (De Souza Machado et al., 2018; Rachman, 2018). Moreover, previous researches on MPs were mainly about their distribution and ecotoxicology (Rillig and Lehmann, 2020). Considering that MPs with a hydrophobic surface have been identified as vectors for environmental contaminants, and recognized to provide an ideal habitat for environmental microorganisms (Arias-Andres, 2020; Rummel et al., 2017), the microbe-MPs interactions in the small niches have become an important and new topic in MPs pollution and need further investigation.

The biofilms of MPs are referred to as the plastisphere and research on the microbial community in the plastisphere also initially focused on marine and aquatic ecosystems (Jacquin et al., 2019; Yang et al., 2020). Abundant bacterial groups at a relative high taxonomic level have been observed to be selected and enriched in the plastisphere from the surrounding aquatic environments through the use of sequencing and spectral imaging (Arias-Andres, 2020). An overview of the ecology of the microorganisms living in the plastisphere in the aspect of their diversity, function and fate in the aquatic ecosystems pointed out that generally, the diversity and structural complexity in the plastisphere are increasing during the development of biofilms, and Rhodobacteraceae is abundant at all-time points and plays important roles in biofilm formation, as it is able to produce extracellular polymeric substances and promote the settlement of other microorganisms (Amaral-Zettler et al., 2020). However, few studies have focused on the microorganisms in the plastisphere of soil environments (Chai et al., 2020). Since MP contamination in soil is also severe and soil acts as a long-term sink for MPs (Rillig, 2012), researchers should expand their focus to include the soil environments. Moreover, the soil microbial community is more diverse and the biofilm development in soil would be more complicated. To better understand the behavior of MPs in soil, the microorganisms living in the plastisphere need more attention.

Since MPs can adsorb a variety of inorganic and organic pollutants including heavy metals and antibiotics from surrounding environments (Li et al., 2018; Yu et al., 2020), the microbial community colonizing on MPs may be shaped under the stress of these pollutants, becoming a reservoir of antibiotic resistance genes (ARGs). Experiments under the laboratory conditions and in fields both revealed that MPs were important vectors for a variety of pathogenic bacteria and ARGs in the aquatic ecosystems (Maso et al., 2003; Zettler et al., 2013). Moreover, the surface of MPs, colonized by metabolically complex microbial consortia, has been found to have a greater relative abundance of mobile genetic elements (MGE) and can be a hotpot for horizontal gene transfer in urban rivers (Wang et al., 2020a). More unfortunately, the long distance transport of MPs colonized by potential pathogens with ARGs can further facilitate the migration of ARGs and pathogens (Song et al., 2020). MPs in soil, especially in agricultural soil which is a reservoir of ARGs in environments (Su et al., 2014), would inevitably enrich ARGs in the plastisphere. However, there is still a knowledge gap on the profiles of ARGs in the biofilms of MPs in soil.

Among MPs, tire particles (TPs), released from car and airplane tires, are considered as one of the greatest contributors to MP pollutions (Baensch-Baltruschat et al., 2020). According to a global scale survey, a total of 5,917,515 tons of TPs are emitted into environments per year (Kole et al., 2017). Compared with other types of MPs, TPs are more resistant to physical, chemical and biological degradation and have more carbon black, heavy metals and polycyclic aromatic hydrocarbons embedded as additives (Halle et al., 2020; Wagner et al., 2018). Moreover, TPs have been demonstrated to display strong sorption affinities towards heavy metals and antibiotics (Hueffer et al., 2019), and the ambient environmental factors are regarded as the main factors influencing the microbial community structure in the biofilms of TPs in urban water environments (Wang et al., 2020b). As mentioned above, the biofilms on MPs from soil receive less attention, while this situation is even worse for TPs compared with other types of MPs, although soil is the most dominant reservoir for TPs in environments (Kole et al., 2017). Moreover, considering pollutants including heavy metals could induce co-selection of ARGs in the gut microbiota of collembolans (Ding et al., 2019a), the co-occurrence of TPs and soil pollutants including heavy metals and antibiotics may induce a stress on the bacteria and associated ARGs in the biofilms of TPs. Hence, we need to profile the bacterial community and associated ARGs in the biofilms of TPs from soil under stress of pollutants.

The study represents the first comprehensive characterization of bacterial communities and associated ARGs in the biofilms of TPs and their responses to pollutants in soil. The objects of this study are to: (1) identify the composition and structure of microbiota both in the biofilms of TPs and in the surrounding soils; (2) reveal the effects of heavy metal and antibiotic on the microbiota living on the TPs; (3) profile the ARGs in the biofilms of TPs under the stress of heavy metal and antibiotic; (4) explore the correlations between bacterial taxa and ARGs on TPs. Results from this study are expected to fill an important knowledge by ascertaining the roles TPs play in soil with or without pollution stress and improving our understanding of their environmental behavior and risks.

## 2. Materials and methods

### 2.1. Tire particles preparation

TPs were generated from a second-hand car tire (SUNFULL, China, all season, sidewall markings: 155R12C 8PR 88/86Q) using a stainless-steel grater to scalp their surface. The particle size of TPs ranged from 13 μm to 1400 μm with a medium diameter of 225.6 μm. More detailed information about the compositions of the TPs has been described in our previous study (Ding et al., 2020). To asepticize the TPs, they were soaked in a 10% (v/v) sodium hypochlorite solution for 2 h. Afterward, TPs were washed with sterile water 10 times and freeze-dried for 2 days.

### 2.2. Experimental design

A microcosm experiment was conducted to reveal the biofilm formation on TPs in soil under the stress of heavy metal and antibiotic. Copper ($Cu^{2+}$) and tetracycline (Tet) were selected in the present study as they generally co-occurred in agricultural soil and could be co-absorbed in TPs (Ding et al., 2019a). Through the use of $CuCl_2$ and Tet stock solution, 200 mg $kg^{-1}$ soil for $Cu^{2+}$ and 10 mg $kg^{-1}$ soil for Tet were added into the soil according to environmental quality standards for soils of China (GB 15618-2018) and previous studies (Ding et al., 2019a; Wang et al., 2018). The soil was collected from an agriculture land located in Ningbo, China, and the physicochemical properties of the soil were provided in Table S1. The soil is of high quality according to environmental quality standards for soils of China (GB 15618-2018). A total of four treatments were set: soil without $Cu^{2+}$ and Tet (control), soil with $Cu^{2+}$ only ($Cu^{2+}$), soil with Tet only (Tet) and soil with $Cu^{2+}$ and Tet ($Cu^{2+}$+Tet). The experiments were conducted in sterile 250 mL beakers, with four replicates for each treatment. After the water content of these soils was adjusted to 60% of the maximum water capacity, all the soils were pre-incubated in dark for two weeks to stabilize the soil properties. A nylon mesh bag (30 μm, 5.5 cm * 3.5 cm) containing 100 mg sterile TPs was placed in the surface layer (0–10 cm) of each beaker. The beakers were incubated at 20 ± 1 °C in the dark with a relative humidity of 75%. The water content was adjusted twice a week.

### 2.3. Sample collection, chemical analysis and DNA extraction

After four weeks of incubation, the bags were dug up from soils. 2.0 g soil was collected, and analyzed for Tet according to a previous study (Huang et al., 2013). For DNA extraction, the TPs in each bag were transferred into a 2 mL centrifuge tube and frozen at −20 °C immediately. About 0.5 g soil around each bag was collected and frozen immediately. Total genome DNA from soil (0.25 g for each sample) and TP (0.05 g for each sample) samples was extracted using a DNeasy Powersoil Kit (Qiagen, Hilden, Germany) according to manufacturer's protocols. The obtained DNA was kept at −20 °C until further use.

### 2.4. Characterize the microbiota in TP biofilms and soil

To amplify the V4 regions of the 16S rRNA gene in TP and soil samples, the barcoded primer set 515F and 806R was used. Triplicate PCR reactions were conducted in 50 μL reaction system containing 25 μL TaKaRa ExTap, 1 μL DNA, 2 μL corresponding forward and backward primers, and 22 μL of PCR-grade water. The PCR was performed at 95 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 50 °C for 45 s, 68 °C for 45 s, and a final step at 72 °C for 10 min. Finally, the PCR products were quantified and pooled together. High-throughput sequencing was performed on the purified and pooled samples using an Illumina Hiseq2500 platform (Novogene, Tianjin, China).

QIIME was used to carry out the sequence analysis. The detailed process has been stated in our previous study (Ding et al., 2020). Briefly, after filtering adaptor sequences and removing low-quality reads, ambiguous nucleotides and barcodes, we subsampled the reads to obtain the same number of reads in each sample. The sequences were clustered into operational taxonomic units (OTUs) at a 3% sequence difference identity with UCLUST (Edgar, 2010). Taxonomy classification was assigned by applying Ribosomal Database Project (RDP) database (version 11.5) as the reference. Observed species were calculated to reflect the alpha-diversity of the microbiota, and principal coordinate analysis (PCoA) based on the weighted unifrac distance was conducted to investigate the beta-diversity.

### 2.5. Profile the ARGs in TP biofilms and soil

The Wafergen SmartChip Real-time PCR system was used to reveal the ARG profiles in TP biofilms and soil. A total of 286 primer pairs targeting 285 ARGs and 16S rRNA gene were used in the present study. More detailed information on experimental procedure and data analysis has been described in our previous study (Ding et al., 2019b). To minimize errors due to differences in bacterial abundances between samples, the relative abundance of each ARG was calculated according to the Eqs. (1) and (2). The threshold of the $C_T$ value was set at 31, which was the number of cycles when the fluorescence signal reached the threshold.

$$\text{Relative Gene Copy Number} = 10^{\left(31-C_T\right)/(10/3)} \quad (1)$$

$$\text{The Relative Abundance of ARG} = \text{Relative ARG Copy/Relative 16S rRNA Gene Copy Number} \quad (2)$$

### 2.6. Statistical analysis

*t*-Test was used to evaluate the differences in diversity index and bacterial taxa between TP biofilms and soil samples. One-way analysis of variance (ANOVA) combined with the Tukey HSD test was conducted to reveal the effects of $Cu^{2+}$ and Tet on the relative abundance of ARGs in TP biofilms. Both *t*-test and ANOVA were conducted using the SPSS software (version20, SPSS Inc., Chicago, USA). Adonis and Anoism tests with 999 permutations were used to reveal the differences in bacterial community structure between TP biofilms and soil and between TP biofilm samples from different treatments. Nonmetric multidimensional scaling (NMDS) ordinations based on the Bray-Curtis dissimilarity matrix were performed to visualize the stresses of $Cu^{2+}$ and Tet on the ARG profiles in TP biofilms. These stresses on each bacterial taxa and shared ARGs in TP biofilms were also shown by heatmap. Mantel test, Procrustes test, and co-occurrence network were used to reveal the relationships between microbial taxa and ARGs. The analysis mentioned above was conducted in R3.4.1.

## 3. Results and discussion

### 3.1. Differences of microbiota in TP biofilms and surrounding soils

The average numbers of the identified OTUs at 97% similarity were 3214 and 1381 for soil and TP samples, respectively. Alpha-diversity was used to investigate the community diversity of microbiota and diverse bacterial assemblages were detected on TPs, with a statistically lower diversity compared with the soil microbiota (Fig. 1a, $P < 0.01$). This is consistent with previous findings that less diverse bacteria could colonize on MPs compared with surrounding environments including urban river, seawater and sediments (De Tender et al., 2015; McCormick et al., 2014; Wu et al., 2020; Zettler et al., 2013), suggesting that TPs, as a special type of MPs, might induce selective enrichment of bacteria from surrounding environments like other types of MPs did (Wu et al., 2019). Moreover, 214 OTUs were shared in all TP samples, accounting for 75.5% of the total abundance of all TP samples, and most of them were shared with soil samples (Fig. 1b). The large number of the shared OTUs between TPs and surrounding soils suggested that the bacteria living in the biofilms predominantly originated from the ambient environments. Considering that diverse OTUs were shared by TPs which have been incubated in different water columns (Wang et al., 2020b) and a core microbiota has been proposed to exist in MP biofilms in aquatic environment (Zettler et al., 2013), the results in this study indicated that TP biofilms in soil likely have a core microbiota.

To further reveal the separation pattern in bacterial community between TP biofilm and surrounding soil, PCoA based on the weighted Unifrac metric was conducted and revealed a significant separation (Adonis, $P < 0.01$) along with the primary principal coordinate (36.6%, Fig. 1c). Additionally, the relative abundances of bacterial taxa in family level further confirmed these differences (Fig. 1d). In comparison with the soil samples, 11 families were significantly enriched on TPs, while 17 families were significantly depleted. As differences in bacterial community have been observed between MP or TP biofilms and water columns (Ogonowski et al., 2018; Wang et al., 2020b; Wu et al., 2019), and between MP biofilms and soil (Chai et al., 2020), the significant differences in taxonomic composition (Fig. 1d), coupled with separation pattern (Fig. 1c) in this study revealed that the sorting phenomenon of microbial communities between TPs and surrounding soils is also prevalent.

### 3.2. Soil pollutants shaping the bacterial community in TP biofilms

The taxonomic compositions at phylum and OTU levels were used to describe the changes of bacterial community under the stress of heavy metal and antibiotic (Fig. 2). The most common bacteria in all biofilm samples were Proteobacteria, Actinobacteria and Firmicutes, accounting for 86.8% (Fig. 2a), and these were also the dominant bacteria in the soil of the control group (Fig. S1), indicating that bacteria on TPs were mainly derived from soils. This is consistent with the general observation that most of the bacteria detected in MP biofilms belong to these three phyla (Amaral-Zettler et al., 2020). In addition, these bacteria are commonly biofilm formers and contribute to the settlement of other microorganisms (Amaral-Zettler et al., 2020). Combined exposure to $Cu^{2+}$ and Tet decreased the relative abundance of Proteobacteria from 54.7% to 47.8% in TP biofilms (Fig. 2a), which may be due to that taxa within Proteobacteria in the biofilms are generally sensitive to

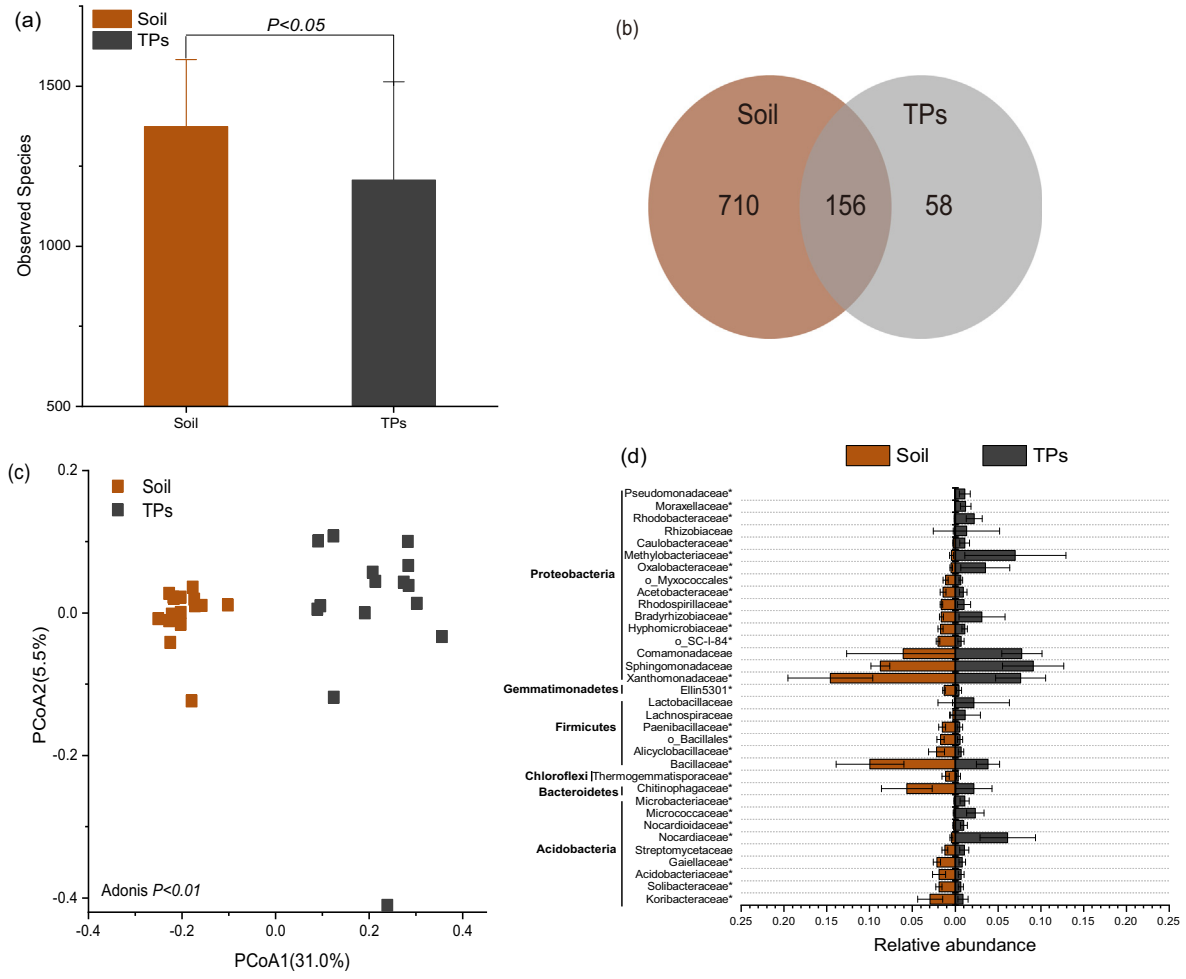


**Fig. 1.** Dissimilar microbiota in TP biofilms and soils. (a) Differences in observed species of bacterial communities in TP biofilm and soil samples (*t*-test, $n = 16$). (b) The number of shared OTUs in TP biofilm and soil samples. (c) Principal coordinate analysis (PCoA) plots of OTUs between soil and TP samples based on the weighted UniFrac distance matrix. (d) A significant difference of bacterial community between soil and TP samples at family level (*t*-test, n = 16). Only the relative abundances >1% were presented.

$Cu^{2+}$ and Tet (Corcoll et al., 2019; Lin et al., 2016). PCoA further demonstrated that both in TP biofilms and soils, bacterial community was significantly shaped after pollutant exposure ($P < 0.05$, Fig. 2b and Fig. S2), and the combined effect of $Cu^{2+}$ and Tet on bacterial community was stronger than these of individual component alone, indicating a synergistic effect (Fig. 2b and Fig. S2). One probable mechanism is that the shifts in soil microbial community induced by $Cu^{2+}$ and Tet stress might drive the bacterial community in TPs, since soil microbiota determined the microbiota living on TPs. Besides, considering it has been reported that antibiotic absorption by MPs might be the main reason driving the bacterial community structure on MPs (Wang et al., 2020b) and TPs have sorption affinities towards heavy metals and antibiotics (Lian et al., 2013), the shift in bacterial community on TPs could be attributed to the synergistic stress induced by sorption of $Cu^{2+}$ and Tet on the TPs. Similarly, the taxonomic compositions at OTU level also indicated a synergistic effect of $Cu^{2+}$ and Tet on microbiota on TPs, and the abundances of some genus such as *Sphingomonas* and *Pseudomonadaceae* were significantly increased in Tet and $Cu^{2+}$+Tet treatments (Fig. 2c). The increase in the abundances of these specific genera were likely due to their metabolic versatility, genome plasticity, and intrinsic resistance to Tet (Butiuc-Keul et al., 2021; Khan et al., 2016; Zhang et al., 2018), which will enable them to withstand the presence of these stressors and grow in various environments. Interestingly, Tet concentrations decreased after four weeks of incubation (Fig. S3), and this is consistent with the increased abundances of bacteria capable of degrading organic compounds. These bacteria are generally the dominant genus in the biofilms of bioreactors under the stress of antibiotics (Qiu et al., 2013), further revealing that antibiotic could be absorbed onto the surface of TPs and induce specific selection on the microbiota. To date, the formation mechanism of biofilms in MPs has rarely been studied, and location specific characteristic is considered as the most important determinant than time-specific and substrate-specific factors in shaping the bacterial community on MPs (Oberbeckmann et al., 2018; Yang et al., 2020). While previous study found that nutrients in water contributed to the formation of biofilms on TPs (Wang et al., 2020b), the above results of this study confirmed that soil pollutants play an important role in shifting the bacterial community on TPs.

### 3.3. Soil pollutants increasing the abundance of ARGs in TP biofilms

The number of detected ARGs in all TP biofilms was 26, which was lower than that in soil (75 subtype ARGs were detected in all soil samples). However, most of the ARGs in TP biofilms were shared with soil ARGs (Fig. S4), indicating that most ARGs on TPs were derived from soils. It has been reported that MPs are an important reservoir for ARGs in both marine and terrestrial environment (Wang et al., 2020a; Wang et al., 2020c; Yang et al., 2019), and the diversity of ARGs on MPs was higher than that of ARGs from seawater microbiota (Wu et al., 2019). The average detected numbers of ARGs were 11 and 38 in TP biofilm samples and soil samples, respectively, which is consistent with the lower diversity of microbiota in TP biofilms compared with the soil microbiota (Fig. 1a, $P < 0.01$). This could be due to that the soil is

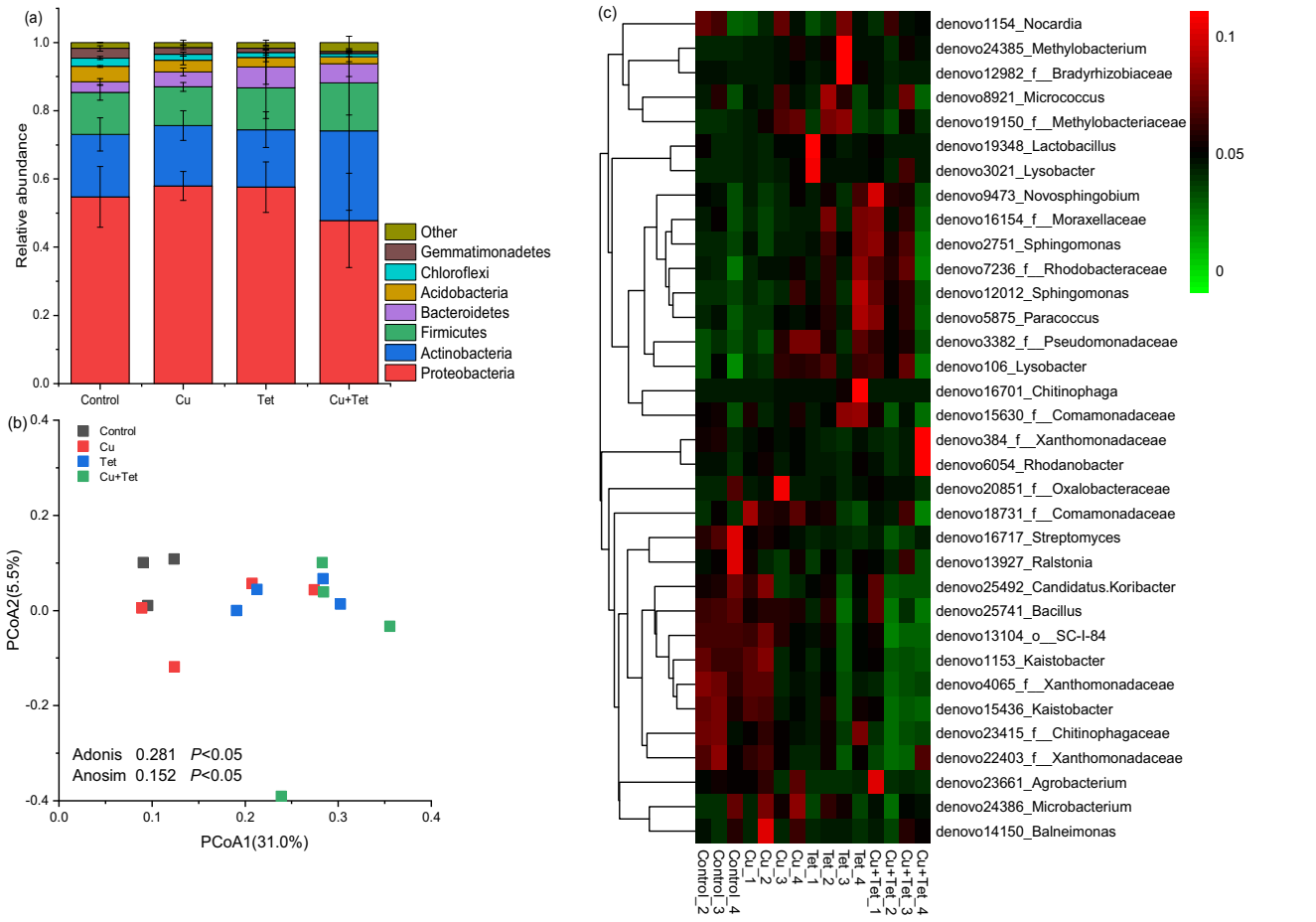


**Fig. 2.** Effects of heavy metal ($Cu^{2+}$) and antibiotic (Tet) amendment on the bacterial community on TPs. (a) Bar plot showing the relative abundance of different bacterial phylum of TP samples from different treatments. (b) Principal coordinate analysis (PCoA) plots of OTUs among different treatments based on the weighted UniFrac distance matrix. (c) Heatmap showing the relative abundance of different bacterial taxa of TP samples from different treatments.

more nutritional than seawater and the poorer nutritional conditions on TPs than soil might limit the enrichment of ARGs from surrounding soils. Notably, compared with the control treatment, pollutant amendment did not increase the abundance of ARGs in soils (Fig. S5), but the stress of $Cu^{2+}$ and Tet, especially their combined stress, increased the abundance of ARGs in TP biofilms (Fig. 3a). To our knowledge, this study for the first time observed that exposure to soils contaminated with heavy metal and antibiotic could increase the abundance of ARGs in TP biofilms. Moreover, the compositions of ARGs in soils and TP biofilms were both shifted due to the stress of $Cu^{2+}$ and Tet (Fig. 3b and Fig. S6). The synergistic effect on ARGs has been observed in soils, water columns and even animal gut (Ding et al., 2019a; Hu et al., 2016; Stepanauskas et al., 2006). Since $Cu^{2+}$ and Tet amendment under the concentrations of the present study did not induce the enrichment of ARGs in soils (Fig. S5), pollutants adsorbed on the TPs were considered as an important factor shaping the composition of ARGs in TP biofilms. As the presence of $Cu^{2+}$ could enhance the sorption of Tet on TPs (Lian et al., 2013), the coadsorption of $Cu^{2+}$ and Tet might further explain their synergistic effect on the ARGs in the biofilms of TPs. The synergistic effect has also been observed in the gut associated ARGs of soil fauna (Ding et al., 2019a). The above results indicated that bacteria living in the special niches of soil such as TP biofilms or gut of soil fauna might suffer a heavier stress of heavy metal and antibiotic than soil microbiota, inducing the enrichment of ARGs. In addition, ARGs with the highest relative abundances in the biofilms of TPs were blaOXY-1, fabK and aadA7 (Fig. S7), and these ARGs have been reported to be widely distributed in different environments and can be disseminated through horizontal gene transfer (Mbelle et al., 2020; Xu and Chen, 2020), indicating the potential risks of TPs to the environment and public health.

To further visualize the specific enrichments of ARGs on TPs, heatmap was conducted and showed that 8 ARGs were shared in all the TP samples, with 6 subtypes of ARGs being significantly increased under the combined stress of $Cu^{2+}$ and Tet (Fig. 3c). Surprisingly, the abundances of some subtypes of ARGs which were not targeted to Tet increased under the stress of Tet (Fig. 3c). For example, the abundances of the ARGs belonging to aminoglycoside resistance and multidrug were up-regulated (Fig. 3c). Considering that aminoglycoside genes were prevalent in different environments and also the dominant ARGs in biofilms (Huyan et al., 2020), and multidrug genes, such as *mef*A were also detected in the biofilms of MPs (Wang et al., 2020a), co-selection of heavy metal and antibiotic might be the dominant driver for the increase and prevalence of non-targeted ARGs. The co-selection has been observed in different environmental media, including freshwater, soil and even the digestive tract of soil fauna (Ding et al., 2019a; Hu et al., 2016; Stepanauskas et al., 2006). The present study for the first time confirmed the co-selection in the biofilms of TPs. Since the potential pathogens are widely distributed in the biofilms, the co-selection may contribute to the emergence of superbacteria and further threaten human health.

### 3.4. Relationships between microbiota and ARGs in TP biofilms

To investigate the relationships between ARG profiles and bacterial community composition, Procrustes analysis and Mantel test were

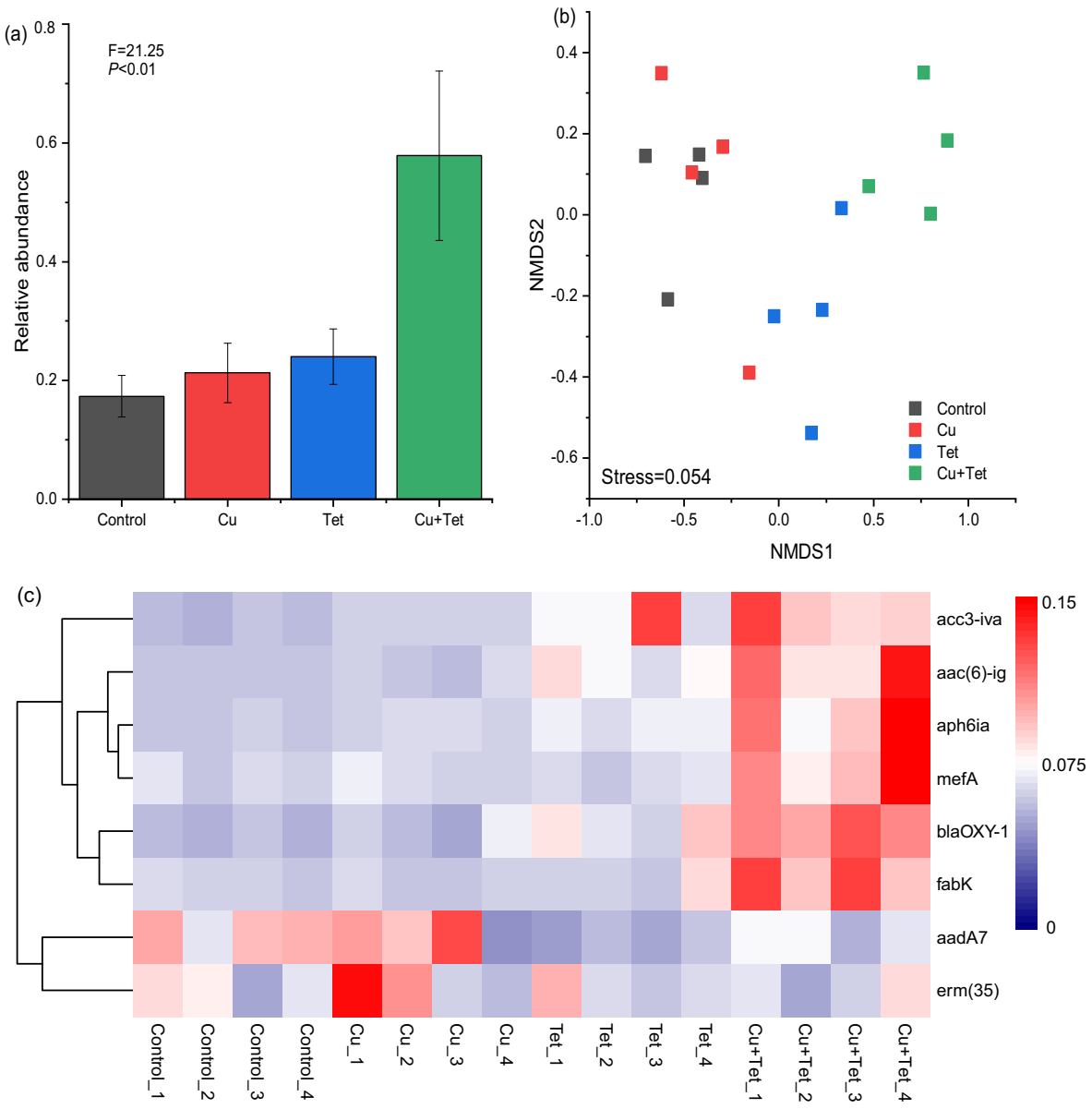


**Fig. 3.** Effects of heavy metal ($Cu^{2+}$) and antibiotic (Tet) amendment on the profiles of ARGs in the biofilms of TPs. (a) Differences in relative abundance of ARGs on TPs after exposure to $Cu^{2+}$ and Tet (one-way ANOVO, $n = 4$). (b) Nonmetric multidimensional scaling revealing the separation of ARG profiles on TPs among different treatments. (c) Heatmap showing the enrichment of shared ARGs in the $Cu^{2+}$ and Tet treated TP biofilms compared with the control.

conducted using Bray-Curtis dissimilarity metrics. Procrustes analysis revealed that there was a significant correlation between ARG profiles and bacterial community composition ($M^2 = 0.296$, $P < 0.001$, Fig. 4a), which was further confirmed by the Mantel test, indicating that the distinct profile patterns of ARGs in different treatments might be potentially linked to the changes of microbiota. As bacterial community has been reported as the main driver of resistome composition in various environments, such as soil, sediment, and the plastisphere (Chen et al., 2016; Wang et al., 2020a; Zhu et al., 2017), the stress induced by antibiotic and heavy metal could shift the microbiota on TPs and synchronously change the profiles of their ARGs.

The close connection between bacterial taxa and ARGs on TPs was further confirmed by the co-occurrence network (Fig. 4b). Positive correlations were generally found in ARGs-ARGs and bacteria-bacteria pairing, however, there were still several links between ARGs and bacterial taxa, such as the correlations between de novo 384 and fabK and between de novo 22,403 and mefA, indicating a potential host for these ARGs in the biofilms of TPs (Fig. 4b). Interestingly, the ARGs on TPs were significantly correlated with Proteobacteria (Fig. 4b and Table S2), indicating that ARGs might be primarily contained in Proteobacteria. The close links between ARGs and Proteobacteria were also observed in the biofilms of other types of MPs in urban rivers (Wang et al., 2020a), indicating MPs including TPs might selectively enrich microorganism containing ARGs from surrounding environments.

## 4. Conclusions

TPs, as an emerging pollutant, widely occurred in soil, and their environmental behavior has attracted a large attention. The present study showed that a variety of soil microorganisms were selectively enriched in the biofilms of TPs and the bacterial community composition on TPs was further shaped by $Cu^{2+}$ and Tet exposure in soil, implying that

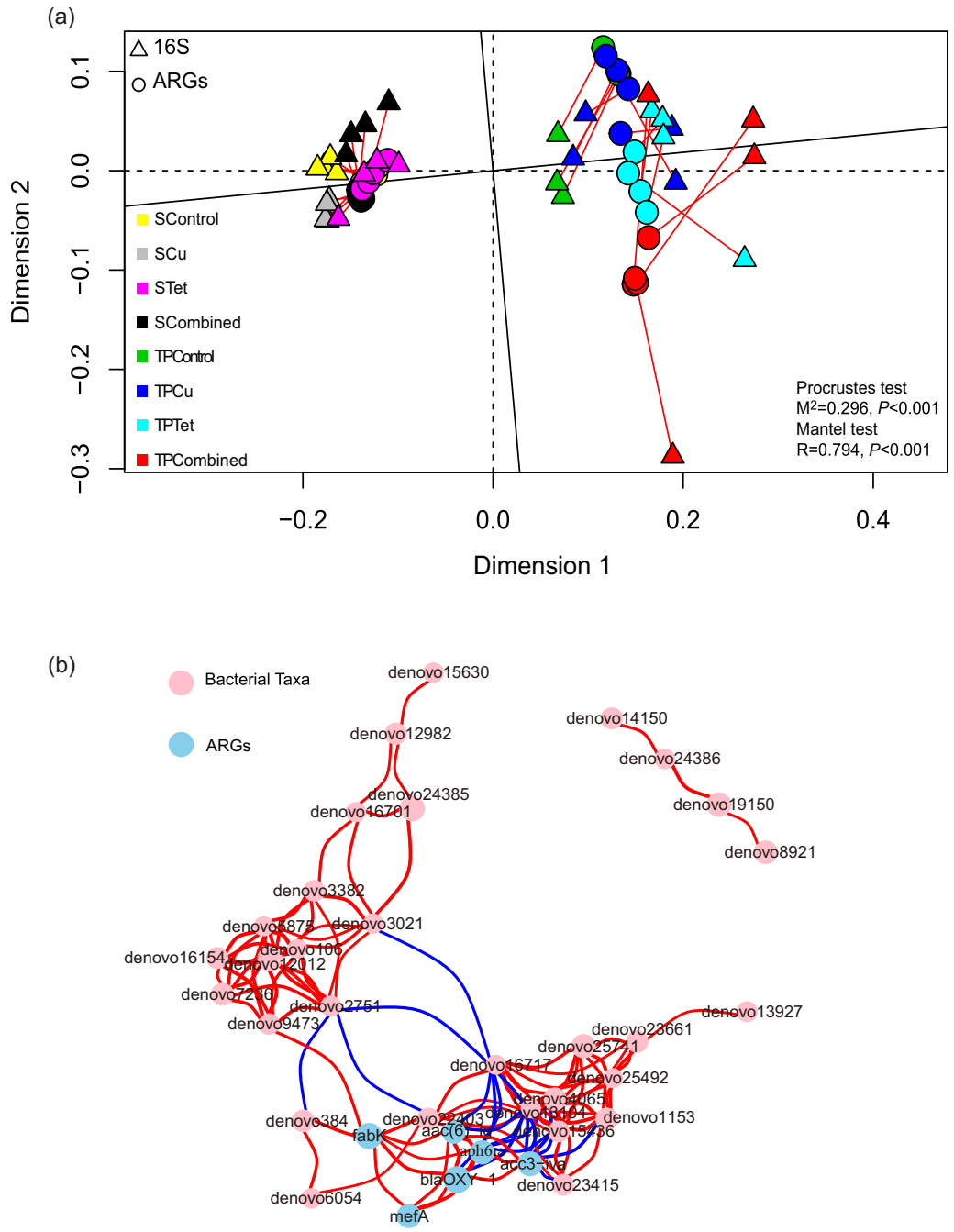


**Fig. 4.** Relationships between microbiota and ARGs in biofilms of TPs. (a) Procrustes test showing significant correlation between ARG profile and bacterial community. (b) Co-occurrence network analysis of the abundant microbiota (relative abundance >1%) and shared ARGs on TPs in response to heavy metal ($Cu^{2+}$) and antibiotic (Tet) amendment.

biofilms of TPs provide a unique niche for particular microorganisms. More importantly, compared with soil, although ARGs on TPs were less diverse, but they were more strongly impacted by soil pollutants, with the abundance of these ARGs in TP biofilms increased, indicating that TPs are important reservoirs for ARGs. Considering that TPs could subsequently transport from soil into surface water along with rainwater, the present study highlights the potential for TPs to act as vector for the spread of ARGs in environments. xNotably, TPs are more durable and have a stronger adsorption towards pollutants than other typical MPs, and they could therefore provide a long-distance transportation of ARGs, further increasing the incidence of superbugs in environments. Hence, TPs in soils may emerge as a preferred habitat and a vector for the disseminating of ARGs in different environments, especially under the stress of pollutants, thus increasing potential risks to ecological safety and human health.

## CRediT authorship contribution statement

**Jing Ding:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Dong Zhu:** Conceptualization, Formal analysis. **Yang Wang:** Investigation. **Hongtao Wang:** Investigation. **Aiping Liang:** Investigation. **Hongwei Sun:** Investigation. **Qinglin Chen:** Formal analysis, Investigation. **Simon Bo Lassen:** Investigation. **Min Lv:** Conceptualization, Investigation, Writing – review & editing, Supervision. **Lingxin Chen:** Resources, Writing – review & editing.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148417.

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