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Machine learning predicts the impact of antibiotic properties on the composition and functioning of bacterial community in aquatic habitats

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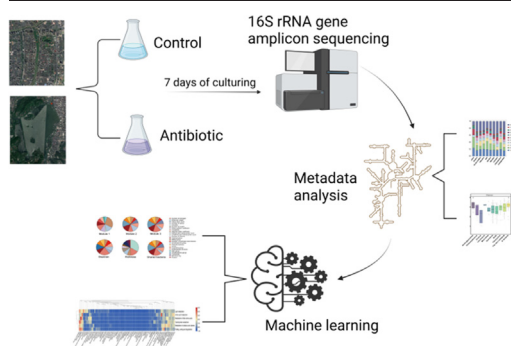
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HIGHLIGHTS

- Aquatic environmental risk was completely evaluated for 50 typical antibiotics.
- Antibiotics disturbed the structure and function of bacterial community.
- Machine learning screened several important factors in affecting bacterial community.

GRAPHICAL ABSTRACT



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ABSTRACT

In the past decades, hundreds of antibiotics have been isolated from microbial metabolites or have been artificially synthesized for protecting humans, animals and crops from microbial infections. Their everlasting usage results in impacts on the microbial community composition and causes well-known collateral damage to the functioning of microbial communities. Nevertheless, the impact of different antibiotic properties on aquatic microbial communities have so far only poorly been disentangled. Here we characterized the environmental risk of 50 main kinds of antibiotics from 9 classes at a concentration of 10 µg/L for aquatic bacterial communities via metadata analysis combined with machine learning. Metadata analysis showed that the alpha diversity of the bacterial community increased only after treatment with aminoglycoside and β-lactam antibiotics, while its structure was changed by almost all tested antibiotics. The antibiotic treatment also disturbed the functions of the bacterial community, especially with regard to metabolic pathways, including amino acids, cofactors, vitamins, xenobiotics and carbohydrate metabolism. The critical characteristics (atom stereocenter count, number of hydrogen atoms in the antibiotic, and the adipose water coefficient) of antibiotics affecting the composition of the bacterial community in aquatic habitats were screened by machine learning. The key characteristics of antibiotics affecting the function bacterial communities were the number of hydrogen atoms, molecular weight and complexity. In summary, by developing machine learning models and by performing metadata analysis, this study provides the relationship between the properties of antibiotics and their adverse impacts on aquatic microbial communities from a macro perspective. The study also provides guidance for the rational design of antibiotics.

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1. Introduction

The term 'antibiotics' refers to substances produced by microorganisms that inhibit reproduction or kill other microorganisms (Clardy et al., 2009). Penicillin was the first antibiotics discovered and successfully used for medical treatment. This was regarded as a milestone in human history to fight against bacteria (Gould, 2016). The rapid revolution in antibiotics has saved countless lives. Apart from their application in medicine, increasing amounts and kinds of antibiotics are also widely used in modern agriculture (Miroshnikova et al., 2021) and animal husbandry (Sarmah et al., 2006). By 2030, the usage of antibiotics for veterinary and clinic use is expected to rise to 105,600 tons per year (Gelband et al., 2015) and 85.45 billion defined daily doses (Klein et al., 2018). Globally, chicken, cattle and pig antimicrobial sales are expected to reach 104,079 tons in 2030, and consumption in Asia is expected to reach 63,062 tons, with all continents expected to increase their antimicrobial use in the future due to the current COVID-19 pandemic (Lardé et al., 2021; Tiseo et al., 2020).

Such everlasting usage of antibiotics is increasing the concentrations of their residues in the environment, which threatens the stability and functioning of ecosystems (Hu et al., 2010). Wastewater from hospitals, aquacultures and livestock containing residual antibiotics commonly passes through wastewater treatment plants before being discharged to freshwater (Diwan et al., 2010; Larsson, 2014; Sim et al., 2011; Zhang et al., 2013). However, wastewater treatment plants cannot completely remove antibiotics (Islam and Gilbride, 2019; Lenart-Boron et al., 2020; Zielinski et al., 2021), which results in antibiotic residues in freshwater. It was reported that the concentration of residual antibiotic was 127–1210 ng/L in Taihu Lake (Xu et al., 2014). China has very high antibiotic detection rates of 100, 98.0 and 96.4% in soil, surface water and coastal water in 2020, respectively (Lyu et al., 2020).

Previous research mainly focused on the impact of single antibiotics on aquatic microorganisms. For example, Janecko et al. (2016) assessed the negative effects of residual fluoroquinolones on metabolic pathways of aquatic organisms. Lu et al. (2019b) exhibited that the classification and function of freshwater plankton communities were induced by ciprofloxacin. Due to the difference of experimental designs, methods, and physico-chemical properties of the aquatic media used, results across reported across various studies were highly heterogeneous and could not be directly compared for the aim of obtaining universal conclusions regarding the impact of antibiotics on microbial ecosystems (Ramirez et al., 2018; Xu et al., 2020). At the same time, the impact of the physical and chemical properties of antibiotics on aquatic microorganisms that are critical for the design of eco-friendly antibiotics is also still unknown. The development of high-throughput sequencing technology and machine learning provides a new perspective to reveal the specific impact of antibiotics on the aquatic microbial community and diversity.

Machine learning is currently the most popular research technique with powerful training and prediction capabilities and has been widely used in robot control, compound synthesis, precision medicine, and microbiome research (Ban et al., 2020; Cammarota et al., 2020; Jordan and Mitchell, 2015; Wright et al., 2021; Yuan et al., 2020; Zhang et al., 2021). At present, machine learning methods including random forest, support vector machine and in the statistical classification meta-algorithm AdaBoost (Wilck et al., 2017). Among the available machine learning techniques, the random forest generally provided both higher accuracy and prediction accuracy compared to the other two approaches (Fernandez-Delgado et al., 2014; Speiser et al., 2019). Specifically, the random forest algorithm was the most common machine learning method in the field of dietary habit typing (Ren et al., 2019), disease diagnosis (Zhang et al., 2019), and plant subspecies prediction (Yuan et al., 2020) due to its high accuracy and robustness in the prediction of highly heterogeneous data. For example, Yuan et al. (2020) revealed the key biological indicators and characteristics of the soil microbial community with regard to the Fusarium wilt disease, and these authors thereby predicted the occurrence of this pathogenic fungus in soil to ensure that measures can be taken to increase the food production. Zhang et al. (2022b) combined meta-data analysis with machine

learning and accurately identified the general patterns of the bacterial community composition in disease-suppressive soils. These authors detected 28 potential beneficial bacteria.

It is known that several factors determine the effects of antibiotics on communities of microorganisms, such as the test concentration (Lundstrom et al., 2016; Marti et al., 2016), exposure time (Corno et al., 2014; Lofmark et al., 2006), and joint effects of multiple antibiotics (Liu et al., 2009; Zhang et al., 2022c). However, few studies focused on the influence of the physical and chemical properties of antibiotics on microorganisms in the aquatic environment. In this study, 16S rRNA gene sequencing data were combined with machine learning to predict the relationship between antibiotic characteristics (such as atom stereocenter count, number of hydrogen atoms, and the adipose water coefficient) and the composition and functioning of aquatic bacterial communities at one specific test concentration (10 µg/L). This concentration was chosen because it showed inhibitory effects on aquatic microorganism in our previous report (Lu et al., 2019b). We studied the changes of composition and function of the aquatic microbial community after the treatment of 50 kinds of antibiotics belonging to 9 classes. The present study mainly aimed to establish a predictive model of antibiotic properties that affect the composition and functioning of aquatic microbial communities. The study could be a contribution to the knowledge of which antibiotic characteristics are harmful for the aquatic ecosystem, and provides a guidance of the further development of eco-friendly antibiotics.

2. Material and methods

2.1. Sample collection and processing

In this experiment, 9 categories of antibiotics commonly used in human and veterinarian were selected. From these 9 categories of antibiotics, we selected 50 commonly used and easily available antibiotics (Huang et al., 2020) (Table S1 and Fig. S1). For subsequent prediction, we obtained their physical and chemical properties from online websites (<https://pubchem.ncbi.nlm.nih.gov>). The properties considered, included: atom stereocenter count, number of hydrogen atoms, adipose water coefficient etc. Considering that some antibiotics are not soluble in water, we used acetone to prepare 1 g/L stock solutions for each antibiotic. The final concentration of acetone in the microcosm was 0.001% and acetone was also added at the same concentration in the control.

A total of 40 L surface water was sampled at a depth of 0.5 m in the Hangzhou West Lake (Zhejiang, China; 30°18'45"N, 120°09'06"E) and the Hangzhou Tiesha River (Zhejiang, China; 30°16'18"N, 120°11'01"E) as representatives of two kinds of freshwater microbial samples. It is known that the water temperature in the West Lake varies from 29.4 °C in summer to 7.1 °C in winter. The lake surface mean pH ranged from 6.5 to 7.3. The Dissolved Oxygen (DO) concentration ranged from 5.21 mg/L to 7.36 mg/L (Song et al., 2017). The annual water temperature of the Tiesha River was between 10.9 °C (winter) and 27.4 °C (summer). The average surface pH of this river ranged from 6.65 to 8.16. The DO concentration in the Tiesha River ranges from 6.31 mg/L to 8.72 mg/L (Shi et al., 2015). The water samples were immediately transported to the laboratory and placed under a 46 µmol/m²/s cool-white fluorescent light with a 12:12 h light to dark photoperiod at 25 ± 0.5 °C. After 2 days of adaptation, the microcosms containing fresh aquatic microbial communities were divided into a control group and antibiotic-treated groups. Each microcosm contained 200 mL of aquatic microbial samples and was cultured in a 250 mL Erlenmeyer flask, with 3 replicates for each group and with the same light intensity and temperature for 7 days. Antibiotics were separately added until the final concentration in each bottle reached 10 µg/L. This could slightly change the composition of the main species of aquatic microbiota present in the samples.

2.2. The 16S rRNA gene amplicon sequencing

For each group, 150 mL of the aquatic medium was filtered through a 0.45 µm membrane to collect samples for 16S rRNA gene amplicon

sequencing after 7 days of culturing. The total DNA of microbes was extracted using the FastDNA SPIN Kit (MP Biomedicals, OH, USA). The primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGAC TACHVGGGTWTCTAAT) were used to amplify the V3-V4 hypervariable region of the 16S rRNA gene via thermocycler a PCR system (GeneAmp 9700, ABI, USA) and they were then sequenced on the Illumina HiSeq2500 platform (Illumina, USA). The original sequencing data was submitted to the NCBI Sequence Read Archive (SRA) database with the BioProject number PRJNA771876.

2.3. Analysis of 16S rRNA gene sequencing

The raw data obtained by sequencing were imported into QIIME2. After removal of the adaptor and primer sequences from the reads by Cutadapt (Martin, 2011), we used DADA2 to merge the paired end reads, to denoise the sequence, and to obtain the feature table and sequence of amplified sequence variants (ASVs) under the default quality threshold. All characteristic sequences were annotated with the full-length 16S rRNA gene SILVA database for subsequent analysis. For over-amplification for different samples, we screened the ASV table according to the following rules: (1) screening the ASVs with reads number less than 10; (2) removing ASVs annotated as mitochondria and chloroplasts; (3) subsampling for each sample to an equal sequencing depth (20,000 reads per sample).

2.4. Functional prediction of the microbial community and co-occurrence network analysis

Functional prediction was performed in this study by PICRUSt2, and functional pathways were classified in the KEGG database (Kyoto Encyclopedia of Genes and Genomes; <https://www.kegg.jp/>). In order to explore the influence of the physicochemical properties of antibiotics on the stability of the microbial network in the aquatic systems, we conducted a co-occurrence network analysis at the genus level. The interactions between aquatic bacteria were calculated using pairwise Spearman's rank correlations (r) by the psych package in R (version 4.0.3). Only strong ($r > 0.8$ or $r < -0.8$) and statistically significant ($p < 0.05$) correlations were used for constructing the co-occurrence network in Gephi (version 0.9.2) as described in our previous study (Zhang et al., 2022a). The modularity of the co-occurrence network was also calculated in Gephi. Bacteria were divided into several modules according to the interaction with each other.

2.5. Predictive random forests model construction

Using the regression model established by the random forest (RF) algorithm, the relationship between the physicochemical properties of antibiotics and the microbial profiles, including the Shannon and Richness indices, the relative abundance of the three microbial modules, the antibiotic-sharing bacteria and the functional profiles was constructed. The physical and chemical properties of antibiotics were input as independent variables, whereas the microbial profiles were the dependent variables. The regression model was verified by a 10-fold cross-validation method using the scikit-learn library (version 0.24.0) in python, and the best model with the highest R^2 values was selected for subsequent analysis. The importance of each physicochemical property of the antibiotics was calculated by the R package RandomForest.

2.6. Visualization and statistics

The vegan and picante packages in R were used to calculate the alpha diversity index of Shannon and Richness. The principal coordinate analysis (PCoA) graph was generated by the Bray-Curtis distance created using the R packages ggplot2 (version 3.3.2) and vegan (version 2.5-6). Permutation multivariate analysis of variance (PERMANOVA) (Adonis, Bray-Curtis conversion data, permutation = 999) was used to determine whether the beta diversity was significantly different between the antibiotic treatment and the control. All histograms and pie charts were constructed with Prism

5.0 software. The heat map was constructed using TBtools (Chen et al., 2020), and the Venn diagrams were performed using the online website jvenn (Bardou et al., 2014). Statistically significant differences ($p < 0.05$) were determined using Kruskal-Wallis test in SPSS software (version 26.0).

3. Results and discussion

3.1. Diversity and composition of the aquatic bacterial community after antibiotic treatment

We analysed the microbial community structure from the two locations with high-intensity human activity. The residual amount of antibiotics could not be detected in the water after 7 d of exposure. Previous reports showed that the physico-chemical properties of the aquatic exposure medium were only slightly changed after antibiotic treatment, compared with the control (Lu et al., 2019b; Deng et al., 2022). Therefore, we ignored the change of water physico-chemical properties, and directly explored the change of the bacterial community composition in microcosm after antibiotic treatment. In the microcosms with water from the Tiesha River, five types of antibiotics (nitroimidazole, quinolone, lincomycin, chloramphenicol and aminoglycosides) significantly increased the Richness of the bacterial community (Fig. S2a) compared to the control, while the Shannon index of the bacterial community increased significantly after the treatment of β -lactams, nitroimidazole, tetracycline and aminoglycosides (Fig. S2b). In microcosms with water from West Lake, the Richness of β -lactams, nitroimidazole, tetracycline and aminoglycosides increased significantly (Fig. S2d) compared to the control, while seven types of antibiotics (nitroimidazole, β -lactams, tetracycline, quinolone, macrolides, lincomycin and aminoglycosides) significantly increased the Shannon index of the bacterial community (Fig. S2e). Principal coordinate analysis (PCoA) combined with the Bray-Curtis distance showed that the composition of the bacterial community of the West Lake and the Tiesha River changed significantly after antibiotic treatments (Fig. S2c and f; Table S1). In general, antibiotics showed more dramatic influences on the Richness and Shannon indices in the microcosms established by water from the West Lake than in the water of the Tiesha River (Fig. 1a). At the phylum level, the abundance of *Patescibacteria* increased slightly in West Lake (Fig. S3a), but decreased in the Tiesha River (Fig. S3c) after Macrolides treatment. At the genus level, *Elstera* was significantly decreased in water from the West Lake and from the Tiesha River after macrolides, tetracycline and sulfa treatment (Fig. 1b). Our findings showed that after antibiotic treatment the diversity of both water samples had increased with the change of community composition.

In order to illustrate the universal influence of antibiotics on aquatic microbiota, we combined the data from rivers and lakes to exclude the possible influence of environmental factors. The combination of the data for the river and lake showed that the Shannon index and Richness of the bacterial community in the microcosms were significantly increased after the treatment of aminoglycoside and β -lactam (Fig. 2a and b, $p < 0.05$), but were not significantly affected by any of the other tested antibiotics. We obtained antibiotic-sharing bacteria using the Venn diagram, in which 247 genera of bacteria were present in both the control and the antibiotic treatment. However, the number of bacterial groups in the different types of antibiotics treatment exceeded the numbers of bacterial groups in the control (Fig. S4). The increased diversity could be attributed to stimulative effects of these two types of antibiotics on the growth of specific aquatic microorganisms and enriched some tolerant bacteria, as tetracycline increased the relative abundance of tetracycline resistant bacteria at 10 $\mu\text{g/L}$ in biofilms of aquatic bacterial communities (Deng et al., 2020; Lundstrom et al., 2016). Principal coordinate analysis (PCoA) combined with the Bray-Curtis distance showed that the composition of the bacterial community changed significantly after antibiotic treatments (Fig. 2c, $R^2 = 0.06615$, $p < 0.001$, Adonis). At the family level, the top bacteria of all samples were mainly composed of *Comamonadaceae*, *Flavobacteriaceae*, *Oxalobacteraceae* and *Methylophilaceae* (Fig. 2d). The numbers of some bacterial families, for example: the abundance of *Azospirillaceae*, were

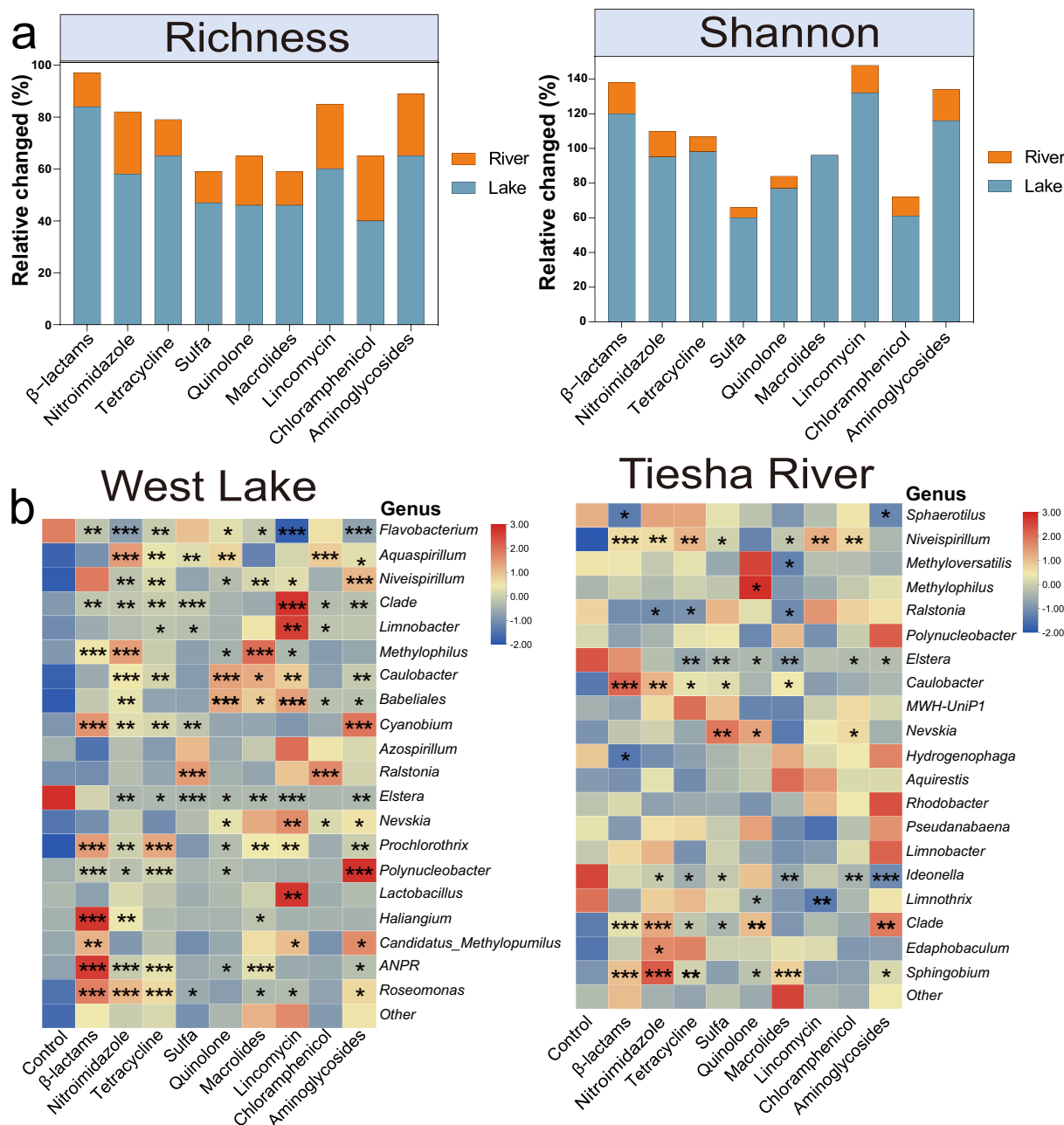


Fig. 1. The influence of antibiotics on the diversity and classification of microbial communities in the West Lake and in the Tiesha River. (a) Growth rates of the Richness and Shannon indices (b) Changes in genus (top 20) of microbial communities ("Other" refers to all the remaining genus except the top 20 were collected). * represents statistically significant differences from a one-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

significantly higher than in the control after the treatments of β -lactams ($p < 0.05$), nitroimidazole ($p < 0.05$), tetracycline ($p < 0.05$), and sulfa ($p < 0.05$), lincomycin ($p < 0.001$) and chloramphenicol ($p < 0.05$), while the relative abundance of *Burkholderiaceae* under quinolone treatment was lower than that of the control ($p < 0.05$; Fig. 2d).

The composition of aquatic microbial community depended on environmental factors, including biotic and abiotic factors (Lu et al., 2019a; Lu et al., 2021; Zhang et al., 2022b). Altogether, antibiotics treatment dramatically influenced the composition of the bacterial community. The possible reason is that addition of antibiotics in the aquatic system exerted selective pressure on the microbial community, with the resistant microbes being able to survive and further influence other microorganisms via species interactions (An et al., 2018; Rodriguez-Mozaz et al., 2020). At the same time, different kinds of antibiotics exhibited strain-specific activity,

which inhibited or irreversibly killed specific members of the microbial community, and hence, impacted on community composition (Maier et al., 2021).

The abundance of antibiotic resistance genes (ARGs) in samples could change and explain the effects of antibiotics on aquatic microbiota. Zhang et al. (2022e) detected 2561 ARGs that collectively conferred resistance to 24 classes of antibiotics from various habitats (560 sites, 4572 samples, including aquatic habitats) by public metagenomic data, and demonstrated that the abundance of ARGs is high in the area with high-intensity anthropogenic activity, such as in two sampling sites in this study. ARGs accumulate resistant microbes after antibiotic treatment, and inhibit the growth of antibiotic-sensitive bacteria, and therefore change the structure of the microbial community (Grenni et al., 2018; Lu et al., 2019b). The mechanism underlying the impact of antibiotics on microbiota communities should

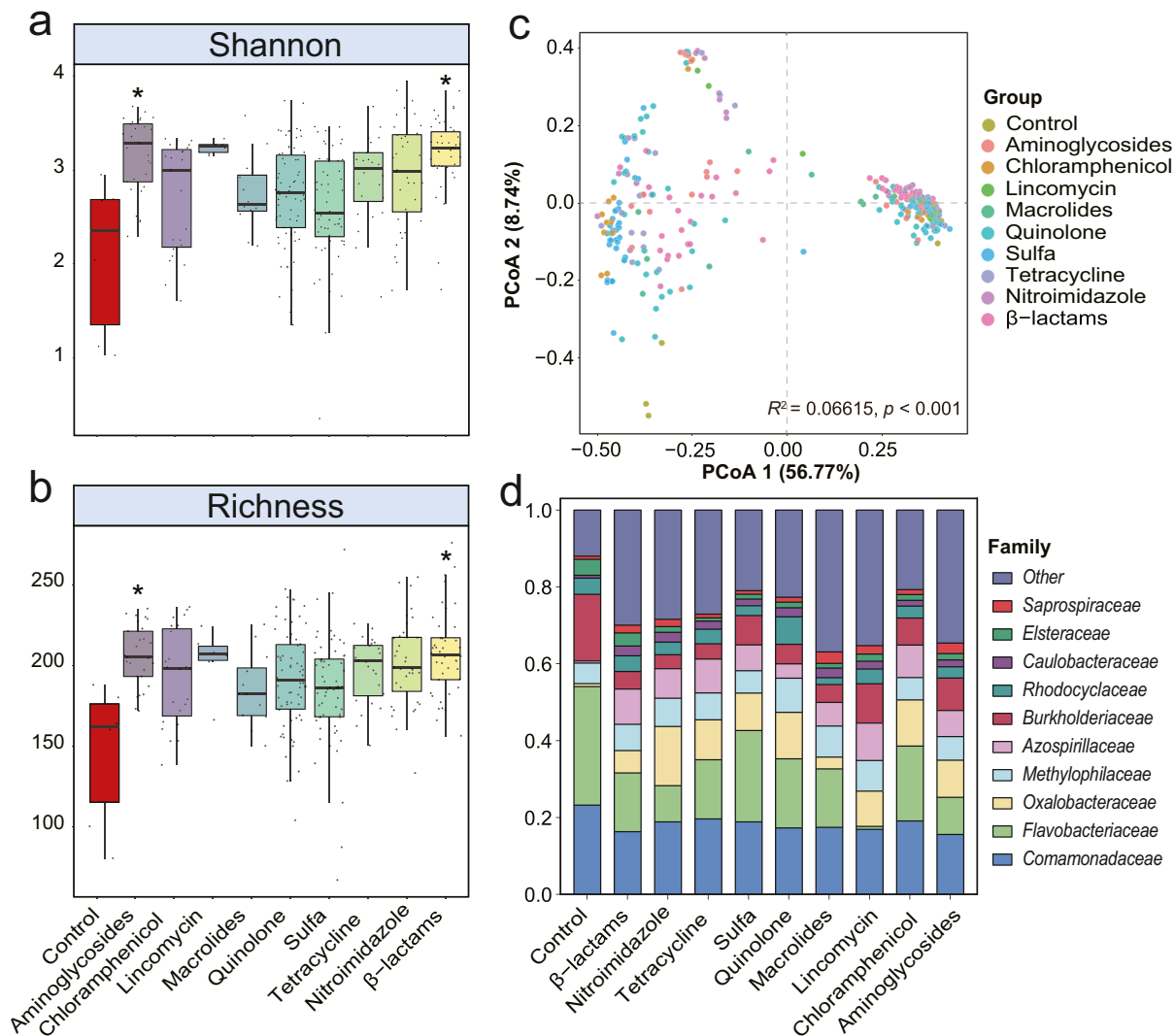


Fig. 2. Influence of antibiotics on the diversity and classification of microbial communities. (a) Shannon index (b) Richness index (c) Principal coordinate analysis (PCoA) score (d) Classification comparison of the 10 most abundant families in the antibiotic treatment group and the control group. * represents statistically significant differences at $p < 0.05$ from a one-way ANOVA.

be disentangled in future work with ARGs research, and explain the dissemination of ARGs upon use of antibiotics.

3.2. Antibiotic treatments changed functions of bacterial community

In order to understand the functional profiles of the aquatic bacterial community after antibiotic treatment, PICRUSt2 software was used to predict the function of the 16S rRNA sequence, and annotated these functional pathways from the KEGG database. A total of 137 functional pathways were detected in the Tiesha River and in the West Lake. Most functional pathways were shared between the antibiotic treatment and the control group (Fig. S5). However, the bacterial functional pathways were significantly changed after treatment with different kinds of antibiotics. For example, there were 80 functions up-regulated and 57 functions down-regulated in the Tiesha River, while 75 functions were up-regulated and 62 functions down-regulated in West Lake, under aminoglycosides treatment. Our results indicated that Tiesha River and West Lake also had differences in the functions of antibiotic-treated microorganisms (Fig. S6). A total of 38 functional pathways referred to 15 categories were dramatically changed in antibiotic treatments and selected for further analysis (Table S3, Fig. 3). After antibiotic treatment the metabolism of cofactors and vitamins

were upregulated. This could influence the heme biosynthesis and NAD biosynthesis (Obornik and Green, 2005; Xu et al., 2017). The former could be beneficial to the synthesis of photosynthetic pigments to improve the photosynthetic efficiency (Gong et al., 2017) and the latter could maintain cellular functions under environmental stress (Hashida et al., 2009; Liang et al., 2017). Therefore, the up-regulation of cofactors and vitamins metabolism could be a useful strategy for bacteria to cope with the stress of antibiotics. Xenobiotics biodegradation and metabolism, carbohydrate metabolism, and amino acid metabolism (such as glycine, serine and threonine, histidine and tyrosine metabolism) were down-regulated under antibiotic treatment. These metabolic pathways played an important role in the degradation of complex organic molecules (Widada et al., 2002; Qiu et al., 2022), energy conversion (Spriet, 2014), and protein synthesis to control life processes (Morot-Gaudry et al., 2001; Wagenmakers, 1998; Zhang et al., 2022a–e). Their down-regulation indicated that the growth and proliferation of bacteria in the microcosms was inhibited under antibiotic treatment. Our study showed that 38 functional pathways were significantly changed after treatment with β -lactams (14), nitroimidazole (23), macrolides (17), lincomycin (17), and aminoglycosides (13) compared with the control (Fig. 3).

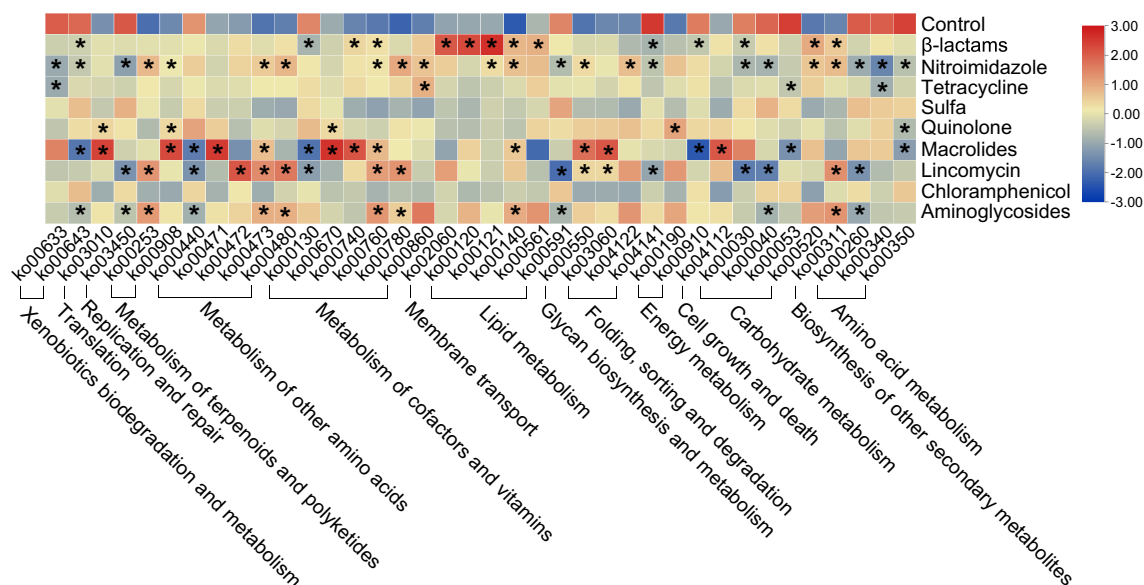


Fig. 3. The effect of antibiotics on bacterial functional pathways analysed by the PICRUSt2 software and the KEGG database. Relative expression values are scaled by Z-score per transcript. * represents statistically significant differences at $p < 0.05$ from a one-way ANOVA.

3.3. Development of models to correlate antibiotic characteristics with bacterial community traits

Antibiotic treatment affected the diversity and composition of the aquatic bacterial community. The unclarified question is: which dominated antibiotic properties played a decisive role in this study? We established a regression model to correlate the antibiotic characteristics with microbial community traits via machine learning with the Random Forest (RF) algorithm, implicating the influence of antibiotic characteristics on bacterial community structure and the diversity and relative abundance (Fig. S7). A total of 76 physicochemical properties of the antibiotics were collected as independent variables in the model (Table S1). To simplify the composition profiles of the bacterial communities, a co-occurrence network divided the whole bacterial community into several modules based on the species interaction, and three modules comprising the highest numbers of bacteria were chosen for bacterial community prediction (Fig. 4a). In order to ensure the credibility of the prediction accuracy and appropriate fitness, we used ten-fold cross validation to verify our three models with the predictions (mean R^2 in each model ranged from 57 to 78%) (Fig. 4b). The effects of the physicochemical properties of antibiotics on the changes of the three modules of bacterial communities were different. In Module 1, the adipose water coefficient explained 24.3% of the microbial community change under antibiotic treatment, while the second and third most important contributions were the atom stereocenter count (13.7%) and the number of hydrogen atoms (12.2%). Hydroxyl and dimethylamino groups were the most important factors in Module 2, which could explain 14.7 and 14.8% of the microbiological differences, followed by the atom stereocenter count (12.7%). In Module 3, the numbers of hydrogen and dimethylamino groups were the most important factors, which could explain 15.8 and 15.6% of the microbial differences. The impact of the physicochemical properties of antibiotics on the Richness and Shannon index of aquatic microbial community was also different. The number of hydrogen atoms in antibiotic was the most important factor in the Shannon index changes, which accounted for 20.8%, followed by the dimethylamino group (12.6%). In the Richness, the antibiotic solubility is the largest influencing factor, accounting for 33.3% of the variance in the data, followed by the number of hydrogen atoms (15.9%). In the influence on the average relative abundance of antibiotic-sharing bacteria, the main factors were number of hydrogen atoms and number of dimethylamino groups (Fig. 3c).

Totally, atom stereocenter count, number of hydrogen atoms and the adipose water coefficient of antibiotics were the most important factors to determine the changes of microorganisms (Fig. 3d). It was worth noting that in many single studies, the type and concentration of antibiotics was considered as the most important factors affecting aquatic microbial community (Ding and He, 2010; Liu et al., 2012; Zhao et al., 2019). In antibiotic treatment, our model clarified that three physical and chemical properties including atom stereocenter count, number of hydrogen atoms and the adipose water coefficient were more important potential factors to shape microbial community, because these factors could affect the absorption of drugs by biota (Barton et al., 1997; Martinez and Amidon, 2002; Raevsky and Schaper, 1998; Walker, 2014). Therefore, it might better to reduce the number of these three factors to design environmental friendly antibiotics.

3.4. Establishing a model to connect the characteristics of antibiotics with the functioning of a bacterial community

In order to clarify the specific impact of the characteristics of antibiotics on bacterial community function, we used RF to construct a non-linear regression model between the specific physicochemical properties of antibiotics. Our results indicated that six main functions (cofactors and vitamins, lipid, folding, sorting and degradation, carbohydrate and amino acid metabolism) were the largest proportion of secondary channels. Through the 10-fold cross-prediction model, the R^2 value was found to range between 66 and 69%, implicating the influence of antibiotic characteristics on the function of bacterial community (Fig. S8). The number of hydrogen atoms was the most important factor to impact the changes of the metabolism of cofactors and vitamins, lipid metabolism, folding, sorting and degradation, and carbohydrate metabolism (Fig. 5a). Within the changes of other amino acids metabolism, molecular weight was the most important factor under antibiotics treatment, accounting for 19.7% of the variance in the data. Molecular weight may affect bacterial proteins, such as some products with low molecular weights acting with potency and specificity at protein receptors (Clardy and Walsh, 2004). Among the effects of amino acid metabolism in the treatment of antibiotics, the number of sulfur atoms had the largest contribution rate, accounting for 17.5%.

By building machine learning models, we could infer that the number of hydrogen atoms, molecular weight, and complexity had universal effects on bacterial functions (Fig. 5b). The number of hydrogen atoms was also an

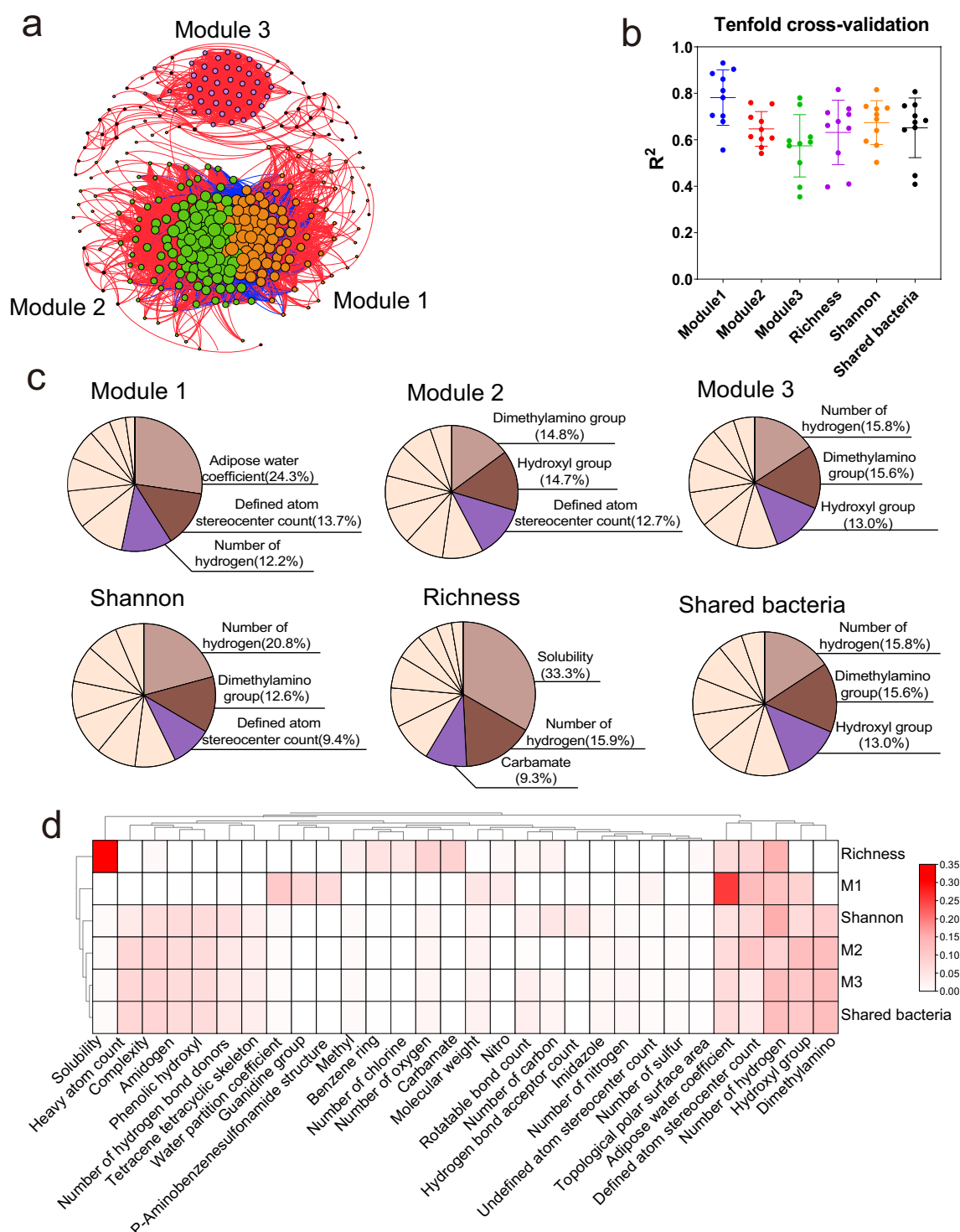


Fig. 4. Effects of antibiotic properties on the diversity and structure of microbial communities. (a) Modular classification of the ecological network of microbial communities in artificial waters (In Gephi, bacteria were divided into Module 1, Module 2 and Module 3 according to the interaction with each other) treated with antibiotics. (b) Validation of the R^2 value in the model by ten-fold cross-validation evaluation. (c) The contribution of antibiotic characteristics to the microbial characteristics of the aquatic microbial community Richness, Shannon, the relative abundance of the three microbial modules and the average relative abundance of antibiotic-sharing bacteria. (d) The contribution rate of the physicochemical properties of antibiotics (select the top 30 contribution rates) to M1, M2, M3, Richness, Shannon, and antibiotic sharing bacteria.

important factor in affecting the composition of aquatic microbial communities, as described above. Therefore, in the design of antibiotics, the number of hydrogens could be changed in order to reduce the impact of antibiotics on the function of aquatic microorganisms.

4. Conclusion

In summary, this study showed that exposure to 50 kinds of antibiotics at a concentration of 10 $\mu\text{g/L}$ had different degrees of influence on the

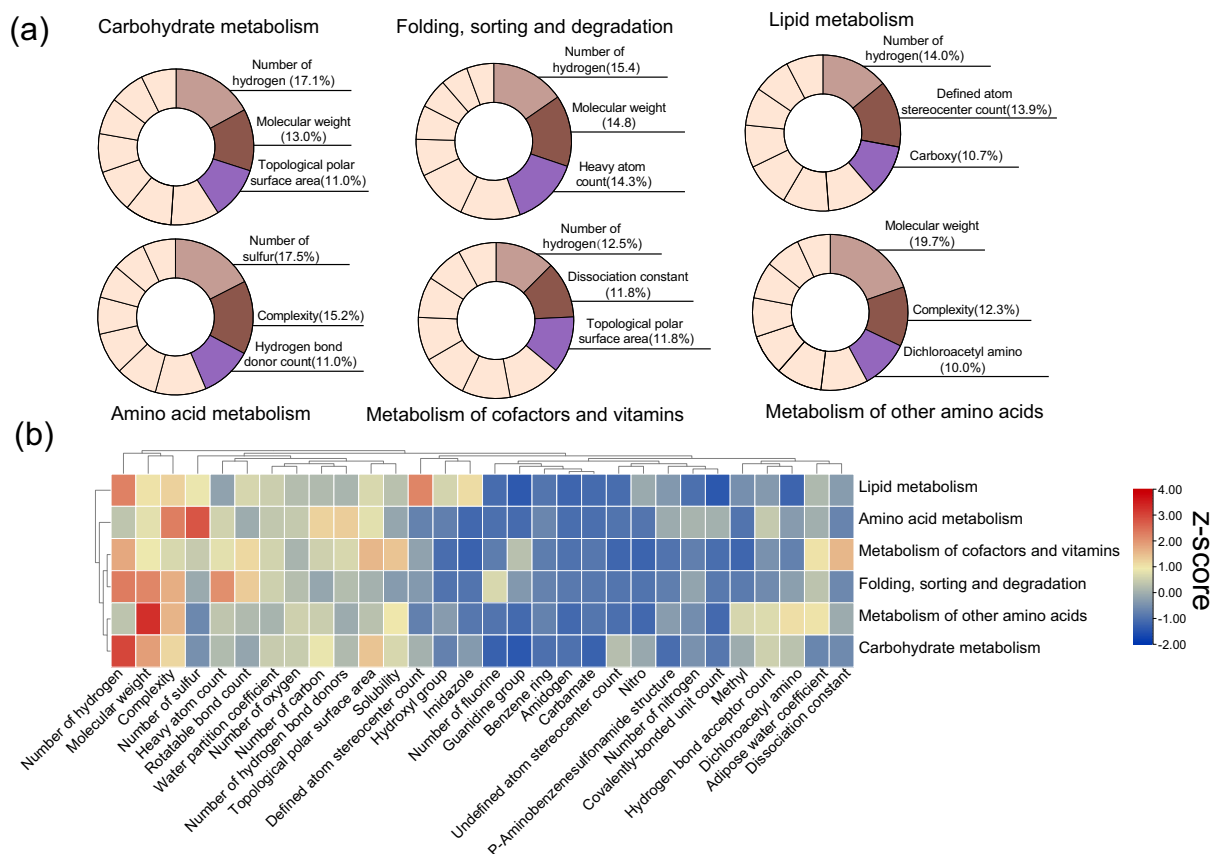


Fig. 5. The effect of antibiotic properties on the main bacterial metabolism (amino acids, metabolism of cofactors and vitamins, lipid metabolism, folding, sorting and degradation, carbohydrate metabolism and amino acid metabolism) analysed by the KEGG database. (a) Antibiotic properties contributed to the six metabolic pathways of aquatic bacteria: metabolism of other amino acids, metabolism of cofactors and vitamins, lipid metabolism, folding, sorting and degradation, carbohydrate metabolism and amino acid metabolism. (b) Contribution rate of the physical and chemical properties of antibiotics (select the top 30 contribution rates) to the six metabolisms of aquatic bacteria. Values are scaled by their Z-score.

composition and function of the aquatic microbial community, which in turn may affect the stability of aquatic ecosystems in different ways. The present study revealed that the characteristics of antibiotics, such as defined atom stereocenter count, number of hydrogen atoms and adipose water coefficient, play a significant role in shaping the composition of the microbial community in aquatic environments. At the same time, the number of hydrogen atoms, the molecular weight of antibiotics and complexity also were key factors in the functional traits of bacteria. This research developed an accurate prediction model to establish specific links between antibiotic characteristics, biodiversity and ecosystem functions of aquatic communities, and will guide the design and optimization of antibiotic molecules to minimize environmental risks. However, the research did not take into account the effect of antibiotic concentration in the present study, since we focused on the physical and chemical characteristics of the antibiotics. It is still interesting and meaningful to figure out the impact of various antibiotics with different concentration gradients on the water microbial community, as this can guide the impact of the rational use of antibiotic concentrations in the environment.

CRediT authorship contribution statement

Jian Kang: Methodology, Investigation, Writing – original draft, Writing – review & editing. **Zhenyan Zhang:** Investigation, Writing – original draft. **Yiling Chen:** Formal analysis, Writing – original draft. **Zhigao Zhou:** Methodology, Investigation. **Jinfeng Zhang:** Methodology, Software. **Nuohan Xu:** Methodology, Formal analysis. **Qi Zhang:** Methodology, Validation. **Tao Lu:** Investigation, Writing – original draft. **W.J.G.M. Peijnenburg:** Writing – original draft. **Haifeng Qian:** Conceptualization, Writing – review & editing, Funding acquisition.

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.2022.154412>.

References

- An, X.L., Su, J.Q., Li, B., Ouyang, W.Y., Zhao, Y., Chen, Q.L., Cui, L., Chen, H., Gillings, M.R., Zhang, T., Zhu, Y.G., 2018. Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR. *Environ. Int.* 117, 146–153.
- Ban, Z., Yuan, P., Yu, F.B., Peng, T., Zhou, Q.X., Hu, X.G., 2020. Machine learning predicts the functional composition of the protein corona and the cellular recognition of nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 117 (19), 10492–10499.
- Bardou, P., Mariette, J., Escudie, F., Djemiel, C., Klopp, C., 2014. jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics* 15, 293.
- Barton, P., Davis, A.M., McCarthy, D.J., Webborn, P.J.H., 1997. Drug-phospholipid interactions.2. Predicting the sites of drug distribution using n-octanol/water and membrane/water distribution coefficients. *J. Pharm. Sci.* 86 (9), 1034–1039.
- Camarota, G., Ianaro, G., Ahern, A., Carbone, C., Temko, A., Claesson, M.J., Gasbarrini, A., Tortora, G., 2020. Gut microbiome, big data and machine learning to promote precision medicine for cancer. *Nat. Rev. Gastroenterol. Hepatol.* 17 (10), 635–648.

- Chen, C.J., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y.H., Xia, R., 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13 (8), 1194–1202.
- Clardy, J., Walsh, C., 2004. Lessons from natural molecules. *Nature* 432 (7019), 829–837.
- Clardy, J., Fischbach, M.A., Currie, C.R., 2009. The natural history of antibiotics. *Curr. Biol.* 19 (11), R437–R441.
- Corno, G., Coci, M., Giardina, M., Plechuk, S., Campanile, F., Stefani, S., 2014. Antibiotics promote aggregation within aquatic bacterial communities. *Front. Microbiol.* 5, 297.
- Deng, C.X., Liu, X.W., Li, L.L., Shi, J.H., Guo, W., Xue, J.H., 2020. Temporal dynamics of antibiotic resistant genes and their association with the bacterial community in a water-sediment mesocosm under selection by 14 antibiotics. *Environ. Int.* 137, 105554.
- Deng, Y., Debognies, A., Zhang, Q., Zhang, Z., Zhou, Z., Zhang, J., Sun, L., Lu, T., Qian, H., 2022. Effects of ofloxacin on the structure and function of freshwater microbial communities. *Aquat. Toxicol.* 244, 106084.
- Ding, C., He, J.Z., 2010. Effect of antibiotics in the environment on microbial populations. *Appl. Microbiol. Biotechnol.* 87 (3), 925–941.
- Diwan, V., Tamhankar, A.J., Khandal, R.K., Sen, S., Aggarwal, M., Marothi, Y., Iyer, R.V., Sundblad-Tonderski, K., Stalsby-Lundborg, C., 2010. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health* 10, 414.
- Fernandez-Delgado, M., Cernadas, E., Barro, S., Amorim, D., 2014. Do we need hundreds of classifiers to solve real world classification problems? *J. Mach. Learn. Res.* 15, 3133–3181.
- Gelband, H., Miller-Petrie, M., Pant, S., Gandra, S., Levinson, J., 2015. The State of the World's Antibiotics 2015. 8. Wound Healing Southern Africa, pp. 30–34.
- Gong, W.D., Browne, J., Hall, N., Schrueth, D., Paerl, H., Marchetti, A., 2017. Molecular insights into a dinoflagellate bloom. *ISME J.* 11 (2), 439–452.
- Gould, K., 2016. Antibiotics: from prehistory to the present day. *J. Antimicrob. Chemother.* 71 (3), 572–575.
- Grenni, P., Ancona, V., Barra Caracciolo, A., 2018. Ecological effects of antibiotics on natural ecosystems: a review. *Microchem. J.* 136, 25–39.
- Hashida, S.N., Takahashi, H., Uchimiya, H., 2009. The role of NAD biosynthesis in plant development and stress responses. *Ann. Bot.* 103 (6), 819–824.
- Hu, X.G., Zhou, Q.X., Luo, Y., 2010. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environ. Pollut.* 158 (9), 2992–2998.
- Huang, F.Y., An, Z.Y., Moran, M.J., Liu, F., 2020. Recognition of typical antibiotic residues in environmental media related to groundwater in China (2009–2019). *J. Hazard. Mater.* 399, 122813.
- Islam, G.M., Gilbride, K.A., 2019. The effect of tetracycline on the structure of the bacterial community in a wastewater treatment system and its effects on nitrogen removal. *J. Hazard. Mater.* 371, 130–137.
- Janecko, N., Pokladova, L., Blahova, J., Svobodova, Z., Literak, I., 2016. Implications of fluoroquinolone contamination for the aquatic environment - a review. *Environ. Toxicol. Chem.* 35 (11), 2647–2656.
- Jordan, M.I., Mitchell, T.M., 2015. Machine learning: trends, perspectives, and prospects. *Science* 349 (6245), 255–260.
- Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. U. S. A.* 115 (15), E3463–E3470.
- Lardé, H., Francoz, D., Roy, J.-P., Archambault, M., Massé, J., Paradis, M.E., Dufour, S., 2021. Comparison of quantification methods to estimate farm-level usage of antimicrobials in medicated feed in dairy farms from Québec, Canada. *Microorganisms* 9 (9), 1834.
- Larsson, J., 2014. Antibiotics in the environment. *Ups. J. Med. Sci.* 119, 108–112.
- Lenart-Boron, A., Prajsnar, J., Guzik, M., Boron, P., Chmiel, M., 2020. How much of antibiotics can enter surface water with treated wastewater and how it affects the resistance of waterborne bacteria: a case study of the Bialka river sewage treatment plant. *Environ. Res.* 191, 110037.
- Liang, M., Yin, X.L., Wang, L.Y., Yin, W.H., Song, N.Y., Shi, H.B., Li, C.Y., Yin, S.K., 2017. NAD plus attenuates bilirubin-induced hyperbilirubinemia in the ventral cochlear nucleus by inhibiting excitatory neurotransmission and neuronal excitability. *Front. Cell. Neurosci.* 11, 21.
- Liu, H., Zhang, G.P., Liu, C.Q., Li, L., Xiang, M., 2009. The occurrence of chloramphenicol and tetracyclines in municipal sewage and the Nanming River, Guiyang City, China. *J. Environ. Monit.* 11 (6), 1199–1205.
- Liu, F., Wu, J.S., Ying, G.G., Luo, Z.X., Feng, H., 2012. Changes in functional diversity of soil microbial community with addition of antibiotics sulfamethoxazole and chlortetracycline. *Appl. Microbiol. Biotechnol.* 95 (6), 1615–1623.
- Lofmark, S., Jernberg, C., Jansson, J.K., Edlund, C., 2006. Clindamycin-induced enrichment and long-term persistence of resistant *Bacteroides* spp. and resistance genes. *J. Antimicrob. Chemother.* 58 (6), 1160–1167.
- Lu, T., Zhang, Q., Lavoie, M., Zhu, Y.C., Ye, Y.Z., Yang, J., Paerl, H.W., Qian, H.F., Zhu, Y.G., 2019a. The fungicide azoxystrobin promotes freshwater cyanobacterial dominance through altering competition. *Microbiome* 7 (1), 128.
- Lu, T., Zhu, Y.C., Ke, M.J., Peijnenburg, W., Zhang, M., Wang, T.Z., Chen, J., Qian, H.F., 2019b. Evaluation of the taxonomic and functional variation of freshwater plankton communities induced by trace amounts of the antibiotic ciprofloxacin. *Environ. Int.* 126, 268–278.
- Lu, T., Zhang, Q., Zhang, Z.Y., Hu, B.L., Chen, J.M., Chen, J., Qian, H.F., 2021. Pollutant toxicology with respect to microalgae and cyanobacteria. *J. Environ. Sci.* 99, 175–186.
- Lundstrom, S.V., Ostman, M., Bengtsson-Palme, J., Rutgersson, C., Thoudal, M., Sircar, T., Blanck, H., Eriksson, K.M., Tysklind, M., Flach, C.F., Larsson, D.G.J., 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. *Sci. Total Environ.* 553, 587–595.
- Lyu, J., Yang, L.S., Zhang, L., Ye, B.X., Wang, L., 2020. Antibiotics in soil and water in China: a systematic review and source analysis. *Environ. Pollut.* 266, 115147.
- Maier, L., Goemans, C.V., Wirbel, J., Kuhn, M., Eberl, C., Pruteanu, M., Muller, P., Garcia-Santamarina, S., Cacace, E., Zhang, B., Gekeler, C., Banerjee, T., Anderson, E.E., Milanese, A., Lober, U., Forslund, S.K., Patil, K.R., Zimmermann, M., Stecher, B., Zeller, G., Bork, P., Typas, A., 2021. Unravelling the collateral damage of antibiotics on gut bacteria. *Nature* 299, 120–124.
- Marti, E., Huerta, B., Rodriguez-Mozaz, S., Barcelo, D., Balcazar, J.L., Marce, R., 2016. Effects of subinhibitory ciprofloxacin concentrations on the abundance of qnrS and composition of bacterial communities from water supply reservoirs. *Chemosphere* 161, 470–474.
- Martin, M., 2011. CUTADAPT removes adapter sequences from high-throughput sequencing reads. *EMBnetJ.* 17, 200.
- Martinez, M.N., Amidon, G.L., 2002. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. *J. Clin. Pharmacol.* 42 (6), 620–643.
- Miroshnikova, M.S., Miroshnikova, E.P., Arinzhanov, A.E., Kilyakova, Y.V., 2021. Application of antibiotics in agriculture and alternatives of their use. *Agrar. Sci. J.* 5, 65–70.
- Morot-Gaudry, J.-F., Job, D., Lea, P.J., 2001. In: Lea, P.J., Morot-Gaudry, J.-F. (Eds.), *Plant Nitrogen*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 167–211.
- Obornik, M., Green, B.R., 2005. Mosaic origin of the heme biosynthesis pathway in photosynthetic eukaryotes. *Mol. Biol. Evol.* 22 (12), 2343–2353.
- Qiu, D.Y., Ye, Y.Z., Ke, M.J., Xu, N.H., Zhang, Z.Y., Zhang, F., Kang, J., Yu, Y.T., Lu, T., Qian, H.F., 2022. Effects of chiral herbicide dichlorprop on Arabidopsis thaliana metabolic profile and its implications for microbial communities in the phyllosphere. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-021-17936-y>.
- Raevsky, O.A., Schaper, K.J., 1998. Quantitative estimation of hydrogen bond contribution to permeability and absorption processes of some chemicals and drugs. *Eur. J. Med. Chem.* 33 (10), 799–807.
- Ramirez, K.S., Knight, C.G., de Hollander, M., Brearley, F.Q., Constantinides, B., Cotton, A., Creer, S., Crowther, T.W., Davison, J., Delgado-Baquerizo, M., Dorrepaal, E., Elliott, D.R., Fox, G., Griffiths, R.L., Hale, C., Hartman, K., Houlden, A., Jones, D.L., Krab, E.J., Maestre, F.T., McGuire, K.L., Monteux, S., Orr, C.H., van der Putten, W.H., Roberts, I.S., Robinson, D.A., Rocca, J.D., Rowntree, J., Schlaeppi, K., Shepherd, M., Singh, B.K., Straathof, A.L., Bhatnagar, J.M., Thion, C., van der Heijden, M.G.A., de Vries, F.T., 2018. Detecting macroecological patterns in bacterial communities across independent studies of global soils. *Nat. Microbiol.* 3 (2), 189–196.
- Ren, Z.G., Li, A., Jiang, J.W., Zhou, L., Yu, Z.J., Lu, H.F., Xie, H.Y., Chen, X.L., Shao, L., Zhang, R.Q., Xu, S.Y., Zhang, H., Cui, G.Y., Chen, X.H., Sun, R.R., Wen, H., Lerut, J.P., Kan, Q.C., Li, L.J., Zheng, S.S., 2019. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 68 (6), 1014–1023.
- Rodriguez-Mozaz, S., Vaz-Moreira, I., Della Giustina, S.V., Llorca, M., Barcelo, D., Schubert, S., Berendonk, T.U., Michael-Kordatou, I., Fatta-Kassinos, D., Martinez, J.L., Elpers, C., Henriques, I., Jaeger, T., Schwartz, T., Paulshus, E., O'Sullivan, K., Parnanen, K.M.M., Virta, M., Do, T.T., Walsh, F., Manaia, C.M., 2020. Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. *Environ. Int.* 140, 105733.
- Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65 (5), 725–759.
- Shi, X.L., Sun, Z.Q., Liu, G.J., Xu, H.L., 2015. Insights into community-based discrimination of water quality status using an annual pool of phytoplankton in mid-subtropical canal systems. *Environ. Sci. Pollut. Res.* 22 (2), 1199–1206.
- Sim, W.J., Lee, J.W., Lee, E.S., Shin, S.K., Hwang, S.R., Oh, J.E., 2011. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. *Chemosphere* 82 (2), 179–186.
- Song, H., Xu, J.H., Lavoie, M., Fan, X.J., Liu, G.F., Sun, L.W., Fu, Z.W., Qian, H.F., 2017. Biological and chemical factors driving the temporal distribution of cyanobacteria and heterotrophic bacteria in a eutrophic lake (West Lake, China). *Appl. Microbiol. Biotechnol.* 101 (4), 1685–1696.
- Speiser, J.L., Miller, M.E., Tooze, J., Ip, E., 2019. A comparison of random forest variable selection methods for classification prediction modeling. *Expert Syst. Appl.* 134, 93–101.
- Spriet, L.L., 2014. New insights into the interaction of carbohydrate and fat metabolism during exercise. *Sports Med.* 44, 87–96.
- Tiseo, K., Huber, L., Gilbert, M., Robinson, T.P., Van Boeckel, T.P., 2020. Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics* 9 (12), 918.
- Wagenmakers, A.J.M., 1998. 1998 In: Holloszy, J.O. (Ed.), *Exercise and Sport Sciences Reviews*. vol. 28, pp. 287–314.
- Walker, M.A., 2014. Novel tactics for designing water-soluble molecules in drug discovery. *Expert Opin. Drug Discov.* 9 (12), 1421–1433.
- Widada, J., Nijiri, H., Omori, T., 2002. Recent developments in molecular techniques for identification and monitoring of xenobiotic-degrading bacteria and their catabolic genes in bioremediation. *Appl. Microbiol. Biotechnol.* 60 (1–2), 45–59.
- Wilck, N., Matus, M.G., Kearney, S.M., Olesen, S.W., Forslund, K., Bartolomeaus, H., Haase, S., Mahler, A., Balogh, A., Marko, L., Vedenskaya, O.V., Kleiner, F.H., Tsvetkov, D., Klug, L., Costea, P.I., Sunagawa, S., Maier, L., Rakova, N., Schatz, V., Neubert, P., Fratz, C., Krannich, A., Gollasch, M., Grohme, D.A., Corte-Real, B.F., Gerlach, R.G., Basic, M., Typas, A., Wu, C., Titz, J.M., Jantsch, J., Boschmann, M., Dechend, R., Kleiweitfeld, M., Kempa, S., Bork, P., Linker, R.A., Alm, E.J., Muller, D.N., 2017. Salt-responsive gut commensal modulates T(H)17 axis and disease. *Nature* 551 (7682), 585–589.
- Wright, R.J., Langille, M.G.I., Walker, T.R., 2021. Food or just a free ride? A meta-analysis reveals the global diversity of the Plastisphere. *ISME J.* 15 (3), 789–806.
- Xu, J., Zhang, Y., Zhou, C.B., Guo, C.S., Wang, D.M., Du, P., Luo, Y., Wan, J., Meng, W., 2014. Distribution, sources and composition of antibiotics in sediment, overlying water and pore water from Taihu Lake, China. *Sci. Total Environ.* 497, 267–273.
- Xu, N., Ye, C., Chen, X.L., Liu, J., Liu, L.M., 2017. Genome-scale metabolic modelling common cofactors metabolism in microorganisms. *J. Biotechnol.* 251, 1–13.
- Xu, N.H., Qu, Q., Zhang, Z.Y., Yuan, W.T., Cui, H.Z., Shen, Y.J., Lin, W., Lu, T., Qian, H.F., 2020. Effects of residual S-metolachlor in soil on the phyllosphere microbial communities of wheat (*Triticum aestivum* L.). *Sci. Total Environ.* 748, 141342.

- Yuan, J., Wen, T., Zhang, H., Zhao, M.L., Penton, C.R., Thomashow, L.S., Shen, Q.R., 2020. Predicting disease occurrence with high accuracy based on soil macroecological patterns of *Fusarium* wilt. *ISME J.* 14 (12), 2936–2950.
- Zhang, Y., Xie, J.P., Liu, M.M., Tian, Z., He, Z.L., van Nostrand, J.D., Ren, L.R., Zhou, J.Z., Yang, M., 2013. Microbial community functional structure in response to antibiotics in pharmaceutical wastewater treatment systems. *Water Res.* 47 (16), 6298–6308.
- Zhang, J.Y., Liu, Y.X., Zhang, N., Hu, B., Jin, T., Xu, H.R., Qin, Y., Yan, P.X., Zhang, X.N., Guo, X.X., Hui, J., Cao, S.Y., Wang, X., Wang, C., Wang, H., Qu, B.Y., Fan, G.Y., Yuan, L.X., Garrido-Oter, R., Chu, C.C., Bai, Y., 2019. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat. Biotechnol.* 37 (6), 676–684.
- Zhang, Q., Zhang, Z., Lu, T., Yu, Y., Penueles, J., Zhu, Y.-G., Qian, H., 2021. Gammaproteobacteria, a core taxon in the guts of soil fauna, are potential responders to environmental concentrations of soil pollutants. *Microbiome* 9 (1), 196.
- Zhang, Q., Yu, Y.T., Jin, M.K., Deng, Y., Zheng, B.Y., Lu, T., Qian, H.F., 2022a. Oral azoxystrobin driving the dynamic change in resistome by disturbing the stability of the gut microbiota of *Enchytraeus crypticus*. *J. Hazard. Mater.* 423, 127252.
- Zhang, Z.Y., Zhang, Q., Cui, H.Z., Li, Y., Xu, N.H., Lu, T., Chen, J., Penueles, J., Hu, B.L., Qian, H.F., 2022b. Composition identification and functional verification of bacterial community in disease-suppressive soils by machine learning. *Environ. Microbiol.* <https://doi.org/10.1111/1462-2920.15902>.
- Zhang, Z.Y., Zhang, Q., Lu, T., Zhang, J.Y., Sun, L.W., Hu, B.L., Hu, J., Penueles, J., Zhu, L.Z., Qian, H.F., 2022c. Residual chlorine disrupts the microbial communities and spreads antibiotic resistance in freshwater. *J. Hazard. Mater.* 423, 127152.
- Zhang, Z.Y., Zhang, Q., Wang, T.Z., Xu, N.H., Lu, T., Hong, W.J., Penueles, J., Gillings, M., Wang, M.X., Gao, W.W., Qian, H.F., 2022d. Assessment of global health risk of antibiotic resistance genes. *Nature Communications* (Accepted) (in press).
- Zhang, M.W., Zhou, Z.G., Zhang, J.F., Yu, Y.T., Sun, L.W., Lu, T., Qian, H.F., 2022e. Metagenomic ecotoxicity assessment of trace difenoconazole on freshwater microbial community. *Chemosphere* 294, 133742.
- Zhao, R.X., Feng, J., Liu, J., Fu, W.J., Li, X.Y., Li, B., 2019. Deciphering of microbial community and antibiotic resistance genes in activated sludge reactors under high selective pressure of different antibiotics. *Water Res.* 151, 388–402.
- Zielinski, W., Korzeniewska, E., Harnisz, M., Drzyma, J., Felis, E., Bajkacz, S., 2021. Wastewater treatment plants as a reservoir of integrase and antibiotic resistance genes - an epidemiological threat to workers and environment. *Environ. Int.* 156, 106641.