Anthropogenic Activities Destabilized Bacterial Co-occurrence Networks in a Subtropical River

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Original Article

Keywords: bacterial community, anthropogenic activities, stability of ecological co-occurrence network, nutrient concentrations, subtropical river

Posted Date: February 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-188152/v1>

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Abstract

Previous studies suggested that strong positive correlations between microbial taxa destabilized microbial co-occurrence network, because network members which had strong positive correlations with each other tended to decrease in abundance synchronously. Anthropogenic activities have strong influences on microbial community composition and diversity in riverine ecosystems, but how they influence the stability of microbial co-occurrence network remain unclear. In this study, we used nutrient concentrations (nitrogen and phosphorus) as an indicator for anthropogenic activities, and explored the effects of anthropogenic activities on the stability of bacterial co-occurrence networks in a subtropical river, Xiyuan River. The nutrient concentrations were higher in midstream and downstream areas than in upstream area of Xiyuan River. The average proportion and correlation coefficient of positive correlations were higher in midstream and downstream networks than in upstream networks, indicating frequent anthropogenic activities destabilized the bacterial co-occurrence networks. To further explore the mechanisms, we found that the changes of network stabilities were associated with the changes of bacterial functions. Anthropogenic activity tolerant bacteria (e.g. nutrient removal, aromatic degradation and pathogen bacteria) and their linked bacterial members formed the large and strong positive modules in the midstream and downstream networks, and thus destabilized the networks. Based on network perspective, our results provide a new insight in the mechanisms of how anthropogenic activities alter riverine microbial communities.

Introduction

Since the middle of the last century, the anthropogenic activities have increased sharply, resulting in degradation of water quality and loss of biodiversity in aquatic ecosystems such as rivers, lakes and coastal areas [1–3]. One of the most negative effects by anthropogenic activities is nutrient pollution (excess nitrogen and phosphorus) [4–6]. Since the 1960s, excess nutrients have been discharged into aquatic environments due to the anthropogenic activities, leading to eutrophication in aquatic environments worldwide [7, 8]. Eutrophication has been recognized as one of the major threats to global aquatic ecosystems [9]. Previous studies in assessment of global rivers indicated that nutrients and pathogens were two principal categories of human-induced pollutants within rivers [1]. Therefore, we used nutrient concentrations as an important indicator of anthropogenic activities.

The increasing anthropogenic activities in water is likely to profoundly affect the aquatic community composition and function [10–13], and further have the potential consequences for co-occurrence networks of aquatic communities [14–16]. A co-occurrence network represents a set of nodes (microbial taxa or environmental factors) connected by directed or undirected edges (correlations) based on spearman' correlation, SparCC, eLSA or other methods [14, 17, 18]. Previous studies have used co-occurrence network to explore the potential interactions between microbial taxa (e.g. mutualism, competition, predation and parasitism) [19–21]. Comparing with the studies about microbial community composition and diversity, we still know little about the multiple interactions between microbial taxa. Recently, an increasing number of studies have examined the microbial co-occurrence networks along the

environmental gradients, indicating environmental factors strongly drive the shapes of networks such as centrality of nodes, proportion of positive and negative links [22, 23]. However, the effects of anthropogenic activities on microbial co-occurrence network are rarely studied.

In addition, previous studies suggested that ecological networks (e.g. food web, cooperative and competitive networks) that were mainly composed of negative links were more stable than those mainly consisted of positive links under disturbances [24–26]. This is because in a community with a large proportion of strong and positive links between members, the members may respond in tandem to the changes of environmental conditions, resulting in co-oscillation [24]. In this case, if a key member in the network becomes extinct, other members will have extinct risk due to their strong and positive correlations with this key member [24]. However, negative links might decrease co-oscillation in communities and promote stability of networks [24]. Co-occurrence network analysis can investigate the correlations between taxa in a community. Although the caution is that edges (correlations) in co-occurrence networks cannot exactly represent the interactions (e.g. cooperation, competition and predation) between microbial taxa, the edges in networks can indicate the links (positive or negative correlations) between microbial taxa. Recently, based on this theoretical framework, de Vries et al. indicated that soil bacterial co-occurrence networks having higher strong and positive correlations were less stable than fungi co-occurrence networks under drought [27]. However, it remains unknown the response in stability of microbial co-occurrence network to anthropogenic activities in aquatic ecosystems.

In this study, 16S rRNA gene high-throughput sequencing was used to investigate the bacterioplankton communities in a subtropical river (Xiyuan River) along an anthropogenic gradient for one year. We used nutrient concentrations as an indicator of anthropogenic activities. The nutrient concentrations were higher in midstream and downstream areas than in upstream area of Xiyuan River due to the frequent anthropogenic activities in midstream and downstream areas. We assume that the strong environmental pressure from anthropogenic activities tends to select some specific bacteria. For example, antibiotics largely increased the richness and abundance of antibiotic resistance bacteria [28, 29]. These bacteria co-vary with the anthropogenic activities, and therefore promote the positive correlations in networks (increase the proportion and strength of positive correlations). In contrast, bacterial communities tend to randomly assemble under low environmental pressure [30]. As a result, we hypothesize that the stability of bacterial co-occurrence networks will decline in the midstream and downstream areas of Xiyuan River where are effected by frequent anthropogenic activities.

Material And Methods

Study area and sampling

The Xiyuan River was located in Fuzhou, Southeast China with a total length of approximately 40 km. It is characterized by subtropical marine monsoon climate with an annual mean temperature of 19–20 °C and an annual mean precipitation of 1000–1600 mm. The rainfall is concentrated from March to September.

Surface water were collected along the Xiyuan River (26.008–26.078°N, 119.127–119.238°E) in 10 sites every month from October 2018 to September 2019 (10 sites × 12 months = 120 samples) (Fig. 1). The latitude and longitude of sampling sites were shown in Table S1. Sites 1 to 3 are located at the upstream area near by a drinking water source protection area. This area is dominated by dense forest and is exposed to slight anthropogenic activities. Sites 4 to 7 are located at the midstream area which passes through a college town. This area is impacted by intensive anthropogenic activities such as domestic wastewater and agricultural activities. Sites 8 to 10 are located at the downstream area which passes through a newly urbanized zone. This area is also impacted by intensive anthropogenic activities. The photos of sampling sites were shown in Fig. S1. After sampling, the water samples transported immediately to the laboratory.

Analyses of environmental variables

Water temperature and pH were measured *in situ*. Total nitrogen (TN) (ultraviolet spectrophotometry after digestion by potassium peroxodisulfate), ammonium nitrogen (NH₄-N) (Nessler's reagent spectrophotometry), nitrate nitrogen (NO₃-N) (ultraviolet spectrophotometry), total phosphorus (TP) (molybdenum blue spectrophotometry after digestion by potassium peroxodisulfate) and PO₄-P (molybdenum blue spectrophotometry) were analyzed according to standard methods [31]. Water samples for chlorophyll *a* (chl *a*) measurement were filtered with 0.45 µm pore-size membranes. Then, the chl *a* in the membranes were extracted by acetone solution and quantified by spectrophotometry [32]. Dissolved organic carbon (DOC) was analyzed using a TOC-L total organic carbon analyzer (Shimadzu, Kyoto, Japan) after water samples were filtered with 0.45 µm pore-size membranes.

Bacterial community collection and Illumina sequencing

Approximately 300–500 mL water sample was filtered using 0.2 µm pore-size polycarbonate membrane (47 mm diameter, Millipore, Billerica, USA) for bacterial community DNA extraction. Total DNA of bacterial communities was extracted from the membrane using the FastDNA SPIN Kit (MP Biomedicals, Santa Ana, USA) according to the manufacturer's instructions. The hypervariable V3-V4 region of bacterial 16S rRNA gene were amplified with the 16S primer pair 341F (5'- CCT AYG GGR BGC ASC AG -3') and 806R (5'- GGA CTA CNN GGG TAT CTA AT -3') [33]. The PCR procedures were according to our previous study [18]. PCR products from triplicates reactions per sample were pooled and gel-purified. Finally, the sequencing libraries were generated based on the pooled PCR reactions and sequenced on the Illumina Hiseq platform (Illumina Inc., San Diego, USA) using a paired-end strategy.

Bioinformatics

Raw paired-end reads were processed using a DADA2 pipeline (an open-source R package) with default settings [34, 35]. DADA2 uses a model-based approach that is amplicon sequence variants (ASVs) for correcting amplicon errors without constructing operational taxonomic units (OTUs) [36]. Purified sequences were assigned taxonomy by the SILVA reference database version 128. Eukaryotic, archaea, chloroplast, mitochondria and unknown sequences were eliminated from the bacterial sequence data. For our data analyses, we used a randomly selected subset of same number reads from each sample to

standardize sequencing effort (21425 sequences for each sample, respectively). All sequence data from this study have been deposited in the public NCBI Sequence Read Archive (SRA) database under the accession number PRJNA633223.

Analyses of bacterial community composition

The Bray-Curtis similarity matrices were created based on the Hellinger transformed bacterial sequence number. Then, non-metric multidimensional scaling (NMDS) ordinations were used based on these matrices [37]. Analysis of similarities (ANOSIM) was used to investigate the differences in bacterial community composition. The ANOSIM global R statistic ranges from 0 to 1 and indicates the overall degree of separation between groups of sites, and no separation is indicated by $R = 0$, whereas $R = 1$ suggests complete separation [37].

Analyses of relationships between bacterial community composition and environmental variables

Canonical correspondence analysis (CCA) was performed to explore the relationships between bacterial community composition and environmental variables. This method was chosen because preliminary detrended correspondence analysis on bacterial community data revealed that the longest gradient lengths were longer than 3.0, indicating that the majority of species exhibited unimodal responses to the environmental variation [38].

Bacterial co-occurrence network construction

We constructed 10 bacterial co-occurrence networks, and each network represents each sampling site. We constructed bacterial co-occurrence networks based on the Spearman's rank correlation to explore the relationships that were consistent among the bacterial ASVs using "picante" R package [18, 39]. To reduce noise and thus false-positive predictions, we restricted our analysis to bacterial ASVs present in $> 2/3$ samples. Only statistically significant ($P < 0.01$) correlations were included in the networks, therefore our networks include both weak and strong but significant correlations. Network visualization and degree calculation were made with Gephi version 0.9.1. Degree is the number of paths that connect the local node to other nodes (node is the bacterial ASVs). The bacterial networks were compared with 1000 Erdős-Rényi random networks, which have the identical number of nodes and edges (edge is the connection between nodes) as the real networks, were generated in the igraph R package [39]. All bacterial networks were significantly more clustered than random networks.

We also constructed the combined bacterial co-occurrence networks for upstream, midstream and downstream respectively. For example, the combined network in upstream contained the total numbers of correlations and nodes (remove the repetitive nodes) from the networks of sites 1, 2 and 3.

Stability of bacterial co-occurrence network

The networks are more stable, if the networks have higher proportion and stronger correlation coefficients of positive edges (correlations). Previous studies suggested that strong negative correlations between

species acted to promote the strong consumer–resource correlations in food webs [40–42]. The strong consumer–resource correlations have potential unstable effects on ecological communities, because these correlations increased spatiotemporal variation in species abundance. However, in this study, the potential consumer–resource correlations between bacterial taxa is scarce, therefore we did not consider the influences of strength (correlation coefficients) of negative correlations between bacterial taxa on the stability of bacterial co-occurrence networks.

Definition of anthropogenic activity tolerant and sensitive bacterial ASVs in bacterial co-occurrence networks

The anthropogenic activity tolerant/sensitive bacterial ASVs in bacterial co-occurrence networks were defined as the bacterial ASV in networks had significant positive/negative Spearman's rank correlation ($P < 0.01$, $n = 120$) with the TN, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, TP or $\text{PO}_4\text{-P}$. We used all data to construct these correlations ($n = 120$), therefore, these correlations combined the spatial and seasonal response of bacterial ASVs to nutrients. We did not find that a bacterial ASV belongs to both anthropogenic activity tolerant bacteria and anthropogenic activity sensitive bacteria.

General statistical analyses

The data were checked for normality, homogeneity of variance and log or square root transformed if necessary. Analysis of variance (ANOVA) was used in combination with Student-Newman-Keuls multiple-comparison test to examine differences among the parameters of the sampling sites.

Results

Spatiotemporal dynamics of environmental variables

The concentrations of TN, $\text{NH}_4\text{-N}$, TP, $\text{PO}_4\text{-P}$, chl *a* and DOC were highest in the midstream area, followed by the downstream area, and were lowest in the upstream area (Figs. 2, S2 and S3). However, the $\text{NO}_3\text{-N}$ was highest in the downstream area, followed by the midstream area, and was lowest in the upstream area (Fig. S2).

For midstream and downstream areas, the concentrations of TN, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, TP, $\text{PO}_4\text{-P}$ and chl *a* were higher in winter (October - January) than in spring (February - May) and summer (June - September), except the TP in downstream area. However, the nutrients and chl *a* did not show regular seasonal patterns in upstream area (Figs. 2 and S2). We found the precipitation showed significant negative correlations with TN, $\text{NH}_4\text{-N}$, TP, $\text{PO}_4\text{-P}$ and chl *a* in midstream area, and showed significant negative correlations with TN, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and chl *a* in downstream area. However, the precipitation did not show significant correlations with nutrient concentrations and chl *a* in upstream area (Table S2 and Fig. S3).

Spatiotemporal dynamics of bacterial community composition and richness

NMDS showed that bacterial communities were divided into two clusters according to spatial dynamics of community composition (cluster 1: upstream, cluster 2: midstream and downstream) (Fig. 3A). However, three clusters of bacterial communities were detected according to temporal dynamics of community composition (cluster 1: summer, cluster 2: winter and cluster 3: spring) (Fig. 3B).

ANOSIM showed that the bacterial community compositions in midstream and downstream areas were apparently different from those in upstream area (global R: upstream vs. midstream = 0.307, $P < 0.01$; upstream vs. downstream = 0.370, $P < 0.01$). However, the bacterial community compositions in midstream area were similar with those in downstream area (global R: midstream vs. downstream = 0.016, $P = 0.20$). In contrast, the bacterial community compositions were apparently distinguished between spring, summer and winter seasons (global R: summer vs. winter = 0.680, $P < 0.01$; summer vs. spring = 0.667, $P < 0.01$; winter vs. spring = 0.515, $P < 0.01$).

Overall, for the spatial dynamic, the species richness was lower in the midstream and downstream than in the upstream areas. For the temporal dynamic, the species richness was lower in winter than in spring and summer (Fig. S4).

Factors that associated with dynamics of bacterial community composition

According to the CCA analysis, TN and $\text{NH}_4\text{-N}$ had the strongest influences on bacterial community composition at both spatial and temporal scales (Fig. S5). Moreover, water temperature had strong influence on bacterial community composition at temporal scale (Fig. S5).

Stability of bacterial co-occurrence network

When considering significant correlations ($P < 0.01$), we found that the proportions of positive correlations were larger in midstream and downstream networks than in upstream networks (Fig. 4A), and the positive correlations were stronger in midstream and downstream networks than in upstream networks (Figs. 4B and 4C). This supports our expectation that bacterial co-occurrence networks in midstream and downstream areas were less stable than in upstream area. The properties of the networks were shown in Table S3. When considering all correlations (include non-significant correlations), again we found that midstream and downstream networks contained more positive correlations and these positive correlations were stronger than in upstream networks.

Anthropogenic activity tolerant/sensitive bacteria in networks

In total, 87 bacterial ASVs were classified to anthropogenic activity tolerant bacteria, 97 bacterial ASVs were classified to anthropogenic activity sensitive bacteria, and 72 bacterial ASVs were classified to non-indicator bacteria in the networks (Figs. 5A and 5B). We found that regardless of the combined positive networks (only included positive correlations) or combined negative networks (only included negative correlations), the proportion of degree for anthropogenic activity tolerant bacteria increased and the

proportion of degree for anthropogenic activity sensitive bacteria decreased from the combined upstream networks to combined midstream and downstream networks (Figs. 5A and 5B). The mean proportions of degree for anthropogenic activity tolerant bacteria in combined upstream, midstream and downstream positive/negative networks were 14.5%/9.2%, 39.4%/50.3%, and 46.4%/40.9%, respectively. The mean proportions of degree for anthropogenic activity sensitive bacteria in combined upstream, midstream and downstream positive/negative networks were 58.7%/62.9%, 29.6%/24.7%, and 24.2%/29.6%, respectively. However, the proportions of degree for non-indicator bacteria were similar between the combined upstream, midstream and downstream networks (Figs. 5A and 5B). The mean proportions of degree for non-indicator bacteria in combined upstream, midstream and downstream positive/negative networks were 26.8%/27.9%, 31.0%/24.9%, and 29.5%/29.5%, respectively.

Bacteria that drove the stability of networks

The mean number of positive correlations increased largely from the combined upstream (261) positive network to combined midstream (483) and downstream (439) positive networks (Fig. 5C). However, the mean number of negative correlations were similar between the combined upstream (142), midstream (140) and downstream negative networks (192) (Fig. 5D). Therefore, we focused on the positive networks, and found that comparing with the edges (correlations) that connected with anthropogenic activity sensitive or non-indicator bacteria, the edges that connected with the anthropogenic activity tolerant bacteria increased substantially from the upstream positive networks to midstream and downstream positive networks (Fig. 6A). Similarly, we found that comparing with the edges that connected with anthropogenic activity sensitive or non-indicator bacteria, the correlation coefficients of edges that connected with the anthropogenic activity tolerant bacteria increased substantially from the upstream positive networks to midstream and downstream positive networks (Fig. 6B). This indicated that the anthropogenic activity tolerant bacteria played the important roles in increase of proportion and strength of positive edges in the midstream and downstream networks.

Moreover, the relative abundance (read number) of anthropogenic activity tolerant bacteria increased, while the relative abundance of anthropogenic activity sensitive bacteria decreased from the upstream positive networks to midstream and downstream positive networks (Figs. S6A and S6B). However, the relative abundance of non-indicator bacteria was similar between the upstream, midstream and downstream positive networks (Fig. S6C).

Most of anthropogenic activity tolerant bacterial ASVs in the positive networks were affiliated to genus 12up (mean relative abundance was 9.04% of the total bacteria in midstream and downstream areas), followed by genera *Mycobacterium* (2.56%) and *Novosphingobium* (2.20%) (Figs. 7A and S6A). In contrast, most of nutrient sensitive bacteria in the positive networks were affiliated to genus hgcl_clade (mean relative abundance was 8.70% of the total bacteria in upstream area), followed by genera *Limnohabitans* (8.66%) and CL500-29 (4.69%) (Figs. 7B and S6B).

Discussion

Given that the stable bacterial communities are considered to provide sustainable functions and services for aquatic ecosystems [30, 43, 44], it is important to understand the effects of environmental disturbance on the stability of bacterial communities. However, the effects of anthropogenic activities on the stability of bacterial co-occurrence networks are largely unknown [27]. In this study, we found that the stability of bacterial co-occurrence networks decreased due to the frequent anthropogenic activities in midstream and downstream areas of Xiyuan River.

In Xiyuan River, more than a dozen college campuses and a large number of villages are located in the midstream area. Also, a newly urbanized zone is located in downstream area of Xiyuan River. Intensive anthropogenic activities increased the discharge of domestic and agricultural wastewater, and therefore increased the concentrations of nutrients and other pollutants in midstream and downstream of Xiyuan River. Moreover, the negative correlations between nutrient concentrations and precipitation in midstream and downstream areas of Xiyuan River indicated that the nutrients were further concentrated due to the rare rainfall in winter season [45, 46]. In contrast, only slight anthropogenic activities occur in the upstream area, and thus the nutrient concentrations were low in this area (Fig. 2).

Interestingly, we found that the bacterial co-occurrence networks in midstream and downstream areas were characterized by properties that indicate low stability. The proportions of positive correlations were higher and the positive correlations were stronger in the midstream and downstream networks than in the upstream networks (Fig. 4). However, the networks in upstream had properties that suggest high stability (the proportions of positive correlations were lower and the positive correlations were weaker in the upstream networks than in the midstream and downstream networks) (Fig. 4). Previous studies indicated that increased negative correlations between species could promote ecological network stability due to the compensatory dynamics (i.e. decrease in the abundance of one species is associated with increase in the abundance of another species) [24–26]. These compensatory dynamics reflect species may be redundant in the ecosystem functions they provide. An ecosystem function can be provided stably by many mutually compensatory species with the same or similar function [47, 48]. In contrast, strong positive correlations between species are thought to weaken the compensatory dynamics. Moreover, in a community with a large proportion of strong and positive links between members, the members may respond in tandem to variation of environmental conditions, resulting in co-oscillation [18]. In this case, if a key member in network becomes extinct, other members will have extinct risk due to their strong and positive correlations with this key member [24]. Therefore strong positive correlations destabilized microbial co-occurrence network. A recent study using co-occurrence networks also indicated that bacterial networks were more stable than fungi networks under drought, because bacterial networks had larger proportion of strong positive correlations than fungi networks [27].

We further explored the mechanisms of network stability decline in midstream and downstream areas of Xiyuan River. Given that the numbers of negative correlations were similar between the upstream, midstream and downstream networks (Fig. 5C), we focused on the positive correlations in the networks (positive networks) (Fig. 5D). We found that the anthropogenic activity sensitive bacteria had higher proportion of degree in the positive networks of upstream area. However, the anthropogenic activity

tolerant bacteria had higher proportion of degree in the positive networks of midstream and downstream areas (Fig. 5A). Furthermore, we found that comparing with the edges (correlations) linked to the anthropogenic activity sensitive bacteria and non-indicator bacteria, the number and strength of edges linked to the anthropogenic activity tolerant bacteria increased largely from the upstream positive networks to midstream and downstream positive networks (Fig. 6). This indicated that the anthropogenic activity tolerant bacteria and their linked bacterial members formed a large and strong co-variational module in midstream and downstream positive networks, and these bacteria played the most important role in destabilizing midstream and downstream networks. To explore the potential functions of bacteria in the networks, we found that among the positive network members, the relative abundance (number of reads) of anthropogenic activity tolerant bacteria was substantially higher in midstream and downstream than in upstream areas, and the most abundant anthropogenic activity tolerant bacterial genera were 12up, *Mycobacterium* and *Novosphingobium* (Fig. 7A). Genus 12up is affiliated to family Rhodocyclaceae. Rhodocyclaceae was reported as the core family with responsibility for denitrifying [49], phosphorus removal [50] and aromatic degradation processes [51, 52] in activated sludge systems of wastewater treatments. Interestingly, genus *Novosphingobium* is also an important bacteria degrading aromatic compounds such as phenol, aniline, nitrobenzene and phenanthrene, and was commonly found in wastewater or activated sludge [38, 53, 54]. *Mycobacterium* is one of the most common bacterial pathogens and was frequently found in domestic wastewater [55, 56]. For example, *Mycobacterium paratuberculosis* had the ability to cause Johne's disease in cattle [57]. Previous studies found that *Mycobacterium* such as *M. avium* subsp. *paratuberculosis*, *M. fortuitum* and *M. ratisbonense* persisted in influents or activated sludge of wastewater treatment plant [58, 59], perhaps reflecting the high proportion of animal waste was received by these wastewater treatment plants [57]. In contrast, among the positive network members, the relative abundance (number of reads) of anthropogenic activity sensitive bacteria was substantially higher in upstream than in midstream and downstream areas, and the most abundant anthropogenic activity sensitive bacterial genera were hgcl clade, *Limnohabitans* and CL500-29 (Fig. 7B). The hgcl_clade, also known as the acl lineage, is globally distributed in freshwater ecosystems [60]. Glockner *et al.* suggested that genus hgcl_clade had competitive advantage over other bacteria in an oligotrophic freshwater [61]. Previous study suggested that the genus *Limnohabitans* represented an average of 12% of freshwater bacterioplankton, with global distribution in a broad range of habitats [32, 62]. Another study found that the relative abundance of *Limnohabitans* was significantly higher in low anthropogenic activity area than in high anthropogenic activity area of Songhua River, northeast of China [63]. The CL500-29 marine group was also frequently determined in freshwater rivers, lakes and drinking water systems [64–66].

As same as the anthropogenic activity tolerant bacteria, the anthropogenic activity sensitive bacteria had higher abundance, node number and degree in the positive networks of upstream area. However, the co-variational module which was formed by anthropogenic activity sensitive bacteria in the upstream positive networks was smaller and weaker than the co-variational module which was formed by anthropogenic activity tolerant bacteria in the midstream and downstream positive networks. We assume that the anthropogenic activity tolerant bacteria in the midstream and downstream networks were

specific to anthropogenic activities. For example, antibiotics largely increased the richness and abundance of antibiotic resistance bacteria [28, 29]. As a result, the anthropogenic activity tolerant bacteria might co-vary with the anthropogenic activities, and thus promoted the positive correlations in the networks. In contrast, the anthropogenic activity sensitive bacteria in the upstream networks tended to randomly change because of the low selection pressure [30]. Moreover, the abundance of anthropogenic activity sensitive bacteria was lower than the anthropogenic activity tolerant bacteria, because the habitats with low anthropogenic activities normally have low organic matters and nutrients. Therefore, the potential interactions between anthropogenic activity sensitive bacteria were less frequent than the anthropogenic activity tolerant bacteria due to the chances of encounter.

Conclusions

To date, the influences of anthropogenic activities on the stability of riverine bacterial co-occurrence network are largely unknown. As a result, our findings have important implications for understanding how complex riverine microbial communities respond to anthropogenic activities. We found that the bacterial co-occurrence networks were unstable in the midstream and downstream areas where were effected by frequent anthropogenic activities. To further explore the mechanisms, we found that bacteria having similar functional traits which positively responded to anthropogenic activities (e.g. nutrient removal, aromatic degradation and pathogen bacteria) formed the large and strong positive modules in the midstream and downstream networks, and therefore destabilize the networks. Given that the effects of anthropogenic activities on aquatic ecosystems over the coming century will probably be intensified, it is urgent to understand the correlations between aquatic ecosystem stability and anthropogenic activity.

Declarations

Funding

This work was funded by the National Natural Science Foundation of China (31971469) and the Qishan Scholar Program of Fuzhou University (GXRC-20061).

Conflicts of interest

All authors have no conflict of interest to declare.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All sequence data from this study have been deposited in the public NCBI Sequence Read Archive (SRA) database under the accession number PRJNA633223.

Code availability

Not applicable

Authors' contributions

LL and JC conceived the idea and designed the experiments. LL wrote the paper. SW and LL performed the experiments. LL and SW analyzed the data. JC and LL contributed the reagents/materials/analysis tools. All authors read and approved the final manuscript.

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Supplementary Information

Fig. S1 Photos of sampling sites along Xiyuan River

Fig. S2 Spatiotemporal variation of NO₃-N and PO₄-P in Xiyuan River (U or Up – upstream, M or Mid – midstream, D or Down – downstream; Sp – spring, Su – summer, Wi – winter; mean ± s.e., different letters indicate significant differences testing by one-way ANOVA).

Fig. S3 Spatiotemporal variation of DOC (dissolved organic carbon) (A), precipitation (B) and water temperature (C) in Xiyuan River. Mean ± s.e., different letters indicate significant differences testing by one-way ANOVA.

Fig. S4 Spatiotemporal variation of bacterial species richness in Xiyuan River (mean ± s.e.).

Fig. S5 CCA ordination showing bacterial community composition in relation to environmental factors for spatial scale (A) and temporal scale (B).

Fig. S6 Anthropogenic activity tolerant (A, 85 bacterial ASVs), anthropogenic activity sensitive (B, 95 bacterial ASVs) and non-indicator (C, 69 bacterial ASVs) bacterial ASVs in the positive networks, and their relative abundance (read number) in upstream, midstream and downstream areas (bacterial ASVs were classified into genes level, NA – unidentified genera, Others – other genera).

Table S1 Latitude and longitude of sampling sites along Xiyuan River

Table S2 Spearman’s rank correlations showing precipitation in relation to nutrient concentration and chl a.

Table S3 Properties of the ten networks from the ten sampling sites (networks contained only the significant correlations)

Figures

Figure 1

Map showing the location of 10 sampling sites along Xiyuan River (Upstream: S1-S3, Midstream: S4-S7, Downstream: S8-S9). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Spatiotemporal variation of total nitrogen (TN), total phosphorus (TP), NH₄-N and Chl a in Xiyuan River (U or Up – upstream, M or Mid – midstream, D or Down – downstream; Sp – spring, Su – summer, Wi – winter; mean ± s.e., different letters indicate significant differences testing by one-way ANOVA).

Figure 3

Non-metric multidimensional scaling (NMDS) ordination showing the spatial (A) and temporal (B) variations of bacterial community composition in Xiyuan River.

Figure 4

Proportion (A) and correlation coefficients (B) of positive correlations in networks from upstream, midstream and downstream areas. Mean $\pm$ s.e., different letters indicate significant differences testing by one-way ANOVA. Frequency distributions of positive correlation coefficients in networks from upstream, midstream and downstream areas(C).

Figure 5

Combined bacterial co-occurrence networks in upstream (3 networks), midstream (4 networks) and downstream (3 networks) areas (A: networks consist of positive correlations, B: networks consist of negative correlations) showing anthropogenic activity tolerant, anthropogenic activity sensitive and non-indicator bacteria. Number of positive correlations (C) and negative correlations (D) in combined upstream, midstream and downstream networks. Mean $\pm$ s.e., different letters indicate significant differences testing by one-way ANOVA.

Figure 6

Number (A) and correlation coefficients (B) of edges (correlations) that connected with anthropogenic activity tolerant, anthropogenic activity sensitive and non-indicator bacteria in upstream, midstream and

downstream bacterial positive networks. Mean $\pm$ s.e., different letters indicate significant differences testing by one-way ANOVA.

Figure 7

Dominated anthropogenic activity tolerant (A) and anthropogenic activity sensitive (B) bacterial ASVs in the positive networks, and their relative abundance (read number) in upstream, midstream and downstream positive areas (bacterial ASVs were classified into genes level).

Supplementary Files

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