Bacterial community responses to tourism development in the Xixi National Wetland Park, China

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HIGHLIGHTS

- The impacts of tourism development on bacterial communities were clarified.
- Bacterial communities were sensitive to tourism projects.
- Tourism reduced α-diversity of but increased β-diversity of bacterial communities.
- Potential pathogens were enriched in wetland park watershed with human activities.
- TOC of water and TC of sediment were the main drivers of bacterial assemblage.

GRAPHICAL ABSTRACT
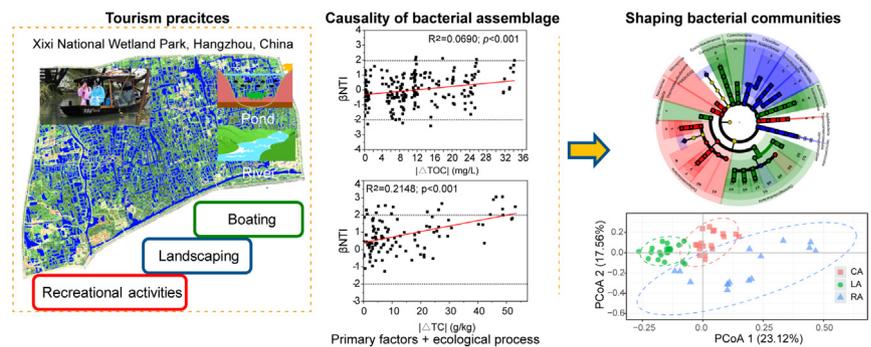
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ABSTRACT

A large number of urban wetland parks have been established, but knowledge about the effects of tourism development on the microbial diversity and ecosystem functioning remains limited. This study aimed to clarify the responses of bacterial communities to tourism development targeted the Xixi National Wetland Park, China. By analyzing the diversity, composition, assembly pattern, and environmental drivers of bacterial communities, we found that tourism development considerably affected the water quality, which further decreased the α-diversity but increased the β-diversity in open areas for landscaping and recreation. Specifically, there was higher Simpson dissimilarity across functional wetland areas, indicating that species replacement mainly explained β-diversity patterns of bacterial communities. RDA analysis and ecological processes quantification further suggested that TOC and TC were the major factors in the open areas driving bacterial communities in water and sediment, respectively. Also, typical anti-disturbance taxa (*Gammaproteobacteria*) and potential pathogens (*Bacillus*) were enriched in the wetlands under more anthropogenic disturbances. Findings of the present study highlighted the effects of tourism development on bacterial communities resulted in obvious spatial variation in the Xixi National Wetland Park. This study gives us useful information for ecological assessments of urban wetlands, and further can provide references in making appropriate strategies to manage wetland ecosystems.

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1. Introduction

Urban wetlands provide us considerable ecosystem services from the supportive, regulatory and cultural aspects (Costanza et al., 1997; Russi et al., 2013; Yu et al., 2018). To more fully utilize their environmental and economic benefits (Stålhammar and Pedersen, 2017), a large number of urban wetland parks have been established all over the world (Arsić et al., 2018; Chatterjee et al., 2015; Mitsch et al., 2014; Wahlroos et al., 2015). For example, China has 1699 wetland parks by the end of 2017, and 898 of which were national wetland parks (http://www.shidi.org/unit.html). These wetland parks are primarily to protect wetland resources and effectively utilize their economic benefits. However, less attention has been paid to ecological benefits during the development of urban wetlands. Increasing tourism accelerates waste generation, nutrient inputs, water pollution and habitat destruction in the wetlands (Pan et al., 2018). Such disturbances from tourists' activities have considerably affected the diversity of aquatic organisms (Geneletti and Dawa, 2009; Markogianni et al., 2016). Microorganisms are essential members of urban wetland ecosystems due to their abilities in pollutants purification, nutrient cycling, and energy conversion (Wu et al., 2013a; Xu et al., 2014). Although the microbial composition and diversity are so important for the wetland ecosystem health (Baulch et al., 2011; Cavicchioli et al., 2019; Raymond et al., 2013), we know little about how microorganisms respond to tourism development in wetland ecosystems.

Increasing evidence indicated that microorganisms are closely related to their surrounding environments (Battin et al., 2016). The microbial diversity and community structure are sensitive to the environmental changes caused by human activities such as dam construction, tunnel excavation and amusement projects (Nogales et al., 2007; Wu et al., 2019a; Wu et al., 2013b; Xiao et al., 2019). The degree of environmental variation in water and sediment is acknowledged as the major factors affect microbial communities in wetlands (Battin et al., 2016; Wu et al., 2013a). For example, the concentration of NH^{+}-N and redox potential found to be important regulators of bacterial communities in watersheds (Ding et al., 2015; Peralta et al., 2014). Also, plants could alter bacterial communities (Li et al., 2019; Zeng et al., 2012). So, abiotic or biotic conditions could affect ecosystem functions indirectly by shaping bacterial communities, which in turn can affect wetland functions (Judd et al., 2006). However, there is little understanding of factors influencing assembly patterns of bacterial communities in wetland parks under intense human disturbances.

Wetland parks usually involve in a variety of commercial development such as ecological sightseeing and recreation services in the open area. In contrast with the undisturbed state in conservation areas, artificial landscaping (covering aquatic plants) altered microhabitats, and therefore influenced the abundance and diversity of bacterial communities (Mentes et al., 2017; Zeng et al., 2012). Surface runoffs with complex organic and/or inorganic pollutants could also affect the bacterial communities (Hu et al., 2017; Méndez-García et al., 2014). Additionally, environmental factors can affect microbial functions (Amend et al., 2016). Substantial efforts have been paid to reveal the potential microbial functions of energy metabolism, xenobiotic biodegradation, signaling molecules and interaction in aquatic environments (Bier et al., 2015; Kuang et al., 2016; Wang et al., 2018b). Peter and Sommaruga (2016) found that ecosystem functioning involving in carbon, nitrogen and phosphorus cycles could shift during the transition between turbid and clear conditions. Such sensitive responses of microorganisms to environmental changes, including pH, nitrogen, phosphorus, metal concentrations, and other nutrients or contaminants, have been proposed to monitor ecosystem health (Wu et al., 2016; Wu et al., 2015; Zhang et al., 2016a). Potential microbial indicators could regard as an additional reference for water quality evaluation (Sims et al., 2013). Recently, an increasing number of pleasure-boat projects have been promoted in wetland parks for tourists, which could influence the water and sediment properties (Herbert et al., 2009; Lenzi et al.,

2013). This can further affect water-dependent wildlife (Rodgers Jr and Schwikert, 2002; Velando and Munilla, 2011). However, we have much less understanding of their impacts on microorganisms. So, it is necessary to get a comprehensive understanding of bacterial communities and their responses to serious anthropogenic disturbances in wetland parks.

Recently, β-diversity has been proposed to be incorporated into management decisions, which could improve assessments and predictions of ecosystem status (Mori et al., 2018; Socolar et al., 2016). One way is to partition total β-diversity into its turnover and nestedness components, which may give additional insights into the underlying causes of spatial variability (Baselga, 2010). A previous study indicated that species turnover measures similar to the total β-diversity, and higher contribution of community turnover attribute to larger environmental heterogeneity (Soininen et al., 2018). Another way is using the β-nearest taxon index (βNTI) as a key index to determine the main driving factor of bacterial assembly (Stegen et al., 2016). For example, with βNTI values regressed against environmental factors of pH, NH_4^+-N and ORP, results indicated that these factors could mediate the balance between stochastic and deterministic assembly of bacterial communities (Tripathi et al., 2018; Zhang et al., 2019). However, application of these approaches in urban wetland parks has not been reported.

This study aimed to explore how tourism development influences water and sediment bacterial communities. We hypothesized that nutrients associated with landscaping and leisure projects would be an important impetus for altering bacterial communities in the wetland watershed. To test this hypothesis, three different wetland functional areas (conservation, landscaping, and recreation areas) and two kinds of rivers (with or without pleasure-boats) were selected to analyze bacterial communities and their responses to environmental factors. This study intended to (i) explore the impact of tourism development on the bacterial community diversity, composition, and assemblage patterns; (ii) identify typical microbial taxa under different disturbance conditions; and (iii) reveal the main environmental factors driving the assembly of microbial communities. This study provides insights into dynamics of bacterial communities under different degrees of tourism disturbances, and helps to improve the management and protection of wetland parks.

2. Materials and methods

2.1. Study area and sampling procedures

The Xixi National Wetland Park, which is the earliest constructed national wetland park in China, located in Hangzhou with a total area of 11.5 km². Since its opening in 2003, tourism projects and tourists are increasing gradually. The whole park can be divided into conservation areas and open areas. According to specific tourism projects, the open areas are greatly disturbed by human activities, which can be further divided into landscaping areas and recreational areas. The watersheds in landscaping areas were mainly affected by the plants, whereas recreational areas were mainly affected by surface runoff carrying complex pollutants. To evaluate the possible ecological effects resulting from the tourism development, we set up 18 ponds (three samples were collected from the left, center and right of each pond) across three functional regions (conservation areas, CA; landscaping areas, LA; recreation areas, RA). Also, 6 sites within two kinds of rivers (boating river, BR; no-boating river, NR) in the wetland park (Figs. 1 and S1) were sampled in May 2018. A total of 120 water and sediment samples were collected from Xixi National Wetland Park. The water samples were stored in sterile plastic bottles. The upper layer of sediment (0–10 cm) collected using a Peterson grab sampler was kept in a sterile plastic bag. The samples were transported in a cooler box to the laboratory for further analysis.

2.2. Physicochemical measurements

Dissolved oxygen (DO), pH, and conductivity (Cond) in water were determined in situ using a multiprobe meter (6600V2-4 Sonde, YSI, USA). Chlorophyll-*a* was measured using a fluorometer (Turner Designs, CA, USA). Water chemical characteristics including total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and total phosphorus (TP) were measured using standard methods according to Cai et al. (2016). Total organic carbon (TOC) in water was determined with TOC Elemental analyzer (TOC-L, Shimadzu, Japan). After drying to constant weight at 30 °C, sediment chemical characteristics including total nitrogen (TN) and total carbon (TC) were determined with an Elemental analyzer (EA3000, EuroVector, Geman). Total phosphorus (TP), Cu, Zn, K, and Mg were measured by an inductively coupled plasma spectrometry (ICP) instrument (Prodigy7, Leeman Labs, USA). Conductivity and pH of sediment were determined according to Wu et al. (2017). All samples were analyzed in three replicates.

The comprehensive water quality identification (CWQI) index (Xu, 2005a; Xu, 2005b) was calculated based on five levels of the environmental quality standard for surface water of China, GB 3838-2002 (SEPA, 2002). The high value means more serious pollution (see details in Supplementary material). DO, NH_4^+-N, TP, TOC, and TN were chosen as the primary organic pollution and nutrient variables.

2.3. DNA extraction, PCR amplification and high throughput sequencing

The metagenomic DNA was extracted using the PowerWater DNA Extraction kit (water) or PowerSoil DNA Extraction kit (sediment) (Mo Bio, CA, USA) according to the manufacturer's instructions. The extracted DNA was dissolved in 50 μL elution buffer. DNA concentrations were determined using a NanoDrop-2000 fluorospectrometer (Thermo Fisher Scientific, MA, USA), and all of the DNA samples were then stored at −20 °C.

The V3-V4 region of the 16S rRNA gene was amplified by using the primer pairs 388F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Xu et al., 2017). PCR amplification was performed in a total volume of 50 μL, which contained 10 μL buffer, 0.2 μL Q5 High-Fidelity DNA Polymerase, 10 μL High GC Enhancer, 1 μL dNTP, 10 μM of each primer and 60 ng genome DNA. Thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 15 cycles at 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 7 min. The PCR products from the first step PCR were purified through VAHTSTM DNA Clean Beads. A second round PCR was then performed in a 40 μL reaction which containing 20 μL 2 × Phμsion HF MM, 8 μL ddH2O, 10 μM of each primer and 10 μL PCR products from the first step. Thermal cycling conditions were as follows: an initial denaturation at 98 °C for 30 s, followed by 10 cycles at 98 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Finally, the concentration of the purified PCR amplicons was determined by Quant-iT™ dsDNA HS Reagent, and these were then used for sequencing on an Illumina Hiseq 2500 platform in Beijing Biomarker Technologies Corporation.

All sequence reads were assembled using FLASH (Magoč and Salzberg, 2011), and the quality was checked using Trimmomatic software (Lohse et al., 2012). UCHIME was used for identifying chimeras (Edgar et al., 2011). The poorly overlapped and low-quality sequences such as those with length <75% of the tags length and moving-window (50 bp) quality score < 20 were eliminated before downstream analysis. Then, the good quality sequences were clustered into operational taxonomic units (OTUs) based on a 97% similarity cutoff by using the UPARSE (Edgar, 2013). Sequences were taxonomically aligned against the SILVA reference alignment database (Quast et al., 2012). To reduce the influence of sequencing depth on treatment effects, all the samples were resampled to the same sequence depth. All the raw reads were deposited in the NCBI under the accession number PRJNA592258.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, version 1.0.0 pipeline) analysis was performed to predict functional characteristics of bacterial communities as described previously (Langille et al., 2013; Wu et al., 2019b). The predicted relative abundances of genes for selected pathways representing microbial functions were plotted in R using the pheatmap package (Team, 2014).

2.4. Statistical analysis

The physic-chemical data were compared through multiple sample comparisons using one-way ANOVA analysis with Student-Newman-Keuls test. The species diversity, richness, and coverage were calculated using the Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010). Principle components analysis (PCoA) was performed based on Bray-Curtis distance of bacterial communities and the significance was tested by permutational multivariate analysis of variance (ADONIS). The relationship between community structure and
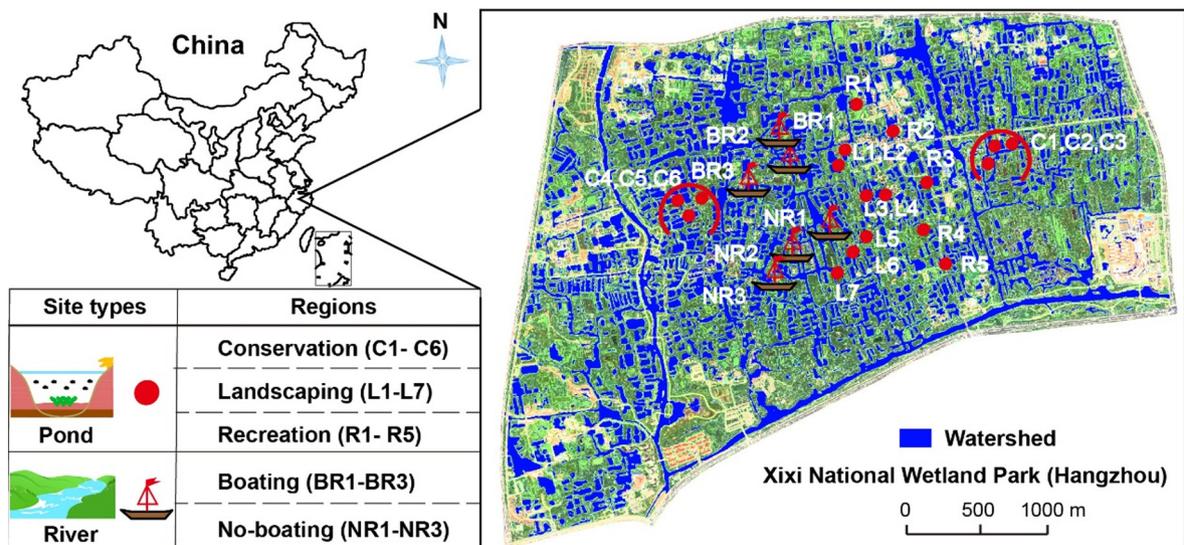
Fig. 1. Distribution of sampling sites in the Xixi National Wetland Park. C1-C6, L1-L7, and R1-R5 denote sites in conservation areas, landscaping areas and recreation areas; BR1-BR3, and NR1-NR3 denote sites in river with- or without-boating.

environmental factors was assessed by redundancy analysis (RDA) via Monte Carlo permutation testing with 999 permutations. The significantly discriminant taxa in each group were identified by the linear discriminant analysis (LDA) effect size pipeline (LEfSe), which employs the factorial Kruskal-Wallis rank-sum test ($\alpha = 0.05$) to determine taxa with significantly different relative abundances among groups (Segata et al., 2011). Multiple-site dissimilarities proposed by Baselga (see Supplementary material), including the Sørensen dissimilarity index (β_{SOR}), the Simpson dissimilarity index (β_{SIM}) and the nestedness-resultant dissimilarity index (β_{NES}) were employed to characterize the causality of the processes underlying the observed bacterial community patterns (Baselga, 2010).

The assembly of bacterial communities in the ponds was analyzed according to Stegen et al. (2013). The weighted beta nearest taxon index (βNTI) was computed by the ecological process analysis pipeline program at http://mem.rcees.ac.cn:8080/, which was in combination with the Bray-Curtis based Raup-Crick (RC$_{bray}$) (Feng et al., 2017; Stegen et al., 2013). The ecological processes of drift, selection and dispersal were further quantified, including (i) βNTI values that fell above $+2$ and below -2 showed that observed turnover between a pair of communities is regulated by heterogeneous and homogeneous selection, respectively (Dini-Andreote et al., 2015); (ii) The relative contribution of dispersal limitation was assessed as the percentage of pairwise comparisons with $|\beta$NTI$| < 2$ and RC$_{bray} > 0.95$, whereas that of homogenizing dispersal was assessed as the percentage of pairwise comparisons with $|\beta$NTI$| < 2$ and RC$_{bray} < -0.95$. The value of all pairwise comparisons with $|\beta$NTI$| < 2$ and $|$RC$_{bray}| < 0.95$ assesses the effect of drift (Stegen et al., 2013). To fully evaluate the variation in community assembly processes along a gradient of relevant environmental factors under disturbance intensity, the βNTI values were regressed against Euclidean distance matrices of the selected environmental factors (Stegen et al., 2016).

3. Results

3.1. Physicochemical properties of water and sediments

The physicochemical analysis showed that pH in ponds and rivers ranging from 7.21 to 8.99 and 7.36 to 7.91, respectively (Tables S1 and S2). Significant differences in water conductivity, DO (from <2 to >8 mg/L) and TOC (from <10 to >40 mg/L) content were observed among ponds ($p < 0.05$), whereas there was no significant difference between rivers (Table S1). Higher concentrations of TOC, Chlorophyll-a and TP were detected in LA and RA compared to CA. Also, the higher content of TP (0.74 g/kg) and TN (2.87 g/kg) was observed in sediments than that in water (Table S2). The content of Cu and Zn in the sediments within open areas were also higher. The highest concentration of Mg was observed in LA (Table S2).

The CWQI index showed that the water quality of the Xixi National Wetland Park was generally maintained at grade III. Specifically, the CWQI value of ponds gradually increased from CA (2.38), LA (3.22) to RA (3.48) (Fig. 2), indicating that tourism activity negatively affected water quality ($p < 0.01$). The water quality in BR was a little better than that of NR. These results indicated that tourism disturbances led to an overall decline in water quality.

3.2. Bacterial diversity, structure, composition and predicted functions

The high-quality sequences from all the 120 samples were clustered into 629 and 1913 operational taxonomic units (OTUs) from water and sediments, respectively. The observed OTUs and Shannon diversity of sediment bacteria from ponds were relatively higher in the conservation areas (CA) than that of open areas (LA and RA) (Fig. S2). Moreover, the bacterial diversity was negatively correlated with the CWQI values, indicating that species diversity decreases as water deteriorates caused by the increase of anthropogenic disturbance. Similarly, the average

number of observed bacterial species and Shannon indices in BR sediments (1127 and 4.92) were lower than those in NR (1159 and 5.72) ($p < 0.05$) attributed to long-term pleasure-boat disturbances in BR (Fig. S2).

The overall patterns of bacterial communities as visualized by the PCoA indicated that the first two components explained 33.53% and 40.68% of variances, respectively (Fig. 3A and C). Adonis test further confirmed that the bacterial communities in the water and sediment were significantly different among three functional regions ($p = 0.001$). According to PCoA analysis, bacterial community structures between in rivers with and without pleasure boats were mainly separated by the second axis (Fig. 3B and D).

The dominant bacterial phyla in water were *Proteobacteria* (38.4%), *Actinobacteria* (35.8%), *Bacteroidetes* (13.7%), and *Cyanobacteria* (4.1%) (Fig. 4A). From conservation areas to open areas, the dominant group shifted from *Actinobacteria* to *Proteobacteria*, mainly due to the notable increase in the order taxa *Betaproteobacteriales* (Fig. 4B). The most dominant phylum in sediments was *Proteobacteria* (36.0%) (Fig. 4C), followed by *Chloroflexi* (19.8%), *Acidobacteria* (8.8%), *Actinobacteria* (7.5%), *Epsilonbacteraeota* (5.3%) and *Nitrospirae* (5.2%). The sediment samples from ponds within RA had higher *Proteobacteria* (39.7%) than the other two areas. *Chloroflexi* (21.5%) and *Acidobacteria* (11.3%) were relatively abundant in the sediments from CA. In the river, sediment samples from BR had higher *Proteobacteria* (32.6%) abundance but lower *Chloroflexi* (15.8%) than that of NR. At the orders level, the most abundant orders across all ponds were *Betaproteobacteriales* (10.2%), *Anaerolineales* (9.1%) and *Campylobacterales* (5.3%). The sediment from RA had higher abundance of *Betaproteobacteriales* (14.9%) and *Campylobacterales* (6.4%), while the dominant orders were *Anaerolineales* (10.8%) and *Pseudomonadales* (6.4%) in CA (Fig. 4D).

A LEfSe analysis showed the significant different bacterial taxa in relation to anthropogenic disturbance modes (Figs. S3 and 5). Specifically, 9 phyla, 17 orders and 16 families were screened across all samples of pond sediments (Fig. 5A). *Chloroflexi*, *Bacteroidetes* and *Verrucomicrobia* were the most abundant within CA. *Nitrospinae* and *Nitrospirae* represented the abundant phyla within LA, whereas *Cyanobacteria* phylum, *Sulfuricurvum* and *Arthrobacter* genera in RA were significantly increased. In the water, *Candidatus_Planktoluna*, *Sediminibacterium* and *Mycobacterium* within CA were the most abundant, while the abundance of *Deinococcus, Paenibacillus, Rhodoluna* and *Pseudomonas* were significantly increased in the open areas (LA and RA) (Fig. S3A). As for river systems, there were 2 phyla, 14 orders and 13 families showed significant differences in sediments (Fig. 5B). Specifically, *Romboutsia* and *Chlorobaculum* in NR were significantly increased, while the abundance of *Gammaproteobacteria*, *Actinobacteria* and *Bacilli* were significantly higher in BR than that of NR. Similarly, more abundant pathogens were found in water samples within BR (Fig. S3B).

The PICRUSt analysis showed that the NSTI scores for all samples ranged from 0.06 to 0.24, suggesting an acceptable prediction. Specifically, cell growth and death, cell motility and signal transduction of water bacteria from ponds within the open areas were highest functional abundance ($p < 0.05$) (Fig. S4A), while these functions generally showed no significant differences among rivers (Fig. S4B). In pond sediments, samples from LA and RA had significantly higher abundances of functional genes compared with CA, including energy metabolism, glycan biosynthesis and metabolism ($p < 0.05$). Due to stimulation by extra nutrients, genes related to energy metabolism (e.g., nitrogen metabolism and photosynthesis) significantly increased from conservation areas to open areas (Fig. S4C). In addition, higher abundances of genes related to xenobiotics biodegradation and metabolism were also observed in ponds and rivers with anthropogenic disturbance (Fig. S4C and D).

3.3. Causality of the bacterial assemblage underlying biodiversity

The dissimilarity of bacterial communities in water and sediments were grouped by functional regions (Fig. S5). Specifically, the β-
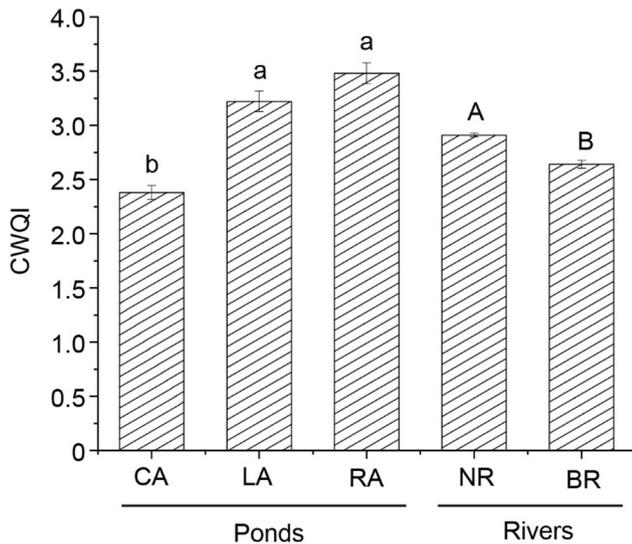
Fig. 2. The comprehensive water quality identification (CWQI) index of surface water was used to indicate water quality. Different small letter means the statistical difference among functional regional ponds, different capital letter means the statistical difference between river sites ($p < 0.05$). CA - conservation areas; LA - landscaping areas; RA - recreation areas; BR - boating river; NR - no-boating river.

diversity (β_{SOR}) of bacterial communities in RA was 0.49 (water) and 0.39 (sediment), where a strong contribution of species turnover ($\beta_{SIM} = 0.36$ and $\beta_{SIM} = 0.28$, respectively) and a small contribution of nestedness ($\beta_{NES} = 0.13$ and $\beta_{NES} = 0.11$, respectively) were observed. In parallel, the β_{SOR} of bacterial communities within LA and CA compose of β_{SIM} with the high value and β_{NES} with the low value. Dissimilarity indices of bacterial community indicated that the values of β_{SOR} in water and sediment of RA were higher than those in other areas (Fig. S5A). Similarly, the values of β_{SIM} within open areas were higher (LA = 0.41, RA = 0.36) than that in conservation areas (0.35). It was also true in river systems (Fig. S5B). Overall, these results indicated that both species turnover and nestedness contributed to the construction of bacterial communities, while the β-diversity patterns in ponds and rivers were mainly driven by species replacement. Further ecological process quantification indicated that dispersal limitation dominated (contributing about 80% in both water and sediment) the

phylogenetic turnover of bacterial communities across all pond samples, the heterogeneous selection increased from CA to RA (from 0 to >10%) (Table S3).

3.4. Identifying primary drivers controlling deterministic processes

RDA was performed to illustrate the relationship between environmental factors and bacterial communities. The DO, TP, TOC, NH$_4^+$-N, TN and chlorophyll-*a* showed close relationship with water bacterial communities (Fig. 6A), and pH, conductivity, TP, TC, Zn and Mg were the factors to explain the bacterial community variation in sediments of ponds (Fig. 6C). These factors could explain 17.90% and 16.97% of the total variation in water and sedimental bacterial communities, respectively. TP was a major predictor of bacterial community in both water and sediment environments. In river systems, the first two axes could explain 72.3% and 73.7% of the variations in bacterial community structure in water (Fig. 6B) and sediments (Fig. 6D), respectively.

Based on the RDA results, factors significantly related to microbial communities were selected as potential drivers for further identification in different functional regional ponds. The relationships between βNTI and the potential drivers were studied to infer different influences of ecology processes along the gradient of different environmental factors changes. DO content was affiliated with the βNTI within CA, whereas βNTI within LA was related to TOC concentration (Fig. 7). As for the sedimentary environment, TC and conductivity were more strongly associated with the βNTI within RA than those in other areas (Fig. 7). Specifically, the βNTI distribution gradually shifted with increasing difference in TC concentration, from primarily consistent with stochastic process ($-2 < \beta$NTI$ < 2$) to consistent with heterogeneous selection (βNTI$ > 2$) in sediment samples.

4. Discussion

Understanding the impacts of tourism development on wetland microbial communities and ecosystem functioning is an important topic in ecology and environmental management. We found that the wetland watershed within open areas had lower α-diversity and higher β-diversity than that of conservation areas, and the tourism development in open areas lead to enrichments of typical tolerant species and potential pathogens. Such changes were mainly dominated by nutrient input and macrophyte coverage associated with tourism development. These results revealed that the tourism development shaped bacterial
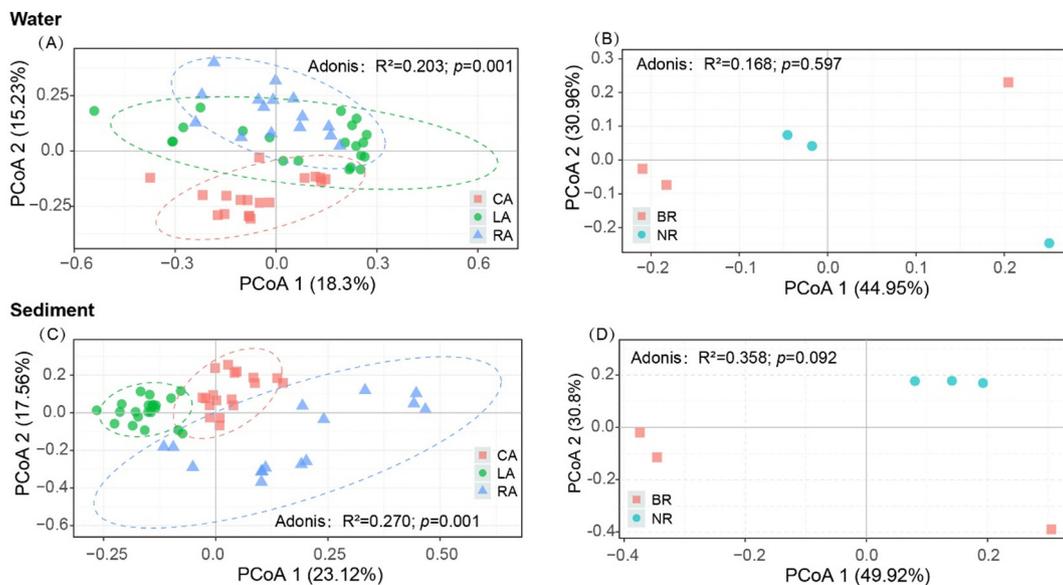
Fig. 3. Bacterial community composition in different functional areas of ponds (A, C), and river sites (B, D) as visualized by the principal components analysis (PCoA) plots. The 95% ellipses were presented to illustrate groups CA, LA and RA. CA - conservation areas; LA - landscaping areas; RA - recreation areas; BR - boating river; NR - no-boating river.

Fig. 4. The bar chart shows the bacterial community composition of the Xixi National Wetland Park. Relative abundances of bacteria were presented at the phylum (A, C), and order level (B, D). The CA, LA, RA, BR and NR at the top represent sampling sites from conservation areas, landscaping areas, recreation areas, boating river and no-boating river, respectively.

communities, and could provide useful information for improving eco-logical evaluation and for urban wetland management.

    Tourism could affect the aquatic environment through direct litter, fecal waste and nutrients or indirect surface runoff delivering these pol-lutants, which may result in nutrient enrichment and algal blooms, and pathogens. Furthermore, the microbial community diversity and com-position will be influenced (Halpern et al., 2007; Nogales et al., 2011). Similar to previous studies in lakes (Ye et al., 2009), we found the OTU richness of sediments was considerably higher than that of the corre-sponding water. Lower sediment bacterial α-diversity in the open areas (RA and LA) than conservation areas (CA) may attribute to the en-richment of special taxa adapted to tourism disturbance. The higher

bacterial β-diversity in the open areas may be due in part to the differ-ential intrinsic resistance capacity of bacterial communities to distur-bance stressors. This is consistent with a previous study indicated that α-diversity of microbiome significantly decreased and β-diversity in-creased across a range of environmental stressors (Rocca et al., 2018). Moreover, the heterogeneous biogeochemistry caused by tourism prac-tices across different functional regions can also affect the succession of microbial communities (Raymond et al., 2013; Roberto et al., 2018). As reported, the patterns of bacterial communities were significantly dif-ferent between the closed areas and the open areas during the construc-tion of a tunnel in the lake (Xiao et al., 2019). Understanding the assemblage of microbial communities is beneficial for a feasibility
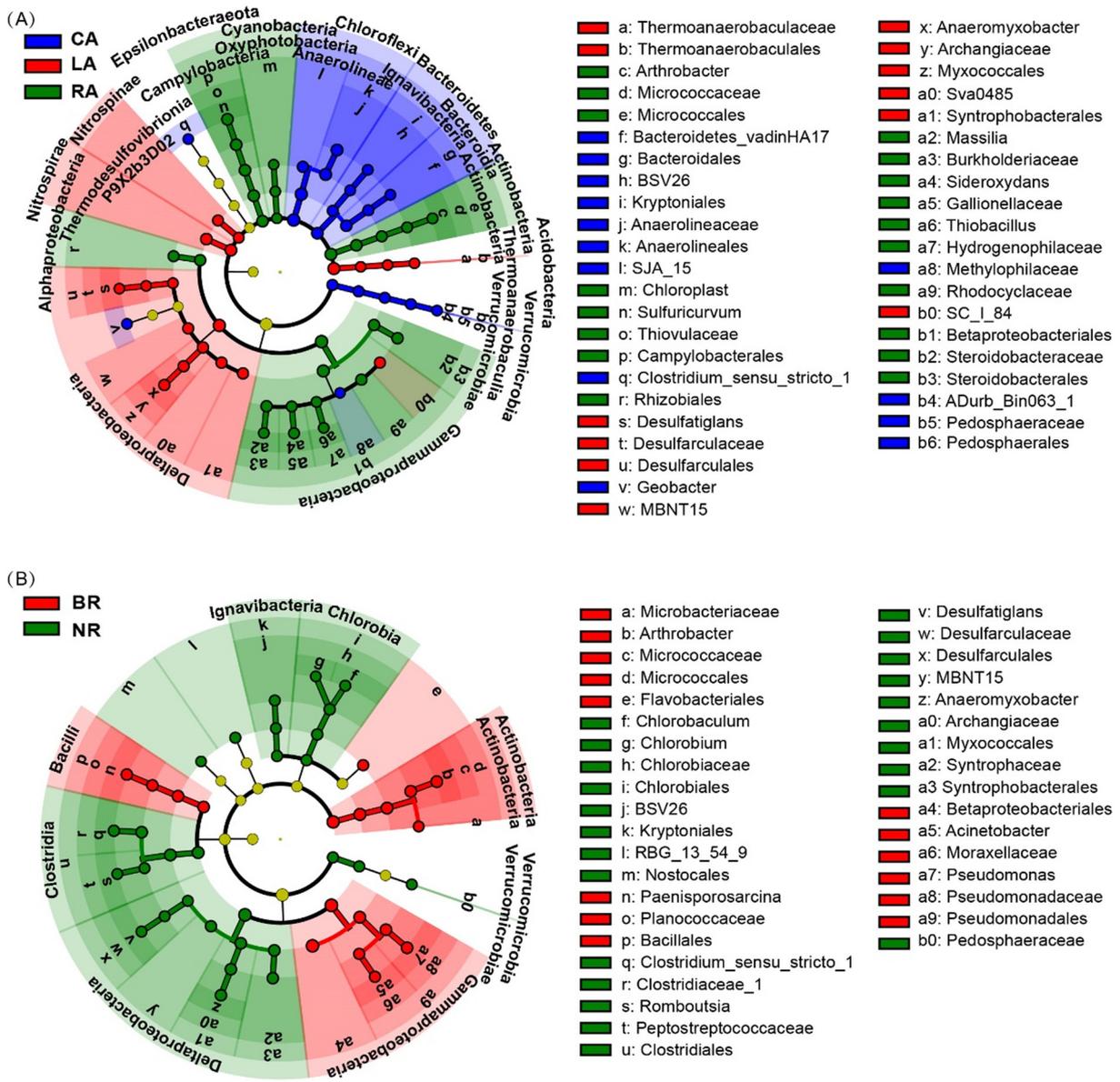
Fig. 5. linear discriminant analysis (LDA) effect size taxonomic cladogram comparing bacterial communities in sediment among three functional regional ponds (A) and two kinds of river sites (B). Taxon nodes in yellow are not significantly discriminant. The branch areas are shaded according to the highest-ranked variety for that taxon. If the taxon is not significantly differentially represented among the sample groups throughout the lineage, the corresponding nodes are not shown in the cladogram. CA - conservation areas; LA - landscaping areas; RA - recreation areas; BR - boating river; NR - no-boating river.

assessment of management measures and important for maintaining urban wetlands ecosystem health (Li et al., 2018; Sims et al., 2013; Socolar et al., 2016).

Our finding of higher *Gammaproteobacteria* in open areas showed positive response to increased nutrient concentrations, which is in agreement with the response of marine microorganisms to anthropogenic perturbations (Nogales et al., 2011). Moreover, the presence of the highest *Cyanobacteria* abundance in sediments within RA due to tourism practices introducing extra nutrients may increase the possibility of algal blooms. Due to the high correlation between *Massila* and energy metabolism (Liang et al., 2020), eutrophication may be the steering factor for the dominant of *Massila* in RA. Similarly, pollutants discharged from pleasure-boats, like a moving source of disturbance along the river, resulting in the higher abundance of *Flavobacteria*, which could consume various forms of organic matter within BR (Teeling and Amann, 2012; Williams et al., 2013). The increase of organic matter also promotes the growth of *Acinetobacter* in BR. One possible explanation is

that *Acinetobacter* was associated with carbohydrate and lipid metabolism in polluted wetlands (Liang et al., 2020).

In the landscaping areas, the growth and decay of macrophytes affect the surrounding environments by supplying carbon and nutrient source for the microbes (Mentes et al., 2017), promoting the colonization of functional bacteria. If the macrophytes are not harvested and maintained in time, they will directly increase the nutrient elements by decomposition products of plant litter (Heilmayr, 2014; Wang et al., 2018a). On the one hand, with the development of landscaping projects, water quality is getting worse compared to conservation areas. On the other hand, the representatives phylum of *Acidobacteria* involved in the degradation of plant-derived compounds (e.g., cellulose) (Eichorst et al., 2011) and *Anaeromyxobacter* genus responsible for anaerobic decomposition of carbohydrates via fermentation (He et al., 2011) were promoted in LA. In turn, microorganisms also accelerated the related biogeochemical processes such as carbon cycling modulated by those species utilizing plant degradation products
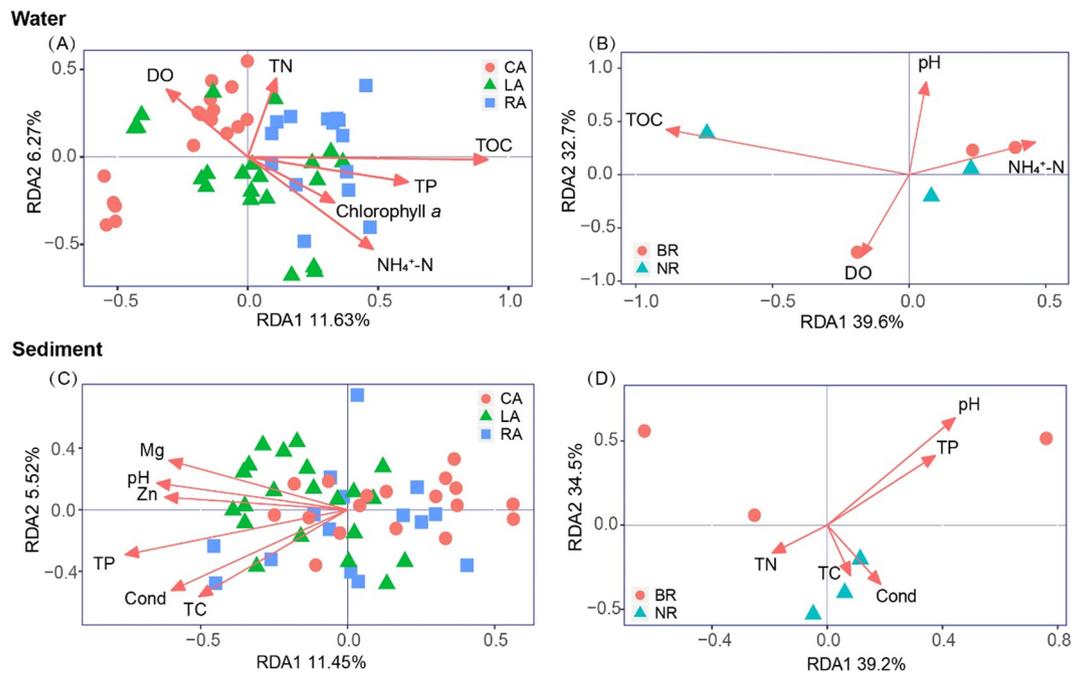
Fig. 6. RDA of the bacterial community and the most significant physiochemical variables shaping bacterial community composition in ponds (A, C), and river (B, D). Cond - conductivity.

or humic substances (Mentes et al., 2017; Wu et al., 2016). The representatives phyla of *Nitrospinae* and *Nitrospirae* involved in nitrogen cycling, and the high abundance of *Desulfatiglans* involved in sulfur cycling were widespread in plant cover areas. This is consistent with previous studies (Pang et al., 2016; Song et al., 2015; Wang et al., 2017; Yun et al., 2017). The enhancement of biogeochemical cycle was also consistent with the results of PICRUSt analysis, which showed the high relative abundance of genes related to energy metabolism and xenobiotic biodegradation within open areas. It has been advised that there were correlations between the abundance of xenobiotic degradation genes and xenobiotic biodegradation rates (Pagé et al., 2015). Therefore, tourism activities had an important impact on aquatic physicochemical property, bacterial community composition and functioning in the urban wetlands.

Partitioning of β-diversity is increasingly considered to explain the variation of species composition among communities to reveal their underlying ecological mechanisms (Baselga, 2010; Podani and Schmera, 2016). In our study, anthropogenic disturbances increased the estimated total β-diversity observed in ponds and river systems. Dividing the β-diversity into its turnover and nestedness components showed that the contribution of species replacement was significantly higher than that of species loss, suggesting a high proportion of endemics present within different functional regions influenced by tourism projects. A similar conclusion was obtained in a study on assembly patterns of bacterial communities in a heavily polluted urban river (Zhang et al., 2019). Higher contributions of species turnover to community variability attribute to increasing environmental filtering, which was confirmed by our results on the ecological processes with an enhancement of heterogeneous selection from conservation areas to open areas. At present, a thorough understanding of the distribution pattern and driving mechanism of various microbial communities in different spatial-temporal dimensions is still developing through β-diversity partitioning combining with species function and phylogenetic information. Thus, identifying this difference in β-diversity of bacterial communities under different anthropogenic disturbance may provide the groundwork in knowing about the extent to which the differential response of microorganisms can reflect ecosystem conditions and how much information is available

for protecting wetland biodiversity and conservation planning (Socolar et al., 2016).

Traditional analysis (e.g., RDA) based on OTU abundant and environmental factors indicated that DO, TP, TOC, NH_4^+-N, TN and chlorophyll-*a* influenced the bacterial communities, which is consistent with previous studies (Berry et al., 2017; Li et al., 2011; Shade et al., 2012; Zhang et al., 2015). Factors affecting bacterial community assembly were more accurately identified through the analysis of correlation between βNTI and gradients of physicochemical factors (Stegen et al., 2016; Zhang et al., 2019). In our study, TC (sediment) was the most important determining factor significantly related to the changes in bacterial communities within RA. It implied that the water body received carbon sources from restaurants, snack streets and hotels, and may further speed up the consumption of dissolved oxygen in waterbodies, where long-term low dissolved oxygen status reshaped microbial communities (Xu et al., 2018; Yannarell and Triplett, 2005). TOC (water) contributed significantly to the shift of microbial community composition within LA, suggesting that the macrophytes planting needs to be carefully managed to avoid the release of excess plant-derived organic matter.

Accurately, identifying key factors shaping microbial communities is the main strategy to construct comprehensive tools for wetland health monitoring and evaluation (Sims et al., 2013). In this study, the dominance of *Acidobacteria*, *Nitrospirae* and *Desulfarculales* involved in carbon, nitrogen and sulfur cycling reflected high nutrient-laden conditions in ponds. Moreover, the abundant pathogen (e.g., *Bacillus*) may indicate a risk of contamination by human activities. The βNTI metric was proved to be advantageous for accurately determining the drivers of bacterial communities through the analysis of the correlation between βNTI value and gradients of physicochemical factors (Zhang et al., 2016b). In our study, the growing dissimilarity between bacterial communities was recognized through the increasing of βNTI value with the increased difference in TC. The deterministic process becomes the dominant mechanism of bacterial assemblage in the recreational area ponds when the difference in TC > 50. Notably, the more deterministic assembly means phylogenetically the more clustered bacterial communities in a nutritious state (Tripathi et al., 2018). This implied that continuous input of carbon sources with tourism development may
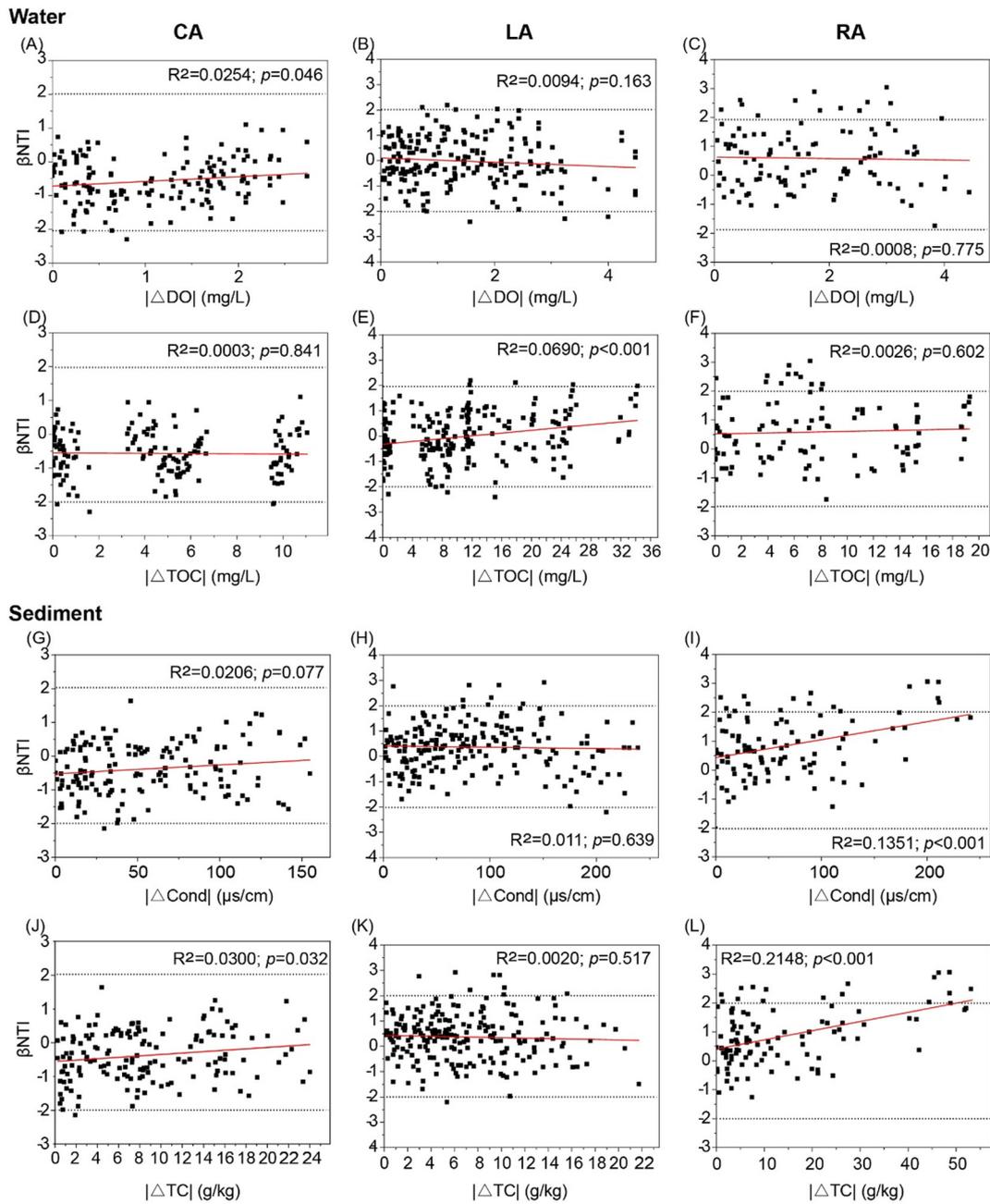
Fig. 7. Relationships between βNTI and differences in DO and TOC from water environment (A–F), and conductivity and TC from sediment (G–L). CA - conservation areas; LA - landscaping areas; RA - recreation areas. Horizontal dashed lines indicate upper and lower significance thresholds at βNTI = +2 and −2, respectively.

further reduce bacterial α-diversity and result in the regime shift of microbial communities, and further affect the ecological functions of wetland parks.

5. Conclusions

This study revealed that tourism development in the urban wetlands affected the water quality and subsequently decreased the bacterial α-diversity. The enhancement of environmental filtering increased the microbial β-diversity in the watershed of wetland in open areas. The TOC of water and TC of sediment were the main drivers determining bacterial community assemblages in open areas. The abundant pathogenic bacteria in highly disturbed water bodies indicated aquatic deterioration. Our findings indicated that taxonomic diversity and ecosystem functional stability of bacterial communities may face a threat with tourism development.

CRediT authorship contribution statement

Binhao Wang: Conceptualization, Methodology, Software, Writing - original draft.**Xiafei Zheng:**Methodology, Writing - review & editing. **Hangjun Zhang:**Conceptualization, Resources.**Fanshu Xiao:**Data curation.**Hang Gu:**Visualization.**Keke Zhang:**Investigation.**Zhili He:** Conceptualization, Writing - review & editing.**Xiang Liu:**Investigation. **Qingyun Yan:**Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

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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.2020.137570.

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