LIMNOLOGY and OCEANOGRAPHY

Riverine bacterioplankton and phytoplankton assembly along an environmental gradient induced by urbanization

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Abstract

Longitudinal environmental heterogeneity and directionality of the water movement are key features that may exert contrasting forces on riverine plankton assembly. Directionality strengthens dispersal-driven assembly, but this can be masked by urbanization-induced environmental heterogeneity along the river continuum. In the light of this contrast, we aimed at delineating the relative importance of assembly processes generating distribution patterns of bacterioplankton and phytoplankton communities in a river draining an urbanizing watershed in Southeast China. We applied variation partitioning analysis, neutral community model, and quantitative process estimate on molecular and morphological plankton data obtained over the years 2012–2016. Despite a relatively short distance between sampling sites (< 20 km), plankton community similarity decreased with increasing distance from the upstream pristine site toward the downstream urban area, and formed clusters that roughly corresponded to five habitat patches, predefined based on hydrology and longitudinal landscape change. These distribution patterns were predominantly driven by deterministic and stochastic processes for phytoplankton and bacterioplankton, respectively, indicating a balance between dispersal due to fluvial connectivity and local selective pressures. Considering the global loss of river connectivity due to downstream fragmentation and flow regulation, our findings imply that plankton-based ecological approaches could be useful to hedge against an uncertain future of rivers draining urbanizing watersheds in an ecologically sustainable way.

Human activities are reshaping the distribution patterns of aquatic communities across the globe through habitat alteration (Vitousek et al. 1997; Yamazaki and Trigg 2016; Anderson et al. 2020). In particular, there is a very long history of habitat alteration of rivers in China; and more recently, the severity and frequency of these disturbances has highlighted a need to explore their effects on plankton communities (Peng et al. 2020; Yang et al. 2022). However, these studies remain rare, especially for riverine ecosystems under human pressure, yet they are among ecosystems undergoing major biodiversity loss (Vörösmarty et al. 2010; McCluney et al. 2014; Anderson et al. 2020). In rivers draining urbanizing watersheds, the most frequent types of disturbances include channelization, reservoir and dam construction, allochthonous biotic and abiotic inputs, and changing hydrological regimes (Wenger et al. 2009; Bai et al. 2017), these could affect ecological feedbacks including those involving plankton (Erős and Lowe 2019; Uchida et al. 2021). Thus, unraveling the processes controlling the community assembly of riverine plankton in urbanizing watersheds is crucial for both theoretical

and applied freshwater ecology, and for the goal of maintaining riverine ecosystem function.

Understanding ecological processes that control distribution patterns of microbial communities is a longstanding question in microbial ecology (Hanson et al. 2012). A key idea has been the importance of what are referred to as "deterministic processes," where aspects of the organism environment—abiotic or biotic—explain which organisms can survive at a particular site (Warming 1909; Hanson et al. 2012; Vass and Langenheder 2017; Ning et al. 2019). An alternative approach is to view community assembly as driven by stochastic processes such as differences among taxa in birth, death, and migration, independent of species traits (Hubbell 2001; Chen et al. 2019). This idea makes assumption that all the species have the same fecundity, independent of their environmental conditions, so that recruitment is solely proportional to the relative abundance of the individuals of a given species (May et al. 2007). Deterministic and stochastic processes are not mutually exclusive; it is the relative importance of either process that is hypothesized to alter microbial diversity and its biogeochemical function (Graham et al. 2016; Graham and Stegen 2017).

Over the past 10 years, a comprehensive framework viewing assembly processes as a result of an interplay of four processes—selection, drift, speciation, and dispersal—governing microbial populations and macroorganisms has been developed (Vellend 2010; Hanson et al. 2012). This framework has offered ways to address a multiplicity of the processes that simultaneously underlie community assembly in natural and engineered ecosystems (Hanson et al. 2012; Nemergut et al. 2013; Goldford et al. 2018). In this context, the outcomes of the recent studies on microbial communities have been highly context-specific. Whereas the studies by Wang et al. (2013), Liao et al. (2017), and Yan et al. (2017) indicated the dominance of deterministic processes in structuring aquatic microbial community, the studies by Liu et al. (2018), Chen et al. (2019), and Tang et al. (2020) showed that mainly stochastic processes structure the aquatic microbial community. In the aquatic ecosystems specifically, different groups of organisms may be structured by different assembly mechanisms. For instance, Wang et al. (2020) found that stochastic processes were less pronounced for archaeal than for bacterial communities in a subtropical river-bay system, and Gad et al. (2020) found that deterministic processes were primarily governing the microeukaryotic habitat specialists while stochastic processes were more important for habitat generalists in an anthropogenically impacted river. Changes in hydrological regimes could also influence the relative importance of deterministic and stochastic processes, as stable hydrological periods tend to allow aquatic communities to assemble deterministically (Isabwe et al. 2018; Larsen et al. 2019).

In the context of rivers in watersheds under rapid urbanization, hypothetically, longitudinal environmental heterogeneity and unidirectional water flow may exert counteracting effects on the assembly of the plankton community. On the one hand, dispersal-driven processes (e.g., dispersal homogenization) may be strengthened by flow and directionality especially because plankton are easily washed downstream (Chen et al. 2019), with no obvious way for them to move upstream against the current. On the other hand, the effects of dispersal and other neutral processes can be overwhelmed by environmental heterogeneity, resulting in more dissimilar communities than expected under the assumptions of the neutral theory (Vályi et al. 2016). For instance, a recent study by Huber et al. (2020) challenged the general view that the strength of selection was weakened due to dispersal homogenization by showing the major effect of environmental heterogeneity in determining the ecological processes of bacterioplankton in Paraná River, South America.

Many of the above studies employed solely multivariate, neutral, or null model approaches to determine the relative importance of deterministic and stochastic processes structuring microbial communities (Zhou and Ning 2017; Chen et al. 2019). However, each of these approaches has its own assumptions, strengths, and caveats. For instance, models built on empirical randomization algorithms may lack clear biological mechanism, and most approaches used to infer environmental selection were insufficient to distinguish it from the outcome of biotic interactions (Cadotte and Tucker 2017; Zhou and Ning 2017). Given that inferences drawn from different approaches are less likely to be artifacts of a particular approach, integrating multiple models synergistically could improve the assessment of the relative importance of different assembly processes.

Based on the assumption that local microbial communities generally have large site-to-site variation in species composition when environmental conditions are different (Zhou et al. 2014), we hypothesized a decreasing plankton community similarity with increasing longitudinal distance between sites along a river draining an urbanizing watershed. Regarding inferences that disturbance increases the importance of deterministic processes (Chase 2007; Jiang and Patel 2008; Peng et al. 2020), we also hypothesized that deterministic processes would primarily shape patterns of plankton community, even in the presence of a strong unidirectional flow of water. To test these hypotheses, we used an observational approach on Houxi River, a river system in southeast China that exhibits a range of different environments along its length (Fig. 1a). We used the variation partitioning analysis (VPA), the neutral community model (NCM), and the quantitative process estimate (QPE) to show that riverine plankton (bacterioplankton and phytoplankton) communities were closely associated with longitudinal environmental heterogeneity induced by urbanization, and that stochastic processes were more important in structuring the bacterioplankton community.
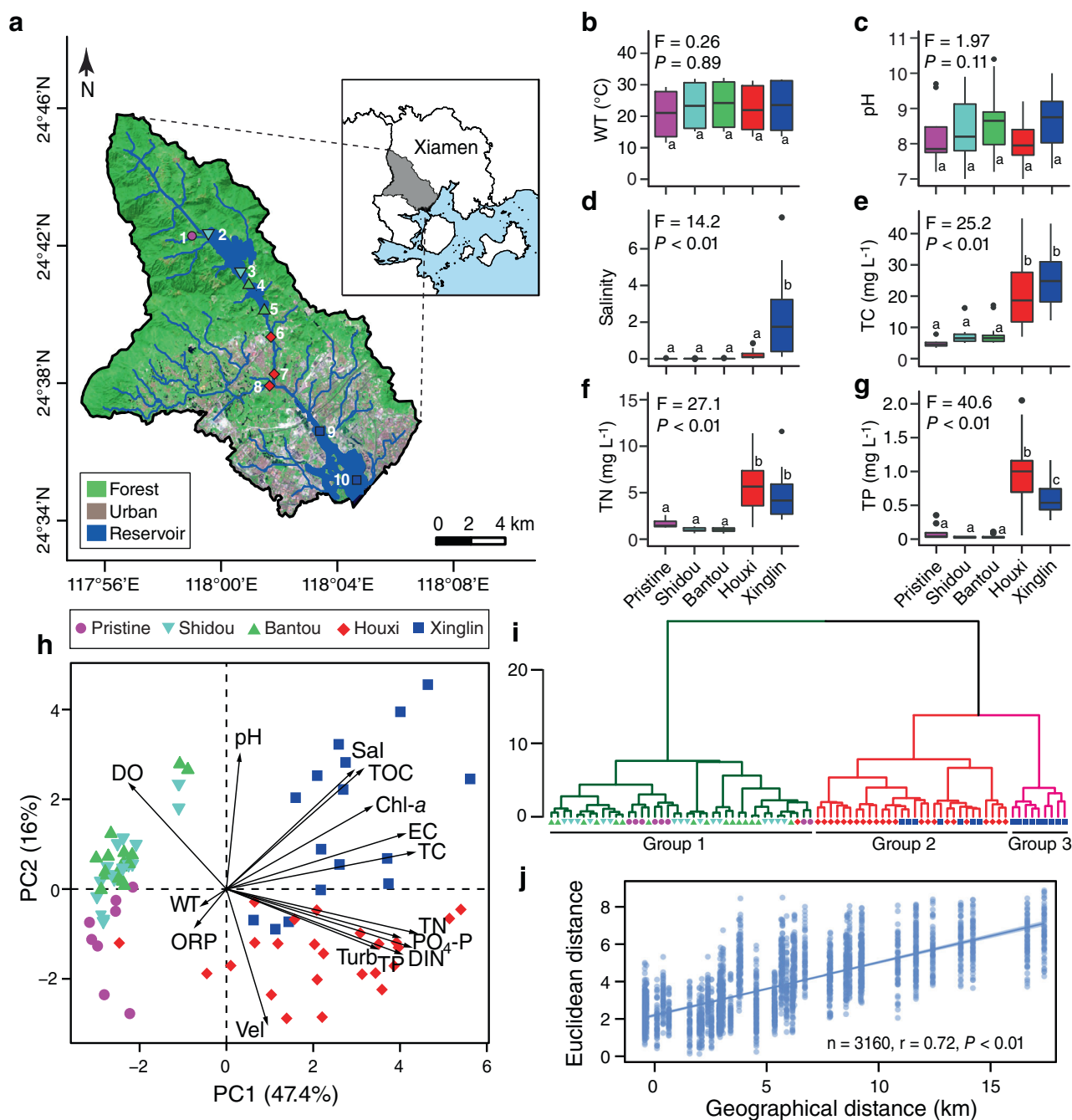
Fig. 1. Study area and longitudinal changes of environmental variables along Houxi River. (**a**) Map of the Houxi River watershed (Xiamen city, China) showing the 10 sampling sites grouped into five different habitats. (**b–g**) Variation of six selected environmental variables including the water temperature (WT), pH, salinity, TC, TN, and TP. Note that other environmental variables were shown in Supporting Information Fig. S3. (**h**) PCA of all determined environmental variables and (**i**) enhanced hierarchical clustering revealing three distinct clusters. From left to right, the 1st cluster (Group 1) includes samples from the upstream pristine and reservoir habitats (Pristine, Shidou, and Bantou), the 2nd cluster (Group 2) was mainly composed of samples from the Houxi sites and a few samples from Xinglin, and the 3rd cluster (Group 3) consisted of only nine samples from the downstream urban area (Xinglin). (**j**) The correlation between a matrix of geographical distance and a matrix of the Euclidean distance of the environmental variables between any two sampling sites.

Materials and methods

Study area and sampling

Houxi River is the 2nd largest river in Xiamen city, Fujian province, southeast China (Yang et al. 2022). The total length

of the river is approximately 25 km and it drains a watershed of around 205 km^2 of which 29% were urbanized in 2013 (Yu et al. 2014). This ecosystem has a subtropical monsoon climate with an annual mean temperature of 20.7°C and a mean

annual precipitation of 1335.8 mm (Peng et al. 2020). The samples were collected from the surface layer (< 0.5 m) of the river water. Sampling was conducted twice a year over the period of 5 yr (2012–2016) at 10 sites selected along the river continuum (Fig. 1a), making a total of 100 samples. The timing of sampling was established based on the annual rainfall pattern, under low (January) and high (July) water flow periods, respectively.

The 10 sites form a northwest to southeast transect, starting at a small stream in the hills and finishing where the river flows through parts of urban northwest Xiamen (Supporting Information Fig. S1). In this study, the Houxi River was divided into five longitudinal habitats according to the hydrology and landscape characteristics. Site 1 (Pristine) was located in the upstream headwater flowing through forested hills and discharging into Shidou Reservoir where two sites (sites 2 and 3) were selected in the riverine and lacustrine zones of the reservoir. The sites 4 and 5 were located in Bantou Reservoir, immediately downstream of Shidou Reservoir. Both reservoirs are located in a forested landscape. The sites 6, 7, and 8 were located in river section passing through Houxi town and both sites 7 and 8 received wastewater discharges via connected pipes with small-scale agricultural activities along the river. Site 8 was located in an adjacent tributary to the main river. The remaining two sites (9 and 10) were located in an urban reservoir of the urban area of Jimei New Town, Xiamen with an influence of seawater intrusion.

Environmental data collection

Water temperature (WT), turbidity, pH, salinity, electrical conductivity (EC), chlorophyll *a* (Chl *a*), and oxidation reduction potential (ORP) were collected in situ by using a Hydrolab DS5 water quality analyzer (Hach Company, Loveland, CO, USA). Current velocity was measured using SonTek Flow Tracker (Handheld-ADV® YSI, San Diego, CA, USA). The concentrations of total nitrogen (TN), total carbon (TC), and total organic carbon (TOC) were determined using TOC/TN-VCPH analyzer (Shimadzu, Kyoto, Japan). Total phosphorus (TP) concentration was measured using a spectrophotometric method after digestion; while ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), and phosphate phosphorus (PO_4-P) were determined following our previous procedure (Yang et al. 2012; 2017; Isabwe et al. 2018). The concentrations of NH_4-N, NO_3-N, and NO_2-N were summed to obtain dissolved inorganic nitrogen (DIN).

Phytoplankton species enumeration

A total of 2.5 L of surface water was collected at each sampling site and preserved in Lugol's iodine solution immediately after sampling. The samples were concentrated from an original volume of 2.5 L to a final volume of 30 mL after sedimentation for 48 h (Lv et al. 2014). A 100-μL aliquot was smeared on a counting chamber for species identification and enumeration using an inverted microscope (Motic AE31, Xiamen, China)

under 400× magnification. At least 500 phytoplankton individuals in each sample were identified using identification keys of Hu and Wei (2006). The number of cells per liter for each species was estimated and used for further analyses. Following common practice in freshwater biology, cyanobacteria, as photosynthetic microbes, were included within the phytoplankton community (Yang et al. 2012, 2017).

DNA extraction and quantitative PCR (qPCR)

Immediately after sampling, 150–500 mL of water samples were prefiltered through a 200-μm pore-size sieve to remove large particles and then filtered through 0.22-μm pore-size polycarbonate filters (Liu et al. 2015). The filters were stored at −80°C until DNA extraction. Filters were cut into small pieces using flame-disinfected scissors in a sterilized biological safety cabinet (Airtech) and packed in Lysing Matrix tubes for further steps of DNA extraction with the FastDNA® Spin Kit and the FastPrep Instrument (MP Biomedicals) following the manufacturer's instructions. The quality of the extracted DNA was checked by using NanoDrop 2000 (Thermo Scientific), assuming that good DNA extracts should have OD260/280 purity of 1.8–2.0 and a concentration > 20 ng μL^{-1}. In addition, 16S rRNA gene was quantified through qPCR as summarized in the Supporting Information Text S1.

Illumina sequencing and bioinformatics

A 20-μL DNA sample was amplified using bacterial primer pair 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) (Hugerth et al. 2014) and subjected to sequencing of the V3–V4 region of the 16S rRNA gene. The libraries were prepared as described in the Supporting Information Text S2 and sequenced on Illumina HiSeq platform (Illumina, Inc.) using paired-end method. Paired-end reads were merged with FLASH (Magoč and Salzberg 2011), and quality control (barcode and primer sequence removal) was carried out in QIIME 1.9 (Caporaso et al. 2010).

The bioinformatics pipeline was carried out using VSEARCH (Rognes et al. 2016). Briefly, merged sequences were filtered to find a representative sequence of one or more sequences in the data. Then the unoise3 algorithm with its default *minsize*8 was used to denoise sequences, remove chimeras from a set of unique sequences, and generate biologically correct sequences (i.e., zero-radius operational taxonomic units [zOTUs]). These sequences were used for operational taxonomic unit (OTU) counts across all samples using the *usearch_global* command. Taxonomy assignment was done by using the *sintax* algorithm on query sequences mapped against the Greengenes v13_8 database (DeSantis et al. 2006). Furthermore, a Newick-formatted phylogenetic tree was generated from the zOTUs *fasta* file using *cluster_agg* command. The resulting OTU table was subjected for the deletion of singletons, archaea, and chloroplasts, and was rarefed to the lowest sequences per sample, that is, 16,854 sequences per sample.

General statistical analyses

Statistical analyses were carried out in R version 4.0.3 (R Core Team 2019). Sampling sites were classified into the predefined five habitat types as this has been previously shown to be a useful approach for addressing microbial distribution (Martiny et al. 2006; Aguirre de Cárcer 2019; Peng et al. 2020). This classification was based on a twofold factor: landscape pattern and hydrology, iterating major consequences of urbanization on riverine ecosystems (Supporting Information Fig. S1). Prior to hypothesis testing, we sought to establish whether environmental variables across the five predefined habitat patches were statistically different to validate the appropriateness of the predicted heterogeneity along the river. This analysis was performed using a one-way ANOVA followed by Tukey's HSD means comparisons with samples grouped by different predefined habitats. Furthermore, the principal component analysis (PCA) and enhanced hierarchical clustering of the $\log(x + 1)$-transformed environmental variables (except the pH) were used to detect the overall sample clustering. The Euclidean distance of the environmental variables was regressed against a matrix of spatial distance between the sampling sites. These analyses were carried out and visualized by using the R packages *factoextra* (Kassambara and Mundt 2017), *ggplot2* (Wickham 2016), *ggpubr* (Kassambara 2018), and *ecodist* (Goslee et al. 2020). Plankton diversity indices were computed using the *vegan* package (Oksanen et al. 2013) and were used for detecting compositional patterns. To test whether community Bray–Curtis similarities were significantly similar or different across habitat types and time (i.e., season or year), the analysis of similarities (ANOSIM) was used with 999 permutations, and the data were visualized in two-dimensional nonmetric multi-dimensional scaling (NMDS) plots. Distance–decay relationships were supplemented by Mantel tests, using the Spearman correlation coefficient, to check whether the geographical distance between sampling sites could correlate with community similarity as a means to evaluate distribution patterns. Both the community alpha diversity (richness) and beta diversity (estimated using the 1st NMDS axis) were used as response variables in generalized additive model (GAM) to evaluate the influence of environmental factors.

VPA, NCM, and QPE

Three models that fully or partially depict the relative importance of the assembly processes were used sequentially. First, the relative importance of local environmental effects and spatial effects on community variation was assessed by using the VPA based on redundancy analysis (RDA). Spatial effects were represented by principal coordinates of neighbor matrices (PCNMs) generated by using geographical coordinates and the *pcnm* function of the *vegan* package (Liu et al. 2013). Local environmental effects were the measured environmental variables. Prior to their use as predictors, these environmental variables were log-transformed, with the

exception of the pH, to improve data homoscedasticity and normality. A forward selection procedure was run on both groups of variables in order to reduce multicollinearity among all predictors. Second, the fit to the NCM by which random immigrations, births, and deaths are assumed to determine the relative abundance of taxa in a community was used (Sloan et al. 2006). This model predicts a relationship between occurrence frequency of individuals (OTUs/species) in a set of local communities and their relative abundance across the wider metacommunity, with N describing the metacommunity size and m indicating the immigration rate (Burns et al. 2016; Chen et al. 2019). Third, a QPE (*sensus* Vass et al. 2020) was used to quantify the process of each pairwise turnover for randomized bacterial OTUs data. In this approach, it is assumed that the phylogenetic signal occurs when more closely related species are more ecologically similar and quantitatively estimates the percentage of compositional turnover linked to deterministic (selection) and stochastic processes (Stegen et al. 2013; Zhou and Ning 2017). The percentage of pairwise comparisons whose beta-nearest taxon indices (*ßNTIs*) were > 2 and < -2 were denoted as variable selection and homogeneous selection, respectively. The remaining pairwise comparisons were denoted as homogenizing dispersal and dispersal limitation if their modified Raup–Crick for Bray–Curtis (RC_{Bray}) coefficients were < -0.95 or > 0.95, respectively. Pairwise comparisons with RC_{Bray} metric between -0.95 and 0.95 were classified in the "undominated fraction" (Supporting Information Fig. S2). This exercise was completed on samples grouped by year of sampling (190 pairwise comparisons from 20 samples collected each year) and when all samples were considered as a single metacommunity (4950 pairwise comparisons from 100 samples). The three models including VPA, NCM, and the QPE were run by using the R scripts developed, respectively, by Bahram et al. (2016), Burns et al. (2016), and Stegen et al. (2013).

Results

Longitudinal environmental heterogeneity

There were no statistically significant differences ($p > 0.05$) in physicochemical variables, namely, the WT, pH, and ORP across the five predefined habitats. However, nutrient-related variables such as the TC, TN, TP, DIN, and PO_4-P differed significantly across the five predefined habitats (Fig. 1b–g; Supporting Information Fig. S3a–h). Spatial differences in nutrient-related variables were substantial between the suburban sites of Houxi, urban sites of Xinglin and the rest of the sites in the upstream habitats. These less-disturbed sites comprising a fast flowing rocky stream (pristine site) and two adjacent reservoirs of Shidou and Bantou had no significant differences in all environmental variables between them ($p > 0.05$) with the exception of water velocity. When all environmental variables were pooled together in a PCA, they highly replicated the five predefined habitats (Fig. 1h). By

using enhanced hierarchical clustering, similar sample grouping was observed with an exception of samples from the pristine site, which were clustered together with those from the two adjacent reservoirs of Shidou and Bantou (Fig. 1i). Furthermore, by regressing the Euclidean distance of all sampled environmental variables, we found that site-to-site difference among environmental variables increased with increasing distance from the upstream towards the downstream sites (Fig. 1j). Spatial differences (between different habitats) in environmental variables were robustly maintained across the 5 yr of sampling within which significant seasonal differences were only obvious for WT (Supporting Information Fig. S4).

## Patterns of plankton community distribution across different habitats

In total, 10,330 bacterial OTUs and 305 phytoplankton species were identified in this study, and the rarefaction curves indicated that the majority of bacterioplankton and phytoplankton taxa had been recovered (Supporting Information Fig. S5). The highest number of bacterial OTUs was observed in the upstream pristine site, whereas the lowest was observed in reservoir habitats of Shidou and Bantou. In contrast, the highest richness in phytoplankton community was found in the Shidou and Bantou reservoirs and the lowest in Xinglinwan Reservoir habitat (Fig. 2). About 37.1% bacterioplankton (3834 OTUs) and only 7.9% phytoplankton (24 species) taxa were shared by all habitats (Supporting

Information Fig. S6). Generally, alpha diversity differed across the five predefined habitat patches (Supporting Information Fig. S7) and the taxonomic composition of both bacterioplankton and phytoplankton communities varied spatially (Supporting Information Fig. S8; Text S3).

Both bacterioplankton and phytoplankton communities exhibited significant distance–decay relationships despite a relatively short distance between the sampling sites. More importantly, the ordination space of the community similarity returned sample clusters based on habitats types rather than seasonality or years of sampling (Fig. 3; Supporting Information Fig. S9). The global R of the ANOSIM was higher than 0.5 for both bacterioplankton ($R = 0.525$, $p < 0.01$; Fig. 3a) and phytoplankton ($R = 0.542$, $p < 0.01$; Fig. 3c) across the five habitats. There was a low but significant clustering level when communities were grouped by the years of sampling ($R = 0.227$, $p = 0.01$ for bacterioplankton and $R = 0.106$, $p = 0.02$ for phytoplankton, Supporting Information Fig. S9). The significant correlation coefficient between bacterioplankton community similarity and geographical distance ($r = -0.17$, $p < 0.01$; Fig. 3b) confirmed the upstream–downstream bacterioplankton community variation. The phytoplankton community followed a similar trend but with a slightly lower correlation coefficient ($r = -0.13$, $p < 0.01$; Fig. 3d). The results of GAM indicated that water velocity was a key environmental variable shaping the diversity and structure of both bacterioplankton and phytoplankton
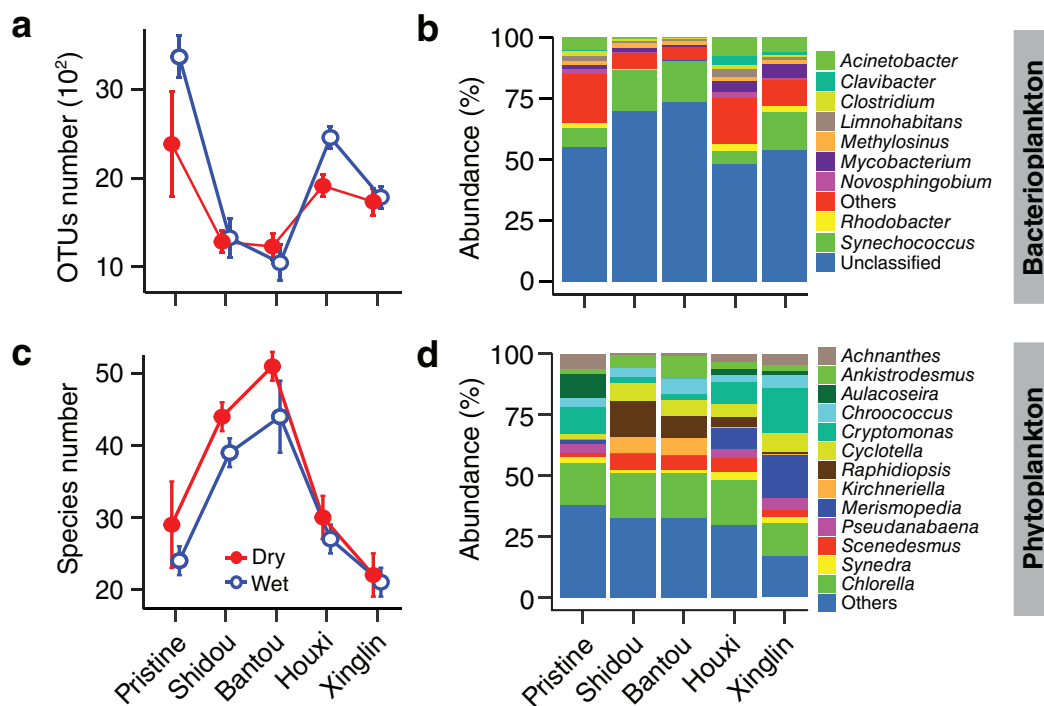
Fig. 2. Plankton community changes across the five distinct habitats along the Houxi River over 5 yr. The number of bacterial OTUs and the mean relative abundance at the genus level (**a,b**). The number of phytoplankton species and their corresponding mean relative abundance at the genus level (**c,d**).
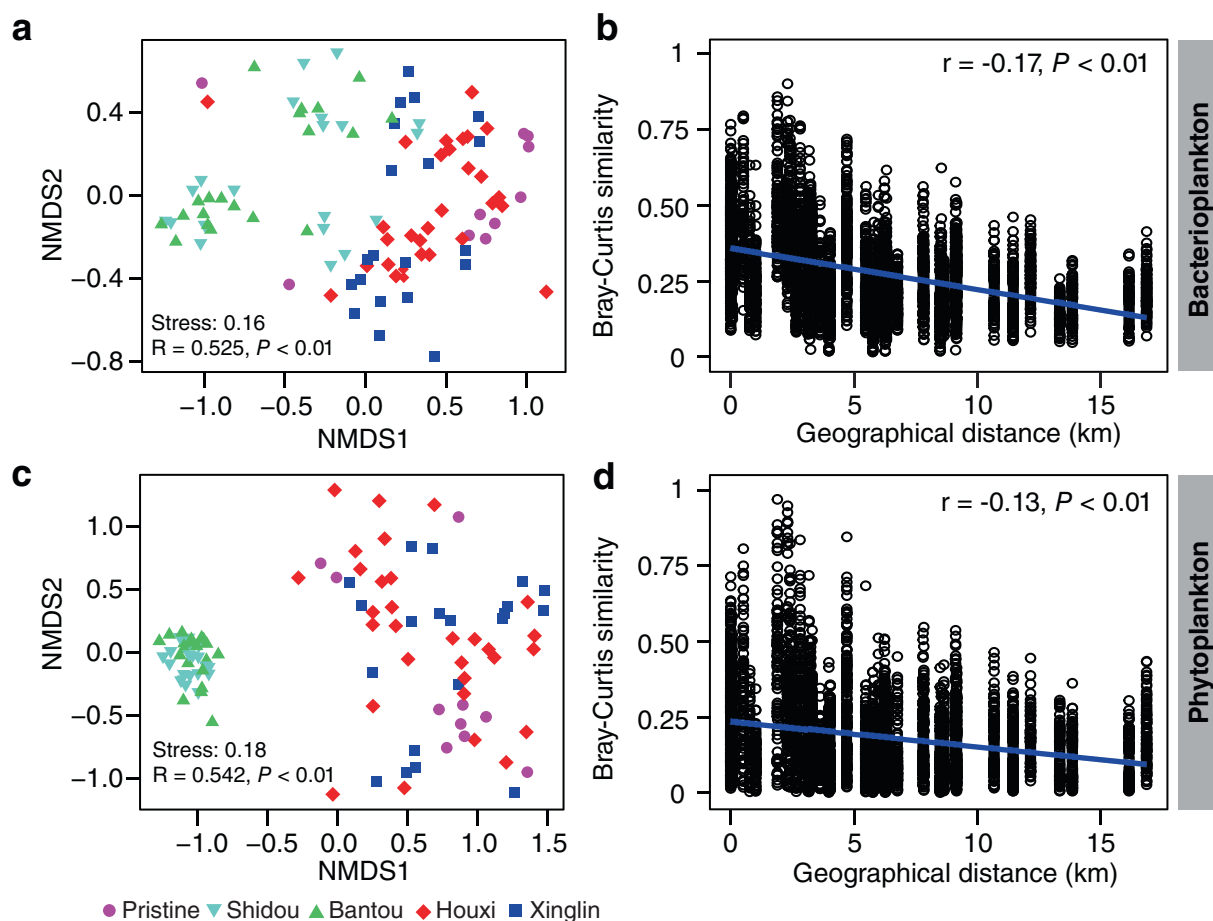
Fig. 3. Patterns of bacterioplankton and phytoplankton community similarity along the Houxi River, southeast China. Non-metric multidimensional scaling (NMDS) graphs visualizing (**a**) bacterioplankton and (**c**) phytoplankton communities based on Bray–Curtis similarity. The global R (> 0.5) and its significant level ($p < 0.01$) indicate the overall degree of sample separation across the five habitats along the river continuum over 5 yr. The distance–decay relationships of (**b**) bacterioplankton and (**d**) phytoplankton communities indicating the variation of community similarity along the geographical distance.

communities (Supporting Information Table S1). In addition, the importance of NO_2-N and TOC was significant for phytoplankton community structure.

Ecological processes underlying plankton community patterns

The VPA results showed that both local environmental and spatial effects explained a small percentage of the variance (3.2% and 3.8%), leaving more than 90% variance unexplained for the whole bacterioplankton community from 2013 to 2016 (Table 1). The variance explained by local environmental effects (40.5%) was greater than that explained by spatial effects (14.7%) for the overall phytoplankton community (Table 1). These results suggested the importance of neutral dynamics and environmental selection structuring bacterioplankton and phytoplankton communities, respectively. By evaluating the fit to NCM, we found a higher fit for bacterioplankton ($R^2 = 0.62$; Fig. 4a) than for phytoplankton

($R^2 = 0.25$; Fig. 4b) communities. The parameter m (describing the migration rate) was 0.195 for bacterioplankton and less than 0.00001 for phytoplankton. Furthermore, by partitioning the phylogenetic distance among bacterial OTUs using the QPE, we found that most site-to-site βNTI ranged between -2 and $+2$, echoing that phylogenetically conserved selection had only a small effect in structuring bacterioplankton community (Fig. 5a). This model also indicated that assembly processes were primarily from the dispersal limitation (41.5%) followed by variable selection (30.2%) for all bacterioplankton communities from 2012 to 2016 (Fig. 5b).

Successional changes of the plankton community assembly processes

In 2012, we observed a strong distance–decay of bacterioplankton community similarity without controlling for the environmental change ($|r| = 0.45$, $p < 0.01$; Supporting Information Fig. S10). Similar findings were observed in 2013, but

Table 1. VPA based on RDA estimating the local environment and spatial effects on bacterioplankton and phytoplankton communities, respectively.

|	Variance explained (%)						Forward selected variables	
	S	E	S|E	S∩E	E|S	U	Spatial variables (S)	Environmental variables (E)
Bacterioplankton								
2013	19.8	40.0	12.0	7.8	32.2	48.0	PCNM2, PCNM1, PCNM3	WT, Chl *a*, TP, EC, NO$_3$-N
2014	32.1	36.2	17.5	14.6	21.6	46.3	PCNM1, PCNM5, PCNM4, PCNM2	WT, Vel, TN
2015	—	20.8	0.4	—	31.0	79.6	ALL PCNMs	WT, pH
2016	—	27.8	−0.04	5.7	33.5	76.2	ALL PCNMs	WT, Turb, NO$_2$-N, TC
All	3.2	3.8	0.9	2.4	1.4	95.4	PCNM2	DO, pH, Vel, TC, NH$_4$-N
Phytoplankton								
2013	24.6	27.5	6.5	18.1	9.5	66.0	PCNM1, PCNM2	Turb, NO$_3$-N
2014	21.2	24.5	4.8	16.4	8.1	70.7	PCNM1, PCNM5, PCNM4, PCNM2	WT, Chl *a*, TN
2015	17.7	73.2	0.8	16.9	56.2	26.1	PCNM1	pH, NO$_2$-N, TC, EC, Vel
2016	26.7	43.5	4.2	22.5	20.9	52.3	PCNM1, PCNM2, PCNM5	TN, Vel, TC, NH$_4$-N, TP, WT
All	14.7	40.5	3.4	11.3	29.2	56.1	PCNM1, PCNM5, PCNM4, PCNM2	EC, pH, ORP, Vel, TOC, NO$_3$-N, NO$_2$-N

Vel: velocity, Turb: turbidity.
Two explanatory matrices were used: the spatial matrix was formed of PCNMs as a proxy for spatial effects (*S*) and the matrix of the environmental variables represented the local environmental effects (*E*). For simplicity, the explanations which were < 1% were not shown. *S*|*E*, variance explained uniquely by spatial effects. *E*|*S*, the variance explained uniquely by local environmental effects. *S*∩*E* is an intersection of the variance explained by spatial and local environmental effects. R stands for the residual variance. "All" indicates all data for the years 2013–2016. Note that the data of the year 2012 were not shown because some environmental variables were not available.

the correlation coefficient reduced upon controlling for the environment (|*r*| = 0.19, *p* < 0.01; Supporting Information Fig. S10). In 2013, the importance of local environmental effects (40.0%) was greater than that of spatial effects (19.8%) in structuring the bacterioplankton community (Table 1). The R^2 of the NCM fit for bacterioplankton was 0.58–0.67 for the first two years (2012 and 2013) of sampling (Fig. 4c). Interestingly, homogeneous selection was the main assembly process structuring bacterioplankton community in 2012, and it was replaced by dispersal limitation in 2013 (Fig. 5b).

For the phytoplankton community, a steeper trend of community similarity along the geographical distance was observed in 2013 (|*r*| = 0.44, *p* < 0.01) compared with that of the previous year (|*r*| = 0.27, *p* < 0.01; Supporting Information Fig. S10). After controlling for the environmental factors, the correlation coefficient between phytoplankton community similarity and geographical distance reduced but remained significant (|*r*| = 0.29, *p* < 0.01) in 2013. Meanwhile, the fit to the NCM was slightly greater in 2012 (R^2 = 0.22) than in 2013 (R^2 = 0.16; Fig. 4c), and the variance explained by local environmental effects (27.5%) was slightly higher than that explained by spatial effects (24.6%) in the year 2013 (Table 1).

Compared with other timescales, 2014 had the smallest |*r*| value of the distance–decay relationships for bacterioplankton
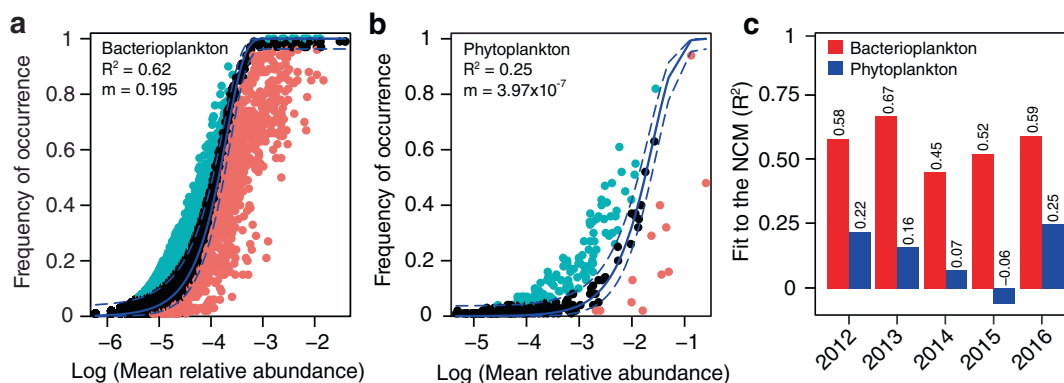
Fig. 4. Fit to the NCM for both bacterioplankton and phytoplankton. All bacterioplankton (**a**) and all phytoplankton (**b**) in a 5-yr period from 2012 to 2016. The coefficients of fit to the neutral model (R^2) and the metacommunity size (m) are shown. The points indicating fits at 95% confidence intervals are colored in black, while the points above and below predictions are colored in cyan and orange, respectively. (**c**) The fit to the neutral model for bacterioplankton and phytoplankton communities in each year of sampling.

a

b
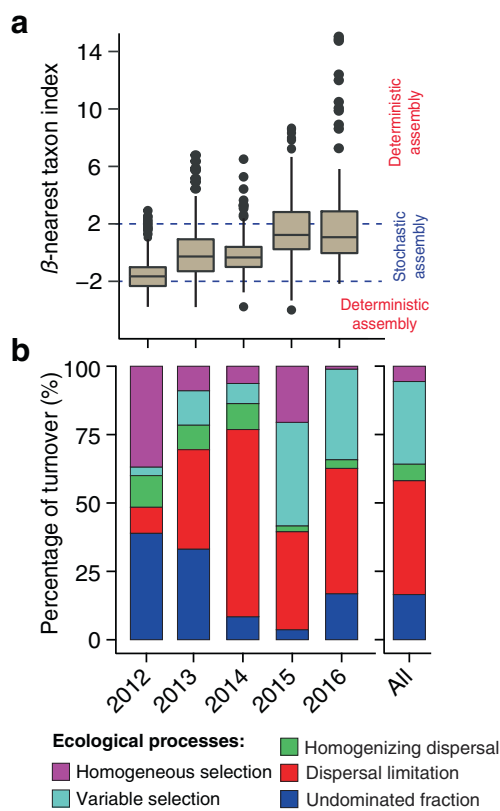
Fig. 5. Community assembly processes inferred by the QPE for bacterioplankton community. (**a**) Boxplots illustrating variation in beta-nearest taxonomic index (ßNTI) from the years 2012–2016. The fraction of pairwise comparisons with significant βNTI values (|βNTI| > 2) is the estimated influence of selection or deterministic assembly. (**b**) The percentage of bacterioplankton compositional turnover governed by various ecological processes. The value of Bray–Curtis-based Raup–Crick (RC$_{Bray}$) characterizes the magnitude of deviation between BC$_{obs}$ and BC$_{null}$; and a value of |RC$_{Bray}$| > 0.95 is considered significant. Each of the number of pairwise comparisons with RC$_{Bray}$ > 0.95, the number with RC$_{Bray}$ < −0.95 and the number with |RC$_{Bray}$| < 0.95 were divided by the total number of all pairwise comparisons and the resulting fractions estimated the influence of the dispersal limitation, homogenizing dispersal, and the undominated fraction (ecological drift), respectively.

community change along the distance before and after controlling for the environmental factors (|r| = 0.39 and |r| = 0.13, p < 0.01; Supporting Information Fig. S10) and the lowest fit to the NCM ($R^2 = 0.45$; Fig. 4c). The QPE inferred a high dominance of the dispersal limitation (> 60%; Fig. 5b) and VPA indicated that the relative contribution of local environmental and spatial effects was almost equal for bacterioplankton in 2014 (Table 1). The predominant effect of deterministic processes for both bacterioplankton and phytoplankton was consistently revealed by the three models in the years 2015–2016. During this period, the |r| values for distance–decay relationships were higher than those observed in the previous years (Supporting Information Fig. S10). In 2015, the percentage variance explained by local environmental effects

(20.8% for bacterioplankton and 73.2% for phytoplankton) was larger than that explained by spatial effects. In 2016, the variance explained by local environmental effects was 27.8% and 43.5% for the bacterioplankton and phytoplankton, respectively (Table 1). Whereas ecological processes related to variable selection, dispersal limitation and homogeneous selection prevailed in 2015 for the bacterioplankton community, variable selection coupled with dispersal limitation dominated in the year 2016 (Fig. 5b).

Relationships among different models

Linear regressions between the data obtained from the distance–decay relationships (Mantel correlation coefficients) and the three models (Fig. 6) indicated that there was a positive relationship between the fit to NCM and the absolute values of the Mantel correlation coefficients for bacterioplankton ($R^2 = 0.54$), but this relationship was much weaker for phytoplankton ($R^2 = 0.07$). After controlling for the environmental variables, however, the relationship between the Mantel test coefficients and the fit to the NCM reduced significantly for bacterioplankton ($R^2 = 0.19$), but increased for phytoplankton ($R^2 = 0.20$; Fig. 6). The fit to the NCM was negatively correlated with the percentage variance explained by both local environment ($R^2 = 0.16$) and spatial effects ($R^2 = 0.11$) for phytoplankton and bacterioplankton, respectively, and it was positively related to the residual variance ($R^2 = 0.15$) for phytoplankton. In addition, the percentage contribution of dispersal limitation, inferred within the QPE, was negatively correlated with the fit to the NCM for bacterioplankton community ($R^2 = 0.35$), and the percentage contribution of the undominated fraction was positively correlated with the fit to the NCM ($R^2 = 0.43$). The fit to the NCM did not show strong correlation with the percentage contribution of homogeneous selection, variable selection and homogenizing dispersal (Fig. 6).

Discussion

Evaluating the distribution patterns and the relative contribution of deterministic and stochastic processes in structuring plankton communities over spatial and temporal scales is crucial for understanding how these communities respond to local, regional, and global changes (Hanson et al. 2012). Our system can be thought of as a natural experiment, where the treatments are the different levels of urbanization along the course of the river. The results indicated that plankton community similarity decreased with increasing geographical distance from the upstream pristine to downstream urban sites of Houxi River, southeast China. Ordination and clustering methods revealed distinct plankton community changes that roughly corresponded to the urbanization-induced environmental heterogeneity along the river continuum (Fig. 2), and complementary models evaluating processes underlying the observed patterns showed that, although it was small, the
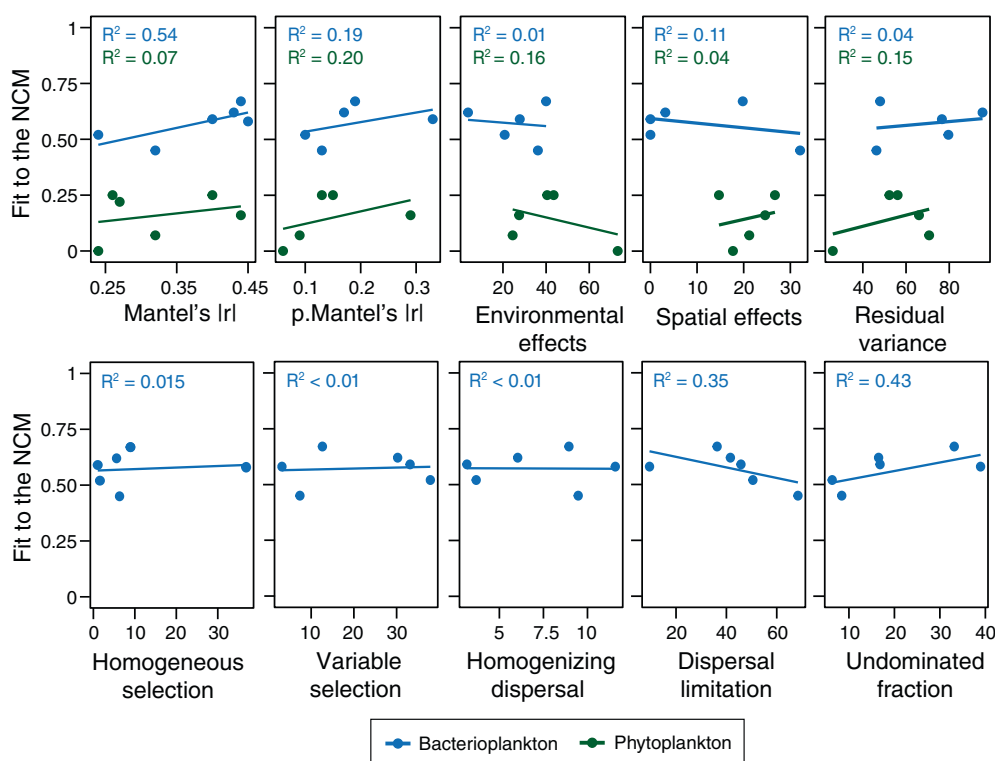
Fig. 6. Relationships among the results of different models depicting the relative importance of the assembly processes. The top-left panels illustrate the linear regression model showing the relationship between the fit to the NCM and the absolute values of the Mantel's |r|, and partial Mantel's |r| for the distance–decay relationships of both bacterioplankton and phytoplankton. The top-right panels show linear regressions between the fit to the NCM and the variance explained by local environmental effects, spatial effects and the residual variance estimated through the VPA. The bottom panels indicate the relationships between the fit to the NCM and the percentage of pairwise comparisons resulting from homogenous selection, variable selection, homogenizing dispersal, dispersal limitation and the "undominated" fraction as revealed by the QPE.

variance explained by local environmental variables was greater than that explained by spatial variables for the whole plankton community and for communities in each year of sampling (Table 1). The NCM results indicated that stochastic processes were more important in structuring bacterioplankton than phytoplankton communities (Fig. 4). This result was further confirmed by the QPE for bacterioplankton (Fig. 5), a model that showed an overall dominance of dispersal limitation, which increased with a decreasing fit to the NCM ($R^2 = 0.35$) over the 5 yr of the study (Fig. 6).

Urbanization amplified environmental heterogeneity along the river continuum

Nutrients were significantly higher in the downstream urban sites than the upstream pristine site indicating human signatures through increasing abiotic inputs downstream (Yang et al. 2022). Previously, we showed that a similar prevalence of different processes generating the core and random subset of plankton communities was a result of concordant responses to environmental change in the Houxi River (Isabwe et al. 2019). In addition, Yu et al. (2014) revealed urbanization effects on metal loadings and Peng et al. (2020) demonstrated increasing richness and abundance of antibiotic

resistant genes from rural to urban sections of Houxi River watershed. The upstream presence of drinking water reservoirs (Shidou and Bantou) for downstream urban population also highlighted human-induced hydrological change (Yang et al. 2017). Taken alone, the results of this study confirm that human activities including construction of drinking water reservoirs and nutrient enrichment downstream could amplify upstream–downstream environmental heterogeneity in urbanizing watersheds (Bai et al. 2017; Pickett et al. 2017).

Plankton community varied with longitudinal environmental heterogeneity

The predicted (based on landscape and hydrological changes) and observed environmental heterogeneity corresponded to a decrease in plankton community similarity with increasing geographical distance from the upstream pristine towards the downstream urban areas. These observations partially corroborated a previous conclusion that local environmental drivers from discharge events, associated with urbanization, shaped bacterial community structure in an urban stream (Tinkers Creek, Cuyahoga River watershed, Ohio, USA; Roberto et al. 2018). Some previous studies have also demonstrated that hydrological change could greatly

influence the distribution patterns of plankton assemblages (Yang et al. 2017; Graco-Roza et al. 2020). In another subtropical river (Jiulong River, Fujian, China), we found that a stronger influence of local environmental than spatial effects was intensified during the dry season under low water-flow conditions (Isabwe et al. 2018). Moreover, both bacterioplankton and phytoplankton communities differed more across habitat types in the watershed than by seasonality. From this, we inferred that the well-known seasonal patterns of plankton in freshwater ecosystems (Sommer et al. 2012; Diao et al. 2017; Nyirabuhoro et al. 2020) were overwhelmed by site-to-site longitudinal disparity as previously shown by Ren et al. (2018) for the amoeba community. Concomitantly, Griffin et al. (2017) showed that microbial dispersal was successful within but not between different habitats in an agriculture-dominated watershed. However, this result runs counter to Hanashiro et al. (2019) who found that bacterioplankton communities inhabiting shallow urban ponds in Belgium were less affected by environmental conditions resulting from urbanization although six local environmental variables significantly explained variation in bacterioplankton community composition. These contrasting findings warrant further studies, because not only environmental heterogeneity and connectivity but also microbial adaptation, their dispersal rates and metabolic versatility could influence the relative importance of different assembly processes (Langenheder and Lindström 2019).

The interplay between deterministic and stochastic processes shaped plankton distribution patterns

Observational studies investigating questions of community assembly can be hard to fully interpret. Whereas experimental studies are typically more informative about mechanisms, they are difficult to carry out at anything but very small scales (Goldford et al. 2018). Indeed, variation in biotic and abiotic characteristics is often greater and more complex in natural than in most experimental systems. In this study, pronounced urbanization-induced environmental variation can be considered a "natural experiment"—if deterministic processes or differences in niche preferences dominate, repeatable and predictable species turnover should be observed. In contrast, more or less repeatable high species turnover should prevail if stochastic processes were mainly at play. The interplay between deterministic and stochastic processes observed in this study indicated that directional dispersal via water flow could not override local selective pressures and biotic interactions, thereby confirming that ecological selection increases compositional differences whereas high dispersal minimizes this effect (Hanson et al. 2012). However, directional dispersal was constrained by lentic habitats in the two drinking water reservoirs in the upstream of the Houxi River. Physical properties such as the size and volume of the reservoir may determine whether a unique microbial community emerges at the lentic sites due to prolonged or short hydraulic retention times (Niño-García et al. 2016). In

turn, the emerging taxa may exert significant impact on the downstream microbial communities upon the reservoir opening as previously observed in the Ebro River and the Yangtze River (Ruiz-González et al. 2013; Gao et al. 2021). Although the hydraulic retention times of these reservoirs were relatively long throughout the year (Yang et al. 2017), we found that the reservoir lentic habitats were characterized by an increase in phytoplankton diversity and a decrease in bacterial diversity compared with the lotic habitats suggesting that habitats with strongest selection shift depending on the hydrological conditions (Stadler and del Giorgio 2021). Recently, Chen et al. (2021) found that longitudinal patterns of microbial community in the Lancang River with cascade dams were not significantly different with those in the parallel Nujiang River without any dam. This result highlights the potential for downstream community coalescence (Mansour et al. 2018) which should be further explored prior to respond to a major question of whether building a series of small reservoirs instead of a single large one is ecologically and environmentally preferable (Maavara et al. 2020). Interestingly, our GAM results indicated that water velocity was a key driver of the differences in plankton alpha and beta diversities along the Houxi River (Supporting Information Table S1). Several studies have shown that the dynamics of plankton communities were related to flow velocity and water discharge (Wu et al. 2018*a*, *b*; Qu et al. 2019; Chen et al. 2021; Stadler and del Giorgio 2021). Flow velocity can potentially influence riverine plankton assemblages by altering nutrient inputs and enhancement of dilution effect (Yang et al. 2017). Other factors that control the growth of phytoplankton include light availability. Light penetration limited phytoplankton in downstream waters as transparency therein was much lower than in the upstream reservoirs (Yang et al. 2022). The multi-dimensionality of plankton responses to environmental drivers is well known, and even an environmental driver with small relative influence might impose its strong influence via its indirect effects on other drivers (Kakouei et al. 2022).

Phytoplankton were predominantly shaped by deterministic processes while bacterioplankton were mainly governed by stochastic processes

Responding to a call for the assessment of the relative importance of assembly processes for different types of microorganisms (Martiny et al. 2006), Soininen et al. (2007) found that spatial effects were more important than local environmental factors in shaping zooplankton than phytoplankton distribution in boreal wetland ponds. In this study, phytoplankton community was determined by deterministic rather than stochastic processes. These results align with those of Liu et al. (2015) who reported that phytoplankton communities exhibited a stronger response to environmental changes than bacterioplankton in three subtropical reservoirs, but contrast sharply with Logares et al. (2018) who found a more pronounced influence of salinity-induced selection in bacterial

communities than microeukaryotes in Eastern Antarctica. Previously, Astorga et al. (2012) questioned if the distance–decay of community similarity is caused by spatially limited dispersal or by niche-related factors and concluded that it is not simply a function of organism size but other traits such as dispersal capacity. Compared with bacterioplankton, phytoplankton communities exhibit a wide array of responses to environmental heterogeneity (Liu et al. 2015). This has brought about a need to evaluate the relative role of the assembly processes between bacterioplankton and phytoplankton (or microeukaryotes). Indeed, different plankton communities have different dispersal potential and metabolic activities. While microeukaryotic communities are metabolically versatile, bacterioplankton are metabolically less flexible (Logares et al. 2018). Thus, the major role of stochastic processes in structuring the bacterioplankton community in this study might be related to the size-related trait plasticity, a hypothesis which assumes that smaller organisms are less environment-filtered than larger organisms (Farjalla et al. 2012; Wu et al. 2018*a*,*b*). Nevertheless, this hypothesis could be further tested through experimental studies, because observational studies might be limited by other factors such as the scale-dependency and sampling resolution.

The QPE model inferred dispersal limitation for bacterioplankton at a small spatial scale

Empirical studies addressing the scale dependency of ecological processes have shown that large spatial scale encompasses greater diversities of habitat types, allowing for a greater diversity of organisms to coexist through ecological selection (Martiny et al. 2006; Kent et al. 2007; Hanson et al. 2012). Large spatial scales can also impart synchronous behavior among microbial populations, leading to a more pronounced influence of deterministic assembly (Hanson et al. 2012). In contrast, stochastic assembly of the plankton community could dominate at small spatial scales with a large species pool, high productivity, low disturbance, and low predation (Zhou and Ning 2017). In riverine ecosystems, however, downstream dispersal is not significantly restricted (Chen et al. 2019). Patterns of compositional turnover may be different to those observed in communities inhabiting lentic and terrestrial habitats. For instance, a recent study at the large spatial scale of the Yangtze River showed that bacterial community composition clustered according to the local landforms, and the stochastic process of turbulent flow generated homogeneous habitats for bacterioplankton (Liu et al. 2018). Despite this, dispersal limitation was the dominant process of microbial community assembly in other rivers such as the Jinsha River (Chen et al. 2020*a*) and the Yangtze River (Chen et al. 2020*b*). In our study, the distance–decay of plankton community similarity along the geographical distance in the Houxi River was highest when there was a lesser effect of the local environment indicating that these plankton communities could be either locally adapted or dispersal limited (Hanson et al. 2012). In their early review, Hanson et al.

(2012) argued that microorganisms can show dispersal limitation either if their movement to a new location is restricted, or if the establishment of individuals in a new location is hindered. Since the water flow is unidirectional and plankton are unlikely to be returned via a random process, the second scenario might have taken place in the Houxi River. This is supported by the fact that a large percentage of taxa shared across the sites at all time-points were observed in the community potentially dominated by stochastic but not deterministic processes (i.e., bacterioplankton). This process is often referred to as the 'drift paradox' in studies of macroorganisms in rivers (Humphries and Ruxton 2001; Hänfling and Weetman 2006). Considering the loss of river connectivity due to downstream fragmentation and flow regulation globally, the aforementioned second scenario may be happening in other rivers draining urbanizing watersheds and deserves further considerations.

Stronger fit to the NCM corresponded with less dispersal limitation for bacterioplankton in this study and the relationship between the NCM and the relative importance of local environment and spatial effects revealed concordant insights. This indicated that even though year-to-year environmental variation and intrusion by invading species may exert spurious correlations when disentangling the effects of selection in VPA models (Clappe et al. 2018), observations through multiple complementary models could help microbial ecologists to accurately and precisely depict the main community assembly processes in future studies. We observed a positive relationship between the NCM fit and Mantel test coefficients indicating that longitudinal distribution patterns of bacterioplankton, through distance–decay could be related to random immigration, birth, and death (Sloan et al. 2006). However, the magnitude of this correlation was reduced after controlling for environmental factors, inferring that environmental effect contributes partially to a decreasing community similarity. In contrast, there was a weak relationship between NCM fit and Mantel test that increased after controlling for environmental variables for phytoplankton. Finally, the good positive relationship between the fit to the NCM and the "undominated" fraction inferred with the QPE indicated the major role of the ecological drift in shaping bacterioplankton communities (Stegen et al. 2013).

Conclusion

In this study, we integrated three models (i.e., VPA, NCM, and the QPE) to distinguish community assembly resulting from environmental heterogeneity and neutral (stochastic) dynamics for riverine plankton in an urbanizing watershed. The results showed that urbanization with the construction of drinking water reservoirs and nutrient inputs being the primary drivers of environmental heterogeneity along river, caused the establishment of distinct but longitudinally connected habitats for the riverine plankton communities. The observed plankton distribution patterns were driven by an interplay between deterministic and stochastic processes, however the

phytoplankton community appeared to be more influenced by the environmental heterogeneity than the bacterioplankton community. These results imply that understanding riverine plankton response to urbanization-induced environmental changes is essential because ecological and evolutionary processes are shaped by both the water movement and the environment. Since microbial plankton responses to ecological disturbances vary markedly across the planet, future studies comparing patterns and processes in rivers draining urbanizing watersheds and those in purely natural settings will improve our understanding of the riverine microbial community assembly in a changing world.

Data availability statement

Bacterioplankton sequence data were uploaded to NCBI under the project number PRJNA383082 and SRA number SRP104354. Phytoplankton data are available from the corresponding author upon request.

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Conflict of Interest

None declared.