Temporal variations of benthic diatom community
and its main influencing factors in a subtropical river, China

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Abstract Benthic diatoms are the main component in many aquatic ecosystems such as streams, creeks and rivers, and they function as important primary producers and chemical modulators for other organisms in the ecosystems. In this study, the composition of benthic diatoms was investigated and further explored the primary physicals and chemicals affecting their temporal variations in the upper Han River, China. There were seasonal variations in physical and chemical variables in waters over the sampling period of 2007–2010. Water temperature (*t*), chemical oxygen demand, total nitrogen, dissolved organic carbon (DOC), silica and fluoride were much higher in the high flow season (i.e., July or August) than these in the base flow season. Three species *Achnanthydium minutissimum* (composed of 10.7 % of the total diatom abundance), *Achnanthydium pyrenaicum* (11.9 %), and *Achnanthydium subatomus* (12.7 %) accounting for more than 5 % of the total diatom abundance were persistently dominant in all seasons, while the other two prostrate and motile species including *Eolimna minima* and *Nitzschia dissipata* also dominant in the base flow season. The species richness always peaked in autumn with significant difference with summer ($p<0.01$), and density of benthic diatom varied and peaked in April. Analyses indicated that the temporal variation in benthic diatom communities was strongly related to *t*, nitrogen, organic pollutants (indicated by COD and DOC), and hydrological regime. The research will expand the understanding of water chemistry monitoring, and improve watershed-

scale management and conservation efforts in the upper Han River, China.

Keywords Benthic algae · Disturbance · Temporal dynamics · Watershed management · Water quality

Introduction

Benthic algae are the main component in many aquatic ecosystems, and they are functioning as important primary producers, chemical modulators, and important habitats for other organisms in those aquatic ecosystems (Stevenson et al. 1996). Also, they are the major source of energy and carbon for aquatic consumers in aquatic ecosystems such as arid zone floodplain rivers, where any change of the algae lead to the alterations in (Bunn et al. 2003). Generally, diatoms (Bacillariophyta) are the dominant taxonomic group in freshwater benthic algae, comprising of more than 80 % of the total abundance and biovolume (Stevenson et al. 1996), therefore it is vital to study the benthic diatom in aquatic ecosystem with the exacerbation of nutrient pollution from human activities into surface waters.

Benthic diatom assemblage structure and spatial distribution respond sensitively to a range of environmental variables including chemicals such as nutrient supply, ionic strength (Blinn and Bailey 2001; Leland and Porter 2000; Leland et al. 2001; Potapova and Charles 2003); physicals involving temperature, climate change, hydrologic regime, stream substrate and sediment (Biggs 1995; Stevenson, 1997; Corns et al. 2010), and even geology, land use (Pan et al. 2000; Cooper et al. 2012). Meanwhile, algal taxa with different growth forms vary in response to those environmental physical and chemical conditions. Therefore, structural metrics of community is indispensable for estimating the change of benthic diatom responding to the environment.

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A dramatic change of ambient environmental factors in aquatic ecosystem including water quality are direct and conspicuous consequences of human activities. Seasonal change of benthic diatom affected by interacting factors such as physicals, chemicals, and biological factors in aquatic habitat can lead to the succession of algae community (Rosemond et al. 2000; Romanov and Kirillov 2012). A large number of studies have been focusing on the effects of these environmental variables on spatial distribution of benthic algal assemblages (Biggs 1995; Leland and Porter 2000; Leland et al. 2001; Potapova and Charles 2003; Carr et al. 2005). However, current studies primarily focus on seasonal variation of physical and chemical factors or periphyton in lakes, glacier rivers, and forest streams or the rivers ecosystems in temperate and tropical zones (Mosisch and Bunn 1997; Haydée et al. 2002; Davies et al. 2008), and relatively little is known regarding the community succession of benthic diatom and its likely primary driver in Asian subtropical rivers.

The upper Han River is located on the upstream of the Danjiangkou Reservoir, the water source for the Middle Route of China's South–North Water Transfer Project, diverting water to North China, alleviating water scarcity for people in highly water-threatened regions (Zhang 2009), so any degradation of water quality and ecological conditions in the river will jeopardize the benefits of the interbasin water transfer project. Previous studies have related to water quality (Li et al. 2008, 2009a, b; Zhang 2009), and investigated the spatial patterns of diatom community (Tan et al. 2013) and adequate diatom indices for water quality assessment in the upper Han River (Tan et al. 2012). However, there is little study regarding succession of benthic diatom communities which is one of the most vital primary producers.

The objectives of this study are therefore threefold: (1) to study the seasonal variation of physical and chemical variables such as temperature, nutrient and ions in the high flow and base flow seasons; (2) to identify the benthic diatom community structure and succession including dominant species, biomass and biodiversity in the upper Han River basin; and (3) to investigate the main driver for seasonal variation of benthic diatom assemblages. These will be helpful to develop water resource conservation strategy for the upper Han River, China.

Materials and Methods

Study area

The upper Han River drains a land area of 95,200 km² with a length of 925 km in a north subtropical monsoon climate zone (Fig. 1). The Danjiangkou Reservoir is located in the junction of the Dan River and Han River. Annual mean precipitation is 700–1800 mm, of which 80 % is concentrated in the time

period from May to October. According to the hydrologic regime, the high flow season is the period from May to October (spring and summer), and the base flow season is the period from November to March of the next year (autumn and winter). There are large areas of crop cultivation including maize, wheat, rice, cassava, vegetables, and fruit plantation like citrus in the basin (Li et al. 2008, 2009a, b). Surface geology is controlled by carbonates (Li and Zhang 2008).

In previous studies, we have characterized variations of water quality variables and its relation with land use and land cover in their respective sub-basins in the upper Han River (Li et al. 2008, 2009a, b, 2012). In this study, seven rivers with large variations in water quality variables, similar cobble beds and totally open riparian coverage were selected (Table 1). They have the similar physical habitat with a depth around 0.5 m at the river center and are about 15 m wide spread and all are in close proximity to small towns. To minimize the effects of light availability on benthic diatom (Hill et al. 1995), the selected sites have a canopy cover of 0–1.0 % and are saturated by light. Site 1 is on the tributary (i.e., Laoguan River) of the Dan River, and the other six sites are located in the Han River and spread on Hanzhong plain and Ankang corridor. In terms of their respective land use compositions, the seven sites represent a good gradient of agriculture intensities in their upstream areas. Urban area ranges from 0.04 to 0.71 %, agriculture percentage between 6.31 and 28.88 %, and the percentage of vegetation from 68.88 to 90.38 %. Therefore, these seven sites are representative in terms of geological setting, physical characteristics and water quality of the upper Han River basin.

Benthic algae and water samples collection

Samples including epilithic diatoms and water samples were collected from seven tributaries in the riverine network (Table 1, Fig. 1) in November 2007, July and November 2008, April, July and November 2009, and January 2010. Three rocks (<25 cm) were collected randomly from left, middle and right (if possible) in the running waters at each sampling site. The rocks were thoroughly scrubbed off in an area delimiter (i.e., a circular area with a diameter 7 cm) with a tough toothbrush, and periphyton from three rocks was rinsed with distilled water into one container as replicates. The algae samples of 100 ml were then preserved in acid-washed bottles with 40 % formaldehyde to final formaldehyde concentration in algae samples of 4 %.

Water samples of 500 ml was collected mixing with 10 % (v/v) sulfuric acid (final pH <2 in the samples) for analysis of total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD_{Mn}). Another sample of about 300 ml were collected and filtered using cellulose nitrate membrane filters (Whatman, 0.45 μm) for the measurement of DOC, soluble reactive phosphorus (SRP) and ions. Among the samples of

Fig. 1 Sampling sites in upper Han River basin, China

300 ml, one aliquot of the water sample was stored to determine DOC and SRP with 10 % (v/v) sulfuric acid added to make sure the final pH <2 in water sample. The second portion was unacidified for measuring anions and the third portion was acidified with ultra-pure nitric acid to pH <2 for cations determination. All the water samples were stored at 4 °C in acid-washed bottles as soon as possible.

The structural metrics of diatoms

An aliquot of the benthic algal suspension was acid-cleaned using sulfuric acid and nitric acid in turn and mounted in NaphraxTM. Species were identified and a minimum of 400 valves was counted per slide at 1000× magnification using a microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan). Diatom identification was carried out according to Prygiel and Coste (2000), Kobayasi (1997), Krammer and Lange-Bertalot (1986, 1991a, b), Krammer (2002), and (Zhu and Chen 2000). Density was estimated according to the number counted on slides and the volume (i.e., 40 μl) used for mounting each slide.

Physical and chemicals analysis

Water temperature (*t*), pH, total dissolved solids (TDS), turbidity (TURB), electrical conductivity (EC), dissolved oxygen (DO), ammonium-nitrogen (NH₄-N), and nitrate-nitrogen (NO₃-N) were measured in situ using YSI 6920. Major cations (Na⁺, K⁺, Ca²⁺, Mg²⁺ and Si) were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (IRIS Intrepid II XSP DUO, Thermo Fisher Scientific, Waltham, MA, USA). HCO₃⁻ was determined using titration using hydrochloric acid in situ. Other anions (i.e., F⁻, Cl⁻, NO₃⁻, and SO₄²⁻) were measured using Dionex Ion Chromatograph (IC) (Dionex Corporation, Sunnyvale, USA). TP and SRP were measured using ammonium

molybdate spectrophotometric method, TN was determined using alkaline potassium persulfate digestion–UV spectrophotometric method, and COD_{Mn} was determined using potassium permanganate index method (Chinese State Environment Protection Bureau CSEPB 2002). DOC was measured using TOC analyzer (Shimadzu Corporation, Kyoto, Japan). Canopy cover was determined using spherical densiometer (convex, D-Model).

Land use data

Interpretation of Landsat Thematic Mapper imagery was used to calculate land use composition in the respective area (Shen et al. 2006). The four main land cover categories are (1) urban, including industrial and residential areas; (2) agriculture, including paddy field, dry land and orchard; (3) vegetation, including forests (coniferous, deciduous, mixed coniferous and broad-leaved), bushes and herb; (4) bare lands, including small gravels, bare ground and rocks. The software ENVI 4.8, ArcGIS10.0 and Excel were used to achieve the composition of land use.

Data analysis

Pearson correlation analysis was used to test the relationship among the environmental variables prior to further analysis. TDS was correlated strongly to EC with correlation coefficient *R*² of 0.95, so TDS was removed from the following analysis. Shannon–Wiener diversity index (Shannon and Weaver 1949), Pielou evenness (Pielou 1966), and species richness of the diatom assemblages were calculated. Dominant species was defined as its relative abundance greater than 5 %.

One-way analysis of variance (ANOVA) was used to test the significance of the differences of variables (environmental variables, structural attributes of benthic diatom) among

Table 1 Characteristics of selected seven sites in the upper 1 Han River basin, China

Site	River	Latitude	Longitude	Altitude (m)	Depth (m)	Width (m)	Canopy coverage (%)	Land use characteristics in upstream (%)				
								Urban	Agriculture	Bare land	Vegetation	Others
1	Laoguan River	33°09'	111°27'	186	0.4	15	0.2	0.66	11.59	5.82	81.09	0.84
2	Jinshui River	33°15'	107°52'	314	0.4	15	1.2	0.31	15.92	0.45	82.57	0.75
3	Xushui River	33°09'	107°22'	480	0.8	20	0.2	0.42	8.53	0.60	89.97	0.48
4	Jushui River	33°07'	106°32'	582	1	10	0.2	0.71	9.77	0.29	88.75	0.48
5	Yue River	32°42'	108°56'	258	0.7	18	0.2	0.28	28.88	1.59	68.88	0.37
6	Shu River	32°56'	109°41'	254	0.5	10	1.2	0.04	6.31	2.23	90.38	1.04
7	Jiangjun River	32°45'	110°15'	183	0.25	15	0.2	0.11	7.24	2.96	88.94	0.75

different seasons. Data were transformed using square root prior to the further analysis if they were not normally distributed Nonparametric test (i.e., Kolmogorov–Smirnov Test) was employed to test the significance of differences if data could not be transformed to normal distribution. . The Least Significant Difference test was applied to test the difference for the data with the homogeneity of variances (the Levene test). Otherwise, the significance was tested using Tamhane’s in Post hoc multiple comparisons.

Normally, non-metric multidimensional scaling (MDS) was used to examine the pattern of assemblage structure (Pan et al. 2000; Snell and Irvine 2013). MDS using the Euclidean distance was performed to test the similarities among sampling sites based on the physical and chemical variables here. All the physical and chemical variables were square root transformed and normalized before processing MDS. Non-parameter multivariate analysis using permutation tests was carried out to explore the main environmental determinants influencing the temporal dynamics of benthic algae. Distance-based redundancy analysis (db-RDA) were regarded as a most reasonable approach to differentiate the variability in the data according to a complex design or model and based the analysis on a multivariate distance measure for ecological data sets (Legendre and Anderson 1999; Mcardle and Anderson 2001). Relative abundance was square root transformed before conducting db-RDA. The distance measure method was the semi-metric Bray–Curtis distance measure and the criterion of selection procedures was Akaike's Information Criterion. All of these statistical analyses were carried out using the PRIMER software (Clarke and Gorley 2002). ANOVA was done using the software SPSS16.0.

Results

Spatial–temporal dynamics of physical and chemicals

There were site-specific large variations in *t*, EC, TURB, nitrogen (NH₄⁺-N, NO₃-N, and TN), COD_{Mn}, and DOC from 2007 to 2010. For example, *t* showed a great variation from 3.19 to 26.77 °C, and EC varied from 247.00 to 604.60 μs/cm at site 1 (Laoguan River). Considerable variation was also observed for NO₃-N from 0.33 to 3.80 mg/L at site 3 (Xushui River). Similarly, COD_{Mn} and TN showed variation of 0.97–8.27 mg/L and 0.60–3.22 mg/L at site 2 (Jinshui River), respectively. In contrast, the variations were moderate for TP (0.03–0.13 mg/L) and SRP (0.00–0.05 mg/L) at site 1 (Laoguan River).

Non-metric MDS indicated the difference among sampling sites and seasonality (Fig. 2). For example, samples in site 2 spread on the lower right corner, and samples in site 1 were located on the upper left hand side. Meanwhile, seasonality of the main physicals and chemicals were also evident. Samples

Fig. 2 Distribution of sites using non-metric multidimensional scaling based on physical and chemical variables. The first two digits indicate year, and digit in parenthesis indicates sampling site

in winter were located in the upper right corner, and samples in summer were in the bottom. The values of the variables such as t , COD_{Mn} , TN, DOC, Si, and F^- in the high flow season were significantly higher than those in the base flow season ($p<0.01$) (Fig. 3). Comparatively, the major ions such as HCO_3^- , SO_4^{2-} , Ca^{2+} , Mg^{2+} and Na^+ reached to the maximum in November (base flow season) (did not display in Fig. 3).

Benthic diatom community and indices

A total of 185 taxa were identified. There were 3 prostrate and motile dominant species, i.e., *Achnanthydium minutissimum* (10.7 %), *Achnanthydium pyrenaicum* (11.9 %), and *Achnanthydium subatomus* (12.7 %), accounting for more than 5 % in all examined samples (Table 2), other two prostrate species *Eolimna minima* and *Nitzschia dissipata* also prevailed in the base flow season. Few more species such as *Cocconeis placentula* var. *euglypta*, *Diatoma vulgare*, *Encyonema minutum*, *Gomphonema minutum*, *Melosira varians*, and *Navicula capitatoradiata* were abundant at least in one season.

Density of benthic diatom in summer was lower than that in other seasons, but only the difference between summer and autumn, the one between summer and spring were significant ($p<0.05$) (Fig. 4). The Shannon–Wiener diversity, evenness, and species richness of the benthic diatom varied among seasons, but only the difference in evenness between autumn and winter, and species richness between autumn and summer were significant ($p<0.01$) (Fig. 5). The minimum of Shannon–Wiener of 1.57 occurred in April and the maximum was 3.46 in November, and the minimum of evenness was 0.54 in November and the maximum was 0.86 in April. Species

richness, ranging from 14 in July to 61 in November with an average of 30, always peaked in November which was significantly higher than that in summer ($p<0.05$). The maximum density of benthic diatom was 3.10×10^{10} cells/ m^2 occurring in April 2009 and the minimum 1.4×10^9 cells/ m^2 occurred in August, 2009 ($p<0.05$).

The main drivers based on db-RDA analysis

The main physicals and chemicals influencing the seasonal change of benthic diatom were derived using db-RDA analysis (Fig. 6). The first two axes explained from 45.9 % to 65.0 % of the total variation in benthic diatom assemblage in the seven selected rivers. Organic pollution (indicated by COD_{Mn} and DOC), nitrogen, ions and t were the primary factors explaining benthic diatom community composition, even though their relative importance varied among the sampling sites. The first two axes accounted for 45.9 % of variation of diatom community and NH_4^+-N , t and ions were the primary factors in Laoguan River (Site 1). The first two axes explained 65.0 % of total variation, and NH_4^+-N , COD_{Mn} and ions were the main factors to explain variation of benthic diatom communities in the Jinshui River (Site 2). Similarly, benthic diatom community was affected by TN and ions such as Si, Mg^{2+} in Xushui River (Site 3), and the first two axes explained 50.3 % of total variation. As for Jushui River (Site 4), the main drivers accounting for the variation of the benthic diatom community were COD_{Mn} , t and ions (SO_4^{2-} , Na^+). EC and DOC were the main factors influencing the diatom community in Yue River (Site 5). In addition to pH or F^- , NH_4^+-N , and TN were the main influencing environmental factors in Shu River (Site 6) and Jiangjun River (Site 7).

Fig. 3 The seasonal variation of environmental variables in the selected sampling sites of the upper Han River, China

Table 2 The average of relative abundance in benthic diatoms at the 7 sites in the upper Han River basin from 2007 to 2010

Dominant species	Growth form ^a	Base flow	High flow	Spring (April)	Summer (July)	Autumn (November)	Winter (January)
<i>Achnanthes minutissimum</i> (Kuezing) Czarnecki	P, M	10.56	10.97	10.64	10.97	10.70	10.34
<i>Achnanthes pyrenaicum</i> (Hustedt) H. kobayasi	P, M	11.88	12.54	12.65	12.54	11.18	11.82
<i>Achnanthes subatomus</i> Lange-Bertalot	P, M	11.06	14.6	10.66	14.6	13.16	9.36
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehr.) Grun	P	4.18	1.48	3.11	1.48	7.29	2.15
<i>Diatoma vulgare</i> Bory	U	2.10	0.14	0.43	0.14	0.61	5.27
<i>Encyonema minutum</i> (Hilse) D.G. Mann	S	2.50	0.71	5.14	0.71	0.63	1.74
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	P	5.00	3.60	3.56	3.60	8.29	3.15
<i>Gomphonema minutum</i> (Ag.) Agardh f. <i>minutum</i>	S	4.06	2.12	3.47	2.12	2.25	6.45
<i>Melosira varians</i> Agardh	U	3.99	1.98	6.37	1.98	2.70	2.89
<i>Navicula capitatoradiata</i> Germain	P	4.44	1.19	4.37	1.19	3.85	5.09
<i>Nitzschia dissipata</i> Hustedt	P, M	9.49	0.36	8.87	0.36	6.07	13.54
<i>Sellaphora seminulum</i>	P			0.08	0.13	0.05	0.00

Values in italics mean the relative abundance of species accounting for more than 5.0 % of total abundance

^a P prostrate, S stalked, U unattached, M motile (Wang and Stevenson 2005)

Discussions

As a primary producer in aquatic ecosystems, benthic diatoms are functioning as an important component in sustaining health aquatic ecosystems. A number of studies have reported that composition and structure of benthic diatom are responsive to various environmental variables (e.g., chemicals and physicals) and their changes in the aquatic environment (Rosemond et al. 2000; Blinn and Bailey 2001; Leland et al. 2001; Potapova and Charles 2003), primarily the effects on spatial distribution of benthic algal assemblages in lakes, glacier rivers, and forest streams in temperate and tropical zones (Mosisch and Bunn 1997; Leland et al. 2001; Haydée et al. 2002; Carr et al. 2005; Davies et al. 2008).

Shannon–Wiener diversity index reflect the abundance of the species richness and take into account the evenness of

individuals as well (Shannon and Weaver 1949). There is no significant difference in the Shannon–Wiener diversity among the four seasons, but the difference in the evenness and species richness are significant (Fig. 5). It means that even diversity index are stable, but the abundance of individual species differ among seasons. Similarly, the species abundance is significantly lower in summer than that in autumn. It implies that the high nutrient concentration like NO₃-N, DOC, TN and Si (necessary for diatom grow and reproduce) cannot guarantee a large number of species. So we speculate that other variables such as flood may have greater influence on diatom community indicating by the lowest density in high flow season (Fig. 4).

In riverine systems, in particular in the monsoon environment, hydrologic regime such as current flow or spate interval is probably one of the most important factors influencing the assemblage structure, spatial distribution, and succession of diatom community (Gregory and Rounick 1981; Biggs 2000; Bergey and Resh 2006). However, the db-RDA analysis does not indicate that flow velocity in the sampling streams is a primary factor influencing benthic diatoms in the upper Han River (Fig. 6). We speculate that the diatom community has been more responding to flood pulse in particular in flooding season (Biggs and Close 1989; Bergey and Resh 2006). Also, the minimum of density in summer (Fig. 4) is consistent with this results that the flood can dramatically decrease the standing crop of benthic algae because of the flush on substratum and contributes to the variation of species in terms of growth form (Biggs 1995; Bergey and Resh 2006; Yang et al. 2009).

Hydrological regime like flood or current velocity determines the growth form such as adnate or prostrate, erect of dominant species to some extent (Wang and Stevenson 2005; Mosisch and Bunn 1997), and it contributes to the succession

Fig. 4 Comparison of density and chlorophyll *a* content of benthic algae among different seasons

Fig. 5 Comparison of the Shannon–Weaver diversity index, evenness and richness of benthic diatom among different seasons

Fig. 6 db-RDA plots of first and second axes relating environmental drivers over benthic diatom assemblage structure

of benthic diatom assemblages (Yang et al. 2009). The genus *Achnanthes* is cosmopolitan and normally prevails among diatoms in subtropical rivers or lakes (Tang et al. 2006; Yang et al. 2009; Tan et al. 2012). There is no exception in this study, and three species, *A. minutissimum*, *A. pyrenaicum* and *A. subatomus* are the dominant species, i.e., accounting for more than 5 % of the diatom abundance, and also they are the only species dominating in summer (i.e., high flow season) (Table 2). Comparatively, few more species including *Diatoma vulgare* (unattached) or *Gomphonema minutum* (stalked) are also rich in winter and spring (base flow period) (Table 2), because their erect type attaching to the substrate by stalks or stems are not able to dominate in high flow period (Dodds and Biggs 2002; Wang and Stevenson 2005). Thus, it seems that dominance of some diatom species is determined by the interaction of hydrological regime and other factors such as nutrient concentration as following we would discuss. The importance of hydrological regime has also been evidence by the seasonal variations in density of benthic diatom. The density of benthic diatoms and species richness are higher in the base flow period than those in high flow period (Figs. 4, 5; Yang et al. 2009). While in spring, increase in *t* may have resulted in the higher density of benthic diatoms, compared to other seasons (Fig. 4).

A number of chemicals have also been reported to contribute to the temporal dynamics of benthic diatom community, and variation of benthic diatom community is attributable to the changes in chemical variables including $\text{NO}_3\text{-N}$, COD_{Mn} , TN, DOC, and ions (Biggs 2000; Uehlinger et al. 2010; Vörösmarty et al. 2010; Tan et al. 2013). In the upper Han River, there are large variations in water chemicals (Figs. 2, 3; Li et al. 2008, 2009a). Diatom composition has been responding to the variabilities in water chemicals including nitrogen, organic pollutants, *t*, pH, EC by the db-RDA analysis (Fig. 6) and diatom based indices (Tan et al. 2013). However, the responses of diatom composition to the chemicals are site-specific due to the spatial variations in water chemicals among the sampling sites (Fig. 3).

Nitrogen including either $\text{NH}_4^+\text{-N}$, NO_3^- , or TN is the primary factors influencing diatom composition in all sampling sites excepting sites 4 and 5 (Jushui River and Yue River). Organic pollutions (e.g., COD_{Mn} and DOC) are one of the primary determinants of diatom structure in Jinshui River, Jushui River, and Yue River. Other factors such as *t*, pH, and EC also influence benthic diatom community composition in few sites, even though their relative importance varies among the sampling sites. Surprisingly, phosphorus has been the limiting factor for algae growing, but they are not significantly affecting diatom composition in the upper Han River, which is probably due to their small variability.

The intensive anthropogenic activities such as fertilizer practices from agriculture practices in its respective upstreams contribute to the nitrogen loading (Matson et al.

1997; Li et al. 2009b; Kireta et al. 2012). Also, leaching from the soil in the cultivated land in high flow season determines interannual variations of nitrogen in waterways to some extent. Land use and land cover as an explanatory variable has been shown to affect the structure, biomass of benthic algae (Carr et al 2005; O'Brien and Wehr 2010; Montuelle et al. 2010; Cooper et al. 2012), and in most cases, its effects were indirect through inducing changes in the aquatic environment (Blinn and Bailey 2001; Schmitt 2005). The results in this study agree with the previous studies. The ions such as Ca^{2+} , HCO_3^- , Mg^{2+} and SO_4^{2-} are mainly controlled by the natural weathering in the study area (Li et al. 2008, 2009a). Therefore, the main determinants of benthic diatom communities variation result from a combination of natural weathering process, land use change caused by human activities, especially seasonal agricultural practices, and flood pulse in their respective upstream uplands.

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